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DEVELOPMENT OF INNOVATIVE AND HIGH NUTRITIONAL VALUE FOODS FROM NATIVE LEGUME SPECIES

DOCTORADO EN TÉCNICAS AVANZADAS EN INVESTIGACIÓN Y DESARROLLO AGRARIO Y ALIMENTARIO

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Dª. PERLA AZUCENA GÓMEZ DI MARCO, Directora, y D. FRANCISCO DE ASÍS ARTÉS HERNÁNEZ, Codirector, de la Tesis doctoral "DEVELOPMENT OF INNOVATIVE AND HIGH NUTRITIONAL VALUE FOODS FROM NATIVE LEGUME SPECIES".

INFORMAN:

Que la referida Tesis Doctoral, ha sido realizada por Dª. ELENA COLLADO MARÍN, dentro del Programa de Doctorado TÉCNICAS AVANZADAS EN INVESTIGACIÓN Y DESARROLLO AGRARIO Y ALIMENTARIO, dando nuestra conformidad para que sea presentada ante el Comité de Dirección de la Escuela Internacional de Doctorado para ser autorizado su depósito.

X Informe positivo sobre el plan de investigación y documento de actividades del doctorando/a emitido por el Director/ Tutor (**RAPI**).

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INDEX

ABSTRACT

There is nowadays an important trend in the habits of human food consumption in which natural, healthy, ready-to-eat and safe elaborates are required. An example of this trend is the development of fresh-cut or minimally processed fruit and vegetables. Legumes are an excellent source of many essential nutrients, including proteins and aminoacids, minerals, fiber, vitamins, slow-assimilation carbohydrates and other bioactive compounds, and are low in calories and fat. However, the bioavailability of some nutrients can be reduced by several legume compounds, which are considered to be antinutritional compounds. Nevertheless, such compounds have also been reported to have beneficial health properties. Therefore, the consumption of legumes should be promoted by looking for new ways of presentation in where the product can be consumed fresh and cooked in a suitable container.

Legumes are usually consumed as fresh seeds. However, different cooking methods can also be used. This food preparation may cause changes in texture, colour, flavour, or bioactive content. One of these methods is microwave cooking, in which a healthy product could be obtained in a short time without loosing quality.

One of the most critical steps in the development of fresh-cut products is disinfection. Although the use of NaOCl is widespread in the industry due to its antimicrobial activity and low cost, it is examined due to the appearance of toxic by-products. Therefore, the study of alternative sanitizing methods is necessary. Others disinfectants, like acidified sodium chlorite (ASC) have been largely applied for the prevention of enzymatic and non-enzymatic browning and, in less extent, to reduce microbial growth at levels that did not adversely affect the sensory quality. Furthermore, UV light can also be an alternative, useful for superficial decontamination, because UV light acts as an antimicrobial agent directly by damaging the microbial DNA and indirectly due to the stimulation of defence mechanisms in vegetables against pathogens, retarding decay and delaying senescence. In addition, edible antimicrobial films or coatings can avoid enzymatic browning and improve quality, safety, shelf life and functionality of food products by reducing moisture transfer, respiration rate and oxidative processes, while minimizing both spoilage and pathogenic microorganisms.

The general objective of this Thesis was to optimize processing steps to develop new fresh and processed foods from native varieties of three legumes species (faba beans, peas and cowpeas). Such legumes are well adapted to several European climates and with high nutritional quality and high content in bioactive compounds. In that way, food of local origin with high protein content could be easily included in the human diet.

For fresh produce disinfection, the effects of NaOCl (100 ppm) or alternatively ASC (300 ppm) stored under modified atmosphere packaging at 1 or 4ºC, on quality of fresh-cut immature pea seeds were evaluated. Disinfection with ASC resulted in better sensory quality, higher content of vitamin C and lower psychrofiles counts. Immature pea seeds could be stored up to 14 days at 1–4ºC under MAP with only minor quality changes.

Subsequently, the effect of different sanitizers (NaOCl (150 ppm), NaOCl + an edible coating based of sucrose fatty acid esters (EC) and UV–C (3 kJ m^{-2}, 90s)) on quality changes of minimally processed faba seeds stored for 10 days at 4ºC were studied. Periodically, samples were microwaved (700 W, 1 min) to obtain a ready to eat food. The EC treatment showed a positive effect on vitamin C, total phenolics content and tannins content retention, whereas UV-C treated samples showed the highest sugars content values. Additionally, EC or UV-C treatments extended the shelf life of fresh-cut faba seeds from 7 to 10 days at 4ºC regarding NaOCl treatment. As expected, microwaving decreased the concentration of bioactive compounds, but retained the quality of faba seeds allowing to obtain a ready to eat tasteful food.

Furthermore, the quality of fresh-cut cowpea, prepared to be eaten raw (immature seeds) or microwaved (seeds and pods), was also evaluated. Fresh cowpea pods were washed with NaClO (150 ppm, pH 6.5, 2 min, 4ºC) and stored for 21 days at 8°C under modified atmosphere packaging (23 kPa CO_2/1.5 kPa O_2 and 19 kPa CO_2/1.2 kPa O_2). Additionally, seeds obtained from hulled pods were also disinfected, packaged and stored for 7 days at 4ºC. Total phenolic content (TPC), total equivalent antioxidant capacity (TEAC), sugars and sensory attributes, were evaluated. TPC and TEAC increased after microwaving (700 W, 1 min) for both seeds and pods. Concentration of sucrose and glucose increased after microwaving, while raffinose was not detected after cooking. According to sensory quality, fresh and microwaved seeds maintained all the

evaluated attributes above the limit of usability after 7 days at 4ºC, while pods were edible up to 14 days at 8ºC.

Finally, the effects of a UV-C treatment (3 kJ m^{-2}), compared with non-illuminated beans, were studied on the sensory and microbial quality and bioactive and anti-nutritional content of fresh-cut and then microwaved faba beans. UV-C treatment extended the fresh-cut faba bean shelf life from 7 to 10 days at 5ºC. Nevertheless, UV-C improved the condensed tannins reductions through storage compared with non-irradiated samples. Microwaving reduced the phytic acid and condensed tannins contents.

Since the general objective of this research is to optimize several processes to develop new fresh and processed foods from native varieties of three legume species (faba, pea and cowpea), to stimulate the consumption of these in the daily human diet, both for fresh and microwave consumption, It can be said that with the use of various minimal processing techniques using NaOCl alone or in combination with edible coatings, or alternatively with chemical (ASC) or physical (UV-C) disinfectants, vegetable products with high nutritional quality and high content of bioactive compounds, fresh and ready to eat, can be obtained.

RESUMEN

Hoy en día existe una importante tendencia en los hábitos de consumo caracterizada por una demanda creciente de alimentos naturales, más sanos, listos para el consumo y seguros. Un ejemplo de esta tendencia es el desarrollo de frutas y hortalizas mínimamente procesadas. Dentro de estas últimas, las legumbres son una excelente fuente de muchos nutrientes esenciales, incluyendo proteínas y aminoácidos, minerales, fibra, vitaminas, carbohidratos de asimilación lenta y otros compuestos bioactivos, y son bajas en calorías y grasas. Sin embargo, la biodisponibilidad de algunos nutrientes puede verse reducida por diversos compuestos presentes en las legumbres, considerados como compuestos antinutricionales. No obstante, muchos de estos compuestos también han sido identificados como beneficiosos para la salud. Por lo tanto, se debe promover el consumo de legumbres buscando nuevas formas de presentación en las que el producto pueda consumirse fresco y además poder cocinarse en un envase adecuado.

Las legumbres se consumen generalmente en forma de semillas frescas, como productos mínimamente procesados. Sin embargo, también se pueden utilizar diferentes métodos de cocción. Esta preparación de alimentos puede causar cambios en la textura, color, sabor o en el contenido de compuestos bioactivos. Uno de estos métodos es la cocción en microondas, en la que se puede cocinar un producto sano en poco tiempo sin perder mucha calidad.

Uno de los pasos más críticos en el desarrollo de los productos mínimamente procesados es la desinfección. Aunque la utilización del hipoclorito sódico (NaOCl) está muy extendida en la industria debido a su actividad antimicrobiana y su bajo costo, su uso se encuentra en entredicho debido a la formación de subproductos tóxicos. Por lo tanto, es necesario el desarrollo de métodos alternativos de desinfección. Otros desinfectantes, como el clorito sódico acidificado (ASC), se han aplicado ampliamente para la prevención del pardeamiento enzimático y no enzimático y, en menor medida, para reducir el crecimiento microbiano a niveles que no afecten negativamente al sabor y el aroma de los productos vegetales. Además, la utilización de luz UV también puede ser una alternativa al NaOCl, ya que es efectiva para la descontaminación superficial, debido a que actúa como agente antimicrobiano tanto directamente, dañando el ADN microbiano, como indirectamente debido a la estimulación de los mecanismos de

defensa de las hortalizas contra los patógenos, retardando la descomposición y retrasando la senescencia. Así mismo, las películas o recubrimientos antimicrobianos comestibles pueden evitar el pardeamiento y mejorar la calidad, la inocuidad, la vida útil y la funcionalidad de los productos alimenticios al reducir la transferencia de humedad, la frecuencia respiratoria y los procesos oxidativos, al tiempo que se reducen al mínimo tanto el deterioro como los microorganismos patógenos.

El objetivo general de esta investigación fue optimizar los principales procesos para desarrollar nuevos alimentos frescos y procesados a partir de variedades nativas de tres especies de leguminosas (habas, guisantes y caupí), bien adaptados a diferentes climas europeos y con alta calidad nutricional y alto contenido en compuestos bioactivos. De este modo, alimentos ricos en proteína de origen local podrían ser incorporados más fácilmente a la dieta, mejorando asó la diversidad de las zonas productoras.

En este trabajo se evaluaron los efectos de la desinfección con NaOCl (100 ppm) o alternativamente con clorito sódico acidificado (ASC) (300 ppm), y el envasado en atmósfera modificada pasiva (MAP) a 1 o 4ºC, sobre la calidad general de las semillas de guisantes inmaduras mínimamente procesadas. La desinfección con ASC resultó en una mejor calidad sensorial, un mayor contenido de vitamina C y un menor recuento en recuento de psicrófilos. Las semillas de guisantes inmaduras pueden almacenarse durante 14 días a 1-4ºC bajo MAP, con leve impacto en su calidad.

Posteriormente, se estudió el efecto de diferentes desinfectantes (NaOCl (150 ppm), NaOCl + recubrimiento comestible a base de ésteres de ácidos grasos de sacarosa (EC), y UV-C (3 kJ m^{-2}, 90s)) sobre los cambios de calidad de las semillas de haba mínimamente procesadas almacenadas durante 10 días a 4ºC. Periódicamente, las muestras se cocinaron en microondas (700 W, 1 min) para obtener un alimento listo para el consumo. El tratamiento con EC mostró un efecto positivo sobre la vitamina C, el contenido total de fenoles (TPC) y la retención del contenido de taninos, mientras que las muestras tratadas con UV mostraron los valores más altos de contenido en azúcares. Además, los tratamientos de EC y UV extendieron la vida útil de las semillas de haba mínimamente procesadas de 7 a 10 días a 4ºC con respecto al tratamiento con NaOCl. Como era de esperar, el microondas redujo la concentración en compuestos bioactivos,

pero mantuvo la calidad de las semillas de haba, lo que permitió obtener un alimento sabroso listo para consumir.

Otro de los objetivos de este trabajo fue evaluar la calidad de caupí mínimamente procesado listo para su consumo en crudo (semillas inmaduras) o cocinado en microondas (semillas y vainas). Las vainas de caupí frescas se lavaron con NaOCl (150 ppm) y se almacenaron durante 21 días a 8°C bajo atmósfera modificada (23 kPa CO_2/1.5 kPa O_2 and 19 kPa CO_2/1.2 kPa O_2), mientras que las semillas también fueron desinfectadas, envasadas y almacenadas durante 7 días a 4ºC. Se evaluó el TPC, la capacidad antioxidante equivalente total (TEAC), los azúcares y la calidad sensorial. El TPC y el TEAC aumentaron sus concentraciones después del cocinado con microondas tanto para las semillas como para las vainas. La concentración de sacarosa y glucosa aumentó después del microondado, mientras que la rafinosa no se detectó después de la cocción. Con respecto a la calidad sensorial, las semillas frescas y microondadas mantuvieron todos los atributos evaluados por encima del límite de aceptabilidad después de 7 días a 4ºC, mientras que las vainas fueron comestibles hasta 14 días a 8ºC.

Finalmente, se estudiaron los efectos del tratamiento UV-C (3 kJ m^{-2}), en comparación con semillas no tratadas, sobre la calidad sensorial y microbiana, y el contenido bioactivo y de antinutricionales de las semillas de haba mínimamente procesadas en fresco y cocinadas en microondas. El tratamiento UV-C extendió la vida útil de las semillas de haba frescas de 7 a 10 días a 5ºC. Además, el UV-C redujo la concentración de taninos condensados en comparación con las muestras no irradiadas. Así mismo, el cocinado con microondas redujo el contenido de ácido fítico y de taninos condensados.

Teniendo en cuenta que el objetivo general de esta investigación es optimizar varios procesos para desarrollar nuevos alimentos frescos y procesados a partir de variedades nativas de tres especies de legumbres (habas, guisantes y caupí), para estimular el consumo de éstas en la dieta humana diaria, tanto para su consumo en fresco como microondado, se puede decir que con el uso de diversas técnicas de procesamiento mínimo utilizando NaOCl solo o en combinación con recubrimientos comestibles, o alternativamente con desinfectantes químicos (ASC) o físicos (UV-C), se pueden obtener productos vegetales con alta calidad nutricional y alto contenido de compuestos bioactivos, frescos y listos para consumir.

LIST OF ABBREVIATIONS

®: registered trade Mark

µM: micromolar

µmol: micromole

λmax: maximum w avelength

µg: microgram

µm: micrometer

AA: ascorbic acid

ABTS: 2,2´-azino-bis (3-thylbenzothiazoline-6-sulphonic acid)

AITC: allyl isothiocyanate

ANOVA: analysis of variance

ASTM: American Society for Testing and Materials

AOAC: Association of Official Agricultural Chemists

ASC: acidified sodium chlorite

b.C: before Christ

BITC: benzylisothiocyanate

BOPP: bi–oriented polypropylene

C*: chroma

C: Celsius

CA: citric acid

CFU: colony forming units

CIE Lab: Lab colour space, International Commission on Illumination

cm: centimeters

CNPq: Council for Scientific and Technological Development (Brazil)

CTRL: control

cvs: cultivars

DAD: diode array detector

DHA: dehydroascorbic acid

DKG: diketogulonic acid

DNA: desoxyribonucleic acid

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

EC: edible coating

EU: European Union

FAO: Food and Agriculture Organization of the United Nations

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

FRAP: ferric reducing antioxidant power

fw: fresh weight

g: gram

GAE: gallic acid equivalents

GC: gas chromatograph

GMP: good manufacturing practices

GRAS: generally regarded as safe

H : hue

HPLC: high-performance (pressure) liquid chromatography

IBV: Instituto de Biotecnología Vegetal (Cartagena, Spain)

ISO: International Organization for Standardization

kg: kilogram

kJ: kilojoules

L*: lightness

LC: liquid chromatography

log: logarithm

MAGRAMA: Ministerio de Agricultura, Alimentación y Medio Ambiente (Spain)

MAP: modified atmosphere packaging

MP: minimally processed

mg: milligram

min: minute

mL: milliliter

mm: millimeter

mM: millimolar

MW: microwave

NAD: dinucleotide phosphate

NADH: monodehydroascorbate reductase

NADPH: nicotinamida adenina dinucleótido fosfato

NaOCl: sodium hypoclorite

rpm: revolutions per minute

PCA: plate count modified agar

pH: potential hydrogen

RD: Real Decreto (Spain)

RH: relative humidity

ROS: reactive oxygen species

SAR: specific absorption rate

SD: standard deviation

TAC: total antioxidant capacity

TEAC: trolox equivalent antioxidant capacity

TPC: total phenolic content

TPTZ: 2,4,6-tripyridyl-s-triazine

UHPLC: ultra high-performance (pressure) liquid chromatography

UPCT: Universidad Politécnica de Cartagena

UK: United Kingdom

USA: United States of America

UV: UV-C light

var: variety

VRBD: violet red bile dextrose

WHO: World Health Organization

Y+M: yeasts and moulds

INTRODUCTION

INTRODUCTION

1. BOTANICAL AND AGRONOMICAL CHARACTERISTICS OF FABA BEANS, PEAS AND COWPEA

1.1. Faba beans

Faba bean (*Vicia faba* L.) is a legume originated in western Asia, from where it was spread to Europe, Africa and central Asia by humans. This legume has been known since Neolithic and served as food for the Mediterranean basin. In northern countries, it was later used in the bronze and iron age. It is an annual plant, not very resources demanding and highly productive. It presents wide morphological diversity and the different cultivars show these variations. The seeds (1-3 cm diameter) are oblong to ovoid in shape (Figure 1). The color of the seeds ranges from brown to red or green. They are typical plants of warn zones for autumn and winter crops and in cold ones they must be sown in spring (Leguminosae, 2012).

Figure 1. Plant of Vicia faba (*in Otto Wilhelm Thomé, Flora von Deutschland, Österreich und der Schweiz, 1885*).

To obtain seeds aimed for fresh consumption, the pods must be harvested at their optimum stage, before they become too lignified. Pods must be around 20 cm long, 1.5 cm thick, bright green, fresh in appearance and without very pronounced bumps that would indicate over-maturity (Figure 2). Seeds must be attached to the pod and when separated from each other they must show a green hilium (the presence of black color indicates over-maturity). The seeds must be soft and should have a *testa* with little amount of fiber (Leguminosae, 2012).

Faba bean is widely grown for its nutritious seeds and pods. As well as being an important food source for humans, the high protein content of faba beans means that it is used for animal feed. The area of production of faba beans in Spain was 22.800 ha in 2019, with a total production of 31.000 t (MAGRAMA, 2019).

Figure 2. Flowers (left) and pods (right) of Vicia faba

1.2. Peas

Peas (*Pisun sativum* L.), a very polymorphic specie that appeared in the Mediterranean or in the Middle East. Quoted by Columela, the roman empire´s main agricultural popularize, there are traces of its non-domesticated consumption by hunter-gatherers from Central Europe during the late Neolithic period. Peas, like many legumes, have the

ability to fix nitrogen from the air through a symbiotic relationship with bacteria housed in root nodules.

Pisum sativum L. is a climbing annual plant, with pods oblong-obovate, whitish or yellowish when ripe, and with 6-10 globular seeds in each pod (Figure 3), harvested, for fresh consumption at immature physiological stage, when the pods are almost round and with the seeds 70% of full size. Their quality indices include that peas should be uniformly bright green, fully turgid, clean and free from any damage (Anurag et al., 2016)

The cultivated area of peas in Spain was 144.900 ha in 2018, with a production of 174.000 t (MAGRAMA, 2019).

Figure 3. Plant of Pisum sativum (in *Otto Wilhelm Thomé, Flora von Deutschland, Österreich und der Schweiz, 1885*).

1.3. Cowpea

Cowpea (*Vigna unguiculata L.*) was first domesticated in West Africa 5.000-6.000 years ago and was introduced to Europe around 300 b.C. The Spanish introduced

cowpea to tropical America in the 17th century and now is grown widely in USA, the Caribbean region and Brazil. Cowpea can be either annual or perennial plant and some types are erected while other are climbing plants (Figure 4). Their fruits are cylindrical seeds with 8 to 30 cm long pods (in some cases 120 cm long), pale brown when ripe and bears 8-30 seeds. The seeds are oblong to globose 0.5-1 cm long and can be black, brown, pink or white (Figure 5). The helium is oblong, covered by a white tissue with a blackish edge. It is considered a rustic and resistant plant, mainly due to its tolerance to drought, pests and fungi and not very demanding in fertilizers (Carvalho et al., 2017).

Cowpea can be harvested at three different stages of maturity: a) green snaps, b) green-mature, and c) dry. Depending on temperature and fresh-market demand, peas are ready for harvest 16 to 17 days after bloom (60 to 90 days after planting). Cowpea characteristics vary widely being the grain size the most important single factor influencing price.

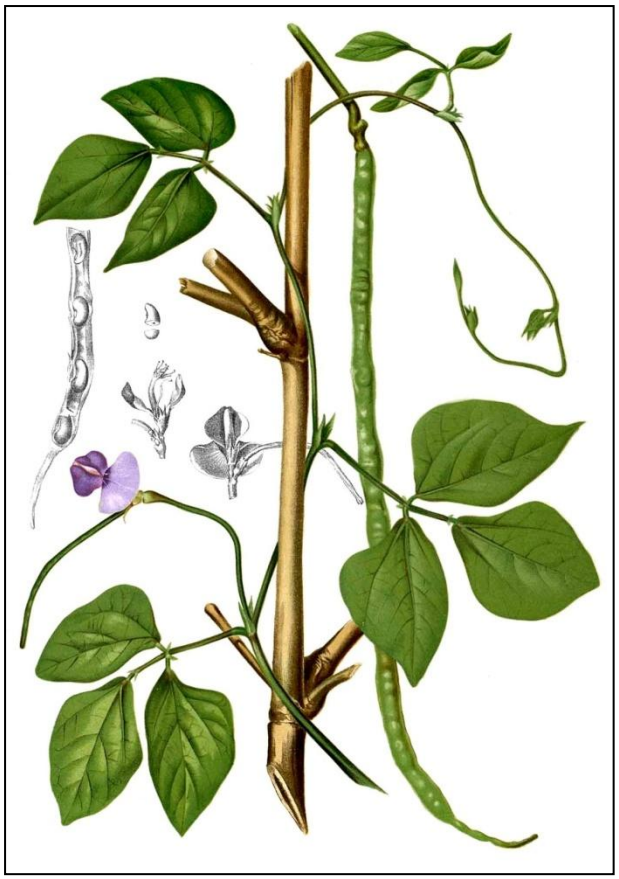

Figure 4. Plant of Vigna unguiculata (http.bibdigital.rjb.csic.esspaindex.php)

Figure 5. Flowers (left), fresh pods (centre) and dry pods (right) of Vigna unguiculata L.

2. NUTRITIONAL, BIOACTIVE AND NON-NUTRITIONAL COMPOSITION OF FABA BEANS, PEAS AND COWPEA

2.1. Nutritional compounds of legumes

Legumes have been an essential part of the human diet for centuries. They are quite similar in their composition but they vary in compounds concentration among different pulse species and varieties (Table 1). They are an excellent source of many essential nutrients, including proteins and aminoacids, minerals, fiber, vitamins, slow assimilation carbohydrates and other bioactive compounds, and are low in calories and fat (Dahl et al., 2012; Bouchenak et al., 2013; Singh et al., 2017). They are considered nutritionally recommended considering their composition. For that reason, legumes should be consumed as a part of a healthy diet to combat obesity and to prevent diseases like diabetes, heart disease and cancer (Tharanathan et al., 2003; Trinidad et al., 2010).

Table 1. Main constituents of faba beans, peas and cowpea. Data obtained from the USDA-ARS (2018).

Constituents	Content (per 100 g^{-1} fw)		
	FABA BEANS	**PEAS**	**COWPEA**
Energy (kcal)	50.4	90.7	116
Water (g)	85.1	76.0	80
Proteins (g)	5.4	6.9	7.73
Carbohydrates (g)	4.2	11.3	20.76
Fibre (g)	5.1	4.9	6.5
Fats (g)	0.2	3.6	0.53
Vitamins			
Vitamin B1 (mg)	0.2	0.29	0.202
Vitamin B2 (mg)	0.1	0.16	0.055
Vitamin B3 (mg)	4.1	4.1	0.495
Vitamin B6 (mg)	0.06	0.17	0.1
Vitamin C (mg)	24	21.9	2.5
Vitamin E (mg)	0.46	0.23	0.28
Vitamin K (µg)	43	29	1.7
Minerals			
Ca (mg)	23	25.4	24
Fe (mg)	1.8	1.9	2.51
Mg (mg)	28	32.2	53
Zn (mg)	0.9	0.7	1.29
Na (mg)	18	2	4
K (mg)	210	247	278
P (mg)	98	113	156
Mn (µg)	0.22	0.35	0.475

However, the consumption is limited due to the presence of a series of non-nutritional compounds (Table 2) that could adversely affect the digestibility of proteins and carbohydrates, interfering with mineral bioavailability, inhibiting some enzymes, making nutrients unavailable, producing intestinal gas and can also be toxic in some cases (Bouchenak et al., 2013). These compounds have historically been known as 'antinutritional factors'. Nevertheless, nowadays, there are evidences that they can play

a beneficial role for human health when they are consumed in the right proportion and frequency (Singh et al., 2017). Therefore, in recent times these compounds are also known as 'non-nutritive' or 'non-nutritional' factors'.

Some of these substances play an important role in plant defence mechanisms or as reserve compounds. They also provide several biological properties including antioxidant, anti-inflammatory, anti-atherogenic and antimicrobial effects (Balasundran et al., 2006). In essence, non-nutritional factors in pulses can potentially have beneficial effects in human health.

Table 2. Main non-nutritive factors of faba beans, peas and cowpea and their effect on humans.

NON-NUTRITIONAL FACTOR	PHYSIOLOGYCAL EFFECTS	BENEFITS	CONCENTRATION (in faba beans)
SAPONINS	- Decreases nutrient absorption - Depression of growth	- Antimicrobial effect - Decreases cholesterol concentration - Positive effect on the intestinal tract	0.4 g 100 g^{-1} Savage and Deo (1989)
TANNINS	- Decreases availability of minerals, proteins and starch	- Antioxidant activity - Decreased blood glucose concentration - Antimicrobial effect	5 - 10 g kg^{-1} Vilariño et al. (2009)
RAFFINOSE	- Gas formation in the colon	- Prebiotic effect - Increased solubility of minerals	4.03 mg g^{-1} Goyoaga et al. (2011)
PHYTIC ACID	- Decreases availability of minerals, proteins and starch	- Toxic metal bonding - Delayed glycemic response	3.20 mg g^{-1} Adamidou et al. (2011)

2.2. Main nutritive and bioactive compounds of faba beans, peas and cowpea

2.2.1. Vitamin C

Vitamin C or ascorbic acid (AA) is one of the simplest structured vitamins, as it is the lactone of an acid-sugar. Ascorbic acid is needed in the diet of a few vertebrates, including humans. This is due to the lack of the enzyme flavoenzyme L-guluno-1,4-

lactone oxidase (Davey et al., 2000; Nelson et al., 2005). Deficiencies of AA are responsible of scurvy.

It is a potent reducer, it is stable in dry form but in solution it easily loses hydrogen atoms and transforms into dehydroascorbic acid (DHA), which also possesses vitamin C activity. However, vitamin activity is lost when the laconic ring of dehydroascorbic acid is hydrolyzed to 2,3-diketogulonic acid (2,3-DKG) (Figures 5 and 6). Various factors such as concentration, temperature, light, pH, etc., influence on the oxidation of L-AA and ADHA hydrolysis. ADHA and specially 2,3-DKG acid have very limited antiscorbutic activity and in some cases do not even have it. L-AA, however, has three types of biological activity: enzyme co-factor, free radical neutralizer, and as a donor/catcher in electron transport in plasma membranes or chloroplasts (Davey et al., 2000). It is the main antioxidant present in plant cells, fulfilling a vital function in the elimination of reactive oxygen species (ROS) by means of both enzymatic and non-enzymatic detoxification.

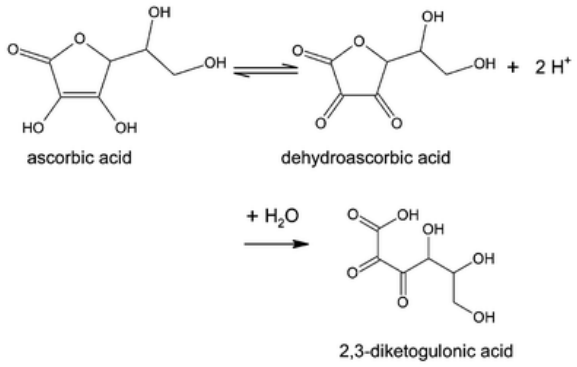

Figure 6. Chemical formulations of ascorbic acid, dehydroascorbic acid and 2,3-diketogulonic acidCITA?

Large amounts of ascorbic acid can be found in vegetable tissues, being one of the major vitamins in beans, peas and cowpea.

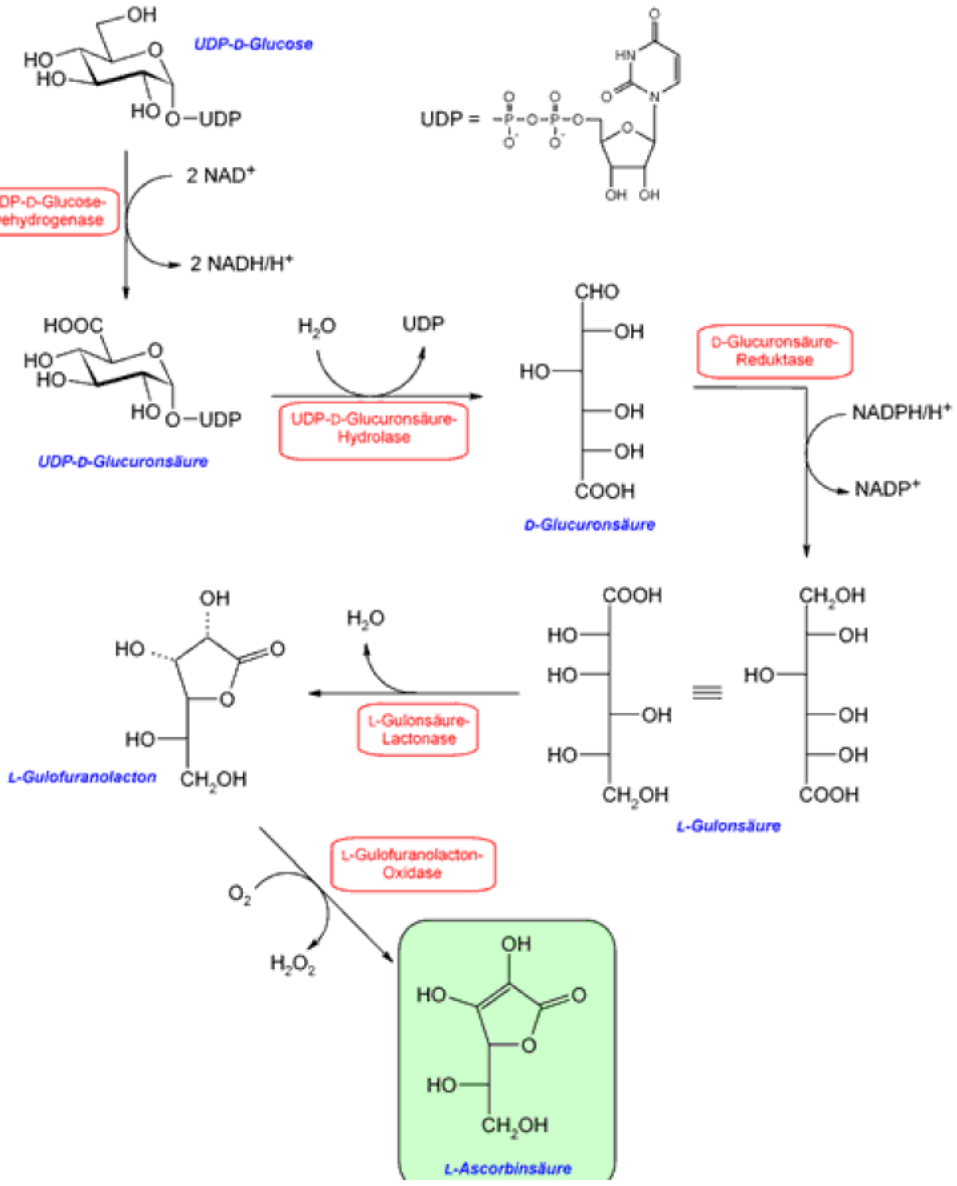

Figure 7. Biosynthetic pathway of ascorbic acid (www. acidoascorbico.com)

2.2.2. Phenolic compounds

Phenolic compounds or polyphenols constitute a broad group of chemical substances characterized by having a benzene ring with one or more hydroxyl groups. In nature, phenols are found together with sugars and organic acids (Cartea et al., 2011). Therefore, the most common way to find them in nature is in the form of glycosides (Martínez-Valverde et al., 2000). Concentration of some phenolic compounds in immature seeds of faba bean, peas and cowpea is shown in Tables 3, 4 and 5.

Its main functions in plant cells are to act as essential metabolites for the growth and reproduction of plants, and as a defense mechanism against pathogens. Phenolic

compounds have a wide effect in human health as these can contribute towards the antioxidant activities and they have many bioactive properties such as antimicrobial, anticancer, anti-inflammatory and reduction in heart and brain diseases (Chaieb et al., 2011; Singh et al., 2017). Phenolic compounds are related to the sensory quality of fruit and vegetables, as their oxidation causes enzymatic browning in food, a phenomenon of great importance as a quality parameter. Likewise, a type of phenolic compounds, condensed tannins, is responsible for the astringency of many vegetable foods (Martínez-Valverde et al., 2000).

Phenolic acids and their derivate flavanols, flavan-3-ols, anthocyanins, and condensed tannins are the main polyphenol compounds present in legumes. Flavonoids, phenolic acids and procyanidins are the dominant phenolic compounds present in peas and faba beans (Zhang et al., 2015).

Phenolic compounds can be grouped, depending on their basic chemical structure, into flavonoids and non-flavonoids. The former are characterized by sharing a basic structure of two benzene rings linked by a heterocyclic ring of pyrone C, (flavonols, flavones, falvan-3-ols, anthocyanidins, flavanones, isoflavones and others), and the latter by being a more heterogeneous group (phenolics acids, hydroxycinnamates, stilbenes and others) (Tomás-Barberán et al., 2003). The biosynthetic pathway of some phenolic compounds is showed in Figure 8. The most important nutritional factors are briefly described below (Martínez-Valverde et al., 2000; Crozier et al., 2006; Cartea et al., 2011):

- Phenols and phenolic acids: the simple phenols (phenol, crucible, thymol, resorcinol) are widely distributed among all plant species, as are phenolic acids (galic, vanillinic), which are abundant in upper plants and ferns. Phenolics acids have one carboxylic acid group and may be present in plants in free and bound forms.
- Cynamic acids and coumarins: cinamic acids (caffeic, p-coumaric and synaptic) are rarely found free. On the other hand, coumarins are generally found in the form of glycosides.
- Lignans: metabolites of low molecular weight, formed by 2 units of phenylpropane linked by a hydrogen bridge.

- Flavonoids: they are the most important group within this classification, being the polyphenols most distributed in plants. They have a structure of 15 carbon atoms (C6-C3-C6). The aromatic ring A is derived from the acetate/malonate pathway, and ring B is derived from phenylalanine through the shikimate pathway. Variations in substitution patterns to ring C result in 13 flavonoid classes, being the most important flavonols, flavones, isoflavones, flavanones, flavanols (also called flavan-3-ols) and anthocyanidins or anthocyanins.
- Tannins: water-soluble compounds with a molecular weight between 500-3000 Da, able to bind proteins and other macromolecules. They can be classified into two groups: hydrolyzable tannins and non-hydrolizable or condensed tannins (also known as protoanthocyanidins).

Table 3. Concentration of some phenolic compounds (mg kg^{-1}fw) in immature seeds of faba bean (Baginsky et al., 2013).

COMPOUND	CONCENTRATION (mg kg^{-1} fw)
Prodelphinidin dimer	199.01
(+)-Catechin	643.90
Procyanidin dimer	78.41
Procyanidin trimer	146.52
(-)-Epicatechin	653.67
Procyanidin dimer	195.41
Total proanthocyanidins	**1916.96**
Quercetin 3-O-rutinoside	56.91
Apigenin 7-O-galactoside	20.66
Apigenin 7-O-galactoside	31.13
Quercetin 3-O-galactoside	82.12
Myricetin 3-O-glucoside	25.28
Quercetin derivative	19.06
Quercetin 3-O-glucoside	22.73
Myricetin	119.49
Total flavonols + flavones	**377.38**

Table 4. Concentration of some phenolic compounds (mg kg^{-1} fw) in immature seeds of peas (Stanisavljević et al., 2015).

COMPOUND	CONCENTRATION (mg kg^{-1} fw)
Gallic acid	1.96
Protocatechuic acid	127.22
Gentisic acid	5.01
Chlorogenic acid	1.59
Cafeic acid	2.49
p-Hydroxyphenylacetic acid	3.49
p-Coumaric acid	2.14
Ferulic acid	5.33
Sinapic acid	4.56
Syringic acid	1.87
Rosmarinic acid	1.81
Catechin	3.08
Epicatechin	14.59
Catechin gallate	2.69
Epigallocatechin	5.96
Quercetin	13.66
Rutin	11.51
Kaempferol	6.49
Galangin	4.19
Morin	4.93
Luteolin	9.40
Apigenin	3.62
Naringin	1.68
Hesperetin	3.15
Pinocembrin	3.44

Table 5. Concentration of some Phenolic compounds (µg g^{-1} fw) in immature seeds of cowpea (Adelakun et al., 2017).

COMPOUND	CONCENTRATION (µg g^{-1} fw)
Gallic acid	378.00
Protocatechuic acid	493.60
4-hydroxybenzoic acid	81.60
Vanillic acid	13.39
Caffeic acid	11.90
Syringic acid	920.40
p-coumaric acid	5.20
Ferulic acid	3.80
Quercetin dihydrate	442.80
Hesperidin	
Naringin	617.40
Fisetin	403.30
Kaempferol	
Kaemferol glucoside	184.80
Taxifolin	430.30
Catechin	8,799.00
Epicatechin	1,545.20
Tannic acid	7,031.90

2.2.3. Antioxidant compounds

Active molecules containing O_2 are called reactive oxygen species (ROS). The ROS include oxygen ion (O_2), free radicals (O_2, -OH, NO-, etc.) and peroxides (H_2O, ONOO-, etc.). They have great reactive capacity due to the presence of odd valence membrane electrons. ROS are formed as by-products of the metabolism of O_2 and have an important role in cell signaling and homeostasis (Taverne et al., 2013). However, under stress, whether exogenous (exposure to heat, ultraviolet light, O_3, contaminants, additives, etc.) or endogenous (mono-electronics reduction of O_2, auto-oxidation of C, catalytic activation of numerous enzymes, etc.), the levels of ROS can increase greatly and cause accumulated damage to the cellular structure, which is known as oxidative stress (Devasagayam et al., 2004).

Antioxidants are compounds that, at low concentrations compared to the substrate, delay or prevent oxidation during oxidative stress (Devasagayam et al., 2004). According to their origin, these compounds are classified as enzymatic and non-enzymatic (Figure 9). Total antioxidant capacity (TAC) is influenced by physiological factors (such as maturity, senescence) and technological factors such as storage or processing conditions (Tarazona-Díaz, 2011). Biosynthetic pathway of some antioxidant enzymes are shown in Figure 10.

Diets rich in fruits and vegetables have been shown to reduce the risk of cardiovascular and other chronic and degenerative diseases associated with oxidative damage to cells (Dragsted, 2003; Balasundram et al., 2006). This protective effect of fruit and vegetable consumption has been associated to the presence of antioxidants such as polyphenols and vitamin C in their composition (Scalbert et al., 2005).

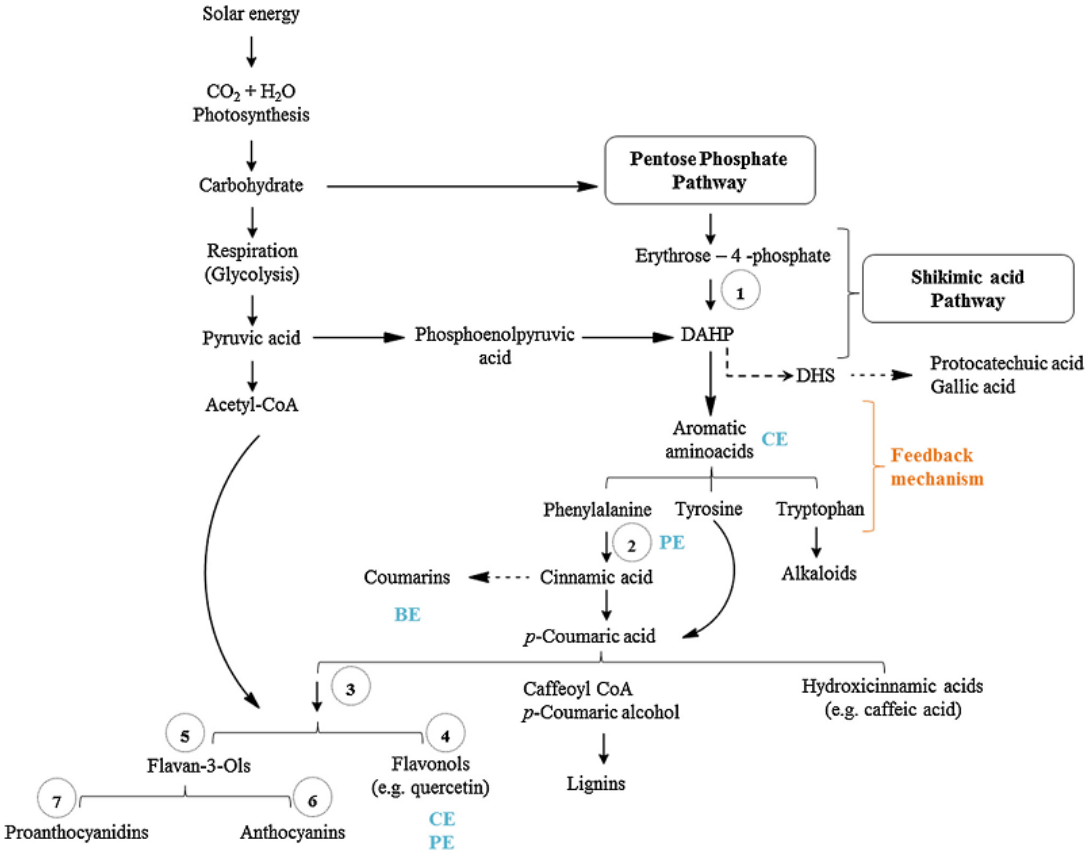

*Figure 8 . Biosynthetic pathway of some phenolic compounds and the influence of elicitationCO2—carbon dioxide; H2O—water; Acetil-CoA—AcetilCoenzyme A; DAHP—3-Deoxy-O-arabino-heptulosonate phosphate; DHS—3-Dehydroquinate; BE—biological elicitation;*CE—chemical elicitation; PE-Physical elicitation; Enzymes involved in the biosynthesis are marked with rounded dashed black forms: 1-DAHP synthase (3-Deoxy-O-arabino-heptulosonate phosphate synthase); 2-PAL (Phenylalanine ammonia-lyase); 3-CHS (Chalcones synthase), CHI (Chalcones isomerase), F3H (Flavanone-3-hydroxylase); 4-FLS(Flavonol synthase); 5-LAR (Leucoanthocyanidin reductase); 6-LDOX (Leucoanthocyanidin dioxygenase). (*Dias et al., 2016*)

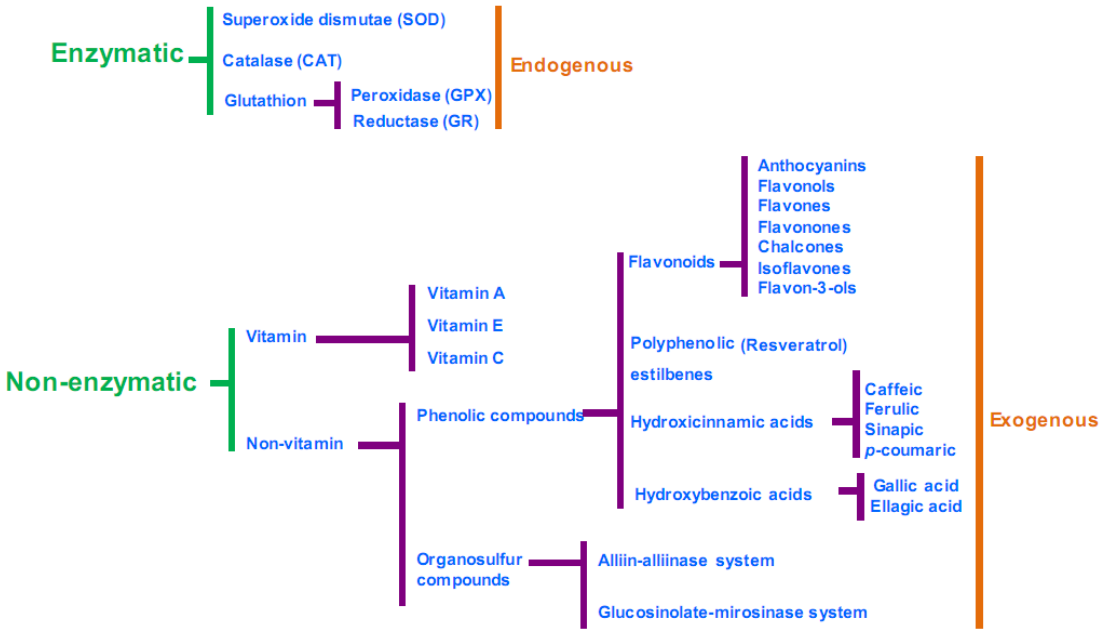

Figure 9. Antioxydant compounds classification (Artés-Hernández et al., 2009).

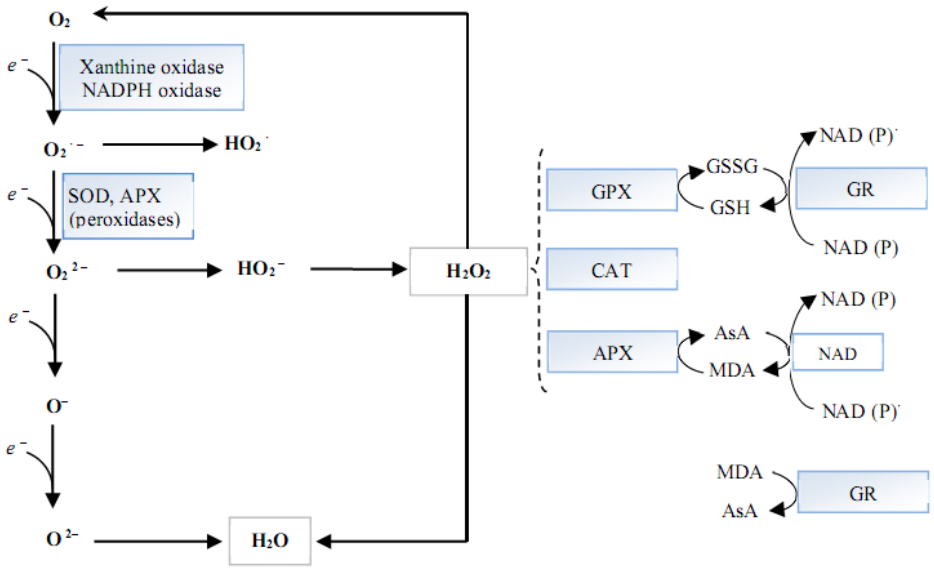

Figure 10 Antioxidant enzymes pathways (Gärtner and Wese, 1986). AsA: ascorbate; APX: ascorbate peroxidase; CAT: catalase; GPX: guaiacol peroxidase; GR: gluaiacol reductase; GSH: glutathione; GSSG: glutathione disulfide; MDA: monodehydroascorbate; NAD: nicotinamide adenine dinucleotide; NADPH: nicotinamide adenine dinucleotide phosphate; SOD: superoxide dismutase.

2.3. Main non-nutritional factors in legumes

2.3.1. Condensed tannins

Tannins are water-soluble polyphenolic compounds with high molecular weight (500-3000 Da). They are associated with plant defense mechanism against mammalian herbivores, birds and insects (Hassanpour et al., 2011) due to its astringent property, and against bacteria, viruses and fungi, because its ability to inhibit enzymes. In addition, they participate in the nodulation of legumes, generating important quantities of nitrogen (Shirley et al., 1996).

Depending on their chemical structure and properties, tannins can be divided into two main groups: hydrolysable and condensed tannins (Figure 11), in which each form has different nutritional and toxic effects (Nikmaram et al., 2017). The hydrolysable type of tannins is prone to hydrolysis during the digestion process. They are molecules which contain a carbohydrate, generally D-glucose, as a central core. These carbohydrates are esterified with phenolic groups. Hydrolysable tannins are usually found in lower concentration in plants than condensed tannins (Hassanpour et al., 2011).

In contrast, condensed type of tannins is more resistant to hydrolysis; for that, it is neither hydrolyzed nor absorbed during digestion. They are the most common type of tannins found in legumes (Barry and MacNabb, 1999). The first researchers that detect them in seed of faba beans were Barker and Morris (1968). Condensed tannins consist of flavonoid units (flavan-3-ol and/or flavan-3,4-diol) linked by carbon-carbon bonds. In presence of heat and acid medium, they release anthocyanidins, hence they are also called proanthocyanidins.

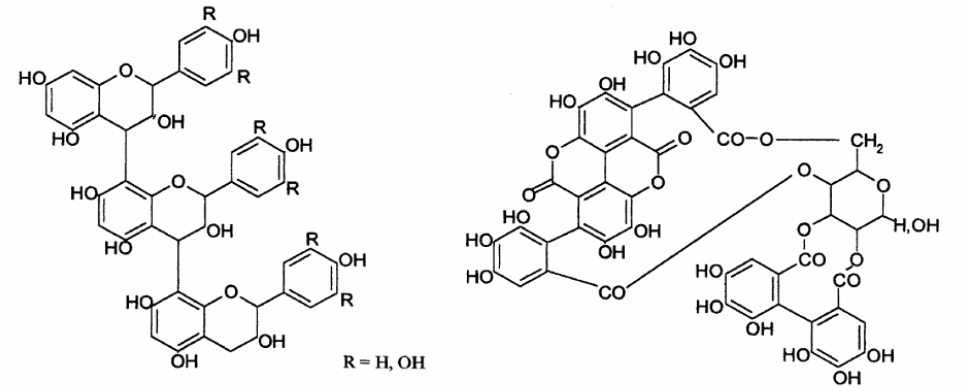

Figure 11. Chemical structure of condensed tannins (left) and hydrolysable tannins (right) (Hassanpour et al., 2011).

Condensed tannins have a variety of chemical structures affecting their physical and biological properties. Its biological effects are specially related to its polymerization degree, and the number of phenolic groups present in the molecule is the basis of its binding to proteins, carbohydrates, metal cations and free radicals (Santos-Buelga and Scalbert, 2000).

The anti-nutritional effects of tannins include interference with digestion by binding to proteins or minerals. The tannin-protein complex (Figure 12) is the consequence of multiple hydrogen bonds forming between the hydroxyl group of tannins and the carbonyl group of proteins (Raes et al., 2014). However, because of this, they also have therapeutic or preventive properties against gastrointestinal diseases due to the binding with proteins of the intestinal mucous membrane, forming a protective film (Saito et al., 1998). Tannins interfere with the assimilation of disaccharides by a strong inhibition of maltases, sacarases and lactases. They also inhibit the active transport of glucose through the intestine and reduce digestion and absorption of starch. This leads to a reduction in blood glucose and an increase in insulin, being useful as a treatment for diabetes and obesity control (Thompson et al., 1993).

Protocyanidines can cause a deficiency of Fe^{+2}, sometimes triggering anemia. They also reduce the bioavailability of Ca^{+2}, Mg^{+2} and Zn^{+2}, while having a little affinity with Cu^{+3} increase their bioavailability. Thanks to the reducing of the condensed tannins, oxidative damage is avoided and anti-cancer and antimutagenic activity is developed (Santos-Buelga and Scalbert, 2000). In addition, condensed tannins are antimicrobial

agents that act against a large number of bacteria, viruses and fungi (Sakanaka, et al., 1990). On the other hand, it has also been observed that regular ingestion of large amounts of tannins increases the risk of developing tumor diseases due to irritation and cellular damage (Singleton, 1981).

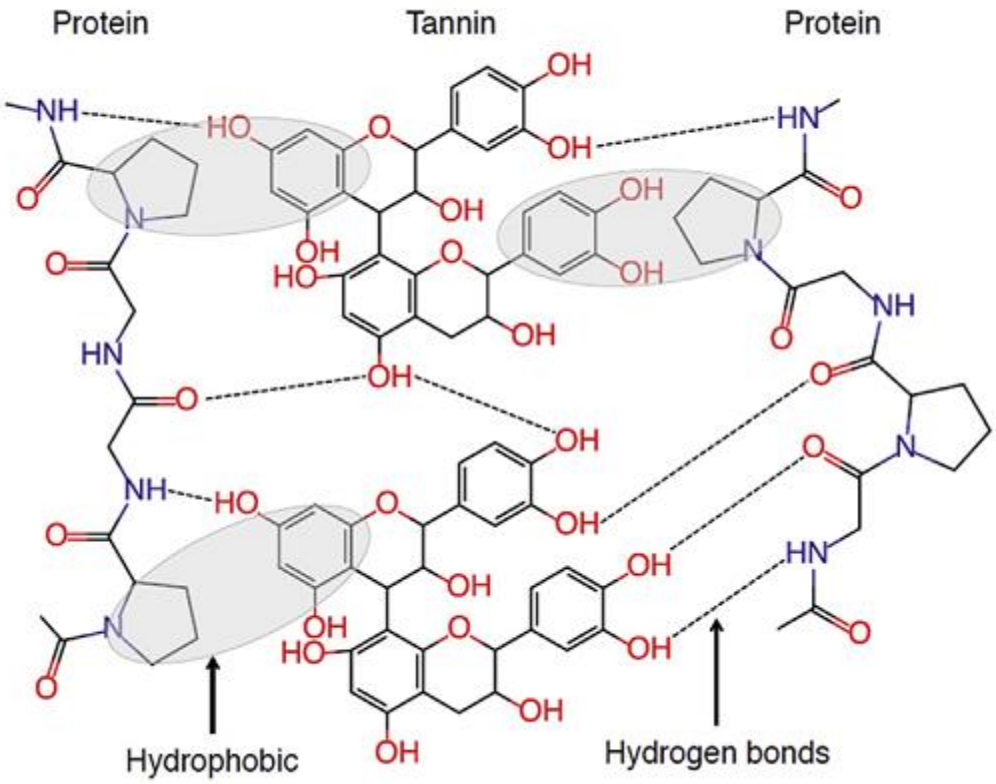

Figure 12. Interaction of tannin with protein (Nikmaram et al., 2017)

2.3.2. Saponins

Saponins are secondary metabolites which are widely distributed in the plant kingdom. They derive their name from their ability to form stable soap-like foams in aqueous solutions and they have been commonly used for centuries as household detergent due to its amphiphilic nature with the presence of a lipid-soluble aglycone and water-soluble chain in their structure (Guclu-Ustundag and Mazza, 2007).

Saponins may be considered a part of plants´ defense systems since they act as a chemical barrier countering pathogens and herbivores (Cheok et al., 2014). Many of them are known to be amtimicrobial, to inhibit mould and to protect plants from insect attack (Francis et al., 2002). They are a very heterogeneous group of amphipathic

glycosides. They consisted of non-polar aglycones (triterpenoid or steroid) attached to one or more polar monosaccharide chains (hexose, pentose or uronic acid) (Nikmaram et al., 2017). The common sugars in saccharide chains are D-glucose, D-galactose, L-arabinose, D-xylose, L-rhamnose and D-lucoronic acid (Du et al., 2002). Saponins have a polycyclic ring system in their aglycone (either 27 carbon sterol or 30 carbon triterpene). The sugar moiety in a saponin molecule is attached to aglycone at one or two glycosylation sites by glycosidic linkage. Saponins are glycosylated compounds or glycosides that are divided into three main groups according to the carbon skeleton of non-polar aglycone region: triterpenoidal glycosides, steroidal glycosides and steroid-alkaloidal glycosides. Saponins that have been characterized and commonly identified in pulses are the triterpene glycosides (Barakat et al., 2015).

They are categorized as monodesmosides (having a single sugar chain linked to carbon-3 of the aglycone) and bidesmosides (having two sugar chains separately linked to carbon-3 and carbon-22 of aglycone) (Lásztity et al., 1998). These compounds show a variation in their structure among different plant species, depending upon the type and composition of the aglycone and saccharide chains. Saponins are categorized into A, B and E group according to their aglycone structures (Wu and Kang, 2011) (Figure 13). The group A have sapogenol A as the triterpenoid moiety with a hydroxyl group at carbon-21 and two saccharide chains attached to carbon-3 and carbon-22.

The group B contains sapogenol B in their structure and differs from group A by having a hydrogen atom at carbon-21 of its aglycone. They are monodesmosides with one glycoyl group attached at carbon-3 position of aglycone and are named as soyasaponin I (Bb), II (Bc), III (Bb´), IV (Bc´) and V (Ba) (Wu and Kang, 2011). Group B saponins with 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) group at carbon-22 are denoted as the DDMP saponins. DDMP saponins are named as soyasaponin αa, αg, βa, βg, γa and γg (Berhow et al., 2002). DDMP saponins are unstable under commonly used food processing conditions such as high temperature, acidic and alkaline pH and subsequently get converted into saponin B after losing their DDMP moiety (Heng et al., 2006). Group E are monodesmosides that resemble group B with a single saccharide chain attached to carbon-3 of triterpenoid moiety, but differ from saponins B by having a carbonyl group at carbon-22 of aglycone (Wu and Kang, 2011).

Saponins are commonly identified in the seeds of edible legumes, and have a significant importance, mainly because of their biological activities. Several researchers have documented that legume seeds are the main saponin containing foods in the human diet (Price et al., 1986; Khalil and El-Adawy, 1994; Lásztity et al., 1998). Two kinds of saponins are identified in faba beans and peas, soyasaponin I and soyasaponin βg (Ha et al., 2014; Reim and Rohn 2015; Singh et al., 2017). In cowpea, in addition to this two types of saponins, have also been identified soyasaponin II and soyasaponin αg (Ha et al., 2014).

The ingestion of large amounts of saponins can cause irritation of the intestinal epithelium, but they do enter to the bloodstream through lesions, and can cause hepatic damage, hemolysis, respiratory failure and coma. Saponins are also capable of hydrolyzing other cells, such as those in the intestinal mucous membrane, interfering with the absorption of nutrients. In addition, they inhibit metabolic and digestive enzymes such as proteases, amylases or lipases (Price et al., 1987; Thompson, 1993).

However, small concentrations of saponins absorbed by the intestine exert a positive effect on the intestinal tract. Their biological effects have been attributed as they can enhance the permeability of intestinal mucosal cells, inhibit the transport of active mucosal and facilitate the uptake of substances that are normally not absorbed (Couto et al., 2015). Presence of saponins in food has other beneficial effects on protein digestion, cholesterol metabolism and immune and nervous systems (Francis et al., 2002). Also, they decreased bloods lipids and lowered blood glucose response, have importance in reducing the risk of cancer and contribute towards the antioxidant and anti-inflammatory properties (Singh et al., 2017).

2.3.3. Raffinose

The oligosaccharides of the raffinose family (RFO): raffinose, stachyose and verbascous, are α-galactosides that are within the group of galactosyl sucrose oligosaccharides (Figure 15). These non-reducing sugars of low molecular weight soluble in water and solutions of water-alcohol, are reserve compounds present in varying quantities in organs and in seeds of numerous plants, including leguminous plants. These oligosaccharides are also called ‘flatulence factors’ because when

fermented by intestinal microflora, release considerable amounts of gases (Kadlec et al., 2000a).

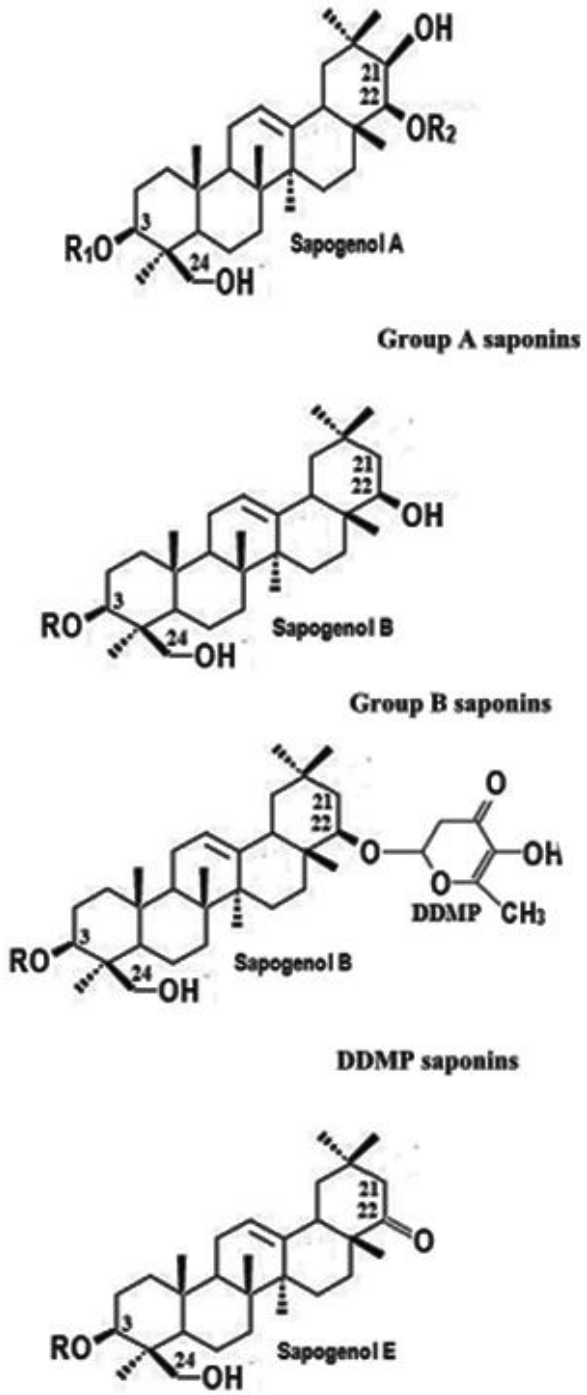

Figure 13. *Chemical structures of groups A, B, E and DDMP saponins (glcUA: β-D-Glucuronopyranosyl, gal: β-D-Galactopyranosyl, glc: β-D-Glucopyranosyl, ara: α-L-Arabinopyranosyl, xyl: β-D-Xylopyranosyl, rha: α-L-Rhamnopyranosyl) (Singh et al., 2017).*

RFOs have been proposed to act as protective agents during desiccation and storage of seeds in the dry state, as transport sugar in phloem sap and as storage sugars (Peterbauer et al., 2001; Downie et al., 2003). The mechanisms of action through which these sugars could confer to the seed a tolerance of drying and acclimatization against cold are based on the stabilization of cellular components. They participate in several cellular functions such as transport and storage of carbon, signal transduction (Xue et al., 2007), membrane trafficking (Thole and Nielsen, 2008), and mRNA export (Okada and Ye, 2009). They also act as signaling molecule following pathogen attack and wounding (Couée et al., 2006; Kim et al., 2008).

RFOs are α-1,6-galactosyl extensions of sucrose. The galactosyl group of RFOs is donated by galactinol (Gol; 1-O-α-D-galactopyranosyl-L-myo-inositol). Synthesis of Gol is a key and absolute requirement for entering into the pathway of RFO biosynthesis (Sengupta et al., 2015) (Figure 14).

One of the phenomena produced by legume consumption is the formation of gases at the colon level, due to the absence of the enzyme α-(1-6)-galactosidase necessary to hydrolyse the α-galactosides. These sugars, because they cannot be digested and do not pass through the intestinal wall, pass intact to the colon, where they are metabolized by bacteria. This metabolic process produces short-chain fatty acids that reduce pH, as well as carbon dioxide, hydrogen and methane. These gases are responsible for flatulence, which manifests itself in the form of nausea, abdominal pain, cramps, constipation or diarrhea.

Although RFOs have long been regarded as antinutritional factors in human nutrition, RFOs have a beneficial role as prebiotics (Voragen, 1998; Aranda et al., 2000), promoting the proliferation of bifidobacteria and lactobacilli that generate health benefits (Rubio et al., 2005). Monogastric animals cannot digest these sugars, leaving them available in the colon to be used by beneficial intestinal bacteria as a source of carbon and energy for maintenance and growth. The large number of bifidobacteria and lactobacilli generated in the colon synthesize antibiotic substances and produce high levels of short-chain fatty acids that reduce the pH of the medium. Through these mechanisms, these beneficial bacteria reduce the proliferation of pathogenic bacteria (Delzenne and Roberfroid, 1994).

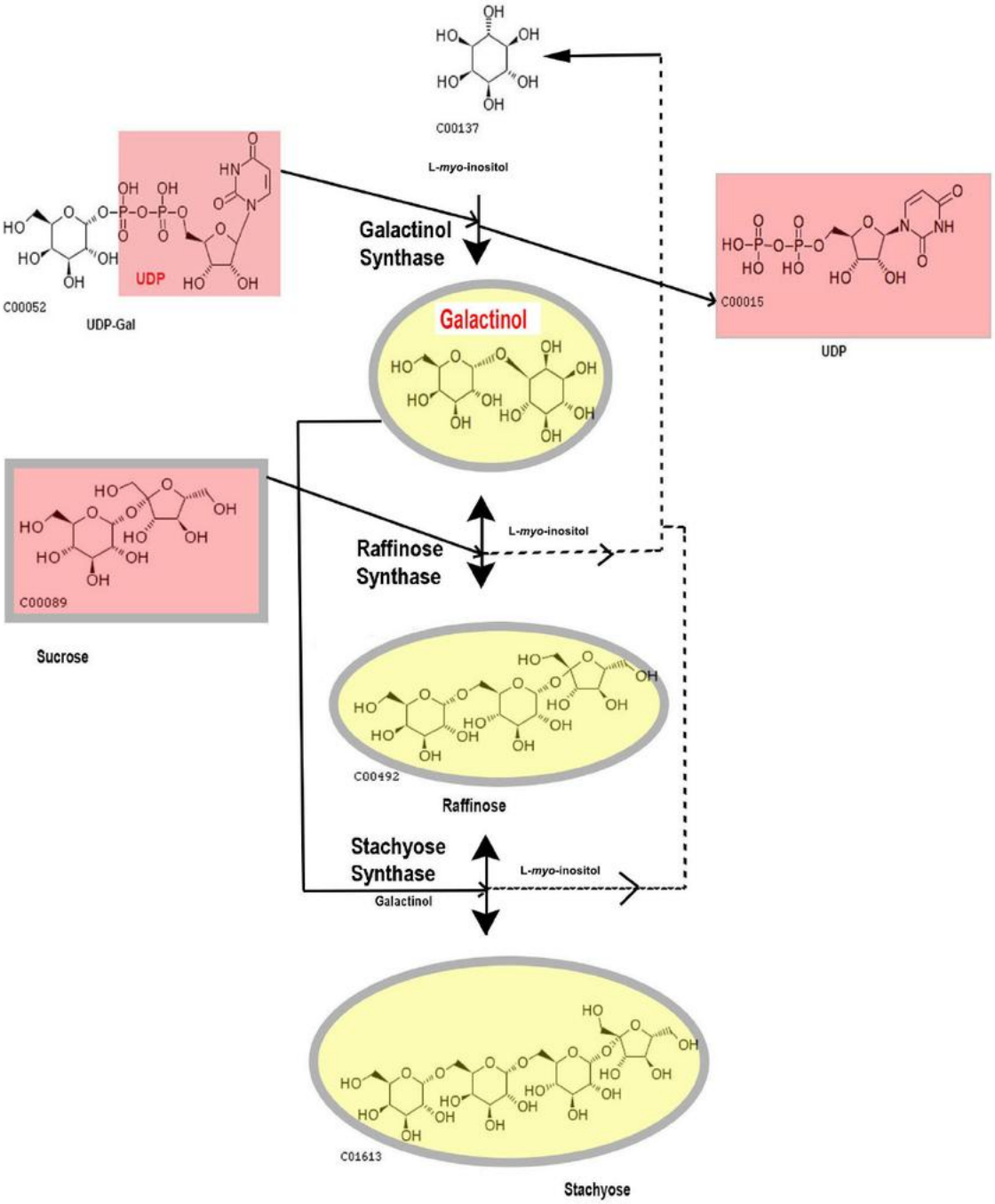

Figure 14. The biochemical pathway of RFO synthesis (Sengupta et al., 2015)

With this drop in pH, the solubility of minerals such as Ca, Mg or Fe is also increased, so that oligosaccharides could be used in the prevention of osteoporosis, in Mg deficiency states or in anemia situations (Grizard and Barthomeuf, 1999). The increase in bacterial mass favors a larger fecal bolus size, reducing the possibility of colon cancer and constipation (Delzenne and Roberfroid, 1994).

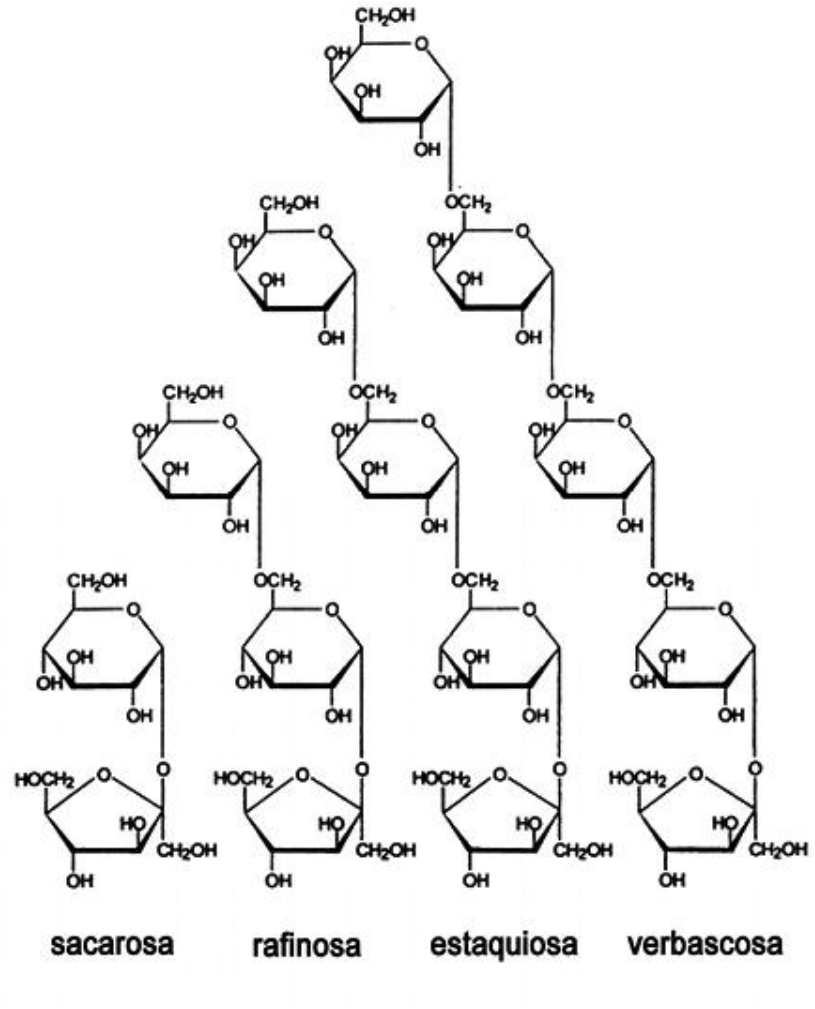

Figure 15. Chemical structure of sucrose and RFO (Goyoaga, 2005)

2.3.4. Phytic acid

Phytic acid (IP6) (Figure 16) is a natural antioxidant that is found in vegetables, where it serves as the storage form of phosphorus and represents 60% to 90% of the total phosphorus present in the seeds used in food and feed (Wu et al., 2009; Silva and Bracarense, 2016). It is a compound widely distributed in legume seeds, which can be degraded by phytases. These enzymes belong to the phosphatase group, and are able to hydrolyze sequentially IP6 to myo-inositol esters with fewer orthophosphate groups (myinositol pentachis-, tetrakis-, tri-, di- and monophosphate) and inorganic phosphate (Goyoaga, 2005).

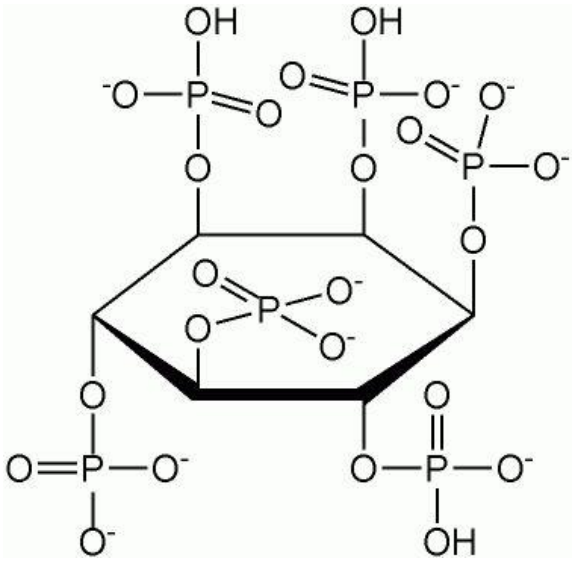

Figure 16. Chemical structure of phytic acid (Wu et al., 2009)

IP6 could be a seed dormancy inducer, since by binding to multivalent cations necessary for the control of cellular processes; metabolism slows down (Gibson and Ullah, 1990). In addition, because of its antioxidant properties, IP6 could preserve seed viability during the dormancy period (Graf et al., 1987). It has traditionally been considered as an antinutrient because of its binding to proteins, minerals and starch, with which it forms complexes that cannot be assimilated (Figure 17).

Phytic acid has a strong ability to form a complex with multivalent metal ions, especially zinc, calcium and iron. This binding can result in very insoluble salts with poor bioavailability of the minerals (Wu et al., 2009). Monogastric animals, including humans, have few phytases in the stomach and small intestine, so that they cannot hydrolyze the phytic acid molecule and use the phosphorus found in its structure, nor the minerals with which it forms salts (Steer and Gibson, 2002), causing severe mineral ions deficiency.

Another nutrient limitation is the ionic interaction of phytic acid with proteins forming protein-phytate complexes at acid pH and protein-mineral-phytate at basic pH. Because of these phytate-enzyme protein interactions, phytic acid inhibits digestive enzymes

such as lipases, proteases or α-amylases, paralyzing enzyme reactions at the digestive level (O`Dell and Boland, 1976; Knuckles and Betschart, 1987).

The interactions of phytic acid with cations can sometimes be beneficial, as in the case of its binding to toxic metals such as Cd^{2+} or Al^{3+} to be excreted by faeces (Evans and Martin, 1988). It also has anti-cancer properties, since thanks to its combination with Zn^{2+} and Mg^{2+}, it reduces the bioavailability of these minerals necessary for DNA synthesis, preventing cell proliferation (Steer and Gibson, 2002).

Inositol hexaphosphate acts as a plasma hypolipidemic agent, minimizing the risk of cardiovascular disease. This is due to its greater affinity for Zn^{2+} versus Cu^{2+} (Zulet and Martínez, 2001). Phytic acid can slow down digestion and absorption of starch, resulting in delayed glucemic response, so less insulin is required and reduces the risk of diabetes (Pallauf and Rimbach, 1997).

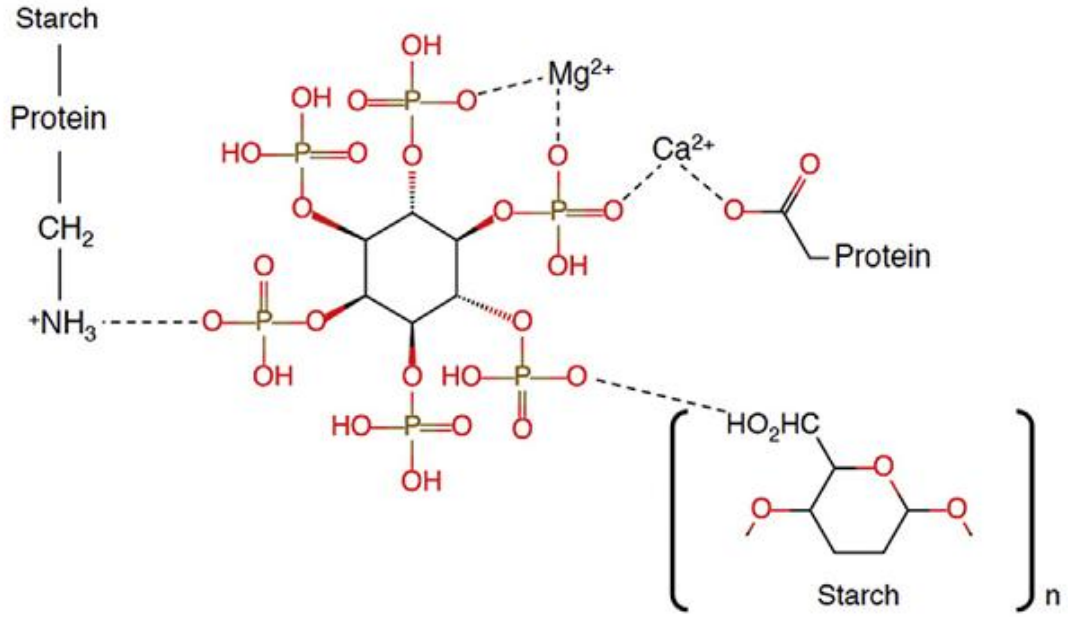

Figure 17. Interaction of phytate with minerals, proteins and starch.

3. MINIMAL PROCESSING OF LEGUMES

3.1. Overview

The current life style, with little time to cook balanced meals and the increase of interest about healthy food, has driven the demand towards natural, fresh and ready-to-eat vegetable products, as the fresh cut or minimally fresh processed (MFP) fruit and vegetables. In addition, there are increasing demands for extension of shelf life to better meet longer and more global distribution chains, and minimally processed products can meet that demand. In addition, after storage and commercialization, that product could be consumed fresh or directly microwaved if packaged in a suitable container (Klug et al., 2108a). Given that, a healthy cooked food with high organoleptic quality would be quickly obtained.

Fresh and fresh-cut products continue all metabolic processes, and are susceptible to quality deterioration and microbial infestation mainly due to increase in respiration and transpiration, that play a significant role in the postharvest quality of fresh-cut vegetables. Passive modified atmosphere packaging (MAP) can be generated inside the package depending on respiration and film permeability to attain the desired gas composition over time. MAP offers the possibility to extend the shelf life of fresh product, by reducing respiration rate by decreasing O_2 concentration around the fresh produce. Decreasing respiration rate and lowering temperature delays enzymatic degradation of complex substrates and reduces sensibility to ethylene synthesis (Artés et al., 2012).

Plastic films of selective permeability must be selected taking into account the permeability to gases of physiological interest (O_2, CO_2, C_2H_4 and water vapour). The optimal balance of gases inside the package is created by diffusion of these gases, which are generated by the breath of the product and its interaction with the permeability of the polymer (Artés et al., 2012; Kader et al., 2002). Minimum O_2 and maximum CO_2 concentration must be attained, but considering that excessively low O_2, below 1%, may result in anaerobic respiration leading to tissue deterioration as well as production of off-odors and off-flavors (Caleb et al., 2013). Also, maintaining high levels of RH and reduced water loss are a common outcome of MAP which can prove beneficial for many fruit and vegetables (Wilson et al., 2017).

For fresh-cut products, sanitation is one of the most critical steps in their production process, due to the effects it has on microbial load and, consequently, on quality, safety and shelf-life (Otón et al., 2015). In general, the industry has widely used sodium hypochlorite (NaOCl) due to its antimicrobial activity and low cost. However, it is controversial because it can be potentially harmful due to the formation of toxic by-products like trihalomethanes and chloramines (Otón et al., 2014; Artés et al., 2009), and because several studies have reported that NaOCl could be insufficient to reduce normal microflora in fresh-cut products (Foley et al., 2004). Therefore, others disinfectants, both chemical (acidified sodium chlorite, ascorbic acid, citric acid, etc.) and physical (UV light), as well as edible coatings, are increasingly being studied as alternative to NaOCl disinfection (Otón et al., 2016; Artés-Hernández et al., 2014 and 2017).

Fresh-cut industries are continuously looking for and applying new technologies to extend the commercial life of their products keeping the best sensory, nutritional and microbial quality. All these aspects, in addition to their high nutritional value, confer great advantages for consumers and food services (Artés y Artés-Hernández, 2012; Artés-Hernández et al., 2017):

- Reduces preparation time, as they are ready for consumption
- They have characteristics very similar to the original product
- Present uniform and consistent high quality
- Encourage the supply of healthy products
- Reasonably priced
- Easy to store
- Produce little or no waste

3.2. Current state of the fresh-cut market

These companies were born in the early 1970s in the U.S. to meet the demand for fresh salads in fast food shops. During the 1980s, they spread to European countries such as Germany or Switzerland, and years later followed their development in the United Kingdom, France, the Netherlands and Italy. In Spain, MFP products did not appear on the market until the early 1990s (Artés and Artés-Hernández, 2003).

The European fresh-cut industry has shown exponential growth since its appearance in the early 1980s. UK is the major fresh-cut produce consumer because the ready-to-eat product culture is deeply established in that country. In countries like Germany and Spain, in which fresh-cut fruit and vegetables market is still emerging, the market growth in the last years was higher than other countries in which this market is already established, for instance Italy and the Netherlands. In the 2015-2016 campaign, the fresh-cut market in Spain increased 15%. Concerning the features of the fresh-cut market, packaged salads appear to be the leader of fresh-cut products, in fact they hold about 50% of total fresh-cut market volume. The other 50% is shared by the fresh-cut fruit (10%) and the other categories as ready-to-cook, *crudités* and other with 40% (MAGRAMA, 2019).

3.3. Units operations during minimally fresh product processing

The units operations during fresh-cut products production depend on the fruit or vegetable used for each case. However, the general steps as unit operations in the fresh-cut products elaboration are presented in Figure 18 and subsequently described.

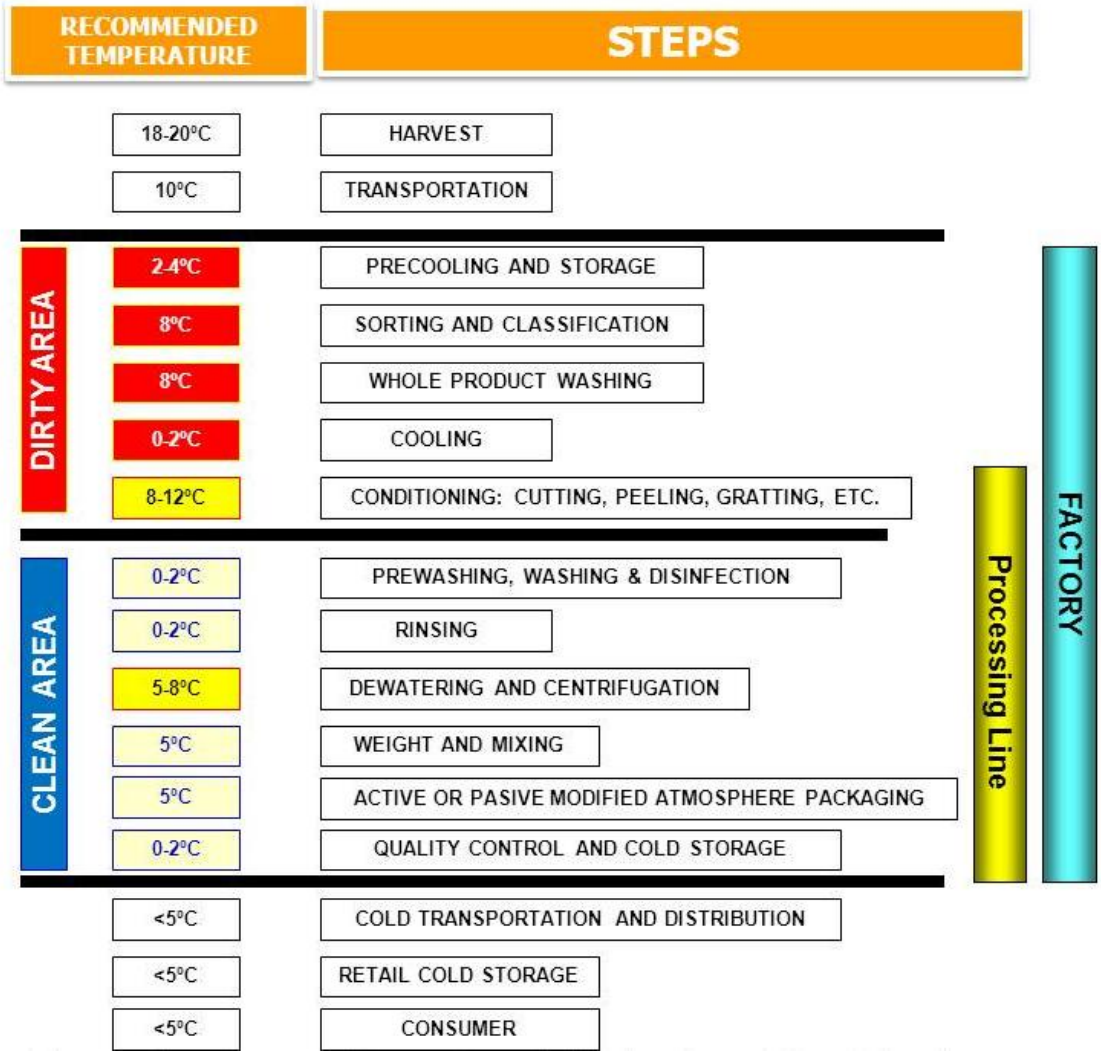

Figure 18. Flow chart of the general minimal processing steps and recommended temperatures (Artés-Hernández et al., 2013)

3.3.1. Raw material

The physiological behavior and the suitability for the fresh cut vegetable processing that ensure a good postharvest quality and shelf–life may be influenced by the following preharvest factors (Yildiz et al., 1994):

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

When referred to legumes, pods must be picked up at the appropriate ripening stage (Figure 19), as if they are harvested too early, the economic yield will not be as high as can be expected and, if harvesting is delayed, the pods will harden and the product will be much less valuable (MAPAMA, 2019). The harvest season depends on the variety and climatic conditions. Green grain harvesting can be done manually or mechanically with combine harvesters, when the grains have an average moisture content between 70-75% (in case of peas) or when the pod reaches 3/4 of the final size and before the hilum turns black (in case of beans). These values are for open field crops.

Pods should be uniformly green (light to deep green but not yellow-green), fully turgid, clean, and free from damage (thrips injury, broken pods). The stem and calyxes should be green and there should be very few blossoms attached to the pods (Suslow and Cantwell, 1998).

The green pods, if they are intended for fresh consumption, must be quickly classified, packaged and marketed in order to avoid losses in quality and losses in production.

Figure 19.Growing filed of faba beans (left) and collected beans (right)

3.*3.2. Transportation and reception*

It should take less than an hour between harvesting and arrival at the processing plant. A refrigerated transport (5ºC) is recommended if distance is far. When the raw material arrives at the processing plant it is selected according to quality standards. If it is inadequate or deficient, it must be rejected and it will not be possible to process this

way. In this step, the product is also weighed once it has been classified, both for control during processing, product formulation and quality control.

3.3.3. Precooling and storage

Pre-cooling should be performed to rapidly decrease product temperature. This is one of the most important steps to extend the shelf–life of these high–metabolic–rate vegetables, slows down the stress generated during the processing stages and maintains better product quality. Pre-cooling also has the advantages of reducing (Artés-Hernández et al., 2013):

- weight loss and withering
- microbial growth
- deterioration
- the emission of and sensitivity to ethylene

Raw material is usually pre-cooled with forced air and stored between 0 to 4ºC and 95% RH for a few hours or a day to regulate the supply to the processing line before processing (Artés-Hernández et al., 2013).

3.3.4. Sorting, classification and peeling

Good sorting and classification will facilitate subsequent processes, increasing line productivity and the quality of the final product. The selected seeds must be of a similar size, be of an intense green color, uniformly developed, and free of damages and pathogens (Acuña, 2011). The selection is manually made by choosing or separating grains of another colour, broken, deformed or immature, and other materials such as pods or leaves, to obtain a clean and quality product.

Legume seeds have one of the highest respiration rates (RR) among fruit and vegetables (27-38 mL CO_2 kg^{-1} h^{-1} at 5ºC in peas) (Suslow and Cantwell, 1998). The peeling of pods increases the RR, ethylene emission and tissue damage of the product and therefore accelerates the velocity of senescence of the tissue and reduces resistance to microbial contamination (Artés et al., 2009).

In order to minimize these undesirable effects of shelling, the operation must be done in a cold room (5 ± 1°C) and the obtained seeds must be immediately immersed in cold water at 4 ± 1°C. Pods can be manually or mechanically shelled in a chamber previously disinfected (Figure 20). The cutting equipment must be cleaned, disinfected and sharpened at regular intervals every working day.

The conditioned product must be directly conducted from the dirty to the clean area for further processing.

Figure 20. Peeling of faba beans (left), peas (center) and cowpea (right) in a cold room

3.3.5. Washing, disinfection and rinsing

Washing and disinfection reduces the microbial load of the product (Suslow, 1997; Artés et al., 2009; Otón et al., 2015), since fresh products that have not undergone any heat treatment may transmit infectious diseases (Leistner and Gould, 2002; Harris et al., 2003; Allende and Artés, 2005). Also, microbial load affects postharvest life, since it might cause produce deterioration and senescense. Chlorine (in various forms) has been widely used as a disinfectant in the fresh cut industry. NaClO is the most commonly used disinfectant in the fresh cut industry due to its strong oxidizing properties, antimicrobial activity and low cost. In this way, the washing and disinfection of legumes are made with cold chlorinated water (4°C; 150 mg L^{-1} free Cl_2), which is acidified (pH 6.5–7.5) with citric acid to increase 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). 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 et al., 2017).

Sodium hypochlorite is controversial because it can be potentially harmful due to the formation of toxic by-products like trihalomethanes and chloramines (López-Gálvez et al., 2010, Otón et al., 2014), 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). Also several studies have reported that NaOCl could be insufficient to reduce normal microflora in fresh-cut products (Foley et al., 2004). These negative aspects related to chlorine have induced some European countries (Germany, The Netherlands, Denmark, Switzerland and Belgium) to forbid the use of NaClO for disinfection of fresh cut produces (Artés et al., 2011).

Consequently, the food industry is now looking for alternatives to chlorine which may assure the safety of the fresh cut 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. Among these techniques UV–C light, edible coatings and alternative acids can be considered.

3.3.5.1. UV-C radiation

The use of non-ionizing, germicidal, artificial and most energetic fraction of the UV spectra UV light (wavelength of 190–280 nm, corresponding to the UV-C range) could be effective for surface decontamination of fresh cut products using germicidal lamps (254 nm) (Selma et al., 2008, Artés et al., 2009).

UV-C affects several physiological processes in plant tissues and damages microbial DNA. UV–C acts indirectly by stimulating plant defense mechanisms, but also UV light promotes photo-oxidative reactions in plants producing reactive oxygen species (ROS). The major ROS are singlet oxygen, hydrogen peroxide and hydroxyl radicals. The free radicals generated from UV radiation can target cell membranes, nucleic acids, cell walls and enzymes, inducing the acceleration of senescence (Turtoi, 2013). For this reason, it is very important to find a safe dose which greatly weakens microbial development without damaging the product (Artés-Hernández et al., 2010).

The antimicrobial effect of UV-C light is due to its ability to damage microbial DNA and to a lesser extend denatures proteins. The damage caused by UV-C probably involves specific target molecules and a dose in the range from 0.5 to 20 KJ m^{-2} leads to lethality by directly altering microbial DNA (Bintsis et al., 2000). Furthermore, it induces the formation of pyrimidine dimers, which distort the DNA helix and block cell replication, compromising cellular functions and eventually leading to cell death. (Manzocco et al., 2011).

The effectiveness of UV–C seems to be independent of the temperature in the range of 5–37°C but depends on the incident irradiation, as determined by the structure and surface of treated product (Bintsis et al., 2000; Gardner and Shama, 2000; Lado and Yousef, 2002). Furthermore, the germicidal action of UV light is strongly dependent on the natural resistance of the microorganisms. Also, 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). The germicidal effect occurs over relatively short time that is essentially limited to the time of exposure of the microorganism to the UV source (Turtoi et al., 2013).

The use of UV-C light has been proposed for surface disinfection of fresh cut fruit and vegetables. Some studies have reported that UV-C inhibited microbial growth, delaying decay and senescence. In zucchini squash slices UV-C exposure reduced microbial activity and deterioration during subsequent storage at 5 or 10 ◦C (Erkan et al., 2001). 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). Similarly, exposure to UV-C doses (4.5–9 kJ m^{-2}) of kalian-hybrid broccoli reduced mesophilic loads by approximately 1.2 log units while enterobacteria and psychrophilic were unaffected (Martínez-Hernández et al., 2011, 2013d).

It has been reported that abiotic stresses such as that from UV-C light may enhance the nutraceutical content of fresh fruit and vegetables. It would affect secondary metabolism of fresh produce and would increase synthesis of phytochemicals with nutraceutical activity or reduce synthesis of undesirable compounds (Cisneros-Zevallos, 2003).

Treatment with ultraviolet energy offers several advantages to food processors as it does not leave any residue, is easy to use and lethal to most types of microorganisms (Bintsis et al., 2000), the equipment is relatively inexpensive (Yaun et al., 2004) and does not have legal restrictions since Food and Drug Administration approved UV-C light as a disinfectant technology for surface treatment of food (USDA-FDA, 2002).

The best equipment for application of the UV–C technique in the fresh cut industry are 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 21 shows a faba beans sanitation in a UV–C hood.

Figure 21. Faba beans sanitation with UV–C light at a pilot plant scale (discontinuous system).

3.3.5.2. Edible coatings

Edible antimicrobial films or coatings (EC) can avoid browning and improve the quality, safety, shelf life and functionality of foods products by reducing moisture transfer, respiration rate and oxidative processes, while minimizing both spoilage and pathogenic microorganisms (Raybaudi-Massilia et al., 2016).

Edible coatings are defined as a thin layer of material that covers the surface and can be ingested as part of the entire product. Its composition must comply with all regulations that apply to the product used (Guilbert et al., 1995). In accordance with the European Guidelines (ED, 1995; 1998) and FDA (2006), edible coatings must be made from

ingredients suitable for food consumption. Ingredients that can be incorporated into their formulations include: karaya and Arabic resin, pectins, shellac, beeswax and carnauba wax, lecithin, polysorbates, fatty acids and fatty acid salts (ED, 1995 and 1998).

The functional characteristics required for a coating depends on the product matrix and the deterioration processes that the product is subjected to. However, edible coatings must meet a number of functional requirements (Kester and Fennema, 1986):

- Sensory properties: transparent, tasteless and odourless.
- Barrier properties: suitable permeability to water vapour and solutes and selective permeability to gases and volatile compounds.

In addition, formulations should be microbiologically safe, suitable for human consumption and the cost of technology and materials used during processing should be relatively low.

The use of EC in food applications and especially highly perishable products such as horticultural ones, is conditioned by the achievement of diverse characteristics such as cost, availability, functional attributes, mechanical properties (flexibility, tension), optical properties (brightness and opacity), the barrier effect against gases flow, structural resistance to water and microorganisms and sensory acceptability (Falguera et al., 2011).
Edible coatings can create a modified atmosphere by modifying internal gas composition retarding ripening and reducing decay. However, a certain degree of oxygen and carbon dioxide permeability is necessary to avoid anaerobic respiration, which would result in physiological disorders and a rapid loss of quality. (Moldao-Martins et al., 2003). Application of EC on faba bean seeds at pilot plant scale is showed in Figure 22.

Edible coatings and films are usually classified according to their structural material. In this way, films and coatings are based on proteins, lipids, polysaccharides or composite. For example, a composite film may consist of an oil-in-water emulsion.

Edible coatings can affect the quality of the product very differently, as different biological mechanisms are involved. These include control of moisture loss in the product, loss of chemical compounds such as antimicrobials, sugar compounds or antioxidants, reduction of partial pressure of O_2 within the product with a consequent decrease in its metabolism, as well as some restructuring of its internal structure (Shaidi et al., 1999). Some of the effects observed in products to which edible films have been applied include a reduction in RR (Gaouth et al., 1991; Wong et al., 1994), weight loss (Baldwin et al., 1999), enzymatic browning (Baldwin et al., 1999; McHugh and Senesi, 2000; Le Tien et al., 2001) and overall, a significant increase in product shelf life.

Figure 22. Faba beans sanitation with an edible coating (Naturcover-P, Decco Iberica, Spain) (left) at a pilot plant scale, and seeds drying after the treatment (right)

3.3.5.3. Organic acids and acidified solutions

Sanitizing agents are usually added to process water to reduce microbial populations and prevent cross-contamination of products. As alternatives to chlorine, some organic acids such as citric acid (CA) and ascorbic acid (AA), and other chemicals, as acidified sodium chlorite (ASC), have been proposed. These compounds have been applied largely for the prevention of enzymatic and non-enzymatic browning (Sapers, 1993), texture deterioration (Rosen and Kader, 1989) and microbial growth (Yildiz, 1994) at concentrations that did not adversely affect taste and flavour of plant commodities. Application of organic acids on faba bean seeds at pilot plant scale is showed in Figure 23.

The ASC is approved by FDA as a secondary additive in the food industry. The antimicrobial activity of ASC is attributed to the oxidative effect of chlorous acid ($HClO_2$), which is derived from the conversion of chlorite ion into its acid form under strong acidic conditions (Tomás-Callejas et al., 2012). When ASC comes into contact with organic matter, a number of oxychlorous antimicrobial intermediates are formed. These substances are broad-spectrum germicides that act by breaking oxidative bonds on cell membrane surfaces. The fundamental nonspecific oxidative mode of action of this chemistry is thought to also minimize the potential problem of acquired resistance that often arises in bacterial populations following prolonged exposure to antimicrobial procedures (Yousuf et al., 2018).

Inatsu et al. (2005) evaluated the efficacy of ASC in reducing the load of pathogenic microorganisms in lightly fermented chinese cabbage: washing the inoculated leaves with distilled water reduced the load of E. coli O157: H7 in less than 1 UFC g^{-1} log while a dilution of 0.5 g L^{-1} ASC reduced the population by more than 2 log UFC g^{-1}. Allende et al. (2009) found a reduction of 3 log UFC g^{-1} in the population of E. coli O157: H7 in washed MFP cilantro with 1 g ASC L^{-1} compared to control. Also, Tomás-Callejas et al. (2012) observed that after using ASC (300mg L^{-1}) on fresh-cut tatsoi baby leaves the total aerobic mesophilic bacteria remained stable throughout storage for 11 days at 5ºC.

Organic acids such as CA or AA, which are in GRAS status, have been described as strong antimicrobial agents against psychrophilic and mesophilic microorganisms in fresh-cut fruit and vegetables (Uyttendaele et al., 2004; Bari et al., 2005). The antimicrobial action of organic acids is due to environment pH reduction, disturbance of membrane transport and/or permeability, anion accumulation, or a reduction in internal cellular pH (Neal et al., 2012). Less direct antibacterial activities include interference with nutrient transport, cytoplasm membrane damage resulting in leakage, disruption of outer membrane permeability, and influence on macromolecular synthesis (Beuchat, 1998; Inatsu et al., 2005; Miller et al., 2009).

Fresh cut 'Amarillo' melon dipped in 0.52 mM CA for 30 s before MAP reached a shelf-life of 10 d at 5ºC. This treatment maintained microbial safety and avoided

translucency and discoloration. Compared to the control (1.4 mM NaClO), CA increased lightness and improved visual appearance of melon pieces (Aguayo et al., 2003). Dipping green celery crescents in a 0.5 M ascorbic and 0.1 M citric acid solution was as effective as 100 mg L^{-1} NaClO for reducing microbial counts and improving consumer acceptability (Gómez and Artés, 2004).

Figure 23. Faba beans (left), peas (centre) and cowpea (right) sanitation with alternative acids

3.3.6. Dewatering

The water from the product surface must be removed after rinsing to reduce product moisture and not promote microbial growth and enzyme activity (Simons and Sanguansri, 1997; Soliva-Fortuny and Martín-Belloso, 2003). The drying systems include vibrating belts, hydrofoils, centrifuges, tunnel dryers with forced air. Artés-Hernández et al. (2013) recommend using cold air injections on a perforated conveyor belt, although its efficiency may be low for large productions.

3.3.7. Weight and packaging

After drying and the possible application of some coating to the product, the desired quantity is weighed and packaged in trays or bags. The most common packaging method for fresh cut is modified atmosphere (MAP). Aspect of faba, peas and cowpea seeds under MAP are showed in Figure 24.

Figure 24. Details of Faba beans (left), peas (centre) and cowpea (right) under MAP.

It is important avoiding O_2 concentrations exceeding the permissible lower limits, as anaerobic disease can occur inside the package and cause tissue damage and foreign flavours and flavours (Watada et al., 1996).

The main benefits of MAP are (Artés-Hernández et al., 2017):

- 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.

Finally, the containers must be properly labelled including the processing date, expiration date, net weight, producer details and the lot code, which will ensure a good traceability of the final product.

3.3.8. Quality control and cold storage

Before the shipment of the product, it must pass a thorough control to ensure the safety and compliance of all quality specifications. In addition, a procedure of product recall when the specifications are not meet must be accomplished. These control measures are normally carried out with machines that have to be adjusted, checked and continuously calibrated.

The bags or trays with the final product shall be placed in boxes which must be quickly stored between 1 and 4ºC until their distribution, normally within a period of less than one day.

3.3.9. Cold transportation and distribution

The recommendable temperature range throughout distribution chain is 0 to 5ºC. 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 fresh cut products. For retail sale, the fresh cut products must be placed in special display cabinets at 0–5ºC (although 0–1ºC is preferable) in the food supply chains. However, it has already been demonstrated that fresh cut products are often subjected to temperature abuse of about 12ºC in the display cabinets of supermarkets. The inadequate temperature management during distribution and marketing, together 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 fresh cut 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 fresh cut vegetables

The expected characteristics of fresh cut products by consumers are freshness, optimum overall quality (general appearance, sensory quality –texture/firmness, aroma and taste– and nutritional quality) and safety. However, during fresh cut processing and retail period some physiological, physical and nutritional changes may occur, reducing the expected quality attributes. Furthermore, some pathological disorders can appear in the fresh cut products, which can highly limit the shelf–life and safety of the fresh cut product (Artés–Hernández et al., 2013).

3.4.1. Physiological, physical and pathological disorders

The fresh-cut processing steps may increase the metabolism of the plant material, reflected in higher respiration rates (RR) and C_2H_4 emission, which usually leads to a faster deterioration rate (Artés et al., 2007). Temperature is the most important factor that affects the metabolism of these products, and the optimal conservation will depend on the type of product, the cultivar and exposure time. They are also very sensitive to weight loss, because when peeling, seeds are exposed to the absence of protection of the pod.

However, RH is generally very high within the package and dehydration is not a big problem. MAP can be beneficial for keeping RH and maintaining the product quality (Artés et al., 2009).

The action of the enzyme lipoxygenase, which catalyzes peroxidation reactions, should also be taken into account as it can lead to the formation of aldehydes and ketones that are responsible for off–odours during the product's lifetime. These changes in the metabolism of fresh cut legume seeds may be reflected in the following physiological disorders (Suslow and Cantwell, 1998).

- Freezing injury. Appears as water-soaked areas, which subsequently deteriorate and decay. Freezing injury occurs at temperatures of -0.7°C or below (Figure 25).

Physical disorders like rough handling at harvest or damage from shipping containers that can result in translucent areas that are susceptible to decay, should be also minimized (Suslow and Cantwell, 1998).
Common postharvest decay organisms on green seeds are the fungi *Pythium, Rhizopus*, and *Sclerotinia,* all of which may occur as ’nests’ of decay or on broken or damaged seeds.

3.4.2. Nutritional and bioactive compounds changes

The fresh cut 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 fresh cut fruit and vegetables is the temperature management.

Storage conditions strongly influence the stability of postharvest seed colour in many types of beans. In other legumes there is some evidence that temperature, relative humidity (RH), seed moisture content (SMC) and light are the main factors that affect the stability of seed colour during storage (Hughes and Sandsted, 1975; Nordstorm and Sistrunk, 1977; Nozzolillo and De Bezada, 1984; Park and Maga, 1999).

3.4.3. Use of microwaves for cooking

After storage and marketing, the product could be consumed fresh or directly microwaved if packaged in a suitable container, providing a product of high sensory quality and intact nutritional properties. However, cooking methods may affect the nutrient content and health–promoting compounds of fresh cut products, such as vitamin C, polyphenols and glucosinolates (Martínez-Hernández et al., 2013). Nonetheless, Microwaving is more efficient than conventional cooking methods (boiling, high pressure boiling, steaming, etc.) since it takes shorter cooking times with consequent lower nutritional and sensory losses (Castillejo et al., 2018; Martínez-Hernández et al., 2013a, 2013b), so cooking in a microwave oven could be an interesting alternative to conventional cooking due to high efficiency and faster processing time (Alajaji and El-Adawy 2006). It has been reported that microwaved faba beans (6 min) achieved the same anti-nutritional reductions as conventional boiling (30 min) (Luo and Xie, 2013). Also, LasoYadav et al. (2018) studied the impact of microwave (MW) cooking (800 W, 15 min) and boiling (90 min) on the total phenolic compounds of cowpea dry seeds and observed a higher preservation in MW-treated samples regarding boiling samples.

3.4.4. Safety aspects of fresh cut vegetables

The regulation of substances that are used to reduce the microbial load of fresh fruits and vegetables is complex and in some areas uncertain. In each country, the regulatory status of sanitizing solutions is different. The definition of the product used to disinfect wash water depends on 1) the type of product to be washed, and in some cases, 2) to the location where the disinfectant is used (IFPA, 2001). In the USA, the wash water disinfectants used for fresh-cut produce are regulated by the FDA as a secondary direct food additive, unless they are considered to be Generally Recognized As Safe (GRAS) (Gil et al., 2009).

The European Council Directive 94/34/EC (amending Directive 89/107/EEC), on food additives comprises the lists of substances which may legally be added to food if they perform a useful purpose, are safe and do not mislead the consumer. The detailed controls made under the Framework Directive are implemented into the national law of each EU member state and stipulate which food additives are permitted for use, the

specific purity criteria and conditions of use, including maximum levels for specific additives (Gil et al., 2009).

Spain has adopted the EU legislation (94/34/EC) with the RD 3177/1983 approving the Technical-Sanitary Regulation on Food Additives, RD 111/1991 amending the RD 3177/1983, and RD 1359/1998 approving the procedure for incorporating into the Spanish positive lists of additives authorized in other Member States of the EU which are not included in the Spanish lists, or in doses different from those permitted in these lists.

When the mentioned programs are not properly applied, outbreaks may occur with disastrous consequences (Table 6). According to this, these outbreaks associated with fresh cut products have pointed microbiological safety as the major issue of concern in the fresh cut industry. The microbiological risks which may occur in the fresh cut products can be classified into two categories (Hurst et al., 2002):

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

Table 6. Outbreaks linked to fresh and fresh cut produce from 2005 to 2011 (Olaimat and Holley, 2012).

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

EU regulation establishes some pathogenic microorganisms as the unique microbiological criteria. Then, fresh-cut and fruit and vegetables beverages with mild heat treatments or non-thermal treatments are regulated by the EU Regulation

1441/2007 (2007). Table 7 includes the applicable microbial criteria of the latter Regulation.

Table 7. Food safety criteria applied to the fresh-cut products food (Regulation EC 1441/2007, 2007).

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

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

OBJECTIVES

OBJETIVES

The general objective of this research is to optimize several processes to develop new fresh and processed human food from native varieties of three legumes species (faba beans, peas and cowpeas) with high nutritional quality and high bioactive compounds content. Such legumes species are well adapted to several European climates. In that way, legumes of local origin and rich in proteins could be easily included in daily human diet.

The general objective can be achieved through the following specific objectives:

1. Optimize minimal processing and packaging technologies for faba beans, peas and cowpeas to be microwaved during the refrigerated shelf life serving as a 'ready to cook and eat product'.
2. Evaluate the new elaborates in relation of their physical, biochemical, microbiological and sensory characteristics throughout a refrigerated shelf life.
3. Guarantee the nutritional value of these foods, as well as to reduce or eliminate the presence of anti-nutritional factors.
4. Study of the use of alternative chemical and physical disinfectants to NaOCl during legumes minimal processing.
5. Study of the use of edible coatings to avoid quality looses in the developed minimally processed products.

CHAPTERS

Chapter I:

Immature pea seeds: Effect of storage under modified atmosphere packaging and sanitation with acidified sodium chlorite

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ABSTRACT

Peas (*Pisum sativum* L. var. saccharatum) are an important source of protein, carbohydrates, vitamins and minerals. Pods are harvested before physiological maturity and stored at temperatures near 0°C. Due to their very high respiration rate, and even when classified as non-climacteric product, loss of quality is fast. Most studies conducted on fresh peas have dealt with the fresh pod but very little information is available on the optimum storage conditions of immature pea seeds, which are well adapted to be prepared as a minimally processed product. Appropriate sanitation is a priority for extending the shelf life and promoting the consumption of immature pea seeds, as processing accelerates quality deterioration and microbial growth. The effects of sanitation with chlorine (100 ppm, pH 6.5) or alternatively with acidified sodium chlorite (300 ppm, pH 1.8) and passive modified atmosphere packaging on overall quality of fresh pea seeds (var. Lincoln) were assessed during storage at 1 and 4°C. After 12 days, atmospheres within packages were 8 kPa CO_2 / 12 kPa O_2 and 11 kPa CO_2 / 10 kPa O_2 at 1 and 4°C, respectively. Compared with the initial microbial load, samples stored at 1°C showed an increase of 1 log CFU g^{-1} in psychrophiles when treated with NaOCl, whereas no increase of note occurred with ASC. In general, microbial counts were always below 3 log CFU g^{-1} for all the treatments. Greenness and vitamin C had decreased, especially in the NaOCl-disinfected samples. Total phenols and antioxidant capacity were not affected by disinfection. Proteins levels fell by around 27%, regardless of the sanitizer and storage temperature. Low temperature storage allowed obtaining a high quality product even after 12 days of storage, being ASC a good alternative to chlorine. In conclusion, immature pea seeds could be stored for 14 days at 1–4°C under MAP with only minor quality changes. Disinfection with ASC resulted in better sensory quality, higher content of vitamin C and lower psychrophile counts.

Research Article

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Immature pea seeds: effect of storage under modified atmosphere packaging and sanitation with acidified sodium chlorite

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Abstract

BACKGROUND: Appropriate sanitation is a priority for extending the shelf life and promoting the consumption of immature pea seeds, as processing accelerates quality deterioration and microbial growth.

RESULTS: The combined effect of disinfection with acidified sodium chlorite (ASC) or sodium hypochlorite (SH) and packaging under a passive modified atmosphere (MAP) at 1 or 4 °C on quality was analysed. After 14 days, greenness and vitamin C had decreased, especially in the SH-disinfected samples. Total phenols and antioxidant capacity were not affected by disinfection. Proteins levels fell by around 27%, regardless of the sanitizer and storage temperature. Compared with the initial microbial load, samples stored at 1 °C showed an increase of 1 log CFU g^{-1} in psychrophiles when treated with SH, whereas no increase of note occurred with ASC. In general, microbial counts were always below 3 log CFU g^{-1} for all the treatments.

CONCLUSION: Immature pea seeds could be stored for 14 days at 1–4 °C under MAP with only minor quality changes. Disinfection with ASC resulted in better sensory quality, higher content of vitamin C and lower psychrophile counts. More research is needed to analyse the effect of these treatments on other quality parameters.

Keywords: *Pisum sativum* L.; fresh cut; sanitation; microbial counts; antioxidant capacity; vitamin C

INTRODUCTION

Legumes are an important source of proteins, carbohydrates, vitamins and minerals. In particular, green peas are a legume rich in proteins (~24%), complex carbohydrates, vitamins and minerals considered important for humans.[1] For that reason, peas should be consumed as part of a healthy diet to combat obesity and to help prevent diseases such as diabetes, heart disease and cancer.[2,3]

Our current lifestyles, with little time left to prepare balanced meals, combined with an increased interest in healthy food, has led consumers to demand natural, fresh and ready-to-eat vegetable products, as represented by fresh-cut or minimally fresh processed (MFP) fruits and vegetables.[4] The development of minimally processed immature pea seeds, therefore, would be a new format to promote the consumption of legumes. However, processing causes physiological stress, resulting in an increased respiration rate, membrane deterioration, water loss and higher susceptibility to microbial contamination. Few studies have dealt with the physiological response of peas to minimal processing,[5–7] and there are no recent references to immature fresh seeds. Nevertheless, in the few past years, there has been increased interest in this legume, especially concerning its behaviour during storage, susceptibility to oxidation, dehydration and loss of colour, for example.

Sanitation is one of the most critical steps in fresh-cut production, owing to the effects of microbial load on the quality, safety and shelf life of the final product. The industry has widely used sodium hypochlorite (SH) as a sanitizer because of its antimicrobial activity and low cost.[8] However, its use has been questioned because of the risk of trihalomethane synthesis resulting from contact with organic matter and its potentially harmful effect on health.[9]

As an alternative, it is possible to disinfect fresh-cut products with acidified sodium chlorite (ASC), which is obtained by lowering the pH of a sodium chlorite solution ($NaClO_2$), since it has none of the above-mentioned negative effects and is recognized as safe. ASC can be used[10] on raw agricultural commodities at chlorite concentrations of 500–1200 mg L^{-1}. Moreover, ASC has been seen to be effective at inactivating pathogens like *Escherichia coli* O157:H7 and *Salmonella*.[11]

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The objective of this work was to study MFP pea seeds disinfected with ASC, packaged under passive modified atmosphere and stored at two temperatures (1 and 4 °C). Disinfection with SH was used as control. During storage, physicochemical, physiological, microbiological and sensory characteristics, as well as changes in bioactive components (total phenols and vitamin C), proteins and antioxidant capacity, were assessed.

EXPERIMENTAL

Plant material

Pea pods (var. Lincoln) were collected from an open field crop (Balsapintada, Cartagena, Spain) at immature physiological stage, when the pods were almost round (70% of seeds' full size[7]) and with a harvest temperature of 15 ± 2 °C. They were transported cold (5 ± 1 °C, 30 min) to the laboratory, where they were kept in darkness at 1 °C and 90–95% relative humidity (RH).

Processing, packaging and storage

The next day, the peas were shelled by hand in a cold room (5 ± 1 °C) and the obtained peas were immersed in cold water at 4 ± 1 °C. They were then sanitized by immersion in sodium hypochlorite (100 mg L^{-1}, pH 6.5, 2 min, 4 °C) or in ASC (300 mg L^{-1}, pH 1.8, 2 min, 4 °C) before rinsing with cold tap water (4 ± 1 °C, 1 min). Seeds (about 125 g) were packaged in 35 µm polypropylene bags (15 × 15 cm) with an O_2 permeability of 900 cm^3 m^{-2} d^{-1}, CO_2 permeability of 1100 cm^3 m^{-2} d^{-1} at 23 °C and 0% RH. This film was selected based on earlier studies by our research group. Bags were previously sterilized with UV-C light (8 kJ m^{-2}) to prevent any kind of microbial contamination due to the packaging.

The bags were heat sealed to generate a passive modified atmosphere and stored at different temperatures (1 ± 0.5 and 4 ± 0.5 °C) and 90% RH for 14 days. At fixed times (0, 4, 8 and 14 days), samples were removed from storage and analysed. Five replicates per treatment and day of analysis were used.

Atmosphere composition within packages

Throughout storage, the gas composition (O_2 and CO_2) inside the packages was monitored using the method developed by Rodríguez-Hidalgo *et al.*[12] For this, 1 mL of package headspace was extracted with a syringe, through a silicone septum placed over the film, and analysed in a gas chromatograph (7820A GC, Agilent Technologies, Waldbroon, Germany), using three replicates per treatment and per evaluation day. Samples were taken on days 1, 3, 5, 7 and 11 of storage.

Physical quality

Pea colour was analysed using a Minolta colorimeter (CR-300 series, Ramsey, NJ, USA), obtaining a^*, b^* and L^* parameters. Hue angle ($H°$) and total colour difference (ΔE) during storage were compared to their respective initial values.[13] The pH was analysed by pH-meter (GLP 21, Crison, Barcelona, Spain).

To evaluate the bioactive compounds (total phenolic and vitamin C), protein content and antioxidant capacity, samples were frozen in liquid N_2 and stored at −80 °C until analysis.

Vitamin C

Vitamin C, in the form of ascorbic acid (AA) and dehydroascorbic acid (DHA), was measured according to the method of Zapata and Dufour,[14] with slight modifications. Derivatized samples (20 µL) were injected into a Gemini NX (250 mm × 4.6 mm, 5 µm) C18 column (Phenomenex, Torrance CA, USA), using a high-performance liquid chromatograph (Series 1100 Agilent Technologies) equipped with a G1322A degasser, G1311A quaternary pump, G1313A autosampler, G1316A column heater and G1315B photodiode array detector. The system was controlled by Chem Station Agilent v.08.03 software. AA and DHA were quantified using commercial standards (Sigma, St Louis, MO, USA). Total vitamin C, calculated as the sum of AA and DHA, was expressed as grams per kilogram, fresh weight basis (FW). All samples were tested in triplicate.

Total phenolic content (TPC)

TPC was determined using the method developed by Singleton and Rossi,[15] with some modifications introduced by Martínez-Hernández *et al.*[16] Briefly, 2 g frozen samples was placed in glass bottles, and 3 mL methanol was added. The extraction was carried out in an orbital shaker (Stuart, Stone, UK) for 1 h at 200 × *g* in darkness inside a polystyrene box with an ice bed. The extracts were transferred to tubes and centrifuged at 15 000 × *g* for 10 min at 4 °C. Then, 19 µL TPC extract was placed in a well plate, and 29 µL of 1 mol L^{-1} Folin–Ciocalteu reagent was added. The mixture was incubated for 3 min in darkness at room temperature. Then, 192 µL of a solution containing Na_2CO_3 (0.4%) and NaOH (2%) was added. After 1 h of incubation at room temperature in darkness, the absorbance was measured at 750 nm with a multiscan plate reader (Infinite M200, Tecan, Männedorf, Switzerland). TPC was expressed as grams of gallic acid equivalents (GAE) per kilogram FW. All samples were tested in triplicate.

Total antioxidant capacity (TAC)

TAC was determined by the FRAP (fluorescence recovery after photobleaching) method.[17] The absorbance measurement was performed at 593 nm using a multiscan plate reader (Infinite M200). Briefly, a reaction solution containing sodium acetate buffer (pH 3.6), 10 mmol L^{-1} TPTZ solution (in 40 mmol L^{-1} HCl) and 20 mmol L^{-1} $FeCl_3$ was prepared daily in a proportion of 10:1:1 (v/v/v) and incubated at 37 °C for 2 h in darkness. Then, 6 µL TAC extract was allowed to react with 198 µL of the FRAP solution for 40 min at room temperature in darkness. TAC was measured by the decrease in absorbance at 593 nm using the multiscan plate reader. The results were expressed as grams of Trolox equivalents per kilogram FW. All samples were tested in triplicate.

Protein

To determine the protein content, 5 µL of the extract prepared for determining TAC was placed on a polystyrene plate (Greiner Bio-One, Frickenhausen, Germany), to which 250 mL Bradford reagent was added and allowed to react for 30 min in the dark and at room temperature. The absorbance at 595 nm was measured with the multiscan plate reader. The protein content was expressed as gram equivalents of albumin per kilogram FW. All samples were tested in triplicate.

Sensory evaluation

A panel of seven people (aged 24–50), trained in sensory quality analysis, performed the evaluation. Before running the experiments, a consensus was reached among the panellists on those

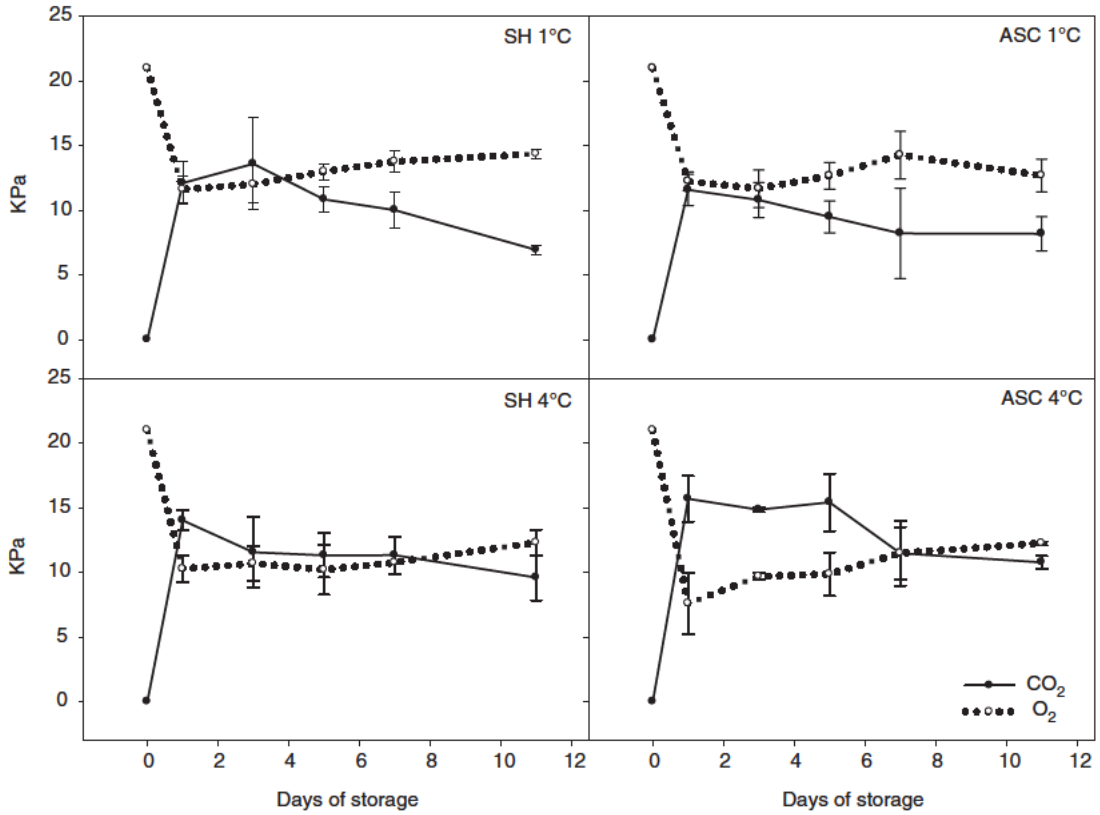

Figure 1. Changes in gas composition within packages of fresh pea seeds washed with different sanitizers (SH, sodium hypochlorite; ASC, acidified sodium chlorite) and stored in MAP for up to 14 days at 1 and 4 °C. Data are mean ± standard deviation ($n = 3$).

attributes that best described sensory changes. Samples (about 30 g) were served in randomly coded transparent glasses. Sensory analyses were performed according to international standards (ASTM STP 913 1986). Still mineral water was used as palate cleanser. Sensory quality was evaluated on the processing day and after 4, 8 and 14 days of storage at both 1 and 4 °C. A 9-point hedonic scale was scored for visual symptoms of dehydration (9 = none; 5 = limit of usability; 1 = extreme), and other parameters, such as visual appearance, flavour, aroma, texture, colour and overall quality, were scored as follows: 1 = extremely bad; 5 = limit of usability; 9 = excellent.

Microbial growth

To determine the mesophilic and psychrophilic bacteria, enterobacteria, and yeast and mould growth, standard enumeration methods were used.[18] Samples of 10 g were homogenized in 90 mL sterile peptone saline solution (pH 7; Scharlau Chemie SA, Barcelona, Spain) for 30 s 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, tenfold dilution series were prepared in 9 mL sterile peptone saline solution. Mesophilic, enterobacteria and psychrotrophiles 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) for mesophilic and psychrotrophilic aerobic bacteria, incubated at 30 °C for 48 h and at 5 °C for 7 days, respectively; violet red bile dextrose agar (Scharlau Chemie) for enterobacteria, incubated at 37 °C for 48 h; and rose Bengal agar (Scharlau Chemie) 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}). Each of the three replicates was analysed in duplicate.

Statistical analysis

Analysis of variance was performed to compare the sanitizing treatments, storage times and temperatures at a significance level of $P \leq 0.05$, using PASW Statistics 23 for Windows (SPSS Inc., Chicago, IL, USA). In some cases, when significant differences were observed, the Tukey HSD (honestly significant difference) test was applied.

RESULTS

Atmosphere composition within packages

The generated passive modified atmosphere (MAP) was analysed to detect whether the sanitizers had any influence on the gas composition (Fig. 1). During the first day of storage there was an increase in the partial pressure of CO_2 and a decrease in O_2, both remaining constant from that time onwards (8 kPa CO_2/12 kPa O_2 and 11 kPa CO_2/10 kPa O_2 at 1 and 4 °C). No differences in the O_2 and CO_2 concentrations during storage were observed between sanitizing solutions. However, changes were influenced by the storage temperature. The concentration of O_2 was lower in the packs kept at 4 °C than at 1 °C, whereas the CO_2 concentration was always higher when stored at 4 °C than at 1 °C (Fig. 1).

Physical quality analysis

No significant differences in pH were observed between sanitizing treatments, although a significant decrease was observed

Table 1. pH and colour of fresh pea seeds washed with different sanitizers (S) (SH, sodium hypochlorite; ASC, acidified sodium chlorite) and stored in MAP for up to 14 days at 1 and 4 °C. Data are mean ± standard deviation ($n = 3$)

t	*T*	*S*	pH	*L**	*a*	*b*	Δ*E*
0	1 °C	SH	6.60 ± 0.00	44.53 ± 7.75	−14.47 ± 1.31	29.86 ± 1.90	0.00 ± 0.00
		ASC	6.63 ± 0.06	44.53 ± 7.75	−14.47 ± 1.31	29.86 ± 1.90	0.00 ± 0.00
	4 °C	SH	6.60 ± 0.00	44.53 ± 7.75	−14.47 ± 1.31	29.86 ± 1.90	0.00 ± 0.00
		ASC	6.63 ± 0.06	44.53 ± 7.75	−14.47 ± 1.31	29.86 ± 1.90	0.00 ± 0.00
4	1 °C	SH	6.40 ± 0.10	52.20 ± 4.13	−13.43 ± 0.99	28.01 ± 2.04	6.54 ± 0.81
		ASC	6.37 ± 0.06	47.55 ± 5.04	−13.10 ± 0.97	26.65 ± 2.38	5.14 ± 0.69
	4 °C	SH	6.37 ± 0.12	51.85 ± 4.61	−13.68 ± 0.78	28.09 ± 1.60	5.23 ± 1.30
		ASC	6.33 ± 0.06	51.00 ± 5.93	−12.89 ± 1.05	26.30 ± 1.91	6.11 ± 0.31
8	1 °C	SH	6.33 ± 0.15	50.62 ± 6.67	−14.51 ± 1.31	29.67 ± 2.98	6.96 ± 0.97
		ASC	6.37 ± 0.06	47.63 ± 4.28	−14.08 ± 0.79	28.20 ± 1.26	6.16 ± 0.74
	4 °C	SH	6.73 ± 0.40	49.24 ± 6.18	−13.67 ± 1.22	27.97 ± 2.41	5.67 ± 1.30
		ASC	6.43 ± 0.12	51.78 ± 5.57	−14.08 ± 1.64	29.14 ± 3.50	6.51 ± 1.85
14	1 °C	SH	6.17 ± 0.06	46.01 ± 3.34	−13.28 ± 1.31	28.86 ± 3.07	6.51 ± 1.09
		ASC	6.17 ± 0.06	50.01 ± 4.13	−13.29 ± 0.66	27.92 ± 0.97	8.75 ± 1.20
	4 °C	SH	6.13 ± 0.06	48.47 ± 5.28	−12.40 ± 0.92	27.42 ± 2.33	7.20 ± 0.78
		ASC	6.23 ± 0.06	42.18 ± 7.81	−12.01 ± 1.43	27.81 ± 2.42	6.19 ± 0.67
Sanitizer (*S*)			ns	ns	ns	ns	ns
Time (*t*)			***	***	***	***	***
Temperature (*T*)			ns	ns	ns	ns	ns
S × *t* × *T*			ns	ns	ns	ns	ns
S × *t*			ns	ns	ns	ns	ns
S × *T*			ns	ns	ns	ns	ns
t × *T*			ns	ns	ns	ns	ns

during storage (Table 1), with no notable difference between temperatures.

Colour is one of the most important visual attributes of peas. In this respect, storage time had a significant effect on lightness (*L**), colour difference (Δ*E*) and *a** and *b** parameters after 14 days of cold storage. As regards Δ*E*, which represents the colour change perceptible to consumers, its value increased with time, with no differences between sanitizing treatments or temperatures. Lightness also tended to increase, whereas *a** and *b** values tended to decrease, with no differences between treatments or temperatures. Colour changes were related to a loss in green colour and brightness, both corroborated by the sensory panel.

Vitamin C

Vitamin C (Fig. 2) decreased during storage with both sanitizing treatments and at both temperatures. The decrease was from 707 to 267 g kg^{-1} FW at 1 °C and from 707 to 306 mg kg^{-1} FW at 4 °C when SH was used, which was about 30% less drastic than in in the case of ASC (from 643 to 446 and 466 mg kg^{-1} FW at 1 and 4 °C, respectively). In this way, at the end of the storage time, the vitamin C content had decreased to a greater extent after using SH, without any important effect of temperature being observed. For all cases, the concentration of DHA was always higher than that of AA. This fact is consistent with the results obtained by Martínez-Sánchez *et al.*[19]

Total phenolic content

A tendency towards a slight increase (around 15%) was observed for all the treatments as storage time progressed (Fig. 3). On the processing day, the TPC was very similar for both SH and ASC washing solutions: 25.18 ± 4.73 and 26.95 ± 6.62 mg GAE 100 g^{-1} FW, respectively, with no significant differences between them. At the end of storage, the TPC values were 30.83 ± 1.91 and 31.02 ± 6.08 mg GAE 100 g^{-1} FW for seeds treated with SH at 1 and 4 °C, respectively, and 30.81 ± 6.37 and 30.46 ± 9.00 mg GAE 100 g^{-1} FW for those treated with ASC at 1 and 4 °C.

In general, the TPC of fresh peas was not affected by the washing solutions. Moreover, the storage time did not have any detrimental effect either. However, although not significant, TPC increased between days 4 and 8 in the product treated with ASC at 4 °C before falling, to reach the same values as the other treatments.

Total antioxidant capacity

The initial TAC of fresh-cut peas treated with SH and ASC was similar (27.41 ± 0.13 and 28.01 ± 1.04 mg Trolox equivalents 100 g^{-1} FW, respectively). At the end of storage it had slightly decreased to 23.80 ± 3.98 and 27.40 ± 2.54 mg Trolox equivalents 100 g^{-1} FW for SH-treated samples at 1 and 4 °C, respectively, and to 25.90 ± 5.20 and 24.04 ± 1.73 mg Trolox equivalents 100 g^{-1} FW for ASC-treated samples at 1 and 4 °C, respectively.

Independently of this decreasing trend, and, as can be seen in Fig. 4, the TAC determined by FRAP method did not generally show any significant ($P > 0.05$) change during storage, with no differences between treatments or temperatures. However, although not significant, TAC increased during the first 4 days for the product treated with ASC at 4 °C and then fell to the same values as the other treatments.

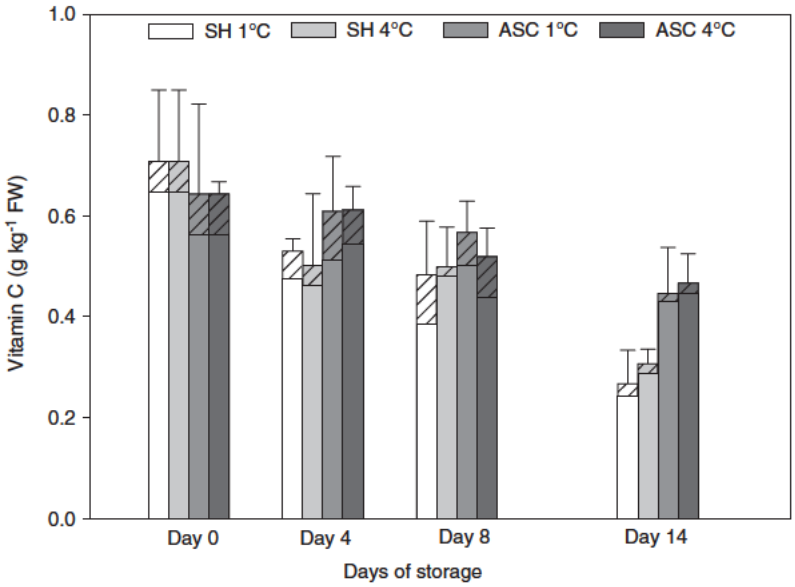

Figure 2. Evolution of vitamin C (AA + DHA) in MAP-stored immature pea seeds (1 and 4 °C) previously sanitized with sodium hypochlorite (SH) or acidified sodium chlorite (ASC) (DHA, dehydroascorbic acid; AA, ascorbic acid). Data are mean ± standard deviation ($n = 3$).

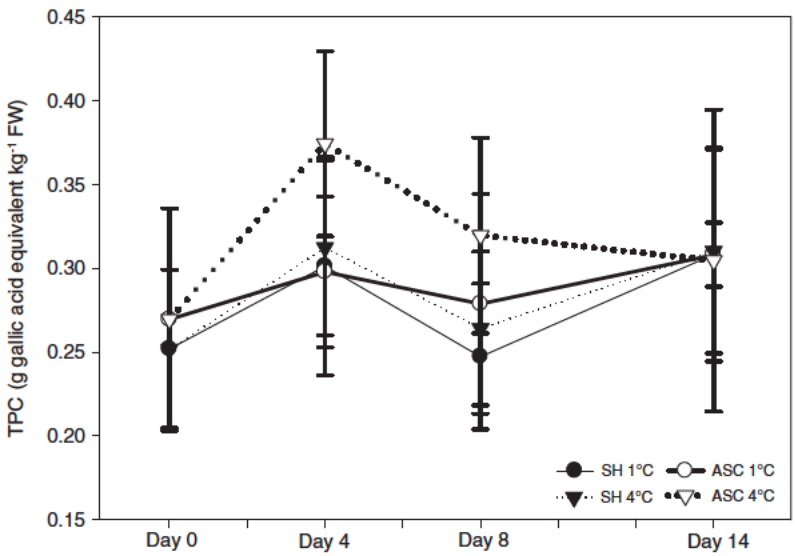

Figure 3. Evolution of total phenolic content (TPC) in MAP-stored immature peas seeds (1 and 4 °C), previously sanitized with sodium hypochlorite (SH) or acidified sodium chlorite (ASC). Data are mean ± standard deviation ($n = 3$).

Proteins

The initial protein concentration was 23.35 mg albumin equivalents 100 g^{-1} FW for the seeds treated with SH, and 21.49 albumin equivalents 100 g^{-1} FW for the group treated with ASC, while the final concentrations were 14.77 and 13.78 albumin equivalents 100 g^{-1} FW in the group treated with SH at 1 and 4 °C, respectively, and 13.43 and 11.98 mg albumin equivalents 100 g^{-1} FW for those treated with ASC at 1 and 4 °C, respectively. As shown in Fig. 5, the protein concentration fell during storage, regardless of the type of sanitizer used and storage temperature. This downward trend was more pronounced from day 4 onwards.

Sensory evaluation

The effects of the different washing treatments on the sensory quality of immature seeds are shown in Fig. 6. The mean scores for all sensory attributes at day 0 indicated an optimal quality, with no differences between the washing solutions as regards overall quality, taste and colour. During storage, overall quality, taste and colour declined, although all the scores indicated good sensory quality with no significant differences between washing treatments. From day 8, the peas showed symptoms of dehydration. At day 14, especially in the case of samples stored at 4 °C, those sanitized with ASC presented a better sensory quality than those treated with SH. Dehydration and taste were worse for SH than ASC. However, all the samples were scored as being above the acceptable limit for fresh consumption.

Microbial analysis

The total mesophilic counts of fresh peas stored at 1 and 4 °C are shown in Fig. 7. Initial values were relatively low: 1.81 and 1.70 log CFU g^{-1} for SH and ASC, respectively.

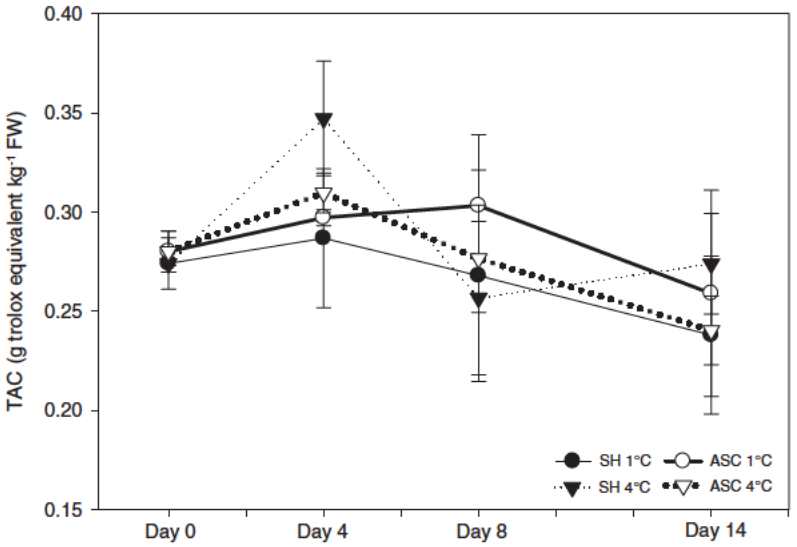

Figure 4. Evolution of total antioxidant capacity (TAC) measured by FRAP method in MAP-stored immature peas seeds (1 and 4 °C) previously sanitized with sodium hypochlorite (SH) or acidified sodium chlorite (ASC). Data are mean ± standard deviation ($n = 3$).

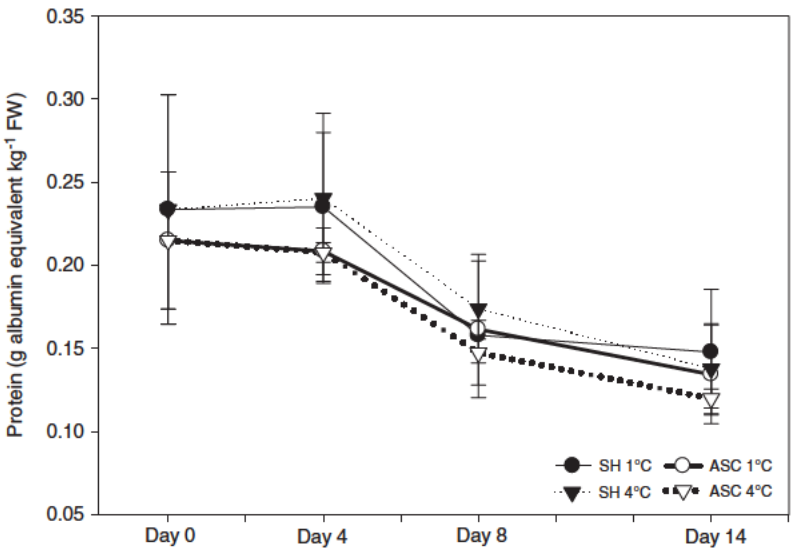

Figure 5. Evolution of proteins in MAP-stored immature peas seeds (1 and 4 °C) previously sanitized with sodium hypochlorite (SH) or acidified sodium chlorite (ASC). Data are mean ± standard deviation ($n = 3$).

During the first week of storage, mesophilic bacteria counts remained almost unchanged, but after 8 days they increased for all the samples. After 14 days at 1 °C the mesophilic counts reached 2.90 and 2.63 log CFU g^{-1} for SH and ASC, respectively, and 3.00 and 2.99 log CFU g^{-1} for SH and ASC at 4 °C, respectively. A comparison of the antimicrobial effect of ASC and SH after washing provided similar results, and no significant differences were observed during storage.

Psychrophile counts for samples stored at 4 °C remained unchanged until day 4 for samples treated with ASC, and until day 8 for those sanitized with SH, both then increasing by 2 log units. By contrast, samples stored at 1 °C showed an increase of just 1 log unit at the end of storage for SH, and no noticeable increase for the ASC treatment.

Mould and yeast counts remained constant (2.00 log CFU g^{-1}) throughout the storage period, whereas no enterobacteria were detected in any treatment (values below the detection limit).

DISCUSSION

Peas are a highly perishable product and their shelf life and visual quality greatly depend on the storage conditions, including temperature and atmosphere composition.[5,20,21] A high storage temperature increases the respiration rate, leading to greater decay.[22] Green beans have intense respiration and heat emission, which limits their postharvest shelf life to 3–4 weeks maximum. This can be partly attributed to the intense metabolic activity of immature seeds inside the pods.[22] The results presented here show that a cold temperature and modified atmosphere can extend the shelf life of peas. Changes in gas composition inside the packages were influenced by the temperature. These data are consistent with those of Martínez-Sánchez *et al.*[23] The atmospheric composition within the packages obtained in our study can be considered as appropriate for this product, in accordance with previous studies.[6] However, those studies were based on pea pods, whereas the results presented here refer to immature seeds.

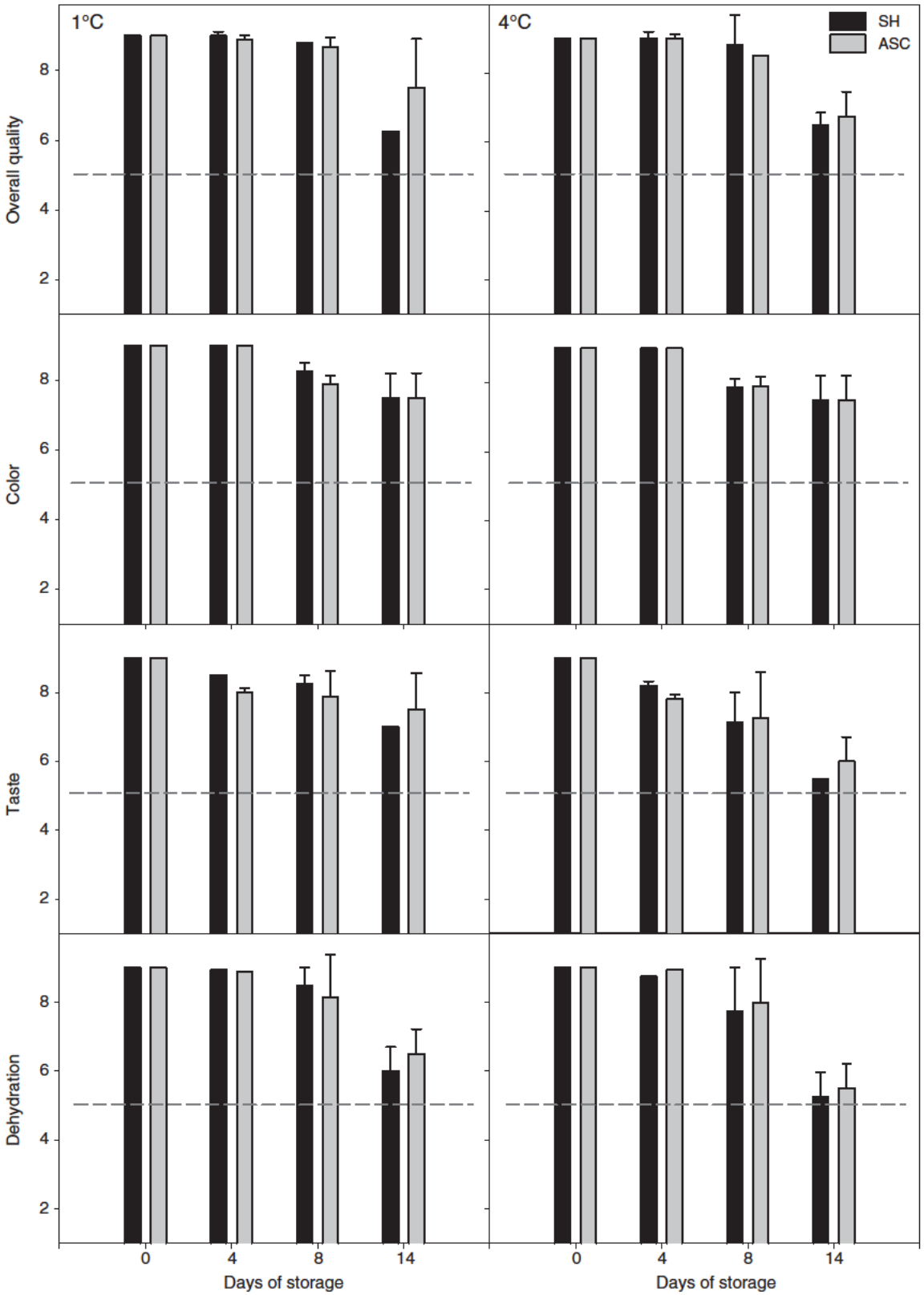

Figure 6. Evolution of sensory quality of MAP-stored immature pea seeds (1 and 4 °C) previously sanitized with sodium hypochlorite (SH) or acidified sodium chlorite (ASC) at 1 and 4 °C. Dashed horizontal line represents the limit of marketability. Data are mean ± standard deviation ($n = 3$).

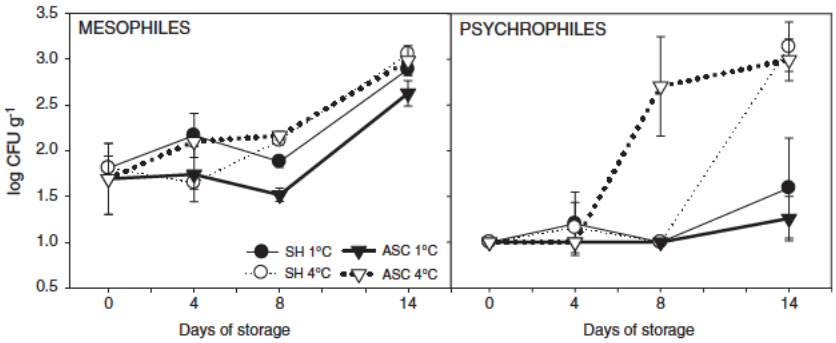

Figure 7. Evolution of mesophiles and psychrophiles in MAP-stored immature peas seeds (1 and 4 °C) previously sanitized with chlorine (SH) or acidified sodium chlorite (ASC). Data are mean ± standard deviation ($n = 3$).

Colour is one of the most important quality parameters determining consumer acceptance. ΔE levels gradually increased during storage. However, storing fresh immature pea seeds at the lower temperature (1 °C) and with a suitable adequately modified atmosphere induced lower colour changes. The maintenance of colour during storage was also confirmed by sensory analyses, since the scores for colour and overall quality were above the acceptance limit. In addition, no significant differences in colour between ASC and SH treated samples were observed.

The seeds presented a low initial degree of microbial contamination, which was probably due to the fact that they were within the pods and, consequently, protected from external bacteria. Moreover, good management practices during processing preserved the safety of the product. The antimicrobial effect of sanitizers after application is very important, but the maintenance of their antimicrobiological effect during storage is also essential. In this study, aerobic microflora, psychrophiles and moulds and yeasts were below 3 log CFU g^{-1} at the end of cold storage, independently of the sanitizer used. In shredded carrots, Ruiz-Cruz *et al.*[24] observed that aerobic bacteria grew rapidly during cold storage for all the concentrations of ASC used, while Escalona *et al.*[25] detected a fast increase in mesophilic bacteria on minimally processed summer-harvested watercress washed with ASC (250 mg L^{-1}). Our results agree with those reported by Tomás-Callejas *et al.*,[26] who observed that after using ASC (300 mg L^{-1}) on fresh-cut tatsoi baby leaves the total aerobic mesophilic bacteria remained stable throughout storage for 11 days at 5 °C. The antibacterial capacity of ASC is attributed to chlorous acid, which is formed by the acidification of chlorite.[27] Chlorous acid gradually decomposes to form chlorate ions, chlorine dioxide and chloride ions. These reactive intermediates are highly oxidative, with broad-spectrum germicidal activity.[28] Moreover, the low pH of ASC solutions (~1.8) probably affected the ability of cells to maintain pH homeostasis, disrupting substrate transport and inhibiting metabolic pathways.[29]

Phenolic compounds in plants are largely responsible for metabolism and defensive mechanisms. The induction of phenolic compounds is a physiological response to infections or injuries. Any changes in the environment surrounding the product during storage stimulate the cells to induce more phenols in an attempt to initiate the response of the defence mechanism.[7] In general, the total phenolic content of pea seeds was not affected by the washing solutions or temperature (Fig. 3), but did show an upward trend during storage, which could be attributed to the stressful condition during processing. Similar trends were reported by Anurag *et al.*[7] and Selcuk and Erkan,[30] in this last case for pomegranate arils.

The retention in antioxidant capacity observed in our study could be attributed to the retention of total phenols. Similar FRAP values were observed for different sanitizers and temperatures, with no significant changes. In agreement with this, a very slight decrease in antioxidant activity was recorded in broccoli samples, irrespective of treatments, with the advancement of storage time.[31]

The present results related to the significant retention of phenolic compounds and the antioxidant capacity are important, since phenolic compounds have therapeutic properties for various diseases such as diabetes, bacterial and viral infections, hypercholesterolaemia, gastrointestinal ulcers, cancer and cardiovascular diseases due to their antiradical properties.[32]

Ascorbic acid (AA) is considered to be highly sensitive to processing and shelf life conditions and it is often used as a marker for product quality deterioration.[7] In our study, vitamin C levels fell during the storage period. This decrease may be associated with the accelerated rate of biochemical changes caused by processing, which can sometimes increase even at low temperatures,[33] and is consistent with previous studies.[7,31,34,35] Most of the vitamin C content was due to DHA (Fig. 2), perhaps because during processing pea seeds suffer oxidative stress that transforms AA to DHA. The vitamin C content of the fresh peas treated with ASC was higher at the end of storage, when a considerably lower decrease (30%) was evident compared to the value attained with SH. The low pH of the ASC washing solution could favour that trend.

Statistical analysis of the obtained data revealed, as reported by Sánchez-Mata *et al.*,[22] significant variations in the total protein content during storage. In most of the analysed samples, a general and significant decrease in the protein content was observed in the final stages of storage (Fig. 5), especially in peas stored at 4 °C, probably due to the more intense protein and amino acid degradation that occurs in this period. Other authors have reported a general decrease in total solids during shelf life, as indicated by Kinyuru *et al.*[35] for snap bean.

CONCLUSION

Immature green pea seeds can be stored for 14 days under MAP at temperatures between 1 and 4 °C without any noticeable quality loss. The use of ASC as a sanitizer during processing presents itself as a good alternative to SH since it led to seeds with a better sensory quality and a higher content of vitamin C. More research is needed in the future to analyse the effect of these treatments on other important aspects of pea quality, such as the possible presence of antinutritional factors.

ACKNOWLEDGEMENTS

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REFERENCES

1 Dahl WJ, Foster LM and Tyler RT, Review of the health benefits of peas (*Pisum sativum* L.). *Br J Nutr* **108**:S3–S10 (2012).
2 Tharanathan RN and Mahadevamma S, Grain legumes, a boom to human nutrition. *Trends Food Sci Technol* **14**:507–518 (2003).
3 Trinidad P, Mallillin AC, Loyola AS, Sagum RS and Encabo RR, The potential health benefits of legumes as a good source of dietary fibre. *Br J Nutr* **103**:569–574 (2010).
4 Artés-Hernández F, Aguayo E, Gómez PA and Artés F, Innovaciones tecnológicas para preservar la calidad de los productos vegetales mínimamente procesados o de la 'cuarta gama'. *Hortic Int* **69**:52–57 (2009).
5 Pariasca JAT, Miyazaki T, Hisaka H, Nakagawa H and Sato T, Effect of modified atmosphere packaging (MAP) and controlled atmosphere (CA) storage on the quality of snow pea pods (*Pisum sativum* L. var. saccharatum). *Postharvest Biol Technol* **21**:231–223 (2001).
6 Cantwell M and Saltveit M, Tolerance of sugar snap peas to modified atmospheres with high concentrations of carbon dioxide. *Acta Hortic* **1071**:619–625 (2015).
7 Anurag RK, Manjunatha M, Narayan JS and Kumari L, Storage quality of shelled green peas under modified atmosphere packaging at different storage conditions. *J Food Sci Technol* **53**:1640–1648 (2016).
8 Artés F, Gómez P, Aguayo E, Escalona V and Artés-Hernández F, Sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities. *Postharvest Biol Technol* **51**:287–296 (2009).
9 Ölmez H and Kretzschmar U, Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *Food Sci Technol* **42**:686–693 (2009).
10 Food and Drug Administration, Code of Federal Regulations Title 21. [Online]. Available: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Databases/ucm135680.htm [6 July 2016].
11 Ruiz-Cruz S, Islas-Osuma MA, Sotelo-Mundo RR, Vázquez-Ortiz F and González-Aguilar GA, Sanitation procedure affects biochemical and nutritional changes of shredded carrots. *J Food Sci* **72**:146–152 (2007).
12 Rodríguez-Hidalgo S, Artés-Hernández F, Gómez PA, Fernández J and Artés F, Quality of fresh-cut baby spinach grown under floating trays system as affected by N fertilization and innovative package treatments. *J Sci Food Agric* **90**:1089–1097 (2010).
13 Walkling-Ribeiro M, Noci F, Cronin DA, Lyng JG and Morgan DJ, Shelf life and sensory attributes of a fruit smoothie-type beverage processed with moderate heat and pulsed electric fields. *Food Sci Technol* **43**:1067–1073 (2010).
14 Zapata S and Dufour JP, Ascorbic, dehydroascorbic and isoascorbic and simultaneous determinations by reverse phase ion interaction HPLC. *J Food Sci* **57**:506–511 (1992).
15 Singleton VL and Rossi JA, Colorimetry of total phenolics with phospomolobdic–phosphotungstic acid reagents. *Am J Enol Vitic* **16**:144–158 (1965).
16 Martínez-Hernández GB, Gómez PA, Pradas I, Artés F and Artés-Hernández F, Moderate UV-C pretreatment as a quality enhancement tool in fresh-cut Bimi® broccoli. *Postharvest Biol Technol* **62**:327–337 (2011).
17 Benzie IFF and Strain JJ, Ferric reducing antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol* **299**:15–27 (1999).
18 Castillejo N, Martínez-Hernández GB, Gómez PA, Artés F and Artés-Hernández F, Red fresh vegetables smoothies with extended shelf life as an innovative source of health-promoting compounds. *J Food Sci Technol* **53**:1475–1486 (2016).
19 Martínez-Sánchez A, Allende A, Bennett RN, Ferreres F and Gil MI, Microbial, nutritional and sensory quality of rocket leaves as affected by different sanitizers. *Postharvest Biol Technol* **42**:86–97 (2006).
20 Kader AA, Postharvest biology and technology: an overview and modified atmosphere during transport and storage, in *Postharvest Technology of Horticultural Crops*, ed. by Kader AA. Publication 3311, University of California, Davis, CA, pp. 15–17, 85–92 (1992).
21 Elwan MWM, Nasef IN, El-Seifi SK, Hassan AM and Ibrahim RE, Storability, shelf-life and quality assurance of sugar snap peas (cv. super sugar snap) using modified atmosphere packaging. *Postharvest Biol Technol* **100**:205–211 (2015).
22 Sánchez-Mata MC, Camara M and Diez-Marques C, Extending shelf-life and nutritive value of green beans (*Phaseolus vulgaris* L.), by controlled atmosphere storage: macronutrients. *Food Chem* **80**:309–315 (2003).
23 Martínez-Sánchez A, Allende A, Cortés-Galera Y and Gil MI, Respiration rate response of four baby leaf *Brassica* species to cutting at harvest and fresh-cut washing. *Postharvest Biol Technol* **47**:382–388 (2008).
24 Ruiz-Cruz S, Luo Y, Gonzalez RJ, Tao Y and González GA, Acidified sodium chlorite as an alternative to chlorine to control microbial growth on shredded carrots while maintaining quality. *J Sci Food Agric* **86**:1887–1893 (2006).
25 Escalona VH, Hinojosa A, Char C, Villena P, Bustamante A and Saenz C, Use of alternative sanitizers on minimally processed watercress harvested in two different seasons. *J Food Process Pres* **39**:1287–1298 (2015).
26 Tomás-Callejas A, López-Velasco G, Artés F and Artés-Hernández F, Acidified sodium chlorite optimization assessment to improve quality of fresh-cut tatsoi baby leaves. *J Sci Food Agric* **92**:877–885 (2012).
27 Warf CC, The chemistry and mode of action of acidified sodium chlorite, in *IFT Annual Meeting*, pp. 1–91 (2001).
28 Food and Drug Administration, Guide to Minimize Microbial Food Safety Hazards of Fresh-cut Fruits and Vegetables 2007. [Online]. Available: http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM169112.pdf [6 July 2016].
29 Beales N, Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH and osmotic stress: a review. *Compr Rev Food Sci Food Saf* **3**:1–20 (2004).
30 Selcuk N and Erkan M, Changes in antioxidant activity and postharvest quality of sweet pomegranates cv. Hicrannar under modified atmosphere packaging. *Postharvest Biol Technol* **92**:29–36 (2014).
31 Nath A, Bagchi B, Misra LK and Deka BC, Changes in post-harvest phytochemical qualities of broccoli florets during ambient and refrigerated storage. *Food Chem* **127**:1510–1514 (2011).
32 Sibul F, Orcic D, Vasic M, Anackov G, Nadpal J, Savic A *et al.*, Phenolic profile, antioxidant and anti-inflammatory potential of herb and root extracts of seven selected legumes. *Ind Crops Prod* **83**:641–653 (2016).
33 Martínez-Damián MT, Cruz-Álvarez O, Beryl Colinas-León MT, Rodríguez-Pérez JE and Ramírez-Ramírez SE, Actividad enzimática y capacidad antioxidante en menta (*Mentha piperita* L.) almacenada bajo refrigeración. *Agronomía Mesoamericana* **24**:57–69 (2013).
34 Zenoozian MS, Combined effect of packaging method and temperature on the leafy vegetables properties. *Int J Environ Sci Dev* **2**:124–127 (2011).
35 Kinyuru JN, Kahenya KP, Muchui M and Mungai H, Influence of post-harvest handling on the quality of snap bean (*Phaseolus vulgaris* L.). *J Agric Food Technol* **1**:43–46 (2011).

Chapter II:

Nutritional and quality changes of minimally processed faba (Vicia faba L.) beans during storage: Effects of domestic microwaving

Collado, E., Venzke Klug, T., Martínez-Hernández, G.B Artés-Hernández, F., Martínez-Sánchez, A., Aguayo, E., Artés, F., Fernández, J.A., Gómez, P.A.

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ABSTRACT

Numerous studies have shown that regular consumption of vegetables is associated with beneficial properties for human health. At the same time, today, there by consumers an interest in functional, healthy and ready to eat foods. Currently they are developing new ways of presenting the bean as minimally processed fresh food as well as its cooked in microwave, trying to encourage consumption, given its advantages as part of a nutritious and healthy diet. Disinfection prior to packaging fresh immature seeds is done usually with sodium hypochlorite. In this study the effect of two alernative treatments were analyzed: the effect of different sanitizers (an edible coating (EC) based on sucrose fatty acid esters, and UV–C (3 kJ m^{-2}), compared with a control consisting of a conventional NaOCl washing (CTRL)) on the quality changes of fresh–cut faba (*Vicia faba* L.) seeds stored for 10 d at 4 °C. Additionally, domestic cooking of samples was assessed by periodically microwaving (3 min, 700 W) during fresh–cut samples storage to obtain a ready–to–eat product. Sensorial attributes, microbial growth, and evolution of vitamin C, total phenolics content (TPC), sugars and tannins were studied on uncooked and cooked faba beans. These analyzes were performed for minimally processed product during storage at 5°C for 10 days, so to this same product immediately after microwave cooking. The modified atmosphere gas composition at the steady was the same for all treatments. Sensorial attributes were above the limit of acceptability for fresh and microwaved beans, subjected to treatment with UV-C light and antibrowing edible coating Naturcover P until the last day of storage. Beans treated with sodium hypochlorite maintained their sensory acceptance until day 7, both in fresh and microwaved product. The EC treatment better retained vitamin C, total phenolics content (TPC) and tannins, while UV–C better maintained the sugars levels of samples. EC and UV–C controlled mesophilic and enterobacteria growth with 1 and 2–log units lower contents than CTRL after 10 d at 4°C. Microwaving reduced the microbial loads below detection limits. EC or UV–C treatments extended the shelf–life of fresh–cut faba seeds from 7 to 10 days at 4°C comparing with CTRL. As expected, microwaving decreased the bioactive compounds contents, but retained the quality of faba seeds allowing to obtain a ready–to–eat tasteful food. A UV–C pretreatment (3 kJ m^{-2}) or a conventional NaOCl sanitizing step plus an edible coating (Naturcover® P; EC) during processing of fresh–cut faba seeds could be considered as important tools to improve their sensory, microbial and nutritional quality. Our results suggest that a typical quality loss during shelf–life can be reduced by using such coadjutants in the processing steps.

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Nutritional and quality changes of minimally processed faba (*Vicia faba* L.) beans during storage: Effects of domestic microwaving

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Edible coatings
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ABSTRACT

The present study evaluated the effect of different sanitizers (an edible coating (EC) based on sucrose fatty acid esters, and UV–C (3 kJ m ^{2}), compared with a control consisting of a conventional NaOCl washing (CTRL)) on the quality changes of fresh–cut (FC) faba (*Vicia faba* L.) seeds stored for 10 d at 4 °C. Additionally, domestic cooking of samples was assessed by periodically microwaving (3 min, 700 W) during FC samples storage to obtain a ready–to–eat product. The modified atmosphere gas composition at the steady was the same for all treatments. The EC treatment better retained vitamin C, total phenolics content (TPC) and tannins, while UV–C better maintained the sugars levels of samples. EC and UV–C controlled mesophilic and enterobacteria growth with 1 and 2–log units lower contents than CTRL after 10 d at 4 °C. Microwaving reduced the microbial loads below detection limits. EC or UV–C treatments extended the shelf–life of fresh–cut faba seeds from 7 to 10 d at 4 °C comparing with CTRL. As expected, microwaving decreased the bioactive compounds contents, but retained the quality of faba seeds allowing to obtain a ready–to–eat tasteful food.

1. Introduction

Legumes are one of the most essential food in the human diet with a per capita consumption of 2.56 kg year ^{1} in Europe, while the world average is 7.21 kg year ^{1} (FAOSTAT, 2013). Most of legumes, like faba beans, are rich in proteins, fats, carbohydrates, antioxidants, fibre, vitamins and minerals (Multari et al., 2015). Faba beans are rich in many bioactive compounds, such as phenolic compounds (mainly flavonoids), with related high antioxidant, anti inflammatory and anti diabetic properties (Siah et al., 2014; Turco et al., 2016). Nevertheless, faba seeds contain some metabolites known as anti nutritional factors that can limit the interest on these legumes (Revilla, 2015). One of the most important are tannins, which are phenolic compounds that can cross link with proteins, carbohydrate and vitamins making them unavailable during digestion (Revilla, 2015). Contrary, tannins have been linked with several health promoting properties (anticarcinogenic, antioxidant, etc.) together with antimicrobial properties (Chung et al., 1998).

The current lifestyle, with limited time for food preparation and the interest in healthy food, has driven the food demand towards natural, fresh, and ready to eat vegetable products. That is the case of the fresh cut (FC) or minimally processed fruit and vegetables (Artés et al., 2009). It is necessary to optimize the processing and preservation methods to maintain the beneficial properties of FC products (Artés et al., 2009). Accordingly, the production of FC immature faba seeds would be an alternative to promote the legumes consumption. Bitter compounds (e.g. tannins) of faba beans may highly limit the consumer acceptance of this legume as a FC product. Nevertheless, bitter flavour is widely known to be masked by sweetness. Accordingly, sweeter faba beans cultivars (as Palenca and Muchamiel.) are preferred for FC faba beans being of high importance the changes in sugars content along storage.

In addition, FC immature faba seeds could be consumed either fresh or directly after domestic cooking. Then, a healthy ready to eat food with high sensory quality would be quickly obtained. However, the studies on immature faba seeds subjected to minimal processing, preservation and cooking are limited.

Sanitation is one of the most critical steps of FC processing in order

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to reduce microbial loads of the product ensuring its safety. Consequently, sanitation aims to maintain the product quality ex tending its shelf life. The FC industry has widely used sodium hypo chlorite (NaOCl) due to its high antimicrobial activity and low cost (Artés Hernández et al., 2009). However, NaOCl may be potentially harmful due to the formation of toxic by products like trihalomethanes and chloramines (López Gálvez et al., 2010). Furthermore, several studies have reported that NaOCl could be insufficient to reduce normal microflora in FC products (Foley et al., 2004).

UV C sanitation has been proposed as an alternative to NaOCl (Artés et al., 2009). UV C radiation damages microbial DNA and, in directly, stimulates the defence mechanisms of plants, retarding decay and senescence processes. In addition, UV C is considered an eco sus tainable sanitation method, due to the absent of residues, with rela tively low cost (Rico et al., 2007; Artés et al., 2009).

Edible coatings (EC) can improve the quality, safety, shelf life and functionality of FC products by reducing moisture transfer, respiration rate and browning, while minimizing both spoilage and pathogenic microorganisms depending of the EC type (Raybaudi Massilia et al., 2016). Particularly, EC based on sucrose fatty acid esters improved the moisture barrier properties and maintained the quality of FC broccoli (Navarro Rico et al., 2015).

Cooking methods may affect the nutrient content and health pro moting compounds of FC products, such as vitamin C, polyphenols and glucosinolates (Martínez Hernández et al., 2013). Furthermore, cooking of legumes reduces the content of several anti nutritional factors (Revilla, 2015). Cooking techniques with short cooking times, such as microwaves, may have beneficial effects on food quality when compared with other long cooking techniques (boiling, high pressure boiling, etc.) (Revilla, 2015).

Nevertheless, there are no studies on quality changes of FC faba beans, which may be consumed fresh or after domestic cooking. This study aimed to evaluate the effects of a conventional NaOCl washing, used as control (CTRL), alone or combined with an EC based on sucrose fatty acid esters, or alternatively after a UV C pretreatment on the overall quality of FC immature faba seeds, which were also periodically microwaved, during refrigerated storage.

2. Materials and methods

2.1. Plant material

Faba beans (*Vicia faba* var. Palenca) were grown in the Southeast of Spain (Balsapintada, Murcia) according to integrated pest management cultural practices. Faba beans were harvested on February at immature physiological stage when the pods were almost round (seeds 70% of full size; Anurag et al., 2016). Beans were cold transported (5 ± 1 °C) ≈28 km to the Pilot Plant of the Institute of Plant Biotechnology. Then, beans were stored at 1 °C and 90 95% relative humidity (RH) until the next day when they were processed.

2.2. Processing, packaging and storage

The next day, faba beans pods were shelled by hand in a cold room (5 °C) and the obtained seeds were immersed in cold water (4 °C) to slow down the seeds metabolism. The obtained seeds were then sani tized using the following treatments:

- CTRL: A standard industrial sanitation with NaOCl washing (150 mg L^{-1}; 2 min; 4 °C; pH 6.5 ± 0.1) was used as the sanitizing control treatment. NaOCl was prepared from a stock solution (50 g L^{-1}; Panreac, Spain). A ratio of 1 kg plant material:2 L disinfectant (*w:v*) was used. The sanitized material was rinsed for 1 min with tap water (4 °C) and subsequently drained for 1 min.
- EC: An EC based on sucrose fatty acid esters was prepared diluting (1:10) Naturcover P® (oil in water emulsion of sucrose fatty acid esters at 230 g L^{-1}; Decco Ibérica S.A.U., Spain) in tap water. Prior to EC, samples were treated by the standard CTRL treatment. Subsequently, samples were immersed for 2 min in the prepared EC and allowed to drain for 5 min in a perforated stainless steel basket.
- UV C: The UV C treatment chamber (Artés Hernández et al., 2009) consisted of a reflective stainless steel chamber with two UV C banks of 15 unfiltered germicidal emitting lamps (> 80% emitted spectrum at λ = 254.7 nm; TUV 36 W/G36 T8, Philips, The Neth erlands) each one. One bank was horizontally suspended 17.5 cm over the radiation vessel and the other was placed 17.5 cm below it. The applied UV C dose (3 kJ m^{-2}; 90 s) was calculated as the mean of 18 UV C readings on each side of the net using a VLX 254 radiometer at λ = 254 nm (Vilber Lourmat, France). Thus, both sides received the same UV C intensity. The UV C light intensity was kept constant and the applied dose was varied by altering the exposure time at the fixed distance. The UV C dose was selected based on our previous experiments.

Treated seeds (≈125 g) were packaged in a bioriented poly propylene (BOPP; 25 + 25 μm) bag (150 × 150 mm) and then heat sealed to generate a modified atmosphere packaging. O_2 permeability of the BOPP film was 700 cm^3 m^{-2} d^{-1} at 23 °C 0% RH according to the supplier (Plásticos del Segura, Murcia, Spain). The film was selected based on earlier studies performed by our research group. Empty bags were previously sanitized with UV−C light (8 kJ m^{-2}) to avoid any kind of microbial contamination. Bags with samples were stored in darkness at 4 ± 0.5 °C and 90% RH up to 10 d. Sampling days were 0 (proces sing day), 3, 7 and 10. Four bags were taken on each sampling time (per treatment) to be analysed as fresh product while another four bags (per treatment) were taken and microwaved (700 W, 1 min; Moulinex BH7, France). Thirty holes (0.2 mm Ø) were done on each bag prior to mi crowaving to allow an adequate vapour gas flushing. A total of 128 bags (4 treatments × 2 cooking treatments × 4 sampling times × 4 replicates) were prepared. All fresh and microwaved samples were analysed for physicochemical determinations on the same sampling day while sample portions for nutritional/anti nutritional contents were frozen (−80 °C) until analysed.

2.3. Gas analysis within modified atmosphere packages

Headspace gas composition (O_2 and CO_2) inside the packages was monitored throughout storage. Samples of 1 mL were taken with a syringe and analysed in a gas chromatograph (GC; 7820 A GC Agilent Technologies, Germany). The GC conditions for CO_2/O_2 determination were: oven at 80 °C, injector and detector at 250 °C, and H_2 and air as gas carriers at 35 and 350 mL min^{-1}, respectively. A PorapakQ GC column (1/8″, 80/100 mesh size; Supelco Inc., Bellefonte PA, USA) was used. Gases calibration was done by comparison with an external CO_2/O_2 standard (Praxair, Spain). Gas sampling was made after 1, 3, 5, 7 and 10 d.

2.4. Microbial analyses

Standard enumeration methods were used to determine mesophilic, psychrophilic, enterobacteria and yeast and mould growth as indicated by Castillejo et al. (2016). All used microbial media was acquired from Scharlau Chemie (Barcelona, Spain). The following media and incuba tion conditions were used: Plate Count Modified Agar for mesophilic and psychotropic aerobic bacteria with incubations of 30 °C/48 h and 5 °C/7 d, respectively; Violet Red Bile Dextrose Agar for enterobacteria with an incubation of 37 °C/48 h; and Rose Bengal Agar for yeasts and moulds (Y + M) with an incubation of 22 °C/7 d. All microbial counts were reported as log colony forming units per gram of product (log CFU g^{-1}). Each of the four replicates was analysed in duplicate.

2.5. Sensory evaluation

The sensory evaluation of samples was performed by a sensory panel of seven people (24 50 years) trained for sensory quality analyses. The evaluations were done in a sensory room according to international standards (ASTM STP 9133, ASTM, 1986). Still mineral water was used as a palate cleanser. A 9 points hedonic scale was scored for visual symptoms of browning and dehydration (9 = none; 5 = limit of usability; 1 = extreme), and the rest of parameters (visual appearance, flavour, aroma, texture, colour and overall quality) were scored as: 1 = extremely bad; 5 = limit of usability; 9 = excellent.

2.6. Total vitamin C

Total vitamin C was analysed according to Zapata and Dufour (1992) with slight modifications. Briefly, 5 *g* of frozen sample was homogenised (UltraTurrax T25 basic, IKA, Germany) with 10 mL cold (5 °C) buffer (0.1 M citric acid, 1.7 mM ethylenediaminetetraacetic acid, 4 M sodium fluoride and 50 mL L^{-1} methanol) for 10 s. The latter extract was filtered (four layers cheesecloth) and the pH was adjusted (6 M HCl) to 2.35 2.40. Then, it was centrifuged (14,000 × g, 15 min, 4 °C) and the supernatant was further purified with solid phase extraction (SPE) C18 columns (Waters, Ireland). The purified extracts were filtered with 0.45 µm polytetrafluoroethylene (PTFE) membrane filters. Derivatisation was conducted to quantify dehydroascorbic acid (DHA). Briefly, 750 µL of the previous extract was derivatised with 250 µL of 18.5 mM 1,2 phenylenediamine for 37 min in darkness at room temperature. Immediately after derivatisation, 20 µL of derivatised sample was analysed in a high performance liquid chromatograph (HPLC). Chromatographic separation was achieved with a Gemini NX (250 mm × 4.6 mm, 5 µm) C18 column (Phenomenex, Torrance CA, USA). The HPLC (Series 1100 Agilent Technologies, Germany) was equipped with a G1311 A quaternary pump, a G1313 A autosampler and a G1315B photodiode array detector. Ascorbic acid (AA) and DHA were quantified at 261 and 348 nm, respectively, using commercial standards (Sigma, USA). Total vitamin C was calculated as the sum of AA and DHA, being expressed as mg kg^{1} on fresh weight basis. Each of the four replicates was analysed in triplicate.

2.7. Total phenolic content

Total phenolic content (TPC) was determined according to Singleton et al. (1999) with some modifications (Martínez Hernandez et al., 2011). Briefly, 2 *g* of ground frozen samples was extracted with 3 mL of methanol for 1 h in an orbital shaker (Stuart, UK) on an ice bed. The obtained extracts were centrifuged at 15,000 × g for 10 min at 4 °C. Then, 19 µL of the TPC extract was placed in a flat − bottom polystyrene 96 − wells plate (Greiner Bio − One, Germany) and 29 µL of 1 N Folin Ciocalteu reagent was added. The latter mix was incubated for 3 min in darkness at room temperature. Subsequently, 192 µL of a mix solution (40 mM Na_2CO_3 and 0.1 M NaOH) was added. Finally, the absorbance was measured at 750 nm with a microplate reader (Tecan Infininte M200, Switzerland) after 1 h of incubation at room temperature in darkness. The TPC was expressed as gallic acid equivalents in g kg^{1} on fresh weight basis. Each of the four replicates was analysed in triplicate.

2.8. Total tannins content

Total tannins content was determined by the modified vanillin method described by Price et al. (1978). Briefly, ground frozen samples (1 *g*) were extracted with 5 mL of methanol for 20 min in a water bath at 30 °C and then centrifuged (15,000 × g, 10 min, 4 °C). Subsequently, 1 mL of the supernatant was mixed with 2.5 mL of a 65.7 mM vanillin (Sigma Chemical, USA) solution and 2.5 mL of 1 M HCl, being then incubated at 30 °C for 20 min. Finally, the absorbance was measured at 500 nm using the microplate reader. Results were expressed as catechin (Sigma Chemical, USA) equivalents in g kg^{1} on fresh weight basis. Each of the four replicates was analysed in triplicate.

2.9. Sugars

The sugars contents were analysed as described by Klug et al. (2018) with some modifications. Frozen samples (3 *g*) were homogenized with milliQ water (10 mL) for 40 s. The extracts were filtrated (four layers cheesecloth) and then centrifuged (12,000 × g, 20 min, 4 °C). The supernatant was purified by SPE with a Strata C18 E column (55 um, 70 A; Phenomenex, UK) and the purified extract was finally filtered with a 0.45 µm PTFE syringe filter. Subsequently, 20 µL were injected on an ultra high performance liquid chromatography instrument (UHPLC; Shimadzu, Japan) equipped with a DGU 20 A degasser, LC 30 CE quaternary pump, SIL 30 AC autosampler, CTO 10AS column heater and refractive index detector. Chromatographic separation was carried out with a RAM Carbohydrate Ag^{+} column (100 mm × 4.6 mm, 2.6 µm particle size; Phenomenex, UK). MiliQ water was used as the mobile phase in an isocratic flow rate of 0.6 mL min^{1}. Quantification was made with commercial standards (Sigma Chemical, USA). Results were expressed as g kg^{1} on fresh weight basis. Each of the four replicates was analysed in triplicate.

2.10. Statistical analysis

Analysis of variance (ANOVA) was performed to compare the sanitizing treatments during storage time for both, fresh and microwaved, samples at a significant level of $P \leq 0.05$ using PASW Statistics 23 for Windows (SPSS Inc., Chicago, IL, USA). When significant differences were observed, the Tukey´s HSD (Honestly Significant Difference) test was applied.

3. Results

3.1. Gas analysis within modified atmosphere packages

The steady state was approximately reached on day 1 with CO_2/O_2 partial pressures of 14/6, 19/3 and 16/4 kPa for CTRL, EC and UV C, respectively. CO_2 was significantly lower in CTRL samples compared with UV C and EC samples throughout storage (Fig. 1).

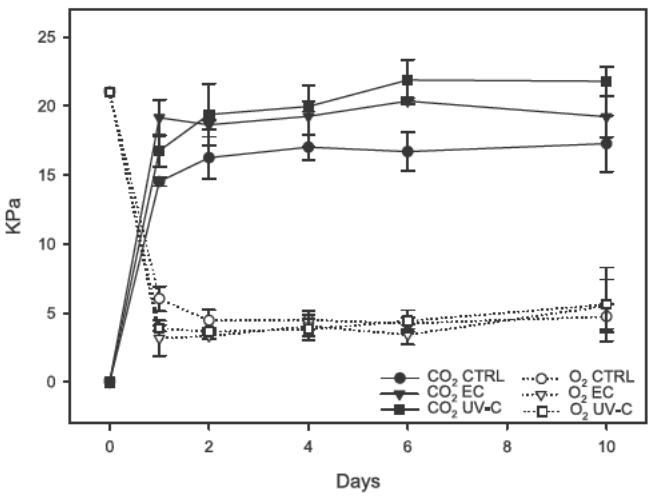

Fig. 1. Gases partial pressures within packages of fresh–cut faba beans treated with different sanitizers up to 10 d at 4 °C (n = 4 ± SD).

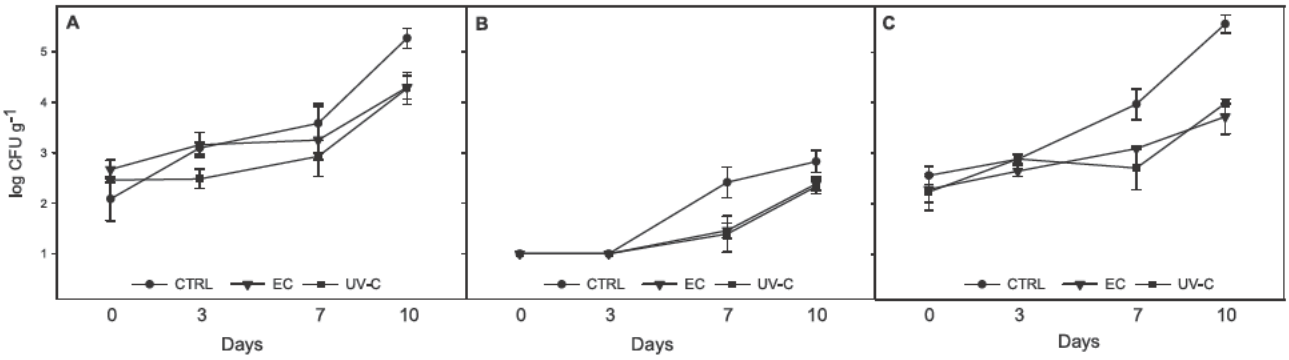

Fig. 2. Mesophilic (A), psychrophilic (B) and enterobacteria (C) loads (log CFU g ^{1}) of fresh–cut faba beans treated with different sanitizers up to 10 d at 4 °C (n = 4 ± SD).

3.2. Microbial analyses

The initial mesophilic, psychrophilic, enterobacteria and Y + M counts of CTRL samples were 2.1 2.7, 1.0, 2.2 2.6 and 2.0 log CFU g ^{1}, respectively (Fig. 2). As expected, microbial loads increased throughout storage. Particularly, mesophilic loads of CTRL samples showed 1 log unit higher loads than EC (4.3 ± 0.2 log CFU g ^{1}) or UV−C (4.3 ± 0.2 log CFU g ^{1}) after 10 d at 4 °C (Fig. 2). The psychrophilic loads of fresh faba seeds remained unchanged (1.0 ± 0.1 log CFU g ^{1}) until day 3, independently of the treatment. However, these counts increased more in CTRL samples compared with EC or UV−C after 7 d of storage. Such differences were maintained until the end of storage. On the other hand, enterobacteria counts increased rapidly in CTRL samples after 7 d with significant ($p < 0.05$) differences in relation to the other treatments. Finally, Y + M counts (data not shown) remained unchanged with low levels (2.0 ± 0.2 log CFU g ^{1}) until the end of storage. Microbial loads of microwaved samples remained under the detection limit (2 log CFU g ^{1} for Y + M and 1 log CFU g ^{1} for the rest of microbial groups) throughout storage (data not shown).

3.3. Sensory evaluation

Most of the evaluated sensory parameters were well maintained after 7 d at 4 °C (Fig. 3). Significant ($p < 0.05$) differences were observed for overall quality, visual appearance and taste after 10 at 4 °C comparing to initial scores. Hence, CTRL samples were under the limit of usability for visual appearance and overall quality. Similar results were observed for aroma, showing CTRL samples the lowest scores at the end of storage (data not shown). Among microwaved samples after 10 d, CTRL was the only treatment below the limit of usability for all sensory parameters. Even when browning increased with the storage time, CTRL samples were the worst scored. Microwave processing did not cause an increase in browning in relation to the fresh seeds. In addition, any symptoms of dehydration were observed along storage. Therefore, samples treated with CTRL showed a reduced shelf life (7 d at 4 °C) regarding samples treated with UV C or EC, which shelf life was established in 10 d at 4 °C.

3.4. Total vitamin C

Total vitamin C significantly decreased during storage in both fresh and microwaved samples, independently of the treatment (Fig. 4). Initial vitamin C values for fresh samples ranged from 154.6 (CTRL) to 163.78 mg kg ^{1} (UV C). Vitamin C decreases of approximately 35 (CTRL), 24 (EC) and 41% (UV C) was observed in fresh seeds after 10 d. Microwaving of 0 D samples reduced total vitamin C contents by ≈ 61, 70 and 72% for CTRL, EC and UV C, respectively. On the other hand, microwaving of 10 D samples reduced total vitamin C contents by ≈ 44, 47 and 53% for CTRL, EC and UV C samples, respectively. No significant ($p > 0.05$) total vitamin C differences were observed between sanitizing treatments for both fresh and microwaved samples.

3.5. Total phenolic content

TPC was similar for all fresh seeds on processing day, independently ($p > 0.05$) of the sanitizing treatment, ranging from 2.13 ± 0.23 g kg ^{1} (CTRL) to 2.58 ± 0.01 g kg ^{1} (EC) (Fig. 5). Those values were maintained for approximately 4 d. Then, TPC decreased until the end of storage (Fig. 5) with TPC reductions of approximately 30%. Contrary, microwaved samples maintained the same TPC during storage. Microwaved samples showed TPC levels approximately 39% lower than those of fresh samples. Particularly, EC better preserved the TPC, especially for the fresh samples, while no significant differences were observed between CTRL or UV C treated samples.

3.6. Total tannins content

Tannins content of fresh samples significantly decreased during storage, while microwaved samples showed unchanged tannins contents during storage (Fig. 6). Total tannins content decreased by 3.13, 1.68 and 3.15 g kg ^{1} after 10 d for fresh CTRL, EC and UV C samples, respectively. Microwaving caused a decrease of approximately 30, 56 and 60% in EC, UV C and CTRL samples, respectively, comparing with fresh samples. EC seeds amounted the highest total tannins content for both fresh and microwaved samples, while UV C induced the lowest total tannins contents (Fig. 6).

3.7. Sugars content

Sucrose and fructose were the main sugars found in faba beans, while raffinose was not detected in any sample (Fig. 7). Sucrose content decreased in fresh samples with no significant differences between treatments (Fig. 7A). For microwaved samples, the storage time significantly affected the sucrose content, but in a minor proportion comparing to fresh samples. UV C treated fresh samples showed the highest sucrose content. Fructose significantly increased after microwaving (Fig. 7B). The highest fructose content was observed in UV C treated samples. After microwaving, sucrose content decreased approximately 44, 27 and 25% in CTRL, EC and UV C samples, respectively. In contrast, microwaving increased fructose content of CTRL, EC and UV C samples by approximately 300, 400 and 350%, respectively.

4. Discussion

Very scarce information is available about the effect of alternative treatments to NaOCl on nutritional and quality parameters of FC faba beans. Since FC faba beans is a product with a high respiration rate, the

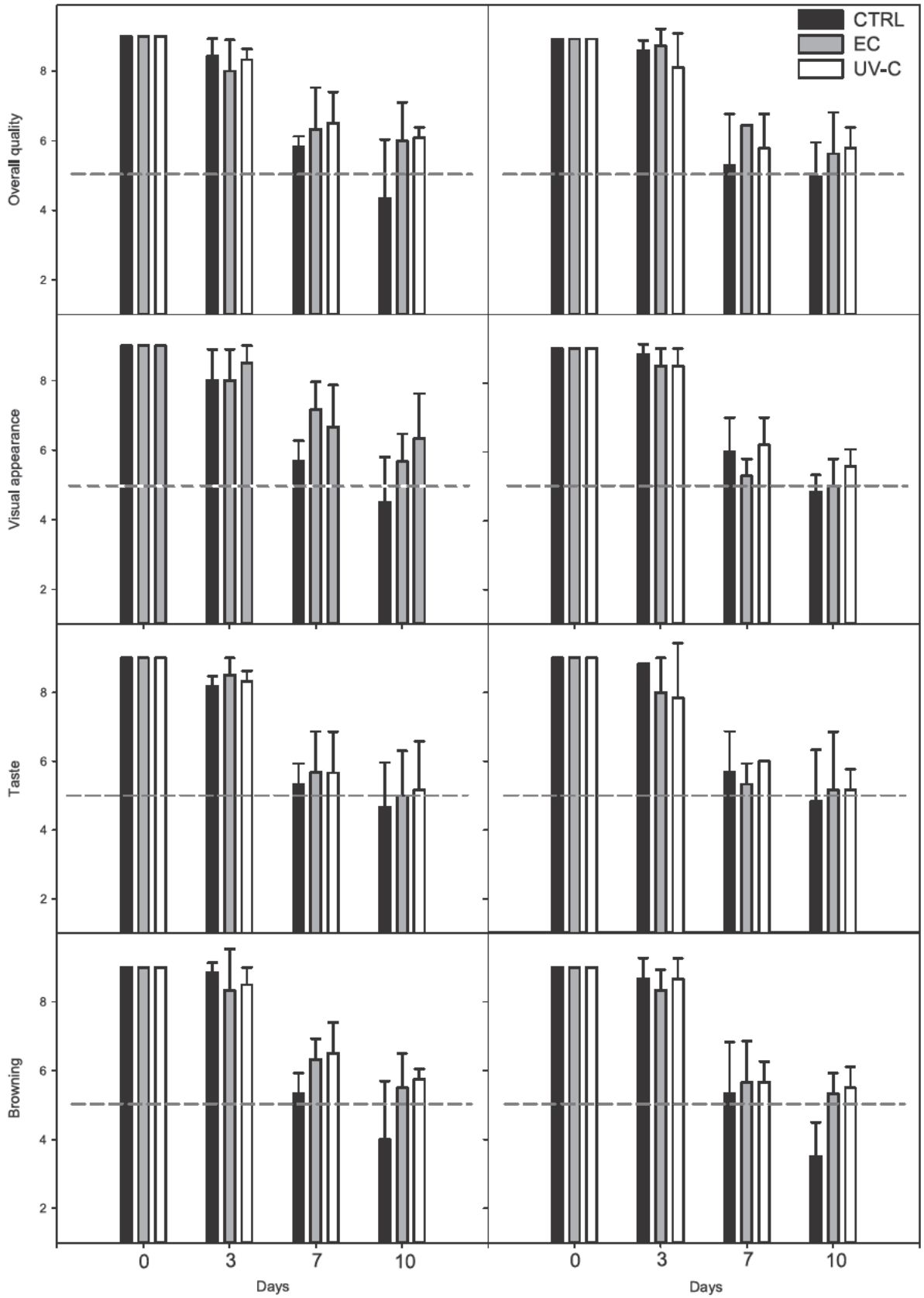

Fig. 3. Sensory quality of fresh–cut (first column) and microwaved (second column) faba beans treated with different sanitizers up to 10 d at 4 °C (n = 4 ± SD).

gas partial pressures within packages could have an effect on their main quality attributes changes (Sánchez Mata et al., 2003). CTRL samples achieved the steady state 1 d later than the remaining treatments, indicating a lower respiration rate. Covering of different FC products with edible coatings has been reported as a protective barrier reducing the respiration rate owed to a modification of the internal gas

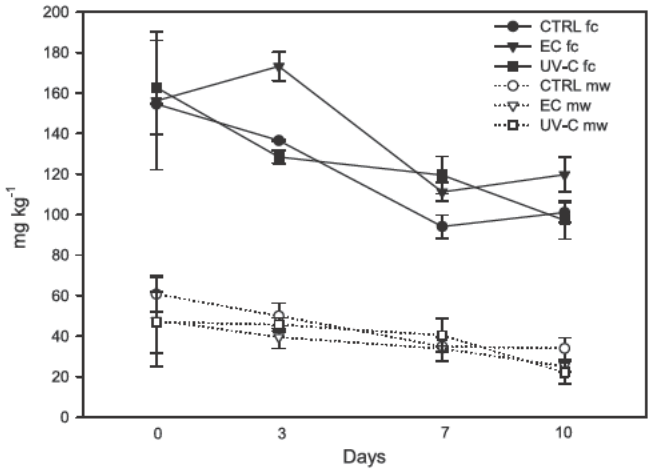

Fig. 4. Total vitamin C of fresh–cut (fc) and microwaved (mw) faba beans treated with different sanitizers up to 10 d at 4 °C (n = 4 ± SD).

atmosphere of the fruit and vegetables surface. Nevertheless, sucrose fatty acid esters and chitosan used in EC have been reported to act as chemical elicitors leading to a metabolic response in the plant cells due to such abiotic stress (Artés Hernández et al., 2017; Devlieghere et al., 2004; Smith and Stow, 1984). On the other side, previous studies have also reported the lowest respiration rate in samples washed with NaOCl (Klaiber et al., 2005), while others authors described unchanged respiration rates when washed with different sanitizers (Beltrán et al., 2005).

Our data shows that MAP did not avoid the important vitamin C loss during storage. Frequently, vitamin C loss is used as an index of nutrient degradation and quality of food products due to its high degradation due to oxidation and thermal treatments. The higher vitamin C preservation in coated samples may be explained by the antioxidant properties of the sucrose fatty acid esters, as observed in coated (including sucrose fatty acid esters) FC guava (Thommohaway et al., 2007). Heating occurred during cooking highly reduce vitamin C of products, although faster cooking methods like microwaving might induce lower vitamin C losses due to a more effective heating (Castillejo et al., 2017; Martínez Hernández et al., 2013; Vallejo et al., 2002). Masrizal et al. (1997) also reported a higher vitamin C retention in green beans with microwaving compared to conventional boiling. In our experiment, temperature reached within the packages was 70 °C, enough for producing an important vitamin C degradation, even when microwaving was performed for a short period of time (1 min). A cooking treatment of at least 80 °C (10 min) was recommended in FC broccoli florets to highly inactivate the AA oxidase enzyme reducing then the AA to DHA oxidation (Munyaka et al., 2010). Then, an intense cooking treatment would be of high interest to reduce the DHA formation, since this compound is greatly unstable being degraded to other compounds without health promoting properties.

The TPC of CTRL samples (2.13 ± 0.23 g kg ^{1}) was higher than the content previously reported by Baginsky et al. (2013) in immature seeds from different faba beans varieties (0.81 ± .0.07 to 1.33 ± 0.08 g kg ^{1}). UV C treatment has been associated with the enhancement of bioactive compounds, such as vitamins, carotenoids and phenolic compounds in several fruit and vegetables (Martínez Hernández et al., 2011). However, UV C treatment (3 kJ m^{-2}) did not affect the TPC of fresh faba seeds compared with CTRL, although UV C samples showed lower TPC than EC on days 3 and 10. The phenolics content of faba seeds was adversely affected by MAP. These findings are in agreement with previous studies, where a high reduction of phenolic compounds was observed when carbon dioxide partial pressure was above 10 kPa (Gil et al., 1998). On the other hand, the TPC decrease observed after microwaving agrees to the 30% reduction of hydrolysable polyphenol content observed by Dolinsky et al. (2016) in microwaved (1000 W, 5 min) green beans. Hithamani and Srinivasan (2014) also observed that microwaving (450 W, 4 min) reduced TPC of green gram (*Vigna radiate)* by 25%. Such TPC reductions after cooking may be due explained since 1) polyphenols are affected by high temperatures and/or 2) to the formation of new compounds caused by cooking (Xu and Chang, 2008). Non significant differences were observed between CTRL and UV C treatments. On the contrary, EC samples showed the highest TPC during storage, especially for fresh samples. The latter finding may be explained by the reported antioxidant properties of sucrose fatty acid esters, which probably reduce the phenolic compounds oxidation.

Faba seeds contain different compounds that may exert an antinutritional effect. Microwave processing decreased ≈ 30 60% the tannins content on processing day. These results agree with previous cooking studies on different legumes where microwaving was the most effective treatment to reduce tannins contents (Klug et al., 2018; Revilla, 2015). EC showed the highest tannins content of fresh seeds during storage. Such tannins preservation could be related to the barrier effect of EC against external factors, such as oxygen that might oxidize these phenolic compounds. Chitosan, one of the most studied edible coatings, also shows a similar oxyprotective effect on phenolic compounds of fruit as reviewed (Kerch, 2015).

The initial sugars content of samples ranged from 68 (EC) to 83% (CTRL and UV C) on dry weight basis. The content and composition of carbohydrates considerably varies depending on the bean cultivar (Goyoaga et al., 2011). A significant sucrose increment was observed on

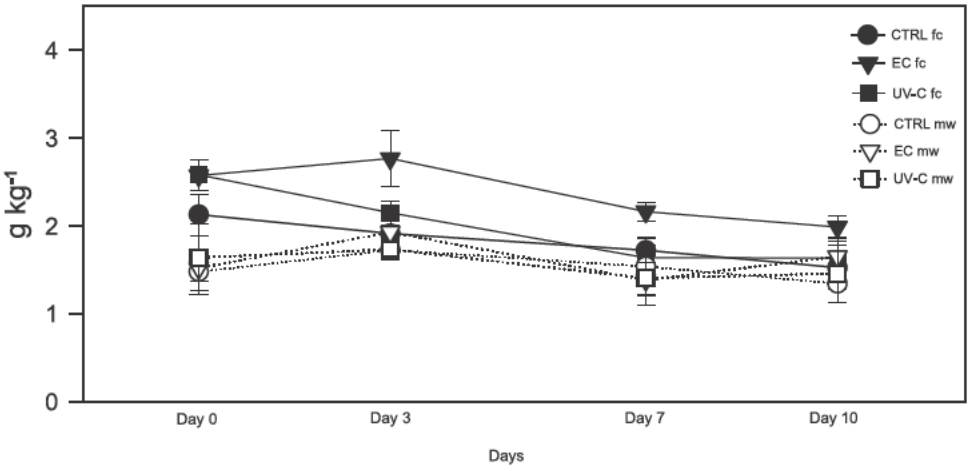

Fig. 5. Total phenolic content of fresh–cut (fc) and microwaved (mw) faba beans treated with different sanitizers up to 10 d at 4 °C (n = 4 ± SD).

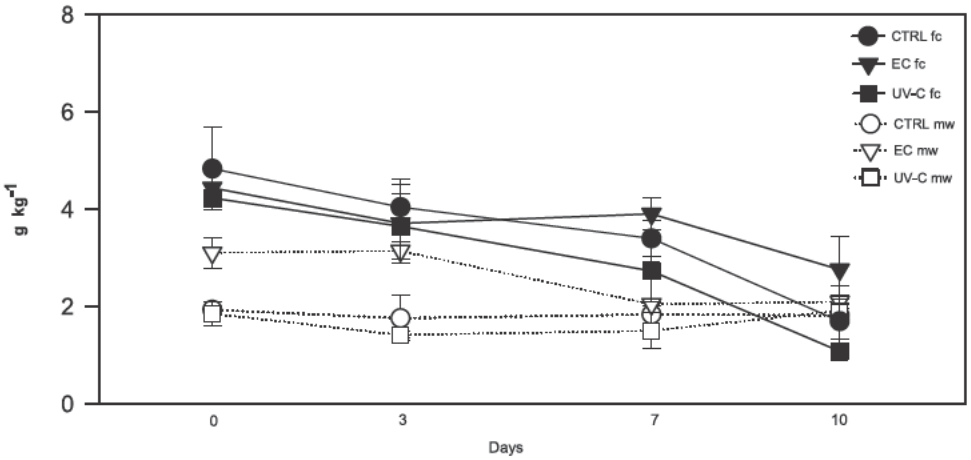

Fig. 6. Total tannins content of fresh–cut (fc) and microwaved (mw) faba beans treated with different sanitizers up to 10 d at 4 °C (n = 4 ± SD).

day 3 in UV C fc samples although such enhanced sucrose content was decreased through storage to other monosaccharides (i.e. fructose). It has been reported that sucrose synthesis may still occur in stored green beans as a consequence of starch hydrolysis and some residual synthesis after product harvest (Bognår et al., 1990). UV has been also considered as an abiotic stress able to increase the activity of enzymes during postharvest life of plant products (Cisneros Zevallos, 2003). Accord ingly, the observed sucrose increment in UV C FC samples may be due to an activation of those enzymes involved in the sucrose formation. Sucrose, which decreased during storage, seemed to be hydrolysed to fructose, whose concentration increased significantly after micro waving. Raffinose was not detected in any of the samples as previously reported (Revilla, 2015).

Sensory quality is one of the most important aspects related to the quality of FC products. Essentially, consumers appreciate five main sensory attributes: visual appearance and colour, followed by taste, aroma and texture (Barrett et al., 2010). According to our results, the shelf life of FC faba seeds was at least 10 d at 4 °C for EC and UV C samples, whereas it was reduced to 7 d for CTRL samples. Browning was the parameter affecting adversely visual appearance. The better appearance of EC microwaved samples can be attributed to the already mentioned antioxidant effect of sucrose fatty acid esters. Accordingly, emulsion based coatings were effective in preventing the browning of apple slices, depending on the composition of the continuous phase (Khan et al., 2014). Our findings also agree with previous reports that showed a positive effect of UV C treatments on the sensory quality of peppers (Vicente et al., 2005) and broccoli (Costa et al., 2006). In our case, browning for UV C microwaved samples was always than for the CTRL. Any reference could be found in the literature related to a re duced browning in microwaved fresh products. More studies are needed on this aspect. In the meantime, Navarro Rico et al. (2015) stated the advantages of EC based on sucrose fatty acid esters in FC broccoli. Additionally, microwaving of FC faba beans still led to a ready to eat product with good sensory properties.

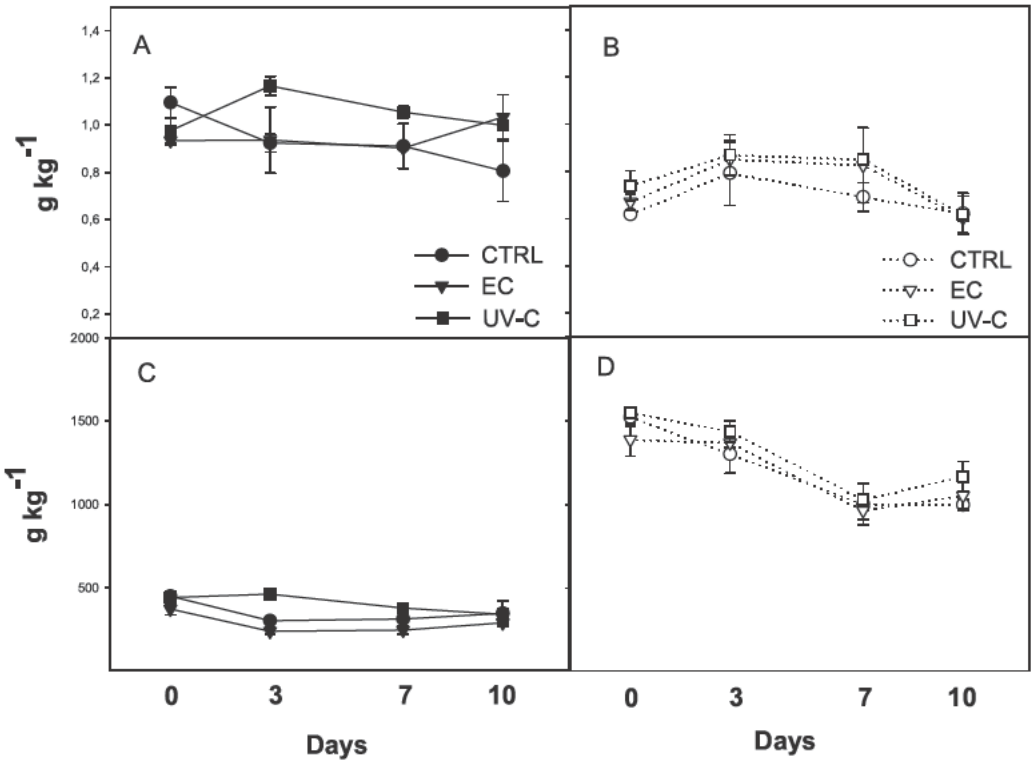

Fig. 7. Sucrose (A, B) and fructose (C, D) of fresh–cut (A, C) and microwaved (B, D) faba beans treated with different sanitizers up to 10 d at 4 °C (n = 4 ± SD).

Microbial quality of samples was highly controlled during storage with microbial loads below 6 log units at the end of product shelf life. On processing day, all sanitizing treatments achieved similar ($p < 0.05$) reductions among all studied microbial groups. CTRL registered the highest microbial growth during storage of samples. However, the lower microbial loads of EC indicates its synergetic effect when combined with sanitizers such as NaOCl. Similar results were reported by Navarro Rico (2015) for FC broccoli. Additionally, UV−C treatment showed a similar effect to EC (including NaOCl). UV−C (4.93 and 9.86 kJ m^{-2}) also showed an important reduction of aerobic bacterial counts in FC zucchini squash slices (Erkan et al., 2001). Moreover, Graça et al. (2017) reported that UV−C treatment (2.5 10 kJ m^{-2}) was more effective to reduce *Escherichia coli*, *Salmonella enterica* and *Listeria* spp. than NaOCl sanitation, without highly affecting the quality of FC Rocha pears.

5. Conclusion

A UV C pretreatment (3 kJ m^{-2}) or a conventional NaOCl sanitizing step plus an edible coating (Naturcover® P; EC) during processing of fresh cut faba seeds could be considered as important tools to improve their sensory, microbial and nutritional quality. Both fresh and microwaved EC samples showed the highest vitamin C and phenolics content retention after 10 d at 4 °C, while UV treated samples presented the highest sugars content. Therefore, these samples (EC and UV treated) achieved the highest sensory quality. Our results suggest that a typical quality loss during shelf life can be reduced by using such coadjutants in the processing steps. However, further studies are needed to optimize the UV C treatment conditions for enhancing the antioxidant compounds content. As hereby proposed, fresh seeds can be microwaved within the same package obtaining a very tasteful product with reduced tannins content.

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References

Anurag, R.K., Manjunatha, M., Jha, S.N., Kumari, L., 2016. Storage quality of shelled green peas under modified atmosphere packaging at different storage conditions. J. Food Sci. Technol. 53, 1640–1648. https://doi.org/10.1007/s13197-015-2066-y.

Artés, F., Gómez, P., Aguayo, E., Escalona, V., Artés–Hernández, F., 2009. Sustainable sanitation techniques for keeping quality and safety of fresh–cut plant commodities. Postharvest Biol. Technol. 51, 287–296. https://doi.org/10.1016/j.postharvbio.2008.10.003.

Artés-Hernández, F., Escalona, V., Robles, P.A., Martínez-Hernández, G.B., Artés, F., 2009. Effects of UV–C radiation on quality of minimally processed spinach leaves. J. Sci. Food Agric. 89, 414–421. https://doi.org/10.1002/jsfa.3460.

Artés-Hernández, F., Formica-Oliveira, A.C., Artés, F., Martínez-Hernández, G.B., 2017. Improved quality of a vitamin B12-fortified 'ready to blend' fresh-cut mix salad with chitosan. Food Sci. Technol. Int. 23, 513–528. https://doi.org/10.1177/1082013217705036.

ASTM, 1986. Physical Requirements Guidelines for Sensory Evaluation Laboratories (Vol. 913, ASTM Special Technical Pub. 913). American Society for Testing Materials, Philadelphia. https://doi.org/10.1520/E2299-13.

Baginsky, C., Peña-Neira, A., Cáceres, A., Hernández, T., Estrella, I., Morales, H., Pertuzé, R., 2013. Phenolic compound composition in immature seeds of faba bean (*Vicia faba* L.) varieties cultivated in Chile. J. Food Anal. 31, 1–6. https://doi.org/10.1016/j.jfca.2013.02.003.

Barrett, D.M., Beaulieu, J.C., Shewfelt, R., 2010. Color, flavor, texture, and nutritional quality of fresh–cut fruit and vegetables: desirable levels, instrumental and sensory measurement, and the effects of processing. Crit. Rev. Food Sci. Nutr. 50, 369–389. https://doi.org/10.1080/10408391003626322.

Beltrán, D., Selma, M.V., Tudela, J.A., Gil, M.I., 2005. Effect of different sanitizers on microbial and sensory quality of fresh cut potato strips stored under modified atmosphere or vacuum packaging. Postharvest Biol. Technol. 37, 37–46. https://doi.org/10.1016/j.postharvbio.2005.02.010.

Bognår, A., Bohling, H., Fort, H., 1990. Nutrient retention in chilled foods. In: Gormley, T.R. (Ed.), Chilled Foods. The State of the Art. Elsevier Applied Science, London, pp. 305–336.

Castillejo, N., Martínez-Hernández, G.B., Gómez, P.A., Artés, F., Artés-Hernández, F., 2016. Red fresh vegetables smoothies with extended shelf–life as an innovative source of health–promoting compounds. J. Food Sci. Technol. 53, 1475–1486. https://doi.org/10.1007/s13197-015-2143-2.

Castillejo, N., Martínez-Hernández, G.B., Lozano-Guerrero, A.J., Pedreño-Molina, J.L., Gómez, P.A., Aguayo, E., Artés, F., Artés-Hernández, F., 2017. Microwave heating modelling of a green smoothie: Effects on glucoraphanin, sulforaphane and*S*-methyl cysteine sulfoxide changes during storage. J. Sci. Food Agric. 98, 1863–1872. https://doi.org/10.1002/jsfa.8665.

Chung, K.-T., Wong, T.Y., Wei, C.-I., Huang, Y.-W., Lin, Y., 1998. Tannins and human health: a review. Crit. Rev. Food Sci. Nutr. 38, 421–464. https://doi.org/10.1080/10408699891274273.

Cisneros-Zevallos, L., 2003. The use of controlled postharvest abiotic stresses as a tool for enhancing the nutraceutical content and adding-value of fresh fruits and vegetables. J. Food Sci. 68, 1560–1565. https://doi.org/10.1111/j.1365-2621.2003.tb12291.x.

Costa, L., Vicente, A.R., Civello, P.M., Chaves, A.R., Martínez, G.A., 2006. UV–C treatment delays postharvest senescence in broccoli florets. Postharvest Biol. Technol. 39, 204–210. https://doi.org/10.1016/j.postharvbio.2005.10.012.

Devlieghere, F., Vermeulen, A., Debevere, J., 2004. Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. Food Microbiol. 21, 703–714. https://doi.org/10.1016/j.fm.2004.02.008.

Dolinsky, M., Agostinho, C., Ribeiro, D., Rocha, G.S., Barroso, S.G., Ferreira, D., Polinati, R., Ciarelli, G., Fialho, E., 2016. Effect of different cooking methods on the polyphenol concentration and antioxidant capacity of selected vegetables. J. Culinary Sci. Technol. 14, 1–12. https://doi.org/10.1080/15428052.2015.1058203.

Erkan, M., Wang, C.Y., Krizek, D.T., 2001. UV–C radiation reduces microbial populations and deterioration in *Cucurbita pepo* fruit tissue. Environ Exper. Bot. 45, 1–9. https://doi.org/10.1016/S0098-8472(00)00073-3.

FAOSTAT, 2013. Production–Crops, Pulses. Accessed 20 April 2018. https://goo.gl/ccjQUY.

Foley, D., Euper, M., Caporaso, F., Prakash, A., 2004. Irradiation and chlorination effectively reduces *Escherichia coli* o157:h7 inoculated on cilantro (*Coriandrum sativum*) without negatively affecting quality. J. Food Prot. 67, 2092–2098. https://doi.org/10.4315/0362-028X-67.10.2092.

Gil, M.I., Castañer, M., Ferreres, F., Artés, F., Tomás–Barberán, F.A., 1998. Modified–atmosphere packaging of minimally processed 'Lollo Rosso' (*Lactuca sativa*): Phenolic metabolites and quality changes. Eur. Food Res. Technol. 206, 350–354. https://doi.org/10.1007/s002170050.

Goyoaga, C., Burbano, C., Cuadrado, C., Romero, C., Guillamón, E., Varela, A., Pedrosa, M.M., Muzquiz, M., 2011. Content and distribution of protein, sugars and inositol phosphates during the germination and seedling growth of two cultivars of Vicia faba. J. Food Anal. 24, 391–397. https://doi.org/10.1016/j.jfca.2010.11.002.

Graça, A., Santo, D., Quintas, C., Nunes, C., 2017. Growth of *Escherichia coli*, *Salmonella enterica* and *Listeria* spp., and their inactivation using ultraviolet energy and electrolyzed water, on Rocha fresh–cut pears. Food Control 77, 41–49. https://doi.org/10.1016/j.foodcont.2017.01.017.

Hithamani, G., Srinivasan, K., 2014. Bioaccessibility of polyphenols from wheat (*Triticum aestivum*), Sorghum (*Sorghum bicolor*), Green Gram (*Vigna radiata*), and Chickpea (*Cicer arietinum*) as influenced by domestic food processing. J. Agric. Food Chem. 62, 11170–11179. https://doi.org/10.1021/jf503450u.

Kerch, G., 2015. Chitosan films and coatings prevent losses of fresh fruit nutritional quality: a review. Trends Food Sci. Technol. 46, 159–166. https://doi.org/10.1016/j.tifs.2015.10.010.

Khan, M., Cakmak, H., Tavman, S., Schutyser, M., Schroën, K., 2014. Anti-browning and barrier properties of edible coatings prepared with electrospraying. Innov. Food Sci. Emerg. Technol. 25, 9–13. https://doi.org/10.1016/j.ifset.2013.10.006.

Klaiber, R.G., Baur, S., Wolf, G., Hammes, W.P., Carle, R., 2005. Quality of minimally processed carrots as affected by warm water washing and chlorination. Innov. Food Sci. Emerg. Technol. 6, 351–362. https://doi.org/10.1016/j.ifset.2005.03.002.

Klug, T.V., Collado, E., Martínez-Sánchez, A., Gómez, P.A., Aguayo, E., Otón, M., Artés, F., Artés-Hernández, F., 2018. Innovative quality improvement by continuous microwave processing of a faba beans pesto sauce. Food Bioproc. Technol. 11, 561–571. https://doi.org/10.1007/s11947-017-2024-y.

López-Gálvez, F., Allende, A., Truchado, P., Martínez-Sánchez, A., Tudela, J.A., Selma, M.V., Gil, M.I., 2010. Suitability of aqueous chlorine dioxide versus sodium hypochlorite as an effective sanitizer for preserving quality of fresh–cut lettuce while avoiding by–product formation. Postharvest Biol. Technol. 55, 53–60. https://doi.org/10.1016/j.postharvbio.2009.08.001.

Martínez-Hernández, G.B., Gómez, P.A., Pradas, I., Artés, F., Artés-Hernández, F., 2011. Moderate UV–C pretreatment as a quality enhancement tool in fresh–cut Bimi® broccoli. Postharvest Biol. Technol. 62, 327–337. https://doi.org/10.1016/j.postharvbio.2011.06.015.

Martínez-Hernández, G.B., Artés-Hernández, F., Colares-Souza, F., Gómez, P.A., García-Gómez, P., Artés, F., 2013. Innovative cooking techniques for improving the overall quality of a kailan–hybrid broccoli. Food Bioproc. Technol. 6, 2135–2149. https://doi.org/10.1007/s11947-012-0871-0.

Masrizal, M.A., Giraud, D.W., Driskell, J.A., 1997. Retention of vitamin C, iron, and carotene in vegetables prepared using different cooking methods. J. Food Qual. 20,

403–418. https://doi.org/10.1111/j.1745-4557.1997.tb00483.x.
Multari, S., Stewart, D., Russell, W.R., 2015. Potential of fava bean as future protein supply to partially replace meat intake in the human diet. Compr. Rev. Food Sci. Food Saf. 14, 511–522. https://doi.org/10.1111/1541-4337.12146.
Munyaka, A.W., Makule, E.E., Oey, I., Van Loey, A., Hendrickx, M., 2010. Thermal stability of L-ascorbic acid and ascorbic acid oxidase in broccoli (*Brassica oleracea* var. italica). J. Food Sci. 75, 336–340. https://doi.org/10.1111/j.1750-3841.2010.01573.x.
Navarro-Rico, J., Martínez-Hernández, G.B., Artés, F., Artés-Hernández, F., Gómez, P.A., 2015. Effect of edible coatings and electrolyzed water sanitation on fresh–cut 'bimi' broccoli quality. Acta Hortic. 1071, 463–469. https://doi.org/10.17660/ActaHortic.2015.1071.59.
Price, M.L., Scoyoc, S.V., Butler, L.G., 1978. A critical evaluation of the vanillin reaction as an assay for tannin on sorghum grain. J. Agric. Food Chem. 26, 1214–1218. https://doi.org/10.1021/jf60219a031.
Raybaudi-Massilia, R., Mosqueda-Melgar, J., Soliva-Fortuny, R., Martín-Belloso, O., 2016. Combinational edible antimicrobial films and coatings. In: Barros-Velázquez, J. (Ed.), Antimicrobial Food Packaging. Elsevier, Amsterdam, pp. 633–646. https://doi.org/10.1016/B978-0-12-800723-5.00052-8.
Revilla, I., 2015. Impact of thermal processing on faba bean (*vicia faba*) composition. In: Preedy, V. (Ed.), Processing and Impact on Active Components in Food. Elsevier, Amsterdam, pp. 337–343. https://doi.org/10.1016/B978-0-12-404699-3.00040-8.
Rico, D., Martín-Diana, A.B., Barat, J.M., Barry-Ryana, C., 2007. Extending and measuring the quality of fresh–cut fruit and vegetables: a review. Trends Food Sci. Technol. 18, 373–386. https://doi.org/10.1016/j.tifs.2007.03.011.
Sánchez-Mata, M.C., Camara, M., Diez-Marques, C., 2003. Extending shelf–life and nutritive value of green beans (*Phaseolus vulgaris* L.), by controlled atmosphere storage: macronutrients. Food Chem. 80, 309–315. https://doi.org/10.1016/S0308-8146(02)00265-0.
Siah, S., Wood, J.A., Agboola, S., Konczak, I., Blanchard, C.L., 2014. Effects of soaking, boiling and autoclaving on the phenolic contents and antioxidant activities of faba beans (*Vicia faba* L.) differing in seed coat colours. Food Chem. 142, 461–468. https://doi.org/10.1016/j.foodchem.2013.07.068.
Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods Enzymol. 299, 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1.
Smith, S.M., Stow, J.R., 1984. The potential of a sucrose ester coating material for improving the storage and shelf-life qualities of Cox's Orange Pippin apples. Ann. Appl. Biol. 104, 383–391. https://doi.org/10.1111/j.1744-7348.1984.tb05624.x.
Thommohaway, C., Uthairatanakij, A., Jitareerat, P., 2007. Effects of sucrose fatty acid ester on the quality of fresh–cut guava (*Psidium guajava*). Acta Hortic. 746, 455–460. https://doi.org/10.17660/ActaHortic.2007.746.56.
Turco, I., Ferretti, G., Bacchetti, T., 2016. Review of the health benefits of faba bean (*Vicia faba* L.) polyphenols. J. Food Nutr. Res. 55, 283–293.
Vallejo, F., Tomás-Barberán, F.A., García-Viguera, C., 2002. Glucosinolates and vitamin C content in edible parts of broccoli florets after domestic cooking. Eur. Food Res. Technol. 215, 310–316. https://doi.org/10.1007/s00217-002-0560-8.
Vicente, A.R., Pineda, C., Lemoine, L., Civello, P.M., Martínez, G.A., Chaves, A.R., 2005. UV–C treatments reduce decay, retain quality and alleviate chilling injury in pepper. Postharvest Biol. Technol. 35, 69–78. https://doi.org/10.1016/j.postharvbio.2004.06.001.
Xu, B., Chang, S.K.C., 2008. Effect of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes. Food Chem. 110, 1–13. https://doi.org/10.1016/j.foodchem.2008.01.045.
Zapata, S., Dufour, J.P., 1992. Ascorbic, dehydroascorbic and isoascorbic and simultaneous determinations by reverse phase ion interaction HPLC. J. Food Sci. 57, 506–511. https://doi.org/10.1111/j.1365-2621.1992.tb05527.x.

Chapter III:

Quality changes in nutritional traits of fresh-cut and then microwaved cowpea seeds and pods

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ABSTRACT

Numerous studies show that regular vegetable consumption is associated with beneficial properties for human health. At the same time, there is an interest in functional, healthy and ready-to-consume foods from consumers. In this sense, cowpea (*Vigna unguiculata*) is an excellent source of many essential nutrients, including proteins and aminoacids of vegetable origin, complex carbohydrates, minerals, fiber, vitamins, and other bioactive compounds, and is low in calories and fat. Dry grains are the most common produce used for human food, but leaves and immature seeds and pods are also consumed.In this context, the consumption of cowpea beans as a fresh food minimally processed, as well as, cooked in microwaves, can be alternatives to stimulate the consumption of legumes. The objective of this work was to evaluate the quality of fresh-cut cowpea prepared to be eaten raw (immature seeds) or microwaved (seeds and pods). Fresh cowpea pods were washed with NaOCl (150 mg L^{-1}, pH 6.5) and stored for 21 days at 8°C under modified atmosphere packaging. Additionally, seeds obtained from hulled pods were equally disinfected, packaged, and stored for 7 days at 4°C. The total phenolic content (TPC), total antioxidant capacity (TAC), sugars (raffinose, sucrose, glucose), and sensory attributes were evaluated in microwaved pods (700 W, 1 min), fresh seeds, and microwaved seeds. TPC and TAC increased after microwaving in both seeds and pods. Sucrose and glucose concentrations increased after microwaving, while raffinose was not detected after cooking. According to sensory quality, fresh (4°C) and microwaved seeds maintained all the above attributes above the limit of usability until day 7, while pods were edible for up to 14 days if kept at 8°C. These results indicate that cowpea seeds and pods (fresh-cut and then microwaved) are feasible and practical products to stimulate legume consumption from local landraces, especially in the absence of raffinose, which improves product digestibility.

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Quality Changes in Nutritional Traits of Fresh-Cut and Then Microwaved Cowpea Seeds and Pods

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Abstract

There is increasing interest in healthy and ready-to-eat natural horticultural products. In this sense, fresh-cut and then microwaved cowpea can be considered a good option to stimulate legume consumption, since they are a source of proteins and bioactive compounds. The objective of this work was to evaluate the quality of fresh-cut cowpea prepared to be eaten raw (immature seeds) or microwaved (seeds and pods). A Spanish landrace (accession BGE038474), which is well adapted to the cropping area, was selected. Fresh cowpea pods were washed with NaOCl (150 mg L^{-1}, pH 6.5) and stored for 21 days at 8 °C under modified atmosphere packaging. Additionally, seeds obtained from hulled pods were equally disinfected, packaged, and stored for 7 days at 4 °C. The total phenolic content (TPC), total antioxidant capacity (TAC), sugars (raffinose, sucrose, glucose), and sensory attributes were evaluated in microwaved pods (700 W, 1 min), fresh seeds, and microwaved seeds. TPC and TAC increased after microwaving in both seeds and pods. Sucrose and glucose concentrations increased after microwaving, while raffinose was not detected after cooking. According to sensory quality, fresh (4 °C) and microwaved seeds maintained all the above attributes above the limit of usability until day 7, while pods were edible for up to 14 days if kept at 8 °C. These results indicate that cowpea seeds and pods (fresh-cut and then microwaved) are feasible and practical products to stimulate legume consumption from local landraces, especially in the absence of raffinose, which improves product digestibility.

Keywords *Vigna unguiculata* · Legumes · Minimally processed · Raffinose · Microwave cooking · Nutrition

Introduction

Cowpea (*Vigna unguiculata*), an annual crop adapted to semi-arid conditions of heat and drought, is an excellent source of many essential nutrients, including proteins and amino acids, complex carbohydrates, minerals, fiber, vitamins, and other bioactive compounds; it is also low in calories and fat (Bouchenak and Lamri-Senhadji 2013; Gonçalves et al. 2016; Singh et al. 2017). Dry grains are the most common produce used for human food, but leaves and immature seeds and pods are also consumed (Frota et al. 2008; Xu and Chang 2012). Cowpea is of scarce importance in Europe (0.4% of worldwide production), but it is very important in Africa, where 95.8% of worldwide production takes place (FAOStat 2017). In Europe, cowpea is cultivated on a small scale in many parts of southern Europe (Domínguez-Perles et al. 2015) and its cultivation is mostly based on landraces, of the cv. Greek *Unguiculata* and cv. Greek *Sesquipedalis*, which are cultivated for their seeds and fresh pods (like French beans), respectively (Karapanos et al. 2017). Thus, local genetic resources represent an important material for genetic variability, which can be used either as a source of new cultivars with improved features or as a gene pool of useful traits for breeding programs. However, such agrobiodiversity could disappear from farmers' fields for a variety of reasons. Among landraces, a Spanish landrace of cowpea (BGE038474) belonging to the cv. Greek *Sesquipedalis* has been previously studied and characterized for its agronomic and nutritional parameters (Martos-Fuentes et al. 2017; Karapanos et al. 2017), demonstrating a great potential for commercial exploitation due to its high yields under standard and stressing growing conditions and short pods, which makes it very suitable for minimal processing.

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There is a growing consumer demand for natural, fresh, and ready-to-eat vegetable products, as those represented by fresh-cut or minimally fresh processed fruits and vegetables (Artés-Hernández et al. 2009). In light of this, the development of fresh-cut immature cowpea pods and seeds may help in promoting the consumption of this vegetable. In addition, after storage and marketing, the product could be consumed fresh or directly microwaved if packaged in a suitable container, providing a product of high sensory quality and intact nutritional properties. However, it is known that cooking methods can affect both bioactive and nutritional components (Yasmin et al. 2008; Deng et al. 2015). Thus, cooking in a microwave oven could be an interesting alternative to conventional cooking (e.g., boiling) due to high efficiency and faster processing time (Alajaji and El-Adawy 2006). Yadav et al. (2018) studied the impact of microwave (MW) cooking (800 W, 15 min) and boiling (90 min) on the total phenolic compounds of cowpea dry seeds cv. *Kohinoor* and observed a higher preservation (39.1%) in MW-treated samples regarding boiling samples (27.3%).

Although there are many studies dealing with the nutritional properties of the most common legumes, there is limited information about the nutritional value of fresh cowpea. Promoting its inclusion in diets is important because of its protein content and also because of other functional properties, such as its phenolic content and high antioxidant activity. Therefore, the objective of this study was to evaluate quality changes of fresh-cut cowpea during storage at 4 °C for 7 days (green seeds) and at 8 °C for 21 days (pods). Then, seeds and pods were microwaved at certain fixed times and the resulting changes in sensory attributes, total phenols, antioxidant capacity, and sugars content were assessed.

Material and Methods

Plant Material

Cowpea pods from a local European landrace (accession BGE038474) were harvested in the Agri-food Experimental Station Tomás Ferro (La Palma, Cartagena, Spain) at an immature physiological stage. This landrace, which was selected based on previous experiments carried out at the UPCT, is notable for its high yield (226.45 ± 60.19 g m^{-2}) and having rather small seeds (14.59 ± 1.79 g 100^{-1} seeds) and short pods (16.14 ± 1.18 cm) (Martos-Fuentes et al. 2017), which makes it very suitable for minimal processing. It also has a high protein content (24.35 ± 1.63 g 100^{-1} g d.w.) and it is well adapted to the local climate (Martos-Fuentes et al. 2017). Immediately after harvest, pods were cold transported (7 ± 1 °C, 20 min) to the laboratory, where they were kept in darkness at 8 °C and 90–95% relative humidity (RH).

Processing, Packaging, and Storage

The following day, half of the pull of pods was shelled by hand in a cold room (5 °C) and the seeds were immersed in cold water at 4 ± 1 °C, 5 min, until disinfection. The seeds and the remaining unshelled pods were sanitized by immersion in NaOCl (150 mg L^{-1}, pH 6.5, 2 min, 4 °C), and subsequently rinsed in cold tap water for 1 min.

Fifty ± 5 g of seeds and pods were packaged in 50-μm-thick polypropylene bags (O_2 permeability, 700 cm^3 m^{-2} d^{-1}; CO_2 permeability, 1100 cm^3 m^{-2} d^{-1}, at 23 °C and 0% RH). This film was selected based on own previous studies performed by the research group. Before packaging, the bags (15 × 30 cm for pods and 15 × 15 cm for seeds) were sterilized with UV-C light (8 kJ m^{-2}) to prevent microbial contamination.

The bags were heat-sealed to generate a passive modified atmosphere (PMA) and stored at 90% RH for 7 days at 4 ± 0.5 °C (seeds) and for 21 days at 8 ± 0.5 °C (pods) to avoid chilling injury. After 0, 3, and 7 (seeds) and 0, 7, 14, and 21 days (pods) of storage, four bags of seeds and pods were analyzed for different quality parameters. Another four bags of seeds and pods were also removed and microwaved (700 W, 1 min). Thirty holes (0.2-mm diameter) were made in the bags before microwaving. These samples were then analyzed for the same quality parameters as for the fresh product, as described below. Except for sensory quality, all the remaining quality parameters for both seeds and pods were assessed on frozen samples stored at − 80 °C until analysis.

Headspace Analysis

Throughout storage, headspace gas composition (O_2 and CO_2) within the packages was monitored following the method described by Rodríguez-Hidalgo et al. (2010). Samples of 1 mL were taken with a syringe and analyzed in a gas chromatograph (GC) (7820A GC Agilent Technologies, Waldbroon, Germany) with a molecular sieve Molsieve 5A 80/100 (Teknokroma, Barcelona, Spain) and Hayesep Q 80/100 column (Teknocroma, Barcelona, Spain). GC was fitted with a thermal conductivity detector (TCD), using He as carrier gas, with an oven temperature of 80 °C and a detector temperature of 200 °C. Calibration was done based on external standard (O_2 10%, CO_2 10%, N_2 80%). Atmospheric pressure was measured (Brooks Instruments BV, Netherlands) to convert percentage readings to kilopascal. Three replicates were used per treatment and evaluation day. Samples were taken on days 1, 3, 4, and 5 of storage in the case of seeds and on days 0, 3, 7, 11, 13, 17, and 19 for pods.

Total Phenolic Content

The total phenolic content (TPC) was determined by the method developed by Singleton and Rossi (1965) with some

modifications introduced by Martínez-Hernández et al. (2011). Briefly, frozen samples (2 g) were placed in glass bottles, and 3 mL of methanol 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. The extracts were centrifuged at 15,000×*g* for 10 min at 4 °C. Supernatant samples (19 μL) and 1 N Folin–Ciocalteu reagent (29 μL) were mixed in a 96-well plate and incubated for 3 min at room temperature in darkness. Then, 192 μL of a solution containing Na_2CO_3 (0.4%) and NaOH (2%) was added. After 1 h of incubation at room temperature in darkness, the absorbance was measured at 750 nm with a multiscan plate reader (Tecan Infininte M200, Männedorf, Switzerland). The TPC was expressed as milligrams of gallic acid equivalents (GAE) per kilogram fresh weight. All samples were tested in triplicate.

Total Equivalent Antioxidant Capacity

The same sample extracts to determine TPC were used to evaluate the antioxidant capacity (TAC) by 2,2-diphenyl-1-picrylhydrazil (DPPH) (Brand-Williams et al. 1995) and ferric reducing antioxidant power (FRAP) (Benzie and Strain 1999) assays:

- DPPH assay: the free radical scavenging activity using the free radical DPPH was evaluated by measuring the decrease in absorbance at 515 nm for 30 min at room temperature, with measurements every 5 min to determine the right time to measure TAC. A volume (194 μL) of DPPH solution (0.7 mM) was added to each extract sample (21 μL).
- FRAP assay: the freshly made up FRAP solution containing sodium acetate buffer (pH 3.6), 10 mM TPTZ solution (in 40 mM HCl), and 20 mM $FeCl_3$ was prepared in a *v*/*v*/*v* proportion of 10:1:1. A volume (198 μL) of FRAP solution was added to each extract sample (6 μL) and left to stand for 45 min at room temperature in darkness, measuring the increase in absorbance at 593 nm.

The antioxidant activity for the DPPH and FRAP assays was expressed as milligrams of Trolox equivalent antioxidant capacity (TAC) per kilogram fresh weight.

Sugars

The raffinose, glucose, and sucrose content was analyzed as described by Flores et al. (2012) with some modifications. Samples (3 g) were homogenized (Ultra-turrax T-25, Ika-Labortechnik, Staufen, Germany) with 10 mL of MilliQ water for 40 s. The extracts were filtered through cheesecloth and centrifuged at 12,000×*g* for 20 min. The supernatant was purified by passing through a solid-phase extraction column in a parallel Strata C18-E column (55 um, 70 A; Phenomenex, Macclesfield, UK) and then filtered through a 0.45-μm PTFE syringe filter. Then, 20 μL was injected in an ultra-high-performance liquid chromatograph (Shimadzu, Kyoto, Japan). Chromatographic analyses were carried out in a RAM-Carbohydrate Ag^+ column (100 mm 4.6 mm, 2.6 mm particle size; Phenomenex, Macclesfield, UK). The mobile phase used was MilliQ water with an isocratic flow rate of 0.6 mL min^{-1}. Quantification and assignment of peaks were based on the peak areas and retention times of known standard curves for fructose, sucrose, and raffinose (Sigma, St Louis, MO, USA). Results were expressed as milligrams per 100 g fresh weight. All samples were tested in triplicate.

Sensory Evaluation

Sensory analyses were carried out according to international standards (ASTM 1986). Tests were conducted in a standard room (ISO 2012) equipped with ten individual taste boxes using white light. Samples (about 20 g) of fresh and microwaved seeds and microwaved pods were served at room temperature. Still mineral water was used as palate cleanser. The evaluation of fresh and microwaved samples was performed by a panel of seven people (aged 24–60) trained in sensory quality analysis. A 9-point hedonic scale was scored for visual symptoms of browning and dehydration (9 = none; 5 = limit of usability; 1 = extreme), and the rest of the parameters, i.e., flavor, aroma, visual appearance, texture, lightness, and overall quality, were scored as 1 = extremely bad, 5 = limit of usability, and 9 = excellent.

Statistical Analyses

Analysis of variance (ANOVA) was performed to compare the differences at different storage times for fresh (at days 1, 3, 4, and 5 for seeds and on days 0, 3, 7, 11, 13, 17, and 19 for pods) and microwaved cowpea, at a significance level of $\alpha \leq 0.05$ using PASW Statistics 23 for Windows (SPSS Inc., Chicago, IL, USA). When significant differences were observed, Tukey's HSD (honestly significant difference) test was applied.

Results and Discussion

Headspace Analysis

The generated passive atmosphere was analyzed to monitor the gas composition within the PMA packages (Fig. 1). As expected, there was an increase in the partial pressure of CO_2 and a decrease in that of O_2 from the first day of storage, reaching a steady state at day 3 (23 kPa CO_2/1.5 kPa O_2 and 19 kPa CO_2/1.2 kPa O_2, for fresh seeds and pods, respectively).

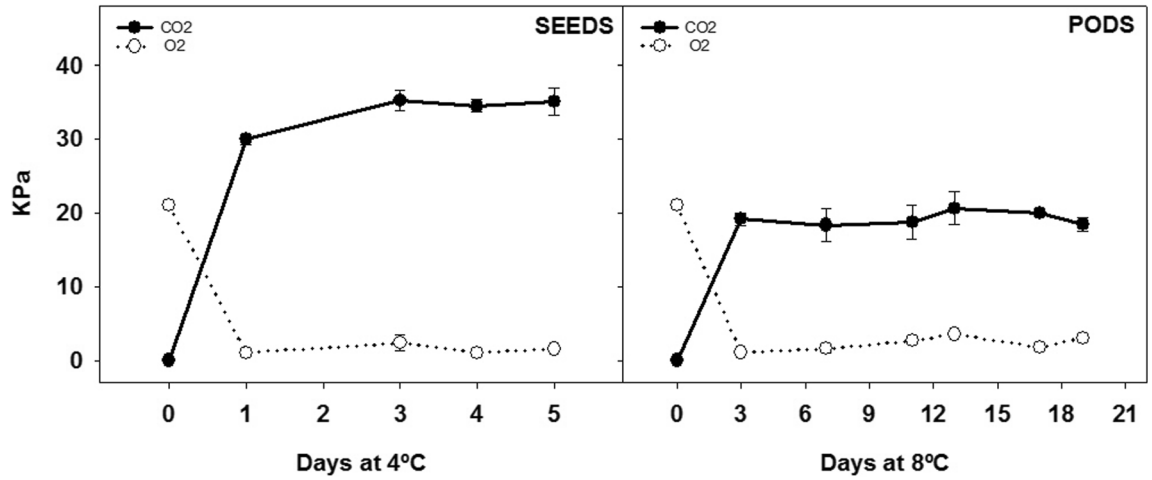

Fig. 1 Gas changes within packages of fresh cowpea seeds (left) and pods (right) stored in PMA for 7 and 21 days, respectively. Data are mean ($n = 3$) ± standard deviation

Limited information is available about the effect of processing and cooking on the nutritional and quality parameters of fresh-cut cowpea seeds and pods. The immature seeds have a short shelf life due to rapid dehydration (they have a high water content of around 50–70%) (Oliveira et al. 2001) and a high respiration rate (Pinto and Morais 2000). Both aspects could have an effect on main quality changes that can be reduced by using a package with suitable permeability to generate a passive modified atmosphere. In the experiments presented here, bags containing seeds had a higher CO_2 partial pressure than those with pods, indicating their higher respiration rate. As the respiration is an indicator of metabolic activity, it is expected that seeds will deteriorate faster. Reducing respiration is one of the key benefits from cooling and PMA. The high CO_2 partial pressure reached within packages probably had a bacteriostatic activity against the fungi and bacteria responsible for deterioration. However, it did not affect sensory attributes, since no off-flavors were detected by the panelists.

Total Phenolic Content

On day 0, the TPC (Fig. 2) was slightly higher for fresh seeds than for pods (950 ± 28.85 mg GAE kg ^{1} fw and 885 ± 8.55 mg GAE kg ^{1} fw respectively). Variable results have been found in the literature about the total phenolic content of different cowpea cultivars, with TPC values reported from 465 to 14,000 mg GAE kg ^{1} (Deng et al. 2013; Zia-Ul-Haq et al. 2013; Adjei-Fremah et al. 2015). Values found here were higher than those previously reported by Adjei-Fremah et al. (2015) (465 mg GAE kg ^{1}) and lower than those reported by Deng et al. (2013), who measured up to 8280 mg GAE kg ^{1}. The TPC content found here partially agree with the contents reported by Ali et al. (2014) for cowpea seeds (1230 mg GAE kg ^{1} fw) and Karapanos et al. (2017) in cowpea pods (839.40 ± 39.5 mg GAE kg ^{1} fw). Genetic factors and environmental conditions can influence the concentration of TPC in vegetables (Hoeck et al. 2000) and the variability observed in our results may be attributed to these factors. Due to their potential positive effect on consumer's health, it is convenient to consider phenolic content as a quantitative trait of cowpea in cultivar development programs.

When microwaved on day 0, TPC increased by around 57% for seeds, and about 13% for pods. This agrees with the increase of more than 100% in the total phenolic content observed by Chumyam et al. (2013) and Sharma et al. (2015) after heating eggplants (microwaving, 5 min) and onions (oven 80 °C, 30 min). Moreover, Zhou et al. (2016) observed that domestic microwaving increased the total phenolic content from 12.8 to 29.0% in defatted avocado puree in comparison with untreated samples. This increase could be due to the fact that heat treatments probably facilitate the extraction of phenolic compounds after cooking. Heating might cause severe fractures to the seed physical structures and could facilitate the release of cell components, making them more available. On the contrary, Hithamani and Srinivasan (2014) found that microwaving (450 W, 4 min) significantly decreased (25%) the total polyphenol content in green gram (*Vigna radiate*).

Fresh seeds maintained similar TPC values throughout storage, whereas the TPC of fresh pods decreased by 55% after 21 days. The decreasing trend observed was also detected when microwaved, with TPC always higher in cooked pods than in the fresh ones. At the end of storage, non-significant differences were observed between fresh and microwaved seed samples, and significant differences between fresh and microwaved pods, but very close one to the other.

Total Antioxidant Capacity

The TAC of fresh cowpea seeds and pods as evaluated by FRAP and DPPH assays is presented in Tables 1 and 2,

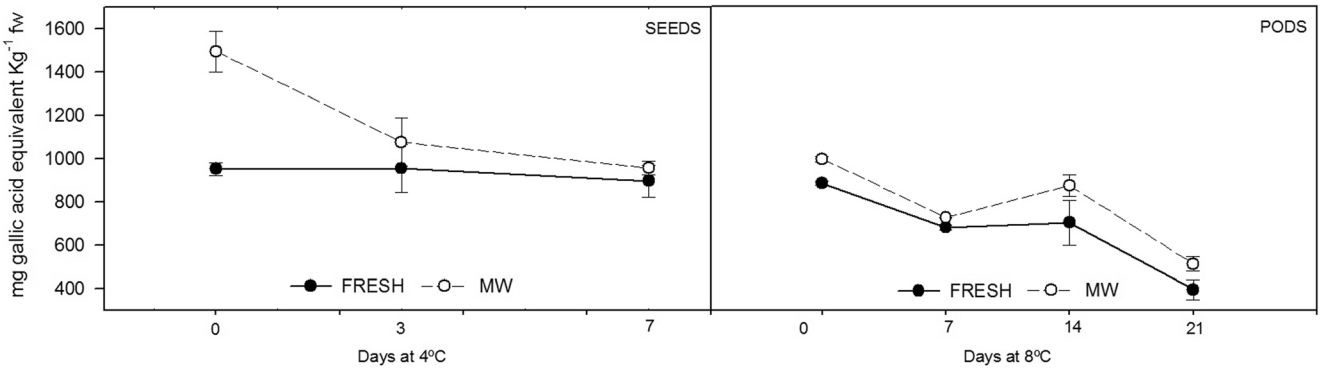

Fig. 2 Total phenolic content of fresh cowpea seeds (left) and pods (right) stored under PMA and then microwaved. Full lines represent fresh samples while dotted lines represent microwaved samples. Data are mean ($n = 3$) ± standard deviation

respectively. In the FRAP assay, the initial TAC value of fresh seeds was 1383.9 ± 130.7 mg TEAC kg ^{1} fw. After 1 min of microwaving, the TAC of fresh seeds increased by 52%. At the end of storage, a decrease of 56% was observed for both fresh and MW seeds, with significant differences between them. With regard to fresh pods, the TAC value was 751.1 ± 78.7 mg TEAC kg ^{1} fw, which increased by 60% after processing in the microwave. However, at day 21, a decrease of 84% and 62% was observed for fresh and MW cowpea pods, respectively, related to the initial day.

The TAC evaluated by DPPH assay showed an increase of 56% and 23% after microwave processing in seeds and pods, respectively. After 7 days of storage, a decrease of 50% was observed in fresh seeds, with non-significant differences between fresh and microwaved seeds. The TAC values decreased by 90% and 80% at the end of 21 days of storage in fresh and MW pods, respectively, the MW pods showing higher values of TAC with the DPPH assay than by FRAP.

Table 1 Total antioxidant capacity measured by FRAP and DPPH assays (mg TAC kg ^{1} fw) of cowpea seeds stored under PMA at 4 °C and then microwaved

Day	Sample	FRAP	DPPH
0	Fresh seeds	1383.0 ± 130.8 Ba	2079.4 ± 180.4 Ba
	MW seeds	2108.0 ± 163.3 Aa	3243.6 ± 49.1 Aa
3	Fresh seeds	999.4 ± 97.8 NSab	1418.6 ± 133.1 Bb
	MW seeds	1180.9 ± 46.0 NSb	2116.3 ± 191.4 Ab
7	Fresh seeds	606.1 ± 41.7 Bb	1105.3 ± 56.6 NSb
	MW seeds	920.3 ± 77.8 Ab	1527.1 ± 162.3 NSb
Treatments		***	***
Days		***	***
Days × treatments		*	*

FRAP, ferric reducing antioxidant power; *DPPH*, 2,2 diphenyl 1 picrylhydrazil; *NS*, non significant. Data are mean ± standard deviation ($n = 3$). Different capital letters within the same column show significant differences between fresh and microwaved samples. Different lowercase letters within the same column show significant differences between stor age times. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$

The values obtained for the antioxidant capacity were much higher than those found in the literature (Adjei-Fremah et al. 2015; Nassourou et al. 2016), perhaps due to differences among varieties of cowpea. The results reported here regarding the considerable retention of phenolic compounds and antioxidant capacity are health-relevant since phenolic compounds have therapeutic properties in different diseases. As with the phenolic content, the concentration of TAC increased after microwaving, possibly due to the increased extraction of the compounds after heating and to the moisture loss taking place during cooking. The higher antioxidant capacity observed in our study related to that cited in the references could be attributed to the low degradation of total phenolics. A decrease in antioxidant activity was recorded in pea seeds (Collado et al. 2017) as storage progressed.

At the end of storage, both seeds and MW pods showed lower losses of antioxidant capacity and total phenols compared to fresh samples. This may be due to the thermal inactivation of enzymes responsible for their degradation.

Sugars

The concentration of the three most relevant sugars (raffinose, sucrose and glucose) of cowpea seeds and pods is presented in Tables 3 and 4, respectively. Values for sucrose and glucose were higher in pods than in seeds, while for raffinose the concentration was very similar in fresh seeds and pods.

Few data have been published on the effects of fresh-cut processing on the soluble sugars of cowpea. Of importance in this context is raffinose, which is considered a non-nutritional and "flatulence factor" because, when fermented by intestinal microflora, it releases considerable amounts of gases (Singh and Kayastha 2013). The concentration at day 0 in fresh seeds and pods was very similar (83 mg 100 g ^{1} fw). Our results agree with previous data on cowpea seeds. Khattab and Arntfield (2009), Sreerama et al. (2012), and Kalpanadevi and Mohan (2013) reported concentrations of 84, 103, and 68 mg 100 g ^{1}, respectively. After microwaving, raffinose

Table 2 Total antioxidant capacity measured by FRAP and DPPH assays (mg TAC kg ^{1} fw) of cowpea pods stored under PMA conditions at 8 °C and then microwaved

Day	Sample	FRAP	DPPH
0	Fresh pods	751.1 ± 78.8 Ba	1883.2 ± 164.2 NSa
	MW pods	1205.2 ± 136.0 Aa	2315.7 ± 16.8 NSa
7	Fresh pods	203.5 ± 22.5 Bb	363.7 ± 6.0 Bc
	MW pods	361.00 ± 13.9 Ab	502.9 ± 18.1 Ab
14	Fresh pods	727.4 ± 96.3 Ba	1313.6 ± 118.0 Bb
	MW pods	1112.1 ± 66.8 Aa	2142.6 ± 140.1 Aa
21	Fresh pods	118.4 ± 2.2 Bb	149.0 ± 16.4 Bc
	MW pods	453.3 ± 12.0 Ab	502.8 ± 6.3 Ab
Treatments		***	***
Days		***	***
Days × treatments		NS	**

FRAP, ferric reducing antioxidant power; *DPPH*, 2,2 diphenyl 1 picrylhydrazil; *NS*, non significant. Data are mean ± standard deviation ($n = 3$). Different capital letters within the same column show significant differences between fresh and microwaved samples. Different lowercase letters within the same column show significant differences between stor age times. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$

was not detected in any sample. This reduction in the raffinose content is a relevant factor for a legume-based product. Onigbinde and Akinyele (1983) proposed that the decrease in raffinose during cooking may be attributed to heat hydrolysis or raffinose to disaccharides and monosaccharides or to the formation of other compounds. The raffinose content decreased during storage and was more affected by storage time than the other sugars. We have not found any clear relationship between the decrease in raffinose and the increase in glucose and fructose. Probably, it could be related, at least partially, to moisture loss occurring in cooking. In fresh seeds, there was a decrease of 45% of raffinose concentration from day 0 to day 3, while it was undetected on the last day of storage. In the case of fresh pods, the raffinose concentration had fallen by 65% after 14 days with respect to day 0 and was undetectable at day 21 of storage.

The observed sucrose content (64.75 ± 21.48 and 178.83 ± 44.48 mg 100 g ^{1} fw in seeds and pods, respectively) (Tables 3 and 4) was in agreement with previously reported data. Tchiagam et al. (2011) evaluated the sucrose content in cowpea seeds, finding values of 53.2 ± 5.7 mg 100 g ^{1} fw. Moreover, Karapanos et al. (2017) reported a sucrose concentration of 194.2 ± 5.20 mg 100 g ^{1} fw in cowpea pods. The sucrose content increased by 43% and 17% after microwaving seeds and pods, respectively, on day 0 (Tables 3 and 4). Klug et al. (2018) evaluated the sucrose content of a faba bean pesto sauce, after cooking in a microwave (11 kW, 30 s), and observed an increase of 77%. On the contrary, Alajaji and El-Adawy (2006) showed that the content of sucrose of chickpea seeds after microwave cooking was reduced by 28%. In the experiments presented here, even when some moisture loss occurred, it was an increasing trend. As storage progressed, a significant decrease in the sucrose content of around 75% in fresh and MW seeds was observed, while for pods there was a decrease of about 80% and 63% for the fresh and MW, respectively.

Karapanos et al. (2017) studied the glucose content of 37 varieties of cowpea pods, finding that it ranged from 247 to 1082 mg 100 g ^{1} fw. This wide variation could be due to different pre-harvest factors but it agrees, in general, with our results (300.74 ± 35.53 mg 100 g ^{1} fw) (Table 4). After microwaving at day 0, the glucose concentration increased by 67% and 17%, in seeds and pods, respectively. The glucose concentration was significantly affected throughout storage in the case of seeds, where the reduction in both the fresh and MW products was around 85%. In the case of fresh pods, the decrease was about 40% and only 3.5% for microwaved pods (Tables 3 and 4).

Table 3 Sugar (raffinose, sucrose, and galactose) content (mg 100 g ^{1} fw) of cowpea seeds stored under PMA at 4 °C and then microwaved

Day	Sample	Raffinose	Sucrose	Glucose
0	Fresh seeds	83.8 ± 22.3 Aa	64.8 ± 21.5 NS a	170.2 ± 13.2 NS a
	MW seeds	ND B ns	92.5 ± 15.2 NS a	285.1 ± 25.6 NS a
3	Fresh seeds	45.4 ± 4.8 Ab	43.0 ± 4.6 NS a	39.1 ± 14.5 NS b
	MW seeds	ND B ns	66.8 ± 9.3 NS a	130.6 ± 119.0 NS b
7	Fresh seeds	ND NS C	15.6 ± 1.8 NS b	26.7 ± 4.2 NS b
	MW seeds	ND NS ns	24.3 ± 2.8 NS b	47.6 ± 10.3 NS b
Treatments			*	*
Days			***	***
Days × treatments			NS	**

ND, non detectable; *NS* or *ns*, non significant. Data are mean ± standard deviation ($n = 3$). Different capital letters within the same column show significant differences between fresh and microwaved samples. Different lower case letters within the same column show significant differences between storage times.***$P < 0.001$; **$P < 0.01$; *$P < 0.05$

Table 4 Sugars (raffinose, sucrose, glucose) content (mg 100 g ^{1} fw) of cowpea pods stored under PMA at 8 °C and then microwaved

Day	Sample	Raffinose	Sucrose	Glucose
0	Fresh pods	83.61 ± 3.68 Aa	178.83 ± 44.48 NS a	300.74 ± 35.54 B ns
	MW pods	ND B ns	209.49 ± 21.89 NS a	352.78 ± 20.76 A ns
7	Fresh pods	71.07 ± 9.93 Aa	64.04 ± 3.38 NS b	230.63 ± 15.14 B ns
	MW pods	ND B ns	63.10 ± 6.23 NS b	387.54 ± 69.54 A ns
14	Fresh pods	20.40 ± 6.35 Ab	33.58 ± 0.03 Bb	224.53 ± 93.10 B ns
	MW pods	ND B ns	64.04 ± 5.23 Ab	314.87 ± 34.70 A ns
21	Fresh pods	ND NS b	32.01 ± 2.34 Bb	183.44 ± 17.41 B ns
	MW pods	ND NS ns	76.52 ± 3.34 Ab	340.45 ± 22.24 A ns
Treatments			NS	*
Days		*	***	NS
Days × treatments			**	NS

ND, non detectable; *NS* or *ns*, non significant. Data are mean ± standard deviation ($n = 3$). Different capital letters within the same column show significant differences between fresh and microwaved samples. Different lowercase letters within the same column show significant differences between storage times. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$

Sensory Evaluation

Sensory quality is one of the most important aspects for fresh-cut products. Basically, consumers appreciate five main attributes, typically visual appearance and color, followed by taste, aroma, and texture Barrett et al. 2010. As storage progressed, a significant decrease in the score of the different sensory parameters was observed for MW pods (Fig. 3), reaching values below the limit of marketability by the end of storage period for all sensory parameters, providing a shelf life of 14 days.

In both fresh and MW seeds, values were always over the limit of marketability even at the end of storage for all the sensory parameters. For fresh samples, the parameter values were very similar, with non-significant differences, at day 7 with respect to day 3. Microwave processing did not cause any change to the visual appearance, flavor, aroma, texture, or

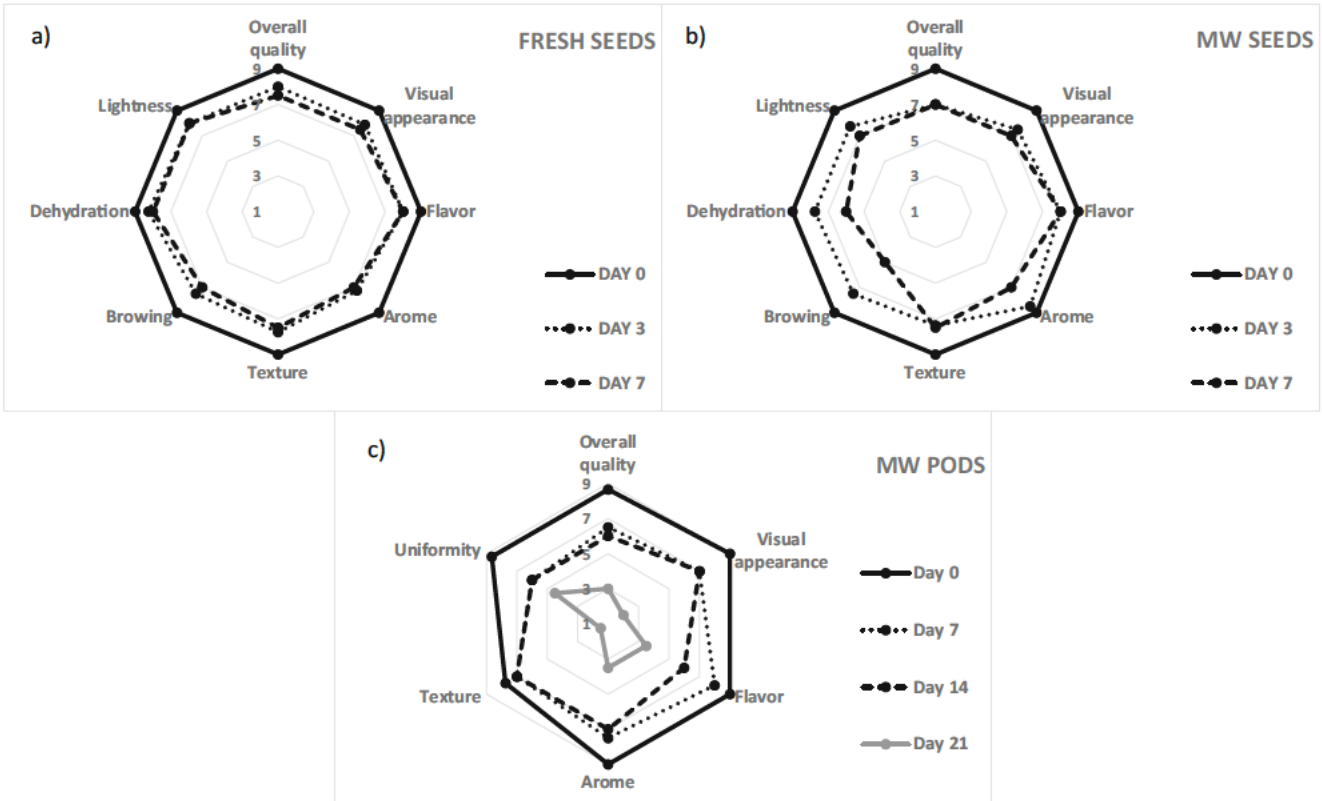

Fig. 3 Sensory quality of **a** fresh cowpea seeds stored under PMA, **b** microwaved seeds, and **c** microwaved pods. Data are mean ($n = 7$)

overall quality even of fresh seeds, at the end of storage, although there was a slight decrease in visual quality due to browning, dehydration, and loss of lightness.

According to the results obtained in the sensory evaluation, the shelf life of fresh-cut cowpea seeds, both fresh and microwaved, was of at least 7 days at 4 °C, although on the last day of storage the acceptance was higher for fresh seeds compared to MW seeds. For all cases, microwaving after storage led to good sensory properties, making cowpea an interesting product for both the food industry and the consumers.

Conclusions

Immature cowpea seeds and pods, from a local, well-adapted, and highly productive cowpea landrace, could be stored for at least 7 and 14 days, respectively, at 4 and 8 °C under MAP. However, shelf life of pods was shorter than initially expected (21 days vs 14 days) mostly due to sensory quality loss. Despite this fact, even when there were noticeable losses on total antioxidant capacity, and sugar and phenolic content, the nutritional value was quite acceptable after storage. Both seeds and pods can be seen as promising for promoting cowpea consumption, thereby helping to reduce the loss of agrodiversity in legume species. Heating slightly negatively affected the total phenolic content and antioxidant capacity. Despite that, microwaving can be still considered a good alternative for cowpeas. In addition, the degradation of raffinose due to microwave heating is a positive consequence since it reduces the possible adverse effects of this non-nutritional factor. Although the results are promising and could be helping in preserving cowpea biodiversity, more research is needed to analyze the effect of heating on other important aspects of cowpea quality.

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References

Adjei Fremah, S., Jackai, L., & Worku, M. (2015). Analysis of phenolic content and antioxidant properties of selected cowpea varieties tested in bovine peripheral blood. *American Journal of Animal and Veterinary Sciences, 10*(4), 235 245. https://doi.org/10.3844/ajavsp.2015.235.245.

Alajaji, S. A., & El Adawy, T. A. (2006). Nutritional composition of chickpea (*Cicer arietinum* L.) as affected by microwave cooking and other traditional cooking methods. *Journal of Food Composition and Analysis, 19*(8), 806 812. https://doi.org/10.1016/j.jfca.2006.03.015.

Ali, A., Al Saady, N. A., Waly, M. I., Bhatt, N., Al Subhi, A., & Khan, A. J. (2014). Evaluation of indigeous Omani legumes for their nutritional quality, phytochemical composition and antioxidant properties. *International Journal of Postharvest Technology and Innovation, 3*(4), 333 346. https://doi.org/10.1504/IJPTI.2013.060267.

Artés Hernández, F., Aguayo, E., Gómez, P. A., & Artés, F. (2009). Innovaciones tecnológicas para preservar la calidad de los productos vegetales mínimamente procesados o de la "cuarta gama". *Horticultura Internacional, 69*, 52 57.

ASTM. (1986). *Physical requirements guidelines for sensory evaluation laboratories (Vol. 913, ASTM Special Technical Pub. 913)*. Philadelphia: American Society for Testing Materials.

Barrett, D. M., Beaulieu, J. C., & Shewfelt, R. (2010). Color, flavor, texture, and nutritional quality of fresh cut fruits and vegetables: desirable levels, instrumental and sensory measurement, and the effects of processing. *Critical Reviews in Food Science and Nutrition, 50*(5), 369 389. https://doi.org/10.1080/10408391003626322.

Benzie, I. F., & Strain, J. J. (1999). Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology, 299*, 15 27. https://doi.org/10.1016/S0076 6879(99)99005 5.

Bouchenak, M., & Lamri Senhadji, M. Y. (2013). Nutritional quality of legumes, and their role in cardiometabolic risk prevention: a review. *Journal of Medicinal Food, 16*(3), 185 198. https://doi.org/10.1089/jmf.2011.0238.

Brand Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *LWT Food Science and Technology, 28*(1), 25 30. https://doi.org/10.1016/S0023 6438(95)80008 5.

Chumyam, A., Whangchai, K., Jungklang, J., Faiyue, B., & Saengnil, K. (2013). Effects of heat treatments on antioxidant capacity and total phenolic content of four cultivars of purple skin eggplants. *Science Asia, 39*(3), 246 251. https://doi.org/10.2306/scienceasia1513 1874.2013.39.246.

Collado, E., Klug, T. V., Martínez Sánchez, A., Artés Hernández, F., Aguayo, E., Artés, F., Fernández, J. A., & Gómez, P. A. (2017). Immature pea seeds: effect of storage under modified atmosphere packaging and sanitation with acidified sodium chlorite. *Journal of the Science of Food and Agriculture, 97*(13), 4370 4378. https://doi.org/10.1002/jsfa.8513.

Deng, G., Lin, X., Xu, X., Gao, L , Xie, J., & Li, H. (2013). Antioxidant capacities and total phenolic contents of 56 vegetables. *Journal of Functional Foods, 5*(1), 260 266. https://doi.org/10.1016/j.jff.2012.10.015.

Deng, Y., Padilla Zakour, O , Zhao, Y., & Tao, S. (2015). Influences of high hydrostatic pressure, microwave heating, and boiling on chemical compositions, antinutritional factors, fatty acids, in vitro protein digestibility, and microstructure of buckwheat. *Food and Bioprocess Technology, 8*(11), 2235 2245. https://doi.org/10.1007/s11947 015 1578 9.

Domínguez Perles, R., Carnide, V., Marques, G., de Castro, I., & Rosa, E. (2015). Relevance, constraints and perspectives of cowpea crops in the Mediterranean Basin. *Legume Perspectives, 10*, 40 42.

FAOStat. (2017). Food and Agriculture Organization (FAO), World Production. Available at: http://faostat3.fao.org/browse/Q/QC/E. Accessed 12 Dec 2017.

Flores, P., Hellín, P., & Fenoll, J. (2012). Determination of organic acids in fruits and vegetables by liquid chromatography with tandem mass spectrometry. *Food Chemistry, 132*(2), 1049 1054. https://doi.org/10.1016/j.foodchem.2011.10.064.

Frota, K. M. G., Mendonça, S., Saldiva, P. H. N., Cruz, R. J., & Arêas, J. A. G. (2008). Cholesterol lowering properties of whole cowpea seed

and its protein isolate in hamsters. *Journal of Food Science, 73*(9), 235 240. https://doi.org/10.1111/j.1750 3841.2008.00953.x.

Gonçalves, A., Goufo, P., Barros, A., Domínguez Perles, R., Trindade, H., Rosa, E. A. S., Ferreira, L., & Rodrigues, M. (2016). Cowpea (*Vigna unguiculata* L. Walp), a renewed multipurpose crop for a more sustainable agri food system: Nutritional advantages and con straints. *Journal of the Science of Food and Agriculture, 96*(9), 2941 2951. https://doi.org/10.1002/jsfa.7644.

Hithamani, G., & Srinivasan, K. (2014). Bioaccessibility of polyphenols from wheat (*Triticum aestivum*), Sorghum (*Sorghum bicolor*), green gram (*Vigna radiata*), and chickpea (*Cicer arietinum*) as influenced by domestic food processing. *Journal of Agricultural and Food Chemistry, 62*(46), 11170 11179. https://doi.org/10.1021/jf503450u.

Hoeck, J. A., Fehr, W. R., Murphy, P. A., & Welke, G. A. (2000). Influence of genotype and environment on isoflavone contents of soybean. *Crop Science, 40*(1), 48 51. https://doi.org/10.2135/cropsci2000.40148x.

ISO (2012). Sensory analysis general guidelines for the selection, train ing and monitoring of selected assessors and expert sensory asses sors. In I. O. f. S. (ISO) (Ed.), *8586*:2012. Geneva.

Kalpanadevi, V., & Mohan, V. R. (2013). Effect of processing on antinutrients and in vitro protein digestibility of the underutilized legume, *Vigna unguiculata* (L.) Walp subsp. Unguiculata. *LWT Food Science and Technology, 51*(2), 455 461. https://doi.org/10.1016/j.lwt.2012.09.030.

Karapanos, I., Papandreou, A., Skouloudi, M., Makrogianni, D., Fernández, J. A., Rosa, E., Ntatsi, G., Bebeli, P. J., & Savvas, D. (2017). Cowpea fresh pods a new legume for the market: assess ment of their quality and dietary characteristics of 37 cowpea acces sions grown in southern Europe. *Journal of the Science of Food and Agriculture, 97*(13), 4343 4352. https://doi.org/10.1002/jsfa.8418.

Khattab, R. Y, & Arntfield, S. D. (2009). Nutritional quality of legume seeds as affected by some physical treatments 2. Antinutritional factors. *LWT Food Science and Technology, 42*(6), 1113 1118. https://doi.org/10.1016/j.lwt.2009.02.004.

Klug, T. V., Collado, E., Martínez Sánchez, A., Gómez, P. A., Aguayo, E., Otón, M., Artés, P., & Artés Hernández, F. (2018). Innovative quality improvement by continuous microwave processing of a faba beans pesto sauce. *Food and Bioprocess Technology, 11*(3), 561 571. https://doi.org/10.1007/s11947 017 2024 y.

Martínez Hernández, G. B., Gómez, P. A., Pradas, I., Artés, F., & Artés Hernández, F. (2011). Moderate UV C pretreatment as a quality enhancement tool in fresh cut Bimi® broccoli. *Postharvest Biology and Technology, 62*(3), 327 337. https://doi.org/10.1016/j.postharvbio.2011.06.015.

Martos Fuentes, M., Fernández, J., Ochoa, J., Carvalho, M., Carnide, V., Rosa, E., Pereira, G., Barcelos, C. J., Bebeli, P., & Egea Gilabert, C. (2017). Genotype by environment interactions in cowpea (*Vigna unguiculata* L. Walp.) grown in the Iberian Peninsula. *Crop and Pasture Science, 68*(11), 924 931. https://doi.org/10.1071/CP17071.

Nassourou, M. A., Njintang, Y. N., Noubissié, T. J. B., Nguimbou, R. M., & Bell, J. M. (2016). Genetics of seed flavonoid content and anti oxidant activity in cowpea (*Vigna unguiculata* L. Walp.). *The Crop Journal, 4*(5), 391 397. https://doi.org/10.1016/j.cj.2016.05.011.

Oliveira, A. P., Araújo, J. S., Alves, E. U., Noronha, M. A. S., Cassimiro, C. F., & Mendonça, F. G. (2001). Yield of cowpea beans cultivated with bovine manure and mineral fertilization. *Horticultura Brasileira, 19*(1), 81 84. https://doi.org/10.1590/S0102 05362001000100017.

Onigbinde, A., & Akinyele, I. O. (1983). Oligosaccharide content of 20 varieties of cowpeas in Nigeria. *Journal of Food Science, 48*(4), 1250 1254. https://doi.org/10.1111/j.1365 2621.1983.tb09203.x.

Pinto, P. M. Z., & Morais, A. M. M. B. (Eds.). (2000). *Boas práticas para a conservação de produtos horto frutícolas* (1st ed.pp. 1 39). Porto: AESBUC: Associação para a Escola Superior de Biotecnologia de Universidade Católica.

Rodríguez Hidalgo, S., Artés Hernández, F., Gómez, P. A., Fernández, J., & Artés, F. (2010). Quality of fresh cut baby spinach grown under floating trays system as affected by N fertilization and innovative package treatments. *Journal of the Science of Food and Agriculture, 90*(6), 1089 1097. https://doi.org/10.1002/jsfa.3926.

Sharma, K., Ko, E. Y., Assefa, A. D., Ha, S., Nile, S. H., Lee, E. T., & Park, S. W. (2015). Temperature dependent studies on the total phe nolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. *Journal of Food and Drug Analysis, 23*(2), 243 252. https://doi.org/10.1016/j.jfda.2014.10.005.

Singh, N., & Kayastha, A. M. (2013). A novel application of Cicer α galactosidase in reduction of raffinose family oligosaccharides in soybean flour. *Journal of Plant Biochemistry and Biotechnology, 22*(3), 353 356. https://doi.org/10.1007/s13562 012 0173 7.

Singh, B., Singh, J. P., Shevkani, K., Singh, N., & Kaur, A. (2017). Bioactive constituents in pulses and their health benefits. *Journal of Food Science and Technology, 54*(4), 858 870. https://doi.org/10.1007/s13197 016 2391 9.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phospomolobdic phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144 158.

Sreerama, Y. N., Sashikala, V. B., Pratape, V. M., & Singh, V. (2012). Nutrients and antinutrients in cowpea and horse gram flours in com parison to chickpea flour: Evaluation of their flour functionality. *Food Chemistry, 131*(2), 462 468. https://doi.org/10.1016/j.foodchem.2011.09.008.

Tchiagam, J. B. N., Youmbi, E., Njintang, N. Y., Bell, J. M., & Nassourou, M. A. (2011). Generation means analysis of seed su crose content in cowpea (*Vigna unguiculata* L. Walp.). *Asian. Journal of Agricultural Sciences, 3*, 475 480.

Xu, B., & Chang, S. K. C. (2012). Comparative study on antiproliferation properties and cellular antioxidant activities of commonly consumed food legumes against nine human cancer cell lines. *Food Chemistry, 134*(3), 1287 1296. https://doi.org/10.1016/j.foodchem.2012.02.212.

Yadav, N., Kaur, D., Malaviya, R., Singh, M., Fatima, M., & Singh, L. (2018). Effect of thermal and non thermal processing on antioxidant potential of cowpea seeds. *International Journal of Food Properties, 21*(1), 437 451. https://doi.org/10.1080/10942912.2018.1431659.

Yasmin, A., Zeb, A., Khalil, A. W., Paracha, G. M., & Khattak, A. B. (2008). Effect of processing on anti nutritional factors of red kidney bean (*Phaseolus vulgaris*) grains. *Food and Bioprocess Technology, 1*(4), 415 419. https://doi.org/10.1007/s11947 008 0125 3.

Zhou, L , Tey, C. Y., Bingol, G., & Bi, J. (2016). Effect of microwave treatment on enzyme inactivation and quality change of defatted avocado puree during storage. *Innovative Food Science & Emerging Technologies, 37*, 61 67. https://doi.org/10.1016/j.ifset.2016.08.002.

Zia Ul Haq, M., Ahmad, S., Amarowicz, R., & De Feo, V. (2013). Antioxidant activity of the extracts of some cowpea (*Vigna unguiculata* (L) Walp.) cultivars commonly consumed in Pakistan. *Molecules, 18*(2), 2005 2017. https://doi.org/10.3390/molecules18022005.

Chapter IV:

UV-C pretreatment of fresh-cut faba beans (Vicia faba) for shelf life extension: Effects of domestic microwaving for consumption

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ABSTRACT

Faba beans (*Vicia faba* L.) are an important source of protein, carbohydrates, vitamins and minerals. However, there are not many studies about the physiological behavior of immature fabas subjected to minimal processing, preservation and cooking. Currently they are developing new ways of presenting the faba been seeds as minimally processed fresh food as well as its cooked in microwave, trying to encourage consumption, given its advantages as part of a nutritious and healthy diet. Sanitation is one of the most critical steps in the fresh-cut production, due to the effects of microbial load on quality, safety and shelf-life of the final product. The present studied evaluated the effect of different sanitizers (NaOCl, and UV-C (3 kJ m^{-2}, UV) and passive modified atmosphere packaging on the sensory and microbial quality, and bioactive and anti-nutritional content of fresh-cut faba beans during storage at 5ºC. After 10 days, the atmospheres within the packages were 12.5 kPa CO_2 / 5.3 kPa O_2 in the case of seeds treated with NaOCl and 22.5 KPa CO_2 / 4.2 KPa O_2, in the case of seeds treated with UV-C. Sensorial attributes were above the limit of acceptability for fresh and microwaved faba bean, subjected to treatment with UV-C light until the last day of storage. Beans treated with NaOCl maintained their sensory acceptance until day 7, both in fresh and microwaved product. The microbial load was low at the end of storage, although NaOCl showed the highest microbial counts for mesophiles, psycrophiles and enterobacteria. UV-C did not negatively affect the total antioxidant capacity of samples during storage. The phytic acid and raffinose contents decreased after 10 days, without influence of the UV-C treatment. Microwaving reduced the phytic acid and condensed tannins contents in those samples stored for up to six days, with low microwaving effect in the last storage days. Nevertheless, UV-C improved the condensed tannins reductions through storage compared with non-irradiated samples. In conclusion, the UV-C treatment of fresh-cut faba beans extended shelf life to 10 days without affecting the antioxidant capacity and with a reduction in anti- nutritional compounds achieved after domestic microwaving.

Article

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UV-C pretreatment of fresh-cut faba beans (*Vicia faba*) for shelf life extension: Effects of domestic microwaving for consumption

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Abstract

Faba beans have a short shelf life which is even reduced after fresh-cut processing mainly due to browning and dehydration. In that sense, the effects of a UV-C treatment (3 kJ m^{-2}), compared with non-exposed beans (CTRL), were studied on the sensory and microbial quality, and bioactive and anti-nutritional content of fresh-cut faba beans (cv. *Muchamiel*) during storage at 5 °C. The effect of a domestic microwaving (3 min, 900 W) on bioactive and anti-nutritional compounds of fresh seeds prior to consumption at each sampling time was also studied. UV-C treatment extended the fresh-cut faba bean shelf life from 7 to 10 days with browning score (the main sensory parameter adversely affected) of 8 and 1 log unit lower than CTRL at day 10. UV-C did not negatively affect the total antioxidant capacity of samples during storage. The phytic acid and raffinose contents decreased by 30/40%, respectively, after 10 days, without influence of the UV-C treatment. Microwaving reduced the phytic acid and condensed tannins contents by 30% in those samples stored for up to six days, with low microwaving effect in the last storage days. Nevertheless, UV-C improved the condensed tannins reductions through storage (≈30%) compared with non-irradiated samples.

Keywords

Vicia faba L., microwave, phytic acid, tannins, raffinose, antioxidants

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INTRODUCTION

Faba beans (*Vicia faba* L.) are one of the oldest crops with an annual production of ≈5 Mt in 2017, out of a total pulses production of ≈55 Mt (FAOSTAT, 2017). Pulses are an important worldwide staple food due to their low cost and high nutritional contents (proteins, fibre, carbohydrates, vitamins, etc.) (Crépon et al., 2010). Nevertheless, the bioavailability of some nutrients (proteins, minerals and vitamins) may be reduced due to several compounds of pulses (e.g. phytic acid, tannins, among others like saponins, trypsin inhibitors, amino acid analogues and alkaloids with anti-vitamin effects), which have been considered as anti-nutritional compounds (Soetan and Oyewole, 2009; Wang et al., 2009). Nevertheless, such compounds have been also reported to have health-promoting properties (antioxidant, anticancer, prebiotics, hypocholesterolemic,

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antimicrobial activities) (Chung et al., 1998; Revilla, 2015; Soetan, 2002; Zartl et al., 2018).

Fresh-cut products are highly appreciated by consumers due to their convenience and ready-to-use properties (Artés et al., 2009). The faba bean cv. *Muchamiel* might be a valued fresh-cut product due to its sweety flavour (Parra-Galant, 2009), contrary to most faba beans cvs., which highly mask the typical bitter flavour (mainly due to tannins) of this pulse. The main quality loss of fresh faba beans is due to browning, flavour deterioration and dehydration, which lead to a short postharvest life. Due to fresh-cut processing, such quality losses are increased while microbial growth is also enhanced. Accordingly, several postharvest techniques, such as modified atmosphere packaging (MAP) and alternative sanitizing treatments to conventional NaOCl washing (which might produce carcinogenic by-products), like UV-C radiation, may extend the shelf life of fresh-cut products (Artés et al., 2009).

The microbicidal effect of UV-C ($\lambda = 190–280$ nm) radiation is due to the formation of pyrimidine dimers, which alters the microbial DNA helix and blocks the microbial cell replication (Nakajima et al., 2004). UV-C radiation reduces the enzymatic (mainly polyphenoloxidase (PPO)) browning of fresh-cut products through protein aggregation (Manzocco et al., 2009). The low atmospheric oxygen concentrations (5–10%) achieved during MAP also minimize the enzymatic browning of fresh-cut products. UV-C radiation has been also reported to reduce the contents of several anti-nutritional compounds in soybeans (Maetens et al., 2018).

Some consumers still prefer cooking of fresh faba beans prior to consumption to reduce the concentration of oligosaccharides derived from raffinose (Revilla, 2015), which have been widely known to cause flatulence in humans (Rao and Belavady, 1978). Microwaving is more efficient than conventional cooking methods (boiling, high pressure boiling, steaming, etc.) since it takes shorter cooking times with consequent lower nutritional and sensory losses (Castillejo et al., 2018; Martínez-Hernández et al., 2013a, 2013b). It has been reported that microwaved faba beans (6 min) achieved the same anti-nutritional reductions as conventional boiling (30 min) (Luo and Xie, 2013).

The aim of this study was to evaluate the use of UV-C light, as an alternative sanitizing treatment to NaOCl, to extend the shelf life of fresh-cut faba beans (cv. *Muchamiel*) during storage for up to 10 days at 5 °C. The content of the main nutritional and anti-nutritional compounds was also studied. Furthermore, the effects of a domestic microwaving prior to consumption (at each sampling time: 0, 3, 7 and 10 days) were also studied. The experiment layout is described in Figure 1.

MATERIALS AND METHODS

Plant material

Faba beans (*V. faba* L., var. *Muchamiel*) were grown in the Agri-food Experimental Station Tomás Ferro

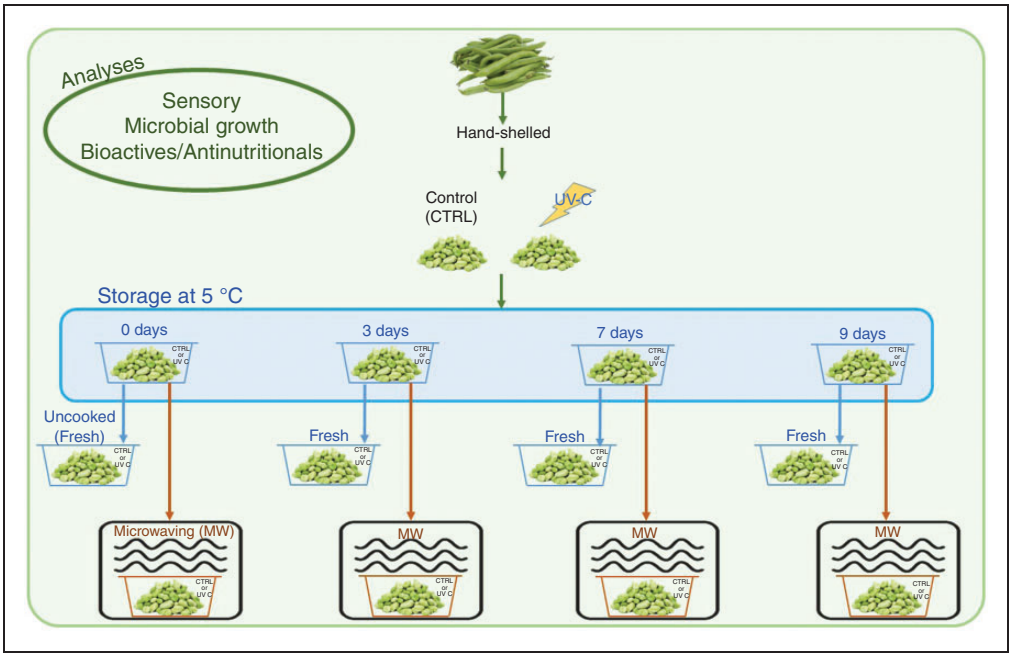

Figure 1. Flow diagram of the experiment indicating the different treatments for fresh cut and then microwaved immature faba seeds.

(La Palma, Cartagena, Spain) following integrated pest management cultural practices. The beans were manually harvested in April 2016 when the pods were almost round and at an immature physiological stage with at least 70% of their seeds in full size. Then, plant material was transported $\approx$30 km to the pilot plant of the Institute of Plant Biotechnology in polystyrene boxes with top icing to maintain a low transport temperature. Upon arrival, faba beans were stored at 1 °C, and $\approx$90–95% relative humidity (RH), until the next day, when they were processed.

Processing, packaging and storage

Faba pods were hand-shelled in a cold room (5 °C) and the obtained seeds were immediately immersed in cold water (4 ± 1 °C) to slow down the seed metabolism. The obtained faba seeds were then subjected to the following treatments:

- Control (CTRL): Sanitation with chlorinated water (150 mg l^{-1} NaOCl; pH 6.5; 4 °C; 2 min; plant material:washing solution, 1 kg:2 l), as commonly used in the fresh-cut industry. After the sanitation treatment, samples were rinsed for 1 min with cold water (4 °C) and finally drained with a manual spinner.
- UV-C: The UV-C treatment was carried out using a UV-C chamber from the Research group as previously described (Formica-Oliveira et al., 2016). Briefly, it consisted of 30 (15 top and 15 bottom) unfiltered germicidal emitting lamps ($\approx$80% of emitted spectrum at λ = 254.7 nm; TUV 36 W/G36 T8, Philips, Eindhoven, The Netherlands) with a measured UV-C intensity of 33.3 W m^{-2} (VLX 254 UV-C radiometer, Vilber Lourmat, Marne la Vallee, France). A 3 kJ m^{-2} dose (which meant 90 s of exposure) was selected as the optimum dose to extend the product's shelf life according to previous experiments (data not published).

Treated samples ($\approx$150 g) were then packaged in oriented polypropylene (OPP; 35 µm; 150 mm × 150 mm) bags, which were heat-sealed to generate a MAP. The O_2 and CO_2 permeabilities of the used OPP film were 900 and 1100 cm^3 m^{-2} day^{-1} (23 °C, 0% RH), respectively, as described by the supplier (Plásticos del Segura S.L., Murcia, Spain). Atmosphere composition within packages was analysed with a portable gas analyser (CheckPoint, PBI Dansensor, Ringsted, Denmark). Packaged samples were stored in darkness at 4 ± 0.5 °C and 90% RH up to 10 days.

Sampling was done after 0 (processing day), 3, 7 and 10 days. Eight bags (four CTRL bags and four UV-C bags) were taken at each sampling time. Four of them were analysed as fresh samples (fresh) while the other four bags were heated on a domestic microwave, as described below (see also Figure 1). Four replicates per treatment (CTRL, UV-C, fresh and microwaving (MW) treatments) and sampling time (four sampling times) were prepared (total samples number = 64).

MW treatment was carried out at 700 W for 1 min using a domestic microwave (Model BH7, Moulinex, Écully, France). All bags were perforated (30 holes (0.2 mm Ø) per bag) prior to the MW treatment to allow vapour gas flushing during heating.

Sensory evaluation

Sensory quality was assessed by a trained panel composed of seven people (aged 24–50 years). The evaluations were completed in a sensory room according to international standards (ASTM, 1986). Still mineral water was used as a palate cleanser. A 9-point hedonic scale was scored for visual symptoms of browning and dehydration (9 = none; 5 = limit of usability; 1 = extreme). The remaining parameters, such as visual appearance, flavour, aroma, texture, colour and overall quality were scored as follows: 1 = extremely bad; 5 = limit of usability; 9 = excellent.

Microbial growth

Standard enumeration methods were used to determine mesophilic, psychrophilic, enterobacteria and yeast and mould growth as previously described (Castillejo et al., 2016). All microbial media were acquired from Scharlau Chemie (Barcelona, Spain). The following media and incubation conditions were used: plate count modified agar for mesophilic (incubation: 30 °C/48 h) and psychotropic (5 °C/7 days) aerobic bacteria, violet red bile dextrose agar for enterobacteria (37 °C/48 h), and rose bengal agar for yeasts and moulds (Y+M) (3–5 days/22 °C). All microbial counts were reported as log of colony forming units per gram of product (log CFU g^{-1}). Each of the four replicates was analysed in duplicate.

Phytic acid

Phytic acid was determined using a commercial kit (K-Phyt kit, Megazyme, Bray, Ireland), based on the measurement of the inorganic phosphate formed after the enzymatic hydrolysis of phytic acid (McKie and McCleary, 2016). Briefly, 1 g of frozen ground samples was extracted with 20 ml of 0.66 M HCl and then stirred overnight at room temperature. The latter extract was then centrifuged (13,000 × g, 10 min, 4 °C) and 0.5 ml of the obtained supernatant was neutralized with 0.75 M NaOH (0.5 ml). The neutralized extract

was then enzymatically dephosphorylated (phytase followed by alkaline phosphatase; supplied by the kit). Subsequently, 1 ml of the hydrolysed extract was mixed with 0.5 ml of the colouring reagent (0.47 M ascorbic acid, 8.43 mM ammonium molybdate, 0.8 M sulphuric acid) and incubated at 40 °C for 1 h. The absorbance of incubated samples was then measured at 655 nm using a Multiscan plate reader (Tecan Infinite M200, Männedorf, Switzerland). Phytic acid was quantified using an authentic standard, supplied by the kit, being expressed as $g\,kg^{-1}$ on a fresh weight (fw) basis. Each of the four replicates was analysed in duplicate.

Total condensed tannins (TCT) content

The TCT content was determined based on the modified vanillin method (Price et al., 1978). Briefly, 2 g of frozen ground samples was extracted with 5 ml of methanol for 20 min in a water bath at 30 °C followed by centrifugation ($15{,}000 \times g$, 10 min, 4 °C). Then, 1 ml of the supernatant was mixed with 2.5 ml of a vanillin solution (1%, Sigma-Aldrich, St Louis MO, USA) and 2.5 ml HCl 8%, before incubation in the water bath (30 °C) for 20 min. Finally, the absorbance of the incubated samples was measured at 500 nm using the already cited microplate reader. Results are expressed as g catechin (Sigma-Aldrich, St Louis MO, USA) equivalents kg^{-1} fw. Each of the four replicates was analysed in triplicate.

Total phenolic content (TPC)

TPC was determined as previously described (Martínez-Hernández et al., 2011; Singleton and Rossi, 1965). Briefly, 0.1 g of frozen ground samples was extracted with 2 ml of methanol for 1 h in an orbital shaker ($200 \times g$) in darkness on an ice bed. The latter extracts were then centrifuged ($15{,}000 \times g$, 10 min, 4 °C) and the supernatants were used as TPC and total antioxidant capacity (TAC) extracts. Subsequently, 19 µl of the TPC extract was placed in a flat-bottom polystyrene 96-wells plate (Greiner Bio-One, Frickenhausen, Germany) and 29 µl of 1 N Folin–Ciocalteu reagent was added. The mix was incubated for 3 min in darkness at room temperature. Then, 192 µl of a solution containing Na_2CO_3 ($4\,g\,l^{-1}$) and NaOH ($20\,g\,l^{-1}$) was added and incubated for 1 h at room temperature in darkness. The absorbance of the incubated samples was measured at 750 nm using the microplate reader. The TPC was expressed as g gallic acid (Sigma-Aldrich, St Louis MO, USA) equivalents kg^{-1} fw. Each of the four replicates was analysed in triplicate.

Raffinose

For the raffinose analysis, 3 g of frozen ground samples was homogenized (UltraTurrax T-25, Ika-Labortechnik, Staufen, Germany) with 10 ml of nanopure water for 40 s. The latter extracts were filtered through a four-layer cheesecloth and subsequently centrifuged ($12{,}000 \times g$, 20 min, 4 °C). The supernatants were purified by solid phase extraction (SPE) mini-columns (C18-E SPE 55 µm, 500 mg; Phenomenex, Macclesfield, UK) and filtered through a 0.45 µm polytetrafluoroethylene syringe filter. An ultra-high-performance liquid chromatography (Shimadzu, Kyoto, Japan), equipped with a DGU-20A degasser, LC-170 30AD quaternary pump, SIL-30AC autosampler, CTO-10AS column heater, refractive index detector and SPDM-20 A diode array detector, was used. Chromatographic separation was carried out using a Rezex Carbohydrate Ag^+ column (100 mm × 4.6 mm, 2.6 µm; Phenomenex, Macclesfield, UK). Nanopure water was used as the mobile phase at a flow rate of $0.6\,ml\,min^{-1}$. The injection volume was set at 20 µl. Raffinose was identified and quantified with an authentic commercial standard (Sigma, St Louis MO, USA) and expressed as $g\,kg^{-1}$ fw. Each of the four replicates was analysed in triplicate.

TAC

The TAC of samples was analysed following the ferric reducing antioxidant power (FRAP) method as previously described (Benzie and Strain, 1999; Rodríguez-Verástegui et al., 2016). Briefly, a daily reaction solution containing sodium acetate buffer (pH 3.6), 10 mM TPTZ solution (in 40 mM HCl) and 20 mM $FeCl_3$ was prepared in a *v:v:v* proportion of 10:1:1, and incubated at 37 °C for 2 h in darkness. Then, 6 µl of the TAC extract was allowed to react with 198 µl of the FRAP solution for 40 min at room temperature in darkness. The TAC of samples was measured by the absorbance decrease (Multiscan plate reader) at 593 nm and expressed as g of Trolox equivalents kg^{-1} fw. Each of the four replicates was analysed in triplicate.

Statistical analysis

An analysis of variance was performed to compare the sanitizing (CTRL and UV-C) and cooking treatments (MW and fresh) during storage time (four sampling times) at a significant level of $p < 0.05$ using the SPSS software (IBM, Chicago, IL, USA). The Tukey's Honestly Significant Difference test was applied when significant differences were observed.

RESULTS AND DISCUSSION

Sensory and microbial quality

Sensory analysis of CTRL samples showed overall quality scores of 7.0 after 7 days, while such samples were scored below the limit of usability after 10 days with a value of 4.4 (Figure 2). However, the overall quality scores of UV-C samples were scored with 7.6 after still 10 days. The most affected sensory parameter was browning followed by brightness loss and, consequently, scores with low visual appearance. Browning is due to enzymatic (mainly PPO) reactions, which may be controlled by the reduction of oxygen concentrations within the MAP of fresh-cut products. Here, the MAP steady state was reached on day 1 with CO_2/O_2 partial pressures of 14–16/3–6 kPa, respectively (data not shown). In that sense, the CTRL samples still showed browning scores (6.5) above the limit of usability after 7 days, although such samples received a browning score of 3.3 after 10 days. On the other side, browning of UV-C samples was scored above the limit of usability (8) after 10 days of cold storage. The browning inhibition by UV-C has been reported to occur due to a PPO inactivation through protein aggregations (Manzocco et al., 2009). Additionally, the UV-C samples showed lower dehydration, and consequently a better texture than CTRL with scores of 7.8 and 6.8, respectively, at day 10. The lower dehydration of UV-C samples may be explained by their lower microbial loads at the end of storage, as shown below. Furthermore, the UV-C samples were scored with better flavour values than CTRL since bitter compounds, other than tannins, were probably degraded to a higher degree in these samples due to the UV-C-activation of specific degradative reactions. Microwaving did not highly affect the sensory quality of the samples. Nevertheless, the browning scores of UV-C samples, and consequently visual appearance, were reduced by ≈ 1.5 units although such scores were still above 6.5 at day 10.

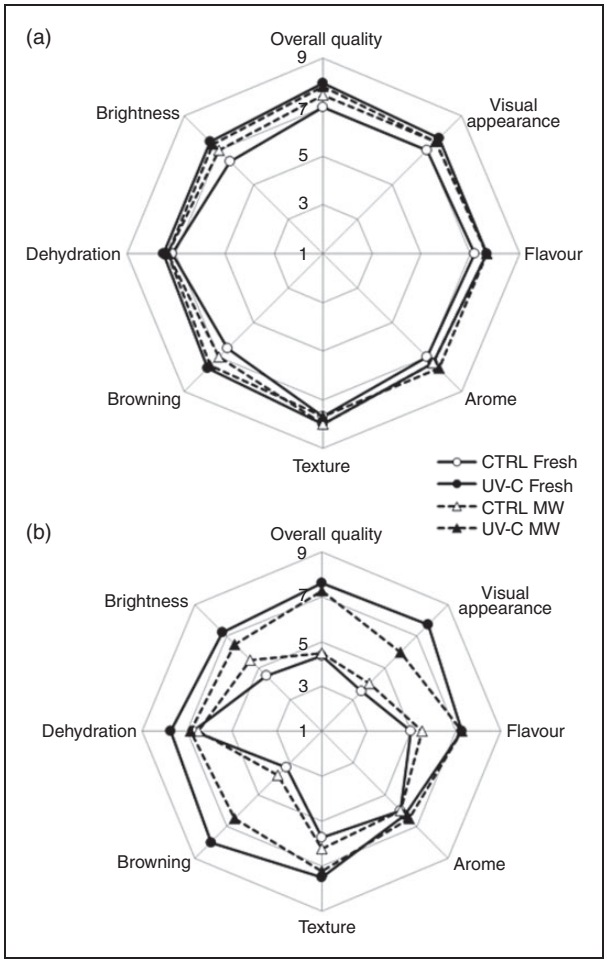

Figure 2. Sensory scores of UV-C treated or non-treated (CTRL) fresh-cut faba beans, after (a) 7 and (b) 10 days at 5°C and then microwaved (MW treatment).

The initial mesophilic and enterobacteria loads of CTRL samples were 2.0 ± 0.2 and 2.6 ± 0.1 log CFU g^{-1}, respectively, on processing day (Figure 3). However, the psychrophilic and Y+M loads were below the detection limits on processing day (1 and 2 log CFU g^{-1}, respectively). The UV-C treatment did not produce ($p > 0.05$) an immediate microbial reduction on processing day although the UV-C samples showed lower microbial growth during storage (Figure 3). Accordingly, the UV-C samples displayed enterobacteria, mesophilic and psychrophilic loads 1.5, 1.0 and 0.5 log units lower than CTRL at day 10, respectively. The Y+M loads of samples remained below detection throughout the storage period due to the low growing rate of these microbial groups at refrigerated temperatures (data not shown). The low microbial growth in UV-C samples may be explained by the UV-C-induced damage of the microbial DNA, the microbial cell replication mechanism being blocked (Nakajima et al., 2004). As expected, MW reduced the microbial loads to below the detection limits (data not shown).

In conclusion, the UV-C samples showed an excellent sensory quality after 10 days at 5 °C, together with lower microbial loads than CTRL, while the CTRL samples were not accepted after 7 days.

Phytic acid

Fresh-cut faba beans showed an initial phytic acid content of 0.94 ± 0.08 g kg^{-1} prior to UV-C (Figure 4). Phytic acid has been reported to decrease as a result

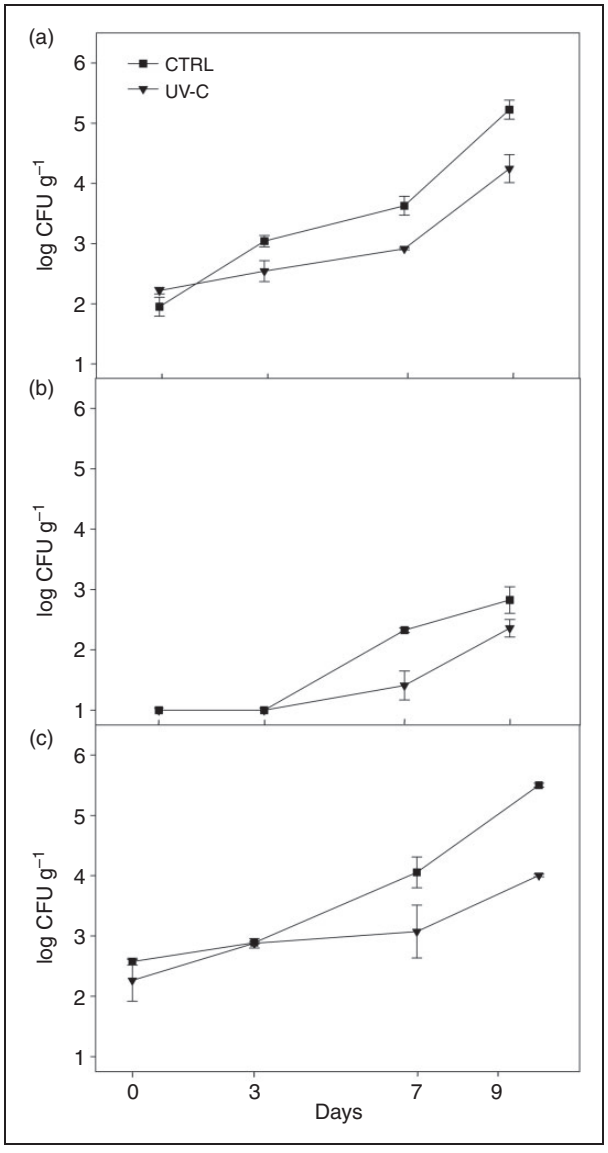

Figure 3. Microbial loads ((a) mesophiles, (b) psychrophiles, (c) enterobacteria) (log CFU g^{-1}) of UV-C treated or non-treated (CTRL) fresh-cut faba beans during storage at 5 °C and then microwaved (MW treatment) at days 0, 3, 7 and 10 (n 4 ± SD).

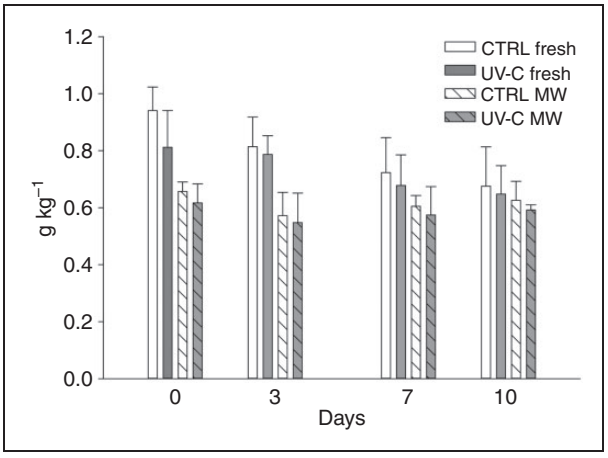

Figure 4. Phytic acid content (g kg^{-1} fw) of UV-C treated or non-treated (CTRL) fresh-cut faba beans during storage at 5 °C and then microwaved (MW treatment) at days 0, 3, 7 and 10 (n 4 ± SD).

of radiolysis since the radicals produced during irradiation may remove phosphorus from the structure, thus lowering the concentration (Ahn et al., 2003; Demir and Elgün, 2013). Indeed, UV-C (non-ionizing radiation) and γ-irradiation (ionizing radiation) applied in wheat flour and faba beans, respectively, as stabilization treatments, were found to successfully reduce the phytic acid content (Al-Kaisey et al., 2003; Demir and Elgün, 2014). Nevertheless, data presented here indicated that UV-C did not significantly ($p > 0.05$) affect the phytic acid content (Figure 4). Most of the phytic acid is located in the 'protein bodies' of the seed cotyledon (Alonso et al., 2000; Campos-Vega and Loarca-Piña, 2010). However, UV-C irradiation of fresh-cut products has been reported to affect only at the produce surface (Formica-Oliveira et al., 2016). The unaffected phytic acid content after UV-C treatment may be due to the low UV-C transmittance to the inner faba bean tissues where most of the phytic acid is located. However, phytic acid of a wheat flour was degraded by UV-C due to the high UV-C incidence on such powder (Demir and Elgün, 2014). Furthermore, phytic acid of faba beans was only degraded by γ radiation when a more penetrating radiation was used (Al-Kaisey et al., 2003).

The phytic acid of CTRL samples was reduced by ≈30% after 10 days (Figure 4). Phytic acid is the major storage form of phosphorous in leguminous, cereals, oilseeds and nuts (Vats and Banerjee, 2004). Accordingly, the phytic acid content of fresh-cut faba beans falls during storage since it is probably used as the main phosphorous source for the different metabolic reactions occurring during faba bean postharvest life. The UV-C samples presented a degradation trend as the CTRL, with no ($p > 0.05$) UV-C × storage interaction, probably because the inner part remained unaffected by the UV-C treatment, which mainly acts on surface.

Microwaved samples had a reduced phytic acid content of around 25–30, 15–6 and 7–9% at days 0–3, 6 and 10, respectively (Figure 4). Phytic acid reduction by exposure to thermal treatments has been conventionally used for dried faba beans and other pulses (Revilla, 2015) and is hypothesized to occur due to thermal degradation, as well as changes in the chemical reactivity or the formation of insoluble complexes (Alonso et al., 2000). The CTRL and UV-C samples showed similar ($p > 0.05$) phytic acid reductions

after MW, but tended to diminish as the storage time increased. This might be explained by the fact that phytic acid molecules contained in the 'proteins bodies' of the outer parts of cotyledons are degraded early during storage while the phytic acid from the inner 'protein bodies' is more resistant to the subsequent thermal degradation. Similarly, microwaving (6 min) of dried faba beans only reduced the phytic acid content by ≈8% probably due to the already decreased phytic acid concentrations in the dried plant material (Luo and Xie, 2013). As conclusion, microwaving of <6-day-old fresh-cut faba beans may obtain the same phytic acid reduction as in fresh-cut faba beans during storage for 10 days.

TCT content

The initial TCT content of fresh-cut faba beans was 2.20 ± 0.01 g CaE kg^{-1} (Figure 5). Condensed tannins are mainly concentrated in the seed coat (Revilla, 2015) and a 92% reduction of TCT was reported when the seed coats of faba beans are removed (Alonso et al., 2000). Nevertheless, the TCT contents of the samples remained unaffected ($p > 0.05$) after the UV-C treatment. Similarly, the tannin contents of persimmon and grapes were unaffected after similar UV-C treatments (Khademi et al., 2012; Pinto et al., 2016). Many phenolic compounds are commonly present as covalently bound forms (Peleg et al., 1991) and the applied UV-C dose is usually insufficient to produce the breakdown of such bond phenolic compounds. The TCT content of the samples decrease by ≈40% after three days and remained unchanged ($p > 0.05$) until the end of storage. This effect of storage time on the TCT content – and the absence of any effect of UV-C – has been previously reported in persimmon fruit (Khademi et al., 2012). The observed TCT reduction during storage may be attributed to the complex formation between the pectin released from the cell walls and tannins (Taira et al., 1997).

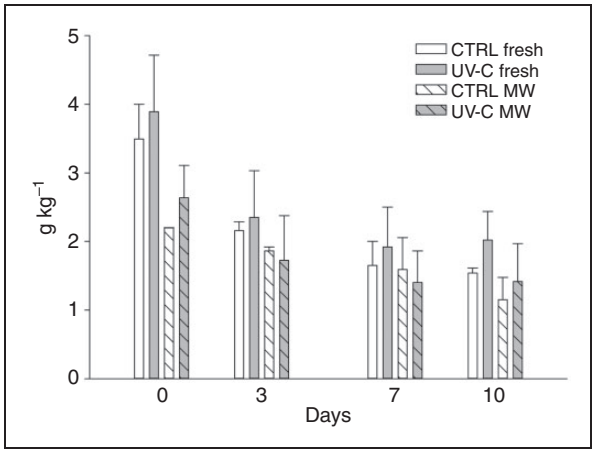

Figure 5. Tannin content (g kg^{-1}) of UV-C treated and non-treated (CTRL) fresh-cut faba beans during storage at 5 °C and then microwaved (MW treatment) at days 0, 3, 7 and 10 (n 4 ± SD).

Microwaving reduced the TCT content of all the samples by ≈30% on the processing day (Figure 5). That reduction of tannins has been reported to occur due to thermal degradation or interaction with other seed components, such as proteins, to form insoluble complexes (Nithya et al., 2007). Nevertheless, the TCT contents after MW were reduced in a lower degree when compared to the decrease of tannin contents of CTRL samples through storage. The latter finding may be explained due to the higher thermal resistance of the remaining tannins as the storage continued. However, the UV-C-treated faba beans presented a ≈30% TCT reduction for the whole storage period when they were microwaved. This may be explained if the UV-C treatment triggered an initial plant cell disruption, thus making tannins more available for the subsequent thermal degradation. In summary, the TCT content of fresh-cut faba beans was significantly reduced after three days, being further lowered after microwaving the UV-C treated samples.

TPC

The TPC of CTRL samples on processing day was 2.04 ± 0.11 g kg^{-1} (Table 1). It slightly increased (15%) after the UV-C treatment due to an already reported increase in extractability of these compounds when UV-C-plant cell disruption is induced (Formica-Oliveira et al., 2017). The TPC of samples also decreased during storage, regardless of UV-C or CTRL. TPC levels dropped ≈20% by seven days and remained at this reduced concentration until the end of storage. Microwaving led to a thermal degradation of the phenolic of ≈30%. The TPC of dried faba beans was similarly reduced by ≈30% after a thermal extrusion treatment (≈150 °C, feeder speed of 384 g min^{-1}) (Alonso et al., 2000). As previously observed for tannins, the TPC reduction after microwaving was minimized as the storage time increased.

Raffinose content

The initial raffinose content of fresh-cut faba beans was 6.64 ± 0.87 g kg^{-1} prior to UV-C (see Supplementary data). Raffinose has been reported to be absent in dried faba beans since it is probably degraded during long drying treatments (Khalil and Mansour, 1995). Higher raffinose concentrations have been reported in other pulses such as velvet bean, which fell by ≈40% after microwaving (4 min, 900 W) (Kala and Mohan, 2012).

Table 1. Total phenolic content (TPC) ($g\,kg^{-1}$) and total antioxidant capacity (TAC) ($g\,TrE\,kg^{-1}$ fw) of UV-C treated and non-treated (CTRL) fresh-cut faba beans during storage at 5°C and then microwaved (MW treatment) at days 0, 3, 7 and 10 (n 4 ± SD).

Storage time (days)	Treatment		TPC	TAC
0	CTRL	Fresh	203.68 ± 11.29	189.93 ± 24.74
		MW	151.70 ± 24.72	258.92 ± 19.95
	UV-C	Fresh	234.31 ± 3.09	258.68 ± 18.60
		MW	164.80 ± 16.45	21.36 ± 3.05
3	CTRL	Fresh	195.63 ± 13.84	157.23 ± 18.00
		MW	166.83 ± 10.48	251.96 ± 0.56
	UV-C	Fresh	213.75 ± 11.52	168.70 ± 8.77
		MW	182.55 ± 14.53	263.03 ± 25.66
7	CTRL	Fresh	161.42 ± 19.71	44.93 ± 7.63
		MW	155.92 ± 24.16	217.09 ± 5.18
	UV-C	Fresh	149.23 ± 29.75	49.41 ± 7.81
		MW	158.68 ± 14.83	154.32 ± 15.15
10	CTRL	Fresh	156.10 ± 20.20	27.35 ± 8.80
		MW	131.88 ± 3.82	147.54 ± 11.38
	UV-C	Fresh	177.89 ± 18.24	39.68 ± 12.00
		MW	143.54 ± 11.71	125.98 ± 43.56
Treatment			ns	ns
Cooking			*	**
Storage time			ns	**
Treatment × cooking			ns	ns
Treatment × storage time			ns	ns
Cooking × storage time			**	**
Treatment × cooking × storage time			ns	*

ns: not significant; **$p < 0.001$; *$p < 0.01$.

The raffinose content of samples analysed here was not affected after UV-C or MW treatments. It is also convenient to avoid excessive raffinose degradation since this oligosaccharide is considered a necessary prebiotic for the gut microbiota (Zartl et al., 2018). Furthermore, the negative health effects of raffinose have been historically related to its capacity to produce flatulence, which may not be strictly considered as an anti-nutritional effect.

The raffinose content fell during storage since sugars are used as an energy source for keeping postharvest life of plant products. In general, the raffinose content decreased by 15–30 and 30–40% after three and six days, respectively, compared to the initial concentrations, with only slight changes in the last four days of storage. Neither UV-C nor MW affected the raffinose changes during storage.

TAC

Fresh-cut faba beans showed an initial TAC that ranged from 1.98 to 2.36 $g\,kg^{-1}$, with no differences ($p > 0.05$) among UV-C or MW treatments (Table 1). The TAC of samples decreased during storage, the values being 20–30, 75–80 and 80–85% lower compared to their initial values after 3, 7 and 10 days, respectively. No differences ($p > 0.05$) in the decrease in TAC were observed between CTRL and UV-C samples at any time during storage. The observed decrease may be explained by the degradation of antioxidant compounds (i.e. phenolic compounds, vitamin C, etc.) during the storage of samples, as commonly occurs in other fresh-cut products (Ansah et al., 2018). As regards microwaving, the TAC concentrations were 0.9 and 1.2–1.7 $g\,kg^{-1}$ higher on days 3 and 7/10, respectively, due to the increase in antioxidant compounds extractability, as previously mentioned. Such higher TAC after MW was not observed on day 0 samples that can be explained since processing of fresh-cut products implies several abiotic stresses (shelling, UV-C, sanitizing washing treatments, etc.) (Cisneros-Zevallos, 2003), which lead to a high consumption of antioxidant compounds, resulting in the observed reduced TAC concentrations after MW.

Phenolic compounds are widely recognized to have important in vitro and in vivo antioxidant properties (Sahidi and Ambigaipalan, 2015). Several compounds in faba beans (tannins (Riedl et al., 2002), phytic acid (Graf and Eaton, 1990), raffinose (ElSayed et al., 2014; Ende, 2013), etc.), historically recognized as antinutritionals, have been reported with antioxidant properties. In that sense, TAC was highly correlated to TPC (fresh/MW $R^2 = 0.88/0.74$) followed by phytic acid ($R^2 = 0.19/0.45$), while raffinose and TCT were poorly correlated ($R^2 < 0.1$) (data not shown). In summary, the TAC of samples was not affected by the UV-C treatment and TAC degradation during storage was compensated after MW.

CONCLUSIONS

The studied UV-C dose ($3\,kJ\,m^{-2}$) to disinfect fresh-cut faba beans was appropriated since the shelf life of the immature faba seeds increased from 7 (when conventionally treated with $150\,mg\,l^{-1}$ NaOCl for 2 min) to 10 days. Furthermore, a reduction of 30% of condensed tannins was observed after microwaving UV-C-treated samples previously stored for 10 days. Nevertheless, the UV-C treatment did not negatively affect the TAC of samples during storage compared to the non-UV-C exposed samples. Moreover, the phytic acid, raffinose and condensed tannins contents decreased by 30, 40 and 50% after 10 days. In conclusion, the UV-C treatment of fresh-cut faba beans extended shelf life to 10 days without affecting the antioxidant capacity and with a reduction in antinutritional compounds achieved after domestic microwaving.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online.

REFERENCES

Ahn HJ, Kim JH, Yook HS and Byun MW. (2003). Irradiation effects on free radical scavenging and antioxidant activity of phytic acid. *Journal of Food Science* 68(7): 2221 2224.

Al Kaisey MT, Alwan A KH, Mohammad MH and Saeed AH. (2003). Effect of gamma irradiation on antinutri tional factors in broad bean. *Radiation Physics and Chemistry* 67(3 4): 493 496.

Alonso R, Aguirre A and Marzo F. (2000). Effects of extru sion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. *Food Chemistry* 68(2): 159 165.

Ansah FA, Amodio ML and Colelli G. (2018). Quality of fresh cut products as affected by harvest and postharvest operations. *Journal of the Science of Food and Agriculture* 98(10): 3614 3626.

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

ASTM. (1986). *Physical Requirement Guidelines for Sensory Evaluation Laboratories*. Eggert J and Zook K (eds). Philadelphia, PA: ASTM International. Available at: https://books.google.es/books/about/Physical_Requireme nt_Guidelines_for_Sens.html?id NphlAKGkfLYC& redir_esc y (accessed 19 October 2018).

Benzie IF and Strain JJ. (1999). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology* 299: 15 27.

Campos Vega R and Loarca Piña G. (2010). Minor compo nents of pulses and their potential impact on human health. *Food Research International* 43(2): 461 482.

Castillejo N, Martínez Hernández GB, Gómez PA, Artés F and Artés Hernández. (2016). Red fresh vegetables smoothies with extended shelf life as an innovative source of health promoting compounds. *Journal of Food Science and Technology* 53(3): DOI: 10.1007/s13197 015 2143 2.

Castillejo N, Martínez Hernández GB, Lozano Guerrero AJ, Pedreño Molina JL, Gómez PA, Aguayo E, et al. (2018). Microwave heating modelling of a green smoothie: Effects on glucoraphanin, sulforaphane and S methyl cysteine sulfoxide changes during storage. *Journal of the Science of Food and Agriculture* 98(5): 1863 1872.

Chung K T, Wong TY, Wei C I, Huang Y W and Lin Y. (1998). Tannins and human health: A review. *Critical Reviews in Food Science and Nutrition* 38(6): 421 464.

Cisneros Zevallos L. (2003). The use of controlled posthar vest abiotic stresses as a tool for enhancing the nutraceut ical content and adding value of fresh fruits and vegetables. *Journal of Food Science* 68(5): 1560 1565.

Crépon K, Marget P, Peyronnet C, Carrouée B, Arese P and Duc G. (2010). Nutritional value of faba bean (*Vicia faba* L.) seeds for feed and food. *Field Crops Research* 115(3): 329 339.

Demir MK and Elgün A. (2013). Stabilization of whole wheat flour branny fractions with special emphasis on internal and external characteristic of whole wheat. *Food Science and Technology Research* 19(2): 195 200.

Demir MK and Elgün A. (2014). Comparison of autoclave, microwave, IR and UV C stabilization of whole wheat flour branny fractions upon the nutritional properties of whole wheat bread. *Journal of Food Science and Technology* 51(1): 59 66.

ElSayed AI, Rafudeen MS and Golldack D. (2014). Physiological aspects of raffinose family oligosaccharides in plants: Protection against abiotic stress. *Plant Biology* 16(1): 1 8.

Ende W Van den. (2013). Multifunctional fructans and raf finose family oligosaccharides. *Frontiers in Plant Science* 4.

FAOSTAT. (2017). FAOSTAT. Available at: http://www.fao.org/faostat/en/#data (accessed 17 October 2018).

Formica Oliveira AC, Martínez Hernández GB, Aguayo E, Gómez PA, Artés F and Artés Hernández F. (2016). UV C and hyperoxia abiotic stresses to improve healthiness of carrots: Study of combined effects. *Journal of Food Science and Technology* 53(9): 3465 3476.

Formica Oliveira AC, Martínez Hernández GB, Díaz López V, Artés F and Artés Hernández F. (2017). Use of post harvest UV B and UV C radiation treatments to revalor ize broccoli byproducts and edible florets. *Innovative Food Science & Emerging Technologies* 43: 77 83.

Graf E and Eaton JW. (1990). Antioxidant functions of phytic acid. *Free Radical Biology & Medicine* 8(1): 61 69.

Kala B and Mohan V. (2012). Effect of microwave treatment on the antinutritional factors of two accessions of velvet bean, *Mucuna pruriens* (L.) DC. var. *utilis* (Wall. ex Wight) Bak. ex Burck. *International Food Research Journal* 19(3): 961 969.

Khademi O, Zamani Z, Poor Ahmadi E and Kalantari S. (2012). Effect of UV C radiation on postharvest physi ology of persimmon fruit (*Diospyros kaki* Thunb.) cv. 'Karaj' during storage at cold temperature. *International Food Research Journal* 20(1): 247 253.

Khalil AH and Mansour EH. (1995). The effect of cooking, autoclaving and germination on the nutritional quality of faba beans. *Food Chemistry* 54(2): 177 182.

Luo Y W and Xie W H. (2013). Effect of different processing methods on certain antinutritional factors and protein digestibility in green and white faba bean (*Vicia faba* L.). *CyTA Journal of Food* 11(1): 43 49.

McKie VA and McCleary BV. (2016). A novel and rapid colorimetric method for measuring total phosphorus and phytic acid in foods and animal feeds. *Journal of AOAC International* 99(3): 738 743.

Maetens E, Hettiarachchy N, Dewettinck K, Horax R and Moens K. (2018). Reductions of anti nutritional factors of germinated soybeans by ultraviolet and infrared treat ments for snack chips preparation. *LWT Food Science and Technology* 90: 513 518.

Manzocco L, Quarta B and Dri A. (2009). Polyphenoloxidase inactivation by light exposure in model systems and apple derivatives. *Innovative Food Science & Emerging Technologies* 10(4): 506 511.

Martínez Hernández GB, Artés Hernández F, Colares Souza F, Gómez PA, García Gómez PA and Artés F. (2013a). Innovative cooking techniques for improving the overall quality of a kailan hybrid broccoli. *Food and Bioprocess Technology* 6(8): 2135 2149.

Martínez Hernández GB, Artés Hernández F, Gómez PA and Artés F. (2013b). Induced changes in bioactive compounds of kailan hybrid broccoli after innovative processing and storage. *Journal of Functional Foods* 5(1): 133 143.

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

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

Nithya KS, Ramachandramurty B and Krishnamoorthy VV. (2007). Effect of processing methods on nutritional and anti nutritional qualities of hybrid (COHCU 8) and trad itional (CO7) Pearl Millet varieties of India. *Journal of Biological Sciences* 7(4): 643 647.

Parra Galant J. (2009). El haba muchamiel. *Horticultura Internacional* 70: 32 33.

Peleg H, Naim M, Rouseff RL and Zehavi U. (1991). Distribution of bound and free phenolic acids in oranges (*Citrus sinensis*) and Grapefruits (*Citrus paradisi*). *Journal of the Science of Food and Agriculture* 57(3): 417 426.

Pinto EP, Perin EC, Schott IB, da Silva Rodrigues R, Lucchetta L, Manfroi V and Rombaldi CV. (2016). The effect of postharvest application of UV C radiation on the phenolic compounds of conventional and organic grapes (*Vitis labrusca* cv. 'Concord'). *Postharvest Biology and Technology* 120: 84 91.

Price ML, Van Scoyoc S and Butler LG. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry* 26(5): 1214 1218.

Rao PU and Belavady B. (1978). Oligosaccharides in pulses: Varietal differences and effects of cooking and germin ation. *Journal of Agricultural and Food Chemistry* 26(2): 316 319.

Revilla I. (2015). Impact of thermal processing on faba bean (*Vicia faba*) composition. In: Preedy V (ed.) *Processing and Impact on Active Components in Food*. UK: Elsevier Academic Press, pp.337 343.

Riedl KM, Carando S, Alessio HM, McCarthy M and Hagerman AE. (2002). Antioxidant activity of tannins and tannin protein complexes: Assessment in vitro and in vivo. In: Morello MJ, Shahidi F and Ho C T (eds) *Free Radicals in Food*. ACS Symposium Series, Washington, DC: American Chemical Society, pp.188 200.

Rodríguez Verástegui LL, Martínez Hernández GB, Castillejo N, Gómez PA, Artés F and Artés Hernández F. (2016). Bioactive compounds and enzymatic activity of red vegetable smoothies during storage. *Food and Bioprocess Technology* 9(1): 137 146.

Sahidi F and Ambigaipalan P. (2015). Phenolics and poly phenolics in foods, beverages and spices: Antioxidant activity and health effects A review. *Journal of Functional Foods* 18: 820 897.

Singleton VL and Rossi JA. (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16(3): 144 158.

Soetan KO. (2002). Pharmacological and other beneficial effects of antinutritional factors in plants A review. *African Journal of Biotechnology* 7(25): 4713 4721.

Soetan KO and Oyewole OE. (2009). The need for adequate processing to reduce the anti nutritional factors in plants used as human foods and animal feeds: A review. *African Journal of Food Science* 3(9): 223 232.

Taira S, Ono M and Matsumoto N. (1997). Reduction of persimmon astringency by complex formation between pectin and tannins. *Postharvest Biology and Technology* 12(3): 265 271.

Vats P and Banerjee UC. (2004). Production studies and cata lytic properties of phytases (myo inositolhexakispho sphate phosphohydrolases): An overview. *Enzyme and Microbial Technology* 35(1): 3 14.

Wang N, Hatcher DW, Toews R and Gawalko EJ. (2009). Influence of cooking and dehulling on nutritional compos ition of several varieties of lentils (*Lens culinaris*). *LWT Food Science and Technology* 42(4): 842 848.

Zartl B, Silberbauer K, Loeppert R, Viernstein H, Praznik W and Mueller M. (2018). Fermentation of non digestible raffinose family oligosaccharides and galactomannans by probiotics. *Food & Function* 9(3): 1638 1646.

CONCLUSIONS

CONCLUSIONS

In order to develop new legume products for both fresh and microwave cooking, several minimal processing methods and techniques have been studied, evaluating the use of NaOCl alone or in combination with edible coatings, or alternatively with chemical (ASC) or physical (UV-C) disinfectants.

The main general conclusions of the current PhD Dissertation are as follows:

- Faba beans:

- A pretreatment with UV–C (3 kJ m^{-2}, 90 s) or a conventional NaOCl (150 ppm) sanitizing step plus an EC based of sucrose fatty acid esters during fresh-cut processing of faba seeds could be important technological tools to improve their sensory, microbial and nutritional quality.
- Fresh seeds can be microwaved within the package used for fresh storage under MAP. Microwaving (700 W, 3 min) decreased the concentration of bioactive compounds, but retained the quality of seeds allowing to obtain a ready to eat tasteful food.
- Fresh-cut faba seeds treated with EC or UV (3 kJ m^{-2}, 90 s) treatments extended the shelf life of from 7 to 10 days at 4ºC regarding NaOCl (150 ppm) treatment.
- UV-C (3 kJ m^{-2}, 90 s) treatment in fresh-cut faba bean samples showed lower microbial loads compared to disinfection with NaOCl, after 10 days at 5ºC.
- A conventional NaOCl (150 ppm) sanitizing step plus an EC reduced the microbial load in fresh faba beans seeds after 10 days at 4ºC, although NaOCl samples showed the highest natural microflora counts.
- The UV-C treatment did not negatively affect the TAC of faba bean seeds during storage compared to the non-UV-C exposed samples, and showed the highest sugars content values compared to samples treated with NaOCl or EC.
- The EC treatment showed a positive effect on vitamin C, TPC and tannins content retention in both fresh and microwaved faba bean seeds.
- The phytic acid, raffinose and condensed tannins contents decreased in faba beans samples after 10 days of storage.
- UV-C improved the condensed tannins reductions through storage compared with non-irradiated faba bean seeds samples.

- A reduction of condensed tannins and raffinose was observed after microwaving UV-C-treated faba bean seeds previously stored for 10 days.
- Microwaving reduced the phytic acid and condensed tannins contents in faba bean seeds.

- Peas:

- The use of ASC (300 ppm) as a sanitizer during fresh-cut processing of fresh pea seeds is a good alternative to NaOCl (100 ppm, pH 6.5, 2 min, 4ºC) since it led to better sensory quality and a higher nutritional quality.
- Immature green pea seeds disinfected with NaOCl (100 ppm) or ASC (300 ppm) can be stored for 14 days under MAP (8 kPa CO_2 / 12 kPa O_2 and 11 kPa CO_2 / 10 kPa O_2 at 1 and 4ºC) at temperatures between 1 and 4°C without any noticeable quality loss.
- Disinfection of fresh pea seeds with ASC (300 ppm) showed a lower psychrophile counts than disinfection with NaOCl (100 ppm).
- ASC disinfection in pea seeds not affected the TAC and TPC, while the vitamin C content was higher.

- Cowpea:

- Cowpea seeds and pods (fresh-cut and then microwaved) are feasible and practical products to stimulate legume consumption.
- Immature cowpea seeds and pods, disinfected with NaOCl, could be stored for at least 7 and 14 days, respectively, at 4 and 8°C under MAP (23 kPa CO_2 / 1.5 kPa O_2 and 19 kPa CO_2 / 1.2 kPa O_2 for fresh seeds and pods, respectively).
- Fresh seeds and pods can be microwaved within the package used for fresh storage under MAP. Microwaving (700 W, 1 min) decreased the concentration of bioactive compounds, but retained the quality of seeds and pods allowing to obtain a ready to eat tasteful food.
- TPC and TAC increased after microwaving in both cowpea seeds and pods. Sucrose and glucose concentrations increased after microwaving, while raffinose was not detected after cooking.
- Heating negatively affected the total phenolic content and antioxidant capacity in cowpea seeds and pods.

Since the general objective of this research is to optimize several processes to develop new fresh and processed foods from native varieties of three legume species (faba, pea and cowpea), to stimulate the consumption of these in the daily human diet, both for fresh and microwave consumption, It can be said that with the use of various minimal processing techniques using NaOCl alone or in combination with edible coatings, or alternatively with chemical (ASC) or physical (UV-C) disinfectants, vegetable products with high nutritional quality and high content of bioactive compounds, fresh and ready to eat, can be obtained.

SCIENTIFIC PUBLICATIONS DERIVED FROM THIS PhD DISSERTATION

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

- **Collado, E.**, Artés-Hernández, F., Navarro, L., Artés, F., Aguayo, E., Fernández, J.A., Gómez, P.A. (2016). Overall quality of minimally processed pea seeds. In: Proc. III ISHS International Conference on Fresh-cut Produce. University of California, Davis, California, USA. Eds.: M. Cantwell. Edit: ISSH. ISBN: 978-94-6261-129-0. ISSN: 0567-7572. **Acta Horticulturae.** 1441: 137-144. https://doi.org/10.17660/ActaHortic.2016.1141.15.

- **Collado, E.**, Klug, T.V, Martínez-Sánchez, A., Artés-Hernández, F., Aguayo, E., Artés, F., Fernández, J.A. and Gómez, P.A. (2017). Immature pea seeds. Effect of storage under modified atmosphere packaging and sanitation with acidified sodium chlorite. **Journal of the Science of Food and Agriculture**. 97: 4370-4378. http://dx.doi.org/10.1002/jsfa.8513.

- **Collado, E.,** Artés-Hernández, F., Navarro, L., Artés, F., Aguayo, E., Fernández, J.A. and Gómez, P.A. (2018). Overall quality of minimally processed faba bean seeds stored in MAP. **Acta Horticulturae**. 1194: 513-518. https://doi.org/10.17660/ActaHortic.2018.1194.75.

- **Collado, E.,** Venzke Klug, T., Artés-Hernández, F., Aguayo, E., Artés, F., Fernández, J.A., Gómez, P.A. (2019). Quality changes in nutritional traits of fresh-cut and then microwaved cowpea seeds and pods. **Food and Bioprocess Technology**. 12: 338-346. http://dx.doi.org/10.1007/s11947-018-2214-2.

- **Collado, E.**, Venzke Klug, T., Martínez-Hernández, G.B., Artés-Hernández, F., Martínez-Sánchez, A., Aguayo, E., Artés, F., Fernández, J.A., Gómez, P.A. (2019). UV-C pretreatment of fresh-cut faba beans (*Vicia faba*) for shelf life extension: Effects of domestic microwaving for consumption. **Food Science and Technology International**. In press. https://doi.org/10.1177/1082013219873227.

- **Collado, E.,** Venzke Klug, T., Martínez-Hernández, G.B Artés-Hernández, F., Martínez-Sánchez, A., Aguayo, E., Artés, F., Fernández, J.A., Gómez, P.A. (2019). Nutritional and quality changes of minimally processed faba (*Vicia faba* L.) beans during storage: Effects of domestic microwaving. **Postharvest Biology and Technology**. https://doi.org/10.1016/j.postharvbio.2019.01.008.

SCIENTIFIC PUBLICATIONS RESULTING FROM THE TRAINING PERIOD

- Klug, T.V., Martínez-Sánchez, A., Gómez, P.A., **Collado, E**., Aguayo, E., Artés, F. and Artés-Hernández, F. (2017). Improving quality of an innovative pea puree by high hydrostatic pressure. **Journal of the Science of Food and Agriculture**. 97: 4362-4369, doi: 10.1002/jsfa.8454.

- Klug, T.V., **Collado, E**., Martínez-Sánchez, A., Gómez, P.A., Aguayo, E., Otón, M., Artés, P. and Artés-Hernández, F. (2018). Innovative quality improvement by continuous microwave processing of a faba beans pesto sauce. **Food and Bioprocess Technology.** 11:561–571, doi: 10.1007/s11947-017-2024-y.

- Klug, T.V., Martínez-Hernández, G.B., **Collado, E**., Artés, F. and Artés-Hernández, F. (2018). Effect of microwave and high pressure processing on quality of an innovative broccoli hummus. **Food and Bioprocess Technology.** 11: 1464-1477, doi: 10.1007/s11947- 018-2111-8.

- Klug, T.V., **Collado, E**., Martínez-Hernández, G., Artés, F. and Artés-Hernández, F. (2018). Effect of stevia supplementation of kale juice spheres on their quality changes during refrigerated shelf life. **Journal of the Science of Food and Agriculture**. 99 (5): 2384-2392, doi: 10.1002/jsfa.9445.

Original scientific papers published in JCR-ISI not included journals

- Gómez, P.A., **Collado, E**., Venzke Klug, T., Artés-Hernández, F., Aguayo, E., Martínez-Sánchez, A., Artés, F., Fernández, J.A. (2017). Nuevos alimentos de leguminosas autóctonas para promover su consumo seguro y de calidad en un entorno sostenible. Accepted by the popular magazine: CTC. Revista de Agroalimentación e Industrias afines.

International Congress

- Artés-Hernández, F., **Collado, E**., Klug, T.V., Aguayo, E., Artés, F., Gómez, P.A. 2018. Evolución de la calidad de semillas y vainas de Caupí mínimamente procesadas en fresco y microondadas. **XII Simposio Nacional y X Ibérico de Maduración y Postcosecha**. Badajoz, 4-7 junio 2018.

- **Collado, E.,** Artés-Hernández, F., Navarro, L., Artés, F., Aguayo, E., Fernández, J.A., Gómez, P.A. 2016. Overall quality of minimally processed pea seeds. In: Proc. **III ISHS International Conference on Fresh-cut Produce**. University of California, Davis, California, USA. Eds.: M. Cantwell. Edit: ISSH. ISBN: 978-94-6261-129-0. ISSN: 0567-7572. Acta Hort., 1441. Pp. 137-144. doi 10.17660/ActaHortic.2016.1141.15.

- **Collado, E**., Artés-Hernández, F., Aguayo, E., Navarro, L., Artés, F., Fernández, J.A. and Gómez, P.A. 2016. Overall quality of minimally processed faba bean seeds stored in MAP. **International Postharvest Symposium. Cartagena**, Spain, 21-24/6/2016.

- **Collado E.,** Armero, E., Gómez, P.A. Development of innovative food with high nutritional value from native legumes. In: Proceedings of the **4th Workshop on Agri-Food research: WiA.15**. Cartagena: Universidad Politécnica de Cartagena, CRAI Biblioteca, 2016. ISBN: 978-84-608-5399-2. Pp 236-238. Open access: http://repositorio.upct.es/handle/10317/5290

- **Collado, E.,** Armero, E., Artés-Hernández, F., Aguayo, E., Artés, F., Fernández, J.A., Gómez, P.A. 2017. Vitamin C and microbial quality of minimally fresh processed pea seeds stored in modified atmosphere packaging. In: Proceedings of the **5th Workshop on Agri-Food Research WiA-16**. Eds: F. Artés-Hernández, J.A. Fernández-Hernández, J. Calatrava, E. Aguayo, J.J. Alarcón, J.E. Cos. Editorial: CRAI Biblioteca, Universidad Politécnica de Cartagena, Murcia, España. ISBN: 978-84-16325-29-0. 223 pp. Pp. 48-51. Open access: http://hdl.handle.net/10317/5558.

- **Collado E.,** Fernández J.A., Vivancos N., Moya F. and Armero E. 2016. Pea straw: an advantageous co-product in dairy goat diets **Second International**

Legume Society Conference. 2016: Legumes for a sustainable world. Troia, Portugal, 12-14/10/2016

- **Collado, E.,** Artés Hernández, F., Artés, F., Aguayo, E., Fernández, J.A. and Gómez, P.A. 2016. Sustainable sanitation methods for keeping overall quality of minimally processed faba seeds. **Second International Legume Society Conference** 2016: Legumes for a sustainable world. Troia, Portugal, 12-14/10/2016

- **Collado, E**., Artés-Hernández, F., Aguayo, E., Artés, F. and Gómez, P.A. 2016. Quality changes of minimally processed fresh and microwave cooking of faba bean seeds. **IX Simpósio Ibérico Maturaçao e pós-colheita**, Libro de Resumos. ISBN: 978-972-8936-24-2. p. 82. Lisboa, Portugal, 2-4/11/2016.

- **Collado, E**., Klug, T. V., Artés-Hernández, F., Aguayo, E., Artés, F., Fernández, J.A. and Gómez, P.A. 2017. Avaliação da qualidade de grãos de feijão-fava minimamente processados e microondados durante armazenamento. **1° Congresso Luso-Brasileiro de Horticultura.** Lisboa, Portugal, 1-4/11/2017.

- **Collado, E**., Klug, T.V., Artés-Hernández, F., Aguayo, E., Artés, F., Fernández, J.A. and Gómez, P.A. 2017. Quality changes of fresh and then microwaved minimally processed faba seeds. **International Conference Legato-Eurolegume**. Novi Sad, Serbia, 27-28/9/2017.

- **Collado, E.,** Klug, T.V., Artés-Hernández, F., Aguayo, E., Artés, F., Fernández, J. and Gómez, P.A. 2017. Cambios en la calidad de guisantes mínimamente procesados en fresco y su respuesta al cocinado con microondas. **IX Congreso Iberoamericano de Tecnología Postcosecha y Agroexportaciones**. Santiago, Chile, 29/11-01/12/2017.

- **Collado, E.,** Venzke Klug, T., Artés-Hernández, F., Aguayo, E., Martínez-Sánchez, A., Artés, F., Gómez, P. 2018. Cambios en la calidade de semillas de haba mínimamente procesadas y microondadas. In: Proceedings of the **6th Workshop on Agri-Food Research WiA-17**. Editores: F. Artés-Hernández, J.E. Cos, J.A. Fernández-Hernández, J. Calatrava, E. Aguayo, J.J. Alarcón, M. Egea. Editorial: CRAI Biblioteca, Universidad Politécnica de Cartagena,

Murcia, España. ISBN: 978-84-16325-64-1. 208 pp Pp. 19-22. Open access: http://hdl.handle.net/10317/6706

- **Collado, E.,** Venzke Klug, T., Artés-Hernández, F., Aguayo, E., Martínez-Sánchez, A., Artés, F., Gómez, P. 2019. Cambios en la calidad de semillas de haba mínimamente procesadas tratadas con diferentes desinfectantes. In: Proceedings of the **7th Workshop on Agri-Food Research WiA-18**. Editors: Artés-Hernández, F; Fernández-Hernández, J.A.; Cos, J.E., Alarcón, J.J.; Egea-Cortines. M.; Aguayo, E. Editorial: Editorial: CRAI Biblioteca, Universidad Politécnica de Cartagena, Cartagena, Murcia, Spain. ISBN: 978-84-16325-89-4. 34-37. 167 pp. Open Access. http://hdl.handle.net/10317/7652

- Klug, T.V, **Collado, E**., Martínez-Sánchez, A., Artés-Hernández, F., Gómez, P.A., Aguayo, E., Artés, F. 2016. Sensory and microbial quality of a pea puree processed under conventional or microwave treatment. **VI Congreso Internacional de Ingeniería Química, Biotecnológica y Alimentaria** (CIIQBA, 2016), La Habana, Cuba. 21-25/11/2016.

- Klug, T.V., **Collado, E.,** Martínez-Sánchez, E., Artés-Hernández, F., Gómez, P.A., Aguayo, E. and Artés, F. 2017. Avaliação da qualidade de feijão caupi minimamente processado e microondado. **1° Congresso Luso-Brasileiro de Horticultura**. Lisboa, Portugal, 1-4/11/2017.

LIST OF REFERENCES

Acuña, M.L. (2011). Evaluación agronómica de la fecha de siembra en habas determinadas (*Vicia faba* L. var. Major) en Valdivia, Región de Los Ríos. Tesis Doctoral. Escuela de Agronomía. Universidad Austral de Chile. 259 pp.

Adamidou, S., Nengas, I., Grigorakis, K., Nikolopoulou, D. and Jauncey, K. (2011). Chemical composition and antinutritional factors of field peas (*Pisum sativum*), chickpeas (*Cicer arietinum*), and faba beans (*Vicia faba*) as affected by extrusion preconditioning and drying temperatures. Cereal Chemistry. 88(1): 80–86.

Adelakun, O. and Duodu, G. (2017). Identification and Quantification of Phenolic Compounds and Bioactive Properties of Sorghum-cowpea-based Food Subjected to an In vitro Digestion Model. European Journal of Nutrition & Food Safety. 7: 57-66.

Adjei-Fremah, S., Jackai, L.E.N. and Worku, M. (2015). Analysis of phenolic content and antioxidant properties of selected cowpea varieties tested in bovine peripheral blood. Journal of Animal and Veterinary Science. 10(4): 235.245.

Aguayo, E., Allende, A. and Artés, F. (2003). Keeping quality and safety of minimally fresh processed melon. European Food Research and Technology. 216: 494–499.

Agüero, M., Pereda, J., Roura, S., Moreira, M. and del Valle, C. (2005). Sensory and biochemical changes in Swiss chard (*Beta vulgaris*) during blanching. Lwt - Food Science and Technology. 38: 772-778.

Ahn, H.J., Kim, J.H., Yook, H.S. and Byun, M.W. (2003). Irradiation effects on free radical scavenging and antioxidant activity of phytic acid. Journal of Food Science. 68(7): 2221–2224.

Alajaji, S.A. and El-Adawy, T.A. (2006). Nutritional composition of chickpea (*Cicer arietinum* L.) as affected by microwave cooking and other traditional cooking methods. Journal of Food Composition and Analisys. 19(8): 806-812.

Ali, M., Al-Saady, N.A., Waly, M.I., Bhatt, N., Al-Subhi, A.M. and Khan, A.J. (2014). Evaluation of indigeous Omani legumes for their nutritional quality, phytochemical composition and antioxidant properties. International Journal of Postharvest Technology and Innovation. 3: 333-346.

Al-Kaisey, M.T., Alwan, A.K.H., Mohammad, M.H. and Saeed, A.H. (2003). Effect of gamma irradiation on antinutritional factors in broad bean. Radiation Physics and Chemistry. 67(3–4): 493–496.

Allende, A. and Artés, F. (2003). Combined ultraviolet-C and modified atmosphere packaging treatments for reducing microbial growth of fresh processed lettuce. LWT– Food Science Technology. 36: 779–786.

Allende, A. and Artés, F. (2005). Processing lines and alternative techniques to prolong the shelf–life of minimally fresh processed leafy vegetables. European Journal of Horticultural Science. 70: 231–245.

Allende, A., McEvoy, J., Tao, Y. and Luo, Y. (2009). Antimicrobial effect of acidified sodium chlorite, sodium chlorite, sodium hypochlorite, and citric acid on *Escherichia coli* O157:H7 and natural microflora of fresh-cutcilantro. Food Control. 20: 230–234.

Alonso, R., Aguirre, A. and Marzo, F. (2000). Effects of extrusión and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. Food Chemistry. 68(2): 159–165.

Ansah, F.A., Amodio, M.L. and Colelli, G. (2018). Quality of fresh-cut products as affected by harvest and postharvest operations. Journal of the Science of Food and Agriculture. 98(10): 3614–3626.

Anurag, R.K., Manjunatha, M., Narayan, J.S. and Kumari, L. (2016). Storage quality of shelled green peas under modified atmosphere packaging at different storage conditions. Journal of Food Science and Technology. 53: 1640.

Aranda, P., Dostalova, J., Frias, J., Lopez-Jurado, M., Kozlowska, H., Pokorny, J., Urbano, G., Vidal-Valverde, C. and Zdyunczyk, Z. (2000). Nutrition. In CL Hedley, ed, Carbohydrates in Grain Legume Seeds: Improving Nutritional Quality and Agronomic Characteristics, CAB International, Wallingford, UK, pp 61–87.

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

Artés, F., Gómez, P.A. and Artés-Hernández F. (2007). Physical, physiological and microbial deterioration of minimally fresh processed fruits and vegetables. Food Science and Technology International. 13(3): 177–188.

Artés, F. and Artés-Hernández, F. (2012). Innovaciones en técnicas de procesado para facilitar el consumo de hortalizas y frutas. CTC. Revista de Agroalimentación e Industrias afines. 51: 9-15.

Artés–Hernández, F., Aguayo, E., Gómez, P.A. and Artés, F. (2009a). Innovaciones tecnológicas para preservar la calidad de los productos vegetales mínimamente procesados o de la "cuarta gama". Horticultura Internacional. 69: 52–57.

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

Artés-Hernández, F., Robles, P.A., Gómez, P.A., Tomás-Callejas, A. and Artés, F. (2010). Low UV-C illumination for keeping overall quality of fresh-cut watermelon. Postharvest Biology & Technology. 55: 114–120.

Artés-Hernández, F., Gómez, P. and Artés, F. (2013). Unit processing operations used in minimally processed horticultural products: quality and safety preservation. In: Food Quality and Safety. Ed: G.P.P. Lima; F. Vianello. Edit: Springer Verlag. ISBN: 978-3-7091-1639-5. Pags: 35-52.

Artés-Hernández, F., Gómez, P., Aguayo, E., Tomás-Callejas A. and Artés F. (2014). Sustainable Processing of Fresh-Cut Fruit and Vegetables. In: Sustainable Food Processing. Ed: B.K. Tiwari, T. Norton, N.M. Holden. Edit: John Wiley & Sons, Ltd. ISBN: 978-0-4706-7223-5. Chapter 10: 219-268.

Artés–Hernández, F., Formica-Oliveira, A.C., Artés, F., and Martínez–Hernández, G.B. (2017). Improved quality of a vitamin B12-fortified 'ready to blend' fresh-cut mix salad with chitosan. Food Science and Technology International. 23: 513–528.

Artés-Hernández, F., Martínez-Hernández, G.B., Aguayo, E., Gómez, P. and Artés, F. (2017). Fresh-cut fruit and vegetables: emerging eco-friendly techniques for preserving quality and safety. In: Postharvest Handling, Editorial: InTechOpen. Open access.

ASTM (1986). Physical requirements guidelines for sensory evaluation laboratories (Vol. 913, ASTM Special Technical Pub. 913). Philadelphia, USA: American Society for Testing Materials.

Baginsky, C., Peña–Neira, A., Cáceres, A., Hernández, T., Estrella, I., Morales, H. and Pertuzé, R. (2013). Phenolic compound composition in immature seeds of faba bean (*Vicia faba* L.) varieties cultivated in Chile. Journal of Food composition and Analysis. 31: 1–6.

Bahçeci, K.S., Serpen, A., Gökmen, V. and Acar, J. (2005). Study of lipoxygenase and peroxidase as indicator enzymes in green beans: change of enzyme activity, ascorbic

acid and chlorophylls during frozen storage. Journal of Food Engineering. 66: 187–192.

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

Baldwin, E.A., Burns, J.K., Kazokas, W., Brecht, J.K., Hagenmaier, R.D., Bender, R.J. and Pesis, E. (1999). Effect of two edible coatings with different permeability characteristics on mango *Mangifera indica* L. ripening during storage. Postharvest Biology and Tecnhology. 17: 215–226.

Baljeet, S.Y., Roshanlal, Y. and Ritika, B.Y. (2016). Effect of cooking methods and extraction solvents on the antioxidant activity of summer squash (*Cucurbita pepo*) vegetable extracts. International Food Research Journal. 23(4): 1531–1540.

Barakat, H., Reim, V. and Rohn, S. (2015). Stability of saponins from chickpea, soy and faba beans in vegetarian, broccoli-based bars subjected to different cooking techniques. Food Research International. 76(1): 142-149.

Bari, M.L., Ukuku, D.O., Kawasaki, T., Inatsu, Y., Isshiki,K. and Kawamoto, S. (2005). Combined efficacy of nisin and pediocin with sodium lactate, citric acid, phytic acid, and potassium sorbate and EDTA in reducing the *Listeria monocytogenes* population of inoculated fresh-cut produce. Journal of Food Protection. 68: 1381-1387.

Barker, P.A. and Morris, D.J.M. (1968). Leucoanthocyanins in the Broad Bean barker. 170. 49. 79-86.

Barrett, D.M., Beaulieu, J.C., and Shewfelt, R. (2010). Color, flavor, texture, and nutritional quality of fresh–cut fruit and vegetables: desirable levels, instrumental and sensory measurement, and the effects of processing. Critical Reviews in Food Science and Nutrition. 50:369–389.

Barry T.N. and McNabb, W.C. (1999). The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. British Journal of Nutrition. 81: 263-272.

Beales, N. (2004) Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH and osmotic stress: A review. Comprehensive Reviews in Food Science and Food Safety. 3:1–20.

Beltrán, D., Selma, M.V., Tudela, J.A. and Gil, M.I. (2005). Effect of different sanitizers on microbial and sensory quality of fresh cut potato strips stored under

modified atmosphere or vacuum packaging. Postharvest Biology and Technology. 37: 37-46.

Benlloch-Tinoco, M., Igual-Ramo, M., Rodrigo-Aliaga, MD., Martínez-Navarrete, N. (2013). Comparison of microwaves and conventional thermal treatment on enzymes activity and antioxidant capacity of kiwifruit puree. Innovative Food Science and Emerging Technologies. 19: 166-172.

Benzie, I.F.F. and Strain, J.J. (1999). Ferric reducing antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymology. 299: 15-27.

Berhow, M.A., Cantrell, C.L., Duval, S.M., Dobbins, T.A., Maynes, J. and Vaughn, S.F. (2002). Analysis and quantitative determination of group B saponins in processed soybean products. Phytochemical Analysis. 13: 343–348.

Beuchat, L.R., Nail, B.V., Adler, B.B. and Clavero, M.R.S. (1998). Efficacy of spray application of chlorine in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. Journal of Food Protection. 61: 1305-1311.

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

Bognår, A., Bohling, H., Fort, H. (1990). Nutrient retention in chilled foods, in: Gormley, T.R. (Ed.), Chilled foods. The state of the art. Elsevier Applied Science, London, pp. 305–336.

Bouchenak, M. and Myriem, L.S. (2013). Nutritional quality of legumes and their role in cardiometabolic risk prevention: A Review. Journal of medicinal food. 16(3): 185-198.

Boukhenouf, S., Louaileche, H. and Perrin, D. (2016). Phytochemical content and in vitro antioxidant activity of faba bean (*Vicia faba* L.) as affected by maturity stage and cooking practice. International Food Research Journal. 23(3): 954-961.

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

Caleb, O.J., Mahajan P.V., Al-Said F.A.J. and Opara U.L. (2013). Modified Atmosphere Packaging Technology of Fresh and Fresh-cut Produce and the Microbial Consequences—A Review. Food Bioprocess Technology. 6: 303–329.

Campos-Vega, R. and Loarca-Piña, G. (2010). Minor components of pulses and their potential impact on human health. Food Research International, 43(2): 461–482.

Cantwell, M., and Saltveit, M. (2015). Tolerance of sugar snap peas to modified atmospheres with high concentrations of carbon dioxide. Acta Horticulturae. 1071:619-625.

Cartea, M.E., Francisco, M., Soengas, P. and Velasco, P. (2011). Phenolic Compounds i n Brassica. Vegetables. Molecules. 16(1): 251.

Carvalho, M., Lino-Neto, T., Rosa, E. and Carnide, V. (2017). Cowpea: a legume crop for a challenging environment. Journal of the Science of Food and Agriculture. 97(13): 4273–4284.

Castillejo, N., Martínez–Hernández, G.B., Gómez, P.A., Artés, F. and Artés–Hernández, F. (2015). Red fresh vegetables smoothies with extended shelf life as an innovative source of health–promoting compounds. Journal of Food Science and Technology.

Castillejo, N., Martínez–Hernández, G.B., Gómez, P.A., Artés, F. and Artés–Hernández, F. (2016). Red fresh vegetables smoothies with extended shelf–life as an innovative source of health–promoting compounds. Journal of Food and Science Technology. 53: 1475–1486.

Castillejo, N., Martínez-Hernández, G.B., Mónaco, K., Gómez, P.A., Aguayo, E., and Artés, F. (2017). Preservation of bioactive compounds of a green vegetable smoothie using short time-high temperature mild thermal treatment. Food Science and Technology International. 23(1): 46-60.

Castillejo, N., Martínez-Hernández, G.B., Lozano-Guerrero, A.J., Pedreño-Molina, J.L., Gómez, P.A., Aguayo, E., Artés, F. and Artés–Hernández, F. (2018). Microwave heating modelling of a green smoothie: Effects on glucoraphanin, sulforaphane and S-methyl cysteine sulfoxide changes during storage. Journal of the Science of Food and Agriculture. 98(5): 1863–1872.

Chaieb, N., González, L.J., Lopez-Mesas, M., Bouslama, M. and Valiente, M. (2011). Polyphenols content and antioxidant capacity of thirteen faba bean (*Vicia faba* L.) genotypes cultivated in Tunisia. Food Research International. 44: 970-977.

Cheok, C.Y., Salman, H.A.K. and Sulaiman, R. (2014). Extraction and quantification of saponins: A review. Food Research International. 59: 16-40.

Chitravathi, K., Chauhan, O.P. and Raju, P.S. (2015). Influence of modified atmosphere packaging on shelf-life of green chillies (*Capsicum annuum* L.). Food Packaging and Shelf Life. 4: 1-9.

Chumyam, A., Whangchai, K., Jungklang, J., Faiyue, B. and Saengnil, K. (2013). Effects of heat treatments on antioxidant capacity and total phenolic content of four cultivars of purple skin eggplants. Science Asia. 39: 246–251.

Chung, K.T., Wong, T.Y., Wei, C.I., Huang, Y.W. and Lin, Y. (1998). Tannins and human health: A Review. Critical Reviews in Food Science and Nutrition. 38: 421–464.

Cisneros-Zevallos, L. (2003). The use of controlled postharvest abiotic stresses as a tool for enhancing the nutraceutical content and adding-value of fresh fruits and vegetables. Journal of Food Science. 68: 1560–1565.

Collado, E., Venzke Klug, T., Martínez-Sánchez, A., Artés-Hernández, F., Aguayo, E., Artés, F., Fernández, J.A. and Gómez, P.A. (2017). Immature pea seeds. Effect of storage under modified atmosphere packaging and sanitation with acidified sodium chlorite. Journal of Science of Food Agriculture. 97(13): 4370-4378.

Contreras, C., Benlloch–Tinoco, M., Rodrigo, D. and Martínez–Navarrete, N. (2017). Impact of microwave processing on traditional, sensory, and other quality attributes. The Microwave Processing of Foods 2nd Edition. Editors: Marc Regier Kai Knoerzer Helmar Schubert. pp 65-99.

Costa, L., Vicente, A.R., Civello, P.M., Chaves, A.R., and Martínez, G.A. (2006). UV–C treatment delays postharvest senescence in broccoli florets. Postharvest Biology and Technology. 39: 204–210.

Couée, C., Sulmon, G., Gouesbet, A. and Amrani, E. (2006). Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. Journal of Experimental Botany. 57: 449–459.

Couto, A., Kortner, T.M., Penn, M., Østby, G., Bakke, A.M., Krogdahl, Å. (2015). Saponins and phytosterols in diets for European sea bass (*Dicentrarchus labrax*) juveniles: Effects on growth, intestinal morphology and physiology. Aquaculture Nutrition. 21(2): 180-193.

Crandall, P.G. (2012). Comparison of multiple chemical sanitizers for reducing *Salmonella* and *Escherichia coli* O157:H7 on spinach (*Spinacia oleracea*) leaves. Food Research International. 45(2): 1123-1128.

Crépon. K., Marget, P., Peyronnet, C., Carrouée, B., Arese, P. and Duc, G. (2010). Nutritional value of faba bean (*Vicia faba* L.) seeds for feed and food. Field Crops Research. 115(3): 329–339.

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

Dahl, W.J., Foster, L.M. and Tyler, R.T. (2012). Review of the health benefits of peas (*Pisum Sativum* L.). British Journal of Nutrition. 108: 3–10.

Davey, M.W., Montagu, M.V., Inzé, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I.J.J., Strain, J.J., Favell, D. and Fletcher, J. (2000). Plant L–ascorbic acid: Chemistry, function, metabolism, bioavailability and effects of processing. Journal of the Science of Food and Agriculture. 80: 825–860.

Delchier, N., Reich, M. and Renard, C.M.G.C. (2012). Impact of cooking methods on folates, ascorbic acid and lutein in green beans (*Phaseoluos vulgaris*) and spinach (*Spinacea oloracea*). Food Science and Technology. 49: 197–201.

Delzenne N.M. and Roberfroid M.R. (1994). Physiological effects of non-digestible oligosaccharides. Lebensmittel-Wissenschaft und Technologie. 27: 1-6.

Demir, M.K., and Elgün, A. (2013). Stabilization of whole wheat flour branny fractions with special emphasis on internal and external characteristic of whole wheat. Food Science and Technology Research. 19(2): 195–200.

Demir, M.K. and Elgün, A. (2014). Comparison of autoclave, microwave, IR and UV-C stabilization of whole wheat flour branny fractions upon the nutritional properties of whole wheat bread. Journal of Food Science and Technology. 51(1): 59–66.

Deng, G., Lin, X., Xu, X., Gao, L., Xie, J. and Li, H. (2013). Antioxidant capacities and total phenolic contents of 56 vegetables. Journal of Functional Foods. 5: 260-266.

Deng, Y., Padilla-Zakour, O., Zhao, Y. and Tao, S. (2015). Influences of high hydrostatic pressure, microwave heating, and boiling on chemical compositions, antinutritional factors, fatty acids, in vitro protein digestibility, and microstructure of buckwheat. Food and Bioprocess Technology. 8(11): 2235–2245.

Devasagayam, T.P.A., Tilak, J., Boloor, K.K., Sane, K.S., Ghaskadbi, S.S. and Lele, R. D. (2004). Free radicals and antioxidants in human health: current status and future prospects. Journal of the Association of Physicians of India. 52: 794–804.

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

Dias, M.I., Sousaa, M.J., Alves, R.C. and Ferreira, I. (2016). Exploring plant tissue culture to improve the production of phenolic compounds: A review. Industrial Crops and Products. 82: 9-22.

Dolinsky, M., Agostinho, C., Ribeiro, D., De Souza Rocha, G., Girão Barroso, S., Ferreira, D., Polinati, R., Ciarelli, G. and Fialho, E. (2016). Effect of different cooking methods on the polyphenol concentration and antioxidant capacity of selected vegetables. Journal of Culinary Science & Technology. 14: 1-5.

Domínguez-Perles, R., Carnide, V., Marques, G., de Castro, I. and Rosa. E. (2015). Relevance, constraints and perspectives of cowpea crops in the Mediterranean Basin. Legume Perspectives. 10: 40-42.

Downie, B., Gurusinghe, S., Dahal, P., Thacker, R.R., Snyder, J.C. and Nonogaki, H. (2003). Expression of a galactinol synthase gene in tomato seeds is up-regulated before maturation desiccation and again after imbibitions whenever radicle protrusion is prevented. Plant Physiology. 131: 1347–1359.

Dragsted, L.O. (2003). Antioxidant actions of polyphenols in humans. International Journal for Vitamin and Nutrition Research. 73, 112–119.

Du, M., Cherian, G., Stitt, P.A. and Ahn, D.U. (2002). Effect of dietary sorghum cultivars on the storage stability of broiler breast and thigh meat. Poultry Science. 81: 1385–1391.

Duranti, M. and Gius, C. (1997). Legume seeds: protein content and nutritional value. Field Crops Research. 53: 31–45.

ED-European Parliament and Council Directive No 40/2008 (2008). On food additive other than colors and sweeteners. https://www.fsai.ie/uploadedFiles/S.I_No_40_of_2008.pdf. Accessed 25.09.19.

El Gaouth, A., Arul, J., Ponnampalam, and R., Boulet, M. (1991). Chitosan coating effect on storability and quality of fresh strawberries. Journal of Food Science. 12: 1618–1632.

ElSayed, A.I., Rafudeen, M.S. and Golldack, D. (2014). Physiological aspects of raffinose family oligosaccharides in plants: Protection against abiotic stress. Plant Biology. 16(1): 1–8.

Elwan, M.W.M., Nasef, I.N., El-Seifi, S.K., Hassan, A.M. and Ibrahim, R.E. (2015). Storability, shelf-life and quality assurance of sugar snap peas (cv. super sugar snap) using modified atmosphere packaging. Postharvest Biology and Technology. 100: 205-211.

Erkan, M., Wang, C.Y. and Krizek, D.T. (2001). UV–C radiation reduces microbial populations and deterioration in Cucurbita pepo fruit tissue. Environmental and Experimental Botany. 45: 1–9.

Escalona, V.H., Hinojosa, A., Cha,r C., Villena, P., Bustamante, A. and Saenz, C. (2015). Use of alternative sanitizers on minimally processed watercress harvested in two different seasons. Journal of Food Processing and Preservation. 39:1287–1298.

Evans, W.J. and Martin, C.J. (1988). Heat of complex formation of Al (III) and Cd (II) with phytic acid. IX. J. Inorganic Biochemical. 34: 11-18.

Fabbri, A.D.T. and Crosby, G.A. (2016). A review of the impact of preparation and cooking on the nutritional quality of vegetables and legumes. International Journal of Gastronomy and Food Science. 3: 2–11.

Falguera, V., Quintero, J.P., Jimenez, A., Aldemar Muñoz, J. and Ibarza, A. (2011). Edible films and coatings: Structures, active functions and trends in their use. Trends in Food Science & Technology. 22: 292-303.

FAOStat. (2017). Food and Agriculture Organization (FAO), World Production. Available at: http://faostat3.fao.org/browse/Q/QC/E (accessed 17 October 2018).

Flores, P., Hellín, P. and Fenoll, J. (2012). Determination of organic acids in fruit and vegetables by liquid chromatography with tandem–mass spectrometry. Food Chemistry. 132: 1049–1054.

Foley, D., Euper, M., Caporaso, F. and Prakash, A. (2004). Irradiation and chlorination effectively reduces *Escherichia coli* O157:H7 inoculated on cilantro (*Coriandrum sativum*) without negatively affecting quality. Journal of Food Protection. 67(10): 2092–2098.

Food and Drug Administration. Guide to Minimize Microbial Food Safety Hazards of Fresh-cut Fruits and Vegetables 2007. http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM169112.pdf.

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

Formica-Oliveira, A.C., Martínez-Hernández, G.B., Díaz-López, V., Artés, F. and Artés-Hernández, F. (2017). Use of postharvest UV-B and UV-C radiation treatments to revalorize broccoli byproducts and edible florets. Innovative Food Science & Emerging Technologies. 43: 77–83.

Francis, G., Kerem, Z., Makkar, H. P. and Becker, K. (2002). The biological action of saponins in animal systems: A review. British Journal of Nutrition. 88: 587-605.

Francis, G.A., Gallone, A., Nychas, G.J., Colelli, G., Amodio, M.L., Spano, G. and Sofos, J.N. (2012). Factors affecting quality and safety of fresh–cut produce. Critical Reviews in Food Science and Nutrition. 52: 595–610.

Frota, K.M.G., Mendonça, S., Saldiva, P., Cruz, R. and Areas, J. (2008). Cholesterol-Lowering Properties of Whole Cowpea Seed and Its Protein Isolate in Hamsters. Journal of Food Science. 73(9): 235-240.

Gardner, D.W. and Shama, G. (2000). Modeling UV-induced inactivation of microorganisms on surfaces. Journal of Food Protection. 63: 63–70.

Gärtner, A. and Wese, U. (1986). Molecular and functional aspects of superoxide dismutases. In: Vögtle, F., Weber, E. (Eds.). Biomimetic and Bioorganic Chemistry II. Vol. 132. Springer–Verlag, Berlin, Germany. 1–61.

Gawłowska, M., Święcicki, W.K., Lahuta, L.B. and Kaczmarek Z. (2016). Raffinose family oligosaccharides in seeds of *Pisum* wild taxa, type lines for seed genes, domesticated and advanced breeding materials. Genetic Resources and Crop Evolution. 64: 569-578.

Gdala, J. and Buraczewska, L. (1997). Chemical composition and carbohydrate content of several varieties of faba bean and pea seeds. Journal of Animal and Feed Sciences. 6: 123-135.

Gibson D.M. and Ullah A.B.J. (1990). Phytases and their action on phytic acid. Inositol Metabolism in Plants. Morre, Biss, Loewus (Ed.), Wiley-Liss, NY, EEUU, 77-92.

Gil, M.I., Castañer, M., Ferreres, F., Artés, F. and Tomas–Barberan, F.A. (1998). Modified–atmosphere packaging of minimally processed 'Lollo Rosso' (*Lactuca sativa*). European Food Research and Technology. 206(5): 350-354.

Gil, M.I., Selma, M.V., López-Gálvez, F. and Allende, A. (2009). Fresh-cut product sanitation and wash water disinfection: Problems and solutions. International Journal of Food Microbiology. 134: 37–45.

Gómez, P. and Artés, F. (2004). Ascorbic and citric acids to preserve quality of minimally processed green celery. In: Proceedings of IV Postharvest Iberian Symposium, Oeiras, Portugal, pp. 369–373.

Gómez, P.L., Alzamora, S.M., Castro, M.A. and Salvatori, D.M. (2010). Effect of ultraviolet–C light dose on quality of cut–apple: microorganism, color and compression behaviour. Journal of Food Engineering. 98: 60–70.

Gonçalves, A., Piebiep, G., Barros, A., Domínguez-Perles, R., Trindade, H., Rosa, E., Ferreira, L. and Rodrigues, M. (2016). Cowpea (*Vigna unguiculata* L. Walp) a renewed multipurpose crop for a more sustainable agri-food system. Nutritional advantages and constraints. Journal of Science and Food Agriculture. 96(9): 2941-2951.

Goyoaga, C. (2005). Estudio de factores no nutritivos en "*Vicia faba* l.": influencia de la germinación sobre su valor nutritivo. Tesis Doctoral. Facultad De Farmacia. Universidad Complutense De Madrid. 198 pp.

Goyoaga, C., Burbano, C., Cuadrado, C., Romero, C., Guillamón, E., Varela, A., Pedrosa, M.M. and Muzquiz, M. (2011). Content and distribution of protein, sugars and inositol phosphates during the germination and seedling growth of two cultivars of *Vicia faba*. Journal of Food Composition and Analysis. 24: 391–397.

Graça, A., Santo, D., Quintas, C.E. and Nunes, C. (2017). Growth of *Escherichia coli*, *Salmonella enterica* and *Listeria* spp., and their inactivation using ultraviolet energy and electrolyzed water, on Rocha fresh–cut pears. Food control. 77: 41–49.

Graf, E. and Eaton, J.W. (1990). Antioxidant functions of phytic acid. Free Radical Biology & Medicine. 8(1): 61–69.

Graf, E., Empson, K.L. and Eaton, J.W. (1987). Phytic acid. A natural food antioxidant. Journal of Biological Chemistry. 262: 11647-11650.

Grizard, D. and Barthomeuf, C. (1999). Non-digestible oligosaccharides used as prebiotic agents: mode of production and beneficial effects on animal and human health. Reproduction Nutrition Development. 39: 563-588.

Güçlü-Üstündağ, Ö. and Mazza, G. (2007). Saponins: Properties, Applications and Processing. Critical reviews in food science and nutrition. 47: 231-58.

Gui-Fang. D., Xi, L., Xiang-Rong, X., Li-Li, G., Jie-Feng, X. and Hua-Bin, L. (2013). Antioxidant capacities and total phenolic contents of 56 vegetables. Journal Functional Food. 5: 260-266.

Guilbert, S., Gontard, N. and Cuq, B. (1995). Technology and applications of edible protective films. Packaging Technology and Science. 8: 339–346.

Ha, T.J., Lee, B.W., Park, K.H., Jeong, S.H., Kima H.T., Ko J.M., Baek I.Y. and Lee J.H. (2014). Rapid characterisation and comparison of saponin profiles in the seeds of Korean Leguminous species using ultra performance liquid chromatography with photodiode array detector and electrospray ionisation/mass spectrometry (UPLC–PDA–ESI/MS) analysis. Food Chemistry. 146: 270–277.

Han, H. and Baik, B.K. (2008). Antioxidant activity and phenolic content of lentils (*Lens culinaris*), chickpeas (*Cicer arietinum* L.), peas (*Pisum sativum* L.) and soybeans (*Glycine max*), and their quantitative changes during processing. International Journal of Food Science & Technology. 43: 1971 - 1978.

Harris, L.J., Farber, J.N., Beuchat, L.R., Parish, M.E., Suslow, T.V., Garret, E.H. and Busta, F.F. (2003). Outbreaks associated with fresh produce: Incidence, growth, and survival of pathogens in fresh and fresh–cut produce. Comprehensive Reviews in Food Science and Food Safety. 2: 78–141.

Hassanpour, S., Maheri-Sis, N., Eshratkhah, B. and Baghbani Mehmandar, F. (2011). Plants and secondary metabolites (Tannins): A Review. International Journal of Forest, Soil and Erosion. 1: 47-53.

Hefnawy, H. (2011). Effect of processing methods on nutritional composition and anti-nutritional factors in lentils (*Lens culinaris*). Annals Agriculture Science. 56(2): 57-61.

Heng, L., Vincken, J.P., Hoppe, K., Koningsveld, G.A., Decroos, K., Gruppen, H., Boekel, M.A.J.S. and Voragen, A.G.J. (2006). Stability of pea DDMP saponin and the mechanism of its decomposition. Food Chemistry. 99: 326–334.

Heredia, J.B. and Cisneros-Zevallos, L. (2009). The effects of exogenous ethylene and methyl jasmonate on the accumulation of phenolic antioxidants in selected whole and wounded fresh produce. Food Chemistry. 115 (4): 1500-1508.

Hithamani, G., and Srinivasan, K. (2014). Bioaccessibility of polyphenols from wheat (*Triticum aestivum*), sorghum (*Sorghum bicolor*), green gram (*Vigna radiata*), and chickpea (*Cicer arietinum*) as influenced by domestic food processing. Journal of Agriculture and Food Chemistry. 62(46): 11170–9.

Hoeck, J.A., Fehr, W.R., Murphy, P.A. and Welke, G.A. (2000). Influence of genotype and environment on isoflavone contents of soybean. Crop Science. 40: 48-51.

Hrudey, S.E. (2009). Chlorination disinfection by–products, public health risk tradeoffs and me. Water Research. 43: 2057–2092.

Hughes, P.A. and Sandsted, R.F. (1975). Effect of temperature, relative humidity and light on the color of 'California Light Red Kidney' bean seeds during storage. Hortscience. 10: 421–423.

Hurst, W.C. (2002). Safety aspects of fresh-cut fruits and vegetables. Fresh-Cut Fruits and Vegetables. Science, Technology and Market. 45–90.

Inatsu, Y., Maeda, Y., Bari, M.L., Kawasaki, S.W. and Kawamoto, S. (2005). Prewashing with acidified sodium chlorite reduces pathogenic bacteria in lightly fermented Chinese cabbage. Journal of Food Protect. 68: 999–1004.

ISO (2012). Sensory analysis -- General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors. In I. O. f. S. (ISO) (Ed.), 8586:2012. Geneva.

Jain, A., Singh, A., Singh, S., Sarma, B.K. and Singh, H.B. (2015). Biocontrol agents-mediated suppression of oxalic acid induced cell death during *Sclerotinia sclerotiorum*-pea interaction. Journl of Basic Microbiology. 55(5): 601-606.

Kader, A.A. (2002). Modified atmospheres during transport and storage. Kader. A. A. (Ed.). Postharvest technology of horticultural crops. Publication 3311. 3rd edition. Edit. University of California, Agriculture and Natural Resources, Davis USA. 135–145.

Kadlec, P., Kaasová, J., Bubník, Z. and Pour, V. (2000b). Effect of germination and microwave treatment on chemical composition of pea. Microwave Chemistry. 241–243.

Kadlec, P., Rubecová, A., Réblová, Z., Starhová, H. and Bubník, Z. (2000a). Influence of germination on the carbohydrate composition of pea. Czech Journal of Food Sciences. 18: 9-13.

Kala, B. and Mohan, V. (2012). Effect of microwave treatment on the antinutritional factors of two accessions of velvet bean, *Mucuna pruriens* (L.) DC. var. utilis (Wall. ex Wight) Bak. ex Burck. International Food Research Journal 19(3): 961–969.

Kalpanadevi, V. and Mohan, V.R. (2013). Effect of processing on antinutrients and in vitro protein digestibility of the underutilized legume, *Vigna unguiculata* (L.) Walp subsp. Unguiculata. LWT – Food Sci Technol. 51(2): 455-461.

Kannan, U., Sharma, R., Gangola, M.P. and Chibbar, R. N. (2018). Improving Grain Quality in Pulses: Strategies to Reduce Raffinose Family Oligosaccharides in Seeds. Ekin Journal of Crop Breeding and Genetics. 4: 70-88.

Karapanos, I., Papandreou, A., Skouloudi, M., Makrogianni, D., Fernández, J.A., Rosa, E., Ntatsi, G., Bebeli, P.J. and Savvas, D. (2017). Cowpea fresh pods - a new legume for the market: assessment of their quality and dietary characteristics of 37 cowpea accessions grown in southern Europe. Journal of Science and Food Agriculture. 97(13): 4343-4352.

Kemp, G., Aldrich, M.L. and Waldroup, A.L. (2000). Acidified Sodium Chlorite Antimicrobial Treatment of Broiler Carcasses. Journal of food protection. 63: 1087-92.

Kerch, G. (2015). Chitosan films and coatings prevent losses of fresh fruit nutritional quality: A review. Trends in Food Science & Technology. 46: 159-166.

Kester, J.J. and Fennema, O.R. (1986). Edible films and coatings: A review. Food Technology. 40: 47–59.

Khademi, O., Zamani, Z., Poor Ahmadi, E. and Kalantari, S. (2012). Effect of UV-C radiation on postharvest physiology of persimmon fruit (*Diospyros kaki Thunb*.) cv. 'Karaj' during storage at cold temperature. International Food Research Journal. 20(1): 247–253.

Khalil, A.H. and Mansour, E.H. (1995). The effect of cooking, autoclaving and germination on the nutritional quality of faba beans. Food Chemistry. 54(2): 177–182.

Khalil, A.H. and El-Adawy, T.A. (1994). Isolation, identification and toxicity of saponin from different legumes. Food Chemistry. 50(2): 197-201.

Khan, M., Cakmak, H., Tavman, S., Schutyser, M. and Schroën, K. (2014). Anti-browning and barrier properties of edible coatings prepared with electrospraying, Innov. Innovative Food Science and Emerging Technologies. 25: 9-13.

Khattab, R.Y. and Arntfield, S.D. (2009). Nutritional quality of legume seeds as affected by some physical treatments 2. Antinutritional factors. LWT – Food Science Technology. 42(6): 1113-1118.

Kim, M.S., Cho, S.M., Kang, E.Y., Im, Y.J., Hwangbo, H. and Kim, Y.C. (2008). Galactinol is a signaling component of the induced systemic resistance caused by Pseudomonas chlororaphis O6 root colonization. Molecular Plant-Microbe Interactions Journal. 21: 1643–1653.

Kinyuru, J.N., Kahenya, K.P., Muchui, M. and Mungai, H. (2011). Influence of Post-harvest Handling on the Quality of Snap Bean (*Phaseolus vulgaris* L.). Journal of Agriculture and Food Technology. 1(5): 43-46.

Klaiber, R.G., Baur, S., Wolf, G., Hammes, W. P. and Carle, R. (2005). Quality of minimally processed carrots as affected by warm water washing and chlorination. Innovative Food Science and Emerging Technologies. 6: 351-362.

Klug, T., Martínez-Hernández, G., Collado, E., Artés, F. and Artés-Hernández, F. (2018a). Effect of Microwave and High-Pressure Processing on Quality of an Innovative Broccoli Hummus. Food and Bioprocess Technology. 1-14.

Klug, T.V., Collado, E., Martínez-Sánchez, A., Gómez, P.A., Aguayo, E., Otón, M., Artés, P. and Artés-Hernández, F. (2018b). Innovative quality improvement by continuous microwave processing of a faba beans pesto sauce. Food Bioprocess Technology. 11(3): 561-571.

Knuckles, B.E., Kuzmicky, D.D. and Betschart, A.A. (1982). HPLC analysis of phytic acid in selected foods and biological samples. Journal of Food Science. 49: 1267-1268.

Kwon, S.J., Kim, D.G., Kim, J.M., Kang, K.Y., Lee, M.K., Hong, M.J., Kim, J.B., Eom, S.H., Kang, S.Y., Ha, B.K. and Ryu J. (2018). Phytochemical Compounds and Antioxidant Activity in the Grain of Selected Faba Bean (*Vicia faba*) Genotypes. Plant Breeding and Biotechnology. 6(1): 65-73.

Lado, B. and Yousef, A. (2002). Alternative food-preservation technologies: efficacy and mechanisms. Microbes Infection. 4: 433–440.

Lásztity, R., Hidvégi, M. and Bata, Á. (1998). Saponins in food. Food Reviews International. 14(4): 371–390.

Le Tien, C., Vachon, C., Mateescu, M. A. and Lacroix, M. (2001). Milk protein coatings prevent oxidative browning of apples and potatoes. Journal of Food Science. 66: 512–516.

Lee, S.K. and Kader. A.A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biology and Technology. 20 (3): 207-220.

Leguminosae, V. (2012). Faba Bean. Genetics, Genomics and Breeding of Cool Season Grain Legumes, 27(Latokartanonkaari 5), 50–97.

Leistner, L, and Gould, G. (2002). Hurdle technologies: combination treatments for food stability, safety and quality. Kluwer Academic/Plenum Publishers, New York, USA. 24–25.

López–Gálvez, F., Allende, A., Truchado, P., Martínez-Sánchez, A., Tudela, J.A., Selma, M.V. and Gil, M.I. (2010). Suitability of aqueous chlorine dioxide versus sodium hypochlorite as an effective sanitizer for preserving quality of fresh–cut lettuce while avoiding by–product formation. Postharvest Biology and Technology. 55(1): 53–60.

López–Gálvez, F., Allende, A., Truchado, P., Martínez–Sánchez, A., Tudela, J.A., Selma, M.V. and Gil, M.I. (2010). Suitability of aqueous chlorine dioxide versus sodium hypochlorite as an effective sanitizer for preserving quality of fresh–cut lettuce while avoiding by–product formation. Postharvest Biology and Technology. 55: 53–60.

Luo, Y.W. and Xie, W.H. (2013). Effect of different processing methods on certain antinutritional factors and protein digestibility in green and white faba bean (*Vicia faba* L.). CyTA – Journal of Food. 11(1): 43–49.

Maetens, E., Hettiarachchy, N., Dewettinck, K., Horax, R. and Moens, K. (2018). Reductions of anti-nutritional factors of germinated soybeans by ultraviolet and infrared treatments for snack chips preparation. LWT – Food Science and Technology. 90: 513–518.

MAGRAMA. (2019). https://www.mapa.gob.es/es/agricultura/temas/producciones-agricolas/anexo_proteaginosas_oct2019_tcm30-521009.pdf

Manzocco, L., Quarta, B. and Dri, A. (2009). Polyphenoloxidase inactivation by light exposure in model systems and apple derivatives. Innovative Food Science & Emerging Technologies. 10(4): 506–511.

Manzocco, L., Da Pieve, S. and Maifreni, M. (2011). Impact of UV-C light on safety and quality of fresh-cut melon. Innovative Food Science and Emerging Technologies. 12: 13–17.

Martínez-Damián, M.T., Cruz-Álvarez, O., Beryl Colinas-León M.T., Rodríguez-Pérez, J.E. and Ramírez-Ramírez, S.E. (2013). Actividad enzimática y capacidad antioxidante en menta (*Mentha piperita* L.) almacenada bajo refrigeración. Agronomía mesoamericana. 24:57-69.

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

Martínez-Hernández, G.B., Artés-Hernández, F., Colares-Souza, F., Gómez, P.A., García-Gómez, P. and Artés, F. (2013a). Innovative Cooking Techniques for Improving the Overall Quality of a Kailan-Hybrid Broccoli. Food and Bioprocess Technology. 6(8): 2135–2149.

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

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

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

Martínez-Hernández, G., Amodio, M., and Colelli, G. (2015). Potential use of microwave treatment on fresh-cut carrots: Physical, chemical and microbiological aspects. Journal of the science of food and agriculture. 96: 2063-2072.

Martínez-Sánchez, A., Allende, A., Bennett, R.N., Ferreres, F. and Gil, M.I. (2006). Microbial, nutritional and sensory quality of rocket leaves as affected by different sanitizers. Postharvest Biology and Technology. 42: 86–97.

Martínez-Sánchez, A., Allende, A., Cortés-Galera, Y. and Gil, M.I. (2008). Respiration rate response of four baby leaf Brassica species to cutting at harvest and fresh-cut washing. Postharvest Biology and Technology. 47: 382-388.

Martínez-Valverde, I., Periago, M.J., Gordon, Provan, G. and Chesson, A. (2000). Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). Journal of the Science and Food Agriculture. 82: 323-330.

Martos-Fuentes, M., Fernandez, J., Ochoa, J., Carvalho, M., Carnide, V., Rosa, E., Pereira, G., Barcelos, C. J., Bebeli, P. and Egea-Gilabert, C. (2017). Genotype by environment interactions in cowpea (*Vigna unguiculata* L. Walp.) grown in the Iberian Peninsula. Crop Pasture Science. 68: 924-931.

Masrizal, M.A., Giraud, D.W. and Driskell, J.A. (1997). Retention of vitamin C, iron, and carotene in vegetables prepared using different cooking methods. Journal of Food Quality. 20: 403-418.

McHugh, T.H. and Senesi, E. (2000). Apple wraps: A novel method to improve the quality and extend the shelf life of fresh-cut apples. Journal of Food Science. 65: 480–485.

McKie, V.A. and McCleary, B.V. (2016). A novel and rapid colorimetric method for measuring total phosphorus and phytic acid in foods and animal feeds. Journal of AOAC International. 99(3): 738–743.

Miller, L.A., Gordon, J. and Davis, E.A. (1991). Dielectric and thermal transition properties of chemically modified starches during heating. Journal of Cereal Chemistry. 68: 441-448.

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

Miller, F.A., Ramos, B., Gil, M.M., Brandão, T.R.S., Teixeira, P. and Silva, C.L.M. (2009). Influence of pH, type of acid and recovery media on the thermal inactivation of *Listeria innocua*. International Journal of Food Microbiology. 133(1–2): 121–128.

Moldao-Martins, M., Beirao-da-Costa, S.M. and Beirao-da-Costa, M.L. (2003). The effects of edible coatings on postharvest quality of the "Bravo de Esmolfe" apple. European Food Research and Technology. 217: 325–328.

Multari, S., Stewart, D. and Russell, W.R. (2015). Potential of fava bean as future protein supply to partially replace meat intake in the human diet. Comprehensive Reviews in Food Science and Food Safety. 14: 511–522.

Munyaka, A.W., Makule, E.E., Oey, I., Van Loey, A. and Hendrickx, M. (2010). Thermal stability of L-ascorbic acid and ascorbic acid oxidase in broccoli (*Brassica oleracea* var. italica). Journal of Food Science. 75: 336–340.

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

Nassourou, M.A., Njintang, Y.N., Noubissié, T.J.B., Nguimbou, R.M. and Bell, J.M. (2016). Genetics of seed flavonoid content and antioxidant activity in cowpea (*Vigna unguiculata* L. Walp.). Crop Journal. 4(5): 391-397.

Nath, A., Bagchi, B., Misra, L.K. and Deka, B.C. (2011). Changes in post-harvest phytochemical qualities of broccoli florets during ambient and refrigerated storage. Food Chemistry. 127:1510–1514.

Navarro–Rico J. (2015). Optimización del procesado mínimo y biodisponibilidad de compuestos bioactivos de brócoli bimi y convencional (Tesis Doctoral). Universidad Politécnica de Cartagena, España.

Navarro–Rico, J., Martínez–Hernández, G.B., Artés, F., Artés–Hernández, F. and Gómez, P.A. (2015). Effect of edible coatings and electrolyzed water sanitation on fresh–cut 'bimi' broccoli quality. Acta Hort. (ISHS). 1071:463–469.

Neal, J.A., Marquez-Gonzalez, M., Cabrera-Diaz, E., Lucia, L.M., O'Bryan, C.A. and Crandall, P.G. (2012). Comparison of multiple chemical sanitizers for reducing *Salmonella* and *Escherichia coli* O157: H7 on spinach (*Spinacia oleracea*) leaves. Food Research International. 45(2): 1123-1128.

Negueruela, A. I. (2012). Chapter 9: Is the color measured in food the color that we see? In J. L. Caivano, & M. P. Buera (Eds.), Color in food: Technological and psychophysical aspects (pp. 171e180). Boca Raton, FL: CRC Press.

Nelson, D.L., Cuchillo, Foix, C.M., Lehninger, A.L. and Cox, M.M. (2005). Lehninger: Principios de Bioquímica (4a. ed.). Barcelona: Omega.

Nieuwenhuijsen, M.J., Toledano, M.B. and Elliot, P. (2000). Uptake of chlorination disinfection by–products; a review and a discussion of its implications for exposure assessment in epidemiological studies. Journal of Exposure Analysis and Environmental Epidemiology. 10: 586–599.

Nikmaram, N., Leong, S.Y., Koubaa, M., Zhu, Z., Barba, F.J., Greiner, R., Oey, I. and Roohinejad, S. (2017). Effect of extrusion on the anti-nutritional factors of food products: An overview. Food Control. 79: 62-73.

Nithya, K.S., Ramachandramurty, B. and Krishnamoorthy, V.V. (2007). Effect of processing methods on nutritional and anti-nutritional qualities of hybrid (COHCU-8) and traditional (CO7) Pearl Millet varieties of India. Journal of Biological Sciences. 7(4): 643–647.

Nordstorm, C.L. and Sistrunk, W.A. (1977). Effect of type of bean, soak time, canning media and storage time on quality attributes and nutrient content of canned dry beans. Journal of Food Science. 42: 795–798.

Noubissie Tchiagam, J.B., Emmanuel, Y., Nicolas, N., Bell, J.M., and Nassourou, A. (2011). Generation Means Analysis of Seed Sucrose Content in Cowpea (*Vigna unguiculata* L. Walp.). Asian Journal of Agriculture Science. 3: 475-480.

Nozzolillo, C. and De Bezada, M. (1984). Browning of lentil seeds, concomitant loss of viability, and the possible role of soluble tannins in both phenomena. Canadian. Journal of Plant Science. 64: 815–824.

Ntatsi, G., Egea-Cortines, M., Karapanos, I., Barros, A., Weiss, J., Balliu, A., Augusto dos Santos Rosa, E. and Savvas, D. (2018). The quality of leguminous vegetables as influenced by preharvest factors. Scientia Horticulturae. 232: 191-205.

O`Dell, B.L. and de Boland, A. (1976). Complexation of phytate with proteins and cations in corn and oilseed meals. Journal of Agricultural and Food Chemistry. 24: 804-808.

Ofuya, Z.M. and Akhidue, V. (2005). The Role of Pulses in Human Nutrition: A Review. Journal of Applied Sciences and Environmental Management. 9(3): 99 – 104.

Ogunlade, I., Ogunleye, R.T. and Osasona, I. (2014). Chemical composition, antioxidant capacity and total phenolic content of the flours obtained from cowpea (*Vigna unguiculata*) varieties commonly consumed in Nigeria. British Journal of Applied Science & Technology. 4(12): 1729-1734.

Okada, M. and Ye, K. (2009). Nuclear phosphoinositide signaling regulates messenger RNA export. RNA Biology. 6: 12–16.

Olaimat, A.N. and Holley, R.A. (2012). Factors influencing the microbial safety of fresh produce: a review. Food Microbiology. 32: 11–19.

Oliveira, A.P., Araújo, J.S., Alves, E.U., Noronha, M.A.S., Cassimiro, C.F., and Mendonça, F.G. (2001). Yield of cowpea-beans cultivated with bovine manure and mineral fertilization. Horticultura Brasileira. 19: 81-84.

Ölmez, H. and Kretzschmar, U. (2009). Potencial alternative disinfection methods for organic fresh–cut industry for minimizing water consumption and environmental impact. Lebensmittel-Wissenschaft und Technologie. 42: 686–693.

Onigbinde, A. and Akinyele, I.O. (1983). Oligosaccharide content of 20 varieties of cowpeas in Nigeria. Journal of Food Science. 48: 1250-1254.

Otón, M., Artés, F. and Artés-Hernández, F. (2014). Efecto de agentes desinfectantes alternativos al hipoclorito sódico en la calidad de habas mínimamente procesadas en fresco. En: Actas del III Workshop en Investigación Agroalimentaria. Editores: F. Artés-Hernández, M. Egea-Cortines, A. Palop-Gómez, S. Bañón-Arias, P. Bielza. Editorial: Universidad Politécnica de Cartagena. ISBN: 978-84-697-1358-7. Cartagena, Murcia, España. 58-61.

Otón, M., Artés, F. and Artés-Hernández, F. (2016). Optimization of chlorine sanitation to preserve quality of minimally processed *Vicia faba* seeds. En: Proceedings of the 4th Workshop on Agri-Food Research. Editors: F. Artés-Hernández, M. Egea-Cortines, J. Fernández-Hernández, A. Baile, J. Calatrava. Editorial: Universidad Politécnica de Cartagena. ISBN: 978-84-608-5399-2. Cartagena, Murcia, España. Mayo 2015. 51-54.

Otón, M., Artés, F. and Artés-Hernández, F. (2017). Antibrowning washing agents effect to preserve quality of minimally processed *Vicia faba* seeds. En: Proceedings of the 5th Workshop on Agri-Food Research. Editors: F. Artés-Hernández, J.A. Fernández-Hernández, J. Calatrava, E. Aguayo; JJ Alarcón, JE Cos. Editorial: Universidad Politécnica de Cartagena. ISBN: 978-84-16325-29-0. Cartagena, Murcia, España. Mayo 2016. 223 pp. 40-43.

Pallauf J. and Rimbach G. (1997). Nutritional significance of phytic acid and phytase. Archives of Animal Nutrition. 50: 301-319.

Pariasca, J.A.T, Miyazaki, T., Hisaka, H., Nakagawa, H. and Sato, T. (2001). Effect of modified atmosphere packaging (MAP) and controlled atmosphere (CA) storage on the quality of snow pea pods (*Pisum sativum* L. var. saccharatum). Postharvest Biology and Technology. 21:231-223.

Park, D. and Maga, J.A. (1999). Dry bean (*Phaseolus vulgaris*) color stability as influenced by time and moisture content. Journal of Food Processing and Preservation. 23(6): 515–522.

Parra-Galant, J. (2009). El haba muchamiel. Horticultura Internacional 70: 32–33.

Peleg, H., Naim, M., Rouseff, R.L. and Zehavi, U. (1991). Distribution of bound and free phenolic acids in oranges (*Citrus sinensis*) and Grapefruits (*Citrus paradisi*). Journal of the Science of Food and Agriculture. 57(3): 417–426.

Peng, W., Ji-Chun T., Walker, C.E. and Feng-Cheng, W. (2009). Determination of phytic acid in cereals - A brief review. International Journal of Food Science and Technology. 44: 1671 - 1676.

Peterbauer, T. and Richter, A. (2001). Biochemistry and physiology of raffinose family oligosaccharides and galactosylcyclitols in seeds. Seed Science Research. 11: 185–197.

Pinto, E.P., Perin, E.C., Schott, I.B., da Silva Rodrigues, R., Lucchetta, L., Manfroi, V. and Rombaldi, C.V. (2016). The effect of postharvest application of UV-C radiation on the phenolic compounds of conventional and organic grapes (*Vitis labrusca* cv. 'Concord'). Postharvest Biology and Technology. 120: 84–91.

Pinto, P.M.Z. and Morais, A.M.M.B. (Eds.). (2000). Boas práticas para a conservação de produtos horto frutícolas. 1 st. Ed. AESBUC: Associação para a Escola Superior de Biotecnologia de Universidade Católica: Porto, p. 39.

Price, M.L., Van Scoyoc, S., and Butler, L.G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. Journal of Agricultural and Food Chemistry. 26(5): 1214–1218.

Price, K., Curl, C., and Fenwick, G. (1986). The saponin content and sapogenol composition of the seed of 13 varieties of legume. Journal of the Science of Food and Agriculture. 37: 1185-1191.

Price, K.R., Johnson, I.T. and Fenwick, G.R. (1987). The chemistry and biological significance of saponins in foods and feeding stuff. CRC Critical Reviews in Food Science and Nutrition. 26: 27-131.

Price, M.L., Socoyoc, S.V. and Butler, L. (1978). A certical evaluation of the vanillin reaction as an assay for tannin on sorgum graim. Journal of Agricultural and Food Chemistry. 26: 1214–1218.

Raes, K., Knockaert, D., Struijs, K. and Van Camp, J. (2014). Role of processing on bioaccessibility of minerals: Influence of localization of minerals and anti-nutritional factors in the plant. Trends in Food Science and Technology. 37(1): 32-41.

Rao, P.U. and Belavady, B. (1978). Oligosaccharides in pulses: Varietal differences and effects of cooking and germination. Journal of Agricultural and Food Chemistry. 26(2): 316–319.

Raybaudi–Massilia, R., Mosqueda–Melgar, J., Soliva–Fortuny, R. and Martín–Belloso, O. (2016). Combinational edible antimicrobial films and coatings, in: Barros–Velázquez, J. (Ed.), Antimicrobial food packaging. Elsevier, Amsterdam, pp. 633–646.

Regulation 1441/2007 (2007) https://www.boe.es/diario_boe/xml.php?id=DOUE-L-2007-82244.

Reim, V. and Rohn, R. (2015). Characterization of saponins in peas (*Pisum sativum* L.) by HPTLC couple to mass spectrometry and a hemolysis assay. Food Research International. 76: 3–10.

Revilla, I. (2015). Impact of thermal processing on faba bean (*Vicia faba*) composition, in: Preedy, V. (Ed.), Processing and impact on active components in food. Elsevier, Amsterdam, pp. 337–343.

Rico, D., Martín–Diana, A.B., Barat, J.M. and Barry–Ryana, C. (2007). Extending and measuring the quality of fresh–cut fruit and vegetables: a review. Trends in Food Science & Technology. 18: 373-386.

Riedl, K.M., Carando, S., Alessio, H.M., McCarthy, M. and Hagerman, A.E. (2002). Antioxidant activity of tannins and tannin-protein complexes: Assessment in vitro and in vivo. In: Morello MJ, Shahidi F and Ho C-T (eds) Free Radicals in Food. ACS Symposium Series, Washington, DC: American Chemical Society, pp.188–200.

Robles, P., de Campos, A., Artés–Hernández, F., Gómez, P., Calderón, A., Ferrer, M. and Artés, F. (2007). Combined effect of UV–C radiation and controlled atmosphere storage to preserve tomato quality. V Congreso Iberoamericano de Tecnología Postcosecha y Agroexportaciones. Cartagena, Spain.

Rodríguez-Hidalgo, S., Artés-Hernández, F., Gómez, P.A., Fernández, J. and Artés, F. (2010). Quality of fresh-cut baby spinach grown under floating trays system as affected by N fertilization and innovative package treatments. Journal of Science and Food Agriculture. 90: 1089-1097.

Rodríguez-Verástegui, L.L., Martínez-Hernández, G.B., Castillejo, N., Gómez, P.A., Artés, F. and Artés-Hernández, F. (2016). Bioactive compounds and enzymatic activity of red vegetable smoothies during storage. Food and Bioprocess Technology. 9(1): 137–146.

Rosen, J. and Kader, A., (1989). Postharvest physiology and quality maintenance of sliced pear and strawberry fruits. Journal of Food Science. 54: 656–659.

Rubio, L.A., Pedrosa, M.M., Pérez, A., Cuadrado, C., Burbano, C. and Muzquiz, M. (2005). Ileal digestibility of defatted soybean, lupin and chickpea seed meals in cannulated iberian pigs: II. Fatty acids and carbohydrates. Journal of the Science of Food and Agriculture. 85: 1322-1328.

Ruiz-Cruz, S., Islas-Osuma, M.A., Sotelo-Mundo, R.R., Vázquez-Ortiz, F. and González-Aguilar, G.A. (2007). Sanitation procedure affects biochemical and nutritional changes of shredded carrots. Journal of Food Science. 72:146-152.

Ruiz-Cruz, S., Luo, Y., Gonzalez, R.J., Tao, Y. and González, G.A. (2006). Acidified sodium chlorite as an alternative to chlorine to control microbial growth on shredded carrots while maintaining quality. Journal of the Science of Food and Agriculture. 86:1887–1893.

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

Saini, P., Singh, P., Dubey, S. and Srivastava, A. (2016). Effect of different processing methods on polyphenolic content and antioxidant activity of broad beans (*Vicia faba*). International journal of Critical Research and Review. 8: 6-11.

Saito M., Hosoyama H., Ariga T., Kataoka S. and Yamaji N. (1998). Antiulcer activity of grape seed extract and procyanidins. Journal of Agricultural and Food Chemistry. 46: 1460-1464.

Sakanaka S., Sato T., Kim M. and Yamamoto T. (1990). Inhibitory effects of green tea polyphenols on glucan synthesis and cellular adherence of cariogenic streptococci. Agricultural and Biological Chemistry. 54(11): 2925-2929.

Sánchez–Mata, M.C., Camara, M. and Diez–Marques, C. (2003). Extending shelf–life and nutritive value of green beans (*Phaseolus vulgaris* L.), by controlled atmosphere storage: macronutrients. Food Chemistry. 80: 309–315.

Santos-Buelga C., and Scalbert A. (2000). Proanthocyanidins and tannin-like compounds-nature, occurrence, dietary intake and effects on nutrition and health. Journal of the Science of Food and Agriculture. 80: 1094-1117.

Sapers, G. (1993). Scientific status summary browning of foods: control by sulphites, antioxidants and other means. Food Technology. 47: 75–84.

Savage, G. P, and Deo, S. (1989). The Nutritional Value of Peas (*Pisum sativum*). A Literature Review. Nutrition Abstracts and Reviews. 59 (2): 66-89.

Scalbert, A., Manach, A., Morand, C., Remesy, C. and Jiménez, L. (2005). Dietary polyphenols and the prevention of diseases. Critical Reviews in Food Science and Nutrition. 45: 287–306.

Selcuk, N. and Erkan. M. (2014). Changes in antioxidant activity and postharvest quality of sweet pomegranates cv. Hicrannar under modified atmosphere packaging. Postharvest Biology and Technology. 92:29–36.

Selma, M., Allende, A., Lopez Galvez, F., Conesa, M.A. and Gil, M. (2008). Disinfection potential of ozone, ultraviolet-C and their combination in wash water for the fresh-cut vegetable industry. Food microbiology. 25: 809-14.

Sengupta, S., Mukherjee, S., Basak, P. and Majumder, A.L. (2015). Significance of galactinol and raffinose family oligosaccharide synthesis in plants. Frontiers in Plant Science. 6: 656.

Shaidi, F., Arachchi, J.K.V. and Jeon, Y.J. (1999). Food applications of chitin and chitosan. Trends in Food Science and Technology. 10: 37–51.

Sharma, K., Ko, E.Y., Assefa, A.D., Ha, S., Nile, S.H., Lee, E.T. and Park, S.W. (2015). Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. Journal of Food Drug Analisys. 23(2): 243-252.

Shimelis, E. and Rakshit, S. (2005). Effect of microwave heating on solubility and digestibility of proteins and reduction of anti-nutrients of selected common bean (*Phaseolus Vulgaris* L.) varieties grown in Ethiopia. Italian Journal of Food Science. 17(4): 407–418.

Shirley, B., Kubasek, W.L., Storz, G., Bruggemann, E., Koorneef, M. and Ausubel, F.M. (1995). Analysis of Arabidopsis mutants deficient in flavonoid biosynthesis. The Plant Journal. 8: 659–671.

Siah, S., Wood, J.A., Agboola, S., Konczak, I. and Blanchard, C.L. (2014). Effects of soaking, boiling and autoclaving on the phenolic contents and antioxidant activities of faba beans (*Vicia faba* L.) differing in seed coat colours. Food Chemistry. 142: 461–468.

Sibul, F., Orcic, D., Vasic, M., Anackov, G., Nadpal, J., Savic, A. and Mimica-Dukic, N. (2016). Phenolic profile, antioxidant and anti-inflammatory potential of herb and root extracts of seven selected legumes. Industrial Crops Production. 83: 641–653.

Silva, E.O. and Bracarense A.P. (2016). Phytic Acid: from antinutritional to multiple protection factor of organic systems. Journal of Food Science. 81(6): 1357-1362.

Simões, A.D.N., Tudela, J.A., Allende, A., Puschmann, R. and Gil, M.I. (2009). Edible coatings containing chitosan and moderate modified atmospheres maintain quality

and enhance phytochemicals of carrot sticks. Postharvest Biology and Technology. 51: 364–370.

Simons, L.K. and Sanguansri, P. (1997). Advances in the washing of minimally processed vegetables. Food Australia. 49: 75-80.

Singh, N. and Kayastha, A. M. (2013). A novel application of Cicer α-galactosidase in reduction of raffinose family oligosaccharides in soybean flour. Journal of Plant Biochemistry and Biotechnology. 22(3): 353-356.

Singh, B., Singh, J.P., Kaur, A. and Singh, N. (2017). Phenolic composition and antioxidant potential of grain legume seeds: A review. Food Research International. 101: 1-16.

Singh, B., Singh, J.P., Shevkani, K., Singh, N. and Kaur, A. (2017). Bioactive constituents in pulses and their health benefits. Journal of Food Science and Technology. 54(4): 858-870.

Singh, B., Singh, Jatinder, P., Shevkani, K., Singh, N. and Kaur, A. (2017). Bioactive constituents in pulses and their health benefits. Journal of Food Science and Technology. 54: 1-13.

Singleton, V.L. and Rossi, J.A. (1965). Colorimetry of total phenolics with phospomolobdic–phosphotungstic acid reagents. American Journal of Enology and Viticulture. 16: 144–158.

Singleton, V.L. (1981). Naturally occurring food toxicants: phenolic substances of plant origin common in foods. Advances in Food Research. 27:149-242.

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

Smith, S.M. and Stow, J.R. (1984). The potential of a sucrose ester coating material for improving the storage and shelf-life qualities of Cox's Orange Pippin apples. Annals of Applied Biology. 104: 383–391.

Soetan, K.O. and Oyewole, O.E. (2009). The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds: A review. African Journal of Food Science. 3(9): 223–232.

Soetan, K.O. (2002). Pharmacological and other beneficial effects of antinutritional factors in plants – A review. African Journal of Biotechnology. 7(25): 4713–4721.

Soliva-Fortuny, R.C. and Martín-Belloso, O. (2003). New advances in extending the shelf-life of fresh-cut fruits: a review. Trends in Food Science & Technology. 14(9): 341-353.

Sreerama, Y.N., Sashikala, V.B., Pratape, V.M. and Singh, V. (2012). Nutrients and antinutrients in cowpea and horse gram flours in comparison to chickpea flour: Evaluation of their flour functionality. Food Chemistry. 131(2): 462-468.

Stancheva, I., Nedyalkova, K., Geneva, M., Donkova, R., Hristozkova, M., Perfanova, I., Sichanova, M., Petkova, G., Djonova, E. and Valchovski, H. (2016). Nutritional value of cowpea (*Vigna unguiculata* L. Walp) grain grown under different soil moisture as affected to the dual inoculation with nitrogen fixing bacteria and arbuscular mycorrhizal fungi. Bulgarian Journal of Soil Science. 1: 112-121.

Stanisavljević, N., Ilic, M., Jovanović, Ž., Cupic, T., Dabić, D., Natić, M., Tesic, Z. and Radovic, S. (2015). Identification of seed coat phenolic compounds from differently colored pea varieties and characterization of their antioxidant activity. Archives of Biological Sciences. 67: 42-42.

Steer, T.E. and Gibson, G.R. (2002). The microbiology of phytic acid metabolism by gut bacteria and relevance for bowel cancer. International Journal of Food Science and Technology. 37: 783-790.

Suslow, T. (1997). Postharvest chlorination. Basic properties and key points for effective disinfection. Publication 8003. University of California. Division of Agriculture and Natural Resources, Davis CA, USA.

Suslow, T and Cantwell M. (1998). Peas: Snow and Snap Pod: Recommendations for Maintaining Postharvest Quality. http://postharvest.ucdavis.edu/Commodity_Resources/Fact_Sheets/Datastores/Vegetables_English/?uid=26&ds=799 (Accessed January 18, 2018).

Taira, S.., Ono, M. and Matsumoto, N. (1997). Reduction of persimmon astringency by complex formation between pectin and tannins. Postharvest Biology and Technology. 12(3): 265–271.

Tarazona-Díaz, M.P., Viegas, J., Moldao-Martins, M. and Aguayo, E. (2011). Bioactive compounds from flesh and by-product of fresh-cut watermelon cultivars. Journal of the Science of Food and Agriculture. 91:805–812.

Taverne, Y.J., Bogers, A.J., Duncker, D.J. and Merkus, D. (2013). Reactive oxygen species and the cardiovascular system. Oxidative medicine and cellular longevity

Tchiagam, J.B.N., Youmbi, E., Njintang, N.Y., Bell, J.M. and Nassourou, M.A. (2011). Generation Means Analysis of Seed Sucrose Content in Cowpea (*Vigna unguiculata* L. Walp.). Asian Journal of Agricultural Sciences. 3: 475-480.

Tharanathan, R.N. and Mahadevamma, S. (2003). Grain legumes, a boon to human nutrition. Trends Food Science and Technology. 14: 507-518.

Thole, J.M. and Nielsen, E. (2008). Phosphoinositides in plants: novel functions in membrane trafficking. Current Opinion in Plant Biology. 11: 620–631.

Thommohaway, C., Uthairatanakij, A. and Jitareerat, P. (2007). Effects of sucrose fatty acid ester on the quality of fresh–cut guava (*Psidium guajava*). Acta Horticulturae. 746: 455–460.

Thompson L.U. (1993). Potential health benefits and problems associated with antinutrients in foods. Food Research International. 26: 131-149.

Tiong, N., Prasad, K., Yang, B. and Ismail. A. (2010). Bioactive substance contents and antioxidant capacity of raw and blanched vegetables. Innovative Food Science & Emerging Technologies. 11: 464-469.

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

Tomás-Barberán, F. (2003). Los polifenoles de los alimentos y la salud. Alimentación Nutrición y Salud. 10 (2): 41-53.

Tomás-Callejas, A., López-Velasco, G., Artés, F. and Artés-Hernández, F. (2012). Acidified sodium chlorite optimization assessment to improve quality of fresh-cut tatsoi baby leaves. Journal of Science of Food and Agriculture. 92: 877–885.

Trinidad, P., Mallillin, A.C., Loyola, A.S., Sagum, R.S. and Encabo, R.R. (2010). The potential health benefits of legumes as a good source of dietary fibre. British Journal of Nutrition. 103: 569–574.

Turco, I., Ferretti, G. and Bacchetti, T. (2016). Review of the health benefits of faba bean (*Vicia faba* L.) polyphenols. Journal of Food and Nutrition Research. 55: 283–293.

Turtoi, M. (2013). Ultraviolet light treatment of fresh fruits and vegetables surface: A review. Journal of Agroalimentary Processes and Technology. 19: 325-337.

USDA Nutrient Database for Standard Reference, Release 28. (2018). USDA-ARS, Beltsville, MD. Nutrient Data Laboratory Home Page. Available online at: https:// ndb.nal.usda.gov/ndb/search [03 March 2018].

Uyttendaele, M., Neyts, K., Vanderswalmen, H., Notebaert, E. and Debevere, J. (2004). Control of Aeromonas on minimally processed vegetables by decontamination with lactic acid, chlorinated water, or thyme essential oil solution. International Journal of Food Microbiology. 90: 263-271.

Vallejo, F., Tomas-Barberan, F.A. and Garcia-Viguera, C. (2002). Glucosinolates and vitamin C content in edible parts of broccoli florets after domestic cooking. European Food Research and Technology. 215(4): 310–316.

Van den Ende, W. (2013). Multifunctional fructans and raffinose family oligosaccharides. Frontiers in Plant Science. 4(247): 247.

Vats, P. and Banerjee, U.C. (2004). Production studies and catalytic properties of phytases (myo-inositolhexakisphosphate phosphohydrolases): An overview. Enzyme and Microbial Technology. 35(1): 3–14.

Vicente, A.R., Pineda, C., Lemoine, L., Civello, P.M., Martínez, G.A. and Chaves, A.R. (2005). UV–C treatments reduce decay, retain quality and alleviate chilling injury in pepper. Postharvest Biology and Technoly. 35: 69–78.

Vidal–Valverde, C., Frias, J., Sotomayor, C., Diaz–Pollan, C., Fernandez, M. and Urbano, G. (1998). Nutrients and antinutritional factors in faba beans as affected by processing. Zeitschrift für Lebensmitteluntersuchung und -Forschung A. 207: 140–145.

Vidal-Valverde, C., Frías, J., Hernández, A., Martín-Álvarez, P.J., Sierra, I., Rodríguez, C., Blázquez, I. and Vicente, G. (2003). Assessment of nutritional compounds and antinutritional factors in pea (*Pisum sativum*) Seeds. Journal of the Science of Food and Agriculture. 83: 298 - 306.

Vilariño, M., Métayer, P., Crépon, J., Katell and Duc, G. (2009). Effects of varying vicine, convicine and tannin contents of faba bean seeds (*Vicia faba* L.) on nutritional values for broiler chicken. Animal Feed Science and Technology. 150. 114-121.

Voragen, A.G.J. (1998). Technological aspects of function food-related carbohydrates. Trends in Food Science & Technology. 328–335.

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

Wang, N., Hatcher, D.W., Toews, R. and Gawalko, E.J. (2009). Influence of cooking and dehulling on nutritional composition of several varieties of lentils (Lens culinaris). LWT – Food Science and Technology. 42(4): 842–848.

Warf, C.C. (2001). The chemistry and mode of action of acidified sodium chlorite. IFT Annual Meeting pp. 1–91.

Warriner, K., Huber, A., Namvar, A., Fan, W. and Dunfield, K. (2009). Recent advances in the microbial safety of fresh fruits and vegetables. Advances in food and nutrition research. 57: 155–208.

Watada, A.E., Ko, N.P. and Minott, D.A. (1996). Factors affecting fresh–cut horticultural products. Postharvest Biology and Technology. 9: 115–125.

Wen, T.N., K. Prasad, N., Yang, B. and Ismail, A. (2010). Bioactive substance contents and antioxidant capacity of raw and blanched vegetables. Innovative Food Science & Emerging Technologies. 11(3): 464-469.

Wilhelm Thomé O. (1885). Flora von Deutschland, Österreich und der Schweiz.

Wilson, M., Stanley, R.A., Eyles, A. and Ross, T. (2017). Innovative processes and technologies for modified atmosphere packaging of fresh and fresh-cut fruits and vegetables: A review. Critical Reviews in Food Science and Nutrition. 59(3):00-00.

Wong, D.W.S., Tillin, S.J., Hudson, J.S. and Pavlath, A.E. (1994). Gas exchange in cut apples with bilayer coatings. Journal of Agricultural and Food Chemistry. 42: 2278–2285.

Wongputtisin, P., Ramaraj, R., Unpaprom, Y., Kawaree, R. and Pongtrakul, N. (2015). Raffinose family oligosaccharides in seed of Glycine max cv. and potential source of prebiotic substances. International Journal of Food Science & Technology. 50: 1-7.

Wu, X. and Kang, J. (2011). Phytochemicals in soy and their health effects. In I. Rasooli (Ed.), Phytochemicals – bioactivities and impact on health (pp. 43–76). InTech.

Xiong, S., Yao, X. and Li, A. (2013). Antioxidant Properties of Peptide from Cowpea Seed. International Journal of Food Properties. 16: 1245-1256.

Xu, B. and Chang, S.K. (2008). Effect of soaking, boiling, and steaming on total phenolic contentand antioxidant activities of cool season food legumes. Food Chemistry. 110(1): 1–13.

Xu, B. and Chang, S.K.C. (2012). Comparative study on antiproliferation properties and cellular antioxidant activities of commonly consumed food legumes against nine human cancer cell lines. Food Chemistry. 134(3): 1287-96.

Xu, Y., Cartier, A., Obielodan, M., Jordan, K., Hairston, T., Shannon, A. and Sismour, E. (2016). Nutritional and anti–nutritional composition, and in vitro protein digestibility of Kabuli chickpea (*Cicer arietinum* L.) as affected by differential processing methods. Journal of Food Measurement and Characterization. 10: 625–633.

Xue, H., Chen, X. and Li, G. (2007). Involvement of phospholipids signaling in plant growth and hormone effects. Plant Biology. 10: 483–489.

Yadahally, N., Sreerama, N.Y., Vadakkoot, B., Sashikala, V., Pratape M. and Vasudeva S. (2012). Nutrients and antinutrients in cowpea and horse gram flours in comparison to chickpea flour: Evaluation of their flour functionality. Food Chemistry. 131(2): 462-468.

Yadav, N., Kaur, D., Malaviya, R., Singh, M., Fatima, M. and Singh, L. (2018). Effect of thermal and non-thermal processing on antioxidant potential of cowpea seeds. International Journal of Food Properties. 21(1): 437–451.

Yasmin, A., Zeb, A., Khalil, A. W., Paracha, G. M. and Khattak, A. B. (2008). Effect of processing on anti-nutritional factors of red kidney bean (*Phaseolus vulgaris*) grains. Food and Bioprocess Technology. 1(4): 415-419.

Yaun, B., Sumner, S., Eifert, J. and Marcy, J. (2004). Inhibition of pathogens on fresh produce by ultraviolet energy. International Journal of Food Microbiology. 90: 1–8.

Yildiz, F. (1994). Initial preparation, handling and distribution of minimally processed refrigerated fruits and vegetables. In: Wiley, R.C. (Ed.). Minimally Processed Refrigerated Fruits and Vegetables. Chapman & Hall, New York, USA, pp. 15–49.

Youngmok, K., Jeffrey, B.K. and Stephen, T.T. (2007). Antioxidant phytochemical and fruit quality changes in mango (*Mangifera indica* L.) following hot water immersion and controlled atmosphere storage. Food Chemistry. 105 (4): 1327–1334.

Yousuf, B., Qadri, O. and Srivastava, A. (2018). Recent developments in shelf-life extension of fresh-cut fruits and vegetables by application of different edible coatings: A review. LWT- Food Science and Technology. 89.

Zapata, S. and Dufour, J.P. (1992). Ascorbic, dehydroascorbic and isoascorbic and simultaneous determinations by reverse phase ion interaction HPLC. Journal of Food Science. 57: 506–511.

Zartl, B., Silberbauer, K., Loeppert, R., Viernstein, H., Praznik, W. and Mueller, M. (2018). Fermentation of non-digestible raffinose family oligosaccharides and galactomannans by probiotics. Food & Function. 9(3): 1638–1646.

Zenoozian, M.S. (2011). Combined effect of packaging method and temperature on the leafy vegetables properties. International Journal of Environmental Science and development. 2:124-127.

Zhang, D. and Hamauzu, Y. (2004). Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food Chemistry. 88: 503–509.

Zhang, B., Deng, Z., Ramdath, D. D., Tang, Y., Chen, P. X., Liu, R., and Tsao, R. (2015). Phenolic profiles of 20 Canadian lentil cultivars and their contribution to antioxidant activity and inhibitory efects on α-glucosidase and pancreatic lipase. Food Chemistry. 172: 862–872.

Zhou, L., Tey, C.Y., Bingol, G. and Bi, J. (2016). Effect of microwave treatment on enzyme inactivation and quality change of defatted avocado puree during storage. Innovative Food Science and Emerging Technologies. 37: 61-67.

Zia-Ul-Haq, M., Ahmad, S., Amarowicz, R. and De Feo, V. (2013). Antioxidant activity of the extracts of some cowpea (*Vigna unguiculata* (L) Walp.) cultivars commonly consumed in Pakistan. Molecules. 18: 2005-2017.

Zulet, M.A., and Martínez, J.A. (2001). Dieta mediterránea: legumbres y colesterolemia. Revista chilena de nutrición. 28(2): 312-320.

APPENDIX

COPIES OF THE E-MAILS OF ACCEPTANCE OF THE PUBLICATIONS OF WHICH THE THESIS CONSISTS AND IMPACT INDEX

- **Collado, E**., Klug, T.V, Martínez-Sánchez, A., Artés-Hernández, F., Aguayo, E., Artés, F., Fernández, J.A. and Gómez, P.A. 2017. Immature pea seeds. Effect of storage under modified atmosphere packaging and sanitation with acidified sodium chlorite. **Journal of the Science of Food and Agriculture**. 97: 4370-4378. http://dx.doi.org/10.1002/jsfa.8513.

Accept - Journal of the Science of Food and Agriculture - Manuscript JSFA-16-2696.R1

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Dom 25/06/2017 23:35

JSFA-16-2696.R1

Dear Dr Gómez,

I am delighted to inform you that your manuscript, "IMMATURE PEA SEEDS. EFFECT OF STORAGE UNDER MODIFIED ATMOSPHERE PACKAGING AND SANITATION WITH ACIDIFIED SODIUM CHLORITE", is now accepted for publication in the Journal of the Science of Food and Agriculture. Within the next few days the corresponding author will receive an email from Wiley's Author Services system which will ask them to log in and will present them with the appropriate license for completion. Your article cannot be published until the publisher has received the appropriate signed license agreement.

The article will be published online within the next few days as an Accepted Article. This will be an unedited version but will be fully citable and will constitute the paper's official publication date.

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Yours sincerely,

Dr Carol Wagstaff
Associate Editor
Journal of the Science of Food and Agriculture
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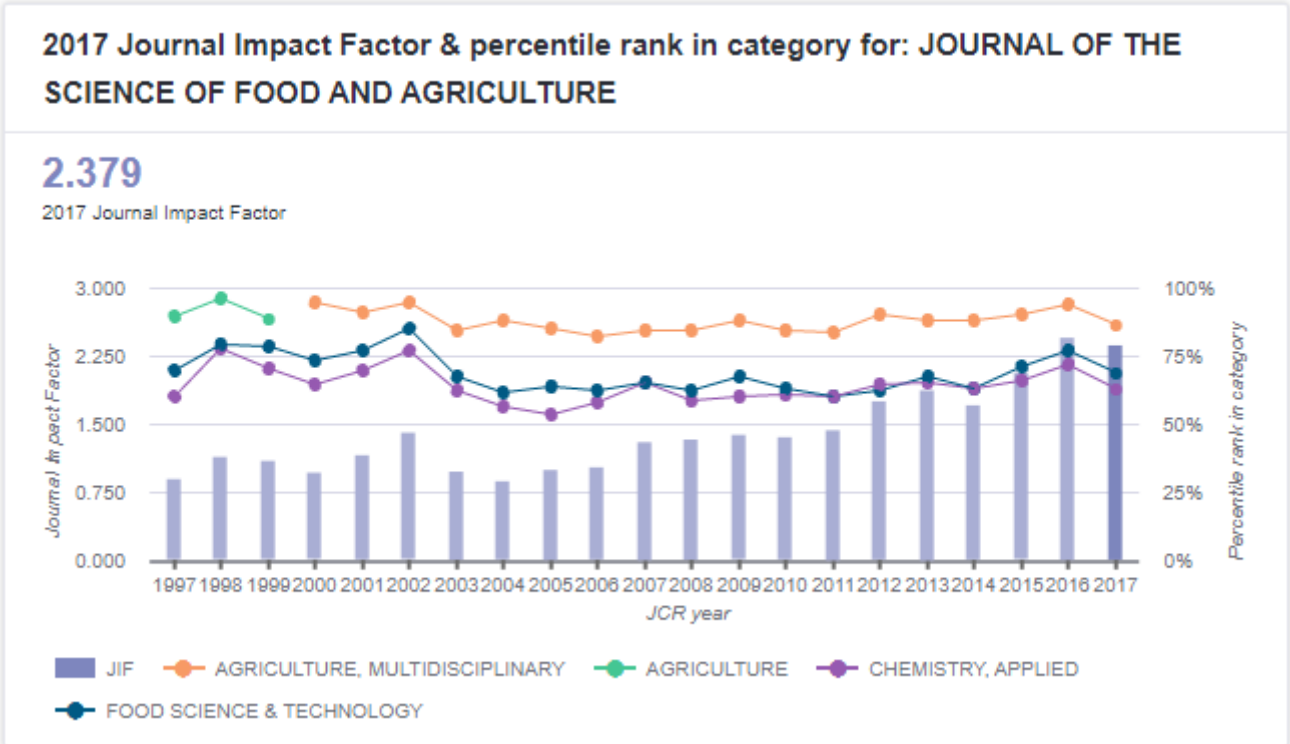


- **Collado, E.**, Venzke Klug, T., Martínez-Hernández, G. B., Artés-Hernández, F., Martínez-Sánchez, A., Aguayo, E., Artés, F., Fernández, J.A., Gómez, P.A. (2019). UV-C pretreatment of fresh-cut faba beans (*Vicia faba)* for shelf life extension: Effects of domestic microwaving for consumption. **Food Science and Technology International**. In press. https://doi.org/10.1177/1082013219873227.

FSTI-19-0097.R1; Final clean files required for production

Food Science and Technology
International <onbehalfof@manuscriptcentral.com>
Mar 30/07/2019 12:53

FSTI-19-0097.R1 - UV–C PRETREATMENT OF FRESH–CUT FABA BEANS (*Vicia faba*) FOR SHELF LIFE EXTENSION. EFFECTS OF DOMESTIC MICROWAVING FOR CONSUMPTION

Dear Dr. Gomez Di Marco:

Hope you are doing well.

I am glad to inform you that as your manuscript is accepted for production, we require final clean accepted files.

(a) Please provide clean accepted editable manuscript and title page.

(b) Please provide figures in TIFF/EPS/JPEG/XLS formats only. Tables should be in excel/word format.

In addition, while checking the manuscript the citation for figure 2 was not present. Request you to update the manuscript with the same.

Look forward to receiving the files.

Sincerely,
Shivani Barthwal
Food Science and Technology International

- **Collado, E.,** Venzke Klug, T., Artés-Hernández, F., Aguayo, E., Artés, F., Fernández, J.A., Gómez, P.A. (2019). Quality changes in nutritional traits of fresh-cut and then microwaved cowpea seeds and pods. **Food and Bioprocess Technology**. 12: 338-346. http://dx.doi.org/10.1007/s11947-018-2214-2

Decision on your manuscript #FABT-D-18-00821R2

Food and Bioprocess Technologies <" <em@editorialmanager.com>">

Lun 12/11/2018 14:22

Dear Dr. Gómez:

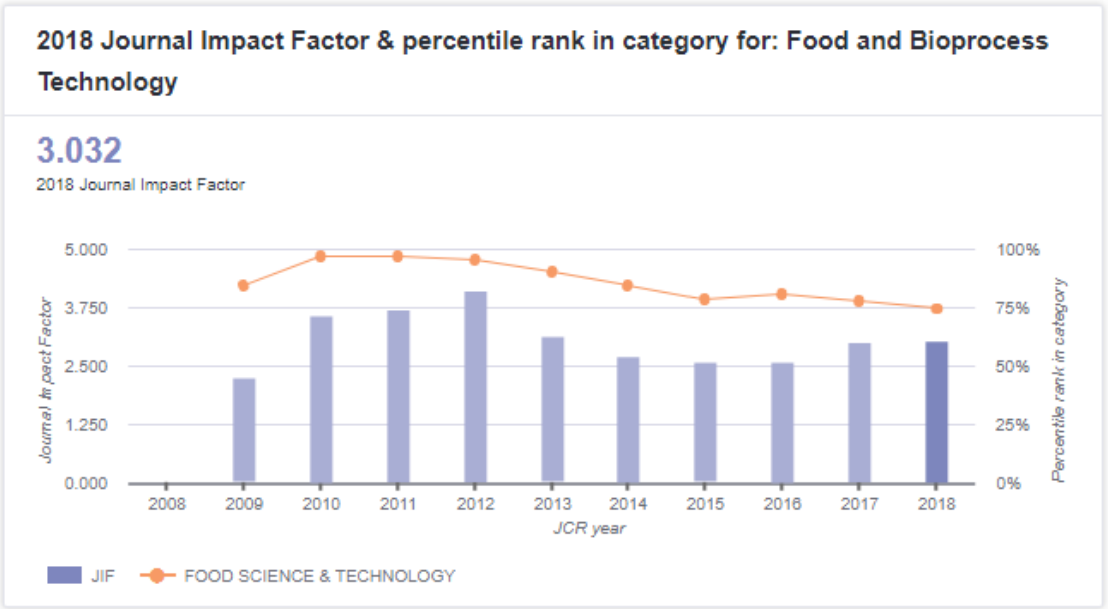
We are pleased to inform you that your manuscript, "QUALITY CHANGES IN NUTRITIONAL TRAITS OF FRESH-CUT AND THEN MICROWAVED COWPEA SEEDS AND PODS" has been accepted for publication in Food and Bioprocess Technology.

For queries regarding your accepted paper, please click the following link http://www.springer.com/11947; then click on "Contacts", and then "Production Editor", complete the query form and click "Submit".

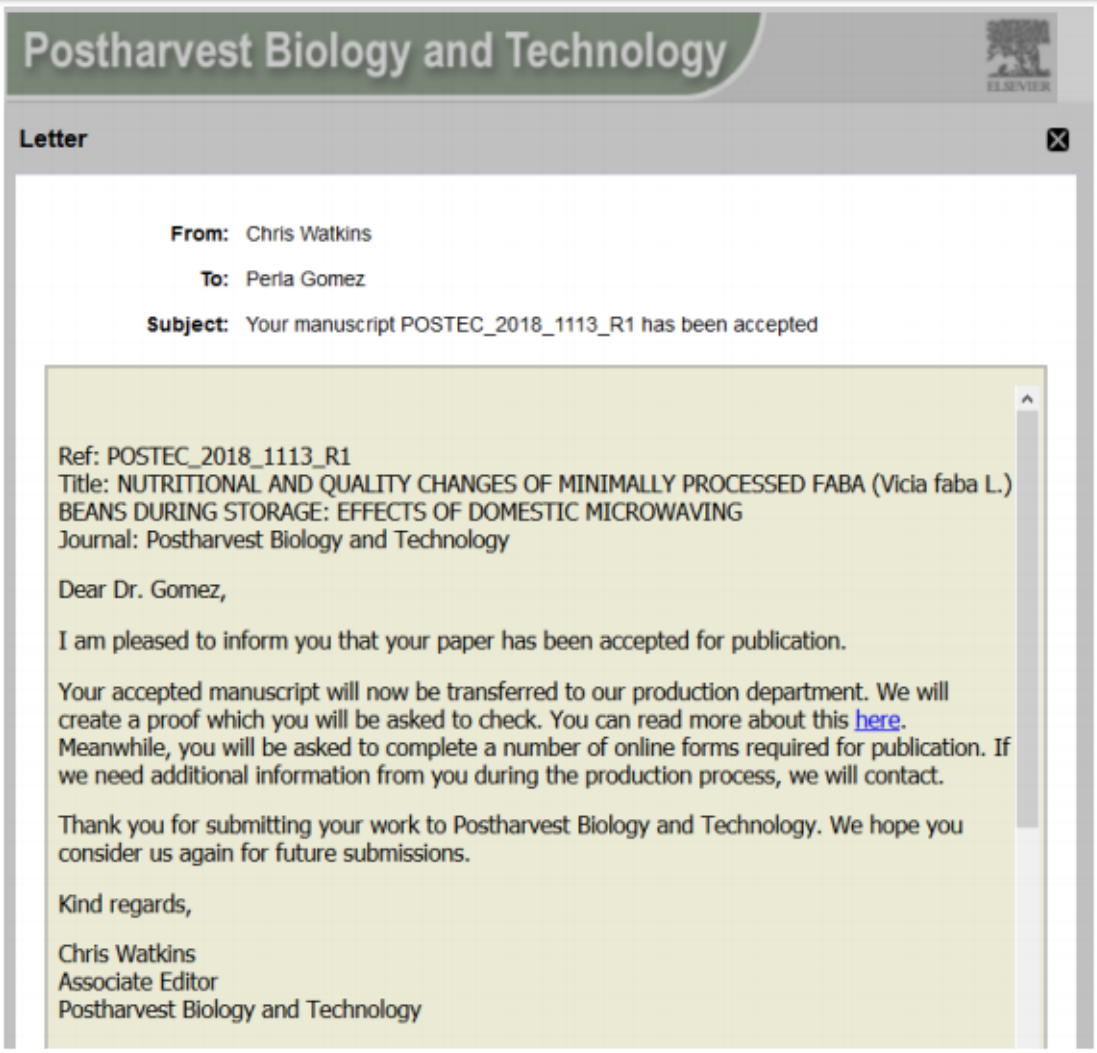
Please remember to always include your manuscript number, #FABT-D-18-00821R2, whenever inquiring about your manuscript. Thank you.

Best regards,

Jorge Barros-Velazquez, Ph.D.
Food and Bioprocess Technology



Collado, E., Venzke Klug, T., Martínez-Hernández, G. B Artés-Hernández, F., Martínez-Sánchez, A., Aguayo, E., Artés, F., Fernández, J.A., Gómez, P.A. 2019. Nutritional and quality changes of minimally processed faba (*Vicia faba* L.) beans during storage: Effects of domestic microwaving. **Postharvest Biology and Technology**. https://doi.org/10.1016/j.postharvbio.2019.01.008



Postharvest Biology and Technology

Letter

From: Chris Watkins

To: Perla Gomez

Subject: Your manuscript POSTEC_2018_1113_R1 has been accepted

Ref: POSTEC_2018_1113_R1
Title: NUTRITIONAL AND QUALITY CHANGES OF MINIMALLY PROCESSED FABA (Vicia faba L.) BEANS DURING STORAGE: EFFECTS OF DOMESTIC MICROWAVING
Journal: Postharvest Biology and Technology

Dear Dr. Gomez,

I am pleased to inform you that your paper has been accepted for publication.

Your accepted manuscript will now be transferred to our production department. We will create a proof which you will be asked to check. You can read more about this here. Meanwhile, you will be asked to complete a number of online forms required for publication. If we need additional information from you during the production process, we will contact.

Thank you for submitting your work to Postharvest Biology and Technology. We hope you consider us again for future submissions.

Kind regards,

Chris Watkins
Associate Editor
Postharvest Biology and Technology