Universitat de Lleida

Preserving natural attributes of mango products by non-thermal technologies

Blanca Salinas-Roca

Universitat de Lleida

Departament de Tecnologia d'Aliments

Preserving natural attributes of mango products by non-thermal processing technologies

Dissertation to fulfill the requirements for degree of doctor of philosophy

Doctoral program in Agricultural and Food Science and Technology

by

Blanca Salinas-Roca

Directed by

Olga Martín-Belloso PhD

Jorge Welti-Chanes PhD

Lleida, 2017

Preserving natural attributes of mango products by non-thermal processing technologies

by

Blanca Salinas-Roca

A dissertation submitted to the department of Food Science and Technology of the University of Lleida in partial fulfilment of the requirements for the degree of doctor of philosophy

Thesis committee

Isabel Odriozola-Serrano. PhD
Universitat de Lleida

Marta Capellas Puig. PhD
Universitat Autònoma de Barcelona

Alejandra Rojas-Grau. PhD
PROINEC

Other committee members

Gemma Oms-Oliu. PhD
Universitat de Lleida

Jordi Saldo Periago. PhD
Universitat Autònoma de Barcelona

The present research work was carried out in the laboratory of Novel Technologies on Food Processing, Department of Food Technology of the University of Lleida, Spain, under the supervision of Prof. Olga Martín Belloso and Prof. Jorge Welti-Chanes.

This work was supported by the University of Lleida (Spain) and financed by Tecnológico de Monterrey (Research Chair Funds CAT-200 and CDB081).

The studies of food processing by high hydrostatic pressure were carried out in the Centro de Biotecnología FEMSA, Escuela de Ingeniería y Ciencias, Tecnológico de Monterrey, Av. Eugenio Garza Sada 2501 Sur, Col. Tecnológico, 64849 Monterrey, México

The difference between giving everything
and almost everything is infinite.

Marc Vilarassau sj.

Table of contents

SUMMARY

Previous studies suggest an inverse association between the bioactive compounds content in fruits and the risk of health disorders such as cardiovascular disease and several cancers. In particular, a rich source of bioactive compounds such as phenols and vitamins has reported in mango fruit. In addition, consumers are currently demanding safe, healthy and novel fruit products, such as ready-to-eat, with natural attributes. Non-conventional processing technologies have emerged as interesting alternatives to obtain fruit products with natural attributes. Therefore, the aim of the present doctoral thesis was to assess the impact of non-thermal treatments (coating, dipping, pulsed light, high intensity pulsed electric fields and high hydrostatic pressure) on quality properties (microbiological, enzymatical, physicochemical and biochemical) of various mango products: fresh-cut, juice and puree.

The effect of different polysaccharide-based coatings, alginate (AL), chitosan (CH), pectin (PE) and carboxymethylcellulose (CMC) was evaluated on the quality preservation of fresh-cut mango. The optimal coating in terms of microbial safety was CH, which maintain microbial population below 1 log CFU/g. Despite all coatings maintained the pH, soluble solids content and colour after 14 days of storage at 4 ˚C, only AL preserved firmness (2.5 N) and overall acceptance (90.2 %). Mangoes coated with AL and CH showed the maximal antioxidant capacity and phenolics content. Also in fresh-cut mango, the influence of pulsed light (PL), combined with AL coating and malic acid (MA) dipping on the inactivation of Listeria innocua, the physicochemical properties and the phenolics content were determined. After 14 days, the combination of PL (8 J•cm^{-2}), AL (2 %) and MA (2 %) had an additive effect on the reduction of the microbial load and preserved the fresh-like colour and firmness of mangoes. In addition, the combination of PL, AL and MA treatments enhanced the phenolics content of fresh-cut mango along the storage compared with those untreated.

The influence of high intensity pulsed electric fields (HIPEF) treatment on Listeria innocua load was tested in mango juice. HIPEF at 35 kV/cm, 4 μs- bipolar pulses, 200 Hz and 1800 μs was effective in the reduction of Listeria innocua population to

pasteurization levels while enzymatic activities of polyphenol oxidase, lipoxygenase and peroxidase were significantly declined after 59 days. Also, the physicochemical attributes were preserved and an increase of phenolic compounds in HIPEF-treated mango juice was obtained after 59 days. Although, antioxidant capacity and carotenoid content of HIPEF-treated mango juice decreased gradually throughout storage period and better preservation was observed in thermally-treated mango juice.

The impact of high hydrostatic pressure (HHP) treatments (400, 450, 500 MPa for 0 to 16 min at 34 or 59 °C) on physicochemical and enzymatic properties (pectinmethylesterase, polyphenol oxidase and peroxidase) of mango puree (Tommy Atkins and Manila varieties) was evaluated. HHP-treated mango purees at 34°C had higher enzymatic activity than those processed at 59°C. HHP-treated mango puree kept pH, aw, and soluble solids as the untreated puree; nonetheless, there were significant differences between varieties. The viscosity of Tommy Atkins puree increased with increasing pressure and holding time during the HHP treatment. Higher yellow index and lower lightness were noted in Tommy Atkins mango puree after 550 MPa and 59 °C compared with Manila variety. Total carotenoid preservation in HHP-treated samples varied from 77 to 98 %. HHP treatments increased the phenolic content up to 34 % (550 MPa, 59°C and 4 min) compared to the initial content, probably due to the compression effect that enhance phenolics delivery from cells.

The results obtained in the present doctoral thesis showed that non-thermal technologies minimize the detrimental effects in mango products while preserving the natural attributes of this tropical fruit. Moreover, the current research represents an insight to the processing of mango, in the way of increasing the value of mango products, diminishing fruit waste and enabling non-producing countries to incorporate mango products with fresh-like attributes to the diet.

RESUM

Estudis recents suggereixen una relació inversa entre el contingut de compostos bioactius en fruites i el risc de patir problemes de salut com malalties cardiovasculars o alguns càncers. En particular, el mango s'ha descrit com a font de compostos bioactius entre els quals hi ha fenols i vitamines. Actualment, els consumidors demanden nous productes a base de fruites -com els a punt per menjar-, segurs, saludables i amb propietats similars als naturals. Les tecnologies no convencionals de processat suposen alternatives interesants per obtenir productes a base de fruites amb atributs naturals. Així, l'objecte d'estudi d'aquesta tesi doctoral fou avaluar l'impacte de tractaments no tèrmics (recobriments, *dipping*, llum polsada, polsos elèctrics d'alta intensitat de camp i alta pressió hidrostàtica) en propietats de qualitat (microbiològiques, enzimàtiques, fisicoquímiques i bioquímiques) de diversos productes de mango: tallat, suc i puré.

L'efecte de diferents recobriments a base de polisacàrids, alginat (AL), quitosan (CH), pectina (PE) i carboximetilcel·lulosa (CMC), es va avaluar en la conservació de la qualitat del mango tallat. El recobriment òptim pel que fa a seguretat microbiana va ser el CH, que va mantenir la població microbiana per sota de 10 UFC/g. Malgrat que tots els recobriments van mantenir el pH, el contingut en sòlids solubles i el color al llarg de 14 dies d'emmagatzematge a 4 °C, sol l'AL va mantenir la fermesa (2,5 N) i l'acceptació en el test sensorial (90,2 %). Els mangos recoberts amb AL i CH van mostrar la màxima capacitat antioxidant i contingut en fenols. D'altra banda, es va determinar la influència dels polsos de llum (PL) combinats amb el recobriment AL i la immersió en àcid màlic (MA) sobre la inactivació de *Listeria innocua*, les propietats fisicoquímiques i el contingut de compostos fenòlics en el mango tallat. Després de 14 dies, es va poder observar un efecte additiu de la combinació de PL (8 J • cm^{-2}), AL (2 %) i MA (2 %) en la reducció de la càrrega microbiana i en la conservació del color i la fermesa dels mangos tallats. A més, la combinació dels tractaments PL, AL i MA va millorar el contingut de compostos fenòlics en el mango tallat si es compara amb el no tractat durant l'emmagatzematge.

La influència del tractament de polsos elèctrics d'alta intensitat de camp (HIPEF) en la càrrega de *Listeria innocua* va ser estudiada en suc de mango. HIPEF a 35 kV/cm, 4 μs- pols bipolar, 200 Hz i 1800 μs va ser efectiu en la reducció de població de *Listeria innocua* es van obtenir nivells de pasteurització alhora que l'activitat enzimàtica de polifenoloxidasa, lipoxigenasa i peroxidasa va ser significativament reduïda després de 59 dies. Tanmateix, el suc de mango tractat per HIPEF va conservar els atributs fisicoquímics i el contingut de compostos fenòlics va augmentar després de 59 dies. Malgrat això, la capacitat antioxidant i el contingut de carotens del suc tractat per HIPEF va disminuir gradualment al llarg de l'emmagatzematge a diferència del suc tractat tèrmicament.

L'impacte del tractament d'alta pressió hidrostàtica (HHP) (400, 450, 500 MPa durant 0 a 16 min a 34 o 59°C) es va estudiar en les propietats fisicoquímiques i enzimàtiques (pectinmetilesterasa, polifenoloxidasa i peroxidasa) de puré de mango (varietats Tommy Atkins i Manila). El puré de mango tractat per HHP a 34°C va tenir activitat enzimàtica més alta que els processats a 59°C. El tractament per HHP va permetre mantenir el pH, l'a_w, i els sòlids solubles similars al puré de mango no tractat; nogensmenys, hi van haver diferències significatives entre varietats. La viscositat del puré de mango Tommy Atkins va augmentar a mesura que s'incrementava la pressió i el temps de tractament. Tanmateix, l'índex de color grogós en el mango va augmentar i la lluentor va disminuir en el puré de mango Tommy Atkins després del tractament a 550 MPa i 59 °C si es compara amb la varietat Manila. Els carotens totals es van conservar entre el 77 i el 98 % després del tractament de HHP. El contingut de fenols va augmentar fins a un 34 % després del processat amb HHP (550 MPa, 59°C i 4 min), probablement a causa de l'efecte de compressió que millora l'alliberació de fenols de les cèl·lules.

Els resultats obtinguts en aquesta tesi doctoral posen de manifest que les tecnologies no tèrmiques minimitzen els efectes de deteriorament en els productes de mango alhora que preserven els atributs naturals d'aquesta fruita tropical. Aquesta recerca representa una aproximació al processat del mango, al qual se li dona valor afegit, a més de permetre als països no productors incorporar en la dieta productes de mango amb propietats similars al producte fresc.

RESUMEN

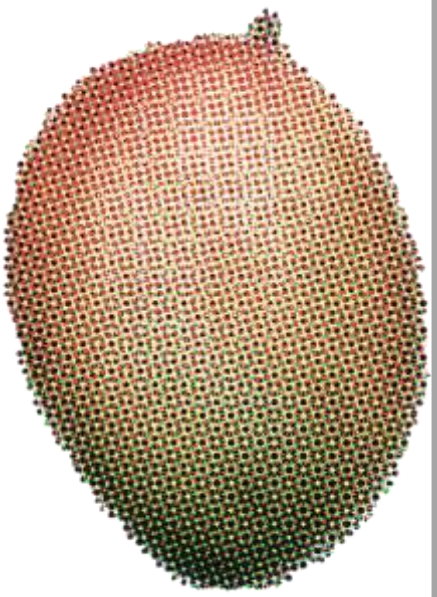
Recientes estudios sugieren una relación inversa entre el contenido de compuestos bioactivos de las frutas y el riesgo de padecer ciertos problemas de salud, como enfermedades cardiovasculares o algunos cánceres. En particular, el mango está descrito como fuente de compuestos bioactivos (compuestos fenólicos y vitaminas). Actualmente, los consumidores piden nuevos productos a base de frutas listos para el consumo, seguros, saludables y con propiedades similares a los naturales. Las tecnologías no convencionales de procesado suponen alternativas interesantes para obtener productos a base de frutas con atributos naturales. Por lo tanto, el objetivo de esta tesis doctoral fue evaluar el impacto de los tratamientos no térmicos (recubrimientos, *dipping*, pulsos de luz, pulsos eléctricos de alta intensidad de campo y altas presiones hidrostáticas) en los atributos de calidad (microbiológica, enzimática, fisicoquímica y bioquímica) de diversos productos de mango: cortado, zumo y puré.

Se evaluó el efecto de diferentes recubrimientos a base de polisacáridos, alginato (AL), quitosano (CH), pectina (PE) y carboximetilcelulosa (CMC) sobre la conservación de la calidad del mango cortado. El recubrimiento óptimo a nivel de seguridad microbiana fue el CH, el cual mantuvo la población microbiana por debajo de 10 UFC/g. A pesar que los recubrimientos estudiados mantuvieron el pH, el contenido de sólidos solubles y el color después de 14 días de almacenamiento a 4 ˚C, sólo el AL mantuvo la firmeza (2.5 N) y la aceptación sensorial (90.2 %). El mango recubierto con AL y CH mostró la máxima capacidad antioxidante y contenido en fenoles. Por otro lado, se determinó la influencia de los pulsos de luz (PL) combinado con el recubrimiento AL y la inmersión en ácido málico (MA) sobre la inactivación de *Listeria innocua*, las propiedades fisicoquímicas y el contenido de compuestos fenólicos en el mango cortado. Después de 14 días, en los mangos cortados se observó un efecto sumatorio de la combinación de PL (8 $J \cdot cm^{-2}$), AL (2 %) y MA (2 %) sobre la reducción de la carga microbiana, la conservación del color y firmeza. La combinación de los tratamientos PL, AL y MA mejoró el contenido de compuestos fenólicos en el mango cortado comparándolo con el no tratado.

En el zumo de mango, se estudió la influencia del tratamiento de pulsos eléctricos de alta intensidad de campo (HIPEF) en la carga de *Listeria innocua*. HIPEF a 35 kV/cm, 4 μs- pulso bipolar, 200 Hz y 1800 μs resultó ser efectivo para reducir la población de *Listeria innocua* a valores similares a la pasteurización. Además, tras 59 días, la actividad enzimática de polifenoloxidasa, lipoxigenasa y peroxidasa se redujo significativamente. El zumo de mango tratado por HIPEF conservó los atributos fisicoquímicos y el contenido en compuestos fenólicos se incrementó tras 59 días. La capacidad antioxidante y el contenido de carotenos en el zumo tratado por HIPEF, a diferencia del zumo tratado térmicamente, disminuyeron gradualmente a lo largo del almacenamiento.

En el puré de mango (variedades Tommy Atkins y Manila) se estudió el impacto del tratamiento de altas presiones hidrostáticas (HHP) (400, 450, 500 MPa de 0 a 16 min a 34 ó 59°C) sobre las propiedades fisicoquímicas y enzimáticas (pectinmetilesterasa, polifenoloxidasa y peroxidasa). El puré de mango tratado por HHP a 34 °C tuvo actividad enzimática más alta que el procesado a 59 °C. El tratamiento por HHP permitió mantener los valores de pH, la a_w y los sólidos solubles similares al puré de mango no tratado; sin embargo hubo diferencias significativas entre variedades. La viscosidad del puré de mango variedad Tommy Atkins aumentó a medida que se incrementó la presión y el tiempo de tratamiento. En el puré de mango variedad Tommy Atkins, comparado con el Manila, después de aplicar el tratamiento de HHP a 550 MPa y 59 °C disminuyó el índice de color amarillento aumentó y la luminosidad. Entre el 77 y 98 % de los carotenos totales se conservaron después del tratamiento de HHP. El contenido de compuestos fenólicos aumentó un 34 % tras el procesado con HHP (550 MPa, 59 °C y 4 min), probablemente debido al efecto de compresión que mejora la liberación de compuestos fenólicos de las células.

Los resultados obtenidos en la presente tesis doctoral mostraron que las tecnologías no térmicas minimizan los efectos de deterioro en los productos de mango a la vez que mantienen los atributos naturales de esta fruta tropical. Este trabajo aborda el procesado del mango, y los resultados contribuyen a darle un valor añadido, gestionar eficientemente su producción, pudiendo incorporar en la dieta de países no productores productos a base de mango con características similares al fruto fresco.



1. GENERAL INTRODUCTION

1 GENERAL INTRODUCTION

1.1. The importance of mango: from market trends to processing

Mango (*Mangifera indica* L.) is an economically important tropical fruit in the world. According to the FAO statistics (FAO, 2012), 93 countries were harvesting mango around the world. Mango cultivation is believed to originate over 1000 years ago, mostly by individual households for family consumption (Campbell & Ledesma, 2015). During the last decades, nine countries account for more than 80 % of total world production. India with 13.6 million tons is the main producer followed by China, Indonesia, Mexico, Thailand, Pakistan, Brazil and Nigeria (Naidua & Naidu, 2009) (figure 1). In addition, mango crop has been adapted in other countries such as Spain in which small production of 3500 ha is occurring.

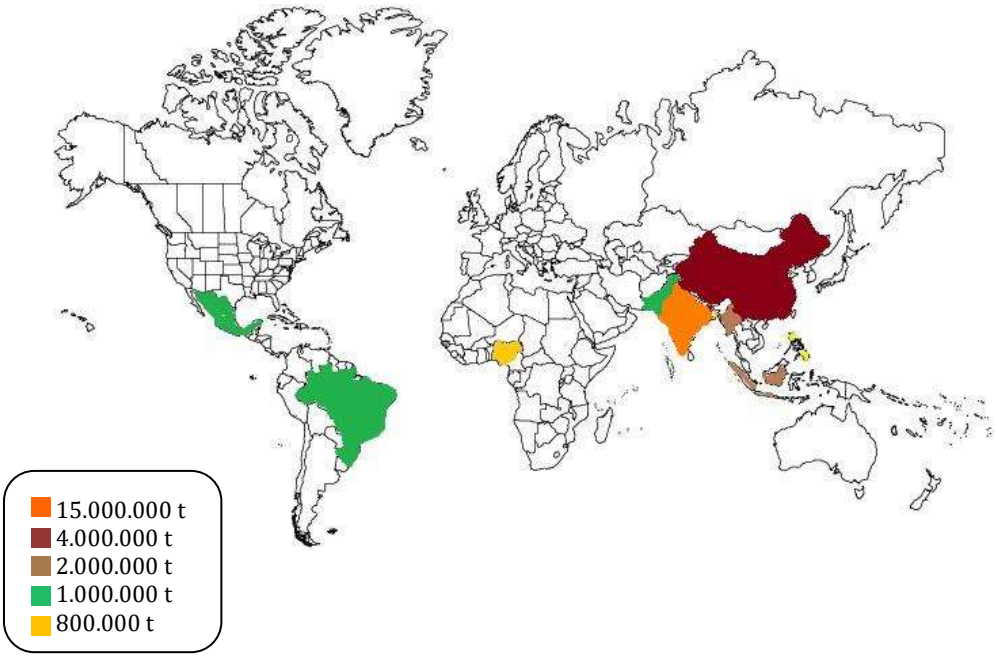


Figure: 1. Worldwide map of the main mango production countries (Saúco, 2013)

Total importation in EU had increased over time: the 120.000 t in 1997 was doubled by 2006 (Saúco, 2013). Nevertheless, market studies indicate several weaknesses in the EU market, where only United Kingdom, France, Germany, Portugal and Spain import quantities over 10.000 t/ year. In 2008, Spain started to be an important supplier of the EU market distributing 6.000 t of autumn-harvested mangoes- *cvs*. Keitt, Kent and Osteen (Sayadia & Calatravab, 2009).

There are hundreds of mango cultivars in the tropic and subtropical areas. Each country or region has also their own selected cultivars. Preferences for cultivars vary in different regions of the world. India, with more than 1000 named cultivars, has provided most of the germplasm for mango varieties development. Nevertheless, other areas such as Florida in US have developed a large number of cultivars, using mainly those from India. The Florida varieties are also dominant in international trade and include Tommy Atkins, Haden, Kent, Keitt and Van Dyke (Siddiq, Ahmed, Lobo, & Ozadali, 2012).

Mango varieties Tommy Atkins, Haden and Palmer are commonly found in global markets for fresh consumption, as they keep yellowish colour, texture and fruity flavour throughout at least 2-3 weeks of storage at 5-10 °C. Apart from these cultivars, Manila, Alphonso and Irwin, among others, are important in local markets since they show excellent hedonic properties as fleshy texture, juicy, sweetness and highly aromatic flavour, although they have shorter storage life than other varieties (Sinha 2012; Ribeiro et al. 2008).

Many processed products such as pulp, puree, juice and nectar, canned slices in syrup, chutneys, dried and jams are present in the markets. Compared to fresh fruit trade, the amount of processed mango that reaches the world market is insignificant, but is nevertheless worthy of mention since mango products are becoming more demanded by consumers. For instance, the demand for pulp and concentrates has grown drastically in recent years (FAO, 2003). Also, data on juice exportation have reported 7.778 t in 2006 and 12.079 t in 2007 (Campbell & Ledesma, 2015). Thailand is the main exporter of processed mangoes except for mango juices in which Egypt is the principal supplier. Otherwise, canned mango is the second best-selling single-fruit product in the world. Despites of the wide variety type of mango processed products,

mango industry is a rather young and small sector as compared to those of other fruits as banana or pineapple sector even the apple and orange sector (Saúco, 2013). However, domestic demand for different fruit products is a driving force for the mango industry to grow further (Jahurul et al., 2015; Ravani & Joshi, 2013).

The consumption of fruits and vegetables has been widely recommended in human diet in the last decades (González-Aguilar et al., 2008; Mitra, Devi, & Debnath, 2014). Particularly, Spanish government has included fruit in the main daily intake as it is shown in the food pyramid. Despite the fruit and vegetables recommendation, fruit intake by Spanish population has decreased from 2013 to 2014 (102.48 kg/person/day) (MAGRAMA, 2014).

Over the last few decades, the rapid socio-economic development and industrialization, accompanied by an increase in the urbanization, market globalization and time scarcity have transformed traditional lifestyle of most population, which has driven consumers away from healthy dietary habits as fruit consumption (Kamphuis et al., 2006; Nicklas, Baranowski, Cullen, & Berenson, 2001; Pearson, Atkin, Biddle, & Gorely, 2010). Consumers are requesting new fruit product that facilitate to introduce fruit in the diet. For instance, they are demanding products being easier to store and prepare, fresher, more natural, and less reliant on additive preservatives than foods that were previously available. Strategies to increase availability, affordability and shelf life will provide more options and opportunities for people to meet dietary recommendations.

In particular, previous researches described that population consumes mango because of its pleasant taste and flavour without much thought about the content of minerals, vitamins, lipids and amino acids (Barreto et al., 2008; Ribeiro et al., 2008; Sogi, Siddiq, Roidoung, & Dolan, 2012). However, mango is an excellent source of provitamin A and considered a fair source of vitamin C, although this varies greatly among cultivars, with a range between a low of 7 mg and as high as 172 mg/100 g of fresh mango (Gorinstein et al., 2011). Subsequently, the study of processing processes to maintain health compounds of mango as well as the safety of its derivatives is a current challenge.

1.2.Post-harvest mango quality

Mango is a climacteric fruit and therefore its respiration rate rises as it reaches full maturity and then begins to be over-ripened (Siddiq et al., 2012). After reaching the climacteric peak, respiration behaviour tends to decline until cell death occurs. Once detached from the plant, mangoes, as living tissues, suffer a cascade of metabolic reactions that can result in accelerated ripening, physical changes and, finally, senescence. To undergo an appropriate handling and subsequent processing of mango, the fruit should be harvested at pre-climacteric or very early climacteric stages (Sinha 2012). Although the ripening of whole mango depends on the variety, this fruit is best harvested in a mature but unripe stage, about 2-4 moths from blooming and usually transported in a firm green pre-climacteric stage. Moreover, whole mangoes could be stored under refrigeration (5-10 °C) to keep acceptable characteristics up to 2-3 weeks. The optimum relative humidity (RH) during storage is between 85- 90 % RH, although for short storage periods (5-7 days) values of 90-95 % RH could be also accepted (Siddiq et al., 2012).

Mango ripe pulp is also used for the beverage industry and for food formulations as flavouring ingredient. This fruit is a valuable source for medicinal, nutritional and industrial purposes (Rymbai, Srivastav, Sharma, Patel, & Singh, 2013) (table 1). During postharvest, an optimal quality regarding physical aspects is needed. Thus, mango fruit with 72-86 % of moisture, 14-23 ° Brix and pH of 3-4.5 would be sufficient for further mango processing, although differences among varieties should be considered (Siddiq et al., 2012).

Quality can be defined in terms of microbial, nutritional and physicochemical stability. The complex perception of many attributes that are objective or subjectively evaluated conform the quality of food (FAO, 2004). Depending on the type of fruit and nutritional composition, processing quality loss could happen. Therefore, preservation treatments are important to maintain fresh-like characteristics of fruit along the shelf-life (Sinha, Hui, Evranuz, Siddiq, & Ahmed, 2010). The quality targets to be preserved in fruit products include microbiological, enzymatic, physical

and biochemical parameters on which an accurate study of the adequate processing must be addressed. Table 2 shows the most common changes on fruit during postharvest considering physical, microbial enzymatic and biochemical attributes. Reducing the loss of quality attributes, consumers would have access to fruit products such as fresh-cut, juice or puree with fresh-like characteristics.

Table 1: Mango ripe pulp composition in 100 g fresh weight (FW)

Component	Content (100 g^{-1} FW)
Water (g)	78.90 – 82.80
Carbohydrate (g)	16.20- 17.18
Protein (g)	0.36 – 0.40
Fat (g)	0.3 - 0.53
Fibre (g)	0.85 – 1.6
Calcium (mg)	6.10 – 12.8
Ascorbic Acid (mg)	7.80 – 172.00
Vitamin A (mg)	0.13 – 1.87
α – Tocopherol (mg)	0.90 - 1.12

Source (Wall-Medrano et al., 2015)

Table 2: Common changes in quality attributes of fruits during post-harvest and processing

Quality attributes	Post-harvest changes of fruit
Physical	- Compound leaching - Loss of crisp texture - Loss of flavours - Freeze damage
Microbial	-Growth of toxicogenic microorganism - Growth or presence of infective microorganism - Growth of spoilage microorganism
Enzymatic	- Hydrolytic reactions catalysed by lipases, proteases and other enzymes - Rancidity catalysed by lipoxygenases -Enzymatic browning -Deterioration of nutrients
Biochemical	- Oxidative rancidity - Oxidative and reductive discoloration - Non-enzymatic browning - Destruction of nutrients

1.2.1. Physical aspects

1.2.1.1. Colour

Mango fruit has an appreciated yellow colour, although differences on the yellowish can be observed among varieties. Studies in Tommy Atkins, Ataulfo and Kent mango varieties has described very similar L* values around 60 and b* values ranging from 30 to 40 (Gonzalez-Aguilar et al., 2008). Browning phenomena, which promotes the loose of mango characteristic colour, appears when an increase of L* and a* parameters occurred simultaneously with a decrease of b*. For instance, in fresh-cut Ataulfo mango, a* and b* values changed from 4.02 to 6.59 and 69 to 49 after 15 days, respectively, hence, a loss of yellow colour occurred (Robles-Sánchez, 2009). Enzymatic browning, which is caused by enzymes liberated from tissues due to mechanical operations, is mainly caused by polyphenoloxidase enzyme (PPO), which in the presence of oxygen and a substrate, converts phenolic compounds into dark-coloured pigments (Martin-Belloso & Fortuny, 2010). Also, light and high oxygen availability has an effect on the oxidative reactions occurring in the surface of fresh-cut mango leading to the change of colour. Nevertheless, the characteristic yellowish colour was maintained throughout 9 days in minimally processed mango under refrigerated storage (5 °C) (Gil, Aguayo, & Kader, 2006; Robles-Sánchez, 2009).

1.2.1.2. Texture

Mango has been described as a fibrous fruit with firmness values between 5- to 8 N, (Salinas-Hernández, González-Aguilar, & Tiznado-Hernández, 2013). Texture is a quality attribute that changes easily in postharvest of mango because of wounding effect (González-Aguilar, Wang, & Buta, 2000). Generally, mango firmness might be reduced in postharvest processing of mango during storage. On one hand, it has been shown that pectinolytic and proteolytic enzymes may be the main factors affecting softening when they are delivered from bruised cells during slicing operations. Cutting operation increases the activity of pectinmethylesterase among other pectinolitic enzymes, thereafter the pectin content and cell turgor are reduced (Skibsted, Risbo, & Andersen, 2010). On the other hand, mechanical operations cause

moisture losses in mango pieces that lead to loss of turgidity and an unacceptable appearance. The softening effect has been delayed by the use of some preservation treatments as it is represented in figure 2, where a decrease of firmness was observed in three different mango varieties (Gonzalez-Aguilar et al., 2008). Furthermore, the use of calcium dipping and low storage temperatures (5- 10 ˚C) minimize the effects of mechanical injuries because they allow to reduce enzymatic activity and water loss reduction.

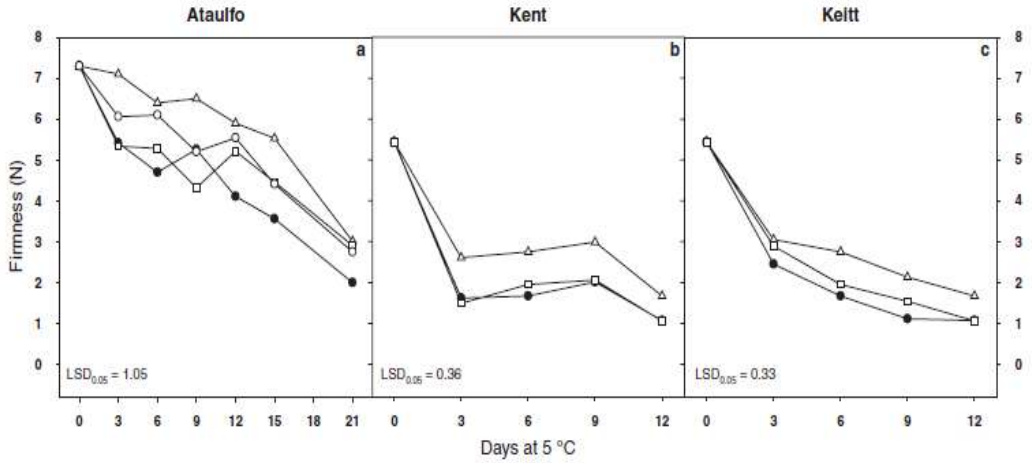


Figure 2: Firmness of fresh-cut (a) Ataulfo, (b) Kent and (c) Keitt mangoes treated with antioxidants or edible films (Gustec or SemperFresh) during storage at 5 °C. For all figures, antioxidants: 2 % ascorbic acid + 2 % citric acid; edible coatings: 2% gustec or simper fresh. Data from Semper Fresh are not shown in panels b and c as those were not significantly different from control Source: Gonzalez-Aguilar et al., 2008

1.2.1.3. Flavour

Naturally ripped mangoes have characteristic taste and flavour. Munafo et al. (2014) reported that ethyl butanoate, HDMF and 3-(methylthio) propanal and 4-hydroxy-2,5-dimethyl-3(2H)-furanone are the major volatile components in five different mango varieties. The flavour in mangoes could be affected by oxidative reactions leading to off-odour. Overall, fresh-cut mangoes preserve its original aroma better and appearance is kept similar to that of the fresh mango when the loss of water is diminished. The dehydration process during non-adequate storage might trigger to

loss of the characteristic mango flavour. Plotto et al. (2010) observed an improvement of the sensory profile (sweetness, tartness and fruity flavour) of fresh-cut mango with different preservation treatments such as edible coatings containing organic acids. They noted that the sweet taste was reduced after 5 days, although fruity taste flavour was increased. Preservation treatments and adequate packaging help in maintaining the volatile compounds that give the characteristic mango flavour.

1.2.2. Microbiological aspects

The microflora of fruits comprises microorganisms on produce peel and pulp. The normal microflora of the outer surface of fresh fruits would vary according to the microbiologically safe conditions/practices followed during post-harvest: handling, processing, storage, and distribution. The foodborne diseases are caused by a wide range of agents with different degrees of severity ranging from mild indisposition to chronic or life-threating illness. Thus, the presence of some microorganisms in mango products must be controlled in order to assure food safety.

Mango can be a vehicle for pathogenic bacteria. In the last years numerous outbreaks of salmonellosis and listeriosis in mangoes or foods elaborated with this fruit have been reported (Penteado, de Castro, & Rezende, 2014). In spite of the protection offered by the fruit peel, pathogenic bacteria can survive in a wide range conditions. In fruits of low acidity, in which mango is included, *Listeria innocua* also find the conditions to survive and multiply. Growth and survival of these bacteria was already reported in other fruits and their derivatives. Thus, it is important to know the behaviour of the bacteria under different processing and storage conditions.

Nowadays, the major concern of food preservation is the control of microbial growth to ensure product safety and to prevent spoilage, which makes the fruit products undesirable and then discarded. Concerning mango products, over the years 3 groups of microorganisms: aciduric bacteria, moulds and yeasts have been reported as the most important ones since they are acid tolerant.

Prevention and control of microbiological hazards could be achieved by the application of food-processing technologies that can prevent or reduce the use of

chemicals in food. Processed foods may be safer than fresh foods and extend shelf-life of fruit products by removing, inhibiting or killing pathogenic microorganism.

1.2.3. Enzymatic activity

Enzymes are proteins whose catalytic activity relies on the native configuration of their active site and the conformation of the surrounding proteins (Hendrickx, Ludikhuyze, Van den Broeck, & Weemaes, 1998). Enzymes are very important for growth and ripening of fruits. Nevertheless, some enzymes have also undesirable effects on colour, flavour, texture and nutritional value of fruit derivatives (Jamsazzadeh Kermani et al., 2015; Quitão-Teixeira, Aguiló-Aguayo, Ramos, & Martín-Belloso, 2007). Thus, activities of endogenous deteriorative enzymes considerably shorten the shelf life of fruits and vegetable products.

Two main groups of enzymes are common in mango fruit: oxidative and pectinolitic enzymes. Activity of lipoxygenase, phenoloxidases and peroxidise are related to adverse oxidative effects on browning colour, flavour, taste and nutritional quality. On the other hand, mangoes contain pectic substances that have major effects on the texture. The activity of pectinases such as pectinmethylesterase and polygalacturonase causes fruit softening. α – amylase degrade starch to shorter polymeric fragments affecting textural integrity of fruit and fruit products (Navarro, Izquierdo, Carbonell, & Sentandreu, 2014; Vivar-Vera, Salazar-Montoya, Calva-Calva, & Ramos-Ramírez, 2007).

The inactivation of the quality deteriorative enzymes is very important during fruit processing. Consumer demand for convenient, yet healthy, food products has spurred development of pre-peeled or fresh-cut produce. Enzymatic browning dimminish visual acceptability (Aguiló-Aguayo, Soliva-Fortuny, & Martín-Belloso, 2007). The challenge for food scientists is to find an enzyme inhibitor that will not adversely affect sensory properties at safe and effective use levels. Generally, thermal treatment is used for industrial inactivation of enzymes (Raso & Barbosa-Cánovas, 2003). Nevertheless, novel technologies such as pulsed electric fields, high pressure and

ultrasound or a combination of these technologies can also be used as alternatives to thermal treatment.

1.2.4. Bioactive compounds

Mango contains phenolic compounds, carotenoids and ascorbic acid that have antioxidant capacity and therefore they are a powerful defence against degenerative oxidative diseases in the fruit (Watson & Preedy, 2009). Nutritional composition of mangoes varies among cultivar, harvest conditions, maturity, postharvest conditions and processing. During minimally processing, a decrease of antioxidant bioactive content can occur due to the wounding response and other deleterious reactions (Sinha, 2012). Although a slight loss of the content in bioactive compounds comparing whole and fresh-cut mango exists, processed mango keeps being a rich source of polyphenolic compounds, carotenoids and vitamins (table 3) (Robles-Sánchez et al., 2011; Sarkiyayi, 2013).

Table 3: Total phenols (TP), β- carotene, vitamin C and vitamin E in whole (WM) and fresh-cut mango (FCM) stored at 5 °C for 10 days (Robles-Sánchez et al., 2011).

Samples	**TP** (mg GAE·100g^{-1} FW)	**β-carotene** (mg·100g^{-1} FW)	**VitaminC** (mg·100g^{-1} FW)	**VitaminE** (mg·100g^{-1} FW)
WM	110.7 ± 1.1^{a}	4.47 ± 0.31^{b}	93.5 ± 4.5^{b}	0.92 ± 0.02^{a}
FCM	116.0 ± 1.2^{b}	3.03 ± 0.08^{a}	86.7 ± 6.3^{a}	0.93 ± 0.03^{a}

Values are means ± SD of five measurements. Means in columns without letters in common differ significantly ($p \leq 0.05$).

1.2.4.1. Phenolic compounds

Phenolic compounds have an important contribution to the antioxidant capacity of mango (Sogi et al., 2012). Mangiferin, gallic acid, quercitin, vanillic acid and catechin are among the polyphenolic compounds identified in mango flesh (Masibo & He, 2008) (figure 3). Phenolic compounds are affected by peeling but cutting could trigger their degradation. Wounding response stimulates two main enzymes: phenylalanine ammonio-lyase (PAL) and PPO, which are directly involved in phenolic compounds pathways (Martin-Belloso & Fortuny, 2010). The PAL

activation can elicit by reactive oxygen species (ROS) with further production of phenolic compounds. On the other hand, the decompartimentation of fruit membrane cells allows the oxidation of phenolic compounds by PPO, thus, browning of cut mango surface can occur (Charles, Vidal, Olive, Filgueiras, & Sallanon, 2013). Moreover, phenolics are degraded by exposition to light, oxygen and high temperatures throughout storage.

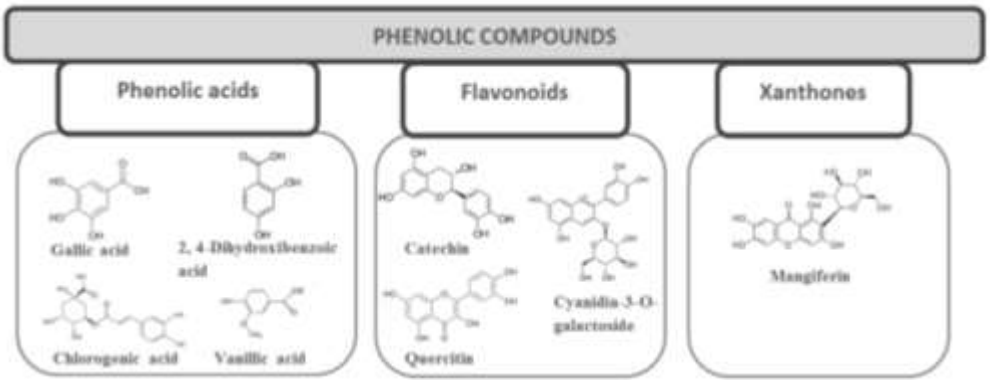


Figure 3: Chemical structure of main phenolic compounds in mango fruit

An increase of gallic acid equivalent concentration in mango products comparing with just harvested fruit is generally observed due to the release of the compounds from the cell after mechanical operations. Nevertheless, phenolic compounds loss could happen along the postharvest and storage. Preservation techniques could enable to maintain phenolic compounds concentration in mango derivative after 10 days under refrigeration (Robles-Sánchez et al., 2011). However, mango showed a slight decrease from 11 to 9 mg/100g fw in the total phenolics content from day 0 to 3 respectively, but the content was maintained thereafter for 9 days under refrigerated storage (Gil et al., 2006).

1.2.4.2. Carotenoids

The predominant carotenoid in mango is β–carotene, which is affected by variety, ripening, cutting processing and storage conditions (figure 4) (Mercadante & Rodriguez-Amaya, 1998; Pierson et al., 2014). Mango ripening produces an increase in β–carotene content, which is more significant at room temperature than under refrigeration, and may be due to an increase in mevalonic acid and geraniol syntheses, which lead to higher levels of total carotenes (Gonzalez-Aguilar et al., 2008). Minimally processed mango preserves the original carotenoid concentration between 4-5 mg/ 100g fw for at least 5 days at 5 ˚C (Robles-Sánchez, 2009), although other studies have shown longer stability (2 weeks) (Gil et al., 2006; Robles-Sánchez et al., 2011). The loss of carotenoids during storage could be influenced by temperature and oxygen concentration within the package, variables that allow the acceleration of this metabolism.

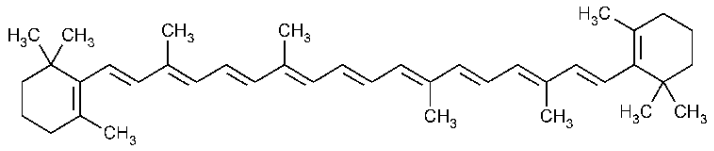


Figure 4: Chemical structure of beta-carotene

1.2.4.3. Vitamins

Vitamin C is present in high concentration in mango. The concentration in ready-to-eat Ataulfo mango (75 mg/ 100g fw) does not differ significantly from the fresh flesh (77 mg/ 100g fw) during the first week of storage (Gil et al., 2006). Nevertheless, a decrease, which could be promoted by processing, has been observed during storage of 15-20 days at 5-10 °C by Robles-Sánchez et al. (2009). Thus, after mechanical operations such as fresh-cut mango processing, the decrease of water content and oxidation reactions is associated with significant losses of vitamin C.

Also, it has been reported 1.3 mg/ 100g fw of vitamin E in mango, which is mainly present as α-tocopherol form (Robles-Sánchez, 2009). Vitamin E can easily suffer lipidic oxidation by light effect throughout the storage (Robles-Sánchez, 2009). The incorporation of antioxidant compounds in the surface of fresh mango is used to prevent losses of hydrophilic and lipophilic vitamins.

1.3.Minimally processed mango

Requirements of consumers toward food have drastically changed over the last decade, demanding less processed, more convenient and fresher as well as natural and healthier food products. Consequently, there is an increasing demand to produce fruit products with superior quality and long shelf life. For this reason, the study of postharvest mango requirements and processing conditions are needed to get high-quality minimal processed mango with fresh-like attributes throughout storage.

Minimal processing is defined as the treatment to achieve least changes in final product quality characteristics as well as during storage and distribution (Ohlsson & Bengtsson, 2002). According to this, minimal processed mango has to keep previously described quality attributes of fresh fruit with minimum variations throughout the storage. The integration of all aspects from field to fork including postharvest handling operations can determine the final quality of processed products.

Mango undergoes physiological changes during handling and processing, increasing its respiration rate, oxidation reactions, colour changes and losses of nutrients because of diverse biochemical and enzymatic reactions. Furthermore, metabolic changes are accelerated when the natural barrier of skin is removed by peeling and cutting. Then, microbial load could be modified and even, when handling is not adequate, microbial growth could highly reduce its shelf-life compared with the whole mango. Therefore, during minimal processing of mango products, two main objectives are sought: to maintain the fresh-like characteristics of flesh (color, flavor, texture and nutritional

value) and to assure the safety of the product as well as to extend shelf-life long enough to allow marketing of fresh-cut mango.

1.3.1. Mango products

Mango is fragile, and significant postharvest losses occur in producing countries due to ripening, inadequate handling and transportation and storage practices. Because of the short shelf life of fresh mango, which limits its marketability, to process mango products into various shelf-stable derivatives in order to minimize post-harvest losses has an interest. Therefore, mango products such as fresh-cut, juice and puree that represent a major potential market can be designed and manufactured using emerging technologies.

1.3.1.1. Fresh-cut mango

According to the International Fresh Produce Association (IFPA), fresh-cut fruits are those peeled, cut in pieces and ready-to-eat. These products have also been referred as lightly processed or minimally processed (Sinha et al., 2010).

In figure 5, stages or processes for the production of fresh-cut mango are shown. Fresh-cut mango processing includes three basic mechanical operations: peeling, seed removal and cutting. All of these operations provoke increasing in the respiration rate and the production of ethylene after one hour of processing, accelerating the maturity and senescence of fresh-cut mango (Souza, Durigan, Donadon, Teixeira, & Durigan, 2005). Moreover, wounding effect undergoes the degradation of tissues by the cell wall enzymes, mainly pectinmethylesterase and polygalacturonase, which consequently induces physicochemical changes. Also, the phenolic production and wound-healing metabolism is initiated during the mechanical operations of fresh-cut mango processing (Siddiq et al., 2012).

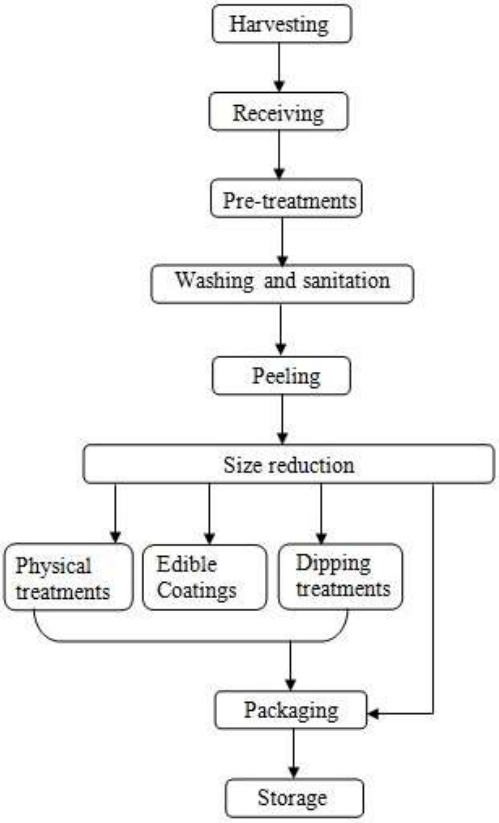


Figure: 5: Flow diagram of fresh-cut mango produce adapted from Sinha (2012)

The deleterious processes as wounding effect and fresh-cut mango metabolism acceleration have an influence on the final product. Thus, quality changes in fresh-cut mango, including softening, loss of sugars, bioactive compounds content, moisture and weight, appearance of undesired flavour and colour, occur. Furthermore, fresh-cut mango, could suffer chilling as well as water and heat stress during processing. To avoid the undesired changes and obtain a high-quality final product, the cold chain during whole processing must be maintained. Temperature conditions during mechanical operations (10-20 ˚C) and storage (4-5 ˚C) need to be controlled. A refrigerated storage enables fresh-cut mango to reduce the physiological deterioration and be marketable after 9 days (Gil et al., 2006). On the other hand, preservation methods and package conditions could also reduce the deleterious reactions triggered

by processing operations and extend the shelf-life of fresh-cut mango (Rojas-Graü, Tapia, Rodríguez, Carmona, & Martin-Belloso, 2007).

1.3.1.2. Juice and puree of mango

Juice and purees represent a very important segment of total processed fruit industry. Moreover, juices are being marketed as refrigerated, shelf-stable and frozen in a variety of packages with increased emphasis on functionality, health attributes, new flavours or blends, and in some cases fortified with vitamins and minerals (Koutchma, Popović, Ros-Polski, & Popielarz, 2016). In order to satisfy consumer demand for juices with fresh-like characteristics, fresh-juices have become highly popular beverages in the global food market despite their very short shelf-life. Also, novel processing methods to extend shelf-life while preserving natural attributes on juice and pure products are being seek.

The handling and processing for juice and purees production begins with the freshly mango harvested the field or taken from refrigerated or frozen storage. After the storage, mango is sanitized that rely on hygienic conditions to ensure the safety of perishable products. In juice products fruit is completely destroyed and transformed by the extraction (pitting, crushing, heating and enzymatic treatment). On the other hand, puree, which is the principal product obtained from mango, is commonly used to prepare nectars, juices and other foodstuffs (Jahurul et al., 2015).

1.3.2. Processing technologies for mango products

Throughout the years, processing technologies for food preservation have been in constant development in order to meet current consumers' claims. Prolonged shelf-life in juices has traditionally been associated with thermal processing alone or in combination with chemical preservation. Heat processing, however, tends to reduce product quality and freshness (Ohlsson & Bengtsson, 2002b). Thus, non-thermal liquid food preservation technologies have been developed as alternatives to heat

treatments in order to obtain products that maintain their nutritional and sensorial attributes as unchanged as possible.

In this section both thermal and non-thermal processing technologies are presented. Therefore, dipping, edible coating, pulsed light, high pulsed electric fields and high hydrostatic pressure processing at either ambient or mild temperature are among the emerging technologies that can help in leading high-quality mango products.

1.3.2.1. Thermal treatments

Thermal treatments (TT) are extensively used for the preservation and preparation of foods. The classical approach to overcome or at least minimise these undesirable quality changes in thermal processing is the high temperature short time concept.

The application of heat on food products will give the rapid inactivation of microorganisms and enzymes. The effectiveness of TT is based on the fact that the inactivation of microorganisms, whereas many undesirable quality changes depend on the duration of the heat treatment.

Foods which are thermally treated can be either solid or liquid. Thermal processes vary considerably in their intensity, ranging from mild process (i.e. pasteurisation) through to more severe conditions (sterilisation). Currently fruit juices are treated using pasteurisation. The process of pasteurisation applies mild temperatures (90-95 ˚C) during short times (15-60 seconds) in order to inactivate pathogenic microorganism and reduce microbial growth and enzymatic activity (Gonzalez & Barrett, 2010). Nevertheless, undesired chemical, physical, phytochemical and sensorial changes in fruit products such as orange juice have been observed (Cortés, Esteve, & Frígola, 2008).

1.3.2.2. Non-thermal treatments

Over the recent years, non-thermal processes have gained importance as a potential technology to replace or complement the traditional thermal processing of foods. The non-thermal processing at low processing temperature and short residence times

allow a highly effective microbial and enzymatic stability while retaining product quality (Moody, Marx, Swanson, & Bermúdez-Aguirre, 2014). Many fruit products treated with non-thermal technologies have recently drawn the interest of the scientific community as they might preserve fresh-like characteristics of fruits. For instance, fruit juices such as: apple, orange and carrot treated by non-thermal technologies showed better preservation of sensorial and nutritional characteristics than conventional treatments (Chen, Yu, & Rupasinghe, 2013).

Dipping treatments with antimicrobials, antibrowning agents and texture preservatives as well as edible coating application, among other preservative methods, have been studied to assure the quality of minimally processed mango. The combination of different preservation methods could also be a feasible way to achieve better fresh-cut product quality. In this sense, the combination of dipping, edible coating and physical treatments have been proven to achieve a higher reduction of microbial load in fresh-cut mango keeping fresh-like quality parameters, thus, leading to extend the shelf-life of the product (Salinas-Roca et al. 2014).

On the other hand, the application of high intensity pulsed electric field (HIPEF) and high hydrostatic pressure (HHP) processing are among the emerging non-thermal technologies undergoing intensive scientific evaluation for liquid food products. HIPEF and HHP have reported to allow microbiologically stable fruit juices and purees as well as better preservation of sensorial and nutritional characteristics than conventional treatments (Bermúdez-Aguirre & Barbosa-Cánovas, 2010; Y. Chen et al., 2013). Despite of the noteworthy literature using HIPEF treatment for fruit juices quality preservation, no studies comparing the effects of HIPEF and thermal treatment on quality changes of mango juice have been found.

1.3.2.2.1. Dipping treatments

The incorporation of chemical agents in dipping solutions is the most common way to control deleterious phenomena such as browning or microbial growth. The antibrowning and antimicrobial action of organic acids and essential oils generally regarded as safe (GRAS) allow extending shelf-life of fresh-cut fruits (Oms-Oliu et

al. 2010). Organic acids can also be effective in preventing fresh-cut mango browning (González-Aguilar et al., 2000). The use of citric acid is widely extended as it helps to keep colour and the pH reduction in fresh-cut mango, which makes possible to extend its shelf-life by avoiding microbial growth (Chiumarelli, Ferrari, Sarantópoulos, & Hubinger, 2011). Nevertheless, a 9 % of weight loss in citric acid dipped fresh-cut mangoes was observed. Also, vanillic acid has been tested in fresh-cut mango against spoilage microorganisms, the use of 80 mM of vanillic acid delays the growth of microorganisms up to 14 days (Ngarmsak et al., 2006). On the other hand, other chemicals such as calcium chloride can be incorporated in dipping treatments to maintain the turgidity of the cell membrane allowing a texture similar to the fresh-fruit throughout the storage (Oms-Oliu et al. 2010). In addition, the combination of chemical agents could even enhance the final quality in fresh-cut mango. Siddiq et al. (2013) determined that ascorbic, citric acid and calcium chloride at 1, 1 and 0.5%, respectively, maintained similar colour, firmness and soluble solids content than fresh-cut mango during 12 days at 4 °C.

1.3.2.2.2. Edible coatings

Edible coatings (EC) are materials such as polysaccharides, proteins and lipids that are either applied to or formed directly on fresh-cut fruit surface. EC used in minimally processed mango can act as a barrier to external elements, protecting the fruit and reducing the changes on its characteristic aroma and weight (figure 6).

Common coatings used in fresh-cut mango are cassava starch (Chiumarelli & Hubinger, 2012), sodium alginate (Robles-Sánchez et al., 2013), carboxymethylcellulose, chitosan and carrageenan (Plotto et al., 2010). The use of sodium alginate in mango pieces using calcium chloride as a cross-linker let maintain fresh-like characteristics as texture and colour (Chiumarelli et al., 2011). Table 4 shows EC as alginate, chitosan, carboxymethylcellulose and pectin which can preserve texture, colour, pH and acidity of fresh-cut fruits in concentration between 0.5 to 2 % (w/v) (Djioua et al., 2009; Ducamp-Collin, Reynes, Lebrun, & Freire, 2009; Plotto et al., 2010). Although no antimicrobial effects were observed for most EC, chitosan used as a coating in fresh-cut mango could reduce microbial growth.

Hence, chitosan maintains both physicochemical and microbiological quality extending the shelf-life of mango pieces up to 10 days (Campaniello, Bevilacqua, Sinigaglia, & Corbo, 2008; Chien, Sheu, & Yang, 2007). In order to assure high-quality fresh-cut fruit, the incorporation of antimicrobial essential oils or organic acids to EC is recommended (Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2009).

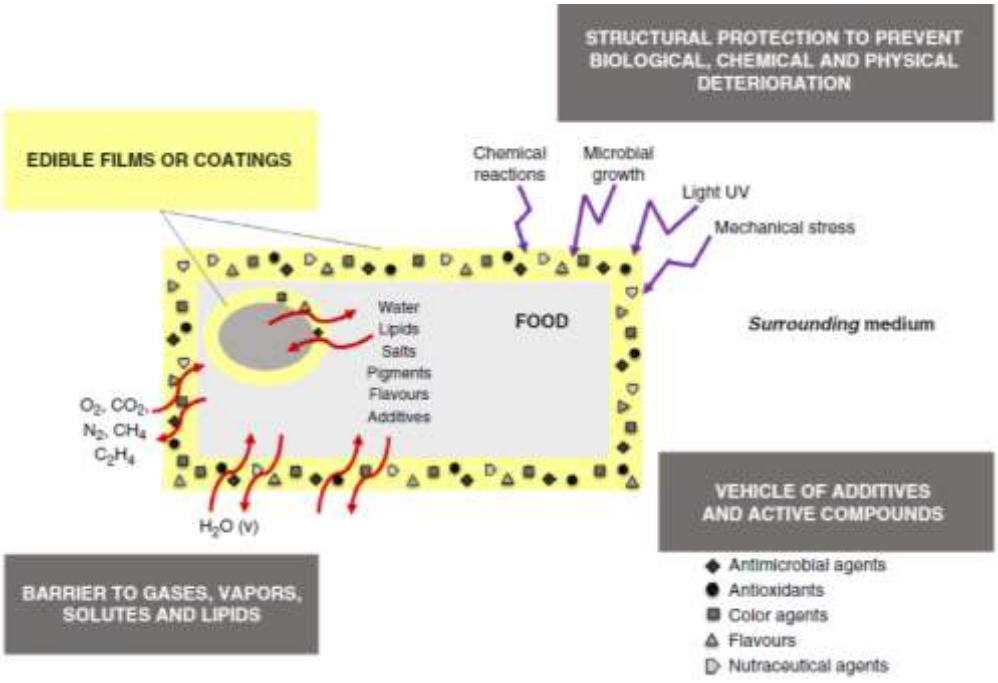
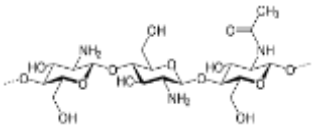
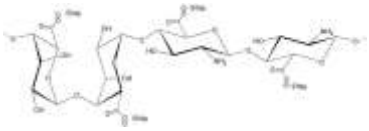
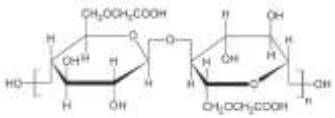
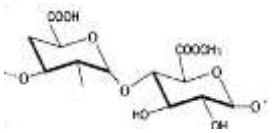


Figure 6: Main functions of edible films and coatings in food. Source: Salgado et al. (2015)

Table 4: Physical properties of main polysaccharide-based edible coatings

Polysaccharide – based edible coatings	Edible coating properties		
	Structure	Barrier properties	References
Chitosan		Low WVP 8.60 $gm^{-1}{\cdot}s^{-1}{\cdot}Pa^{-2}$ Less hidrophilic	(Silva-Weiss, Ihl, Sobral, Gómez-Guillén, & Bifani, 2013)
Alginate		30 $gmmkPa^{-1}{\cdot}d^{-1}{\cdot}m^{-2}$	(Wang, Liu, Holmes, Kerry, & Kerry, 2007) (Silva-Weiss et al., 2013)
Carboxymetilcellulose		60 $gmmkPa^{-1}{\cdot}d^{-1}{\cdot}m^{-2}$ High barrier o2, co2 Low WVR	(Bonilla, Atarés, Vargas, & Chiralt, 2012)
Pectin		Low $0.06 \cdot 10^{-13}$ g/msPa	(Espitia, Du, Avena-Bustillos, Soares, & McHugh, 2014)

1.3.2.2.3. Pulsed light

Pulsed light (PL) treatments is an emerging postharvest process developed initially as non-thermal sterilization technology to maintain safety of vegetable products free of any foodborne. Figure 7 shows a schematic representation of PL chamber, which consists mainly of a light flash, a sample shelf and a control panel. The target sample is placed on the sample shelf and a filter may be located in other tray above. PL

works with xenon lamps that can produce intense and short time pulses (100 – 400 μs) of broad spectrum "white light", from ultraviolet wavelengths of 180 nm to infrared wavelengths of 1100 nm. The power is magnified many times by storing energy in a high power capacitor over relatively long times (fractions of a second) and releasing it over a short period of time producing several high energy pulses per second. This phenomenon increases the instantaneous energy intensity that contributes to the inactivation of both spoilage and pathogenic microbial cells (González-Aguilar et al. 2007). Decontamination is attributed to a cell membrane damage in the microorganism, hence the growth of microbial population is limited and the shelf-life extended (Oms-Oliu, Martín-Belloso, & Soliva-Fortuny, 2008). Several critical parameters should be considered to assess the suitability of PL, such as type of microorganism, energy dose supplied, the number of pulses and the depth of the samples (Jiménez-Sánchez, Lozano-Sánchez, Segura-Carretero, & Fernández-Gutiérrez, 2017a; Pataro, Sinik, Capitoli, Donsì, & Ferrari, 2015). The effectiveness of the PL treatment is limited by its low degree of penetration or because of a shadow effect.

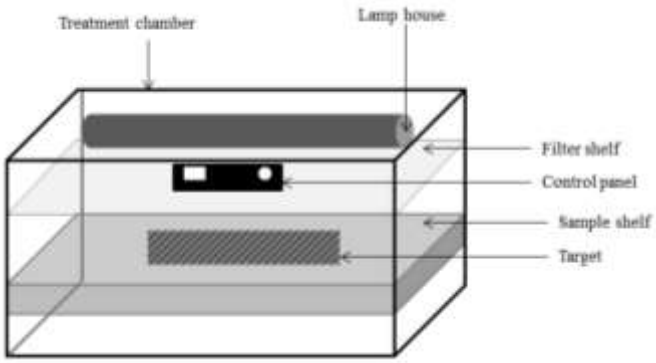


Figure 7: Schematic representation of a bench experimental unit of pulsed light

The first work on disinfection with flash lamps was performed in the late 1970s in Japan (Gómez-López, Ragaert, Debevere, & Devlieghere, 2007). The application of

PL has two main benefits: the decontamination and the activation of the bioactive compounds pathway. The lethal effect of PL on microorganisms is attributed mostly to the photochemical action of ultraviolet part of the spectrum emitted by the flash lamp. Additionally, light at low wavelength (λ = 100- 280 nm), preserve phenolic content in fresh-cut mango from 0.34 mg/g fw at day 0 to 0.46 mg/g fw after 15 days (González-Aguilar et al. 2007).

1.3.2.2.4. High intensity electric fields

High intensity electric fields (HIPEF) have shown to be a potential technology to preserve liquid food with fresh-like characteristics. The concept of applying pulsed discharges of high voltage across two electrodes to inactivate microorganisms in foods was firstly investigated in 1950s.

Basically, HIPEF involves the application of high-voltage pulses (20-80 kV/cm) for short periods of time (ms or μs) to a product confined or flowing between two electrodes (Vega-Mercado et al., 1997). As is shown in figure 8, a typical HIPEF unit consists of a pulse generator, treatment chambers, a fluid-handing system and monitoring and control devices. The HIPEF treatment chamber, which is one of the key factors for achieving the highest effectiveness of the process, is used to house the electrodes and deliver the high voltage to the food (Morales-de la Peña, Elez-Martínez, & Martín-Belloso, 2011). Besides treatment chambers, other parameters such as: electric field strength, treatment time, temperature, pulse frequency, width and polarity are critical factors that have an influence on process effectiveness.

Experiments conducted revealed that HIPEF can be mainly used for liquid food but also for mass transfer improvement prior to dehydration, extraction or pressing. It is generally accepted that using HIPEF in food can cause: breakdown of cell membrane, stress reactions and secondary metabolite biosynthesis, which can be desirable food constituents (Caminiti, Noci, Morgan, Cronin, & Lyng, 2012; Timmermans et al., 2014b). Based on the phenomenon called electroporation, microbial membrane and enzymes could be damaged and even inactivated when irreversible electroporation occurs. The process of pore formation occurred without any appreciable temperature

rise or chemical or physical changes in some food materials (Huang, Tian, Gai, & Wang, 2012; Ivorra, Villemejane, & Mir, 2010).

HIPEF is based on the properties of liquid food to conduct electricity due to their high concentration of ions and to their capability to transport electric charges. The effectiveness of HIPEF processing depends on factors coming from the parameters related to the treatment, as well as the intrinsic features of the product (Timmermans et al., 2014b) . Consequently, electrical conductivity, pH, ionic strength, viscosity, content of nutrients and suspended particles are among the intrinsic characteristics of the product that determine the effectiveness of HIPEF treatment. Scientific studies have demonstrated that HIPEF treatment keeps better product quality than conventional sterilization, but it does not effectively destroy spores or other thermal resistant microorganisms (Vervoort et al., 2011).

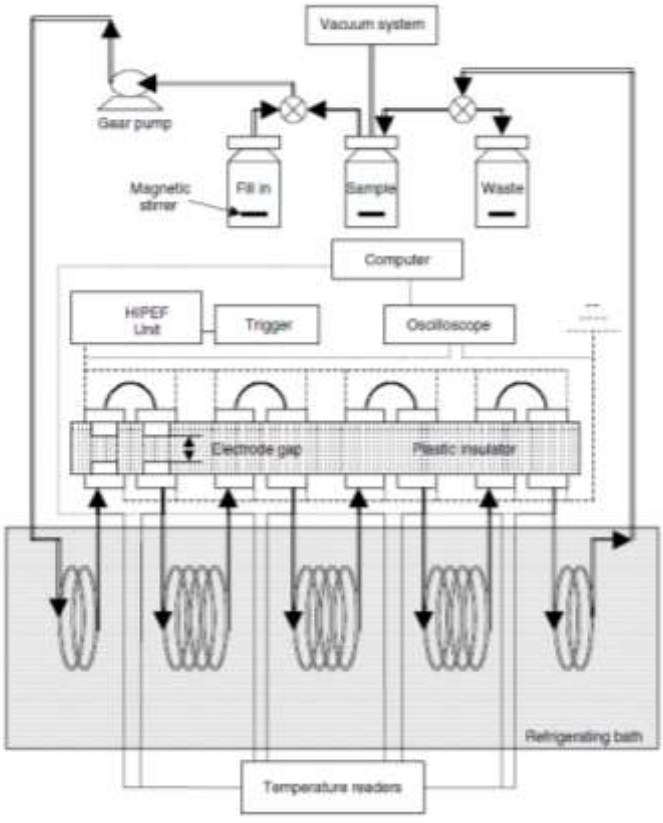


Figure 8: Diagram of the high-intensity pulsed electric field bench-scale processing unit (Morales-de la Peña et al., 2011).

1.3.2.2.5. High hydrostatic pressure

An alternative to the thermal treatments is also high hydrostatic pressure (HHP), which is based on the use of pressure to compress food located inside a pressure vessel (figure 9). HHP processing is based on Pascal's law, meaning that pressure is instantaneously and homogenously transmitted throughout the food regardless the size or shape of the sample (Hendrickx & Knorr, 2001). This implies that all atoms and molecules in the food are subjected to the same pressure at exactly the same time, unlike heat processing where temperature gradients are established. The second key feature of HHP arising from Le Chatelier-Braun principle suggests that a system in equilibrium could be disrupted by the increase of pressure, hence the volume may reduce. Thus, pressure alters interatomic distances, acting only on those weak interactions, for which bon energy is distance-dependent, such as van der Waals forces, electrostatic forces and hydrogen bonding (Bermúdez-Aguirre & Barbosa-Cánovas, 2010).

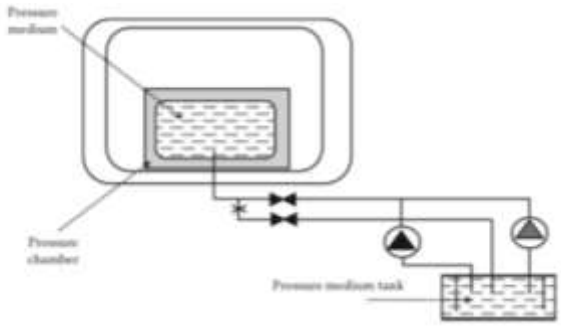


Figure 9: Diagram of the high pressure processing by indirect compression (Ibarz & Barbosa-Canovas, 2014).

HHP can be generated by direct or indirect compression, or by heating the pressure medium. In overall, the pressure level ranges from 100 to 600 MPa and the pressure-transmitting medium is usually water. The treatment chamber is loaded with the product and closed, then the pressure-transmitting medium is placed and degassed

and the pressure is generated by a pump. The presence of air in the food increases the time of pressurization. Although HHP is a non-thermal food preservation technology, the increased pressure led to a small adiabatic rise in temperature (San Martín, Barbosa-Cánovas, & Swanson, 2002).

Studies on the application of high-pressure processing in different food systems have shown that HP in the range 3000 – 8000 bars can reduce microorganisms and enzymes without the degradation in flavour and nutrients associated to thermal treatment (Martinez-Monteagudo & Saldaña, 2014). Detrimental microorganism in HHP -treated food could be destroyed without changing food characteristics. In addition, HHP can either activate or inactivate enzymes depending on the treatment pressure and temperature (Chakraborty, Kaushik, Rao, & Mishra, 2014). Minimal effects of HHP on the colour of fruit products have been reported by a number of researchers (Tadapaneni, Daryaei, Krishnamurthy, Edirisinghe, & Burton-Freeman, 2014). HHP is considered to be a promising technology for the food industry as it can result in the development of new shelf stable foods and/or extension of the shelf life of foods while preserving nutritional value and excellent organoleptic characteristics.

1.3.3. Regulation aspects

The consumer demand for mildly processed products having good organoleptic properties and retention of nutritional quality has driven the development of new preservation methods. Non-thermal treatments are able to inactivate a range of microorganisms and some enzymes, resulting in acceptable products with a reasonable shelf-life. Even though the potential benefits of the use of non-thermal processing in food products are arising with high expectations for the food industry, the associated risk that they may lead are still a concern. Before novel technologies can be applied for fruit products use, it is essential that they are shown to be safe in use and have the necessary regulatory approval. This section is on the relevant existing considerations for possible approaches on the legislation to gain the regulatory approval.

Foods treated by a process that does not have a significant history of safe use in food production fall within the scope of the European Regulation on Novel Foods (EU nº 2283/2015). This regulation will apply 1st January of 2018. Concenrning non-thermal treatments if it is possible to demonstrate that the processing applied to the food is not sufficient to break chemical bonds in the food during processing, then the potential for generate new compounds would be decreased. Thus, how the novel food or novel food ingredient may be substantially equivalent to an existing food or food ingredient as regards to its: (a) composition (such as the source organism and preparation method), (b) nutritional value, (c) metabolism, (d) intended use (such as a food ingredient or supplement) and (e) level of undesirable substances (such as contaminants, mycotoxins and allergens). If it is not possible to demonstrate the lack of nutritional and toxicological change in fruit product after treatment then the new process should be notified to a competent authority in an EU member state. Moreover, the process must be assessed for microbial safety and the results also submitted to the competent authority in one of the EU member states to initiate approval under the novel foods procedure (EC 2073/2005).

Most regulations in the United States and the European Union concern exclusively safety and microbiological quality. Before the commercialization of processed foods by a non-thermal technology can take place, each process must comply with appropriate safety regulations set forth by the FDA according to the type of product. However, the FDA does not approve processes *per se*, but rather the use of substances or components used in the process. A food safety standard has been introduced by a regulation of the Food and Drug Administration for minimally processed juices requiring a minimum reduction of 5.0 log units of microorganisms (FDA, 2004).

Consequently, novel thermal and non-thermal technologies have been designed to meet the required food product safety or shelf-life demands while minimizing the effects on a product's nutritional and quality attributes. There is a general consensus among the regulatory agencies that novel technologies are of benefit to both food processors and consumers alike. Although at present there is no specific legislation for fruit products. The Commission of the *Codex Alimentarius* of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United

Nations developed a document entitled Processing of fresh-cut tropical fruits and vegetables: a technical guide (FAO 2011). Also, some review papers have been published to describe the current situation according to the regulation aspects of non-thermal processing such as pulsed electric fields or high hydrostatic pressure (Góngora-Nieto, Sepúlveda, Pedrow, Barbosa-Cánovas, & Swanson, 2002; Heinz & Buckow, 2010).

Concerning the non-thermal treatments in the United States of America, there is a specific regulation with respect of pulsed light treatment. So far, The Food and Drug Administration (FDA) have released a guide concerning pulsed light treatment. On April 2016, they published the items to take into account for food light radiation. FDA considered pulsed light treatment for microorganism's inactivation when a maximum of 12 $J \cdot cm^{-2}$ is used (FDA, 2016).

In order to be granted the information to consumers on October 2011, the European Parliament and the Council of the European Union published the Regulation EU nº 1169/2011. This new regulation stated that the food shall include or be accompanied by particulars as to the physical condition of the food or the specific treatment which it has undergone (for example, powdered, refrozen, freeze-dried, quick-frozen, concentrated, smoked, …) in all cases where omission of such information could mislead the purchaser.

1.4.References

Aguiló-Aguayo, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2007). Comparative study on color, viscosity and related enzymes of tomato juice treated by high-intensity pulsed electric fields or heat. European Food Research and Technology, 227(2), 599–606.

Barreto, J. C., Trevisan, M. T. S., Hull, W. E., Erben, G., De Brito, E. S., Pfundstein, B., … Owen, R. W. (2008). Characterization and quantitation of polyphenolic compounds in bark, kernel, leaves, and peel of mango (Mangifera indica L.). Journal of Agricultural and Food Chemistry, 56(14), 5599–5610.

Bermúdez-Aguirre, D., & Barbosa-Cánovas, G. V. (2010). An Update on High Hydrostatic Pressure, from the Laboratory to Industrial Applications. Food Engineering Reviews, 3(1), 44–61.

Bonilla, J., Atarés, L., Vargas, M., & Chiralt, A. (2012). Edible films and coatings to prevent the detrimental effect of oxygen on food quality: Possibilities and limitations. Journal of Food Engineering, 110(2), 208–213.

Caminiti, I. M., Noci, F., Morgan, D. J., Cronin, D. A., & Lyng, J. G. (2012). The effect of pulsed electric fields, ultraviolet light or high intensity light pulses in combination with manothermosonication on selected physico-chemical and sensory attributes of an orange and carrot juice blend. Food and Bioproducts Processing, 90(3), 442–448.

Campaniello, D., Bevilacqua, A., Sinigaglia, M., & Corbo, M. R. (2008). Chitosan: Antimicrobial activity and potential applications for preserving minimally processed strawberries. Food Microbiology, 25(8), 992–1000.

Campbell, R. J., & Ledesma, N. (2015). Mango cultivars with potential for commercial development. In Acta Horticulturae (Vol. 1075, pp. 33–40). International Society for Horticultural Science.

Chakraborty, S., Kaushik, N., Rao, P. S., & Mishra, H. N. (2014). High-Pressure Inactivation of Enzymes: A Review on Its Recent Applications on Fruit Purees

and Juices. Comprehensive Reviews in Food Science and Food Safety, 13(4), 578–596.

Charles, F., Vidal, V., Olive, F., Filgueiras, H., & Sallanon, H. (2013). Pulsed light treatment as new method to maintain physical and nutritional quality of fresh-cut mangoes. Innovative Food Science & Emerging Technologies, 18, 190–195.

Chen, Y., Yu, L. J., & Rupasinghe, H. P. V. (2013). Effect of thermal and non-thermal pasteurisation on the microbial inactivation and phenolic degradation in fruit juice: a mini-review. Journal of the Science of Food and Agriculture, 93(5), 981–6.

Chien, P.-J., Sheu, F., & Yang, F.-H. (2007). Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. Journal of Food Engineering, 78(1), 225–229.

Chiumarelli, M., Ferrari, C. C., Sarantópoulos, C. I. G. L., & Hubinger, M. D. (2011). Fresh cut Tommy Atkins mango pre-treated with citric acid and coated with cassava (Manihot esculenta Crantz) starch or sodium alginate. Innovative Food Science and Emerging Technologies, 12(3), 381–387.

Chiumarelli, M., & Hubinger, M. D. (2012). Stability, solubility, mechanical and barrier properties of cassava starch – Carnauba wax edible coatings to preserve fresh-cut apples. Food Hydrocolloids, 28(1), 59–67.

Cortés, C., Esteve, M. J., & Frígola, A. (2008). Color of orange juice treated by High Intensity Pulsed Electric Fields during refrigerated storage and comparison with pasteurized juice. Food Control, 19(2), 151–158.

Djioua, T., Charles, F., Lopez-Lauri, F., Filgueiras, H., Coudret, A., Jr, M. F., … Sallanon, H. (2009). Improving the storage of minimally processed mangoes (Mangifera indica L.) by hot water treatments. Postharvest Biology and Technology, 52(2), 221–226.

Ducamp-Collin, M.-N., Reynes, M., Lebrun, M., & Freire, M. (2009). Fresh cut mango fruits: Evaluation of edible coatings. Acta Horticulturae.

Espitia, P. J. P., Du, W.-X., Avena-Bustillos, R. de J., Soares, N. de F. F., & McHugh, T. H. (2014). Edible films from pectin: Physical-mechanical and antimicrobial properties - A review. Food Hydrocolloids, 35, 287–296.

European Union., 2005, Commision regulation (EC) No 2073/2005 of 15 November 2005 on the microbiological criteria of foodstuffs. Off J Eur Union

Euorpean Union.,Comision regulation (EU) No 2283/2015 of 25 November 2001 on the novel foods. Off J Eur Union

Food Agricultural Organization of the United Nations. (2003). Tropical fruits.

Food Agricultural Organization of the United Nations. (2004). Manual for the preparation and sale of fruits and vegetables. Rome.

Food Agricultural Organization of the United Nations. (2012). FAOSTAT.

Food Agricultural Organization of the United Nations (2011). Processing of fresh-cut tropical fruits and vegetables: a technical guide.

Food and Drug Administration of the United States of America. (2004). Guidance for industry: Juice HACCP hazards and controls guidance first edition. Retrieved from http://www.fda.gov/Food/%0AGuidanceComplianceRegulatoryInformation/GuidanceDocuments/Juice/%0Aucm072557.htm#ftn1

Food Drug Administration of the United States of America (2016). Irradiation in the production, processing and handling of food. Code of Federal Regulations. 21CFR179.41.

Gil, M. I., Aguayo, E., & Kader, A. A. (2006). Quality changes and nutrient retention in fresh-cut versus whole fruits during storage. Journal of Agricultural and Food Chemistry, 54(12), 4284–4296.

Gómez-López, V. M., Ragaert, P., Debevere, J., & Devlieghere, F. (2007). Pulsed light for food decontamination: a review. Trends in Food Science & Technology, 18(9), 464–473.

Góngora-Nieto, M. M., Sepúlveda, D. R., Pedrow, P., Barbosa-Cánovas, G. V., & Swanson, B. G. (2002). Food Processing by Pulsed Electric Fields: Treatment Delivery, Inactivation Level, and Regulatory Aspects. LWT - Food Science and Technology, 35(5), 375–388.

Gonzalez-Aguilar, G. A., Celis, J., Sotelo-Mundo, R. R., De La Rosa, L. A., Rodrigo-Garcia, J., & Alvarez-Parrilla, E. (2008). Physiological and biochemical changes of different fresh-cut mango cultivars stored at 5 °C. International Journal of Food Science & Technology, 43(1), 91–101.

González-Aguilar, G. A., Villegas-Ochoa, M. A., Martínez-Téllez, M. A., Gardea, A. A., & Ayala-Zavala, J. F. (2007). Improving antioxidant capacity of fresh-cut mangoes treated with UV-C. Journal of Food Science, 72(3), S197-202.

González-Aguilar, G. A., Wang, C. Y., & Buta, J. G. (2000). Maintaining Quality of Fresh-Cut Mangoes Using Antibrowning Agents and Modified Atmosphere Packaging. Journal of Agricultural and Food Chemistry, 48(9), 4204–4208.

González-Aguilar, G. A., Zavaleta-Gatica, R., & Tiznado-Hernández, M. E. (2007). Improving postharvest quality of mango "Haden" by UV-C treatment. Postharvest Biology and Technology, 45(1), 108–116.

González-Aguilar, G., Robles-Sánchez, R., Martínez-Téllez, M., Olivas, G., Alvarez-Parrilla, E., & de la Rosa, L. (2008). Bioactive compounds in fruits: health benefits and effect of storage conditions. Stewart Postharvest Review, 4(3), 1–10.

Gonzalez, M. E., & Barrett, D. M. (2010). Thermal, high pressure, and electric field processing effects on plant cell membrane integrity and relevance to fruit and vegetable quality. Journal of Food Science, 75(7), R121-30.

Gorinstein, S., Poovarodom, S., Leontowicz, H., Leontowicz, M., Namiesnik, J., Vearasilp, S., … Tashma, Z. (2011). Antioxidant properties and bioactive constituents of some rare exotic Thai fruits and comparison with conventional fruits. Food Research International, 44(7), 2222–2232.

Heinz, V., & Buckow, R. (2010). Food preservation by high pressure. Journal Für Verbraucherschutz Und Lebensmittelsicherheit, 5(1), 73–81

Hendrickx, M. E., & Knorr, D. (2001). Ultra High Pressure Treatment of Foods.

Hendrickx, M., Ludikhuyze, L., Van den Broeck, I., & Weemaes, C. (1998). Effects of high pressure on enzymes related to food quality. Trends in Food Science & Technology, 9(5), 197–203.

Huang, K., Tian, H., Gai, L., & Wang, J. (2012). A review of kinetic models for inactivating microorganisms and enzymes by pulsed electric field processing. Journal of Food Engineering, 111(2), 191–207.

Ibarz, A., & Barbosa-Canovas, G. V. (2014). Introduction to Food Process Engineering. CRC Press.

Ivorra, A., Villemejane, J., & Mir, L. M. (2010). Electrical modeling of the influence of medium conductivity on electroporation, 12(34), 10055–10064.

Jahurul, M. H. A., Zaidul, I. S. M., Ghafoor, K., Al-Juhaimi, F. Y., Nyam, K.-L., Norulaini, N. A. N., … Mohd Omar, A. K. (2015). Mango (Mangifera indica L.) by-products and their valuable components: A review. Food Chemistry, 183, 173–180.

Jamsazzadeh Kermani, Z., Shpigelman, A., Houben, K., ten Geuzendam, B., Van Loey, A. M., & Hendrickx, M. E. (2015). Study of mango endogenous pectinases as a tool to engineer mango purée consistency. Food Chemistry, 172, 272–282.

Jiménez-Sánchez, C., Lozano-Sánchez, J., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2017). Alternatives to conventional thermal treatments in fruit-juice processing. Part 1: Techniques and applications. Critical Reviews in Food Science and Nutrition, 57(3), 501–523.

Koutchma, T., Popović, V., Ros-Polski, V., & Popielarz, A. (2016). Effects of Ultraviolet Light and High-Pressure Processing on Quality and Health-Related Constituents of Fresh Juice Products. Comprehensive Reviews in Food Science and Food Safety, 15(5), 844–867.

M. Kamphuis, C. B., Katrina Giskes, Bruijn, G.-J. de, Wanda Wendel-Vos, Johannes Brug, & Lenthe, F. J. van. (2006). Environmental determinants of fruit and vegetable consumption among adults: a systematic review. British Journal of Nutrition, 96(4), 620–635.

MAGRAMA. (2014). Informe del consumo de alimentación en España. Retrieved January 13, 2016, from http://www.magrama.gob.es/es/alimentacion/temas/consumo-y-comercializacion-y-distribucion-alimentaria/informeconsumoalimentacion2014_tcm7-382148.pdf

Martin-Belloso, O., & Fortuny, R. S. (2010). Advances in Fresh-Cut Fruits and Vegetables Processing.

Martinez-Monteagudo, S. I., & Saldaña, M. D. A. (2014). Chemical Reactions in Food Systems at High Hydrostatic Pressure. Food Engineering Reviews, 6(4), 105–127.

Masibo, M., & He, Q. (2008). Major mango polyphenols and their potential significance to human health. Comprehensive Reviews in Food Science and ….

Mercadante, A. Z., & Rodriguez-Amaya, D. B. (1998). Effects of Ripening, Cultivar Differences, and Processing on the Carotenoid Composition of Mango. Journal of Agricultural and Food Chemistry, 46(1), 128–130.

Mitra, S. K., Devi, H. L., & Debnath, S. (2014). Tropical and subtropical fruits and human health. In Acta Horticulturae (Vol. 1024, pp. 39–48). International Society for Horticultural Science.

Moody, A., Marx, G., Swanson, B. G., & Bermúdez-Aguirre, D. (2014). A comprehensive study on the inactivation of Escherichia coli under nonthermal technologies: High hydrostatic pressure, pulsed electric fields and ultrasound. Food Control, 37, 305–314.

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

Munafo, J. P., Didzbalis, J., Schnell, R. J., Schieberle, P., & Steinhaus, M. (2014). Characterization of the major aroma-active compounds in mango (Mangifera indica L.) cultivars Haden, White Alfonso, Praya Sowoy, Royal Special, and Malindi by application of a comparative aroma extract dilution analysis. Journal of Agricultural and Food Chemistry, 62(20), 4544–51.

N. Sinha, et al. (2012). Handbook of Vegetables and Vegetable Processing.

Naidua, G. M., & Naidu, G. R. (2009). Marketing strategies for exporting mangoes and mango products from India. In Acta Horticulturae (Vol. 820, pp. 79–95).

Navarro, J. L., Izquierdo, L., Carbonell, J. V., & Sentandreu, E. (2014). Effect of pH, temperature and maturity on pectinmethylesterase inactivation of citrus juices treated by high-pressure homogenization. LWT - Food Science and Technology, 57(2), 785–788.

Ngarmsak, M., Delaquis, P., Toivonen, P., Ngarmsak, T., Ooraikul, B., & Mazza, G. (2006). Antimicrobial activity of vanillin against spoilage microorganisms in stored fresh-cut mangoes. Journal of Food Protection, 69(7), 1724–1727.

Nicklas, T. A., Baranowski, T., Cullen, K. W., & Berenson, G. (2001). Eating patterns, dietary quality and obesity. Journal of the American College of Nutrition.

Ohlsson, T., & Bengtsson, N. (2002). Minimal processing technologies in the food industry. Cambridge: Woodhead Publishing limited.

Oms-Oliu, G., Martín-Belloso, O., & Soliva-Fortuny, R. (2008). Pulsed Light Treatments for Food Preservation. A Review. Food and Bioprocess Technology, 3(1), 13–23.

Oms-Oliu, G., Rojas-Graü, M. A., González, L. A., Varela, P., Soliva-Fortuny, R., Hernando, M. I. H., … Martín-Belloso, O. (2010). Recent approaches using chemical treatments to preserve quality of fresh-cut fruit: A review. Postharvest Biology and Technology, 57(3), 139–148.

Pataro, G., Sinik, M., Capitoli, M. M., Donsì, G., & Ferrari, G. (2015). The influence of Post-harvest UV-C and Pulsed Light treatments on quality and antioxidant

properties of tomato fruits during storage. Innovative Food Science & Emerging Technologies, 30, 103–111.

Pearson, N., Atkin, A. J., Biddle, S. J. H., & Gorely, T. (2010). A family-based intervention to increase fruit and vegetable consumption in adolescents: a pilot study. Public Health Nutrition, 13(6), 876–85.

Penteado, A. L., de Castro, M. F. P. M., & Rezende, A. C. B. (2014). Salmonella enterica serovar Enteritidis and Listeria monocytogenes in mango (Mangifera indica L.) pulp: growth, survival and cross-contamination. Journal of the Science of Food and Agriculture, 94(13), 2746–2751.

Pierson, J. T., Monteith, G. R., Roberts-Thomson, S. J., Dietzgen, R. G., Gidley, M. J., & Shaw, P. N. (2014). Phytochemical extraction, characterisation and comparative distribution across four mango (Mangifera indica L.) fruit varieties. Food Chemistry, 149, 253–63.

Plotto, A., Narciso, J. A., Rattanapanone, N., & Baldwin, E. A. (2010). Surface treatments and coatings to maintain fresh-cut mango quality in storage. Journal of the Science of Food and Agriculture, 90(13), 2333–41.

Quitão-Teixeira, L. J., Aguiló-Aguayo, I., Ramos, A. M., & Martín-Belloso, O. (2007). Inactivation of Oxidative Enzymes by High-Intensity Pulsed Electric Field for Retention of Color in Carrot Juice. Food and Bioprocess Technology, 1(4), 364–373.

Raso, J., & Barbosa-Cánovas, G. V. (2003). Nonthermal preservation of foods using combined processing techniques. Critical Reviews in Food Science and Nutrition, 43(3), 265–85.

Ravani, A., & Joshi, D. (2013). Mango and it's by product utilization–a review. Trends in Post Harvest Technology.

Ribeiro, S. M. R., Barbosa, L. C. A., Queiroz, J. H., Knödler, M., & Schieber, A. (2008). Phenolic compounds and antioxidant capacity of Brazilian mango (Mangifera indica L.) varieties. Food Chemistry, 110(3), 620–626.

Robles-Sánchez, M., Astiazarán-García, H., Martín-Belloso, O., Gorinstein, S., Alvarez-Parrilla, E., de la Rosa, L. A., González-Aguilar, G. A. (2011). Influence of whole and fresh-cut mango intake on plasma lipids and antioxidant capacity of healthy adults. Food Research International, 44(5), 1386–1391.

Robles-Sánchez, R. M., Rojas-Graü, M. A., Odriozola-Serrano, I., González-Aguilar, G., & Martin-Belloso, O. (2013). Influence of alginate-based edible coating as carrier of antibrowning agents on bioactive compounds and antioxidant activity in fresh-cut Kent mangoes. LWT - Food Science and Technology, 50(1), 240–246.

Robles-Sánchez, R. (2009). Quality Index, Consumer Acceptability, Bioactive Compounds, and Antioxidant Activity of Fresh-Cut "Ataulfo" Mangoes (Mangifera Indica L.) as Affected by Low-temperature storage. Journal of Food Science, 74(3), S126–S134.

Rojas-Graü, M. A., Soliva-Fortuny, R., & Martín-Belloso, O. (2009). Edible coatings to incorporate active ingredients to fresh-cut fruits: a review. Trends in Food Science & Technology, 20(10), 438–447.

Rojas-Graü, M. A., Tapia, M. S., Rodríguez, F. J., Carmona, A. J., & Martin-Belloso, O. (2007). Alginate and gellan-based edible coatings as carriers of antibrowning agents applied on fresh-cut Fuji apples. Food Hydrocolloids, 21(1), 118–127.

Rymbai, H., Srivastav, M., Sharma, R. R., Patel, C. R., & Singh, A. K. (2013). Bio-active compounds in mango (Mangifera indica L.) and their roles in human health and plant defence - A review. Journal of Horticultural Science and Biotechnology.

Salgado, P. R., Ortiz, C. M., Musso, Y. S., Di Giorgio, L., & Mauri, A. N. (2015). Edible films and coatings containing bioactives. Current Opinion in Food Science, 5, 86–92. http://doi.org/10.1016/j.cofs.2015.09.004

Salinas-Hernández, R. M., González-Aguilar, G. A., & Tiznado-Hernández, M. E. (2013). Utilization of physicochemical variables developed from changes in sensory attributes and consumer acceptability to predict the shelf life of fresh-cut mango fruit. Journal of Food Science and Technology, 52(1), 63–77.

Salinas-Roca, B., Soliva-Fortuny, R., Welti-Chanes, J., Martín-Belloso, O. (2014). Quality properties of coated mango slices treated by intense light pulses. EFFOST 2014.

San Martín, M. F., Barbosa-Cánovas, G. V, & Swanson, B. G. (2002). Food processing by high hydrostatic pressure. Critical Reviews in Food Science and Nutrition, 42(6), 627–45.

Sarkiyayi, S. (2013). Comparative analysis of nutritional and anti nutritional contents of some varieties of mango (Mangifera indica) in Kaduna Metropolis-Nigeria. Research Journal of Applied Sciences, Engineering and Technology.

Saúco, V. G. (2013). Worldwide mango production and market: Current situation and future prospects. Acta Horticulturae, 992, 37–48.

Sayadia, S., & Calatravab, J. (2009). Structure and productivity of spanish mango industry: A farmers survey analysis. In Acta Horticulturae (Vol. 820, pp. 47–56).

Siddiq, M., Ahmed, J., Lobo, M. G., & Ozadali, F. (2012). Tropical and Subtropical Fruits. (M. Siddiq, Ed.)Tropical and Subtropical Fruits: Postharvest Physiology, Processing and Packaging. Oxford, UK: Wiley-Blackwell.

Siddiq, M., Sogi, D. S., & Dolan, K. D. (2013). Antioxidant properties, total phenolics, and quality of fresh-cut "Tommy Atkins" mangoes as affected by different pre-treatments. LWT - Food Science and Technology, 53(1), 156–162.

Silva-Weiss, A., Ihl, M., Sobral, P. J. A., Gómez-Guillén, M. C., & Bifani, V. (2013). Natural Additives in Bioactive Edible Films and Coatings: Functionality and Applications in Foods. Food Engineering Reviews, 5(4), 200–216. http://doi.org/10.1007/s12393-013-9072-5

Sinha, N., Hui, Y. H., Evranuz, E. Ö., Siddiq, M., & Ahmed, J. (2010). Handbook of Vegetables and Vegetable Processing. (Wiley-Blackwell, Ed.) (Vol. 2010). Wiley-Blackwell.

Skibsted, L. H., Risbo, J., & Andersen, M. L. (2010). Chemical deterioration and physical instability of food and beverages. Chemical Deterioration and Physical Instability of Food and Beverages. Elsevier Inc.

Sogi, D. S., Siddiq, M., Roidoung, S., & Dolan, K. D. (2012). Total Phenolics, Carotenoids, Ascorbic Acid, and Antioxidant Properties of Fresh-cut Mango (Mangifera indica L., cv. Tommy Atkin) as Affected by Infrared Heat Treatment. Journal of Food Science.

Souza, B. S., Durigan, J. F., Donadon, J. R., Teixeira, G. H. A., & Durigan, M. F. B. (2005). Respiratory and storage behavior of fresh cut "Tommy Atkins" mango. In Acta Horticulturae (Vol. 682, pp. 1909–1916).

Tadapaneni, R. K., Daryaei, H., Krishnamurthy, K., Edirisinghe, I., & Burton-Freeman, B. M. (2014). High-Pressure Processing of Berry and Other Fruit Products: Implications for Bioactive Compounds and Food Safety. Journal of Agricultural and Food Chemistry, 62(18), 3877–3885.

Timmermans, R. A. H., Nierop Groot, M. N., Nederhoff, A. L., van Boekel, M. A. J. S., Matser, A. M., & Mastwijk, H. C. (2014). Pulsed electric field processing of different fruit juices: impact of pH and temperature on inactivation of spoilage and pathogenic micro-organisms. International Journal of Food Microbiology, 173, 105–11.

Vega-Mercado, H., Martín-Belloso, O., Qin, B.-L., Chang, F. J., Marcela Góngora-Nieto, M., Barbosa-Cánovas, G. V., & Swanson, B. G. (1997). Non-thermal food preservation: Pulsed electric fields. Trends in Food Science & Technology, 8(5), 151–157.

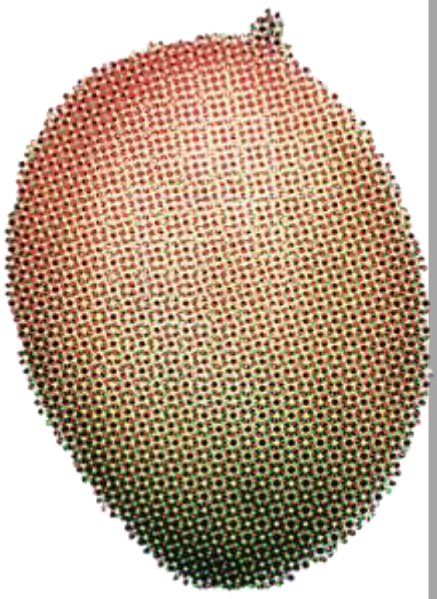
Vervoort, L., Van der Plancken, I., Grauwet, T., Timmermans, R. A. H., Mastwijk, H. C., Matser, A. M., … Van Loey, A. (2011). Comparing equivalent thermal, high pressure and pulsed electric field processes for mild pasteurization of orange juice: Part II: Impact on specific chemical and biochemical quality parameters. Innovative Food Science & Emerging Technologies, 12(4), 466–477.

Vivar-Vera, M. A., Salazar-Montoya, J. A., Calva-Calva, G., & Ramos-Ramírez, E. G. (2007). Extraction, thermal stability and kinetic behavior of pectinmethylesterase from hawthorn (Crataegus pubescens) fruit. LWT - Food Science and Technology, 40(2), 278–284.

Wall-Medrano, A., Olivas-Aguirre, F. J., Velderrain-Rodriguez, G. R., González-Aguilar, A., de la Rosa, L. A., López-Díaz, J. A., & Álvarez-Parrilla, E. (2015). [Mango: agroindustrial aspects, nutritional/functional value and health effects]. Nutrición Hospitalaria, 31(1), 67–75.

Wang, L. Z., Liu, L., Holmes, J., Kerry, J. F., & Kerry, J. P. (2007). Assessment of film-forming potential and properties of protein and polysaccharide-based biopolymer films. International Journal of Food Science & Technology, 42(9), 1128–1138.

Watson, R., & Preedy, V. (2009). Bioactive foods in promoting health: fruits and vegetables. London: Academic Press.



2. HYPOTHESIS AND OBJECTIVES

2 HYPOTHESIS AND OBJECTIVES

The present Doctoral thesis assumed that the non-thermal preservation technologies: coatings, dipping, pulsed light, high intensity pulsed electric fields and high hydrostatic pressure are feasible for obtaining fresh-cut, juice or puree safe mango products with fresh-like characteristics. Thus, the general objective of the present doctoral thesis was to evaluate the feasibility of coatings, dipping, pulsed light, high intensity pulsed electric fields and high hydrostatic pressure as non-thermal treatments for three different mango products: fresh-cut, juice and puree; considering microbial, enzymatical, physicochemical and biochemical quality attributes.

Aiming to achieve the main goal, the following targets and specific objectives were proposed:

Section i: Preservation of fresh-cut mango quality by edible coatings and pulsed light treatment

I. To evaluate the effect of the polysaccharide–based coatings (alginate, pectine, carboxymethylcellulose and chitosan) on microbial stability (moulds and yeasts and psychrophilic bacteria), physicochemical parameters (pH, soluble solids, colour and firmness), bioactive compounds (total phenolic and carotenoid content) and antioxidant capacity in fresh-cut mango throughout refrigerated storage.

II. To elucidate the effectiveness of combining pulsed light, alginate coating and malic acid dipping on the reduction of *Listeria innocua* population as well as to evaluate microbial growth and physicochemical parameters (pH, soluble solids, colour and firmness) of mango slices over refrigerated storage.

III. To estimate possible the effect of combining pulsed light, alginate coating and malic acid dipping treatment in the content of individual phenolic compounds of mango to improve the antioxidant compounds content in fresh-cut mango along storage.

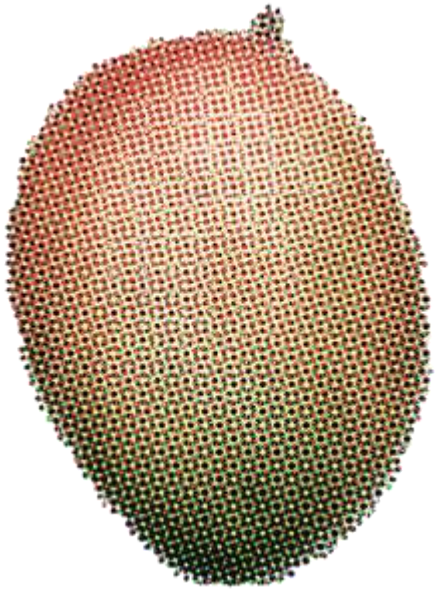
Section ii: Maintain the high intensity pulsed electric fields (HIPEF) treated mango juice stability along the storage

IV. To assess the impact of high intensity pulsed electric fields treatment time on the inactivation of *Listeria innocua* population and enzymatic activity (polygalacturonase, pectinmethylesterase, polyphenoloxidase, peroxidase and lipoxigenase) while preserving sensorial attributes.

V. To study the feasibility of high intensity pulsed electric fields treatment in extending quality stability in terms of microbial, enzymatic activity and bioactive compounds content along the storage comparing with conventional treatment as pasteurization.

Section iii: Determination of the high hydrostatic pressure (HHP) conditions for mango puree treatment

VI. To determine the influence of high hydrostatic pressure treatment conditions (pressure, temperature and time) on enzymatic activity (pectinmethylesterase, polyphenoloxidase and peroxidase), physicochemical changes (pH, colour, water activity, soluble solids and viscosity) and bioactive compounds (phenolics and carotenoids content and antioxidant capacity) of mango puree with the purpose to minimize the product deterioration compared with fresh product.



3. MATERIALS AND METHODS

3 MATERIALS AND METHODS

3.1.Experimental scheme

Logical sequence of actions carried out to fulfil the research objectives is depicted and the experimental design as well as material methods described in the following sections (Figure 1).

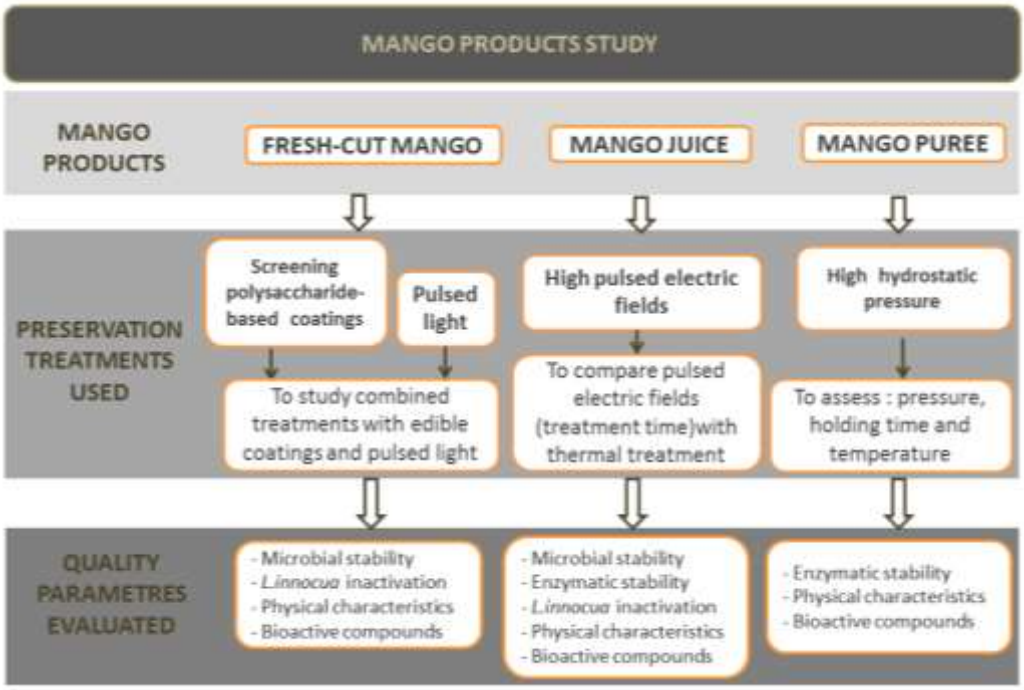


Figure 1: General scheme of experimental methodology.

3.2.Mango products preparation

The raw material used for processing was the same in all mango products. Mangoes (*Mangifera indica* L.) cv. Tommy Atkins were purchased at commercial maturity from a local wholesale market (Lleida, Spain).

3.2.1. Mango slices

Whole mangoes were washed with an aqueous solution of sodium hypochlorite (300μL/L) and then peeled and cut to obtain 5 mm-thick slices (35±1g). Sliced mangoes were treated, placed into transparent polypropylene trays and stored (4 ± 1 ºC) until analysis at days 0, 3, 7, 10 and 14.

3.2.2. Mango juice

Mangoes were washed, drained, peeled and the seed was discarded. The pulp was squeezed and then centrifuged at 6000 rpm during 5 min at 4°C to obtain mango juice (MJ) (AVANTITM J-25 Beckman; Instruments Inc; Fullerton, CA) and vacuum filtered.

3.2.3. Mango puree

Two varieties were used for mango puree studies. Mangoes (cv. Manila and Tommy Atkins) were purchased from local market in Monterrey (Mexico). Fruits were washed; hand peeled, sliced, pulped using a household blender and packaged in plastic bags. Bags of two different varieties of mango puree were stored at - 35 °C until processing.

3.3.Processing treatments

3.3.1. Edible coatings

Coating forming solutions were prepared similarly to Rojas-Graü et al (2007a) by dissolving powders of alginate (AL) and pectin (PE) at 2 % (w/v) and carboxymethylcellulose (CMC) at 0.5 % (w/v) in distilled water while stirring until the solution became clear. In addition, chitosan (CH) at 0.5 % (w/v)

coating was prepared following Jiang & Li (2001) who dissolved powders in acetic acid and adjust pH at 5.0 to assure CH solubility. Ascorbic acid (AA) 1% was added as anti-browning agent in all edible coating solutions (Robles-Sánchez, Rojas-Graü, Odriozola-Serrano, González-Aguilar, & Martín-Belloso, 2009). Fresh-cut mango dipped in AL solution had a second dipping in calcium chloride solution (1 %) for the formation of the cross-linking of the carbohydrate polymer with calcium.

3.3.2. Pulsed light

Pulsed light (PL) treatments were carried out with a XeMaticA-2L System (SteriBeam Systems GmbH, Germany). The experiments were performed at a charging voltage of 2.5 kV. Pulses of 0.3 ms with a radiant fluence of 0.4 $J \cdot cm^{-2}$ were emitted from one lamp situated 8.5 cm above the sample holder. The total light energy was measured according to the calibration of the equipment with a standard light source estimated by photodiode readings and manufacturer's directions. The emitted spectrum ranged from 180-1100 nm. To evaluate the effect of the wavelength of PL on the inactivation of *L.innocua*, two types of UV filters were used: a 2 mm-thick Pyrex glass filter that cuts off wavelengths below 305 nm hence allowing to pass some UVB, all UVA, visible light (V) and infrared (IR) wavelengths (89 % of the emitted energy); and Makrolon polycarbonate plastic filter that cuts all light below 400 nm, thus allowing only V and IR light to pass through (83 % of the emitted energy). In addition, treatments with increasing number of pulses (0, 10, 15, 20, 25, and 30) were assayed in order to evaluate the inactivation of *L.innocua* as affected by PL.

3.3.3. High intensity pulsed electric fields

High intensity pulsed electric fields (HIPEF) treatments were performed using a continuous flow bench scale system (OSU-4F, Ohio State University, Columbus, OH), that generates squared wave pulses. The flow rate was 60 mL/ min and it was controlled by a speed pump (model 752210-25, Cole Palmer Instrument Company, Vermon Hills, IL). The treatment chamber device consisted of eight co-linear chambers disposed in series and each pair of chambers had a thermocouple to control temperature. The outlet treatment temperature was kept below 40 °C using a cooling coil, which was connected before and after each pair of chambers and submerged in an ice-water shaking bath. In the present study constant electric field strength (35 kV/ cm), pulse frequency (200Hz) and width (4 μs) applied in bipolar mode were used while different treatment times were assayed (50, 100, 200, 400, 800, 1200, 1600, 1800 and 2000 μs). HIPEF fluid handling system was cleaned with 4 % NaOH and then with 10 % chloride and 20 % ethanol distillate water solutions prior to processing. Moreover, the first 200 mL treated were discarded to ensure stationary treatment conditions. MJ analysed during storage was treated at 1800 μs, which was the needed HIPEF treatment time to assure 5 log reductions of *L. innocua* and low enzymatic activity.

3.3.4. High hydrostatic pressure

Mango puree (MP) was thawed and 20 g were placed in polyethylene bags, air evacuated and heat sealed (Torrey EVD4, Guadalupe, Mexico). MP was treated by high hydrostatic pressure (HHP) at 25 and 55 °C using an isostatic system (Avure Technologies, Middeltown, OH, USA). Different pressures: 400, 450 and 550 MPa and holding times: 2, 4, 8 and 16 min were assayed. The come-up times (CUT) required to reach each pressure were 2.28 ± 0.11, 1.33 ± 0.15 and 1.44 ± 0.16 min at 55 °C and 1.46 ± 0.11, 1.33 ± 0.12 and 1.13

± 0.04 at 25 °C for 400, 450 and 500 MPa, respectively. A non-treated MP was considered as control.

3.3.5. Thermal treatment

MJ was heat- treated (90°C, 60 s) in order to compare the thermal effect on quality parameters of MJ with that of HIPEF treatment. The juice was pumped with a peristaltic pump (model D-21V, Dinko, Barcelona, Spain) and passed through a tubular stainless steel heat exchange coil system (University of Lleida, Lleida, Spain). After the processing the tubular stainless steel was immediately immersed in an ice-water bath and thereafter MJ was packaged.

3.4. Physicochemical determination

3.4.1. pH and total soluble solids content

The pH (pH-meter Crison Instruments S.A. Barcelona, Spain) and total soluble solids content (TSS) (refractometer, Haake RS 80) were determined in a homogenate obtained from crushed mango slices (20 g). Triplicate analyses were carried out and results were expressed as mean and standard deviation.

3.4.2. Colour parameters

Colour was expressed as *L**, *a** and *b**, which indicate luminosity, chromaticity on a green (-) to red (+) axis, and chromaticity on a blue (-) to yellow (+) axis, respectively. Lightness (*L**) was determined with a tri-stimulus Minolta CR-400 colorimeter (Konica Minolta Sensing, INC, Osaka, Japan) using a D65 illuminant and an observation angle of 10º. For reference, a standard white tile (Y= 94.00, x= 0.3158, y= 0.3322) was used. Based on the CIE *L**, *a** and *b** values Hue angle (h°)

was calculated (eq. 1). Colour parameters were obtained as the mean of three determinations.

$h° = \tan^{-1}(b^*/a^*)$ eq.1

Browning index (BI) was calculated with eq.2 (Pathare, Opara, & Al-Said, 2012). Numerical values of L*, a* and b* parameters were obtained as the mean of three measurements.

$$BI = 100\,x\,(X - 0.31/0.17) \quad \text{eq.2}$$

where

$$X = \frac{(a^* + 1.75L^*)a^*}{(5.645L^* + a^* - 3.012b^*} \quad \text{eq.3}$$

3.4.3. Firmness

Firmness of sliced mangoes was analysed with a TA-XT2 Texture Analyser (Stable Micro Systems Ltd., England, UK) by measuring the area of the penetration force with a 4 mm diameter steel rod. The test speed was 4 mm/s and the distance of penetration was 4 mm. Results were the mean of six measurements per sample and given as the delta firmness in N·s.

3.5.Microbial analyses

3.5.1.*L.innocua* inoculation

L. innocua IPL 1.17 (Institute Pasteur de Lille; Lille, France), as a surrogate of the pathogenic *L. monocytogenes*, were provided from the culture collections of the Department of Food Technology (University of Lleida, Spain). Stock culture of *L.innocua* was grown in tryptone soy broth (TSB) with 0.6% yeast extract (Bioakar Diagnostic; Beauvais, France) and incubated at 35 °C with continuous agitation at 200 rpm for 15 h to obtain cells in stationary growth phase (10^8 - 10^9 CFU/mL).

Mango slices (35g) were inoculated by spreading 100 µL of *L.innocua* stock cultures over the entire upper surface with a sterile micropipette before treatment and packaging (Ramos-Villarroel et al., 2011).

3.5.2.Moulds and yeasts and psychrophilic bacteria

Sliced mangoes (10 g) were placed into sterile plastic bags with 90 mL of saline peptone water (Bioakar Diagnostic; Beauvais, France) and homogenized for 1 min in a stomacher blender (IUL Instruments, Barcelona, Spain) for microbial analyses. Serial dilutions were made and 100 µL were placed in Palcam agar plates (Bioakar Diagnostic; Beauvais, France) and spread with a Drigalsky handle. The evaluation was made by duplicate for each dilution and the plates were incubated for 48 h at 37ºC. Enumeration of psychrophilic microorganisms on sliced mango was carried out by agar plate counting (PCA) (Biokar Diagnostic; Beauvais, France), after incubation at 4°C ± 1°C for 10 days, following the ISO 17410 (2001) method. Mould and yeast counts were determined by the ISO 7954 (1987) method using chloramphenicol glucose agar (CGA) (Biokar Diagnostic; Beauvais, France) and incubating for 4 days at 25 °C ± 1°C.

Microbial population was evaluated and the results expressed as $\log_{10}$ CFU/ g for fresh-cut mango and CFU/mL for mango juice.

3.6.Enzyme activity assays

3.6.1.Pectinolitic enzymes

The pectinolitic enzyme PME was determined by potentiometric titration based on the method described by (Kimball, 1991) with minor modifications. MP (10 g) was mixed with 10 mL of NaCl 2N. The mix was homogenised and centrifuged (5000 rpm, 20 min and 4 °C) and 5 mL of supernatant was mixed with 30 mL of 1% p/v pectin solution at 30 °C. The pH was adjusted to 7 with 1 N and 0.1 N NaOH and once set, 50 µL of NaOH 0.1 N were added. The time required for the solution to

return to pH 7 was measured. Equation 4 was used to calculate the PME activity (AE_{PME}) where [NaOH] is the NaOH concentration (0.1 N), V_{NaOH} is the volume of NaOH 0.1 N solution (0.05 mL), V_{sample} is the volume of the supernatant (5 mL) and *t'* is the time in minutes required for the solution to return to pH 7 after the addition of NaOH 0.1 N.

$$AE_{PME/mL} = \frac{[NaOH] \cdot V_{NaOH}}{V_{sample} \cdot t'} \qquad \text{eq.4}$$

3.6.2. Oxidative enzymes

Peroxidase (POD)

POD activity in MJ was determined using the method described by Elez-Martínez, Aguiló-Aguayo et al. (2006) with some modifications. The enzyme extract for POD activity measurement was obtained by the homogenization of 10 mL of juice and 20 mL of sodium phosphate buffer 0.2 M at pH 6.5. The homogenate was centrifuged at 24000g for 15 min at 4°C (AVANTI™ J-25, Beckman Instruments Inc; Fullerton, CA, USA). The supernatant was filtered throughout a no 1 Whatman paper and the resulting liquid constituted the enzymatic extract. POD activity was assayed spectrophotometrically in a 1 cm path cuvette by adding at 0.1 mL of enzymatic extract 2.7 mL of sodium phosphate buffer (0.05 M, pH 6.5), 0.1 mL phenylenediamine (1 %) and 0.1 mL hydrogen peroxide (1.5 %) as oxidant. The oxidation of p-phenylenediamine was determined at 470 nm measuring the absorbance every 10 seconds during 3 min. The activity of POD was determined from the slope of the linear portion of the curve. One unit of POD activity was assumed as the change of one unit in absorbance per minute and millilitre of enzymatic extract at 22ºC.

Polyphenoloxidase (PPO)

PPO activity was determined by the method of Vásquez-Caicedo et al., (2007) with some modifications. For the extraction of the enzyme, 5 g of MJ were mixed with 0.5

g polyvinylpolypyrrolidone (PVPP) and 4.5 g McIlvaine buffer solution (pH 6.5) consisting of 35 % of 0.1 M citric acid and 75 % 0.2 M disodium phosphate. The mixture was homogenised and centrifuged at 23000 g for 15 min at 4 °C (Centrifuge AVANTITM J-25, Beckman Instruments Inc; Fullerton, CA). The supernatant was filtered with no 1 Whatman paper to obtain the enzyme extract. PPO activity was measured using a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd, Cambridge, UK) at 400 nm by adding 100 µL enzyme extract and 3 mL of 0.5 M cathecol solution and obtaining the absorbance every 10 seconds during 3 min. The activity was obtained from the slope of the linear portion of the curve; one unit of PPO activity was defined as a change of one unit of absorbance per minute and millilitre of enzyme extract at 22 °C.

Lipoxigenase (LOX)

LOX activity was determined by the method described by Anthon & Barrett (2003) with modifications. The enzyme extract was obtained by mixing 20 mL of MJ with 5 mL of a solution containing 0.5 M phosphate buffer (pH 6.5) and 0.5% Triton X-100 and centrifuging 10 min at 10000 g at 4 °C (Centrifuge AVANTI^{TM} J-25, Beckman Instruments Inc; Fullerton, CA). The pellet was discarded and the supernatant was filtered with a n° 1Whatman paper. LOX activity was measured by the formation of conjugated dienes from linoleic acid (Axelrod, Cheesbrough, & Laakso, 1981). The activity of the enzyme was measured by mixing 40 µL linoleic acid and 2 mL phosphate buffer 0.1 M (pH 6.5) and adding 100 µL enzymatic extract. The reaction was measured with a spectrophotometer at 234 nm each 10 seconds during 3 min. The activity was calculated from the slope of the linear portion of the curve. One unit of LOX activity was defined as a change of 0.1 units of absorbance per minute and per millilitre of enzyme extract. Oxidative enzymes: peroxidase (POD), polyphenoloxidase (PPO) and lipoxigenase (LOX) were measured spectrophotometrically (CECIL CE 2021 spsectrophotometer Cecil Instruments Ltd, Cambridge, UK)

Enzymatic activities were expressed as percentage of residual activity (RA %) which was calculated by the quotient between the enzyme activity of treated sample (*AEt*) and the untreated MJ (AE_O) (equation 5).

$$RA(\%) = \frac{AEt}{AE_O} \cdot 100 \qquad \text{eq.5}$$

3.7.Bioactive compounds determination

3.7.1. Total carotenoids content

Total carotenoids (TC) were determined according to Robles-Sánchez, Islas-Osuna, et al., (2009). THF (20 mL) was added to mango slices (5 g), homogenized with an Ultra-Turrax T 25 (IKA® WERKE, Germany) and filtered by Whatman paper (n°. 1). The supernatant was used to determine the total content of carotenoids determined by a spectrophotometer (CECIL CE 2021 Cecil Instruments Ltd, Cambridge, UK) at 470 nm. TC content was calculated on the basis of a standard curve of β-carotene and represented as the relative content using the quotient between the concentration at different days and the concentration at day 0.

3.7.2. Total phenolic content

The methanolic extract for total phenolic compounds (TP) and antioxidant capacity was obtained according to Ribeiro, Barbosa, Queiroz, Knödler, & Schieber (2008) adding 10 mL of MeOH 85% to 20 g of mango slices. The mixture was homogenised and centrifuged at 10000 rpm, 15 min at 4 °C. The supernatant was filtered using Whatman paper (n°. 1) to obtain the extract. TP was determined according to the Folin-Ciocalteu colorimetric method described by Singleton, Orthofer, & Lamuela-Raventós (1998) with slight modifications. MJ extract (0.5 mL) was mixed and homogenised with saturated sodium carbonate solution (10 mL) and Folin-Ciocalteu reagent (10 mL) in ultra turrax homogenized. After one hour in dark storage, absorbance was measured at 765 nm. TP content was calculated on the basis of a

standard curve of gallic acid and represented as the relative content using the quotient between the concentration at different days and the concentration at day 0.

3.7.3. Individual phenolic compounds

3.7.3.1. Sample preparation

Preparation of the mango methanolic extract to determine the individual phenolic compounds were determined according to Palafox-Carlos, Yahia, & González-Aguilar, (2012) with slightly modifications. A portion of 0.5 g freeze-dried mango slice was mixed with 20 mL of methanol (62.5 %), terbuthilhidroxiquinone (TBHQ) (2 g/L) and 5 mL HCl (6 M). The mixture in amber glass was sonicated for 3 min previously to be refluxed in acid hydrolysis (90 °C, 2 h), cooled and diluted to 25 mL with methanol (100%). Finally, the extracts were sonicated 3 min, filtered (0.20 µm membrane) and stored at - 45 °C up to the analysis.

3.7.3.2. High-performance liquid chromatography (HPLC) analysis

Phenolic profile was determined by HPLC, using the methodology reported by Hertog, Hollman, & Venema, (1992) and Ribeiro et al., (2008) with slight modifications. The HPLC system was equipped with a 600 Controller and a 2996 diode array detector (Waters Corporation, Milford, MA, USA) which were set to scan from 200 to 600 nm. Separations were performed on a reverse-phase C18 Spherisorb ODS2 (5 µm) stainless steel column (4.6 mm x 250 mm) operating at room temperature with a flow rate of 1 mL/min. A gradient elution was employed with a mixture of two solvents: (A) 2.5 % of acetic acid in water and (B) 2.5 % acetic acid in methanol as follows: linear gradient from 5 % to 13 % B, 0- 15 min; linear gradient from 13 % to 15 % B, 15-20 min; linear gradient from 15 % to 30 % B, 28- 32 min; isocratic elution 45 % B, 32- 35 min; linear gradient 45- 90 % B, 35- 40 min; isocratic elution 90 % B, 40- 45 min; linear gradient to reach the initial conditions after 5 min; post-time 10 min before the next injection.

3.7.3.3. Identification and quantification

Each phenolic compound was identified by comparing its retention time and spectrum with the external standards (gallic, dihydroxybenzoic and chlorogenic acid; mangiferin and quercetin). Quantification of individual phenols was carried out integrating the peak areas and using calibration curves (R^2 in the range of 0.96- 0.99), Results were expressed as mg of phenolic compounds/ kg of dry weight (dw).

3.7.4. Antioxidant capacity

3.7.4.1. ABTS assay

The percentage of radical-scavenging activity (RSA) was carried out using ABTS radical cation decolorization assay based on the method described by (Siddiq, Sogi, & Dolan, 2013) with slight modifications. An aliquot of 10 µL of the extract obtained was mixed with 3.9 mL of ABTS solution, which was prepared 14 h before usage, with 7.4 mmol/L ABTS and 0.2 mmol/L potassium sulphate. The absorption of the samples was measured with a spectrophotometer (CECIL CE 2021 Cecil Instruments Ltd, Cambridge, UK) at 734 nm after 6 min kept in dark place. A blank of methanol was used without the extract. The results were expressed as percentage of RSA (eq.6).

$$RSA(\%) = \frac{Ao - As}{Ao} x100 \qquad \text{eq.6}$$

3.7.4.2. DPPH assay

The antioxidant capacity was determined using a colorimetric method slightly modified reported by (De Ancos, Sgroppo, Plaza, & Cano, 2002), which is based on the RSA of antioxidant compounds against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The reaction mixture with 10 µL of supernatant, 3.9 mL of methanolic DPPH (0.0025 gL-1) and 90 µL of distilled water was carried out. The samples were shaken vigorously and kept in the dark for 30 min. The absorption of the samples was measured with a spectrophotometer (CECIL CE 2021 Cecil Instruments Ltd, Cambridge, UK) at 515 nm against a blank of methanol without DPPH. The results

were expressed as percentage of RSA as shown in eq.1, where A_o is the absorbance of the reagent and A_s is the absorbance of the sample.

3.8.Sensory evaluation

Sensory evaluation for fresh-cut mango and mango juice were performed. An incomplete block experimental balanced design was used to provide the consumers with three random samples of the edible coatings used for fresh-cut mango (alginate, pectin, carboxymethilcellulose and chitosan) were studied and compared with control and ascorbic acid dipping for each storage period. Eighty-one non-trained consumers, 60 % female and 40 % male, aged 18-60 years old were recruited at University of Lleida for fresh-cut mango sensorial evaluation. On the other hand, thirty non-trained panellists were requested to assess the consumer preference of mango juice treated by thermal or HIPEF treatment.

Mango derivates were served at 16 ± 1 °C in transparent cup coded with three digits randomly numbered. Moreover, a glass containing potable water and a piece of non-salted cracker were provided to panellists to eliminate the residual taste between samples (Mosqueda-Melgar, Raybaudi-Massilia, & Martín-Belloso, 2012). Consumers were asked to score: colour, taste, texture and overall acceptance. For the sensorial attribute evaluation, a structured hedonic scale from 0 (dislike very much) to 10 (like very much) was used. The percentage of consumer acceptance was calculated by the number of consumers that like the sample (score> 5) divided by the total number of consumers tasting that sample and multiplying by 100 (Ngamchuachit, Sivertsen, Mitcham, & Barrett, 2014).

3.9. Statistical analyses

The treatments were assayed in duplicate and two replicate analyses were carried out on each parameter in order to obtain mean values. Statistical analyses were performed using the Statgraphics v.5.1 software (Manugistics, Inc. Rockville, MA, USA) and JMP 11 program for Windows (SAS software; SAS Institute, NC, USA). The results were compared by analysis of variance (ANOVA) followed by Tukey's multiple comparison test to determine differences among means with a significance level of 5 %.

3.10. References

Anthon, G. E., & Barrett, D. M. (2003). Thermal inactivation of lipoxygenase and hydroperoxytrienoic acid lyase in tomatoes. Food Chemistry, 81(2), 275–279.

Axelrod, B., Cheesbrough, T. M., & Laakso, S. (1981). Lipoxygenase from soybeans: EC 1.13.11.12 Linoleate:oxygen oxidoreductase. Methods in Enzymology, Volume 71, 441–451.

Elez-Martínez, P., Aguiló-Aguayo, I., & Martín-Belloso, O. (2006). Inactivation of orange juice peroxidase by high-intensity pulsed electric fields as influenced by process parameters. Journal of the Science of Food and Agriculture, 86(1), 71–81.

Hertog, M. G. L., Hollman, P. C. H., & Venema, D. P. (1992). Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. Journal of Agricultural and Food Chemistry, 40(9), 1591–1598.

ISO 17410. (2001). Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of psychrotrophic microorganisms.

ISO 7954. (1987). Microbiology feneral guidance for enumeration of yeasts and moulds and colony count technique at 25 oC.

Kimball, D. A. (1991). Citrus processing: quality control and technology.

Mosqueda-Melgar, J., Raybaudi-Massilia, R. M., & Martín-Belloso, O. (2012). Microbiological shelf life and sensory evaluation of fruit juices treated by high-intensity pulsed electric fields and antimicrobials. Food and Bioproducts Processing, 90(2), 205–214.

Ngamchuachit, P., Sivertsen, H. K., Mitcham, E. J., & Barrett, D. M. (2014). Effectiveness of calcium chloride and calcium lactate on maintenance of textural and sensory qualities of fresh-cut mangos. Journal of Food Science, 79(5), C786-94.

Palafox-Carlos, H., Yahia, E. M., & González-Aguilar, G. A. (2012). Identification and quantification of major phenolic compounds from mango (Mangifera indica, cv. Ataulfo) fruit by HPLC-DAD-MS/MS-ESI and their individual contribution to the antioxidant activity during ripening. Food Chemistry, 135(1), 105–111.

Pathare, P. B., Opara, U. L., & Al-Said, F. A.-J. (2012). Colour Measurement and Analysis in Fresh and Processed Foods: A Review. Food and Bioprocess Technology, 6(1), 36–60.

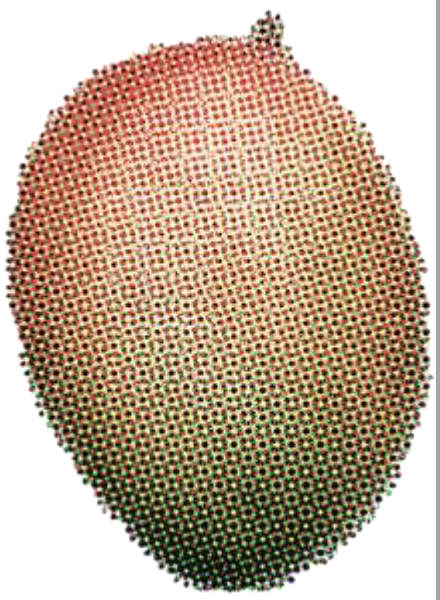
Ramos-Villarroel, A. Y., Martín-Belloso, O., & Soliva-Fortuny, R. (2011). Bacterial inactivation and quality changes in fresh-cut avocado treated with intense light pulses. European Food Research and Technology, 233(3), 395–402.

Ribeiro, S. M. R., Barbosa, L. C. A., Queiroz, J. H., Knödler, M., & Schieber, A. (2008). Phenolic compounds and antioxidant capacity of Brazilian mango (Mangifera indica L.) varieties. Food Chemistry, 110(3), 620–626.

Robles-Sánchez, R. M., Islas-Osuna, M. A., Astiazarán-García, H., Vázquez-Ortiz, F. A., Martín-Belloso, O., Gorinstein, S., & González-Aguilar, G. A. (2009). Quality index, consumer acceptability, bioactive compounds, and antioxidant activity of fresh-cut “ataulfo” mangoes (mangifera indica L.) as affected by low-temperature storage. Journal of Food Science, 74(3), S126-34.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1998). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology.

Vásquez-Caicedo, A. L., Schilling, S., Carle, R., & Neidhart, S. (2007). Effects of thermal processing and fruit matrix on β-carotene stability and enzyme inactivation during transformation of mangoes into purée and nectar. Food Chemistry, 102(4), 1172–1186.



4. RESULTS

CHAPTER I: Improving quality of fresh-cut mango using polysaccharide-based edible coatings

CHAPTER II: Combined effect of pulsed light, edible coating and malic acid dipping to improve microbial stability and quality of fresh-cut mango

CHAPTER III: Effect of pulsed light and edible coating on the phenolic profile and antioxidant potential of fresh-cut mango

CHAPTER IV: Quality changes in mango juice treated by high-intensity pulsed electric fields throughout the storage

CHAPTER V: Effect of high hydrostatic pressure on enzymatic activity and quality attributes in mango puree varieties (cv. Tommy Atkins and Manila)

CHAPTER I

International Journal of Food Science and Technology (under revision)

Improving quality of fresh-cut mango using polysaccharide-based edible coatings

B. Salinas-Roca, A. Guerreiro, J. Welti-Chanes, M.D.C. Antunes and O. Martín-Belloso

ABSTRACT

This study was aimed at evaluating the effect of alginate (AL), pectin (PE), carboxymethyl cellulose (CMC) or chitosan (CH) on microbial stability, physicochemical attributes, total phenolics and carotenoids content, antioxidant capacity and sensory properties of fresh-cut mango during 14 days at 4 ± 1 ˚C. Coated fresh-cut mango kept microbial counts below 6 logs CFU/g, being CH-coated fresh-cut mango those that exhibited the lowest microbial counts (1 log CFU/g) along entire storage. AL, PE and CMC coatings maintained yellow colour of fresh-cut mango throughout storage. AL and CH coatings, which have different monomers in their chain, improved the content of antioxidant compounds in fresh-cut mango as related to uncoated. AL-coated fresh-cut mangoes were the toughest, among those coated, during 14 days. The highest consumer acceptance was achieved in AL (90.2 %) coated fresh-cut mango. CH would be the most suitable coating to extend the quality of fresh-cut mango throughout storage.

Key words: Fresh-cut mango, polysaccharide-based coatings, quality and sensory attributes.

1. INTRODUCTION

Mango (Mangifera indica L.) is a tropical fruit with attractive colour, texture, taste, and high content of bioactive compounds such as carotenoids and phenolics. These bioactive compounds are good antioxidants (R. M. Robles-Sánchez et al., 2013) and their intake has been related to prevention of cardiovascular diseases and cancer (Jahurul et al., 2015). Since consumers demand ready-to-eat products with fresh-like characteristics, the study of preservation techniques to maintain physicochemical and nutritional attributes of mango is required.

Fresh-cut fruits (FCF), which are minimally processed products, fit with the food market demand of ready-to-eat products. However, peeling and cutting operations needed, to obtain FCF, accelerate the metabolic activities of plant tissues, making these minimally processed products more perishable than fresh fruits (R. M. Robles-Sánchez, Rojas-Graü, Odriozola-Serrano, González-Aguilar, & Martín-Belloso, 2009). Besides cold storage, FCF require the use of preservation treatments to reduce respiration rate, surface damage and browning, hence to extend their shelf-life (Antunes, Gago, Cavaco, & Miguel, 2012). Polysaccharides-based edible coatings may be a strategy to preserve quality of ready-to-eat fruits due to their barrier properties that help to have low oxygen permeability and decrease the respiration rate of FCF (Campos, Gerschenson, & Flores, 2011).

Structural differences in polysaccharides-based coatings might determine the preservation of FCF. Alginate, pectin, carboxymethyl cellulose and chitosan are commonly used polysaccharide-based coatings with different structure (Silva-Weiss et al., 2013). Alginate (AL) derived from sea brown algae (Phaeophyceae), is a linear unbranched polysaccharide containing mannuronic and guluronic acids residues. AL can form coatings in the presence of divalent ions, such as calcium, egg-box structures by ionic crosslinking (Tavassoli-Kafrani, Shekarchizadeh, & Masoudpour-Behabadi, 2015). Pectin (PE), extracted from apple or citrus fruits, is a homopolymeric linear chain of galacturonic acid units that form strong coatings when gelation occurs (Moalemiyan, Ramaswamy, & Maftoonazad, 2012). Carboxymethyl cellulose (CMC) is obtained from plant cell walls composed of linear anionic chains of glucopyranosyl units. (Campos et al., 2011) Low concentrations of CMC form a

rigid coating on the food matrix. Chitosan (CH), which is the deacetylated form of chitin composed of glucosamine and N-acetyl-D-glucosamine units, allows the formation of strong coatings. Its efficacy extending shelf life of CH-coated fresh-cut fruits has been previously observed (Plotto et al., 2010).

The potential use of some polysaccharides-based edible coatings have been tested in various FCF to preserve fresh quality throughout storage (Falguera, Quintero, Jiménez, Muñoz, & Ibarz, 2011). Also, the use of coatings such as cassava to preserve physicochemical characteristics in fresh-cut mango during short storage (9 days) has been investigated (Chiumarelli, Ferrari, Sarantópoulos, & Hubinger, 2011) However, to the best of our knowledge, a systematic study that evaluate the quality, safety, antioxidant activity and sensorial acceptance of fresh-cut mango using different polysaccharide-based coatings has not been performed before. Thus, the aim of the present work was to compare the feasibility of AL, PE, CMC or CH coatings on fresh-cut mango quality attributes by evaluating microbial stability, firmness, colour, antioxidant activity, total carotenoid and phenolic content as well as sensory characteristics in coated and uncoated fresh-cut mango along 14 days of refrigerated storage (4±1ºC).

2. MATERIALS AND METHODS

2.1.Mango fruits

Mature-green mangoes (*Mangifera indica*. *cv*. Tommy Atkins) of size 12.1 ± 2.2 x 7.5± 2.3 cm and 388.7 ± 35.6 g of weight were purchased at commercial maturity from a local wholesale market in Lleida, Spain. The initial values of mango pH (3.41 ± 0.04) (Crison 2001 pH-meter; Crison Instruments S.A; Barcelona, Spain), total soluble solids (13.4 ± 0.5 °Brix) (Atago RX-1000 refractometer, Atago Company Ltd; Japan) and firmness (4.3 ± 0.8 N) (Texture Analyzer TA-XT2 Stable Micro Systems Ltd., Surrey, England, UK) were determined prior to treatments.

2.3 Coating forming solutions

Coating forming solutions were prepared similarly to (M A Rojas-Graü, Raybaudi-Massilia, et al., 2007) by dissolving powders of sodium alginate (AL) (Manucol LD, FMC-biopolymers, USA)and high-methoxyl pectin (PE) (Across Organics, Fair Lawn, NJ, USA) at 2% (w/v) and carboxymethyl cellulose (CMC) and chitosan (CH) (Sigma-Aldrich Co. Steinhein, Germany) at 0.5% (w/v) in distilled water while stirring until the solution became clear. In addition, CH solution was prepared following the procedure described by Jiang and Li (2001) who dissolved powders in glacial acetic acid (50 mL/L) (Scharlau Chemie SA, Sentmenat, Barcelona, Spain) and adjust pH at 5.0 with sodium hydroxide (0.1 M) to assure CH solubility. Ascorbic acid (AA) 1% was added as anti-browning agent in all coating forming solutions (R. M. Robles-Sánchez, Rojas-Graü, et al., 2009). Coating forming solutions were homogenised with an Ultra Turrax T25(IKA WERKE, Germany).

2.4 Fresh-cut mango coating

Mangoes were sanitized in a solution of sodium hypochlorite 0.1% for 3 min, manually peeled and cut to obtain 5 mm-thick slices. Mango slices were dipped into the coatings forming solution for 2 min. The excess of coating material was dripped off for 30s. A second dipping in calcium chloride solution (1%) (Sigma-Aldrich Chemic, Steinhein, Germany) for 2 min in mango slices coated with AL was performed. Fresh mango slices were dipped in a solution containing 1% of ascorbic acid (Scharlau, Barcelona, Spain) (uncoated-AA) or in distilled water (uncoated). Mango slices were placed in polypropylene plastic trays and stored at 4±1 ºC until analysis on days 0 (day of processing), 3, 7, 10 and 14.

2.5 Microbial evaluation

Moulds and yeasts counts were determined with the ISO 7954 (ISO 7954, 1987) method using CGA and incubating for 4 days at 25±1°C. Record of psychrophilic microorganisms on sliced mango was carried out after incubation at 4±1°C for 10 days using PCA (Biokar Diagnostic. Beauvais, France), following the ISO 17410 (ISO 17410, 2001) method. Microbial counts were calculated for each gram of cut

fruit and expressed as survival fraction of microorganism log10 (N/No), where N is the total population and No the initial counts.

2.6 Physicochemical determination

Four physicochemical parameters were evaluated: pH, total soluble solids content (TSS), colour and firmness. The pH and TSS content were determined from the squeezed mango slices. Colour of mango slices was measured with a spectrophoto-colorimeter (Minolta Chroma Meter Model CR- 400, Minolta, Tokyo, Japan). The CIE L* (lightness), a* (red-green) and b* (yellow-blue) parameters were read using an illuminant D65 and 10º observer angle, and calibrated using a standard white reflector plate (Y=94.00, x=0.3158, y=0.3322). Colour saturation was calculated by Chroma as C* = (a*2 + b*2)0.5 (McGuire, 1992; Pathare et al., 2012). Firmness was measured as the maximum penetration force expressed in Newton (N) using a texture analyzer TA-XT2 (Stable Micro Systems Ltd., Surrey, England, UK) with a 4 mm diameter steel rod. The downward distance was set at 20 mm at a rate of 4 mm s-1 and automatic return.

2.7 Bioactive compounds determination

Total carotenoids (TC) were obtained according to Robles-Sánchez et al, (2009b). TC content was measured by a UV-VIS spectrophotometer (CECIL CE 2021 Cecil Instruments Ltd, Cambridge, UK) at 470nm. TC content for each day of analysis was calculated on the basis of a standard curve of β-carotene (r^2= 0.99). The results were represented as the relative content of carotenoids using the quotient between the concentration at different days and the concentration at day 0.

The methanolic fraction of fresh-cut mango for total phenolic compounds (TP) and antioxidant capacity analysis was obtained according to (Ribeiro et al., 2008) TP was determined according to the Folin-Ciocalteu colorimetric method described by (Singleton et al., 1998) with slight modifications. The methanolic extract with saturated sodium carbonate solution and Folin-Ciocalteu reagent was mixed in an Ultra-Turrax. After one hour in dark storage, absorbance was measured at 765nm. TP

content for each day was calculated on the basis of a standard curve of gallic acid (r2=0.94). The results were represented as the relative content using the quotient between the concentration at different days and the concentration at day 0.

Antioxidant activity of mango slices was analysed by radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Fluka Sigma-Aldrich Chemic. Steinhein, Germany) method (Ribeiro et al., 2008). A 10µL of supernatant extract was mixed with 90 µL of distilled water and 3.9mL of a DPPH solution (0.0634mM in meOH), stirred vigorously and placed in a dark place for 30min. A blank without extract was prepared. Changes in the absorbance of the samples were measured at 515nm. Radical-scavenging activity (RSA) was expressed as DPPH inhibition percentage and was calculated using the equation 1, where Ao was the absorbance of the methanolic solution of the reagent without extract, and As was the absorbance of the test sample.

$$RSA(\%) = \frac{Ao - As}{Ao} x100 \qquad \text{(eq.1)}$$

2.8 Sensory evaluation

An incomplete block experimental balanced design was conducted to evaluate uncoated, uncoated-AA and the coated mango slices by AL, PE, CMC and CH at days 0, 7 and 14. Eighty-one non-trained consumers, 60 % female and 40 % male, aged 18-60 years old were recruited at the University of Lleida. Three random samples of the treatments assayed were provided to the consumers with three random samples of the treatments assayed. Consumers were asked to score: colour, taste, texture and overall acceptance. For the sensorial attribute evaluation, a structured hedonic scale from 0 (*dislike very much*) to 10 (*like very much*) was used. The percentage of consumer acceptance was calculated by the number of consumers that like the sample (score> 5) divided by the total number of consumers tasting that sample and multiplying by 100 (Ngamchuachit et al., 2014).

2.9 Statistical analysis

Two independent runs with three repetitions of each analysed parameter were performed, except for firmness where six measurements were performed. Statistical

analyses were carried out with JMP 11 program for Windows (SAS software; SAS Institute, NC, USA). Two-way ANOVA and Duncan's Multiple-Range Test ($p<0.05$) were used to compare differences between edible coatings over time. The means of firmness, L*, C* and sensory variables obtained for each treatment were subjected to principal component analysis (PCA) to evaluate relationships among them.

3. RESULTS AND DISCUSSION

3.1 Microbial stability

The use of coatings, excepting CMC, reduced moulds and yeasts and psychrophilic bacteria counts of mango slices compared with uncoated at day of processing (table 1). Moulds and yeasts and psychrophilic bacteria population increased in all mango slices during 14 days of storage, although they did not exceed the upper safety limit of 6 logs CFU/g established for fresh-cut products (IFPA, 2003) (figure 1).

During storage, mango slices coated with AL, PE and CMC showed significantly higher moulds and yeasts counts than those coated with CH (figure 1a). PE-coated mango slices achieved the maximum moulds and yeasts population, increasing up to 3 log cycles at day 14 (figure 1a). On the other hand, psychrophilic bacteria growth on mango slices coated with AL and PE did not differ from uncoated throughout storage (figure 1b). CMC-coated mango slices had significantly less microbial growth the first week of storage, whereas those CH-coated maintained microbial population below 1 log cycles during 14 days.

Table.1. Initial moulds and yeasts (MY) and psychrophilic bacteria (PS) population of uncoated, ascorbic acid-dipped (uncoated-AA), alginate-coated (AL), pectin-coated (PE), carboxymethylcellulose-coated (CMC) and chitosan-coated (CH) mango slices.

Treatment	MY (log CFU/g)	PS (log CFU/g)
uncoated	2.7 ± 0.2^{a}	2.84 ± 0.25^{a}
uncoated-AA	2.13 ± 0.11^{a}	1.35 ± 0.11^{b}
AL	1.8 ± 0.4^{b}	1.65 ± 0.45^{b}
PE	1.35 ± 0.55^{b}	1.2 ± 0.1^{bc}
CMC	2.4 ± 0.5^{ab}	2.2 ± 0.5^{ab}
CH	1.0 ± 0.2^{c}	1.0 ± 0.1^{d}

Values represent the mean ± standard deviation. Values of the same parameter in a column followed by the same lower case letter are not significantly different by Duncan's multiple range test ($p<0.05$).

According with other studies, the antimicrobial efficiency of CH against spoilage microorganisms was demonstrated (Chien et al., 2007; Plotto et al., 2010). The antimicrobial activity of CH was attributed to the polycation nature of the molecule, which enables interaction and formation of polyelectrolyte complex with the acidic polymers produced in the surface of the bacterial cell (Falguera et al., 2011). Thus, CH can cause alterations in cell membrane function through its strong interaction with electronegative surface charge, leading to permeability changes. Moreover, CH structure containing glucosamine can trigger the synthesis of phytoalexins, which reduce the microbial growth throughout storage (Tamer & Çopur, 2010).

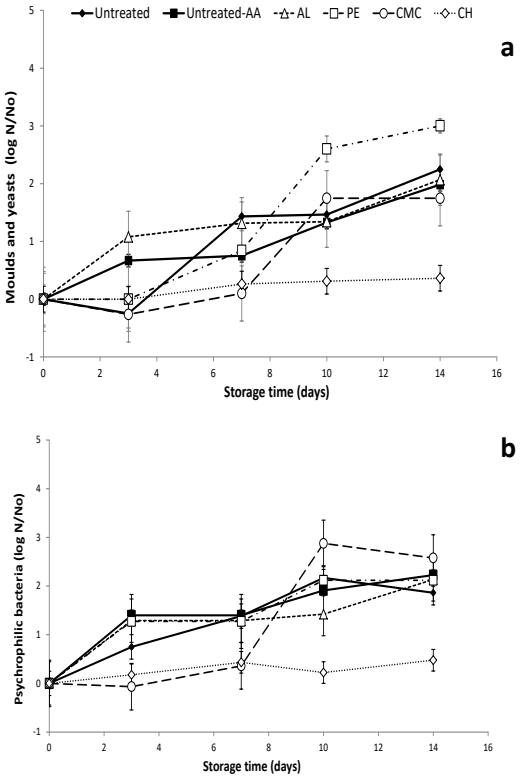


Fig.1. Moulds and yeasts (a) and psychrophilic bacteria (b) growth in uncoated, ascorbic acid-dipped (uncoated-AA), alginate-coated (AL), pectin-coated (PE), carboxymethylcellulose-coated (CMC) and chitosan-coated (CH) mango slices throughout 14 days of storage at 4 ± 1 °C.

When comparing the rest of EC, the cross-linking matrix induced by calcium in AL coating may stabilize the cell wall, reduce water vapour permeability and hence, improve the resistance of mango tissue to microbial growth along the storage (Silva-Weiss et al., 2013). Differently, moulds and psychrophilic bacteria could use cellulose and pectin as nutrients (Plotto et al., 2010) and consequently, they created proper growth conditions for microorganisms.

3.2 Colour

At day 0, coated and uncoated mango slices had similar lightness (L*) (table 2). No differences of L* in uncoated mango slices were observed throughout storage. Otherwise, differences in L* values among coatings were observed at day 14. CMC-coated mango slices maintained L* values similar to those uncoated and uncoated-AA. According to Villalobos, Chanona, Hernández, Gutiérrez, & Chiralt (2005), who described the glossy and low opacity character of methylcellulose film, the use of CMC coating enabled to keep L* in mango slices as uncoated. AL and PE coated mango slices presented lower L* values compared to those CMC- coated, which could be explained by the higher opacity of AL and PE coating solutions (Espitia et al., 2014). According with other authors a decrease in L* values in CH-coated fresh-cut mango along storage was observed (Plotto et al., 2010). Since mango has high content of reducing sugars such as fructose, and CH chain contains amino groups, Maillard reaction could occur (Liu et al., 2013). In this sense, Maillard reaction leads to a decrease in L* values in mango slices by the generation of brown products as melanoidins (Phisut & Jiraporn, 2013).

The b* and C* parameters, which followed similar pattern along storage, described yellowish colour in mango slices (table 2). b* values of coated mango slices were maintained along the storage, whereas it decreased in uncoated and uncoated-AA mango slices after day 14 of storage. According to the present results, Robles-Sánchez et al., (2013) described no deterioration of C* in AL-coated mango slices containing ascorbic acid (1%) along storage. The barrier effect of coatings to carbon dioxide and oxygen allowed diminishing the production of ethylene. The releasing of

pigments from the cell wall might conduct the maintenance and increase of yellow in coated mango slices (Moalemiyan et al., 2012).

3.3 Firmness

Firmness of uncoated and coated mango slices declined along 14 days of refrigerated storage. Nevertheless, coated mango slices maintained greater resistance to rupture compared to those uncoated along storage (table 2). The firmness of mango slices might change via two main mechanisms. The first mechanism involves the continuous mango respiration, which leads the breakdown of complex carbohydrate polymers present in the cell wall triggering to softening (Plotto et al., 2010). The second mechanism involves the loss of water that changes the cell turgidity and thus firmness. AL-based coating kept significantly ($p<0.05$) higher firmness values of the mango slices compared with other coatings. AL-based coating, as a linear un-branched polysaccharide with carboxylate functional group accommodated calcium in its structure creating a more rigid film that reduced gas exchange and rate of respiration (Dang, Singh, & Swinny, 2008; Qi, Hu, Jiang, Tian, & Li, 2011). On the other hand, CMC and PE coatings, which contains carboxylic group bounded with hydroxyl groups, might diminish the water binding properties of coatings and the surface adhesion with mango slices (Bonilla et al., 2012; Silva-Weiss et al., 2013)

3.4 Carotenoids and phenolic compounds content

Initial total carotenoid content in CMC-coated, uncoated and uncoated-AA mango slices were significantly higher than in those AL, PE and CH coated (table 1). Similar respiratory coefficient between uncoated and CMC-coated mango slices has been previously described (Ducamp-Collin et al., 2009). Indeed, Bonilla et al., (2012) reported CMC as the most O2 permeable coating (0.097mL·µm/ m2·d·Pa), compared with AL and CH coatings. Thus, gas barrier and respiration rate differences among coatings might affect carotenoids preservation in mango slices. During storage, uncoated and CMC-coated mango slices declined an 18% the initial carotenoid content as is shown in fig 2a. On the other hand, the initial content of total

carotenoids in fresh-cut mango was increased by AL, PE and CH coatings until 107%, 110% and 120%, respectively, at day 10. The changes along the storage could be attributed to carotenoid metabolism along ripening (Chiumarelli & Pereira, 2010). Coatings with low oxygen permeability such as AL, PE and CH could retain carotenoids from oxidation along the storage (Gil et al., 2006).

Table.2. Initial content of carotenoids and phenolics of uncoated, ascorbic acid-dipped (uncoated-AA), alginate-coated (AL), pectin-coated (PE), carboxymethylcellulose-coated (CMC) and chitosan-coated (CH) mango slices.

Treatment	Total carotenoids (μg β-cartone/100g fw)	Phenolic compounds (mg gallic acid/100g fw)
uncoated	847.5 ± 97.8 [a]	7.6± 0.5 [a]
uncoated-AA	745.37 ± 42.17 [b]	9.7± 0.2 [b]
AL	624.9 ± 93.9 [b]	9.9± 0.5 [b]
PE	583.9 ± 35.4 [c]	12.9± 0.2 [c]
CMC	856.7± 21.9 [a]	13.83± 0.1 [d]
CH	608.2 ± 11.6 [bc]	9.5 ± 0.4 [b]

Values represent the mean ± standard deviation. Values of the same parameter in a column followed by the same lower case letter are not significantly different by Duncan's multiple range test ($p<0.05$).

Total phenolic content of coated and uncoated-AA mango slices was significantly higher than those uncoated at the processing day (table 1). This could be related with the acidification of mango slices with ascorbic acid. It has been reported that the polyphenoloxidase activity, decreased as the pH of food matrix decreased since the optimal pH is between 5 and 7 (Liu et al., 2013). Hence, the pH of mango slices coating solutions, which ranged from 3.5 in CMC to 5.1 in CH, could delay or avoid the action of polyphenoloxidase. During storage, an increase of phenolic compounds in uncoated and coated mango slices, except using CMC and PE, was observed until day 10 of storage. The wounding stress during the processing could activate phenylpropanoid pathways up to day 10, when a significant decrease of phenolics started (fig. 2b). Despites the decrease in total phenolics in all mangoes slices from days 10 to 14, AL and CH coatings presented higher phenolics concentration at the end than at the beginning of storage. Other authors reported no changes on phenolic

compounds in uncoated fresh-cut mango along 12 days (R. M. Robles-Sánchez, Rojas-Graü, et al., 2009). Results in this study confirm that selecting adequate coatings contribute to depletion of the respiration rate as they could act as a protective barrier, and hence, retain carotenoids and phenolics compounds.

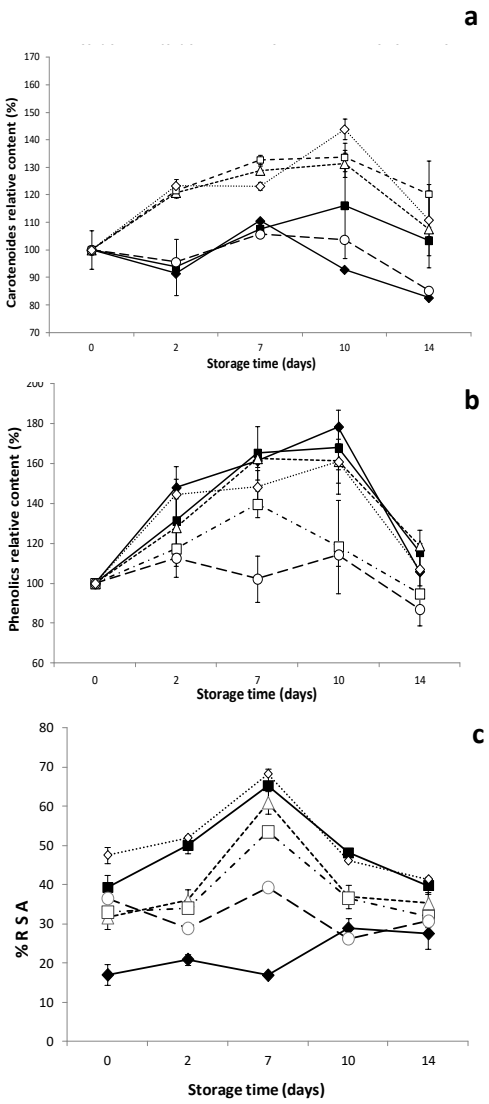


Fig.2. Carotenoids relative content (a), phenolics relative content (b) and residual scavenging activity-RSA (c) of uncoated, ascorbic acid-dipped (uncoated-AA), alginate-coated (AL), pectin-coated (PE), carboxymethylcellulose-coated (CMC) and chitosan-coated (CH) mango slices throughout 14 days of storage at 4 ± 1 °C.

3.5 Antioxidant capacity

At day 0, the highest antioxidant capacity measured as radical scavenging activity (RSA) was observed in mango slices coated with CH (47.6±2.1% RSA) followed by uncoated-AA (39.3±3.1% RSA) with no significant differences among RSA values of AL, PE and CMC-coated mango slices (fig. 2c). The wounding response to initial stress in tissues (peeling and cutting) could activate the antioxidant compounds metabolism (Sogi et al., 2012). A decline in antioxidant capacity of coated mango slices at day 7 might indicate that the delivery of antioxidants from the cell vacuoles was stopped. At the end of storage, all the treatments containing ascorbic acid maintained their initial RSA. Similarly to other works, the addition of ascorbic acid had a contribution not only as antibrowning but also preserving (R. M. Robles-Sánchez et al., 2013; M. Siddiq et al., 2013). This work suggested that long heteropolymeric chain EC, such as AL and CH with functional groups in the structure as amine groups (CH) or carboxylate (AL) could contribute to maintain the antioxidant activity as well as adhesion, cohesion and permeability to water vapour, which had an effect in the preservation of bioactive compounds (Silva-Weiss et al., 2013; Villalobos et al., 2005).

3.6 Physicochemical and sensorial changes

TSS and pH in coated and uncoated mango slices from day 0 to the end of storage were not significant. The mean values of pH and TSS values in coated mango slices were 3.5±0.1 and 13.1±0.3°Brix, respectively. Other authors observed similar values of TSS in CMC-coated fresh-cut mango throughout storage (Plotto et al., 2010).

Sensorial properties as colour, texture, taste and overall acceptance were maintained similar among uncoated, uncoated-AA and coated mango slices at day of processing (fig. 3). Furthermore, all mango slices were well-appreciated as the consumer acceptance ranged from 73.3 to 96.7%. Coated mango slices increased significantly the acceptance compared with those uncoated and uncoated-AA throughout the storage. AL-coated mango slices achieved a 90.2% of acceptance at day 14 followed by those CH-coated (86.5%). This indicates that coatings were a good alternative for

organoleptic preservation of mango slices, since they maintained and even improved the sensory acceptance after 14 days. Other fresh-cut fruits such as melon (Raybaudi-Massilia, Mosqueda-Melgar, & Martín-Belloso, 2008a) coated with AL have been reported to be well-accepted after 8 days of storage, respectively. Texture was significantly higher in mango slices coated with AL (6.6±1.5) at day 14. This fact may be explained by ripeness, which had a more gradual cell wall degradation and lead better firmness for the consumer (Dang et al., 2008). The highest taste value was 6.4±2.1 for AL-coated mango slices at day 14. Colour of mango slices was well-appreciated during the whole storage except for uncoated mango slices that diminished the score from 6.3±0.4 at day 7 to 4.3±1.3 at day 14. AL-coated mango slices had the highest colour score (7.3±1.6) at day 14.

Two principal components (PC1 and PC2) were calculated. PC1 and PC2 account with a 70.3% of the variability in the original data. Instrumental parameters L* and firmness were not associated with sensorial variables, whereas C* explained in a 52.9% the sensorial colour evaluation. Taste was the sensory variable that best explained overall acceptance of mango slices ($p<0.001$). Thus, the coating preference of panellist could be described considering taste evaluation. Sensorial colour and overall acceptance had significantly negative correlation ($r2=-0.78$) with L* and firmness, respectively. Thus, the most accepted mango slices were described to be those with low firmness and high L*. The relationship between sensorial evaluation and physicochemical parameters, which has not been previously observed by other authors in mango slices, allowed describing the attributes most appreciated by consumers.

Table.3. Physicochemical parameters: lightness (L*), b*, chroma (C*) and firmness (N) of uncoated, ascorbic acid-dipped (uncoated-AA), alginate-coated (AL), pectin-coated (PE), carboxymethylcellulose-coated (CMC) and chitosan-coated (CH) mango slices throughout 14 days of storage at 4 ± 1 °C

Parameter	Day	uncoated	uncoated-AA	AL	PE	CMC	CH
L*	0	76.7 ± 1.6 aA	75.8 ± 2.3 aA	75.1 ± 2.3 aA	75.2 ± 0.2 aA	74.74 ± 0.42 aA	74.8 ± 2.6 aA
	3	77.3 ± 1.5 aA	75.0 ± 2.6 aA	68.1 ± 0.2 aA	76.1 ± 0.1 aA	76.7 ± 1.2 aA	73.8 ± 0.4 aA
	7	76.3 ± 2.1 aA	76.0 ± 0.9 aA	71.9 ± 8.1 aA	76.7 ± 0.3 aA	78.56 ± 0.15 aA	75.94 ± 1.48 bA
	10	77.4 ± 4.4 aA	66.0 ± 10.4 aA	69.9 ± 2.8 aA	67.5 ± 13.1 aA	80.7 ± 1.6 aA	77.6 ± 1.9 bA
	14	78.94 ± 0.02 aA	78.8 ± 0.9 aA	73.6 ± 0.8 aB	73.2 ± 1.1 aB	77.7 ± 1.3 aA	70.3 ± 0.3 cC
b*	0	56,7 ± 0,8 aC	58,1 ± 5,4 aC	46,5 ± 9,4 aABC	58,99 ± 2,58 aC	51,5 ± 7,6 aBC	50,83 ± 4,01 aABC
	3	54,5 ± 1,8 aC	57,7 ± 7,1 aA	55,2 ± 3,6 aA	54,99 ± 3,05 aA	54,7 ± 3,3 aA	56,4 ± 0,9 aA
	7	53,03 ± 1,18 aC	51,4 ± 3,6 bC	55,7 ± 1,2 aA	50,6 ± 9,8 aC	53,1 ± 0,9 aA	48,3 ± 0,4 aC
	10	51,09 ± 5,47 aC	53,6 ± 3,5 aA	52,8 ± 2,5 aA	57,02 ± 4,94 aA	46,98 ± 0,08 aA	50,17 ± 4,3 aA
	14	47,6 ± 0,9 bBC	42,8 ± 0,6 cE	48,8 ± 5,2 aDE	57,3 ± 0,4 aA	47,8 ± 1,5 aCDE	48,248 ± 5,7 aABC
C*	0	56.88 ± 0.85 aC	58.2 ± 5.3 aABC	46.8 ± 9.1 aABC	59.2 ± 0.6 aA	51.8 ± 7.4 aBC	51.1 ± 3.9 bABC
	3	54.74 ± 1.75 aC	57.8 ± 7.1 aA	55.2 ± 3.6 aA	55.1 ± 2.9 aA	54.8 ± 3.3 aA	56.44 ± 1.01 aA
	7	53.1 ± 1.1 aC	51.5 ± 3.6 bC	55.8 ± 1.3 aA	50.7 ± 9.7 bC	53.26 ± 1.01 aA	48.4 ± 4.3 aC
	10	51.2 ± 5.3 aC	53.7 ± 3.4 aA	52.8 ± 2.5 aA	57.1 ± 4.9 aA	47.12 ± 0.01 aA	50.2 ± 4.3 aA
	14	47.8 ± 0.8 bBC	42.9 ± 0.6 bE	48.9 ± 5.1 aDE	57.4 ± 0.4 aA	48.0 ± 1.4 aCDE	48.26 ± 5.74 aABC
Firmness (N)	0	4.3 ± 0.8 aABC	4.2 ± 0.3 aABC	3.4 ± 0.2 aAB	1.7 ± 0.2 aCD	1.9 ± 0.2 aCD	2.0 ± 0.5 aBCD
	3	3.4 ± 0.1 bA	3.9 ± 0.1 bA	2.7 ± 0.3 bAB	1.63 ± 0.12 aBC	1.7 ± 0.1 aBC	1.9 ± 0.1 aBC
	7	3.8 ± 0.2 bA	3.9 ± 0.2 bA	2.6 ± 0.8 bB	1.5 ± 0.1 aC	1.6 ± 0.1 aC	1.6 ± 0.1 bC
	10	3.8 ± 0.2 bA	3.8 ± 0.6 bA	2.6 ± 0.7 bB	1.7 ± 0.1 aC	1.6 ± 0.1 aC	1.4 ± 0.1 bC
	14	3.7 ± 0.2 bA	3.6 ± 0.5 bA	2.5 ± 0.4 bB	1.7 ± 0.1 aC	1.5 ± 0.2 aC	1.3 ± 0.2 bC

Values represent the mean ± standard deviation. Values of the same parameter in a column followed by different lower case letter, and in a row followed by different upper case letter are significantly different by Duncan's multiple range test ($p < 0.05$).

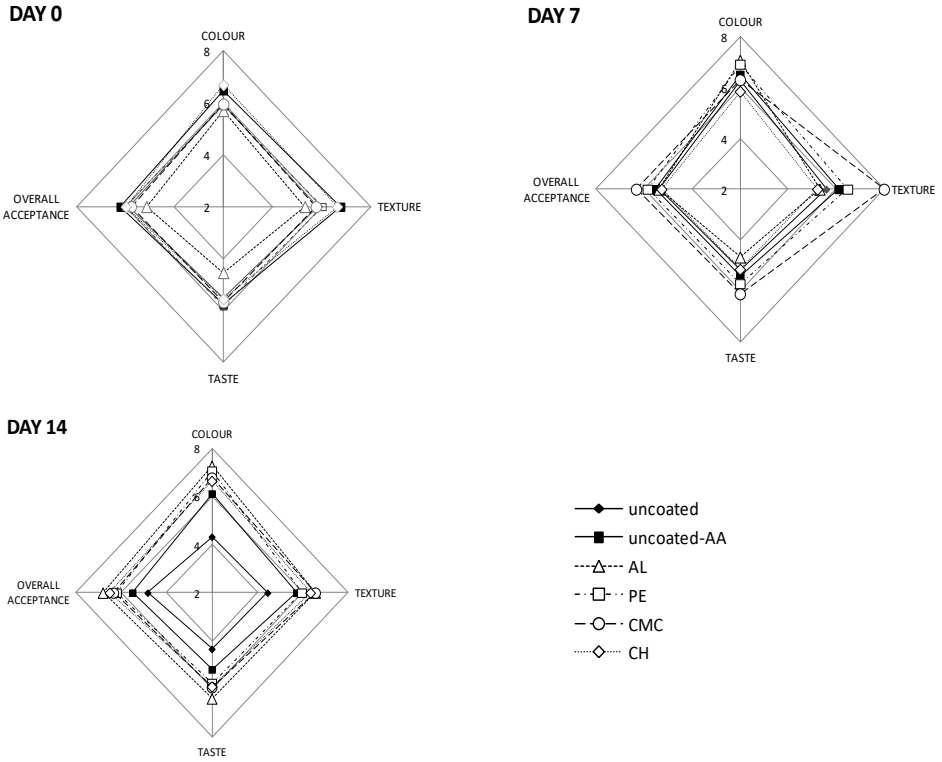


Fig.3. Sensorial evaluation (colour, texture, taste and overall acceptance) of uncoated, ascorbic acid-dipped (uncoated-AA), alginate-coated (AL), pectin-coated (PE), carboxymethylcellulose-coated (CMC) and chitosan-coated (CH) mango slices at 0, 7 and 14 days of storage at 4 ± 1 °C

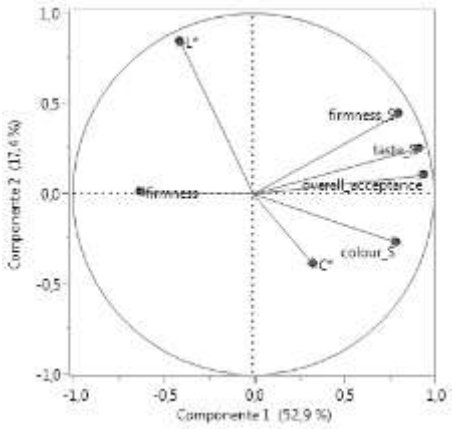


Fig.4. Principal Components of the firmness, lightness (L*), chroma (C*) and sensory variables: taste (taste_S), firmness (firmness_S), colour (colour_S) and overall acceptance of coated mango slices.

4. CONCLUSION

The use of polysaccharide-based coatings enabled lower microbial counts compared with uncoated mango slices at day of processing and no-one exceeded the upper safety limit of 6 logs CFU/g during 14 days. In addition, an antimicrobial effect of chitosan coating was observed. Yellow colour in mango slices was maintained throughout storage when coatings were used. Alginate coating retarded the firmness decay in mango slices whereas softening easily appeared in mango sliced when other coatings, which did not form an ionic crosslinking in presence of calcium, were applied. Regarding bioactive compounds content, especially alginate and chitosan coatings improved fresh mango phenolics and carotenoids content. Sensory evaluation of mango slices confirmed that coated mango slices were better accepted than those uncoated after 14 days of storage. Considering the obtained results, chitosan would be the most satisfactory coating since microbial growth was avoided and quality, nutritive value and consumer acceptance of mango slices were maintained throughout storage.

ACKNOWLEDGMENTS

This work was supported by the University of Lleida (Spain) and financed by Tecnológico de Monterrey (Research Chair Funds CAT-200 and CDB081).

5. REFERENCES

Antunes, M.D., Gago, C.M., Cavaco, A.M. & Miguel, M.G. (2012). Edible coatings enriched with essential oils and their compounds for fresh and fresh-cut fruit. Recent Patents on Food, Nutrition and Agriculture, 4, 114–122.

Bonilla, J., Atarés, L., Vargas, M. & Chiralt, A. (2012). Edible films and coatings to prevent the detrimental effect of oxygen on food quality: Possibilities and limitations. Journal of Food Engineering, 110, 208–213.

Campos, C.A., Gerschenson, L.N. & Flores, S.K. (2011). Development of Edible Films and Coatings with Antimicrobial Activity. Food and Bioprocess Technology, 4, 849–875.

Chien, P.-J., Sheu, F. & Yang, F.-H. (2007). Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. Journal of Food Engineering, 78, 225–229.

Chiumarelli, M., Ferrari, C.C., Sarantópoulos, C.I.G.L. & Hubinger, M.D. (2011). Fresh cut "Tommy Atkins" mango pre-treated with citric acid and coated with cassava (Manihot esculenta Crantz) starch or sodium alginate. Innovative Food Science & Emerging Technologies, 12, 381–387.

Chiumarelli, M. & Pereira, L. (2010). Cassava Starch Coating and Citric Acid to Preserve Quality Parameters of Fresh-Cut "Tommy Atkins" Mango. Journal of Food Science.

Dang, K.T.H., Singh, Z. & Swinny, E.E. (2008). Edible coatings influence fruit ripening, quality, and aroma biosynthesis in mango fruit. Journal of Agricultural and Food Chemistry, 56, 1361–1370.

Ducamp-Collin, M.-N., Reynes, M., Lebrun, M. & Freire, M. (2009). Fresh cut mango fruits: Evaluation of edible coatings. Acta Horticulturae.

Espitia, P.J.P., Du, W.-X., Avena-Bustillos, R. de J., Soares, N. de F.F. & McHugh, T.H. (2014). Edible films from pectin: Physical-mechanical and antimicrobial properties - A review. Food Hydrocolloids, 35, 287–296.

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

Gil, M.I., Aguayo, E. & Kader, A.A. (2006). Quality changes and nutrient retention in fresh-cut versus whole fruits during storage. Journal of Agricultural and Food Chemistry, 54, 4284–4296.

IFPA. (2003). Guía de seguridad alimentaria para la industria de productos vegetales frescos cortados. Alexandria, Virginia.

ISO 17410. (2001). Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of psychrotrophic microorganisms.

ISO 7954. (1987). Microbiology feneral guidance for enumeration of yeasts and moulds and colony count technique at 25 oC.

Jahurul, M.H.A., Zaidul, I.S.M., Ghafoor, K., Al-Juhaimi, F.Y., Nyam, K.-L., Norulaini, N.A.N., Sahena, F. & Mohd Omar, A.K. (2015). Mango (Mangifera indica L.) by-products and their valuable components: A review. Food Chemistry, 183, 173–180.

Jiang, Y. & Li, Y. (2001). Effects of chitosan coating on postharvest life and quality of longan fruit. Food Chemistry, 73, 139–143.

Liu, F.-X., Fu, S.-F., Bi, X.-F., Chen, F., Liao, X.-J., Hu, X.-S. & Wu, J.-H. (2013). Physico-chemical and antioxidant properties of four mango (Mangifera indica L.) cultivars in China. Food chemistry, 138, 396–405.

McGuire, R. (1992). Reporting of objective color measurements. HortScience, 27, 1254–1255.

Moalemiyan, M., Ramaswamy, H.S. & Maftoonazad, N. (2012). Pectin-based edible coating for shelf-life extension of ataulfo mango. Journal of Food Process Engineering, 35, 572–600.

Ngamchuachit, P., Sivertsen, H.K., Mitcham, E.J. & Barrett, D.M. (2014). Effectiveness of calcium chloride and calcium lactate on maintenance of textural and sensory qualities of fresh-cut mangos. Journal of food science, 79, C786-94.

Pathare, P.B., Opara, U.L. & Al-Said, F.A.-J. (2012). Colour Measurement and Analysis in Fresh and Processed Foods: A Review. Food and Bioprocess Technology, 6, 36–60.

Paula, G.A., Benevides, N.M.B., Cunha, A.P., Oliveira, A.V. de, Pinto, A.M.B., Morais, J.P.S. & Azeredo, H.M.C. (2015). Development and characterization of edible films from mixtures of κ-carrageenan, ι-carrageenan, and alginate. Food Hydrocolloids, 47, 140–145.

Phisut, N. & Jiraporn, B. (2013). Characteristics and antioxidant activity of Maillard reaction products derived from chitosan-sugar solution. International Food Research Journal, 20, 1077–1085.

Plotto, A., Narciso, J.A., Rattanapanone, N. & Baldwin, E.A. (2010). Surface treatments and coatings to maintain fresh-cut mango quality in storage. Journal of the science of food and agriculture, 90, 2333–41.

Qi, H., Hu, W., Jiang, A., Tian, M. & Li, Y. (2011). Extending shelf-life of Fresh-cut "Fuji" apples with chitosan-coatings. Innovative Food Science and Emerging Technologies, 12, 62–66.

Raybaudi-Massilia, R.M., Mosqueda-Melgar, J. & Martín-Belloso, O. (2008). Edible alginate-based coating as carrier of antimicrobials to improve shelf-life and safety of fresh-cut melon. International journal of food microbiology, 121, 313–27.

Ribeiro, S.M.R., Barbosa, L.C.A., Queiroz, J.H., Knödler, M. & Schieber, A. (2008). Phenolic compounds and antioxidant capacity of Brazilian mango (Mangifera indica L.) varieties. Food Chemistry, 110, 620–626.

Robles-Sánchez, R.M., Rojas-Graü, M.A., Odriozola-Serrano, I., González-Aguilar, G.A. & Martín-Belloso, O. (2009). Effect of minimal processing on bioactive compounds and antioxidant activity of fresh-cut "Kent" mango (Mangifera indica L.). Postharvest Biology and Technology, 51, 384–390.

Robles-Sánchez, R.M., Rojas-Graü, M.A., Odriozola-Serrano, I., González-Aguilar, G. & Martin-Belloso, O. (2013). Influence of alginate-based edible coating as

carrier of antibrowning agents on bioactive compounds and antioxidant activity in fresh-cut Kent mangoes. LWT - Food Science and Technology, 50, 240–246.

Rojas-Graü, M.A., Raybaudi-Massilia, R.M., Soliva-Fortuny, R.C., Avena-Bustillos, R.J., McHugh, T.H. & Martín-Belloso, O. (2007). Apple puree-alginate edible coating as carrier of antimicrobial agents to prolong shelf-life of fresh-cut apples. Postharvest Biology and Technology, 45, 254–264.

Siddiq, M., Sogi, D.S. & Dolan, K.D. (2013). Antioxidant properties, total phenolics, and quality of fresh-cut "Tommy Atkins" mangoes as affected by different pre-treatments. LWT - Food Science and Technology, 53, 156–162.

Silva-Weiss, A., Ihl, M., Sobral, P.J.A., Gómez-Guillén, M.C. & Bifani, V. (2013). Natural Additives in Bioactive Edible Films and Coatings: Functionality and Applications in Foods. Food Engineering Reviews, 5, 200–216.

Singleton, V.L., Orthofer, R. & Lamuela-Raventós, R.M. (1998). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology.

Sogi, D.S., Siddiq, M., Roidoung, S. & Dolan, K.D. (2012). Total Phenolics, Carotenoids, Ascorbic Acid, and Antioxidant Properties of Fresh-cut Mango (Mangifera indica L., cv. Tommy Atkin) as Affected by Infrared Heat Treatment. Journal of Food Science.

Tamer, C.E. & Çopur, O.¨ U. (2010). Chitosan: An edible coating for fresh-cut fruits and vegetables. In: Acta Horticulturae. Pp. 619–626.

Tavassoli-Kafrani, E., Shekarchizadeh, H. & Masoudpour-Behabadi, M. (2015). Development of edible films and coatings from alginates and carrageenans. Carbohydrate Polymers, 137, 360–374.

Villalobos, R., Chanona, J., Hernández, P., Gutiérrez, G. & Chiralt, A. (2005). Gloss and transparency of hydroxypropyl methylcellulose films containing surfactants as affected by their microstructure. Food Hydrocolloids, 19, 53–61.

CHAPTER II

Food Control 66 (2016) 190e197

Combined effect of pulsed light, edible coating and malic acid dipping to improve fresh-cut mango safety and quality

B. Salinas-Roca, R. Soliva-Fortuny, J. Welti-Chanes and O. Martín-Belloso

ABSTRACT

The impact of pulsed light (PL), alginate coating (ALC) and malic acid dipping (MA) treatments on quality and safety aspects of fresh-cut mango was studied. Fresh-cut mangoes were inoculated with *L.innocua* and then subjected to PL (20 pulses at fluence of 0.4 J•cm^{-2}/ pulse), ALC (2 %) or MA (2 %) treatments. Moreover, different combinations of PL, ALC and MA were assayed to evaluate possible synergisms among treatments. Microbial stability and quality parameters (colour, pH, soluble solids and firmness) of fresh-cut mango were examined throughout 14 days of storage at 4 °C. Results show that MA-PL and PL-ALC-MA treatments additively reduced *L.innocua* counts by 4.5 and 3.9 logs, respectively. Microbial population in fresh-cut mango remained below 6 log CFU/g over 14 days. Differences between firmness values of untreated and treated fresh-cut mangoes were evident throughout storage. Namely, firmness of alginate-coated slices sharply increased and progressively decreased over storage. Colour parameters and total soluble solids content decreased in all treated mango slices throughout 14 days, while pH was kept similar to that of the fresh tissue. An optimal combination of different treatments enables to ensure safety of fresh-cut mango with minimal quality deterioration throughout storage.

Key words: Fresh-cut mango; combined treatments, *L.innocua*, pulsed light, edible coatings.

1. INTRODUCTION

The growing demand for ready-to-eat fruits and vegetables has led to an increasing interest in the study of methods that enhance their safety while preserving freshness. Although fruits do not generally pose a safety hazard, peeling and cutting operations make fresh-cut fruits more susceptible to microbial attack. *Listeria sp* can be a hazardous contaminant of fresh-cut fruits as it is able to survive in a wide range of pH and temperature conditions. In fruits of low acidity, in which mango is included, *Listeria sp* may also find the conditions to survive and multiply (Penteado et al., 2014). Among fresh-cut melon, apple and pineapple are the most commonly consumed and studied; however the demand for other fruits such as mango is continuously growing (M. Siddiq et al., 2013). Mango (*Mangifera indica L*.) is one of the most harvested tropical fruits (FAO, 2012). It is widely demanded for its yellow colour, fleshy texture and unique flavour. Freshness and appearance are the primary criteria determining consumer satisfaction. Produce safety is also critical to maintain consumer confidence. Therefore, developing adequate treatments to obtain fresh-cut mango could help to promote its consumption and enable industry to satisfy the market trends.

Recent research in preservation methods for fresh-cut fruits has focused on assuring safety and maintaining original characteristics of fruit, while avoiding the undesired effects caused by handling and processing (Caminiti et al., 2011; Moody et al., 2014; Proctor, 2010). Pulsed light (PL) treatments are being studied as a feasible alternative to conventional preservative processes (Oms-Oliu, Martín-Belloso, & Soliva-Fortuny, 2008). This technology involves the application of very short high-intensity pulses of broad spectrum light: (180 - 1100 nm). The composition of the spectrum and the energy density has been shown to play an important role in microbial cell death by PL (Keklik, Demirci, Puri, & Heinemann, 2012; Ramos-Villarroel, Martín-Belloso, & Soliva-Fortuny, 2011). Different studies have proposed the use of PL treatments for the decontamination of fresh-cut fruits; however, applications for fresh-cut tropical fruits are scarce. As far as we know, literature offers only a prospective study regarding the application of PL for the decontamination of fresh-cut mango (Charles, Vidal, Olive, Filgueiras, & Sallanon, 2013). On the other hand, edible coatings based

on polysaccharides such as sodium alginate have been proposed for extending the shelf-life of fresh-cut fruits (M A Rojas-Graü, Tapia, et al., 2007). These coatings are commonly formed as a thin layer on the cut surface of fruits, acting as a barrier against gas exchange and transpiration. Edible coatings enable to retard the physiological response to mechanical stress and other physical disorders leading to moisture and solutes migration, gas exchange, respiration and increased oxidative phenomena that have a deleterious impact on the product quality (Oms-Oliu et al., 2010; Raybaudi-Massilia, Mosqueda-Melgar, & Tapia, 2010). However, their effects in preventing microbial inactivation are scarce (Raybaudi-Massilia et al., 2008a). The use of organic acids is another strategy which could be used to ensure safety of fresh-cut fruits. Malic acid dips have been shown to enable a decrease in microbial loads, thus ensuring safety and extending quality of fresh-cut produce over storage (Gómez et al., 2012; Raso & Barbosa-Cánovas, 2003; M A Rojas-Graü, Raybaudi-Massilia, et al., 2007; Tapia et al., 2007; Valencia-Chamorro, Palou, Del Río, & Pérez-Gago, 2011). Their antimicrobial activity could be attributed to the reduction of the medium pH, decrease of the intracellular pH by ionization of undissociated acid molecules.

As these strategies do not individually succeed in guaranteeing safety and quality maintenance of fresh-cut fruits, a combined methods approach stands as a good alternative to achieve this goal. Hence, the aim of the present work was to assess the effectiveness of combining PL, alginate coating and malic acid on the reduction of *Listeria innocua* population as well as to evaluate microbial growth and physicochemical parameters (pH, soluble solids, colour and firmness) of mango slices over refrigerated storage.

2. MATERIALS AND METHODS

2.1. Mango slices preparation

'Tommy Atkins' mangoes were purchased from a local market (Lleida, Spain) at commercial maturity. Mango pH (3.46 ± 0.01) (Crison 2001 pH-meter; Crison Instruments S.A; Barcelona, Spain), total soluble solids (13.9 ± 0.2 °Brix) (Atago RX-1000 refractometer, Atago Company Ltd; Japan) and firmness (5.74 ± 0.7 N·s)

(Texture Analyzer TA-XT2 Stable Micro Systems Ltd., Surrey, England, UK) of the fruit flesh were determined before processing. Whole mangoes were washed with an aqueous solution of sodium hypochlorite (300μL/L) and then peeled and cut to obtain 5 mm-thick slices. Sliced mangoes were inoculated and/or subjected to the different treatments, as described in the following sections. Once treated, slices (35±1g) were placed into transparent polypropylene trays and stored (4 ± 1 ºC) until analysis at days 0, 3, 7, 10 and 14.

2.2. *Listeria innocua* culture and inoculation

L. innocua IPL 1.17 (Institute Pasteur de Lille; Lille, France), as a surrogate of the pathogenic *Listeria monocytogenes*, were provided from the culture collections of the Department of Food Technology (University of Lleida, Spain). Stock culture of *L.innocua* was grown in tryptone soy broth (TSB) with 0.6 % yeast extract (Bioakar Diagnostic; Beauvais, France) and incubated at 35 °C with continuous agitation at 200 rpm for 15 h to obtain cells in stationary growth phase (10^8 - 10^9 CFU/mL). Mango slices (35 g) were inoculated by spreading 100 μL of *L.innocua* stock cultures over the entire upper surface with a sterile micropipette before treatment and packaging (Ramos-Villarroel et al., 2011).

2.3. Pulsed light treatment

Pulsed light (PL) treatments were carried out with a XeMaticA-2L System (SteriBeam Systems GmbH, Germany). The experiments were performed at a charging voltage of 2.5 kV. The system is equipped with a lamp situated 8.5 cm above the sample holder. The lamp delivered pulses of 0.3 ms with an overall radiant fluence of 0.4 J•cm^{-2} at the sample level. The total light energy was measured according to the calibration of the equipment with a standard light source estimated by photodiode readings and manufacturer's directions. The emitted spectrum ranged from 180 - 1100 nm. To evaluate the effect of the wavelength of PL on the inactivation of *L.innocua*, two types of UV filters were used: a 2 mm-thick Pyrex glass filter that cuts off wavelengths below 305 nm hence allowing to pass some

UVB, all UVA, visible light (V) and infrared (IR) wavelengths (89 % of the emitted energy); and Makrolon polycarbonate plastic filter that cuts all light below 400 nm, thus allowing only V and IR light to pass through (83 % of the emitted energy). In addition, treatments with increasing number of pulses (0, 10, 15, 20, 25, and 30) were assayed in order to evaluate the inactivation of *L.innocua* as affected by PL.

2.4. Alginate coating

Film-forming solutions were prepared by dissolving 20 g of alginate coating (ALC) (FMC Biopolymer Ladybum Works, USA) into 1000 mL of distilled water and homogenised with an Ultra Turrax T25 (IKA WERKE, Germany). Calcium chloride (20 g) was dissolved into 1000 mL of distilled water to be used as a crosslinking agent (Sigma-Aldrich Chemic. Steinhein, Germany). Mango slices were dipped into the sodium alginate solution (2 % w/v) during 2 minutes and the excess was removed thereafter. A second dipping in calcium chloride (2 % w/v) solution was performed for ALC-coated mango slices.

2.5. Malic acid solution

DL-Malic acid (20 g) (MA) (Fluka; Steinhein, Germany) was dissolved by stirring into 1000 mL of distilled water. Mango slices were dipped into MA solution during 2 minutes. It must be noted that MA was incorporated to the calcium chloride solution when combined with the edible coating.

2.6. Combined treatments

Different combinations of PL (20 pulses of broad-spectrum light), ALC (2 % w/v) and MA (2 %) were evaluated to elucidate possible synergistic, additive or antagonist effects. The evaluated treatments were: ALC followed by PL (ALC-PL), MA followed by PL (MA-PL), ALC followed by MA (ALC-MA), PL followed by ALC and MA (PL-ALC-MA) and ALC followed by MA and PL (ALC-MA-PL). Untreated

mango slices dipped in distilled water were considered as a control reference treatment (C).

2.7. Microbiological analyses

Sliced mangoes (10 g) were placed into sterile plastic bags with 90 mL of saline peptone water (Bioakar Diagnostic; Beauvais, France) and homogenized for 1 min in a stomacher blender (IUL Instruments, Barcelona, Spain) for microbial analyses. Serial dilutions were made and 100 μL were placed in Palcam agar plates (Bioakar Diagnostic; Beauvais, France) and spread with a Drigalsky handle. The evaluation was made by duplicate for each dilution and the plates were incubated for 48 h at 37 ºC. Microbial population was evaluated and the results expressed as $\log_{10}$ CFU/g.

Enumeration of psychrophilic microorganisms on sliced mango was carried out by agar plate counting (PCA) (Biokar Diagnostic; Beauvais, France), after incubation at 4 ± 1 °C for 10 days, following the ISO 17410 (2001) method. Mould and yeast counts were determined by the ISO 7954 (1987) method using chloramphenicol glucose agar (CGA) (Biokar Diagnostic; Beauvais, France) and incubating for 4 days at 25 ± 1 °C. Counts were expressed as $\log_{10}$ CFU/ g.

2.8. Physicochemical determinations

2.8.1. pH and total soluble solids

The pH (pH-meter Crison Instruments S.A. Barcelona, Spain) and total soluble solids (TSS) (refractometer, Haake RS 80) were determined in a homogenate obtained from crushed mango slices (20 g). Triplicate analyses were carried out and results were expressed as mean and standard deviation.

2.8.2. Colour parameters

Colour was expressed as L*, a* and b*, which indicate luminosity, chromaticity on a green (-) to red (+) axis, and chromaticity on a blue (-) to yellow (+) axis,

respectively. Lightness (L*) was determined with a tri-stimulus Minolta CR-400 colorimeter (Konica Minolta Sensing, INC, Osaka, Japan) using a D65 illuminant and an observation angle of 10º. For reference, a standard white tile (Y=94.00, x=0.3158, y=0.3322) was used. Based on the CIE L*, a* and b* values Hue angle (h°) was calculated (eq. 1). Colour parameters were obtained as the mean of three determinations.

$$h° = \tan^{-1} (b^*/a^*) \quad \text{eq.1}$$

2.8.3. Firmness

Firmness of sliced mangoes was analysed with a TA-XT2 Texture Analyser (Stable Micro Systems Ltd., England, UK) by considering the impulse required to penetrate the fruit flesh with a 4 mm diameter steel rod. To this purpose the area beneath the force-time curve was recorded. The test speed was 4 mm/s and the distance of penetration was 4 mm. Results were the mean of six measurements per sample and given as the delta firmness in N·s.

2.9. Data analysis

The treatments were conducted in duplicate; hence data were representative of two independent experimental runs. Statistical analyses were performed using the Statgraphics v.5.1 software (Manugistics, Inc. Rockville, MA, USA). The results were compared by analysis of variance (ANOVA) followed by Tukey's multiple comparison test to determine differences among means with a significance level of 5 %.

3. RESULTS AND DISCUSSION

3.1. Inactivation of *L. innocua* on mango slices

3.1.1. Effect of PL

The effect of PL spectral range and pulse number on the *L. innocua* population on mango slices is shown in Fig. 1. Inactivation was higher as pulse number increased. However, no additional inactivation was observed for treatments above 20 full spectrum pulses. Under those conditions, corresponding to an energy of 8 $J \cdot cm^{-2}$, 3.15 log reductions of *L. innocua* population were achieved. Similarly, Ramos-Villarroel, Martín-Belloso, & Soliva-Fortuny, (2011) and Ramos-Villarroel, Aron-Maftei, Martín-Belloso, & Soliva-Fortuny (2014) reported 2.61 and 2.97 log reductions of *L. innocua* population on fresh-cut avocado after treatments of 15 and 30 pulses, respectively. In addition, microbial inactivation rates decreased for intense treatments, probably due to the "shadow" effects caused by the formation of biofilms, the product geometry and the internalization of microorganisms in the fruit tissue. Furthermore, significant differences ($p < 0.05$) were observed between treatments using full spectrum PL and spectral ranges in which the UV component was removed, either partially (λ= 305 - 1100 nm) or completely (λ= 400 - 1100 nm). This is in line with other studies describing a higher bactericidal effect for light wavelengths in the range of 250 - 270 nm than for those above 305 nm. Higher inactivation levels achieved when UV light was used are related to the induction of DNA strand breaks and formation of pyrimidine dimers (Guerrero-Beltrán, 2004; Keyser, Műller, Cilliers, Nel, & Gouws, 2008). However, inactivation was yet significant for wavelengths above 400 nm, denoting a weak effect of light on *L. innocua*. Although high-energy pulse light treatments present several drawbacks due to the generation of heating on the product surface, no thermal effect was attributed to the decrease of *L. innocua* population. Thus, inactivation was related with the amount of energy received at the sample surface, wavelength and the microorganism type.

3.1.2. **Effect of combined methods**

The reduction of *L. innocua* counts on treated mango slices as affected by the combination of PL, ALC and MA treatments is shown in Fig. 2. The assayed treatments led to a significant ($p< 0.05$) reduction of the *L. innocua* survival fraction compared to those of untreated mango slices. The three preservation factors applied individually led to a substantial reduction of the initial counts. MA treatment was

more effective than PL and ALC, in this order, leading to 2.9, 2.5 and 1.9 log reductions, respectively. Beyond the already discussed photochemical effect of PL (Ross, Griffiths, Mittal, & Deeth, 2003), the antimicrobial effects of malic acid have been reported to decrease the intracellular pH by ionization of un-dissociated molecules (Ramos-Villarroel et al., 2014; Rathnayaka, 2013). Our results are in line with those reported by Raybaudi-Massilia, Mosqueda-Melgar, Sobrino-López, Soliva-Fortuny, & Martín-Belloso, (2009), who achieved 4 log reductions of *Listeria monocytogenes* counts in fresh-cut apples dipped with MA. On the other hand, although sodium alginate has not been reported to possess any antimicrobial effect, ALC treatments could remove part of microbial load or even limit their ability to grow on the product surface.

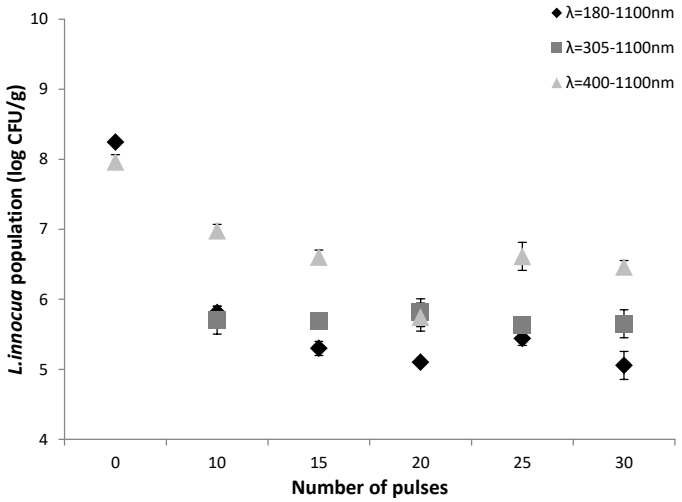


Figure 1: Influence of the spectral range on survival of *L. innocua* inoculated in mango slices treated by pulsed light at different number of pulses. The values are the mean of four determinations ± SD.

Regarding the combined treatments, no synergistic effects were observed. However, the effect of the combination of MA and PL was additive, leading to a maximum reduction of 4.49 log cycles. It is noteworthy that the combination of the three preservation factors provided lower inactivation levels, which at the same time depended on the order of the treatments application. Hence, PL-ALC-MA and ALC-MA-PL treatments led to 3.92 and 3.03 log reductions of the *L. innocua* populations, respectively. This fact was attributed to the antagonistic action of the ALC factor, which may limit the effectiveness of the PL and MA factors (Raybaudi-Massilia et al., 2010a). Moreira, Tomadoni, Martín-Belloso, & Soliva-Fortuny, (2015) observed that a gellan gum-based coating hindered the effectiveness of PL applied to fresh-cut apples due to the blockage of a significant part of the UV-C radiation, thus reducing the extent of photochemical inactivation. In this sense, our results are consistent and suggest that PL treatments should be applied before alginate coating and malic acid treatments. Although the feasibility of applying a combined methods strategy for reducing the *L. innocua* populations growing on mango slices was demonstrated, a further experiment was carried out to evaluate the impact of the assayed treatments on quality aspects throughout refrigerated storage.

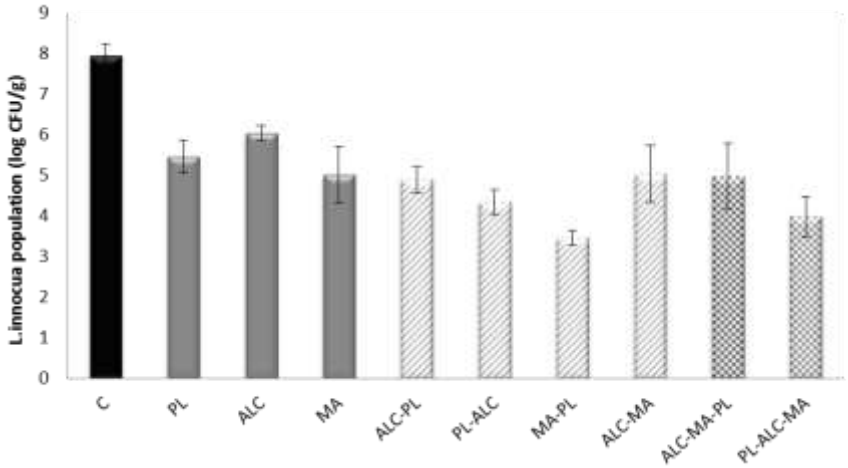


Figure 2: Influence of different individual or combined treatments on survival of *L. innocua* inoculated in mango slices. PL: pulsed light (20 pulses at λ = 180- 1100 and 0.4 J·cm^{-2}/pulse); ALC: alginate coating (2 %); MA: malic acid (2 %) and C: untreated. The values are the mean of four determinations ± SD.

3.2. Effects of combined treatments during storage on quality parameters of fresh-cut mango

3.2.1. Microbial stability

Among the naturally-occurring microbiota of untreated fresh-cut mangoes, moulds and yeasts were initially predominant (Figure 3). However, after cutting, mould and yeast counts remained almost stable, whereas psychrophilic bacteria abruptly increased. After the application of the different treatments, both fungi and psychrophilic bacteria were reduced. On the one hand, mould and yeast counts on the freshly cut untreated mango slices were significantly ($p < 0.05$) reduced after the application of any of the individual or combined treatments (fig. 3a). The lowest mould and yeast loads just after processing were observed for mango slices subjected to PL or PL-ALC-MA, with reductions of 2.07 and 2.09 log cycles, respectively. Mould and yeast inactivation achieved with the other treatments ranged from 1 to 1.5 log cycles. On the other hand, psychrophilic bacteria were less affected by the treatments (fig. 3b). The highest inactivation of psychrophilic bacteria was obtained with the PL treatment (1.37 log cycles), followed by the PL-ALC-MA treatment (1.09 logs cfu/g). Mould and yeast counts observed after each treatment were maintained without substantial change during the first week of storage. This lag period coincided with an increase in the psychrophilic bacteria counts (Figure 3b) and might be directly attributed to the environmental changes promoted by minimal processing. In general, psychrophilic bacteria counts increased during the days thereafter processing but then they did not significantly change during the storage. In contrast, an increase in the moulds and yeasts populations was observed subsequently regardless the treatment applied. Microbial quality of mango slices was best maintained after the application of the PL-ALC-MA treatment. Hence, those samples exhibited the lowest counts for moulds and yeasts as well as for psychrophilic bacteria at the end of the studied period.

In accordance with the results obtained for *L. innocua* inactivation, microbial growth was influenced by the order of treatments combination. In this sense, PL application before coating enabled light to more efficiently decontaminate mango surface from microorganisms. Consistently, ALC, ALC-MA and ALC-PL treatments presented the

highest microbial growth over storage. However, when applied in the adequate order, alginate coatings could help to maintain the integrity of damaged fruit tissues, thus limiting the presence of exudates and the consequent proliferation of microorganisms, as in the case of mango slices subjected to the PL-ALC-MA treatment.

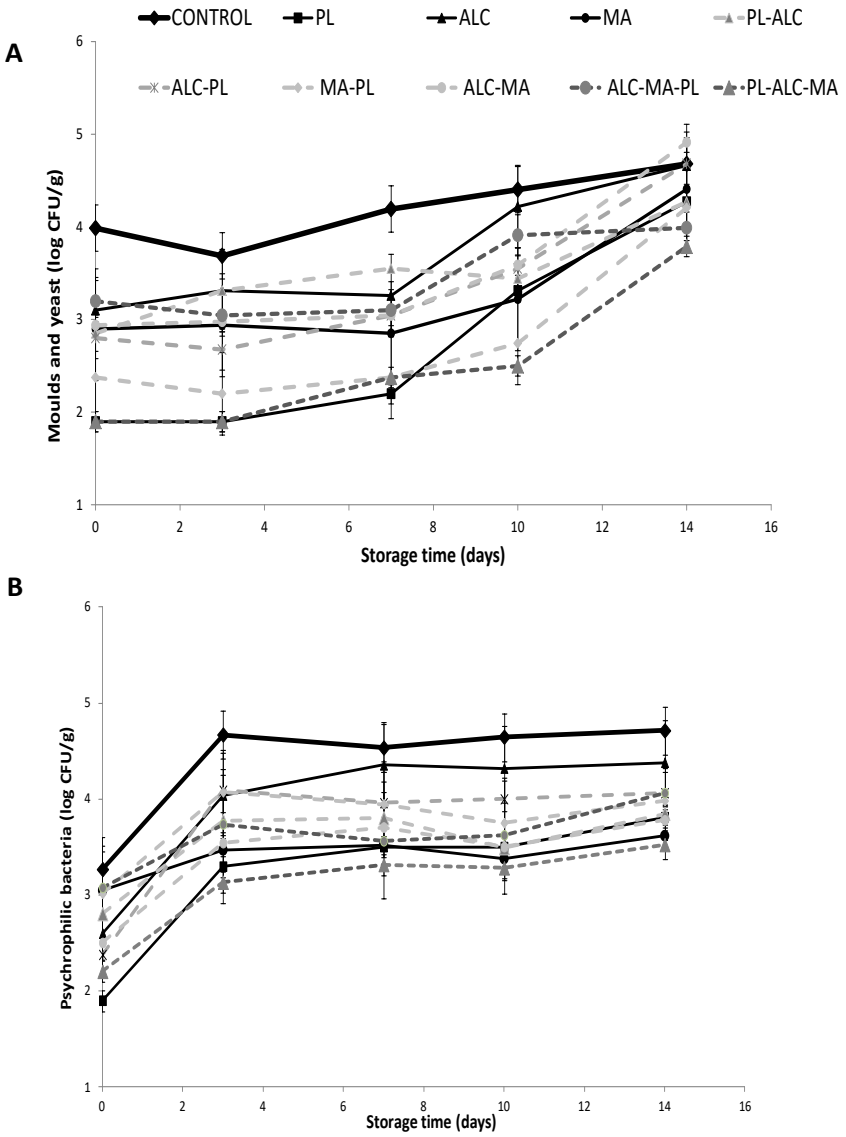


Figure 3: Growth of moulds and yeasts (A) and psychrophilic bacteria (B) on sliced mango submitted to different treatments throughout 14 days of storage at 4 °C. PL: pulsed light (20 pulses at λ = 180- 1100 and 0.4 J·cm^{-2}/pulse); ALC: alginate coating (2 %); MA: malic acid (2 %) and C: untreated. Data shown are the mean of two replicate measurements obtained from two replicate packages ± standard deviation.

Table 1: Changes in mango slices total soluble solids (TSS) and pH during 14 days of storage (4 °C) after treatments PL: pulsed light (20 pulses at λ = 180-1100 nm and 0.4 J·cm^{-2}/ pulse); ALC: alginate coating (2 %); MA: malic acid (2 %) and C: untreated. Data shown are the mean of two replicate measurements ± standard deviation. Values within a column followed by the same lowercase letter are not significantly different ($p < 0.05$). Values within the same line followed by the same uppercase letter are not significantly different ($p < 0.05$).

TSS														
	0			3			7			10			14		
CONTROL	12.9	±	0.6^{Aa}	12.1	±	0.3^{Aa}	11.9	±	0.1^{ABa}	11.5	±	0.1^{Ba}	11.1	±	0.1^{Ba}
PL	12.6	±	0.9^{Aa}	12.6	±	0.8^{Aa}	12.4	±	0.1^{Ab}	13.1	±	0.5^{Bb}	13.6	±	0.1^{Ab}
ALC	11.3	±	0.9^{Abb}	11.3	±	0.7^{Aab}	10.6	±	0.2^{Ab}	10.9^{A}	±	0.1^{Ac}	10.1	±	0.1^{Aa}
MA	11.3	±	0.2^{Abb}	10.9	±	0.1^{Bb}	11.0	±	0.1^{Ba}	10.4	±	0.1^{Bc}	10.5	±	0.1^{Ba}
PL-ALC	12.0	±	1.1^{Aa}	11.3	±	0.1^{Bab}	11.4	±	0.1^{Ba}	11.2	±	0.4^{Ba}	11.0	±	0.1^{Aa}
ALC-PL	10.9	±	0.2^{Ab}	11.5	±	0.8^{Aab}	11.1	±	0.1^{Aa}	10.9	±	0.5^{Ac}	10.4	±	0.1^{Bc}
MA-PL	12.0	±	0.9^{Aab}	12.0	±	0.6^{Aa}	11.3	±	0.1^{Ba}	11.6	±	0.1^{Ba}	11.5	±	0.1^{Aab}
ALC-MA	11.4	±	0.8^{Ab}	12.4	±	0.6^{Aa}	11.4	±	0.1^{Aa}	11.5	±	0.4^{Aa}	11.9	±	0.1^{Aac}
ALC-MA-PL	10.6	±	0.8^{Ab}	10.6	±	0.6^{Ab}	11.2	±	0.2^{Aa}	10.3	±	0.2^{Ac}	10.0	±	0.1^{Aa}
PL-ALC-MA	11.3	±	0.5^{Aab}	11.3	±	0.3^{Aab}	11.1	±	0.1^{Aa}	10.8	±	0.1^{Ac}	11.2	±	0.3^{Aa}

pH														
	0			3			7			10			14		
CONTROL	3.46	±	0.01^{Aa}	3.48	±	0.04^{Aa}	3.6	±	0.0^{Ba}	3.46	±	0.01^{Aa}	3.33	±	0.01^{Ca}
PL	3.37	±	0.05^{Aa}	3.45	±	0.09^{Ba}	3.5	±	0.0^{Ba}	3.33	±	0.01^{Ab}	3.56	±	0.01^{Bb}
ALC	3.48	±	0.03^{Aa}	3.48	±	0.1^{Aa}	3.57	±	0.02^{Ba}	3.39	±	0.01^{Cb}	3.54	±	0.01^{Bb}
MA	3.28	±	0.03^{Ab}	3.13	±	0.1^{Bc}	3.41	±	0.05^{Cb}	3.36	±	0.01^{Ab}	3.57	±	0.01^{Cc}
PL-ALC	3.6	±	0.04^{Ac}	3.7	±	0.1^{Ab}	3.60	±	0.01^{Aa}	3.45	±	0.03^{Ba}	3.44	±	0.01^{Bc}
ALC-PL	3.69	±	0.01^{Ac}	3.63	±	0.06^{Ab}	3.61	±	0.01^{Aa}	3.51	±	0.02^{Bc}	3.64	±	0.01^{Acd}
MA-PL	3.22	±	0.03^{Ab}	3.22	±	0.02^{Ac}	3.3	±	0.0^{Ac}	3.51	±	0.01^{Bc}	3.53	±	0.02^{Cb}
ALC-MA	3.3	±	0.1^{Ab}	3.2	±	0.2^{Ac}	3.41	±	0.01^{Bb}	3.46	±	0.02^{Ba}	3.5	±	0.02^{Bb}
ALC-MA-PL	3.27	±	0.05^{Ab}	3.21	±	0.15^{Ac}	3.3	±	0.01^{Ac}	3.32	±	0.03^{Ab}	3.40	±	0.01^{Bc}
PL-ALC-MA	3.10	±	0.01^{Ab}	3.2	±	0.08^{Ac}	3.3	±	0.01^{Ac}	3.65	±	0.01^{Bcd}	3.45	±	0.01^{Bc}

Table 2: Changes in mango slices lightness (L*) and hue angle (h°) during 14 days of storage (4 °C) after treatments PL: pulsed light (20 pulses at λ = 180-1100 nm and 0.4 J·cm^{-2}/ pulse); ALC: alginate coating (2 %); MA: malic acid (2 %) and C: untreated. Data shown are the mean of two replicate measurements ± standard deviation. Values within a column followed by the same lowercase letter are not significantly different ($p < 0.05$). Values within the same line followed by the same uppercase letter are not significantly different ($p < 0.05$).

	L*											
	0			3			7			10			14		
CONTROL	73.42	±	2.53^{Aabc}	71.99	±	2.58^{Aa}	74.86	±	3.97^{Aa}	77.2	±	2.4^{Ba}	77.3	±	3.5^{Ba}
PL	76.41	±	1.96^{Ac}	73.57	±	0.19^{Ba}	72.04	±	2.31^{Ba}	78.4	±	0.3^{Ca}	76.57	±	2.37^{Aa}
ALC	73.85	±	1.05^{Aabc}	67.2	±	2.9^{Bab}	75.10	±	0.54^{Cb}	71.7	±	1.1^{Db}	72.2	±	2.1^{ADb}
MA	77.6	±	2.7^{Aabc}	72.7	±	1.4^{ABa}	77.20	±	0.04^{Ac}	75.6	±	2.0^{Aa}	76.8	±	4.1^{Aba}
PL-ALC	72.98	±	0.08^{Aab}	64.57	±	1.93^{Bab}	72.40	±	0.13^{Aa}	70.0	±	2.5^{Ab}	71.1	±	1.0^{Ab}
ALC-PL	69.61	±	1.68^{Aabc}	66.4	±	1.2^{Bab}	66.80	±	1.54^{Ac}	68.2	±	0.7^{Bc}	67.1	±	1.8^{A}
MA-PL	78.01	±	3.15^{Aabc}	71.26	±	2.66^{Bab}	71.7	±	3.9^{Aa}	72.47	±	0.78^{Ab}	72.90	±	2.46^{Ab}
ALC-MA	72.8	±	1.2^{Aabc}	65.9	±	1.9^{Bab}	68.5	±	1.9^{Bc}	66.89	±	1.70^{Bc}	72.73	±	2.32^{Ab}
ALC-MA-PL	71.33	±	4.87^{Aa}	65.22	±	1.29^{Bab}	70.70	±	3.37^{Aa}	71.8	±	6.4^{Ab}	69.85	±	1.31^{Ac}
PL-ALC-MA	71.6	±	3.5^{Abc}	67.13	±	2.64^{Bab}	71.73	±	4.28^{Ba}	68.6	±	0.2^{Bc}	70.16	±	0.06^{Ac}

	h°											
CONTROL	93.3	±	1.1^{Ac}	97.15	±	2.47^{Ba}	93.7	±	1.1^{Aa}	95.24	±	1.08^{Ba}	95.8	±	1.6^{Ba}
PL	94.2	±	0.2^{Aab}	94.83	±	1.20^{Ab}	94.14	±	2.36^{Aa}	93.86	±	0.55^{Bb}	92.6	±	0.8^{Bb}
ALC	94.81	±	0.44^{Aab}	97.01	±	0.23^{Ba}	94.74	±	0.17^{Aa}	92.94	±	0.04^{Cb}	95.51	±	1.67^{Aa}
MA	95.3	±	0.5^{Aa}	97.33	±	0.55^{Aa}	96.5	±	0.4^{Bb}	92.84	±	0.26^{Cb}	95.23	±	2.16^{Aa}
PL-ALC	94.03	±	0.88^{Abc}	96.55	±	0.04^{Bc}	94.09	±	0.06^{Aa}	93.85	±	0.88^{Abc}	96.5	±	0.1^{Ba}
ALC-PL	95.9	±	2.3^{Aa}	99.72	±	1.97^{Bad}	94.95	±	0.35^{Ca}	95.9	±	0.5^{Aa}	95.01	±	0.06^{Aa}
MA-PL	95.20	±	3.22^{Aa}	97.37	±	2.78^{Aa}	92.97	±	0.45^{Bab}	93.03	±	0.59^{BCbc}	94.95	±	1.10^{Ca}
ALC-MA	92.35	±	1.45^{Ac}	97.01	±	3.28^{Ba}	92.77	±	3.10^{Ab}	92.6	±	1.8^{Ab}	93.39	±	2.05^{Ab}
ALC-MA-PL	94.7	±	1.9^{Abc}	98.25	±	0.93^{Bad}	95.2	±	1.5^{Aab}	95.8	±	3.2^{Ba}	95.65	±	2.85^{Ba}
PL-ALC-MA	93.9	±	1.3^{Aa}	97.26	±	1.68^{Bad}	94.2	±	0.8^{Aab}	94.0	±	1.3^{Aab}	94.5	±	0.5^{Aa}

3.2.2. Physicochemical parameters

3.2.2.1. Total soluble solids (TSS) and pH

Regarding TSS, significantly lower values, compared with the untreated, were observed just after the processing with the exception of mango samples subjected to the PL treatment (Table 1). These differences may be attributed to sugars lixiviation from the fruit slices when immersed into the dipping solutions used in the ALC and MA treatments (M Chiumarelli et al., 2011; Hodges & Toivonen, 2008). Initial TSS values were kept almost constant over storage although a slight decrease, probably caused by microbial spoilage, was observed specially in untreated mango slices over the second week of storage.

Concerning pH values, these were significantly affected by the type of treatment applied. Malic acid dips resulted into a reduction of the natural pH of mango. However, this reduction was not considered to play a significant role on quality stability, as the greatest pH change, as much as 0.36 units, occurred after the PL-ALC-MA treatment. The decrease in pH may be explained by the acidification of the cytoplasm which can be promoted by the production of CO_2. As it is produced, this gas is partially dissolved in the water of the cellular tissues with the consequent decrease of pH medium (A. Y. Ramos-Villarroel et al., 2011). PL applied individually was not found to cause any pH modification. In contrast, alginate coated mango slices exhibited increased pH values in comparison with those obtained for untreated sliced mango. This may be attributed to the pH of the sodium alginate solution (pH = 4.3), which is higher than that of untreated mango (pH = 3.5).

3.2.2.2. Colour

Lightness (L*) and hue angle (h°), of mango slices as affected by combined treatments are displayed in Table 2. L* is an indicative parameter associated with the enzymatic browning of fruit and vegetables. ALC treatments, either individually applied or in combination led to decreased L* values compared to the colour of untreated mango slices (73.42 ± 2.53) or with other combined treatments. L* values

of mango slices stored 14 days were similar to those of the just processed although untreated mango slices increased L* values up to 77.3 ± 3.5. On the other hand, mango slices had similar h° value between untreated (93.3 ± 1.1) and treated mango slices at day 0. From then on, differences on h° values between treated and untreated mango slices were not observed or were really scarce.

According to Chiumarelli et al., (2011) and Ramos-Villarroel et al. (2011) a decrease of L* and h° parameter in fresh-cut mango and avocado respectively was observed due to PL treatment. Moreover, other authors suggested that mango cubes could develop undesirable colour as a consequence of the exposure to visible light due to a decompartmentalization process allowing colour substances such as phenolic compounds and carotenoids to come in contact with oxidative enzymes (Charles et al., 2013; Gómez et al., 2012). Despite of this, the present results indicated no signs of browning in treated mango slices. In this regard, the chlorine wash may reduce the browning effect as reported by Chen, Zhu, Zhang, Niu, & Du (2010) for fresh-cut lettuce. In addition, mango slices containing ALC preserved natural pigments of mango, such as carotenoids, confined in the cells, thus, the oxidation was avoided throughout the storage.

3.2.2.3. Firmness

Figure 4 shows the changes in firmness of mango slices as affected by combined treatments and storage time. Treated mango slices, except those subjected to PL, ALC-MA and ALC-MA-PL treatments, had higher firmness after processing than untreated mango slices. Fruits are likely to soften mainly due to hydrolysis of the pectic acids found in cell walls. Nevertheless, a protective effect of alginate and PL treatments against texture loss in mango slices was observed during storage. This fact was attributed to the action of calcium ions, which enable the crosslinking effect between the alginate polymer and calcium. Also, PL could lead to the increase of polyamines, which could be related with a limitation of the accessibility to the cell wall of the deleterious enzymes that promote softening (Charles et al., 2013). Furthermore, an additive effect was observed when PL and alginate were combined. In this sense, coating applied after PL treatment may have more influence on mango

surface texture than alone since pulsed light may have already increased the permeabilization of the cell wall. Similarly, Gómez et al., (2012) observed, by light microscopy, that apple discs treated by PL and dipping solution of ascorbic acid and calcium chloride increased the resistance to rupture compared with untreated apple disc. This is in line with different studies that described PL as a feasible treatment for firmness enhancement in fresh-cut fruits (Gonzalez-Aguilar, Wang, Buta, & Krizek, 2001; Manzocco, Da Pieve, & Maifreni, 2011; Ramos-Villarroel et al., 2011).

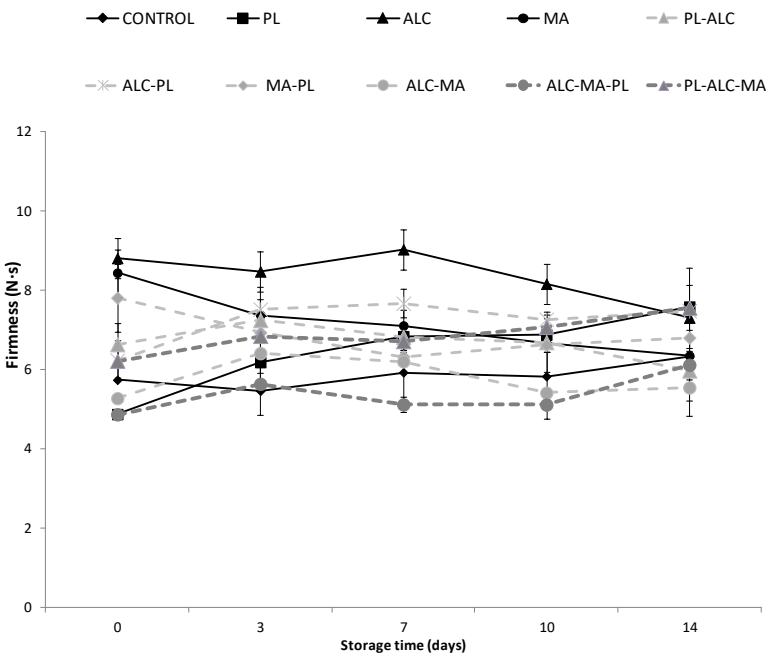


Figure 4: Changes of firmness in stored mango slices after expose them to different treatments. PL: pulsed light (20 pulses at λ = 180- 1100 nm and 0.4 J·cm^{-2}/ pulse); ALC: alginate coating (2 %); MA: malic acid (2 %) and C: untreated. Data shown are the mean of six measurements ± SD.
from two replicate packages ± standard deviation.

4. CONCLUSIONS

A PL treatment of 20 pulses of broad-spectrum light (λ= 180 - 1100 nm) with an overall energy 8 $J \cdot cm^{-2}$ was most suitable for controlling the growth of *L. innocua* on fresh-cut mango. The reduction could still be improved when MA-PL and PL-ALC-MA treatments were used.

Moulds and yeasts and pshychrophilic bacteria counts of mango slices were below 1 x 10^6 CFU/g after 14 days. In addition, the results suggested an additive effect on microbial load reduction by treatments combination. In that sense, low microbial counts were obtained in mango slices treated by those combined treatments where PL was applied first. PL, ALC, MA and their combinations contributed to maintain the colour parameters of sliced mango for 14 days. Mango slices had high resistance to rupture when PL treatment was individually applied or combined with both ALC and MA. Beyond confirming that PL plays an important role on fresh-cut mango preservation, the present study indicated better quality parameters and microbial stability in PL-ALC-MA treated mango slices.

ACKNOWLEDGMENTS

This work was supported by the University of Lleida (Spain) and financed by Tecnológico de Monterrey (Research Chair Funds CAT-200 and CDB081)

5. REFERENCES

Caminiti, I. M., Noci, F., Muñoz, A., Whyte, P., Morgan, D. J., Cronin, D. A., & Lyng, J. G. (2011). Impact of selected combinations of non-thermal processing technologies on the quality of an apple and cranberry juice blend. Food Chemistry, 124(4), 1387–1392.

Charles, F., Vidal, V., Olive, F., Filgueiras, H., & Sallanon, H. (2013). Pulsed light treatment as new method to maintain physical and nutritional quality of fresh-cut mangoes. Innovative Food Science & Emerging Technologies, 18, 190–195.

Chen, Z., Zhu, C., Zhang, Y., Niu, D., & Du, J. (2010). Effects of aqueous chlorine dioxide treatment on enzymatic browning and shelf-life of fresh-cut asparagus lettuce (Lactuca sativa L.). Postharvest Biology and Technology, 58(3), 232–238.

Chiumarelli, M., Ferrari, C. C., Sarantópoulos, C. I. G. L., & Hubinger, M. D. (2011). Fresh cut Tommy Atkins mango pre-treated with citric acid and coated with cassava (Manihot esculenta Crantz) starch or sodium alginate. Innovative Food Science and Emerging Technologies, 12(3), 381–387.

FAO. (2012). FAOSTAT.

Gómez, P. L., García-Loredo, A., Nieto, A., Salvatori, D. M., Guerrero, S., & Alzamora, S. M. (2012). Effect of pulsed light combined with an antibrowning pretreatment on quality of fresh cut apple. Innovative Food Science & Emerging Technologies, 16, 102–112.

Gonzalez-Aguilar, G. A., Wang, C. Y., Buta, J. G., & Krizek, D. T. (2001). Use of UV-C irradiation to prevent decay and maintain postharvest quality of ripe "Tommy Atkins" mangoes. International Journal of Food Science and Technology, 36(7), 767–773.

Guerrero-Beltran, J. A. (2004). Advantages and Limitations on Processing Foods by UV Light. Food Science and Technology International, 10(3), 137–147.

Hodges, D. M., & Toivonen, P. M. A. (2008). Quality of fresh-cut fruits and vegetables as affected by exposure to abiotic stress. Postharvest Biology and Technology, 48(2), 155–162.

ISO 17410. (2001). Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of psychrotrophic microorganisms.

ISO 7954. (1987). Microbiology feneral guidance for enumeration of yeasts and moulds and colony count technique at 25 oC.

Keklik, N. M., Demirci, A., Puri, V. M., & Heinemann, P. H. (2012). Modeling the inactivation of Salmonella Typhimurium, Listeria monocytogenes, and Salmonella Enteritidis on poultry products exposed to pulsed UV light. Journal of Food Protection, 75(2), 281–8.

Keyser, M., Műller, I. A., Cilliers, F. P., Nel, W., & Gouws, P. A. (2008). Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. Innovative Food Science & Emerging Technologies, 9(3), 348–354.

Manzocco, L., Da Pieve, S., & Maifreni, M. (2011). Impact of UV-C light on safety and quality of fresh-cut melon. Innovative Food Science & Emerging Technologies, 12(1), 13–17.

Moody, A., Marx, G., Swanson, B. G., & Bermúdez-Aguirre, D. (2014). A comprehensive study on the inactivation of Escherichia coli under nonthermal technologies: High hydrostatic pressure, pulsed electric fields and ultrasound. Food Control, 37, 305–314.

Moreira, M. R., Tomadoni, B., Martín-Belloso, B., & Soliva-Fortuny, R. (2015). Preservation of Fresh-cut Apple Quality Attributes by Pulsed Light in Combination with Gellan Gum-Based Prebiotic Edible Coatings. LWT - Food Science and Technology, 64(2), 1130–1137.

Oms-Oliu, G., Martín-Belloso, O., & Soliva-Fortuny, R. (2008). Pulsed Light Treatments for Food Preservation. A Review. Food and Bioprocess Technology, 3(1), 13–23.

Oms-Oliu, G., Rojas-Graü, M. A., González, L. A., Varela, P., Soliva-Fortuny, R., Hernando, M. I. H., … Martín-Belloso, O. (2010). Recent approaches using chemical treatments to preserve quality of fresh-cut fruit: A review. Postharvest Biology and Technology, 57(3), 139–148.

Penteado, A. L., de Castro, M. F. P. M., & Rezende, A. C. B. (2014). Salmonella enterica serovar Enteritidis and Listeria monocytogenes in mango (Mangifera indica L.) pulp: growth, survival and cross-contamination. Journal of the Science of Food and Agriculture, 94(13), 2746–2751.

Proctor, A. (2010). Alternatives to Conventional Food Processing.

Ramos-Villarroel, A., Aron-Maftei, N., Martín-Belloso, O., & Soliva-Fortuny, R. (2014). Bacterial inactivation and quality changes of fresh-cut avocados as affected by intense light pulses of specific spectra. International Journal of Food Science & Technology, 49(1), 128–136.

Ramos-Villarroel, A. Y., Martín-Belloso, O., & Soliva-Fortuny, R. (2011). Bacterial inactivation and quality changes in fresh-cut avocado treated with intense light pulses. European Food Research and Technology, 233(3), 395–402.

Raso, J., & Barbosa-Cánovas, G. V. (2003). Nonthermal preservation of foods using combined processing techniques. Critical Reviews in Food Science and Nutrition, 43(3), 265–85.

Rathnayaka, R. M. U. S. K. (2013). Antibacterial Effect of Malic Acid Against Listeria monocytogenes, Salmonella enteritidis and Escherichia coli in Mango, Pineapple and Papaya Juices. American Journal of Food Technology, 8(1), 74–82.

Raybaudi-Massilia, R. M., Mosqueda-Melgar, J., & Martín-Belloso, O. (2008). Edible alginate-based coating as carrier of antimicrobials to improve shelf-life and safety of fresh-cut melon. International Journal of Food Microbiology, 121(3), 313–27.

Raybaudi-Massilia, R. M., Mosqueda-Melgar, J., & Tapia, M. S. (2010). Edible coatings as carriers of food additives on fresh-cut fruits and vegetables. Stewart Postharvest Review, 6(3), 1–7.

Raybaudi-Massilia, Rosa Mosqueda-Melgar, J., Sobrino-López, A., Soliva-Fortuny, R. C., & Martín-Belloso, O. (2009). Use of malic acid and other quality stabilizing compounds to assure the safety of fresh-cut "Fuji" apples by inactivation of Listeria Monocytogenes, Salmonella Enteritidis and Escherichia Coli O157:H7. Journal of Food Safety, 29(2), 236–252.

Rojas-Graü, M. A., Raybaudi-Massilia, R. M., Soliva-Fortuny, R. C., Avena-Bustillos, R. J., McHugh, T. H., & Martín-Belloso, O. (2007). Apple puree-alginate edible coating as carrier of antimicrobial agents to prolong shelf-life of fresh-cut apples. Postharvest Biology and Technology, 45(2), 254–264.

Rojas-Graü, M. A., Tapia, M. S., Rodríguez, F. J., Carmona, A. J., & Martin-Belloso, O. (2007). Alginate and gellan-based edible coatings as carriers of antibrowning agents applied on fresh-cut Fuji apples. Food Hydrocolloids, 21(1), 118–127.

Ross, A. I. V., Griffiths, M. W., Mittal, G. S., & Deeth, H. C. (2003). Combining nonthermal technologies to control foodborne microorganisms. International Journal of Food Microbiology, 89(2-3), 125–138.

Siddiq, M., Sogi, D. S., & Dolan, K. D. (2013). Antioxidant properties, total phenolics, and quality of fresh-cut "Tommy Atkins" mangoes as affected by different pre-treatments. LWT - Food Science and Technology, 53(1), 156–162.

Tapia, M. S., Rojas-Graü, M. A., Rodríguez, F. J., Ramírez, J., Carmona, A., & Martin-Belloso, O. (2007). Alginate- and gellan-based edible films for probiotic coatings on fresh-cut fruits. Journal of Food Science, 72(4), E190–6.

Valencia-Chamorro, S. A., Palou, L., Del Río, M. A., & Pérez-Gago, M. B. (2011). Antimicrobial edible films and coatings for fresh and minimally processed fruits and vegetables: a review. Critical Reviews in Food Science and Nutrition, 51(9), 872–900.

CHAPTER III

Journal of Food Processing and Preservation (under revision)

Effect of pulsed light and edible coating on the phenolic profile and antioxidant potential of fresh-cut mango

B. Salinas-Roca, R.Soliva-Fortuny, J. Welti-Chanes and O. Martín-Belloso

ABSTRACT

This work aimed at evaluating the individual or combined effect of pulsed light (PL) treatment, alginate (AL) coating and malic acid (MA) dip on antioxidant capacity, phenolic compounds content and colour of fresh-cut mango throughout 14 days. Results showed that PL-AL-MA and AL-MA-PL treatments prevented browning of fresh-cut mango over the storage. Moreover, those treatment combinations resulted into the greatest radical scavenging activity (RSA), which was 42.2 % RSA_{ABTS} in fresh-cut mango at day 14. Total phenolic compounds in fresh-cut mango subjected to AL, AL-MA-PL and PL-AL-MA treatments showed maximal concentration of 1664.46, 1367.58 and 1745.71 mg GAE/kg, respectively, at day 3, when the concentration began to decline until day 7. Although five individual phenolics were identified at day of processing, only gallic acid, mangiferin and quercetin were maintained in treated fresh-cut mango during entire storage. Indeed, MA and AL-MA-PL treatments enhanced mangiferin content in fresh-cut mango for 14 days at 4 ˚C. Phenolic compounds content might be improved by the effect of PL, AL and MA treatments inducing a stress response in fresh-cut mango. Thus, combination of PL, AL and MA could help in preserving colour of fresh-cut mangoes and increase their content of phenolic compounds and antioxidant capacity.

Key Words: fresh-cut mango, combined treatments, pulsed light, phenols, antioxidant potential and colour

1. INTRODUCTION

Mango (*Mangifera indica L.*) is one of the most important tropical fruits worldwide and is greatly valued for its sensorial and nutritional properties. High content in phenolic compounds and antioxidant capacity have been reported in mango (Barreto et al,. 2008; Ribeiro, Barbosa, Queiroz, Knödler, & Schieber, 2008; Robles-Sánchez, 2009). Mangiferin, quercetin, gallic acid, benzoic acid, kaempferol, anthocyanins and protocatechuic acid are among the major phenolic compounds found in mango (Palafox-Carlos et al., 2012). Those phenolics have been related to the inhibition of the oxidation of living cells by free radicals and therefore prevent cardiovascular diseases, artherosclerosis and decrease the risk of some types of cancer (Daud et al,. 2010; Hoang et al,. 2015). Thus, regular consumption of mango may provide significant health benefits (Masibo & He, 2008).

Mango is commercialized not only as whole fresh fruit, but also as a fresh-cut product (Campbell & Ledesma, 2015). Nevertheless, post-harvest mango processing induces biochemical changes, besides increasing product respiration rate, hence, leading to a reduction of shelf-life. Thus, preservation treatments are needed to diminish deleterious changes in quality and nutritive value and extend the shelf-life of fresh-cut mango (Ibarra-Garza, Ramos-Parra, Hernández-Brenes, & Jacobo-Velázquez, 2015). In that sense, surface treatments may inhibit or slow down physiological changes affecting mango appearance, thus maintaining quality throughout fresh-cut fruit storage. Among the most lately studied treatments, pulsed light and edible coatings may be used to extend the shelf-life of fresh-cut mango (Bonilla, Atarés, Vargas, & Chiralt, 2012; Oms-Oliu et al., 2010). Pulsed light (PL) treatments involve the application of very short high-intensity pulses of broad spectrum light (180- 1100 nm). The feasibility of maintaining quality of mango cubes (*cv.* Kent) after PL treatment have been described (Charles, Vidal, Olive, Filgueiras, & Sallanon, 2013). On the other hand, the use of alginate (AL)-based coating delay physiological changes and oxidative reaction rates in fresh-cut fruits (Rojas-Graü, Tapia, Rodríguez, Carmona & Martin-Belloso, 2007). Also, malic acid (MA) dips have shown to act as antimicrobial extending shelf-life of fresh-cut fruits (Raybaudi-Massilia, Mosqueda-Melgar, & Tapia, 2010b; Valencia-Chamorro et al., 2011).

Although the study of PL, AL and MA as individual treatments in fresh-cut fruits quality preservation has been already carried out, scarce information on the effect of their combination has been found. Recently, a study suggested an additive effect in microbial load reduction by PL, AL and MA treatments combination as well as a noted preservation of physicochemical properties in sliced mango along 14 days (Salinas-Roca et al., 2016). They described in fresh-cut mango treated by PL-AL-MA a significant reduction of *L.innocua* population and microbial load below 6 logs CFU/g over 14 days. In addition, fresh-cut mango improved its firmness when PL was individually applied or combined with both AL and MA (Salinas-Roca et al., 2016). Nevertheless, from our knowledge, the effects of combining PL, AL or MA treatments on bioactive compounds content in fresh-cut mango have not been described yet.

Consequently, this work aimed to study the effect of PL, AL, MA treatments and their combinations (PL-AL-MA and AL-MA-PL) on total and individual phenolic compounds, antioxidant capacity, and colour of fresh-cut Tommy Atkins *cv.* mango through 14 days of refrigerated storage.

2. MATERIALS AND METHODS

2.1. Mango slices preparation

Mangoes (cv. Tommy Atkins) were purchased from a local market and stored for one day at 23 ± 1 ºC prior to processing. The whole fruits were cleaned with an aqueous solution of sodium hypochlorite (300µL/L) and then peeled and cut to obtain 5 mm-thick slices (35 ± 1g). Sliced mangoes were treated and placed into transparent polypropylene trays, which were stored at 4 ± 1 ºC until analysis.

2.2. Pulsed light treatment

Pulsed light (PL) treatments were carried out with a XeMaticA-2L System (SteriBeam Systems GmbH, Germany). The treatment chamber system was equipped with a lamp situated 8.5 cm above the sample holder. Treatment conditions were

based on previous studies by Salinas-Roca et al. (2016), the lamp delivered 20 pulses of 0.3 ms with a radiant fluence of 0.4 J•cm^{-2} per pulse at the sample level. The total light energy was measured with a standard light source estimated by photodiode readings following the manufacturer's directions. The emitted spectrum ranged from 180 - 1100 nm. Temperature inside the treatment chamber was controlled with a Thermometer Temp 7 PT 100 (Lab Process, Barcelona, Spain) and never exceeded 40 °C during the treatments.

2.3. Alginate coating

Edible coating forming solution was prepared by dissolving 20 g of alginate (AL) (FMC Biopolymer Ladybum Works, USA) into 1000 mL of distilled water and homogenising with an Ultra Turrax T25(IKA WERKE, Germany). Calcium chloride (20 g·Kg^{-1}) provided by Sigma-Aldrich Chemic (Steinhein, Germany) was used as a crosslinking agent. Mango slices were dipped into the sodium alginate solution (20 g·Kg^{-1}) during 2 minutes and the excess was removed thereafter. A second dipping in calcium chloride (20 g·Kg^{-1}) solution was performed for AL-coated mango slices in order to allow the cross linkage of the polysaccharide chains.

2.4. Malic acid solution

DL-Malic acid (20 g) (MA) (Fluka; Steinhein, Germany) was dissolved by stirring into 1000 mL of distilled water. Mango slices were dipped into a 20 g·Kg^{-1} MA solution during 2 minutes and the excess was removed thereafter. It must be noted that MA was incorporated to the calcium chloride solution when combined with the edible coating.

2.5. Combined treatments

Combined treatments were selected considering the previous results by Salinas-Roca, Soliva-Fortuny, Welti-Chanes, & Martín-Belloso (2016), who described PL-AL-MA and AL-MA-PL as the greatest combination for microbial load reduction and

physicochemical attributes preservation. Thus, PL-AL-MA, AL-MA-PL treatment combinations were assayed. AL-MA-PL treatment was assayed by exposing trays containing alginate-coated and malic acid dipped mango slices to pulsed light. Otherwise, PL-AL-MA combination was performed by coating PL-treated mango slices with alginate solution and a second dipping containing malic acid. Untreated mango slices dipped in distilled water were considered as a reference treatment. Treated and untreated mango slices were stored until the analysis.

2.6. Preparation of mango extracts

Mango extracts to be analysed for total and individual phenolic compounds and antioxidant capacity were prepared according to the method reported by Robles-Sánchez et al., (2011) with slight modifications. Briefly, freeze-dried mango slices (10 g) were crushed and homogenized with 20 mL of methanol (800 $g \cdot Kg^{-1}$) and sonicated during 15 min before centrifugation at 15000 g for 15 min at 4°C in a Centrifuge AVANTI J-25 (Beckman Instruments Inc; Fullerton, CA, USA). The supernatant was filtered to obtain the extract, which was used for antioxidant capacity and total phenolic content determination.

2.7. Antioxidant capacity

The antioxidant capacity of fresh-cut mango was studied through the evaluation of the free radical scavenging activity (RSA) towards the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) and 1-diphenyl-2-picrylhydrazyl (DPPH) radicals.

2.7.1.1. ABTS assay

The RSA was evaluated using the ABTS radical cation decolorization assay based on the method described by Siddiq, Sogi, & Dolan (2013) with slight modifications. An aliquot of 0.01 mL of the extract obtained was mixed with 0.09 mL of distilled water and 3.9 mL of ABTS solution, which was prepared 14 h before usage, with 7.4 mmol/L ABTS and 0.2 mmol/L potassium sulphate. The absorption at 734 nm of the

ABTS solution with the antioxidant mango extract after 6 min kept in a dark place was measured with a spectrophotometer (CECIL CE 2021 Cecil Instruments Ltd, Cambridge, UK). A blank of methanol was used without the extract. The results were expressed as percentage of RSA of the absorbance of test sample (A_s) related to the absorbance of the reagent (A_o) (methanolic solution of the reagent without extract), as shown in eq.1.

$$RSA\,(\%) = \frac{Ao - As}{Ao} \cdot 100 \qquad \text{eq.1}$$

2.7.1.2. DPPH assay

The RSA_{DPPH} assay was carried out according to the methodology proposed by De Ancos, Sgroppo, Plaza, & Cano, (2002). The reaction mixture was carried out by adding 0.01 mL of supernatant, 3.9 mL of methanolic DPPH (0.0025 $g \cdot L^{-1}$) and 0.09 mL of distilled water. The homogenate was mixed by shaking and kept in darkness for 30 min at room temperature. The absorption at 515 nm of the mixture was measured against a blank of methanol without DPPH with a spectrophotometer (CECIL CE 2021 Cecil Instruments Ltd, Cambridge, UK). The results were expressed as percentage of RSA (eq.1).

2.8. Determination of total phenolic content

The content of total phenolic compounds (TP) was determined according to the Folin-Ciocalteu colorimetric method described by Singleton, Orthofer, & Lamuela-Raventós (1998) with slight modifications. The phenolic extract (0.5 mL) was mixed and homogenizated with a saturated sodium carbonate solution (10 mL) and Folin-Ciocalteu reagent (0.5 mL). The mixture was kept during one hour in dark storage. Afterwards, the absorbance of the mixture was measured at 765 nm. TP content was calculated on the basis of a standard curve of gallic acid and expressed as gallic acid equivalents (GAE) on dry weight basis.

2.9. Individual phenolic compounds

2.9.1. Sample preparation

Individual phenolic compounds were identified and quantified according to Palafox-Carlos, Yahia, & González-Aguilar, (2012) with slight modifications. An aliquot of 0.5 g freeze-dried mango was mixed with 20 mL of methanol (625 $g \cdot Kg^{-1}$), 0.05 g terbuthilhidroxiquinone (TBHQ) (970 $g \cdot Kg^{-1}$) and 5 mL HCl (6 M). The mixture was placed in amber glass, sonicated for 3 min and thereafter refluxed for acid hydrolysis (90 °C, 2 h), cooled and diluted to 25 mL with pure methanol. Finally, the extracts were sonicated during 3 min, filtered using 0.20 µm membranes and stored at - 45 °C up to analysis.

2.9.2. High-performance liquid chromatography (HPLC) analysis

Phenolic profile was determined using the methodology reported by Hertog, Hollman, & Venema, (1992) and Ribeiro et al., (2008) with slight modifications. The HPLC system was equipped with a 600 Controller and a 2996 diode array detector (Waters Corporation, Milford, MA, USA) which was set to scan from 200 to 600 nm. Separations were performed on a reverse-phase C18 Spherisorb ODS2 (5 µm) stainless steel column (4.6 mm x 250 mm) operating at room temperature with a flow rate of 1 mL/min. A gradient elution was employed with a mixture of two solvents: (A) 2.5 % of acetic acid in water and (B) 2.5 % acetic acid in methanol as follows: linear gradient from 5 % to 13 % B, 0- 15 min; linear gradient from 13 % to 15 % B, 15-20 min; linear gradient from 15 % to 30 % B, 28- 32 min; isocratic elution 45 % B, 32- 35 min; linear gradient 45- 90 % B, 35- 40 min; isocratic elution 90 % B, 40-45 min; linear gradient to reach the initial conditions after 5 min; post-time 10 min before the next injection.

2.9.3. Identification and quantification

Each phenolic compound was identified by comparing its retention time and spectrum with external standards (gallic, dihydroxybenzoic and chlorogenic acid; mangiferin and quercetin). The concentration of each individual phenolic compound was based on the peak area of the standard solutions at known concentration. Results were expressed as mg of the phenolic compound on a dry weight (dw) basis.

2.10. Colour determination

The colour of mango slices was measured with a spectrophoto-colorimeter (Minolta Chroma Meter Model CR- 400, Minolta, Tokyo, Japan). The CIE L* (lightness) CIE a* (red- green) and CIE b* (yellow- blue) coordinates were read using an illuminant D65 and 10º observer angle and calibrated using a standard white reflector plate. Browning index (BI) was calculated with eq.2 (Pathare et al., 2012).

$$BI = 100 \cdot (X - 0.31/0.17) \quad \text{eq.2}$$

where

$$X = \frac{(a^* + 1.75L^*)a^*}{(5.645L^* + a^* - 3.012b^*} \quad \text{eq.3}$$

2.11. Data analysis

The experiment was conducted in duplicate independent runs and each analysis was performed at least three times. Statistical analyses were carried out with JMP 11 program for Windows (SAS software; SAS Institute, NC). Experiment data were analysed by ANOVA. In addition, a Duncan multi-range test was applied to determine differences among means with a significance level of 5 %. Pearson correlation test was used to evaluate relationships among analysed parameters (antioxidant capacity, total phenolic content, individual phenolics, L*, a* and b* colour parameters and browning index) for each treatment.

3. RESULTS

3.1. Antioxidant capacity

The antioxidant capacity of mango slices, expressed as % of radical scavenging activity (RSA), is shown in figure 1. RSA values of mango slices determined with the ABTS method were higher than those obtained with the DPPH method the day of processing. PL and PL-AL-MA treated mango slices exhibited 10 % more RSA_{ABTS} than those untreated thereafter processing (Fig. 1a). The application of PL-AL-MA

and AL-MA-PL treatments resulted into the highest RSA_{ABTS} (42.2 ± 0.4 % RSA) at the end of the storage. No significant differences in the initial DPPH scavenging capacity of mango slices regardless the applied treatment were observed (Fig. 1b). The RSA_{DPPH} of treated and untreated mango slices was maintained or even increased from day 3 to day 10 and subsequently dramatically declined to 22.5 ± 0.4 % RSA at day 14.

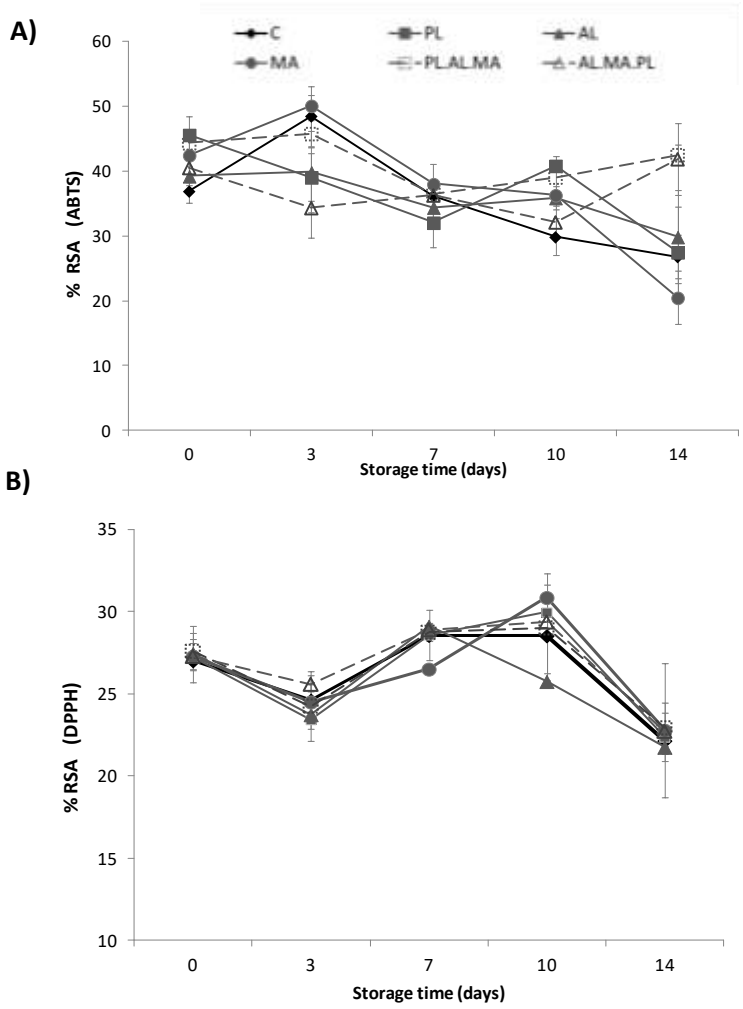


Figure 1: Radical scavenging activity (RSA) of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt ($ABTS^{+}$) (A) and 1-diphenyl-2-picrylhydrazyl (DPPH) (B) of mango slices throughout 14 days of storage at 4 °C. PL: pulsed light (20 pulses at λ = 180- 1100 and 0.4 $J \cdot cm^2$/pulse); AL: alginate coating (20 $g \cdot Kg^{-1}$); MA: malic acid (20 $g \cdot Kg^{-1}$) and C: Untreated. Each assay was performed in triplicate on two separate experimental runs. Data represents mean values ± standard deviation.

3.2. Total phenolic content

Untreated mango slices exhibited 960.4 ± 100.2 mg GAE/ kg just after processing, whereas, the content of total phenolic in treated mango slices ranged from 955.1 ± 152.1 to 1762.2 ± 138.2 mg GAE/ kg. PL and MA treatments did not have a significant effect on phenolic content of mango slices compared with untreated (Fig. 2). Differently, mango slices treated with AL, AL-MA-PL and PL-AL-MA exhibited a higher content of phenolic compounds than those subjected to other treatments just after processing. A dramatic loss of phenolic compounds in any of the mango slices, either treated or untreated was observed from day 3 to day 7. Afterwards, no changes on total phenolic concentration during the storage time were observed. Despite phenolics were reduced throughout the first week of storage, AL and AL-MA-PL-treated mango slices yet exhibited the highest content of phenolic compounds at the end of the evaluated storage period.

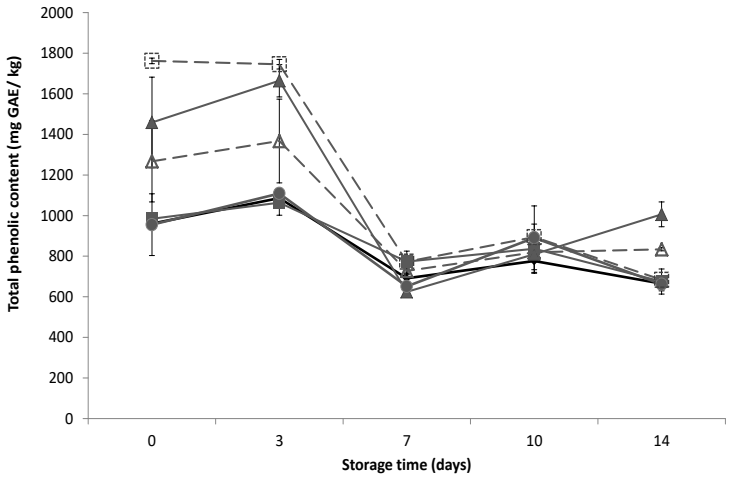


Figure 2: Total phenolic content (expressed as mg of gallic acid equivalent) of mango slices throughout 14 days of storage at 4 °C. PL: intense light pulses (20 pulses at λ = 180- 1100 and 0.4 $J \cdot cm^2$/pulse); AL: alginate coating (20 $g \cdot Kg^{-1}$); MA: malic acid (20 $g \cdot Kg^{-1}$) and C: untreated. Each assay was performed in triplicate on two separate experimental runs. Data represents mean values ± standard deviation.

3.3. Individual phenolic compounds

Principal phenolic compounds identified in untreated and treated mango slices just after processing were phenolic acids (gallic, dehidroxibenzoic and chlorogenic), xanthones (mangiferin) and flavonoids (quercetin) (Table 1). The concentration of most of the individual phenolic compounds identified in treated mango slices after processing increased throughout storage. Gallic acid was the most abundant phenolic compound in any of the analysed mango slices, followed by quercetin and mangiferin. Initial content of gallic acid in mango slices was 571.2 ± 134.5 mg/ kg. It must be noted that gallic acid concentration at day 14 in PL-AL-MA-treated mango slices was 3-fold higher than that observed just after processing. The initial concentration of mangiferin (59.81 ± 1.26 mg/ kg) declined in untreated mango slices along the storage, whereas quercetin content (140.2 ± 12.6 mg/ kg) was maintained. MA-treated mango slices concentration achieved 140.4 ± 33.5 and 161.7 ± 26.3 mg/ kg for mangiferin and quercetin, respectively, at day 14. The sum of all phenolic compounds concentrations in mango slices obtained by quantitative HPLC was compared with the results obtained from the Folin-Ciocalteau assays, generally considered to estimate total phenol contents in plant extract. No difference in the sum of phenolics content was found between Folin-Ciocalteau and HPLC methods regarding untreated mango slices, whereas variance among those treated was found along the entire storage.

3.4. Colour

Colour is a well-appreciated characteristic for consumer's purchase and it is related with antioxidant quality of fresh-cut mango. After processing, the L* of untreated mango slices (73.4 ± 2.5) was maintained (Table 2). The a* parameter exhibited significantly lower values in treated mango slices compared with those untreated at the beginning of storage. On the other hand, b* and BI values in untreated mango slices were higher than in those treated at day of processing. During storage, slight but significant differences in colour parameters between untreated and treated mango slices were observed. The a*, b* and BI values of untreated mango slices decreased throughout storage. On the other hand, a* and b* values in PL-treated mango slices

increased up to -2.7 ± 1.3 and 59.38 ± 1.84, respectively, at day 14. Indeed, b* values in PL and PL-AL-MA treated (56.1 ± 0.3) mango slices were higher than in those treated by AL-MA-PL (52.6 ± 0.2).

Table 1: Effects of pulsed light (PL), alginate (AL) and malic acid (MA) on phenolic compounds content (mg/Kg) of mango slices stored at 4 °C. Each assay was performed in triplicate on two separate experimental runs. Data represents mean values ± standard deviation. Values within a column followed by different lowercase letter are significantly different along storage ($p < 0.05$). Values within a column followed by different uppercase letter are significantly different among treatments ($p< 0.05$).

Treatment	Time (day)	Gallic acid	Dihydroxybenzoic	Chlorogenic	Mangiferin	Quercetin
Untreated	**0**	571.2 ± 134.5 ^{aA}	24.3 ± 1.9 ^{aA}	13.7 ± 10.8 ^{aA}	59.81 ± 1.26 ^{aA}	140.2 ± 12.6 ^{aA}
	3	427.6 ± 135.6 ^{aA}	48.7 ± 2.5 ^{bA}	nd ± nd	36.82 ± 5.37 ^{bA}	167.02 ± 11.11 ^{aA}
	7	509.01 ± 92.51 ^{aA}	36.5 ± 2.2 ^{aA}	nd ± nd	18.41 ± 2.68 ^{cA}	83.51 ± 5.55 ^{bA}
	10	590.3 ± 49.4 ^{bA}	nd ± nd	nd ± nd	10.3 ± 2.2 ^{cA}	102.5 ± 15.8 ^{cA}
	14	373.84 ± 16.67 ^{cA}	nd ± nd	nd ± nd	5.7 ± 7.1 ^{cA}	149.4 ± 3.3 ^{aA}
PL	**0**	313.57 ± 84.01 ^{aB}	24.87 ± 0.23 ^{aA}	6.02 ± 0.04 ^{aB}	65.7 ± 1.2 ^{aA}	179.3 ± 3.2 ^{aB}
	3	327.1 ± 167.3 ^{aB}	26.1 ± 0.3 ^{aB}	nd ± 0	143.6 ± 83.2 ^{bB}	150.8 ± 0.4 ^{aA}
	7	489.8 ± 169.4 ^{aB}	n.d ± n.d	n.d ± n.d	71.8 ± 41.6 ^{bB}	75.41 ± 0.18 ^{bA}
	10	652.6 ± 171.3 ^{bA}	nd ± nd	nd ± nd	71.8 ± 41.4 ^{bB}	86.4 ± 0.1 ^{bB}
	14	876.6 ± 76.2 ^{bB}	nd ± nd	nd ± nd	26.51 ± 7.03 ^{cB}	160.8 ± 7.3 ^{cC}
AL	**0**	237.1 ± 101.1 ^{aB}	26.46 ± 0.14 ^{aA}	6.21 ± 0.07 ^{aB}	82.1 ± 6.3 ^{aB}	150.5 ± 0.5 ^{aA}
	3	367.8 ± 14.7 ^{aB}	59.32 ± 2.06 ^{bA}	nd ± nd	59.1 ± 2.9 ^{bC}	156.56 ± 2.67 ^{aA}
	7	448.53 ± 35.43 ^{aB}	nd ± nd	nd ± nd	nd ± nd	nd ± nd
	10	529.2 ± 56.1 ^{bA}	nd ± nd	nd ± nd	nd ± nd	nd ± nd
	14	681.01 ± 91.26 ^{bB}	nd ± nd	nd ± nd	nd ± nd	nd ± nd
MA	**0**	519.24 ± 46.66 ^{aA}	25.5 ± 0.65 ^{aA}	20.1 ± 9.8 ^{aA}	46.3 ± 15.6 ^{aA}	174.3 ± 41.3 ^{aB}
	3	536.45 ± 36.61 ^{aA}	31.9 ± 9.6 ^{bA}	nd ± nd	43.5 ± 3.4 ^{aA}	151.2 ± 0.7 ^{aA}
	7	559.7 ± 177.4 ^{aA}	28.7 ± 5.19	nd ± nd	91.9 ± 18.4 ^{bC}	156.53 ± 13.54 ^{aB}
	10	582.98 ± 8.14 ^{aA}	nd ± nd	nd ± nd	77.5 ± 12.2 ^{bB}	160.62 ± 16.56 ^{aC}
	14	654.7 ± 200.5 ^{bB}	nd ± nd	nd ± nd	140.4 ± 33.5 ^{cC}	161.76 ± 26.31 ^{aB}
AL.MA.PL	**0**	448.55 ± 27.64 ^{aA}	26.5 ± 1.1 ^{aA}	6.14 ± 0.17 ^{aB}	60.4 ± 12.3 ^{aA}	161.12 ± 14.03 ^{aB}
	3	332.51 ± 20.91 ^{aB}	41.2 ± 16.9 ^{bA}	nd ± nd	66.8 ± 22.3 ^{bC}	150.88 ± 1.28 ^{aA}
	7	541.6 ± 23.1 ^{aA}	33.84 ± 9.03 ^{aA}	nd ± nd	69.62 ± 17.6 ^{bB}	157.4 ± 6.4 ^{aB}
	10	750.7 ± 25.4 ^{bB}	nd ± nd	nd ± nd	59.3 ± 12.5 ^{bB}	159.8 ± 11.6 ^{aC}
	14	658.1 ± 110.4 ^{bB}	nd ± nd	nd ± nd	60.6 ± 20.9 ^{cD}	152.7 ± 13.3 ^{aA}
PL.AL.MA	**0**	259.9 ± 31.9 ^{aB}	24.79 ± 0.08 ^{aA}	16.7 ± 15.2 ^{aA}	62.9 ± 5.95 ^{aA}	164.7 ± 19.6 ^{aB}
	3	244.62 ± 74.55 ^{aB}	30.8 ± 8.5 ^{bA}	nd ± nd	35.6 ± 44.6 ^{aA}	152.9 ± 3.1 ^{aA}
	7	543.7 ± 89.8 ^{bA}	27.8 ± 4.2 ^{aA}	nd ± nd	17.8 ± 22.3 ^{cA}	76.5 ± 1.5 ^{bA}
	10	842.7 ± 105.1 ^{cB}	nd ± nd	nd ± nd	nd ± nd	92.5 ± 3.5 ^{bA}
	14	794.9 ± 232.1 ^{cB}	nd ± nd	nd ± nd	12.5 ± 3.2 ^{cA}	137.8 ± 3. 8 ^{aA}

Table 2: Changes in mango slices lightness (L*) a*, b* and browning index (BI) during 14 days of storage (4 °C). PL: pulsed light (20 pulses at λ = 180- 1100 and 0,4 J·cm^2/ pulse); AL: alginate coating (20 g·Kg^{-1}) and MA: malic acid (20 g·Kg^{-1}). Each assay was performed in triplicate on two separate experimental runs. Data represents mean values ± standard deviation. Values within a column followed by different lowercase letter are significantly different along storage ($p < 0.05$). Values within a column followed by different uppercase letter are significantly different among treatments ($p< 0.05$).

Treatment	Time (day)	L*			a*			b*			BI		
Untreated	**0**	73.4	±	2.5 ^{aA}	-1.9	±	0.1 ^{aA}	58.2	±	0.7 ^{aA}	204.3	±	2.7 ^{aA}
	3	71.9	±	2.6 ^{aA}	-4.76	±	1.81 ^{bA}	57.6	±	1.5 ^{aA}	192.3	±	1.7 ^{bA}
	7	74.8	±	4.1 ^{aA}	-3.47	±	1.12 ^{bA}	55.34	±	0.11 ^{bA}	202.21	±	2.54 ^{aA}
	10	77.2	±	2.4 ^{bA}	-4.9	±	0.8 ^{bA}	54.3	±	2.9 ^{bA}	199.3	±	3.1 ^{aA}
	14	77.3	±	3.5 ^{bA}	-5.1	±	1.1 ^{bA}	51.6	±	4.8 ^{cA}	197.41	±	3.24 ^{aA}
PL	**0**	76.4	±	2.1 ^{aB}	-4.9	±	0.1 ^{aB}	57.5	±	0.3 ^{aA}	201.9	±	0.8 ^{aA}
	3	73.57	±	0.24 ^{bA}	-3.16	±	0.62 ^{aA}	57.82	±	2.04 ^{aA}	193.7	±	1.5 ^{bA}
	7	72.1	±	2.4 ^{bA}	-3.7	±	1.8 ^{aA}	53.7	±	4.9 ^{aA}	201.66	±	4.01 ^{aA}
	10	78.4	±	0.3 ^{aA}	-3.9	±	0.4 ^{aA}	57.4	±	2.7 ^{aB}	201.3	±	1.7 ^{aA}
	14	76.57	±	2.42 ^{aA}	-2.7	±	1.3 ^{aB}	59.38	±	1.84 ^{bC}	204.07	±	0.91 ^{bB}
AL	**0**	73.85	±	1.24 ^{aA}	-3.92	±	1.74 ^{aB}	55.41	±	0.04 ^{aB}	201.5	±	1.6 ^{aA}
	3	67.2	±	2.9 ^{bB}	-4.3	±	0.1 ^{aB}	54.8	±	1.8 ^{aB}	192.34	±	0.31 ^{bA}
	7	75.1	±	0.5 ^{aA}	-4.4	±	0.2 ^{aA}	52.48	±	0.04 ^{aA}	199.4	±	0.3 ^{aA}
	10	71.7	±	1.1 ^{aB}	-2.7	±	0.1 ^{bB}	55.1	±	1.6 ^{bA}	203.64	±	0.33 ^{aA}
	14	72.2	±	2.1 ^{aB}	-5.1	±	1.1 ^{aA}	54.4	±	3.6 ^{aB}	200.6	±	3.6 ^{aB}
MA	**0**	77.6	±	2.7 ^{aB}	-5.5	±	0.1 ^{aB}	54.5	±	0.6 ^{aB}	200.46	±	0.03 ^{aA}
	3	72.7	±	5.4 ^{bA}	-4.2	±	0.1 ^{bA}	52.9	±	2.1 ^{bB}	191.8	±	0.8 ^{bA}
	7	77.2	±	0.1 ^{aB}	-5.7	±	0.4 ^{aB}	50.6	±	0.1 ^{bB}	197.7	±	0.4 ^{bB}
	10	75.6	±	2.2 ^{bA}	-2.8	±	0.1 ^{bB}	55.61	±	3.54 ^{aB}	204.51	±	1.04 ^{aB}
	14	76.8	±	4.1 ^{bA}	-4.6	±	1.6 ^{aA}	51.02	±	4.32 ^{bA}	199.8	±	5.3 ^{aA}
AL. MA.PL	**0**	71.3	±	4.9 ^{aA}	-5.2	±	1.5 ^{aB}	53.39	±	2.04 ^{aB}	201.14	±	2.54 ^{aA}
	3	65.2	±	1.3 ^{bB}	-4.75	±	0.55 ^{aA}	52.7	±	0.1 ^{aB}	191.04	±	0.82 ^{bA}
	7	70.7	±	3.4 ^{aC}	-4.8	±	1.2 ^{aA}	52.8	±	1.9 ^{aB}	200.7	±	3.6 ^{aA}
	10	71.75	±	6.44 ^{aB}	-4.9	±	2.2 ^{aA}	50.4	±	5.3 ^{aB}	199.09	±	7.21 ^{aA}
	14	69.85	±	3.32 ^{aB}	-5.04	±	2.04 ^{aA}	52.6	±	0.2 ^{aA}	200.6	±	6.7 ^{aA}
PL.AL.MA	**0**	71.5	±	3.5 ^{aA}	-2.5	±	1.1 ^{aA}	56.2	±	1.1 ^{aB}	203.32	±	3.06 ^{aA}
	3	67.1	±	2.6 ^{aB}	-4.47	±	1.03 ^{bA}	55.4	±	0.5 ^{aB}	192.4	±	1.9 ^{bA}
	7	71.7	±	4.3 ^{aC}	-4.1	±	0.8 ^{bA}	55.1	±	0.3 ^{aB}	202.19	±	2.53 ^{aA}
	10	68.6	±	0.3 ^{aB}	-3.7	±	1.1 ^{bA}	54.59	±	1.84 ^{aB}	203.45	±	2.32 ^{aB}
	14	70.2	±	3.3 ^{aB}	-4.3	±	0.4 ^{bA}	56.1	±	0.3 ^{aB}	202.9	±	0.6 ^{aB}

Table 3: Correlation coefficients of physical and chemical properties in treated mango slices throughout 14 days of storage at 4 °C.

Determinations	AC	TP	GA	DHB	CHL	MGF	QRC	L*	a*	b*	BI
AC		0.4479	-0.3746	0.5549	0.2943	0.0467	0.2075	0.2969	0.21	0.0294	0.29
TP	0.4479		-0.6488	0.5788	0.3425	0.3008	0.0988	0.2661	0.0946	0.0033	-0.4603
GA	-0.3746	0.6488*		-0.5941	-0.2727	-0.3554	0.1179	-0.2746	0.082	0.0818	0.4423
DHB	0.5549*	0.5788*	-0.5941*		0.1983	0.1445	0.0091	0.5091	0.0786	-0.0949	-0.5555*
CHL	0.2943	0.3425	-0.2727	0.1983		0.1334	0.043	0.3565	-0.0082	0.147	0.2497
MGF	0.0467	0.3008	-0.3554	0.1445	0.1334		-0.1258	0.1258	0.1398	0.1161	0.0613
QRC	0.2075	0.0988	0.1179	0.0091	0.043	-0.12558		-0.0315	-0.0552	-0.2159	-0.0041
L*	0.2969	0.2661	-0.2746	0.5091	0.3565	0.1258	-0.0315		-0.0112	0.5155	-0.1524
a*	0.21	0.0946	0.082	0.0786	-0.0082	0..1398	-0.0552	-0.0112		0.2199	-0.1764
b*	0.0294	0.0033	0.0818	-0.0949	0.147	0.1161	-0.2159	0.5155	0.2199		0.0116
BI	-0.29	-0.4603	0.4423	-0.5555	0.2497	0.0613	-0.0041	-0.1524	-0.1764	0.0116	

* Significant correlation ($p < 0.001$). Antioxidant capacity (AC), total phenolics (TP), galic acid (GA), dehydroxibenzoic acid (DHB), chlorogenic acid (CHL), mangiferin (MGF), quercetin (QRC) and L*, a* and b* colour parametres and browning index (BI).

4. DISCUSSION

Radical scavenging activity (RSA) is one of the relevant roles of antioxidants since free radicals promote the oxidation of biological membranes. According to Prior, Wu, & Schaich, (2005), there is no a simple universal method to measure RSA accurately. The reactivity of the antioxidant compounds in mango slices with ABTS or DPPH radicals varied. Thus, different trends regarding the RSA of mango slices have been perceived depending on the assay. In accordance to these results, Gülçin (2012) reported higher RSA measured by the ABTS than by the DPPH method in foods with a significant noticeable phenolic compound content, which can quench the ABTS radical by two mechanisms: hydrogen atom transfer and single electron donation. The

scavenging activity of mango slices might be therefore mainly attributed to their outstanding phenolic acids content since a significant correlation ($r= 0.55$, $p<0.001$) was found between them, which is in agreement with other authors (Ma et al., 2011). The structure of phenolic compounds contains multiple hydroxyl groups and phenol rings. This could enable to increase the ionisation potential of the phenolics, hence, improving their reaction with ABTS (Müller, Fröhlich, & Böhm, 2011). Also, different RSA was observed depending on the treatment applied to mango slices. The use of PL-AL-MA and AL-MA-PL treatments leads to greater RSA_{ABTS} in mango slices after 14 days of storage. Other authors have reported higher antioxidant activity by ABTS assay in pulp of PL-treated mangoes compared with those untreated (Lopes et al., 2016a). PL caused a photochemical and photophysical effects on mango tissue. Therefore, PL treatment might conduct the disruption of the cell vacuoles, which may release antioxidant compounds such as phenolics (Rojas-Graü, Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2009). Furthermore, an additional effect on the antioxidant compounds production could be promoted in fruit tissue combining PL with AL and MA.

In the present study, a higher concentration of phenolic compounds in AL, AL-MA-PL and PL-AL-MA treated mango slices in comparison to those untreated was observed throughout the first week of storage. The increase of phenolic compounds in fresh-cut mangoes is commonly related with the abiotic stress (Becerra-Moreno et al., 2015). The wounding promotes the synthesis of phenolics, which is controlled by the phenylpropanoid metabolism through the phenylalanine ammonia lyase (PAL) enzyme. However, a dramatical decline of phenolic compounds in AL, AL-MA-PL and PL-AL-MA treated mango slices after 3 days of storage could indicate the suspension of phenylpropanoid metabolism and probably the increase of oxidative reactions. The decay of phenolics was interrupted after one week of storage, when no changes were observed in treated and untreated mango slices. Differences in total phenolics among combined treatments at the end of storage denote greater preservation effect of the AL coating compared to the PL treatment. Thus, different preserving mechanisms for phenolics could be attributed to each treatment. Firstly, PL treatment might cause a stress on fruit cells, hence, starting phenylpropanoid metabolism and enabling to maintain and even increase total phenolic compounds

(Charles et al., 2013). Indeed, PL may reduce the oxidative enzymes activity by changing its structure and preventing further loss of phenolic compounds (Pataro, Donsi, & Ferrari, 2015). Secondly, AL coating could reduce the phenolic compounds degradation by providing a partial barrier to moisture and oxygen, which contributed to control the internal atmosphere of mango. Moreover, coating may help to reduce enzymatic activity by preventing substrate to react with active site of the enzyme (Dang, Singh, & Swinny, 2008; Robles-Sánchez, Rojas-Graü, Odriozola-Serrano, González-Aguilar, & Martin-Belloso, 2013).

In agreement with other authors, gallic acid has been identified as predominant phenolic compound in mango flesh of different varieties (Palafox-Carlos et al., 2012). Otherwise, mangiferin and quercetin, which have been identified in lesser concentration than gallic acid in the present study, are mostly present in mango peels and kernels (Berardini et al., 2005; Dorta, González, Lobo, Sánchez-Moreno, & de Ancos, 2014). Differences in the phenolic compounds profile compared to other studies could be attributed to the high variability and strong influence of maturity stage and variety (Palafox-Carlos et al., 2012; Ribeiro et al., 2008). Furthermore, PL, AL and MA treatments could promote variation on total phenolics content of mango slices due to the appearing of isomer molecules at non-detected concentrations. This study demonstrated the effect of PL, AL and MA treatments on preserving phenolic profile of mango slices. PL-AL-MA treatments stimulated higher content especially of gallic acid, which is induced in plant tissues under stressful conditions in order to alleviate damage caused by wounding. Similarly, it was previously observed that PL enhanced the accumulation of phenolics, principally flavonoids in figs (Rodov, Vinokur, & Horev, 2012). The biosynthesis of mangiferin and quercetin in mango slices could be stimulated by MA and PL treatments. The mechanism by which MA may lead to an increment on the content of xanthones and flavonoids is not well-known, although other organic acids have been reported to act by suppressing the accumulation of reactive oxygen species, hence, reducing ascorbic and glutathione acid oxidation when oxalic acid is applied on mango fruit (Ding, Tian, Zheng, Zhou, & Xu, 2007). As far as we know, this is the first study in which xanthones and flavonoids were identified in flesh mango treated by PL, MA and AL.

Colour of mango slices did not change after applying PL, AL or MA treatments because of the great phenolic content achieved. Indeed, combined treatments maintained mango slices with fresh-like colour along 14 days. In addition, Pearson analysis indicated that browning appearance in mango slices was negatively related to the phenolic content. Particularly, phenolic acids contributed to avoid browning, since a high significant correlation ($p < 0.001$) between total phenolics content and lightness of mango slices was found (table 3). On one hand, the loss of membrane integrity and de-compartmentation process allowing phenolic compounds to come into contact with oxidative enzymes might be reduced in treated mango slices. Thus, brown pigment resulting from the reaction between polyphenoloxidase and phenolics was scarce (Saltveit, 2000). On the other hand, a high antioxidant capacity in mango slices might avoid oxidative reactions affecting colour. Therefore, non-enzymatic browning in mango slices was eluded using PL, AL and MA treatments capable to maintain great antioxidant capacity along storage.

5. CONCLUSIONS

The use of pulsed light treatment as well as the application of alginate-based edible coatings and malic acid dipping has been proven to be feasible for preserving antioxidant compounds of fresh-cut mango. AL and AL-PL-MA treatments enabled to reach the highest total phenolic content in fresh-cut mango at day 14. Gallic acid was detected as the most concentrated among the five phenolic compounds found in mango slices regardless the treatment applied. The use of MA, PL-AL-MA and AL-MA-PL treatments maintained the presence of mangiferin and quercetin compounds in mango slices throughout storage. As treated fresh-cut mango has been shown to contain a great concentration of phenolic compounds and high antioxidant capacity, browning colour was avoided along 14 days of storage at 4 °C. In summary, PL-AL-MA and AL-MA-PL treatments seemed to enhance phenolic compounds content of fresh-cut mango while preserving both antioxidant capacity and colour. These results suggest that PL, AL and MA treatments stimulated the antioxidant compounds metabolism enabling the preservation of other fresh-cut mango attributes. Thereby, further studies should focus on analysing the role of combined treatments in the

response mechanisms of other bioactive compounds present in mango and their health impact.

ACKNOWLEDGMENTS

This work was supported by the University of Lleida (Spain) and financed by Tecnológico de Monterrey (Research Chair Funds CAT-200 and CDB081).

6. REFERENCES

Barreto, J. C., Trevisan, M. T. S., Hull, W. E., Erben, G., De Brito, E. S., Pfundstein, B., … Owen, R. W. (2008). Characterization and quantitation of polyphenolic compounds in bark, kernel, leaves, and peel of mango (Mangifera indica L.). Journal of Agricultural and Food Chemistry, 56(14), 5599–5610.

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

Berardini, N., Knödler, M., Schieber, A., & Carle, R. (2005). Utilization of mango peels as a source of pectin and polyphenolics. Innovative Food Science & Emerging Technologies, 6(4), 442–452.

Bonilla, J., Atarés, L., Vargas, M., & Chiralt, A. (2012). Edible films and coatings to prevent the detrimental effect of oxygen on food quality: Possibilities and limitations. Journal of Food Engineering, 110(2), 208–213.

Campbell, R. J., & Ledesma, N. (2015). Mango cultivars with potential for commercial development. In Acta Horticulturae (Vol. 1075, pp. 33–40). International Society for Horticultural Science.

Charles, F., Vidal, V., Olive, F., Filgueiras, H., & Sallanon, H. (2013). Pulsed light treatment as new method to maintain physical and nutritional quality of fresh-cut mangoes. Innovative Food Science & Emerging Technologies, 18, 190–195.

Dang, K. T. H., Singh, Z., & Swinny, E. E. (2008). Edible coatings influence fruit ripening, quality, and aroma biosynthesis in mango fruit. Journal of Agricultural and Food Chemistry, 56(4), 1361–1370.

Daud, N. H., Aung, C. S., Hewavitharana, A. K., Wilkinson, A. S., Pierson, J.-T., Roberts-Thomson, S. J., … Parat, M.-O. (2010). Mango extracts and the mango component mangiferin promote endothelial cell migration. Journal of Agricultural and Food Chemistry, 58(8), 5181–6.

De Ancos, B., Sgroppo, S., Plaza, L., & Pilar Cano, M. (2002). Possible nutritional and health-related value promotion in orange juice preserved by high-pressure treatment. Journal of the Science of Food and Agriculture, 82(8), 790–796.

Ding, Z. S., Tian, S. P., Zheng, X. L., Zhou, Z. W., & Xu, Y. (2007). Responses of reactive oxygen metabolism and quality in mango fruit to exogenous oxalic acid or salicylic acid under chilling temperature stress, 130(1), 112–121.

Dorta, E., González, M., Lobo, M. G., Sánchez-Moreno, C., & de Ancos, B. (2014). Screening of phenolic compounds in by-product extracts from mangoes (Mangifera indica L.) by HPLC-ESI-QTOF-MS and multivariate analysis for use as a food ingredient. Food Research International, 57, 51–60.

Gülçin, İ. (2012). Antioxidant activity of food constituents: an overview. Archives of Toxicology, 86(3), 345–91.

Hertog, M. G. L., Hollman, P. C. H., & Venema, D. P. (1992). Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. Journal of Agricultural and Food Chemistry, 40(9), 1591–1598.

Hoang, V. L. T., Pierson, J.-T., Curry, M. C., Shaw, P. N., Dietzgen, R. G., Gidley, M. J., … Monteith, G. R. (2015). Polyphenolic contents and the effects of methanol extracts from mango varieties on breast cancer cells. Food Science and Biotechnology, 24(1), 265–271.

Ibarra-Garza, I. P., Ramos-Parra, P. A., Hernández-Brenes, C., & Jacobo-Velázquez, D. A. (2015). Effects of postharvest ripening on the nutraceutical and physicochemical properties of mango (Mangifera indica L. cv Keitt). Postharvest Biology and Technology, 103, 45–54.

Lopes, M. M. A., Silva, E. O., Canuto, K. M., Silva, L. M. A., Gallão, M. I., Urban, L., … Miranda, M. R. A. (2016). Low fluence pulsed light enhanced phytochemical content and antioxidant potential of “Tommy Atkins” mango peel and pulp. Innovative Food Science & Emerging Technologies, 33, 216–224.

Ma, X., Wu, H., Liu, L., Yao, Q., Wang, S., Zhan, R., … Zhou, Y. (2011). Polyphenolic compounds and antioxidant properties in mango fruits. Scientia Horticulturae, 129(1), 102–107.

Masibo, M., & He, Q. (2008). Major mango polyphenols and their potential significance to human health. Comprehensive Reviews in Food Science and ….

Müller, L., Fröhlich, K., & Böhm, V. (2011). Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (αTEAC), DPPH assay and peroxyl radical scavenging assay. Food Chemistry, 129(1), 139–148.

Oms-Oliu, G., Rojas-Graü, M. A., González, L. A., Varela, P., Soliva-Fortuny, R., Hernando, M. I. H., … Martín-Belloso, O. (2010). Recent approaches using chemical treatments to preserve quality of fresh-cut fruit: A review. Postharvest Biology and Technology, 57(3), 139–148.

Palafox-Carlos, H., Yahia, E. M., & González-Aguilar, G. A. (2012). Identification and quantification of major phenolic compounds from mango (Mangifera indica, cv. Ataulfo) fruit by HPLC-DAD-MS/MS-ESI and their individual contribution to the antioxidant activity during ripening. Food Chemistry, 135(1), 105–111.

Pataro, G., Donsi, G., & Ferrari, G. (2015). Post-harvest UV-C and PL irradiation of fruits and vegetables. Chemical Engineering Transactions, 44, 31–36.

Pathare, P. B., Opara, U. L., & Al-Said, F. A.-J. (2012). Colour Measurement and Analysis in Fresh and Processed Foods: A Review. Food and Bioprocess Technology, 6(1), 36–60.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry, 53(10), 4290–302.

Raybaudi-Massilia, R. M., Mosqueda-Melgar, J., & Tapia, M. S. (2010). Edible coatings as carriers of food additives on fresh-cut fruits and vegetables. Stewart Postharvest Review, 6(3), 1–7.

Ribeiro, S. M. R., Barbosa, L. C. A., Queiroz, J. H., Knödler, M., & Schieber, A. (2008). Phenolic compounds and antioxidant capacity of Brazilian mango (Mangifera indica L.) varieties. Food Chemistry, 110(3), 620–626.

Robles-Sánchez, M., Astiazarán-García, H., Martín-Belloso, O., Gorinstein, S., Alvarez-Parrilla, E., de la Rosa, L. A., … González-Aguilar, G. A. (2011). Influence of whole and fresh-cut mango intake on plasma lipids and antioxidant capacity of healthy adults. Food Research International, 44(5), 1386–1391.

Robles-Sánchez, R. M., Rojas-Graü, M. A., Odriozola-Serrano, I., González-Aguilar, G., & Martin-Belloso, O. (2013). Influence of alginate-based edible coating as carrier of antibrowning agents on bioactive compounds and antioxidant activity in fresh-cut Kent mangoes. LWT - Food Science and Technology, 50(1), 240–246.

Robles-Sánchez, R. (2009). Quality Index, Consumer Acceptability, Bioactive Compounds, and Antioxidant Activity of Fresh-Cut "Ataulfo" Mangoes (Mangifera Indica L.) as Affected by Low-temperature storage. Journal of Food Science, 74(3), S126–S134.

Rodov, V., Vinokur, Y., & Horev, B. (2012). Brief postharvest exposure to pulsed light stimulates coloration and anthocyanin accumulation in fig fruit (Ficus carica L.). Postharvest Biology and Technology, 68, 43–46.

Rojas-Graü, M. A., Oms-Oliu, G., Soliva-Fortuny, R., & Martín-Belloso, O. (2009). The use of packaging techniques to maintain freshness in fresh-cut fruits and vegetables: a review. International Journal of Food Science & Technology, 44(5), 875–889.

Rojas-Graü, M. A., Tapia, M. S., Rodríguez, F. J., Carmona, A. J., & Martin-Belloso, O. (2007). Alginate and gellan-based edible coatings as carriers of antibrowning agents applied on fresh-cut Fuji apples. Food Hydrocolloids, 21(1), 118–127.

Salinas-Roca, B., Soliva-Fortuny, R., Welti-Chanes, J., & Martín-Belloso, O. (2016). Combined effect of pulsed light, edible coating and malic acid dipping to improve fresh-cut mango safety and quality. Food Control. http://doi.org/10.1016/j.foodcont.2016.02.005

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

Siddiq, M., Sogi, D. S., & Dolan, K. D. (2013). Antioxidant properties, total phenolics, and quality of fresh-cut “Tommy Atkins” mangoes as affected by different pre-treatments. LWT - Food Science and Technology, 53(1), 156–162.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1998). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology.

Valencia-Chamorro, S. A., Palou, L., Del Río, M. A., & Pérez-Gago, M. B. (2011). Antimicrobial edible films and coatings for fresh and minimally processed fruits and vegetables: a review. Critical Reviews in Food Science and Nutrition, 51(9), 872–900.

CHAPTER IV

Journal of Food BioprocessTechnology (under revision)

Quality changes in mango juice treated by high-intensity pulsed electric fields throughout the storage

B. Salinas-Roca, P.Elez-Martínez, J. Welti-Chanes and O. Martín-Belloso

ABSTRACT

The effect of high-intensity pulsed electric fields (HIPEF) processes on *Listeria innocua* inhibition, physicochemical parameters and activity of oxidative enzymes of mango juice was evaluated to set the optimal HIPEF treatment time. Quality parameters, microbial population and bioactive compounds of HIPEF-treated (35 kV/cm, 1800 µs) and thermally-treated (TT) (90°C, 60 s) mango juices were studied and compared with those non-treated during 75 days of storage at 4 ± 1 °C. HIPEF treatment for 800 µs ensured 5 log reductions of *L. innocua*. Polyphenoloxidase (PPO), lipoxygenase (LOX) and peroxidase (POD) residual activities were significantly reduced to 70, 53 and 44%, respectively, at treatment times of 1800 µs. Similar sensory properties compared with fresh mango juice was attained at product treated at 1800 µs. Moreover, fresh mango juice colour (L*= 38.79 ± 0.01, h°= 106.57 ± 0.26) was preserved after HIPEF treatment throghout storage. Moulds and yeasts and psychrophilic bacteria counts in HIPEF-treated (1800 µs) mango juice remained below 6 log cycles CFU/mL up to 2 months of refrigerated storage. The content of total phenolic compounds in those HIPEF-treated increased from 333 to 683 µg of gallic acid equivalent/mL from day 0 to the end of storage. Hence, the application of HIPEF may be a feasible treatment in order to ensure microbiological stability, high bioactive compounds content and fresh-like characteristics of mango juice.

Key words: mango juice, *L. innocua*, high intensity pulsed electric fields, thermal treatment, quality attributes

1. INTRODUCTION

Mango (*Mangifera indica L.)*, one of the most harvested tropical fruits, is widely used to produce juices due to its well-appreciated sensorial attributes (FAO, 2003, 2012; Nanjundaswamy, 1998). Furthermore, this fruit is a rich source of bioactive compounds such as phenolics and carotenoids, hence mango consumption could have health benefits in preventing degenerative diseases (Rawson et al., 2011; Schieber, Ullrich, & Carle, 2000).

Mango juice can undergo quality-degrading reactions triggered by microbial growth population and quality-degrading enzymes, among others. Therefore, preservation treatments are required to ensure its safety and quality stability. On one hand, thermal treatment is commonly used in the juice industry because of its well-known effectiveness in the inactivation of microorganisms and quality-degrading enzymes (Mercadante & Rodriguez-Amaya, 1998; Soliva-Fortuny, Balasa, Knorr, & Martín-Belloso, 2009). However, undesired chemical, physical and sensorial changes as well as reduction of bioactive compounds content have been observed in thermally-treated juices (Sánchez-Moreno et al., 2005; Wibowo, Grauwet, Gedefa, Hendrickx, & Van Loey, 2015). On the other hand, non-thermal treatments allow to obtain microbiologically stable fruit juices but also a better preservation of sensorial and nutritional characteristics than conventional treatments (Y. Chen et al., 2013). Hence, high-intensity pulsed electric fields (HIPEF) technology has been considered as a feasible non-thermal technique for the preservation of liquid foods. The electric field strength and treatment time are reported as the main parameters of HIPEF treatment to induce an electric potential across cell membrane conducting the cell damage (Morales-de la Peña, Salvia-Trujillo, Rojas-Graü, & Martín-Belloso, 2010).

Several studies have proved the efficiency of HIPEF on the inactivation of microorganisms such as *Listeria innocua*, which is one of the main foodborne microorganisms in fruit juices (Huang et al., 2012; Mosqueda-Melgar, Raybaudi-Massilia, & Martín-Belloso, 2007; Timmermans et al., 2014a). Nevertheless, published data evidenced that the degree of microbial inactivation is strongly dependent on the HIPEF conditions (Jiménez-Sánchez et al., 2017a). With regard to enzyme activity, peroxidase (POD), polyphenoloxidase (PPO) and lipoxygenase

(LOX) catalyse some reactions affecting sensory and nutritional properties in fruit juices. HIPEF treatments from 20 to 35 kV/cm have halved enzymatic activity in tomato and orange juices (Aguiló-Aguayo, Soliva-Fortuny, & Martín-Belloso, 2010; Vervoort et al., 2011). Moreover, HIPEF seems to maintain quality characteristics including colour, soluble solids and viscosity as well as retain bioactive compounds of fruit juices (Buckow, Ng, & Toepfl, 2013; Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2008).

Despite of the noteworthy literature using HIPEF treatment for fruit juices quality preservation, no studies comparing the effects of HIPEF and thermal treatment on quality changes of mango juice have been found. Therefore, the objectives of the present work were firstly to select the HIPEF treatment time capable to inactivate *L. innocua* and to reduce enzymatic activity in mango juice while preserving its fresh-like sensorial attributes. Secondly, to compare the effect of HIPEF and thermal treatments on microbial stability, activity of oxidative enzymes, total carotenoids and phenolics content, antioxidant capacity and physicochemical properties in mango juice throughout 75 days of refrigerated storage.

2. MATERIAL AND METHODS

2.1. Mango juice

Mangoes (*Mangifera indica L.*) *cv*. *Tommy Atkins* were purchased from a local wholesale market (Lleida, Spain). Each fruit was washed, dried, peeled and the seed was discarded. The pulp was squeezed and then centrifuged at 5400 g during 5 min at 4°C (AVANTI^{TM} J-25 Beckman; Instruments Inc; Fullerton, CA) and vacuum filtered to obtain mango juice (MJ). MJ electric conductivity (1.54 ± 0.02 mS/cm), soluble solids (12.77 ± 1.11 °Brix) and pH (3.67 ± 0.14) were measured.

2.2. HIPEF treatments

HIPEF treatments were performed using a continuous flow bench scale system (OSU-4F, Ohio State University, Columbus, OH), that generates squared wave pulses. The

flow rate was 60 mL/min controlled by a speed pump (model 752210-25, Cole Palmer Instrument Company, Vermon Hills, IL). The treatment chamber device consisted of eight co-linear chambers disposed in series and each pair of chambers had a thermocouple to control temperature. The outlet treatment temperature of juice was kept below 40 °C using a cooling coil, which was connected before and after each pair of chambers and submerged in an ice-water shaking bath. Based on previous literature, constant electric field strength (35 kV/cm), pulse frequency (200Hz) and width (4 μs) were kept to apply pulses in bipolar quadratic mode, while different treatment times were assayed (50, 100, 200, 400, 800, 1200, 1600, 1800 and 2000 μs). According to the results of microbial and enzymatic inactivation of HIPEF-treated mango juice, the treatment conditions were set for subsequently study of preservation of mango juice along the storage.

2.3. Thermal treatment

MJ was heat-treated at 90°C for 60 s. The juice was pumped with a peristaltic pump (model D-21V, Dinko, Barcelona, Spain) and passed through a tubular stainless steel heat exchange coil system (University of Lleida, Lleida, Spain). Immediately after heating, the tubular stainless steel was immersed in an ice-water bath at 4 °C and thereafter MJ was packaged (Odriozola-Serrano, Soliva-Fortuny, Hernández-Jover, & Martín-Belloso, 2009).

2.4. Packaging and storage

Treated MJ was bottled directly from the treatment systems in sterilized 100 mL polypropylene bottles and leaving the minimum headspace volume. Non-treated MJ was bottled thereafter the juice preparation. Once filled, the containers were tightly closed and stored in darkness under refrigeration (4 ± 1°C) until analysis. Non-treated and treated MJ were analysed twice a week the first 3 weeks and once a week until day 75.

2.5. *Listeria innocua* culture, inoculation and enumeration

L. innocua IPL 1.17 (Institute Pasteur de Lille; Lille, France) was cultured in tryptone soy broth (TSB) with 0.6 % yeasts extract (Bioakar Diagnostic; Beauvais, France) and incubated at 35 °C with continuous agitation at 200 rpm for 15 h to obtain cells in stationary growth phase. The final concentration reached in the culture was 10^8- 10^9 colonies forming unit per mL (CFU/mL). MJ was inoculated with *L. innocua* to have an initial concentration of 10^7- 10^8 CFU/mL and then HIPEF-treated. Treated and non-treated MJ was serially diluted in saline peptone water (Bioakar Diagnostic; Beauvais, France), for *L. innocua* enumeration; the cells were spread on Palcam agar plates (Bioakar Diagnostic; Beauvais, France) and incubated at 35 °C for 24-48 h as stated by ISO 11290-2 method (1998). Colonies were counted and the results were expressed as $\log_{10}$ CFU/ mL.

2.6. Microbial evaluation during storage

Enumeration of psychrophilic microorganisms in MJ on plate count agar (PCA) (Biokar Diagnostic; Beauvais, France) was carried out after the incubation at 5°C ± 1°C for 10 days (ISO 17410, 2001 Method). Moulds and yeasts counts were determined with the ISO 7954, 1987 Method using chloramphenicol glucose agar (CGA) (Biokar Diagnostic; Beauvais, France) and incubating 2-4 days at 25 °C ± 1°C. Colonies were counted and the results were expressed as $\log_{10}$ CFU/ mL. Counts below the detection limit (1.0 log CFU/mL) were considered no detectable colonies. The criterion for completing the storage study was established as the time at which a microbial population of 10^6 CFU/ mL (Salvia-Trujillo, Morales-de la Peña, Rojas-Graü, & Martín-Belloso, 2011).

2.7. Physicochemical analysis

Electric conductivity (Testo 240 conductivity-meter; Testo GmBh & Co; Lenzkirch, Germany), pH (Crison 2001 pH-meter; Crison Instruments S.A; Barcelona, Spain), soluble solid content (Atago RX-1000 refractometer; Atago Company Ltd; Japan), viscosity using a spindle SP61 at 100 rpm and 5 °C (Brookfield, Stoughton, MA) and

colour (Minolta CR-400; Konica Minolta Sensing, Inc., Osaka, Japan) of MJ were measured. Colour equipment was set up for illuminate D65 and 10º observer angle and calibrated using a standard white reflector plate. MJ (10mL) were placed in petri dishes (3.5cm x 3.5cm) and colour was measured using the CIE *L*, a*, b** scale. Additionally, Hue angle (h°) was calculated as the *arctan* of the b* and a* quotient (mesure of red =0 or 36°, yellow= 90°, green = 180°) (Hunter, 1987).

2.8. Enzyme activity evaluation

Peroxidase (POD)

POD activity was determined using the method described by Elez-Martínez, Aguiló-Aguayo, Martín-Belloso, 2006) with some modifications. The enzyme extract for POD activity measurement was obtained by the homogenization of 10 mL of MJ and 20 mL of sodium phosphate buffer 0.2 M at pH 6.5. The homogenate was centrifuged at 24000g for 15 min at 4°C (AVANTI^{TM} J-25, Beckman Instruments Inc; Fullerton, CA, USA). The supernatant was filtered throughout a Whatman paper (no. 1) and the resulting liquid constituted the enzymatic extract. POD activity was assayed spectrophotometrically (CECIL CE 2021 spectrophotometer Cecil Instruments Ltd, Cambridge, UK) in a 1 cm path cuvette by adding at 0.1 mL of enzymatic extract 2.7 mL of sodium phosphate buffer (0.05 M, pH 6.5), 0.1 mL phenylenediamine (1 %) and 0.1 mL hydrogen peroxide (1.5 %). The oxidation of p-phenylenediamine was determined at 470 nm measuring the absorbance every 10 seconds during 3 min. The absorbance values were referred to a sample blank containing all reagents except hydrogen peroxide, which was substituted by distilled water. POD activity was obtained from the slope of the linear portion of the curve. One unit of POD activity was defined as the change of absorbance per minute and millilitre of enzymatic extract at 22ºC.

Polyphenoloxidase (PPO)

PPO activity was determined by the method of Vásquez-Caicedo et al., (2007) with some modifications. For the extraction of the enzyme, 5 g of MJ were mixed with 0.5 g polyvinylpolypyrrolidone (PVPP) and 4.5 g McIlvaine buffer solution (pH 6.5)

consisting of 35 % of 0.1 M citric acid and 75 % 0.2 M disodium phosphate. The mixture was homogenised and centrifuged at 23000 g for 15 min at 4 °C (Centrifuge AVANTI^{TM} J-25, Beckman Instruments Inc; Fullerton, CA). The supernatant was filtered with Whatman paper (no. 1) to obtain the enzyme extract. PPO activity was measured using a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd, Cambridge, UK) at 400 nm by adding 100 µL enzyme extract and 3 mL of 0.5 M cathecol solution and obtaining the absorbance every 10 seconds during 3 min. A blank of cathecol without extract was used. The PPO activity was obtained from the slope of the linear portion of the curve; one unit of PPO activity was defined as a change of one unit of absorbance per minute and millilitre of enzyme extract at 22 °C.

Lipoxigenase (LOX)

LOX activity was determined by the method described by Anthon & Barrett (2003) with modifications. The enzyme extract was obtained by mixing 20 mL of MJ with 5 mL of a solution containing 0.5 M phosphate buffer (pH 6.5) and 0.5% Triton X-100 and centrifuging 10 min at 10000 g at 4 °C (Centrifuge AVANTI^{TM} J-25, Beckman Instruments Inc; Fullerton, CA). The pellet was discarded and the supernatant was filtered with Whatman paper (No. 1). The LOX activity of the enzyme was measured by mixing 2 mL phosphate buffer 0.1 M (pH 6.5), 40 µL linoleic acid and adding 100 µL enzymatic extract. The reaction was measured with a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd, Cambridge, UK) at 234 nm each 10 seconds during 3 min. The activity was calculated from the slope of the linear portion of the curve. A blank was prepared with 2 mL phosphate buffer 0.1 M mixed with 1 mL linoleic. One unit of LOX activity was defined as a change of one unit of absorbance per minute and per millilitre of enzyme extract at 22 °C.

Enzymatic activity was expressed as percentage of residual activity (RA %) which was calculated by the quotient between the enzyme activity of treated (*AEt*) and the non-treated (AE_O) MJ.

2.9. Bioactive compounds and antioxidant activity determination

Total carotenoids

The determination of total carotenoids was performed according to Robles-Sánchez, Rojas-Graü, Odriozola-Serrano, González-Aguilar, & Martín-Belloso (2009). MJ (5 mL) were added to 20 mL of tetrahydrofuran (THF) and homogenized with an Ultra-Turrax T 25 basic (IKA® WERKE, Germany). An aliquot was filtered throughout a no 1 Whatman paper. Total carotenoids were measured spectrophotometrically (CECIL CE 2021 spectrophotometer; Cecil Instruments Ltd, Cambridge, UK) at 470 nm, quantified using β-carotene as an external standard and expressed as μg of β-carotene equivalent per MJ/mL.

Total phenolic

The content of total phenolic compounds (TP) was determined according to the Folin-Ciocalteu colorimetric method described by Singleton, Orthofer, & Lamuela-Raventós (1998) with slight modifications. MJ (0.5 mL) was mixed and homogenised with saturated sodium carbonate solution (10 mL) and Folin-Ciocalteu reagent (10 mL). After one hour in dark storage, absorbance was measured at 765 nm (CECIL CE 2021 spectrophotometer; Cecil Instruments Ltd, Cambridge, UK). TP content was calculated on the basis of a standard curve of gallic acid and expressed as μg of gallic acid equivalent (GAE) per MJ mL.

Antioxidant Capacity

Antioxidant capacity was determined by a radical-scavenging activity (RSA) assay evaluated as bleaching of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. MJ (10 mL) was centrifuged at 3500 g, 20 min and 4°C in a Centrifuge AVANTI^{TM} J-25 (Beckman Instruments Inc; Fullerton, CA, USA). The reaction mixture constituted of 10 μL of supernatant, 3.9 mL of methanolic DPPH (0.0025 gL^{-1}) and 90 μL of distilled water was carried out. The samples were shaken vigorously and kept in the dark for 30 min. The absorption of the samples was measured with a spectrophotometer (CECIL CE 2021 Cecil Instruments Ltd, Cambridge, UK) at 515 nm against a blank of methanol without DPPH (Odriozola-Serrano et al., 2008). The results were expressed as percentage of DPPH inhibition as shown in equation 1 where A_o is de absorbance of DPPH reagent and *As* is the absorbance of the MJ sample reaction with DPPH.

$$DPPH\ inhibition(\%) = \frac{Ao - As}{Ao} \cdot 100 \qquad \text{eq.1}$$

2.10. Sensory evaluation

A total 30 non-trained panellists participated in the sensory test of treated and non-treated MJ at day of processing. A hedonic scale from 0 (dislike) to 10 (extremely like) was used to rate the colour, flavour and overall acceptance. MJ (30 mL) processed by HIPEF (35 kV, 1800 µs, 200Hz, 4µs), heat (90ºC, 60 s) and non-treated (NT) were served at 16 ± 1 °C in transparent cup coded with three digits randomly numbered. Moreover, a glass containing potable water and a piece of non-salted cracker were provided to panellists to eliminate the residual taste between samples (Mosqueda-Melgar, Raybaudi-Massilia, & Martín-Belloso, 2012)

2.11. Statistical analysis

All the treatments were assayed in duplicate and two replicate analyses were carried out for each sample to obtain the mean values and standard deviations (SD) for each analysed parameter. The analysis of variance (ANOVA) and Least Significant Differences (LSD) was performed in order to find statistical differences ($p \leq 0.05$). All statistical analyses were conducted with Statgraphics plus Centurion XV software Version 15.1.02 (StatPoint Technologies, Inc.).

3. RESULTS AND DISCUSSION

3.1. Effect of HIPEF treatment on mango juice

3.1.1. L. innocua inactivation

A maximal reduction of *L.innocua* survival of 5.7 log units was achieved after applying HIPEF (35 kV/ cm, 200 Hz, 4µs) for 800 µs to MJ (Figure 1). The longer is the HIPEF treatment time up to 800 µs, the higher the decrease of microbial population. As described in Figure 1, no significant differences in the *L.innocua*

inactivation levels were appreciated at HIPEF treatments from 800 to 2000 µs. According to microbiological criteria proposed by FDA (2004) for fruit juices, 5 log reductions of target microorganisms should be accomplished for obtaining safe product. Similarly, Mosqueda-Melgar, Raybaudi-Massilia, & Martín-Belloso (2007) achieved 5 log reduction of *L. innocua* population in melon juice treated by HIPEF (35 kV/cm, bipolar square wave, 4 µs pulses and 200 Hz) as treatment times increased up to 1250 µs. Previous research explained the effect of increasing HIPEF treatment time on microbial inactivation by the formation of membrane pores triggering to membrane destabilization and cell rupture (Vega-Mercado et al., 1997). Although the efficacy of HIPEF (20 kV/cm, 90 Hz and 130 L/h) against *L.innocua* was also proved in orange juice (Timmermans et al., 2014a), less studies have been found to reduce more than 5 log at 800 µs. The low pH (4.1) and conductivity (1.71 mS/cm) of MJ could cause *L. innocua* cells more sensible to damage. Indeed, Amiali, Ngadi, Raghavan, & Nguyen, (2006) reported that lowering ionic concentration cause an increase of the treatment chamber resistance, which could enhance the microbial inactivation levels. Wouters, Dutreux, Smelt, & Lelieveld (1999) observed better reduction of *L. innocua* in solutions with low pH (4.0) and conductivity (2.7 mS/cm) than in alkaline solutions.

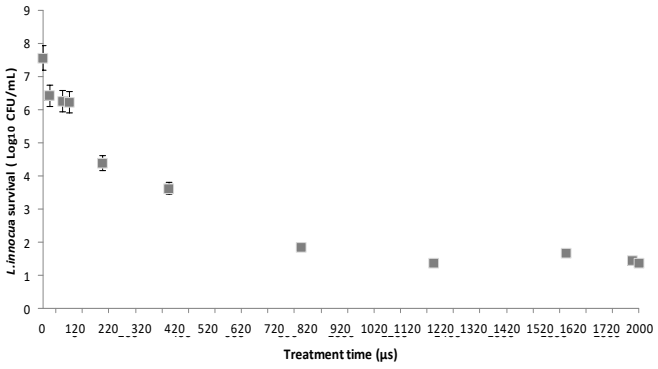


Figure 1: Survival of *L.innocua* population inoculated in mango juice treated by HIPEF (35 kV/cm, 4-µs bipolar pulses at 200 Hz) at different times (µs).

3.1.2. Enzyme activity

HIPEF treatment applied for 1800 µs reduced at 70, 53 and 44 % PPO, LOX and POD activity in the MJ, respectively (Figure 2). Differently, at treatment times below 1800 µs, when no significant reduction of enzymatic activity was observed, various deleterious reactions affecting loss of nutritive value and yellow colour might occur in MJ.

A reduction of the RA as increasing HIPEF treatment time has been also reported by Aguiló-Aguayo, Sobrino-López, Soliva-Fortuny, & Martín-Belloso (2008) and Aguiló-Aguayo et al. (2010), who reached 10 and 30 % of RA for PPO and LOX, respectively, in strawberry treated by HIPEF (35 kV/ cm, 1000 µs, 200 Hz and 4µs). HIPEF treatment, that is known to conduct to cell electroporation, might benefit the contact between enzyme and substrate released from the cell, hence, no complete inactivation was achieved in MJ (Huang et al., 2012). The effect of HIPEF at 1800 µs might cause an irreversible conformational change of the globular protein chain of enzymes in MJ. An enzyme denaturation might be a feasible reason for enzymatic activity reduction (Luo, Zhang, Wang, Chen, & Guan, 2010).

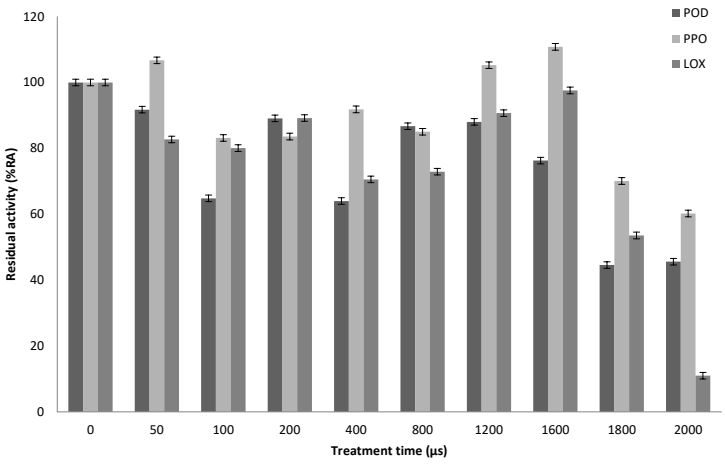


Figure 2: Effect of HIPEF (35 kV/cm, 4-µs bipolar pulses at 200 Hz) at different treatment time in residual activity of oxidative enzymes: peroxidase (POD) (♦), polyphenoloxidase (PPO) (■) and lipoxigenase (LOX) (▲).

The studied oxidative enzymes followed similar pattern of inactivation. Nevertheless, differences on the RA between LOX and the other oxidative enzymes in MJ at the longest treatment time were observed (Figure 2). This could indicate a different level of HIPEF effect on the enzymatic structure. PPO and POD structure contains a prosthetic group, thereby, the influence of electric fields on changing the structure of copper-containing enzyme has been reported scarcely since it can be considered tightly bound organic molecules (Sharma et al., 2013). Otherwise, conformational changes in LOX structure, with no prosthetic group, could occur easily. Moreover, other authors have reported that charges separation of tertiary structure occurred in LOX native conformation leading almost complete inactivation of LOX, when long treatments and high voltage are used in enzymatic solution, but not in PPO (Luo et al., 2010). In agreement with the available scientific literature, electrochemical effect of HIPEF may affect the local electrostatic fields in proteins and disrupt electrostatic interactions of peptide chains leading to conformational changes in enzymes (Buckow et al., 2013). Therefore, HIPEF treatment had greatest degree of activity reduction on LOX compared with PPO and POD in HIPEF-treated MJ at 2000 µs.

3.1.3. Physicochemical parameters

HIPEF treatment had no significant effect ($p \geq 0.05$) on pH and conductivity of MJ when different treatment times were applied. Average values in pH and conductivity of treated-MJ were 4.1 ± 0.1 and 1.71 ± 0.01 mS/cm, respectively. In a similar way, Zhang, Gao, Zhang, Shi, & Xu (2010) and Aguilar-Rosas, Ballinas-Casarrubias, Nevarez-Moorillon, Martin-Belloso, & Ortega-Rivas, (2007) reported that both HIPEF-processed longan and apple juice, did not show pH differences with the non-treated products. Other reports indicated no change of conductivity after HIPEF treatment (Mosqueda-Melgar et al., 2012; Vega-Mercado et al., 1997). Although no effect of HIPEF treatment time on TSS content or viscosity of MJ was observed, differences between HIPEF-treated and non-treated MJ were detected. Non-treated MJ (10.8 ± 0.7 °Brix and 4.0 ± 0.3 mPa·s) presented lower average values of TSS and viscosity compared with HIPEF-treated (35 kV/ cm, 200 Hz, 4µs and 2000 µs) MJ (12.9 °Brix ± 0.6 and 5.4 mPa·s ± 1.1). Cserhalmi, Sass-Liss, Tóth-Markus &

Lechner (2006) and Falade, Babalola, Akinyemi, & Ogunlade (2004) reported an increase in TSS and viscosity of citrus juices treated by HIPEF (28 kV/cm, 100 µs, 2 µs-bipolar pulses at 100 Hz), which were attributed to the breakdown cell effect releasing soluble solids from the cell. Moreover, changes in HIPEF-treated MJ compared with the non-treated might be also attributed to a decline of the pectinolitic enzyme activity, which could enable to maintain pectin content in MJ and hence higher TSS and viscosity (Espachs-Barroso, Van Loey, Hendrickx, & Martín-Belloso, 2006).

Figure 3 shows a non-significant changes of L* value of MJ from 50 to 2000 µs. Similarly, the h° value was maintained in the range of 74.5 to 73.9 in HIPEF-treated MJ as treatment time increased (Figure 3). Thus, HIPEF treatment preserved characteristic colour of MJ. The significant reduction of enzymatic activity in HIPEF-treated MJ might prevent quality degrading oxidative reactions (Pathare et al., 2012). The present results are aligned with previous studies, where colour of HIPEF-treated orange (Cortés et al., 2008) and carrot juice (Luciano J. Quitão-Teixeira et al., 2007) were preserved as in fresh juices. Carrot, orange and mango juice have similar yellow colour tonality, which could be mainly attributed to carotenoid compounds. Thus, yellow colour might be preserved whether great content of natural pigments such as carotenoids is maintained.

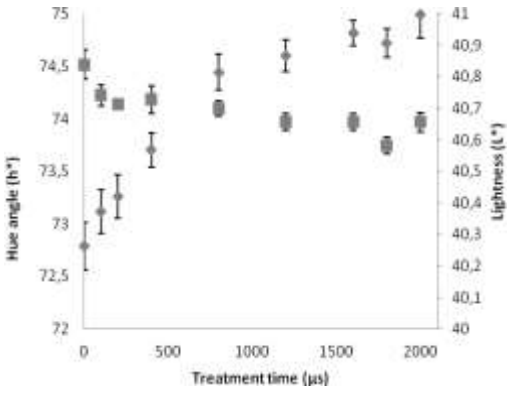


Figure 3: Colour parametres Lightness (L*) (♦) and hue angle (h˚) (■) of mango juice treated by HIPEF (35 kV/cm, 4-µs bipolar pulses at 200 Hz) at different treatment times.

3.2. Sensory evaluation of mango juice

Figure 4 shows the influence of HIPEF (35 kV/cm, 1800 µs, 200 Hz, 4 µs) and TT (90 ˚C, 60 s) on sensorial attributes (colour, flavour and overall acceptance) of MJ compared with the non-treated. Similar overall acceptance and flavour between treated and non-treated MJ were observed. Mosqueda-Melgar, Raybaudi-Massilia, Martín-Belloso, (2012) observed no differences in flavour and overall acceptance comparing fresh fruit juices and those treated by HIPEF and TT. On the other hand, colour values in HIPEF and thermally-treated MJ were alike. Nevertheless, significant differences ($p \leq 0.05$) in colour perception of non-treated MJ (5.6 ± 1.6) compared with the HIPEF-treated (7.2 ± 1.8) were detected. The reduction of oxidative enzyme activity in HIPEF-treated MJ might avoid the loss of colour. Also, the possible release of natural pigments due to the electroporation effect in HIPEF treatment could explain the great colour score of HIPEF-treated MJ given by the consumers.

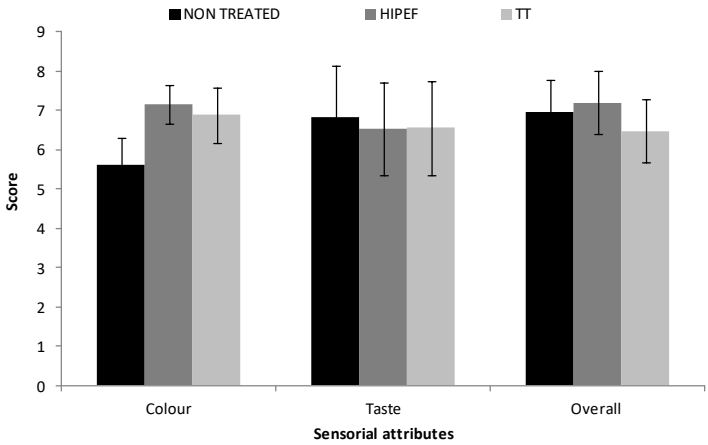


Figure 4: Effect of high intensity pulsed electric fields treatment (HIPEF) (35 kV/cm, 1800 µs, 4-µs bipolar pulses at 200 Hz), thermal treatment (TT) (90 ° C, 60 s) and non-treated conditions on sensorial attributes: colour, taste and overall acceptance of mango juice.

Since HIPEF-treated MJ at 1800 µs led to a significant reduction of *L. innocua* population and enzymatic activity as well as fresh-like physicochemical

characteristics, sensory evaluation of MJ treated by HIPEF and TT at day of processing, and further quality analysis along the storage were carried out at 35 kV/cm, 1800 µs, 200 Hz, 4 µs.

3.3. Storage stability of mango juice

3.3.1 Microbial evaluation

Initial counts of moulds and yeasts in non-treated MJ were 4.22 ± 0.58 $\log_{10}$ CFU/mL, while those of psychrophilic bacteria were 1.74 ± 0.15 $\log_{10}$ CFU/mL. HIPEF or TT effectively reduced microbial loads of the juice up to the detection limit just after processing (day 0) (Figure 5). During storage, it was observed that moulds and yeasts population increased earlier than psychrophilic bacteria in treated and non-treated MJ. No microbial growth in HIPEF-treated MJ was detected during the first two weeks of storage, while the TT-MJ did not show microbial growth along the entire storage time. Microbial counts for HIPEF-treated and thermal-treated MJ was lower than 6 $\log_{10}$ CFU/mL until day 59 and 75, respectively, whereas non-treated MJ exceed those counts at day 23.

Diverse studies have suggested that microorganisms are inactivated because of electroporation and electrofusion phenomena during the HIPEF treatment (Buckow et al., 2013). Nevertheless, a microbial growth in HIPEF-treated MJ could be attributed to a non-complete inactivation of microorganisms (Mosqueda-Melgar et al., 2007). HIPEF treatment enabled to extend the lag phase of MJ microbial population, hence, the recovery of injured microorganisms and germination of those sporulated was delayed. Timmermans et al., (2011) and Elez-Martínez, Soliva-Fortuny, Martín-Belloso, (2006) observed no growth of moulds and yeasts in HIPEF-treated orange juice at 25 and 35 kV/ cm, respectively, during 20 and 56 days. Although, Timmermans et al., (2011) achieved similar microbial stability compared with the present study, it must be noted that the treatment temperature used was 56 °C, whereas present results were obtained without exceeding 40 °C.

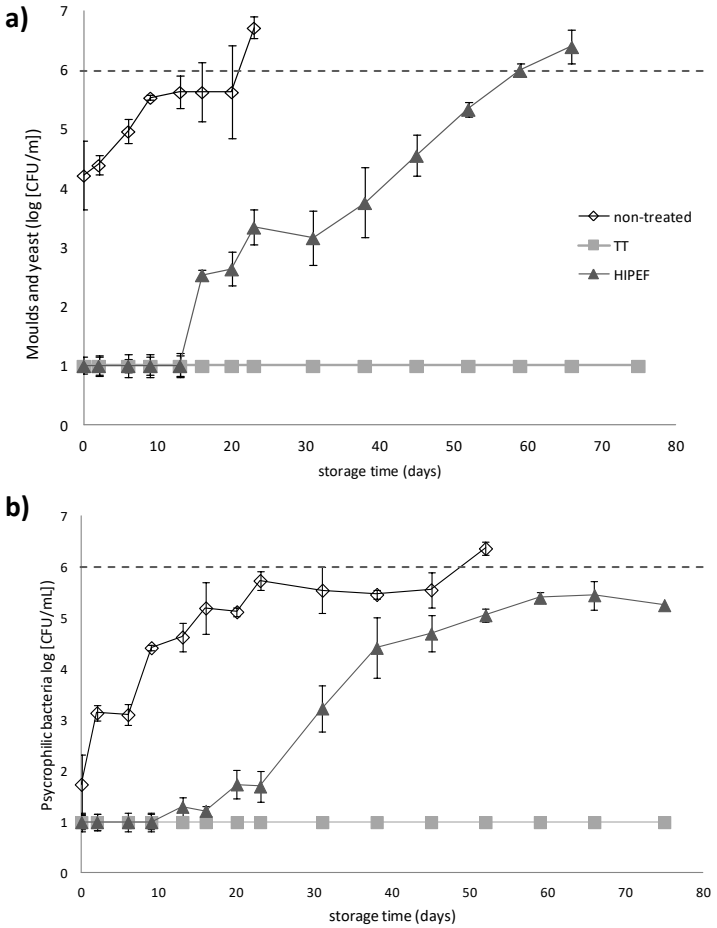


Figure 5: Moulds and yeasts (a) and psychrophilic bacteria (b) growth in mango juice treated by HIPEF treatment (35 kV/cm, 1800 µs, 4-µs bipolar pulses at 200 Hz) or thermal treatment (90 ° C, 60 s) compared with the non-treated throughout storage at 4 °C during 75 days. Limit of microbial shelf-life at 6 log CFU/mL (----).

3.3.2. Enzyme activity

At the beginning of storage, RA of HIPEF-treated (35 kV/cm for 1800 μs with bipolar pulses of 4 μs at 200Hz) MJ were 70.0 ± 5.1; 69.9 ± 4.9 and 46.3 ± 10.2 % for PPO, LOX and POD, respectively. The application of thermal treatment to MJ significantly reduced activity of PPO and POD up to 55.5 ± 0.5 and 20.7 ± 1.0 at day 0 (table 1). The PPO and POD molecular structure, which contains a prosthetic group in their structure, has been reported to be specially affected by pH, temperature and electric fields (Luo et al., 2010). Otherwise, RA_{LOX} after thermal treatment increased at day of processing, LOX appeared to be less thermo-sensible. During storage, a severe increase of RA_{POD} in non-treated MJ was observed, whereas PPO and LOX activities were slightly reduced. Probably, the increase of POD activity might be assigned to the cell release of POD substrate (organic hydroperoxides), which enable the enzyme-substrate contact (Vervoort et al., 2011).

Both electrochemical and thermal effects associated with HIPEF and TT could result in changes in the structure and conformation of enzymes, which may lead to inactivation (Huang et al., 2012; Timmermans et al., 2011). However, the appearing of isoenzymes and uncomplete inactivation might explain the fluctuations of enzymatic activity in TT and HIPEF-treated MJ along the storage. RA of PPO and POD in MJ treated by TT and HIPEF had a drastically decrease from day 16 until the end of storage. Among oxidative enzymes, RA_{POD} of 25.1 ± 3.5 % (day 75) and 17.0 ± 4.4 % (day 49) was the lowest in MJ treated by TT and HIPEF, respectively. Consistently, literature has reported that POD seemed to be more susceptible to HIPEF than other enzymes and is associated with the modification of the α-helix structure (Leong & Oey, 2014). These results are inconsistent with the complete POD inactivation during 56 days reported by Elez-Martínez, Soliva-Fortuny, et al. (2006) in orange juice after HIPEF treatment (35 kV/cm for 1000 μs with bipolar pulses of 4 μs at 200 Hz). However, other authors described a progressive decrease of RA_{POD} in HIPEF-treated orange juice (23 kV/cm, 90 Hz, monopolar pulses of 2 μs and 130 L/h) along 58 days (Vervoort et al., 2011).

In contrast, significant RA_{LOX} reduction in treated MJ required long storage time. Both TT and HIPEF treatments reduced significantly more than a 50 % the initial

activity of LOX at the end of storage. Similar to other studies a retarded decrease of the RA_{LOX} was observed (Espachs-Barroso et al., 2006; Zhao, Yang, Lu, Tang, & Zhang, 2007). According to Aguiló-Aguayo, Soliva-Fortuny & Martín-Belloso (2010), LOX protein chain could undergo changes and a development of resistant isoforms in HIPEF-treated fruit juices. Thus, the conformational changes in LOX structure might delay the reduction of the activity throughout storage time.

It is known that HIPEF and thermal enzyme inactivation mechanisms are related to the unfolding of proteins due to changes in their secondary structure (Salvia-Trujillo et al., 2011). Also, a weak affinity of enzyme-substrate complex might describe the decrease of RA in HIPEF-treated MJ during the storage. Another hypothesis for reducing enzymatic activity in HIPEF-treated MJ throughout storage would be the formation of aggregates as a result of a strong polarization of the protein molecules and hydrophobic interactions or covalent bonds (Luo et al., 2010). Therefore, the protein aggregation along the storage could reduce the enzymatic reaction by avoiding the substrate from fitting the active site of the enzyme.

Table 1: Effect of HIPEF (35 kV/cm, 1800 µs and 200Hz) and TT (90°C 60 s) on residual activities (RA) of polyphenoloxidase (PPO), peroxidase (POD) and lipoxigenase (LOX) enzymes in mango juice throughout 75 days of storage at 4˚C.

	RA $_{PPO}$ (%)			**RA $_{POD}$ (%)**			**RA $_{LOX}$ (%)**		
Days	NT	TT	HIPEF	NT	TT	HIPEF	NT	TT	HIPEF
0	100 ± 1.0 ^{aA}	55.5 ± 0.5 ^{deE}	70.0 ± 5.1 ^{cdC}	100 ± 5.9 ^{bA}	20.7 ± 1.0 ^{aB}	46.3 ± 10.2 ^{aC}	100 ± 11.1 ^{abA}	120.9 ± 26.7 ^{aB}	69.9 ± 4.9 ^{aB}
2	61.1 ± 6.4 ^{bA}	66.1 ± 1.0 ^{fgA}	39.2 ± 21.9 ^{defB}	127.1 ± 0.0 ^{bA}	37.2 ± 8.9 ^{dB}	56.0 ± 0.0 ^{bC}	91.5 ± 4.4 ^{bA}	143.7 ± 29.1 ^{aB}	119.5 ± 8.4 ^{bC}
6	42.7 ± 0.0 ^{efA}	63.8 ± 15.1 ^{efgB}	92.0± 8.7 ^{bcC}	128.21 ± 12.8 ^{aA}	46.2 ± 16.6 ^{eB}	32.2 ± 8.3 ^{cC}	109.9 ± 12.4 ^{aA}	155.4 ± 11.3 ^{aB}	163.1 ± 22.0 ^{bB}
9	45.4 ± 0.0 ^{deA}	78.3 ± 20.3 ^{ghB}	98.7 ± 14.1 ^{jC}	127.4 ± 32.0 ^{cdA}	40.2 ± 3.5 ^{eB}	34.5 ± 6.1 ^{deC}	94.4 ± 7.1 ^{bA}	84.5± 13.7 ^{aB}	109.9 ± 0.1 ^{cC}
13	57.2 ± 1.7 ^{cA}	72.4 ± 1.6 ^{fgB}	106.8 ± 19.1 ^{jC}	134.5 ± 3.3 ^{bcA}	40.0 ± 11.6 ^{eB}	23.7 ± 3.2 ^{efC}	72.3 ± 7.4 ^{cA}	92.7 ± 17.3 ^{aB}	102.6 ± 2.4 ^{cdC}
16	51.4 ± 8.5 ^{cdA}	96.9 ± 20.8 ^{hB}	56.0 ± 16.0 ^{iA}	154.5 ± 12.1 ^{bcA}	40.1 ± 6.4 ^{eB}	20.7 ± 0.0 ^{efC}	49.3 ± 12.9 ^{efA}	76.5 ± 20.3 ^{bA}	103.6 ± 3.7 ^{cdA}
20	38.7 ± 0.0 ^{efA}	52.1 ± 0.0 ^{cdB}	52.1 ± 0.0 ^{iC}	168.7 ± 21.7 ^{dA}	28.8 ± 1.8 ^{bcdB}	22.0 ± 3.9 ^{defB}	44.6 ± 10.4 ^{fghA}	77.3 ± 0.0 ^{aB}	105.7 ± 0.0 ^{cC}
23		38.7 ± 11.9 ^{bA}	38.3 ± 11.9 ^{defgA}		32.6 ± 11.4 ^{cdB}	24.3 ± 2.3 ^{deB}		75.4 ± 2.4 ^{aB}	92.9 ± 5.7 ^{eC}
31		56.9 ± 2.6 ^{deB}	51.9 ± 11.2 ^{hiB}		22.0 ± 0.0 ^{bB}	22.4 ± 2.4 ^{defB}		75.9 ± 0.0 ^{aB}	108.7 ± 9.6 ^{cC}
38		40.4 ± 7.7 ^{bcB}	45.2 ± 3.7 ^{fghiB}		22.4 ± 3.2 ^{bB}	20.2 ± 7.4 ^{efB}		74.6 ± 13.3 ^{aB}	96.3 ± 3.9 ^{deC}
45		60.6 ± 9.37 ^{efB}	46.8± 14.3 ^{defgA}		24.8 ± 0.8 ^{bcB}	26.8 ± 3.1 ^{dB}		80.0 ± 0.0 ^{aB}	80.3 ± 1.5 ^{fB}
52		48.8 ± 15.9 ^{bcdB}	28.2 ± 4.8 ^{cdeA}		28.6 ± 16.2 ^{cdB}	23.5 ± 0.8 ^{deB}		52.3 ± 13.8 ^{aB}	55.8 ± 11.4 ^{gAB}
59		52.1 ± 6.1 ^{cdB}	45.7 ± 11.8 ^{ghiB}		24.8 ± 4.7 ^{bcB}	17.0 ± 4.4 ^{fgB}		49.6 ± 7.7 ^{aB}	43.4 ± 6.3 ^{ghB}
66		17.9 ± 11.2 ^{aB}			21.9 ± 1.9 ^{bB}			51.6 ± 11.5 ^{aA}	
75		12.1 ± 8.7 ^{aB}			25.1 ± 3.5 ^{bcB}			40.6 ± 6.1 ^{aB}	

NT: non-treated mango juice

Values represent the mean ± standard deviation. Values in a column followed by the same lower case letter and in a row followed by the same upper case letter are not significantly different ($p > 0.05$).

Table 2: Effect of HIPEF (35 kV/cm, 1800 µs and 200Hz) and TT (90°C 60 s) on lightness (L*) and hue angle (h°) colour parameters s in mango juice throughout 75 days of storage at 4˚C.

	L*			h°		
Days	NT	TT	HIPEF	NT	TT	HIPEF
0	38,79 ± 0,01 ^{Aa}	40,34 ± 0,25 ^{Ba}	38,87 ± 0,52 ^{Aa}	106,57 ± 0,26 ^{Aa}	107,4 ± 0,6 ^{Aa}	108,03 ± 0,38 ^{Aa}
2	32,52 ± 0,21 ^{Ab}	37,78 ± 0,00 ^{CBb}	38,73 ± 0,01 ^{Ca}	107,97 ± 1,31 ^{Aa}	110,3 ± 0,5 ^{Ab}	108,75 ± 0,11 ^{Aa}
6	32,21 ± 0,21 ^{Ab}	37,59 ± 0,09 ^{Cb}	38,56 ± 0,05 ^{Ca}	104,00 ± 2,31 ^{Aa}	110,6 ± 0,7 ^{Ab}	108,86 ± 0,16 ^{Aa}
9	32,05 ± 0,01 ^{Ab}	37,15 ± 0,61 ^{Cb}	36,28 ± 3,03 ^{Ca}	103,90 ± 2,80 ^{Ab}	109,98 ± 0,47 ^{Ab}	110,08 ± 0,57 ^{Aa}
13	31,91 ± 0,05 ^{Ab}	37,32 ± 0,03 ^{Cb}	32,76 ± 0,11 ^{Cb}	103,22 ± 3,24 ^{Ab}	109,64 ± 0,66 ^{Ab}	108,52 ± 0,09 ^{Aa}
16	31,91 ± 0,01 ^{Ab}	37,07 ± 0,04 ^{Cb}	32,7 ± 0,0 ^{Cb}	102,3 ± 3,4 ^{Ab}	108,96 ± 0,78 ^{Ab}	107,27 ± 0,05 ^{Aa}
20	31,6 ± 0,2 ^{Ab}	37,00 ± 0,46 ^{Cb}	31,9 ± 0,3 ^{Cb}	101,2 ± 3,8 ^{Cb}	107,04 ± 1,61 ^{Ab}	105,75 ± 0,77 ^{Bb}
23	31,13 ± 1,35 ^{Ab}	36,90 ± 0,39 ^{Cb}	32,43 ± 0,21 ^{Cb}	100,4 ± 4,1 ^{Cb}	107,3 ± 1,3 ^{Ab}	104,86 ± 0,07 ^{Bb}
31		36,9 ± 0,1 ^{Cb}	32,52 ± 0,01 ^{Cb}		112,3 ± 1,5 ^{Dc}	109,9 ± 0,2 ^{Aa}
38		36,82 ± 0,05 ^{Cb}	32,42 ± 0,09 ^{Cb}		112,6 ± 1,6 ^{Dc}	109,4 ± 0,2 ^{Aa}
45		36,22 ± 0,57 ^{Cb}	32,63 ± 0,01 ^{Cb}		110,7 ± 1,3 ^{Dc}	108,09 ± 0,02 ^{Aa}
52		36,8 ± 0,0 ^{Cb}	32,3 ± 0,2 ^{Cb}		111,2 ± 2,2 ^{Dc}	105,86 ± 0,09 ^{Ab}
59		35,77 ± 1,73 ^{Cb}	31,8 ± 0,2 ^{Cc}		111,8 ± 1,2 ^{Dc}	105,67 ± 0,09 ^{Ab}
66		38,3 ± 1,3 ^{Cb}			126,3 ± 1,7 ^{Dd}	
75		40,8 ± 0,0 ^{Ca}			102,5 ± 1,8 ^{De}	

NT: Non-treated mango juice

Values represent the mean ± standard deviation. Values in a column followed by the same lower case letter and in a row followed by the same upper case letter are not significantly different

3.3.3. Physicochemical parameters

pH and TSS values remained stable throughout the storage and no statistical differences among treatments were observed. pH average values for non-treated, TT and HIPEF-treated MJ were 3.7 ± 0.1, 3.76 ± 0.04 and 3.7 ± 0.1, respectively. The mean values of TSS for non-treated, TT and HIPEF-treated MJ were 9.4 ± 0.9, 10.72 ± 0.52 and 8.53 ± 1.62, respectively. In contrast to the obtained results, Timmermans et al., (2011) observed a TSS increase in HIPEF-treated orange juice (23 kV/cm and 90 Hz) after 58 days of refrigerated storage. Differences might be attributed to the use of lower electric field compared with that of the present study; hence, less reduction of enzymatic activity might lead deleterious quality process as increment of turbidity and TSS.

L* values of the non-treated, HIPEF-treated and TT MJ at day 0 were 39.78 ± 0.01, 38.87 ± 0.52 and 40.34 ± 0.25, respectively (table 2). During storage, non-treated MJ rapidly declined L*, whereas a slightly decreased in HIPEF-treated MJ was observed. L* values of thermal-treated MJ were preserved along the storage. On the other hand, initial h° values of non-treated (106.57 ± 0.26), TT (107.4 ± 0.6) and HIPEF-treated (108.03 ± 0.38) MJ were not significantly different. Along the storage, h° of non-treated MJ decreased; hence loss of yellow colour might occur. TT and HIPEF treatment maintained similar h˚ in MJ throughout the storage. The loss of L* and h˚ could be associated with the formation of dark colour compounds and reduction of yellow colour in beverages due to the non-enzymatic browning reactions (Pathare et al., 2012). According to other studies, the loss of colour in non-treated MJ might be related with the oxidative reactions mostly triggered by residual activity of POD and PPO (Timmermans et al., 2011; Wibowo et al., 2015). In this sense, the increase of RA_{POD} observed in non-treated MJ probably conducted the deterioration of colour. Differently, all treated MJ significantly reduced the activity of POD and PPO; hence, enzymatic browning was avoided. Therefore, treated MJ preserved the yellow colour of fresh mango juice.

3.3.4. Bioactive compounds and antioxidant activity

The effects of processing and storage time on bioactive compounds and antioxidant activity of MJ are shown in Figure 6. Considering total carotenoid content, TT and HIPEF-treated MJ showed a decrease of 17 and 13 %, respectively, compared with non-treated MJ at the beginning of the storage (Figure 6a). Carotenoids compounds are thermo-labile; hence, heat processing leads to significant higher losses in TT MJ than those HIPEF-treated. Differently, an electroporation on the cell membrane, which enable the releasing of carotenoids among other compounds, in HIPEF-treated MJ could occur. Oxidative reactions promoted by enzymes, light or oxygen could affect rapidly the carotenoids released in TT or HIPEF-treated MJ, which could explain the subsequently decline of carotenoids content (Soliva-Fortuny, Balasa, Knorr & Martín-Belloso, 2009). According to Odriozola-Serrano, Soliva-Fortuny, Hernández-Jover, & Martín-Belloso (2009) oxidation may occur by self-oxidation, where alkylperoxyl radicals are formed and these radicals attack the double bonds resulting in formation of epoxides. Thus, the severity of oxidation depends on the structure of carotenoids and the environmental conditions. However, during storage period, HIPEF-treated MJ reached 2.2 times more carotenoids than those heat-treated (Figure 6a). Similarly, other studies described great retention of carotenoids in HIPEF-treated compared to heat-treated orange juice during storage (Buckow et al., 2013). Total phenolic compounds in MJ varied from 560.1 ± 17.9 (non-treated) to 333.8 ± 27.8 (HIPEF-treated) and 529.6 ± 15.4 (TT) μg of gallic acid/ mL at processing day (day 0). Similarly to Santhirasegaram, Razali, George, & Somasundram, (2015), no significant difference in the phenolics concentration after thermal treatment compared with non-treated MJ was observed immediately after processing. Although other authors have also reported that after HIPEF treatment he phenolic content is reduced, the mechanism is not well known (Rawson et al., 2011). The interaction with other compounds such as solutes resulting from the high electric field and long treatment time applied could create aggregations reducing the content of phenolic compounds (Soliva-Fortuny et al., 2009).

Total phenolics decreased in non-treated MJ along the storage (Figure 6b). Otherwise, total phenolic compounds concentration increased in MJ treated by HIPEF throughout

the storage. Indeed, HIPEF-treated MJ (683.79 ± 0.50 µg GAE/mL) showed the highest phenolics concentration compared with TT MJ at day 59. Phenolic compounds are formed in plant products via the action of phenylalanine ammonia-lyase (PAL) in the phenylpropanoid metabolism (Patthamakanokporn, Puwastien, Nitithamyong, & Sirichakwal, 2008). This response is initiated when the plant recognizes a stimulus at the cellular level. It could be hypothesized that HIPEF induced PAL activity and may influence the voltage-gated ion channels and increase the membrane permeability for Ca^{2+} at the cellular level, followed by a rapid influx of Ca^{2+} through cation channels. Through this process, Ca^{2+}-dependent protein kinase phosphorylates PAL, which regulates the phenylpropanoid metabolism (Vallverdú-Queralt et al., 2012). On the other hand, the loss of phenolic compounds in non-treated fruit juice was also observed by Patthamakanokporn, Puwastien, Nitithamyong, & Sirichakwal (2008) who attributed the decrease of phenolics during the storage to deleterious enzymes such as PPO. After analyzing the data obtained in this work, it was observed that there was a negative correlation (r= - 0.74) between the activity of PPO and the content of phenolics. This result seems to indicate the importance of TT and HIPEF treatment in reducing RA_{PPO}. Thus, decreasing of PPO activity, which uses phenolic compounds for the oxidative processes to trigger on quinones, was mainly associated with increasing in phenolics (Cheema & Sommerhalter, 2015).

Initial antioxidant capacity in MJ was 20.4 ± 1.3, 18.7 ± 0.4 and 17.9 ± 0.4 % of DPPH inhibition for HIPEF, TT and non-treated MJ, respectively. The enhancement of radical scavenging activity in HIPEF-treated MJ might be attributed to the stress response of antioxidant compounds. During storage, the antioxidant capacity of MJ depleted irrespective of the treatment applied (Figure 6c). It is remarkable that both total carotenoids content and antioxidant capacity rapidly decreased in TT and non-treated MJ along the storage. Our results for HIPEF-treated MJ were in accordance with Odriozola-Serrano et al. (2008) who observed a significant loss of antioxidant capacity as storage time increased in HIPEF-treated tomato juice (35 kV/ cm, 100 Hz and 1500 µs of treatment time). In many plant species, a good relationship between antioxidant activity and total phenolics was noted. Contrarily, no correlation between total phenolic compounds and antioxidant capacity in treated MJ was observed. Thus,

antioxidant capacity in MJ during refrigerated storage could be related to other bioactive compounds such as vitamin C, which could be easily affected by oxidative deleterious reactions (Buckow et al., 2013).

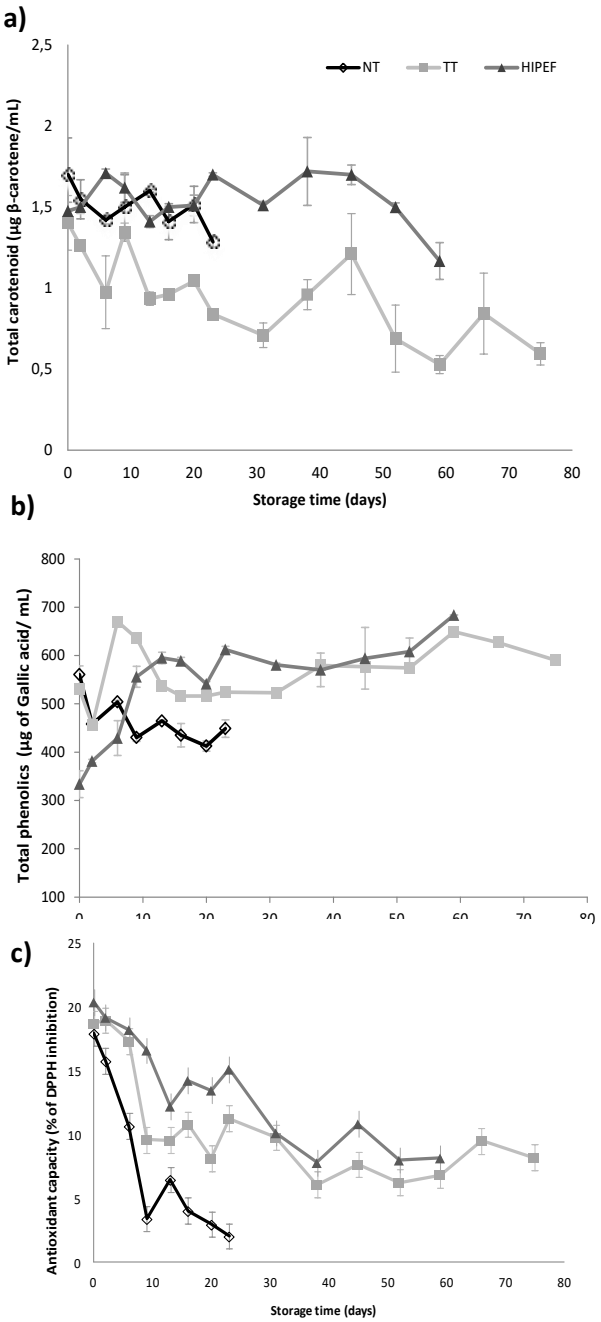


Figure 6: Changes on bioactive compounds during stored mango juice HIPEF (35 kV/cm, 1800 µs, 4-µs bipolar pulses at 200 Hz),TT (90 ° C, 60 s) and non-treated (NT) . a: total carotenoid concentration, b: total phenolics and c: antioxidant capacity

4. CONCLUSIONS

HIPEF treatment at 35 kV/cm, 4 μs- bipolar pulses, 200 Hz and 1800 μs proved to be feasible in the reduction of *L. innocua* population to pasteurization levels in mango juice while enzymatic activity of PPO, LOX and POD was reduced up to 70, 53 and 44 % RA, respectively, and fresh-like physicochemical properties maintained. The native flora stability of HIPEF-treated mango juice was assured throughout 59 days at 4 °C. On the other hand, LOX activity of HIPEF- treated mango juice was halved along the storage. Also, the POD and PPO enzymatic activity in HIPEF-treated mango juice was lower than in those untreated throughout storage. The reduction of PPO enabled a significant increase of the phenolic content in HIPEF-treated mango juice during 59 days. Differently, antioxidant capacity and carotenoid content of all evaluated mango juices decreased gradually throughout storage period. However, bioactive compounds in mango juice were better retained after HIPEF than thermal treatments. The beneficial effect of the HIPEF treatment was noticeable over the storage period with enhanced phenolic content and maintaining fresh-like characteristics of mango juice.

ACKNOWLEDGMENTS

This work was supported by the University of Lleida (Spain) and financed by Tecnológico de Monterrey, Mexico (Research Chair Funds CAT-200 and CDB081).

5. REFERENCES

Aguilar-Rosas, S. F., Ballinas-Casarrubias, M. L., Nevarez-Moorillon, G. V., Martin-Belloso, O., & Ortega-Rivas, E. (2007). Thermal and pulsed electric fields pasteurization of apple juice: Effects on physicochemical properties and flavour compounds. Journal of Food Engineering, 83(1), 41–46.

Aguiló-Aguayo, I., Sobrino-López, Á., Soliva-Fortuny, R., & Martín-Belloso, O. (2008). Influence of high-intensity pulsed electric field processing on lipoxygenase and β-glucosidase activities in strawberry juice. Innovative Food Science & Emerging Technologies, 9(4), 455–462.

Aguiló-Aguayo, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2010). High-intensity pulsed electric fields processing parameters affecting polyphenoloxidase activity of strawberry juice. Journal of Food Science, 75(7), C641–C646.

Amiali, M., Ngadi, M. O., Raghavan, V. G. S., & Nguyen, D. H. (2006). Electrical Conductivities of Liquid Egg Products and Fruit Juices Exposed to High Pulsed Electric Fields. International Journal of Food Properties, 9(3), 533–540.

Anthon, G. E., & Barrett, D. M. (2003). Thermal inactivation of lipoxygenase and hydroperoxytrienoic acid lyase in tomatoes. Food Chemistry, 81(2), 275–279.

Buckow, R., Ng, S., & Toepfl, S. (2013). Pulsed electric field processing of orange juice: A review on microbial, enzymatic, nutritional, and sensory quality and stability, 12(5), 455–467.

Cheema, S., & Sommerhalter, M. (2015). Characterization of polyphenol oxidase activity in Ataulfo mango. Food Chemistry, 171, 382–7.

Chen, Y., Yu, L. J., & Rupasinghe, H. P. V. (2013). Effect of thermal and non-thermal pasteurisation on the microbial inactivation and phenolic degradation in fruit juice: a mini-review. Journal of the Science of Food and Agriculture, 93(5), 981–6.

Cortés, C., Esteve, M. J., & Frígola, A. (2008). Color of orange juice treated by High Intensity Pulsed Electric Fields during refrigerated storage and comparison with pasteurized juice. Food Control, 19(2), 151–158.

Cserhalmi, Z., Sass-Kiss, Á., Tóth-Markus, M., & Lechner, N. (2006). Study of pulsed electric field treated citrus juices. Innovative Food Science & Emerging Technologies, 7(1–2), 49–54.

Elez-Martínez, P., Aguiló-Aguayo, I., & Martín-Belloso, O. (2006). Inactivation of orange juice peroxidase by high-intensity pulsed electric fields as influenced by

process parameters. Journal of the Science of Food and Agriculture, 86(1), 71–81.

Elez-Martínez, P., Soliva-Fortuny, R. C., & Martín-Belloso, O. (2006). Comparative study on shelf life of orange juice processed by high intensity pulsed electric fields or heat treatment. European Food Research and Technology, 222(3–4), 321–329.

Espachs-Barroso, A., Van Loey, A., Hendrickx, M., & Martín-Belloso, O. (2006). Inactivation of plant pectin methylesterase by thermal or high intensity pulsed electric field treatments. Innovative Food Science & Emerging Technologies, 7(1–2), 40–48.

Falade, K. O., Babalola, S. O., Akinyemi, S. O. S., & Ogunlade, A. A. (2004). Degradation of quality attributes of sweetened Julie and Ogbomoso mango juices during storage. European Food Research and Technology, 218(5), 456–459.

FAO. (2003). Tropical fruits.

FAO. (2012). FAOSTAT.

Food and Drug Administration of the United States of America. (2004). Guidance for industry: Juice HACCP hazards and controls guidance first edition. Retrieved from http://www.fda.gov/Food/%0AGuidanceComplianceRegulatoryInformation/GuidanceDocuments/Juice/%0Aucm072557.htm#ftn1

Huang, K., Tian, H., Gai, L., & Wang, J. (2012). A review of kinetic models for inactivating microorganisms and enzymes by pulsed electric field processing. Journal of Food Engineering, 111(2), 191–207.

Hunter, R. S. (1987). The Measurement of Appearance (Vol. 5).

Jiménez-Sánchez, C., Lozano-Sánchez, J., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2017). Alternatives to conventional thermal treatments in fruit-juice processing. Part 1: Techniques and applications. Critical Reviews in Food Science and Nutrition, 57(3), 501–523.

Leong, S. Y., & Oey, I. (2014). Effect of pulsed electric field treatment on enzyme kinetics and thermostability of endogenous ascorbic acid oxidase in carrots (Daucus carota cv. Nantes), 146, 538–547.

Luo, W., Zhang, R. B., Wang, L. M., Chen, J., & Guan, Z. C. (2010). Conformation changes of polyphenol oxidase and lipoxygenase induced by PEF treatment, 40(2), 295–301.

Mercadante, A. Z., & Rodriguez-Amaya, D. B. (1998). Effects of Ripening, Cultivar Differences, and Processing on the Carotenoid Composition of Mango. Journal of Agricultural and Food Chemistry, 46(1), 128–130.

Morales-de la Peña, M., Salvia-Trujillo, L., Rojas-Graü, M. A., & Martín-Belloso, O. (2010). Impact of high intensity pulsed electric field on antioxidant properties and quality parameters of a fruit juice-soymilk beverage in chilled storage. LWT - Food Science and Technology, 43(6), 872–881.

Mosqueda-Melgar, J., Raybaudi-Massilia, R. M., & Martín-Belloso, O. (2012). Microbiological shelf life and sensory evaluation of fruit juices treated by high-intensity pulsed electric fields and antimicrobials. Food and Bioproducts Processing, 90(2), 205–214.

Mosqueda-Melgar, J., Raybaudi-Massilia, R., & Martín-Belloso, O. (2007). Influence of treatment time and pulse frequency on Salmonella Enteritidis, Escherichia coli and Listeria monocytogenes populations inoculated in melon and watermelon juices treated by pulsed electric fields. International Journal of Food Microbiology, 117(2), 192–200.

Nanjundaswamy, A. M. (1998). The mango botany, production and uses (509-544) . (R. E. Litz, Ed.). Wallingford, UK: CAB International.

Odriozola-Serrano, I., Soliva-Fortuny, R., Hernández-Jover, T., & Martín-Belloso, O. (2009). Carotenoid and phenolic profile of tomato juices processed by high intensity pulsed electric fields compared with conventional thermal treatments. Food Chemistry, 112(1), 258–266.

Odriozola-Serrano, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2008). Changes of health-related compounds throughout cold storage of tomato juice stabilized by thermal or high intensity pulsed electric field treatments. Innovative Food Science & Emerging Technologies, 9(3), 272–279.

Pathare, P. B., Opara, U. L., & Al-Said, F. A.-J. (2012). Colour Measurement and Analysis in Fresh and Processed Foods: A Review. Food and Bioprocess Technology, 6(1), 36–60.

Patthamakanokporn, O., Puwastien, P., Nitithamyong, A., & Sirichakwal, P. P. (2008). Changes of antioxidant activity and total phenolic compounds during storage of selected fruits. Journal of Food Composition and Analysis, 21(3), 241–248.

Quitão-Teixeira, L. J., Aguiló-Aguayo, I., Ramos, A. M., & Martín-Belloso, O. (2007). Inactivation of Oxidative Enzymes by High-Intensity Pulsed Electric Field for Retention of Color in Carrot Juice. Food and Bioprocess Technology, 1(4), 364–373.

Rawson, A., Patras, A., Tiwari, B. K., Noci, F., Koutchma, T., & Brunton, N. (2011). Effect of thermal and non thermal processing technologies on the bioactive content of exotic fruits and their products: Review of recent advances. Food Research International, 44(7), 1875–1887.

Robles-Sánchez, R. M., Rojas-Graü, M. A., Odriozola-Serrano, I., González-Aguilar, G. A., & Martín-Belloso, O. (2009). Effect of minimal processing on bioactive compounds and antioxidant activity of fresh-cut “Kent” mango (Mangifera indica L.). Postharvest Biology and Technology, 51(3), 384–390.

Salvia-Trujillo, L., Morales-de la Peña, M., Rojas-Graü, M. A., & Martín-Belloso, O. (2011). Microbial and enzymatic stability of fruit juice-milk beverages treated by high intensity pulsed electric fields or heat during refrigerated storage. Food Control, 22(10), 1639–1646.

Sánchez-Moreno, C., Plaza, L., Elez-Martínez, P., De Ancos, B., Martín-Belloso, O., & Cano, M. P. (2005). Impact of high pressure and pulsed electric fields on bioactive compounds and antioxidant activity of orange juice in comparison with

traditional thermal processing. Journal of Agricultural and Food Chemistry, 53(11), 4403–4409.

Santhirasegaram, V., Razali, Z., George, D. S., & Somasundram, C. (2015). Effects of Thermal and Non-thermal Processing on Phenolic Compounds, Antioxidant Activity and Sensory Attributes of Chokanan Mango (Mangifera indica L.) Juice. Food and Bioprocess Technology. http://doi.org/10.1007/s11947-015-1576-y

Schieber, A., Ullrich, W., & Carle, R. (2000). Characterization of polyphenols in mango puree concentrate by HPLC with diode array and mass spectrometric detection. Innovative Food Science and Emerging Technologies, 1(2), 161–166.

Sharma, S., Singh, A. K., Kaushik, S., Sinha, M., Singh, R. P., Sharma, P., … Singh, T. P. (2013). Lactoperoxidase: structural insights into the function, ligand binding and inhibition. Int J Biochem Mol Biol, 4(3), 108–128.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1998). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology.

Soliva-Fortuny, R., Balasa, A., Knorr, D., & Martín-Belloso, O. (2009). Effects of pulsed electric fields on bioactive compounds in foods: a review. Trends in Food Science and Technology, 20(11–12), 544–556.

Timmermans, R. A. H., Mastwijk, H. C., Knol, J. J., Quataert, M. C. J., Vervoort, L., der Plancken, I. Van, … Matser, A. M. (2011). Comparing equivalent thermal, high pressure and pulsed electric field processes for mild pasteurization of orange juice. Part I: Impact on overall quality attributes. Innovative Food Science & Emerging Technologies, 12(3), 235–243.

Timmermans, R. A. H., Nierop Groot, M. N., Nederhoff, A. L., van Boekel, M. A. J. S., Matser, A. M., & Mastwijk, H. C. (2014). Pulsed electric field processing of different fruit juices: Impact of pH and temperature on inactivation of spoilage and pathogenic micro-organisms, 173, 105–111.

Vallverdú-Queralt, A., Oms-Oliu, G., Odriozola-Serrano, I., Lamuela-Raventos, R. M., Martín-Belloso, O., & Elez-Martínez, P. (2012). Effects of pulsed electric fields on the bioactive compound content and antioxidant capacity of tomato fruit. Journal of Agricultural and Food Chemistry, 60(12), 3126–34.

Vásquez-Caicedo, A. L., Schilling, S., Carle, R., & Neidhart, S. (2007). Effects of thermal processing and fruit matrix on β-carotene stability and enzyme inactivation during transformation of mangoes into purée and nectar. Food Chemistry, 102(4), 1172–1186.

Vega-Mercado, H., Martín-Belloso, O., Qin, B.-L., Chang, F. J., Marcela Góngora-Nieto, M., Barbosa-Cánovas, G. V., & Swanson, B. G. (1997). Non-thermal food preservation: Pulsed electric fields. Trends in Food Science & Technology, 8(5), 151–157.

Vervoort, L., Van der Plancken, I., Grauwet, T., Timmermans, R. A. H., Mastwijk, H. C., Matser, A. M., … Van Loey, A. (2011). Comparing equivalent thermal, high pressure and pulsed electric field processes for mild pasteurization of orange juice: Part II: Impact on specific chemical and biochemical quality parameters. Innovative Food Science & Emerging Technologies, 12(4), 466–477.

Wibowo, S., Grauwet, T., Gedefa, G. B., Hendrickx, M., & Van Loey, A. (2015). Quality changes of pasteurised mango juice during storage. Part I: Selecting shelf-life markers by integration of a targeted and untargeted multivariate approach. Food Research International, 78, 396–409.

Wouters, P. C., Dutreux, N., Smelt, J. P. P. M., & Lelieveld, H. L. M. (1999). Effects of pulsed electric fields on inactivation kinetics of Listeria innocua. Applied and Environmental Microbiology, 65(12), 5364–5371.

Zhang, Y., Gao, B., Zhang, M., Shi, J., & Xu, Y. (2010). Pulsed electric field processing effects on physicochemical properties, flavor compounds and microorganisms of longan juice. Journal of Food Processing and Preservation, 34(6), 1121–1138.

Zhao, W., Yang, R., Lu, R., Tang, Y., & Zhang, W. (2007). Investigation of the mechanisms of pulsed electric fields on inactivation of enzyme: lysozyme. Journal of Agricultural and Food Chemistry, 55(24), 9850–8.

CHAPTER V

(Under revision)

Effect of high hydrostatic pressure on enzymatic activity and quality attributes in mango puree varieties (cv. Tommy Atkins and Manila)

M. Morales-de la Peña, B. Salinas-Roca, O. Martín-Belloso, Z. Escobedo-Avellaneda and J. Welti-Chanes

ABSTRACT

The effect of High Hydrostaic Pressure (HHP) treatments on pectinmethylesterase (PME), peroxidase (POD), polyphenoloxidase (PPO) residual activity (RA) and physicochemical parameters: pH, water activity (aw), total soluble solids (TSS), viscosity and colour of Tommy Atkins and Manila mango purees was evaluated at 400, 450, 500 MPa/0-16 min/25 or 55°C. HHP-treated mango purees at 25°C had higher enzyme RA than those processed at 55°C. The lowest RA of PME (30.5%) and PPO (28%) was achieved in Tommy Atkins treated during 16 min at 400 MPa and 550 MPa, respectively. Manila mango puree processed at 550 MPa and 8 min achieved the lowest RA in POD (46%). There were no significant changes in pH, aw, and TSS values of both HHP-treated mango purees (Tommy Atkins and Manila) compared to those untreated. It was observed that viscosity of HHP-treated Tommy Atkins mango puree augmented 2.1 times as pressure and treatment time increased. Otherwise, slightly changes in viscosity of HHP-treated Manila mango puree were identified. Higher yellow index (122.4 ± 3.3) and lower lightness (37.3 ± 5.3) were observed in Tommy Atkins mango puree after 550 MPa and 55°C compared with Manila cultivar. According to the results, HHP processing could be a feasible treatment to reduce enzymatic activity and preserve fresh-like quality attributes in mango puree.

Key words: mango puree, high hydrostatic pressure, enzymatic activity and physicochemical attribute

1. INTRODUCTION

Mango (*Mangifera indica*) is one of the main tropical fruits harvested worldwide being Tommy Atkins and Manila varieties the most produced (Saúco, 2013). While Tommy Atkins is well valued for preserving its quality along the postharvest handling; Manila is most appreciated for its taste but less popular in international markets due to its perishability (FAO, 2012). In the last decade, the consumption of mango fruit and its derivatives have increased, although scarce products preserving their fresh-like characteristics can be found overseas (FAO, 2003). Paste and purees belong to a class of convenience food for seasonal and perishable fruits, such as mango (Nath et al., 2016). In this sense, mango purees can be used to produce juices, nectars, jams, and jellies, among other products (Kaushik, Kaur, Rao, & Mishra, 2014). Commercialization of mango purees would enable the consumers worldwide to avail the benefits of this exotic fruit. Beyond the microbial growth, one of the main challenges in mango puree preservation is endogenous enzymatic activity, which is directly related to the loss of its fresh properties (Chakraborty et al., 2014). Therefore, to obtain high-quality and safety mango puree with fresh-like attributes, the study of alternative preservation treatments is required.

Currently, thermal treatments are widely used in food industries to preserve juices, purees and other fruit-based products. Nevertheless, the high temperatures achieved during processing can alter fruit quality attributes such as colour and texture and reduce bioactive compounds content (Liu, Li, Wang, Bi, & Liao, 2014; Liu, Wang, Li, Bi, & Liao, 2013). A non-thermal alternative to avoid detrimental effects of heat is the HHP treatment. Different studies have proved that HHP processing is highly effective in destroying pathogens and spoilage microorganisms in different fruit products (Hiremath & Ramaswamy, 2012; Moody, Marx, Swanson, & Bermúdez-Aguirre, 2014; Serment-Moreno, Barbosa-Cánovas, Torres, & Welti-Chanes, 2014; Valdramidis et al., 2009; Welti-Chanes et al., 2009). Furthermore, HHP exerts irreversible damage in the microbial cell allowing to extend the product shelf-life (Gonzalez & Barrett, 2010). On the other hand, it is well known that viscosity and colour of fruit products are dependant of enzymatic activity (Hendrickx, Ludikhuyze, Van den Broeck, & Weemaes, 1998; Kaushik et al., 2014; F. Liu et al., 2014, 2013;

Tribst, Franchi, de Massaguer, & Cristianini, 2011). Oxidative enzymes like polyphenoloxidase (PPO) and peroxidase (POD) are responsible for the deterioration of colour and nutritional value. Likewise, pectinmethylesterase (PME) is a pectinolitic enzyme involved in the breakdown of pectin, affecting textural properties of the final product. Hence, their inactivation during processing is relevant to achieve high-quality fruit-based products. Numerous studies have demonstrated that PPO, POD and PME enzymes are in general resistant to HHP, although their stability depends on their origin and matrix composition (Terefe et al., 2014). To achieve higher levels of enzymatic inactivation, recently, the combination of high pressures and mild temperatures (30 - 60°C) has been proposed (Tejada, Ortigoza et al., 2015).

The aim of this research was to evaluate the influence of HHP treatment at different conditions of pressure, temperature and time, on enzymatic activity (PME, PPO and POD) and physico-chemical parameters of Tommy Atkins and Manila mango purees.

2. MATERIALS AND METHODS

2.1. Mango puree preparation

Mangoes (*Mangifera indica* cv. Manila and Tommy Atkins) were purchased from a local market (Monterrey, Mexico) at commercial maturity. Fruits were washed; hand peeled, sliced and pulped using a household blender. Obtained mango puree (MP) was packaged in plastic bags (3 Kg/ bag) and stored at -35°C until processing. pH, water activity and total soluble solids values were 3.54 ± 0.01, 0.99 ± 0.01 and 14.5 ± 0.13, respectively, in Tommy Atkins, and 3.89 ± 0.05, 0.99 ± 0.00, 16.3 ± 0.20, respectively, in Manila MP.

2.2. High hydrostatic pressure processing

MP of each variety was thawed and samples of 20 g were placed in polyethylene bags, air evacuated and heat sealed (Torrey EVD4, Guadalupe, Mexico). MP of Tommy Atkins and Manila were HHP treated at 25 and 55°C using an isostatic system (Avure Technologies, Middeltown, OH, USA). HHP processes at 400, 450 and 550 MPa and holding times: CUT, 2, 4, 8 and 16 min were assayed. The come-up time (CUT) required to reach each pressure was 2.28 ± 0.11, 1.33 ± 0.15 and 1.44 ±

0.16 min at 55 °C and 1.46 ± 0.11, 1.33 ± 0.12 and 1.13 ± 0.04 at 25 °C for 400, 450 and 550 MPa, respectively. Untreated samples of MP were considered as control. Treatments were conducted in duplicate and three replicate analyses were carried out for each sample.

2.3. Physicochemical properties

Total soluble solids (TSS) of untreated and HHP-treated MP were measured using a refractometer (PR-101Atago USA, Inc. Bellevue, WA), and expressed as Brix degrees (°Bx), while pH was determined at 25°C with a pH-meter (Orion Research Inc., model 420, Boston, MA). MP viscosity was measured using a rotary viscosimeter (model DV-I, Brookfield, Stoughton, MA) with a SP61 spindle at 100 rpm and 5 °C. Water activity (a_w) analysis was carried out with an AquaLab 4TE equipment (Washington, USA) at 23°C. Finally, colour of the control and processed MP was measured at room temperature with a tri-stimulus Minolta CR-400 colorimeter (Konica Minolta Sensing, INC, Osaka, Japan). CIE L*a*b* values were determined and yellow index (YI) was calculated with eq. (1).

$$YI = 142.86\ (b^*/L^*) \qquad \text{eq. (1)}$$

2.4. Enzyme activity determination

Pectin methyl esterase (PME)

PME activity was determined by potentiometric titration based on the method described by Kimball (1991). MP (10 g) was mixed with 10 mL of NaCl 2N. The mix was homogenised and centrifuged (3800 g, 20 min and 4 °C) and 5 mL of supernatant was mixed with 30 mL of 1% (p/v) pectin solution at 30 °C. The pH was adjusted to 7 with 1 N and 0.1 N NaOH and once set, 50 µL of NaOH 0.1 N were added. The time required for the solution to return to pH 7 was measured.

Peroxidase (POD)

POD activity in MP was measured using the method described by Elez-Martínez, et al. (2006) with some modifications. Enzyme extract was obtained by the homogenization of 10 g of MP and 20 ml of sodium phosphate buffer 0.2 M at pH

6.5. The homogenate was centrifuged (18000 g, 20 min at 4°C) (Beckman Coulter, Avanti J-XP. CA USA). The supernatant was filtered throughout a no. 1 Whatman paper and the resulting liquid constituted the enzymatic extract. POD activity was assayed spectrophotometrically in a 1 cm path cuvette by adding 0.1 mL of enzymatic extract with 1.8 mL of sodium phosphate buffer (0.05 M, pH 6.5), 0.2 mL *ρ*-phenylenediamine (1 %) and 0.1 mL hydrogen peroxide (1.5 %) as oxidant. The oxidation of *ρ*-phenylenediamine was measured the absorbance at 470 nm at 25 °C evaluating every 10 seconds during 3 min using a Genesys 10S UV-VIS spectrophotometer (Thermo Scientific, Madison, USA).

Polyphenoloxidase (PPO)

PPO activity was determined following the procedure of Vásquez-Caicedo et al., (2007) with minor modifications. The enzyme extract was obtained by mixing 5 g of MP with 0.5 g Polivinilpolipirolidona (PVPP) and 4.5 g McIlvaine buffer solution (pH 6.5) consisting of 35% of 0.1 M citric acid and 75 % 0.2 M disodium phosphate. The mix was homogenised and centrifuged at 12500 rpm, 15 min and 4 °C (Beckman Coulter, Avanti J-XP. CA USA). The supernatant was filtered with no. 1 Whatman paper to obtain the enzyme extract. PPO activity was measured using a Genesys 10S UV-VIS spectrophotometer (Thermo Scientific, Madison, USA) at 400 nm. The reaction started by mixing 100 µL enzyme extract and 3 mL of 0.5 M cathecol solution in a 1 cm path cuvette at 25 °C. The activity was determined from the slope of the linear portion of the curve and obtaining the absorbance every 10 seconds during 2 min. One unit of PPO activity was defined as a change of 0.1 units of absorbance per minute and millilitre of enzyme extract at 25 °C.

The percentage of residual activity (RA %), was calculated as the ratio of the enzyme activity of treated MP (AEt) and the untreated MP (AE_O) multiplied by 100.

2.5. Statistical analysis

All the analyses were carried out in triplicate to obtain the mean and standard deviations (SD) values for each parameter. Analysis of variance (ANOVA) to compare treatments and Pearson's correlation test were carried out using JMP 11

program for Windows (SAS software; SAS Institute, NC). Confidence interval was set at 0.95 ($p < 0.05$) for analysis and procedures.

3. RESULTS

3.1. pH, water activity and total soluble solids

The two MP of the varieties studied, had significant differences ($p < 0.05$) in pH, and TSS nonetheless, a_w values were similar in both samples (Table 1). The high concentration of organic acid compounds such as vitamins and phenolic acids in Manila cultivar (Sarkiyayi, 2013) could be related with the observed differences.

As can be observed in Table 1, there were significant effects in pH of HHP-treated Tommy Atkins MP at 450 MPa/25°C and 550 MPa/55°C, as well in HHP-processed Manila MP at 550MPa/25°C. Boyton et al. (2002) reported slightly changes in pH values of Tommy Atkins mangoes after HHP treatment of 300 and 600 MPa. Likewise, the pH of Manila mango after HHP at 75 and 150 MPa at room temperature was significantly different compared to the untreated mangoes (Vargas-Ortiz et al., 2013). According to Kaushik et al. (2014a, 2014b), the small changes in pH of HHP processed fruit-based products might be due to the change in ionization of water molecules within the fruit, which increases the H^+ ion concentration leading to shift in pH levels.

Regarding TSS, no significant changes were observed in Tommy Atkins and Manila MP after HHP processing, irrespectively of the treatment applied; except, Tommy Atkins MP processed at 550 MPa/25°C and 450-550MPa/55°C, which showed lower TSS values than the untreated MP (Table 1). However, observed differences deviated from the untreated MP to a maximum of 4%. Minimal effects of HHP treatment in TSS of different fruit and vegetable products have already been reported (Liu et al., 2012; Swami et al., 2017; Nath et al., 2016; Ahmed et al., 2005; Vervoot et al., 2011). On the other hand, water activity of both MP was kept constant after HHP treatment, regardless of the processing conditions applied (Table 1).

Table 1. Effects of HHP treatments on pH, TSS and aw in Tommy Atkins and Manila MP.

		Tommy Atkins			Manila		
Treatment	**Time**	**TSS**	**pH**	**aw**	**TSS**	**pH**	**aw**
Control	**-**	14.5 ± 0.13a	3.54 ± 0.01a	0.99 ± 0.00a	16.3 ± 0.20a	3.89 ± 0.05a	0.99 ± 0.00a
400MPa-25℃	**CUT**	14.3 ± 0.36a	3.53 ± 0.01a	0.99 ± 0.00a	16.4 ± 0.14a	3.85 ± 0.01a	0.99 ± 0.00a
	2	14.3 ± 0.17a	3.56 ± 0.01a	0.99 ± 0.00a	16.5 ± 0.07a	3.91 ± 0.02a	0.99 ± 0.00a
	4	14.4 ± 0.26a	3.55 ± 0.04a	0.99 ± 0.00a	16.4 ± 0.14a	3.87 ± 0.02a	0.99 ± 0.00a
	8	14.3 ± 0.21a	3.54 ± 0.01a	0.99 ± 0.00a	16.3 ± 0.07a	3.86 ± 0.01a	0.99 ± 0.00a
	16	14.2 ± 0.24a	3.55 ± 0.01a	0.99 ± 0.00a	16.4 ± 0.28a	3.86 ± 0.01a	0.99 ± 0.00a
450MPa-25℃	**CUT**	14.4 ± 0.38a	3.41 ± 0.01b	0.99 ± 0.00a	15.8 ± 0.30a	3.85 ± 0.01a	0.99 ± 0.00a
	2	14.2 ± 0.33a	3.38 ± 0.01b	0.99 ± 0.00a	16.0 ± 0.10a	3.88 ± 0.02a	1.00 ± 0.01a
	4	14.5 ± 0.35a	3.36 ± 0.04b	0.99 ± 0.00a	16.2 ± 0.10a	3.86 ± 0.02a	0.99 ± 0.00a
	8	14.3 ± 0.32a	3.41 ± 0.01b	0.99 ± 0.00a	15.4 ± 0.70a	3.86 ± 0.01a	0.99 ± 0.00a
	16	14.2 ± 0.28a	3.39 ± 0.01b	0.99 ± 0.00a	16.0 ± 0.10a	3.81 ± 0.03a	0.99 ± 0.00a
550MPa-25℃	**CUT**	13.9 ± 0.24b	3.52 ± 0.01a	0.99 ± 0.00a	15.6 ± 0.70a	3.85 ± 0.01a	0.99 ± 0.01a
	2	14.2 ± 0.26a	3.58 ± 0.02b	0.99 ± 0.00a	16.1 ± 0.10a	3.81 ± 0.00b	0.99 ± 0.00a
	4	14.3 ± 0.12a	3.54 ± 0.01a	0.99 ± 0.00a	16.2 ± 0.20a	3.82 ± 0.00b	0.99 ± 0.01a
	8	14.2 ± 0.10b	3.53 ± 0.01a	0.99 ± 0.00a	16.1 ± 0.10a	3.82 ± 0.00b	0.99 ± 0.00a
	16	14.1 ± 0.13b	3.51 ± 0.01b	0.99 ± 0.00a	16.4 ± 0.10a	3.80 ± 0.01b	1.00 ± 0.01a
400MPa-55℃	**CUT**	14.5 ± 0.13a	3.55 ± 0.00a	0.99 ± 0.00a	16.3 ± 0.00a	3.95 ± 0.00b	1.00 ± 0.01a
	2	14.5 ± 0.00a	3.57 ± 0.01b	0.99 ± 0.00a	16.2 ± 0.10a	3.91 ± 0.01a	1.00 ± 0.01a
	4	14.3 ± 0.00b	3.56 ± 0.01a	0.99 ± 0.00a	16.3 ± 0.10a	3.92 ± 0.05a	1.00 ± 0.01a
	8	14.5 ± 0.05a	3.55 ± 0.01a	0.99 ± 0.00a	16.2 ± 0.00a	3.94 ± 0.01a	1.00 ± 0.01a
	16	14.4 ± 0.00a	3.57 ± 0.01b	0.99 ± 0.00a	16.2 ± 0.06a	3.94 ± 0.01a	1.00 ± 0.01a
450MPa-55℃	**CUT**	14.4 ± 0.06a	3.54 ± 0.01a	0.99 ± 0.00a	16.4 ± 0.06a	3.92 ± 0.01a	1.00 ± 0.01a
	2	14.3 ± 0.06a	3.55 ± 0.01a	0.99 ± 0.00a	16.1 ± 0.10a	3.94 ± 0.01a	1.00 ± 0.01a
	4	14.3 ± 0.00b	3.54 ± 0.01a	1.00 ± 0.01a	15.9 ± 0.40a	3.94 ± 0.00a	1.00 ± 0.01a
	8	14.3 ± 0.00b	3.53 ± 0.00a	0.99 ± 0.00a	16.0 ± 0.20a	3.92 ± 0.01a	0.99 ± 0.01a
	16	14.3 ± 0.13a	3.51 ± 0.01b	0.99 ± 0.00a	16.2 ± 0.00a	3.95 ± 0.01a	0.99 ± 0.01a
550MPa-55℃	**CUT**	13.9 ± 0.24b	3.64 ± 0.01b	0.99 ± 0.00a	16.4 ± 0.30a	3.91 ± 0.01a	0.99 ± 0.01a
	2	14.2 ± 0.26a	3.61 ± 0.01b	0.99 ± 0.00a	16.0 ± 0.00a	3.92 ± 0.01a	0.99 ± 0.00a
	4	14.3 ± 0.12a	3.64 ± 0.02b	0.99 ± 0.00a	15.8 ± 0.10b	3.95 ± 0.00b	0.99 ± 0.00a
	8	14.2 ± 0.10b	3.59 ± 0.02b	0.99 ± 0.00a	16.0 ± 0.10a	3.92 ± 0.00a	0.99 ± 0.00a
	16	14.1 ± 0.13b	3.6 ± 0.01b	0.99 ± 0.00a	16.0 ± 0.10a	3.92 ± 0.01a	0.99 ± 0.00a

3.2. Effects of HHP processing in enzyme activity

PME activity

Figures 1a and 2a show PME residual activity of mango purees after HHP treatments. The lowest RA_{PME} achieved in Tommy Atkins MP was 30.5 ± 3.8 % at 400 MPa/16 min/55°C. Otherwise, Manila MP had the lowest RA_{PME} (34.6 ± 1.1 %) after HHP treatment at 550 MPa/16 min/55°C. Different studies on PME inactivation by HHP treatment have been reported during the last decade. Similar to our results, Liu et al. (2012) reported 23.2% RA_{PME} of HHP-processed watermelon juice at 600MPa/60min (room temperature). Swami et al. (2014) also observed a significant inactivation of PME (30%) in *Aloe vera* treated by HHP at different processing conditions (60 – 740MPa, 3 – 40min). Likewise, Riahi & Ramaswamy (2003) showed significant reduction of the PME activity in apple juice up to 50 % at 400MPa/25min/25°C. Recently, Terefe et al. (2015) found that pear PME was relatively sensitive to HHP process with about 50% of enzyme inactivation after 3 min treatment (600 Mpa/20°C) of pear slices. Endogenous PME enzyme in mango purees may have primary protein structure with covalent bonds in which pressure has slight effects. The increase of holding times and pressures could provoke changes in the PME structure and inevitably promote the enzyme denaturalization leading to the reduction of RA (Bayindirli, 2010). The residual PME activity after the application of high pressure in the different evaluated products could be due to a pressure resistant isoform of the enzyme, which has been identified in many fruits and vegetables (Terefe et al., 2015). Pressure resistant and labile PME isoenzymes, which seem to be similar to those heat resistant and labile, have already been reported in orange, grape fruit, apple and tomato (Goodner et al., 1998; Baron et al., 2006; Plaza et al., 2007).

As seen in Figure 2a, processing conditions at 400 and 450 MPa (25°C) during CUT up to 16 min induce activation of PME in Manila MP. Just after HHP processing at mentioned conditions, the RA_{PME} of Manila MP ranged between 102.8 – 120.9%. It has been reported that some enzymes, such as PME have been activated by HHP treatment (Liu et al., 2012). Cano et al. (1997) observed PME activation in orange juice treated by HHP between 200 and 400 MPa at room temperature. According to

Liu et al. (2012) the activation of enzymes could be attributed to binding of isoenzymes with the added chemical reactive or partial denaturation to released isoenzymes with higher activity. Comparing the effects of HHP treatment in both MP varieties (Fig. 1a and 2a), it was observed that Manila MP had higher RA_{PME} (34.6 – 120.9%) than Tommy Atkins MP (32.8 – 91.8%). It might be possible that pH differences among Tommy Atkins MP (3.55 ± 0.05) and Manila MP (3.89 ± 0.01) are related to the lower RA achieved in Tommy Atkins MP. Kaushik et al., (2015) mentioned that at lower pH, the higher concentration of ion pairs surrounding the enzyme may experience more electrostrictive effects of different charges which are mainly influenced by high pressure, contributing to significant enzyme inactivation levels. Likewise, Chakraborty et al. (2014) highlighted that besides the effect of pressure, time and temperature reducing PME activity in mango puree, the pH may have an influence on the PME conformational structure(Chakraborty et al., 2014).

POD and PPO activity

Figure 1b illustrates the effect of processing conditions (pressure, holding time and temperature) on RA_{POD} in Tommy Atkins mango puree. HHP treatment of 450 MPa/16 min/55°C lead to the lowest RA_{POD} (53.4%). In the case of Manila MP (Fig. 2b), the lowest RA_{POD} was 46.6%, achieved after HHP processing of 550MPa/16 min/55°C. As can be seen in both figures (1ba and 2b), RA_{POD} decreased as treatment time increased. In addition, it was observed that POD activity in Manila MP was clearly influenced by pressure combined with mild temperature (55°C), with RA_{POD} values of 76.3 - 80.6% at 400 - 450 MPa compared to 46.5% at 550 MPa after 16 min of holding time (fig. 2b). Other authors reported that higher pressure levels (600 MPa) were required to obtain 20 and 40% of RA_{POD} in watermelon (Liu, Zhao, Zou, & Hu, 2013) and kiwi (Fang, Jiang, & Zhang, 2008a) juices, respectively. Furthermore, it can be elucidated that POD in both MP was thermo-sensible, since significant differences among 25 and 55 °C were observed. In agreement to Chakraborty et al.(2014) and Hendrickx et al. (1998), an enhancement in the reduction of RA_{POD} in mango puree treated by HHP processing at 55 °C compared with 25 °C was recognized. In addition it has been reported that HHP process at the level used in

commercial food processing (p > 500 MPa) has in general synergistic effects with temperature on inactivation of enzymes (Ludikhuyze et al., 2003; Terefe et al., 2014).

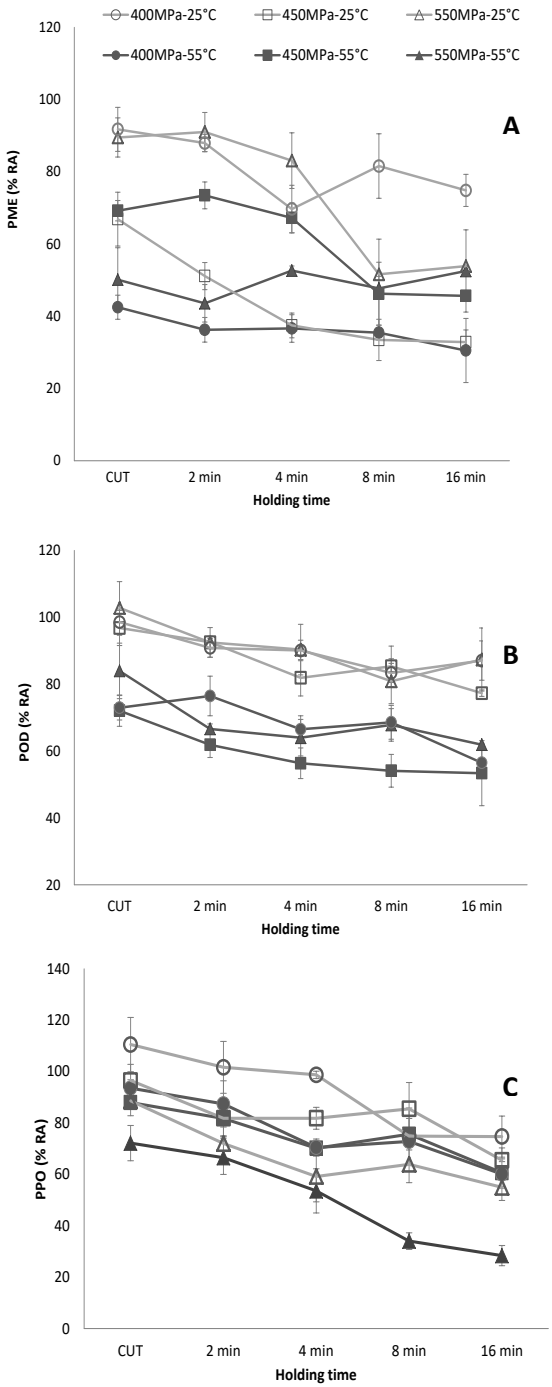


Figure 1: Influence of processing conditions (pressure, temperature and holding time) on A: pectinmethylesterase (PME), B: peroxidase (POD) and C: polyphenoloxidase (PPO) on Tommy Atkins mango pulp.

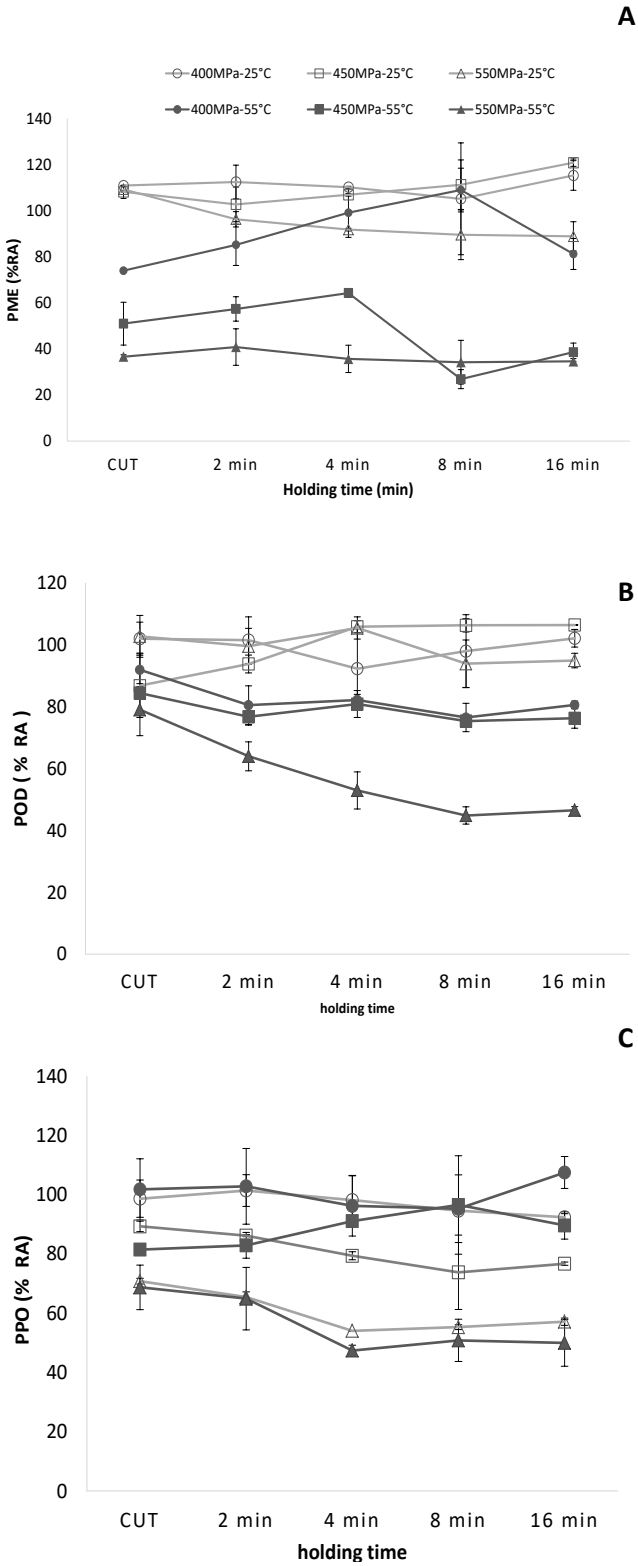


Figure 2: Influence of processing conditions (pressure, temperature and holding time) on A: pectinmethylesterase (PME), B: peroxidase (POD) and C: polyphenoloxidase (PPO) on Manila mango puree.

Otherwise, the lowest RA_{PPO} were 28.4 and 47.4% in Tommy Atkins mango puree (550 MPa/16 min/55 °C) and in Manila mango puree (550 MPa/4 min/55 °C), respectively (fig 1c, 2c). Obtained results indicate that the highest the pressure applied, the better the enzyme inactivation. It is well known that enzymes are proteins in nature with their active sites responsible for the entire biological activity, and thus pressure inactivation of enzymes is possible through the disruption of these active sites (Nath et al., 2016). Just like our results, Guerrero-Beltran et al. (2005) reported that HHP processing (379 – 586 MPa, 0 – 20 min, room temperature) of natural mango puree inhibited the initial PPO activity 26% for all combinations of pressure ant time, highlighting that the higher the treatment time, the higher the inhibition of PPO activity.

As seen in figures 1c and 2c, the effect of temperature and holding time over RA_{PPO} was different in both MP varieties. Firstly, Manila mango puree reached the minimum RA_{PPO} at 4 min of holding time whereas long treatments in Tommy Atkins were needed. Secondly, observed results suggested that PPO enzyme in Tommy Atkins mango puree was more thermo-sensible than Manila variety. The endogenous characteristics could have an interaction with the enzyme bonds (Hendrickx et al., 1998).

It has been reported that HHP process combined with mild heat (150-350MPa/20-30°C) produced significant reduction of POD and PPO at various degrees in tomato puree (Hernandez and Cano, 1998). Likewise, Liu et al. (2012) showed that residual activity of POD and PPO in watermelon juice decreased with treatment time and pressure. These authors observed that at each pressure, the residual activity decreased drastically during the initial stage and slowly after 5 min with increase in treatment time. After HHP treatment of 30 MPa for 60 min, they achieved a PPO and POD residual activity of 12.3% and 57.6%, respectively. In a recent study, Marszalek et al. (2015) evaluated the effect of HHP on PPO and POD in strawberry puree, and observed that processing at 500 MPa combined with 50°C during 15 min was more effective in reducing PPO activity up to 72%. Nonetheless, POD was more resistant to the treatment, achieving only 37% of inactivation. The discrepancy among the

obtained results in this study compared to those observed by other authors could be attributed to the characteristics of the enzyme from different sources and processing parameters applied. According to Swami et al. (2017 HHP4), the extent of inactivation of POD and PPO in various fruit-based products is dependent on the source and treatment medium. Likewise, Guerrero-Beltran et al. (2004 HHP5) elucidate that HHP process can inactivate deteriorative enzymes; however, the extent of such inactivation will depend on pressure, treatment time and the type of enzymes.

The present results confirmed that oxidative enzymes required a threshold pressure and temperature for reduction activity process to begin. Therefore, it could be assumed that the conformation changes caused in the enzymes by the HHP treatment could enable the preservation of colour, which could be deteriorated by the action of oxidative enzymes in mango puree.

3.3. Viscosity

As shown in figure 3 all treated MP had higher viscosity values than the control of Tommy Atkins (792.85 ± 24.8 mPa·s) and Manila (843.3 ± 56.7 mPa·s) varieties. The effect of pressure, holding time and temperature on MP was significantly different between both varieties. On one hand, viscosity values of HHP-treated Tommy Atkins mango puree raised as pressure and holding times increased, regardless of the temperature applied (figure 3a). The maximum viscosity value in treated-Tommy Atkins MP was achieved at 550 MPa/16 min/55 °C (1708.5 ± 67.5 mPa·s). On the other hand, viscosity in Manila mango puree slightly changed after moderate pressurization (400 and 450 MPa) compared to viscosity of control MP (figure 3b). Interestingly, a significant augment in viscosity values was observed in HHP-treated Manila MP at 550 MPa and 55°C from 4 min up to 16 min of holding time (Fig. 3b). Same results have been reported by Liu et al. (2012), who observed that viscosity of watermelon juice increased significantly after HHP treatment. According to them, HHP processing might stimulate protopectin action in pulp, transforming into water-soluble pectin and leading to the increase in viscosity of pressurized products. Also, HHP conducted a homogenization effect to the mango puree, which may trigger two mechanisms enhancing viscosity: firstly, the increase of soluble pectin leached from

cell walls and secondly, the increment of cell structure compactness (Chakraborty et al., 2014). Although information about mango puree viscosity at moderate pressures (300-400 MPa) is available (Yu et al., 2013), no results have been reported regarding higher pressurization (550 MPa) in mango puree

Other works conducted in apricot juice (Patrignani, Tabanelli, Siroli, Gardini, & Lanciotti, 2013) and carrot puree (Moelants et al., 2012) have demonstrated that there is a relation between enzymatic activity, viscosity and high molecular weight carbohydrates content in fruit. Hence, the residual activity of PME in both MP, Tommy Atkins (30.5 ± 3.8%) and Manila (34.6 ± 1.1 %), might be contributing to the changes in viscosity values after HHP processing.

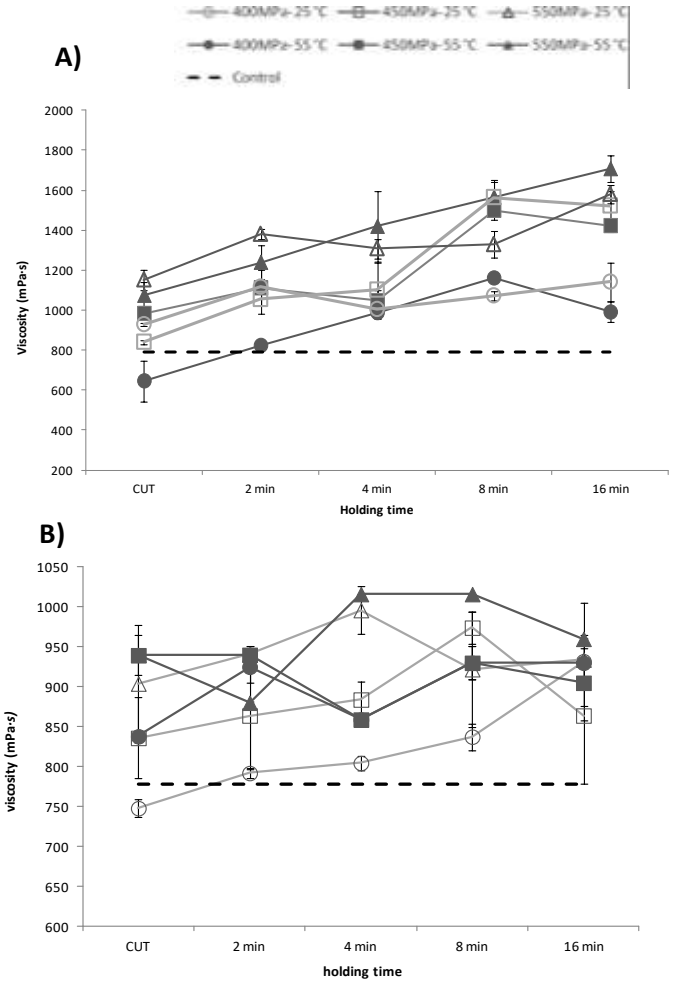


Figure 3: Influence of processing conditions (pressure, temperature and holding time) on viscosity of mango puree cv. Tommy Atkins (A) and Manila (B) compared with control.

3.4. Colour

Lightness (L*) and yellow index (YI) were determined after HP treatments (figure 4). The HP treatments promoted colour changes in both mango puree varieties. Tommy Atkins mango puree changed L* and YI when pressurisation and different temperature conditions were applied but no effect regarding holding times was observed. A significant decrease of L* in HP-treated Tommy Atkins mango puree at 55 °C and 550 MPa was observed comparing with untreated mango puree although no differences were observed among 400-450 MPa at which remained similar to control. Otherwise, there was an increase of L* in all HP-treated Tommy Atkins mango puree at 25 °C compared with untreated while no differences among pressures were perceived. Besides the enzymatic and the pressure contribution to colour changes, the loss of colour as the temperature increases was observed by Kaushik et al. (2014), who attributed the colour difference in mango pulp to the adiabatic compression. YI of HP-treated Tommy Atkins mango puree increased significantly ($p < 0.05$) only when 550 MPa was applied up to 8 min of holding time. The decrease of YI at longest holding time could be attributed to a starting of degradation of antioxidant compounds and the POD activity. Although the present study was not focused on the antioxidant compounds relation with colour parameters, Liu et al. (2013) observed in HP-treated watermelon juice a relation among bioactive compounds content such as carotenoids and lycopene and the colour changes. Therefore, high L* values in Tommy Atkins mango puree could be related with a high content of bioactive compounds.

On the other hand, Manila mango puree treated by HP showed higher L* than control. Nevertheless, no significant differences in mango puree colour regarding treatment temperatures and holding times. Slight differences in L* and YI with respect to processing pressure (400, 450 and 550 MPa) were observed. Likewise, Guerrero-Beltran (2005) observed colour differences in mango puree from 0 to 15 min of treatment using pressures from 300-500 MPa. It must be noted that pressurization may have less effect on colour variation in Manila variety due to less content of oxidative enzymes. PPO is a soluble enzyme predominantly localized in plant cells,

where phenolic compounds are confined to vacuoles and spatially separated from the enzyme. When the cell is disrupted, enzyme and substrate are brought together and phenolic oxidation products are formed, resulting in the formation of brown complexes, which trigger changes in colour.

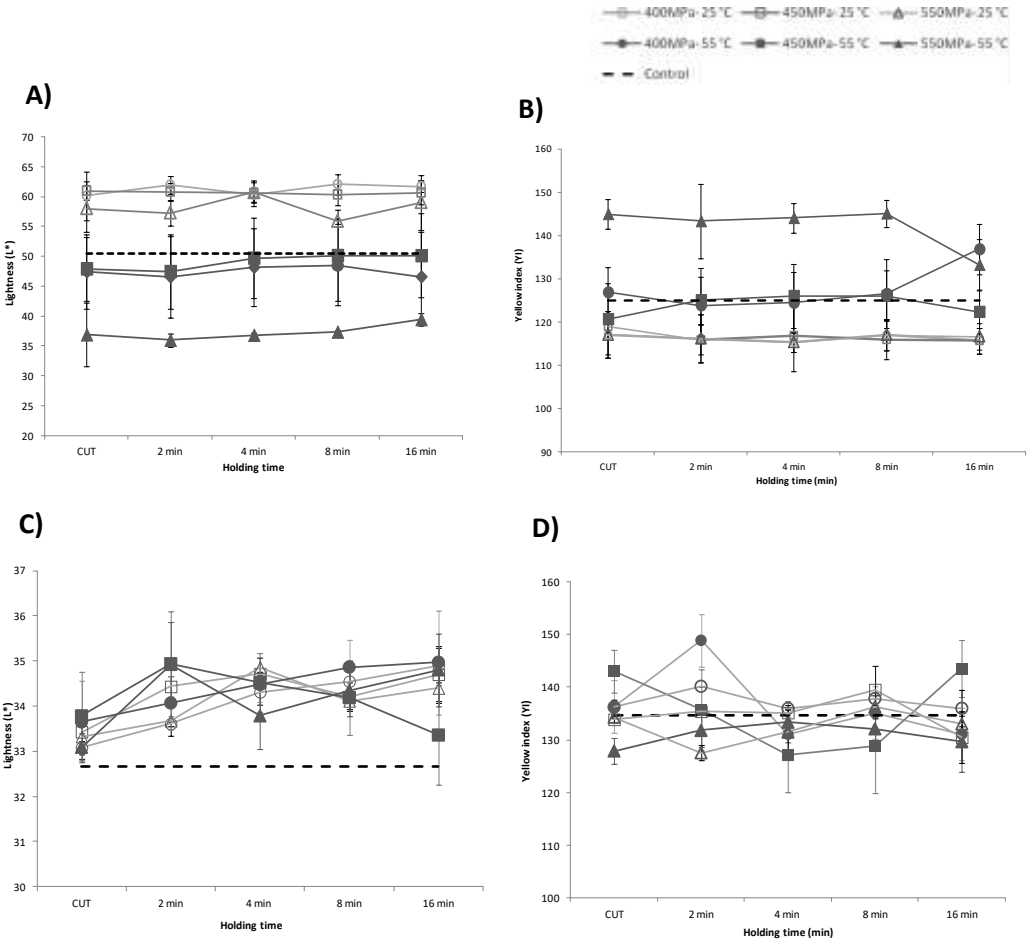


Figure 4: Influence of processing conditions (pressure, temperature and holding time) on lightness (L*) and yellow index (YI) of mango puree cv. Tommy Atkins (a, b) and Manila (c, d) compared with their control.

According to the present results, Ahmed et al. (2005) and (Liu et al. (2013) observed constant colour parameters in mango pulp after HP treatment at low and moderate pressures (100- 400 MPa) without exceeding 40 °C. Carotenoids have been reported to be pressure stable, so the decrease in YI might not have been caused by a decrease

in b* (yellow colour) values, but rather by an increase in L* values of pulp which have resulted from expulsion of air from pulp tissue due to pressurization, thus making it lighter and opaque (Kaushik et al., 2014). Overall pressurization was found to induce moderate enhance in yellowish colour of mango puree, hence preserving its fresh-like appearance.

4. **CONCLUSIONS**

Physicochemical parameters (pH, TSS and a_w) of Tommy Atkins and Manila MP remained with no significant changes after HHP processing at different pressures, temperatures and holding times. Values of PME activity were reduced below 42 % of residual activity in both MP varieties under HHP 400 and 550 MPa and mild temperature. Residual activity of POD decreased as HHP treatment time increase. Furthermore, POD activity in Manila MP was influenced by pressure combined with mild temperature (55°C). Regarding PPO inactivation by HHP treatment, it was observed that the highest the pressure applied in both MP, the better the enzyme inactivation. On the other hand, viscosity of Tommy Atkins mango puree raised as holding time and pressure increased. Similarly, colour parameters (lightness and yellow index) of both mango puree varieties changed after pressurisation but no effect of holding time was observed. Hence, yellowish in HP-treated mango purees after pressurization at 450 or 550 MPa were higher than those untreated. Then, it can be assumed that HHP treatment at 450 MPa and mild temperature constitutes an effective technology to decrease the activity of deleterious enzymes in mango puree maintaining fresh-like quality and improving yellowish. Nevertheless, further research during storage of HHP-treated mango purees is required in order to better understand the effects of HHP on quality parameters of MP.

5. REFERENCES

Ahmed, J., Ramaswamy, H. S., & Hiremath, N. (2005). The effect of high pressure treatment on rheological characteristics and colour of mango pulp. International Journal of Food Science and Technology, 40(8), 885–895.

Bayindirli, A. (2010). Enzymes in Fruit and Vegetable Processing: Chemistry and Engineering Applications.

Chakraborty, S., Kaushik, N., Rao, P. S., & Mishra, H. N. (2014). High-Pressure Inactivation of Enzymes: A Review on Its Recent Applications on Fruit Purees and Juices. Comprehensive Reviews in Food Science and Food Safety, 13(4), 578–596.

Elez-Martínez, P., Aguiló-Aguayo, I., & Martín-Belloso, O. (2006). Inactivation of orange juice peroxidase by high-intensity pulsed electric fields as influenced by process parameters. Journal of the Science of Food and Agriculture, 86(1), 71–81.

Fang, L., Jiang, B., & Zhang, T. (2008). Effect of combined high pressure and thermal treatment on kiwifruit peroxidase. Food Chemistry, 109(4), 802–807.

FAO. (2003). Tropical fruits.

FAO. (2012). FAOSTAT.

Gonzalez, M. E., & Barrett, D. M. (2010). Thermal, high pressure, and electric field processing effects on plant cell membrane integrity and relevance to fruit and vegetable quality. Journal of Food Science, 75(7), R121–30.

González-Cebrino, F., Durán, R., Delgado-Adámez, J., Contador, R., & Ramírez, R. (2013). Changes after high-pressure processing on physicochemical parameters, bioactive compounds, and polyphenol oxidase activity of red flesh and peel plum purée. Innovative Food Science & Emerging Technologies, 20, 34–41.

Guerrero-Beltran, J. A. (2005). High Hydrostatic Pressure Processing of Mango Puree Containing Antibrowning Agents. Food Science and Technology International, 11(4), 261–267.

Hendrickx, M. E., & Knorr, D. (2001). Ultra High Pressure Treatment of Foods.

Hendrickx, M., Ludikhuyze, L., Van den Broeck, I., & Weemaes, C. (1998). Effects of high pressure on enzymes related to food quality. Trends in Food Science & Technology, 9(5), 197–203.

Hiremath, N. D., & Ramaswamy, H. S. (2012). High-pressure destruction kinetics of spoilage and pathogenic microorganisms in mango juice. Journal of Food Processing and Preservation, 36(2), 113–125.

Jamsazzadeh Kermani, Z., Shpigelman, A., Houben, K., ten Geuzendam, B., Van Loey, A. M., & Hendrickx, M. E. (2015). Study of mango endogenous pectinases as a tool to engineer mango purée consistency. Food Chemistry, 172, 272–282.

Kaushik, N., Kaur, B. P., Rao, P. S., & Mishra, H. N. (2014). Effect of high pressure processing on color, biochemical and microbiological characteristics of mango pulp (Mangifera indica cv. Amrapali). Innovative Food Science & Emerging Technologies, 22, 40–50.

Kaushik, N., Nadella, T., & Rao, P. S. (2015). Impact of pH and Total Soluble Solids on Enzyme Inactivation Kinetics during High Pressure Processing of Mango (Mangifera indica) Pulp. Journal of Food Science, 80(11), E2459–70.

Kimball, D. A. (1991). Citrus processing: quality control and technology.

Kubo, M. T. K., Augusto, P. E. D., & Cristianini, M. (2013). Effect of high pressure homogenization (HPH) on the physical stability of tomato juice. Food Research International, 51(1), 170–179.

Landl, A., Abadias, M., Sárraga, C., Viñas, I., & Picouet, P. A. (2010). Effect of high pressure processing on the quality of acidified Granny Smith apple purée product. Innovative Food Science & Emerging Technologies, 11(4), 557–564.

Liu, F., Li, R., Wang, Y., Bi, X., & Liao, X. (2014). Effects of high hydrostatic pressure and high-temperature short-time on mango nectars: Changes in microorganisms, acid invertase, 5-hydroxymethylfurfural, sugars, viscosity, and cloud. Innovative Food Science & Emerging Technologies, 22, 22–30.

Liu, F., Wang, Y., Bi, X., Guo, X., Fu, S., & Liao, X. (2012). Comparison of Microbial Inactivation and Rheological Characteristics of Mango Pulp after High

Hydrostatic Pressure Treatment and High Temperature Short Time Treatment. Food and Bioprocess Technology, 6(10), 2675–2684.

Liu, F., Wang, Y., Li, R., Bi, X., & Liao, X. (2013). Effects of high hydrostatic pressure and high temperature short time on antioxidant activity, antioxidant compounds and color of mango nectars. Innovative Food Science & Emerging Technologies.

Liu, Y., Zhao, X. Y., Zou, L., & Hu, X. S. (2013). Effect of high hydrostatic pressure on overall quality parameters of watermelon juice. Food Science and Technology International = Ciencia Y Tecnología de Los Alimentos Internacional, 19(3), 197–207.

Moelants, K. R. N., Jolie, R. P., Palmers, S. K. J., Cardinaels, R., Christiaens, S., Van Buggenhout, S., … Hendrickx, M. E. (2012). The Effects of Process-Induced Pectin Changes on the Viscosity of Carrot and Tomato Sera. Food and Bioprocess Technology, 6(10), 2870–2883.

Moody, A., Marx, G., Swanson, B. G., & Bermúdez-Aguirre, D. (2014). A comprehensive study on the inactivation of Escherichia coli under nonthermal technologies: High hydrostatic pressure, pulsed electric fields and ultrasound. Food Control, 37, 305–314.

Patrignani, F., Tabanelli, G., Siroli, L., Gardini, F., & Lanciotti, R. (2013). Combined effects of high pressure homogenization treatment and citral on microbiological quality of apricot juice. International Journal of Food Microbiology, 160(3), 273–81.

Riahi, E., & Ramaswamy, H. S. (2003). High-pressure processing of apple juice: kinetics of pectin methyl esterase inactivation. Biotechnology Progress, 19(3), 908–14.

Sarkiyayi, S. (2013). Comparative analysis of nutritional and anti nutritional contents of some varieties of mango (Mangifera indica) in Kaduna Metropolis-Nigeria. Research Journal of Applied Sciences, Engineering and Technology.

Saúco, V. G. (2013). Worldwide mango production and market: Current situation and future prospects. Acta Horticulturae, 992, 37–48.

Serment-Moreno, V., Barbosa-Cánovas, G., Torres, J. A., & Welti-Chanes, J. (2014). High-pressure Processing: Kinetic Models for Microbial and Enzyme Inactivation. Food Engineering Reviews, 6(3), 56–88.

Tejada-Ortigoza, V., Escobedo-Avellaneda, Z., Valdez-Fragoso, A., Mújica-Paz, H. & Welti-Chanes, J. (2015) Combined Effect of High Hydrostatic Pressure and Mild Heat Treatments on The Pectin Methylesterase (PME) Inactivation in Comminuted Orange. Journal of the Science of Food and Agriculture. 95 (12), 2438-2444.

Tribst, A. A. L., Franchi, M. A., de Massaguer, P. R., & Cristianini, M. (2011). Quality of mango nectar processed by high-pressure homogenization with optimized heat treatment. Journal of Food Science, 76(2), M106–10.

Valdramidis, V. P., Graham, W. D., Beattie, A., Linton, M., McKay, A., Fearon, A. M., & Patterson, M. F. (2009). Defining the stability interfaces of apple juice: Implications on the optimisation and design of High Hydrostatic Pressure treatment. Innovative Food Science & Emerging Technologies, 10(4), 396–404.

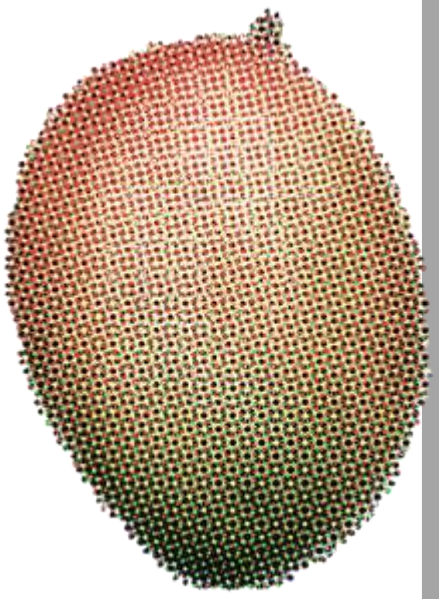
Vásquez-Caicedo, A. L., Schilling, S., Carle, R., & Neidhart, S. (2007). Effects of thermal processing and fruit matrix on β-carotene stability and enzyme inactivation during transformation of mangoes into purée and nectar. Food Chemistry, 102(4), 1172–1186.

Vervoort, L., Van der Plancken, I., Grauwet, T., Timmermans, R. A. H., Mastwijk, H. C., Matser, A. M., … Van Loey, A. (2011). Comparing equivalent thermal, high pressure and pulsed electric field processes for mild pasteurization of orange juice: Part II: Impact on specific chemical and biochemical quality parameters. Innovative Food Science & Emerging Technologies, 12(4), 466–477.

Vivar-Vera, M. A., Salazar-Montoya, J. A., Calva-Calva, G., & Ramos-Ramírez, E. G. (2007). Extraction, thermal stability and kinetic behavior of pectinmethylesterase from hawthorn (Crataegus pubescens) fruit. LWT - Food Science and Technology, 40(2), 278–284.

Welti-Chanes, J., Ochoa-Velasco, C. E., & Guerrero-Beltrán, J. Á. (2009). High-pressure homogenization of orange juice to inactivate pectinmethylesterase. Innovative Food Science & Emerging Technologies, 10(4), 457–462.

Yu, Y., Xiao, G., Wu, J., Xu, Y., Tang, D., Chen, Y., … Fu, M. (2013). Comparing characteristic of banana juices from banana pulp treated by high pressure carbon dioxide and mild heat. Innovative Food Science & Emerging Technologies, 18, 95–100.



5. GENERAL DISCUSSION

5 GENERAL DISCUSSION

The overall aim of the present doctoral thesis was to assess whether mango products treated by non-thermal technologies, could be safe while keeping fresh-like characteristics. Three mango products: fresh-cut, juice and puree were assayed. The following goals were considered in mango products: i) to assure a reduction of microbial population and enzymatic activity; ii) to maintain or increase the content of bioactive compounds; iii) to maintain the physicochemical and sensorial characteristics along the storage. In this section, a comprehensive discussion of the results obtained is presented. Figure 1 summarizes the optimal non-thermal treatments and the proposed phenomena that may explain their effect on obtaining high-quality mango products.

5.1. Edible coatings and pulsed light as non-thermal treatments for processing fresh-cut mango

Polysaccharide-based EC, such as alginate (AL), carboxymethylcellulose (CMC), pectin (PE) and chitosan (CH) are considered feasible coatings for cut fruit preservation. Polysaccharide-based EC can act as a barrier to deleterious reactions in fresh-cut mango. Moreover, fresh-cut fruits can be dipped in organic acids solutions such as malic acid (MA) or ascorbic acid (AA) with antimicrobial or antioxidant function. In addition, pulsed light (PL) treatment is being studied as an alternative to extend shelf-life of fresh-cut fruits. In the current work, the combination of AL, PL and MA as a hurdle technology approach was assayed in order to improve preservation of fresh-cut mango.

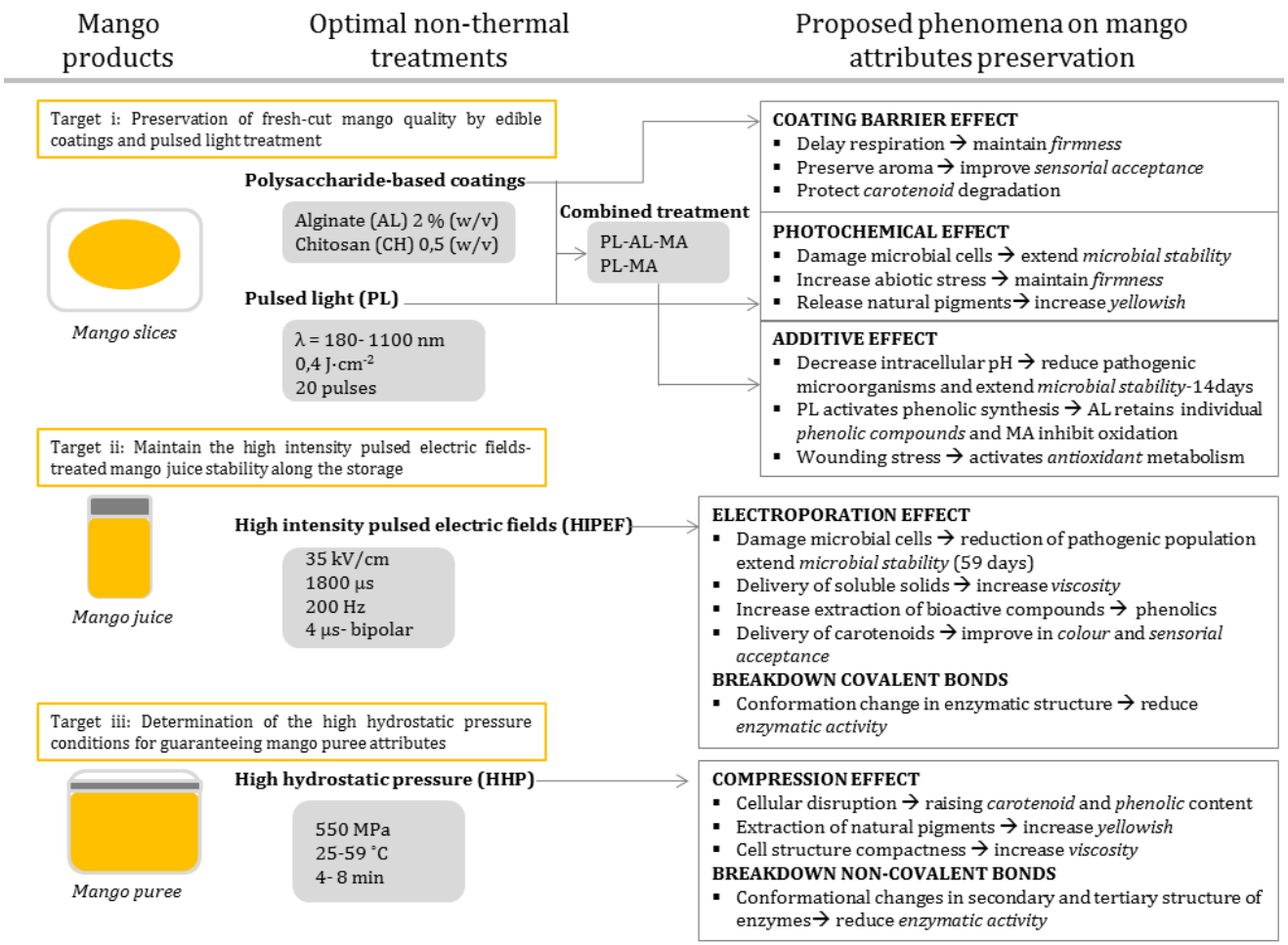


Figure 1: Overview of main findings in the processing of mango products by non-thermal technologies

5.1.1. Reduction of microbial population in fresh-cut mango

Microbial growth

The microbial growth of fresh-cut mango is an important aspect to consider when a preservation treatment is applied. The microbiological stability of mango slices treated by polysaccharide-based coatings, PL or a combination of PL, AL and malic acid (MA) was studied to assure the safety throughout 14 days. The end of the microbiological stability was established according to the microbiological criterion for fresh-cut fruits and vegetables, which must not to exceed 10^6 CFU/g on the expiry date (IFPA, 2003).

In this work, none of coated mango exceeded the microbial load of 10^6 CFU/g neither at the day of processing (day 0) nor at the end of storage (day 14). The use of polysaccharide-based coatings: AL, PE and CH reduced moulds and yeasts and psychrophilic bacteria counts of mango slices compared with uncoated at day of processing. Probably, coatings could be a barrier for microorganisms to reach nutrients as organic compounds. Otherwise, CMC showed similar initial microbial content than uncoated mango slices, hence, CMC might be used as a nutrient for microbial growth.

During storage, all coated mango slices, except CH, showed significant microbial growth. Mango slices coated with CH maintained below 1 log cycles moulds and yeasts and psychrophilic population along the storage. According to other studies, our results denoted the antimicrobial efficiency of CH against moulds and yeasts and bacteria (Chien et al., 2007; Flores-López, Cerqueira, de Rodríguez, & Vicente, 2015; Plotto et al., 2010). The antimicrobial activity of CH was attributed to the polycation nature of the molecule, which enables interaction and formation of polyelectrolyte complex with the acidic polymers produced in the surface of the bacterial cell (Falguera et al., 2011). PE and CMC coatings in mango slices might be used as a nutrient by microorganisms, hence, they created conducive growth conditions for moulds and yeasts and psychrophilic bacteria achieving counts over 4 log cycles at

day 14. On the other hand, microbial population of AL-coated mango slices at the end of storage remained below 4 log cycles. The crosslinking matrix induced by calcium in AL coating may stabilize the cell wall, reduce the oxygen permeability and increment the resistance to microbial growth of mango tissue (Narsaiah et al., 2015). Therefore, the incorporation of MA as antimicrobial in AL coating was assayed in combination with PL to enhance microbial stability of mango slices.

The application of the different treatments individually (PL, AL, MA) or combined (AL-PL, PL-AL, MA-PL, AL-MA, AL-MA-PL and PL-AL-MA) reduced microbial growth. Mango slices treated by PL-AL-MA, exhibited the lowest counts for moulds and yeasts (3.7 logs CFU/g) as well as for psychrophilic bacteria (3.5 logs CFU/g) at the end of the studied period. In this sense, PL application before coating enabled light to more efficiently decontaminate mango surface from microorganisms. The use of MA as antimicrobial in AL coatings was not enough to achieve a reduction greater than those combined with PL. The use of combined treatments showed a defined lag phase in moulds and yeasts from day 0 to day 10 and in psychrophilic bacteria from day 3 to day 14. Differently, no lag phase in microbial growth in AL-coated mango slices was identified. Therefore, microorganisms might easily adapt themselves to growth conditions with the presence of AL rather than PL, which hindered the maturity of microbial cells damaging its DNA (Narsaiah et al., 2015; Raybaudi-Massilia, Mosqueda-Melgar, & Martín-Belloso, 2008b).

Mango slices subjected to PL, AL and MA treatments enabled to reduce the proliferation of microorganism by two main effects. Firstly, the photochemical effect of UV performed by PL and secondly the protective effect of AL coatings on the integrity of damaged fruit tissues limiting the presence of exudates. Differently, Moreira, Tomadoni, Martín-Belloso, & Soliva-Fortuny (2015), who applied gellan-gum coating and PL treatment in apple slices, obtained similar microbial growth in PL-treated apple slices and those after gellan gum and PL. This could indicate an influence of the opacity properties of coating, which may act as barrier for PL.

L. Innocua

L.innocua was selected as the target microorganism to inactive. To assess the effect of AL, MA and PL treatment on *L.innocua* inactivation, a concentration of 10^8 CFU/g was inoculated on mango slices and subsequently treated.

PL had a significant effect on reducing the *L.innocua* population in mango slices. The effect of different wavelengths and number of pulses allowed determining the most PL suitable conditions for a maximum *L.innocua* reduction. On one hand, significant differences ($p< 0.05$) were observed among treatments depending on the wavelength emitted by equipment. The most successful results were obtained using full spectrum (180 - 1100 nm) PL compared with the spectral ranges in which the UV component was removed either partially (λ= 305 – 1100 nm) or completely (λ= 400 – 1100 nm). According with other studies, a higher bactericidal effect of light wavelengths in the range of 250 - 270 nm than those above 305 nm was noticed in mango slices (Guerrero-Beltrán, 2004; Keyser, Műller, Cilliers, Nel, & Gouws, 2008).

On the other hand, inactivation was higher as the pulse number increased. However, no additional inactivation was observed for treatments above 20 full spectrum pulses, when 3.15 log reductions of *L. innocua* population on mango slices was attained. Ramos-Villarroel, Martín-Belloso, & Soliva-Fortuny, (2011) and Ramos-Villarroel, Aron-Maftei, Martín-Belloso, & Soliva-Fortuny (2014) who reported 2.61 and 2.97 log reductions of *L. innocua* population on fresh-cut avocado after treatments of 15 and 30 pulses, respectively. Therefore, PL treatment in mango slices exerted a photochemical effect on the reduction of the *L.innocua* population when UV light was used since an induction of DNA strand breaks and formation of pyramidine dimers could occurred (Guerrero-Beltran, 2004).

According to the results obtained in the present thesis, PL treatment has been reported to play an important role in microbial cell death (Keklik, Demirci, Puri, & Heinemann, 2012; Ramos-Villarroel, Martín-Belloso, & Soliva-Fortuny, 2011). Nonetheless, as a result of the non-complete *L.innocua* log reduction in PL-treated mango slices, the combination with AL coating and MA dipping was assayed in order to assess an added effect between them (Leistner, 2000). The PL, AL and MA preservation treatments applied individually led to a substantial reduction of the initial

counts. MA treatment was more effective than PL and AL, in this order, leading to 2.9, 2.5 and 1.9 log reductions of *L.innocua*, respectively. Beyond the discussed photochemical effect of PL (Ross et al., 2003), the antimicrobial effect of MA has been reported to decrease the intracellular pH by ionization of un-dissociated molecules (Ramos-Villarroel et al., 2014; Rathnayaka, 2013).

Regarding the combined treatments, no synergistic effect but additive was observed. Combining MA and PL a maximum reduction of 4.49 log cycles was achieved. It is noteworthy that the combination of the three preservation factors provided lower inactivation levels, which at the same time depended on the order of the treatments application. Hence, PL-AL-MA and AL-MA-PL treatments led to 3.92 and 3.03 log reductions of the *L. innocua* populations, respectively. Consequently, an antagonistic action of AL coating was observed when it was applied before PL. The coating hindered the effectiveness of PL applied to mango slices due to the blockage of a significant part of the UV-C radiation, causing the reduction of photochemical inactivation due to a shadow effect. Previous study described 3.3 log reductions of *L.innocua* in fresh-cut avocado after PL treatment combined with antibrownings and calcium lactate (Ramos-Villarroel et al., 2011). Although, no description considering the order of application was previously done before, the present thesis suggests that PL treatments should be applied before alginate coating and malic acid treatments.

5.1.2. Physicochemical and sensorial attributes in fresh-cut mango

pH and total soluble solids content

The use of polysaccharide-based EC did not significantly affect initial values of pH and total soluble solids (TSS) of mango slices along 14 days of storage. Usually, it has been reported that quality attributes as pH and TSS of coated fresh-cut fruits remain unaltered (Plotto et al., 2010). Nonetheless, the combination of AL coating with MA dipping and/or PL treatment led to a significant decrease of pH and TSS in all the assays except when mango slices were treated first with PL. On the other hand, MA dips resulted into a reduction of the natural pH of mango. However, this reduction was not considered to play a significant role on quality stability, as the

greatest pH change, as much as 0.36 units, occurred after the PL-AL-MA treatment. The decrease in pH is attributed to the addition of an organic acid (MA). Also, the decrease of pH could be explained by the acidification of the cytoplasm can be promoted by the production of CO_2, which is partially dissolved in the water of the cellular tissues (Ramos-Villarroel et al., 2011). It is remarkable that although an organic acid (ascorbic acid) was incorporated in polysaccharide-based coated mango slices, no decrease in pH values was observed. Ascorbic acid (pKa = 4.1) had higher acid dissociation constant than malic acid (pKa = 1.9), hence, different effect on mango slices tissue acidity occurred. Similarly, Calder, Kash, Davis-Dentici, & Bushway (2011) observed differences between potatoes dipped with sodium acid sufate (pKa = 1.99) and citric acid (pKa = 3.14).

Firmness

Firmness is one of the major attributes affecting sensory quality in fresh-cut fruits. Mango slices suffered mechanical operations such as cutting, which enhances water loss, wound responses and increase in respiration rate. Firmness values in AL-coated mango slices were significantly higher than that of other coated mango slices from the beginning of storage (3.4 ± 0.2 N) until day 14 (2.5 ± 0.4 N). AL coating solution, as a linear un-branched homopolymer with carboxylate functional group accommodated calcium in its structure creating a more rigid coating that reduced softening. However, uncoated mango slices were the toughest compared with those coated. Consequently, cell walls of uncoated mango slices may be more compact due to a loss of water and therefore they had higher firmness values along entire storage (Raybaudi-Massilia, Mosqueda-Melgar, & Martín-Belloso, 2008).

The softening in mango slices was not completely avoided by polysaccharide-based coatings. Furthermore, mechanical injury during post-harvest handling may provoke enzymatic hydrolysis of cell wall and promote a loss of firmness (Robles-Sánchez, Islas-Osuna, et al., 2009). Since, only the use of AL coating had firmness close to uncoated mango slices, further combinations with PL were performed. A protective effect of AL and PL treatments against texture loss in mango slices during storage was observed. Moreover, the effect of PL treatment in mango slices might increase

polyamines, which could be related with a limitation of the accessibility to the cell wall of the deleterious enzymes that promote softening (Charles et al., 2013). Along the storage, less softening in mango slices under combined treatments except for PL-AL remained constant until day 14. This suggested that combined treatment might extend the toughest effect of alginate when they are combined in the following order: PL-AL-MA.

Sensorial evaluation

Mango slices with polysacharide-based coatings were well-appreciated as the consumer acceptance ranged from 73.3 to 96.7 %. Particularly, AL and CH-coated mango slices increased significantly the acceptance throughout the storage, while uncoated mango slices decreased. Indeed, taste was the sensory parameter that best correlated with overall acceptance of mango slices ($p < 0.001$). In this sense, the selection of a coating would be taken depending on taste consumer perception. Probably taste could be intensified along the processing of fresh-cut mango since barrier effect of AL coating retains volatile compounds and prevent the decay of sensorial acceptance (Mastromatteo, Mastromatteo, Conte, & Del Nobile, 2011). The handling of fresh-cut mango triggered abiotic stress effects such as synthesis of volatile compounds related with mango flavour such as: terpenes, aldehydes and lactone (Gonzalez-Aguilar, Villa-Rodriguez, Ayala-Zavala, & Yahia, 2010). Aldehydes increased in fruits at harvest whereas they stabilised in ripe fruits indication that the synthesis of these aromas occurred during the first phase of the climacteric process (Joas, Vulcain, Desvignes, Morales, & Léchaudel, 2012). Nevertheless, coated mango slices may delay the ripening stage and therefore aldehydes content may be greater than those uncoated at day 14. Also, alcohol and terpenes content was low at harvest and then increased during ripening, particularly for well-exposed fruits to abiotic stress (Lum, Shelp, DeEll, & Bozzo, 2016). Therefore, wounding and postharvest treatments could preserve and even enhance the taste of fresh-cut mango.

5.1.3. Bioactive compounds and colorimetric parameters in fresh-cut mango

The stability of bioactive compounds such as phenolics and carotenoids lead to a more nutritive product but also enable the preservation of colour, which is one of the critical attributes for consumer acceptance.

Total phenolic compounds of coated mango slices were kept as fresh-like at day 14. The use of coatings may accelerate the metabolism of phenolic compounds due to the wounding stress in the processing which activates phenylpropanoid pathways (Becerra-Moreno et al., 2015). Differently, total phenolic content of mango slices declined from day 0 to day 7 when combined treatments were used. The main phenolic compounds identified in untreated and combined-treated mango slices just after processing: phenolic acids (gallic, dehidroxibenzoic and chlorogenic), xanthones (mangiferin) and flavonoids (quercetin); were preserve by combined treatments. The changes observed on the phenolic profile of PL-AL-MA- treated mango slices, which includes mangiferin and quercetin, could be due to some of the following reasons: i) Metabolic reactions due to abiotic stress which trigger to activate the antioxidant system of plant cell and phenolic synthesis pathways. ii) Pulsed light effect on cell membranes and in releasing of phenolic compounds. iii) Reduction of enzymatic activity specially polyphenoloxidase and phenilamino lyase by promoting their denaturalization (Buckow et al., 2013; Lopes et al., 2016b).

It must be noted that gallic acid concentration in mango slices treated by PL-AL-MA presented three fold higher concentration at day 14 compared with day of processing. The biosynthesis of mangiferin and quercetin may increase mainly because of pulsed light, which explained the absence of chlorogenic, mangiferin and quercetin in AL-treated mango slices from day 7 to 14 (Agati et al., 2011). Mangiferin and quercetin, which were positive correlated with a* and lightness, contributed to avoid browning colour. Indeed, among individual phenolic compounds negative correlation with browning was observed in quercetin compared with gallic acid. PL-AL-MA-treated mango slices, which b* value (56.1) was the highest among combined treatments, might stimulate higher production of photoprotective phenolic compounds. Therefore,

three combined treatments should induce an added stressful due to damage caused by wounding in plant tissues (Pataro, Donsi, et al., 2015).

On the other hand, AL, PE and CH coatings promoted the carotenoid biosynthesis and the antioxidant capacity in mango slices as they increased the content after 14 days at 4 ˚C. The changes along the storage could be attributed to carotenoid metabolism. Also, Chiumarelli & Pereira (2010) described that chlorophyll decomposition raises in fresh-cut mango and a transformation of chloroplasts into chromoplasts may occur, implying the carotenoids formation, during storage. In that sense, coatings could protect carotenoids release acting as barrier for light as well as having low oxygen permeability (Silva-Weiss et al., 2013).

For the most part, in coated mango slices, except in those with CH coating, the loss of yellow colour was avoided mainly by two mechanisms. Firstly, the oxygen barrier properties of coatings kept away from oxidative deleterious reactions (Espitia et al., 2014; Paula et al., 2015). Secondly, natural antioxidant compounds of mango slices were kept over 30 % of residual scavenging properties using coatings. Normally, the antioxidant capacity of fresh-cut fruits is related to the composition and concentration of bioactive compounds such as phenols and carotenoids (Gülçin, 2012). The presence of antioxidant compounds has been previously described to enable lesser browning in fruits tissue in coated fresh-cut fruits (Siddiq et al., 2013) In that sense, the present thesis indicated that pulsed light had a key role avoiding browning. Pulsed light might stimulate antioxidant and phenylpropanoid metabolism and alginate avoided the contact with oxygen. Furthermore, combined treatments achieved to kept mango slices with fresh-like colour and phenolic compounds. So, our results confirmed that although the minimally processing of fruits accelerates the senescence of fresh-cut tissues, selecting adequate coatings might protect fresh-cut mango against the oxidative reactions and be useful to maintain the total carotenoid content.

5.2. High intensity pulsed electric fields treatment as non-thermal processing for mango juice

High intensity pulsed electric fields (HIPEF) can be used to process liquid foods such as juices. The treatment time conditions for mango juice processing were first stablished in order to assure microbiological safety, low enzymatic activity and fresh-like physicochemical attributes of mango juice was required. Subsequently, HIPEF-treated mango juice stability along 75 days was observed and compared with thermal treatment.

5.2.1. HIPEF treatment time in mango juice processing

Setting the adequate HIPEF treatment conditions such as treatment time to obtain a safe product is required. HIPEF-treated mango juice reached the FDA requirement (at least 5 log reductions of target microorganisms) for *L.innocua* safe fruit beverages at treatment time of 800 µs. The longer is the HIPEF (35 kV/ cm, 200 Hz, 4µs) treatment time up to 800 µs, the higher the decrease of *L.innocua* population.

Obtained results indicated a damage of *L.innocua* cells in HIPEF-treated mango juice that could be attributed to different mechanisms. On one hand, the increase of treatment time enabled to intensify the dielectric effect on mango juice. Consequently, the formation of pores in cell membranes could conduct their rupture performing electroporation effect. The reversible or irreversible cell rupture may depends on other HIPEF treatment parameters as treatment time or electrical field (Vega-Mercado et al., 1997). The inactivation of microorganism did not follow a linear trend but there was a point along the treatment time where the microbial reductions become gradual. This effect could be due to the accumulation of dead cells in mango juice during the processing. Thus, the difficulty to inactivate the survivor microorganisms by electric fields increases. In this sense, treatment time is an important parameter that should be well controlled during HIPEF treatment in order to avoid over-processing and possibly save energy costs. On the other hand, intrinsic

factors of mango juice as conductivity and pH might have a contribution to the inactivation levels achieved in mango juice. Since fresh mango juice had a low pH (3.67) and conductivity (1.54 mS/cm) 5 log reductions of *L.innocua* were achieved at low HIPEF treatment times. Probably, a low ionic concentration cause an increase of the treatment chamber resistance, which could enhance the microbial inactivation (Amiali et al., 2006).

Oxidative (peroxidase, polyphenoloxidase and lipoxigenase) and pectinolitic (pectinmethylesterase) enzymatic activities in mango juice treated by HIPEF reduced their activity as treatment time increased (Appendix I). Polygalacturonase (PG) was activated from 100 μs whereas pectinmethylesterase (PME) was sensible to HIPEF treatment decreasing its activity up to 20 % of residual activity at 1800 μs. Differently, peroxidase (POD), polyphenoloxidase (PPO) and lipoxygenase (LOX) after HIPEF treatment at 2000 μs was 60, 45 and 10%, respectively. Divergence among results could be attributed to the initial contents of enzyme and substrate in different juices and to the sensitivity of enzymes facing pulsed electric fields (Buckow et al., 2013). The native structure of the enzymatic active site and the conformation of the enzymatic protein might change at 1800 μs, when significant differences in residual activities were found. HIPEF could cause a protein unfolding and denaturation, breakdown of covalent bonds and oxidation reduction action (Luo et al., 2010).The HIPEF conditions to reduce enzymatic activity are more severe than those for microorganism inactivation. Other authors have suggested that depending on the isoforms and the resistant fraction of the structure the enzymatic reduction could be reversible (Ingrid Aguiló-Aguayo, Soliva-Fortuny, & Martín-Belloso, 2009).

On the other hand, the electrocompressive forces inducing membrane pores in HIPEF-treated mango juice as increased treatment time might explain the physicochemical changes. Although no effect of rising HIPEF treatment time on TSS content or viscosity of mango juice was observed, HIPEF-treated presented higher values than non-treated. The breakdown cell effect releasing soluble solids from the cell contributed to change viscosity of mango juice. At treatment times of 1800 μs, colour parameters indicating yellow (b^*) and lightness (L^*) of HIPEF-treated mango juice increased compared with untreated. Confirming our results, other authors

observed colour enhancement in HIPEF-treated orange (Cortés et al., 2008) and carrot juice (Luciano J. Quitão-Teixeira et al., 2007). Therefore, HIPEF treatment may reduce the synthesis of browning compounds. The pore formation in cell membrane could trigger the release bioactive compounds. In this sense, the content of carotenoids, which are considered a natural pigment in mango, in HIPEF-treated mango juice may increase as treatment time. Various biochemical reactions as hydroxylation, methylation, isoprenylation, dimerization and/or glucosilation could occur among them and with other components (e.g. enzymes or sugars) of the mango juice when subjected to stress conditions such as HIPEF (Oms-Oliu et al., 2010). These reactions may lead a subsequently degradation of colour whether antioxidant compounds would decrease in further storage.

5.2.2. HIPEF-treated mango juice during storage

The feasibility of HIPEF treatment (35 kV/ cm, 1800 μs, 200 Hz and 4 μs of width in bipolar mode) in preservation of quality attributes in mango juice along 75 days was studied and compared with thermal treatment having untreated juice as reference.

5.2.2.1. Microbial growth

Untreated mango juice had an initial population of moulds and yeasts (4.2 logs CFU/mL) higher than that of psychrophilic bacteria (1.7 logs CFU/mL). The predominance of moulds and yeasts in this kind of products might be attributed to the low pH and high level of sugar. The application of HIPEF or thermal treatments effectively maintained microbial loads of the juice below the detection limit just after processing (day 0). A high temperature damages cell membrane fluidity and stability leading the microbial death more than HIPEF. Consequently, heat treated mango juice extended microbial stability throughout 75 days whereas HIPEF reached 6 logs CFU/mL, which is established as the upper safety limit, at day 59 (Salvia-Trujillo, Morales-de la Peña, Rojas-Graü, & Martín-Belloso, 2011).

Considering that mango juice never exceeds 40 ˚C during the HIPEF processing, the microbial load reductions observed in HIPEF treated juice were only attributed to the

effects of the pulsed electric fields. The mechanism of microbiological inactivation by HIPEF is the electroporation of the cell membranes, which is described as the pore formation of the membrane causing the loss of its functionality and leakage of intracellular content and, depending on the intensity, this effect could be reversible or irreversible (Wouters et al., 1999). The observed microbial growth during storage of HIPEF-treated mango juice might be attributed to the recovery of the injured microorganisms. In this sense, remaining cells could have the ability to adapt to the applied stress and slight damaged cells might recover the membrane structure affected by electroporation (Timmermans et al., 2014b)

5.2.2.2. Enzymatic activity

Oxidative enzymes have been identified as deleterious enzymes of fruit juices. In addition, their presence in fruit juices might generate rancid off-flavours and loss of juice consistence (Hossain, Rana, Kimura, & Roslan, 2014). Hence, the activity of oxidative enzymes should be reduced in order to avoid loss of quality attributes. Both thermal and HIPEF treatment reduced POD, PPO and LOX activities of mango juice, whereas that untreated increased enzymatic activity throughout storage.

HIPEF treatment for 1800 μs reduced the initial enzymatic activity after the processing but also along storage. The principal mechanisms of enzyme inactivation by HIPEF treatment is based on denaturation of the enzyme by the breakdown of covalent bonds. Nevertheless, other authors suggested that changes in enzymatic conformation lead to avoid the substrate from reaching the active site, hence preventing the conversion of substrates into products (Quitão-Teixeira, Odriozola-Serrano, Soliva-Fortuny, Mota-Ramos, & Martín-Belloso, 2009). Also, the hypothesis for reducing enzymatic activity throughout storage would be the formation of aggregates as a result of a strong polarization of the protein molecules and hydrophobic interactions or covalent bonds (Aguiló-Aguayo, Soliva-Fortuny, & Martín-Belloso, 2007).

The molecular structure of enzymes contributed to explain differences in reduction of residual activity. The PPO and POD enzymes has been reported to be specially

affected by pH, temperature and electric fields but only changes on α-helical structure occurred, whereas, LOX easily changes its tertiary structure (Luo et al., 2010). Probably, the presence of a prosthetic group in PPO and POD structure could explain the difficulty to conduct changes in tertiary structure. During storage, a severe increase of RA_{POD} in non-treated MJ was observed, whereas PPO and LOX activities were slightly reduced. Probably, the increase of POD activity might be assigned to the cell release of POD substrate (organic hydroperoxides), which enable the enzyme-subtrate contact (Vervoort et al., 2011). Consistently, literature has reported that POD seemed to be more susceptible to HIPEF than other enzymes and is associated with the modification of the α-helix structure (Leong & Oey, 2014).

5.2.2.3. Physicochemical changes and sensorial aspects

HIPEF or heat processing did not significantly affect initial values of pH and total soluble solids of mango juice; nonetheless differences in colour of mango juice were observed between treatments along the storage. Also, the mango juice sensorial evaluation at day of processing indicated highly valued colour in treated mango juice compared with untreated. However, no differences in taste and overall acceptance of treated and untreated mango juice was reported. In agreement with other authors, HIPEF treatment can enhance colour while preserving pH and soluble solids (Zhang et al., 2010). Probably, physicochemical attributes remain unchanged since no temperature was applied in HIPEF treatment (Santhirasegaram et al., 2015).

During storage, lightness and b* value decreased in HIPEF-treated mango juice. The formation of dark colour compounds, which is commonly described in fruit beverages, is attributed to browning reactions, ascorbic acid oxidation or phenolase browning (Ebiloma, Arobgba, & Aminu, 2011). The loss of yellow colour was related with the content of oxidative enzymes in mango. It is known that PPO activity uses phenolic compounds for the oxidative processes to trigger on quinones, which are responsible of brown pigmentation (Cheema & Sommerhalter, 2015). Thermal and HIPEF treatments can reduce the action of oxidative enzymes preserving colour attributes. No differences in overall acceptance and taste between treated and non-treated mango juice was observed. As it has also been observed in previous

experiments, no differences in taste and overall acceptance comparing fruit juices treated by HIPEF and thermal treatments (Mosqueda-Melgar et al., 2012).

5.2.2.4. Bioactive compounds and antioxidant capacity

Carotenoids

Carotenoids are responsible for the colour of the fruit products due to the content of provitamin A (e.g. α-carotene, β-carotene and β-cryptoxanthin) and also they can contribute to the antioxidant capacity (e.g. β-carotene and β-cryptoxanthin, zeaxanthin and lutein) (Gülçin, 2012).

Total carotenoid concentration of HIPEF and thermal treated mango juice showed a decrease of 17 and 13 %, respectively, compared with non-treated MJ at the beginning of the storage. However, HIPEF-treated mango juice content of carotenoids was significantly higher than thermal treatments. Because of their highly saturated conformation, carotenoids are prone to oxidation and isomerization during processing and storage (Vervoort et al., 2011). These phenomena may explain the gradual loss of yellow colour of HIPEF-treated mango juice along the storage. Exposure to oxygen in head-space of the packaging could predominantly leads to the formation of the cis-isomer. In most carotenoid-containing fruits, ripening is accompanied by enhanced carotenoid biosynthesis (Humayoun Akhtar & Bryan, 2008).

Phenolic compounds

Phenolic compounds are strongly influenced by the maturity stage, growing areas, variety and storage conditions. The polyphenols degrade, oxidise or polymerise quickly during processing and storage of fruit products. Therefore, total phenolic content could be one of the most important indicators of the quality in fruit juices (Y. Chen et al., 2013). Phenolic compounds of mango juice varied from 560.1 ± 17.9 (non-treated) to 333.8 ± 27.8 (HIPEF-treated) and 529.6 ± 15.4 (TT) μg of gallic acid/ mL at day 0. Thermal processing in mango juice caused complex physical and chemical reactions affecting the phenolic composition, including the release of

phenolic compounds from their bonded forms, the degradation of polyphenols and the breakdown and transformation of phenolics. Similarly to Santhirasegaram, Razali, George, & Somasundram, (2015), no significant difference in the phenolics concentration after thermal treatment compared with non-treated MJ was observed at day of processing. The interaction with other compounds creating aggregations resulting from the high electric field and long treatment time applied might reduce the detection of initial content of phenolic compounds (Soliva-Fortuny et al., 2009).

Polyphenols are formed in plant products via the action of phenylalanine ammonia-lyase (PAL) in the phenylpropanoid metabolism. It could be hypothesized that HIPEF induced stress and increased PAL activity, thus enhancing phenolics content. This stress response is initiated when the plant recognizes a stimulus at the cellular level. On the other hand, the loss of phenolic compounds in non-treated fruit juice was also observed by Patthamakanokporn, Puwastien, Nitithamyong, & Sirichakwal (2008) and Policegoudra & Aradhya (2007), who attributed the decrease of phenolics during the storage to deleterious enzymes such as PPO. A positive association between the increase activity of PPO and the loss of phenolics was observed. This result seems to indicate that lowering PPO activity, which uses phenolic compounds for the oxidative processes to trigger on quinones, was mainly associated with increasing in phenolics in HIPEF-treated mango juice (Cheema & Sommerhalter, 2015).

Antioxidant activity

In general, a decrease of antioxidant capacity of mango juice was observed throughout time. Our results for HIPEF-treated mango juice were in accordance with Odriozola-Serrano et al. (2008) who observed a significant loss of antioxidant capacity as storage time increased in HIPEF-treated tomato juice (35 kV/ cm, 100 Hz and 1500 μs of treatment time). However, treated mango juice had antioxidant capacity higher than untreated. HIPEF and thermal treated mango juice decreased a 10 % the antioxidant activity during the first two weeks of storage but then it was maintained up to the end of storage. The antioxidant capacity in HIPEF-treated mango juice might be stimulated by the stress response which could potentially make the extraction of antioxidant compounds, such as phenolics, more efficient. Similar

response in other fruit juices have also been observed (Oms-Oliu, Aguiló-Aguayo, Martín-Belloso, & Soliva-Fortuny, 2010; Soliva-Fortuny et al., 2009). In this sense, antioxidant capacity in mango juice during refrigerated storage could be related to other bioactive compounds such as vitamin C, which could be easily affected by oxidative deleterious reactions (Buckow et al., 2013).

5.3. High hydrostatic pressure treatment as non-thermal processing for mango puree

Application of high hydrostatic pressure (HHP) for food preservation is a growing concern among food scientists and manufacturers. Although bacterial inactivation efficiency of HHP have been already proved to have a direct relation with pressure level, holding time and treatment temperature in mango pulp (Liu et al., 2012) less information on the effect of HHP in enzymatic activity and bioactive compounds of mango puree have been found. This thesis covers the effect of different HHP processing conditions in both oxidative and pectinolitic enzymes, physicochemical attributes and bioactive compounds of mango puree.

5.3.1. Enzymatic activity

The action of enzymes leading to deterioration of sensory aspects in fruit products arise the need to minimize their activity. Mango puree has inherent advantage of being acidic, which can be another potential hurdle for enzymatic inactivation. Pectin methylesterase (PME), which is a pectinolitic enzyme, reduced the activity in HHP-treated mango puree at 550 MPa pressure, temperature of 59 °C and short processing times from come-up-time to 2 min of holding time. Otherwise, peroxidase (POD) enzyme in HHP mango puree decreased residual activity at 550 MPa and 16 min of holding pressure time whether treatment temperature was 59 °C. Polyphenoloxidase

(PPO) was highly affected by pressure and holding pressure time at both treatment temperatures 25 and 59 °C. Both mango puree varieties (Tommy Atkins and Manila) decreased enzymatic activity after pressurisation, especially when holding time and temperature were increased. Similarly, other studies observed a decline in enzymatic activity at long holding times and high pressures (550 MPa) for PME (Bayindirli, 2010) and an enhancement in the reduction of POD activity in mango puree treated by HP processing at 59 °C (Chakraborty et al., 2014). The reduction of enzymatic activity in mango puree only occurs under specific conditions. Some possible explanations might be related with the sensitivity of enzymatic structure to high pressure. Therefore, changes in protein conformational structure, in enzyme-substrate interaction or changes in the cell membrane could occur (Bermúdez-Aguirre & Barbosa-Cánovas, 2010).

The combination of mild temperature and pressure has resulted in the highest inactivation of enzyme (Fang, Jiang, & Zhang, 2008b). Indeed, the effect of HHP processing on enzymatic inactivation could be mainly attributed to the effect of pressure on enzyme stability. An instantaneous disruption or disorientation in enzymatic conformation resulting in reduced activity might happen. Less contribution of the isobaric effect during the holding pressure period on the enzymatic activity reduction was considered (Chakraborty et al., 2014; Kaushik, Nadella, & Rao, 2015).

In some cases, high pressure can modify the microstructure of a food, especially when the food contains entrapped air molecules, causing changes in the tissue. These changes result from damage in the cell structure, deforming the cell walls and releasing cell serum into the medium. Such changes in cell permeability allow the interchange of water and substrate for enzymatic reactions in the microstructure of the product (Chakraborty et al., 2014). The correct combination of pressure and moderate temperature (59 ˚C) must be adequately chosen for baroresistant enzymes like pectin methylesterase (Kaushik et al., 2015).

5.3.2. Physicochemical attributes

Quality characteristic related with fruit-based products are colour, appearance, and texture. These characteristics are negatively affected by most of traditional processing methods currently used in the food industry. Differently, no changes of pH in HHP-treated mango puree were observed. Probably, the less changes in a_w the better preservation of H_20 molecules, hence, scarce ionization and no increase of H^+ occurred. Nevertheless, significant differences ($p< 0.05$) in studied physicochemical attributes between Tommy Atkins and Manila mango were identified. The high composition of organic acid compounds such as vitamins and phenolic acids in Manila cultivar (Sarkiyayi, 2013) could be related with the difference in the results.

HHP is an adequate option for processing mango puree because of the minimal changes in the colour. The HHP processing conditions assed in the present work improved yellow colour of mango puree. Colour changes in mango puree occur because of enzymatic activity, but improvements in yellow colour due to PPO reduction have been reported using HHP. Furthermore, colour preservation of mango puree can be explained by the partial effects of HHP on the covalent bonds and the great retention of carotenoids and phenolic compounds (Ahmed et al., 2005; Kaushik et al., 2014). The effect of HHP on the colour of a food depends on the processing conditions. However, yellow colour of mango puree diminished at 550 MPa and 59 ˚C. One explanation for this effect is that pressurization and mild temperature generate physical stress in the cell triggering the cell permeabilization and, as a result, released yellow pigments might be oxidized conducting to browning.

Tommy Atkins mango puree viscosity was significantly higher than Manila mango puree variety. According to our results, Ahmed et al. (2005) reported different rheological behaviour among varieties of mango pulp. They attributed the differences to the pressure influence on the soluble solids content. Probably, in the present study the variation of viscosity values of each variety after HHP processing could be caused by the different content of high molecular weight carbohydrates, such as sugar and starch. In addition, the activity of endogenous enzymes as PME was reduced in HHP-treated mango puree, hence pectin among other high molecular weight carbohydrates in mango purees suffered less break-down (Jamsazzadeh Kermani et al., 2015). Other recent researches conducted in apricot juice (Patrignani et al., 2013) and carrot puree

(Moelants et al., 2012) have demonstrated that there is a relation between enzymatic activity, viscosity and high molecular weight carbohydrates content in fruit similar to mango.

5.3.3. Bioactive compounds and antioxidant capacity

The content of carotenoids, phenolic compounds, vitamin C and antioxidant capacity was preserved or even enhance after HHP processing (Appendix II). Low processing pressure (400 MPa) exerted no effect on studied bioactive compounds. Indeed, no changes on vitamin C and carotenoids content were identified as increased pressure. However, an increase on the retention of phenolic compounds and antioxidant capacity in mango puree treated by 450 or 550 MPa was observed. The increase could be related to cellular disruption due to HHP. The effect of pressure may facilitate the extraction of bioactive compounds, such as phenolics. Probably, a disruption of weak bounds with pectin chains, covalent bounds or other electrostatic forces could enable the delivery of bioactive compounds after HHP treatment (González-Cebrino, Durán, Delgado-Adámez, Contador, & Ramírez, 2013; Valdramidis et al., 2009). Furthermore, processing is known to change some physicochemical features of phenolic compounds. For instance, several changes in the phenol structure (hydroxylation, methylation, isoprenylation, dimerization, glycosylation, among others) and/or the formation of phenolic derivatives could occur during HHP processing. A recent paper also evidenced the reduced effect of treatments at ambient temperature and the relevant importance of pressure in releasing phenolic compounds (Jiménez-Sánchez, Lozano-Sánchez, Segura-Carretero, & Fernández-Gutiérrez, 2017b).

Temperature of processing only exerted an influence on declining vitamin C content as treatment time increased. Since vitamin C is highly thermo-labile, HHP-treated (59 ˚C) mango puree reduced its content at long holding pressure times. This results are in agreement with Landl, Abadias, Sárraga, Viñas, & Picouet (2010) who observed a loss of vitamin C in HHP-treated apple juice at 600MPa. Also, the vitamin C loss could be easily found in fruit juice because of endogenous pro-oxidants such as metal ions and enzymes (Kaushik et al., 2014). Similarly, antioxidant activity in HHP-

treated mango puree declined when temperature and treatment time increased. Therefore, it was evidenced that unlike the effect of HHP after cell disruption promoting bioactive compounds delivery, temperature treatment at 59˚C adversely affect the antioxidant compounds, such as vitamin C.

5.4. Concluding remarks

This thesis contributed to support the application of EC, PL, MA, HIPEF or HHP treatments as feasible processing to obtain high-quality mango products. Mango products such as fresh-cut, juice and puree products treated by non-thermal technologies maintain its natural attributes along the storage. The results of this work showed the added effect of surface treatments (EC, PL and MA) on fresh-cut mango to assure microbial safety and phenolic content along the storage. With regards to HIPEF-treated mango juice, the longest storage stability is still attributed to thermal treatments but greater liberation of phenolic compounds under HIPEF treatment. Similarly, HHP treatments combined with moderate temperature enhanced the content of phenolic compounds and antioxidant capacity. The great content on phenolic compounds obtained after some processing conditions could be attributed to cellular disruption causing better extraction for quantification, which is important because it could increase their bioavailability in the human body. The fact that sensory perception of fresh-cut mango and mango juice under non-thermal treatments was maintained or enhanced in comparison to that of untreated demonstrated the preservation potential of non-thermal processing.

This thesis reveals that through non-thermal treatments high-quality mango products can be obtained. In that sense, the application on industrial scale is a still a challenge. Considering the outcomes of the present work, further research on the environmental impact of non-thermal technologies as green energy or the influence of processing on bioavailability of health-related compounds could be useful to reinforce the development of non-thermla processing in food industry. Thereby, the consumers would have access to great nutritive fruit products.

5.5.References

Agati, G., Biricolti, S., Guidi, L., Ferrini, F., Fini, A., & Tattini, M. (2011). The biosynthesis of flavonoids is enhanced similarly by UV radiation and root zone salinity in L. vulgare leaves. Journal of Plant Physiology, 168(3), 204–12.

Aguiló-Aguayo, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2007). Comparative study on color, viscosity and related enzymes of tomato juice treated by high-intensity pulsed electric fields or heat. European Food Research and Technology, 227(2), 599–606.

Aguiló-Aguayo, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2009). Changes in viscosity and pectolytic enzymes of tomato and strawberry juices processed by high-intensity pulsed electric fields. International Journal of Food Science & Technology, 44(11), 2268–2277.

Ahmed, J., Ramaswamy, H. S., & Hiremath, N. (2005). The effect of high pressure treatment on rheological characteristics and colour of mango pulp. International Journal of Food Science and Technology, 40(8), 885–895.

Amiali, M., Ngadi, M. O., Raghavan, V. G. S., & Nguyen, D. H. (2006). Electrical Conductivities of Liquid Egg Products and Fruit Juices Exposed to High Pulsed Electric Fields. International Journal of Food Properties, 9(3), 533–540.

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

Bermúdez-Aguirre, D., & Barbosa-Cánovas, G. V. (2010). An Update on High Hydrostatic Pressure, from the Laboratory to Industrial Applications. Food Engineering Reviews, 3(1), 44–61.

Buckow, R., Ng, S., & Toepfl, S. (2013). Pulsed electric field processing of orange juice: A review on microbial, enzymatic, nutritional, and sensory quality and stability, 12(5), 455–467.

Calder, B. L., Kash, E. A., Davis-Dentici, K., & Bushway, A. A. (2011). Comparison of sodium acid sulfate to citric acid to inhibit browning of fresh-cut potatoes. Journal of Food Science, 76(3), S164-9. http://doi.org/10.1111/j.1750-3841.2011.02082.x

Chakraborty, S., Kaushik, N., Rao, P. S., & Mishra, H. N. (2014). High-Pressure Inactivation of Enzymes: A Review on Its Recent Applications on Fruit Purees and Juices. Comprehensive Reviews in Food Science and Food Safety, 13(4), 578–596.

Charles, F., Vidal, V., Olive, F., Filgueiras, H., & Sallanon, H. (2013). Pulsed light treatment as new method to maintain physical and nutritional quality of fresh-cut mangoes. Innovative Food Science & Emerging Technologies, 18, 190–195.

Cheema, S., & Sommerhalter, M. (2015). Characterization of polyphenol oxidase activity in Ataulfo mango. Food Chemistry, 171, 382–7.

Chen, Y., Yu, L. J., & Rupasinghe, H. P. V. (2013). Effect of thermal and non-thermal pasteurisation on the microbial inactivation and phenolic degradation in fruit juice: a mini-review. Journal of the Science of Food and Agriculture, 93(5), 981–6.

Chien, P.-J., Sheu, F., & Yang, F.-H. (2007). Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. Journal of Food Engineering, 78(1), 225–229.

Chiumarelli, M., & Pereira, L. (2010). Cassava Starch Coating and Citric Acid to Preserve Quality Parameters of Fresh-Cut "Tommy Atkins" Mango. Journal of Food Science.

Cortés, C., Esteve, M. J., & Frígola, A. (2008). Color of orange juice treated by High Intensity Pulsed Electric Fields during refrigerated storage and comparison with pasteurized juice. Food Control, 19(2), 151–158.

Ebiloma, U. G., Arobgba, S. S., & Aminu, O. R. (2011). Some Activities of Peroxidase from Mango (Mangifera indica L. Var. Mapulehu) Kernel. International Journal of Biological Chemistry, 5(3), 200–206.

Espitia, P. J. P., Du, W.-X., Avena-Bustillos, R. de J., Soares, N. de F. F., & McHugh, T. H. (2014). Edible films from pectin: Physical-mechanical and antimicrobial properties - A review. Food Hydrocolloids, 35, 287–296.

Falguera, V., Quintero, J. P., Jiménez, A., Muñoz, J. A., & Ibarz, A. (2011). Edible films and coatings: Structures, active functions and trends in their use. Trends in Food Science & Technology, 22(6), 292–303.

Fang, L., Jiang, B., & Zhang, T. (2008). Effect of combined high pressure and thermal treatment on kiwifruit peroxidase. Food Chemistry, 109(4), 802–807.

Flores-López, M. L., Cerqueira, M. A., de Rodríguez, D. J., & Vicente, A. A. (2015). Perspectives on Utilization of Edible Coatings and Nano-laminate Coatings for Extension of Postharvest Storage of Fruits and Vegetables. Food Engineering Reviews. http://doi.org/10.1007/s12393-015-9135-x

Gonzalez-Aguilar, G. A., Villa-Rodriguez, J. A., Ayala-Zavala, J. F., & Yahia, E. M. (2010). Improvement of the antioxidant status of tropical fruits as a secondary response to some postharvest treatments. Trends in Food Science & Technology, 21(10), 475–482.

González-Cebrino, F., Durán, R., Delgado-Adámez, J., Contador, R., & Ramírez, R. (2013). Changes after high-pressure processing on physicochemical parameters, bioactive compounds, and polyphenol oxidase activity of red flesh and peel plum purée. Innovative Food Science & Emerging Technologies, 20, 34–41.

Guerrero-Beltran, J. A. (2004). Advantages and Limitations on Processing Foods by UV Light. Food Science and Technology International, 10(3), 137–147.

Gülçin, İ. (2012). Antioxidant activity of food constituents: an overview. Archives of Toxicology, 86(3), 345–91.

Hossain, M. A., Rana, M. M., Kimura, Y., & Roslan, H. A. (2014). Changes in biochemical characteristics and activities of ripening associated enzymes in mango fruit during the storage at different temperatures. BioMed Research International, 2014, 232969.

Humayoun Akhtar, M., & Bryan, M. (2008). Extraction and quantification of major carotenoids in processed foods and supplements by liquid chromatography. Food Chemistry, 111(1), 255–261. http://doi.org/10.1016/j.foodchem.2008.03.071

Jamsazzadeh Kermani, Z., Shpigelman, A., Houben, K., ten Geuzendam, B., Van Loey, A. M., & Hendrickx, M. E. (2015). Study of mango endogenous pectinases as a tool to engineer mango purée consistency. Food Chemistry, 172, 272–282.

Jiménez-Sánchez, C., Lozano-Sánchez, J., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2017). Alternatives to conventional thermal treatments in fruit-juice processing. Part 2: Effect on composition, phytochemical content, and physicochemical, rheological, and organoleptic properties of fruit juices, 57(3), 637–652.

Kaushik, N., Kaur, B. P., Rao, P. S., & Mishra, H. N. (2014). Effect of high pressure processing on color, biochemical and microbiological characteristics of mango pulp (Mangifera indica cv. Amrapali). Innovative Food Science & Emerging Technologies, 22, 40–50.

Kaushik, N., Nadella, T., & Rao, P. S. (2015). Impact of pH and Total Soluble Solids on Enzyme Inactivation Kinetics during High Pressure Processing of Mango (Mangifera indica) Pulp. Journal of Food Science, 80(11), E2459-70.

Keklik, N. M., Demirci, A., Puri, V. M., & Heinemann, P. H. (2012). Modeling the inactivation of Salmonella Typhimurium, Listeria monocytogenes, and Salmonella Enteritidis on poultry products exposed to pulsed UV light. Journal of Food Protection, 75(2), 281–8.

Keyser, M., Műller, I. A., Cilliers, F. P., Nel, W., & Gouws, P. A. (2008). Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. Innovative Food Science & Emerging Technologies, 9(3), 348–354.

Landl, A., Abadias, M., Sárraga, C., Viñas, I., & Picouet, P. A. (2010). Effect of high pressure processing on the quality of acidified Granny Smith apple purée product. Innovative Food Science & Emerging Technologies, 11(4), 557–564.

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

Leong, S. Y., & Oey, I. (2014). Effect of pulsed electric field treatment on enzyme kinetics and thermostability of endogenous ascorbic acid oxidase in carrots (Daucus carota cv. Nantes), 146, 538–547.

Liu, F., Wang, Y., Bi, X., Guo, X., Fu, S., & Liao, X. (2012). Comparison of Microbial Inactivation and Rheological Characteristics of Mango Pulp after High Hydrostatic Pressure Treatment and High Temperature Short Time Treatment. Food and Bioprocess Technology, 6(10), 2675–2684.

Lopes, M. M. A., Silva, E. O., Canuto, K. M., Silva, L. M. A., Gallão, M. I., Urban, L., … Miranda, M. R. A. (2016). Low fluence pulsed light enhanced phytochemical content and antioxidant potential of "Tommy Atkins" mango peel and pulp. Innovative Food Science & Emerging Technologies, 33, 216–224. http://doi.org/10.1016/j.ifset.2015.12.019

Lum, G. B., Shelp, B. J., DeEll, J. R., & Bozzo, G. G. (2016). Oxidative metabolism is associated with physiological disorders in fruits stored under multiple environmental stresses. Plant Science : An International Journal of Experimental Plant Biology, 245, 143–52. http://doi.org/10.1016/j.plantsci.2016.02.005

Luo, W., Zhang, R. B., Wang, L. M., Chen, J., & Guan, Z. C. (2010). Conformation changes of polyphenol oxidase and lipoxygenase induced by PEF treatment, 40(2), 295–301.

Mastromatteo, M., Mastromatteo, M., Conte, A., & Del Nobile, M. A. (2011). Combined effect of active coating and MAP to prolong the shelf life of minimally processed kiwifruit (Actinidia deliciosa cv. Hayward). Food Research International, 44(5), 1224–1230.

Moelants, K. R. N., Jolie, R. P., Palmers, S. K. J., Cardinaels, R., Christiaens, S., Van Buggenhout, S., … Hendrickx, M. E. (2012). The Effects of Process-Induced Pectin Changes on the Viscosity of Carrot and Tomato Sera. Food and Bioprocess Technology, 6(10), 2870–2883.

Moreira, M. R., Tomadoni, B., Martín-Belloso, B., & Soliva-Fortuny, R. (2015). Preservation of Fresh-cut Apple Quality Attributes by Pulsed Light in Combination with Gellan Gum-Based Prebiotic Edible Coatings. LWT - Food Science and Technology, 64(2), 1130–1137.

Mosqueda-Melgar, J., Raybaudi-Massilia, R. M., & Martín-Belloso, O. (2012). Microbiological shelf life and sensory evaluation of fruit juices treated by high-intensity pulsed electric fields and antimicrobials. Food and Bioproducts Processing, 90(2), 205–214.

Narsaiah, K., Wilson, R. A., Gokul, K., Mandge, H. M., Jha, S. N., Bhadwal, S., … Vij, S. (2015). Effect of bacteriocin-incorporated alginate coating on shelf-life of minimally processed papaya (Carica papaya L.). Postharvest Biology and Technology, 100, 212–218.

Odriozola-Serrano, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2008). Changes of health-related compounds throughout cold storage of tomato juice stabilized by thermal or high intensity pulsed electric field treatments. Innovative Food Science & Emerging Technologies, 9(3), 272–279.

Oms-Oliu, G., Aguiló-Aguayo, I., Martín-Belloso, O., & Soliva-Fortuny, R. (2010). Effects of pulsed light treatments on quality and antioxidant properties of fresh-cut mushrooms (Agaricus bisporus). Postharvest Biology and Technology, 56(3), 216–222.

Oms-Oliu, G., Rojas-Graü, M. A., González, L. A., Varela, P., Soliva-Fortuny, R., Hernando, M. I. H., … Martín-Belloso, O. (2010). Recent approaches using chemical treatments to preserve quality of fresh-cut fruit: A review. Postharvest Biology and Technology, 57(3), 139–148.

Pataro, G., Donsi, G., & Ferrari, G. (2015). Post-harvest UV-C and PL irradiation of fruits and vegetables. Chemical Engineering Transactions, 44, 31–36.

Patrignani, F., Tabanelli, G., Siroli, L., Gardini, F., & Lanciotti, R. (2013). Combined effects of high pressure homogenization treatment and citral on microbiological quality of apricot juice. International Journal of Food Microbiology, 160(3), 273–81.

Patthamakanokporn, O., Puwastien, P., Nitithamyong, A., & Sirichakwal, P. P. (2008). Changes of antioxidant activity and total phenolic compounds during storage of selected fruits. Journal of Food Composition and Analysis, 21(3), 241–248.

Paula, G. A., Benevides, N. M. B., Cunha, A. P., de Oliveira, A. V., Pinto, A. M. B., Morais, J. P. S., & Azeredo, H. M. C. (2015). Development and characterization of edible films from mixtures of κ-carrageenan, ι-carrageenan, and alginate. Food Hydrocolloids, 47, 140–145.

Plotto, A., Narciso, J. A., Rattanapanone, N., & Baldwin, E. A. (2010). Surface treatments and coatings to maintain fresh-cut mango quality in storage. Journal of the Science of Food and Agriculture, 90(13), 2333–41.

Policegoudra, R. S., & Aradhya, S. M. (2007). Biochemical changes and antioxidant activity of mango ginger (Curcuma amada Roxb.) rhizomes during postharvest storage at different temperatures. Postharvest Biology and Technology, 46(2), 189–194.

Quitão-Teixeira, L. J., Aguiló-Aguayo, I., Ramos, A. M., & Martín-Belloso, O. (2007). Inactivation of Oxidative Enzymes by High-Intensity Pulsed Electric Field for Retention of Color in Carrot Juice. Food and Bioprocess Technology, 1(4), 364–373.

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

Ramos-Villarroel, A., Aron-Maftei, N., Martín-Belloso, O., & Soliva-Fortuny, R. (2014). Bacterial inactivation and quality changes of fresh-cut avocados as affected by intense light pulses of specific spectra. International Journal of Food Science & Technology, 49(1), 128–136. http://doi.org/10.1111/ijfs.12284

Ramos-Villarroel, A. Y., Martín-Belloso, O., & Soliva-Fortuny, R. (2011). Bacterial inactivation and quality changes in fresh-cut avocado treated with intense light pulses. European Food Research and Technology, 233(3), 395–402.

Ramos-Villarroel, A. Y., Martín-Belloso, O., & Soliva-Fortuny, R. (2011). Using antibrowning agents to enhance quality and safety of fresh-cut avocado treated with intense light pulses. Journal of Food Science, 76(9), S528-34. http://doi.org/10.1111/j.1750-3841.2011.02410.x

Rathnayaka, R. M. U. S. K. (2013). Antibacterial Effect of Malic Acid Against Listeria monocytogenes, Salmonella enteritidis and Escherichia coli in Mango, Pineapple and Papaya Juices. American Journal of Food Technology, 8(1), 74–82.

Raybaudi-Massilia, R. M., Mosqueda-Melgar, J., & Martín-Belloso, O. (2008b). Edible alginate-based coating as carrier of antimicrobials to improve shelf-life and safety of fresh-cut melon. International Journal of Food Microbiology, 121(3), 313–27.

Raybaudi-Massilia, R. M., Mosqueda-Melgar, J., & Martín-Belloso, O. (2008a). Edible alginate-based coating as carrier of antimicrobials to improve shelf-life and safety of fresh-cut melon. International Journal of Food Microbiology, 121(3), 313–27.

Robles-Sánchez, R. M., Islas-Osuna, M. A., Astiazarán-García, H., Vázquez-Ortiz, F. A., Martín-Belloso, O., Gorinstein, S., & González-Aguilar, G. A. (2009). Quality index, consumer acceptability, bioactive compounds, and antioxidant activity of fresh-cut “ataulfo” mangoes (mangifera indica L.) as affected by low-temperature storage. Journal of Food Science, 74(3), S126-34.

Ross, A. I. V, Griffiths, M. W., Mittal, G. S., & Deeth, H. C. (2003). Combining nonthermal technologies to control foodborne microorganisms. International Journal of Food Microbiology, 89(2–3), 125–138. http://doi.org/10.1016/S0168-1605(03)00161-2

Salvia-Trujillo, L., Morales-de la Peña, M., Rojas-Graü, M. A., & Martín-Belloso, O. (2011). Microbial and enzymatic stability of fruit juice-milk beverages treated by high intensity pulsed electric fields or heat during refrigerated storage. Food Control, 22(10), 1639–1646.

Santhirasegaram, V., Razali, Z., George, D. S., & Somasundram, C. (2015). Effects of Thermal and Non-thermal Processing on Phenolic Compounds, Antioxidant Activity and Sensory Attributes of Chokanan Mango (Mangifera indica L.) Juice. Food and Bioprocess Technology. http://doi.org/10.1007/s11947-015-1576-y

Sarkiyayi, S. (2013). Comparative analysis of nutritional and anti nutritional contents of some varieties of mango (Mangifera indica) in Kaduna Metropolis-Nigeria. Research Journal of Applied Sciences, Engineering and Technology.

Silva-Weiss, A., Ihl, M., Sobral, P. J. A., Gómez-Guillén, M. C., & Bifani, V. (2013). Natural Additives in Bioactive Edible Films and Coatings: Functionality and Applications in Foods. Food Engineering Reviews, 5(4), 200–216. http://doi.org/10.1007/s12393-013-9072-5

Soliva-Fortuny, R., Balasa, A., Knorr, D., & Martín-Belloso, O. (2009). Effects of pulsed electric fields on bioactive compounds in foods: a review. Trends in Food Science and Technology, 20(11–12), 544–556.

Timmermans, R. A. H., Nierop Groot, M. N., Nederhoff, A. L., van Boekel, M. A. J. S., Matser, A. M., & Mastwijk, H. C. (2014). Pulsed electric field processing of different fruit juices: impact of pH and temperature on inactivation of spoilage and pathogenic micro-organisms. International Journal of Food Microbiology, 173, 105–11.

Valdramidis, V. P., Graham, W. D., Beattie, A., Linton, M., McKay, A., Fearon, A. M., & Patterson, M. F. (2009). Defining the stability interfaces of apple juice: Implications on the optimisation and design of High Hydrostatic Pressure treatment. Innovative Food Science & Emerging Technologies, 10(4), 396–404.

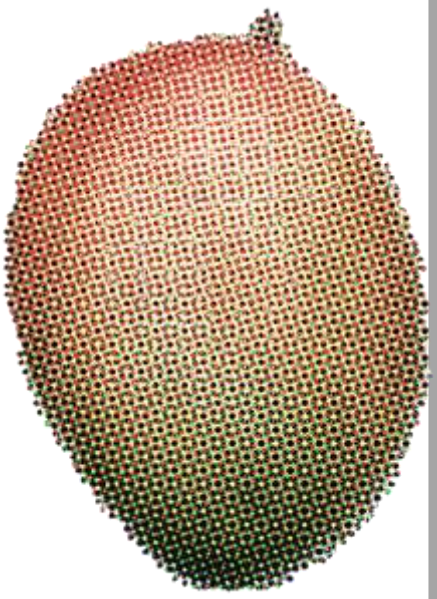
Vega-Mercado, H., Martín-Belloso, O., Qin, B.-L., Chang, F. J., Marcela Góngora-Nieto, M., Barbosa-Cánovas, G. V., & Swanson, B. G. (1997). Non-thermal food preservation: Pulsed electric fields. Trends in Food Science & Technology, 8(5), 151–157.

Vervoort, L., Van der Plancken, I., Grauwet, T., Timmermans, R. A. H., Mastwijk, H. C., Matser, A. M., … Van Loey, A. (2011). Comparing equivalent thermal, high

pressure and pulsed electric field processes for mild pasteurization of orange juice: Part II: Impact on specific chemical and biochemical quality parameters. Innovative Food Science & Emerging Technologies, 12(4), 466–477.

Wouters, P. C., Dutreux, N., Smelt, J. P. P. M., & Lelieveld, H. L. M. (1999). Effects of pulsed electric fields on inactivation kinetics of Listeria innocua. Applied and Environmental Microbiology, 65(12), 5364–5371.

Zhang, Y., Gao, B., Zhang, M., Shi, J., & Xu, Y. (2010). Pulsed electric field processing effects on physicochemical properties, flavor compounds and microorganisms of longan juice. Journal of Food Processing and Preservation, 34(6), 1121–1138.



6. CONCLUSIONS

6 CONCLUSIONS

Considering the objectives proposed and the results obtained in the present thesis, the following conclusions can be asserted:

Section i: Preservation of fresh-cut mango quality by edible coatings and pulsed light treatment

- Alginate, carboxymethylcellulose, pectin and chitosan are feasible polysaccharide-based edible coatings for fresh-cut mango preservation. Coatings maintained fresh-like colour and microbial population below 1 x 10^6 CFU/g along 14 days. An antimicrobial effect and great consumer acceptance can be assigned to chitosan and alginate, respectively.

- The effect of pulsed light treatment (λ = 180- 1100 nm) in mango slices inactivated 3.18 log cycles of *Listeria innocua* population. The reduction was improved by the combination with alginate coating and malic acid dipping (PL-AL-MA) reducing the population up to 4.5 log cycles. It was pointed out that mango slices extended microbial stability along 14 days of refrigerated storage whether alginate coating is applied after pulsed light.

- The use of pulsed light treatment as well as the application of alginate-based coating and malic acid dipping indicated more resistance to rupture and enhancement of yellow colour in mango slices. The combination of PL, AL and MA is a feasible strategy for preserving antioxidant compounds and phenolic profile of mango slices. It is noted that quercetin and mangiferin were identified in mango slices treated by MA-PL and PL-AL-MA after 14 days. This was a step forward in the preservation of interesting health-related compounds in ready-to-eat fruits for consumers.

Section ii: Maintain the high intensity pulsed electric fields (HIPEF) treated mango juice stability along the storage

- HIPEF treatment (35 kV/ cm, 200 Hz, 4µs-bipolar) for 800 µs assured the 5 log reductions of *Listeria innocua* population in mango juice. Nevertheless, significant decrease in enzymatic activity was only found in treatment times above 1800 µs. Pectinmethylesterase was better reduced (81-77 %) than peroxidase (65-47 %), lipoxygenase (50-45 %) or polyphenoloxidase (40-20 %) after HIPEF processing at 1800 µs, whereas polygalacturonase remain unchanged. Considering physicochemical (colour, pH, solid soluble content, viscosity) and sensorial attributes (colour, taste and overall acceptance), the application of HIPEF treatment was suitable for maintaining natural attributes since HIPEF-treated mango juice was highly scored by panellists compared with those untreated thereafter processing.

- HIPEF treatment time at 1800 µs was effective to maintain mango juice safety throughout 59 days. During storage, physicochemical parameters remained unchanged, only a decline in lightness was observed in both HIPEF and heat-treated after 21 days of storage. The enzymatic reduction enable to enhance the total phenolic content up to 700 µg gallic acid equivalent/ mL along the 59 days of storage. Differently, antioxidant capacity and carotenoid content of all evaluated mango juices decreased regardless the treatment applied along the storage.

Section iii: Determination of the high hydrostatic pressure (HHP) conditions for mango puree treatment

- High hydrostatic pressure processing parameters, being pressure, holding time and temperature, significantly influence the enzymatic reduction of mango puree. The highest reduction was obtained when pressure was applied at mild temperature. Pectinmethylesterase and polyphenoloxidase in Tommy

Atkins mango puree decreased up to 70 % and 72 % with a HHP treatment conducted at 400 and 550 MPa, respectively. Manila mango puree achieves the highest reduction of polyphenoloxidase activity (54 %) at 400 MPa. Moreover, holding times of 16 min were needed to achieve the highest reduction of enzymes in mango puree whereas pecinmethylesterase was not significantly affected by holding time.

- The overall pH, a_w and TSS of fresh mango puree were maintained after HHP processing. Otherwise, HHP had significant effect in colour and viscosity of mango puree. HHP at 550 MPa promoted a decline of lightness and yellow index in Tommy Atkins mango puree whereas Manila puree colour was kept as fresh-like regardless the HHP conditions. Also, carotenoids were negatively affected by temperature and 550 MPa due to their sensitivity to temperature. Nevertheless, phenolic compounds and antioxidant capacity of mango puree was enhanced by the application of HHP-processing under moderate temperature (30 ˚C) conditions.

6.1 CONCLUSIONS

Considerant els objectius proposats i els resultats obtinguts en la present tesis, les següents conclusions poden ser establertes:

Secció i: Conservació de la qualitat de mango tallat per recobriments comestibles i tractament de polsos de llum

- L'alginat, la carboximetilcel·lulosa, la pectin i el quitosan son recobriments comestibles a base de polisacàrids viables per a la conservació del mango tallat. Els recobriments van mantenir el color similar al mango fresc i la población microbiana per sota de $1x10^6$ UFC/g durant 14 dies. Es pot asignar al quitosan i l'alginat un efecte antimicrobià i bona acceptació dels panelistes, respectivament.

- L'efecte del tractament per polsos de llum (λ = 180- 1100 nm) va inactivar 3.18 cicles logarítmics la población de *Listeria innocua* en les llestes de mango. La reducció va ser millorada per la convinació amb el recobriment d'alginat i la immersió d'àcid màlic (PL-AL-MA) reduint la población fins a 4.5 cicles logarítmics. A més, les llesques de mango van extendre l'estabilitat microbiológica al llarg de 14 dies en refrigeració sempre hi quan el recobriment comestible d'alginat s'apliqui després del tractament de polsos de llum

- La utilització del tractament de polsos de llum així com l'aplicació del recobriment comestible a base d'alginat i la immersió d'àcid màlic van indicar més resistència a la ruptura i van millorar el color groguent en les llesques de mango. La convinació de PL, AL i MA és una estrategia viable per conservar els compostos antioxidants i el perfil de fenols en les llesques de mango. Cal denotar que la quercitina i la manguiferina van ser

identificades en les llesques de mango tractat per MA-PL i PL-AL-MA després de 14 dies. Això, permet ser un pas endavant en la conservació de compostos amb un interés per la salut en productes llestos per ser consumits.

Seccó ii: Mantenir l'estabilitat del suc de mango tractat per polsos elèctrics d'alta intensitat (HIPEF) al llarg de l'emmagatzematge.

- El tractament de HIPEF (35 kV/ cm, 200 Hz, 4µs-bipolar) durant 800 µs va asegurar 5 reduccions logarítmiques de la población de *Listeria innocua* en el suc de mango. Malgrat això, la reducció significativa de l'activitat enzimática es va detectar únicament en temps de tractament per sobre de 1800 µs. La pectinmetilesterasa es va reduïr més (81-77 %) que la peroxidasa (65-47 %), la lipoxigenasa (50-45 %) o la polifenoloxidasa(40-20 %) després del processat per HIPEF a 1800 µs, mentre que la poligalacturonasa es va mantener sense canvis. Pel que fa als atributs fisicoquímics (color, pH, contingut en sòlids solubles, viscositat) i sensorials (color, gust i acceptació global), l'aplicació del tratament de HIPEF va ser adequada per mantener els atributs naturals ja que el suc de mango tractat va obtener millor puntuació sensorial dels panelistes comparant-ho amb el suc no tractat.

- El temps de tractament per HIPEF a 1800 µs va ser efectiu per mantener la seguretat del suc de mango al llarg de 59 dies. Durant l'emmagatzematge, els paràmetres fisicoquímics es van mantenir sense canvis, únicament la lluminositat del suc de mango i del tractat tèrmicament va decaure després de 21 dies d'emmagatzematge. La reducció enzimática va permetre millorar el contingut total de fenols fins obtenir 700 µg àcid gallic / mL durant 59 dies d'emmagatzematge. D'altra banda, la capacitat antioxidant i el contingut de carotens del suc de mango va decrèixer al llarg de l'emmagatzematge sense tenir en compte el tractament aplicat.

Secció iii: Determinació de les condicions de tractament per altes presions hidrostàtiques (HHP) per puré de mango

- La pressió, el temps de tinença i la temperature essent els paràmetres de processat per altes presions hidrostàtiques, van tenir una influència significativa en la reducció enzimàtica del puré de mango. La reducció més elevada es va obtenir quan es va convinar la pressió amb la temperatura suau. La pectinmetilesterasa i la polifenoloxidasa en el puré de mango Tommy Atkins va ser reduïda fins un 70 % i 72 % amb HHP a 400 i 550 MPa, respectivament. El puré de mango Manila va obtenir a la reducció més elevada de l'activitat de polifenoloxidasa (54 %) a 400 MPa. A més a més, el temps de tinença de 16 min va ser necessari per obtenir la major reducció enzimática en el puré de mango tot i que l'enzim pectinmetilesterasa no va ser significativament afectat pel temps de tinença.

- En general el pH, l'a_w i el contingut total de sòlids del puré de mango fresc es van mantenir després del processat per HHP. De diferent manera, HHP va afectar significativament al color i la viscositat del puré de mango. HHP a 550 MPa va promoure la disminució de la lluminositat i l'índex groguenc en el puré de mango Tommy Atkins mentre que en la varietat Manila el color es va mantenir com el fresc independentment de les condicions de HHP. També els carotenoids van ser afectats negativament per la temperatura i la pressió a 550 MPa ja que tenen alta sensibilitat a la color. No obstant, els compostos fenòlics i la capacitat antioxidant del puré de mango van millorar després de l'applicació del processat per HHP a temperatura moderada (30 ˚C).

6.2 CONCLUSIONES

Considerando los objetivos propuestos y los resultados obtenidos en la presente tesis, se establecen las siguientes conclusiones:

Sección i: Conservación de la calidad de mango cortado mediante recubrimientos comestibles y tratamiento de pulsos de luz

- El alginato, la caroximetilcelulosa, la pectina y el quitosano son recubrimientos comestibles a base de polisacáridos viables para la conservación de mango cortado. Los recubrimientos mantuvieron el color similar al mango fresco y la población microbiana por debajo de $1x10^6$ CFU/g durante 14 días. Se puede asignar al quitosano y al alginato un efecto antimicrobiano y buena aceptación de los panelistas, respectivamente.

- El efecto del tratamiento por pulsos de luz (λ = 180- 1100 nm) inactivó 3.18 ciclos logarítmicos la población de *Listeria innocua* en el mango cortado. La reducción fue mejorada por la combinación con el recubrimiento de alginato y la inmersión de ácido málico (PL-AL-MA) reduciendo la población hasta 4.5 ciclos logarítmicos. Además, las rebanadas de mango extendendieron la estabilidad microbiológica durante 14 días en refrigeración siempre y cuando el recubrimiento comestible de alginato sea aplicado después del tratamiento de pulsos de luz.

- La utilización del tratamiento de pulsos de luz así como la aplicación del recubrimiento comestible a base de alginato y la inmersión de ácido málico denotaron más resistencia a la ruptura y mejoraron el color amarillento en las rebanadas de mango. La combinación de PL, AL y MA es una estrategia viable para conservar los compuestos antioxidantes y el perfil de fenoles en las rebanadas de mango. Cabe destacar que la quercitina y la manguiferina

fueron identificades en las rebanadas de mango tratado por MA-PL y PL-AL-MA después de 14 días. Esto supone un avance en la conservación de compuestos con un interés para la salut en productos listos para consumir.

Seccón ii: Mantener la estabilidad del zumo de mango tratado por pulsos eléctricos de alta intensidad (HIPEF) a lo largo del almacenamiento

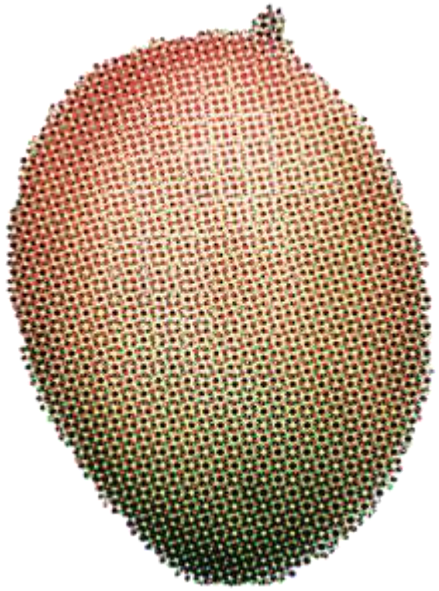
- El tratamiento de HIPEF (35 kV/ cm, 200 Hz, 4µs-bipolar) durante 800 µs aseguró 5 reducciones logarítmicas de la población de *Listeria innocua* en el zumo de mango. A pesar de ello, la reducción significativa de la actividad enzimática se detectó únicamente en tiempos de tratamiento por encima de 1800 µs. La pectinmetilesterasa (81-77 %) se redujo más que la peroxidasa (65-47 %), la lipoxigenasa (50-45 %) o la polifenoloxidasa (40-20 %) después del procesado por HIPEF a 1800 µs, mientras que la poligalacturonasa se mantuvo sin cambios después del tratamiento. Los atributos fisicoquímicos (color, pH, contingut en sòlids solubles, viscositat) y sensoriales (color, gust i acceptació global), la aplicación del tratamiento de HIPEF fue adequada para mantener los atributos naturales ya que el zumo de mango tratado obtuvo mejor puntuación sensorial por parte de los panelistas comparando con el zumo no tratado.

- El tiempo de tratamiento por HIPEF a 1800 µs fue efectivo para mantener la seguridad del zumo de mango a lo largo de 59 días. Durante el almacenamiento, los parámetros fisicoquímicos se mantuvieron sin cambios, únicamente la luminosidad del zumo de mango y del tratado térmicamente cayó después de 21 días de almacenamiento. La reducción enzimática permitió mejorar el contenido total de fenoles hasta obtener 700 µg ácido gallic/ mL durante 59 días de almacenamiento. Por otro lado, la capacidad antioxidante y el contenido de carotenos del zumo de mango decreció a lo largo del almacenamiento sin tener en cuenta el tratamiento aplicado.

Sección iii: Determinación de las condiciones de tratamiento por altas presiones hidrostáticas para puré de mango

- La presión, el tiempo de permanencia y la temperatura son los parámetros de procesado por altas presiones hidrostáticas que tuvieron una influencia signficativa e la reducción enzimática del puré de mango. La reducción más elevada se obtuvo cuando se combinó la presión con la temperatura suave. La pectinmetilesterasa y la polifenoloxidasa en el puré de mango Tommy Atkins fue reducida hasta un 70 y 72 % con HHP a 400 y 550 MPa, respectivamente. El puré de mango Manila obtuvo la reducción más elevada de la actividad de polifenoloxidasa (54 %) a 400 MPa. También el tiempo de permanencia a 16 min fué necesario para obtener la mayor reducción enzimática en el puré de mango a pesar que la enzima pectinmetilesterasa no fue afectada significativamente por el tiempo de tratamiento.

- En general el pH, la a_w y el contenido total de sólidos solubles del puré de mango fresco se mantuvo después del procesado por HHP. Distintamente, HHP afectó significativamente al color y la viscosidad del puré de mango. HHP a 550 MPa promovió la disminución de la luminosidad y el índice amarillento en el puré de mango Tommy Atkins mientras que en la variedad Manila el color se mantuvo cono el fresco independientemente de las condiciones de HHP. También los carotenoides fueron afectados negativamente por la temperatura y la presión a 550 MPa ya que tienen alta sensibilidad al calor. No obstante, los compuestos fenólocos y la capacidad antioxidante del puré de mango mejoraron después de la aplicación del procesado por HHP a temperatura moderada (30 ˚C).



7. APPENDICES

APPENDIX ·I·

Universitat de Lleida

EFFoST

TECNOLÓGICO DE MONTERREY.

Inactivation of *Listeria innocua* and enzymatic changes in mango juice treated by High Intensity Pulsed Electric Field (HIPEF)

*B. Salinas-Roca[1], P. Elez-Martínez[1], J. Welti-Chanes[2], O. Martín-Belloso[1]**

[1]University of Lleida, Lleida Agrotecnio Center Spain

[2]Instituto Tecnológico de Estudios Superiores de Monterrey, Monterrey, Mexico

**omartin@tecal.udl.cat*

INTRODUCTION

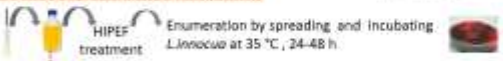
Mango production represents a 20.5% of tropical fruits. Consumers consider mangos as an attractive fruit for its flavor and texture. Moreover, recent studies have shown that mango could be a good source of bioactive compounds. Thus, there is an increasing interest to use mango in different products such as jellies, purees and juices. One of the main goals is finding a technology capable to preserve the quality of fresh mango.

HIPEF treatments have been studied in the last years as an alternative to traditional thermal techniques for fruit juices preservation. However, there is few information available about preservation of tropical juices such as mango by HIPEF treatment.

The aim of this work was to study the effect of HIPEF treatment on the inactivation of *Listeria innocua* and the changes in enzymatic activity in mango juice.

MATERIALS AND METHODS

Inoculation and *L.innocua* counts



Enzymatic determinations

Oxidative enzymes

SAMPLE HOMOGENIZATION + CENTRIFUGATION

ENZIMATIC REACTION determined by spectrophotometry

- **Polyphenoloxidase(PPO)** at 400 nm
- **Peroxidase (POD)** 472 nm
- **Lipoxigenase (LOX)** 234 nm

Pectinolytic enzymes

- **Polygalacturonase (PG):** Sample homogenization → Centrifugation → PG extract incubation at 30° C → Enzimatic reaction determined at 276 nm
- **Pectilmetilesterase (PME):** MJ to pH 7 → Titration reaction determined by potentiometry

HIPEF Treatment conditions

- ✓ 35 kV/ cm
- ✓ 200 Hz
- ✓ 0- 2000 µs
- ✓ < 40 °C
- ✓ 4-µs bipolar square wave pulses

RESULTS AND DISCUSSION

L. innocua inactivation

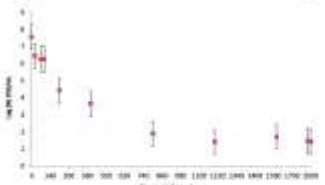
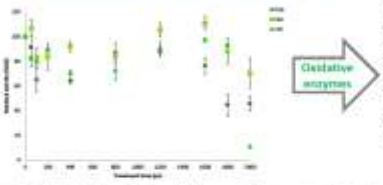


Figure 1: Survival of *L. innocua* population inoculated in mango juice treated by HIPEF at different treatment times (µs).

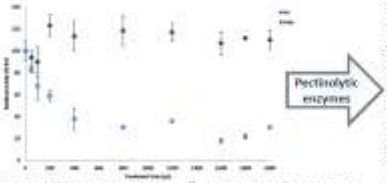
L. innocua population inoculated in mango juice decreased as the HIPEF treatment time increased. A 5 log reduction was achieved at 800 µs. It was observed an effect of treatment time in the *L. innocua* population although no changes were observed from 1200 µs of treatment time. The conditions used in this study and the low pH of mango juice have probably promoted the high log reduction recorded in our work.

Enzymatic activity



Residual activity (RA) of PPO, POD and LOX after HIPEF treatment for 2000 µs was 60, 45 and 10% respectively. The highest oxidative enzyme inactivations were achieved from 1600 µs. LOX had the lowest RA at high treatment times probably due to the easily degradation of the structure.

Figure 2: HIPEF treatment time effect on oxidative enzymes Peroxidase (POD) (●), Polyphenoloxidase (PPO) (○) and Lipoxigenase (LOX) (▲).



PG residual activity was activated from 100 µs. Differently, PME was sensible to HIPEF treatment decreasing its activity a maximum of 80%. This suggest that the inactivation of PG was not possible with HIPEF treatments at the conditions used but it could be possible increasing the temperature above 40 °C.

Figure 3: HIPEF treatment time effect on pectinolitic enzymes Polygalacturonase (PG) (●) and Pectin methylesterase (PME) (○)

AKNOWLEDGMENTS

This work was supported by the University of Lleida (Spain) and financed by the Instituto Tecnológico de Estudios Superiores de Monterrey, Monterrey, Mexico. Prof.Olga Martín-Belloso thanks the Institution for Research and Advanced Studies (ICREA) for the Academia Award 2008 from the Catalan Government.

CONCLUSIONS

HIPEF proved to be efficient in reducing a 5 log CFU/ml (800µs) the initial *L. innocua* population inoculated in mango juice. There was a clearly effective inactivation of PME and LOX decreasing more than 80% its RA. Consequently, mango juice treated by HIPEF at 35 kV/cm, 200Hz, 4 µs bipolar square wave pulses and 1800 µs could be a good alternative to inactivate *Listeria innocua* and to reduce the activity of POD, PPO, LOX and PME enzymes in mango juice.

Appendix I

APPENDIX ·II·

Food Bioprocess Technol
DOI 10.1007/s11947-016-1844-5

ORIGINAL PAPER

High Hydrostatic Pressure and Temperature Applied to Preserve the Antioxidant Compounds of Mango Pulp (*Mangifera indica* L.)

Mariana Camiro-Cabrera[1] · Zamantha Escobedo-Avellaneda[1] · Blanca Salinas-Roca[2] · Olga Martín-Belloso[2] · Jorge Welti-Chanes[1]

Received: 13 July 2016 / Accepted: 30 November 2016

Abstract High hydrostatic pressure (HHP) processes combined with moderate heating can be used to preserve foods while maintaining general quality. The effect of these conditions on the total phenolic (TP), vitamin C (L-ascorbic acid (AA)), carotenoids, and antioxidant activity (AOA) of mango purees was evaluated. Purees were processed at 400–550 MPa/34 and 59 °C at different holding times. Unprocessed puree had TP of 26.6 mg gallic acid/100 g, 21.1 mg L-ascorbic acid/100 g, AOA of 885 μmol trolox equivalents/100 g, and total carotenoids of 6.0 mg β-carotene/100 g. HHP treatments increased the phenolic concentrations up to 34% (550 MPa/59 °C/2 and 4 min) compared with the initial content, probably due to improvement of their extraction. AA content was reduced significantly (10–45%) after all HHP processes performed at 59 °C, while at 34 °C, they were diminished only after 8 and 16 min of treatment (13–26%). At 34 °C and lower times, AA concentration increased in average 18%. Total carotenoid retention in HHP-treated samples varies from 77 to 98%, being the higher the temperature the lower the retention observed. The concentration of most individual carotenoids remains unchanged, but violaxanthin content was reduced (21–26%) and 9-cis-violaxanthin was increased by about 10%. The AOA was also increased (up to 39%) at some processing conditions. A linear correlation between the TP and AOA was obtained. HHP at 550 MPa combined with moderate temperature (34 °C) at processing times up to 8 min is recommended for the maximum retention of the antioxidant compounds of mango puree.

Keywords Mango · High hydrostatic pressures · Vitamin C · Carotenoids · Antioxidant activity · Phenolics

✉ Zamantha Escobedo-Avellaneda
zamantha.avellaneda@itesm.mx

✉ Jorge Welti-Chanes
jwelti@itesm.mx

[1] Tecnologico de Monterrey, Escuela de Ingeniería y Ciencias, Centro de Biotecnología FEMSA, Av. Eugenio Garza Sada 2501 Sur, Col. Tecnológico, 64849 Monterrey, NL, Mexico

[2] Food Science and Technology, University of Lleida, Pl. de Víctor Siurana, 1, E-25003 Lleida, Spain

Published online: 13 December 2016

Introduction

Mango (*Mangifera indica* L.) is one of the most important tropical fruits worldwide. In addition to vitamins, mango contains bioactive compounds such as phenolics and carotenoids that have been related to beneficial effects against chronic and degenerative diseases due in part to their antioxidant activity (AOA) against radicals such as hydroxyle, superoxide, and singlet oxygen (Mesa-Vanegas et al. 2010).

Puree is the principal product obtained from mango, and it is used to prepare nectars, juices, jams, jellies, and other foodstuffs. Extending the shelf life of mango is important to reach different markets and to ensure its availability in all seasons. The food industry is looking for new preservation technologies to ensure safety while avoiding negative changes in sensory, nutritional, physicochemical, and antioxidant characteristics. The use of non-thermal technologies like high hydrostatic pressures (HHP) is having great acceptance due to the minimal modification in food quality (Oey et al. 2008). Some studies have shown that HHP facilitates the liberation of antioxidant compounds from food matrixes due to cellular disruption, being a promising technology for the treatment of foods (Barba et al. 2012; Hernández-Brenes et al. 2013; Queiroz et al. 2010; Saldo et al. 2009).

The combination of HHP with moderate temperature (30–60 °C) has been proposed by some authors to inactivate

baroresistant enzymes like pectin methylesterase (PME) that is present in mango (Tejada-Ortigoza et al. 2015) and to destroy baroresistant spores in low-acid products (FDA: http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm101456.htm).

Many HHP units do not reach the working pressure level instantly. The *come up time* (CUT) is the time necessary to achieve the required pressure, and it depends on the design of the pumping system and size of the HHP units. For comparing purposes, it is important to study the effect of the CUT when performing experiments about HHP (Escobedo-Avellaneda et al. 2015).

Some researchers have applied HHP in puree mango to evaluate antioxidant compounds (Liu et al. 2014), but its effect combined with mild heating has not been investigated, which is important to determine the best processing conditions to retain antioxidant compounds. This work aimed to determine the effect of different HHP conditions combined with moderate heating on the content of total phenolic, total vitamin C, L-ascorbic acid, carotenoids, and AOA of puree mango. The influence of the CUT was also evaluated.

Material and Methods

Reagents

Folin-Ciocalteau phenol reagent (2 N), trichloroacetic acid (TCA), 99% DL-ithiothreitol (DTT), 98% HPLC-grade N-ethylmaleimide (NEM), gallic acid (GA), L-ascorbic acid (AA), α-α′-bipyridyl,2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH), fluorescein sodium salt, β-carotene (βC), β-cryptoxanthin, and trolox (TE) (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were acquired from Sigma-Aldrich Corp. (St. Louis, MO, USA). Acid chloride, potassium phosphate monobasic, potassium phosphate dibasic, sodium chloride, potassium hydroxide, ethanol, and sodium phosphate monobasic were purchased from Fermont-Productos Químicos Monterrey (Monterrey, NL, Mexico). HPLC-grade phosphoric acid (85%), ferric chloride, and HPLC-grade methyl tert-butyl ether (MTBE) were purchased from Fisher Scientific (Monterrey, NL, Mexico). Methanol and phenolphthalein were obtained from Desarrollo de Especialidades Químicas (San Nicolás de los Garza, Nuevo León, Mexico) and zeaxanthin and lutein from Applichem (Darmstadt, Germany). Sodium carbonate and sodium phosphate dibasic were acquired from CTR Scientific (Control Técnico y Representaciones, S.A. de C.V., Monterrey, Nuevo León, Mexico), HPLC-grade methanol from J.T. Baker (Avantor Performance Materials, Center Valley, PA), HPLC-grade isopropanol from Honeywell (Brooklyn, NY, USA), and BHT from Spectrum Quality Products (New Brunswick, NJ, USA). Unless otherwise noted, all solvents and reagents were of analytical grade.

Raw Material

Mango (*M. indica* (L.) Tommy Atkins) was acquired in Central de Abastos, Guadalupe, N.L. (Mexico). Mangoes with yellow peel were selected and washed. The peel was removed and the pulp was ground in a domestic blender (BRLY-Z00-013, Oster, Monterrey, NL, Mexico) to obtain a homogenous puree. Puree was divided in portions of 10 g and placed in 12 × 5-cm polyethylene bags (Filmpack S.A. de C.V., Guadalupe, Nuevo León, Mexico) that were vacuum sealed and used for HHP treatments.

HHP Treatments

Vacuum-sealed samples (five bags for each treatment condition) were treated in a HHP unit (Avure 2 L, Columbus, OH, USA) at 400, 450, and 500 MPa during 2, 4, 8, and 16 min at initial temperature of the pressure transmission medium (water) of 25 and 55 °C. These processing conditions were selected based on previous studies of microbiological and PME inactivation (unpublished data). Due to adiabatic heating during the holding time, water temperatures of 34 ± 3 and 59 ± 4 °C were reached for initial temperatures of 25 and 55 °C, respectively. The effect of the CUT (about 80 s at 400 and 450 and about 86 s at 500 MPa) was also evaluated (time 0). For CUT treatments, samples were treated as previously described, but once the pressure level was reached, runs were interrupted and the system was decompressed instantly. Unprocessed mango puree was used as control. Each run was performed in duplicate and samples maintained at −80 °C while analyzed.

Physicochemical Analysis

The moisture content, water activity (a_w), total soluble solids (TSSs), pH, and titratable acidity (TA) of the raw material were measured. Moisture content was evaluated gravimetrically according to 934.06 AOAC method (1990a). a_w was measured with an AquaLab 4TE (Decagon Devices Inc., Pullman, WA, USA) at 25 °C. TSS and pH were determined using a refractometer (TR 101 01148, Atago Inc., Bellevue, WA, USA) and a potentiometer (Orion 3 star, Thermo Fisher Scientific Inc.), respectively. TA was evaluated according to the 942.15 AOAC method (1990b). Maturity index was calculated as TSS/TA. Mango used in this study had a moisture content of 83.9 ± 0.3%, a_w of 0.980 ± 0.003, TSS of 14.3 ± 0.1°Bx, pH of 3.7 ± 0.1, AT of 1.1 ± 0.1 g citric acid/100 g, and maturity index of 12.7.

Antioxidant Compounds and Antioxidant Activity Evaluation

Total phenolics (TPs), total vitamin C, carotenoids, and AOA were measured as described in Escobedo-Avellaneda et al. (2014).

Total Phenolics

About 100 mg sample was weighed and mixed with 2.5 mL of 1.2 M HCl in methanol:water solution (1:1 *v*/*v* ratio). After heating during 3 h at 90 °C (model 102, Thermo Fisher Scientific Inc., Waltham, MA, USA), sample was centrifuged and the supernatant was recovered and diluted with methanol. Quantification was done with the Folin-Ciocalteau method, and the absorbance was measured at 765 nm in a microplate reader (BioTek Instruments Inc., Bad Friedrichshall, Germany). Three replicate analyses were performed, and results were expressed as a percentage of retention (%) with respect to the initial content (mg gallic acid (GA)/100 g on a wet basis).

Vitamin C

Vitamin C was quantified as total content (L-ascorbic acid + dehydroascorbic acid) and L-ascorbic acid (reduced form of the vitamin C). Samples were mixed with 6% TCA and then centrifuged. Supernatant aliquots (50 μL) were mixed with 25 μL potassium phosphate buffer (75 mM). For the analysis of L-ascorbic acid, 50 μL water was added, while for total vitamin C, 25 μL DTT (10 mM) and 25 μL NEM (0.5%) were used. In all samples, 335 μL of 10% TCA:43% H_3O_4:4% α-α′-bipyridyl:3% $FeCl_3$ was added (33.3:26.7:26.7:13.3% *v*/*v*). After 1-h incubation at 37 °C, the absorbance was read at 525 nm in a microplate reader. Three replicate analyses were performed, and results were expressed as percentage of retention (%) with respect to the initial content (mg AA/100 g on a wet basis).

Carotenoids

About 2 g sample was mixed with 10 mL hexane containing 0.1% BHT and homogenized at room temperature during 1 min at 15,000 rpm. This procedure was repeated twice, the sample was centrifuged, and the supernatant was recovered and saponified with 25 mL of a 10% (*w*/*v*) KOH ethanolic solution. The extract was transferred to a separation funnel, and 30 mL of 30% NaCl solution was added. The hexane phase was then washed with water until free of alkali and evaporated at 35 °C (RV 10 Control, IKA, Staufen, Germany). The final extract was diluted with isopropanol, filtered (0.45-μm PTEF membrane), and analyzed by reversed-phase HPLC-PAD (1200 series, Agilent Technologies Inc., Santa Clara, CA, USA) using a 5-μm 4.6 × 250-mm YMC carotenoid column. Ninety-six-percent methanol (A) and 100% MTBE (B) were used as eluent solvents at flow rate of 1 mL/min using the following gradient: 0 min-5% B, 5 min-20% B, 20 min-30% B, 30 min-47.5% B, 40 min-47.5% B, 44 min-100% B, and 47 min-100% B. Identification was done by comparing the wavelength of maximum absorption (λ_{max}) and the shape of the visible absorption spectrum with those previously reported (Hernández-Brenes et al. 2013; Mercadante and Rodriguez-Amaya 2001; Escobedo-Avellaneda et al. 2014; Mercadante et al. 1997). Triplicate extractions were performed and results were expressed as percentage of retention (%) with respect to the initial content (mg βC/100 g on a wet basis).

Antioxidant Activity

The AOA was measured by the oxygen radical absorbance capacity (ORAC) method. Twenty-five microliters of sample diluted in sodium phosphate-buffered solution (PBS) (75 mM, pH 7.4) was placed in Costar polystyrene black plates with 96 round bottom wells. A microplate reader automatically dispensed 150 μL of 11 M fluorescein followed by 25 μL of 153 mM AAPH after 30 min of incubation at 37 °C. Fluorescence was measured at 485/20-nm excitation wavelength and 528/20-nm emission wavelength. Three replicate analyses were performed, and results were expressed as percentage of retention (%) with respect to the initial content (mg TE/100 g on a wet basis).

Kinetic Degradation of Vitamin C

A first-order kinetic model was applied to evaluate the vitamin C degradation at the process conditions evaluated (Eq. 1).

$$ln\left(\frac{C_t}{C_{CUT}}\right) = -kt \tag{1}$$

where k (min^{-1}) is the reaction rate constant obtained from the slope of the curve of $ln\ (C_t/C_{CUT})$ versus time (t, min) at constant pressure and temperature. C and C_{CUT} are the vitamin C content at each holding time and after each CUT treatment, respectively. The activation volume of the reaction V_a (cm^3/mol) was calculated with the Eyring equation (Eq. 2) (Serment-Moreno et al. 2014).

$$lnk = lnk_0 - \frac{V_a P}{RT} \tag{2}$$

where k_0 is a constant, P is the pressure in MPa, T is the absolute temperature (K), and R is the universal ideal gas constant. The degree of adjustment of the model was evaluated based on the lineal correlation value R^2.

Statistical Analysis

One-way ANOVA (unstacked) with Tukey test was used to determine significant differences between the treated samples and the control by using a significance level of $\alpha = 0.05$.

Results and Discussion

Effect of HHP on Phenolic Content

The total phenolic content in the unprocessed mango puree was 26.6 mg GA/100 g; similar values have been reported by other authors for the *Tommy Atkins* variety (Kim et al. 2009; Manthey and Perkins-Veazie 2009; Sogi et al. 2014). HHP-treated mango puree had contents that varied from 26.8to 35.9 mg GA/100 g, indicating an increase in concentration up to 34% (550 MPa/59 °C/2 and 4 min) as compared with the initial content (Fig. 1). The increase could be related to cellular disruption due to HHP that facilitates extraction of these compounds or to disruption of weak bounds between dietary fiber and phenolic compounds that, in addition to covalent bounds, interact by electrostatic forces and hydrogen bonds (Palafox-Carlos et al. 2011; Quirós-Sauceda et al. 2014). In most cases, the phenolic content of samples treated at 400 MPa was statistically equal to the control independently of temperature and time. The increase of pressure at 450 and 550 MPa had a positive effect on the amount of phenolics quantified. Samples treated at 450 MPa showed about 18% of increase in total phenolic compounds, without important effect of temperature and time. At 550, the average increase was 24%, and for treatments performed at 34 °C, the increment of time from 2 to 8 min had a positive effect on the phenolic content, but a phenolic concentration reduction was observed when time increased to 16 min. In general, the increment of pressure and temperature enhanced the extraction of phenolics after the CUT. At 550 MPa/59 °C/CUT, the phenolic content was increased by 26% (33.6 ± 1 mg GA/100 g). Higher contents in phenolics due to HHP have been reported previously in other fruits and fruit-based products like blackberry, pomegranate juice, litchi juice, and apple juice (Ferrari et al. 2010; Jayachandran et al. 2015; Queiroz et al. 2010).

Effect of HHP on Vitamin C Content

Mango puree had a total vitamin C content of 21.1 mg AA/100 g, and the L-ascorbic acid accounted for about 52% of the total content. Similar results have been reported previously for the same mango variety (Brecht et al. 2014; Manthey and Perkins-Veazie 2009; Oliveira et al. 2010).

Concentration of L-ascorbic acid and total vitamin C decreased significantly after all HHP treatments at 59 °C, while at 34 °C, they diminished only after 8 and 16 min due probably to the combined effect of pressure, time, and temperature. At 34 °C, the amount quantified of L-ascorbic acid increased on average 18% at times up to 4 min, but decreased by approximately 13 to 26% at 8 and 16 min, respectively, independent of the pressure. The total vitamin C content increased by about 10–20% up to 4 min with no significant effect of the pressure level, while at 8 min, it decreased 1, 6, and 11% at 400, 450, and 550 MPa, respectively, and 15, 33, and 38% at 16 min (Fig. 2). The increase in the content of L-ascorbic acid and total vitamin C could be related with cellular disruption that promotes better extraction of both types of vitamin at shorter treatment times, while at higher times, they start to decrease, probably due to oxidative degradation caused by residual dissolved oxygen in the sample or due to enzymatic activity. Escobedo-Avellaneda et al. (2015) also found that HHP treatment increased the release of vitamin C (11.4–43.6 and 21.2–45.3% for total and L-ascorbic acid, respectively) of

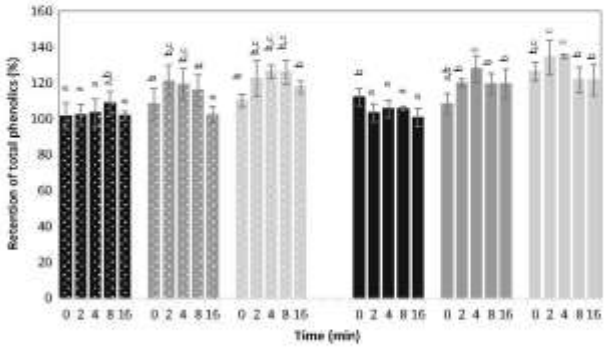


Fig. 1 Effects of HHP treatments on the retention of total phenolic compounds of mango puree with respect to the untreated product. *Bars with the same letters* indicate no significant differences ($p > 0.05$). *Dotted bars* = 34 °C, *solid bars* = 59 °C, *black bars* = 400 MPa, *dark gray bars* = 450 MPa, *light gray bars* = 550 MPa

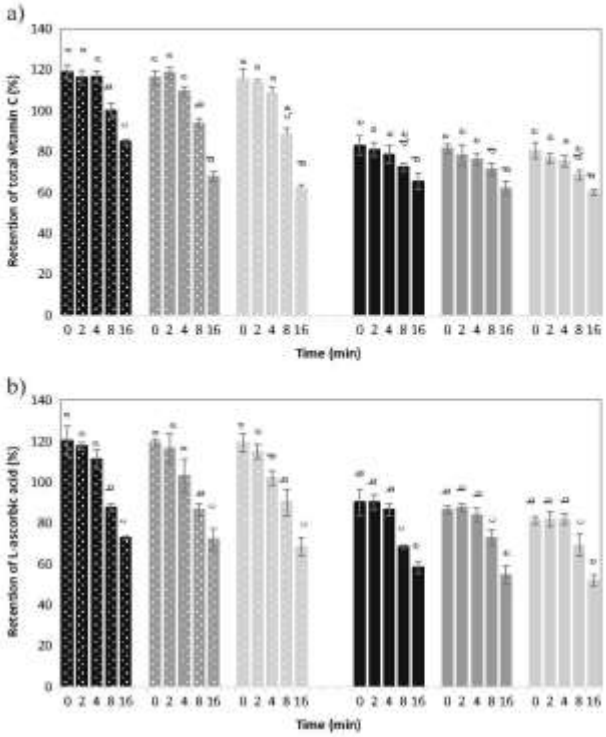


Fig. 2 Effects of HHP treatments on the retention of total vitamin C and L-ascorbic acid of mango puree with respect to the untreated product. *Bars with the same letters* indicate no significant differences ($p > 0.05$). *Dotted bars* = 34 °C, *solid bars* = 59 °C, *black bars* = 400 MPa, *dark gray bars* = 450 MPa, *light gray bars* = 550 MPa

comminuted orange, resulting in significantly higher measured values than for the untreated product. In other studies, the L-ascorbic acid content of HHP-treated red peppers increased about 10–20% (Castro et al. 2008), while in yellow peppers, it increased 11 to 48% (Castro et al. 2011). The vitamin C content of mango pulp increased by 29% after the CUT treatment at 600 MPa (Kaushik et al. 2014).

At 59 °C, the L-ascorbic acid content decreased in average 10–45%, while the total vitamin C content was decreased 17–37% independent of the pressure. On average, L-ascorbic acid decreased 10% up to 4 min and total vitamin C in about 17%. L-ascorbic acid decreased by 30 and 45% at 8 and 16 min, respectively, due to the higher times of exposure to temperature. On the other hand, the total vitamin C content decreased on average 33% at times of 8 and 16 min (Fig. 2). The losses of L-ascorbic acid could be related with the oxidation to dehydroascorbic acid or the complete degradation of L-ascorbic acid to other compounds. The exposure to heat and the action of enzymes like L-ascorbic acid oxidase could be implicated in the degradation process. The reduction of the total vitamin C content reflects both the degradation observed for the L-ascorbic acid in addition to the losses of the dehydroascorbic acid.

Because of its sensitivity to oxygen and temperature, total vitamin C is frequently used as indicator in the design of pasteurization process (Barba et al. 2010; Krebbers et al. 2002). In this way, obtaining kinetic parameters is important for prediction purposes when designing preservation process. First-order kinetic models adequately fit to describe the change of vitamin C content under HHP (R^2 from 0.85 to 0.97), as was shown previously for broccoli-apple juice (Houška et al. 2006). Rate constant increased as the pressure level increased; at 34 °C, k values of 0.022, 0.036, and 0.041 min^{-1} at 400, 450, and 550 MPa, respectively, and of 0.016, 0.017, and 0.018 min^{-1} at 59 °C, were obtained. It is noted that k values at 59 °C differed only slightly when pressure increased, indicating the low effect of pressure on vitamin C degradation. This effect is confirmed by the lower absolute

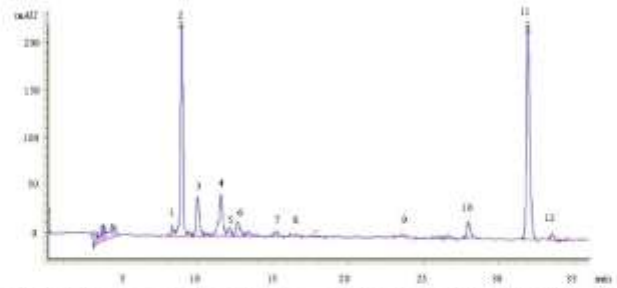


Fig. 3 Carotenoid profiles of untreated mango puree. 1, 2 = violaxanthin (416, 439, 469 nm); 3, 5, 6 = luteoxanthin isomer (399, 422, 448 nm); 4 = 9-cis-violaxanthin (412, 436, 464 nm); 7 = zeaxanthin (421, 451, 478 nm); 8 = not identified (418, 442, 470 nm); 9 = all-trans-β-cryptoxanthin (422, 445, 478 nm); 10 = 13-cis-β-carotene (338, 445, 471 nm); 11 = all trans β-carotene (420, 452, 473 nm); and 12 = 9-cis β-carotene (424, 446, 472 nm)

value of the activation volume calculated for 59 °C (−2.5 cm^3/mol, $R^2 = 0.95$) than for 34 °C (−9.4 cm^3/mol, $R^2 = 0.76$). This value also indicates that the degradation of vitamin C at 59 °C is less pressure dependent than at 34 °C.

Effect of HHP on Carotenoid Content

Unprocessed mango presented a total carotenoid content of 6.0 mg β carotene/100 g. Similar concentration have been previously reported (Hernández-Brenes et al. 2013; Manthey and Perkins-Veazie 2009; Pott et al. 2003). The total content of carotenoids was composed of 30.0% violaxanthin (peaks 1, 2), 12.8% luteoxanthin isomers (peaks 3, 5, 6), 9.5% 9-cis-violaxanthin (peak 4), 3.2% zeaxanthin (peak 7), 2.9% non-identified carotenoid, 3.0% all-trans-β-cryptoxanthin (peak 9), 5.0% 13-cis-β-carotene (peak 10), 30.3% all-trans-β-carotene (peak 11), and 3.2% 9-cis-β-carotene (peak 12) (Fig. 3). Mango *Tommy Atkis* is a good source of provitamin A because of its high content of all-trans-β-carotenes, which is superior to the content found in orange, mandarin, and papaya. The elution order of these carotenoids corresponds with higher or lower polarity as has been previously reported by other authors for the separation of carotenoids by reversed phase chromatography (Hernández-Brenes et al. 2013; Mercadante and Rodriguez-Amaya 2001; Mercadante et al. 1997).

HHP-treated mango had in general the same carotenoid profile as the untreated samples, indicating that the application of high pressure did not contribute to the generation of new carotenoids. Total carotenoid retention in HHP-treated purees varied from 77 to 98% (Fig. 4). The CUT treatments also decreased the total carotenoid content in the mango pulp. The higher the temperature, the higher the losses obtained due to the temperature sensitivity of carotenoids. Samples treated at 400–550 MPa/34 °C/CUT, 550 MPa/34 °C/2 min, and 400 MPa/34 °C/16 min showed the highest retentions (93–98%), but in general, independent of pressure, samples processed at 59 °C during 4 and 8 min retained in average 77% of total carotenoids, while samples treated during the CUT, 2 and 16 min retained 87%. At 34 °C, the same level

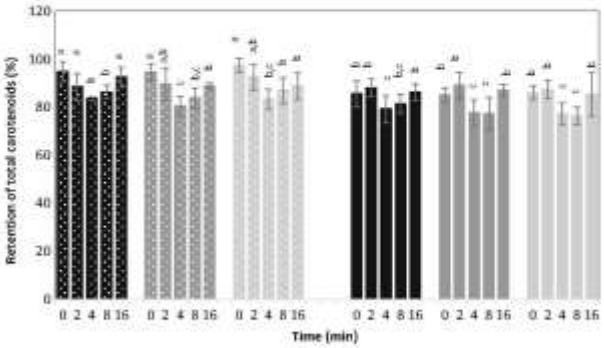


Fig. 4 Effects of HHP treatments on the retention of total carotenoids of mango puree with respect to the untreated product. *Bars with the same letters* indicate no significant differences ($p > 0.05$). *Dotted bars* = 34 °C, *solid bars* = 59 °C, *black bars* = 400 MPa, *dark gray bars* = 450 MPa, *light gray bars* = 550 MPa

Table 1 Effects of HHP at 34 °C on the retention of individual carotenoids of mango puree with respect to the total content

	400 MPa					450 MPa					550 MPa				
Peak	0 min	2 min	4 min	8 min	16 min	0 min	2 min	4 min	8 min	16 min	0 min	2 min	4 min	8 min	16 min
1	3.5 ± 0.1	3.7 ± 0.2	3.7 ± 0.2	3.8 ± 0.2	3.2 ± 0.0	3.6 ± 0.1	3.7 ± 0.1	3.4 ± 0.1	3.4 ± 0.1	3.6 ± 0.2	3.7 ± 0.3	3.9 ± 0.2	3.5 ± 0.0	3.4 ± 0.1	4.5 ± 0.3
2	24.0 ± 1.5	22.9 ± 1.4	22.0 ± 1.1	23.0 ± 1.2	20.6 ± 0.6	24.3 ± 1.8	23.3 ± 2.1	22.0 ± 1.1	22.8 ± 1.1	20.0 ± 0.9	23.8 ± 0.9	22.8 ± 1.2	23.0 ± 1.2	24.1 ± 1.0	17.5 ± 1.3
3	6.2 ± 0.6	3.8 ± 0.2	5.6 ± 0.5	5.7 ± 0.1	6.1 ± 0.3	5.8 ± 0.2	5.1 ± 0.3	6.3 ± 0.3	4.9 ± 0.3	5.5 ± 0.6	6.9 ± 0.6	6.5 ± 3	6.9 ± 0.1	6.1 ± 0.4	6.9 ± 0.6
4	10.2 ± 0.4	11.3 ± 0.8	10.0 ± 0.8	9.8 ± 1.2	12.2 ± 0.2	9.3 ± 0.2	11.1 ± 0.5	10.7 ± 0.5	9.3 ± 0.3	13.9 ± 0.4	9.4 ± 1.1	10.1 ± 0.5	9.6 ± 0.6	9.3 ± 0.5	11.1 ± 0.8
5	3.8 ± 0.2	3.5 ± 0.3	3.9 ± 0.2	3.6 ± 0.1	3.4 ± 0.0	3.6 ± 0.1	3.8 ± 0.2	3.8 ± 0.1	3.6 ± 0.2	3.6 ± 0.1	3.3 ± 0.2	3.0 ± 0.1	3.7 ± 0.3	2.9 ± 0.1	3.8 ± 0.3
6	4.6 ± 0.3	4.5 ± 0.2	3.6 ± 0.5	4.2 ± 0.3	3.9 ± 0.2	3.5 ± 0.1	3.7 ± 0.2	3.8 ± 0.1	3.5 ± 0.4	4.0 ± 0.3	3.9 ± 0.4	4.2 ± 0.2	4.1 ± 0.4	4.1 ± 0.2	3.9 ± 0.4
7	3.5 ± 0.1	3.2 ± 0.2	3.5 ± 0.2	3.4 ± 0.1	3.4 ± 0.0	3.2 ± 0.1	3.2 ± 0.1	3.3 ± 0.1	3. 0 ± .1 4	3.6 ± 0.1	3.0 ± 0.1	3.4 ± 0.2	3.3 ± 0.0	3.2 ± 0.1	3.4 ± 0.0
8	3.4 ± 0.1	3.2 ± 0.1	3.4 ± 0.1	3.5 ± 0.1	3.2 ± 0.1	3.1 ± 0.1	3.0 ± 0.1	3.1 ± 0.1	3.6 ± 0.2	3.3 ± 0.1	3.0 ± 0.2	3.4 ± 0.3	3.2 ± 0.1	3.3 ± 0.2	3.4 ± 0.1
9	3.5 ± 0.2	3.2 ± 0.1	3.6 ± 0.2	3.4 ± 0.1	3.4 ± 0.1	3.4 ± 0.1	3.3 ± 0.1	3.4 ± 0.1	3.8 ± 0.5	3.3 ± 0.2	3.0 ± 0.1	3.5 ± 0.3	3.2 ± 0.1	3.4 ± 0.2	3.3 ± 0.0
10	5.3 ± 0.2	4.9 ± 0.2	5.4 ± 0.3	5.2 ± 0.2	5.4 ± 0.1	5.5 ± 0.0	4.6 ± 0.2	4.8 ± 0.3	5.7 ± 0.1	4.6 ± 0.6	5.0 ± 0.1	5.4 ± 0.3	4.8 ± 0.3	5.3 ± 0.3	5.5 ± 0.2
11	30.5 ± 1.1	32.5 ± 2.4	31.7 ± 0.4	31.2 ± 2.2	32.0 ± 1.8	31.2 ± 1.0	31.9 ± 2.1	31.8 ± 2.2	32.5 ± 1.1	31.1 ± 1.5	31.9 ± 1.9	31.5 ± 1.3	29.8 ± 1.3	31.4 ± 1.7	33.4 ± 2.1
12	3.3 ± 0.1	3.3 ± 0.1	3.4 ± 0.1	3.3 ± 0.0	3.3 ± 0.0	3.3 ± 0.2	3.3 ± 0.1	3.5 ± 0.0	3.5 ± 0.1	3.5 ± 0.0	3.1 ± 0.1	3.3 ± 2	3.5 ± 0.1	3.4 ± 0.1	3.4 ± 0.1

1, 2 = violaxanthin (416, 439, 469 nm); 3, 5, 6 = luteoxanthin isomer (399, 422, 448 nm); 4 = 9-cis-violaxanthin (412, 436, 464 nm); 7 = zeaxanthin (421, 451, 478 nm); 8 = not identified (418, 442, 470 nm); 9 = all-trans-β-cryptoxanthin (422, 445, 478 nm); 10 = 13-cis-β-carotene (338, 445, 471 nm); 11 = all trans β-carotene (420, 452, 473 nm); and 12 = 9-cis β-carotene (424, 446, 472 nm)

Table 2 Effects of HHP at 59 °C on the percentage of retention of individual carotenoids of mango puree with respect to the total content

	400 MPa					450 MPa					550 MPa				
Peak	0 min	2 min	4 min	8 min	16 min	0 min	2 min	4 min	8 min	16 min	0 min	2 min	4 min	8 min	16 min
1	3.7 ± 0.1	3.9 ± 0.2	3.5 ± 0.1	3.5 ± 0.0	4.0 ± 0.1	3.6 ± 0.1	3.3 ± 0.1	3.2 ± 0.2	3.6 ± 0.1	3.9 ± 0.2	3.6 ± 0.1	3.5 ± 0.1	3.7 ± 0.1	3.2 ± 0.0	3.5 ± 0.2
2	22.4 ± 0.7	22.8 ± 1.1	21.9 ± 1.3	21.4 ± 1.3	22.5 ± 1.6	21.2 ± 0.6	20.6 ± 0.8	22.1 ± 1.2	21.9 ± 0.8	21.8 ± 1.3	21.3 ± 0.9	21.9 ± 0.6	21.6 ± 1.4	20.6 ± 0.6	23.2 ± 1.7
3	7.2 ± 0.2	6.5 ± 0.2	5.4 ± 0.1	5.8 ± 0.3	7.1 ± 0.1	6.6 ± 0.4	5.4 ± 0.1	6.5 ± 0.4	6.7 ± 0.4	6.8 ± 0.5	5.6 ± 0.3	4.8 ± 0.0	7.1 ± 0.3	6.1 ± 0.3	6.6 ± 0.3
4	9.6 ± 0.5	7.9 ± 0.2	10.4 ± 0.3	10.5 ± 0.3	11.8 ± 0.6	9.4 ± 0.4	10.7 ± 0.5	10.9 ± 0.5	11.2 ± 0.5	11.2 ± 1.0	10.2 ± 0.5	10.9 ± 0.8	11.4 ± 0.1	12.2 ± 0.2	10.9 ± 0.9
5	3.4 ± 0.1	3.8 ± 0.1	4.1 ± 0.1	3.7 ± 0.1	3.5 ± 0.1	3.6 ± 0.1	3.8 ± 0.1	4.3 ± 0.2	3.4 ± 0.1	3.5 ± 0.2	3.5 ± 0.0	3.7 ± 0.1	4.0 ± 0.2	3.4 ± 0.0	3.4 ± 0.2
6	4.2 ± 0.3	4.1 ± 0.1	4.5 ± 0.4	3.6 ± 0.2	4.7 ± 0.1	4.6 ± 0.3	3.8 ± 0.3	4.0 ± 0.2	4.6 ± 0.2	3.8 ± 0.4	3.9 ± 0.1	4.1 ± 0.3	3.5 ± 0.1	3.9 ± 0.2	4.6 ± 0.2
7	3.4 ± 0.1	3.3 ± 0.1	3.7 ± 0.3	3.4 ± 0.2	3.4 ± 0.1	3.4 ± 0.1	3.8 ± 0.2	3.8 ± 0.2	3.7 ± 0.1	3.4 ± 0.0	3.5 ± 0.0	4.0 ± 0.4	3.8 ± 0.3	3.4 ± 0.0	3.3 ± 0.1
8	3.2 ± 0.0	3.0 ± 0.2	3.7 ± 0.1	3.0 ± 0.1	3.4 ± 0.1	3.4 ± 0.0	3.3 ± 0.1	2.9 ± 0.1	3.2 ± 0.1	3.5 ± 0.2	3.1 ± 0.0	3.3 ± 0.0	3.4 ± 0.1	3.2 ± 0.1	3.4 ± 0.1
9	3.4 ± 0.0	3.9 ± 0.2	3.8 ± 0.1	3.2 ± 0.0	3.4 ± 0.1	3.5 ± 0.3	3.6 ± 0.1	3.5 ± 0.2	3.5 ± 0.1	3.8 ± 0.3	3.4 ± 0.1	3.3 ± 0.1	3.3 ± 0.0	3.4 ± 0.1	3.7 ± 0.1
10	5.7 ± 0.2	4.3 ± 0.2	5.2 ± 0.1	5.7 ± 0.1	5.3 ± 0.3	6.0 ± 0.3	5.5 ± 0.2	5.2 ± 0.3	5.0 ± 0.1	4.9 ± 0.2	5.3 ± 0.1	4.6 ± 0.5	4.5 ± 0.1	5.4 ± 0.1	5.2 ± 0.3
11	30.2 ± 1.8	32.9 ± 1.6	30.6 ± 1.2	32.7 ± 1.1	27.7 ± 1.4	31.4 ± 1.5	32.6 ± 1.5	30.1 ± 2.1	30.1 ± 1.3	30.3 ± 1.0	33.1 ± 1.1	32.3 ± 1.2	30.3 ± 1.1	32.0 ± 1.8	28.7 ± 2.1
12	3.5 ± 0.1	3.6 ± 0.2	3.3 ± 0.1	3.5 ± 0.2	3.3 ± 0.1	3.4 ± 0.2	3.5 ± 0.1	3.5 ± 0.2	3.1 ± 0.2	3.3 ± 0.0	3.4 ± 0.0	3.6 ± 0.0	3.5 ± 0.0	3.3 ± 0.0	3.4 ± 0.1

1, 2 = violaxanthin (416, 439, 469 nm); 3, 5, 6 = luteoxanthin isomer (399, 422, 448 nm); 4 = 9-cis-violaxanthin (412, 436, 464 nm); 7 = zeaxanthin (421, 451, 478 nm); 8 = not identified (418, 442, 470 nm); 9 = all-trans-β-cryptoxanthin (422, 445, 478 nm); 10 = 13-cis-β-carotene (338, 445, 471 nm); 11 = all trans β-carotene (420, 452, 473 nm); and 12 = 9-cis β-carotene (424, 446, 472 nm)

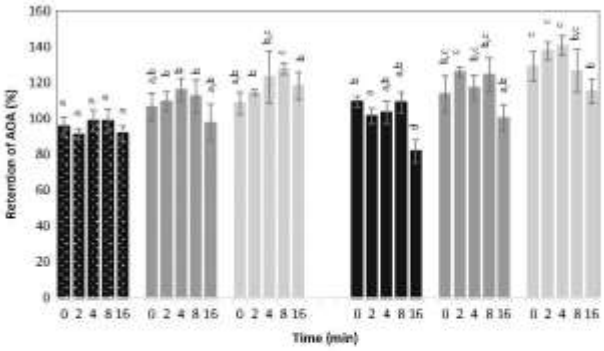


Fig. 5 Effects of HHP treatments on the retention of antioxidant activity of mango puree with respect to the untreated product. *Bars with the same letters* indicate no significant differences ($p > 0.05$). *Dotted bars* = 34 °C, *solid bars* = 59 °C, *black bars* = 400 MPa, *dark gray bars* = 450 MPa, *light gray bars* = 550 MPa

of retention (87%) was obtained at the highest pressure level (550 MPa) during 4–16 min, while at lower times of treatment, an average retention of 97% was achieved. There was no clear effect of time on the percentage of carotenoids retained at 34 °C and pressures of 400 and 450 MPa.

Tables 1 and 2 show the percentage of retention of individual carotenoids of HHP-treated puree. In general, the proportion of each carotenoid with respect to the untreated purees remains unchanged, with the exception of violaxanthin, which was reduced from 21 to 26%, and 9-cis-violaxanthin, which increased about 10%. This indicated that part of the concentration of violaxanthin was isomerized to 9-cis-violaxanthin, while some proportion was degraded to other types of compounds. Studies performed by Ma et al. (2009) and Sánchez-Moreno et al. (2006) demonstrated changes in the content of some individual carotenoids like β-carotene, α-carotene, β-criptoxantin, α-criptoxantin, zeaxantina, and lutein after HHP treatments.

Effect of HHP on Antioxidant Activity

Unprocessed puree mango presented 885 μmol TE/100 g of AOA; this activity could be mainly related with compounds of polar nature like phenolics and vitamin C. Similar AOA values have been previously reported for mango (Kim et al. 2009; Mahattanatawee et al. 2006; Sogi et al. 2012; Zapata et al. 2014). The HHP treatments had diverse effect on the AOA of puree mango. The AOA of most samples treated at 400 MPa was not affected by the treatments independent of the time and temperature, while at 450 and 550 MPa, it increased about 5–39%. These results coincide with the behavior of total phenolic under HHP, in which not significant differences with respect to the control were observed at 400 MPa, while at 450 and 550 MPa, higher phenolic concentrations were quantified. The highest increases of AOA were observed at 550 MPa/59 °C during CUT 4 min, while at higher times, the AOA started to decrease due to the higher times of exposure to temperature. At 400 MPa/59 °C/16 min, AOA was reduced by 18%, but there is no explanation for the reduction observed at these particular conditions (Fig. 5). A linear relationship between the AOA and the content of phenolics was obtained (R^2 of 0.88), confirming that the AOA is highly related with the concentration of phenolics obtained. A correlation between the vitamin C content and the AOA was not found.

Conclusions

Mango *Tommy Atkins* is a good source of vitamin C and carotenoids. Mango contains about 41% of vitamin A precursors with proven health beneficial effects. Most HHP-processing conditions and CUT treatments caused increases in the content of phenolics and AOA of puree mango, while vitamin C and carotenoids were negatively affected at 59 °C due to their sensitivity to temperature. Vitamin C content was increased at 34 °C for times up to 4 min, showing increases up to 20%. The highest contents on functional compounds obtained at some processing conditions could be attributed to cellular disruption causing a better extraction for quantification, which is important because it could increase their bioavailability in the human body, but more studies are necessary to confirm these hypotheses. HHP at 550 MPa combined with moderate temperature (34 °C) up to 8 min can be used to obtain the maximum retention or even enhance the functional compound contents and the antioxidant activity of mango puree.

Acknowledgements Authors acknowledge the financial support from Tecnologico de Monterrey (Research Chair Funds CAT 200 and CDB081) and CONACYT-SEP (Research Project 101700 and Scholarship Program).

References

AOAC. (1990a). Official method 934.06. Moisture in dried fruits. In Official methods of analysis, Vol. 2, Food composition, additives, natural contaminants (15th ed.) (pp. 912). Arlington, VA, USA: *Association of Official Analytical Chemists*.

AOAC (1990b). Official method 942.15. Acidity (titratable) of fruit products. Glass electrode method. In Official methods of analysis, Vol. 2, Food composition, additives, natural contaminants (15th ed.) (pp. 918). Arlington, VA, USA: *Association of Official Analytical Chemists*.

Barba, F. J., Esteve, M. J., & Frigola, A. (2010). Ascorbic acid is the only bioactive that is better preserved by high hydrostatic pressure than by thermal treatment of a vegetable beverage. *Journal of Agricultural and Food Chemistry, 58*, 10070–10075.

Barba, F. J., Jäger, H., Meneses, N., Esteve, M. J., Frigola, A., & Knorr, D. (2012). Evaluation of quality changes of blueberry juice during refrigerated storage after high-pressure and pulsed electric fields processing. *Innovative Food Science & Emerging Technologies, 14*, 18–24.

Brecht, J. K., Sargent, S. A., Kader, A. A., Mitcham, E. J., Maul, F., Brecht, P. E., & Menocal, O. (2014). *Manual de prácticas para el mango poscosecha del mango*. Orlando: UF IFAS Extension.

Castro, S. M., Saraiva, J. A., Domingues, F. M. J., & Delgadillo, I. (2011). Effect of mild pressure treatments and thermal blanching on yellow bell peppers (*Capsicum annuum* L.). *Lebensmittel Wissenschaft und Technologie, 44*, 363–369.

Castro, S. M., Saraiva, J. A., Lopes-da-Silva, J. A., Delgadillo, I., van Loey, A., Smout, C., & Hendrickx, M. (2008). Effect of thermal blanching and of high pressure treatments on sweet green and red bell pepper fruits (*Capsicum annuum* L.). *Food Chemistry, 107*, 1436–1449.

Escobedo-Avellaneda, Z., Gutiérrez-Uribe, J., Valdez-Fragoso, A., Torres, J. A., & Welti-Chanes, J. (2014). Phytochemicals and antioxidant activity of juice, flavedo, albedo and comminuted orange. *Journal of Functional Foods, 6*, 470–481.

Escobedo-Avellaneda, Z., Gutiérrez-Uribe, J., Valdez-Fragoso, A., Torres, J. A., & Welti-Chanes, J. (2015). High hydrostatic pressure combined with mild temperature for the preservation of comminuted orange: effects on functional compounds and antioxidant activity. *Food and Bioprocess Technology: An International Journal, 8*(5), 1032–1044.

Ferrari, G., Maresca, P., & Ciccarone, R. (2010). The application of high hydrostatic pressure for the stabilization of functional foods: pomegranate juice. *Journal of Food Engineering, 100*(2), 245–253.

Hernández-Brenes, C., Ramos-Parra, P. A., Jacobo-Velázquez, D. A., Villarreal-Lara, R., & Díaz-De la Garza, R. I. (2013). High hydrostatic pressure processing as a strategy to increase carotenoid contents of tropical fruits. In B. S. Patil, G. K. Jayaprakasha, C. Osorio Ros, & K. Muhattumtawee (Eds.), *Tropical and subtropical fruits: flavors, color, and health benefits (pp. 29–42)*. American Chemical Society.

Houška, M., Strohalm, J., Kocurová, K., Totušek, J., Lešnerová, D., Tříska, J., Vrchotová, N., Fiedrleová, V., Holasova, M., Gabrovská, D., & Pauličková, I. (2006). High pressure and foods fruit/vegetable juices. *Journal of Food Engineering, 77*(3), 386–398.

Jayachandran, L. E., Chakraborty, S., & Rao, P. S. (2015). Effect of high pressure processing on physicochemical properties and bioactive compounds in litchi based mixed fruit beverage. *Innovative Food Science & Emerging Technologies, 28*, 1–9.

Kaushik, N., Kaur, B. P., Rao, P. S., & Mishra, H. N. (2014). Effect of high pressure processing on color, biochemical and microbiological characteristics of mango pulp (*Mangifera indica* cv. Amrapali). *Innovative Food Science & Emerging Technologies, 22*, 40–50.

Kim, Y., Lounds-Singleton, A. J., & Talcott, S. T. (2009). Antioxidant phytochemical and quality changes associated with hot water immersion treatment of mangoes (*Mangifera indica* L.). *Food Chemistry, 115*(3), 989–993.

Krebbers, B., Matser, A., Koets, M., Bartels, P. V., & van den Berg, R. (2002). High pressure-temperature processing as an alternative for preserving basil. *High Pressure Research, 22*, 711–714.

Ma, Y., Xu, L., Shanggaun, L., & Li, X. (2009). Effect of ultra-high pressure physical energy on carotenoid isomers in carrot juice. *Food Science, 29*(10), 105–108.

Muhattanatawee, K., Manthey, J., Luzio, G., Talcott, S. T., Goodner, K., & Baldwin, E. A. (2006). Total antioxidant activity and fiber content of select Florida-grown tropical fruits. *Journal of Agricultural and Food Chemistry, 54*, 7355–7363.

Manthey, J. A., & Perkins-Veazie, P. (2009). Influences of harvest date and location on the levels of beta-carotene, ascorbic acid, total phenols, the in vitro antioxidant capacity, and phenolic profiles of five commercial varieties of mango (*Mangifera indica* L.). *Journal of Agricultural and Food Chemistry, 57*(22), 10825–10830.

Mercadante, A. Z., & Rodriguez-Amaya, D. B. (2001). Confirmação da identidade da alfa-criptoxantina e incidencia de carotenóides minoritários provitamínicos a em verduras folhosas verdes. *Ciencia e Tecnologia de Alimentos, 21*(2), 216–222.

Mercadante, A. Z., Rodriguez-Amaya, D. B., & Britton, G. (1997). HPLC and mass spectrometric analysis of carotenoids from mango. *Journal of Agricultural and Food Chemistry, 45*(1), 120–123.

Mesa-Vanegas, M., Gaviria, C., Cardona, F., Sáez-Vega, J., Trujillo, S. B., & Rojano, B. (2010). Antioxidant activity and total phenols content from some species of *Calophyllum* genus. *Revista Cubana de Plantas Medicinales, 15*(2), 13–26.

Lin, F., Wang, Y., Li, R., Bi, X., & Liao, X. (2014). Effects of high hydrostatic pressure and high temperature short time on antioxidant activity, antioxidant compounds and color of mango nectars. *Innovative Food Science & Emerging Technologies, 21*, 35–43.

Oey, I., Lille, M., Van Loey, A., & Hendrickx, M. (2008). Effect of high-pressure processing on colour, texture and flavour of fruit- and vegetable-based food products: a review. *Trends in Food Science & Technology, 19*(6), 320–328.

Oliveira, D. D. S., Lobato, A. L., Ribeiro, S. M. R., Santana, A. M. C., Chaves, J. B. P., & Pinheiro Sant'Ana, H. M. (2010). Carotenoids and vitamin C during handling and distribution of guava (*Psidium guajava* L.), mango (*Mangifera indica* L.), and papaya (*Carica papaya* L.) at commercial restaurants. *Journal of Agricultural and Food Chemistry, 58*(10), 6166–6172.

Palafox-Carlos, H., Ayala-Zavala, J. F., & González-Aguilar, G. A. (2011). The role of dietary fiber in the bioaccessibility and bioavailability of fruit and vegetable antioxidants. *Journal of Food Science, 76*(1), R6–R15.

Pott, I., Marx, M., Neidhart, S., Mühlbauer, W., & Carle, R. (2003). Quantitative determination of beta-carotene stereoisomers in fresh, dried, and solar-dried mangoes (*Mangifera indica* L.). *Journal of Agricultural and Food Chemistry, 51*(16), 4527–4531.

Queiroz, C., Moreira, C. F. F., Lavinas, F. C., Lopes, M. L. M., Fialho, E., & Valente-Mesquita, V. L. (2010). Effect of high hydrostatic pressure on phenolic compounds, ascorbic acid and antioxidant activity in cashew apple juice. *High Pressure Research, 30*(4), 507–513.

Quirós-Sauceda, A. E., Palafox-Carlos, H., Sáyago-Ayerdi, S. G., Ayala-Zavala, J. F., Bello-Perez, L. A., Álvarez-Parrilla, E., de la Rosa, L. A., González-Córdova, A. F., & González-Aguilar, G. A. (2014). Dietary fiber and phenolic compounds as functional ingredients: interaction and possible effect after ingestion. *Food & Function, 5*(6), 1063–1072.

Saldo, J., Suárez-Jacobo, Á., Gervilla, R., Guamis, B., & Roig-Sagués, A. X. (2009). Use of ultra-high-pressure homogenization to preserve apple juice without heat damage. *High Pressure Research, 29*(1), 52–56.

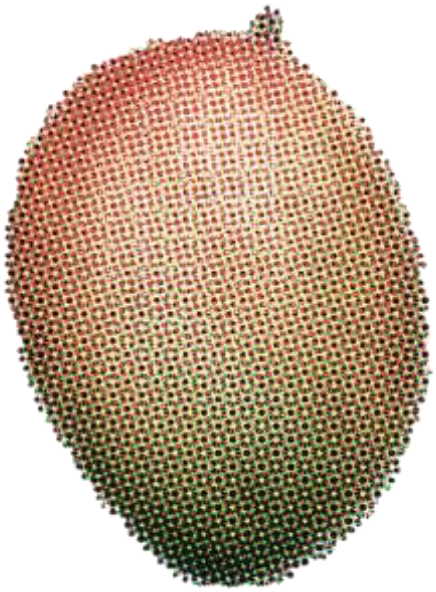
Sánchez-Moreno, C., Plaza, L., de Ancos, B., & Cano, M. P. (2006). Impact of high-pressure and traditional thermal processing of tomato purée on carotenoids, vitamin C and antioxidant activity. *Journal of the Science of Food and Agriculture, 86*(2), 171–179.

Serment-Moreno, V., Barbosa-Cánovas, G., Torres, J. A., & Welti-Chanes, J. (2014). High-pressure processing: kinetic models for microbial and enzyme inactivation. *Food Engineering Reviews, 6*(3), 56–88.

Sogi, D. S., Siddiq, M., Roidoung, S., & Dolan, K. D. (2012). Total phenolics, carotenoids, ascorbic acid, and antioxidant properties of fresh-cut mango (*Mangifera indica L., cv.* Tommy Atkin) as affected by infrared heat treatment. *Journal of Food Science, 77*(11), C1197–C1202.

Tejada-Ortigoza, V., Escobedo-Avellaneda, Z., Valdez-Fragoso, A., Mújica-Paz, H., & Welti-Chanes, J. (2015). Combined effect of high hydrostatic pressure and mild heat treatments on pectin methylesterase (PME) inactivation in comminuted orange. *Journal of the Science of Food and Agriculture, 95*(12), 2438–2444.

Zapata, S., Piedrahita, A. M., & Rojano, B. (2014). Capacidad atrapadora de radicales oxígeno (ORAC) y fenoles totales de frutas y hortalizas de Colombia. *Perspectivas en Nutrición Humana, 16*, 25–36.



ABOUT THE AUTHOR

ABOUT THE AUTHOR

blancasalinasroca@gmail.com
@blancasalroc

Blanca Salinas-Roca, was born on 29th of August 1985 in Lleida (Spain). After finishing her secondary school in her hometown in 2004, Blanca moved to Barcelona (Spain) to study human nutrition and dietetics with focus on food choises and its impact on human health at Hospital Universitari de Bellvitge (Barcelona) interest on food science. Thereafter, Blanca began in Universitat Autònoma de Barcelona (UAB) the studies of Food Science and Technology with emphasis on food composition and novel product developments as a research assistant in Food Technology Department (UAB). In her last year of her Bachelor studies, she moved to Brussels (Belgium) for a stage and she wrote her BSc thesis during a placement in the Research and Development Department of The Coca-Cola Company. Blanca graduated from her BSc in 2009. Thereafter, she enrolled for a Masters in Food and Agriculture Research in University of Lleida (Spain) and performed her MSc thesis about carotenoids content in dryed maize. After obtaining her MSc degree in 2011 and having gotten a taste for research, she was appointed as a PhD candidate at Novel Food Technology research group in a project involving University of Lleida and Instituto Tecnológico de Monterrey. Throughout the PhD programme, she took part on international workshops and conferences. Moreover, she had contributions in book publications and scientific articles.

Cover: Pau Salinas Roca and Blanca Salinas-Roca

Layout: Blanca Salinas-Roca

Printing: Arts Gràfiques Bobalà