Universitat de Lleida

Evaluation of compositional changes in pecan nuts during storage and the use of pulsed electric fields as an aid to enhance oil extraction yield

Lourdes Melisa Rábago Panduro

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Universitat de Lleida

TESI DOCTORAL

Evaluation of compositional changes in pecan nuts during storage and the use of pulsed electric fields as an aid to enhance oil extraction yield

Lourdes Melisa Rábago Panduro

Memòria presentada per optar al grau de Doctor per la Universitat de Lleida
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Evaluation of compositional changes in pecan nuts during storage and the use of pulsed electric fields as an aid to enhance oil extraction yield

A dissertation presented by

Lourdes Melisa Rábago Panduro

Submitted to the
School of Engineering and Sciences
in partial fulfillment of the requirements for the degree of

Doctor

in

Biotechnology

Monterrey Nuevo León, December 15th, 2020

Universitat de Lleida

TECNOLÓGICO DE MONTERREY

Universitat de Lleida
Doctoral School

in collaboration with

Instituto Tecnológico y de Estudios Superiores de Monterrey
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Doctorat en Ciència i Tecnologia Agrària i Alimentària
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The committee members, hereby, certify that have read the dissertation presented by Lourdes Melisa Rábago Panduro and that it is fully adequate in scope and quality as a partial requirement for the degree of *Doctorat en Ciència i Tecnologia Agrària i Alimentària* and *Doctor in Biotechnology*,

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Declaration of Authorship

I, Lourdes Melisa Rábago Panduro, declare that this dissertation titled, *Evaluation of compositional changes in pecan nuts during storage and the use of pulsed electric fields as an aid to enhance oil extraction yield* and the work presented in it are my own. I confirm that:

- This work was done wholly or mainly while in candidature for a research degree at this University.
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- I have acknowledged all main sources of help.
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_____

Lourdes Melisa Rábago Panduro

Monterrey Nuevo León, December 15th, 2020

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Dedication

A quienes confiaron en mí.

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Evaluation of compositional changes in pecan nuts during storage and the use of pulsed electric fields as an aid to enhance oil extraction yield

by

Lourdes Melisa Rábago Panduro

Resumen

La almendra de nuez pecanera es una fuente importante de ácidos grasos mono- y poliinsaturados, fitoesteroles, tocoferoles y compuestos fenólicos. Asimismo, el aceite extraído a partir del prensado de la almendra es considerado un producto con alto potencial funcional debido a su perfil fitoquímico. Sin embargo, el efecto del secado y almacenamiento sobre el contenido de compuestos bioactivos de la almendra de nuez pecanera, así como el mejoramiento de la extracción mecánica de su aceite han sido poco investigados. Una alternativa para mejorar la extracción mecánica del aceite de nuez pecanera es la tecnología de campos eléctricos pulsados (CEP). Esta tecnología se ha empleado como pretratamiento de diferentes semillas incrementando el rendimiento de extracción de aceite (REA) y su contenido de compuestos bioactivos. Por lo tanto, los objetivos de este trabajo de investigación fueron evaluar 1) el efecto del secado y las condiciones de almacenamiento sobre los fitoquímicos y la capacidad antioxidante (CA) de la nuez pecanera y; 2) la aplicación de CEP como pretratamiento para mejorar el REA de la nuez pecanera, así como el efecto de esta tecnología sobre la concentración de compuestos bioactivos en el aceite y en el subproducto también denominado torta.

Para evaluar el efecto del secado sobre la nuez pecanera, se cuantificó el contenido de ácidos grasos, tocoferoles, compuestos fenólicos (CF) y la CA de almendras frescas y secas. El efecto de las condiciones de almacenamiento se investigó monitoreando la concentración de CF y la CA de almendras y nueces con cáscara almacenadas durante 240 días a 4 y 25°C. La evaluación de la aplicación de CEP como pretratamiento se realizó en nuez fresca y seca comparando las almendras tratadas con CEP contra almendras sin tratar y remojadas. Las almendras frescas se trataron a diferentes niveles de energía específica (0.5–17.6 $kJ \cdot kg^{-1}$) y se determinó el REA_{TOTAL}, la acidez y CA del aceite, seguido de la concentración de CF y CA en la torta. En el caso de la nuez seca, los niveles de energía específica aplicados fueron 0.8, 7.8 y 15.0 $kJ \cdot kg^{-1}$ evaluando el REA_{TOTAL} y la microestructura de la almendra. La estabilidad del aceite extraído se analizó mediante la determinación de la acidez, CA, índice de estabilidad oxidativa (IEO), contenido de fitoesteroles y tocoferoles, además de la

actividad enzimática de la lipoxigenasa (LOX). En la torta se evaluó la concentración de CF y CA.

En la primera parte de este trabajo de investigación se observó que el proceso de secado redujo la concentración inicial de CF y CA (33.2 y 22.3%, respectivamente), pero ayudó a conservar las almendras por 240 días sin problemas de deterioro microbiano. Durante el almacenamiento, la concentración de taninos condensados (TC) disminuyó (31.5–41.8%), mientras que la CA se incrementó significativamente (188.4–216.4%) tanto en almendras como en nueces con cáscara. En la segunda parte de la investigación se determinó que el remojo de almendras frescas necesario para la aplicación de CEP provocó una disminución del contenido inicial de aceite (7.3–11.7%). No obstante, el mejor REA_{TOTAL} se alcanzó en las almendras tratadas a 0.5 $kJ \cdot kg^{-1}$ siendo 21.4 y 17.6% mayor que el rendimiento de las almendras sin tratar y remojadas, respectivamente. La aplicación de 0.8 $kJ \cdot kg^{-1}$, en las almendras incrementó la concentración de CF y TC en la torta en comparación con las tortas de almendras sin tratar (9.5 y 30.1%, respectivamente) y remojadas (17.8 y 39.3%, respectivamente). A pesar de este incremento, el pretratamiento con CEP no aumentó la CA de la torta. En relación con las almendras secas tratadas con CEP, también se observó la reducción del contenido inicial de aceite debido al remojo (9.7%). El REA de las almendras sin tratar fue de 88.7%, siendo mayor que el REA_{TOTAL} de las almendras remojadas (76.5–83.0%) y las tratadas con CEP (79.8–85.0%). El análisis de la microestructura de las almendras demostró que la aplicación de 0.8 $kJ \cdot kg^{-1}$ produjo la fusión de los oleosomas dentro del espacio intracelular en el tejido cotiledón de las almendras. No se observaron diferencias entre los valores de acidez, CA e IEO de los aceites extraídos, ni de la actividad de la LOX en las almendras. Mientras que la aplicación de 0.8 $kJ \cdot kg^{-1}$ aumentó la concentración de TC y CA de la torta en comparación con las tortas de las almendras sin tratar (43.5 y 21.8%, respectivamente) y remojadas (27.0 y 24.3%, respectivamente).

Esta tesis doctoral evidenció que tanto el contenido de CF como la CA de la nuez pecanera son alterados por el proceso de secado y el tiempo de almacenamiento lo que podría repercutir en las propiedades funcionales de este alimento. Respecto a la aplicación de CEP para incrementar el REA de la nuez pecanera, es necesario considerar la recuperación del aceite extraído en el agua de remojo. Además, la

efectividad de la tecnología de CEP fue dependiente de la nuez pecanera utilizada incrementando el REA_{TOTAL} en nuez fresca pero no en nuez seca. El análisis microestructural demostró que el tratamiento con CEP modifica la estructura de los oleosomas de las almendras. En cuanto al aceite y la torta, la aplicación de CEP no modificó las características del aceite e incrementó la concentración de TC de la torta aumentando su potencial funcional para considerarse como ingrediente en formulaciones alimentarias.

Palabras clave: **Nuez pecanera, compuestos bioactivos, capacidad antioxidante, almacenamiento, campos eléctricos pulsados, rendimiento de extracción de aceite, aceite, torta**

Resum

Les nous pacanes són una font important d'àcids grassos mono- i poliinsaturats, fitosterols, tocoferols i compostos fenòlics. Així mateix, l'oli de nou pacana, extret habitualment per premsat mecànic, es considera un oli funcional pel seu perfil fitoquímic. Tot i això, l'efecte del processament i emmagatzematge en els compostos bioactius de la nou pacana i la manera de millorar l'extracció mecànica de l'oli de nou pacana han estat poc investigats. La tecnologia de polsos elèctrics (PE) s'està aplicant com a pretractament per millorar l'extracció d'oli de diferents llavors augmentant el rendiment d'extracció d'oli (REO) i millorant el seu perfil fitoquímic. Per tant, l'objectiu d'aquesta tesi va ser avaluar 1) l'efecte de l'assecatge i de les condicions d'emmagatzematge sobre el contingut fitoquímic i la capacitat antioxidant (CA) de la nou pacana i 2) l'aplicació de PE com a pretractament de la nou pacana per millorar l'REO així com el seu efecte sobre l'estabilitat de l'oli i la concentració de compostos bioactius de l'oli i el subproducte generats.

Els àcids grassos, tocoferols, compostos fenòlics (CF) i CA de les nous fresques i seques es van determinar per avaluar l'efecte de l'assecat sobre les nous pacanes, mentre que, CF i CA de les nous sense closca i amb closca van ser emmagatzemats a 4 i 25° C durant 240 dies per estudiar l'efecte de les condicions d'emmagatzematge. L'aplicació de PE es va avaluar sobre les nous fresques i seques, comparant les nous tractades amb PE contra les nous no tractades i les nous remullades. Les nous fresques van ser tractades a diferent nivells d'energia específica (0.5–17.6 $kJ \cdot kg^{-1}$) per avaluar REO_{TOTAL}, l'acidesa i CA de l'oli juntament amb CF i CA del subproducte. Les nous seques es van tractar a 0.8, 7.8 i 15.0 $kJ \cdot kg^{-1}$ per a avaluar REO_{TOTAL} i la microestructura de la nou. L'estabilitat de l'oli de la nou pacana es va valorar mitjançant la determinació d'acidesa, CA, índex d'estabilitat de l'oli (IEO), fitosterols, tocoferols i activitat enzimàtica de la lipoxigenasa (LOX). Els CF i la CA del subproducte també es van analitzar.

Els resultats van demostrar que l'assecat va reduir la concentració inicial de CF i CA (33.2 i 22.3%, respectivament), però va contribuir a preservar les nous pacanes durant 240 dies sense contaminació microbiana. Durant l'emmagatzematge, es va observar una disminució de la concentració de tanins condensats (TC) (31.5–41.8%) i un increment significatiu de CA (188.4–216.4%) tant de la nou sense closca i amb closca.

Pel que fa a l'aplicació de PE a les nous fresques, el remull va disminuir el contingut inicial d'oli de la nou remullada i la tractada amb PE (7.3–11.7%). Tot i això, el REO_{TOTAL} més alt es va obtenir amb el pretractament de 0.5 $kJ \cdot kg^{-1}$, sent un 21.4 i un 17.6% superior als valors de les nous sense tractar i les remullades, respectivament. Es va observar un augment significatiu els CF i la TC en el subproducte de les nous tractades a 0.8 $kJ \cdot kg^{-1}$ en comparació dels subproductes obtinguts de les nous no tractades (9.5 i 30.1%, respectivament) i les remullades (17.8 i 39.3%, respectivament). Tot i la millora dels CF, el pretractament amb PE no va augmentar la CA dels subproductes. Les nous seques tractades amb PE també van presentar una reducció del contingut inicial d'oli (9.7%). El REA de les nous sense tractar va ser de 88.7%, sent més gran que el REA_{TOTAL} de les nous remullades (76.5–83.0%) i les tractades amb CEP (79.8–85.0%). L'anàlisi microestructura de les nous tractades a 0.8 $kJ \cdot kg^{-1}$ va demostrar que el processament de PE va produir la fusió dels oleosomas dins de l'espai intracel·lular. No es van observar diferències entre els resultats d'acidesa, CA i IEP els olis extrets ni de l'activitat LOX en la nou. Mentre que, l'aplicació de 0.8 $kJ \cdot kg^{-1}$ va augmentar TC i CA del subproducte en comparació amb les nous no tractades (43.5 i 21.8%, respectivament) i les nous remullades (27.0 i 24.3%, respectivament).

Aquesta tesi va demostrar que CF i CA de les nous pacanes tenen molta relació amb l'assecat i temps d'emmagatzematge, evidenciant el seu impacte en les seves propietats funcionals. Pel que fa al tractament amb PE, la immersió de les nous en aigua per a l'aplicació de PE va disminuir el contingut inicial d'oli tant en les nous fresques com seques, demostrant que cal tenir en compte l'oli extret en l'aigua de remull. A més, l'efectivitat de la tecnologia de PE va ser depenent de la nou pacana utilitzada incrementant el REA_{TOTAL} a nou fresca però no en nou seca. L'anàlisi microestructura va demostrar que l'aplicació de PE modifica els oleosomas de les nous pacanes. Pel que fa a l'oli i el subproducte, l'aplicació de PE no va modificar les característiques de l'oli i va incrementar la concentració de TC del subproducte augmentant el seu potencial funcional per considerar-se com a ingredient en formulacions alimentàries.

Paraules clau: **Nous pacanes, compostos bioactius, capacitat antioxidant, emmagatzematge, polsos elèctrics, rendiment d'extracció d'oli, oli, subproducte**

Abstract

Pecan nut kernels have been identified as an important source of mono- and polyunsaturated fatty acids, phytosterols, tocopherols, and phenolic compounds. Likewise, the oil commonly extracted by mechanical pressing of pecan nut kernels, is considered a specialty product due to its phytochemical profile. However, the effect of processing and storage on these bioactive compounds and how to improve the mechanical extraction of pecan nut oil have been scarcely investigated. Pulsed electric fields (PEF) are being applied as a pretreatment to improve oil extraction from different seeds increasing the oil extraction yield (OEY) and enhancing the oil phytochemical profile. Therefore, this dissertation aimed to evaluate i) the effect of drying and storage conditions on phytochemical content and antioxidant capacity (AC) of pecan nuts, and ii) the application of PEF as a pecan nuts pretreatment to improve OEY along with its effect on oil stability and bioactive compounds concentration of the oil and by-product generated usually named cake.

Fatty acids, tocopherols, phenolic compounds (PC), and AC of fresh and dry kernels were determined to assess the effect of drying on pecan nuts. PC and AC of kernels and in-shells nuts stored during 240 days at 4 and 25°C were analyzed to study the effect of storage conditions. PEF was evaluated on fresh and dry pecan nuts by comparison of PEF-treated kernels against untreated and soaked kernels. Fresh kernels were pretreated at different levels of specific energy inputs (0.5–17.6 $kJ \cdot kg^{-1}$) to evaluate OEY_{TOTAL}, oil acidity and AC along with cake PC and AC. Dry kernels were pretreated at 0.8, 7.8, and 15.0 $kJ \cdot kg^{-1}$ to assess OEY_{TOTAL} and kernels microstructure. Oil stability was investigated by determination of acidity, AC, oil stability index (OSI), phytosterols, tocopherols, and lipoxygenase (LOX) activity. Cake PC and AC were also analyzed.

The results showed that the drying process reduced the initial concentration of PC and AC (33.2 and 22.3%, respectively), but it helped to preserve pecan nut kernels for 240 days without microbial deterioration. During storage, a decrement of condensed tannins (CT) concentration (31.5–41.8%) and an increment of AC (188.4–216.4%) on both kernels and in-shell nuts were observed. Regarding the evaluation of PEF, water immersion of fresh kernels to PEF pretreatment decreased the initial oil content (7.3–11.7%). Despite that, the highest OEY_{TOTAL} was achieved in kernels pretreated at 0.5

kJ·kg^{-1}, being 21.4 and 17.6% higher than values of untreated and soaked kernels, respectively. A rise of PC content was observed in cakes generated from kernels pretreated at 0.8 kJ·kg^{-1} in comparison with cakes from untreated and soaked kernels (9.5–17.8 and 30.1–39.3% for PC and CT, respectively). Despite PC improvement, the PEF pretreatment did not increase the AC of cakes. Dry kernels water immersion also caused a reduction of the initial oil content (9.7%). OEY of untreated kernels was 88.7%, being higher than OEY_{TOTAL} of soaked (76.5–83.0) and PEF-treated kernels (79.8–85.0%). The microstructural analysis of kernels pretreated at 0.8 kJ·kg^{-1} showed that PEF processing induced oleosomes fusion within the intracellular space of kernels cotyledon tissue. Neither soaking nor PEF processing significantly modified oil acidity, AC, OSI, and LOX activity.In contrast, the application of 0.8 kJ·kg^{-1} to dry kernels increased cake CT and AC by 43.5 and 21.8%, respectively, compared to untreated kernels while in comparison with control kernels was 27.0 and 24.3% higher, respectively.

This dissertation proved that PC and AC of kernels are highly susceptible to drying and storage time, evidencing their impact on the functional properties of pecan nuts. Concerning pretreatment with PEF, it was demonstrated that it is necessary to take into consideration the recovery of the oil retained into the soaking water. Furthermore, the effectiveness of PEF to improve OEY_{TOTAL} depended on whether the pretreatment was applied to fresh or dry pecan nuts. The microstructural analysis showed that the application of PEF modifies kernels oleosomes. Oil extracted from PEF-treated kernels displayed comparable characteristics to pecan nut oil extracted by mechanical and solvent processes. The by-product generated from PEF-treated kernels exhibited an improved CT concentration, making it an attractive ingredient to considerate in food formulations.

Keywords: **Pecan nuts, bioactive compounds, antioxidant capacity, storage, pulsed electric fields, oil extraction yield, oil, cake**

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Chapter 1. Introduction

Chapter 4. Results

Chapter 1

Introduction

Pecan nuts [*Carya illinoinensis* (Wangenh. K. Koch)] are endemic of North America, being Mexico and the United States their main producers (International Nut and Dried Fruit Council 2019). Their kernels are considered an important source of health-related compounds, the lipid fraction is composed by oleic and linoleic acids, γ-tocopherol, α-tocopherol, β-sitosterol, campesterol, and stigmasterol. In addition, the non-lipid fraction contains phenolic compounds such as catechin, epicatechin, proanthocyanidins, and hydrolysable tannins (Chang et al. 2016; Jia et al. 2018; U.S. Department of Agriculture 2017; Villarreal-Lozoya et al. 2007). Consumption of pecan nut kernels has been associated with the reduction of cardiovascular disease risk along with maintenance of body weight and body mass index, while their contribution to the prevention and management of diabetes has been recently demonstrated (Atanasov et al. 2018; Hudthagosol et al. 2011; Kendall et al. 2011; McKay et al. 2018; Santos et al. 2019).

Research concerning pecan nuts has been mainly focused on kernels phytochemical characterization (Flores-Córdova et al. 2017; Robbins et al. 2014) without giving attention to the effects that conservation and storage processes might have on kernels phytochemical quality and stability. The few works related to conservation and storage of pecan nuts have been conducted to evaluate harvest day, roasting, and oil reduction effects on sensory properties of pecan nut kernels throughout storage (Erickson et al. 1994; Herrera 1994; Kanamangala et al. 1999). Likewise, other studies aimed to extend kernels shelf-life by different technologies but relating sensory properties to oil stability (Baldwin and Wood 2006; Oro et al. 2008). About pecan nut oil, Toro-Vazquez et al. (1999) studied oil composition reporting comparable fatty acids profile to olive and sesame oils while Fernandes et al. (2017) reported oleic acid, linoleic acid, and γ-tocopherol as its principal components. Other authors evaluated the effect of the extraction method on oil composition, stating that a high concentration of bioactive compounds is achieved by mechanical extraction (Al Juhaimi et al. 2018; Scapinello et al. 2017). Moreover, Maciel et al. (2020) and Sarkis et al. (2014) determined that the cake generated from oil extraction was rich in carbohydrates, proteins, dietary fiber, and

phenolic compounds. When mechanical oil extraction processes are used, seeds are pressed using a screw press or expeller with advantages such as low cost, simple use, and adaptability to a wide range of raw materials, but the low extraction yield is the major disadvantage (Çakaloğlu et al. 2018; Cockerham et al. 2012; Costa-Singh and Jorge 2015). Drying or enzymatic degradation have been applied as pretreatments to increase oil extraction yield (OEY), increasing the yields but decreasing the bioactive compounds profile of oils as well as altering their physicochemical properties (Jia, Liu, and Ma, 2019; Moussa-Ayoub et al. 2016).

Pulsed electric fields (PEF) is a non-thermal food processing technology based on the dielectric disruption of the cell membrane of microorganisms and foods. Cell exposure to high voltage electric field pulses causes destabilization of the cell membrane by ion accumulation leading to pore formation (Kotniket al. 2019; Kumari et al. 2018). Recently, PEF processing has been employed as a pretreatment to enhance oil and juice extraction from several fruits and vegetables, increasing extraction yield and bioactive compounds content without compromising sensory and physicochemical characteristics of extracted products (El Kantar et al. 2018; Silve et al. 2018; Tehrani et al. 2019). Abenoza et al. (2013) reported that the PEF application during olive oil extraction not only increased OEY by 14.1% but also reduced the temperature of the malaxation step from 26 to 15°C. Similarly, Veneziani et al. (2019) observed an increment in OEY and phenolic compounds concentration in PEF-treated olives while pumpkins pretreated by PEF before purée production contained a higher concentration of lutein, α-carotene, and β-carotene compared to compared to a control (47.1, 41.1, and 34.2%, respectively). Furthermore, PEF have also been used to enhance juice extraction from oranges (El Kantar et al. 2018) and to facilitate tomatoes peeling (Pataro et al. 2018) with bioactive compounds recovery as a side benefit, increasing 18.0 and 188.0% polyphenols and carotenoids extraction from orange and tomato peels, respectively. Manzoor et al. (2019) combined PEF and ultrasound technologies to improve phenolic compounds extraction from the non-lipid fraction of almonds, incrementing its extraction and antioxidant capacity by 33.3 and 41.7%.

Taking into consideration pecan nuts importance as a source of bioactive compounds and the gap of knowledge around how these compounds are affected by

storage conditions, their evaluation is considered crucial in order to direct research efforts towards this area. Likewise, the impact that pulsed electric fields might have to improve the extraction of pecan nut oil and the functional potential of the oil and cake could encourage further research helping the development of products derived from pecan nuts with improved health-benefits.

1.1 Challenges and benefits of using pecan nut kernels, derivatives, and by-products as alternative ingredients in food product development

Pecan [*Carya illinoinensis* (Wangenh. K. Koch)], is a deciduous tree of the *Juglandaceae* family with 20–30 m, 12–20 m and 2 m of height, crown diameter, and trunk circumference, respectively. It is one of the most economically valuable nut crop native from North America (Badenes and Byrne 2012; Grauke et al. 2016). According to the International Nut and Dried Fruit Council (2017), worldwide pecan nuts production for 2016–2017 period was about 118,213 Mt in kernel basis, being Mexico and the United States the main producers with 58,970 Mt and 52,400 Mt, respectively.

The pecan tree produces dehiscent fruits constituted by a shuck and a seed. The seed is divided in shell, middle septum, and kernel, as illustrated in Figure 1.1 (Janick and Paull 2008; Litz 2005; Woodroof and Chapman-Woodroof 1927). During pecan nuts development, biochemical and physical phenomena occur. The kernel, or edible part, starts to produce storage materials. Next, the shuck increases in thickness, and shell hardening begins. According to Byford (2005) and Santerre (1994), the optimum maturation point for pecan nuts harvesting is when the shuck splits into four sutures, having a moisture content of 7.0–12.0%. Immediately after harvesting, pecan nuts are washed, cleaned, dried up to approximately 2.0–4.0% of water content, visually graded, packaged, and stored (Figure 1.1b) (Alasalvar and Shahidi 2008; Doyle and Buchanan 2013; Rábago-Panduro et al. 2020).

Pecan nut kernels, which represent less than the 30.0% of the total mass of the whole nut (Idowu et al. 2017), have been characterized by a significant concentration of macro- and micronutrients, including proteins, polyphenols, and fiber in the non-lipid fraction, and; mono- and polyunsaturated fatty acids, tocopherols, and phytosterols in the lipid fraction. Scientific evidence suggests that these compounds might reduce the risk of non-communicable diseases (NCD) associated with oxidative stress (Caballero et al. 2016; Chang et al. 2016; Tokuşoğlu and Hall III 2011; USDA 2017; Villarreal-Lozoya et al. 2007). Usually, pecan nut kernels are consumed, either raw or roasted, in salads, desserts and as a premium ingredient in bakery goods, candies, and dairy products (Alasalvar and Shahidi 2008; Fernandes et al. 2017; Venkatachalam et al. 2007). Likewise, kernels have been used to oil extraction, which is currently catalogued as a

specialty product due to its unique sensory characteristics and functional profile (Alvarez-Parrilla et al. 2018; Oro et al. 2009).

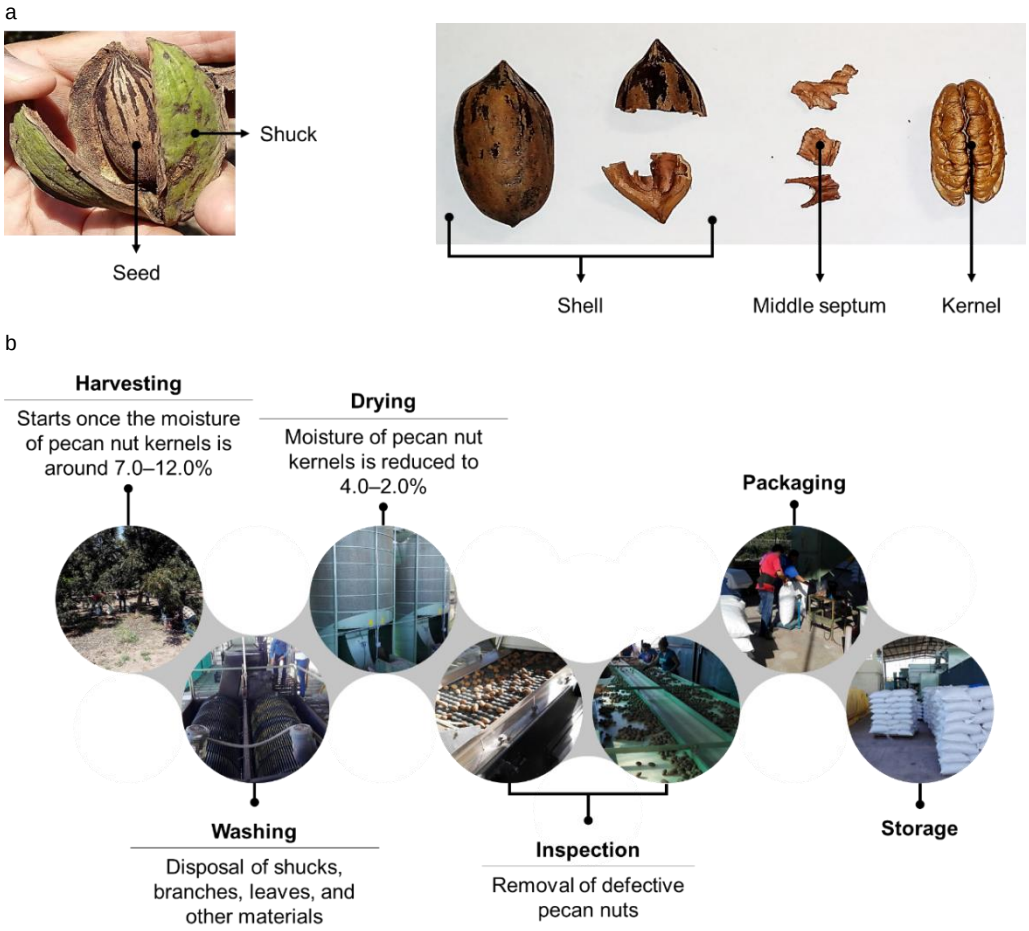

Figure 1.1 Pecan fruit [*Carya illinoinensis* (Wangenh. K. Koch)] and its components (a). Pecan nut processing (b).

Approximately 74.0% of the pecan fruit is not edible. During kernels production, shells and leaves are removed and often discarded; also, a pecan nut cake is obtained as the by-product of the oil extraction process (Figure 1.2). These materials have been considered valuable sources of natural antioxidants for nutraceutical and pharmaceutical applications (Esfahlan et al. 2010). Namely, the most traditional application of pecan nut shells, representing around 40.0 to 50.0% of the whole nut, is the use for infusions claimed for medicinal properties. Complete information of bioactive compounds and health-related attributes of pecan nut kernels, oil, and by-products could be found in a recent review conducted by Álvarez-Parrilla et al (2018).

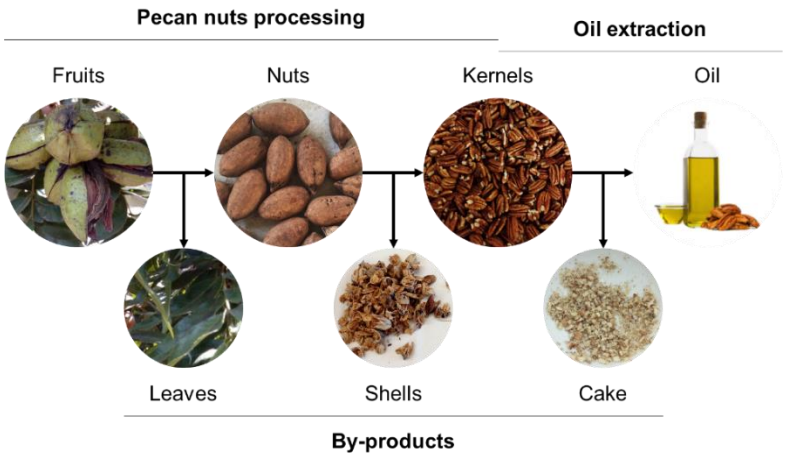

Figure 1.2 By-products from pecan nut kernels production and oil extraction process.

Phytochemical composition and health-beneficial properties associated to pecan nut kernels, oil, and by-products have attracted the attention of scientists for the design of new pecan nut-based products with the potential to counteract health problems. However, their use as alternative ingredients to obtain foods with high quality attributes, represent a great challenge from the technological, physicochemical, and sensorial point of view. Therefore, in this dissertation was compile the most recent information of pecan nuts bioactive compounds, health-related benefits, and techno-functional properties for the development of pecan nut-based products.

1.1.1 Bioactive compounds of pecan nuts

It is well known that nutrient and phytochemical concentration of plant-based products, such as pecan nuts, vary widely ant it is influenced by different factors such as variety, maturation stage, weather conditions, soil characteristics, and processing conditions, among others (Alasalvar and Shahidi 2008; Beuchat and Pegg 2013; Bolling et al. 2011; Byford 2005; Alvarez-Parrilla et al. 2018). The proximal composition of dry kernels reported by the USDA Food Composition Database (2017) is 74.6±0.12 g of lipid, 14.4 g of carbohydrates, 9.9±0.42 g of fiber, 9.5±0.09 g of protein, 3.6±0.12 g of moisture, and 1.5±0.06 g of ash per 100 g in dry basis (db). Flores-Córdova et al. (2016) compared proximate values of Wichita and Western varieties from Mexico and their results were within the ranges reported by the USDA. Recently, Rábago-Panduro et al. (2020a)

evaluated the composition of wet and dry Western pecan nut kernels (Table 4.1.1, *Chapter 4*). Their results indicated that the drying process caused significant changes in the concentration of moisture, lipid, fatty acids, phenolic compounds, and antioxidant capacity.

Due to their high content of macronutrients, especially fatty acids, pecan nuts are considered a high energy dense food with 716 kcal·100 g^{-1} db. The lipid fraction of pecan nuts is mainly composed by fatty acids, tocopherols, and phytosterols. Unsaturated fatty acids are found at the highest concentration (~85.0%), being oleic and linoleic acids the more abundant; while saturated fatty acids represent less than 9.0%, including palmitic, stearic, and arachidic acids (Atanasov et al. 2018; Murray et al. 2003; USDA 2017). Different authors have related the lipid profile of pecan nut kernels to their variety and maturation stage. Rudolph et al. (1992) observed that as the maturation stage was reached, oleic acid concentration increased while that of linoleic acid decreased. Likewise, Bouali et al. (2013) studied lipid composition during Burkett pecan nuts growth and observed a reduction of saturated and polyunsaturated fatty acids concentration (25.0 and 21.1%, respectively) and an increment in monounsaturated fatty acids (13.7%) throughout time. Recently, Flores-Córdova et al. (2016) compared pecan nut kernels of Wichita and Western varieties from Mexico and found that the fatty acid composition differed between both varieties. Namely, oleic acid was found at the highest concentration (45.0%) in Wichita pecan nuts; while in Western, linoleic acid was the predominant with 47.0% of the total content.

Tocopherols, along with tocotrienols, are part of the vitamin E group compounds (Munné-Bosch and Alegre 2002; Murray et al. 2003); being γ-tocopherol the most abundant in pecan nut kernels (~91.0%), followed by α-tocopherol (~5.0%) (USDA 2017). According to Bouali et al. (2013), the Burkett variety has a concentration of 1.4, 0.7, and 25.8 mg·100 g^{-1} oil for α-, δ-, and γ-tocopherol, respectively. Interestingly, tocotrienols are not listed in the USDA Food Composition Database as pecan nuts compounds. However, Pérez-Fernández et al. (2017) identified β- and γ-tocotrienols [<0.025 mg·100 g^{-1} in wet basis (wb)] in Sioux and Pawnee varieties. Regarding phytosterols profile, β-sitosterol (121.3 mg·100 g^{-1} db) along with smaller amounts of Δ-5-avenasterol, campesterol,

stigmasterol, campestanol, and sitostanol have been identified in pecan nut kernels (Phillips et al. 2005; U.S. Department of Agriculture 2017).

On the other hand, kernels non-lipid fraction is mainly characterized by polyphenols, representing more than 97.0% of their total antioxidant capacity (175.2 µmol trolox equivalents·100 g^{-1} wb) (USDA 2017; Wu et al. 2004). It has been reported that, among the diverse types of nuts, pecans contain, after chestnut, the highest total phenolics concentration (1284.0–2016.0 mg gallic acid equivalent·100 g^{-1}) (Chang et al. 2016). According to Bakkalbaşi et al. (2008) and Smeriglio et al. (2017), condensed tannins, specifically procyanidins and prodelphinidins, comprise the majority of polyphenols in pecan nut kernels (~93.0%); while ellagitannins represent a minor component (~5.0%). Still, these nuts along with walnuts are considered a rich source of ellagitannins. Abe et al. (2010) also reported that ellagic acid is one of the most abundant polyphenol found in pecan nut kernels (301.0±7.0 mg·100 g^{-1}).

Pecan nut shells, leaves, and cake also represent a significant source of polyphenolic compounds. It has been stated that shells contain a higher concentration of polyphenols than kernels (Alvarez-Parilla et al. 2018; Villarreal-Lozoya et al. 2007; Flores-Córdova et al. 2016, de la Rosa et al. 2014). Chlorogenic, gallic, and *p*-hydroxybenzoic acids have been identified as the principal phenolic compounds of the shells as well as epigallocatechin, epigallocatechin-3-gallate, and procyanidin with high antioxidant capacity (Engler Ribeiro et al. 2017, do Prado et al. 2014). Otherwise, Hawari et al. (2016) reported that the leaves contain a higher concentration of flavonoids than that reported in the shells. Regarding the pecan nut cake, Sarkis et al. (2014) reported that it contains a similar concentration of phenolic compounds than the whole kernels.

1.1.2 Potential health-related benefits associated with pecan nuts consumption

The high diversity and concentration of bioactive compounds present in pecan nut kernels, oil and by-products have been well-related with health-promoting aspects and antioxidant properties. Most of the scientific evidence available in literature have resulted from epidemiological studies through *in vitro* or *in vivo* tests evaluating the effects of consuming kernels, oil or phenolic compounds extracts (Atanasov et al. 2018; Alvarez-Parrilla et al. 2018). The different assays conducted up today have shown that kernels intake is linked

to the prevention of different NCD such as cardiovascular diseases, diabetes, and cancer. Likewise, the consumption of phenolic compounds extracts could have anti-inflammatory properties (Robbins et al. 2016) and protect cerebellar granule neurons cells from H_2-O_2 scavenging (de la Rosa et al. 2014).

Despite the fact that pecan nuts are catalogued as high energy dense foods, their regular consumption counteract weight gain and obesity (Chang et al. 2016; Grosso and Estruch 2016; Tokusoglu and Hall III 2011; USDA 2017) and, promote a favorable blood lipid profile (Atanasov et al. 2018). The lack of weight gain was associated with the satiating effect after eating and an incomplete digestion of kernels lipids since they are unavailable to digestive enzymes (Ros 2016). Domínguez-Avila et al. (2015) indicated that total body weight of Wistar rats fed with a high fat diet complemented with pecan nut kernels, oil, or phenolic compounds extract, remained unchanged, while the total body fat was significantly higher for all experimental groups, except those rats fed with kernels. According to Zhao et al. (2011), pecan nut oil has a protective effect on hippocampal cell apoptosis by the inhibition of caspase-3 activity, demonstrating that oil intake is able to decrease the cell damage induced by oxidative stress and improve the protection of cell deterioration. Recently, McKay et al. (2018) demonstrated through an *in vivo* study with healthy middle-aged and older adults that the daily incorporation of pecan nut kernels (42.5 g) significantly improved type 2 diabetes biomarkers. Moreover, this practice diminished cardiovascular disease biomarkers compared to an isocaloric control diet. The positive effect of kernels regarding the type 2 diabetes was not only attributed to their high content of unsaturated fatty acids, fiber, and phenolic compounds but also a combination of mechanisms of action such as the improvement of cell permeability to insulin along with the inhibition of carbohydrate digestion and absorption (McKay et al. 2018).

As earlier mentioned, pecan nut shells have been recognized for having a higher concentration of phenolic compounds than kernels. This evidence has positioned them as an interesting matrix for research. Several research works have been conducted to evaluate the functional properties of shells as reported by Alvarez-Parrilla et al. (2018). Namely, Müller et al. (2013) demonstrated that shell extracts have therapeutic attributes on alcoholic liver disease and induce a better blood biochemical profile. Porto et al. (2015) studied genotoxic and mutagenic effects of shell extracts along with their effect on

chemically-induced diabetic and hypercholesterolemic Wistar rats. No genotoxic or mutagenic effects in either experimental group were observed. Biochemical analysis showed that the extract was able to decrease the plasma concentration of glucose, cholesterol, and triglycerides in the animals. Likewise, Dolan et al. (2016) evaluated the toxicological and mutagenic effects of the fiber obtained from pecan nut shells. Their results agreed with those of Porto et al. (2015) concluding that the consumption of the fiber is non-genotoxic and suggesting that this ingredient could be safely consumed.

Based on the scientific evidence presented above, it could be stated that regular consumption of kernels or oil significantly diminish the biomarkers associated with unhealthy conditions and NCD. However, it is important to highlight that these outcomes are dosage dependent and subjected to consumers' health status. Even though there are valid data demonstrating the significant amounts of phytochemicals, specifically phenolic compounds, in pecan nut by-products, they are usually destined to livestock feed and their utilization is not sufficiently profitable. Nonetheless, novel food technologies could be implemented to use these by-products as food ingredients for the development of new food with high functionality. However, further studies evaluating their safety, technological effectiveness, as well as their effects on sensory attributes of the food, must be conducted to assure that safe food with high-quality standards are obtained.

1.1.3 Antimicrobial and techno-functional properties of pecan nut by-products

Antimicrobial and technological properties of any food ingredient are considered as those which are suitable to improve food processing leading to best physicochemical characteristics, sensory properties, longer shelf-life, and increased acceptance of the newly developed food versus the original. Information on those properties of pecan nut by-products is scarce (Table 1.1). However, recent studies have been conducted to evaluate antimicrobial and antioxidant capacity as well as water, oil, emulsifying, and foaming holding capacity of the by-products.

Antimicrobial activity of shell extracts has been studied by different authors, which have found their great potential to be used as a natural preservative able to substitute synthetic antimicrobials used in the food industry. Caxambú et al. (2016) and Yemmireddy et al. (2020) stated that shells aqueous extracts have potential as biopreservative in foods.

Table 1.1 Antimicrobial and techno-functional properties of pecan nut by-products.

By-product	Property	Effect	Application	Reference
Shells				
	Antimicrobial activity	Inactivation of foodborne pathogens	Potential food biopreservative	Caxambú et al. (2016) Yemmireddy et al. (2020)
	Antioxidant capacity	Oxidative stability in fat-rich foods	Alternative to food synthetic antioxidants	Engler-Ribeiro et al. (2017)
Leaves				
	Antimicrobial activity	Inactivation of gram-positive bacteria, gram-negative bacteria, and yeast	Potential food biopreservative	Bottari et al. (2017)
Cake				
	Water and oil absorption capacities	Retention and absorption of water and oil	Texture improver for bakery products, pasta, sausage, and cheese	Marchetti et al. (2017) Maciel et al. (2020)

The extracts were able to inactivate foodborne pathogen bacteria such as *Listeria monocytogenes*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Bacillus cereus*, *Aeromonas hydrophila* and *Pseudomonas aeruginosa*. According to different authors, phenolic compounds exert antimicrobial activity against different bacteria since they are capable of oxidizing microbial cell membranes and inhibit the activity of extracellular enzymes (Prado et al. 2014; Serrano et al. 2009). In addition, Yemmireddy et al. (2020) indicated that there were no sensory quality changes of the foods using shell aqueous extracts as preservative. On the other hand, antioxidant properties of shell extracts were demonstrated by Engler-Ribeiro et al. (2017) when they were added in margarines. The authors incorporated shell extracts to protect and retard the oxidation process of a fat-rich product during storage and compared the effects with those of a commercial rosemary extract and a synthetic antioxidant. No significant differences were observed in quality parameters of margarines supplemented with either the shell extract, rosemary or synthetic antioxidant after 240 days of storage, indicating that shell extracts could be considered as a natural replacement for synthetic antioxidants, such as butylated hydroxytoluene, commonly used to avoid oxidative reactions in oily products. Antimicrobial and antioxidant effects of shell extracts could be attributed to their elevated concentration of phenolic compounds. Nonetheless, more studies should be conducted to define the optimum concentration to be used in different foods in order to achieve the best antimicrobial effects, without affecting physicochemical or sensory properties of food.

Likewise, it has been reported that the lipid fraction (triterpenoids and phytosterols) of the leaves from the pecan tree has antimicrobial activity against *Mycobacterium*

tuberculosis H37Rv. According to Cruz-Vega et al. (2008), 125.0 $mg \cdot mL^{-1}$ of leave extract was suitable to inactivate between 50.0 and 80.0% of the bacteria. Years later, Bottari et al. (2017) suggested that gallic and ellagic acids, rutin, catechin, and epicatechin present in the leaves were the responsible of the antimicrobial activity against gram-positive and gram-negative bacteria as well as yeast, suggesting the potential use of herbal antimicrobials at a concentration ranging between 0.8–25.0 $mg \cdot mL^{-1}$.

Due to their phytochemical profile and techno-functional properties, pecan nut cake is getting the attention of scientists and technologists. Recently, Marchetti et al. (2017) corroborated that kernels are a valuable ingredient to enhance functional properties of formulated foods because of its high content of dietary fiber and protein. It is well known that protein and fiber contain polar and charged side chains with the possibility of establishing hydrophobic interactions and facilitating the link with water and fat (Jitngarmkusol et al. 2008). According to Marchetti et al. (2017), pecan nut cake has high water (1.83±0.06 $g \cdot g^{-1}$) and oil (2.61±0.09 $g \cdot g^{-1}$) absorption capacity, which make it a potential ingredient for bakery products or cereal bars. Maciel et al. (2020) also reported cakes ability to retain and absorb water (1.65±0.1 $g \cdot g^{-1}$) and oil (6.65±0.3 $g \cdot g^{-1}$). However, the authors also detected that cakes exhibited low emulsifying capacity and stability, and poor foaming properties. According to the authors, the high content of fiber might limit the emulsions formation and the low protein-protein interactions and protein solubility would make difficult the formation of foams. Nonetheless, the excellent ability to absorb water and oil of pecan nut cake could be useful to improve the moistness, texture, stability, appearance, and taste of food products such as baked products, snacks, sausages, pasta, and cheese.

Overall, the antimicrobial, antioxidant, and techno-functional attributes found in pecan nuts by-products indicate that they could be potential ingredients for the production of food with high nutritional properties, health-related benefits, and good quality characteristics. Yet, more studies evaluating the techno-functional aspects of the by-products are required to assure their optimal functionality as ingredients or additives in the development of new food products.

1.1.4 Development of new food products from pecan nut kernels, oil, and by-products

Different research groups are currently working on the development of pecan nut-based foods facing the challenge to accomplishing high-quality attributes and long shelf-life (Reyes-Padilla et al. 2018). Obtained results could motivate the consumption of pecan nuts for the consumers benefit from their health-related attributes. However, to the best of the authors' knowledge, this is a recent research subject, and few scientific studies are reported up today (Table 1.2). Reyes-Padilla et al. (2018) used different mixes of kernels and prunes, flaxseed and cranberries, kernels and cranberries, and flaxseed and prunes substituting meat in four formulations to elaborate a bologna-type meat product for the elderly. Physicochemical characteristics, sensory properties, and nutritional quality of the final products were evaluated and compare to a control (conventional product elaborated with meat). In general, moisture, color parameters, and saturated fatty acids content were lower in all bologna-type meat products compared to the control; while fat, total phenolics, unsaturated fatty acids, and antioxidant capacity were higher. Regarding protein, ash, and carbohydrate content including amino acid profile, no statistical differences were found among all formulations. Regarding sensory characteristics, the four formulations showed acceptable scores (>5.4) within an overall satisfaction scale from 0 to 10, but they were significantly lower than the conventional product elaborated with meat. Ranalli et al. (2017) used pecan nut oil as fat source for the development of "dulce de leche" (DL) a caramel-like milk-based spread typical from some Latin American countries. Their results indicated that oil incorporation in the DL formulation not only resulted in a product with similar sensory, physicochemical, and rheological characteristics than those commercially available, but also with an improved unsaturated fatty acids content. Wagener and Kerr (2017) formulated nine formulations of pecan nuts butter with different oil and cake proportions. Authors observed that the high oil content (~70.0%) of pecan nuts makes difficult the production of butter-like products resulting in phase separation and low viscosity. Interestingly, formulations containing between 55.0 and 60.0% of oil got the highest scores in the sensory analysis, with good textural and spreadability attributes.

Table 1.2 The use of pecan nut kernels, oil, and cake for product development.

Ingredient	Product	Effect	Reference
Kernels			
	Bologna-type meat	Increase in fat, total phenolic compounds, unsaturated fatty acids, and antioxidant capacity in comparison to traditional product.	Reyes-Padilla et al. (2018)
Oil			
	"Dulce de leche"	Similar sensory, physicochemical, and rheological characteristics than conventional product.	Ranalli et al. (2017)
Cake			
	Muffins	Increase in yield and high, hardness and browning improvement.	Marchetti et al. (2018)
Oil and cake			
	Pecan butter	Oil content of 50.0–60.0% result in the best sensorial and technological properties (texture and spreadability).	Wagener and Kerr (2017)

In a different study conducted by Marchetti et al. (2018), cake was used to partial replace wheat flour in muffins. The authors evaluated the effect of cake incorporation in baking quality of a typical muffin. It was observed that a replacement of 30.0% of wheat flour by the cake positively influence yield, height, hardness, and browning of muffins. Authors related changes in yield, volume and texture characteristics to the better water and oil absorption capacities of pecan nut cake in comparison with wheat flour. Also, authors indicated that oil and fiber naturally contained in the cake had a plasticizing effect resulting into a matrix modification. Sensory characteristics such as appearance and color were similar to those products elaborated without any replacement. Nonetheless, taste and acceptability were significantly better in pecan nut cake-based muffins.

Designing pecan nut-based products by using pecan nut oil or cake leads to a great opportunity for creating new healthier alternative foods. However, the development of these types of foodstuffs still is in the early stages of research, and further improvement is required to achieve final products with high-quality attributes through optimized processes. To the best of the authors knowledge, there is no information available in literature regarding the use of shell extracts as an ingredient or additive for product development. Knowing that this by-product possesses antimicrobial activity and high content of phenolic compounds with functional properties, further research should be conducted to evaluate the use of this ingredient in medicinal infusions or other products to enhance their quality and health-related attributes.

1.2 Pulsed electric fields for healthy food products

Nowadays consumers are more conscious about what they eat, claiming for safe, natural, fresh, or minimally-processed foods with high quality standards and, furthermore, health-promoting substances that contribute to their well-being. Hence, new food products development involves processes that are focused on not only obtaining innocuous products but also achieving high concentration of health-related compounds. During several decades, thermal pasteurization and sterilization have been the most used technologies for food preservation by the successful inactivation of pathogenic and spoilage microorganisms as well as deteriorative enzymes. However, the high temperatures achieved during processing lead to nutrient losses and detrimental effects in the physicochemical and sensorial characteristics of treated products. As a result, the overall-quality of the product is degraded, thus jeopardizing the acceptance by consumers. In order to satisfy current consumers' demands, the advances in food science and research have given rise to nonthermal food processing technologies, able to render added value products by retaining, or even enhancing, the concentration of their bioactive compounds and nutritional properties without losing the sight of their microbial safety (Ribas-Agustí et al. 2018).

Among nonthermal technologies, pulsed electric fields (PEF) has demonstrated a great potential to be applied for food preservation purposes and as a treatment to assist different processes in the food industry (Wang et al. 2018). Usually, PEF is conducted by applying pulses of high voltage (1.0–45 $kV \cdot cm^{-1}$) to foods placed between two electrodes at ambient, sub-ambient or slightly above ambient temperature for short periods of time (µs). Depending on the voltage applied, PEF can be classified in high intensity (20–45 $kV \cdot cm^{-1}$) or moderate/low intensity (0.1–20 $kV \cdot cm^{-1}$) treatments (Oey et al. 2016; Toepfl et al. 2006). Most of the currently available scientific evidence is related to the effectiveness of high intensity pulsed electric fields (HIPEF) for the cold pasteurization of liquid foods with a minimal impact in their nutritional, functional, and sensory attributes (Odriozola-Serrano et al. 2013; Barba et al. 2015; Gabrić et al. 2015; Elez-Martínez et al. 2017). Nonetheless, during the last decade, different research groups have evaluated the application of moderate intensity pulsed electric fields (MIPEF) as pretreatment to improve oil and juice extraction yields as well to enhance their bioactive compound profile

(Barba et al. 2015; Elez-Martínez et al. 2017; Wang et al. 2018). In addition, the application of MIPEF at the lowest intensities (0.1–3.0 $kV \cdot cm^{-1}$) is currently being researched to prospect its potential as an abiotic elicitor, stimulating the biosynthesis of secondary metabolites in fruit and vegetables (Soliva-Fortuny et al. 2009, 2017; Jacobo-Velázquez et al. 2017).

On the other hand, interesting applications of MIPEF process have been also focused to assist and improve drying, freezing, frying, or osmotic dehydration (OD) treatments. Namely, Traffano-Schiffo et al. (2016, 2017) and Tylewicz et al. (2019) agreed that not only the electroporation mechanism but also a loss of key electrolytes involved in the active transport of the cell membrane such as Ca^{+2} and Na^{+2}, results in an enhancement of mass transfer and decrease of sugar content in OD-kiwifruits. Moreover, apoplastic and symplastic transport phenomena occurring in MIPEF-treated kiwifruit increased the dehydration rate during OD as reported by Traffano-Schiffo et al. (2017). According to Tylewicz et al. (2019) the MIPEF-OD combination improved water loss in strawberry and kiwifruit treated at low intensities (0.2 $kV \cdot cm^{-1}$) regardless of the amount of sugar employed for OD process. The water distribution and water-solid exchange of MIPEF-treated sliced apples prior to freeze-drying was studied by Tylewicz et al. (2016). The authors observed a significant reduction in the initial freezing temperature and freezable water content of MIPEF-treated apples relating it to an increment of cell disintegration index (*z*) which increased along with specific energy input (*W)*.

1.2.1 Basic principles

PEF processing involves the application of short pulses (µs) of electric fields intensity (*E*) varying from 0.1 to 40 $kV \cdot cm^{-1}$, depending on the pursued objective. As seen in Figure 1.3, the increase of product shelf-life by the inactivation of microorganisms and enzymes is usually achieved with HIPEF (20–40 $kV \cdot cm^{-1}$). On the other hand, MIPEF (1–20 $kV \cdot cm^{-1}$) is commonly applied as pretreatments for the improvement/optimization of drying, freezing, and extraction, among other processes. Likewise, MIPEF processing at low *E* (0.1–3 $kV \cdot cm^{-1}$) has demonstrated to be able of inducing stress reactions in horticultural crops generating secondary metabolites (Oey et al. 2016; Toepfl et al. 2006).

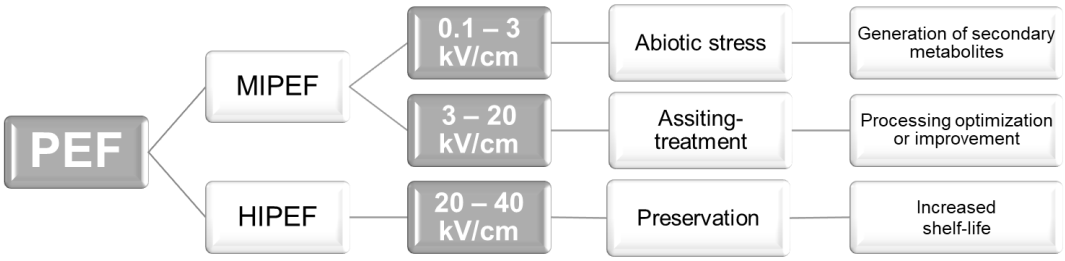

Figure 1.3 PEF at different electric field strengths (E) for food application.

Due to the high intensities achieved during HIPEF processing, its application must be conducted in homogeneous liquid products free of solid particles or gas bubbles in order to prevent dielectric breakdown (Qin et al. 1996), while MIPEF could be used in solid and liquid foods. Regardless of the treatment intensity, the temperature achieved during the process is far below of that commonly used in conventional treatments (Gabrić et al. 2018). This represents a great advantage in front of other technologies, since most heat-sensitive bioactive compounds associated to health benefits are well preserved or even enhanced. Furthermore, total treatment time is very short, which results in saving energy and operational costs.

The main mechanisms associated to PEF are related to electroporation phenomena and electrical breakdown. According to Wan et al. (2018) the phospholipid bilayer and proteins of the cell membrane get unstable under the applied electric fields, causing the formation of holes and increase of cell membrane permeability. As a result, different effects in PEF-treated products could occur, *e.g*. damage to cellular structural integrity of microorganisms leading to microbial death (Barbosa-Cánovas et al. 1998), disruption of plant cell wall improving extraction yield of diverse target compounds (Puértolas et al. 2016; Vinceković et al. 2017; Zhang et al. 2017; Shortskii et al. 2017), or even, a promotion of the antioxidant content in fruits (Soliva-Fortuny et al. 2017). Furthermore, Wan et al. (2018) clearly describe that the application of electric fields can cause modification in electrically-sensitive components, thus accelerating mass and heat transfer in some processes such as drying, freezing, and OD, among other. Hence, some biological effects such as molecular modification, and polarization or realignment of molecules with dipole moments could occur during processing. Depending on treatment conditions, these changes may end up producing reversible or irreversible damage. In

this sense, it has been proved that the effectiveness of PEF processing is related to *a*) operating processing parameters: electric fields intensity ($kV \cdot cm^{-1}$), pulse shape (quadratic or exponential), pulse polarity (monopolar o bipolar), frequency (Hz) and treatment time (µs–ms) and *b*) food matrix properties such as electrical conductivity, pH, and composition (Gabrić et al. 2018). Since foods are complex organic materials, their study under the application of PEF must be conducted specifically in each product to define the optimum processing conditions able to attain the highest quality standards related to safety and health-attributes. In this line, a large number of studies conducted over the last decades have demonstrated the feasibility of PEF application for different purposes in the food industry: microbial or enzyme inactivation, functionality enhancement, extractability increase and recovery of nutritionally valuable compounds in a diverse variety of foods.

1.2.2 Applications

1.2.2.1 HIPEF for the improvement of nutritional and functional properties of liquid foods

HIPEF processing constitutes a potential alternative to traditional thermal treatments to obtain safe products with a long shelf-life and high stability. Since its application is conducted at low or moderate temperatures, usually below 40 °C, most thermolabile compounds, partially degraded when thermal pasteurization or sterilization are applied, are much better retained (Barba et al. 2015). At the same time, it has been corroborated that physicochemical and sensorial properties of HIPEF treated products are minimally affected, resulting in liquid foods with high quality attributes.

Many research efforts have been done to evaluate the influence of HIPEF on different bioactive compounds, such as hydro- and liposoluble vitamins, phenolic compounds, carotenoids, glycosylates, chlorophyll, amino acids, fatty acids, among other, in diverse liquid matrices (Table 1.3). In most cases, available results evidence that the concentration of phytochemicals is well retained, minimally modified, or even increased, immediately after processing and during shelf-life. However, these effects correlate well with the HIPEF process intensity and food matrix characteristics such as physicochemical properties and composition, as well as on the nature of the studied compound. Comprehensive information regarding the potential applications of HIPEF to preserve and

promote bioactive compounds in different foods can be found in Soliva-Fortuny et al. (2009), Odriozola-Serrano et al. (2013), Elez-Martínez et al. (2017) and Gabrić et al. (2018). Some of the most relevant findings related to the effects of HIPEF on health-related compounds are summarized below.

Table 1.3 Effect of HIPEF processing on bioactive compounds contained in liquid foods.

Bioactive compound	Food matrix	HIPEF processing	Effect	Reference
Vitamin C				
	Apple juice	30 kV·cm^{-1}	n.s.	Dziadek et al. (2019)
	Apple cider	28, 32, 36, and 40 kV·cm^{-1}, 25, 50, 100 µs	n.s.	Walkling-Ribeiro et al. (2008)
	Grape juice	65 and 80 kV·cm^{-1} using 20–40 pulses	n.s.	Wu et al. (2005)
B-complex vitamins				
	Milk	18.3, 22.6, and 27.1 kV·cm^{-1}; 47–400 µs	n.s.	Bendicho et al. (2002)
	Milk	15–35 kV·cm^{-1}, 12.5–75 µs	n.s.	Riener et al. (2009)
	Fruit juice-milk beverage	35 kV·cm^{-1}, 1800 µs, 200 Hz, bipolar 4-µs pulses	n.s.	Salvia-Trujillo et al. (2011)
Total phenolic compounds				
	Fruit juice-milk beverage	35 kV·cm^{-1},1400 µs, 200 Hz, bipolar 4-µs pulses	n.s.	Morales-de la Peña et al. (2017a)
	Kombucha	281.6 kJ·kg^{-1}	2-fold significant increase	Vázquez-Cabral et al. (2016)
	Date juice	35 kV·cm^{-1}, 1400 µs, 100 Hz bipolar 4-µs pulses	Increase (18%)	Mtaoua et al., (2017)
Total carotenoids				
	Mango juice	35 kV·cm^{-1}, 1400 µs, 200 Hz, bipolar 4-µs pulses	Decrease (13%)	Salinas-Roca et al. (2017)
	Fruit juice-milk beverage	35 kV·cm^{-1}, 1400 µs, 200 Hz, bipolar 4-µs pulses	Decrease (10%)	Morales-de la Peña et al. (2017b)
Glycosylates				
	Broccoli juice	15–35 kV·cm^{-1}, 2000 µs, 200 Hz, monopolar or bipolar 4-µs pulses	3-fold increase	Franndsen et al. (2014)
Betacyanins				
	Prickly pear juice	35 kV·cm^{-1}, 45 Hz, 3 µs-monopolar pulses	n.s.	Moussa-Ayoub et al. (2017)
Chlorophylls				
	Broccoli juice	25–35 kV·cm^{-1}, 1250 µs	Increase (16.0–20.7%)	Sánchez-Vega et al., (2014)
Iron				
	Fruit juice-milk beverage	35 kV·cm^{-1}, 1400 µs, 200 Hz, bipolar 4-µs pulses	Increase (300%)	Salvia-Trujillo et al. (2017)

n.s., not significant changes.

Effects on vitamins

It is well known that vitamins are vital organic molecules that have an essential role in human metabolism. Furthermore, some of them are well known for their high antioxidant capacity, having a significant impact in food quality due to their reducing nature, as well as in consumers' health. Hence, their retention in processed foods is of high importance.

Since vitamin C is highly sensitive to processing and storage, scientists have studied the impact of HIPEF treatment in vitamin C retention from different products to find the optimal conditions to maintain its highest concentration (Figure 1.4), usually comparing the obtained results with the effects of conventional treatments. A complete investigation on vitamin C structure as affected by HIPEF was conducted by Zhang et al. (2015) using a vitamin C solution to perform the experiments. Their results indicated that HIPEF treatment did not cause any damage to vitamin C and was able to slow down the oxidation process under the experimental conditions (5–35 kV·cm^{-1}, for 800 µs).

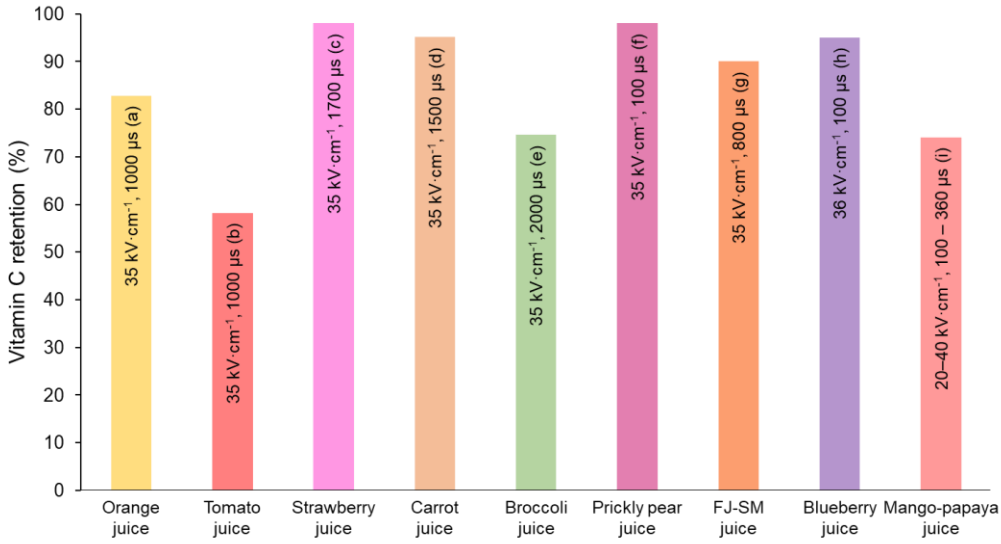

Figure 1.4 Effect of HIPEF on vitamin C retention in different liquid foods. (a) Elez-Martínez et al. (2006) (b) Odriozola-Serrano et al. (2007) (c) Odriozola-Serrano et al. (2008) (d) Quitão-Teixeira et al. (2009) (e) Sánchez-Vega et al. (2015) (f) Moussa-Ayoub et al. (2017) (g) Morales-de la Peña et al. (2010). (h) Barba et al. (2010). (i) Carbonell-Capella et al. (2017). FJ-SM, fruit juice-soymilk.

Besides, the authors observed that HIPEF affected the conformation of vitamin C, promoting transformation of the vitamin C isomer enol-form into keto-form. Interestingly, available data in literature show a greater vitamin C retention in HIPEF-treated fruit juices and mixed beverages compared to those treated by heat-pasteurization (Odriozola-Serrano et al. 2008; Quitão-Teixeira et al. 2009; Morales-de la Peña et al. 2010; Marsellés-Fontanet et al. 2013; Elez-Martínez et al. 2017). Processing parameters such as *E*, pulse shape (σ), treatment time (*t)*, and pulse polarity play an important role in vitamin C retention. Sánchez-Vega et al. (2015) observed that the residual content of

vitamin C in broccoli juice treated by HIPEF ranged from 67.0–90.1 %; achieving the maximum concentration at 35 kV·cm^{-1} and 500 µs with monopolar pulses. Similarly, 98.0 % retention of vitamin C after HIPEF processing (35 kV·cm^{-1}, 3µs-monopolar pulses) of *Opuntia dillenii* juice was reported by Moussa-Ayoub et al. (2017). In a recent study, Lee et al (2018) corroborated that ascorbic acid concentration of a mixed mandarin and Hallabong tangor juice treated at 16 kV·cm^{-1} and 100 kJ/L was not degraded compared to the untreated juice. The best retention of vitamin C in HIPEF-treated products in monopolar mode could be related to the inactivation of the enzymes involved in vitamin C oxidation (Elez-Martínez et al. 2017). According to Zhang et al. (2015), HIPEF processing slows down the involved oxidative reactions. Different authors have attempted to describe the depletion of vitamin C content using exponential response models. Sánchez-Vega et al. (2015) indicated that a second-order polynomial model accurately described (p<0.0001, R^2=0.83) the changes in vitamin C concentration of HIPEF-processed broccoli juice. Likewise, Odriozola-Serrano et al. (2008a) indicated that a Weibull model (R^2_{adj}≥0.84) was able to describe the kinetic changes of vitamin C in tomato and strawberry juices as affected by *E* and *t*, demonstrating an inverse relationship between these parameters and vitamin C retention. Also, Salvia-Trujillo et al. (2011) stated that the Weibull model accurately described (R^2 from 95.5–99.2, A_f from 1.01–1.11) the degradation kinetics of vitamin C during refrigerated storage of HIPEF processed (35 kV·cm^{-1}, 1800 µs, 200 Hz and 4 µs-bipolar pulses) fruit juices-milk mixed beverages. The impact of HIPEF processing on vitamin B complex and fat-soluble vitamins has been mainly studied in milk or milk-based products. Riener et al. (2009) stated that HIPEF treatment ranging from 15–35 kV·cm^{-1} during 12.5 to 75 µs did not affect the concentration of thiamine, riboflavin, retinol, or α-tocopherol of milk immediately after processing. The same effect was reported by Bendicho et al. (2002) when applying HIPEF treatments at different *E* (18.3–27.1 kV·cm^{-1}) in skim milk and simulated milk ultrafiltrate (SMUF). These authors reported that no significant differences in thiamine, riboflavin, cholecalciferol, and tocopherol concentration were observed between HIPEF-processed milks and those untreated. Interestingly, Salvia-Trujillo et al. (2011) and Rivas et al. (2007) observed that pantothenic acid, biotin, and riboflavin levels in HIPEF treated

mixed beverages, containing fruit juices and milk, were higher than those in heated beverages.

Otherwise, few research works have been conducted regarding vitamin A as affected by HIPEF. As reported for other vitamins, vitamin A changes after HIPEF processing are minimal compared to those presented in conventionally pasteurized foods. Torregrosa et al. (2005) and Cortés et al. (2006) found that HIPEF processed orange-carrot and orange juices had higher pro-vitamin A compounds content than heat-treated juices. In view of the existing scientific evidence, it could be stated that these compounds are more heat-sensitive than HIPEF-sensitive, indicating that HIPEF process can be used to preserve the original concentration of vitamins in liquid products.

Effects on phenolic compounds

Phenolic compounds (PC) are the largest group of secondary metabolites distributed in plant kingdom. Due to their high antioxidant capacity, they act as inhibitors of oxidative enzymes, as metal chelators, or as free radical scavengers (Han et al. 2007; Boskou et al. 2006, Gómez-Maqueo et al. 2018). Their retention in processed foods is of great interest since these molecules have demonstrated beneficial effects in human health, either as preventive or even as therapeutic agents for degenerative and chronic diseases (Mtaoua et al. 2017). Due to their high importance, several researchers have evaluated the impact of HIPEF, applied as preservation treatment, on different plant-based beverages including fruit and vegetable juices, mixed beverages and, recently, Kombucha (Elez-Martínez et al. 2017; Vazquez-Cabral et al. 2016).

Interestingly, there is no consensus in the available literature regarding the effects of HIPEF on PC. Different changes in the concentration of phenolic acids (ferulic, *p*-coumaric, caffeic, ellagic and chlorogenic) of tomato, orange, and strawberry juices have been observed immediately after HIPEF processing; however, they were always better retained in comparison to heat processed juices. In addition, observed changes were mainly related to their molecular structure (Odriozola-Serrano et al. 2008b, 2009; Agcam et al. 2014). In accordance to these results, Morales-de la Peña et al. (2017a) highlighted that the application of HIPEF at 35 $kV \cdot cm^{-1}$ during 1800 µs in a fruit juice-milk beverage led to a higher concentration of individual PC than the use of a conventional thermal

processing. On the other hand, Sánchez-Vega et al. (2015) reported that the relative content of total PC of broccoli juice varied from 80.0 to 96.1 % after HIPEF processing at different *E* (15, 25, and 35 $kV \cdot cm^{-1}$) in monopolar and bipolar mode. Similarly, 18.0 and 40.0 % decreases in polyphenol concentrations were detected in apple and mango juices, respectively, after HIPEF processing (Aguilar-Rosas et al. 2007; Salinas-Roca et al. 2017). Sánchez-Vega et al. (2015) suggested that the residual polyphenol oxidase activity might be associated with the degradation of PC during HIPEF processing. By contrast, the concentration of PC in date juice was significantly higher after HIPEF process (569.55 mg/L) in comparison with untreated (483.32 mg/L) and thermally treated (494.35 mg/L) juices (Mtaoua et al. 2017). Likewise, Vázquez-Cabral et al. (2016) demonstrated that lower *W* (246 $kJ \cdot kg^{-1}$) render Kombucha beverage with higher polyphenolic content compared to untreated samples. Moussa-Ayoub et al. (2017) also indicated that HIPEF treatment caused a significant increase in isorhamnetin-3-O-rutinoside, the predominant flavonoid of prickly pear juice, regarding the untreated juice. Diaz-Ribas et al. (2018) investigated the effects of HIPEF processing on herbal infusions and demonstrated that HIPEF application (800 $kJ \cdot kg^{-1}$) increased the amount of free phenolic acids (222 %) showing that HIPEF may be a feasible process for herbal infusions. The influence of HIPEF on the phenolic content of apricot juice was also evaluated by Raham et al. (2020). Authors identified that HIPEF processing caused a slight increase of 3–8 % on the phenolic content of treated juice compared to that without any processing. Raham et al. (2020) suggested that the augment of total phenolic concentration might have happened due to the breakage of the cell wall and release of bound phenolic content due to the effects of the HIPEF treatment. According to Agcam et al. (2014), permeabilization of plant cells and vacuolar membranes is likely to occur during HIPEF processing, thus leading to an increase of PC (Figure 1.5). Furthermore, biochemical, or enzymatic reactions occurring as consequence of processing could also lead to the formation of new compounds, increasing the accumulation of phenolics in HIPEF treated products (Barba et al. 2015; Mtaoua et al. 2017).

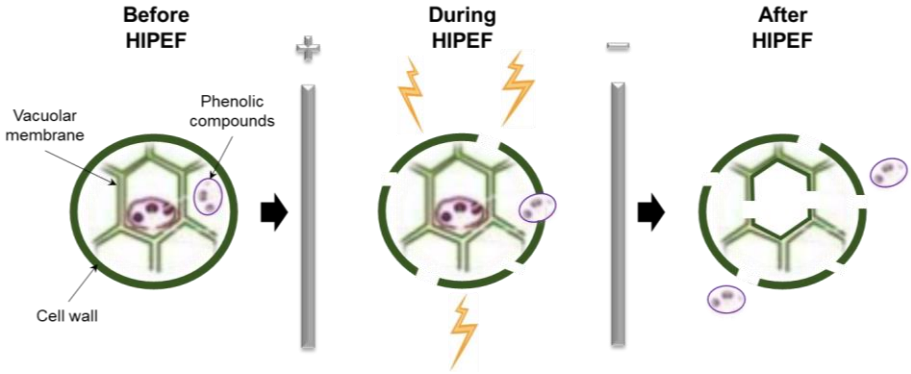

Figure 1.5 Cell wall permeabilization effect during HIPEF treatment and release of phenolic compounds.

Effects on carotenoids

Regular consumption of carotenoids is well correlated to human health because of their antioxidant capacity and pro-vitamin A activity, with β-carotene having the highest activity (van der Berg et al. 2000). These compounds represent a vast group of unsaturated molecules with a large conjugate double-bonds structure, which makes them highly susceptible to chemical reactions such as oxidation and isomerization during processing and storage. Finding the optimal processing conditions in order to better preserve carotenoids in HIPEF treated products has been a great challenge for scientists. Different matrices such as fruit and vegetable juices as well as mixed beverages containing fruit juices and milk, or soymilk have been evaluated considering the impact of HIPEF on their individual or total carotenoids concentration. Obtained results are controversial since not all carotenoids appear to react equally under HIPEF processing. Although the reason for these effects is not well-known up today, it could be speculated that carotenoid conversions are triggered by HIPEF treatments. It has been demonstrated that processing parameters such as *E*, σ, pulse frequency (*f*), *t*, and pulse polarity have a significant impact on carotenoid concentration. Namely, Odriozola-Serrano et al. (2007, 2008a) indicated that bipolar pulses at the highest *E*, σ, and *f* raised lycopene concentration in tomato juices. Likewise, Cortés et al. (2006); Torregrosa et al. (2005), Quitão-Teixeira et al. (2009), Aadil et al. (2015), and Sánchez-Vega et al. (2015) concluded that more intensive HIPEF processes lead to a significant increase in the carotenoids content of plant-based products such as tomato, orange, carrot, orange-carrot, grape, and broccoli juices. Accordingly, Carbonell-Capella et al. (2016) observed

a significant increase in total carotenoid in a papaya-mango juice blend after HIPEF processing at 35 kV·cm^{-1}. It might be possible that HIPEF process causes a disruption of the chloroplast and chromoplast membrane structures and of the protein-carotenoid complex, making carotenoids more accessible for extraction (Nguyen and Schwartz 1999). Otherwise, no significant changes in carotenoid content were observed in a HIPEF treated orange juice or orange juice-milk beverage in comparison with the untreated samples (Plaza et al. 2011; Zulueta et al. 2013). In line with this, Barba et al. (2015) stated that individual carotenoids with antioxidant capacity such as β-carotene, β-cryptoxanthin, zeaxanthin, and lutein were highly stable under HIPEF treatment. Contrarily, Morales-de la Peña et al. (2011a, 2017b) indicated that the initial concentration of most individual carotenoids in fruit juice-mixed beverages containing milk or soymilk was significantly diminished immediately after HIPEF processing at 35 kV·cm^{-1}, 200 Hz and 1800 µs; however, the carotenoid content in HIPEF-processed beverages was higher than that in thermally treated blends. Morales de la Peña et al. (2017b) proposed that different biochemical reactions may take place during HIPEF resulting in an enhancement or a loss of the individual carotenoids in plant-based foods. Interestingly, Sánchez-Vega et al. (2015) observed a significant reduction of 34.5 % and 49.3 % in lutein and β-carotene concentration, respectively, in HIPEF processed broccoli juice at 15 kV·cm^{-1} for 500 µs. Authors inferred that, at these processing conditions, microorganisms and oxidative enzymes present in vegetables are not completely eliminated; hence, eventually causing a decrease in carotenoid concentrations in the final product.

The effects observed during shelf-life studies indicate that different reactions such as isomerization or oxidation could occur throughout the time in HIPEF treated products leading to different changes in carotenoid content. According to Vallverdú-Queralt et al. (2013) the content of *trans*-lycopene significantly depleted as a result of isomerization phenomena, since *trans*-lycopene can be converted to 13-*cys*-lycopene, which can be transformed in other *cis*-isomers. In agreement, Plaza et al. (2011) indicated that the xanthophyll lutein is more susceptible to isomerization or oxidation process than other carotenoids. In this sense, authors observed that the content of this molecule was considerably diminished in a HIPEF treated orange juice after 40 days of refrigerated storage. Nonetheless, higher stability of carotenoid compounds in HIPEF-treated

products in comparison to thermally-treated foods was reported by Odriozola-Serrano et al. (2013). In this line, Morales-de la Peña et al. (2011a) showed that the concentration of lutein, zeaxanthin, and β-cryptoxanthin of a HIPEF-treated fruit juice-soymilk mixed beverage significantly declined during 56 days of storage, although the degradation was lower in comparison to that observed in heated beverages. First-order kinetic models (R^2 ≥ 0.866) successfully fit carotenoid degradation as a function of storage time for HIPEF-processed tomato, carrot, and orange juices, as well as in a mixed beverage prepared with orange juice and milk, with rate constants between $1.4x10^{-2}$ and $2.3x10^{-2}$ $days^{-1}$ (Elez-Martínez et al. 2017). Also, Quitão-Teixeira et al. (2009) indicated that degradation of β-carotene of HIPEF-treated carrot juice (35 $kV \cdot cm^{-1}$ for 1500 µs and frequency of 100 Hz) followed an exponential trend (R_2=0.8007–0.9603) with degradation rates varying from $1.8x10^{-2}$ to $2.2x10^{-2}$ $days^{-1}$.

Effects on amino acids and fatty acids

Amino acids and fatty acids have been also evaluated as affected by HIPEF treatment conditions. Available information is though scarce compared to data reported for other previously described bioactive compounds. It has been reported that the application of HIPEF may cause denaturation of the primary structure of proteins, leading to some effects in the concentration of free amino acids (Bekard and Dunstan 2014). Gardé-Cerdán et al. (2007) reported insignificant changes on the free amino acids profile of grape must after HIPEF processing at 35 $kV \cdot cm^{-1}$ with 4 µs-bipolar pulses at 1000 Hz. The only exceptions were lauric acid, which diminished after processing and histidine, tryptophan, asparagine, and ornithine, which slightly increased. Similarly, Morales-de la Peña et al. (2012) observed that valine concentration significantly augmented in a mixed beverage processed at 35 $kV \cdot cm^{-1}$ with 4 µs-bipolar pulses at 200 Hz during 800 µs. Nonetheless, by increasing the treatment time to 1400 µs the concentrations of glutamic acid, glycine, tyrosine, valine, leucine, and lysine considerably decreased. Authors suggested that some chemical reactions such as desulphurization, deamination, and isomerization resulting in the degradation of some amino acids could occur during processing of the beverages. In a recent study, Sánchez-Vega et al. (2020) observed that a HIPEF processing with monopolar and bipolar pulses at 15–35 $kV \cdot cm^{-1}$ during 500–

2000 µs had a significant influence on the free amino acid concentration of broccoli juice. It was reported that the highest content of free amino acids was obtained when broccoli juice was HIPEF treated at 15 $kV \cdot cm^{-1}$ and 2000 µs in monopolar mode. The authors related the increment of histidine (159.3 %) and lysine (157.8 %) to the increase of enzyme activity associated to their biosynthesis.

The concentration of unsaturated fatty acids in foods is of high importance from both the nutritional and the technological point of view. Hence, the impact of HIPEF treatments on fatty acids has been assessed by different researchers. Zeng et al. (2010) evaluated the effects of HIPEF treatments (20–50 $kV \cdot cm^{-1}$ for 40 µs) in peanut oil and observed slight changes in fatty acids composition. Most studies conducted up today concur that HIPEF processes caused no major modification of most fatty acids in different products such as grape juice, whole milk, and mixed beverages containing fruit juices and milk, or soymilk (Odriozola-Serrano et al. 2006; Zhang et al. 2011; Morales-de la Peña et al. 2011b; Salvia-Trujillo et al. 2017; Garde-Cerdán et al. 2007; Zulueta et al. 2007). Only Morales-de la Peña et al. (2011b) observed that polyunsaturated fatty acids contents, namely linoleic, eicosapentaenoic, and docosahexaenoic acids, were found to decrease after HIPEF process, as it happened in the thermally treated samples. In addition, fatty acids concentrations in HIPEF treated milk and fruit juice-soymilk beverages tended to increase throughout the storage; this effect could be related to the presence of spoilage microorganisms that contribute to fat degradation or to biochemical changes of volatile compounds occurring during processing (Odriozola-Serrano et al. 2006; Morales-de la Peña et al. 2011b).

1.2.2.2 MIPEF as an assisting process

During the last decades, food applications of PEF have evolved from cold pasteurization treatments to assisting treatments of well-established processes in the food industry. Different research studies have demonstrated that the application of MIPEF as a pretreatment may enhance process efficiency by reducing operation times and increasing extraction yields as well as nutritional and nutraceutical properties of extracted food products (Lammerskitten et al. 2019; Traffano-Schiffo et al. 2016). It has been observed that, under the effects of MIPEF treatments, cell membranes may be irreversibly or

reversibly permeabilized (Barba et al. 2015). According to Soliva-Fortuny et al. (2009), MIPEF application has been well associated to a great recovery of high-added value compounds from different matrices, the improvement of mass transfer rates, the enhancement of osmotic dehydration, drying and freezing efficiency, and the increase in oil, juice, and bioactive compounds extraction yields from different media. In this sense, MIPEF processing has the capability of being implemented to assist diverse unit operations, thus allowing the development of high quality products. Relevant information regarding MIPEF-assisted processing in OD, extraction, drying, freezing, and acceleration of winemaking treatment are available in complete reviews conducted by Barba et al. (2015) and Wang et al. (2018).

Juice extraction

Bobinaitė et al. (2014) applied MIPEF processing of different intensity (1.0, 3.0, and 5.0 $kV \cdot cm^{-1}$) to improve juice extraction yield (EY) from blueberries. Their results indicated an increment of *z* along with *E* but independent of *W*. The highest juice EY increase, 31.8 %, was achieved at 3.0 $kV \cdot cm^{-1}$ while the lowest juice EY increase, 23.9 %, was obtained after applying a treatment of 5.0 $kV \cdot cm^{-1}$. This lower increase at 5.0 $kV \cdot cm^{-1}$ was associated with a loss of firmness, which augmented compaction of the press cake closing its capillaries. Recently, El Kantar et al. (2018) determined juice EY of orange, pomelo, and lemon pretreated at 3.0 $kV \cdot cm^{-1}$ and observed that the EY incremented by 25.0, 37.0, and 59.0 %, respectively, compared to the juice EY from untreated citrus fruits. In a different research, Moussa-Ayoub et al. (2016) applied thermal, enzymatic, and MIPEF pretreatments to compare their effect in the juice EY of prickly pears. The processes increased the EY by 85.9 % (thermal), 97.0 % (enzymatic), and 27.9 % (MIPEF) compared to the juice EY from untreated prickly pears. Even though the lowest EY was achieved using MIPEF processing, this technology maintained similar physicochemical characteristics to the juice extracted from untreated prickly pear. Indeed, the combined used of MIPEF with mild thermal or enzymatic treatments presents some interesting applications that are worth of investigation.

In addition to the enhancement of the mechanical extraction process increasing the juice EY from different fruits, it has been observed that MIPEF processing increases

the total phenolics (TC) and total anthocyanin content (TAC) of the extracted juices compared to juices from untreated samples, leading to an improvement of their antioxidant capacity (AC), some interesting results could be consulted in Table 1.4.

Table 1.4 Extraction yield and bioactive compounds profile of juices extracted from fruits treated by MIPEF.

Food matrix	MIPEF processing	EY	TP	TAC	AC	Comparison with untreated fruits	References
Blueberry	*E*, 3.0 kV·cm^{-1}	56.3	109.1	50.2	162.2	Increase of TC, TAC, and AC	Bobinaitė et al. (2014)
Orange Pomelo Lemon	*E*, 3.0 kV·cm^{-1}	60 74 63	95.9 132.8 136.3	-	-	Increase of TC	El Kantar et al. (2018)
Prickly pear	*W*, 5.0 kJ·kg^{-1}	42.6	-	-	-	Increase of isorhamnetin-3-O-rutinoside content Reduction of betaxanthins content and AC	Moussa-Ayoub et al. (2016)

EY, juice extraction yield was expressed as g of juice per 100 g of fruit. TP, total phenolics (mg gallic acid equivalents·100 mL^{-1}); TAC, total anthocyanin content (mg cyanidin-3-glucoside equivalents·100 mL^{-1}); AC, antioxidant capacity (mg trolox equivalents·100 mL^{-1}). *E*, electric field strength; *W*, specific energy input.

Oil extraction

MIPEF have also been used as a pretreatment to increase the oil EY from different products. Abenoza et al. (2013) evaluated oil EY from olive paste treated at *E* of 0–2.0 kV·cm^{-1} with and without malaxation (15–26 °C during 0, 15, and 30 min). They reported an increment of 54.5 % in the oil EY of olive paste treated by MIPEF without malaxation, while the MIPEF processed followed by malaxation at 15 °C during 30 min improved it by 14.1 %. Furthermore, the oils extracted from MIPEF-treated olives maintained without significant changes their sensory and physicochemical properties. Puértolas and Martínez de Marañón (2015) incorporated MIPEF at a pilot scale on olive oil production to investigate its effect on oil EY and oil properties. The application of 2.0 kV·cm^{-1} increased the oil EY and acidity values by 13.3 and 15.8 %, respectively. These results were related to a double effect generated by electroporation of the cell membrane and oil release from the oil-water emulsion produced during oil extraction. Andreou et al. (2017) obtained different oil EY from Amfissis, Manaki, and Tsounati olives treated at a *W* of 20 kJ·kg^{-1}, the highest increment was reported for Manaki olives (19.4 %) while the lowest for Tsounati olives (3.0 %). This effect was associated with the physical characteristics of each variety. Peroxide value, K270 and K232 were similar in all olive oils except for acidity, which was higher in oils extracted from MIPEF-treated olives. Likewise, Veneziani et al.

(2019) reported an increment ranging from 2.3 to 6.0 % in oil EY of Carolea, Ottobratica, and Coratina olives pretreated at 17 $kJ \cdot kg^{-1}$ with no significant differences in oils physicochemical properties and oxidative stability within varieties. The authors concluded that besides olive physical characteristics, oil EY depended on specific composition and agronomic factors, which might interfere in the electrical diffusion.

Similarly, MIPEF has been applied as pretreatments to enhance the oil EY from different oilseeds. Namely, Sarkis et al. (2015) applied MIPEF at different W (40–240 $kJ \cdot kg^{-1}$) as a pretreatment to the oil extraction process in sesame seeds. The authors observed that the application of MIPEF at 40 $kJ \cdot kg^{-1}$ increased oil EY by 4.9 % in comparison to the oil extracted from untreated seeds and, interestingly, the increment of *W* did not cause any *z* increase nor improve oil EY. Recently, Moradi and Rahimi (2018) compared ultrasound (US), MIPEF, and US-MIPEF combination to increase oil extraction from sunflower seeds. According to the authors, MIPEF treatment at 1.1 $kV \cdot cm^{-1}$ was the most suitable technology to enhance the oil EY, increasing it from 30.5 to 47.7 % which was related to a significant increment in *z*. However, similar to the effects observed in oil extracted from MIPEF-treated olives, the MIPEF processing also increased the acidity value of sunflower oil, with no changes in other physicochemical properties. The authors attributed MIPEF effectiveness to a homogeneous application of the electric fields causing pores formation confirmed by scanning electron microscopy (SEM). In another research, Guderjan et al. (2007) studied the effect of MIPEF on hulled and dehulled rapeseed previous to oil extraction by Soxhlet and mechanical pressing. The authors reported an increment in Soxhlet oil EY of hulled rapeseeds pretreated at 84 $kJ \cdot kg^{-1}$ (39.1 %), while no improvement was observed for dehulled seeds. In contrast, it was observed that the application of MIPEF previous to mechanical pressing did not improve the oil EY from hulled or dehulled rapeseeds. Acidity value was the only parameter that significantly increased in oil extracted from MIPEF-treated rapeseeds. According to Guderjan et al. (2007), triglyceride degradation, and a consequent increase in free fatty acid concentrations, is likely to occur after MIPEF by the release of lipase enzymes. It is important to emphasize that improvements in oil EY of different matrix pretreated by MIPEF have been associated with the electroporation mechanism, mainly based on the determination of *z*. Likewise, increments in acidity values have been reported in the

extracted oils being related to an increase in lipase activity (Guderjan et al. 2007). However, there is still a gap of knowledge concerning the MIPEF mechanism on fruits and seeds that could be fulfilled with complementary microscopy and enzymatic activity analysis.

Some authors have employed different staining and microscopy techniques to propose a mechanism that relates MIPEF processing to the enhancement of oil EY from microalgae. Han et al. (2019) applied a MIPEF process at 20 $kV \cdot cm^{-1}$ followed by chloroform/methanol oil extraction to a *Chlorella pyrenoidosa* suspension, leading to a 31.9 % increase in the oil EY in comparison to the oil extracted from the untreated microalga. Moreover, fluorescence and SEM microscopy demonstrated the loss of cell integrity by electroporation in microalgae treated by MIPEF. The authors stated that, rather than release oil from intracellular space, MIPEF process leads to a loss of water and hydrophilic compounds causing oil fusion within the cell facilitating contact between oil and solvents (Figure 1.6).

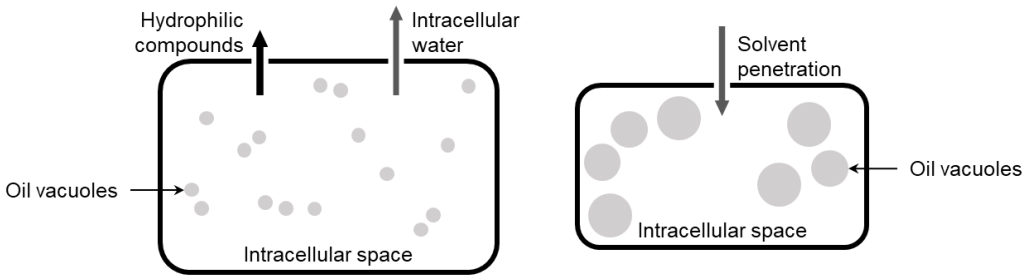

Figure 1.6 Improvement of oil extraction yield (EY) by MIPEF. Adapted from Han et al. (2019).

In another work, Bensalem et al. (2018) evaluated the application of MIPEF, mechanical stressing, and its combination to enhance oil extraction from *Chlamydomonas reinhardtii*. Interestingly, the MIPEF processing at 5.5 $kV \cdot cm^{-1}$ did not improve the oil extraction, but the combination of MIPEF and mechanical stress caused a 50.5 % increase compared to untreated microalgae. These results were associated with an increase in cell permeability provoked by loss of cell viability detected by flow cytometer analysis. Cell lysis was suggested to occur as a result of cell wall rearrangements induced by mechanical stress, while electrical stress contributed through irreversible electroporation and fusion of oil droplets. In addition to the effects of MIPEF observed in

the oil EY of olives and oilseeds, the impact in the bioactive compound profile of the extracted oil has been also evaluated. Interestingly, different studies have reported that TC, chlorophyll, carotenoids, and α-tocopherol content increased in oils extracted from PEF-treated products maintaining, at the same time, their fatty acids profile, γ-tocopherol concentration, and lignans content with no significant changes, as reported in Table 1.5. In this sense, the use of MIPEF as pretreatment of the oil extraction process led to higher yields and, furthermore, to oils with significant contents of bioactive compounds that could be related to health-related benefits. Nonetheless, further research should be conducted in order to evaluate the bioavailability of these compounds immediately after processing and during storage.

Table 1.5 Extraction yield and bioactive compounds profile of oils extracted from olives and oilseeds treated by MIPEF.

Food matrix	MIPEF processing	EY	CC	α-T	TP	Comparison to untreated samples	References
Olive							
	E, 2.0 kV·cm^{-1} w/malaxation	14.1	4.4	247.8	148.9	Increase of α-T Reduction of CC, carotenoids content, and TP	Abenoza et al. (2013)
	E, 2.0 kV·cm^{-1}	22.7	-	143	451 [a]	Increase of α-T, TP, and phytosterols content	Puértolas and Martínez de Marañón (2015)
Amfissis *Manaki* *Tsounati*	*W*, 20 kJ·kg^{-1}	13.2 11.9 25.2	-	-	153.5 341 928.5	Increase of TP	Andreou et al. (2017)
Carolea *Ottobratica* *Coratina*	*W*, 17 kJ·kg^{-1}	15.9 13.1 16.6	-	-	-	Increase of total phenols and oleuropein derivatives	Veneziani et al. (2019)
Sesame seed							
	W, 40 kJ·kg^{-1}	71.6	-	-	-	-	Sarkis et al. (2015)
Sunflower seed							
	E, 1.1 kV·cm^{-1}	47.7	-	602.2	-	Increase of α-T	Moradi and Rahimi (2018)
Rapeseed							
Hulled *Dehulled*	*W*, 84 kJ·kg^{-1}	42.9 36.5	28.6 268.2	185.7 242.9	185.0 [a] -	Increase of CC, α-T, TP, AC, and phytosterols content	Guderjan et al. (2007)

EY, extraction yield (g of oil·100 g^{-1} of food); CC, chlorophyll content (mg pheophitin·kg^{-1}); α-T, α-tocopherol concentration (mg·kg^{-1}); TP, total phenolics (mg gallic acid equivalents·kg^{-1}); AC, antioxidant capacity. *E*, electric field strength; *W*, specific energy input.

[a] TP was expressed as mg of caffeic acid equivalents per kg of oil.

Bioactive compounds extraction

The application of MIPEF to improve phytochemicals extraction has been investigated in different food matrices, such as whole fruits, vegetables, and industrial processing by-products with different purposes. Firstly, MIPEF has been applied on fruits and vegetables

with the aim of increasing their bioactive compounds concentration and thus their functional properties. Secondly, by-products from the food industry could be processed by MIPEF to recover their principal bioactive compounds, develop functional ingredients, and incorporate them into cosmetic or food formulations.

Saldaña et al. (2017) attempted to incorporate MIPEF processing into wine production by investigating the effect of *E*, pulse number (*n*), and *t* on total PC, color, and maceration time of grapes from different varieties. An overall increase in total PC (50.7 %) and color intensity (64.0 %) of grapes from different varieties pretreated with 1 pulse at 5.0 $kV \cdot cm^{-1}$ during 100 µs was observed; however, the maceration time differed among varieties. The combined effect of *E* and *t* was suggested to improve electroporation by effectively reaching the maximum transmembrane voltage at MIPEF treated conditions. Similarly, Maza et al. (2019) explored MIPEF application as a pretreatment to improve the industrial-scale production of Garnacha wine by increasing its phenolic compounds content and quality characteristics. It was concluded that a MIPEF pretreatment at 4.0 $kV \cdot cm^{-1}$ caused a significant increase in total PC, total AC, and condensed tannins (CT) of 29.5, 18.2, and 42.6 %, respectively, after only three days of maceration, compared to untreated grapes. Concerning wine quality characteristics, authors reported that color parameters of wine produced from MIPEF-treated grapes significantly improved and no negative effects on aroma, pH, alcohol content, and total acidity were detected. Maza et al. (2019) stated that color improvement was related to the extraction of condensed tannins from grapes skin rather than seeds. Luengo et al. (2016) compared the impact of MIPEF processes at different *W* (0–70 $kJ \cdot kg^{-1}$), on *z* and betaine extraction from red beet. The authors observed that, up to 20 $kJ \cdot kg^{-1}$, *z* depended on *W* rather than *E* or *t*, while comparable increments in betaine extraction yields of 660.0 % and 720.0 % were reported when MIPEF treatment were applied at 29 and 43 $kJ \cdot kg^{-1}$, respectively. Liu et al. (2019) compared MIPEF processing and drying as pretreatments to enhance phenolic compounds extraction from whole tea leaves and observed that MIPEF at *W* of 22 $kJ \cdot kg^{-1}$ increased 100.0 % and 215.4 % catechin extraction rate in comparison to drying and fresh leaves, respectively, with no differences in the PC profile. The authors attributed the increase in catechin contents to the electroporation phenomena, which was confirmed by SEM images. In another research, a MIPEF process at 2.0 $kV \cdot cm^{-1}$ and 20

pulses was applied to enhance the bioactive compounds content of sliced pumpkin (García-Parra et al. 2018). Lutein, α-carotene, and β-carotene concentrations raised 47.1, 38.9, and 34.2 % compared to untreated pumpkins. Interestingly, pumpkin treated by MIPEF maintained its concentration of total PC, but antioxidant capacity was significantly reduced. Xue and Farid (2015) applied PEF processing combined with thermal treatment to increase PC, proteins, and polysaccharides extraction from white button mushrooms. They reported that the combination of PEF (*E*, 38.4 kV·cm^{-1}; *t,* 272 μs) and thermal treatment (85 °C, 2.6 min) enhanced PC, proteins, and polysaccharides EY by 23.1, 3.8, and 23.4 %, respectively, compared to a conventional aqueous extraction. This improvement was associated to the electroporation phenomena and phospholipids rearrangement in the cell membrane provoked by the thermal and MIPEF treatments.

Recently, different researchers have focused their attention on the effects of PEF at low intensities (0.1–3.0 kV·cm^{-1}) to induce the generation of secondary metabolism as a stress response. Namely, González-Casado et al. (2018a) employed MIPEF processing to induce carotenoids accumulation on whole tomatoes stored at different conditions. Application of 0.02 kJ·kg^{-1} incremented total carotenoids and lycopene content by 58.0 and 150.0 %, respectively, in tomatoes stored at 12 °C for 150 h without affecting its physicochemical properties. Contrarily, the MIPEF treatment applied at higher *W* (0.4 kJ·kg^{-1}) enhanced the concentration of β-carotene (77.0 %), γ-carotene (200.0 %), and lutein (238.0 %) of whole tomatoes stored at 12 °C for 24 h, but compromised tomatoes pH and hardness. In another research, same authors evaluated MIPEF processes at different *E* (0.4, 1.2, and 2.0 kV·cm^{-1}) along with 5, 18, 30 pulses to increase the carotenoids content of whole tomatoes before puree preparation. Total carotenoids and lycopene concentrations in tomato puree improved by 50.0 and 53.0 %, respectively, when MIPEF-treated tomatoes at 2.0 kV·cm^{-1} and 30 pulses were used, suggesting that this treatment elicited a stress response in the whole tomato which was corroborated by an increment of acetaldehyde production and a reduction of ethylene concentration. However, despite the highest increases were obtained under these conditions, a greater bioaccessibility was achieved when lesser pulses were applied (*E*, 2.0 kV·cm^{-1}; *n*, 5) to whole tomatoes. Hence, lycopene, β-carotene, γ-carotene, and lutein bioaccessibility was increased by 132.0, 53.0, 527.0, and 125.0 %, respectively, compared to purees prepared

from untreated tomatoes. These results were related to competitive inhibition among carotenoids and its interaction with cell wall components, both produced by the application of more intense MIPEF treatments (González-Casado et al. 2018b, 2018c). In a recent study, López-Gámez et al. (2020) assessed total PC of whole carrots MIPEF-treated at different *W* (0.1–3.9 $kJ \cdot kg^{-1}$). The authors reported no significant changes in TC immediately after MIPEF treatment, but TC increased by 39.5 and 40.1 % in samples treated at 0.6 and 0.9 $kJ \cdot kg^{-1}$, respectively, after 24 h of refrigerated storage (4 °C). Also, MIPEF processes applied at highest *W* values were directly related to an increment in conductivity and a loss of cell viability. After the obtained evidence from these results, the authors concluded that rather than a release of PC, electropermeabilization of whole carrots induced ions lost drifting in the biosynthesis of PC as a result of the stress response.

Due to the large number of by-products generated by the food industry during the production of fruits, vegetables, grains, and seeds, the application of MIPEF has been resulted of great interest for researchers in order to extract bioactive compounds from shells, peels, bagasse, leaves, and barks, among other, and to develop functional ingredients, having positive impact in the environment. Recent studies have been conducted during the last decade obtaining interesting results, however the available information is scarce compared to other MIPEF applications. Lohani and Muthukumarappan (2016) used MIPEF to enhance total PC and AC of sorghum flour and apple pomace. After a MIPEF process at 2.0 $kV \cdot cm^{-1}$ and 875 µs, total PC and AC of sorghum flour increased 24.8 and 33.9 %, respectively. Likewise, in the apple pomace treated at 2.0 $kV \cdot cm^{-1}$ for 500 µs, a higher increase was observed; the total PC and AC were boosted by 37.4 and 86.0 %, respectively, compared to untreated samples. Authors confirmed through light microscopy and SEM analysis of MIPEF-treated by-products that the observed changes were related to electroporation phenomena. Recently, Kumari et al. (2019) investigated the MIPEF impact on bioactive compounds extraction from brewers' spent grain. MIPEF treatment at 2.8 $kV \cdot cm^{-1}$ and 3000 pulses, caused an increase in protein and reducing sugar concentrations by 5.8 and 4.9 % in light samples along with 37.0 and 53.4 % in dark samples, respectively, while total PC and AC of both grains were not affected by MIPEF. Barbosa-Pereira et al. (2018) used MIPEF to enhance

the extraction of PC from cocoa and coffee by-products of several varieties, geographical origin, and industrial processing. The authors observed an overall improvement in the concentration of total PC of cocoa and coffee samples (75.0 and 83.0 %, respectively) pretreated at 1.4 $kV \cdot cm^{-1}$ and 1000 pulses. However, the MIPEF impact on PC profile differed among samples being associated to particular characteristics of each analyzed by-product. Similarly, PC concentration and AC of yellow onion by-products treated by MIPEF were evaluated (Tehrani et al. 2019). Results indicated that at 4.1 $kV \cdot cm^{-1}$ and 51 pulses, the total PC and quercetin content along with DPPH and FRAP antioxidant capacities improved by 130.3, 71.1, 89.7, and 16.9 %, respectively, compared to a conventional aqueous extraction.

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Chapter 2

Hypothesis, general objective, and specific objectives

2.1 Hypothesis

The drying process and storage conditions of pecan nuts modify their bioactive compounds concentration and antioxidant capacity. Likewise, the application of pulsed electric fields (PEF) on pecan nuts before oil extraction increases oil extraction yield and bioactive compounds concentration of the extracted oil and the by-product generated from oil extraction usually named cake.

2.2 General objective

To evaluate changes in pecan nuts composition and antioxidant capacity due to their drying process and storage conditions as well as to investigate the PEF effect on pecan nuts oil extraction through oil extraction yield, kernels microstructure, oil stability, cake phenolic compounds concentration and antioxidant capacity.

2.3 Specific objectives

The specific objectives of this research are:

2.3.1 To study drying process effect on moisture, oil content, fatty acids profile, tocopherols, and phenolic compounds along with antioxidant capacity of fresh and dry kernels.

2.3.2 To investigate storage temperature and time effects on phenolic compounds and antioxidant capacity of fresh and dry kernels along with dry in-shell nuts stored at 4 and 25°C for 240 days

2.3.3 To evaluate the PEF effect on fresh kernels using electric field strength and pulse number as processing parameters and determining oil extraction yield, oil acidity and antioxidant capacity as well as cake phenolic compounds and antioxidant capacity.

2.3.4 To study PEF effect on dry kernels by analyzing kernels microstructure using light microscopy and oil stability through its acidity, antioxidant capacity, oil stability index, phytosterols, and tocopherols.

Chapter 3

Research plan and general methodology

3.1 Research plan

The experimental work proposed to accomplish each specific objective of this doctoral thesis was:

Compositional analysis of fresh and dry pecan nut kernels:

Independent variables		Dependent variables	Analytical technique
***Pecan nut kernels** **		***Kernels***	
Western variety	Fresh	Moisture	920.151 AOAC
	Dry	a_w	Capacitance method
		Oil content	Solvent extraction
		Lipid fraction	
		Fatty acids profile	GC–FID
		Tocopherols content	HPLC–PDA
		Non–lipid fraction	
		Total phenolics	Folin–Ciocalteu
		Total flavonoids	Flavonoid–$AlCl_3$ complexation
		Condensed tannins	Acidified vanillin
		Antioxidant capacity	DPPH and ORAC

* In-shell nuts of the 2017 harvest from Sonora, Mexico.
a_w, water activity.
920.151 AOAC, AOAC official method 920.151 solids (total) in fruits and fruit products.
GC–FID, gas chromatography with flame–ionization detector.
HPLC–PDA, high performance liquid chromatography with photometric diode array detector.
DPPH, 2,2–diphenyl–1–picrylhydrazyl (DPPH) radical scavenging capacity.
ORAC, oxygen radical absorbance capacity.

Study of the changes in phenolic compounds concentration and antioxidant capacity of fresh and dry kernels and in-shell nuts stored for eight months at two different temperatures (Figure 3.1).

Independent variables		Dependent variables	Analytical technique
***Pecan nut kernels** *		***Non–lipid fraction***	
Western variety	Fresh	Total phenolics	Folin–Ciocalteu
	Dry	Total flavonoids	Flavonoid–$AlCl_3$ complexation
	Dry - In-shell	Condensed tannins	Acidified vanillin
		Antioxidant capacity	DPPH and ORAC
Storage temperature (°C)	4, 25		
Time (days)	240 **		

* In-shell nuts of the 2017 harvest from Sonora, Mexico.
** Samples were analyzed at day 0, 15, 30, 45, 60, 90, 120, 150, 210, and 240.
DPPH, 2,2–diphenyl–1–picrylhydrazyl (DPPH) radical scavenging capacity.
ORAC, oxygen radical absorbance capacity.

a b

Figure 3.1 Fresh and dry kernels (a) along with dry in-shell nuts (b) employed to analyze changes in phenolic compounds concentration and antioxidant capacity of pecan nuts throughout storage.

Evaluation of the PEF effect on oil extraction yield and oil quality as well as phenolic compounds content of the cake by applying different electric field strengths (*E*) and pulse number (*n*) during processing. PEF treatments were expressed as the specific energy input (*W*) applied.

Independent variables		Dependent variables	Analytical technique
Pecan nut kernels *		***Kernels***	
Western variety	Fresh	Moisture	920.151 AOAC
	Control	Oil content	Solvent extraction
	Reference		
		Oil	
E	2.0, 5.0, 7.5	Oil extraction yield	
($kV \cdot cm^{-1}$)		Acidity	940.58 AOAC
		Antioxidant capacity	DPPH
n	10, 55, 100		
		Cake	
W	0.5 –17.6	Oil content	Solvent extraction
($kJ \cdot kg^{-1}$)		Total phenolics	Folin–Ciocalteu
		Condensed tannins	Acidified vanillin
		Antioxidant capacity	DPPH

* In-shell nuts of the 2018 harvest from Sonora, Mexico.
Control, kernels soaked in tap water for 20 min.
Reference, kernels without treatment nor soaking.
920.151 AOAC, AOAC official method 920.151 solids (total) in fruits and fruit products.
940.58 AOAC, AOAC official method 940.28 fatty acids (free) in crude and refined oils.
DPPH, 2,2–diphenyl–1–picrylhydrazyl (DPPH) radical scavenging capacity.

Based on the obtained results, a PEF treatment was selected to evaluate pecan nuts structural and chemical changes as possible factors responsible for modifications of oil extraction yield and oil and cake composition.

Independent variables		Dependent variables	Analytical technique
Pecan nut kernels *		***Kernels***	
	Dry	Moisture	920.151 AOAC
	Control	Oil content	Solvent extraction
	Reference	Microscopy	Light microscopy
		Enzyme activity	Spectrophotometric measurement of lipoxygenase (LOX) and phenylalanine ammonia-lyase (PAL)
E ($kV \cdot cm^{-1}$)	5.0		
		Oil	
n	10	Oil extraction yield	
		Acidity	940.58 AOAC
W ($kJ \cdot kg^{-1}$)	0.8	Antioxidant capacity	DPPH
		Oil stability index	Rancimat method
		Phytosterols	HPLC-ELSD
		Tocopherols	HPLC–PDA
		Cake	
		Oil content	Solvent extraction
		Total phenolics	Folin–Ciocalteu
		Total flavonoids	Flavonoid–$AlCl_3$ complexation
		Condensed tannins	Acidified vanillin
		Antioxidant capacity	DPPH and ORAC

* Kernels halves purchased in a local market in Lleida, Spain.
Control, kernels soaked in tap water for 3 min.
Reference, kernels without treatment nor soaking.
920.151 AOAC, AOAC official method 920.151 solids (total) in fruits and fruit products.
940.58 AOAC, AOAC official method 940.28 fatty acids (free) in crude and refined oils.
HPLC–ELSD, high performance liquid chromatography with evaporative light scattering detector.
HPLC–PDA, high performance liquid chromatography with photometric diode array detector.
DPPH, 2,2–diphenyl–1–picrylhydrazyl (DPPH) radical scavenging capacity.
ORAC, oxygen radical absorbance capacity.

3.2 General methodology

The analytical techniques used throughout this doctoral thesis were performed as listed below according to the studied dependent variable:

3.2.1 Storage experiment

3.2.1.1 Kernels analysis

Moisture content

Moisture (2.5 g) was determined according to the 920.151 AOAC Official Method (AOAC 1996) and expressed as g per 100 g of pecan nut kernels (dry basis, db).

Water activity (a_w)

Pecan nut kernels were ground using a laboratory mortar to determine a_w with an AQUALab-a_w instrument (NY, USA) at 25°C using the capacitance method.

Oil content

Oil extraction was performed as reported by Villarreal-Lozoya et al. (2007) with modifications. Kernels were ground in a laboratory mortar, then samples (3.0 g) were defatted with hexane (1:10 w/v) using a homogenizer adapted with a S 25 N-25G-ST dispersing tool (IKA® T25 Ultra-turrax, IKA, Germany) for 1.5 min at 6,000 rpm. Next, samples were centrifuged (3.273 g, 15 min, 20°C) (Allegra X-12 Centrifuge, Beckman Coulter Inc., USA) and supernatants collected. This procedure was repeated three times. Pooled supernatants were concentrated using a medium-scale vacuum evaporator (2.5 h, 45°C) (Genevac RKCM-12060-SNN, SP Scientific, England). The extracted oil was used to determine oil content gravimetrically in an air oven at 60°C for 24 h following the AOAC 960.39 procedure (AOAC 1996). Oil content was expressed as g per 100 g of pecan nut kernels db.

3.2.1.2 Lipid fraction analysis

Fatty acids profile

Lipid fraction of pecan nut kernels were analyzed using the methodology reported by Christie (1989). Samples (0.1 g) were dissolved in toluene (1:10 v/v), then 2 mL of 2% methanol:sulfuric acid were added. Next, the mixture was heated in a water bath at 80°C for 30 min, the fatty acids methyl esters obtained were recovered with hexane (5 mL), vortexed, and centrifuged (3.273 g, 15 min, 20°C). Aliquots of hexane (1 µL) were used for the identification and quantification of fatty acid methyl esters of pecan nuts lipid fraction. Samples were analyzed using a gas chromatograph system with flame ionization detector (GC-FID) (Agilent 7890A, Agilent Technologies, USA) equipped with a 0.25 µm, 0.25 mm×30 m DB-23 capillary column (Agilent Technologies, USA). The temperature of injection and detector were 250 and 230°C, respectively. The mobile phase was N_2 at a flow rate of 1 mL/min. Fatty acids profile was identified by the retention time of myristic, palmitic, heptadecanoic, stearic, arachidic, palmitoleic, cis-9-heptadecenoic, oleic, behenic, eicosenoic, linoleic, and α-linolenic standards. Results were expressed as g of fatty acid methyl ester per 100 g of pecan nut kernels db.

Tocopherols content

Tocopherols were quantified using a modified procedure reported by Andrés et al. (2014). Lipid fraction samples (25 mg) were reconstituted in a mobile phase consisted of methanol:tetrahydrofuran:water (67:27:6) (1 mL). Aliquots (40 µL) were injected into a Waters HPLC system with a photodiode array detector (HPLC-PDA) equipped with an In-Line Degasser AF, a 600 Controller, a 717 Plus Autosampler, and a 2996 Photodiode Array Detector (Waters Corporation, USA). Tocopherols separation was achieved using a 5 µm, 4.6 mm×250 mm Waters Spherisorb ODS2 column (Waters Corporation, USA) set at 25°C and an isocratic elution at a flow rate of 1 mL/min. The detector was set at 290 nm. For the quantification of δ-, γ-, and α-tocopherol, standard curves were done by preparing a stock solution and diluting it using the mobile phase (0.3–3.5 mM). Results were expressed as mg of tocopherol per 100 g of pecan nut kernels db.

3.2.1.3 Non-lipid fraction analysis

A non-lipid fraction was obtained from the extraction of pecan nut lipid fraction by removing the remaining hexane using a tube-scale vacuum evaporator (2.5 h, 45°C) (Genevac EZ-2.3, SP Scientific, England) and sieving the pellet. Samples were stored at -20°C in 6×15 cm polyethylene bags and vacuum sealed until aqueous and methanolic extractions. Aqueous extracts were used to determine total phenolics (TP), total flavonoids (TF), and antioxidant capacity whereas condensed tannins (CT) was evaluated in methanolic extracts.

Aqueous extraction

Phenolic compounds from non-lipid fraction were extracted as reported by Wu et al. (2004) and Vazquez-Flores et al. (2017). Samples of non-lipid fractions (0.3 g) were placed in 15 mL centrifuge tubes along with an extraction solution (acetone:water:acetic acid, 70:29.5:0.5) in a 1:10 w/v proportion and sonicated in an ultrasonic bath (Ultrasonic Cleaner 97043-970, VWR, China) for 30 min with agitation every 10 min. Next, samples were centrifuged (3.273 g, 15 min, 20°C) and supernatants collected. The extraction process was performed twice. After evaporation of the pooled supernatants, using a tube-scale vacuum evaporator (2.5 h, 45°C), aqueous extracts were diluted to 5 mL with

distilled water in a volumetric flask. Aqueous extracts were stored in 15 mL centrifuge tubes protected from light at -20°C until analyses.

Methanolic extraction

Methanolic extraction was performed according to Villarreal-Lozoya et al. (2007). Non-lipid fraction (0.1 g) and 1% methanol:hydrochloric acid (1:30 w/v) were placed in a water bath (20 min, 30°C) and vortexed at 0, 10, and 20 min of reaction. Afterwards, supernatants were collected by centrifugation (3.273 g, 15 min, 20°C). This procedure was performed once. Methanolic extracts were diluted using 5 mL volumetric flasks with 1% methanol:hydrochloric acid and stored in 15 mL centrifuge tubes protected from light at -20°C until analyses.

Total phenolics

TP analysis was based on the Folin-Ciocalteu method reported by Singleton and Rossi (1965) and adapted by Villarreal-Lozoya et al. (2007). The Folin-Ciocalteu solution (6.25:100 v/v) was prepared using nano pure water. Samples from aqueous extraction (13 µL) were loaded in a 96-well flat bottom plate (Costar® Assay Plate #9017, Corning, USA) along with 221 µL of Folin-Ciocalteu solution and led to react for 3 min in the dark. Next, 26 µL of 0.5 M Na_2CO_3 were added and allowed to react for 2.5 h in darkness. After this time, absorbance was measured at 765 nm using a microplate reader (Synergy HT Plate Reader, Bio-Tek Instruments Inc., VT). A standard curve (0.1–1.0 $mg \cdot mL^{-1}$) was prepared by diluting a stock solution of gallic acid (1.0 $mg \cdot mL^{-1}$) and TP was determined by comparing absorbance of the samples against the standard curve. Results were expressed as µmol gallic acid equivalents per g of pecan nut kernels db.

Total flavonoids

TF were determined as reported by de la Rosa et al. (2011) adapted for microplate reader measurements. Aliquots from aqueous extracts (100 µL) were loaded in a 48-well flat bottom plate (Nunc™ Multidish 48-well plate #150787, Thermo Scientific, Denmark) followed by 420 µL of distilled water and 30 µL of 0.5 M $NaNO_2$. After 5 min, 0.75 M $AlCl_3$ (30 µL) was added and led to react for 3 min. Later, 0.5 M NaOH (30 µL) was pipetted

and the plate was incubated (30 min, 25°C) in darkness. Absorbance measurements were made at 510 nm. A stock solution was prepared using catechin (0.5 mg·mL^{-1}) and the standard curve was done by dilution (0.1–0.5 mg·mL^{-1}). Results were reported as µmol catechin equivalents per g of pecan nut kernels db.

Condensed tannins

CT were quantified using the hydrochloric acid-vanillin method reported by Price et al. (1978) and modified by Herald et al. (2014). A stock solution of vanillin was prepared by dissolving 1.0 g of vanillin in 100 mL of 4% methanol:hydrochloric acid. Before analysis, vanillin solution was diluted (1:1 v/v) with 8% methanol:hydrochloric acid and placed in a water bath at 30°C. Methanolic extracts (30 µL) were pipetted in a 96-well flat bottom plate followed by vanillin dilution (150 µL) and incubated at 30°C for 20 min. A 1% methanol:hydrochloric acid solution was used as blank and absorbance measurements were made at 500 nm. A stock solution of catechin in 1% methanol:hydrochloric acid (3.5 mg·mL^{-1}) was made to prepare a standard curve (1.0–3.5 mg·mL^{-1}) and measure CT content of methanolic extracts. Results were expressed as µmol catechin equivalents per g of pecan nut kernels db.

Antioxidant capacity

To spectrophotometric screening of antioxidant capacity of pecan nuts, two *in vitro* methods based on different mechanisms were selected: DPPH that determines antioxidants ability to transfer one electron to reduce free radicals, and ORAC that measures quenching ability of antioxidants by hydrogen donation (Granato et al. 2018; Shivakumar and Kumar 2018).

DPPH radical scavenging capacity

DPPH was performed according to Villarreal-Lozoya et al. (2007). A 2,2-diphenyl-1-picrylhydrazyl (DPPH) stock solution was prepared by dissolving 0.01 g of DPPH in 25 mL of methanol. Prior to each analysis, the DPPH stock solution was diluted (1.5:10 v/v) in methanol. Aqueous extracts were pipetted (26 µL) into 96 well flat bottom plate. To avoid light exposure, 234 µL of diluted DPPH was added using the dispenser module of

a microplate reader. Absorbance measurements were made at 515 nm and registered every minute until 15 min of reaction. Trolox dissolved in methanol (0.10 mg·mL^{-1}) was used for the standard curve (0.02–0.10 mg·mL^{-1}) and results were expressed as µmol trolox equivalents per g of pecan nut kernels db.

Oxygen radical absorbance capacity

As described by Held (2005), fluorescein solutions, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) solution, and samples dilutions were prepared in 0.075 M phosphate buffer (pH 7.4). Prior to analysis, the fluorescein stock solution (0.003 M) was diluted (1:5000 v/v) and an AAPH solution was prepared (0.15 M). Samples of aqueous extracts (25 µL) were placed in a 96-well black round bottom plate (Costar® Assay Plate #3792, Corning, USA) in addition to fluorescein dilution (150 µL) and incubated (30 min, 37°C) in darkness. After incubation, AAPH solution was added (25 µL) and fluorescence measured at 485 nm for excitation and 520 nm for emission employing a microplate reader. Fluorescence decrement was read every minute during 90 min. Trolox dissolved in the phosphate buffer was used to prepare the standard curve (0.025–0.0025 mg·mL^{-1}). Results were expressed as µmol trolox equivalents per g of pecan nut kernels db.

3.2.2 Pulsed electric fields (PEF) experiment

3.2.2.1 PEF application

The application of PEF was conducted in a batch-equipment with a 0.1 µF capacitor (Physics International, USA) delivering monopolar exponential-wave pulses (pulse width, 4 µs) using a TG-70 gas control unit and a pulse generator (PT-55, Pacific Atlantic Electronics Inc., USA). Kernels halves were manually sectioned in half, immersed in tap water with a conductivity of 463.0 µS·cm^{-1} (1:3 w/w), and placed in parallelepiped methacrylate containers (20×8 cm) equipped with stainless steel parallel electrodes (Figure 3.2). Treatments were performed at different *E* (2.0, 5.0, and 7.5 kV·cm^{-1}) and *n* (10, 55, and 100). The *W*, expressed as kJ per kg of kernels in wet basis (wb), was calculated according to Equation 1 and 2 reported by López-Gámez 2020:

$$V = E \times d \qquad \text{Equation 1}$$

$$W = \frac{V^2 \cdot C \cdot n}{2 \cdot m} \qquad \text{Equation 2}$$

where V is the input voltage (kJ·Coulomb^{-1}), E is the electric field strength (kV·cm^{-1}), d is the distance between electrodes (cm), C is the capacitance of the energy storage capacitor (Coulomb^2·kJ^{-1}), n is the number of pulses, and m is the initial mass of kernels (kg wb).

a b

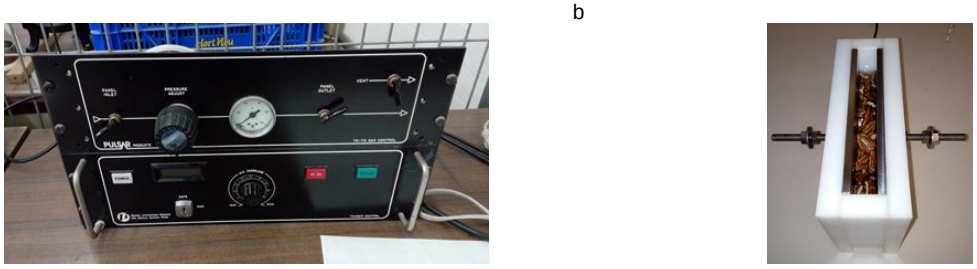

Figure 3.2 Pulse generator (PT-55, Pacific Atlantic Electronics Inc., USA) (a) and containers (b) to application of PEF.

3.2.2.2 Oil extraction

The mechanical extraction of pecan nut oil was performed using an expeller type screw press (YD-ZY-02A, Yoda Europe, China). All samples (85.0 g) were submitted to the same preset conditions while kernels feeding along with oil and cake recovery were standardized to prevent oil and cake loss (Figure 3.3).

Figure 3.3 Screw pressing machine used to oil extraction from fresh and dry kernels pretreated by PEF.

3.2.2.3 Kernels analysis

Moisture and oil content of kernels employed for the application of PEF were performed as previously described (Section 3.2.1.1).

Microscopy

The microstructural analysis of control and PEF-treated kernels was achieved employing a procedure reported by Kendall et al. (2018) with modifications followed by Richardson staining (Richardson et al. 1960). Glutaraldehyde and osmium tetroxide solutions were prepared in 0.1 M phosphate buffer (pH 7.2). Kernels were fixed in 2.5% glutaraldehyde solution and left overnight. Next, samples were washed three times in 0.1 M phosphate buffer and post-fixed in 1% osmium tetroxide solution for 2 h. Subsequently, samples were washed twice with 0.1 M acetate buffer, incubated with 0.5% uranyl acetate for 30 min, and rinsed again two times in 0.1 M acetate buffer. Kernels were dehydrated in an acetonitrile series (30–100%) before embedding in epoxy EMbed 812 resin and polymerizing for 48 h at 60°C. Semithin and ultrathin sections were obtained in a ultramicrotome (Reichert Jung Ultracut E, Leica Microsystems, USA) and stained with Richardson blue. Stained samples were examined at 20× and 100× by light microscope (Olympus BX41, Olympus, USA).

Enzyme activity

Enzyme extraction

Enzymatic extracts were obtained as described by Christopoulos and Tsantili (2015) for lipoxygenase (LOX) and phenylalanine ammonia-lyase (PAL) activities. An extraction solution was prepared by dissolving β-mercaptoethanol (5.0 mM), polyvinylpolypyrrolidone (1:100 w/v), and Triton X-114 (0.05:100 w/v) in 50 mM phosphate buffer (pH 6.6). Kernels (2.0 g) and the extraction solution (10 mL) were homogenized for 40 s at 6,000 rpm (IKA® T25 Ultra-turrax, IKA, Germany), filtered using glass wool, and centrifuged (8,000 rpm, 15 min, 4°C) (Hettich® Universal 320R, Hettich, Germany). Supernatants were collected for determination of enzyme activity in reference, control, and PEF-treated kernels.

Lipoxygenase activity

LOX activity was measured according to the procedure reported by Li et al. (2012) with modifications. Solutions were prepared in 0.2 M borate buffer (pH 9.0). A substrate stock solution was done by dissolving linoleic acid in ethanol and borate buffer (1:1:1000 v/v/v). The stock solution (5 mL) was diluted completely in 20 mL of borate buffer and 5 mL of distilled water. The diluted solution (2 mL) and borate buffer (950 µL) were pipetted in a cell quartz and mixed by inversion. Next, enzymatic extracts (50 µL) were added and mixed by inversion. Absorbance was measured at 234 nm and registered every 10 s until 3 min of reaction in a UV-VIS spectrophotometer (Cecil CE 1010, Cecil Instruments Ltd., England). LOX activity was calculated as reported by Gardner (2001) employing the molar extinction coefficient (ε) of the hydroperoxide 26,800 $M^{-1} \cdot cm^{-1}$ to express it as µmol of hydroperoxide produced per L of LOX per s.

Phenylalanine ammonia-lyase activity

PAL activity was measured as reported by Christopoulos and Tsantili (2015). Solutions were prepared in 0.2 M borate buffer (pH 8.8). Enzymatic extracts (200 µL) mixed with borate buffer (2 mL) and 20 mM L-phenylalanine solution (0.5 mL) were incubated for 30 min at 37°C. Next, 0.5 mL of 6.0 M HCl was added to stop the reaction, and absorbance was measured at 290 nm using a quartz cell in a UV-VIS spectrophotometer (Cecil CE 1010, Cecil Instruments Ltd., England). PAL activity was expressed as µmol of trans-cinnamic acid produced per L of PAL per s using ε of the trans-cinnamic acid 9,568 $M^{-1} \cdot cm^{-1}$.

3.2.2.4 Oil analysis

Oil extraction yield

The oil extraction yield (OEY, %) of reference, control, and PEF-treated kernels was calculated as follows:

$$OEY = \frac{(m_K \times L_K) - (m_C \times L_C)}{(m_K \times L_K)} \times 100 \qquad \text{Equation 3}$$

where m_K and m_C are the mass (g) of freeze-dried kernels and cakes, respectively, while L_K and L_C are the oil content expressed as g of oil per 100 g of freeze-dried kernels and cakes, respectively, all in db.

The oil extracted into the soaking water (o_{SW}) was calculated to determine the total OEY (OEY_{TOTAL}, %) of control and PEF-treated kernels using Equation 4 and 5:

$$o_{SW} = \left(m_K \times L_{Reference}\right) - (m_K \times L_K) \quad \text{Equation 4}$$

where o_{SW} is the mass (g) of oil retained into the soaking water and $L_{Reference}$ is the oil content of reference kernels expressed as g of oil per 100 g of freeze-dried kernels db.

$$OEY_{TOTAL} = \frac{[(m_K \times L_K) - (m_C \times L_C)] + o_{SW}}{(m_K \times L_K)} \times 100 \quad \text{Equation 5}$$

Acidity

Oil acidity was determined following the AOAC 940.28 method (AOAC 1996).

Antioxidant capacity

Oil antioxidant capacity was evaluated using the DPPH radical scavenging capacity method reported by Gao et al. (2019) with modifications. A DPPH solution was prepared by dissolving 0.05 g of DPPH in 250 mL of methanol. Pecan nut oil (200 µL) diluted in ethyl acetate (2 mL) was mixed with the DPPH solution (2 mL). The reaction was left 15 min in darkness and absorbance measured at 515 nm using a UV-VIS spectrophotometer (Cecil CE 1010, Cecil Instruments Ltd., England). Trolox dissolved in methanol was used for the standard curve (0.003–0.030 mg·mL^{-1}) and results were expressed as mg trolox equivalents per 100 g of pecan nut oil.

Oil stability index

Oil stability index (OSI) was measured with a Rancimat 679 apparatus (Metrohm AG, Switzerland) as reported by Tovar et al. (2002). Oil (3.0 g) was heated at 110°C and air was bubbled at a flow rate of 20 L/h. Next, volatile products released during oxidation

were dissolved in deionized water (60 mL) to follow conductivity changes. OSI was expressed in hours (h) and obtained by Metrodata software (Metrohm AG, Switzerland).

Phytosterols

Extraction and quantification of phytosterols were performed according to Domínguez-Avila et al. (2013) and Nair et al. (2006), respectively. HCl and KOH solutions were prepared using ethanol as solvent. Pecan nut oil (0.1 g) was mixed with 0.5 mL of 6 M HCl and incubated for 1 h at 80°C. The mixture was cooled in a water bath after time elapsed. Next, 5 mL of 1.3% KOH solution was added and left to react for 30 min at 80°C. To phytosterols extraction, 2 mL of distilled water and 5 mL of hexane were included to the mixture, vortexed for 1 min, and centrifuged (3,750 rpm, 15 min, 20°C) (Allegra X-12 Centrifuge, Beckman Coulter Inc., USA). The addition of distilled water and hexane was performed twice. Pooled supernatants were recovered and concentrated using a medium-scale vacuum evaporator (2.5 h, 45°C) (Genevac RKCM-12060-SNN, SP Scientific, England). Extracts were reconstituted in 0.5 mL of hexane for chromatographic analysis. A HPLC-ELSD system (Agilent 1200, Agilent Technologies, USA) equipped with a 5 µm, 4.6 mm×500 mm Luna C8 column (Phenomenex, USA) was employed to identify and quantify phytosterols from pecan nut oil. Column and ELSD temperature were maintained at 40°C and 50°C, respectively. Aliquots (10 µL) were analyzed employing a mobile phase consisted of methanol:water (95:5 v/v) at a flow rate of 1 mL/min and the detector set at a gain of 16. Standard curves of β-sitosterol (0.2–1.2 mM), stigmasterol (0.2–1.2 mM), and campesterol (0.05–0.25 mM) were prepared using hexane as solvent by dilutions. Results were expressed as mg per kg of pecan nut oil.

Tocopherols

Tocopherols content of oil extracted by mechanical pressing of pecan nut kernels was determined following the methodology previously described (Section 3.2.1.2). Results were expressed as mg per kg of pecan nut oil.

3.2.2.5 Cake analysis

Oil content, TP, TF, CT, and antioxidant capacity of the cakes obtained from oil extraction were carried out as described for kernels in the storage experiment (Section 3.2.1.1 and 3.2.1.3). Oil content results were expressed as g of oil per 100 g of cake db. TP was expressed as mmol gallic acid equivalents per 100 g of defatted cake db, TF and TC were expressed as catechin equivalents per 100 g of defatted cake db, and DPPH and ORAC were expressed as mmol trolox equivalents per 100 g of defatted cake db.

3.2.3 Statistical analysis

One-way ANOVA was used to evaluate the effect of storage conditions and PEF on pecan nuts. The Tukey test was performed in the storage experiment to determine differences between means, whereas the Dunnett test was applied to determine differences against control kernels in the PEF experiment. Correlation coefficients were calculated to determine relationships between variables. These analyses were performed using Minitab 18 software (Minitab® 18.1, USA).

Chapter 4

Results

4.1 Changes in bioactive compounds content and antioxidant capacity of pecan nuts [*Carya illinoinensis* (Wangenh. K. Koch)] during storage

Abstract

Pecan nut kernels are rich in health-promoting substances such as unsaturated fatty acids, tocopherols, and phenolic compounds. Due to their importance in human diets, it is essential to evaluate the effects of drying, and storage temperature and time, on these phytochemicals. Moisture, water activity, oil content, bioactive compounds concentration, and antioxidant capacity of fresh and dry pecan nuts (Western variety) were determined. Kernels and in-shell nuts were stored (240 days) at 4°C and 25°C, monitoring the changes in tocopherols concentration, phenolic content, and antioxidant capacity. Fresh and dry kernels exhibited similar fatty acids profile and γ-tocopherol concentration (20.37±0.73 and 19.27±1.62 mg·100 g^{-1} kernels in dry basis), whereas phenolic content and antioxidant capacity decreased due to the drying process (33.2 and 22.3%, respectively). Throughout storage, condensed tannins concentration of kernels and in-shell nuts reduced by 31.5 and 41.8%, while DPPH antioxidant capacity improved 216.4 and 188.4%, respectively. These results evidenced that drying and storage time are the most significant variables regarding pecan nuts postharvest processing; nonetheless, further research related to the relationship between condensed tannins degradation and the increments in DPPH antioxidant capacity is needed.

4.1.1 Introduction

Pecan nuts [*Carya illinoinensis* (Wangenh. K. Koch)] are indigenous from Mexico and the United States, together they provide 92% of the worldwide pecan nut production (Bello-Huitle et al. 2010; Corral-Escárcega et al. 2017; International Nut and Dried Fruit Council 2019) The pecan nut kernel, or edible part, has been recognized as a source of bioactive compounds; its lipid fraction contains mono- and polyunsaturated fatty acids, tocopherols, and phytosterols that help diminish plasma cholesterol concentration and act as antioxidants preventing coronary heart disease (Atanasov et al. 2018; Fernandes and Cabral,2007; Gong et al. 2017; U.S. Department of Agriculture 2017), whereas its non-lipid fraction comprises a significant amount of phenolic compounds with reported biological and pharmacological properties (antibacterial, anticancer, anti-inflammatory, antioxidant, antiviral, immunomodulatory, and cardioprotective) (Flores-Martínez et al. 2016; Jia et al. 2018; Smeriglio et al. 2017).

Several research groups have focused on investigating the phytochemical profile of pecan nut kernels from different varieties (Flores-Córdova et al. 2017; Robbins et al. 2014) or growing location (de la Rosa et al. 2014; Domínguez-Avila et al. 2013), while their bioactive compounds changes during maturation are becoming an area of great interest (Bouali et al. 2014; Carrasco-Del Amor et al. 2017; Jia et al. 2018). However, a gap is found regarding the preservation of pecan nuts with outdated investigations directed to evaluate how processing impacts on kernels sensorial quality rather than on their phytochemical composition. For instance, Herrera (1994) investigated sensorial changes in dry pecan nut kernels collected at different harvest dates; Erickson et al. (1994) associated physical and chemical analyses to sensory changes of raw and roasted kernels stored at different conditions, and Kanamangala et al. (1999) analyzed shelf-life of reduced-lipid kernels stored at ambient temperature. There are other studies directed to extend pecan nuts shelf-life by applying edible coatings (Baldwin and Wood, 2006), evaluating different packaging materials (Oro et al. 2008), or using electron-beam irradiation (Villarreal-Lozoya et al. 2009). Therefore, the objective of this research was to investigate the impact of common practices related to pecan nuts processing (drying, storage temperatures, along with kernels and in-shell storage) on their bioactive

compounds content (fatty acids, tocopherols, and phenolic compounds) and antioxidant capacity throughout storage.

4.1.2 Materials and methods

4.1.2.1 Chemicals

Methanol (MeOH), tetrahydrofuran (THF), and water (H_2O) HPLC grade were purchased from Sigma-Aldrich (USA) along with fatty acid methyl esters, δ-, γ-, and α-tocopherols, gallic acid, catechin, Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), fluorescein sodium salt, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), and sodium phosphate dibasic (Na_2HPO_4). Ethanol (EtOH), sodium nitrite ($NaNO_2$), and sodium hydroxide (NaOH) were purchased from DEQ (Mexico). Hexane, hydrochloric acid (HCl), sulfuric acid (H_2SO_4), and aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$) were acquired from Fermont (Mexico). Acetone and sodium carbonate anhydrous (Na_2CO_3) were obtained from Avantor Performance Materials (Mexico) and CTR Scientific (Mexico), respectively. Working solutions were protected from light and stored at -20°C.

4.1.2.2 Pecan nuts

Pecan nuts (*Carya illinoinensis*) from the autumn 2017 harvest (Western variety) were donated by a producer from Cajeme, Sonora, Mexico (27°29’38”N, 109°56’20”W). In-shell fresh pecans were collected directly from the orchard after shucks split, whereas in-shell dry pecans were obtained after a drying process in the producers’ facility.

4.1.2.3 Storage experiment

Fresh and dry in-shell pecans were visually inspected removing damaged or germinated nuts and classified into different experimental groups: fresh and dry kernels stored at 4°C, and in-shell dry pecan nuts stored at 4 and 25°C. A portion of 30 g of kernels and their equivalent of in-shell nuts (100 g) were placed in 12×15 cm polyethylene bags (Filmpack S.A. de C.V., Mexico) and vacuum-sealed (EVD 4, TORREY, Mexico) until sampling day. Moisture, water activity (a_w), oil content, fatty acids profile, tocopherols, total phenolics (TP), total flavonoids (TF), condensed tannins (CT), and antioxidant capacity were

determined for fresh and dry samples. TP, TF, CT, and antioxidant capacity of fresh and dry kernels were evaluated at days 0, 15, 30, 45, 60, 90, 120, 150, 210, and 240 while in-shell nuts were analyzed at day 0, 15, 60, 150, and 240. Tocopherols content of kernels and in-shell pecan nuts was determined at days 0 and 240.

4.1.2.4 Kernels analysis

Moisture content

Moisture (2.5 g) was determined according to the 920.151 AOAC Official Method (AOAC 1996) and expressed as g per 100 g of pecan nut kernels (dry basis, db).

Water activity (a_w)

Pecan nut kernels were ground using a laboratory mortar to determine a_w with an AQUALab-a_w instrument (Novasina, Switzerland) at 25°C.

Oil content

Oil extraction was performed as reported by Villarreal-Lozoya et al. (2007) with modifications; pecan nut kernels were ground in a laboratory mortar, then samples (3.0 g) were defatted with hexane (1:10 w/v) using a homogenizer adapted with a S 25 N-25G-ST dispersing tool (IKA® T25 Ultra-turrax, IKA, Germany) for 1.5 min at 6,000 rpm. Next, samples were centrifuged (3.273 g, 15 min, 20°C) (Allegra X-12 Centrifuge, Beckman Coulter Inc., USA) and supernatants collected, this procedure was repeated three times. Pooled supernatants were concentrated using a medium-scale vacuum evaporator (2.5 h, 45°C) (Genevac RKCM-12060-SNN, SP Scientific, England). The extracted oil was used to determine oil content gravimetrically in an air oven at 60°C for 24 h following the AOAC 960.39 procedure (AOAC, 1996). Oil content was expressed as g per 100 g of pecan nut kernels db. Lipid fractions were stored (-20°C) in 8 mL amber vial with N_2 in the head-space and sealed with parafilm until analyses.

4.1.2.5 Lipid fraction analysis

Fatty acids profile

Lipid fraction of pecan nut kernels were analyzed using the methodology reported by Christie (1989). Samples (0.1 g) were dissolved in toluene (1:10 v/v), then 2 mL of 2% MeOH:H_2SO_4 were added. Next, the mixture was heated in a water bath at 80°C for 30 min, the fatty acids methyl esters obtained were recovered with hexane (5 mL), vortexed, and centrifuged (3.273 g, 15 min, 20°C). Aliquots of hexane (1 µL) were used for the identification and quantification of fatty acid methyl esters of pecan nuts lipid fraction. Samples were analyzed using a gas chromatograph system with flame ionization detector (GC-FID) (Agilent 7890A, Agilent Technologies, USA) equipped with a 0.25 µm, 0.25 mm×30 m DB-23 capillary column (Agilent Technologies, USA). The temperature of injection and detector were 250 and 230°C, respectively. The mobile phase was N_2 at a flow rate of 1 mL/min. Fatty acids profile was identified by the retention time of myristic, palmitic, heptadecanoic, stearic, arachidic, palmitoleic, cis-9-heptadecenoic, oleic, behenic, eicosenoic, linoleic, and α-linolenic standards (CAS 124-10-7, 112-39-0, 1731-92-6, 112-61-8, 1120-28-1, 1120-25-8, 1981-50-6, 112-62-9, 929-77-1, 2390-09-2, 112-63-0, 301-00-8, respectively). Results were expressed as g of fatty acid methyl ester per 100 g of pecan nut kernels db.

Tocopherols content

Tocopherols were quantified using a modified procedure reported by Andrés et al. (2014). Lipid fraction samples (25 mg) were reconstituted in a mobile phase consisted of MeOH:tetrahydrofuran:H_2O (67:27:6) (1 mL). Aliquots (40 µL) were injected into a Waters HPLC system with a photodiode array detector (HPLC-PDA) equipped with an In-Line Degasser AF, a 600 Controller, a 717 Plus Autosampler, and a 2996 Photodiode Array Detector (Waters Corporation, USA). Tocopherols separation was achieved using a 5 µm, 4.6 mm × 250 mm Waters Spherisorb ODS2 column (Waters Corporation, USA) set at 25°C and an isocratic elution at a flow rate of 1 mL/min. Standard curves were prepared (0.3–3.5 mM) for the quantification of δ-, γ-, and α-tocopherol (CAS 119-13-1, 54-28-4, and 59-02-9, respectively), and the detector was set at 290 nm. Results were expressed as mg of tocopherol per 100 g of pecan nut kernels db.

4.1.2.6 Non-lipid fraction analysis

A non-lipid fraction was obtained from the extraction of pecan nut lipid fraction by removing the remaining hexane using a tube-scale vacuum evaporator (2.5 h, 45°C) (Genevac EZ-2.3, SP Scientific, England) and sieving the pellet. Samples were stored at -20°C in 6×15 cm polyethylene bags and vacuum sealed until aqueous and methanolic extraction. Aqueous extracts were used to determine TP, TF, and antioxidant capacity whereas CT was evaluated in methanolic extracts.

Aqueous extraction

Phenolic compounds from non-lipid fraction were extracted as reported by Wu et al. (2004) and Vazquez-Flores et al. (2017). The extraction method was selected after comparison against other procedures (data not showed). Samples of non-lipid fractions (0.3 g) were placed in 15 mL centrifuge tubes along with an extraction solution (acetone:H_2O:CH_3COOH, 70:29.5:0.5) in a 1:10 w/v proportion and sonicated in an ultrasonic bath (Ultrasonic Cleaner 97043-970, VWR, China) for 30 min with agitation every 10 min. Next, samples were centrifuged (3.273 g, 15 min, 20°C) and supernatants collected. The extraction process was performed twice. After evaporation of the pooled supernatants, using the tube-scale vacuum evaporator (2.5 h, 45°C), aqueous extracts were diluted to 5 mL with distilled water in a volumetric flask. Aqueous extracts were stored in 15 mL centrifuge tubes protected from light at -20°C until analyses.

Methanolic extraction

Methanolic extraction was performed according to Villarreal-Lozoya et al. (2007). Non-lipid fraction (0.1 g) and 1% MeOH:HCl (1:30 w/v) were placed in a water bath (20 min, 30°C) and vortexed at 0, 10, and 20 min of reaction. Afterwards, supernatants were collected by centrifugation (3.273 g, 15 min, 20°C). This procedure was performed once. Methanolic extracts were diluted using 5 mL volumetric flasks with 1% MeOH:HCl and stored in 15 mL centrifuge tubes protected from light at -20°C until analyses.

Total phenolics

TP analysis was based on the Folin-Ciocalteu method reported by Singleton and Rossi (1965) and adapted by Villarreal-Lozoya et al. (2007). The Folin-Ciocalteu solution (6.25:100 v/v) was prepared using nano pure water. Samples from aqueous extraction (13 µL) were loaded in a 96-well flat bottom plate (Costar® Assay Plate #9017, Corning, USA) along with 221 µL of Folin-Ciocalteu solution and led to react for 3 min in the dark. Next, 26 µL of 0.5 M Na_2CO_3 were added and allowed to react for 2.5 h in darkness. After this time, absorbance was measured at 765 nm using a microplate reader (Synergy HT Plate Reader, Bio-Tek Instruments Inc., VT). TP was determined by comparing absorbance of the samples against a standard curve of gallic acid (0.1–1.0 $mg \cdot mL^{-1}$). Results were expressed as µmol gallic acid equivalents per g of pecan nut kernels db (µmol gallic acid $EQ \cdot g^{-1}$ db).

Total flavonoids

TF were determined as reported by de la Rosa et al. (2011) adapted for microplate reader measurements. Aliquots from aqueous extracts (100 µL) were loaded in a 48-well flat bottom plate (Nunc™ Multidish 48-well plate #150787, Thermo Scientific, Denmark) followed by 420 µL of distilled water and 30 µL of 0.5 M $NaNO_2$. After 5 min, 0.75 M $AlCl_3$ (30 µL) was added and led to react for 3 min. Later, 0.5 M NaOH (30 µL) was pipetted and the plate was incubated (30 min, 25°C) in darkness. Absorbance measurements were made at 510 nm and a standard curve of catechin (0.1–0.5 $mg \cdot mL^{-1}$) was used to report TF as µmol catechin equivalents per g of pecan nut kernels db (µmol catechin $EQ \cdot g^{-1}$ db).

Condensed tannins

CT were quantified using the HCl-vanillin method reported by Price et al. (1978) and modified by Herald et al. (2014). A stock solution of vanillin was prepared by dissolving 1.0 g of vanillin in 100 mL of 4% MeOH:HCl. Before analysis, vanillin solution was diluted (1:1 v/v) with 8% MeOH:HCl and placed in a water bath at 30°C. Methanolic extracts (30 µL) were pipetted in a 96-well flat bottom plate followed by vanillin dilution (150 µL) and incubated at 30°C for 20 min. A 1% MeOH:HCl solution was used as blank and absorbance measurements were made at 500 nm. A standard curve of catechin (1.0–3.5

mg·mL^{-1}) was made to measure CT content of methanolic extracts. Results were expressed as µmol catechin EQ·g^{-1} db.

Antioxidant capacity

To spectrophotometric screening of antioxidant capacity of pecan nuts, two *in vitro* methods based on different mechanisms were selected: DPPH that determines antioxidants ability to transfer one electron to reduce free radicals, and ORAC that measures quenching ability of antioxidants by hydrogen donation (Granato et al. 2018; Shivakumar and Kumar 2018).

DPPH radical scavenging capacity

DPPH was performed according to Villarreal-Lozoya et al. (2007). To optimize reaction time, preliminary experiments were performed employing the diluted solution of DPPH (data not showed). A DPPH stock solution was prepared by dissolving 0.01 g of DPPH in 25 mL of MeOH. Prior to each analysis, the DPPH stock solution was diluted (1.5:10 v/v) in MeOH. Aqueous extracts were pipetted (26 µL) into 96 well flat bottom plate. To avoid light exposure, 234 µL of diluted DPPH was added using the dispenser module of the microplate reader. Absorbance measurements were made at 515 nm and registered every minute until 15 min of reaction. Trolox was used for the standard curve (0.02–0.10 mg·mL^{-1}) to express the results as µmol trolox equivalents per g of pecan nut kernels db (µmol trolox EQ·g^{-1} db).

Oxygen radical absorbance capacity

As described by Held (2005), fluorescein solutions, AAPH solution, and samples dilutions were prepared in 0.075 M phosphate buffer (pH 7.4). Prior to analysis, the fluorescein stock solution (0.003 M) was diluted (1:5000 v/v) and an AAPH solution was prepared (0.15 M). Samples of aqueous extracts (25 µL) were placed in a 96-well black round bottom plate (Costar® Assay Plate #3792, Corning, USA) in addition to fluorescein dilution (150 µL) and incubated (30 min, 37°C) in darkness. After incubation, AAPH solution was added (25 µL) and fluorescence measured at 485 nm for excitation and 520 nm for emission employing a microplate reader. Fluorescence decrement was read every minute

during 90 min. Trolox was used to prepare the standard curve (0.025–0.0025 mg·mL^{-1}) and results were expressed as µmol trolox EQ·g^{-1} db.

4.1.2.7 Statistical analysis

All measurements along with pecan nut oil extraction were done by triplicate. To determine the effect of storage conditions on bioactive compounds content of pecan nuts, a one-way ANOVA followed by Tukey test for multiple means comparison along with Pearson correlation (*r*) analysis were performed (α=0.05), using Minitab 18 software (Minitab® 18.1, USA).

4.1.3 Results and discussion

4.1.3.1 Effect of the drying process on pecan nuts composition

Moisture, a_w, oil content, fatty acids profile, tocopherols, and phenolic compounds, along with antioxidant capacity of fresh and dry kernels are shown in Table 4.1.1. The drying process, intended to preserve quality attributes of pecan nuts and avoid lipid oxidation (Kader 2013; Olguín Rojas et al. 2019), decreased the moisture content of fresh kernels from 7.10±0.89 to 2.28±0.08 g·100 g^{-1} db with a consequent reduction of a_w (from 0.89 to 0.49) and an increment of oil content (from 67.36±0.07 to 69.60±0.23 g·100 g^{-1} db). The moisture, a_w, and oil content of dry kernels were in the range of values reported by Fernandeset al. (2017), Flores-Córdova et al. (2016), and the U.S. Department of Agriculture (2017). The major fatty acids identified in pecan nut kernels were oleic and linoleic acids representing more than 85.0% of their total oil content: fresh samples contained a higher concentration of monounsaturated fatty acids, specifically oleic acid (17.9%), while no differences between fresh and dry kernels were observed in total saturated and polyunsaturated fatty acids. However, myristic acid was higher in fresh samples (11.8%) and α-linolenic acid on dry samples (16.9%). The effect of drying has been studied in other nuts such as macadamia nuts (Phatanayindee et al. 2012) and walnuts (Fu et al. 2016) which also presented a reduction on oleic acid, whereas α-linolenic acid was not affected. The maintenance of α-linolenic acid concentration was attributed to a decrement in lipoxygenase (LOX) activity (Fu et al. 2016). Regarding tocopherols group, only γ-tocopherol was identified showing a similar content in fresh and

dry kernels. These values (20.37±0.73 and 19.27±1.62 mg·100 g^{-1} kernels db) were comparable to concentrations reported by Yao et al. (1992) for Western Schley pecan nuts and Fernandes et al. (2017) for cold-pressed pecan oil (28.43±1.46 and 27.27±1.78 mg·100 g^{-1} oil for fresh and dry samples, respectively).

Table 4.1.1 Effect of the drying process on moisture, a_w, oil content, fatty acids profile, tocopherols content, phenolic compounds concentration, and antioxidant capacity of pecan nut kernels.

	Fresh		Dry	
Moisture g·100 g^{-1}	7.10 ± 0.89	a	2.28 ± 0.08	b
a_w	0.84 ± 0.01	a	0.49 ± 0.01	b
Oil content g·100 g^{-1}	67.36 ± 0.07	b	69.60 ± 0.23	a
Fatty acids profile g·100 g^{-1}				
Myristic	0.08 ± 0.00	a	0.07 ± 0.00	b
Palmitic	5.67 ± 0.21	a	5.40 ± 0.36	a
Heptadecanoic	0.05 ± 0.00	a	0.05 ± 0.00	a
Stearic	1.83 ± 0.07	a	1.71 ± 0.12	a
Arachidic	0.08 ± 0.00	a	0.08 ± 0.01	a
Total SFA	7.71 ± 0.29	a	7.31 ± 0.49	a
Palmitoleic	0.05 ± 0.00	a	0.05 ± 0.00	a
Cis-9-Heptadecenoic	0.00 ± 0.00	b	0.04 ± 0.00	a
Oleic	35.63 ± 1.36	a	30.18 ± 2.03	b
Behenic	0.68 ± 0.04	a	0.70 ± 0.05	a
Eicosenoic	0.17 ± 0.00	a	0.18 ± 0.01	a
Total MUFA	36.53 ± 1.41	a	31.14 ± 2.10	b
Linoleic	24.99 ± 0.96	a	27.75 ± 1.86	a
α-Linolenic	0.99 ± 0.04	b	1.17 ± 0.08	a
Total PUFA	26.13 ± 1.00	a	28.92 ± 1.94	a
Tocopherols content mg·100 g^{-1}				
δ-Tocopherol	*ND*		*ND*	
γ-Tocopherol	20.37 ± 0.73	a	19.27 ± 1.62	a
α-Tocopherol	*ND*		*ND*	
Phenolic compounds concentration				
Total phenolics µmol gallic acid EQ·g^{-1}	109.52 ± 4.13	a	82.20 ± 3.85	b
Total flavonoids µmol catechin EQ·g^{-1}	31.91 ± 1.34	a	20.72 ± 1.54	b
Condensed tannins µmol catechin EQ·g^{-1}	298.09 ± 20.11	a	182.44 ± 5.58	b
Antioxidant capacity				
DPPH µmol trolox EQ·g^{-1}	62.07 ± 2.71	a	50.75 ± 2.69	b
ORAC µmol trolox EQ·g^{-1}	182.14 ± 2.00	a	124.85 ± 5.42	b

a_w, water activity; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; *ND*, not detected; EQ, equivalents; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity; ORAC, oxygen radical absorbance capacity. Concentrations were expressed in g of pecan nut kernels in dry basis. Means with different letters within rows were significantly different according to the Tukey test (α=0.05).

The drying process produced a decrement on phenolic compounds content (TP 33.2%, TF 54.0%, and CT 63.4%) and antioxidant capacity (DPPH 22.3% and ORAC 45.9%) of pecan nuts. Christopoulos and Tsantili (2012) along with Wang et al. (2018) also reported losses in phenolic compounds of fresh walnuts and hazelnuts after drying. Wang et al. (2018) proposed that moderate temperatures might promote phenolic compounds oxidation by enhancing enzymatic activity, while phenolic compounds could be released at high temperatures. Despite this, TP concentrations were comparable to the values reported by de la Rosa et al. (2014) and Flores-Córdova et al. (2017); besides TF and CT concentrations were 2.1 and 3.1 folds higher than those reported for pecans from Chihuahua, Mexico (de la Rosa et al. 2014). Antioxidant capacity evaluated by DPPH was 2.9 folds higher in fresh and dry samples compared to those reported by Jia et al. (2019) for Western pecan nuts, whereas ORAC values were comparable to the total antioxidant capacity reported for commercial pecans (Wu et al. 2004).

4.1.3.2 Effect of storage conditions on pecan nuts composition

The storage of fresh and dry kernels along with in-shell nuts at 4 and 25°C impacted differently their retention percentage of γ-tocopherol. Better preservation of γ-tocopherol was observed in dry kernels and in-shell pecan nuts than fresh kernels. At day 240, retention percentage of dry kernels was 73.1% while in-shell pecans maintained 58.3 and 69.3% of their initial concentration at 4 and 25°C, respectively. In contrast, fresh kernels retained only 52.8% of their γ-tocopherol concentration after 210 days of storage at 4°C; further analyses were not possible due to fresh samples deterioration by mold growth. Yao et al. (1992) reported a comparable concentration for dry pecan nut kernels stored at 24°C, while Momchilova et al. (2017) observed higher retention of tocopherols in dry kernels than in in-shell dry hazelnuts; this was related to the maintenance of moisture in in-shell hazelnuts inducing an increment on lipids oxidation rate. Fresh samples presented the highest moisture and a_w values that have been associated to oxidative rancidity of nuts (Shahidi and John 2013; Troller and Christian 2014) and might promote degradation of tocopherols.

The effect of storage conditions on the phenolic content and antioxidant capacity of pecan nuts is shown in Figure 4.1.1. Fresh kernels maintained a higher concentration

of phenolic compounds and antioxidant capacity throughout storage with a significant increment of TP (8.7%), TF (7.1%), CT (22.2%), and ORAC (21.3%) on day 15. Similar to these results, Christopoulos and Tsantili (2012) observed an increase in phenolic compounds of fresh walnut kernels after 20 days of storage at 1°C; the authors associated this effect with a rise in phenylalanine ammonia-lyase (PAL) activity due to chilling stress. Likewise, Corral-Escárcega et al. (2017) proposed the abiotic stress as a technique to increase phenolic compounds content in different food matrices. A further study of Christopoulos and Tsantili (2015) related PAL activity to the synthesis of 4-hydroxybenzoic, 2,4-dihydroxybenzoic, syringic, and vanillic acids while no relation to ellagic acid was found. Identification of 4-hydroxybenzoic, syringic, and ellagic acids have been previously reported in pecan nut kernels by Robbins et al. (2015). Hence, the increment in phenolic content of fresh pecan nut kernels might be attributed to the synthesis of phenolic acids. Even though fresh kernels exhibited higher concentrations of phenolic compounds, they developed mold growth after 210 days of storage, with dry samples being more stable during storage. In-shell pecan nuts stored at 4°C preserved a higher content of phenolic compounds compared to other dry samples. This could be related to polyphenols migration from shells to kernels (Santerre 1994). Also, in-shell preservation at low temperatures has been reported to extend nuts shelf-life by preventing skin darkening and lipids oxidation (Gardea et al. 2011; Kader 2013; Shahidi and John 2010). Total phenolics of fresh and dry kernels along with in-shell nuts stored at 4°C remained above 85.54±1.46, 68.14±3.97, and 74.26±2.97 μmol GAE·g^{-1} db, respectively. These were the lowest concentrations observed during pecan nuts storage, representing 78.1, 62.2, and 67.8% of their initial concentration. No changes in the TP of in-shell samples stored at 25°C were observed (Figure 4.1.1a). Fresh kernels preserved 98.8% of their initial total flavonoids content after 210 days of storage, whereas TF increased by 39.4% in dry kernels and 23.9% on in-shell samples at day 240 (Figure 4.1.1b). Contrarily, a marked decrement in condensed tannins was observed during pecan nuts storage (Figure 4.1.1c). Fresh and dry kernels lost 35.2 and 31.5% of CT concentration, respectively, by the end of the storage. CT of in-shell samples were highly influenced by storage temperature decreasing 41.8 and 53.3% at 4 and 25°C, respectively, at day 240.

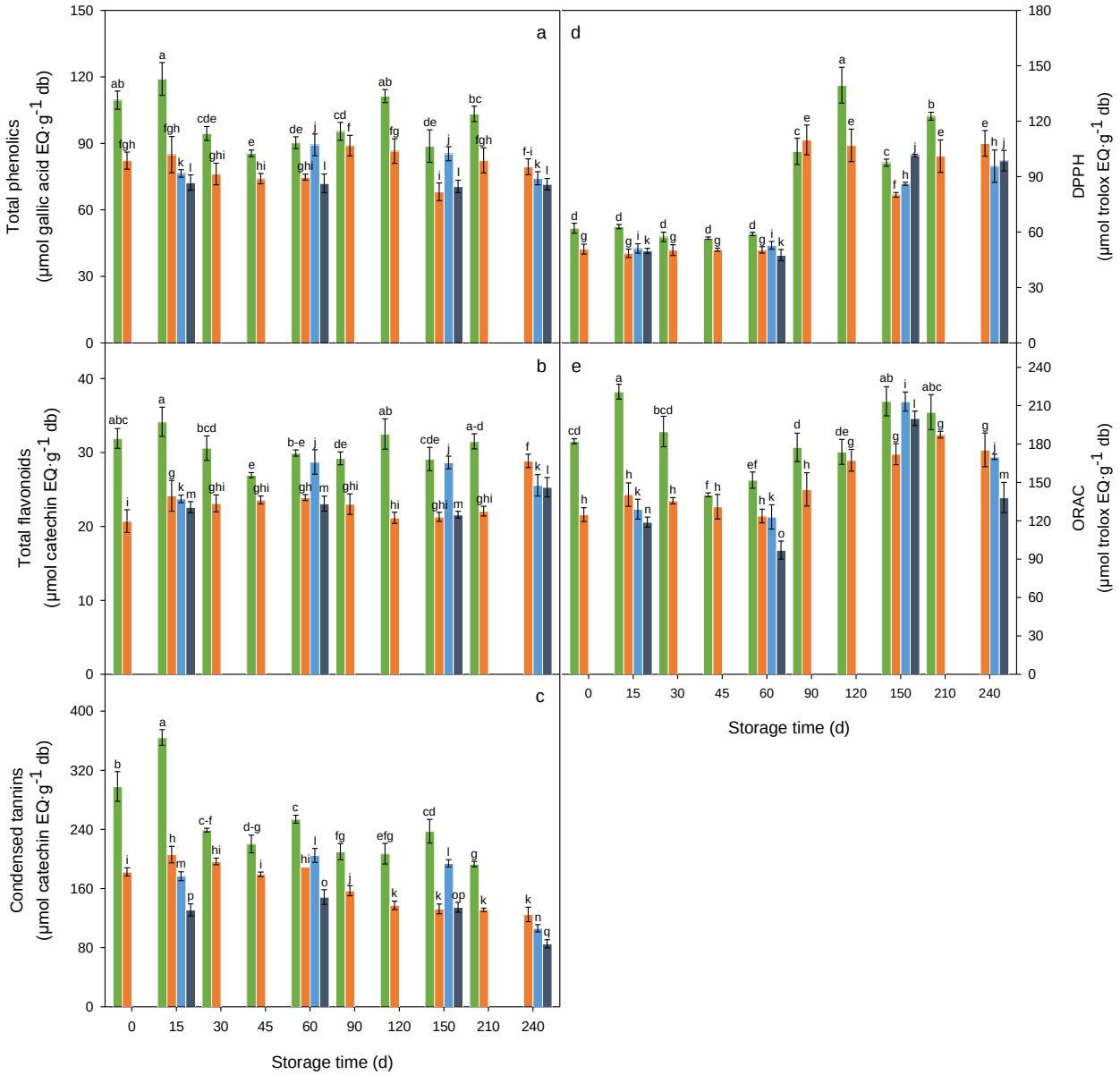

Figure 4.1.1 Changes in total phenolics (a), total flavonoids (b), condensed tannins (c), and antioxidant capacity by DPPH (d) and ORAC (e) of pecan nuts during 240 days (d) of storage at different conditions: fresh kernels at 4°C (■), dry kernels at 4°C (■), in-shell nuts at 4°C (■), and in-shell nuts at 25°C (■). Concentrations were expressed as µmol equivalents (EQ) per g of pecan nut kernels in dry basis (db). DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity; ORAC, oxygen radical absorbance capacity. Means with different letters within storage conditions were significantly different according to the Tukey test (α=0.05).

This decrement could be caused by condensed tannins polymerization throughout storage. Senter and Forbus (1978) reported non-enzymatic polymerization of condensed tannins in pecan nut kernels from Stuart and Western Schley varieties stored at 32°C for 112 days, while do Prado et al. (2013) associated red coloration of spray-dried extracts

of pecan shells with phlobaphenes formation. The determination of CT is based on a reaction between the vanillin reagent and the terminal unit of condensed tannins (Vazquez-Flores et al. 2017), thus a possible explanation for the reduction of CT in stored pecan nuts might be that polymerization of condensed tannins decreases the number of terminal units available to react with vanillin reagent.

Regarding the antioxidant capacity, a similar trend was found between kernels and in-shell pecan nuts: DPPH incremented after 90 days of storage (Figure 4.1.1d). Dry and fresh kernels reached their highest antioxidant capacity at days 90 and 120 improving 216.4 and 224.7% compared to their initial concentrations. Likewise, DPPH values of in-shell nuts increased by 188.4 and 199.8% in samples stored at 4 and 25°C, respectively, towards the end of the storage. ORAC antioxidant capacity differed between pecan nut samples (Figure 4.1.1e) increasing after 150 days of storage in dry kernels (150.1%) and in-shell nuts (170.8 and 160.3% at 4 and 25°C, respectively); while in fresh kernels the highest value was observed at day 15 (121.3%). Pearson correlation coefficients were calculated to associate the decrement in condensed tannins content and the increment in antioxidant capacity of pecan nuts (Table 4.1.2). A negative moderate linear relationship was observed between CT and DPPH of kernels and in-shell pecan nuts, suggesting that antioxidant capacity evaluated by DPPH increases along with the decrement of CT concentrations. As complex polyphenols, condensed tannins are characterized by their amphipathic nature and degree of polymerization, both closely related to their chemical properties (Macías-Cortés et al. 2020; Vazquez-Flores et al. 2017): Nicoli et al. (2002) along with Bors and Michel (2002) reported that the antioxidant capacity of larger polyphenols is related to an increase in their number of reactive hydroxyl groups that allow charge delocalization, while Shivakumar and Kumar (2018) mentioned that condensed tannins possessed higher radical scavenging activity within phenolic compounds. Hence, CT polymerization during storage could be linked to the increment of DPPH antioxidant capacity due to the enhancement of electron-transfer reactions capable to stabilize DPPH radicals. On the contrary, associations between ORAC and CT values were not statistically significant. This method is based on a competitive reaction between antioxidants and a fluorescent probe for the stabilization of AAPH radicals by hydrogen donation (Roy et al. 2010), which could explain the differences in

the antioxidant capacity measurements observed in this study. These findings are an indicative of the antioxidant properties of pecan nuts; at earlier stages of the storage predominate hydrogen donor reactions while, after 90 days of storage, radical-scavenging reactions increase probably due to polymerization of condensed tannins.

Table 4.1.2 Pearson correlation coefficients (*r*) for the relation of condensed tannins content and antioxidant capacity by DPPH and ORAC of kernels and in-shell pecan nuts stored for 240 days (α=0.05).

		Antioxidant capacity			
		DPPH		ORAC	
Condensed tannins	T (°C)	*p-value*	*r*	*p-value*	*r*
Kernels					
Fresh	4	0.001	-0.576	0.105	0.297
Dry	4	0.000	-0.774	0.000	-0.739
In-shell nuts					
Dry	4	0.006	-0.651	0.606	-0.145
Dry	25	0.008	-0.633	0.568	-0.154

DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity; ORAC, oxygen radical absorbance capacity.

4.1.4 Conclusion

From the results of this study, drying process and storage time appear to have the highest impact on phytochemical composition and antioxidant capacity of pecan nuts. The drying process decreased kernels initial content of phenolic compounds (33.2%) and antioxidant capacity (22.3%); however, helped to preserve TP and TF concentration along 240 days despite storage temperature and kernel or in-shell storage. Throughout storage, a decrement in CT content (31.5 and 41.8%) along with a significant increment of DPPH antioxidant capacity (188.4 and 216.4%) were observed in kernels and in-shell nuts. These results indicate the importance of storage conditions on pecan nuts bioactive compounds and their health-related properties. Nevertheless, more research is needed to better understand the polymerization reactions and changes of *in vivo* antioxidant capacity that occurred during pecan nuts storage.

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4.2 Application of pulsed electric fields (PEF) on pecan nuts [*Carya illinoinensis* (Wangenh. K. Koch)]: Oil extraction yield and compositional characteristics of the oil and its by-product

Abstract

Pecan nut oil is conventionally obtained by mechanical extraction characterized by a low oil extraction yield (OEY) compared to solvent extraction. Pulsed electric fields (PEF) has been employed as a pretreatment to enhance OEY from several oilseeds, but no studies have been found regarding tree nuts oil. Hence, PEF was applied at different specific energy inputs (0.5–17.6 kJ·kg-1) to evaluate its impact on OEY, oil acidity and antioxidant capacity (AC), along with total phenolics (TP), condensed tannins (CT), and AC of the by-product generated from oil extraction. Kernels treated by PEF were compared against untreated and soaked kernels due to samples water immersion during PEF processing. The water immersion reduced the initial oil content of soaked and PEF-treated kernels (7.3–11.7%), transferring between 3.8±0.0 and 6.2±0.1 g of oil into the soaking water (o_{SW}). OEY_{TOTAL} of soaked and PEF-treated samples was calculated considering o_{SW}. The application of 0.5 kJ·kg-1 increased OEY_{TOTAL} by 21.4 and 17.6% compared to untreated and soaked kernels, respectively, while oil acidity and AC of PEF-treated kernels were within values reported for pecan nut oil. The highest concentration of TP and CT in the by-product was achieved at 0.8 kJ·kg-1, increasing 9.5 and 30.1%, respectively, compared to untreated kernels. Results evidenced that PEF processing might be a suitable technology to increase OEY from pecan nuts, but the oil extracted during kernels water immersion must be recovered. Furthermore, the by-product of PEF-treated kernels displayed an enhanced content of phenolic compounds increasing its potential as food ingredient.

4.2.1 Introduction

The pecan nut [*Carya illinoinensis* (Wangenh. K. Koch)] is among the most commonly consumed tree nuts worldwide (INC 2020). At being native from North America, pecan nuts are considered an economically important nut crop to Mexico and the United States, being Mexico responsible for almost 50% of pecan nuts worldwide production (Grauke et al. 2016; INC 2020).

Pecan nuts intake has been associated with positive effects on human health due to their significant concentration of phenolic compounds along with mono- and polyunsaturated fatty acids (Alvarez-Parrilla et al. 2018; Atanasov et al. 2018). Kernels phenolic compounds profile is mostly composed by condensed tannins which have been related to pecan nuts antioxidant capacity (Maciel et al. 2020; Rábago-Panduro et al. 2020; Robbins 2012), while kernels fatty acids profile includes oleic, linoleic, and α-linolenic acids (Atanasov et al. 2018; Rábago-Panduro et al. 2020). In comparison to olives, pecan nuts contain a higher concentration of polyunsaturated fatty acids and a lower concentration of saturated fatty acids (USDA 2020). Phytosterols and tocopherols have also been identified as minor components of pecan nuts. As a result, pecan nut oil has been recognized as a specialty oil increasing its commercial value (Alves et al. 2019; Cockerham et al. 2012; Hernandez 2016).

Pecan nut oil is commonly extracted by mechanical processes to preserve its compositional characteristics. In these processes kernels are pressed using a screw press or expeller with the advantages of low cost and simple use (Çakaloğlu et al. 2018; Cockerham et al. 2012). Furthermore, a by-product, usually named cake, rich in carbohydrates, proteins, dietary fiber, and phenolic compounds is obtained from the oil extraction (Alvarez-Parrilla et al. 2018; Sarkis et al. 2014). The cake has been suggested as an ingredient in bakery products with the potential to enhance products' functional properties due to its water and oil absorption capacities along with its phenolic compounds concentration (Maciel et al. 2020; Marchetti et al. 2018). Nevertheless, the main drawback of oil mechanical extraction from pecan nuts is its low oil extraction yield (OEY) compared to solvent extraction (<60.0%) (Cockerham et al. 2012; Costa-Singh and Jorge 2015). In order to increase OEY, drying or enzymatic processes have been applied as pretreatments to oilseeds, modifying the phytochemical profile of the extracted

oil and negatively affecting its physicochemical properties (Çakaloğlu et al. 2018; Liu et al. 2019). As an alternative to improve extraction processes and maintain oil quality, food processing technologies such as ultrasonics, high voltage electrical discharges, and pulsed electric fields (PEF) have been applied as assisting processes (Jia et al. 2019; Puértolas and Barba 2016).

PEF is a nonthermal technology consisting in the application of high-voltage pulses (1–80 $kV \cdot cm^{-1}$) from µs to ms duration (Knorr et al. 2002; Mohamed and Eissa 2012). Its mechanism is based on the cell membrane disruption caused by the increment in the cell membrane conductance leading to pore formation (Barbosa-Canovas et al. 2000). The cell membrane disruption might occur as a reversible or irreversible process depending on the electrical conditions. In a reversible disruption, the cell membrane closes pores by phospholipids and proteins rearrangement. In an irreversible disruption, the cell membrane is not able to close pores, causing the loss of cell integrity (Han et al. 2019). Recently, PEF is being applied as a pretreatment to induce the secondary metabolism in fruit and vegetables as well as to enhance different industrial processes such as drying, freezing, and frying. For instance, López-Gámez et al. (2020) and González-Casado et al. (2018) reported an increment in the concentration of carotenoids in carrots and tomatoes treated by PEF after 24 h of storage. Whereas Traffano-Schiffo et al. (2017) observed that PEF increased the dehydration rate of kiwifruit by electrolytes' loss, and Tylewicz et al. (2016) reported an improvement in the storage stability of freeze-dried apples pretreated by PEF.

Furthermore, the application of PEF to improve mechanical extraction processes of juices, oils, and other products have been reported to enhance the content of the bioactive compound and preserve the sensory characteristics of extracted products (El Kantar et al., 2018; Silve et al., 2018; Tehrani et al., 2019). Veneziani et al. (2019) reported an increment between 2.3 and 6.0% in OEY from olives treated by PEF, producing an oil with a higher concentration of phenolic compounds and no significant changes in its sensory properties. Han et al. (2019) used PEF as a pretreatment to oil extraction from microalgae *Chlorella pyrenoidosa*, reporting an increase of 12.0% in OEY compared to an ultrasound pretreatment. Sarkis et al. (2015) reported a higher OEY in sesame seeds treated by PEF in comparison with untreated seeds. In another research,

Abenoza et al. (2013) evaluated the application of PEF to increase OEY from olive paste reporting an improvement of 13.9%. Guderjan et al. (2007) observed an increment of 39.1% in OEY from rapeseeds treated by PEF. Concerning research relating tree nuts and PEF, Manzoor et al. (2019) combined PEF and ultrasound technologies to improve phenolic compounds extraction from defatted almonds, increasing their extraction and antioxidant capacity by 33.3 and 41.7%, respectively. However, no studies concerning the impact of PEF on OEY and tree nuts oil composition have been found. Therefore, the objective of this work was to apply PEF as a pretreatment to improve oil extraction from pecan nut kernels evaluating the effect of the specific energy input (*W*) on OEY, oil characteristics (acidity and antioxidant capacity), and cake phenolic compounds (total phenolics, condensed tannins, and antioxidant capacity).

4.2.2 Materials and methods

4.2.2.1 Chemicals

Acetone, ethyl acetate, hexane, methanol (MeOH), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), catechin, Folin-Ciocalteu reagent, gallic acid, hydrochloric acid (HCl), potassium hydroxide (KOH) solution (0.1 M), sodium carbonate (Na_2CO_3), acetic acid (CH_3COOH), and vanillin were purchased from Sigma-Aldrich (USA). Solutions were protected from light and stored at 4°C.

4.2.2.2 Pecan nuts

Fresh pecan nuts (*Carya illinoinensis*, Western variety) were harvested in autumn 2018 directly from the orchard [Sonora, Mexico (27°29'38" N, 109°56'20" W)]. In-shell nuts were vacuum-packaged (EVD 4, TORREY, Mexico) and immediately delivered by air to the University of Lleida in Spain where experiments were conducted. In the laboratory, in-shell nuts were shelled, placed in polyethylene bags (Cryovac Europe, Spain), vacuum-sealed (Egarvac® Basic 9, Egarvac S. C. P., Spain), and stored at 4°C until experiments. Kernels halves were manually sectioned in half and divided into three different groups: i) a reference that consisted of kernels without soaking nor PEF treatment; ii) a control of kernels soaked in tap water (1:3 w/w) for 20 min, and; iii) pecan nuts treated by PEF.

Experiments were performed at room temperature and the conductivity of tap water was 463 µS·cm^{-1}. Among PEF treatments, the temperature of the soaking water was randomly measured, not being higher than 30°C. Control and PEF-treated kernels were drained for 10 min then samples were taken to moisture determination. Before oil extraction, reference, control, and PEF-treated kernels were frozen at -16°C for 24 h and freeze-dried (-50°C, 1 mbar) for 72 h (Cryodos 50, Telstar Cryodos, Spain).

4.2.2.3 Pulsed electric fields application

PEF treatments were conducted in a batch-equipment with a 0.1 µF capacitor (Physics International, USA) delivering monopolar exponential-wave pulses (pulse width, 4 µs) using a TG-70 gas control unit and a pulse generator (PT-55, Pacific Atlantic Electronics Inc., USA). Kernels halves were manually sectioned in half, immersed in tap water with a conductivity of 463 µS·cm^{-1} (1:3 w/w), and placed in parallelepiped methacrylate containers (20×8 cm) equipped with stainless steel parallel electrodes. Treatments were performed at different electric field strengths (*E*, 2.0, 5.0, 7.5 kV·cm^{-1}) and pulse number (*n*, 10, 55, 100) (Table 4.2.1). The specific energy input (*W*), expressed as kJ per kg of kernels in wet basis (kJ·kg^{-1} wb), was calculated according to Equation 1 and 2:

$$V = E \times d \qquad \text{Equation 1}$$

$$W = \frac{V^2 \cdot C \cdot n}{2 \cdot m} \qquad \text{Equation 2}$$

where V is the input voltage (kJ·Coulomb^{-1}), E is the electric field strength (kV·cm^{-1}), d is the distance between electrodes (cm), C is the capacitance of the energy storage capacitor (Coulomb^2·kJ^{-1}), n is the number of pulses, and m is the initial mass of kernels (kg wb).

Table 4.2.1 Electric field strength (*E*), pulse number (*n*), and specific energy input (*W*) employed to investigate PEF effect on oil extraction from pecan nut kernels.

E (kV·cm^{-1})	*n*	*W* (kJ·kg^{-1})
2.0	10	0.5
5.0	10	0.8
7.5	10	1.8
2.0	55	2.8
5.0	55	4.3
2.0	100	5.0
5.0	100	7.8
7.5	55	9.7
7.5	100	17.6

4.2.2.4 Oil mechanical extraction

Freeze-dried reference, control, and PEF-treated kernels (85.0 g) were placed in an expeller type screw press (YD-ZY-02A, Yoda Europe, China) for oil mechanical extraction. All samples were submitted to the same preset conditions while kernels feeding along with oil and cake recovery were standardized to prevent oil and cake loss. The extracted oil was stored at -40°C in 50 mL centrifuge tubes avoiding oil oxidation by flushing N_2 in the head-space. The tubes were sealed with parafilm until analyses. The cakes generated from the oil extraction were placed in 12×15 cm polyethylene bags, vacuum-sealed, and stored at -40°C.

4.2.2.5 Kernels analysis

Moisture

The AOAC 920.151 method was employed to moisture determination of reference, control, and PEF-treated kernels (AOAC International 1996). Results were expressed as g·100 g^{-1} db.

Oil content

Oil content of freeze-dried kernels and cakes were determined by solvent extraction as reported by Villarreal-Lozoya et al. (2007) with modifications. Freeze-dried kernels were ground in a laboratory mortar (2.5 g) while cakes were directly weighed (2.5 g). Samples were mixed with hexane (1:10 w/v) for 1.5 min at 6,000 rpm (IKA® T25 Ultra-turrax, IKA, Germany) then centrifuged (8,500 rpm, 15 min, 20°C) (Beckman Avanti™ J-25, Beckman

Instruments Inc., USA) and supernatants collected. This procedure was repeated three times. Pooled supernatants were concentrated using a rotary evaporator (25 rpm, 45°C) (BÜCHI Rotavapor R-3000, BÜCHI Labortechnik AG, Spain), and the extracted oil was used to determine oil content gravimetrically based on the AOAC 960.39 procedure (AOAC International 1996). Oil content of freeze-dried kernels and cakes was expressed as g·100 g^{-1} db.

4.2.2.6 Oil analysis

Oil extraction yield

The oil extraction yield (OEY, %) of reference, control, and PEF-treated kernels was calculated as follows:

$$OEY = \frac{(m_K \times L_K) - (m_C \times L_C)}{(m_K \times L_K)} \times 100 \qquad \text{Equation 3}$$

where m_K and m_C are the mass (g) of freeze-dried kernels and cakes, respectively, while L_K and L_C are the oil content expressed as g of oil per 100 g of freeze-dried kernels and cakes, respectively, all in dry basis (g·100g^{-1} db).

The oil extracted into the soaking water (o_{SW}) was calculated to determine the total OEY (OEY_{TOTAL}, %) of control and PEF-treated kernels using Equation 4 and 5:

$$o_{SW} = \left(m_K \times L_{Reference}\right) - (m_K \times L_K) \qquad \text{Equation 4}$$

where o_{SW} is the mass (g) of oil retained into the soaking water and $L_{Reference}$ is the oil content of reference kernels expressed as g·100g^{-1} db.

$$OEY_{TOTAL} = \frac{[(m_K \times L_K) - (m_C \times L_C)] + o_{SW}}{(m_K \times L_K)} \times 100 \qquad \text{Equation 5}$$

Acidity

Oil acidity was determined following the AOAC 940.28 method (AOAC International 1996), results were expressed as mg KOH per 100 g of pecan nut oil (mg KOH·100 g^{-1}).

Antioxidant capacity

Antioxidant capacity (AC) was evaluated using the DPPH radical scavenging capacity method reported by Gao et al. (2019) with modifications. A DPPH solution was prepared by dissolving 0.05 g of DPPH in 250 mL of MeOH. Pecan nut oil (200 µL) diluted in ethyl acetate (2 mL) was mixed with the DPPH solution (2 mL). The reaction was left 15 min in darkness and absorbance measured at 515 nm using a UV-VIS spectrophotometer (Cecil CE 1010, Cecil Instruments Ltd., England). Trolox was used for the standard curve (0.003–0.030 mg·mL^{-1}) to express results as mg trolox equivalents per 100 g of pecan nut oil (mg trolox EQ·100 g^{-1}).

4.2.2.7 Cake analysis

A defatted cake was obtained after oil content determination by allowing to evaporate overnight the remaining hexane. Defatted cakes were sieved, placed in 6×15 cm polyethylene bags, vacuum sealed, and stored at -40°C. Aqueous and methanolic extractions were performed as described by Rábago-Panduro et al. (2020).

In the aqueous extraction, defatted cake samples (0.3 g) were mixed with an extraction solution consisting of acetone:H_2O:CH_3COOH (70:29.5:0.5) in 1:10 w/v proportion. The mixture was sonicated in an ultrasonic bath for 30 min, centrifuged (8,500 rpm, 15 min, 20°C), and supernatants collected. The extraction process was performed twice. Next, the extraction solution was evaporated using N_2 and the concentrated was diluted to 5 mL with distilled water. Aqueous extracts were stored in 15 mL centrifuge tubes at 4°C until total phenolics (TP) and AC analysis. The methanolic extraction was performed by mixing defatted cakes (0.1 g) with a 1% MeOH:HCl solution (1:30 w/v). The mixture was placed in a water bath (20 min, 30°C). After this time, supernatants were collected by centrifugation (8,500 rpm, 15 min, 20°C) and diluted to 5 mL with 1% MeOH:HCl solution. Methanolic extracts were stored in 15 mL centrifuge tubes at 4°C until condensed tannins (CT) analysis.

Total phenolics

Folin-Ciocalteu method reported by Singleton and Rossi (1965) and adapted by Villarreal-Lozoya et al. (2007) was followed to TP determination. Aqueous extracts (13 µL) were pipetted into a 96-well flat-bottom plate (Costar® Assay Plate #9017, Corning, USA) followed by Folin-Ciocalteu solution (221 µL) and led to react for 3 min in the dark. Next, 0.50 M Na_2CO_3 solution (26 µL) was added and the plate was incubated for 2.5 h in darkness. A microplate reader (Multiskan™ GO, Thermo Scientific™, Finland) was used to absorbance measurement at 765 nm employing a curve of gallic acid (0.1–1.0 $mg \cdot mL^{-1}$) as standard. Results were expressed as mmol gallic acid equivalents per 100 g of defatted cake db (mmol gallic acid EQ·100 g^{-1} db).

Antioxidant capacity

The DPPH radical scavenging capacity method was employed to evaluate cake antioxidant capacity (Villarreal-Lozoya et al. 2007). A DPPH stock solution (1.3 mM) was diluted (1.5:10 v/v) in MeOH. Aqueous extracts (26 µL) were loaded into a 96 well flat bottom plate (Costar® Assay Plate #9017, Corning, USA) along with 234 µL of diluted DPPH. Absorbance measurements were made in the microplate reader at 515 nm and registered every minute until 15 min of reaction. Trolox was used for the standard curve (0.02–0.10 $mg \cdot mL^{-1}$) to express the results as mmol trolox equivalents per 100 g of defatted cake db (mmol trolox EQ·100 g^{-1} db).

Condensed tannins

The HCl-vanillin method reported by Price et al. (1978) and modified by Herald et al. (2014) was employed to analyze CT. From a vanillin stock solution (0.065 M), a dilution (1:1 v/v) was made with 8% MeOH:HCl. Methanolic extracts (30 µL) were pipetted in a 96-well flat-bottom plate (Costar® Assay Plate #9017, Corning, USA) followed by the vanillin dilution (150 µL) and led to react for 20 min at 30°C. The blank was 1% MeOH:HCl and absorbance was measured at 500 nm using the microplate reader. A curve of catechin (1.0–3.5 $mg \cdot mL^{-1}$) was utilized as standard and results expressed as mmol catechin equivalents per 100 g of defatted cake db (mmol catechin EQ·100 g^{-1} db).

4.2.2.8 Soaking water analysis

Given the low water-solubility of condensed tannins, TP was selected to follow the release of the water-soluble phenolic compounds present in pecan nuts into the soaking water (Hemingway and Karchesy 1996). Aliquots of the soaking water (500 µL) were centrifuged (8,000 rpm, 15 min, 20°C) (Hettich® Universal 320R, Hettich, Germany) and supernatants were employed to measure TP as described in Section 2.6.1. Results were expressed as mmol gallic acid equivalents per 100 g of soaking water (mmol gallic acid EQ·100 g^{-1}).

4.2.2.9 Statistical analysis

Reference, control, and PEF processing along with oil and cake analytical determination were performed by duplicate. Results were analyzed through a one-way ANOVA followed by the Dunnett test and calculation of correlation coefficients using Minitab 18 software (Minitab® 18.1, USA). Pearson (*r*) and Spearman (*ρ*) correlation coefficients were determined based on data distribution; *r* for data normally distributed and *ρ* for not normally distributed data or data with outliers (Schober et al. 2018).

4.2.3 Results and discussion

4.2.3.1 Moisture and oil content of pecan nut kernels

Moisture and oil content of reference, control, and PEF-treated kernels are showed in Table 4.2.2. Reference samples contained a moisture and oil content of 3.2±0.1 and 61.2±3.0 g·100 g^{-1} db, respectively. Moisture increased up to 24.9±0.7 g·100 g^{-1} db in control kernels while the moisture content of PEF-treated kernels ranged from 18.7±2.3 to 21.7±2.3 g·100 g^{-1} db (Table 4.2.2). According to the Dunnett test, no significant differences between moisture of control and PEF-treated samples were observed (α=0.05). The oil content of reference kernels was 61.2±3.0 g·100 g^{-1} db decreasing to 54.9±0.8 g·100 g^{-1} db in control kernels; whereas in PEF-treated kernels decreased between 54.0±2.9 and 56.7±1.1 g·100 g^{-1} db. Regarding the o_{SW}, control and PEF-treated samples displayed comparable values ranging from 3.8±0.0 to 6.2±0.1 g with the lowest o_{SW} observed in kernels pretreated at 5.0, 7.8, and 17.6 kJ·kg^{-1} (Table 4.2.2). Moisture and oil content of control and PEF-treated samples were similar suggesting that these changes might be related to the soaking process.

Table 4.2.2 Effect of soaking on moisture and oil content of control and PEF-treated pecan nut kernels.

	Reference	Control	*W* (kJ·kg^{-1})		
			0.5, 0.8, 1.8	2.8, 4.3, 9.7	5.0, 7.8, 17.6
Moisture g·100 g^{-1}	3.2 ± 0.1 *	24.9 ± 0.7	21.3 ± 2.0	21.7 ± 2.3	18.7 ± 2.3
Oil content g·100 g^{-1}	61.2 ± 3.0 *	54.9 ± 0.8	54.3 ± 3.7	54.0 ± 2.9	56.7 ± 1.1

W, specific energy input. Reference, kernels without soaking nor PEF processing. Control, kernels soaked in tap water (1:3 w/w) for 20 min. PEF-treated kernels were categorized according to the pulse number applied: 0.5. 0.8, and 1.8 kJ·kg^{-1} corresponded to 10 pulses; 2.8, 4.3, and 9.7 kJ·kg^{-1} corresponded to 55 pulses; 5.0. 7.8, and 17.6 kJ·kg^{-1} corresponded to 100 pulses. Moisture and oil content were expressed as g per 100 g of kernels in dry basis (db). Means with an asterisk within rows were significantly different from control kernels according to the Dunnett test (α=0.05).

The oil retained into the soaking water could be related to kernels grinding and water immersion, modifying their microstructure. Fatty acids are located in small and spherical structures called oleosomes, these organelles are constituted by a core of triacylglycerols stabilized by a monolayer of phospholipids and proteins found in the cotyledon tissue of pecan nut kernels (Heldt et al. 2011; Rábago-Panduro et al. 2020; Zhang et al. 2017). According to Zhang et al. (2017), it is possible to extract oleosomes from the cotyledon tissue of pecan nuts by grinding and water immersion. In this line, it is suggested that the exposure of cotyledon tissue due to kernels sectioning and the moisture gained during water immersion might facilitate oleosomes transfer to the soaking water.

4.2.3.2 Effect of PEF processing on OEY, acidity, and antioxidant capacity

OEY

The effect of PEF processing on OEY of pecan nut kernels along with those of reference and control kernels are displayed in Figure 4.2.1. The OEY of reference samples was 63.8±1.5% being comparable to OEY reported for the mechanical extraction of pecan nut oil (Costa-Singh and Jorge 2015; Polmann et al. 2019; Scapinello et al. 2017). Water immersion of control kernels decreased OEY to 54.2±2.0%, representing a loss 14.9% compared to reference samples. The reduction of OEY due to water immersion was also reported by Polmann et al. (2019) and Sarkis et al. (2015) for pecan nuts and sesame seeds, respectively. Concerning the application of PEF, kernels treated at 0.5, 1.8, 4.3, 5.0, and 17.6 kJ·kg^{-1} equaled OEY of reference kernels (Figure 4.2.1). No linear relationship between *W* and OEY was observed (Table 4.2.3). The OEY_{TOTAL} estimated

by o_{SW} determination was used to analyze the PEF effect on oil extraction yields. OEY_{TOTAL} of control kernels was 65.8% being comparable to OEY of reference kernels. On the other hand, PEF-treated samples displayed OEY_{TOTAL} that ranged from 68.9 to 77.4% improving oil extraction between 8.0 and 21.4% compared to reference samples (Figure 4.2.1). The application of PEF as a pretreatment to increase oil extraction from pecan nut kernels displayed higher OEY than the enzymatic pretreatment (65.2%) reported by Polmann et al. (2019). Furthermore, the OEY_{TOTAL} of PEF-treated kernels was comparable to OEY achieved in the extraction of pecan nut oil using pressurized CO_2 and *n*-butane (65.3–70.5%) (Alves et al. 2019; Scapinello et al. 2017).

Table 4.2.3 Probability values (*p-value*) of one-way ANOVA (α=0.05) and Pearson correlation coefficients (*r*) of oil and cake from pecan nut kernels pretreated by PEF.

	W (kJ·kg^{-1})	*r*	
Response variables	*p-value*	*p-value*	*r*
Kernels			
Oil extraction yield %	0.006	0.530	-0.158
Total oil extraction yield [a] %	0.008	0.058	-0.455
Oil			
Acidity [b] mg KOH·100 g^{-1}	0.000	0.789	-0.054
Antioxidant capacity mg trolox EQ·100 g^{-1}	0.003	0.033	-0.356
Cake			
Total phenolics mmol gallic acid EQ·100 g^{-1}	0.000	0.014	-0.289
Condensed tannins mmol catechin EQ·100 g^{-1}	0.000	0.037	0.285
Antioxidant capacity mmol trolox EQ·100 g^{-1}	0.080	n.s.	n.s.
Soaking water			
Total phenolics mmol gallic acid EQ·100 g^{-1}	0.000	0.000	0.866

[a] Total oil extraction yield (OEY_{TOTAL}) is the extraction yield considering oil extracted during the soaking process.
[b] The Spearman correlation coefficient was employed to determine the relationship between *W* and acidity.
W, specific energy input; n.s., not significant. Antioxidant capacity was determined by the DPPH radical scavenging capacity method.

The improvement of oil extraction processes after PEF has been reported for maize, olives along with sunflower and sesame seeds, being associated with irreversible cell disruption due to the electroporation mechanism (Guderjan et al. 2005; Moradi and Rahimi 2018; Sarkis et al. 2015). However, it is proposed that rather than irreversible cell

disruption, pecan nut kernels pretreated by PEF might undergo reversible electroporation, producing changes in the cell structure that facilitates oil extraction. Han et al. (2019) suggested that the improvement of OEY by PEF application could be related to the fusion of oil bodies within the cell and the release of intracellular water-soluble compounds. Furthermore, kernels water immersion reduced OEY, demonstrating that not only PEF parameters (*W*, *E*, *n*, pulse shape and width) and food characteristics contribute to the improvement of OEY but also processing steps such as soaking, drying, and grinding. Andreou et al. (2017) observed that more intense PEF pretreatments (≥20 kJ·kg^{-1}) lead to higher OEY, attributing this effect to a combination of cell disruption and demulsification of oil-in-water emulsions formed at the malaxation step during olive oil extraction. In contrast, Guderjan et al. (2005) reported higher OEY of maize germ by combining a PEF processing of 0.6 kJ·kg^{-1} with incubation and drying previous to oil extraction; whereas Sarkis et al. (2015) reported higher OEY from sesame seeds pretreated at 40 kJ·kg^{-1} followed by drying.

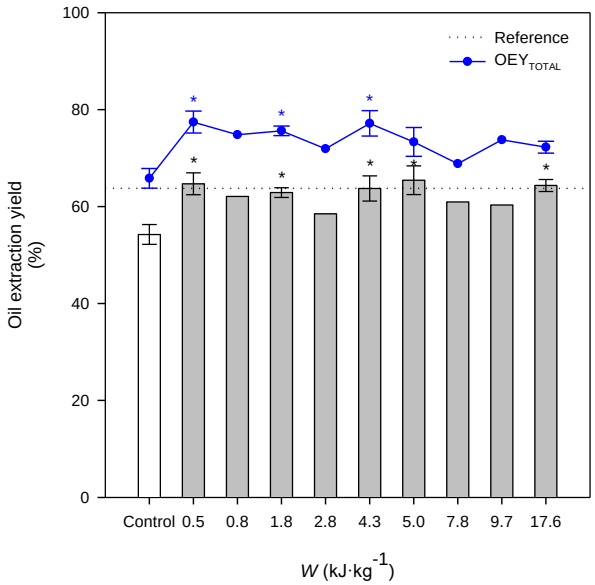

Figure 4.2.1 Effect of PEF pretreatments on oil extraction yield (OEY) of pecan nut kernels. OEY$_{TOTAL}$ is the extraction yield considering oil extracted during the soaking process. *W*, specific energy input. Means with an asterisk were significantly different from the control according to the Dunnett test (α=0.05).

Oil acidity and antioxidant capacity

Acidity and AC of oil extracted from reference, control, and PEF-treated kernels are showed in Table 4.2.4. Oil acidity of PEF-treated kernels varied from 21.3±1.3 to 38.3±1.4 mg KOH·100 g^{-1} being within values reported for cold-pressed and virgin oils of the Codex Standards for Fats and Oils from Vegetable Sources (≤40.0 mg KOH·100 g^{-1} oil) (FAO 2015). Similar results were described by Guderjan et al. (2007), Puértolas and Martínez de Marañón (2015), Andreou et al. (2017), Moradi and Rahimi (2018), and Veneziani et al. (2019) for the acidity of oil extracted from rapeseeds, olives, and sunflower seeds pretreated by PEF. Guderjan et al. (2007) reported that increments in oil acidity of rapeseeds pretreated by PEF might be due to the degradation of triacylglycerols by lipase activity. Likewise, Mohseni et al. (2020) suggested that changes of intracellular materials and cell membrane rupture, as a consequence of PEF application followed by mechanical extraction, might favor the lipid-water interface changes necessary to lipase activation.

Table 4.2.4 Acidity and antioxidant capacity (AC) of the oil extracted from reference, control, and PEF-treated pecan nut kernels.

	Acidity (mg KOH·100 g^{-1})		AC (mg trolox EQ·100 g^{-1})	
Reference	29.0 ± 2.1		55.2 ± 2.3	
Control	28.4 ± 1.0		56.4 ± 1.4	
W (kJ·kg^{-1})				
0.5	27.8 ± 1.9		51.8 ± 2.5	
0.8	38.3 ± 1.4	*	61.4 ± 6.1	
1.8	21.3 ± 1.3	*	56.7 ± 3.2	
2.8	31.7 ± 0.1		55.4 ± 0.8	
4.3	22.2 ± 1.9	*	55.5 ± 4.1	
5.0	30.8 ± 1.1		54.1 ± 1.7	
7.8	26.1 ± 0.1		61.2 ± 0.4	
9.7	27.9 ± 0.1		55.7 ± 2.1	
17.6	21.8 ± 0.9	*	49.4 ± 2.6	*

W, specific energy input. Reference, kernels without soaking nor PEF processing. Control, kernels soaked in tap water (1:3 w/w) for 20 min. AC was determined by the DPPH radical scavenging capacity method. Concentrations were expressed per 100 g of pecan nut oil. Means with an asterisk within rows were significantly different from control kernels according to the Dunnett test (α=0.05).

Concerning oil antioxidant capacity, no significant differences were observed in AC of oil extracted from reference, control, and PEF-treated samples, except at 17.6 kJ·kg^{-1} which produced the lowest AC (49.4±2.6 mg trolox EQ·100 g^{-1}) (Table 4.2.4). AC

reduction at the most intense PEF treatment could be related to the loss of phenolic compounds into the soaking water evidenced by its increment in TP, as discussed below.

4.2.3.3 Effect of PEF processing on TP, CT, and AC of cakes and TP of soaking water

Total phenolics and condensed tannins of the cake generated from oil extraction of reference, control, and PEF-treated samples along with TP of the soaking water are showed in Figure 4.2.2. TP and CT values of the cake of reference kernels were 24.2±1.8 mmol gallic acid EQ·100 g^{-1} db and 24.7±2.9 mmol catechin EQ·100 g^{-1} db, respectively, being comparable to values reported by Maciel et al. (2020) for pecan nut cakes. PEF-treated samples resulted in cakes with similar TP values to those from control samples but below to the reference, except at 0.8 kJ·kg^{-1}. The application of 0.8 kJ·kg^{-1} increased TP by 17.8 and 9.5% compared to control and reference cakes, respectively; whereas PEF pretreatments greater than 0.8 kJ·kg^{-1} led to an increment in TP in the soaking water directly proportional to the *W* applied (*r*=0.866) (Figure 4.2.2a). CT concentration of pecan nut cakes also increased with the specific energy input applied (*W* ≥5.0 kJ·kg^{-1}) except at 0.8 kJ·kg^{-1}, where the highest CT value was achieved (32.1±3.0 mmol catechin EQ·100 g^{-1} db) (Figure 4.2.2b). No significant differences were found between AC of cakes from kernels pretreated by PEF and those from control and reference samples (*p-value*=0.080) (Table 4.2.5).

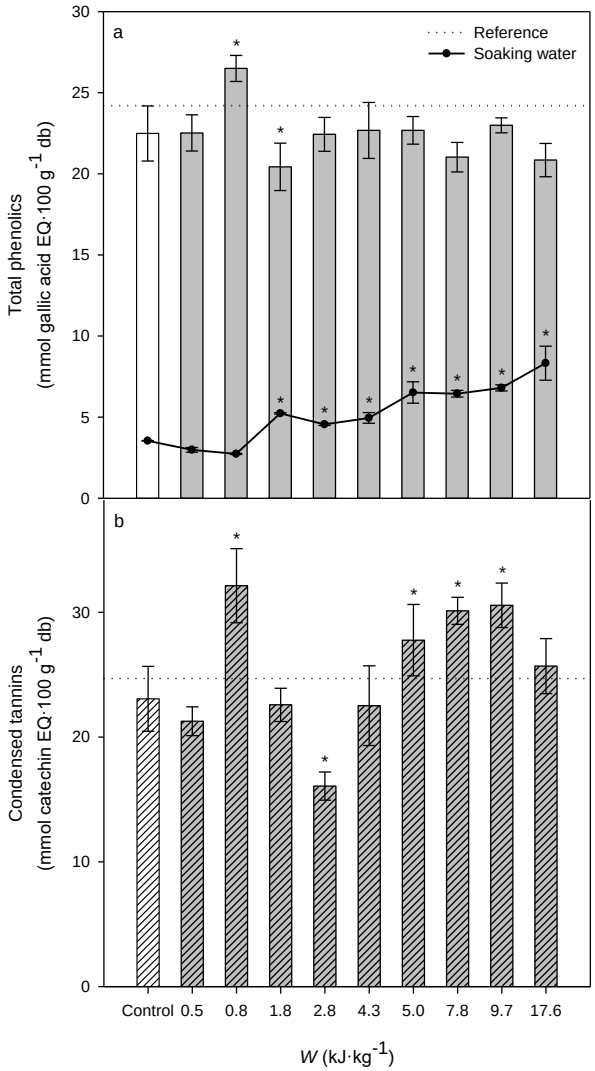

Figure 4.2.2 Effect of PEF pretreatments on total phenolics (TP) (a) and condensed tannins (b) of the generated cakes and TP of the soaking water. Concentrations were expressed as mmol equivalents (EQ) per 100 g of defatted cakes in dry basis (db) and 100 g of soaking water, respectively. Means with an asterisk were significantly different from the control according to the Dunnett test (α=0.05).

Based on the changes of TP and CT concentration of cakes obtained after PEF pretreatment, it is suggested that a rearrangement of intracellular materials (ions and small molecules movement, vacuoles rupture, and enzyme activation) might occur at less intense PEF processing conditions (W<1.8 kJ·kg^{-1}), not being enough the intensity to initiate phenolic compounds release evidenced by TP of the soaking water, and also by the fact that the highest TP and CT values were observed at 0.8 kJ·kg^{-1}. Contrarily, at

higher *W* (≥1.8 kJ·kg^{-1}), the release of phenolic compounds starts increasing along with the specific energy input applied, promoting the interaction between CT and cell wall materials (Renard et al. 2017) and retaining condensed tannins in the cake.

Table 4.2.5 Antioxidant capacity (AC) of cakes from reference, control, and PEF-treated pecan nut kernels.

	AC (mmol trolox EQ·100 g^{-1})
Reference	19.5 ± 0.3
Control	19.6 ± 0.4
W (kJ·kg^{-1})	
0.5	19.5 ± 0.2
0.8	19.7 ± 0.4
1.8	19.3 ± 0.2
2.8	19.7 ± 0.0
4.3	19.5 ± 0.3
5.0	19.6 ± 0.2
7.8	19.1 ± 0.1
9.7	19.5 ± 0.2
17.6	19.3 ± 0.4

AC was evaluated by the DPPH radical scavenging capacity method and expressed as mmol equivalents (EQ) per 100 g of defatted cake in dry basis. No significant differences were determined according to one-way ANOVA (α=0.05).

4.2.4 Conclusion

In this study pecan nut kernels were immersed into water in order to apply PEF processing, which led to an increment of moisture (18.7–24.9 g·100 g^{-1} db) and a decrement of oil content (54.0–56.7 g·100 g^{-1} db). After considering oil extracted into the soaking water, OEY_{TOTAL} of PEF-treated samples increased up to 68.9 and 77.4%. The highest OEY_{TOTAL} was achieved at 0.5 kJ·kg^{-1}, being 21.4 and 17.6% higher than the values of reference and control samples, respectively. The acidity and antioxidant capacity of extracted oils were not affected by PEF processing. Moreover, an increase of TP and CT of 17.8 and 39.3%, respectively, was observed in the cake produced from the oil extraction of kernels pretreated at 0.8 kJ·kg^{-1}. This is probably due to the rupture of condensed tannins vacuoles. The increment of the specific energy input applied (≥1.8 kJ·kg^{-1}) increased phenolic compounds release into the soaking water. These data demonstrate that PEF

technology might be an appropriate pretreatment to enhance mechanical extraction of pecan nut oil with no effect in neither its acidity nor AC, leading to a cake that is a valuable by-product with potential functional properties due to its enhanced content of phenolic compounds. However, oil recovery from the soaking water might be a necessary step to achieve higher OEY employing PEF, affecting the feasibility of PEF as an assisting process in pecan nut oil extraction. Also, further research related to microscopy, enzymatic, and compositional analysis are needed to corroborate the mechanism of PEF and understand kernels microstructural changes involved with the application of PEF to improve oil extraction from pecan nuts.

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4.3 Effect of pulsed electric fields (PEF) on kernels microstructure and oil stability of dry pecan nuts [*Carya illinoinensis* (Wangenh. K. Koch)]

Abstract

Pulsed electric fields (PEF) have been reported to improve the total oil extraction yield (OEY_{TOTAL}) of fresh pecan nuts maintaining oil characteristics and increasing the phenolic compounds concentration of the by-product generated from oil extraction. However, no information is available regarding the effect of PEF on dry pecan nuts as they are usually commercialized. Therefore, dry kernels were treated by PEF at different specific energy inputs (*W*, 0.8, 7.8, and 15.0 $kJ \cdot kg^{-1}$) before oil extraction and results were compared against untreated kernels and kernels soaked at different times (3, 20, and 35 min). OEY_{TOTAL}, kernels microstructure, oil stability [acidity, antioxidant capacity (AC), oil stability index (OSI), phytosterols, tocopherols, and lipoxygenase (LOX) activity], and cake phenolic compounds [total phenolics (TP), condensed tannins (CT), and AC] were evaluated. Untreated kernels yielded 88.7±3.0%, whereas OEY_{TOTAL} of soaked and PEF-treated kernels was 76.5–83.0 and 79.8–85.0%, respectively. The microstructural analysis of kernels pretreated at 0.8 $kJ \cdot kg^{-1}$ showed that PEF processing induced oleosomes fusion within the intracellular space. No differences were found among acidity, AC, and OSI values of oils extracted from untreated, soaked, and PEF-treated kernels. Oil from untreated kernels displayed a low concentration of phytosterols and tocopherols, mainly γ-tocopherol compared to other research works, while LOX activity was not affected by PEF processing. The application of 0.8 $kJ \cdot kg^{-1}$ increased CT and AC of the generated cakes by 27.0 and 24.3%, respectively, compared to cakes from untreated kernels. Results showed that PEF processing is not able to improve OEY_{TOTAL} when it is applied to dry pecan nuts, highlighting that kernels moisture, oil content, and microstructure play an important role in PEF effectiveness.

4.3.1 Introduction

Pecan nut kernels [*Carya illinoinensis* (Wangenh. K. Koch)] are regarded as a source of phenolic compounds, mono- and polyunsaturated fatty acids, phytosterols, and tocopherols (Alvarez-Parrilla et al. 2018; Atanasov et al. 2018). Pecan nut oil differentiates from other tree nut oils for its high concentration of polyunsaturated fatty acids (PUFA), β-sitosterol, and γ-tocopherol. These compounds contribute to the nutritional properties and nutraceutical potential of pecan nut oil (Fernandes et al. 2017; Scapinello et al. 2017). Thus, to extend kernels storage time and avoid oil oxidation, a drying process is carried out reducing kernels moisture to less than or equal to 5.5% (Alasalvar and Shahidi 2008). In a previous study, fresh pecan nut kernels were pretreated by pulsed electric fields (PEF) applying different specific energy inputs (Rábago-Panduro et al. 2020b). Results indicated that kernels immersion in water caused an increase of moisture and a reduction of the initial oil content, decreasing oil extraction yield (OEY) of soaked kernels compared to untreated kernels. Interestingly, when the oil retained into the soaking water was considered to calculate the total OEY (OEY_{TOTAL}), PEF-treated kernels yielded 21.4% more oil than untreated kernels (Rábago-Panduro et al. 2020b). However, the effect of this technology on dry pecan nut kernels has not been reported yet.

PEF consist on the application of intermittent electric fields of moderate to high intensity (0.1–50 $kV \cdot cm^{-1}$) and short duration (Puértolas et al. 2016). This technology has been used as a pretreatment to oil extraction in different seeds enhancing the OEY and increasing oil acidity (Guderjan et al. 2007; Moradi and Rahimi 2018; Sarkis et al. 2015; Veneziani et al. 2019). The improvement of OEY has been attributed to the electroporation phenomenon, while the increase of oil acidity was associated with triacylglycerols hydrolysis by lipase activity; however, studies corroborating both hypotheses are scarce.

The fatty acids of tree nuts are stored as triacylglycerols within intracellular organelles named oleosomes. These organelles are packed in a disk shape within the endoplasmic network of the cotyledon tissue of seeds (Nikiforidis 2019). Oleosomes triacylglycerols are surrounded by a monolayer of phospholipids, proteins, and enzymes like lipoxygenase (LOX). LOX is the key enzyme in triacylglycerol hydrolyzation, leading

to oil oxidative deterioration and modifying its sensory and nutritional characteristics. (Buranasompob et al. 2007; Heldt et al. 2011; Shahidi and Zhong 2020). In this sense, tocopherols are important lipophilic antioxidants of tree nuts oil, slowing down oxidative deterioration of the oil by neutralizing free radicals (Gong et al. 2017; Murray et al. 2003; Shahidi and John 2010). Besides tocopherols, tocotrienols, phenolic compounds, phytosterols, squalene, and phospholipids have also been reported to contribute to oilseeds antioxidant capacity (Gawrysiak-Witulska et al. 2012; Shahidi and John 2010; Wall 2010). During oxidative deterioration, lipids are oxidized to hydroperoxides and then to secondary oxidation products that increment free radicals' concentration; hence, these compounds are considered important biomarkers to measure oil oxidative deterioration (Shahidi and Zhong 2020). Acidity is a measurement of the concentration of free fatty acids produced by the hydrolysis of ester bonds in lipids by either enzyme action, heat, or moisture (Subroto et al. 2015), relating its increment with a loss of oil stability. Another method to evaluate oil stability is the oil stability index (OSI). In the OSI method, oil is oxidized by air and high temperatures, producing volatile acids that are dissolved in water to follow the change in electrical conductivity (Shahidi and Zhong 2020). The OSI value expresses the time necessary to complete oil oxidation at given experimental conditions being indicated by a drop of conductivity. Hence, at higher OSI values more resistance to oxidation of the analyzed oil is observed (Subroto et al. 2015). Therefore, the objective of this study was to investigate the effect of PEF on OEY, OEY_{TOTAL}, and microstructure of dry pecan nuts. In addition, the stability of the extracted oil was evaluated by determination of acidity, AC, OSI, phytosterols, tocopherols, and LOX activity as well as total phenolics, condensed tannins, and AC of the generated cakes.

4.3.2 Materials and methods

4.3.2.1 Chemicals

Acetonitrile, ethyl acetate, methanol (MeOH), hexane, tetrahydrofuran (THF), water (H_2O) HPLC grade, β-mercaptoethanol (β-ME), 2,2-diphenyl-1-picrylhydrazyl (DPPH), boric acid (H_3BO_3), hydrochloric acid (HCl), glutaraldehyde, osmium tetroxide, polyvinylpolypyrrolidone (PVPP), potassium hydroxide (KOH) solution (0.1 M), sodium acetate (CH_3COONa), sodium phosphate dibasic (Na_2HPO_4), sodium phosphate

monobasic (NaH_2PO_4), Triton X-114 and uranyl acetate were purchased from Sigma-Aldrich (USA) along with 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), linoleic acid, δ-, γ-, and α-tocopherols, β-sitosterol, stigmasterol, campesterol, catechin, gallic acid, and vanillin. Epoxy EMbed 812 resin was purchased from Electron Microscopy Sciences (USA). Ethanol (EtOH), sodium carbonate (Na_2CO_3), and KOH were purchased from DEQ (Mexico).

4.3.2.2 Pecan nuts

Dry pecan nuts (*Carya illinoinensis*) from Alesto (Lidl) brand were purchased in a local market in Lleida (Spain) as kernels halves. In order to evaluate PEF processing, PEF-treated kernels were compared against reference and control kernels. No soaking nor processing was performed on reference kernels. Control kernels were soaked in tap water (1:3 w/w) for 3, 20, and 35 min to evaluate the effect of soaking at each PEF processing conditions. Kernels pretreated by PEF were immersed in tap water and processed. Control and PEF-treated kernels were drained for 10 min and samples (0.5 g) were separated for microstructural analysis. Before freeze-drying, reference, control, and PEF-treated kernels (2.0 g) were taken to determination of lipoxygenase (LOX) activity. The remaining kernels were frozen at -16°C for 24 h and freeze-dried at -50°C and 1 mbar for 72 h (Cryodos 50, Telstar Cryodos, Spain). Freeze-dried kernels were stored at -40°C until oil extraction.

4.3.2.3 Pulsed electric fields application

A batch-system equipped with a 0.1 μF capacitator (Physics International, USA), a TG-70 gas control unit, and a pulse generator (PT-55, Pacific Atlantic Electronics Inc., USA) was employed to PEF application. The treatment chamber consisted of a parallelepiped methacrylate container (20×8 cm) and stainless steel parallel electrodes. Kernels were immersed in tap water (1:3 w/w) applying 10, 99, and 192 monopolar exponential-wave pulses at an electric field strength of 5.0 $kV \cdot cm^{-1}$ corresponding to a specific energy input of 0.8, 7.8, and 15.0 kJ per kg of pecan nut kernels in wet basis (wb), respectively.

4.3.2.4 Oil mechanical extraction

Oil was extracted from freeze-dried kernels (85.0 g) employing a domestic screw press (YD-ZY-02A, Yoda Europe, China). Pecan nut oil was stored in 50 mL centrifuge tubes flushing N_2 into the head-space and the generated cakes were placed in polyethylene bags and vacuum-sealed. Oil and cake samples were stored at -40°C until analyses.

4.3.2.5 Kernels analysis

Moisture

Kernels moisture was determined according to the AOAC 920.151 method (AOAC 1996) and results were expressed as g per 100 g of kernels in dry basis ($g \cdot 100\ g^{-1}$ db).

Oil content

Oil content of freeze-dried kernels and cakes was performed as reported by Villarreal-Lozoya et al. (2007) and the AOAC (1996). Samples (2.5 g) were mixed with hexane (25 mL) for 1.5 min at 6,000 rpm (IKA® T25 Ultra-turrax, IKA, Germany) and centrifuged (8,500 rpm, 15 min, 20°C) (Beckman Avanti™ J-25, Beckman Instruments Inc., USA). Supernatants were collected and the procedure repeated three times. Hexane was evaporated from pooled supernatants using a rotary evaporator (25 rpm, 45°C) (BÜCHI Rotavapor R-3000, BÜCHI Labortechnik AG, Spain) and oil content was determined gravimetrically following the AOAC 960.39 method. Oil content was expressed as g per 100 g of freeze-dried kernels and cakes, respectively, both in dry basis ($g \cdot 100\ g^{-1}$ db).

Light microscopy

Control and PEF-treated kernels were fixed employing a procedure reported by Kendall et al. (2018) with modifications followed by Richardson staining (Richardson et al. 1960). Glutaraldehyde and osmium tetroxide solutions were prepared in 0.1 M phosphate buffer (pH 7.2). Kernels were fixed in 2.5% glutaraldehyde solution and left overnight. Next, samples were washed three times in 0.1 M phosphate buffer and post-fixed in 1% osmium tetroxide solution for 2 h. Subsequently, samples were washed twice with 0.1 M acetate buffer, incubated with 0.5% uranyl acetate for 30 min, and rinsed again two times in 0.1 M acetate buffer. Kernels were dehydrated in an acetonitrile series (30–100%) before

embedding in epoxy EMbed 812 resin and polymerizing for 48 h at 60°C. Semithin and ultrathin sections were obtained in a ultramicrotome (Reichert Jung Ultracut E, Leica Microsystems, USA) and stained with Richardson blue. Stained samples were examined at 20× and 100× by light microscope (Olympus BX41, Olympus, USA).

Lipoxygenase activity

Enzymatic extracts were obtained as described by Christopoulos and Tsantili (2015) with modifications. An extraction solution was prepared by dissolving β-ME (5.0 mM), PVPP (1:100 w/v), and Triton X-114 (0.05:100 w/v) in 50 mM phosphate buffer (pH 6.6). Kernels (2.0 g) and the extraction solution (10 mL) were homogenized for 40 s at 6,000 rpm (IKA® T25 Ultra-turrax, IKA, Germany), filtered using glass wool, and centrifuged (8,000 rpm, 15 min, 4°C) (Hettich® Universal 320R, Hettich, Germany). Supernatants were collected for determination of LOX activity in reference, control, and PEF-treated kernels, which was measured according to the procedure reported by Li et al. (2012) with modifications. Solutions were prepared in 0.2 M borate buffer (pH 9.0). Linoleic acid dissolved in EtOH and borate buffer (1:1:1000 v/v/v) was employed as substrate stock solution to measure LOX activity. The stock solution (5 mL) was diluted completely in 20 mL of borate buffer and 5 mL of distilled water. The diluted solution (2 mL) and borate buffer (950 µL) were pipetted in a cell quartz and mixed by inversion. Next, enzymatic extracts (50 µL) were added and mixed by inversion. Absorbance was measured at 234 nm and registered every 10 s until 3 min of reaction in a UV-VIS spectrophotometer (Cecil CE 1010, Cecil Instruments Ltd., England). LOX activity was calculated as reported by Gardner (2001) employing the molar extinction coefficient (ε) of the hydroperoxide 26,800 $M^{-1} \cdot cm^{-1}$ to express it as µmol of hydroperoxide produced per L of LOX per s.

4.3.2.6 Oil analysis

Oil extraction yield

Oil extraction yield (OEY, %) of reference, control, and PEF-treated kernels was calculated based on the mass and oil content of freeze-dried kernels and their respective cakes generated from mechanical extraction. The total oil extraction yield (OEY_{TOTAL}) of

control and PEF-treated kernels was determined by calculating the oil retained into the soaking water (o_{SW}) (Rábago-Panduro et al. 2020b).

Acidity

The AOAC 940.28 method was followed to analyze oil acidity (AOAC, 1996). Results were expressed as mg KOH per g of pecan nut oil (mg KOH·g^{-1}).

Antioxidant capacity

AC was evaluated using the DPPH radical scavenging capacity method reported by Gao et al. (2019) with modifications. Pecan nut oil diluted in ethyl acetate (1:10 v/v) was mixed with 2 mL of DPPH solution (0.50 mM). The mixture was vortexed and left to react in darkness for 15 min. After this time, absorbance was measured at 515 nm with a UV-VIS spectrophotometer (Cecil CE 1010, Cecil Instruments Ltd., England). Trolox was used as standard (0.003–0.030 mg·mL^{-1}) and results were expressed as mg trolox equivalents per 100 g of pecan nut oil (mg trolox EQ·100 g^{-1}).

Oil stability index

Oil stability index (OSI) was measured with a Rancimat 679 apparatus (Metrohm AG, Switzerland) as reported by Tovar et al. (2002). Oil (3.0 g) was heated at 110°C and air was bubbled at a flow rate of 20 L/h. Next, volatile products released during oxidation were dissolved in deionized water (60 mL) to follow conductivity changes. OSI was expressed in hours (h) and obtained by Metrodata software (Metrohm AG, Switzerland).

Phytosterols

Extraction and quantification of phytosterols were performed according to Domínguez-Avila et al. (2013) and Nair et al. (2006), respectively. HCl and KOH solutions were prepared using EtOH as solvent. Pecan nut oil (0.1 g) was mixed with 0.5 mL of 6 M HCl and incubated for 1 h at 80°C. The mixture was cooled in a water bath after time elapsed. Next, 5 mL of 1.3% KOH was added and left to react for 30 min at 80°C. To phytosterols extraction, 2 mL of distilled water and 5 mL of hexane were included to the mixture, vortexed for 1 min, and centrifuged (3,750 rpm, 15 min, 20°C) (Allegra X-12 Centrifuge,

Beckman Coulter Inc., USA). The addition of distilled water and hexane was performed twice. Pooled supernatants were concentrated using a medium-scale vacuum evaporator (2.5 h, 45°C) (Genevac RKCM-12060-SNN, SP Scientific, England). Extracts were reconstituted in 0.5 mL of hexane for chromatographic analysis. A HPLC-ELSD system (Agilent 1200, Agilent Technologies, USA) equipped with a 5 µm, 4.6 mm×500 mm Luna C8 column (Phenomenex, USA) was employed to identify and quantify phytosterols from pecan nut oil. Column and ELSD temperature were maintained at 40°C and 50°C, respectively. Aliquots (10 µL) were analyzed employing a mobile phase consisted of MeOH:H_2O (95:5 v/v) at a flow rate of 1 mL/min and the detector set at a gain of 16. Standard curves of β-sitosterol (0.2–1.2 mM), stigmasterol (0.2–1.2 mM), and campesterol (0.05–0.25 mM) were prepared to quantification. Results were expressed as mg per kg of pecan nut oil.

Tocopherols

The analysis of tocopherols by HPLC coupled to a photodiode array detector (PDA) was performed as described by Andrés et al. (2014) with modifications. Oil samples were diluted in the mobile phase (4:10 w/v). The chromatographic separation was done in a Waters HPLC system (Waters Corporation, USA) using a 5 µm, 4.6 mm×250 mm Waters Spherisorb ODS2 column (Waters Corporation, USA) set at 25°C. Aliquots (40 µL) were injected and separated using a mobile phase of MeOH:THF:H_2O (67:27:6 v/v/v) on isocratic mode at a flow rate of 1 mL/min. Chromatograms were acquired at 290 nm to quantify δ-, γ-, and α-tocopherol based on standard curves (0.3–3.5 mM). Results were expressed as mg per kg of pecan nut oil.

4.3.2.7 Cakes analysis

Cakes defatted portion was used to analyze total phenolics (TP), condensed tannins (CT), and AC of reference, control, and PEF-treated cakes following the procedures reported by Rábago-Panduro et al. (2020b). TP was expressed as mmol gallic acid equivalents per 100 g of defatted cake db (mmol gallic acid EQ·100 g^{-1} db), CT as mmol catechin equivalents per 100 g of defatted cake db (mmol catechin EQ·100 g^{-1} db), and AC as mmol trolox equivalents per 100 g of defatted cake db (mmol trolox EQ·100 g^{-1} db).

4.3.2.8 Statistical analysis

PEF pretreatments, oil extraction, and LOX activity were done by triplicate while oil and cake analysis were performed by duplicate to a total of six replicates per experimental group. One-way ANOVA and Dunnett test were performed using Minitab 18 software (Minitab® 18.1, USA).

4.3.3 Results and discussion

4.3.3.1 Soaking effect on moisture and oil content of pecan nut kernels

Moisture and oil content of dry pecan nut kernels used as reference were 2.4±0.1 and 69.4±0.7 g·100 g^{-1} db, respectively. Despite that, moisture and oil content of reference kernels were within ranges reported for dry pecan nuts (Alasalvar and Shahidi 2008). Moisture content of control kernels at three different soaking times and PEF-treated kernels at three different *W* are presented in Table 4.3.1. Control kernels had moisture values that ranged from 13.5±0.6 to 21.7±0.7 g·100 g^{-1} db with no difference between kernels soaked for 20 and 35 min. Comparable results were observed in PEF-treated kernels, reaching a moisture of 13.5±1.0, 18.0±0.1, and 19.3±1.1 g·100 g^{-1} db at 0.8, 7.8, and 15.0 kJ·kg^{-1} treatments, respectively. The moisture of kernels pretreated at 7.8 and 15.0 kJ·kg^{-1} was significantly lower than their respective control kernels (α=0.05) (Table 4.3.1). These decrements could be associated with the release of intracellular water as a result of electroporation of the cell membrane during PEF processing (Ribas-Agustí et al. 2019; Tylewicz et al. 2016). Regarding the oil content, reference kernels contained 69.4±0.7 g·100 g^{-1} db, decreasing to 62.7±0.0 g·100 g^{-1} db in control kernels and 62.7±0.7 g·100 g^{-1} db in kernels pretreated by PEF. Control and PEF-treated kernels contained 9.7% less oil than reference kernels. This reduction was comparable to that reported for fresh kernels pretreated by PEF (7.3–11.8%) (Rábago-Panduro et al. 2020b). Likewise, the o_{SW} was 5.7±0.0 g for both control and PEF-treated kernels, being within the range of values reported in fresh kernels (3.8–6.2 g of oil) (Rábago-Panduro et al. 2020b). In pecan nuts, fatty acids are located as triacylglycerols in small spherical organelles named oleosomes which, at the same time, are separated by an endoplasmic network to avoid lipid oxidation (Heldt et al. 2011; Perren and Escher 2013; Zhang et al. 2017).

Table 4.3.1 Effect of soaking on kernels, oil, and cakes from pecan nuts pretreated at 0.8, 7.8, and 15.0 kJ·kg^{-1}.

		W (kJ·kg^{-1})					
	Reference	0.8		7.8		15.0	
		Control	PEF	Control	PEF	Control	PEF
Kernels							
Moisture g·100 g^{-1}	2.4 ± 0.1	13.5 ± 0.6	13.5 ± 1.0	21.1 ± 0.9	18.0 ± 0.1 *	21.7 ± 0.7	19.3 ± 1.1 *
Oil							
Acidity mg KOH·100 g^{-1}	21.4 ± 1.1	19.1 ± 0.9	19.9 ± 1.0	20.5 ± 0.1	21.8 ± 0.9	19.0 ± 0.9	21.7 ± 1.0 *
Antioxidant capacity mg trolox EQ·100 g^{-1}	48.6 ± 0.8	45.7 ± 2.2	47.8 ± 1.3	49.0 ± 1.7	48.3 ± 2.8	48.2 ± 1.7	47.1 ± 1.2
Cake							
Total phenolics mmol gallic acid EQ·100 g^{-1}	20.0 ± 1.0	20.6 ± 1.8	16.3 ± 1.0 *	18.3 ± 2.1	17.9 ± 1.2	18.1 ± 0.7	16.5 ± 0.7 *
Condensed tannins mmol catechin EQ·100 g^{-1}	15.4 ± 1.0	17.4 ± 1.6	22.1 ± 2.1 *	13.1 ± 1.3	14.5 ± 0.8 *	13.3 ± 0.8	11.9 ± 1.0 *
Antioxidant capacity mmol trolox EQ·100 g^{-1}	14.7 ± 1.0	14.4 ± 1.7	17.9 ± 1.5 *	18.5 ± 1.2	19.2 ± 0.6	16.3 ± 0.4	18.1 ± 1.3 *

W, specific energy input. Reference, kernels without soaking nor PEF processing. Control, kernels soaked in tap water (1:3 w/w) for 3, 20, and 35 min corresponding to PEF pretreatments of 0.8, 7.8, and 15.0 kJ·kg^{-1}, respectively. Antioxidant capacity was determined by the DPPH radical scavenging capacity method. Moisture was expressed as g per 100 g of kernels in dry basis. Oil acidity and antioxidant capacity were expressed per 100 g of pecan nut oil. Cake total phenolics, condensed tannins, and antioxidant capacity were expressed per 100 g of defatted cake in dry basis. Means with an asterisk were significantly different from the control according to the Dunnett test (α=0.05).

However, to commercialize pecan nuts, in-shell nuts are submitted to a drying process that modifies kernels microstructure by inducing the formation of extracellular pores, destroying the endoplasmic network, and altering the structure of oleosomes (Perren and Escher 2013). Therefore, an increment of oleosomes extraction was expected for dry kernels but, since o_{SW} did not increase compared to fresh kernels, other processes such as kernels rehydration and water-soluble compounds release could be favored over oleosomes extraction.

4.3.3.2 PEF effect on OEY, OEY_{TOTAL}, and microstructure of pecan nut kernels

OEY and OEY_{TOTAL} of reference, control, and PEF-treated kernels are displayed in Figure 4.3.1. The highest OEY was observed for reference kernels, being 88.7±3.0%. This value was higher than those reported for pecan nut oil extracted by hydraulic pressing (56.4–58.9%) (Costa-Singh and Jorge 2015; Polmann et al. 2019; Scapinello et al. 2017) and higher than OEY of fresh pecan nut kernels used as reference in the previous study (63.8%) (Rábago-Panduro et al. 2020b). Control kernels yielded 71.3±1.0, 65.9±3.1, and 72.3±0.7% at 3, 20, and 35 min of soaking, respectively, while OEY of PEF-treated kernels was 74.3±1.1, 69.1±2.1, and 70.6±3.2% at 0.8, 7.8, and 15.0 $kJ \cdot kg^{-1}$ pretreatments, respectively (Figure 4.3.1). No statistical differences were observed between OEY of control and PEF-treated kernels submitted to comparable soaking times (α=0.05). As previously mentioned, dry pecan nuts employed in this study exhibited lower moisture and higher oil content than fresh pecan nuts. Moreover, these nuts are usually air-dried, causing alterations in kernels microstructure (extracellular pores formation, endoplasmic network destruction, and oleosomes burst) in comparison with fresh nuts (Gutiérrez et al. 2008; Jia et al. 2019; Santerre 1994). As a result, dry kernels displayed more structural damage than fresh kernels that might facilitate oil extraction over fresh kernels. Savoire et al. (2013) reviewed oil extraction processes comparing several seeds with different moisture and oil content, reporting an overall improvement of OEY in seeds with lower moisture and higher oil content. They also stated that varietal differences that influence seed characteristics (moisture and oil content, hull and testa thinness, and pore number and size) could affect oil extraction by modifying seed pressing behavior, oil flow, and kernels permeability. After considering the o_{SW}, OEY_{TOTAL} of control kernels

increased up to 82.0±1.0, 76.5±3.1, and 83.0±0.7% for 3, 20, and 35 min of soaking, respectively, and OEY_{TOTAL} of PEF-treated kernels rose to 85.0±1.1, 79.8±2.1, and 81.3±3.2% for 0.8, 7.8, and 15.0 $kJ \cdot kg^{-1}$ pretreatments, respectively (Figure 4.3.1).

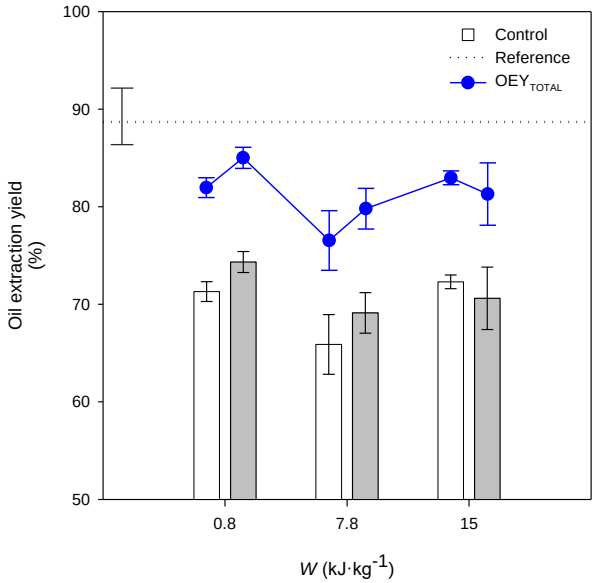

Figure 4.3.1 Effect of PEF on oil extraction yield (OEY) of pecan nut kernels. Controls were kernels soaked in tap water for 3, 20, and 35 min corresponding to PEF pretreatments of 0.8, 7.8, and 15.0 $kJ \cdot kg^{-1}$, respectively. OEY_{TOTAL} is the extraction yield considering oil extracted during the soaking process. *W*, specific energy input. No significant differences were determined according to the Dunnett test (α=0.05).

Although no statistical differences were observed between PEF-treated kernels and their respective controls (α=0.05), the highest OEY_{TOTAL} was achieved at the 0.8 $kJ \cdot kg^{-1}$ pretreatment yielding 3.7% more oil than kernels soaked for 3 min. Interestingly, PEF processing did not improve oil extraction from dry kernels, contrasting with previous findings where the application of 0.8 $kJ \cdot kg^{-1}$ to fresh kernels increased OEY_{TOTAL} up to 74.8% being higher than OEY of kernels without soaking nor treatment (63.8%) (Rábago-Panduro et al. 2020b). A similar effect was reported by Sarkis et al. (2015), drying and grinding of sesame seeds yielded higher values than sesame seeds pretreated by PEF at 40.0 $kJ \cdot kg^{-1}$ (80.4 and 71.6%, respectively). Kernels pretreated at 0.8 $kJ \cdot kg^{-1}$ were

selected for the microstructural analysis because the highest OEY_{TOTAL} was achieved at this treatment conditions.

Light microscopy of testa and cotyledon tissues of control kernels (Figure 4.3.2b, c) and kernels pretreated at 0.8 $kJ \cdot kg^{-1}$ (Figure 4.3.2d, e) are presented in Figure 4.3.2. As reference, a micrograph of the transversal section of dry kernels cotyledon tissue reported by Wakeling et al. (2003) was employed (Figure 4.3.2a). In the reference micrograph, cells delimited by the cell wall containing intracellular oleosomes stained with toluidine blue were showed. In Figure 4.3.2b, cotyledon tissue of control kernels exhibited similarities to the reference micrograph displaying delimited cells and oleosomes within the intracellular space stained with Richardson blue. However, at higher magnification, it appeared that the cotyledon tissue was composed of both intact and damaged cells. Additionally, oleosomes seemed to change their shape and aggregate within the intracellular space (Figure 4.3.2c). Nikiforidis (2019) stated that soaking of seeds causes cell swelling, changing oleosomes shape, and diffusion kinetics. Based on control kernels micrographs, it is proposed that soaking of dry pecan nuts negatively affected oil extraction by two main phenomena: i) the release of oleosomes found in the surface of cotyledon tissue caused by kernels rehydration, and ii) the reorganization of oleosomes remaining within the cotyledon tissue. Thus, even though o_{SW} was considered, the low OEY_{TOTAL} of control kernels might be related to oleosomes trapped within kernels microstructure hindering the oil extraction. Concerning kernels pretreated by PEF, micrographs showed compaction of cells in testa and cotyledon tissues with a loss of delimited inclusions in the intracellular space (Figure 4.3.2d). It seems that PEF processing, rather than inducing the cell rupture, produced the rupture of intracellular inclusions. In Figure 4.3.2e, the higher magnification of PEF-treated kernels cotyledon tissue showed no difference between intact and damaged cells due to oleosomes fusion in the periphery of the cell. Therefore, even though no statistical differences were determined between control and PEF-treated kernels, it is suggested that the fusion and migration of oleosomes could facilitate oil extraction from PEF-treated kernels by increasing oil flow during mechanical extraction in comparison with control kernels. However, the application of PEF on dry kernels was not able to enhance oil extraction beyond OEY of reference kernels probably to the modification of kernels microstructure

and oleosomes properties produced by drying and their further rehydration due to soaking. The combination of both processes could negatively affect kernels compression behavior impeding oil extraction even though PEF-treated kernels were freeze-dried prior to mechanical extraction.

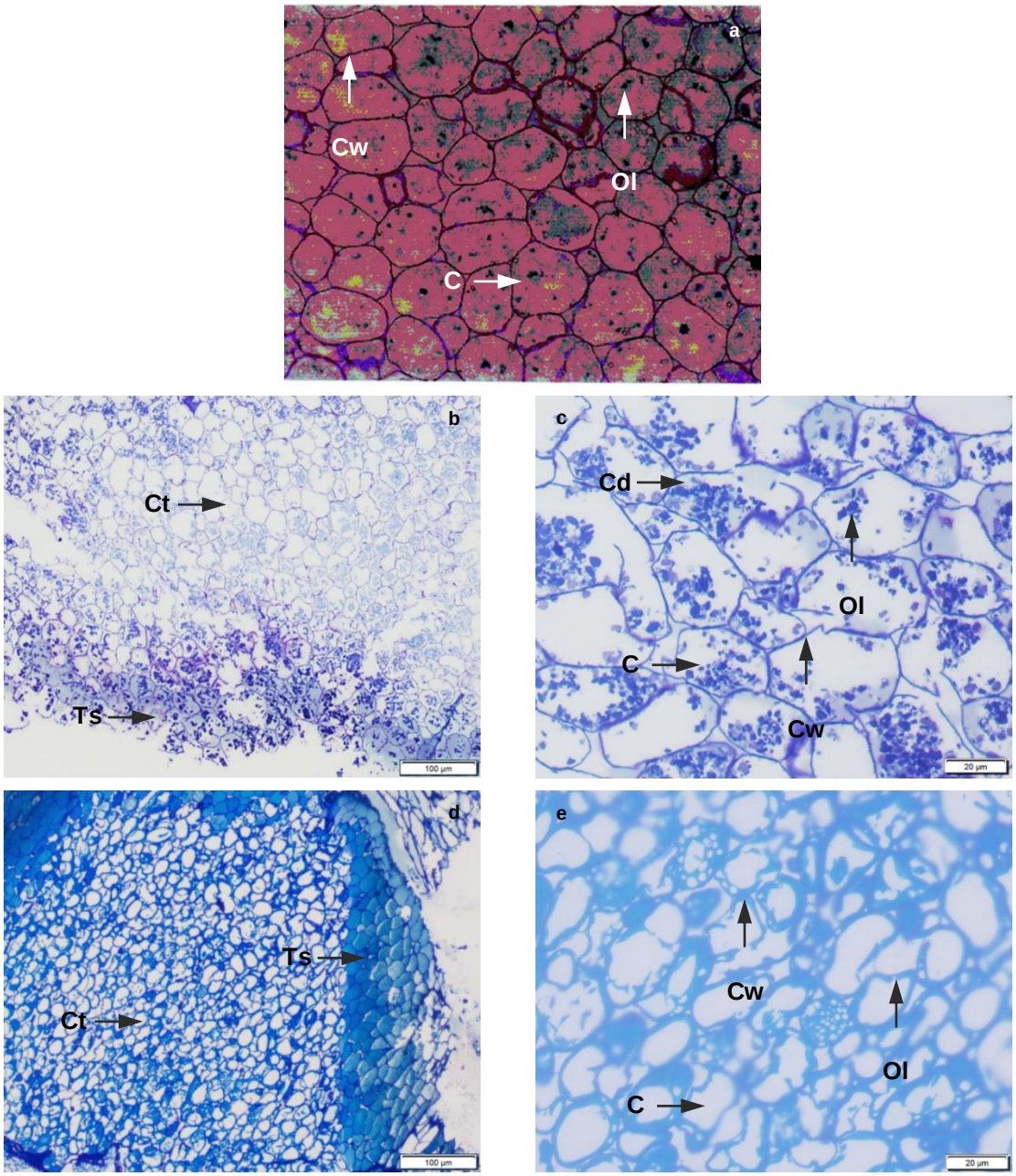

Figure 4.3.2 Light microscopy micrographs of transversal pecan nuts. Cotyledon tissue of pecan nut kernels reported by Wakeling et al. (2003) used as reference (a) along with control (b, c), and PEF-treated (d, e) kernels. Ts, testa; Ct, cotyledon tissue; C, cell; Cd, damaged cell; Cw, cell wall; Ol, oleosomes. Control kernels were soaked in tap water (1:3 w/v) for 3 min while PEF–treated kernels were processed at a specific energy input of 0.8 $kJ \cdot kg^{-1}$.

4.3.3.3 PEF effect on pecan nut oil stability

Acidity of oil extracted from reference, control, and PEF-treated kernels ranged from 19.0±0.9 to 21.8±0.9 mg KOH·100 g^{-1} (Table 4.3.1). These results were within values showed in a previous study oil extracted from fresh kernels (21.3–38.3 mg KOH·100 g^{-1}) (Rábago-Panduro et al. 2020b). Oil acidity did not significantly change between kernels pretreated at 0.8 and 7.8 kJ·kg^{-1}, reference kernels nor their respective control kernels (α=0.05) whereas, at 15.0 kJ·kg^{-1}, the oil from PEF-treated kernels displayed 14.2% higher acidity compared to control kernels (Table 4.3.1). Puértolas and Martínez de Marañón (2015), Andreou et al. (2017), Moradi and Rahimi (2018), and Guderjan et al. (2007) also reported an increment in the acidity of oil extracted from olives, sunflower seeds, and rapeseeds pretreated by PEF at *W* ranging between 11.2 and 48.0 kJ·kg^{-1}. This increment was associated to triacylglycerol degradation due to an increase in lipase activity caused by the application of PEF (Guderjan et al. 2007). Nevertheless, since LOX activation is the first step to initiate triacylglycerol hydrolyzation in oleosomes, it is suggested that the application of 15.0 kJ·kg^{-1} to pecan nut kernels might induce LOX activation rather than lipase activation by modification in kernels microstructure and oleosomes membrane. Despite changes in acidity, oil extracted from PEF-treated kernels was within the acidity range reported for cold-pressed and virgin oils of the Codex Standards for Fats and Oils from Vegetable Sources (FAO 2015).

Concerning AC of extracted oils, it varied from 45.7±2.2 to 49.0±1.7 mg trolox·100 g^{-1} with no significant differences between PEF-treated kernels and their respective controls (α=0.05) (Table 4.3.1). These values were slightly lower than those achieved in the study employing fresh kernels but higher than those reported for pecan nut oil obtained by solvent extraction (49.4–61.4 and 18.5–27.7 mg trolox·100 g^{-1}, respectively) (Domínguez-Avila et al. 2013; Rábago-Panduro et al. 2020b). Acidity and AC of oil extracted from dry kernels were similar to those from fresh kernels when PEF was applied as pretreatment. OSI, phytosterols and tocopherols content, as well as LOX activity of reference and control kernels were compared against kernels pretreated at 0.8 kJ·kg^{-1} (Table 4.3.2). The OSI values of reference, control, and PEF-treated kernels were 10.4±0.4, 10.9±0.3, and 10.6±0.5 h, respectively. Neither soaking nor PEF processing significantly changed the stability index of pecan nut oil (*p-value*=0.303). Oro et al. (2009)

reported a comparable OSI value for pecan nut oil also extracted by mechanical pressing (9.8 h).

Table 4.3.2 Effect of PEF on phytosterols and tocopherols concentration along with lipoxygenase (LOX) activity of pecan nut kernels pretreated at 0.8 kJ·kg^{-1}.

	Reference		Control		PEF	
Phytosterols						
mg·kg^{-1}						
β–Sitosterol	929.0 ± 89.3		858.2 ± 62.6		910.5 ± 132.2	
Stigmasterol	501.5 ± 79.7		352.1 ± 17.8		324.4 ± 48.5	
Tocopherols						
mg·kg^{-1}						
γ–Tocopherol	8.7 ± 0.8	*	1.8 ± 0.2		1.8 ± 0.2	
α –Tocopherol	19.1 ± 2.0	*	6.4 ± 0.8		6.2 ± 0.8	
Enzymatic activity						
LOX	4.52 ± 0.14	*	4.83 ± 0.1		4.41 ± 0.20	*
µmol·L^{-1}s^{-1}						

Reference, kernels without soaking nor PEF processing. Control, kernels soaked in tap water (1:3 w/v) for 3 min. Concentrations were expressed as mg per kg of oil. Means with an asterisk were significantly different from the control according to the Dunnett test (α=0.05).

The main phytosterol quantified in extracted oils was β-sitosterol followed by stigmasterol (Table 4.3.2). Campesterol was not detected despite that it has been reported in low concentration in pecan nuts (Phillips et al. 2005; U.S. Department of Agriculture 2020). Oil from reference, control, and PEF-treated kernels contained a β-sitosterol concentration of 929.0±89.3, 858.2±62.6, and 910.5±132.2 mg·kg^{-1}, respectively, while stigmasterol ranged from 324.4±48.5 to 501.5±79.7 mg·kg^{-1}. β-Sitosterol did not significantly change among extracted oils (*p-value*=0.516), but a significant reduction of stigmasterol was observed in oil from control and PEF-treated kernels compared to reference kernels (Table 4.3.2). Regarding tocopherols' profile, γ- and α-tocopherol were the main tocopherols, while δ-tocopherol was not detected even though it has been reported in pecan nuts (Bouali et al. 2013). Oil extracted from reference kernels displayed a γ- and α-tocopherol concentration of 8.7±0.8 and 19.1±2.0 mg·kg^{-1}, respectively. Interestingly, both γ- and α-tocopherol decreased by 79.3 and 67.0%, respectively, in oil from control and PEF-treated kernels (Table 4.3.2). Oil extracted from reference kernels exhibited lower concentration of phytosterols and tocopherols concentration in comparison with other studies where pecan nut oil was characterized (Al Juhaimi et al. 2018; Fernandes et al. 2017; Gong et al. 2017). Moreover, α-tocopherol was identified as the predominant tocopherol contrasting to the general data

that reported γ-tocopherol as the main homolog and lipophilic antioxidant of pecan nut oil (Costa-Singh and Jorge 2015; Fernandes et al. 2017; Polmann et al. 2019). Therefore, it is possible that the similarities in oil acidity, AC, and OSI of reference, control, and PEF-treated kernels were related to the low concentration of phytosterols and tocopherols, mainly γ-tocopherol, since these phytochemicals have been associated to oil resistance to oxidation (Gawrysiak-Witulska et al. 2012; Shahidi and John 2010; Wall 2010). Reference, control, and PEF-treated kernels displayed a LOX activity of 4.52±0.14, 4.83±0.10, and 4.41±0.20 $\mu mol \cdot L^{-1} s^{-1}$, respectively, with no significant differences between reference and PEF-treated kernels. It seemed that the application of PEF at 0.8 $kJ \cdot kg^{-1}$ did not promote lipid oxidation of pecan nut oil. These results agreed with acidity, AC, and OSI results of the oil extracted from kernels pretreated at 0.8 $kJ \cdot kg^{-1}$ because neither of these values was different from those of oil extracted from reference and control kernels. However, it should not be discarded lipid oxidation via enzyme activation since the acidity of pecan nut oil increased at higher *W*.

4.3.3.4 PEF effect on pecan nut cakes

TP, CT, and AC of cakes generated from reference, control, and PEF-treated kernels are presented in Table 4.3.1. The reference cake showed TP, CT, and AC values of 20.0±1.0 mmol gallic acid EQ·100 g^{-1} db, 15.4±1.0 mmol catechin EQ·100 g^{-1} db, and 14.7±1.0 mmol trolox EQ·100 g^{-1} db, respectively. These results were lower than those reported in the reference cake obtained from fresh kernels used to evaluate PEF (24.2±1.8 mmol gallic acid EQ·100 g^{-1} db, 24.7±2.9 mmol catechin EQ·100 g^{-1} db, and 19.5±0.3 mmol trolox EQ·100 g^{-1} db, respectively) (Rábago-Panduro et al. 2020b). These differences could be due to the effect of drying as this process has been demonstrated to decrease TP, CT, and AC of pecan nuts (Rábago-Panduro et al. 2020a). Regarding cakes from control and PEF-treated kernels, TP varied from 18.1±0.7 to 20.6±1.8 mmol gallic acid EQ·100 g^{-1} db in control kernels while, in PEF-treated kernels, TP decreased by 20.9% at 0.8 $kJ \cdot kg^{-1}$ compared to cakes generated from kernels soaked for 3 min (Table 4.3.1). In contrast, the application of 0.8 and 7.8 $kJ \cdot kg^{-1}$ increased the CT content of cakes by 27.0 and 10.7%, respectively, compared to their respective control kernels. An overall improvement in AC of cakes generated from PEF-treated kernels was observed with the

highest increment achieved at 0.8 kJ·kg^{-1}, increasing by 24.3% in comparison with their control (Table 4.3.1). PEF processing increased not only the CT concentration but also enhanced AC of cakes from PEF-treated kernels. Considering that pecan nuts AC is closely related to their CT concentration, the increment of AC in cakes from PEF-treated kernels could be attributed to the enhancement of CT (Flores-Martínez et al. 2016; Rábago-Panduro et al. 2020a). Simple phenolic compounds are released into the soaking water, while condensed tannins are retained in the cake increasing its AC.

4.3.4 Conclusion

The application of PEF to dry pecan nuts did not improve neither OEY nor OEY_{TOTAL} of pretreated kernels (69.1–74.3% and 79.8–85.0%, respectively) in comparison with kernels without soaking nor PEF processing (88.7%). These results could be due to changes in oleosomes characteristics and their localization within the cotyledon tissue of dry kernels submitted to soaking. Kernels pretreated at 0.8 kJ·kg^{-1} showed a reduction of the cell size and oleosomes fusion in the intracellular space, that probably help oil flow during mechanical extraction. Comparable values of acidity, AC, and OSI between oil extracted from reference, control, and PEF-treated kernels might be linked to the low concentration of phytosterols and tocopherols observed in oil extracted from reference kernels since oil resistance to oxidation is related to these compounds. Furthermore, the application of 0.8 kJ·kg^{-1} increased AC of cakes compared to untreated kernels and kernels soaked for 3 min. These results showed that the effectiveness of PEF to increase OEY_{TOTAL} of pecan nuts is dependent on not only moisture and oil content of kernels but also of drying since rehydration of dry kernels apparently produced a negative effect on kernels microstructure and oleosomes characteristics. Thus, further research focused on these variables is necessary along with other microscopy techniques and analytical procedures to better understand the application of PEF as a pretreatment to oil extraction from pecan nuts.

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Chapter 5

General discussion

In the first part of this dissertation, phytochemical changes of pecan nuts due to drying and storage processes were presented (*Section 4.1*). The drying effect was evaluated by analyzing bioactive compounds concentration of fresh and dry kernels. Conversely, the storage effect was investigated using fresh and dry kernels stored at 4°C and dry in-shell nuts stored at 4 and 25°C, measuring phenolic compounds content and antioxidant capacity of both kernels and in-shell nuts for 240 days. Results indicated that drying process and storage time were the most significant factors affecting phenolic compounds concentration and antioxidant capacity of pecan nuts. The drying process lead to a decrement in total phenolics (TP), total flavonoids (FT), condensed tannins (CT), and antioxidant capacity (AC) – DPPH and ORAC – of 24.9, 35.1, 38.8, 18.8, and 31.5%, respectively. These reductions might be related to oxidation of phenolic compounds by an increment of polyphenol oxidase activity associated with drying temperature (Wang et al. 2018). In general, fresh kernels exhibited the highest concentration of phenolic compounds and AC throughout storage but, after 210 days of storage, developed mold growth reducing kernels shelf-life. In contrast, comparable phenolic compounds concentration and AC were observed in dry pecan nuts despite kernels or in-shell storage and storage temperature. After 15 days of storage, fresh kernels stored at 4°C showed a significant increment of phenolic compounds concentration and AC compared to their initial content (8.7, 7.1, 22.2, and 21.3% for TP, TF, CT, and ORAC, respectively) that could be associated with the synthesis of phenolic compounds due to chilling stress (Christopoulos and Tsantili 2015). During storage, a general decrement of condensed tannins concentration was observed for pecan nuts with a loss of 35.2 and 31.5% for fresh and dry kernels and 41.8 and 53.3% for in-shell nuts stored at 4 and 25°C, respectively. AC measured by DPPH increased by 224.7 and 216.4% in fresh and dry kernels, respectively, after 90 days of storage. Whereas in-shell nuts stored at 4 and 25°C showed an increment of 188.4 and 199.8%, respectively, after 240 days of storage. These results might be attributed to the polymerization of condensed tannins, considering that larger polyphenols have been recognized as great antioxidants due to the high number of

hydroxyl groups that function to charge delocalization (Nicoli et al. 2002; Bors and Michel 2002).

As a second part of this dissertation, the evaluation of PEF application as a pretreatment to increase oil extraction from pecan nuts was performed (*Section 4.2*). Fresh kernels pretreated by PEF were compared against reference and control kernels. In this experiment, reference kernels were neither soaked nor PEF processed while control kernels were soaked in tap water for 20 min. PEF-treated kernels were processed at different specific energy inputs (*W*) ranging from 0.5 to 17.6 kJ·kg^{-1}. Reference kernels contained moisture of 3.2±0.1 g·100 g^{-1} in dry basis (db) increasing 678.1% in control kernels and 484.4–578.1% in kernels pretreated by PEF. Kernels immersion in water also caused the release of oil into the soaking water (o_{SW}). The initial oil content of control kernels reduced by 10.4% while, in PEF-treated kernels, decreased between 7.3 and 11.8% in comparison with the oil content of reference kernels (61.2±3.0 g·100 g^{-1} db). Oil content reduction was associated with grinding and soaking of fresh kernels, increasing the exposure of cotyledon tissue to water, and causing the expansion of the cell wall facilitating oleosomes release (Heldt et al. 2011; Zhang et al. 2017). Oil extraction yield (OEY) of reference kernels was 63.8±1.5% being comparable to yields reported for mechanical extraction of pecan nut oil (Costa-Singh and Jorge 2015; Scapinello et al. 2017; Polmann et al. 2019). A total OEY (OEY_{TOTAL}) was calculated considering the o_{SW} to compare control and PEF-treated kernels against reference kernels. OEY_{TOTAL} of control kernels was 65.8±2.0%, while OEY_{TOTAL} of PEF-treated kernels ranged from 68.9±0.0 to 77.4±3.0%. The application of PEF increased oil extraction between 8.0 and 21.4% compared to reference kernels with the highest increment achieved at the 0.5 kJ·kg^{-1} pretreatment. These improvements were attributed to the reversible electroporation of cells in pecan nut kernels that could induce oleosomes fusion within the cell as well as the release of intracellular hydrophilic compounds that could enhance oil extraction (Han et al. 2019). Acidity of oil extracted from kernels pretreated by PEF ranged from 21.3±1.3 to 38.3±1.4 mg KOH·100 g^{-1} oil being comparable to oil acidity of reference kernels (29.0±2.1 mg KOH·100 g^{-1} oil) and remaining within international standards reported for cold-pressed and virgin oils of the Codex Standards for Fats and Oils from Vegetable Sources (FAO 2015). On the other hand, TP of cakes generated from

PEF-treated kernels varied between 20.4±1.5 and 26.5±0.8 mmol gallic acid EQ·100 g^{-1} defatted cake db with the highest concentration corresponding to 0.8 kJ·kg^{-1} pretreatment. These values were within values obtained from control and reference kernels (24.2±1.8 and 22.5±1.7 mmol gallic acid EQ·100 g^{-1} defatted cake db, respectively). Likewise, CT ranged from 16.1±1.1 to 32.1±3.0 mmol catechin EQ·100 g^{-1} defatted cake db in kernels pretreated by PEF while reference and control values were 24.7±2.9 and 23.1±2.6 mmol catechin EQ·100 g^{-1} defatted cake db, respectively. Comparable behavior was observed for TP and CT of cakes produced from PEF-treated kernels. At 0.5 kJ·kg^{-1}, no difference with control and reference values were noted, but a significant improvement of TP and CT at 0.8 kJ·kg^{-1} treatment (17.8 and 39.3%, respectively) was observed. A mechanism was proposed given that soluble phenolic compounds, including condensed tannins, are found as vacuoles inside the cell (Murad et al. 2019; Renard et al. 2017). At less intense PEF processing conditions, physical rearrangements such as vacuoles rupture might take place inside the cell, but the specific energy input applied is insufficient to initiate phenolic compounds release into the soaking water. Furthermore, a sharp decrease was observed between 1.8 and 2.8 kJ·kg^{-1} followed by a gradual improvement of TP and CT values at higher PEF treatments. These changes could be related to a balance between gain and loss of phenolic compounds within the cell. However, at more intense treatments, the rate of soluble phenolic compounds released into the soaking water increases, while CT are retained in the cell by their interaction with polysaccharides of the cell wall (Renard et al. 2017). Based on these results, dry kernels were pretreated at 0.8, 7.8, and 15.0 kJ·kg^{-1} before oil extraction (*Section 4.3*) and compared against reference kernels. Control kernels soaked for 3, 20, and 35 min to investigate the effect of soaking during PEF processing. Moisture and oil content of reference kernels were 2.4±0.1 and 69.4±0.7 g·100 g^{-1} db, respectively, being within values reported for dry pecan nut kernels (Alasalvar and Shahidi 2008). In control kernels, the moisture content increased by 462.5–804.2% while, in PEF-treated kernels, it augmented 462.5–704.2%. Kernels pretreated at 7.8 and 15.0 kJ·kg^{-1} displayed lower moisture than their respective control kernels. These results might be attributed to the electroporation mechanism causing a release of intracellular water and hydrophilic compounds (Ribas-Agustí et al. 2019; Tylewicz et al. 2016). Comparable oil content and o_{SW} was observed for control and PEF-

treated kernels. The oil content was 9.7% lower than that from reference kernels and o_{SW} was 5.7±0.0 g being within values reported for fresh kernels (Rábago-Panduro et al. 2020 b). Regarding oil extraction, OEY of reference kernels was 88.7±3.0% while OEY_{TOTAL} of control and PEF-treated kernels were 76.5–83.0% and 79.8–85.0%, respectively, with no differences between control and PEF-treated kernels. Interestingly, PEF processing did not improve OEY_{TOTAL} when it was applied to dry kernels. Similar results were reported for sesame seeds pretreated at 40.0 $kJ \cdot kg^{-1}$ (Sarkis et al. 2015). Moreover, the comparison of OEY between same seeds that only differed in their moisture and oil content indicated that seeds with lower moisture and higher oil content displayed higher OEY (Savoire et al. 2013). Therefore, it was proposed that differences in moisture and oil content between fresh and dry kernels along with kernels structural damage caused by drying (Gutiérrez et al. 2008; Jia et al. 2019) facilitated oil extraction in reference kernels. In this sense, PEF processing was not able to equate the structural damage of dry kernels under the specific energy inputs applied, leading to lower OEY_{TOTAL}. Despite these results, the microstructural analysis of kernels pretreated at 0.8 $kJ \cdot kg^{-1}$ showed that the application of PEF modified cell integrity by inducing oleosomes rupture and migration to the cell periphery. These changes could facilitate oil extraction in comparison with control kernels. Oils extracted from reference, control, and PEF-treated kernels displayed comparable acidity values except kernels pretreated at 15.0 $kJ \cdot kg^{-1}$. The oil acidity of these kernels was 14.5% higher than their respective control kernels. The increment might be associated with lipoxygenase (LOX) activation caused by changes in kernels microstructure and oleosomes membrane at more intense PEF treatments. Similar AC and oil stability indexes (OSI) were observed in oil extracted from reference, control, and PEF-treated kernels. Phytosterols and tocopherols of oil extracted from reference kernels were identified in lower concentrations in comparison with pecan nut oil extracted by mechanical and solvent processes (Al Juhaimi et al. 2018; Fernandes et al. 2017; Gong et al. 2017). Moreover, a reduction of stigmasterol, γ–tocopherol, and α–tocopherol was observed in oil extracted from control (29.8, 79.3, and 66.5%, respectively) and PEF-treated kernels (35.3, 79.3, and 67.5%, respectively) compared to reference kernels. Consequently, it was suggested that similarities in acidity, AC, and OSI of extracted oils could be associated with the low concentration of these phytochemicals due to their

association with oil resistance to oxidation (Gawrysiak-Witulska et al. 2012; Shahidi and John 2010; Wall 2010). LOX activity was not significantly affected by neither soaking (3 min) nor PEF processing (0.8 $kJ \cdot kg^{-1}$). TP, CT, and AC of cakes generated from reference kernels were 20.0±1.0 mmol gallic acid EQ·100 g^{-1} db, 15.4±1.0 mmol catechin EQ·100 g^{-1} db, and 14.7±1.0 mmol trolox EQ·100 g^{-1} db, respectively. These results were lower than values reported for cakes generated from fresh kernels (Rábago-Panduro et al. 2020b). The reduction of TP, CT, and AC was associated with the drying process, considering that drying has been related to phenolic compounds decrease in pecan nut kernels (Rábago-Panduro et al. 2020a). The application of PEF at 0.8 $kJ \cdot kg^{-1}$ decreased TP by 20.9% but increased CT and AC by 27.0 and 24.3% in comparison with cakes generated from kernels soaked for 3 min. In this line, it was suggested that low specific energy inputs induce the release of simple phenolic compounds, retaining complex phenolic compounds within the cake leading to an increment of AC (Crozier et al. 2007; Gómez-Maqueo et al. 2016).

Chapter 6

General conclusions

6.1 Conclusions and contributions

This dissertation was focused on study the effect of the drying process and storage conditions on bioactive compounds content and antioxidant capacity of pecan nuts demonstrating that:

I. Drying process and storage time were the most significant factors affecting mainly the phenolic compounds concentration and antioxidant capacity of pecan nuts.

II. Pecan nuts drying decreased both phenolic compounds and antioxidant capacity. Throughout storage, condensed tannins concentration reduced while antioxidant capacity increased. These changes might be related to the polymerization of condensed tannins having a significant impact on the health benefits associated with kernels' consumption.

The second objective of this dissertation was to evaluate the impact of PEF on oil extraction yield of pecan nuts, oil stability, and phytochemical content of the oil and the by-product generated showing that:

III. Kernels water immersion for PEF processing caused oleosomes release into the treatment water. Thus, oil extraction from pecan nuts pretreated by this technology must consider oil recovery from the treatment water in further research.

IV. The application of PEF increased oil extraction yield of fresh kernels, contrarily to its effect on dry kernels, indicating that its effectiveness might depend not only on moisture and oil content but also the drying process of pecan nuts.

V. The microstructural analysis of pecan nuts showed that the application of PEF caused oleosomes rupture and further fusion in the cell perimeter.

VI. The oil extracted from fresh and dry kernels pretreated by PEF displayed acidity values within the range of international standards with no significant changes on its antioxidant capacity.

VII. The PEF pretreatment of fresh and dry kernels generated a by-product with a higher concentration of condensed tannins in comparison with untreated kernels that might enhance its potential as an ingredient for functional foods development.

VIII. The application of PEF at low intense processing conditions (≤0.8 $kJ \cdot kg^{-1}$) was enough to increase oil extraction yield in fresh kernels, maintaining oil stability, and enhancing by-product condensed tannins.

6.2 Future work

Based on the findings from this doctoral dissertation, further research regarding the optimization of drying is crucial to avoid or minimize its impact on bioactive compounds content of pecan nuts. About storage time, the evaluation of the polymerization of condensed tannins throughout storage and its effect on *in vitro* and *in vivo* antioxidant capacity are suggested, considering that both variables are closely related to the health-promoting properties of pecan nuts.

Concerning PEF pretreatment of pecan nuts, kernels initial moisture is an important factor to take into consideration in order to guarantee PEF effectiveness. Therefore, studies relating pecan nuts moisture to OEY_{TOTAL} may help to establish the best initial moisture that could allow the standardization of PEF processing. However, it is necessary to consider compositional and phytochemical analysis of pecan nuts previous to PEF processing to avoid degradation of bioactive compounds. This study should be followed by the phytochemical characterization and physicochemical analysis of the oil and cake. Furthermore, the incorporation of cakes into food formulations must contemplate the compositional and physicochemical analysis of the developed products, as well as the evaluation of their nutraceutical properties by *in vitro* and *in vivo* analysis.

The suggested studies are proposed to increase the knowledge about pecan nuts and PEF, expecting its application in the production of pecan nut oil improving OEY_{TOTAL}

compared to traditional extraction processes and oil nutritional and nutraceutical properties. The development of food products based on pecan nut cakes with better nutraceutical properties is also expected to offer consumers a wide variety of functional foods.

Appendix

Additional data

Table A1 Oxidative stability indexes at different temperatures and extinction coefficients of oil from fresh and dry pecan nut kernels.

	Oxidative stability index (h)										Extinction coefficients	
	Temperature (°C)											
Kernels	90		100		110		120		130		K232	K270
Fresh	47.33 ± 2.26	b	21.21 ± 0.39	b	9.58 ± 0.17	a	4.54 ± 0.05	a	2.23 ± 0.02	a	1.48 ± 0.12	0.07 ± 0.00
Dry	63.47 ± 3.80	a	23.27 ± 0.77	a	8.61 ± 0.39	b	3.83 ± 0.17	b	2.04 ± 0.06	b	1.55 ± 0.05	0.08 ± 0.01

Means with different letters within columns are significantly different according to the Tukey test (0.05).

Table A2 Phenylalanine ammonia lyase (PAL) activity of pecan nut kernels pretreated by PEF at 0.8 kJ·kg^{-1} and phenolic compounds concentration along with antioxidant capacity of cakes from PEF–treated kernels.

	Reference		Control		PEF	
Kernels						
PAL activity µmol·L^{-1}s^{-1}	1.18 ± 0.06		1.17 ± 0.03		1.11 ± 0.07	
Cake						
Phenolic compounds concentration Total flavonoids mmol catechin EQ·100 g^{-1}	4.6 ± 0.4	*	4.3 ± 0.3		3.9 ± 2.2	*
Antioxidant capacity ORAC mmol trolox EQ·100 g^{-1}	46.9 ± 3.7	*	43.2 ± 3.0		42.4 ± 4.9	

ORAC, oxygen radical absorbance capacity. PAL activity was expressed as µmol of trans-cinnamic acid produced per L of PAL in wet basis. Phenolic compounds concentration and antioxidant capacity were expressed mmol equivalents (EQ) per 100 g of defatted cake in dry basis. Means with an asterisk were significantly different from the control according to the Dunnett test (α=0.05).

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