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Dª. Gabriela Lorena Salazar Orbea

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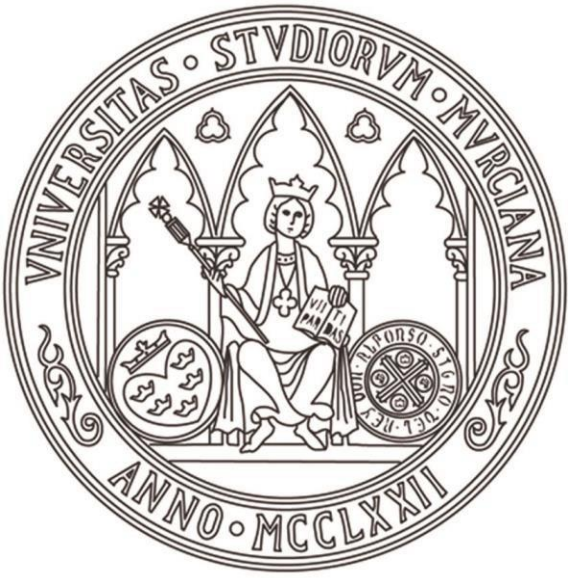

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Aprobado por la Comisión General de Doctorado el 19-10-2022

D./Dña. Gabriela Lorena Salazar Orbea

doctorando del Programa de Doctorado en

Tecnología de los Alimentos, Nutrición y Bromatología

de la Escuela Internacional de Doctorado de la Universidad Murcia, como autor/a de la tesis presentada para la obtención del título de Doctor y titulada:

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The compendium of publications comprises three papers published in journals indexed in Journal Citation Reports (JCR):

1. **Salazar-Orbea, G.**; García-Villalba, R.; Sánchez-Siles, L. M.; Tomás-Barberán, F. A.; García, C. J. Untargeted Metabolomics Reveals New Markers of Food Processing for Strawberry and Apple Purees. Molecules 2022, 7275. https://doi.org/https://doi.org/10.3390/molecules27217275.
2. **Salazar-Orbea, G. L.**; García-Villalba, R.; Bernal, M. J.; Hernández, A.; Tomás-Barberán, F. A.; Sánchez-Siles, L. M. Stability of Phenolic Compounds in Apple and Strawberry: Effect of Different Processing Techniques in Industrial Set Up. Food Chem. 2023, 401 (August 2022). https://doi.org/10.1016/j.foodchem.2022.134099
3. **Salazar-Orbea, G. L.**; García-Villalba, R.; Bernal, M. J.; Hernández-Jiménez, A.; Egea, J. A.; Tomás-Barberán, F. A.; Sánchez-Siles, L.M. Effect of Storage Conditions on the Stability of Polyphenols of Apple and Strawberry Purees Produced at Industrial Scale by Different Processing Techniques. J. Agric. Food Chem. 2023. https://doi.org/10.1021/acs.jafc.2c07828.s.

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Por creer en mí, ser mi ejemplo de superación y guías de vida

A mis hermanos, Katherine y Anthony,

Que a la distancia siempre están en mi vida para darme su apoyo

To Pavel and Matias,

you are my dream come true

my love, my strength to make everything possible.

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Summary

(Poly)phenols are important bioactive compounds with potential beneficial effects on human health, these are commonly found in fruits and vegetables. This research was focused on the (poly)phenols of apple (Malus domestica) and strawberry (Fragaria x ananassa) as these are two of the fruits more consumed worldwide, both fresh and processed (juices, jams, purees, smoothies, dried). Although the consumers increasingly look for less processed products, fruit products need to be offered and available in the market all year long. Therefore, fruit processing is essential to extend their shelf life, together with guaranteeing their nutritional and bioactive content while maintaining their sensory characteristics. Overall, there is a considerable variability among the (poly)phenols, which depends firstly on the matrix, the part of the fruit (e.g., flesh, peel, achenes) and the inherent fruit characteristics (e.g., origin, variety, ripeness degree). However, the different technologies for fruit processing and the storage conditions also play a role in the changes of the (poly)phenols concentration and sensory characteristics of the final product. Simultaneously, the processing technologies and conditions also influence the metabolic profile of both fruits.

The objectives of the present Thesis were to determine to what extent different processing technologies, freezing, thermal treatments, and high-pressure processing, tested at an industrial scale and storage conditions, -20, 4 and 24 ºC for twelve months, affect the (poly)phenols, color and sensory attributes in the selected strawberry and apple products. As well as, to determine how the fruit processing influenced the metabolic profile and identify possible markers that help assess the degree of processing in these products. All this in order to potentially facilitate the use of customized processing and storage conditions to limit the degradation of (poly)phenols while maintaining the best quality and organoleptic characteristics.

For achieving the separation, identification and quantification of the different (poly)phenols compounds characteristics to each fruit, the samples were extracted and analyzed by specific chromatographic methods and conditions. In addition, a non-targeted metabolomics approach based on UPLC-ESI-QTOF-MS was performed for the identification of processing markers. Methods and specific conditions are detailed in CHAPTER III.

Summary

The results reached from this Thesis and included in the CHAPTERS IV, V and VI concluded that :

1. In strawberries, mild and standard thermal treatments showed similar patterns for most phenolic groups; an increase in proanthocyanidins, no change in ellagic acid conjugates, and a major decrease in flavonols and anthocyanins. Nevertheless, the process to obtain strawberry concentrate, vacuum concentration, had a detrimental effect on all the compounds. On the other hand, the storage conditions had a stronger detrimental impact on the phenolic compound levels, as well as on color and sensory attributes than the processing techniques.
2. In apples, mild treatments and high-pressure processing had similar effects in all phenolic groups, with increases in dihydrochalcones, hydroxycinnamics, and proanthocyanidins and decreases in flavonols. However, the standard thermal treatment increased dihydrochalcones and even flavonols concentrations. Thus it can be said that the initial processing techniques exerted a greater influence than storage conditions.
3. In general, proanthocyanidins were the major phenolic group and the most stable during storage, while anthocyanins were the group most affected by both processing and storage.
4. Overall, the storage at -20 and 4 ºC barely affected the phenolic level and the quality characteristics during storage. Nevertheless, storage at 24 ºC highly degraded the polyphenolic content and the quality and sensory attributes in strawberry, although not that drastic in apple products.
5. Through a non-targeted metabolomics approach, seven metabolites were identified and proposed as potentially powerful markers to evaluate the processing degree of strawberry and apple puree products.
6. Pyroglutamic acid, and 2-hydroxy- 5-methoxy benzoic acid were identified as upregulated markers, whereas pteroyl- D-glutamic acid, and 2-hydroxybenzoic acid β-D-glucoside were identified as down regulated markers of thermal processing in strawberry products.
7. Dihydroxycinnamic acid glucuronide, caffeic acid, and lysoPE(18:3(9Z,12Z,15Z)/0:0) were identified as upregulated markers, showing an increasing trend correlated with thermal processing in apple products.

8. The selection of the right variety together with both processing technology and conditions should be customized accordingly by industries, to preserve or even increase the (poly)phenol content in the final product as close as possible to that of fresh fruit.

9. The use of these markers may potentially help to objectively measure the degree of food processing and help to clarify the controversial narrative on ultra-processed foods.

Resumen

Los (poli)fenoles son importantes compuestos bioactivos con posibles efectos beneficiosos para la salud humana, y se encuentran habitualmente en frutas y verduras. Esta investigación se centró en los (poli)fenoles de la manzana (*Malus domestica*) y la fresa (*Fragaria x ananassa*), ya que son dos de las frutas más consumidas en todo el mundo, tanto frescas como procesadas (zumos, mermeladas, purés, batidos). Aunque los consumidores buscan cada vez más productos menos procesados, los productos de fruta deben ofrecerse y estar disponibles en el mercado durante todo el año. Por lo tanto, el procesado de la fruta es esencial para prolongar su vida útil, además de garantizar su contenido nutricional y bioactivo manteniendo sus características sensoriales. En general, existe una variación considerable en la concentración de (poli)fenoles, que depende en primer lugar de la matriz, la parte de la fruta (ej. pulpa, piel, aquenios) y las características inherentes de la fruta (ej. origen, variedad, grado de madurez). Sin embargo, las diferentes tecnologías de procesado de la fruta y las condiciones de almacenamiento también influyen en los cambios de la concentración de (poli)fenoles y en las características sensoriales del producto final. Simultáneamente, las tecnologías y condiciones de procesado también influyen en el perfil metabólico de ambas frutas. Por lo que es importante determinar cómo éstos factores intervienen en la variación de los compuestos biactivos y en las características sensoriales del producto.

Los objetivos de la presente Tesis fueron determinar en qué medida las diferentes tecnologías de procesado, congelación, tratamientos térmicos y procesado por alta presión, ensayadas a escala industrial y condiciones de almacenamiento, -20, 4 y 24 ºC durante doce meses, afectan a los (poli)fenoles, color y atributos sensoriales en los productos seleccionados de fresa y manzana. Así como, determinar cómo influyó el procesado de la fruta en el perfil metabólico e identificar posibles marcadores de procesado térmico que ayuden a evaluar el grado de procesado en estos productos. Todo esto con el fin de potencialmente facilitar el uso de condiciones de procesado y almacenamiento personalizadas para limitar la degradación de los (poli)fenoles manteniendo la mejor calidad y características organolépticas.

Como se mencionó anteriormente, en este trabajo fresa (Fragaria x ananassa) cv. Primoris y manzana (Malus domestica) cv. Golden Delicious provenientes de Huelva y Zaragoza

respectivamente, fueron procesadas mediante diferentes técnicas y condiciones industriales, y almacenadas a -20, 4 y 24 ºC por doce meses. Este trabajo incluye dos estudios, que analizan las técnicas de procesamiento y almacenamiento de la siguiente manera:

Estudio 1, fresas congeladas individualmente (IQF) y purés de fresa obtenidos por trituración en frío sin tratamiento térmico (NT); trituración en frío + tratamiento térmico suave (MT); trituración en caliente + tratamiento térmico estándar (ST); y tratamiento térmico estándar + concentración al vacío (VC). Las fresas IQF se obtuvieron retirando el cáliz de la fruta, seguida de pre-enfriamiento y congelación en dos etapas, primero a -16 ◦C/8 min y finalmente a -26 ◦C/ 8 min. Para obtener los purés de fresa, se utilizaron dos técnicas de extracción: trituración en frío y la trituración térmica estándar. El puré obtenido por trituración en frío y desaireación se dividió en dos partes: una sin tratamiento térmico (NT), y la otra se sometió a un tratamiento térmico suave (MT) a 90 ◦C/ 30 seg. Mientras que para obtener el puré por trituración térmica (ST), las fresas enteras se precalentaron (92 ◦C/ 4 min) y después se trituraron, posteriormente, el puré caliente se sometió a una desaireación térmica (92 ◦C/ 2 min) seguida de una pasteurización a 90 ◦C durante 30 s. Por último, para producir el puré concentrado al vacío, se utilizó como base el puré ST y se sometió a una concentración al vacío (VC) (0,3-0,4 Bar) a 83 ◦C durante 3,5 h.

Estudio 2, purés de manzana obtenidos mediante trituración en frío con inactivación enzimática + procesado por altas presiones (HPP); trituración en frío + tratamiento térmico suave (MT); y trituración en caliente + tratamiento térmico estándar (ST); purés de manzana reprocesados térmicamente (RP) (90 °C/11 min) de las muestras MT y ST (RP.MT y RP.ST). En el caso de la manzana, los purés fueron obtenidos el proceso de trituración en frío se inició con manzanas enteras, que se trituraron directamente sin temperatura, separando en esta fase la piel y las semillas. El puré obtenido tras la trituración se desaireó en frío y se desactivó térmicamente (92 ◦C/ 2 min). A continuación, el puré se dividió en dos partes: una se envasó y se trató con altas presiónes (HPP) (6 bares, 4 ◦C/ 1 min), mientras que la otra parte se desaireó en caliente y se pasteurizó (99 ◦C/ 1 min), obteniéndose un puré ligeramente tratado (MT). Para obtener el puré mediante tratamiento térmico estándar (ST), las manzanas se trocearon, se precalentaron a 92 ◦C durante 5 min y se trituraron en caliente, refinándose finalmente separando la piel y las semillas del puré. El puré obtenido se desaireó en caliente

y se pasteurizó (99 ◦C/ 1 min). Muestras de puré de manzana MT y ST almacenadas a 24 ◦C durante seis meses se reprocesaron con tratamiento térmico (90 ◦C/11 min), obteniéndose purés de manzana reprocesados (RP.MT y RP.ST).

Los purés de fresa y manzana procesados se envasaron asépticamente en bolsas asépticas de doble membrana de baja permeabilidad (<0,02 cc/m2/día) [membrana exterior, PE/Met PET/PE de 102 μm; membrana interior, CoEx PE/EVOH/PA/PE de 90 μm (ARAN Packaging, España)] y se almacenaron durante 12 meses a sus correspondientes temperaturas. Las fresas IQF y el puré de fresa NT se almacenaron únicamente a -20 °C, mientras que el puré de fresa VC se envasó en tarros de cristal sellados al vacío y se conservó a 24 °C. El puré de manzana HPP se conservó sólo a 4 °C. En el caso de los purés de manzana reprocesados, éstos se envasaron al vacío en botes de cristal y se conservaron a 24 °C, que es una norma común del mercado. Todas las muestras almacenadas se analizaron a los 2, 6 y 12 meses. Se analizaron tres réplicas de cada técnica de procesado y condición de almacenamiento. Para eliminar la humedad, todas las muestras se liofilizaron y se trituraron en polvo previamente para homogeneizar la muestra antes de la extracción.

Para lograr la separación, identificación y cuantificación de los diferentes compuestos (poli)fenólicos característicos además de los metabolitos de cada fruta, fresa y manzana, la extracción de las muestras se inició con 50 mg de muestra liofilizada del producto de fruta, las mismas que se extrajeron con 1 mL de metanol/agua/ácido acético (70:29:1, v/v) para la fresa y metanol/agua (70:30, v/v) para la manzana. Las muestras se homogeneizaron en un vórtex durante 1 minuto y se sometieron a sonicación durante 30 minutos a temperatura ambiente. Posteriormente, las muestras se centrifugaron durante 15 min a 20627g a 12 °C (Thermo Scientific™ Sorvall™ ST 16, Alemania). El sobrenadante resultante se filtró a través de un filtro de 0,22 μm. La extracción de proantocianidinas se realizó según Kennedy & Jones (2001), con algunas modificaciones. Se añadió 800 μL de una solución 0.1N HCl en MeOH conteniendo 5 g/L phloroglucinol y 10 g/L de ácido ascórbico a 0.8 g de muestra liofilizada en polvo. La mezcla se incubó a 50 °C durante 20 min con homogenización constante. Posteriormente, se añadió 1 mL de acetato sódico 40 mM para detener la reacción. Por último, las muestras se centrifugaron durante 10 min a 10000 rpm, y el sobrenadante se filtró a través de un filtro de PVDF de 0.22 μm. Todas las muestras se analizaron mediante

métodos y condiciones cromatográficos específicos detallados en el CAPITULO III. Además, se realizó una aproximación metabolómica no dirigida basada en UPLC-ESI-QTOF-MS para la identificación demarcadores de procesado.

Para determinar el cambio de color en las muestras, las muestras se midieron con un cromómetro CR-400. La medición se realizó en el sistema CIEL*a*b*. Los valores se utilizaron para determinar, Chroma, ◦Hue, y la diferencia de color total (ΔE). Por otra parte, el análisis sensorial de los purés se determinó utilizando una escala hedónica de 9 puntos. escala hedónica. Las puntuaciones oscilaron entre 1 y 9 (siendo "1″ de desagrado extremo, y "9" de agrado extremo). Los atributos evaluados fueron color, viscosidad, aroma, sabor y valoración global, que han sido reconocidos como factores que afectan a la aceptación sensorial en productos de fruta.

Para evaluar el efecto de las tecnologías de procesado y el almacenamiento sobre los compuestos fenolicos, los parámetros de color y los atributos sensoriales, se realizó un análisis de varianza (ANOVA) de una vía seguido de comparaciones mediante la prueba de Tukey con un nivel de confianza de 0,05. Además en las muestras almacenadas, se realizó un análisis de componentes principales (PCA) de forma independiente en las fresas y las manzanas para estudiar los patrones de agrupación de las muestras. Todos los análisis de datos se realizaron utilizando el software R (versión 4.0.2).

Mientras que para identificar los metabolitos afectados según el grado de procesamiento, los datos brutos generados por UPLC-ESI-QTOF-MS se pre-procesaron para construir la matriz de datos. Posteriormente, el tratamiento de los datos incluida la transformación logarítmica de los datos y el escalado de Pareto se realizó antes del análisis univariante (ANOVA) y multivariante. En cuanto al análisis multivariante, se realizaron análisis de componentes principales (PCA) y análisis discriminante de mínimos cuadrados parciales (PLS-DA). Finalmente, estándares auténticos, espectros MS/MS de iones se utilizaron para la confirmación de los metabolitos. Mientras que, las bases de datos Metlin y MassBank of Noth America (MoNA) se utilizaron para comprobar la identificación tentativa.

Los resultados alcanzados a partir de esta Tesis e incluidos en los CAPÍTULOS V, VI y VII concluyen que :

1. En las fresas, los tratamientos térmicos suaves y estándar mostraron patrones similares para la mayoría de los grupos fenólicos; un aumento de las proantocianidinas, ningún cambio en los conjugados del ácido elágico, y una disminución importante de los flavonoles y antocianinas. Sin embargo, el proceso de obtención del concentrado de fresa, la concentración al vacío, tuvo un efecto perjudicial sobre todos los compuestos. Por otra parte, las condiciones de almacenamiento tuvieron un mayor impacto perjudicial sobre los niveles de compuestos fenólicos, así como sobre el color y los atributos sensoriales que las técnicas de procesado.
2. En las manzanas, los tratamientos suaves y el procesado a alta presión tuvieron efectos similares en todos los grupos fenólicos, con aumentos en dihidrochalconas, hidroxicinamicos y proantocianidinas y disminuciones en flavonoles. Sin embargo, el tratamiento térmico estándar aumentó las concentraciones de dihidrochalconas e incluso de flavonoles. Por tanto, puede decirse que las técnicas de procesado inicial ejercieron una mayor influencia que las condiciones de almacenamiento.
3. En general, las proantocianidinas fueron el grupo fenólico mayoritario y el más estable durante el almacenamiento, mientras que las antocianinas fueron el grupo más afectado tanto por el procesado como por el almacenamiento.
4. En general, el almacenamiento a -20 y 4 ºC apenas afectó al nivel fenólico y las características de calidad durante el almacenamiento. Sin embargo, el almacenamiento a 24 ºC degradó mucho el contenido polifenólico y los atributos de calidad y sensoriales en fresa, aunque no tan drásticamente en los productos de manzana.
5. A través de un enfoque metabolómico no dirigido, se identificaron siete metabolitos y se propusieron como marcadores potencialmente potentes para evaluar el grado de procesado de los productos de puré de fresa y manzana.
6. El ácido piroglutámico, y el ácido 2-hidroxi- 5-metoxi benzoico fueron identificados como marcadores regulados al alza, mientras que el ácido pteroil- D-glutámico, y el ácido 2-hidroxibenzoico β-D-glucósido fueron identificados como marcadores regulados a la baja del procesado térmico en productos de fresa.
7. El glucurónido del ácido dihidroxicinámico, el ácido cafeico y el lysoPE(18:3(9Z,12Z,15Z)/0:0) se identificaron como marcadores regulados al alza,

mostrando una tendencia creciente correlacionada con el procesado térmico en productos de manzana.

8. La selección de la variedad adecuada, junto con la tecnología y las condiciones de procesado, deben ser adaptadas en consecuencia por las industrias, para preservar o incluso aumentar el contenido de (poli)fenoles en el producto final lo más cerca posible del de la fruta fresca.

9. El uso de estos marcadores podría ayudar a medir objetivamente el grado de procesado de los alimentos y a aclarar la controvertida narrativa sobre los alimentos ultraprocesados**.**

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Figure 1. Scheme of the different processing technologies conditions applied to strawberry and apple. IQF: Individual Quick Freezing; NT: non thermal treated; MT: mild thermal treated; ST: standard thermal treated; VC: Vacuum concentrated; RP.: re-processed; HPP: High pressure processed; * Removal of peel and seeds.

Table 1. Storage conditions applied to strawberry and apple purees obtained after different industrial processing technologies and stored for twelve months. IQF: Individual Quick Freezing; NT: non thermal treated; MT: mild thermal treated; ST: standard thermal treated; VC: Vacuum concentrated; HPP: High pressure processed; RP.: re-processed.

Figure 2. Principal Component Analysis (PCA) of strawberry samples (processed by IQF: Individual Quick Freezing; NT: No Heat Treatment; MT: Mild Treatment; ST: Standard Treatment; VC: Vacuum Concentration) after 2, 6 and 12 months of storage at -20, 4 and 24 ºC.

Figure 3. Principal Component Analysis (PCA) of apple samples (processed by MT: Mild Treatment; HPP: Cold extraction + HPP; and ST: Standard Treatment) after 2, 6 and 12 months of storage at -20, 4 and 24 ºC.

Figure 4. Strawberry purees. Mean values of total polyphenols and the phenolic groups proanthocyanidins, ellagitannins, anthocyanins. Strawberries industrially processed by: IQF: Individual Quick Freezing; NT: No Heat Treatment; MT: Mild thermal Treatment; ST: Standard Treatment; VC: Vacuum concentration. Samples collected just after processing (AP), and after 2, 6 and 12 months of storage at -20 ºC (1), 4 ºC (2) and 24 ºC (3). Different letters within the same phenolic group and storage temperature are significantly different at $p<0.05$.

Figure 5. Apple purees. Mean values of total polyphenols and the phenolic groups, hydroxycinnamic acids, proanthocyanidins, dihydrochalcones, flavonols. Apples industrially processed by MT: mild treatment; ST: standard treatment; HPP: high pressure processing; RP.MT: mild treatment + re-processing; RP.ST: standard treatment + re-processing. Samples collected just after processing (AP) and after 2, 6 and 12 months of storage at -20 ºC (1), 4 ºC (2) and 24 ºC (3). Different letters within the same phenolic group and storage temperature are significantly different at $p<0.05$.

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Figure 1. PCA and PLS-DA model plots. Strawberry: (a) PCA plot, (b) PLS-DA plot. Apple: (c) PCA plot, (d) PLS-DA plot. Strawberry samples: Fresh strawberries (FS), No heat treatment (NT), mild heat treatment (MT), standard thermal treatment (ST), vacuum concentration (VC). Apple samples: Fresh apple (FA), high-pressure processing (HPP), mild heat treatment (MT), standard thermal treatment (ST), reprocessed mild heat treatment (RP.MT), reprocessed standard thermal treatment (RP.ST).

Table 1. Metabolites identified and confirmed by MS/MS in strawberry and apple samples.

m/z.

Figure 2. Bar plot of the metabolites identified in strawberry puree across the processing sample groups. X-axis: total abundance of the metabolite; Y-axis: treatment. (a) Pyroglutamic acid; (b) Pteroyl-D-glutamic acid; (c) 4-hydroxy-3-methoxy benzoic acid; (d) 2-hydroxybenzoic acid β-D- glucoside. Strawberry samples: Fresh strawberries (FS), No heat treatment (NT), mild heat treatment (MT), standard thermal treatment, vacuum concentration (VC).

Figure 3. Bar plot of the metabolites identified in apple puree across the processing sample groups. X-axis: total abundance of the metabolite; Y-axis: treatment; (a) hydroxycinnamic acid glucuronide; (b) Caffeic acid; (c) LysoPE(18:3(9Z,12Z,15Z)/0:0). Apple samples: Fresh apple (FA), High-pressure processing (HPP), mild heat treatment (MT), standard thermal treatment (ST), reprocessed mild heat treatment (RP.MT), reprocessed standard thermal treatment (RP.ST).

Figure 4. Diagram of pyroglutamic acid production.

Figure 5. Diagram of the industrial processing techniques employed to obtain strawberry (Fresh strawberries (FS), No heat treatment (NT), mild heat treatment (MT), standard thermal treatment, vacuum concentration (VC)) and apple (Fresh apple (FA), High-pressure processing (HPP), mild heat treatment (MT), standard thermal treatment (ST), reprocessed mild heat treatment (RP.MT), reprocessed standard thermal treatment (RP.ST)) purees.

CHAPTER I

Introduction

1.1. Phenolic compounds: chemistry, classification and occurrence

As Hippocrates quoted more than two thousand years ago, "*Let food be thy medicine and medicine be thy food*", surprisingly this statement applied since ancient times is now confirmed. After more than twenty years of extensive research it has been established that apart from the already known vitamins, mineral and dietary fiber found in fruits and vegetables there are other bioactive compounds, called phytochemicals, with potential effects on human health [1]. These phytochemicals belong to the plant secondary metabolites and include terpenoids, sulphur-containing compounds, nitrogen containing compounds and phenolic compounds. The phenolic compounds can be synthetized from two metabolic routes, the shikimic acid pathway, which results in phenylpropanoids, and the acetic acid pathway which can produce simple phenols, or the combination of both resulting in the formation of flavonoids [2,3]. Their functions in plants include from contributing to mechanical support, pigmentation, growth and development (as signaling agents), to act as defense agents against physiological and environmental damage, this last one being the main role for which they are highly synthesized [4,5].

Phenolic compounds are widely distributed in plants, with more than 8000 molecules reported [4]. Overall, phenolics are structured by at least one aromatic ring with one or more hydroxyl groups attached. Therefore, these can be found as single aromatic ring compounds with low molecular weight to complex molecules conjugated with sugars or organic acids [4,5]. According to their chemical structure, the number of phenols rings and the structural elements bind to these rings, (poly)phenols can be classified into four groups, flavonoids, phenolic acids, stilbenes and lignans [6,7] (**Figure 1**).

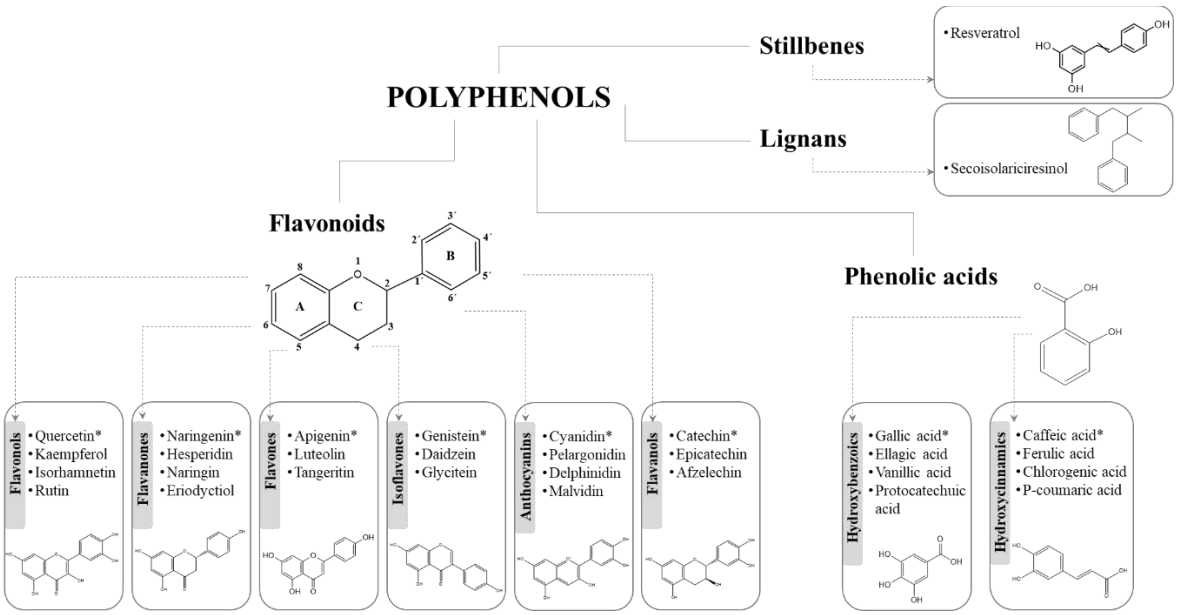

Figure 1. Phenols classes and chemical structures of their main compounds. * Most common representative unit of the phenolic group

1.1.1. Flavonoids. The basic flavonoid (C6-C3-C6) skeleton structure consist of two benzene rings (A and B) linked by an oxygenated heterocycle (ring C) with three carbon atoms. As a result of the variation on the degree and position of hydroxylation and the type of substituent in the heterocycle, these can be further classified in eleven groups. According to their contribution to food content the main subclasses of flavonoids are flavonols, flavanones, flavones, isoflavones, anthocyanins, and flavanols. However there are also some subgroups occurring in lower amounts, such as flavan-3,4-diols, coumarins, chalcones, dihydrochalcones and aurones [8–10]. Flavonoids are widely present in plants, particularly in the epidermis of leaves and the skin of fruits [11]. Their solubility is determined by the substituents they are attached to, which are mainly sugars. However these can also be bounded to hydroxyl and methyl groups, or other substituents such as isopentyl units [4].

Flavonols are the main flavonoids present in the plant kingdom, their production is activated by light. Their structure is characterized by a double bond linking C3-C4, a carbonyl group at the C4 and hydroxyl group at the C3 in the C-ring. The more representative are quercetin, kaempferol, and myricetin, which are predominately found as O-glycosides, however these can also be present as aglycones [4,12]. The main sources of flavanols are yellow onions, curly kale, broccoli, apples, tea, and cloves [8,9,11].

Flavanones, are non-planar molecules with a chiral center at C2. They are commonly glycosylated by a disaccharide (rutinoside or neohesperidoside) at C7 in the A-ring [4]. The conjugation with neohesperidoside [rhamnosyl(1-2)glucoside] exerts a bitter taste, whereas in the presence of rutinoside [rhamnosyl(1-6)glucoside] leads to tasteless flavanones [8]. These are found in high concentrations particularly in citrus fruits, such as grapefruit, oranges and lemons. The most commonly found being naringenin, hesperidin and eriodictyol [9,11].

Flavones share an interesting feature as is their lack of oxygenation at C3. These are represented by luteolin and apigenin, the majority come in the form of 7-O-glycosides [4]. Contrary to flavonols, these are not present in fruits. However, a significant amount has been found in parsley and celery, as well as, in green pepper, broccoli, olives, and some herbs (sage, thyme, oregano) although in lower quantities [9,11,13].

Isoflavones are characterized by having the position of the benzene B-ring attached at C3 [4]. The three main molecules are genistein, daidzein, and glycitein, which are found almost exclusively in legume plants, with the highest concentrations occurring in soy bean and its processed products [8,10].

Anthocyanins, formed by a flavylium cation backbone, are structured by an aglycone unit (anthocyanidin) with a glycoside [14]. The different types of anthocyanidins are determined by the glycosylation nature and position, the number of hydroxylated groups in the aglycone, and the position of these bonds [15]. The six most commonly anthocyanins found are pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin. These are predominantly present in fruits and flowers, but are also found in leaves, stems and roots, and responsible for the red-orange, to blue-violet colors [8,16].

Flavanols are the most structurally complex subclass of flavonoids, including from simple monomers, catechin and its isomer epicatechin, to complex structures called proanthocyanidins comprising dimers, trimers, and oligomers. The monomers are non-planar molecules, with two chiral centers at C2 and C3 of C-ring. These are usually attached to anhydroxyl group at C3, although they may also be esterified with gallic acid [8,17,18]. Unlike most flavonoids, flavanols rarely occur as glycosides. In general, the building blocks of proanthocyanidins are (epi)catechin, (epi)afzelechin and (epi)gallocatechin. Structurally,

for each additional monomer linked, there is an additional chiral center at C4 of C-ring [9,17]. They are called procyanidins when their monomeric are catechin, epicatechin or their gallic esters, propelargonidins when are formed by afzelechin or epiafzelechin and prodelphinidins when the basic units are gallocatechin or epigallocatechin [4,19]. According with the degree of polymerization, procyanidins can be identified as dimers (procyanidin A1-A2, procyanidin B1-B8), trimers (selligueain A and B, procyanidin C1-C2), tretramers and oligomers with a degree of polymerization from 5 to 11 [20]. The main source of flavanols is green tea, as well as cocoa derivatives and all types of fruits such as apples, grapes, blackberries [11,21]. An interesting fact of these compounds is that free catechins exhibit bitterness, and their gallates exert an astringent flavor through the formation of complexes with salivary proteins [21].

Dihydrochalcones are distinguished by having two benzene rings linked together by an open ring structure[4]. Although this is a minor group within the flavonoids, this group will be reviewed because it is an important and almost exclusive constituent of apples [22].

1.1.2. Phenolic acids. They are characterized by a carboxyl group attached to a benzene ring. As a result of the variation in degree and position of the carboxylic group on the aromatic ring or a side propanoic chain, phenolic acids can be subdivided into two groups, hydroxybenzoic acids and hydroxycinnamic acids [23,24]. These contribute to some important organoleptic characteristic of foods, such as color, flavor, astringency and harshness [25].

Hydroxybenzoic acids, are derived from benzoic acid and consisting of a seven-carbon backbone of C6-C1 structure. Naturally present either in their soluble form, conjugated with sugar or organic acids or in their bound form, attached to cell wall constituents as lignin [4,23,24]. The most representative are gallic acid, *p*-hydroxybenzoic acid, vanillic acid, and syringic acid. Gallic acid, a primary hydroxybenzoic acid, can also be part of complex sugar esters such as gallotannins and ellagitannins [8,26]. Although, in general hydroxyl benzoicacids are found in low amounts, these have been reported in red fruit, onions, herbs (oregano and thyme), wine, tea, spices (cloves) and chestnuts [9,24].

Hydroxycinnamic acids are derived from cinnamic acid and formed by a C6-C3 structure. Commonly found conjugated as esters with quinic acids or glucose, or as amides (conjugated

with mono- or polyamines, amino acids, or peptides) [4,23,24]. The most commonly found are caffeic acid, ferulic acid, sinapic acid, and *p*-coumaric acid [8,26]. While, caffeic acid is the most abundant phenolic acid in most fruits, ferulic acid is mainly found in cereal grains, such as wheat, rice, oat, and maize [8].

1.1.3. Stilbenes. These are formed by a C6-C2-C6 structure, consisting of two aromatic rings linked by an ethylene bridge [27,28]. The two rings can have substituents such as sugars, hydroxyl, and methoxyl groups in different positions [29]. Resveratrol and pterostilbene, in their *trans* and *cis* forms, are the most common stilbenes [27]. Their main dietary sources are grapes, berries (blueberries, bilberries, cranberries, mulberries,) almonds, peanuts, grapevine, and wine [27,30].

1.1.4. Lignans. These are structured by two phenyl propane units linked through an 8,8'-hydrogen linkage [31]. The most commonly found in plants are secoisolariciresinol and matairesinol. Dietary lignan compounds are mainly found in seed oils (flax, soy, rapeseed, and sesame) and nuts (sesame, sunflower, cashew), however these can also be found in smaller amounts in some vegetables (curly kale, broccoli, garlic) and fruits (apricot, strawberry, peach) [10,11].

The nature and distribution of the phenolic compounds in our diet depend of the food matrix. Overall, fruits and vegetables could contain up to 200–300 mg of (poly)phenols per 100 g of fresh weight, therefore they can be considered be a great source of bioactive compounds [3]. This Thesis was focused on strawberries and apples since these are two of the most industrially used fruits.

1.2. Phenolic compounds in apple and strawberry

As was observed in the previous section, (poly)phenols are widely spread through the plant kingdom, being found in a variety of fruits, vegetables, herbs, and spices. However, this Thesis was focused on apple (*Malus domestica*) and strawberry (*Fragaria x ananassa*)(poly)phenols as these are two of the fruits more consumed worldwide, both fresh and processed (juices, jams, purees, smoothies, dried), due to their desirable sensory characteristics, nutritional value (**Figure 2**) and bioactive compounds (**Table 1**).

Table 1. Concentration range of phenolic groups (mg/100 g FW) in different fresh strawberry ([S]) and apple ([A]) tissues. Q: quercetin; Cy: cyanidin; Pel: pelargonidin

	Phenolic sub-groups	Phenolic compounds						Ref.
			Flesh	Peel	Seeds	Flesh	Achenes	
Flavonoids	Flavonols	Q [A] Q-3-rutinoside [A] Q-3-galactoside [A] Q-3-glucoside [A] Q-3-xyloside [A] Q-3-arabinoside [A] Q-3-rhamnoside [A] Q-rhamno-glucoside [A] Q-arabinopyranoside [A] Q-arabinofuranoside [A] Q-3-glucuronide [S] Kaempferol 3-glucuronide [S] Kaempferol acetyl-glucoside [S] Isorhamnetin 3-O-glucuronide [S]	0.0 - 0.5 mg	34 - 52 mg	4.6 - 11.8 mg	1.3 - 9.7 mg	6.4 - 7.5 mg	[32–40]
	Anthocyanins	Cy 3-glucoside[S] Cy 3-glucoside-malonate[S] Pel 3-glucoside[S] Pel 3-rutinoside[S] Pel 3-acetylglucoside[S] Pel 3-glucoside-malonate[S]	-	-	-	3.7 - 64.9 mg	8.0 - 12.9 mg	[35–37]
	Flavanols	Catechin [A, S] Epicatechin [A, S] Afzelechin [S] Procyanidin B1, B3, C2 [S] Procyanidin B2 [A]	1.4 - 548.2 mg	41 - 171 mg	1.2 - 2.9 mg	53 – 168 mg	4.7 - 10.4 mg	[32–40]
	Dihydrochalcones	Phloretin-2-xyloglucoside [A] Phloridzin [A] 3-hydroxyphloridzin [A]	0.0 - 16.8 mg	10 - 36 mg	36 - 129 mg	-	-	[32–34,38–40]
Phenolic acids	Hydroxycinnamates	*p*-Coumaric acid glucoside [S] Ferulic acid [S] 1-O-*trans*-cinnamoyl-glucoside [S] Chlorogenic acid [A] Neochlorogenic acid [A] Cryptochlorogenic acid [A] Coumaroyl quinic acid [A] *p*-Coumaroylquinic acid [A] *p*-Coumaric acid [A]	0.7 - 14.3 mg	3.9 - 35.0 mg	2.4 - 5.1 mg	0.7 - 10.6 mg	1.6 - 1.7 mg	[32–40]
	Hydroxybenzoics	Ellagic acid [S] Ellagic acid rhamnoside [S] Ellagic acid pentoside [S] Ellagic acid deoxyhexoside [S]	-	-	-	0.9 - 14.8 mg	11.2 - 11.8 mg	[35–37]
		Galloyl bis-HHDP glucoside [S] Sanguiin-H6 [S] Lambertianin C [S] Agrimoniin [S]	-	-	-	7.2 - 28.9 mg	56.8 - 66.9 mg	[35–37]
		Protocatechuic acid [A]	-	-	0.4 - 1.4 mg			[40]

Nutrition Facts

Apple

Serving size	100 g
Calories	59 kcal
Total fat	0.14 g
Sodium	<1 mg
Total Carbohydrates	13.2 g
Dietary Fiber	2.5 g
Total Sugars	10.6 g
Protein	0.27 g
Calcium	5 mg
Iron	0.07 mg
Magnesium	5.1 mg
Phosporus	10 mg
Potassium	116 mg

Nutrition Facts

Strawberry

Serving size	100 g
Calories	35 kcal
Total fat	0.22 g
Sodium	10 mg
Total Carbohydrates	7.13 g
Dietary Fiber	1.8 g
Total Sugars	5.34 g
Protein	0.64 g
Calcium	12 mg
Iron	0.28 mg
Phosporus	20 mg
Potassium	89 mg
Vitamin C	56 mg
Vitamin A	1 µg

Figure 2. Nutritional facts of raw apple and strawberry according to the USDA

Although, apples and strawberries belong to the Rosaceae Family [41], there is a considerable variability among the phenolic compounds found in both fruits, which depends firstly on the matrix, the part of the fruit (e.g., flesh, peel, achenes) as is shown in **Table 1** and the inherent fruit characteristics (e.g., origin, variety, ripeness degree).

Apple fruits are formed by a number of structures characterized for different tissues including epidermis (peel), cortex (flesh) and an ovary core containing seeds (**Figure 3A**) [42,43]. There are differences in the phenolic compounds derived from their structures, flesh, peel, and seeds. In apple flesh, the major phenols are proanthocyanidins (F3OLs/PACs) and hydroxycinnamic acids (HCAs), being the latter mainly represented by chlorogenic acid. The principal (poly)phenols in apple peel are F3OLs/PACs having even higher concentrations than flesh, followed by flavonols (FOLs). In contrast, apple seeds are a more significant source of dihydrochalcones (DHCs) [38–40,44]. More than 150 apple cultivars have been reported from all over the world, but mainly from China and the United States. The most commercialized cultivars include "Golden Delicious", "Red Delicious", "Jonagold", "Royal Gala", "Fuji", "Granny Smith" and "Idared". However, there is a wide variety of older traditional cultivars produced locally in some countries [39,45]. In 2020, worldwide production of apple was 86.4 million metric tons, being the leading producers, in descending order, China, United States of America, and Turkey [46].

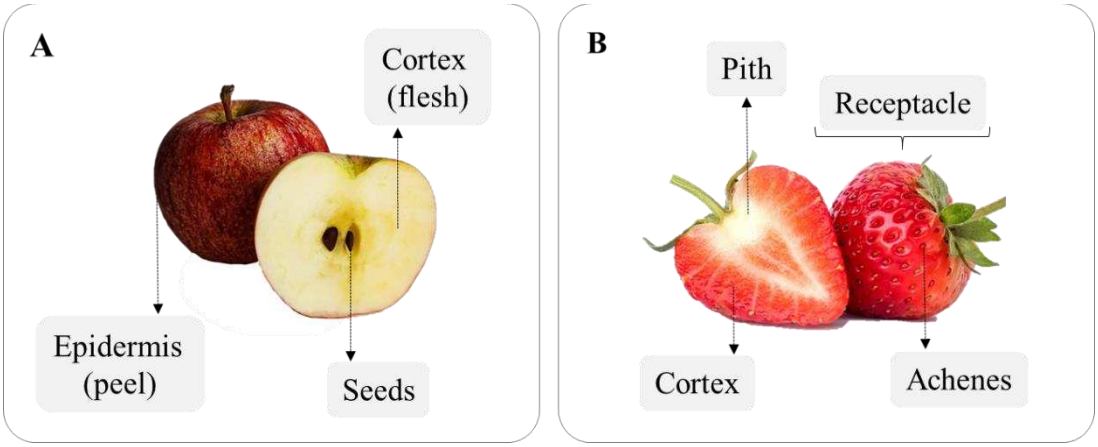

Figure 3. Apple and strawberry structures

In the case of strawberries, this is characterized by a fleshy receptacle consisting on an internal pith, a cortex layer and an epidermal layer where the achenes (commonly known as seeds) are embedded, the latter being the true fruit in this species (**Figure 3B**) [47]. The flesh and achenes have a different (poly)phenol profile (**Table 1**). In the flesh, the major phenols are flavan-3-ols/proanthocyanidins (F3OLs/PACs) and anthocyanins (ATs), while in achenes, the highest concentrations are for ellagitannins (ETs) and ellagic acid (EA) [35–37]. ATs one of the main phenolics in strawberries play an important role on their red bright color, contributing to their organoleptic characteristics. More than 100 strawberry cultivars have been reported so far in studies regarding their nutritional value and polyphenols content [36,48]. Since strawberries are sensitive plants to environmental conditions, the varieties cultivated highly depend on the location. In Spain, the commercial cultivars grown are mainly, "Candonga", "Fortuna", "Primoris", "Sabrina", and "Splendor" [49]. In 2020, the strawberry production worldwide was 8.9 million metric tons. Being the leading producers, in descending order, China, United States of America, and Egypt [46].

1.3. Analysis of phenolic compounds

Since (poly)phenols are abundantly present in fruits, vegetables and grains, and considering their potential health effects, the research on the analytical methodologies used for their identification and quantification has been increasing. (Poly)phenols are an heterogeneous group of plant secondary metabolites that can be found both as simple molecules and as complex condensed polymeric forms, therefore there is not a standard

protocol for their analysis [50]. However, there are some essential steps (extraction, analytical separation and identification) for the determination of (poly)phenols in plants or foodstuff that can be customized according to the required outcome [51]. **Figure 4**Error! Reference source not found. shows a flow diagram with the sequence of steps involved in the determination of phenolic compounds.

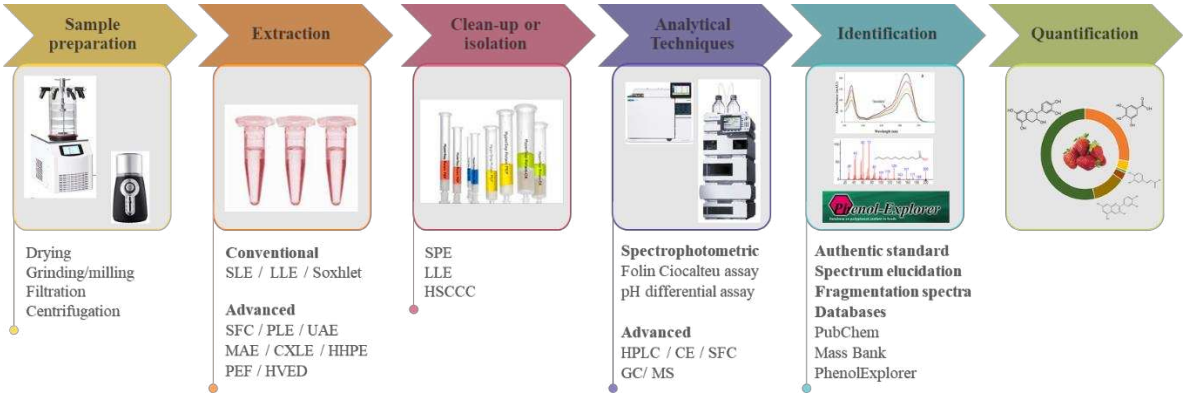

Figure 4. Process flow diagram for the analysis and determination of phenolic compounds in plants and foods samples. **CE**: capillary electrophoresis; **CXLE**: carbon dioxide-expanded liquid extraction; **GC**: Gas chromatography; **HHPE**: high hydrostatic pressure extraction; **HPLC**: high performance liquid chromatography; **HSCCC**: high-speed countercurrent chromatography; **HVED**: high voltage electrical discharge; **LLE**; liquid-liquid extraction; **MAE**: microwave-assisted extraction; **MS**: mass spectrometry; **PC**: plane chromatography; **PEF**: pulse electric field; **PLE**: pressurized liquid extraction; **SFC**: supercritical fluid chromatography; **SLE**: solid-liquid extraction; **SPE**: solid phase extraction; **UAE**: ultrasound assisted extraction.

To start the process of (poly)phenol determination, a pretreatment is required whose selection depends on the sample matrix. In the case of liquid samples, the pretreatment may be centrifugation, filtration or dilution. For solid samples, the pretreatment starts with drying, followed by grinding or crushing and homogenization [52]. The pretreatments reduce the particle size of the solid samples increasing the surface contact between the sample and the extraction solvent, all this in order to improve the extraction yield [53]. Due to the wide variety of chemical structures of (poly)phenols, their polarity, relative amount and the complex interaction in the matrix, the extraction has to be individually designed according to the sample. There are some conventional extraction techniques such as solid-liquid extraction (SLE) and liquid-liquid extraction (LLE). Overall, these techniques may employ a mix of water, methanol, ethanol, or acetic acid in different ratios as solvents [53,54]. Once the solvent is combined with the sample, the extraction process may involve stirring, heating and refluxing at different temperatures and extraction times. Although the conventional

extraction techniques are the most utilized for research, there are new advanced techniques that have been developed to minimize wastes, extraction times, improve specificity and reproducibility [55,56].

There are other more advanced techniques, such as high performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE), supercritical fluid chromatography (SFC)coupled with different detector (UV-Vis, florescence and/or mass spectrometry (MS)), that can be employed for both qualitative and quantitative analysis, providing more specific and detailed information about the individual phenolic compounds [23,57,58]. Subsequently, in order to identify the phenolic compounds, the data can be compared with authentic standards, bibliography or with public databases of phenolic compounds such as Mass Bank (https://massbank.eu/), Pubchem (https://pubchem.ncbi.nlm.nih.gov/), Phenol-Explorer (http://phenol-explorer.eu/) among others. The quantification of different compounds is usually done using calibration curves of the commercial authentic standards. However in the absence of authentic standards, the calibration curve for quantification can be performed using compounds corresponding to the same family or with similar characteristics.

1.4. Phenolic compounds and health

Phenolic compounds are also responsible for some of the organoleptic properties (color, aroma, astringency, bitterness) of apples and strawberries. However, they are mainly known for their beneficial effects on nutrition and human health (antioxidants, anti-inflammatory, gut microbiota modulators, etc.) [15,20]. (Poly)phenols are probably the most investigated compounds of functional interest. Among them, quercetin, catechin, kaempferol, resveratrol, apigenin and luteolin appear to be the most extensively investigated [3]. There is a growing body of literature supporting the beneficial health effects of (poly)phenols, which are mainly based on pre-clinical, epidemiological and observational studies. [59]. These compounds are frequently known for their antioxidant and anti-inflammatory effects [60]. Furthermore, as is shown in **Figure 5**, several studies have recognized that these compounds also prevent or protect against a variety of health problems and chronic diseases [17,61–63]. However, due to their different structure, not all phenolics compounds act in the same way against specific conditions.

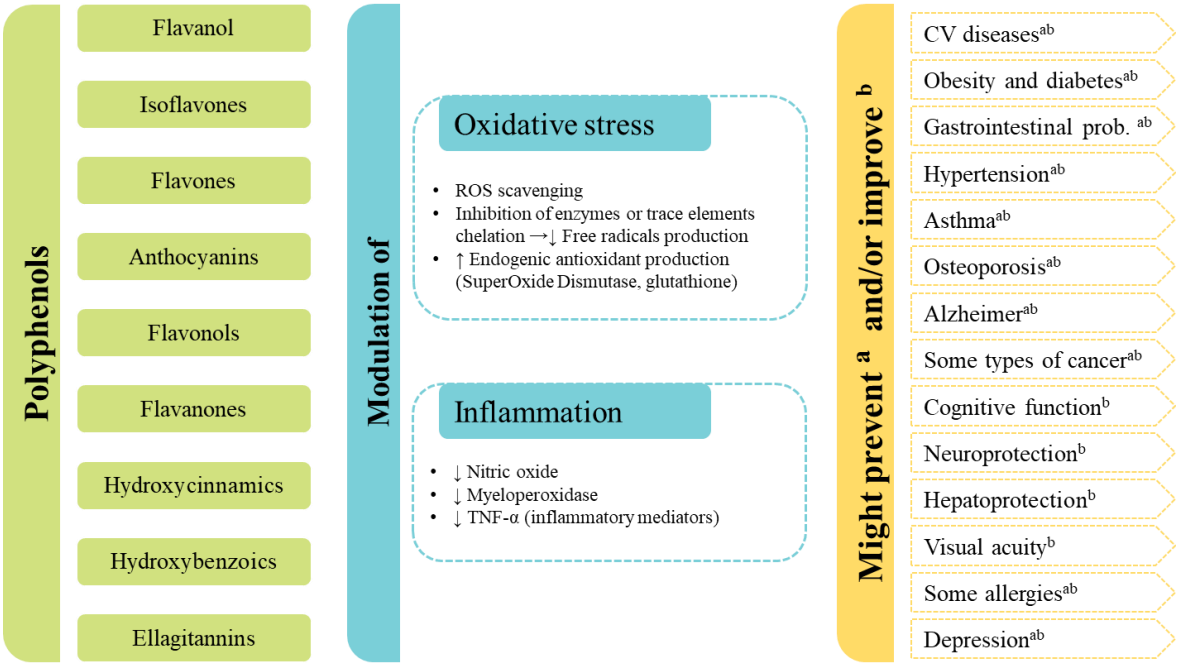

Figure 5. Modulation and action of (poly)phenols on health

The previously mentioned beneficial effects depend on the type of molecule, their structure (aglycone or conjugated form), the interactions between phenolic compounds, the quantity consumed and their bioavailability. Overall, most of the phenolic compounds (e.g. flavonoids, phenolic acids, ellagitannins) exhibit remarkable antioxidant and anticancer properties either as prevention or as support of oncological treatment [19,64–67]. Nevertheless, depending on the phenolic compound they act by targeting different cellular and molecular pathways in different parts of the human body. For instance, quercetin has been proven as anticancer molecule in various types of cancer, such as lymphoma, leukemia, endometrial, cervical, ovarian, breast, oral, pancreatic, gastric, liver, colon, urinary bladder, renal, esophageal and prostate cancer [68]. In addition to those already described, other therapeutic effects, such as bone protection, depression adjuvant and skin protector, have been attributed to some phenolic compounds [69–71].

As have been already stated, the health promoting effects of these compounds depend on their bioavailability, which refers to the fraction finally absorbed in the target tissue or cells where exerts the positive effect [72]. Normally, (poly)phenols are present in the diet as esters, glycosides or polymeric forms are not absorbed directly in the small intestine but they are hydrolyzed by enzymatic reactions before absorption. The absorption of the aglycone in

the small intestine is faster due to the lower molecular weight and higher lipophilicity [60,73]. Once absorbed (poly)phenols are conjugated mainly as glucuronides and sulfates in the small intestine and later in the liver. (Poly)phenols not absorbed in the small intestine, reach the colon where they are further metabolized by the intestinal bacteria [67].

Although, evidence suggest that numerous individual phenolic compounds exert significant positive effects on the prevention of diseases, the mixture of compounds may have either a cumulative or synergistic positive effects compared to a single compound [74]. Since fruits and vegetables are a mixture of various phenolic compounds, their intake boosts the positive effects of these molecules.

Despite the substantial evidence on the potential role of (poly)phenols in the prevention of diseases, there is still insufficient data on their consumption to suggest optimal intake and specific dietary recommendations for these compounds. Although no international organization has published dietary guidelines on the recommended intake, the Chinese Nutrition Society has proposed a specific intake level of 50 mg per day for anthocyanins [16,75]. In addition, and mostly driven by the need to provide essential nutrients but also taking into account the contribution of bioactive compounds, the World Health Organization and the Food Agriculture Organization of the United Nations recommend to consume at least 400 g or 5 portions per day of fruits and vegetables [76]. Additionally, some European countries have developed specific recommendations of fruit intake, in which it is advised to consume 2 to 5 portions per day. However, there is no clear consensus about the portion size. Depending on the country, one portion represents 80 to 160 grams of fresh fruit, 100 to 150 mL of fruit juice, 45 to 100 g of fruit sauce, 20 to 30 g of dried fruits [77].

Overall, the total concentration of phenolic compounds in the human diet might vary significantly depending not only of the food matrix but also and more importantly on intrinsic and extrinsic factors [78,79]. Therefore, several factors can change (poly)phenol levels in foods and their final bioavailability in the human body [3].

1.5. Factors influencing (poly)phenols levels

The phenolic compounds naturally occurring in fruits are highly influenced by intrinsic (such as, plant genetics, cultivar, and maturity state) and extrinsic (such as,

geographic location, climate, soil composition, light hours, as well as, subsequent processing and storage conditions) factors [80,81].

Intrinsic factors. The levels and variety of phenolic compounds present in fruits depend initially on the interspecies variation and then within the same species (fruit) the differences are related at first to plant genetics and/or cultivar [78,82]. For instance, **Table 1** shows the inter- and intra- species variations on phenolic levels of apples and strawberries. It can be seen that some phenolic groups are exclusive of some fruits, as dihydrochalcones that are only present in apples or the anthocyanin pelargonidin 3-glucoside which is almost only present in strawberries. Besides, differences on phenolic levels were found between varieties of the same fruit, while some contain large amounts of phenolics others are very poor in them. Another intrinsic factor influencing the levels of phenolic compounds is the maturity stage of the fruit [83]. In the case of strawberry, significant differences were reported for anthocyanins when comparing nearly ripe, ripe and fully ripe fruits, reporting much higher concentrations in fully ripe fruits [48]. In conclusion, the phenolic levels of fruits rely quantitatively and qualitatively on their genetic/cultivar information and maturity stage at harvest.

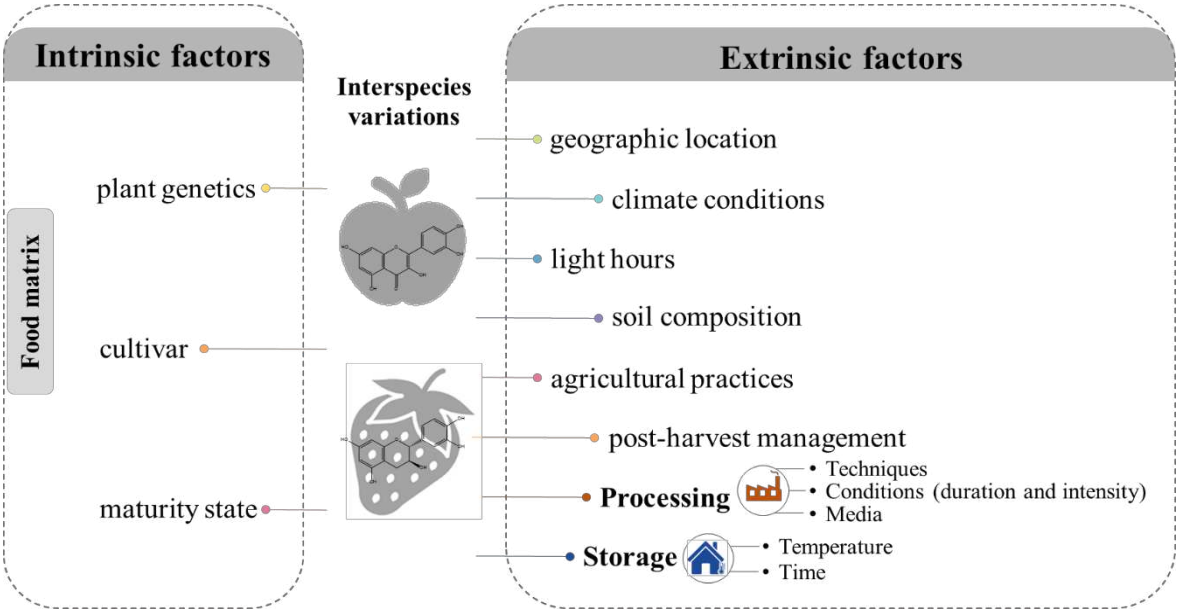

Figure 6. Intrinsic and extrinsic factors influencing phenolics compounds

Extrinsic factors. The biosynthetic pathway of (poly)phenols during plant growth and development determine the concentrations and diversity of these compounds in fruits,

which might be influenced by the geographic location, climate conditions, light hours, soil composition and agricultural practices at which the plants are exposed to [80,81,84]. Numerous studies have published differences in (poly)phenol levels as a result of these extrinsic factors. As the one addressed by Ghorbal et al. [85] where geographical origin, environmental factors and harvest time influenced quantitatively the levels of individual compounds in olive fruits. At the same time, it is noteworthy that not all the compounds are affected in the same manner. For example, in cloudberries, the anthocyanins where highly affected by the climatic conditions resulting in lower levels at high temperatures, whereas the content of ellagic acid showed little response to the temperature change [86].

In addition to the above mentioned extrinsic factors, there are other procedures influencing the stability of phenolic compounds in a greater extent, such as the post-harvest management, and the processing and storage conditions at which the fruits are subjected to extend their shelf life [82,87,88]. Postharvest management conditions include practices like transportation, storage temperature, relative humidity and packaging, which are necessary to reach either the final consumer or the processing center. The control of these postharvest management conditions could impact positively or negatively the levels of phenolic compounds [80,89]. As reported by Arendse et al. [89] in the case of pomegranate fruits, the temperature and storage time influenced the levels of anthocyanins, showing an increase at the beginning of the storage until the third month regardless of temperature. However, a progressive diminution was observed from the third to the fifth month, being more pronounced at 10 ºC.

Finally, two of the factors affecting the most the phenolic levels and on which this Thesis was focused are processing and storage conditions. In order to extend their shelf life, improve sensory characteristics, ensure food safety and offer new options to consumers, fruits are processed into a wide variety of products, such as, dried fruits, purees, juices, jams, baby food, etc. However, the processing techniques needed to obtain these products and the storage conditions applied to the final product, might impact positively or negatively to the phenolic compounds levels [79,88,90]. The next section will give an overview of the food processing techniques commonly used in industry and will intent to analyze how and to what extent these processing techniques could influence the (poly)phenols levels.

1.6. Food processing techniques and their impact on phenolics

Food processing can be defined as any action that substantially alters the raw agricultural products, including initial operations that remove a part of the product such as peeling, pitting or milling, and blanching, as well as operations of heating, smoking, crushing, curing, maturing, drying, marinating, extraction, extrusion or a combination of these processes in order to preserve foods [91,92]. As a result of processing, the structure of food is altered both macro and microstructurally, producing physical, chemical and organoleptic changes [91]. Although, food processing itself barely affect macronutrients (carbohydrates, sugars, and lipids) levels, it can modify the microstructure and distribution of these particles giving as a result new textures, which in some cases will influence their bioavailability and the glycaemic index [93,94]. The alterations in the structure depend on the unit operation or the combinations of processes applied. For example, in the case of peeling, as the peel is a rich source of fibre and bioactives, these will be lost as a result of this unit operation [95]. Another unit operation part of food processing include the use of preservatives and additives, such as sugar, salt and fat, when these are overused the processed food become a high energy dense source that can have negative impact on health [91,96]. Therefore, in order to deliver a nutritious food product, the unit operations during processing and the additives employed have to be carefully chosen.

On the other hand, food processing has a higher and more significant impact on micronutrients, such as vitamins, and phenolic compounds levels. In the case of phenolic compounds, food processing suspend their biosynthesis by cell structure degradation and/or the change of some enzymes such as polyphenol oxidase and peroxidase [80]. In addition, it can also influence the release, transformation, and interactions of these compounds from plant tissue, as well as improve or inhibit their absorption in the gastrointestinal tract [97,98]. For instance, the mechanical disruption provoked by crushing led to the release of phenols from the cell walls, but also of some enzymes that in the presence of oxygen promote oxidation, formation of brown polymers, discoloration and polyphenols degradation [99].

The need of food processing responds to the demand of feeding the increasing world population with nutritious, safe and palatable products throughout the year [79,100]. As many of the fruits are seasonal, the food industry has to ensure their supply. In order to have

a reserve and achieve a proper supply, fruits are processed seasonally, into IQF fruit, purees or concentrates, which eventually will be considered as raw materials and re-processed to obtain the final product. However, depending on the country location and the type of fruit, in some cases raw fruits are directly used to produce the final product. This variation between processing in one step or the combination of processing and re-processing might also have an influence in the final phenolics concentration,

A wide variety of well-established industrial processing technologies are available for food preservation. These are mainly categorized as thermal and non-thermal treatments.

1.6.1. Thermal processing treatments

The application of heat is the most widely and commonly used food preservation technique mainly due to their capacity to deliver a safe, microbiologically stable product with an extended shelf life [79,101]. Nowadays, thermal treatments include conventional techniques such as, drying, pasteurization, sterilization, ultra-high temperature treatment and new thermal treatments such as, ohmic heating and dielectric heating (radiofrequency and microwave) [87,102].

Thermal treatments are basically the transfer of heat to a food product, liquid (e.g. juices, milk, soups), semi-liquid (e.g. sauces, purees, jams) or solid food, at a specific temperature for a certain time trough convection, conduction, radiation, or a combination of them [103,104]. In the case of liquid and semi-liquid products, thermal treatments may be differentiated according to the intensity of heat treatment, into pasteurization (70-80 ºC), sterilization (110-120 ºC) and ultra-high temperature treatment (140-160 ºC) [100]. Industries have developed batch and continuous processing approaches. The most commonly used is the continuous heat processing, which consist in pumping the food product (liquid or semi-liquid) through a system (aseptic, hot filling, or pasteurization process) that will heat it and then cool it down, while is continuously flowing. The heating media in a continuous flow processing could be direct or indirect. Direct heating could be steam injection or steam infusion, whereas indirect heating may be performed through, a plate heat exchanger, tubular exchanger and scraped surface heat exchanger [105]. Regarding solid fruit products, the thermal technique frequently employed is drying. It is an ancient technique used to preserve food, that can be defined as the process by which free and bounded water is removed from

the food usually by mean of heat, with the purpose of reducing their volume and weight, extending its shelf life, ensuring food quality and safety [106]. Based on the drying medium, the techniques used can be convective drying, freeze-drying and osmotic drying.

Although, the application of heat may induce numerous physicochemical changes producing some adverse effects, such as loss of heat sensitive nutrients (e.g. vitamins) and some bioactive compounds, formation of undesirable compounds (e.g. acrylamide, heterocyclic amines and sulphur compounds), and in some cases the degradation of organoleptic characteristics [79,107,108] it also exerts many beneficial effects such as, inactivation of foodborne pathogens, inactivation of toxins and enzymes, polyphenol oxidase, peroxidase, pectin-methyl esterase, improvement of extraction, digestibility and bioavailability of nutrients and bioactive compounds, enhancement of some sensory characteristics (texture and flavor) and extension of shelf life [79,109].

1.6.2. Non-thermal processing treatments

The development of non-thermal techniques was an answer to the customer requirements of healthy, convenient and minimally processed, closer to fresh products [110]. Non-thermal treatments emerged in order to avoid high temperatures during processing and limit the adverse effects of heat on the nutritional and functional properties of the final product. Although these techniques do not involve thermal treatment, as a result of the processing some structural changes are experienced, such as protein alteration or lipid oxidation, giving as a result new textures, changes in sensory properties, improvement of digestibility, water-binding ability, and gelation processes [111]. Non-thermal treatments include high-pressure processing, dehydration, freezing and pulsed electric field, cold plasma, irradiation, ultra sound processing, magnetic field, and ozone [87,102].

One of the most studied non-thermal treatment is High Pressure Processing (HPP). The first attempt to use pressure as a preservation method was initiated around the 1980s, with the aim of preventing milk from souring and extending its shelf life. Subsequent trials concluded that better results were obtained when combining pressure and temperature. These promising results opened up new possibilities for the use of pressure on fruit juices and meat [112]. The first to promote the development of this technology on an industrial scale was the Japanese government in collaboration with some Japanese research centers and companies

[113]. However, its industrial application did not come out until 1997, where the first HPP machine commercially designed for food products was produced in USA, and was designed to deactivate enzymes from guacamole in batches [114]. Nowadays, this technology is used in mainly in liquid (juices) and semi-liquid (purees) products and its principal benefit is that it can be directly applied in the final package, minimizing any post process contamination from the environment [115].

The essential components of an HPP system are the pressure chamber, the pressurizing fluid, and the pressure intensifier pump. In general, the HPP process starts by loading the product that was previously packed in flexible containers into a basket which enters into a pressure chamber. This chamber is then filled with fluid, usually water, and closed. Once it is closed, high pressure is applied to the fluid through pressure intensifier pumps, the pressure transmitted to the fluid is uniformly transmitted to the product for a certain time (seconds or minutes), depending on the type of food. Finally, the pressure is released from the chamber until it reaches atmospheric pressure again [114,116,117]. The more important factors to consider during the process in order to ensure safety and quality in the final product are; pressure, compression time, temperature and decompression time. However, it is also important to considerate the product characteristics such as pH and water activity [118,119]. HPP conditions commonly used at industrial level are between 300 to 600 MPa for 3-5 minutes [116,120].

As a consequence of pressure, there may be some changes in physical properties (solubility, density, viscosity), kinetic reactions (acceleration or delay of reactions rate), as well as, some equilibrium processes (dissociation of weak acids, acid-base equilibria, and ionization) [121]. The effect of pressure on food components depends on the type of bond between their molecules, as well as, the interatomic distance. In general, compounds with covalent bonds (vitamins, minerals, folates, antioxidants, anthocyanins and flavor compounds), are minimally affected by the pressure [102,121,122]. A disadvantage of HPP is that it is less effective in enzyme inactivation than the thermal treatments. Therefore, it triggers oxidation reactions that lead to degradation of phenolic compounds and short shelf life [123,124].

Besides, the positive and negative effects of thermal treatment and HPP on the stability of phenolic compounds from strawberry and apple products have been recently reviewed by Salazar-Orbea [99]. Numerous studies report (poly)phenols thermal degradations considering heat as the main detrimental factor affecting the (poly)phenols levels [125–130]. However, this review revealed that no general conclusion can be drawn about the effect of heat treatment on (poly)phenols, since in addition to the processing conditions, the effect of processing depends on the matrix, the unit operations during processing, and the type of (poly)phenols and their susceptibility [99]. For instance, some phenolic groups such as anthocyanins are definitely affected by thermal treatments. Conversely, thermal treatments influenced positively the levels of other phenolic groups such as flavanols, ellagic acid, dihydrochalcones and flavonols enhancing their extraction from cell tissues of structures as achenes in strawberries and peel and seeds in apples [99].

In order to retain the bioactive compounds of processed foods and their health beneficial properties, food industries should therefore pay further attention at every factor influencing the final phenolic levels, starting by select the appropriate variety, control the agronomical and post-harvest conditions, as well as, select and optimize the processing technology and their relevant unit operations and the storage conditions.

1.7. Metabolomics as a tool to evaluate food processing

Metabolomics is an omic science that study global metabolite profiles found in tissues or cells at a given time as a result of a particular physiological or environmental influence [131]. These metabolites are characterized by being low molecular weight molecules (<1500 Da) that occur at a wide range of concentrations from femtomolar to molar levels [132]. Metabolomics is a sensitive analytical technique widely used in a variety of fields, such as environmental sciences, medicine, agriculture and food sciences among others [133,134]. Since the scope of metabolomics research is quite broad, this section will focus on assessing its use in food science, specifically on how it can be used as a tool for evaluating food processing. Nevertheless, it is also employed in food safety, quality and authentication research [135].

Regarding food processing, metabolomics is an analytical technique that can help us to identify and whenever possible, quantify metabolites associated to chemical changes or

metabolic reactions derived of a particular treatment [135]. This will allow us the identification of processing markers. Depending on their origin, these metabolites can be endogenous or exogenous. Endogenous metabolites are those naturally derived from biosynthesis processes within the food matrix such as carbohydrates, amino acids, lipids, vitamins, (poly)phenols and alkaloids, while exogenous metabolites come from external sources such as food additives, pesticides and toxins [131].

Two different approaches can be followed: targeted and untargeted metabolomics. The targeted metabolomic is based on the analysis of pre-selected known compounds of which authentic standards are available to enable their quantification [136]. On the other hand, the untargeted metabolomic approach is an exploratory technique to detect global and unexpected changes in the metabolome and involves the detection of a more extensive range of analytes [132,137]. Untargeted metabolomic analysis comprises a set of steps that can be divided into two stages, analytical techniques and data analysis (**Figure 7**).

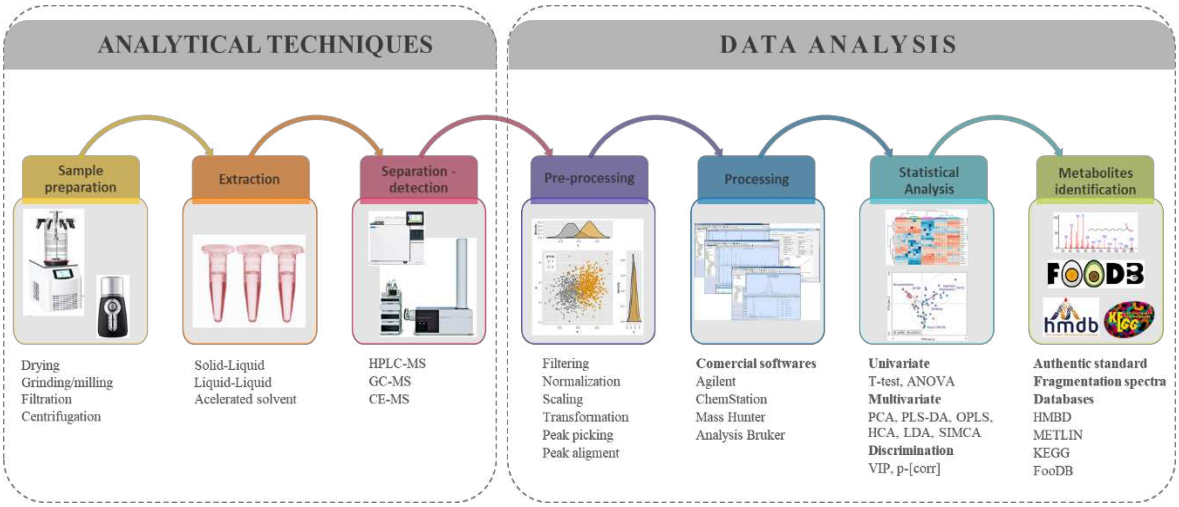

Figure 7. Flow diagram for the untargeted metabolomic analysis **NMR**: Nuclear Magnetic Resonance; **HRMS**: High Resolution Mass Spectrometry; HP**LC**: High Performance Liquid Chromatography; **GC**: Gas Chromatography; **CE**: Capillarity. Electrophoresis, **MS**: coupled with mass spectrometry.

The analytical techniques stage comprises, first, sample preparation and extraction that are selected depending on the type of metabolites that we want to analyze, trying to extract as many compounds as possible.[131]. Then the sample can be analyzed using different techniques mainly based on a separative technique such as Capillary Electrophoresis (CE), Liquid Chromatography (LC), and Gas Chromatography (GC) usually

coupled with Mass Spectrometry MS). Direct analysis by Nuclear Magnetic Resonance (NMR) and High Resolution Mass Spectrometry (HRMS) can be also used [131,132,136].

Since in general metabolomics generate large complex data sets, a crucial step is their analysis. The first approach in order to optimize the analysis is to pre-process these data through commercial software that allow peak definition and alignment, to subsequently filtering, normalizing and scaling the data [132]. In order to extract relevant information from the previously pre-processed data and correlate it with the biological question, further statistical univariate and multivariate (unsupervised and supervised) analysis are needed. In the univariate analysis (T-test, ANOVA) the variables are analyzed separately and can be used to determine significant differences between metabolites of different samples, narrowing down the data for the multivariate analysis [137]. Whereas multivariate analysis, allows to evaluate more than two variables at the same time and draw correlation between them. The first general approach of multivariate analysis is exploratory aiming to visualize trends, grouping and/or outliers, and can be done through unsupervised analysis (Principal Component Analysis (PCA), Hierarchical Clustering Analysis (HCA). On the other hand, supervised multivariate analysis (Hierarchical Linear Discriminant Analysis (HLDA), Partial Least Square-Discriminant Analysis (PLS-DA), Orthogonal Projections to Latent Structures (OPLT), Soft Independent Modeling of Class Analogy (SIMCA)) facilitate the classification or discrimination of groups of metabolites responsible for samples differentiation [131,136,138]. In the case of untargeted metabolomics, the subsequent step is the identification of the metabolites, which can be accomplished through: i) authentic standards and subsequent mass analysis; ii) comparison of similar fragmentation spectra with omics databases such as Human Metabolome Database (HMDB) (https://hmdb.ca), Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.genome.jp/kegg), Food Component Database (FooDB) (https://fdc.nal.usda.gov), METLIN repository (https://metlin.scripps.edu) among others; and iii) comparison of similar fragmentation spectra to already known compounds [131,137]. Despite of the information available to identify metabolites, it is still a labored and challenging process therefore in some cases the metabolites remain unidentified but can be differentiated based on their fragmentation spectra [137].

As consumers demand less processed and healthier products, metabolomic analysis has emerged in response to this requirement to accurately guarantee the degree of food processing. Knowing the degree of processing of fruit products will help assessing the food nutritional values, preservation level of the phenolic compounds and the quality characteristics of the final product [79,132,139]. Targeted and untargeted metabolomics has previously analyzed numerous metabolites focused on the detection and identification of markers that help to assess different food processing technologies in tomato products[140], the effect of thermal processing on black raspberries powder [141], the effect of drying process on nonvolatile compounds of white tea [142], as well as, the modifications due to minimal processing and storage in lettuce [143]. A recent review published by Utpott et al. [132] described the metabolomics application in food processing to identify changes in food metabolic profiles due to different processing techniques, including minimal processing, heat treatments, drying technologies, removal of heat, fermentation, chemical and enzymatic treatments, and some emerging technologies such as cold plasma and high pressure homogenization. Overall, the application of metabolomics used to evaluate food processing allowed to identify and quantify numerous compounds associated to nutrition, sensory characteristics and health properties, as well as toxic or undesirable compounds. At the same time, it was a useful tool to control and evaluate the effect of certain unit operations and industrial processes on the metabolomic profile.

Although all the efforts and investigation performed so far, and the fact that metabolomics-based approaches have demonstrated to be a powerful tool to identify changes in food metabolic profiles due to processing, it is still a challenge for industries and consumers to determine the degree of processing of products. In order to elucidate food processing markers and have an holistic view of the effect of processing, a good strategy would be first the metabolomic analysis on each unit processing operations, which includes the correct study design, the appropriate sample treatment, the use of combining separation techniques, an exhaustive and specialized statistical analysis. Therefore, there is still a need of more studies regarding the influence of processing techniques on the metabolites of products in order to determine common processing markers that help to choose the best conditions and processing techniques to be used and in thus provide useful information to consumers, industries and food governmental regulators.

CHAPTER II

Objectives

2.1. General Objective

Considering the summarized facts given in the introduction: (1) Strawberries and apples are two of the most widely consumed and industrially processed fruits worldwide due to their desirable sensory characteristics, nutritional value and bioactive compounds; (2) (poly)phenols are important bioactive compounds that exert health benefits; (3) (poly)phenols are affected by processing technologies and storage conditions; (4) consumers are increasingly demanding “minimally processed”.

The main objective of the present thesis was to determine to what extent different processing technologies and storage conditions affect the phenolic compounds and other metabolites in the selected strawberry and apple products, in order to potentially facilitate the use of customized processing and storage conditions to limit the degradation of (poly)phenols while maintaining the best quality and organoleptic characteristics.

2.2. Specific Objectives

Conduct a literature review to identify research already performed on the differences between TT and HPP in the impact on (poly)phenol degradation in strawberry and apple products.

Determine to what extent different processing techniques and conditions tested at an industrial scale affect the naturally present phenolic compounds, color and sensory characteristics in strawberries and apple products.

Determine to what extend the storage conditions selected influence the levels of bioactive compounds, color and sensory attributes during the shelf life (12 months) of strawberry and apple products after different processing conditions

Determine potential food processing markers in strawberry and apple subjected to different thermal and non-thermal industrial processing techniques to produce purees through an untargeted metabolomics approach.

CHAPTER III

Materials and Methods

3.1. Chemicals

Methanol, acetic acid, acetonitrile and water 0.1% formic acid (v/v) were from J.T. Baker (Deventer, Netherlands), formic acid from Honeywell (Barcelona, Spain), and hydrochloric acid and sodium acetate from Panreac (Barcelona, Spain). Ascorbic acid was from Acros Organic (Geel, Belgium). Water was deionized using a Milli-Q-system (Millipore, Bedford, MA, USA).

Phloroglucinol and standards of (+)-catechin, (-)-epicatechin, p-coumaric acid, ferulic acid, ellagic acid, quercetin 3-rutinoside, quercetin, cyanidin 3-glucoside and phloridzin used in Chapter IV and V were from Sigma Aldrich (St. Louis, MO, USA). Castalagin was kindly provided by Dr. S. Quideau (Bordeaux, France). Authentic standards of caffeic acid, pyroglutamic acid, vanillic acid, salicylic acid and gentisic acid used in Chapter VI were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A).

3.2. Strawberry and apple samples

3.2.1. Fresh fruit samples

Two of the most industrially used fruits were selected for this work, strawberries (*Fragaria x ananassa*), and apples (*Malus domestica*). For **CHAPTER** I**V**, **V** and **VI** the fruits employed were, strawberries cv. Primoris from Huelva (Spain) harvested ripe (80% red) in June 2018, and apples cv. Golden Delicious from Zaragoza (Spain) harvested ripe in November 2019. The processing conditions for these fruits will be detailed in the following section.

3.2.2. Processing techniques and conditions

Strawberries and apples were industrially processed at AMC Natural Drinks Group and HERO Group (Murcia, Spain) respectively with different technologies commonly employed by the fruit processing industry. The processing techniques employed for strawberry are shown in **Figure 8** and for apple in **Figure 9**.

Strawberry. IQF strawberries were obtained by removing the calyx from the fruit, followed by pre-cooling and freezing in two stages, first at -16 °C/8 min and finally at -26 °C/8 min. In order to obtain strawberry purees two extraction techniques were used; cold pureeing and standard thermal extraction. The puree obtained by cold pureeing and cold

deaeration was divided into two parts: one without thermal treatment (NT), while the other was subjected to a mild thermal treatment (MT) at 90 ºC/ 30 sec. On the other hand, entire strawberries were pre-heated (92 ºC/ 4 min) and then crushed to obtain the puree by standard thermal treatment (ST). The hot mash underwent thermal deaeration (92 ºC/ 2 min) followed by pasteurization at 90 ºC for 30 seconds. Finally, to produce a vacuum concentrated puree, ST puree was subjected to a vacuum concentration (VC) (0.3 - 0.4 Bar) at 83 ºC for 3.5 hours.

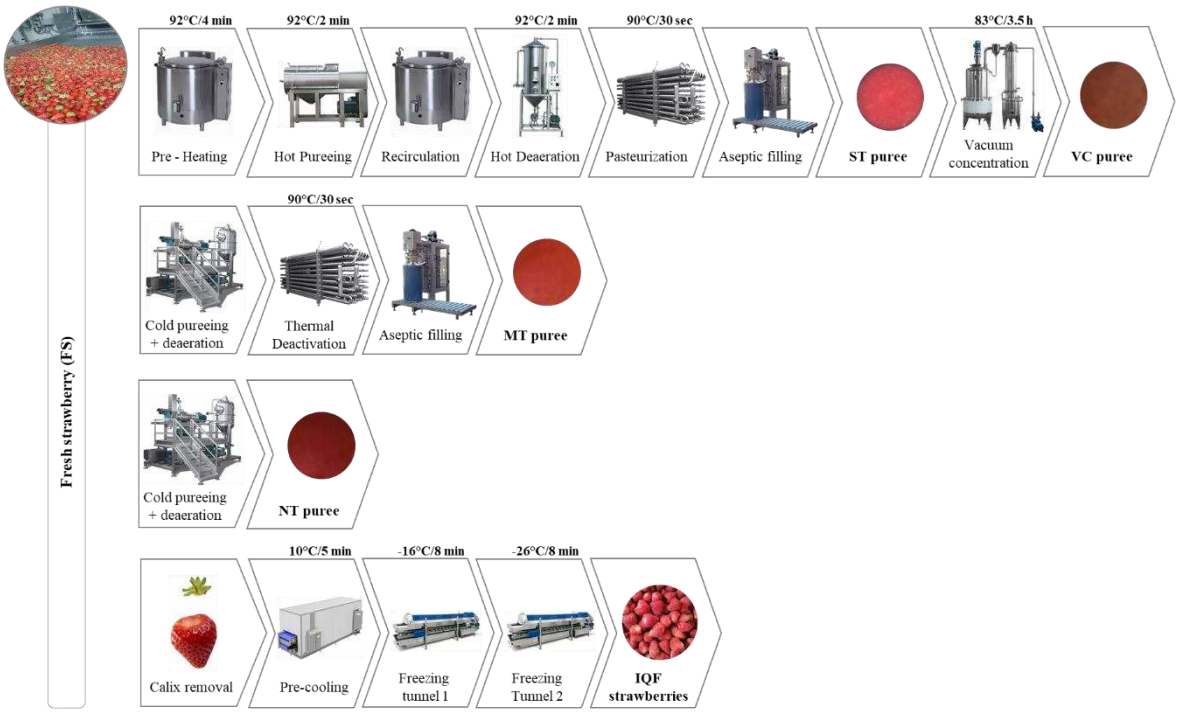

Figure 8. Scheme of the processing technologies and conditions used on strawberry to obtain No Thermal Treated (NT), Mild Thermally Treated (MT), Standard Thermally Treated (ST), Vacuum Concentrated (VC) purees and Individually Quick-Frozen strawberries (IQF).

Apple. Apple purees were also obtained by applying two extraction techniques cold pureeing and standard thermal extraction. The process of cold pureeing started with entire apples, which were directly cold crushed, separating skin and seeds at this stage. The puree obtained after crushing was cold deaerated and thermally deactivated (92 ºC/ 2 min). After that, the puree was divided into two parts: one was packed and treated under high pressure (HPP) (6 bar, 4 ºC/ 1 min), whereas the other part was hot deaerated and pasteurized (99 ºC/ 1 min), obtaining a mildly treated puree (MT). To obtain the puree by standard thermal treatment (ST), apples were chopped, pre-heated at 92 ºC for 5 min and hot crushed, finally being refined separating the skin and seeds from the puree. The obtained puree was hot

deaerated and pasteurized (99 ºC/ 1 min). Samples of MT and ST apple puree stored at 24 ºC for six months were re-processed with thermal treatment (90 ºC/11 min), obtaining re-processed apple purees (RP.MT and RP.ST).

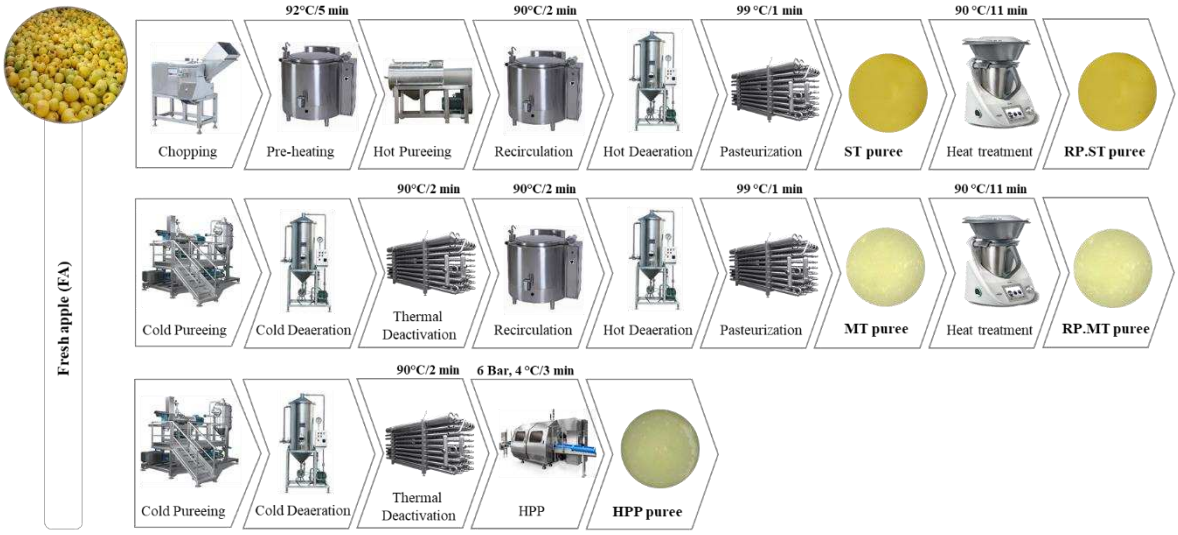

Figure 9. Scheme of the processing technologies and conditions used on apple to obtain High Pressure Processed (HPP), Mild Thermally Treated (MT), Standard Thermally Treated (ST), Reprocessed Mild Thermally Treated (RP.MT) and Reprocessed Standard Thermally Treated (RP.ST) purees.

In both studies, fresh fruits were used as a control. Before extraction, samples of strawberry and apple and their controls were lyophilized to remove the moisture and grounded into powder using a dry bean blender to homogenize the sample.

3.2.3. Storage conditions

Strawberry and apple purees processed by MT and ST were filled into low permeability (< 0.02 cc/m^2/day) double membrane aseptic bags (outer membrane: PE/Met PET/ PE of 102 µm; inner membrane: CoEx PE/EVOH/PA/PE of 90 µm (ARAN Packaging, Spain) and stored for 12 months at -20, 4 and 24 ºC. IQF strawberries and NT strawberry puree were stored only at -20 ºC. While VC strawberry puree was filled into vacuum-sealed glass jars and kept at 24 ºC. HPP apple puree was preserved only at 4 ºC. In the case of the apple re-processed purees, these were vacuum filled in glass jars and stored at 24 ºC. Stored samples were analyzed at 2, 6, and 12 months. In order to remove the moisture, all the samples were lyophilized and grounded into powder using a dry bean blender to homogenize

the sample before extraction. Storage conditions of strawberry and apple samples are shown summarized in **Table 2**.

Table 2. Storage conditions applied to strawberry and apple purees obtained after different industrial processing technologies and stored for twelve months. IQF: Individual Quick Freezing; NT: non thermal treated; MT: mild thermal treated; ST: standard thermal treated; VC: Vacuum concentrated; HPP: High pressure processed; RP.: re-processed.

Fruit	Sample	Storage Temperature		
		-20 ºC	4 ºC	24 ºC
Strawberry	IQF	●		
	NT puree	●		
	MT puree	●	●	●
	ST puree	●	●	●
	VC puree			●
Apple	HPP puree		●	
	MT puree	●	●	●
	ST puree	●	●	●
	RP.MT puree			●
	RP.ST puree			●

3.3. Extraction and analysis of phenolic compounds

50 mg of lyophilized samples were extracted with 1 mL of methanol/water/acetic acid (70:29:1, v/v) for strawberry and methanol/water (70:30, v/v) for apple. The samples were homogenized in a vortex for 1 min and then sonicated for 30 min at room temperature. Subsequently, samples were centrifuged for 15 min at 20627g at 12 °C (Thermo Scientific™ Sorvall™ ST 16, Germany). The resultant supernatant was filtered through a 0.22 µm filter. Extractions were performed and analyzed by triplicate.

(Poly)phenols identification and quantification were completed as previously reported by Buendía et al. [37] with some modifications. Identification was carried out on an Agilent 1100 HPLC system equipped with a photodiode array detector (G1315D) and coupled in series to a HCT Ultra Bruker Daltonics ion trap mass spectrometer through an electrospray ionization (ESI) interface HPLC-DAD-ESI-MS/MS (IT). The chromatographic

conditions for separation, identification and quantification are shown in **Table 3**. In the mass spectrometer, nitrogen was used as drying, nebulizing, and collision gas. The ESI parameters were: nebulizer pressure 65 psi, dry gas flow 11 L/min, and dry gas temperature 350 ºC. The capillary voltage was set at 4 kV, and spectra were acquired in negative ionization mode in the range of *m/z* 100-1500 and target mass 700. Automatic MS/MS mode was applied with fragmentation amplitude 1 V and number of parents, 3. For the quantification, an LC Agilent Series 1290 Infinity system (Agilent, USA) equipped with a diode array detector DAD (Series G4212A) and coupled to a Quadrupole LC/MS System (Agilent 6120 Series) was used. For **CHAPTERS IV** and **V** the results were expressed as mg per 100 g of fresh weight of fruit.

Table 3. Chromatographic conditions for separation, identification and quantification of phenolic compounds

Column	C18-PFP, 3.0 mm x 100 mm, and 2.1 µm particle size, settled at 25 °C	
Stationary phase	A: water/formic acid, 99:1 (v/v) B: acetonitrile	
Elution gradient	0 min, 5% B → 7 min, 18% B → 17 min, 28% B → 22 min, 50% B → 27 min, 90% B → 29-35 min, 5% B	
Flow rate	0.5 mL/min	
Injection volume	5 µL	
Identification	Authentic spectra, UV spectra, retention time, molecular weight, and MS/MS fragmentation pattern.	
	Strawberry	**Apple**
UV detection	280 nm (ellagitannins) 320 nm (hydroxycinnamic acids) 360 nm (flavonols) 520 nm (anthocyanins)	280 nm (dihydrochalcones) 320 nm (hydroxycinnamic acids) 360 nm (flavonols)
Quantification	Castalagin → ellagitannins p-coumaric and ferulic acid → hydroxycinnamic acids Ellagic acid → ellagic acid Quercetin-3-rutinoside → flavonols Cyanidin-3-glucoside → anthocyanins	Phloridzin → dihydrochalcones p-coumaric acid → hydroxycinnamic acids Quercetin → flavonols

3.4. Extraction and analysis of proanthocyanidins

The extraction of proanthocyanidins was performed according to Kennedy & Jones [144] with some modifications. 800 µL of a solution 0.1N HCl in MeOH containing 5 g/L

phloroglucinol and 10 g/L ascorbic acid was added to 0.8 g of lyophilized powdered sample. The mixture was incubated at 50 °C for 20 min with constant steering. Subsequently, 1 mL of sodium acetate 40 mM was added to stop the reaction. Finally, the samples were centrifuged for 10 min at 10000 rpm (Thermo Scientific™ Sorvall™ ST 16, Germany), and the supernatant was filtered through a 0.22 µm PVDF filter. For the analysis, the same HPLC-DAD-ESI-MS/MS (IT) equipment described above was used. Chromatographic separation (**Table 4**) of compounds was performed according to Buendía et al. [37] for strawberry and Díaz-Mula et al. [145] for apple with slight modifications. In the MS detector, ions were recorded in negative mode, with a mass range from *m/z* 100 to 800 and the same parameters previously reported [145]. To calculate the mean degree of polymerization (mDP), all proanthocyanidin products (added flavan-3-ol monomers and phloroglucinol adducts) were divided by the sum of all flavan-3-ol monomers.

Table 4. Chromatographic conditions for separation, identification and quantification of proanthocyanidins

Column	Pursuit XRs C18 250 x 4 mm and 5 µm particle size	
	Strawberry	**Apple**
Stationary phase	A: water/acetic acid, 99:1 (v/v) B: methanol	A: water/acetic acid , 97.5:2.5 (v/v) B: acetonitrile
Elution gradient	0 min, 0.5% B → 0-10 min 10.5% B → 10-20 min, 20% B → 20-40 min, 40% B → 40-45 min, 90% B → 45-48 min, 5% B → 48-55 min, 5% B	0-5 min, 3% B → 5-15 min, 9% B → 15-45 min, 16% B → 45-52, 90% B → 52-57 min, 3% B
Flow rate	0.8 mL/min	
Injection volume	16 µL	
UV detection	280 nm	
Identification	UV spectra, retention time, molecular weight, and MS/MS fragmentation pattern.	
Quantification	Calibration curves of flavan-3-ols (catechin and epicatechin).	

3.5. Analysis of global metabolic profile by UPLC-ESI-QTOF MS

With the aim to extract as many compounds as possible for the untargeted analysis general extraction with hydro-alcoholic mixtures were used in strawberry and apples, similar to those use for the extraction of phenolic compounds: methanol/water/acetic acid (70:29:1,

v/v/v) for strawberries and methanol/water (70:30, v/v) for apples. Three replicates for each condition were extracted and analyzed.

Samples were analyzed using an Agilent 1290 Infinity LC system coupled to the 6550 Accurate- Mass Quadrupole time-of-flight (QTOF) (Agilent Technologies, Waldbronn, Germany) using an electrospray interface (Jet Stream Technology). The optimal conditions of the electrospray interface were as follows: gas temperature 280 °C, drying gas 11 L/min, nebulizer 45 psi, sheath gas temperature 400 °C and sheath gas flow 12 L/min. **Table 5** shown the chromatographic conditions. Spectra were acquired in the m/z range 100–1100 in negative and positive mode, and fragmentor voltage was 100 V. MS/MS product ion spectra were collected at a *m/z* range of 50-1000 using a retention time window of 1 min, collision energy of 10 and 20 eV and an acquisition rate of 4 spectra/s.

Table 5. Chromatographic conditions for the detection of metabolites

Column	C18 column (3.0 mm x 100 mm, 2.1 µm particle size, settled at 30 ºC
Stationary phase	A: 0.1% formic acid (v/v) in water B: 0.1% formic acid (v/v) in acetonitrile
Elution gradient	0–7 min, 5–18 % B → 7–17 min, 18–28 % B → 17–22 min, 28–50 % B → 22-27 min, 50-90% B → 27-29 min the gradient comes back to the initial conditions (5% B) and are maintained for 6 min
Flow rate	0.5 mL/min
Injection volume	5 µL

3.6. Color measurements

Samples' colors were measured using a CR-400 Chromameter (Konica Minolta, Japan), under the conditions, illuminant C, observer 2°, and 8 mm of the illumination area. The instrument was calibrated with a white plate, Y = 86.2, x = 0.3159, y = 0.3224. Puree samples were poured into black plastic cuvettes with an optical path of 15 mm, avoiding the formation of air bubbles. The measurement was made on the CIEL*a*b* system. Values were used to determine, Chroma, °Hue, and the total color difference (ΔE).

3.7. Sensory analysis

The sensory analysis of the purees was determined using a nine-point hedonic scale derived from Sukanya et al [146]. Scores ranged from 1 = "I dislike it extremely" to 9 = "I like it extremely" [147]. The attributes evaluated were color, viscosity, aroma, flavor, and overall evaluation. In general, an overall evaluation higher than 5 was consider as an adequate indicator of acceptability [148]. The evaluations were carried out by a semi-trained sensory panel of ten persons (age 25 - 60 years).

3.8. Statistical analysis

To evaluate the effect of the processing technologies on phenolics, color parameters and sensory attributes, a one-way analysis of variance (ANOVA) followed by comparisons using Tukey test with a confidence level of 0.05 was performed. All data analyses were conducted using R software (version 4.0.2) [147].

In order to assess the effect of storage, the first approach was to perform a principal component analysis (PCA) independently to strawberries and apples to quickly visualize similarities or differences among samples based on their phenolic content and to identify data clustering trends. The data matrix consisted of the samples processed by the different techniques, just after processing (0) and after storage at -20, 4, and 24 ºC for 2, 6 and 12 months, and the total amount of the different polyphenol families as variables. Data were scaled and centered prior to PCA. This standardization to the same scale avoids some variables to become dominant just because of their large amount. PCA biplot graphs were constructed for the visual interpretation of the results. As a second approach, a one-way analysis of variance ANOVA per storage temperature followed by comparisons using Tukey test with a confidence level of 0.05 was performed to evaluate the effect of storage condition (time and temperature) on the phenolic content, color parameters and sensory attributes of the different treated samples. All data analyses were conducted using R software, version 4.0.2 [147].

Whereas to identify the metabolites affected according to the degree of processing the raw data generated by UPLC-ESI-QTOF-MS were exported to Profinder software (Agilent Technologies) for pre-processing procedures and to build the data matrix for further processing and data treatment. Independent data matrixes were created to process and analyze

strawberry and apple samples separately for each negative and positive polarity. The data matrixes were imported in parallel to the Metaboanalyst online platform and Mass Profiler Professional (MPP, Agilent Technologies). Data processing was performed before univariate and multivariate analysis, including data log transformation and Pareto scaling. Regarding multivariate analysis, the principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) models of the final data matrix were created using the Metaboanalyst platform to describe the total variance of the full data set and figure out the discrimination groups under data matrix criteria. The VIP (variable importance in projection) score value (VIP > 1) obtained by the discriminant analysis was used for candidate selection. After the multivariate analysis evaluation, univariate operations were performed in MPP software. Data treatment through MPP software included filters by frequency of the data matrix to reduce the sample variability within each study group and the ANOVA statistics analysis (corrected p-value cut-off: 0.05; p-value computation: Asymptotic; Multiple Testing Correction: Benjamini–Hochberg). The VIP > 1 score and p-value were used to create the candidate list for evaluating the processing treatment. After the selection of the candidates, the authentic standards and the MS/MS spectra data of those ions were used for metabolite confirmation. Metlin and MassBank of Noth America (MoNA) databases were used for checking the tentative identification. In addition to the databases, the competitive fragmentation modeling for metabolite identification (CFM-ID) software was complementary to confirm the metabolites.

CHAPTER IV

Stability of phenolic compounds in apple and strawberry: Effect of different processing techniques in industrial set up

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Stability of phenolic compounds in apple and strawberry: Effect of different processing techniques in industrial set up

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Abstract

Different technologies commonly employed by the fruit processing industry affect the phenolic content. This study aimed to establish the extent to which different processing techniques and conditions, tested at an industrial scale, affect phenolics, color, and sensory attributes of fresh strawberries and apples. The effects of freezing, thermal treatments, and high-pressure processing were investigated. In strawberries, mild and standard thermal treatments showed similar patterns for most phenolic groups; an increase in proanthocyanidins, no change in ellagic acid conjugates, and a major decrease in flavonols and anthocyanins. In apples, mild treatments and high-pressure processing had similar effects in all phenolic groups, with increases in dihydrochalcones, hydroxycinnamics, and proanthocyanidins and decreases in flavonols. However, the standard thermal treatment increased flavonols and dihydrochalcones concentrations. This study shows that each fruit behaves differently, and both technology and processing conditions should be customized accordingly to preserve or even increase the phenolic content.

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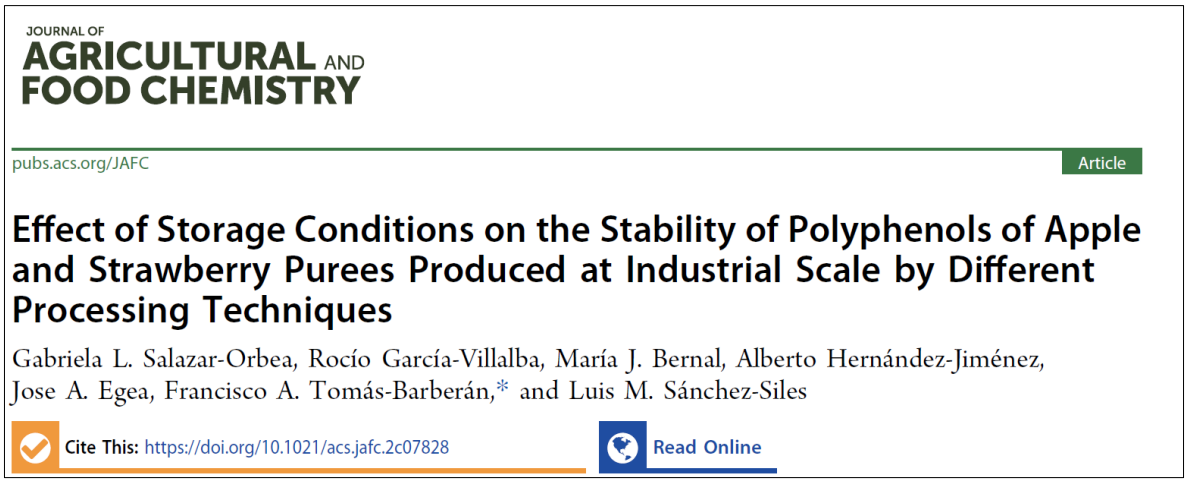
Original research publications

CHAPTER V

Effect of storage conditions on the stability of polyphenols of apple and strawberry samples produced at industrial scale by different processing techniques



Abstract

During the food product life, storage conditions affect its composition on nutrients, bioactive compounds and sensory attributes. In this research, strawberry and apple purees were selected as a model to examine how the storage of various purees industrially produced with different technologies affect the bioactive phenolic compounds, color, and sensory attributes. Specifically, fruit products processed at an industrial scale by different technologies including freezing, thermal treatment (mild and standard), and high-pressure processing were studied, as well as storage for up to 12 months at -20, 4 and 24 ºC. In strawberry puree, storage conditions had a stronger impact on phenolic compound levels, particularly on anthocyanins; whereas in apple puree, the initial processing techniques exerted a greater influence than storage conditions, mainly caused by the hot or cold crushing processes. In general, proanthocyanidins were the major phenolic group and the most stable during storage, while anthocyanins were the group most affected by both processing and storage. Apple flavonols and dihydrochalcones were quite stable, while strawberry ellagitannins suffered higher degradations during storage. Through our analysis, it is found that during storage, the stability of polyphenols in each fruit is different, and processing and storage can be either detrimental or even beneficial. The selection of the ideal storage conditions (time and temperature) is a key factor to maintain the polyphenol content in sensitive fruits as strawberry. However, storage conditions are in some cases more important to minimize the polyphenol losses than how the product is processed.

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Original research publications

CHAPTER VI

Untargeted metabolomics reveals new markers of food processing for strawberry and apple purees

molecules

MDPI

Article

Untargeted Metabolomics Reveals New Markers of Food Processing for Strawberry and Apple Purees

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Abstract

In general, food processing and its conditions affect nutrients, bioactive compounds, and sensory characteristics of food products. This research aims to use a non-targeted metabolomics approach based on UPLC-ESI-QTOF-MS to determine how fruit processing can affect the metabolic profile of fruits and, through a comprehensive metabolic analysis, identify possible markers to assess their degree of processing. The present study uses a real case from the food industry to evaluate markers of the processing of strawberry and apple purees industrially elaborated with different pro- cessing techniques and conditions. The results from the multivariate analysis revealed that samples were grouped according to the type of processing, evidencing changes in their metabolic profiles and an apparent temperature-dependent effect. These metabolic profiles showed changes according to the relevance of thermal conditions but also according to the exclusively cold treatment, in the case of strawberry puree, and the pressure treatment, in the case of apple puree. After data analysis, seven metabolites were identified and proposed as processing markers: pyroglutamic acid, pteroyl- D-glutamic acid, 2-hydroxy-5-methoxy benzoic acid, and 2-hydroxybenzoic acid β-D-glucoside in strawberry and di-hydroxycinnamic acid glucuronide, caffeic acid and lysoPE(18:3(9Z,12Z,15Z)/0:0) in apple purees. The use of these markers may potentially help to objectively measure the degree of food processing and help to clarify the controversial narrative on ultra-processed foods.

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CHAPTER VII

DISCUSSION

General Discussion

A common practice of food industry is the use of fresh, frozen, pureed, or concentrated fruits as raw materials for the production of diverse type of food products such as sauces, compotes, preserves, baby food, among others. This thesis utilized different technologies to produce individually frozen strawberries and apple and strawberry purees. Puree is one of the most traditional food ingredients, commonly elaborated by thermal processing. The uniqueness of this study is that it was executed in an industrial set-up. Therefore, the technologies selected were the commonly employed by the fruit processing industry. In particular, the effects of freezing, thermal treatment (mild and standard), and high-pressure processing were investigated. The aim of this study was to determine the impact of these technologies on the phenolic compounds, color, and sensory attributes of fresh strawberries and apples (**CHAPTER IV**) and the influence of the storage conditions in the strawberry and apple products obtained (**CHAPTER V**).

This study analyzed the impact of the mentioned processing and storage condition on the main phenolic compounds of apples (flavan-3-ols/proanthocyanidins, flavonols, dihydrochalcones and hydroxycinnamic acids) and strawberries (flavan-3-ols/proanthocyanidins, anthocyanins, and ellagic acid).

From the results obtained in **CHAPTER IV** and **V** of this study, it can be established that the effects of processing and storage are highly dependent of the food matrix. This was supported by the review [99] performed by us as a background information, that the effect of the processing techniques and the storage on the stability of (poly)phenols was not dependent only on the processing technique itself, but instead it was influenced by numerous factors, such as, the food matrix (type of fruit, variety and degree of ripening), its characteristics (pH, sugar content, and presence/absence of oxygen) and the behavior of the different phenolic compounds present in the fruit.

Overall, the application of different processing techniques and the storage conditions affected differently the (poly)phenolic profile of strawberry and apple products. For strawberry products, the storage conditions had a higher impact on the degradation of (poly)phenols and quality characteristics than the application of the conventional processing techniques. Whereas, for apple purees, the processing techniques employed impacted more

the phenolic levels than the storage conditions. In general, apple and strawberry samples stored at 24 ºC showed the higher degradation both in phenolic levels and quality attributes, while samples stored at – 20 and 4 ºC were comparable, resulting in levels within the ranges found in fresh fruits from different varieties.

In strawberries, thermal treatments affected more the heat labile compounds such as anthocyanins, but increased other compounds such as ellagic acid and proanthocyanidins. Mild and standard thermal treatments showed similar patterns for most phenolic groups; **i)** an increase in proanthocyanidins, attributed to a higher extraction capacity after thermal treatment and some migration of these compounds from plant tissues, particularly from the achenes, as well as, the cleavage of complex proanthocyanidins into their monomeric units promoted by the heat treatment; **ii)** a slightly increase in ellagic acid conjugates, probably due to an improvement in the extractability of ellagitannins from the achenes and cell walls; **iii)** a reduction in flavonols, that could be due to the breakdown of flavonols during processing and subsequent oxidative reactions; and **iv)** a major decrease in anthocyanins, which could be attributed to the cleavage of covalent bonds, polymerization, derivatization and also to condensations reaction with other phenolics resulting in browning. Regarding anthocyanins, the more severe was the thermal treatment applied (MT<ST<VC) the greater was the degradation. The general severe losses of all the phenolic compounds derived from vacuum concentration, could be attributed to secondary oxidation reactions as a result of the extended heat treatment at which it was subjected to. Surprisingly, a diminution of anthocyanin, ellagic acid and flavonols levels were observed on IQF strawberries, which could be the result of the prior sepals removing. In the case of anthocyanins, leaving the flesh unprotected and in contact with oxygen could have caused oxidation reactions. While for ellagic acid and flavonols, the decrease may be directly related to the elimination of sepals, since both compounds can be found in the sepals. In no thermally treated puree, the effects observed were different depending on the compounds, while **i)** significant losses were registered for anthocyanins, flavonols, ellagitannins and ellagic acid, which could be mainly due to an enzymatic oxidation by PPO; **ii)** substantial increments were observed in proanthocyanidins, probably due to some encapsulation effect in the colloidal matrix (pectin and cell-wall polysaccharides) that could have prevented them.

In apples, in general all the processing treatments lead to positive effects on the phenolic compounds. Mild treatments and high-pressure processing had the same effect in all the phenolic groups, with **i)** increases in dihydrochalcones, hydroxycinnamic acids, and proanthocyanidins attributed to either a higher extraction or migration of these compounds from the cell matrix or to the release of monomeric units from complex proanthocyanidins; and **ii)** a decrease in flavonols, which could be mainly because of the way of processing, as in the initial cold crushing, the peel, rich in flavonols, was discarded during this stage; another reason could be that the low heat treatment applied was not sufficient to inactivate polyphenol oxidase and peroxidase leading to enzymatic oxidative degradation of phenols. However, the standard thermal treatment increased in a greater extent the concentrations of flavonols and dihydrochalcones. This extra positive effect could be mainly attributed to the type of initial extraction. In the case of standard treatment, whole apples (flesh, peel, seeds, and skin) were chopped and pre-heated at 90 ºC for 4 min to subsequently being hot crushed and refined. Apparently, this initial hot extraction favored the release of flavonols, dihydrochalcones and hydroxycinnamic acids from the peel, seeds and flesh respectively. For the case of re-processed samples, the behavior of the phenolic compounds followed the increase or decrease pattern of the principal sample from which these were processed from. In conclusion, for apples, standard treatment was recommended as the technique that obtained the best results preserving the (poly)phenol concentration both after processing and storage.

Regarding proanthocyanidins, significant reductions in their mDP (from 4.5 to 2.5 in strawberry and from 4.0 to 2.7 in apple) were also observed when the above-mentioned processing conditions were applied, indicating a possible reduction in the oligomer size. Since some studies suggested that monomeric and dimeric procyanidins were better absorbed than the oligomers, it can be concluded that the reduction in the mDP in the final products, might help increasing the bioavailability of proanthocyanidins. In general, proanthocyanidins were the major phenolic group in both fruits and the more stable after processing and during storage. Whereas anthocyanins, being thermo-labile compounds were the most affected as a result of processing and storage conditions.

Apart from the effect observed on the phenolic compounds as a result of the different processing technologies, the same samples were analyzed through an untargeted

metabolomic approach (**CHAPTER VI**) in order to find potential markers to evaluate the processing degree of strawberry and apple puree products. Seven metabolites with a temperature-dependent effect were identified, four in strawberry and three in apple: **(1)** pyroglutamic acid, **(2)** pteroyl-D-glutamic acid, **(3)** 2-hydroxy-5-methoxybenzoic acid and **(4)** 2-hydroxybenzoic acid β-D-glucoside in strawberry and **(5)** dihydroxycinnamic acid glucuronide, **(6)** caffeic acid and **(7)** lysoPE(18:3(9Z,12Z,15Z)/0:0) in apple. In strawberry, metabolites **(1)** and **(3)** were identified as upregulated markers, showing an increase trend correlated with the degree of processing, whereas metabolites **(2)** and **(4)** were identified as down regulated markers and found with high intensity in fresh strawberries. In the case of the metabolites identified in apple purees, all of them were upregulated markers, increasing their intensity at the same time that the thermal processing degree. A possible formation pathway was proposed for **(1)** in strawberry, suggesting that **(2)** present in fresh strawberry, is degraded with the temperature, releasing pteroic acid and glutamic acid. Then, glutamic acid is converted into **(1)** as a result of the loss of a water molecule and internal cyclization. The elucidation of these markers might help to define the degree of processing of strawberry and apple products. Thus, these could also be used to optimize the processing conditions and chose the best technique to avoid losses of nutritional and bioactive compounds. Another potential application of these markers is their use as a start point to detect new processing markers in future metabolomic research.

Overall, this study shows that each fruit behaves differently, and both technology and processing conditions should be customized accordingly to preserve or even increase the polyphenol content. Therefore, the impact on the phenolic profile, color and sensory attributes has to be individualized. Furthermore, the metabolites identified as potential processing markers could be used to measure and optimize the degree of fruit processing

CHAPTER VIII

CONCLUSIONS

The conclusions reached from this Thesis are:

1. Each fruit analyzed behaves differently, the selection of right fruit variety togehther with both processing technology and conditions should be customized accordingly by industries, to preserve or even increase the (poly)phenol content of the final product.

2. The processing techniques play a role in the mechanical disruption of fruit tissue which might have improved phenols extractability, potentially leading to a better bio-accessibility.

3. The impact of thermal treatment relied mainly on:

i) The food matrix, being in this case positive by promoting the release of certain phenolic compounds from the fruit structures.

ii) The thermal stability of the different phenolic compounds, affecting some heat labile compounds such as anthocyanins.

4. For apple products, the processing techniques and conditions impacted more the phenolic levels than the storage conditions.

i) Overall, all the processing technologies increased the levels of all phenolic groups.

ii) Standard thermal treatments resulted in a significantly higher additional increase in the levels of dihydrochalcones and flavonols, as well as the better preservation during storage. Therefore, it is recommended as the one that has obtained the best results preserving the (poly)phenol concentration.

5. For strawberry products, the storage conditions had a higher impact on the degradation of (poly)phenols and quality characteristics than the processing techniques employed.

iii) Anthocyanins, being thermo-labile compounds were the most affected as a result of processing and storage conditions.

iv) Proanthocyanidins were the major phenolic group and the more stable after processing and during storage.

v) Standard thermal treatment preserves better heat labile compounds in strawberry products.

6. Regarding storage, apple and strawberry products stored at -20 and 4 ºC barely affected the phenolic level and the quality characteristics during storage. Nevertheless, storage at 24 ºC highly degraded the polyphenolic content and the quality and sensory attributes in strawberry, although not that drastic in apple products.

7. The results of this study has demonstrated the potential of untargeted metabolomics and the data treatment strategies as tools for the discovery new food processing markers and to visualize changes in the metabolic profile of strawberry and apple purees subjected to different processing techniques.

8. The presence or absence of temperature used during the crushing process is crucial in the change of the metabolomic profile of the final product.

9. Seven metabolites were identified and proposed as potentially powerful markers to evaluate the processing degree of strawberry and apple puree products.

10. In apple purees, dihydroxycinnamic acid glucuronide, caffeic acid, and lysoPE(18:3(9Z,12Z,15Z)/0:0) were identified as upregulated markers, showing an increasing trend correlated with thermal processing, except for HPP which increased the metabolites profile.

11. In strawberry purees, pyroglutamic acid, and 2-hydroxy- 5-methoxy benzoic acid were identified as upregulated markers, whereas pteroyl- D-glutamic acid, and 2-hydroxybenzoic acid β-D-glucoside were identified as down regulated markers of thermal processing.

i) Pteroyl-D-glutamic acid could be a marker of non-processed fruits, as it is degraded with the temperature, releasing pteroic acid and glutamic acid.

ii) Glutamic acid is converted into pyroglutamic acid due to the loss of a water molecule and internal cyclization.

References

1. Yeung, A.W.K.; Mocan, A.; Atanasov, A.G. Let food be thy medicine and medicine be thy food: A bibliometric analysis of the most cited papers focusing on nutraceuticals and functional foods. *Food Chem.* **2018**, *269*, 455–465, doi:10.1016/j.foodchem.2018.06.139.

2. Cheynier, V.; Comte, G.; Davies, K.M.; Lattanzio, V.; Martens, S. Plant Physiology and Biochemistry Plant phenolics: Recent advances on their biosynthesis , genetics , and ecophysiology. *Plant Physiol. Biochem.* **2013**, *72*, 1–20, doi:10.1016/j.plaphy.2013.05.009.

3. Rasouli, H.; Farzaei, M.H.; Khodarahmi, R. Polyphenols and their benefits: A review. *Int. J. Food Prop.* **2017**, *20*, 1700–1741, doi:10.1080/10942912.2017.1354017.

4. Crozier, A.; Jaganath, I.B.; Clifford, M.N.; Crozier, A.; Jaganath, B.; Clifford, M.N. Dietary phenolics : chemistry, bioavailability and effects on health. **2009**, *26*, doi:10.1039/b802662a.

5. Belščak-Cvitanović, A.; Durgo, K.; Huđek, A.; Bačun-Družina, V.; Komes, D. *Overview of polyphenols and their properties*; 2018; ISBN 9780128135723.

6. Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jiménez, L. Polyphenols: Food sources and bioavailability. *Am. J. Clin. Nutr.* **2004**, *79*, 727–747, doi:10.1093/ajcn/79.5.727.

7. Di Lorenzo, C.; Colombo, F.; Biella, S.; Stockley, C.; Restani, P. Polyphenols and human health: The role of bioavailability. *Nutrients* **2021**, *13*, 273, doi:10.3390/nu13010273.

8. Herrero, M.; Plaza, M.; Cifuentes, A.; Ibáñez, E. Extraction Techniques for the Determination of Phenolic Compounds in Food. In *Comprehensive Sampling and Sample Preparation*; 2012; Vol. 4, pp. 159–180 ISBN 10.1016/B978-0-12-381373-2.10132-2.

9. Motilva, M.-J.; Serra, A.; Macià, A. Analysis of food polyphenols by ultra high-performance liquid chromatography coupled to mass spectrometry : An overview. *J. Chromatogr. A* **2013**, *1292*, 66–82, doi:10.1016/j.chroma.2013.01.012.

10. Abbas, M.; Saeed, F.; Anjum, F.M.; Afzaal, M.; Tufail, T.; Bashir, M.S.; Ishtiaq, A.; Hussain, S.; Suleria, H.A.R. Natural polyphenols: An overview. *Int. J. Food Prop.* **2017**, *20*, 1689–1699, doi:10.1080/10942912.2016.1220393.

11. Durazzo, A.; Caiazzo, E.; Lucarini, M.; Cicala, C.; Izzo, A.A.; Novellino, E.; Santini, A. Polyphenols : A concise overview on the chemistry , occurrence , and human health. *Phyther. Res.* **2019**, *33*, 2221–2243, doi:10.1002/ptr.6419.

12. Kamiloglu, S.; Tomas, M.; Capanoglu, E. *Dietary Flavonols and O -Glycosides*; 2020; ISBN 9789811317453.

13. Rosillo, M.A.; Alarcón-De-La-Lastra, C.; Sánchez-Hidalgo, M. An update on dietary

phenolic compounds in the prevention and management of rheumatoid arthritis. *Food Funct.* **2016**, *7*, 2943–2969, doi:10.1039/c6fo00485g.

14. Castañeda-Ovando, A.; Pacheco-Hernández, M. de L.; Páez-Hernández, M.E.; Rodríguez, J.A.; Galán-Vidal, C.A. Chemical studies of anthocyanins: A review. *Food Chem.* **2009**, *113*, 859–871, doi:10.1016/j.foodchem.2008.09.001.

15. Mattioli, R.; Francioso, A.; Mosca, L.; Silva, P. Anthocyanins : A Comprehensive Review of Their Chemical Properties and Health Effects on Cardiovascular and Neurodegenerative Diseases. *Molecules* **2020**, *25*, 3809, doi:doi:10.3390/molecules25173809.

16. Wallace, T.; Giusti, M. Anthocyanins. *Am. Soc. Nutr.* **2015**, 620–622, doi:10.3945/an.115.009233.

17. Luo, Y.; Jian, Y.; Liu, Y.; Jiang, S.; Muhammad, D.; Wang, W. Flavanols from Nature : A Phytochemistry and Biological. *Molecules* **2022**, *27*, 719, doi:https://doi.org/10.3390/molecules27030719.

18. Guven, H.; Arici, A.; Simsek, O. Flavonoids in Our Foods : A Short Review. *J. Basic Clin. Heal. Sci.* **2019**, 96–106, doi:https://doi.org/10.30621/jbachs.2019.555.

19. Rauf, A.; Imran, M.; Abu-Izneid, T.; Iahtisham-Ul-Haq; Patel, S.; Pan, X.; Naz, S.; Sanches Silva, A.; Saeed, F.; Rasul Suleria, H.A. Proanthocyanidins: A comprehensive review. *Biomed. Pharmacother.* **2019**, *116*, doi:10.1016/j.biopha.2019.108999.

20. Smeriglio, A.; Barreca, D.; Bellocco, E.; Trombetta, D. Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. *Br. J. Pharmacol.* **2017**, *174*, 1244–1262, doi:10.1111/bph.13630.

21. Terahara, N. Natural Product Communications. *Nat. Prod. Commun.* **2015**, *10*, 521–528, doi:10.1177/1934578X1501000334.

22. Tian, L.; Cao, J.; Zhao, T.; Liu, Y.; Khan, A.; Cheng, G. The bioavailability, extraction, biosynthesis and distribution of natural dihydrochalcone: Phloridzin. *Int. J. Mol. Sci.* **2021**, *22*, 1–15, doi:10.3390/ijms22020962.

23. Arceusz, A.; Wesolowski, M.; Konieczynski, P. Methods for Extraction and Determination of Phenolic Acids in Medicinal Plants: A Review. *Nat. Prod. Commun.* **2013**, *8*, 1821–1829, doi:10.1177/1934578X1300801238.

24. Kumar, N.; Goel, N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnol. Reports* **2019**, *24*, e00370, doi:10.1016/j.btre.2019.e00370.

25. Rashmi, H.B.; Negi, P.S. Phenolic acids from vegetables: A review on processing stability and health benefits. *Food Res. Int.* **2020**, *136*, 109298, doi:10.1016/j.foodres.2020.109298.

26. Heleno, S.A.; Martins, A.; Queiroz, M.J.R.P.; Ferreira, I.C.F.R. Bioactivity of phenolic acids: Metabolites versus parent compounds: A review. *Food Chem.* **2015**,

173, 501–513, doi:10.1016/j.foodchem.2014.10.057.

27. Teka, T.; Zhang, L.; Ge, X.; Li, Y.; Han, L.; Yan, X. Phytochemistry Stilbenes : Source plants , chemistry , biosynthesis , pharmacology , application and problems related to their clinical Application-A comprehensive review. *Phytochemistry* **2022**, *197*, 113128, doi:10.1016/j.phytochem.2022.113128.

28. Shen, T.; Wang, X.; Lou, H. Natural stilbenes : an overview †. **2009**, 916–935, doi:10.1039/b905960a.

29. Navarro-Orcajada, S.; Conesa, I.; Vidal-Sánchez, F.J.; Albaladejo-Maricó, L.; García-Carmona, F.; López-Nicolás, M. Stilbenes : Characterization, bioactivity, encapsulation and structural modifications. A review of their current limitations and promising approaches. *Crit. Rev. Food Sci. Nutr.* **2022**, 1–19, doi:10.1080/10408398.2022.2045558.

30. El Khawand, T.; Courtois, A.; Valls, J.; Richard, T. A review of dietary stilbenes : sources and bioavailability. *Phytochem. Rev.* **2018**, doi:10.1007/s11101-018-9578-9.

31. Li, Y.; Xie, S.; Ying, J.; Wei, W.; Gao, K. Chemical Structures of Lignans and Neolignans Isolated from Lauraceae. *Molecules* **2018**, *23*, 3164, doi:10.3390/molecules23123164.

32. Masi, E.; Taiti, C.; Vignolini, P.; William, A.; Giordani, E.; Heimler, D.; Romani, A.; Mancuso, S. Polyphenols and aromatic volatile compounds in biodynamic and conventional ' Golden Delicious ' apples (Malus domestica Bork .). *Eur. Food Res. Technol.* **2017**, *243*, 1519–1531, doi:10.1007/s00217-017-2861-y.

33. Akagić, A.; Vranac, A.; Gaši, F.; Drkenda, P.; Spaho, N.; Žuljević, O.; Kurtović, M.; Musić, O.; Murtić, S.; Hudina, M. Sugars , acids and polyphenols profile of commercial and traditional apple cultivars for processing. *Acta Agric. Slov.* **2019**, *113*, 2, 239–250, doi:10.14720/aas.2019.113.2.5.

34. Jakobek, L.; García-Villalba, R.; Tomás-Barberán, F.A. Polyphenolic characterisation of old local apple varieties from Southeastern European region. *J. Food Compos. Anal.* **2013**, *31*, 199–211, doi:10.1016/j.jfca.2013.05.012.

35. Aaby, K.; Skrede, G.; Wrolstad, R.E. Phenolic Composition and Antioxidant Activities in Flesh and Achenes of Strawberries (Fragaria ananassa). *J. Agric. Food Chem.* **2005**, *53*, 4032–4040, doi:10.1021/jf048001o.

36. Nowicka, A.; Kucharska, A.Z.; Sokół-Łętowska, A.; Fecka, I. Comparison of polyphenol content and antioxidant capacity of strawberry fruit from 90 cultivars of Fragaria × ananassa Duch. *Food Chem.* **2019**, *270*, 32–46, doi:10.1016/j.foodchem.2018.07.015.

37. Buendía, B.; Gil, M.I.; Tudela, J.A.; Gady, A.L.; Medina, J.J.; Soria, C.; López, J.M.; Tomás-Barberán, F.A. HPLC-MS analysis of proanthocyanidin oligomers and other phenolics in 15 strawberry cultivars. *J. Agric. Food Chem.* **2010**, *58*, 3916–3926, doi:10.1021/jf9030597.

38. Łata, B.; Trampczynska, A.; Paczesna, J. Cultivar variation in apple peel and whole

fruit phenolic composition. *Sci. Hortic. (Amsterdam).* **2009**, *121*, 176–181, doi:10.1016/j.scienta.2009.01.038.

39. Guo, S.; Guan, L.; Cao, Y.; Li, C.; Chen, J.; Li, J.; Liu, G.; Li, S.; Wu, B. Diversity of polyphenols in the peel of apple (Malus sp.) germplasm from different countries of origin. *Int. J. Food Sci. Technol.* **2016**, *51*, 222–230, doi:10.1111/ijfs.12994.

40. Xu, Y.; Fan, M.; Ran, J.; Zhang, T.; Sun, H.; Dong, M.; Zhang, Z.; Zheng, H. Variation in phenolic compounds and antioxidant activity in apple seeds of seven cultivars. *Saudi J. Biol. Sci.* **2016**, *23*, 379–388, doi:10.1016/j.sjbs.2015.04.002.

41. Soundararajan, P.; Won, S.Y.; Kim, J.S. Insight on Rosaceae Family with Genome Sequencing and Functional Genomics Perspective. *Biomed Res. Int.* **2019**, 1–12, doi:https://doi.org/10.1155/2019/7519687.

42. Johnston, J.W.; Hewett, E.W.; Hertog, M.L.A.T.M. Postharvest softening of apple (Malus domestica) fruit : A review. *New Zeal. J. Crop Hortic. Sci.* **2010**, *30*, 145–160, doi:10.1080/01140671.2002.9514210.

43. Janssen, B.J.; Thodey, K.; Schaffer, R.J.; Alba, R.; Balakrishnan, L.; Bishop, R.; Bowen, J.H.; Crowhurst, R.N.; Gleave, A.P.; Ledger, S.; et al. Global gene expression analysis of apple fruit development from the floral bud to ripe fruit. *BMC Plant Biol.* **2008**, *29*, 1–29, doi:10.1186/1471-2229-8-16.

44. Fernández-Jalao, I.; Sánchez-Moreno, C.; De Ancos, B. Effect of high-pressure processing on flavonoids, hydroxycinnamic acids, dihydrochalcones and antioxidant activity of apple 'Golden Delicious' from different geographical origin. *Innov. Food Sci. Emerg. Technol.* **2019**, *51*, 20–31, doi:10.1016/j.ifset.2018.06.002.

45. Feng, S.; Yi, J.; Li, X.; Wu, X.; Zhao, Y.; Ma, Y.; Bi, J. Systematic Review of Phenolic Compounds in Apple Fruits: Compositions, Distribution, Absorption, Metabolism, and Processing Stability. *J. Agric. Food Chem.* **2021**, *69*, 7–27, doi:10.1021/acs.jafc.0c05481.

46. FAO *World Food and Agriculture – Statistical Yearbook 2021*; Rome, 2021; ISBN 9789251343326.

47. Fait, A.; Hanhineva, K.; Beleggia, R.; Dai, N.; Rogachev, I.; Nikiforova, V.J. Reconfiguration of the Achene and Receptacle Metabolic Networks during Strawberry Fruit Development. *Plant Physiol.* **2008**, *148*, 730–750, doi:10.1104/pp.108.120691.

48. Aaby, K.; Mazur, S.; Nes, A.; Skrede, G. Phenolic compounds in strawberry (Fragaria x ananassa Duch.) fruits: Composition in 27 cultivars and changes during ripening. *Food Chem.* **2012**, *132*, 86–97, doi:10.1016/j.foodchem.2011.10.037.

49. Cervantes, L.; Ariza, M.T.; Miranda, L.; Lozano, D.; Medina, J.J.; Soria, C.; Martínez-Ferri, E. Stability of Fruit Quality Traits of Different Strawberry Varieties under Variable Environmental Conditions. *Agronomy* **2020**, *10*, 1242, doi:10.3390/agronomy10091242.

50. Cheynier, V.; Tomás-Barberán, F.A.; Yoshida, K. Polyphenols: From Plants to a Variety of Food and Nonfood Uses. *J. Agric. Food Chem.* **2015**,

doi:10.1021/acs.jafc.5b01173.

51. Plaza, M.; Domínguez-Rodríguez, G.; Castro-Puyana, M.; Marina, M.L. *Polyphenols analysis and related challenges*; Elsevier Inc., 2018; ISBN 9780128135723.

52. Hyötyläinen, T. Critical evaluation of sample pretreatment techniques. *Anal. Bioanal. Chem.* **2009**, *394*, 743–758, doi:10.1007/s00216-009-2772-2.

53. Rijke, E. De; Out, P.; Niessen, W.M.A.; Ariese, F.; Gooijer, C.; Th, U.A. Analytical separation and detection methods for flavonoids. *J. Chromatogr. A* **2006**, *1112*, 31–63, doi:10.1016/j.chroma.2006.01.019.

54. Pyrzynska, K.; Sentkowska, A. Recent Developments in the HPLC Separation of Phenolic Food Compounds. *Crit. Rev. Anal. Chem.* **2015**, *45*, 41–51, doi:10.1080/10408347.2013.870027.

55. Ignat, I.; Volf, I.; Popa, V.I. A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chem.* **2011**, *126*, 1821–1835, doi:10.1016/j.foodchem.2010.12.026.

56. Khoddami, A.; Wilkes, M.; Roberts, T. Techniques for Analysis of Plant Phenolic Compounds. *Molecules* **2013**, *18*, 2328–2375, doi:10.3390/molecules18022328.

57. Capriotti, A.L.; Cavaliere, C.; Foglia, P.; Piovesana, S.; Salvatore, V. Chromatographic Methods Coupled to Mass Spectrometry Detection for the Determination of Phenolic Acids in Plants and Fruits. *J. Liq. Chromatogr. Relat. Technol.* **2015**, *38*, 353–370, doi:10.1080/10826076.2014.941263.

58. Lamuela-Raventós, R.M.; Vallverdú-Queralt, A.; Jáuregui, O.; Martínez-Huélamo, M.; Quifer-Rada, P. Improved Characterization of Polyphenols Using Liquid Chromatography. In *Polyphenols in Plants: Isolation, Purification and Extract Preparation*; Elsevier, 2014; pp. 261–292 ISBN 9780123979346.

59. Cory, H.; Passarelli, S.; Szeto, J.; Tamez, M.; Mattei, J. The Role of Polyphenols in Human Health and Food Systems : A Mini-Review. *Front. Nutr.* **2018**, *5*, 1–9, doi:10.3389/fnut.2018.00087.

60. Sobhani, M.; Farzaei, M.H.; Kiani, S. Immunomodulatory ; Anti-inflammatory / antioxidant Effects of Polyphenols : A Comparative Review on the Parental Compounds and Their Metabolites Polyphenols : A Comparative Review on the Parental Compounds and Their Metabolites. *Food Rev. Int.* **2020**, *00*, 1–53, doi:10.1080/87559129.2020.1717523.

61. Yang, H.; Tian, T.; Wu, D.; Guo, D.; Lu, J. Prevention and treatment effects of edible berries for three deadly diseases: Cardiovascular disease, cancer and diabetes. *Crit. Rev. Food Sci. Nutr.* **2018**, *8398*, 1–10, doi:10.1080/10408398.2018.1432562.

62. Jelena, C.H.; Giorgio, R.; Justyna, G.; Neda, M.D.; Natasa, S.; Artur, B.; Giuseppe, G. *Beneficial effects of polyphenols on chronic diseases and ageing*; 2018; ISBN 9780128135723.

63. Khan, H.; Sureda, A.; Belwal, T.; Çetinkaya, S.; Süntar, İ.; Tejada, S.; Prasad, H.;

Ullah, H.; Aschner, M. Polyphenols in the treatment of autoimmune diseases. *Autoimmun. Rev. J.* **2019**, *18*, 647–657, doi:10.1016/j.autrev.2019.05.001.

64. Musial, C.; Siedlecka-Kroplewska, K.; Kmiec, Z.; Gorska-Ponikowska, M. Modulation of autophagy in cancer cells by dietary polyphenols. *Antioxidants* **2021**, *10*, 1–19, doi:10.3390/antiox10010123.

65. Motilva, M.; Bars-cortina, D.; Sakhawat, A.; Pi, C. Chemopreventive effects of anthocyanins on colorectal and breast cancer : A review. *Semin. Cancer Biol.* **2021**, *6*, 1203, doi:10.1016/j.semcancer.2020.12.013.

66. Al-Harbi, S.A.; Abdulrahman, A.O.; Zamzami, M.A.; Khan, M.I. Urolithins: The Gut Based Polyphenol Metabolites of Ellagitannins in Cancer Prevention, a Review. *Front. Nutr.* **2021**, *8*, 1–15, doi:10.3389/fnut.2021.647582.

67. Kiokias, S.; Proestos, C.; Oreopoulou, V. Phenolic acids of plant origin-a review on their antioxidant activity in vitro (O/W emulsion systems) along with their in vivo health biochemical properties. *Foods* **2020**, *9*, 534, doi:10.3390/foods9040534.

68. Almatroodi, S.A.; Alsahli, M.A.; Almatroudi, A.; Verma, A.K.; Aloliqi, A.; Allemailem, K.S.; Khan, A.A.; Rahmani, A.H. Potential Therapeutic Targets of Quercetin , a Plant Flavonol , and Its Role in the Therapy of Various Types of Cancer through the Modulation of Various Cell Signaling Pathways. *Molecules* **2021**, *26*, 1315, doi:https://doi.org/10.3390/molecules26051315.

69. Contardi, M.; Lenzuni, M.; Fiorentini, F.; Summa, M.; Bertorelli, R.; Suarato, G.; Athanassiou, A. Hydroxycinnamic acids and derivatives formulations for skin damages and disorders: A review. *Pharmaceutics* **2021**, *13*, doi:10.3390/pharmaceutics13070999.

70. Bayes, J.; Schloss, J.; Sibbritt, D. Effects of Polyphenols in a Mediterranean Diet on Symptoms of Depression: A Systematic Literature Review. *Adv. Nutr.* **2020**, *11*, 602–615, doi:10.1093/advances/nmz117.

71. Wong, S.K.; Chin, K.-Y.; Ima-Nirwana, S. Quercetin as an Agent for Protecting the Bone: A Review of the Current Evidence. *Int. J. Mol. Sci.* **2020**, *21*, 6448, doi:10.3390/ijms21176448.

72. Aguilera, J.M. The food matrix: implications in processing, nutrition and health. *Crit. Rev. Food Sci. Nutr.* **2018**, *0*, 1–18, doi:10.1080/10408398.2018.1502743.

73. Kashi, D.S.; Shabir, A.; Boit, M. Da; Bailey, S.J.; Higgins, M.F. The Efficacy of Administering Fruit-Derived Polyphenols to Improve Health Biomarkers, Exercise Performance and Related Physiological Responses. *Nutrients* **2019**, *11*, 2389, doi:10.3390/nu11102389.

74. van Breda, S.G.J.; de Kok, T.M.C.M. Smart Combinations of Bioactive Compounds in Fruits and Vegetables May Guide New Strategies for Personalized Prevention of Chronic Diseases. *Mol. Nutr. Food Res.* **2018**, *62*, 1–12, doi:10.1002/mnfr.201700597.

75. Kozłowska, A.; Dzierzanowski, T. Targeting Inflammation by Anthocyanins as the

Novel Therapeutic Potential for Chronic Diseases: An Update. *Molecules* **2021**, *26*, 4380, doi:https://doi.org/10.3390/molecules26144380.

76. FAO *Fruit and vegetables - your dietary essentials*-; 2020; ISBN 9789251337097.

77. EU Commission Knowledge4Policy Health Promotion and Disease Prevention Knowledge Gateway Available online: https://knowledge4policy.ec.europa.eu/health-promotion-knowledge-gateway/food-based-dietary-guidelines-europe-table-4_en.

78. Nunes, M.A.; Rodrigues, F.; Vinha, A.F.; Alves, R.C.; Oliveira, M.B.P.P. *Nutrigenomics and polyphenols*; Elsevier Inc., 2018; ISBN 9780128135723.

79. van Boekel, M.; Fogliano, V.; Pellegrini, N.; Stanton, C.; Scholz, G.; Lalljie, S.; Somoza, V.; Knorr, D.; Jasti, P.R.; Eisenbrand, G. A review on the beneficial aspects of food processing. *Mol. Nutr. Food Res.* **2010**, *54*, 1215–1247, doi:10.1002/mnfr.200900608.

80. Tomás-Barberán, F.A.; Espín, J.C. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *J. Sci. Food Agric.* **2001**, *81*, 853–876, doi:10.1002/jsfa.885.

81. Nayak, B.; Liu, R.H.; Tang, J. Effect of Processing on Phenolic Antioxidants of Fruits, Vegetables, and Grains—A Review. *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 887–919, doi:10.1080/10408398.2011.654142.

82. Tiwari, U.; Cummins, E. Factors influencing levels of phytochemicals in selected fruit and vegetables during pre- and post-harvest food processing operations. *Food Res. Int.* **2013**, *50*, 497–506, doi:10.1016/j.foodres.2011.09.007.

83. Williner, M.R.; Pirovani, M.E.; Güemes, D.R. Ellagic acid content in strawberries of different cultivars and ripening stages. *J. Sci. Food Agric.* **2003**, *83*, 842–845, doi:10.1002/jsfa.1422.

84. Pereira Lima, G.P.; Vianello, F.; Corrêa, C.R.; Arnoux, R.; Borguini, M.G. Polyphenols in Fruits and Vegetables and Its Effect on Human Health. *Food Nutr. Sci.* **2014**, *5*, 1065–1082, doi:http://dx.doi.org/10.4236/fns.2014.511117.

85. Ghorbal, A. Ben; Leventdurur, S.; Agirman, B.; Boyaci-Gunduz, C.P.; Kelebek, H.; Carsanba, E.; Darici, M.; Erten, H. Influence of geographic origin on agronomic traits and phenolic content of cv . Gemlik olive fruits. *J. Food Compos. Anal.* **2018**, *74*, 1–9, doi:10.1016/j.jfca.2018.08.004.

86. Hykkerud, A.L.; Uleberg, E.; Hansen, E.; Vervoort, M.; Martinussen, I. Seasonal and yearly variation of total polyphenols , total anthocyanins and ellagic acid in different clones of cloudberry (Rubus chamaemorus L .). *J. Appl. Bot. Food Qual.* **2018**, *91*, 96–102, doi:10.5073/JABFQ.2018.091.013.

87. Debelo, H.; Li, M.; Ferruzzi, M.G. Processing influences on food polyphenol profiles and biological activity. *Curr. Opin. Food Sci.* **2020**, *32*, 90–102, doi:10.1016/j.cofs.2020.03.001.

88. Arfaoui, L. Dietary plant polyphenols: Effects of food processing on their content and

bioavailability. *Molecules* **2021**, *26*, doi:10.3390/molecules26102959.

89. Arendse, E.; Fawole, O.; Opara, U. Effects of postharvest storage conditions on phytochemical and radical-scavenging activity of pomegranate fruit (cv . Wonderful). *Sci. Hortic. (Amsterdam).* **2014**, *169*, 125–129, doi:10.1016/j.scienta.2014.02.012.

90. Tomás-Barberán, F.A.; Espín, J.C. Effect of Food Structure and Processing on (Poly)phenol – Gut Microbiota Interactions and the Effects on Human Health. *Annu. Rev. Food Sci. Technol.* **2019**, *10*, 221–238, doi:https://doi.org/10.1146/annurev-food-032818-121615.

91. Knorr, D.; Augustin, M.A. Food processing need, advantages and misconceptions. *Trends Food Sci. Technol.* **2021**, *108*, 103–110, doi:10.1016/j.tifs.2020.11.026.

92. Scholz, R.; Herrmann, M.; Kittelmann, A.; von Schledorn, M.; van Donkersgoed, G.; Graven, C.; van der Velde-Koerts, T.; Anagnostopoulos, C.; Bempelou, E.; Michalski, B. Compendium of Representative Processing Techniques investigated in regulatory studies for pesticides. *EFSA Support. Publ.* **2018**, *EN-1508*, 204, doi:10.2903/sp.efsa.2018.EN-1508.

93. Norton, J.E.; Espinosa, Y.G.; Watson, R.L.; Spyropoulos, F.; Norton, I.T. Functional food microstructures for macronutrient release and delivery. *Food Funct.* **2015**, *6*, 663–678, doi:10.1039/c4fo00965g.

94. Turgeon, S.L.; Rioux, L. Food matrix impact on macronutrients nutritional properties. *Food Hydrocoll.* **2011**, *25*, 1915–1924, doi:10.1016/j.foodhyd.2011.02.026.

95. Kevers, C.; Pincemail, J.; Tabart, J.; Defraigne, J.O.; Dommes, J. Influence of cultivar, harvest time, storage conditions, and peeling on the antioxidant capacity and phenolic and ascorbic acid contents of apples and pears. *J. Agric. Food Chem.* **2011**, *59*, 6165–6171, doi:10.1021/jf201013k.

96. Fardet, A.; Richonnet, C. Nutrient density and bioaccessibility, and the antioxidant, satiety, glycemic, and alkalinizing potentials of fruit-based foods according to the degree of processing: a narrative review. *Crit. Rev. Food Sci. Nutr.* **2019**, *8398*, doi:10.1080/10408398.2019.1682512.

97. Cilla, A.; Rodrigo, M.J.; Ancos, B. De Impact of high-pressure processing on the stability and bioaccessibility of bioactive compounds in Clementine mandarin juice and its cytoprotective. **2020**, 8951–8962, doi:10.1039/d0fo02048f.

98. Shahidi, F.; Pan, Y. Influence of food matrix and food processing on the chemical interaction and bioaccessibility of dietary phytochemicals : A review. *Crit. Rev. Food Sci. Nutr.* **2021**, 1–25, doi:10.1080/10408398.2021.1901650.

99. Salazar-Orbea, G.L.; García-Villalba, R.; Tomás, F.A.; Sánchez-Siles, L.M. Review: High–Pressure Processing vs. Thermal Treatment: Effect on the Stability of Polyphenols in Strawberry and Apple Products. *Foods* **2021**, *10*, 2919, doi:https://doi.org/ 10.3390/foods10122919.

100. Misra, N.N.; Koubaa, M.; Roohinejad, S.; Juliano, P.; Alpas, H.; Inácio, R.S.; Saraiva, J.A.; Barba, F.J. Landmarks in the historical development of twenty first century food

processing technologies. *Food Res. Int.* **2017**, *97*, 318–339, doi:10.1016/j.foodres.2017.05.001.

101. Augusto, P.E.D. Challenges, trends and opportunities in food processing. *Curr. Opin. Food Sci.* **2020**, *35*, 72–78, doi:10.1016/j.cofs.2020.03.005.

102. Pérez-Andrés, J.M.; Charoux, C.M.G.; Cullen, P.J.; Tiwari, B.K. Chemical Modifications of Lipids and Proteins by Nonthermal Food Processing Technologies. *J. Agric. Food Chem.* **2018**, *66*, 5041–5054, doi:10.1021/acs.jafc.7b06055.

103. Aguilera, J.M. Relating Food Engineering to Cooking and Gastronomy. *Compr. Rev. Food Sci. Food Saf.* **2018**, *17*, 1021–1039, doi:10.1111/1541-4337.12361.

104. Liu, J.; Bi, J.; Julian, D.; Liu, X.; Yi, J.; Lyu, J.; Zhou, M.; Verkerk, R.; Dekker, M.; Wu, X.; et al. Impacts of thermal and non-thermal processing on structure and functionality of pectin in fruit- and vegetable- based products : A review. *Carbohydr. Polym.* **2020**, *250*, 116890, doi:10.1016/j.carbpol.2020.116890.

105. Richardson, P. *Thermal technologies in food processing*; Elsevier Inc., 2001; ISBN 978-1-85573-558-3.

106. Calín-Sánchez; Lipan, L.; Cano-Lamadrid, M.; Kharaghani, A.; Masztalerz, K.; Carbonell-Barrachina, A.A.; Figiel, A. Comparison of Traditional and Novel Drying Techniques and Its Effect on Quality of Fruits, Vegetables and Aromatic Herbs. *Foods* **2020**, *56*, 1710–1720, doi:10.3390/foods9091261.

107. Zhang, W.; Shen, Y.; Li, Z.; Xie, X.; Gong, E.S.; Tian, J.; Si, X.; Wang, Y.; Gao, N.; Shu, C.; et al. Effects of high hydrostatic pressure and thermal processing on anthocyanin content, polyphenol oxidase and β-glucosidase activities, color, and antioxidant activities of blueberry (Vaccinium Spp.) puree. *Food Chem.* **2021**, *342*, doi:10.1016/j.foodchem.2020.128564.

108. Agustin, M.; Riley, M.; Stockmann, R.; Bennett, L.; Kahl, A.; Lockett, T.; Osmond, M.; Sanguansri, P.; Stonehouse, W.; Zajac, I.; et al. Role of food processing in food and nutrition security. *Trends Food Sci. Technol.* **2016**, *56*, 115–125, doi:10.1016/j.tifs.2016.08.005.

109. Koszucka, A.; Nowak, A. Thermal processing food-related toxicants : a review. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 3579–3596, doi:10.1080/10408398.2018.1500440.

110. Román, S.; Sánchez-Siles, L.M.; Siegrist, M. The importance of food naturalness for consumers : Results of a systematic review. *Trends Food Sci. Technol.* **2017**, *67*, 44–57, doi:10.1016/j.tifs.2017.06.010.

111. Barbhuiya, R.I.; Singha, P.; Singh, S.K. A comprehensive review on impact of non-thermal processing on the structural changes of food components. *Food Res. Int.* **2021**, *149*, 110647, doi:10.1016/j.foodres.2021.110647.

112. Hite, B.H. The effect of pressure in the preservation of milk: a preliminary report. In *West Virginia Agricultural and Forestry Experiment Station Bulletins*; 1899; Vol. 58.

113. Farr, D. High pressure technology in the food industry. *Trends Food Sci. Technol.*

1990, *1*, 14–16, doi:https://doi.org/10.1016/0924-2244(90)90004-I.

114. Guerrero-Beltrán, J.A.; Barbosa-cánovas, G. V; Swanson, B.G.; Barbosa-cánovas, G. V; Swanson, B.G. High Hydrostatic Pressure Processing of Fruit and Vegetable Products. **2006**, *9129*, doi:10.1080/87559120500224827.

115. Abera, G. Review on high-pressure processing of foods. *Cogent Food Agric.* **2019**, *5*, doi:10.1080/23311932.2019.1568725.

116. Wael, E.; Johari, E.; Yus, Y.; Rosnah, S.; Anvarjon, A. High Pressure Processing Technology and Equipment Evolution: A Review. *J. Eng. Sci. Technol. Rev.* **2015**, *8*, 75–83, doi:10.25103/jestr.085.11.

117. Muntean, M.-V.; Marian, O.; Barbieru, V.; Catunescu, G.; Ranta, O.; Drocas, I.; Terhes, S. High pressure processing in food industry - Characteristics and applications. **2016**, *10*, 377–383, doi:10.1016/j.aaspro.2016.09.077.

118. Govaris, A.; Pexara, A. Inactivation of Foodborne Viruses by High-Pressure Processing (HPP). *Foods* **2021**, *10*, 215, doi:https://doi.org/ 10.3390/foods10020215.

119. Salvi, D.; Gosavi, N.S.; Karwe, M. V; Brunswick, N.; States, U. *High Pressure Cold Pasteurization*; Elsevier, 2016; ISBN 9780081005965.

120. Fam, S.N. High-Pressure Processing in Food. *Biointerface Res. Apllied Chem.* **2021**, *11*, 11553–11561, doi:https://doi.org/10.33263/BRIAC114.1155311561.

121. Martinez-Monteagudo, S.I.; Saldan, M.D.A. Chemical Reactions in Food Systems at High Hydrostatic Pressure. *Food Eng. Rev.* **2014**, *6*, 105–127, doi:10.1007/s12393-014-9087-6.

122. Makita, T. Application of high pressure and thermophysical properties of water to biotechnology. *Fluid Phase Equilib.* **1992**, *76*, 87–95, doi:10.1016/0378-3812(92)85079-N.

123. Aaby, K.; Grimsbo, I.H.; Hovda, M.B.; Rode, T.M. Effect of high pressure and thermal processing on shelf life and quality of strawberry purée and juice. *Food Chem.* **2018**, *260*, 115–123, doi:10.1016/j.foodchem.2018.03.100.

124. Benito-Román; Sanz, M.T.; Illera, A.E.; Melgosa, R.; Beltrán, S. Polyphenol oxidase (PPO) and pectin methylesterase (PME) inactivation by high pressure carbon dioxide (HPCD) and its applicability to liquid and solid natural products. *Catal. Today.* **2019**, *346*, 112–120, doi:10.1016/j.cattod.2018.12.051.

125. Holzwarth, M.; Korhummel, S.; Kammerer, D.R.; Carle, R. Thermal inactivation of strawberry polyphenoloxidase and its impact on anthocyanin and color retention in strawberry (Fragaria x ananassa Duch .) pure. *Eur. Food Res. Technol.* **2012**, *235*, 1171–1180, doi:10.1007/s00217-012-1852-2.

126. Teleszko, M.; Nowicka, P.; Wojdyło, A. Effect of cultivar and storage temperature on identification and stability of polyphenols in strawberry cloudy juices. *J. Food Compos. Anal.* **2016**, *54*, 10–19, doi:10.1016/j.jfca.2016.09.009.

127. Álvarez-fernández, M.A.; Hornedo-ortega, R.; Cerezo, A.B.; Troncoso, A.M.; García-

parrilla, M.C. Effects of the strawberry (Fragaria ananassa) purée elaboration process on non-anthocyanin phenolic composition and antioxidant activity. **2014**, *164*, 104–112, doi:10.1016/j.foodchem.2014.04.116.

128. Kim, A.N.; Lee, K.Y.; Rahman, M.S.; Kim, H.J.; Kerr, W.L.; Choi, S.G. Thermal treatment of apple puree under oxygen-free condition: Effect on phenolic compounds, ascorbic acid, antioxidant activities, color, and enzyme activities. *Food Biosci.* **2021**, *39*, 100802, doi:10.1016/j.fbio.2020.100802.

129. Murtaza, A.; Iqbal, A.; Marszałek, K.; Iqbal, M.A.; Ali, S.W.; Xu, X.; Pan, S.; Hu, W. Enzymatic, phyto-, and physicochemical evaluation of apple juice under high-pressure carbon dioxide and thermal processing. *Foods* **2020**, *9*, 1–15, doi:10.3390/foods9020243.

130. Tian, Y.; Sun, L.; Yang, Y.; Gou, X.; Niu, P.; Guo, Y. Changes in the physicochemical properties , aromas and polyphenols of not from concentrate (NFC) apple juice during production. *CyTA - J. Food* **2018**, *16*, 755–764, doi:10.1080/19476337.2018.1471102.

131. Pedrosa, M.C.; Lima, L.; Heleno, S.; Carocho, M.; Ferreira, I.C.F.R.; Barros, L. Food metabolites as tools for authentication, processing, and nutritive value assessment. *Foods* **2021**, *10*, 2213, doi:10.3390/foods10092213.

132. Utpott, M.; Rodrigues, E.; Rios, A. de O.; Mercali, G.D.; Flôres, S.H. Metabolomics: An analytical technique for food processing evaluation. *Food Chem.* **2022**, *366*, 130685, doi:10.1016/j.foodchem.2021.130685.

133. Santiago-Rodriguez, T.M.; Hollister, E.B. Multi ' omic data integration : A review of concepts , considerations , and approaches. *Semin. Perinatol.* **2021**, *45*, 151456, doi:10.1016/j.semperi.2021.151456.

134. Klevorn, C.M.; Dean, L.L. A metabolomics-based approach identifies changes in the small molecular weight compound composition of the peanut as a result of dry-roasting. *Food Chem.* **2018**, *240*, 1193–1200, doi:10.1016/j.foodchem.2017.08.058.

135. Álvarez-Rivera, G.; Valdés, A.; León, C.; Cifuentes, A. *Foodomics – Fundamentals , State of the Art and Future Trends*; The Royal Society of Chemistry, 2021; ISBN 9781839163005.

136. Valdés, A.; Álvarez-Rivera, G.; Socas-Rodríguez, B.; Herrero, M.; Ibáñez, E.; Cifuentes, A. Foodomics: Analytical Opportunities and Challenges. *Anal. Chem.* **2022**, *94*, 366–381, doi:10.1021/acs.analchem.1c04678.

137. Lacalle-Bergeron, L.; Izquierdo-Sandoval, D.; Sancho, J. V; Francisco, L.; Portolés, T. Chromatography hyphenated to high resolution mass spectrometry in untargeted metabolomics for investigation of food (bio) markers. *Trends Anal. Chem.* **2021**, *135*, 116161, doi:10.1016/j.trac.2020.116161.

138. van den Berg, R.A.; Hoefsloot, H.C.J.; Westerhuis, J.A.; Smilde, A.K.; van der Werf, M.J. Centering, scaling, and transformations: Improving the biological information content of metabolomics data. *BMC Genomics* **2006**, *7*, 142, doi:10.1186/1471-2164-7-142.

139. Lopez-Sanchez, P.; De Vos, R.C.H.; Jonker, H.H.; Mumm, R.; Hall, R.D.; Bialek, L.; Leenman, R.; Strassburg, K.; Vreeken, R.; Hankemeier, T.; et al. Comprehensive metabolomics to evaluate the impact of industrial processing on the phytochemical composition of vegetable purees. *Food Chem.* **2015**, *168*, 348–355, doi:10.1016/j.foodchem.2014.07.076.

140. Lucini, L.; Rocchetti, G.; Kane, D.; Trevisan, M. Phenolic fingerprint allows discriminating processed tomato products and tracing different processing sites. *Food Control* **2017**, *73*, 696–703, doi:10.1016/j.foodcont.2016.09.020.

141. Teegarden, M.D.; Schwartz, S.J.; Cooperstone, J.L. Profiling the impact of thermal processing on black raspberry phytochemicals using untargeted metabolomics. *Food Chem.* **2019**, *274*, 782–788, doi:10.1016/j.foodchem.2018.09.053.

142. Chen, Q.; Shi, J.; Mu, B.; Chen, Z.; Dai, W.; Lin, Z. Metabolomics combined with proteomics provides a novel interpretation of the changes in nonvolatile compounds during white tea processing. *Food Chem.* **2020**, *332*, 127412, doi:10.1016/j.foodchem.2020.127412.

143. Garcia, C.J.; García-Villalba, R.; Garrido, Y.; Gil, M.I.; Tomás-Barberán, F.A. Untargeted metabolomics approach using UPLC-ESI-QTOF-MS to explore the metabolome of fresh-cut iceberg lettuce. *Metabolomics* **2016**, *12*, 138, doi:10.1007/s11306-016-1082-x.

144. Kennedy, J.A.; Jones, G.P. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.* **2001**, *49*, 1740–1746, doi:10.1021/jf001030o.

145. Díaz-Mula, H.M.; Tomás-Barberán, F.A.; García-Villalba, R. Pomegranate Fruit and Juice (cv. Mollar), Rich in Ellagitannins and Anthocyanins, Also Provide a Significant Content of a Wide Range of Proanthocyanidins. *J. Agric. Food Chem.* **2019**, *67*, 9160–9167, doi:10.1021/acs.jafc.8b07155.

146. Wichchukit, S.; O'Mahony, M. The 9-point hedonic scale and hedonic ranking in food science: Some reappraisals and alternatives. *J. Sci. Food Agric.* **2015**, *95*, 2167–2178, doi:10.1002/jsfa.6993.

147. RStudio Team RStudio: Integrated Deevelopment for R Available online: http://www.rstudio.com/.

148. Chen, D.; Pang, X.; Zhao, J.; Gao, L.; Liao, X.; Wu, J.; Li, Q. Comparing the effects of high hydrostatic pressure and high temperature short time on papaya beverage. *Innov. Food Sci. Emerg. Technol.* **2015**, *32*, 16–28, doi:10.1016/j.ifset.2015.09.018.