Centro de Investigación en Alimentación y Desarrollo, A.C.

EFECTO DE LA MATRIZ ALIMENTARIA SOBRE LA BIOACCESIBILIDAD, BIODISPONIBILIDAD Y CAPACIDAD ANTIOXIDANTE DE LOS COMPUESTOS FENÓLICOS PRESENTES EN MANGO CV. 'ATAULFO' (*Mangifera indica* L.)

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CONTENIDO

RESUMEN

El mango (*Mangifera indica* L.) cultivar ‘Ataulfo’ es una fuente rica en compuestos fenólicos (CF), por lo que se le considera un “alimento naturalmente funcional” con efectos benéficos a la salud. El impacto fisiológico que ejercen los CF en el organismo, depende de su bioaccesibilidad a partir del alimento que los contiene y de su biodisponibilidad en el tracto-gastrointestinal humano. Sin embargo, existen factores que afectan estos procesos de liberación/absorción/entrega tales como la composición de la matriz alimentaria (e.g. presencia y tipo de fibra dietaria; estado de madurez) y el procesamiento del alimento. El objetivo general de este estudio fue el *evaluar el efecto de la matriz alimentaria sobre la bioaccesibilidad, biodisponibilidad y capacidad antioxidante de los CF presentes en mango ‘Ataulfo’*. Primeramente se evaluó la bioaccesibilidad, difusión pasiva y capacidad antioxidante de los CF presentes en matrices alimentarias con diferente contenido y tipo de fibra: pulpa de mango ‘Ataulfo’ y salvado de trigo. Posteriormente se evaluó el efecto del estado de madurez de mango (EM1, EM2, EM3) sobre la bioaccesibilidad de los CF presentes. Además, se investigó el efecto del procesamiento de jugo de mango sobre el contenido de CF, capacidad antioxidante y bioaccesibilidad. Finalmente, se realizó un ensayo clínico cruzado para evaluar el efecto del consumo de mango en diferente matriz alimentaria (pulpa y jugo) sobre la biodisponibilidad y capacidad antioxidante de los CF. Se encontró que la bioaccesibilidad *in vitro* de los CF es mayor en mango que en salvado de trigo, y se atribuyó principalmente a la composición de fibras y microestructura de la matriz alimentaria, siendo mayor en aquellas muestras con menor contenido de fibra (mango). También se encontró que la composición de la matriz alimentaria durante los estados de madurez de mango tiene una influencia directa en la bioaccesibilidad y capacidad antioxidante *in vitro* de los compuestos fenólicos presentes; se mostró mayor bioaccesibilidad en el EM3 (maduro). Además, el procesamiento de jugo de mango afecta la forma y composición de la matriz alimentaria, disminuyendo la concentración de CF y aumentando su bioaccesibilidad *in vitro*. Por último, el consumo *in vivo* de pulpa y jugo de mango permite la absorción, metabolismo y excreción de los CF presentes, no habiendo diferencia significativa entre ambas matrices alimentarias. En base a estos resultados se puede inferir que el consumo de pulpa o jugo de mango incrementa la presencia de fenoles en el cuerpo humano, los cuales han demostrado poseer propiedades bioactivas.

Palabras clave: mango, compuestos fenólicos, matriz alimentaria, bioaccesibilidad, biodisponibilidad.

ABSTRACT

‘Ataulfo’ mango is a rich source of phenolic compounds (PC), and therefore it is considered as a “naturally functional food” with benefitial health effects. The physiological impact exerted by PC in the body depends on its bioaccessibility from the food that contains and its bioavailability in the human gastrointestinal-tract. However, several factors affect these release/absorption/delivery processes, such as the composition of the food matrix (e.g. presence and type of dietary fiber stage of ripeness) and food processing. The main objective of this study was to *evaluate the effect of the food matrix on the bioaccessibility, bioavailability and antioxidant capacity of phenolic compounds present in ‘Ataulfo’ mango.* First, the *in vitro* bioaccessibility, passive diffusion and antioxidant capacity of PC present in two plants food with different content and type of dietary fiber: mango flesh and wheat bran, was evaluated. Then, the effect of ripeness mango (“EM1”, EM2”, “EM3”) on the bioaccessibility of the PC was evaluated. Furthermore, the effect of ‘Ataulfo’ mango juice processing on the content of PC, antioxidant capacity and bioaccessibility was investigated. Finally, we carried out a pilot randomized crossover clinical trial to evaluate the effect of consumption of different ‘Ataulfo’ mango food matrix (flesh and juice) on the acute bioavailability of PC. It was found that the bioaccessibility of phenolics in mango is greater that in wheat bran, and was largely attributed to the composition and microstructure of the food matrix, being higher in those samples with lower fiber content (mango). It was also found that the bioaccessibility and antioxidant capacity of mango phenolic compounds is affected by the physical structure and composition of the food matrix during ripening; high bioaccessibility in EM3 (ripe mango). In addition, the processing of mango juice affects the composition of the food matrix, decreasing the concentration of phenolic compounds and increasing its bioaccessibility. Lastly, mango flesh and juice consumption allows the absorption, metabolism and excretion of phenolic compounds, with no significant difference between the two food matrices. Based on these results, it can be inferred that consumption of ‘Ataulfo’ mango flesh or juice increases the presence of phenols in the human body, which have been shown to possess bioactive properties.

Keywords: mango, phenolic compounds, food matrix, bioaccessibility, bioavailability

SINOPSIS

Los compuestos fenólicos son metabolitos secundarios de las plantas de gran interés alimentario, debido a que existen evidencias que respaldan sus efectos bioactivos y su consecuente protección a la salud humana. Su efecto protector se atribuye principalmente a las propiedades antioxidantes que poseen éstas moléculas. Estos compuestos se encuentran ubicuos en la mayoría de las frutas y hortalizas y entre los frutos tropicales destaca el mango (*Mangifera indica* L.*)* 'Ataulfo', un cultivar de origen mexicano preferido por sus consumidores debido a sus excelentes características organolépticas, sensoriales y funcionales. Estudios previos han reportado que la pulpa de mango 'Ataulfo' contiene una alta concentración de compuestos fenólicos, particularmente ácidos fenólicos con una alta capacidad antioxidante. El potencial antioxidante de un fruto está determinado por el tipo y concentración de compuestos fenólicos presentes y otros antioxidantes como la vitamina C. En este sentido, debido a los altos niveles de estos compuestos, esta variedad de mango ha sido considerada como un "alimento naturalmente funcional", ya que su consumo en fresco podría ejercer algunos efectos benéficos a la salud del consumidor.

Para que un compuesto fenólico proporcione efectos bioactivos en el organismo humano debe cumplir con ciertos requerimientos. Primeramente, debe estar; (1) liberado de la matriz alimentaria que lo contiene (bioaccesibilidad); (2) ser absorbido intacto o bio-transformado en compuestos activos por el epitelio gastrointestinal absorción, y por último; (3) debe pasar a la sangre o linfa para ser transportado a otros órganos y/o tejidos donde ejercerá su acción (biodisponibilidad). No obstante, existen numerosos factores externos e internos que afectan la liberación y/o absorción de los compuestos fenólicos, ya sea de forma directa durante su paso por el tracto gastrointestinal o disminuyendo el contenido de éstos en el alimento. Entre los principales factores que afectan estos procesos destacan la composición de la matriz alimentaria (e.g. presencia de fibra dietaria), factores ambientales (e.g. estado de madurez del fruto) y factores relacionados al procesamiento del alimento.

Por lo anterior, no solo es importante cuantificar el contenido de compuestos bioactivos en un alimento, sino profundizar aún más en las posibles interacciones moleculares que podrían estar afectando la bioaccesibilidad y biodisponibilidad de éstos compuestos.

Los compuestos fenólicos y la fibra dietaria son dos constituyentes de gran importancia en los alimentos de origen vegetal. Ambos están asociados con diversos beneficios para la salud. Generalmente, estas moléculas se han estudiado por separado debido a sus diferencias estructurales, fisicoquímicas, biológicas y metabólicas. Sin embargo, en los últimos años ha surgido evidencia científica suficiente que sugiere que los componentes no digeribles (indigestibles) de la fibra dietaria (polisacáridos) pueden secuestrar otros constituyentes de los alimentos, incluyendo a los compuestos fenólicos. Sin embargo, tal fisicoquímica dentro del tracto gastrointestinal puede modificarse por diversos factores incluyendo desarrollo del fruto, su procesamiento o cambios durante el proceso digestivo. Existe controversia sobre los efectos de la interacción de ambas moléculas en términos tecnológicos y nutricionales, ya que se ha reportado que puede llegar a impedir la liberación, absorción y acción de las moléculas antioxidantes.

Durante el desarrollo y maduración del fruto ocurren cambios fisiológicos, que afectan la estructura y composición de la matriz alimentaria. En frutos como la papaya, piña, mango y el aguacate, la concentración de compuestos fenólicos asociados a moléculas indigestibles (conocidos como compuestos fenólicos no-extraíbles, fibra dietaria antioxidante o antioxidantes macromoleculares) disminuye durante la maduración del fruto. Uno de los cambios de mayor importancia durante la maduración del fruto es la modificación de la pared celular en términos de estado físico, estructural y de composición, resultando en la pérdida de firmeza. La modificación de los polisacáridos de la pared celular durante la maduración del fruto conlleva a cambios en el contenido de fibra dietaria, lo cual puede favorecen la liberación y aumentar la bioaccesibilidad de los fenoles, siendo mayor en los frutos maduros. No obstante, el procesamiento de los alimentos (prensando, estrujado, secado, tratamientos térmicos, altas presiones, ultrasonido, etc.) afecta directamente la estructura y composición de la matriz alimentaria. Estos cambios pueden llegar a afectar de forma positiva o negativa la liberación, absorción y las propiedades bioactivas de los compuestos de interés.

El **CAPITULO I** presenta una revisión sobre el uso de los compuestos fenólicos y la fibra dietaria como ingredientes funcionales para enriquecer alimentos como bebidas, pastas, carne, productos lácteos, etc. Se habla también sobre el uso de un material que combina las propiedades de éstas dos moléculas: fibra dietaria antioxidante. Además, describe los tipos de interacción que pueden llegar a surgir entre ambas moléculas y los posibles efectos fisiológicos en el organismo después de su ingesta. El concepto de "fibra dietaria antioxidante" surgió en años recientes y se definió como un concentrado de fibra dietaria que contiene cantidades significativas de antioxidantes naturales (principalmente compuestos fenólicos) asociados a compuestos indigestibles (polisacáridos). Este material cobró popularidad debido a que según algunos estudios combina los efectos funcionales de ambas moléculas y promete aumentar las propiedades tecnológicas y bioactivas de diversos productos. La revisión narrativa explica que la presencia de estas moléculas en un alimento no garantiza su biodisponibilidad y efecto bioactivo en la salud humana debido a las interacciones que pueden surgir entre ellas. Estas interacciones pueden ser puentes de hidrógeno, interacciones covalentes o mediante un atrapamiento físico. No obstante, se ha reportado que aquellos compuestos fenólicos asociados o atrapados en la fibra dietaria pueden ser arrastrados al colon y ejercer ahí efectos biológicos, por lo que pueden jugar un rol importante en la salud intestinal. La revisión termina sugiriendo realizar estudios *in vitro* e *in vivo* que investiguen el efecto de la matriz alimentaria de un alimento sobre la bioaccesibilidad y biodisponibilidad de los compuestos fenólicos, particularmente en presencia de fibra dietaria. Por lo que estudiamos cómo se veía afectada la biodisponibilidad de los fenoles con la presencia del tipo de fibra. Para ello se comparó la fibra presente en mango con la de salvado de trigo, con el fin de comprobar cómo las interacciones moleculares afectaban la biodisponibilidad de fenoles.

Con estos antecedentes, nos planteamos la hipótesis de que la bioaccesibilidad (cantidad y concentración de un compuesto presente en el intestino delgado como consecuencia de su liberación de la matriz alimentaria) y biodisponibilidad (cantidad y velocidad a la que el compuesto se absorbe y llega al lugar de acción) de los compuestos fenólicos presentes en mango cv. 'Ataulfo', puede verse limitada por el tipo de matriz alimentaria, reflejándose directamente en la concentración y propiedad antioxidante de estos compuestos de forma *in vitro*.

El **CAPITULO II** presenta la evaluación de la bioaccesibilidad, difusión pasiva y capacidad antioxidante *in vitro* de los compuestos fenólicos presentes en dos alimentos con diferente tipo y cantidad de fibras dietaria: pulpa de mango 'Ataulfo' y salvado de trigo, utilizando una simulación de digestión *in vitro*. Se examinó la microestructura de las muestras mediante microscopía electrónica de barrido. Por otra parte, los resultados de difusión pasiva, como posible explicación de un fenómeno in vivo de transporte para celular, se ajustaron a un modelo matemático. Al analizar los resultados se encontró que ambas muestras presentan microestructuras diferentes, la pulpa de mango se mostró amorfa, mientras que la de salvado de trigo mostró una red estructural compleja debido a la alta presencia de arabinoxilanos (celulosas). Los tipos de microestructura de los alimentos evaluados se atribuyeron al tipo de fibra predominante en cada muestra: mientras que el salvado de trigo es rico en fibra insoluble, la pulpa de mango se presenta una relación similar de ambos tipos de fibra predominando la soluble pero en menor cantidad total (fibras solubles + insolubles).

La bioaccesibilidad de los compuestos fenólicos fue mayor en la pulpa de mango 'Ataulfo', lo cual se atribuyó al tipo y contenido de fibra dietaria en comparación al salvado de trigo, así, la pulpa de mango presentó menor cantidad de fibra, siendo esta predominantemente fibra soluble. La concentración y capacidad antioxidante de los compuestos fenólicos durante las diferentes etapas de digestión fue menor a la presente en los alimentos sin ser digeridos, lo que indica una pérdida o atrapamiento de éstos. Para finalizar, se encontró que el mecanismo de difusión de los compuestos fenólicos libres fue del tipo Fickiana determinada por el gradiente de concentración y/o por una relajación controlada de la matriz alimentaria. Estos resultados sugirieren que la bioaccesibilidad de los compuestos fenólicos depende en gran medida de la composición y estructura de la matriz del alimento, en la cual se encuentran embebidos, siendo mayor en las matrices con menor contenido de fibra dietaria. Lo anterior coinciden con un estudio previo publicado, en donde el salvado de trigo disminuye en mayor porcentaje el contenido de compuestos fenólicos presentes en un extracto metanólico de diversos frutos tropicales (mango, papaya, piña y guayaba).

Posteriormente, en el **CAPITULO III,** se evaluó el efecto del estado de madurez de mango 'Ataulfo' sobre el contenido total, bioaccesibilidad y capacidad antioxidante de los compuestos fenólicos presentes. Se utilizaron muestras de mango

en 3 estados de madurez de acuerdo a las diferencias entre la cantidad de nutrientes reportados y publicados previamente en el laboratorio, “poco maduro” EM1, “moderadamente maduro” EM2 y “completamente maduro” EM3. Primeramente, se realizó una caracterización fisiológica y química (pH, sólidos solubles totales, ácido cítrico y málico, acidez titulable, firmeza, color, compuestos fenólicos, capacidad antioxidante) de las tres muestras con fines comparativos a un estudio previo pero incluyendo la evaluación del perfil fenólico de la porción no digestible. Además se determinó el contenido de fibra dietaria, se examinó su microestructura mediante microscopía electrónica de barrido y se evaluó su digestibilidad aparente. Los resultados mostraron que el estado de madurez del fruto afecta el contenido de compuestos fenólicos totales (extraíbles + no extraíbles) y su capacidad antioxidante, los cuales disminuyen conforme el fruto madura y se atribuye al proceso de maduración. Además, la composición y estructura de la matriz alimentaria cambia de firme a suave durante la maduración, esto se debe a la actividad de ciertas enzimas como pectin-metil-esterasa, poligalacturonasa, entre otras, involucradas en hidrolizar polisacáridos de la pared celular. Como resultado de la acción de estas enzimas, observamos una reducción en el contenido de fibra dietaria y almidón durante el proceso de maduración.

La evaluación de la bioaccesibilidad de los compuestos fenólicos mostró que estas moléculas presentan mayor bioaccesibilidad en el fruto EM3 en comparación al EM1. Estos resultados se pueden atribuir a las diferencias en la composición y microestructura de las matrices alimentarias. Como se mencionó previamente, el mango EM1 presentó mayor contenido de fibra dietaria y almidón, y se caracterizó por su alta firmeza. Estas características indican que los polisacáridos contenidos en la matriz alimentaria podrían estar interaccionando con los compuestos fenólicos e interfiriendo en su liberación. En cambio, el mango EM3 presentó una menor firmeza y un mayor ablandamiento. La suavidad de las frutas EM3 aumenta la bioaccesibilidad de algunos compuestos, ya que es más fácil que las enzimas digestivas penetren la matriz alimentaria y provoquen su ruptura.

La simulación del proceso de absorción intestinal se llevó a cabo mediante una diálisis de los compuestos fenólicos liberados en las etapas gástrica e intestinal. Se mostró que la cantidad de compuestos fenólicos absorbidos mediante difusión aumenta en el mango EM1, EM2 y EM3, respectivamente. Estos resultados fueron menores al contenido total de fenoles bioaccesibles en la digestión gastrointestinal.

Lo anterior sugirió que los compuestos fenólicos liberados podrían estar interaccionando con otros compuestos resultantes de la digestión del alimento e impidiendo su transporte. Al mismo tiempo la capacidad antioxidante de las muestras digeridas se vio afectada por las condiciones fisicoquímicas de las etapas gastrointestinales. Los resultados claramente mostraron que la composición de la matriz alimentaria durante los diferentes estados de madurez de mango 'Ataulfo', tiene una influencia directa en la bioaccesibilidad de los compuestos fenólicos, fenómeno que hace sinergia con los eventos de modificación gastrointestinal de la matriz alimentaria de este fruto.

Con el objetivo de continuar evaluando diferentes factores que pudieran interferir con la bioaccesibilidad y biodisponibilidad de los compuestos fenólicos, en el **CAPITULO IV** se investigó el efecto del procesamiento de jugo de mango 'Ataulfo' sobre el contenido compuestos fenólicos, capacidad antioxidante y su bioaccesibilidad después de una digestión *in vitro*. Se utilizaron frutos de mango en estado maduro EM3, los cuales se lavaron, pelaron, cortaron y se introdujeron a un extractor de jugos eléctrico. De esta forma se modificó la microestructura alimentaria original de la pulpa de mango y se eliminó una pequeña parte del material insoluble presente en la matriz alimentaria, obteniéndose un material en su mayoría soluble. También se evaluó la composición de carbohidratos presente. Los resultados mostraron que el contenido de fibra dietaria y almidón fue ligeramente menor al presente en la pulpa. Además, el procesamiento de jugo de mango tuvo un impacto en la disminución del contenido de compuestos fenólicos y su capacidad antioxidante. Una posible explicación a este comportamiento pudiera deberse a que la pared celular o membrana del fruto se rompe durante el proceso de extracción, permitiendo la liberación de enzimas que generan reacciones de oxidación y degradación. La concentración de compuestos fenólicos no extraíbles (asociados a otros compuestos) se redujo, lo cual coincide con una disminución de fibra dietaria.

No obstante, al evaluar la bioaccesibilidad y absorción pasiva de los compuestos fenólicos presentes en jugo de mango 'Ataulfo', se encontró que ésta fue ligeramente mayor en comparación a la obtenida en pulpa. Estos resultados coinciden con los reportados por otros autores para diferentes alimentos procesados, indicando que ciertos tipos de procesamiento pueden llegar a incrementar la liberación y/o absorción de algunos antioxidantes. Esto se debe a que algunos tipos de procesamiento eliminan material indigestible de la matriz del alimento,

aumentando la solubilidad de otros compuestos y facilitando su liberación y transporte. Se cree que este fenómeno podría potencializar aún más la bioaccesibilidad de compuestos fenólicos presentes en matrices alimentarias muy complejas y con concentraciones de fibra dietaria muy altas.

Para finalizar, con el objetivo de completar la información obtenida de estudios *in vitro*, en el **CAPITULO V** se realizó un ensayo clínico aleatorizado cruzado para evaluar el efecto del consumo de mango 'Ataulfo' en diferente matriz alimentaria (pulpa y jugo) sobre la biodisponibilidad y capacidad antioxidante de los compuestos fenólicos. Los participantes consumieron en diferentes tiempos una sola cantidad de pulpa y jugo de mango 'Ataulfo' que proporcionaba similar concentración de compuestos fenólicos. En este tipo de estudio cada sujeto ejerce como su propio control y así se consigue mantener equilibradas las variables de confusión que puedan existir. Se monitoreo el metabolismo de los compuestos fenólicos durante 24 h posteriores al consumo del alimento. Se recolectaron muestras de sangre (0, 1, 2, 3, 4, 5 y 6 h) y de orina (0-4, 4-8, 8-12, 12-24 h).

Los resultados arrojaron que el consumo de ambas matrices alimentarias permite la absorción, metabolismo y excreción de los compuestos fenólicos presentes, no habiendo diferencia significativa entre ambas matrices alimentarias. Estos resultados confirman los resultados obtenidos en los capítulos previos, que indican que alrededor del 50% de los compuestos fenólicos presentes en pulpa y jugo de mango 'Ataulfo' son liberados y están potencialmente disponibles para ser absorbidos. A pesar de no haberse encontrado diferencia significativa entre ambas matrices, los compuestos fenólicos presentes en jugo se absorbieron en un ligero menor tiempo y mayor concentración. Un resultado importante fue la identificación de una alta concentración del metabolito microbiano 'pirogalol' en orina, lo cual se atribuye a la metabolización colónica de ácido gálico libre y polimerizado presente en mango cv. 'Ataulfo'.

De acuerdo a los resultados obtenidos se concluye que existen diversos factores, como el contenido de fibra dietaria, el estado de madurez del fruto, así como el procesamiento del alimento, que pueden influir muy ligeramente en la bioaccesibilidad *in vitro* de los compuestos fenólicos presentes en mango 'Ataulfo'. Esta influencia se atribuye principalmente a la composición y forma de la matriz alaimentaria. No obstante, la absorción y metabolismo *in vivo* de los compuestos fenólicos no se ve afectada por el estado de la matriz alimentaria de mango 'Ataulfo'.

Con los resultados presentados en este estudio, el consumo de pulpa y/o mango ‘Ataulfo’ podría considerarse como una fuente potencial para incrementar la presencia de ácidos fenólicos en el organismo humano, los cuales han demostrado poseer propiedades antiinflamatorias y anticancerígenas. Futuras investigaciones podrían estar enfocadas sobre la distribución de los compuestos fenólicos en órganos y tejidos. Además de evaluar su efecto bioactivo sobre diversas enfermedades.

CONCLUSIONES GENERALES

A partir de los resultados derivados de este estudio se tienen las siguientes conclusiones:

1. La bioaccesibilidad *in vitro* de los compuestos fenólicos es mayor en mango ‘Ataulfo’ que en el salvado de trigo, ya que ésta depende en gran medida de la composición y microestructura de la matriz del alimento en la cual se encuentran embebidos, siendo mayor en aquellos con menor contenido de fibra dietaria.

2. La bioaccesibilidad *in vitro* y capacidad antioxidante de los compuestos fenólicos de mango ‘Ataulfo’, es afectada por la estructura física y composición de la matriz alimentaria durante el proceso de maduración del fruto.

3. El procesamiento de jugo de mango ‘Ataulfo’ afecta la forma y composición de la matriz alimentaria, disminuyendo la concentración de compuestos fenólicos y aumentando su bioaccesibilidad *in vitro*.

4. El consumo *in vivo* de pulpa y jugo de mango ‘Ataulfo’ permite la absorción, metabolismo y excreción de los compuestos fenólicos presentes, no habiendo diferencia significativa entre ambas matrices alimentarias.

CAPÍTULO I

Compuestos fenólicos y fibra dietaria como ingredientes funcionales: interacción y posible efecto después de su ingesta

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Dietary fiber and phenolic compounds as functional ingredients: interaction and possible effect after ingestion

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Dietary fiber and phenolic compounds are two recognized dietary factors responsible for potential effects on human health; therefore, they have been widely used to increase functionality of some foods. This paper focuses on showing the use of both substances as functional ingredients for enriching foods, and at the same time, describes the use of a single material that combines the properties of the two types of substances. The last part of the work describes some facts related to the interaction between dietary fiber and phenolic compounds, which could affect the bioaccessibility and absorption of phenolics in the gut. In this sense, the purpose of the present review is to compile and analyze evidence relating to the use of dietary fiber and phenolic compounds to enhance technological and nutritional properties of foods and hypothesize some of the possible effects in the gut after their ingestion.

Introduction

Dietary fiber and phenolic compounds are two plant food constituents that are associated with many health benefits and have been demonstrated to reduce risk for developing cancer and some chronic diseases.[1] Therefore, the intake and the use of these compounds as functional ingredients to enrich foods have been increasing in order to provide health benefits to consumers.[2,3] Dietary fiber has an essential role in intestinal health and appears to be significantly associated with a reduction of cholesterolaemia and modification of the glycaemic response.[4] Furthermore, phenolic compounds have potent antioxidant and free-radical scavenging properties that protect against oxidative damage to important biomolecules.[5] All these properties are associated with the chemical structures of these compounds, which determine their subsequent physiological and nutritional properties as functional ingredients.[6]

Dietary fiber and phenolic compounds are generally studied separately, probably because of differences in their chemical structures, physicochemical and biological properties, and metabolic pathways.[7] However, there is scientific evidence suggesting that indigestible components of dietary fiber (polysaccharides) can associate with other food constituents, such as phenolic compounds.[7,8] This interaction can occur during fruit ripening, food processing or during the gastrointestinal process and can be ascribed to the ability of polysaccharides to bind and trap phenolic compounds at several sites.[7] Therefore, dietary fibers with associated phenolic compounds have become increasingly interesting, as these could be useful for the food industry to enhance the bioactive and technological properties of products.

Some authors have identified dietary fiber with associated phenolic compounds with an exceptional biological antioxidant capacity from mango peels, unripe whole mango, pineapple shells, guava pulp, grape pomace and other vegetable materials.[9–12] This material promises an enhancement in functional properties of foods and at the same time an increase in the antioxidant capacity of the product with exceptional effects on human health.[13–17] However, the *in vivo* antioxidant activity of dietary fibers with associated phenolic compounds is still disputed, because the bioavailability of the antioxidants is no guarantee, due to evidence indicating that dietary fiber may negatively affect the release and absorption of some molecules, including phenolic compounds.[18]

In this context, the aim of this review is to analyze the use of dietary fiber and phenolic compounds as advantageous functional ingredients, as well as to describe the chemical interaction that can arise between these two components (fiber and phenolics), which can provide functionality to the food but may impact the bioactive effects of the compounds after their intake.

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Dietary fiber and phenolic compounds

Dietary fiber and phenolic compounds are two important plant constituents that are associated with multiple physiological effects; so their study, consumption and use as functional ingredients have widely increased.[10] According to AACC[30] and DeVries *et al.*,[31] dietary fiber is defined as "the remnants of the edible part of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine, with complete or partial fermentation in the large intestine". Non-starch polysaccharides are considered the main dietary fiber components, and they are classified by their solubility as insoluble (IDF) and soluble dietary fiber (SDF). IDF includes cellulose, hemicellulose or chitin; however, resistant starch is also consider a type of IDF, whereas the SDF class includes non-starch polysaccharides such as pectins, β-glucans, gums, mucilage, oligosaccharides or inulin.[32] Besides, other indigestible compounds can be considered as part of the dietary fiber structure, such as resistant protein, phenolic compounds, waxes, saponins, phytates, and phytosterols that exist in plant cell structures.[33] In fact, some works hypothesize that several bioactive benefits of dietary fiber are determined by the action of some linked compounds, such as phenolic compounds.[34,35]

High dietary fiber diets are associated with improvement in gastrointestinal health and reduction and treatment of some cardiovascular diseases and some forms of cancer.[36,37] Indeed, a reduction of hyperlipidemia, hypertension, modification of the glucose tolerance and insulin response and increased satiety, and hence some degree of weight management, are other physiological effects associated with dietary fiber consumption in humans.[4] Furthermore, dietary fiber is widely used to enrich foods, because it can impart some functional properties (*e.g.* increase water holding capacity, emulsification and/or gel formation, viscosity, adsorption/binding or fermentability).[4] In this sense, consumption of dietary fiber-rich foods, as well as the use of dietary fiber as a functional ingredient, has increased.

Phenolic compounds are other important bioactive compounds identified in plant foods and represent a wide variety of compounds characterized by a phenolic structure (aromatic ring bearing one or more hydroxyl groups).[38] They are present in all plant organs, having great significance in plant physiology and protecting plants against pathogens, parasites, predators and plagues.[38] Their structures are diverse and can be classified into different groups as a function of the number of phenol rings that they contain and the structural elements that bind these rings to one another.[38] Distinctions are thus made between phenolic acids, flavonoids, lignans, and stilbenes. In addition to this diversity, phenolic compounds may be associated with carbohydrates (simple and complex), lipids, organic acids, and as mentioned, some phenolic compounds can also be linked to cell wall components (cellulose, hemicelluloses, and lignin).[39]

The consumption of foods rich in phenolic compounds is associated with various physiological effects, such as preventing cancer and some chronic diseases, due to the compounds' potent antioxidant properties and free radical scavenging.[40] Thus, the consumption and incorporation of these molecules into foods have been increasing, in order to enhance health.[3] Phenolic compounds are ubiquitous in fruits, vegetables, cereals, nuts and furthermore in plant-based beverages, such as wine, beer and tea.[39] However, the biological properties and health effects of phenolic compounds depend on their respective intake and bioavailability, which can be affected by different factors including the binding of phenolic compounds within the food matrix, especially dietary fiber.[41,42,43] The maximum concentration in plasma rarely exceeds 1 μM after the consumption of 10–100 mg of a single phenolic compound. Nevertheless, the total plasma phenol concentration is probably higher due to the presence of metabolites formed in the body's tissues.[39]

Therefore, dietary fiber and phenolic compounds are two food constituents which present distinct functional properties. However, recent evidence suggests that the presence of phenolics–carbohydrates complexes in food is generally higher than that of simpler compounds, and these types of interactions have been underestimated in many papers mainly due to analytical problems.[44] Due to the importance and interest of different researchers to elucidate the biological role of these complexes, more studies are being developed. Regarding these molecules, Saura-Calixto[45] established a new concept for polyphenols attached to macromolecules such as fiber, which is well described in the next section.

Dietary fiber with phenolic compounds associated: a new concept and a potential food ingredient

Nowadays, the dietary fiber concept, in which healthy effects were previously attributed only to non-starch polysaccharides and lignin components, is changing in order to consider food as a complex matrix capable of carrying other non-digestible food constituents that are resistant to digestion and absorption in the human small intestine, with complete or partial fermentation in the large intestine.[43,45] Phenolic compounds are the most abundant antioxidants in plant foods that can be found chemically associated with the fiber matrix (Fig. 1). In this sense, the concept of "antioxidant dietary fiber" has been recently introduced and was defined as a dietary fiber concentrate, containing significant amounts of natural antioxidants (mainly phenolic compounds) associated with non-digestible compounds.[45] This material combines the physiological properties of both dietary fiber and phenolic compounds and promises to be a potential food ingredient useful in enhancing the bioactive and technological properties of products (Fig. 2).

The most abundant phenolic compounds linked to dietary fiber belong to the chemical class of hydroxycinnamic acids. In fruits, these types of compounds are mainly polymeric tannins, and after hydrolysis the most common phenolic compounds are gallic and ellagic acids.[46] However, in cereals the main compound linked to fiber is ferulic acid, followed by diferulic acids, and then sinapic, *p*-coumaric and caffeic acids.[46] Through this conjunction, it is estimated that around 2.5% of the dietary

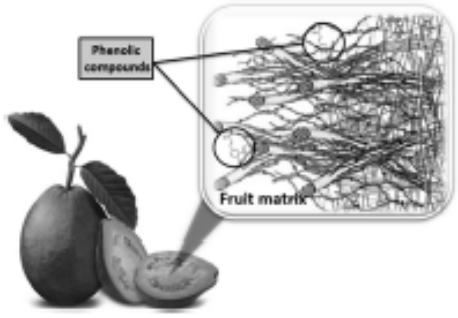

Fig. 1 Phenolic compounds linked to dietary fiber in fruit matrix.

Fig. 2 Incorporation of dietary fiber with antioxidant activity into food to enhance its properties.

fiber content present in fruits is associated with phenolic compounds.[7] Indeed, 95% of grain phenolic compounds are linked to dietary fiber polysaccharides, mainly α-arabinoxylans, as diferulates covalently bound through ester bonds.[34]

There are certain requirements that the material should meet to be considered as an "antioxidant dietary fiber" and a potential food ingredient: (1) dietary fiber content should be higher than 50% on a dry matter basis. (2) One gram of "antioxidant dietary fiber" should have a capacity to inhibit lipid oxidation equivalent to at least 200 mg of vitamin E and a free radical scavenging capacity equivalent to at least 50 mg of vitamin E. (3) The antioxidant capacity must be an intrinsic property, derived from natural constituents of the material. In this context, it can be suggested that phenolic compounds could be dietary fiber constituents in some food matrices, and that these compounds could confer the antioxidant activity attributed to the dietary fiber as a beneficial effect. However, the physiological antioxidant effect of dietary fibers with associated phenolic compounds is still disputed, because the chemical interaction between these two molecules might prevent the release and absorption of phenolics.

Sources of dietary fiber with associated phenolic compounds

In order to take advantage of the properties of this new dietary fiber concept, some authors investigated plant food sources of dietary fiber with phenolic compounds associated. Table 1 shows the difference in total dietary fiber and phenolic compounds content from different whole fruits, byproducts and antioxidant dietary fiber; the last-named being the one that presents the greatest bioactive properties. In recent years, the search for novel sources of dietary fiber with antioxidant properties focused widely on plant food by-products. Jiménez-Escrig[8] reported that the pulp and peel of guava fruit are good sources of natural antioxidants that could be used to obtain dietary fiber with antioxidant activity. In another study, Chantaro[41] reported the feasibility of using carrot peels, a byproduct from the food industry, to produce dietary fiber with antioxidants associated (phenolic compounds and carotenoids), which may be used as a food ingredient. Pineapple shells were reported as a promising source of dietary fiber (composed of 70.6% dietary fiber) containing a high concentration of associated phenolics (mainly

Table 1 Total dietary fiber (TDF) and extractable polyphenols (EP) in raw fruits, fruit byproducts and sources of antioxidant dietary fiber

	TDF (% dry matter)	EP (mg GA g^{-1} dry matter)	Reference
Raw fruit			
White guava	5.3	1.5	
Red guava	2.7	23	
Cas mbola	2.7	22	
Mango	1.8	0.5	36
Papaya (cv. Red Lady)	1.7	0.4	
Blueberries	2.4	5.3	37
Grape	1.5	1.4	
Pineapple	1.4	—	
Apple	1.2	2.1	
Orange	1.1	1.3	
Strawberry	2.3	1.6	38
Durian	1.2	1.0	
Snake fruit	1.1	2.1	
Mangosteen	0.9	1.9	
Byproduct			
Banana peel	7.6	9.2	39
Guava peel	—	58	9
Mango peel	28	70	10
Mango seed	—	117	40
Jack fruit seed	—	27	41
Carrot peel	45	13	42
Pomegranate peel	—	249	45
Grape stem	77	116	
Antioxidant dietary fiber			
Cocoa powder	60	1.3	44
Guava pulp	48	26	
Guava peel	49	77	9
Jamaica	33	61	45
Orange-lime	69	—	10
Pineapple shells	70	—	
Cauliflower	6.0	3.4	48
Mango peel	51	96	49
Cabbage leaf	51	3.4	50
Acai	71	15	51

myricetin) that exhibits antioxidant activity. This property, together with the neutral color and flavor, makes it a suitable fiber for a wide range of applications as a food ingredient.[40] Vergara-Valencia *et al.*[41] obtained a mango dietary fiber concentrate with antioxidant capacity, which could be an alternative for the development of products with balanced dietary fiber components and low glycemic response. Lecumberri *et al.*[42] obtained a dietary fiber powder with intrinsic antioxidant capacity (derived from soluble polyphenols and condensed tannins) from cocoa. The by-products of the Prensal Blanc white grape (*Vitis vinifera*) are an excellent source of dietary fiber with antioxidant properties.[43] Nilnakara *et al.* obtained an antioxidant dietary fiber powder from cabbage outer leaves.[47] Rufino *et al.*[44] reported that BRS-Pará acai fruits can be considered as an excellent source of associated dietary fiber/antioxidants.

In general, there is increasing interest to find new sources of dietary fibers with specific bioactive constituents that may add new healthy properties to the traditionally commercialized products. Fruits, cereals and grains are potential sources of this material. However, as mentioned, by-products, such as peels, seeds and unused flesh, can present similar or even higher contents of these bioactive compounds and have traditionally been undervalued. Nevertheless, it is widely known that plant foods are an excellent source for both isolated phenolic compounds and dietary fiber.

Dietary fiber and phenolic compounds as functional ingredients

In recent years, interest in nutrition and disease prevention has begun to drive consumer demand for value-added foods, or functional foods enriched with an ingredient able to provide or promote a beneficial action for human health.[11] These compounds are the so-called functional ingredients, which provide benefits additional to nutritional and energetic gains; and at the same time are able to improve the technological functionality of a food. The term "functional ingredient" is meant to convey the function of these new ingredients, which is to produce a positive health outcome *via* physiological activity in the body.[13] It has become recognized that these compounds markedly influence quality of life factors, such as modulation of performance or reducing risk of acquiring a variety of diseases, by modifying one or more physiologic processes.[13] There is a diverse group of compounds classified as functional ingredients, for example, carotenoids, flavonoids, dietary fiber, phenolic compounds, allyl compounds, glucosinolates, and peptides, among others.

Dietary fiber and phenolic compounds hold all the characteristics required to be considered as important functional ingredients, due to their physiological roles. Dietary fiber plays several important roles, including increasing the volume of fecal bulk, decreasing the time of intestinal transit, decreasing cholesterol and glycaemia levels, trapping substances that can be dangerous for the human organism (mutagenic and carcinogenic agents), and stimulating intestinal microflora proliferation.[4] Moreover, dietary fiber improves the technological properties of the food, such as water-holding capacity, swelling capacity, and increasing viscosity, texture or gel formation, which is essential in formulating certain food products[14] (Fig. 2). In the case of beverages and drinks, the addition of dietary fiber increases their viscosity and stability. Additionally, fiber-rich byproducts may be incorporated into food products as inexpensive, non-caloric bulking agents for partial replacement of flour, fat or sugar, as enhancers of water and oil retention and to improve emulsion or oxidative stabilities.[14] Also, phenolic compounds are involved in decreasing the risk of chronic diseases, such as cardiovascular disease and cancer, and are useful against lipid peroxidation in food processing.[14,15] There are many benefits that these compounds provide as functional ingredients; therefore, dietary fiber with associated phenolic compounds is a novel promising material for the food processing and nutrition industry, because it combines the properties of both components in a single material.

Various foods, such as bread, meat, fish and beverages,[13,16,17] have been enriched with different sources of dietary fiber and phenolic compounds with satisfactory results (Table 2). The literature contains many reports about the addition of dietary fiber to food products, such as baked goods, beverages, confections, dairy, frozen dairy, meat, pasta and soups. Among the most known and consumed dietary fiber-enriched foods are breakfast cereals and bakery products such as whole-grain breads and cookies,[61,62] as well as milk- and meat-derived products. Some types of soluble fibers, such as pectins, inulin, guar gum and carboxymethyl-cellulose, are utilized in milk products.[63] Guar gum and inulin are added during cheese processing to decrease its percentage fat without losing its organoleptic characteristics. Moreover, for the elaboration of jams and marmalades, the most common added fibers are those consisting of pectins with different degrees of esterification, obtained mainly from fruits and are a factor in keeping the stability of the final product.[64] On the other hand, phenolic compounds as functional ingredients act as antimicrobials, antioxidants, flavorings and thickeners.[3] In general, phenolic compounds are added mainly into meat and fresh-cut fruits and vegetables to avoid enzymatic browning, lipid oxidation, bacterial contamination and increase the antioxidant capacity and health benefits of products. Therefore, delaying lipid oxidation and preventing bacterial cross-contamination are highly relevant to food processors. However, phenolic compounds are also attracting more and more attention not only due to their antioxidant properties, but as anti-carcinogenic and anti-inflammatory agents. Among the most common materials used as sources of phenolic compounds are herb extracts and citrus fruits.[65]

Several patents have been published about the addition of dietary fiber with associated phenolic compounds to increase the health benefits status of the supplemented foods. For example, Myllymaki[73] claimed the formulation of a rye cereal product having higher dietary fiber and phenolic content. This cereal is a good source of dietary fiber (38%) and also contains a significant fructan concentration (7 g/100 g), which according to the suggested new dietary-fiber concept is also a component of dietary fiber.

Table 2 Effect of functional foods enriched with dietary fiber, phenolic compounds and dietary fiber with associated phenolic compounds

Functional food	Functional ingredient	Source	Results	Reference
Cookies and bread	Dietary fiber	Mango	Products with balanced components and low predicted glycemic index response	43
Bologna cooked sausages	Dietary fiber	Lemon albedo	Similar sensory properties to conventional sausages but improvement in the nutritional properties	58
Cookies	Dietary fiber	Extruded wheat bran	Dietary fiber content was increased and the glycemic index was low	59
Yogurt	Dietary fiber	Acacia	Greater therapeutic effects in patients with irritable bowel syndrome	60
Cupcakes	Dietary fiber	Oat and wheat	Addition of 30% dietary fiber improved quality characteristics of cupcakes. Also prolonged the shelf-life of the cakes by delaying the moisture loss and the increase in crumb firmness	61
Fresh potatoes	Phenolic compounds	Oregano	Increase in antioxidant activity and reduction of acrylamide content	65
Bread	Phenolic compounds (proanthocyanidins)	Grape seed	High antioxidant activity and reduce the N(carboxymethyl) lysine formation, related to health risks	66
Cooked pork meat patties	Phenolic compounds	Rapeseed and pine bark	Inhibition of protein oxidation between 42 and 64%	67
Cheese product	Phenolic compounds	Herb extracts (cinnamon stick, oregano, clove, pomegranate peel, and grape seed)	Plant extracts were effective against *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella enterica*. Also, extracts increased the stability of cheese against lipid oxidation	68
Dough biscuits	Dietary fiber with associated phenolic compounds	Mango peel	Dietary fiber and polyphenols content increase 14% and 90%, respectively	70
Cake	Dietary fiber with associated phenolic compounds	By-product of apple juice	Increase the dietary fiber and polyphenols content, to 14% and 7.16 mg g^{-1}, respectively	71
Yogurt and salad dressing	Dietary fiber with associated antioxidants	Wine grape pomace	Increase dietary fiber and total phenolic content, also delay lipid oxidation of samples during refrigeration storage	72
Fish mince horse mackerel	Dietary fiber with associated phenolic compounds	*Fucus vesiculosus* spp.	Prevented lipid oxidation during 5 months of frozen storage at −20 °C. Also, reduced total yield after thawing and cooking after up to 3 months of frozen storage	14
Macaroni products	Dietary fiber with associated phenolic compounds	Mango peel	Enhance nutritional and technological quality. The dietary fiber content increased 9% and exhibited improved antioxidant properties	49

Another patent reports the preparation process and health benefits of a grape antioxidant dietetic fiber concentrate.[74] The powder obtained from black or white grape skins had the following characteristics expressed in dry weight: total dietary fiber content of 65–80%, 15–25% bioactive compounds content (soluble and insoluble condensed tannins, flavonoids, proanthocyanidins and other polyphenols), 11 to 15% protein, and 5 to 8% crude fat.[74] According to these authors, the incorporation of dietary fiber and phenolic compounds can be utilized for the preparation of functional foods presumably with improved health benefits and technological properties. However, these studies need to be accompanied by quality and sensory evaluations.

Dietary fiber with associated phenolic compounds obtained from wine grape pomace was added to yogurt and salad dressings.[72] The addition resulted in a 35–65% reduction of peroxide values in all samples. Total phenolic content and DPPH radical scavenging activity were 958–1340 mg GAE kg^{-1} product and 710–936 mg AAE kg^{-1} product, respectively. The addition was mostly liked by consumers, based on the sensory study. Fiber extract from *Lentinus edodes* mushrooms containing 514 g kg^{-1} of (1–3)-beta-glucans was added to wheat flour.[78] Replacement of a portion of wheat flour with the extract resulted in lower values of pasting parameters and also caused significant changes in starch gelatinization. When the same extract was incorporated into cake formulations, batter viscosity increased with more shear-thinning behavior and elastic properties

improved. However further studies are needed to find the health benefits of this addition and particularly the presence of associated phenolic compounds in edible mushrooms.

The use of dietary fiber with associated phenolic compounds from grapes has been reported to inhibit food lipid oxidation. Sanchez-Alonso *et al.*[43] observed a 57% lipid inhibition measured by TBARS in frozen minced mackerel patties treated with 2% grape antioxidant dietary fiber. The authors reported that this protective effect could be either by the chelation action of fiber over prooxidant metals or the antioxidant capacity of the polyphenols present in the material. Similar results were observed for raw and cooked chicken hamburgers stored 14 days at 4 °C, in which not only the lipid oxidation was inhibited, but also an increase of radical scavenging capacity in the fortified hamburgers was observed.[45] Even though there are no studies of the effect of these protected food products over total plasma oxidative status of consumers, it may be hypothesized that the consumption of these products may exert a beneficial effect over the consumer as a result of less free radical intake.

Although great achievements have been made by using dietary fiber and phenolic compounds as functional ingredients, as well as the material that combines both substances, further investigations about structure and functionality within the food matrices (proteins, lipids and water activity) and the bioavailability effects after intake are needed.

Dietary fiber effect over phenolic compounds in the human digestive tract

As stated above, in recent years, there has been a growing interest among researchers in the formulation of food products with dietary fiber and phenolic compounds due to their linkage to human health. However, consumption of food rich in some nutrients or bioactive compounds does not guarantee their bioavailability in the digestive tract, therefore, the biological effect of the nutrients/bioactive compounds is not insured.[27] The bioavailability or absorption in the gut is in many cases quite uncertain or varies for the same food depending on processing conditions, presence of other compounds, and so on. Furthermore, there are some specific factors that could affect the absorption of the molecules in the gut, such as food microstructure, structure and molecular weight of the compound, and chemical interactions between food constituents.[28] This last factor is very relevant because recent scientific data appear to demonstrate that, in the case of certain nutrients and bioactive compounds, the state of the matrix of natural foods or the microstructure of processed foods may improve or hinder their nutritional response *in vivo*. In fact, it has recently been stated that the generation of functional foods fortified with fiber rich and phenolic compounds could result in a loss of absorption of the antioxidants, because fiber may trap the antioxidant molecules, decreasing the proposed food functionality.[46] However, some evidence suggests that phenolic compounds entrapped into dietary fiber can reach the colon and exert a biological effect, playing an important role in intestinal health.[34]

The next sections describe the possible interactions that may arise between phenolic compounds and dietary fiber and how these interactions can affect the bioavailability of these compounds.

Dietary fiber and phenolic compounds chemical interactions

As previously described, some plant foods are rich sources of dietary fiber that carry putatively bioactive compounds, phenolic compounds in particular, embedded in them; these previous studies indicate that both components are able to interact chemically in the food matrix.[7] Phenolic compounds have both hydrophobic aromatic rings and hydrophilic hydroxyl groups with the ability to bind to polysaccharides and proteins at several sites on the cell wall surface.[7] They are linked by hydrogen bonding (between the hydroxyl group of phenolic compounds and oxygen atoms of the glycosidic linkages of polysaccharides), hydrophobic interactions, and covalent bonds such as ester bonds between phenolic acids and polysaccharides (Fig. 3). Interactions can be dependent on particle size, specific porosity and surface properties, which can restrict the size of the molecules that penetrate. Pore size in the cell wall can range from 4 to 10 nm in diameter, which may restrict penetration of phenolic compounds with molecular masses larger than 10 kDa (equivalent to 34 units to catechin).[28]

Dietary fiber can interact and bind during gastrointestinal digestion with antioxidants present in the food matrix. These interactions can be either hydrogen bonds, strong (covalent) interactions or physicochemical entrapment exerted by dietary fiber.[18] Considering that these bonds are weak, they are stable only above a minimum critical length, and their formation and disruption often occur as sharp, cooperative processes in response to comparatively small changes in, for example, pH or solvent quality in the gastrointestinal tract (that is the nature and concentration of dissolved solids in the chyme). In this context, the possible interactions that may arise between dietary fiber and phenolic compounds can decrease or delay their absorption in the gut, as mentioned in early sections.

Effect on bioaccessibility and bioavailability

Bioavailability is defined as the proportion of a nutrient that is digested, absorbed, and utilized in normal metabolism; bioaccessibility is a commonly used term to describe the amount of an ingested nutrient that is available for absorption in the gut after digestion.[1477] In this sense, bioavailability strictly depends on bioaccessibility, and it is well known that the biological properties of nutrients and bioactive compounds, such as phenolic compounds, depend on this release-absorption process. It has been reported that phenolic compounds are released from the food matrix in the upper area of the gastrointestinal tract by direct solubilization in the intestinal fluids at physiological conditions (37 °C, pH 1–7.5) and/or by the action of digestive enzymes (enzymatic hydrolysis of protein, carbohydrates, and lipids favors the release of phenolics from the food matrix).[34] These accessible compounds (low molecular

weight phenolics) are at least partially absorbed through the small intestine mucosa. However, another group of phenolics are not bioaccessible; these compounds pass undissolved and unaltered through the upper intestine in association with the food matrix, including dietary fiber, which alters the efficiency of the physical, enzymatic, and chemical digestion processes.[76] Moreover, this bioavailability can be even lower for large molecular weight food polyphenols, as is the case of hydrolyzable and condensed tannins and complex flavonoid conjugates with several sugars and acylated with hydroxycinnamic acids. Therefore, it is generally accepted that the bioavailability of phenolics is rather low, even though high variability in the bioavailability of the different polyphenols may be observed, and can be expressed as the relative urinary excretion of the intake range, from 0.3% for anthocyanins to 43% for isoflavones such as daidzin.[60]

Several factors can explain this variability, among them the food matrix, particularly dietary fiber components, plays an important role. There is ample evidence that the physical state of the food polysaccharides plays a key role in the bioaccessibility of many bioactive food components, such as antioxidants.[77,84,85] It is known that dietary fiber can reduce the bioavailability of macronutrients and biomolecules, especially fat, and some minerals and trace elements in human digestion.[60] In general, the two main effects of dietary fiber in the foregut are to prolong gastric emptying time and to retard absorption of nutrients.[86] Both are dependent on the physicochemical characteristics of the fiber, and in particular, its influence on the viscosity of the bolus. Dietary fiber can act in the small intestine in three main physical forms: as soluble polymer chains in solution, as insoluble macromolecular assemblies, and as swollen, hydrated, sponge-like networks.[60] Therefore, the dominant factors involved in the influence of dietary fiber on antioxidant digestion are: (1) physical trapping of antioxidants within structured assemblies, such as fruit tissue, and (2) enhanced viscosity of gastric fluids restricting the peristaltic mixing process that promotes transport of enzymes to their substrates, bile salts to unmicellized fat, and soluble antioxidants to the gut wall.[60] For these reasons, interactions of phenolic compounds with dietary fiber are expected and may affect their releasing during digestion and interfere with absorption in the gut.[14]

In this context, the limited bioavailability of antioxidants associated with dietary fiber is determined by their low bioaccessibility in the small intestine, due to the physical and chemical interactions between antioxidants and the indigestible polysaccharides of the cell wall. However, all non-absorbable antioxidants reach the large intestine and remain in the colonic lumen where they may contribute to a healthy antioxidant environment.[74]

Functional and biological properties in the gut

Dietary fiber associated with phenolic compounds possesses some functional and biological properties, such as antioxidant capacity and colonic fermentation.[87] The appreciable amount of phenolic compounds linked to or entrapped by dietary fiber provides a significant antioxidant capacity that may have pronounced effects on biological systems, such as the gastrointestinal tract. Phenolic compounds associated with dietary fiber may have significant effects in intestinal health. The antioxidant dietary fiber is transported largely unaltered along the small intestine all the way to the colon. The intestinal microbiota ferments the antioxidant dietary fiber matrices, and phenolic compounds are gradually released in the intestinal lumen and partially absorbed by gut epithelial cells. Therefore, non-absorbable phenols and non-fermented phenolic compounds remain in the colonic tissue, scavenging free radicals and counteracting the effects of dietary fiber pro-oxidants[7] (Fig. 4). At the same time, the partial or total fermentation of dietary fiber constituents (e.g. cellulose, hemicelluloses, pectins, resistant starch, fructans, arabinoxylans) releases several beneficial short-chain fatty acids (SCFA), such as phenylacetic, phenylpropionic and phenylbutyric acids.[78] These compounds may exert systemic effects in conjunction with

Fig. 3 Types of interactions between phenolic compounds and dietary fiber.

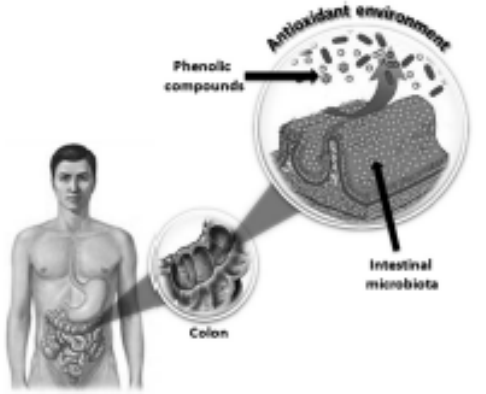

Fig. 4 Colon antioxidant environment formed by the action of intestinal microbiota that ferments the dietary fiber matrices, and phenolic compounds are gradually released at the intestinal lumen and partially absorbed into gut epithelial cells.

phenolic compounds, for example the induction of cellular differentiation and apoptosis.[45,46] Moreover, epidemiological studies have shown an inverse association between dietary fiber with associated antioxidants consumption and colon cancer, mainly due to the effect of SCFA (butyrate hypothesis) on the modulation of genes associated with this disease.[46] Recently, Lizarraga *et al.*[46] analyzed the effect of consumption of grape antioxidant fiber over 26 393 mice genes, observing that 363 genes were upregulated and 641 downregulated. From the analysis of these results, the authors suggested that the beneficial health effect was because the grape antioxidant fiber consumption downregulated nuclear receptor signaling, lipid biosynthesis (TNF and PPARα) and energy metabolism, pathways associated with obesity and cancer. At the same time, antioxidant and detoxification enzymes (Fase I and II) and apoptotic (BFAR and CARD14), immune system and tumor suppression genes (NBL1) were upregulated. These results clearly show the beneficial effect of dietary fiber with associated phenolic compounds. In particular, phenolic compounds, dietary fiber components and their metabolites come into contact with the gut wall for up to several hours (more than 24). For this reason, the antioxidant environment formed in the colon could modulate the incidence of certain kinds of degenerative diseases, such as colon cancer.

Furthermore, the beneficial effect of consumption of dietary fiber with phenolics has been associated with the proliferation of lactobacilli, and to a lesser degree Bifidobacteria, both *in vitro* and *in vivo*, and an inhibition of pathogenic bacteria (*Escherichia coli*, *Clostridium*) that improves the overall gastrointestinal health. This beneficial effect may be explained in terms of the presence of phenolic compounds such as (+)-catechins, (−)-epicatechin and (−)-epicatechin-3-*O*-gallate, and tannins in the material, which exert antimicrobial activity against pathogenic bacteria in the gut. The same authors suggested that dietary fiber with phenolic compounds embedded modifies the gut morphology improving gastrointestinal absorption.

Conclusion and future research

The use of phenolic compounds and dietary fiber as food ingredients is of great interest not only as a means of improving the functionality of food products, but also to formulate functional foods with health benefits, such as reducing cholesterolaemia, modifying the glucaemic response, and preventing the development of cancer and some cardiovascular diseases. Furthermore, the physicochemical association between these two bioactive substances (fiber and phenolic compounds) that has created a new material that combines the functional properties of both fiber and antioxidants (mainly antioxidant capacity) is well known, and in the last few years it has been used as a functional ingredient. However, there is evidence that this association may not only exert beneficial effects, but also some unwanted effects, because dietary fiber may affect the bioaccessibility and bioavailability of phenolic compounds and consequently reduce fiber's healthy and biological effects. Nevertheless, it has been stated that due to these fiber–phenolic compounds interactions, an appreciable amount of phenolic compounds are carried out by dietary fiber through the gastrointestinal tract, producing antioxidant metabolites in the colon and creating an antioxidant environment for the prevention of diseases such as colon cancer. However, future research is needed to verify this hypothesis.

In this context, research on the dietary fiber–phenolic compounds association offers to be a very promising area. Future work is needed to elucidate the real contribution of functional foods enriched with dietary fiber to the well-being of consumers. For this reason, more studies on bioaccessibility and bioavailability, both *in vitro* and *in vivo*, from different formulations in new products and sources of dietary fiber–phenolic compounds are needed. In addition, the role of fiber as a controlled release system of bioactive compounds in the colon must be studied in more detail.

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CAPÍTULO II

Bioaccessibilidad, difusión pasiva y capacidad antioxidante de compuestos fenólicos presentes en mango cv. 'Ataulfo' (Mangifera indica *L*) y salvado de trigo (*Triticum aestivum*), después de una digestión *in vitro*

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Bioaccessibility, passive diffusion and antioxidant capacity of phenolics compounds from 'Ataulfo' mango-flesh (Mangifera indica L) and wheat bran (Triticum aestivum) after in vitro digestion

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Key Words:	Fruit, cereal, phenolic compounds, dietary fiber, SEM, gastrointestinal digestion

Bioaccessibility, passive diffusion and antioxidant capacity of phenolics compounds from 'Ataulfo' mango-flesh (*Mangifera indica* L) and wheat bran (*Triticum aestivum*) after *in vitro* digestion

Running tittle: *In vitro* bioaccessibility and antioxidant activity of 'Ataulfo' mango and wheat bran

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ABSTRACT

BACKGROUND: The aim was to evaluate the bioaccessibility, passive diffusion and antioxidant capacity of total phenolic compounds (PCs) present in plant food samples with different content and type of dietary fiber: 'Ataulfo' mango-flesh and wheat bran, after *in vitro* digestion. Foods microstructure of samples were examined by scanning electron microscopy and dietary fiber content were analyzed. Passive diffusion behavior was fitted to a kinetic model to disentangle how PCs are released and could be absorbed within the gut. **RESULTS**: Mango-flesh (amorphous) and wheat bran (matrix-like) microstructures corresponded to their soluble and insoluble fibers. The accumulated bioaccessible percentage of PCs after intestinal digestion was higher in mango-flesh (less fiber content) than wheat bran, being 49.7% and 22.7%, respectively. Digestion phases showed antioxidant capacity (DPPH and FRAP) apparently due to the amount of PCs released. The potential uptake of PCs available to be absorbed by passive diffusion followed a Fickian's behavior, where mango-flesh had a major interaction with food matrix and consequently a lower concentration of absorption. **CONCLUSION:** The above suggests that bioaccessibility of PCs depends largely on the type and composition of food matrix, being higher for lower fiber content matrices. Also, fiber type affects the absorption diffusion behavior of PCs released.

Keywords: fruit, cereal, phenolic compounds, dietary fiber, gastrointestinal digestion, SEM

INTRODUCTION

Phenolic compounds (PCs) constitute a vast array of secondary plant metabolites and are probably the most investigated molecules of nutraceutical interest due to the strong evidence that supports their bioactive effects that promote health benefits.[1] Several studies address the effects of certain plant foods rich in PCs with specific nutraceutical actions including tea, coffee, red wine, tropical fruits and many cereal grains.[2] However, these studies generally report the content of chemically extractable PCs from plant cell vacuoles, largely underestimating those PCs in conjugated and bounded form (non-extractable PCs, also called macromolecular antioxidants) because they are difficult to release and quantify.[3]

Mexico is the leading mango (*Mangifera indica* L) exporting country (41% of the world market), being its varietal 'Ataulfo' the most exported to United States.[2] 'Ataulfo' mango consumption has been related to functional effects, most of them attributed to its PCs content. *In vitro* studies have reported that 'Ataulfo' mango has the highest content of extractable PCs among several mango varieties, and consequently the highest antioxidant capacity which in turns correlates with the type and quantity of PCs.[4] However, the non-extractable PCs in this varietal has not been extensively investigated so far. Nevertheless, the non-extractable fraction has been analyzed in 'Ataulfo' mango byproducts, such peel and paste.[5, 6]

On the other hand, PCs derived cereal grains have not received as much attention as those from fruits, and their health benefits have been more associated to its dietary fiber content. Nevertheless, recent research has shown that these PCs are commonly found at a higher concentration in many grains, specifically in kernel's outer layer (bran).[7] However, most of these PCs are part of the non-extractable PCs fraction because they are chemically bounded to other food matrix components, mainly dietary fiber.[8] Wheat (*Triticum aestivum*) is one of the most popular cereal grains, and its bran contains an important concentration of non-extractable PCs, which intrinsically contributes to its *in vitro* antioxidant capacity as well as to its health effects.

Although the content and chemical nature of PCs (extractable and non-extractable) from different plant foods has been studied so far, the potential health effects depends, among other factors, to their release from the food matrix (bioaccessibility), its gut absorption and circulation (bioavailability) and peripheral metabolism.[9] However, several factors affect the release-absorption process. It has

been reported that PCs bioaccessibility is mainly affected by the food matrix (fiber content) and physicochemical interaction between food components; while bioavailability is controlled by the presence of absorption inhibitors or enhancers, enzyme actions, host and others related factors.[2]

The aim was to evaluate the bioaccessibility, passive diffusion and antioxidant capacity of total PCs present in two plant foods with different content and type of dietary fiber: 'Ataulfo' mango-flesh and wheat bran.

EXPERIMENTAL

Materials

Mangoes (commercial ripeness stage) and wheat bran were purchased from a local market (Hermosillo, Sonora, Mexico) and transported to the laboratory. Mango-flesh were washed, peeled and the fruit flesh was freeze-dried. Subsequently ground, sifted with a mesh size of 0.5 microns, and stored at room temperature in a desiccator, until analysis.

Mango-flesh and wheat bran extracts

For the quantification of extractable PCs, organic aqueous extraction (0.033 g/mL) was performed on samples with methanol-water (80:20 v/v) solution (sonication for 30 min, Bransonic Ultrasonic Co., Model 2210, Danbury, CT, USA). For non-extractable PCs extraction, residues from the aqueous extraction were dispersed in 20 mL of methanol and 2 mL of H_2SO_4 were added. The extracts were incubated in a shaking water bath at 85 °C for 20 h. They were cooled at room temperature and centrifuged at 3000 rpm for 10 min. Then, the supernatants were recovered. Subsequently, the residue was washed twice with 10 mL of distilled water and the supernatants were mixed in a 50 mL volumetric flask. These extracts were used to analyze total PCs content and antioxidant capacity in raw samples (non-digested).

Total PCs content

Total PCs content is given by the sum of the extractable and non-extractable PCs. The extractable PCs and hydrolysable tannins (part of the non-extractable PCs) were measured by the Folin-Ciocalteau procedure according to Singleton and Rossi *et al.* [10] with some modifications, using a microplate reader. Results were expressed as mg of gallic acid equivalents (GAE)/g of dry weight. Condensed tannins (part of the

non-extractable PCs) were calculated from their absorbance at 550 nm and compared to a standardized anthocyanidin solution prepared with Mediterranean carob pod (*Ceratonia siliqua* L.).[11]

Antioxidant capacity

(2, 2-diphenyl-1-picryl-hydrazyl-hydrate) DPPH: The method was performed as reported by Brand-Williams *et al.*[12], with some modifications. Samples (20 µL) were placed in a microplate and 280 µL of DPPH radical (0.0634 mol/L) were added. The mixture was kept in the dark for 30 min. After, absorbance was read at 515 nm using a microplate reader. **Ferric Reducing Antioxidant Power (FRAP):** The method was carried out according with Benzie and Strain [13]. The reagent (280 µL) and sample solutions (20 µL) were added to each well and mixed thoroughly. The mixture was kept in the dark for 30 min and absorbance was read at of 593 nm. For both assays, trolox (6-hydrozy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was used as a standard and methanol as a blank. Results were expressed as µmol of trolox equivalents (TE)/g of dry weight.

Dietary fiber content

Dietary fiber was analyzed by the AOAC enzymatic-gravimetric method (991.42) as modified by Mañas and Saura-Calixto.[14] Mango flesh and wheat bran (1 g) were treated with heat-stable α-amylase (25 µL, pH 6, 35 min, 100 °C; EC 232-560-9), protease (50µL of 50 mg/mL solution in phosphate buffer 0.08 M, pH 6, 60°C, 35 min; EC 232-752-2) and amyloglucosidase (150 µL, pH 4.5, 60 °C, 25 min; EC 3.2.1.3) to remove protein and available starch. After the enzymatic hydrolysis of samples, they were centrifuged (2615 x g for 15 min, 4 °C) to separate in soluble and insoluble dietary fiber. Supernatants were transferred to cellulose membrane dialysis tubes (12000-14-000 Da) for 24 h. Dialysates (containing soluble fiber) and residues from centrifugation (containing insoluble fiber) were subjected to hydrolysis with 12 M sulfuric acid at 100 °C for 90 min to determine the non-starch polysaccharides following the Englyst and Cummings[15] method, using glucose as standard. Remaining residues from insoluble fiber hydrolysis were quantified gravimetrically as klason-lignin. Insoluble dietary fiber was calculated as total non-starch polysaccharides plus klason-lignin. The total dietary fiber content was considered as the sum of both fractions and expressed as percentage (%) on dry basis.

Total PCs and antioxidant capacity associated to dietary fiber

Total PCs content were determined in the soluble and insoluble dietary fiber, in order to evaluate the associated PCs. Total PCs in the soluble fiber were determined directly in aliquots of this fraction that were taken after dialysis. For the insoluble fiber fraction, a chemical extraction with methanol was performed. Extractable PCs and non-extractable PCs were determined following the procedures described above. Antioxidant capacity was determined by DPPH and FRAP assays described previously.

Microstructural analysis

Mango-flesh and wheat bran morphological differences were examined by scanning electron microscopy using an accelerative voltage of 15 kV with objectives 250X, 500X and 1000X. Prior to the analysis, samples were coated with gold/palladium alloy in order to improve the contrast.

In vitro digestion

The in vitro digestion was performed following the protocols proposed by Saura-Calixto *et al.*[16] and Granfeldt *et al.*[17], with slight modifications. Samples (300 mg) with 10 mL of HCl-KCl solution (pH 1.5) were incubated with a 0.3 mL of pepsin (EC 232-629-3) solution (300 mg/mL) during 1 h at 40 °C with continuous agitation, simulating gastric conditions. Then, 4.5 mL of phosphate buffer (pH 7.5) was added and the pH was adjusted to 7.5. After this step, to simulate intestinal digestion, 1 mL of pancreatin (EC 232-468-9) solution (5 mg/mL) was added, which was further incubated an additional 6 h at 37 °C. At the end of this second incubation, samples were centrifuged (2615 x g for 15 min) and supernatants separated (released PCs). The precipitate corresponds to the indigestible part that reaches the colon; however, supernatants contain soluble PCs for potential uptake. Then, supernatants were transferred into semipermeable cellulose dialysis bags (12000-14-000 Da), sealed with clips, completely immersed into tubes contained phosphate buffer, and dialyzed for 3 h at 37 °C. To monitor the release of PCs at different phases of digestion, aliquots from gastric, intestinal and dialyzable fraction were analyzed, respectively. Antioxidant capacity (DPPH and FRAP assays) was also determined in each phase. Individual experiments were conducted to measure bioaccessible PCs at each of the

different phases of digestion and not affect the sample volume. Bioaccessibility was considered as the concentration of PCs released from the food matrix during the *in vitro* digestion, and was calculated as:

$$\text{Bioaccessibility (\%)} = \left(\frac{\text{PCs digestion phase}}{\text{PCs non} - \text{digested}}\right) x\ 100 \quad (1)$$

where PCs digestion phase correspond to the phenolic concentration at each phase of the *in vitro* digestion. This equation was modified from Blancas-Benitez *et al.*[18]

Passive diffusion kinetic and diffusion behavior

Dynamic dialysis was used to determine the *in vitro* uptake of PCs from mango-flesh and wheat bran throughout a semipermeable membrane. To determine the PCs dialyzed, aliquots of the external liquid were removed at 30 min intervals, and analyzed. The amount of passive diffused PCs was analyzed by Folin-Ciocalteau method and is reported as accumulated diffusive percentage of PCs. In order to examine the mass transport kinetic and the diffusional mechanism, the uptake profile of PCs from mango-flesh and wheat bran were fitted to the following kinetic diffusion models:

First-order model: $\mathbf{\ln (1 - M_t/M_\infty) = - \mathit{k} t}$ (2)

Peppas: $\mathbf{M_t/M_\infty = \mathit{k} t^n}$ (3)

where M_t/M_∞ is a fraction of the bioactive compound released at time t, k is the release rate constant and n is the release exponent. The n value is used to characterize different release for cylindrical shaped matrices. In this model the value of *n* characterizes the release mechanism of PCs, where $0.45 \leq n$ corresponds to a Fickian diffusion mechanism, $0.45 < n < 0.89$ to non-Fickian transport, $n = 0.89$ to Case II (relaxational) transport, and $n > 0.89$ to super case II transport.[19]

Statistical analyses

A completely randomized design was used. Data were statistically analyzed by one-way ANOVA procedure and *post hoc* Tukey-Kramer multiple comparison tests were used at 95 confidence level. Number Cruncher Statistical System version 6.0

software (NCSS, LLC) was used. All analyses were performed by triplicate; means and standard deviations from each determination were calculated.

RESULTS AND DICUSSION

Total PCs content and antioxidant capacity of mango-flesh and wheat bran

The content of total PCs (extractable and non-extractable) for mango-flesh and wheat bran are presented in **Table 1**. Non-extractable PCs fraction are formed by hydrolyzable (phenolic acids polymers) and condensed (flavonoids polymers) tannins,[20] and in both samples only hydrolysable tannins -were detected. Total PCs values were 17.1 and 20.7 mg EAG/g of dry weight for mango-flesh and wheat bran, respectively, no statistically difference ($p < 0.05$) was shown between samples. The value for wheat bran was higher to those reported by Kim *et al.* [21]*;* however, varieties, extraction conditions as well as particle size can be a factors of differences between works. Nevertheless, in both theirs and our results the non-extractable PCs fraction were significantly higher than the extractable, indicating that the major PCs in wheat bran are non-extractable by aqueous methanol, but released upon acid hydrolysis because they are strongly bound to other food matrix components. By other hand [2], the value of total PCs in mango-flesh was higher than those reported by others studies, this may be explain considering that in most works only extractable PCs fraction was reported. The non-extractable PCs fraction for mango-flesh was slightly lower than extractable fraction. In this sense, this is the first work that reported the total PCs concentration in mango-flesh. A previous work reported by Blancas-Benitez *et al.*[22] showed the value of the total PCs of mango by-products (peel and paste), and the same trend was observed, where extractable PCs concentration tends to be higher than the non-extractable. Also, results showed that only hydrolysable tannins were identified as non-extractable PCs in mango-flesh, which matches with previous studies.[23]

Results confirms that cereal grains present higher percentage of bound PCs than fruits, and this could be associated to their higher concentration of polysaccharides and indigestible material that are able to chemically interact with PCs.[24] For this reason, wheat bran showed slightly higher total PCs content in comparison to mango-flesh. From a physiological point of view, it is useful to distinguish and determine the content of extractable and non-extractable PCs, which

present different bioaccessibility in the gastrointestinal-tract. It has been reported that extractable PCs appear to be released and absorbed from the digestive tract and produce systemic effects, while non-extractable PCs are not bioaccessible at all in the small intestine, but may be partially degraded by colonic microbiota.[25]

The antioxidant capacity of the total PCs are shown in **Table 1**. The total activity determined by the DPPH method was 21.8 and 113.3 μmols TE/g of dry weight for mango-flesh and wheat bran, respectively. FRAP assay showed 63.4 and 349.6 μmols TE/g of dry weight for mango-flesh and wheat bran, respectively. FRAP assay showed significant higher values compared to the DPPH assay. FRAP measures the ability of compounds to act as an electron donor while DPPH measures their ability to act as hydrogen donors.[26] Also, antioxidant capacity is influenced by the polarity of radicals, DPPH measured nonpolar species, while FRAP measured polar species. However, both methods presented the same trend in which wheat bran showed higher antioxidant capacity, in agreement with its higher total PCs content.

Dietary fiber content (total PCs and antioxidant capacity associated)

6% and 35% of total dietary fiber content were observed for mango-flesh and wheat bran, respectively (**Table 2**). The results agree that cereal grains have higher total fiber content than fruits, and are largely predominant by insoluble fiber which is formed by cellulose, hemicellulose, quitin and resistant starch.[27] By contrast, fruits are characterized to have soluble dietary fiber type, which includes non-starch polysaccharides such as pectins, β-glucans, gums, mucilages, oligosaccharides or inulin.[24]

As mentioned above, some PCs are able to chemically and physically interact with the components of the food matrix, such as dietary fiber. PCs has hydrophobic aromatic rings and hydrophilic hydroxyl groups that can be highly associated to polysaccharides at several sites on the cell wall (cellulose, hemicellulose and lignin) of different foods.[28] It has been hypothesized that higher fiber content in a food matrix could be associated to a greater interaction among molecules and consequently a reduced bioaccessibility of PCs.[24] **Table 2** shows the total PCs content associated to soluble and insoluble dietary fiber of samples. A minimal concentration of extractable PCs (0.04 mg EAG/g of dry weight) was obtained in the soluble fiber of mango-flesh, while no PCs in the soluble fiber of wheat bran were detected. In contrast, both fractions of PCs (extractables and non-extratables) were

detected in the insoluble fiber of mango-flesh and wheat bran. Wheat bran presented the highest non-extractable PCs concentration (4.0 mg EAG/g of dry weight). This is attributed to the greater presence of fiber in the wheat bran food matrix that leads a higher association of PCs to it; in addition, PCs are primarily linked to carbohydrates that form insoluble fiber, such as arabinoxylans.[29] Based on the obtained values, PCs associated to dietary fiber in mango-flesh and wheat bran corresponds to 0.58% and 19.5%, respectively, of the total PCs present in each food matrix.

Some fibers can exert biological antioxidant capacity due the content of PCs that are linked to it. By DPPH method, the total fiber of mango-flesh and wheat bran showed 0.42 and 18.60 µmols TE/g of dry weight, respectively. FRAP assay showed 0.95 and 45.16 µmols TE/g of dry weight for total dietary fiber of mango-flesh and wheat bran, respectively. Likewise, the greater antioxidant capacity was showed for the dietary fiber of wheat bran that presented higher concentration of PCs in their structure. Also, the high antioxidant capacity value presented by wheat bran could be attributed to ferulolyl oligosaccharides from insoluble dietary fiber.[30] The antioxidant capacity exerted by dietary fiber could possibly be linked to health effects provided to the consumer.

Microstructural analysis

Scanning electron microscopy micrographs of the mango-flesh and wheat bran are shown in **Fig 1**. The images show an amorphous structure of mango-flesh that based on the literature is formed mainly by pectin, hemicellulose and cellulose, with a very low presence of starch and a small granule size (< 10 µm). By contrast, micrographs of wheat bran show a regular network morphology, which represents a higher content of dietary fiber, also a high concentration of crystalline starch granules with a higher granule size (> 20 µm) was observed. Starches presented rounded and oval shapes.

Results agree with those reported by other authors indicating that during mango ripening the starch is degraded. Simão *et al.*[31] reported that starches granules isolated from ripe 'Keitt' mango were approximately 8 to 10 µm in size. Moreover, wheat bran (an important source of fiber) is rich in insoluble fiber but also contains soluble fiber. The main compounds present are arabinoxylan, cellulose and β-glucans. These compounds form the crosslinking matrix characteristic of this food matrix. Furthermore, wheat bran is characterized by present starch in its structure (>16%), which could be observed in the micrographs.[32]

Bioaccessibility of PCs of mango-flesh and wheat bran

The impact of the *in vitro* digestion on release of total PCs of mango-flesh and wheat bran is shown in **Table 2**. In general, results highlight that the higher percentage of PCs bioaccessibility occurs during gastric digestion. This fact could be mainly attributed to the acidic pH and enzymatic activity during this digestive phase, which can induce the hydrolysis of PCs from the food matrix.[33] An additional increase in the PCs bioaccessibility from gastric phase to intestinal phase was observed, for both samples. This increase could be explained by the additional time of extraction (6 h) and/or the effect of intestinal digestive enzymes (pancreatin pool) on the complex food matrices, being able to facilitate the release of more extractable PCs, as well as the release of some non-extractable PCs.[34, 35] The amount of dialysable PCs was found to be lower compared to the concentration released during intestinal digestion. The interaction between PCs and other digested constituents of the food matrix may favor the formation of complexes with loss solubility or large molecular weight, which cannot cross the cellulose dialysis membrane, causing a reduction in released PCs concentration. All amounts were lower when compared to chemical extraction (non-digested sample), indicating incomplete bioaccessibility, release or degradation of PCs. In addition, differences in the bioaccessibility of PCs between samples was observed.

The accumulated bioaccessible percentage of PCs after the intestinal digestion was higher in mango-flesh than wheat bran, being 49.7% and 22.7%, respectively. Conversely, wheat bran (50.6%) showed higher passive absorption of the PCs released than mango-flesh (31.7%). Bouayed *et al.*[35] reported a 75% of PCs release in apple varieties and Chitindingu *et al.*[36] reported PCs bioaccessibily from 20% to 26% in cereal grains. The PCs bioaccessibility largely varied between foods and may be strictly dependent on the composition of the food matrix and chemical interactions among food components. For example, extractable PCs could be released more easily because they are not strongly bounded to food matrix constituents.[34] This is in accordance with the higher bioaccessibility of PCs in mango-flesh and lower released of PCs in wheat bran. Also, results confirm and agree with other authors in that food matrices that are high in dietary fiber content, such as wheat bran, can interact and affect the release of PCs.[36, 37]. Mango-flesh

which contained 6% of fiber (mainly soluble) showed 27% more of PCs bioaccessibility than wheat bran which had 35% of fiber (mainly insoluble). The linked of PCs to the insoluble fiber of wheat bran, apparently is affecting the bioaccessibility of these compounds during digestion. In addition, cereals have higher content of non-digestible carbohydrates, such as resistant starch, which could impedes the release of PCs.

Moreover, the low PCs bioaccessibility and potential uptake presented by both food matrices could be attributed to the dietary fiber behavior during it passage through the gastrointestinal-tract.[38] Dietary fiber can act under the small intestine conditions as soluble polymer chains in solution, as insoluble macromolecular assemblies, and as swollen, hydrated, sponge-like net-works.[38] One physicochemical property of dietary fiber is its viscosity, which is recognized to affect physiological responses. Viscous fibers (gums, pectins, and β-glucans) thicken when mixed with fluids. The degree of thickening depends on the chemical composition and concentration of the polysaccharide.[39] As a result, increasing the viscosity of the gastric fluids restricts the peristaltic mixing process that promotes transport of enzymes to their substrates, as well as affects the absorption of various nutrients (including soluble PCs) to the intestinal wall. In this sense, viscous fibers have been associated with alterations in blood glucose, cholesterol and PCs concentrations, prolonged gastric emptying, and slower transit time through the small intestine.[38, 39] It is expected that foods containing high concentration of insoluble fiber would exhibit lower viscosity because these type of fiber typically have lower water-holding capacity than soluble fiber, however; many insoluble fiber such as those found in wheat bran contain water-soluble arabinoxylans that contribute to water holding capacity and increased viscosity in solutions.[40] By other hand, dietary fiber can act as a physical trapping that prevents the absorption of extractable PCs. Unreleased PCs (associated or entrapped to fiber) are not accessible in the small intestine and cannot be absorbed; however, they can reach the colon and be fermented by microbiota releasing a significant amount of PCs that can create an antioxidant environment.[41]

The antioxidant capacity of the samples decrease from gastric to intestinal *in vitro* digestion phase, decreasing again during dialysis (**Fig 2**). The antioxidant capacity of mango-flesh and wheat bran during the different digestion phases were

lower than those determined by methanolic extracts, presumably due to the lower concentrations of PCs present compared to chemical extraction.

Diffusion behavior

Elucidation of the possible absorption mechanism of PCs from mango-flesh and wheat bran is of special importance in order to assess if the absorption of the PCs embedded in these matrices is affected by interaction with other compounds present in the food matrix. Many mathematical models have been proposed to describe the kinetics transport of molecules from nanoparticles, microparticles, dendrimers based in synthetic and natural polymers. Mathematical kinetic models provide basis for the study of mass transport mechanism of the bioaccessibles PCs in the simulated epithelial barrier. These transport mechanisms are divided into three categories: Fickian diffusion models, collective diffusion models and non-Fickian diffusion models.[42] Four simple empirical models were applied to the data for the explanation of possible absorption kinetics. Modelling analysis was carried out by fitting the accumulated absorption percentage of PCs (**Fig 3**). Coefficient of correlation analysis (r^2) of linear relationship between the amount of PCs absorbed and time were conducted for the four models. Both samples showed the best correlation with the Peppas model with r^2 of 0.93. The diffusion exponent (n) were 0.334 and 0.491 for mango-flesh and wheat bran, respectively (**Table 4**). These results suggest that the absorption mechanism of PCs is primarily governed by a Fickian diffusion. That means that diffusion are determined by both concentration gradient-controlled (moves from a more concentrated to a less concentrated space) and/or relaxation-controlled diffusion.[43] Additionally, our results showed a biphasic diffusion behavior, with a rapid start, following by a slow uptake in both treatments. Apparently, the mango-flesh had a major interaction with food matrix and consequently a lower concentration of absorption was observed. As described before, during the human digestive process the viscosity of the samples in solutions could increase and act as an entrapment network whose prevents the PCs transport. In addition, once the samples were digested, released compounds of the food matrix (free sugars) could be interacting between them and/or with the dialysis membrane, preventing the PCs passage.

CONCLUSIONS

The present work investigated the *in vitro* bioaccessibility, passive diffusion and antioxidant capacity of total PCs present in two plant foods with different content and type of dietary fiber, 'Ataulfo' mango-flesh and wheat bran. Results suggest that the bioaccessibility of PCs are higher in the food matrix with the low content of dietary fiber and starch (mango-flesh). However, the type of fiber present in the food matrix affects the absorption diffusion behavior of PCs released (low absorption at high soluble fiber). This is reflected in the antioxidant capacity of the samples. The absorption mechanism for the bioaccessibility of PCs is primarily by Fickian's diffusion with a possible interaction between phenols and other food matrix components.

Future studies in humans should be carried out in order to confirm the data obtained *in vitro.*

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Illustrations and Tables

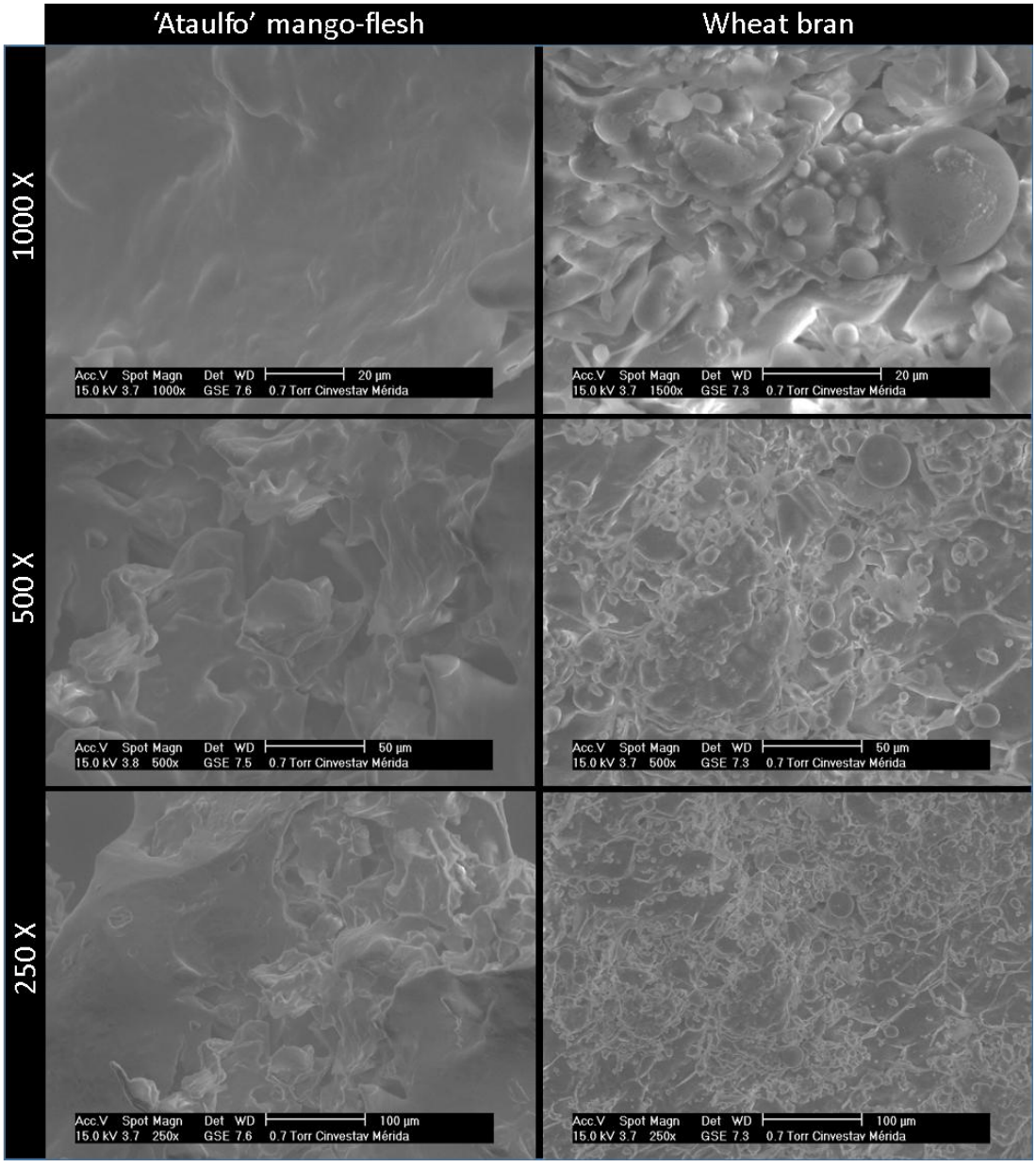

Figure 1. Microstructure of mango-flesh and wheat bran in scanning electron microscopy micrography.

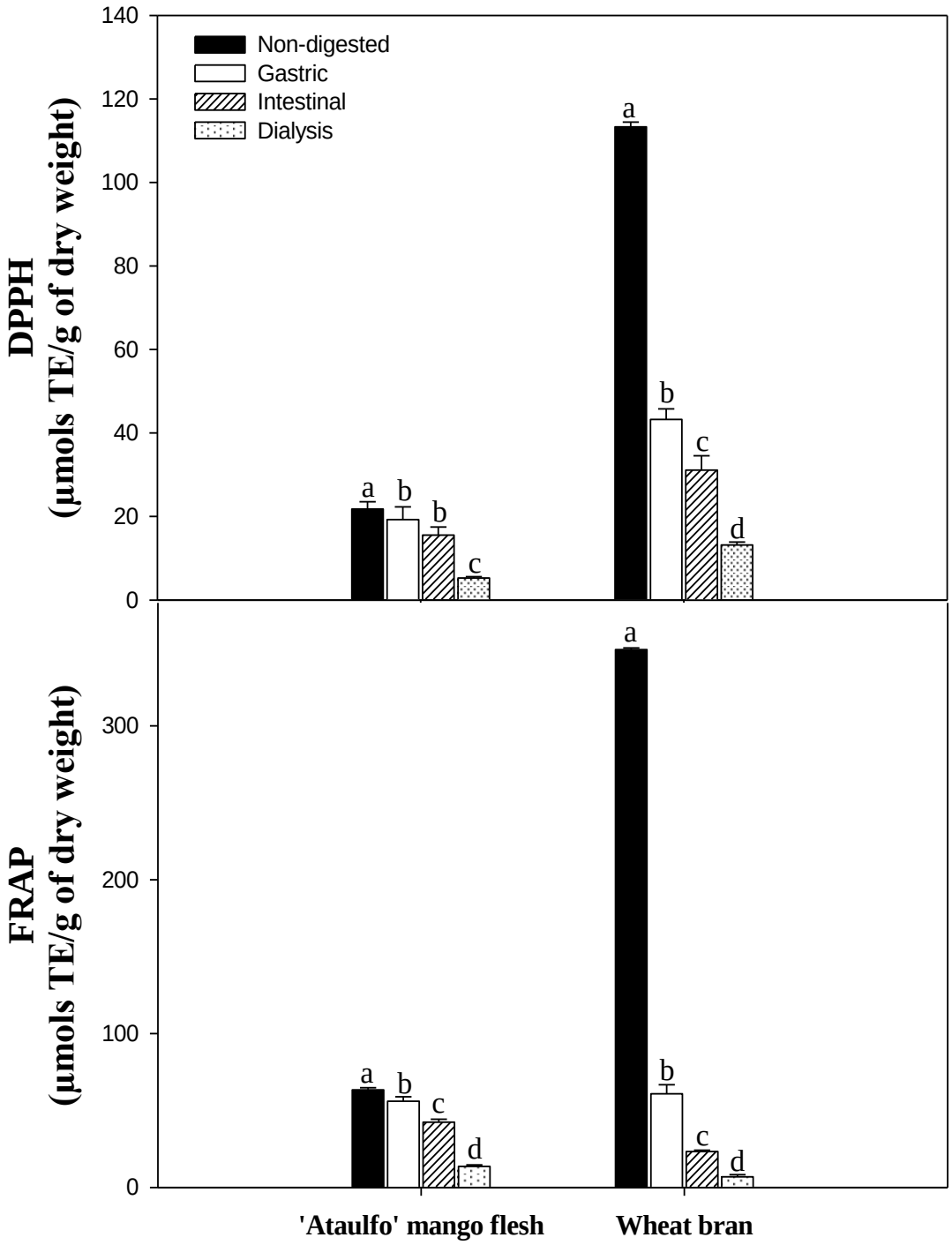

Figure 2. Changes in antioxidant capacity during *in vitro* digestion of mango-flesh and wheat bran. Different letters indicate significant differences (p<0.05) between non-digested sample and digestion phases.

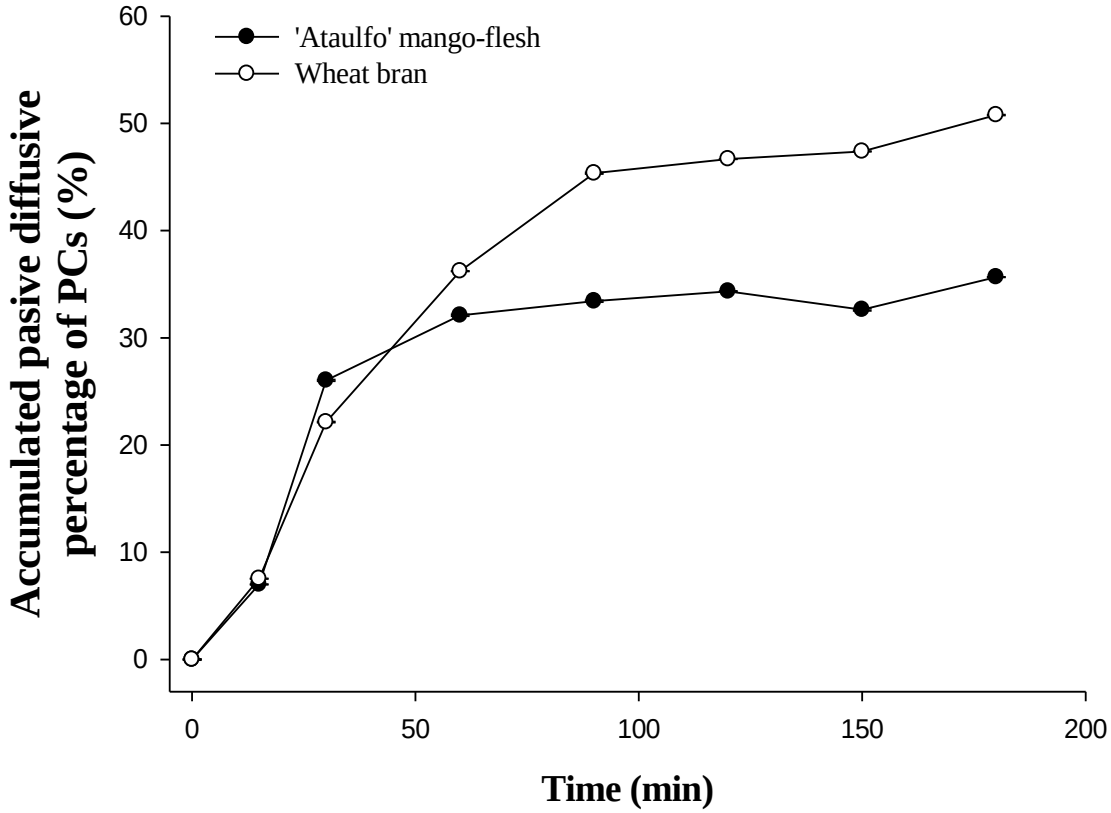

Figure 3. Accumulated absorption percentage of PCs from mango-flesh and wheat bran during dialysis.

Table 1. Total PCs content and antioxidant capacity of mango-flesh and wheat bran.

Parameter	Mango-flesh	Wheat bran
Total PCs (mg GAE/g of dry weight)	**17.1^{A}**	**20.7^{B}**
Extractable PCs	9.32^{aA}	1.05^{aB}
Non-extractable PCs	7.81^{aA}	19.6^{bB}
Hydrolysable tannins	*7.81*	*19.6*
Condensed tannins	*n.d.*	*n.d.*
Antioxidant capacity (µmols TE/g of dry weight)	**21.8^{A}**	**113^{B}**
DPPH	14.6^{aA}	9.70^{aB}
Extractable PCs	7.23^{bA}	103^{bB}
Non-extractable PCs		
FRAP	**63.4^{A}**	**349^{B}**
Extractable PCs	52.0^{aA}	8.2^{aB}
Non-extractable PCs	11.4^{bA}	341^{bB}

Mean (n = 3). *n.d*: not detected. Different lower case letters between rows indicate significant differences (p<0.05). Different upper case letters between columns indicate significant differences (p<0.05).

Table 2. Dietary fiber, PCs and antioxidant capacity associated to it, of mango-flesh and wheat bran.

Parameter	Mango-flesh	Wheat bran
Total dietary fiber (%)	**5.82^{A}**	**35.1^{B}**
Soluble	2.61^{aA}	1.60^{aB}
Insoluble	3.26^{bA}	33.5^{bB}
Total PCs in soluble dietary fiber (mg GAE/g of dry weight)	**0.04**	***n.d.***
Extractable PCs	0.04	*n.d.*
Non-extractable PCs	*n.d.*	*n.d.*
Total PCs in insoluble dietary fiber (mg GAE/g of dry weight)	**0.05^{A}**	**4.00^{B}**
Extractable PCs	0.02^{aA}	0.11^{aB}
Non-extractable PCs	0.03^{aA}	3.82^{bB}
Antioxidant capacity in total dietary fiber (µmols TE/g of dry weight)		
DPPH	0.41^{aA}	18.6^{aB}
FRAP	0.97^{aA}	45.1^{bB}

Mean (n = 3). *n.d*: not detected. Different lower case letters between rows indicate significant differences (p<0.05). Different upper case letters between columns indicate significant differences (p<0.05).

Table 3. Total PCs (mg GAE/g of dry weight) and bioaccessibility (%) of mango-flesh and wheat bran in non-digested sample and during *in vitro* digestion phases.

Digestive phase	Mango-flesh			Wheat bran		
		Bioaccessibility (%)	Accumulated (%)		Bioaccessibility (%)	Accumulated (%)
Non-digested	17.1^{aA}			20.7^{aA}		
Gastric	7.11^{bA}	41.5	41.5	2.60^{bB}	12.5	12.5
Intestinal	8.56^{bA}	8.20	49.7	4.70^{cB}	10.2	22.7
Dialysis*	2.72^{cA}	31.7		2.38^{bA}	50.6	

Mean (n = 3). *n.d*:. Different lower case letters between rows indicate significant differences (p<0.05). Different upper case letters between columns indicate significant differences (p<0.05). *:Percentage of bioaccessibility during dialysis phase was calculated based on PCs released during the intestinal phase.

Table 4. Correlation coefficients (r^2) according to the different models and diffusion/release exponent (n) used for describing the diffusion mechanism of PCs after an *in vitro* digestion of mango-flesh and wheat bran wheat bran.

	First order		**Peppas**	
	r^2	***K***	**r^2**	***n***
Mango-flesh	0.51	.003	.932	.334
Wheat bran	0.86	.005	.939	.451

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CAPÍTULO III

Impacto del estado de madurez de mango cv. 'Ataulfo' (*Mangifera indica* L.) sobre la bioaccessibilidad y capacidad antioxidante de compuestos fenólicos

Preparado: ***Foods***

Impact of the stage of ripening on *in vitro* bioaccessibility and antioxidant capacity of phenolic compounds in 'Ataulfo' mango (*Mangifera indica* L)

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Abstract

Changes occur during fruit ripening that strongly affect fruits' matrices and consequently the bioaccessibility/bioavailability of phenolic compounds. Flesh from 'slightly' (SR), 'moderately' (MR) and 'fully' (FR) ripe 'Ataulfo' mangoes were subject to a physicochemical characterization and a simulated in *vitro* digestion to evaluate the impact of mango ripening on the releasability and passive difussion of its phenolic compounds. Physical analysis changes in pH (+2.4), TSS (+2.7 ^{o}Brix), citric (-5.6 g/100g) and malic (-0.06 g/100g) acids, titratable acidity (-0.36 g citric acid/L) and flesh's tone (97.4 vs. 82.7 ^{o}Hue) during ripening (SR to FR, $p<0.05$). Total (-10.4) and soluble (-7.38) dietary fibers, starch (-5.36) and free glucose (-4.24) declined but total soluble sugars increased (+22) during ripening ($p<0.05$). Total (-5.7) and non-extractable (-8.9) phenolic compounds decline and extractable fraction (+3.2) (mg EAG/ g DW) increases ($p<0.05$), but their antioxidant capacity (TEAC, FRAP) did not vary much during ripening ($p>0.05$). Total digestibility of phenolics increased 28.5% (SR) to 43.4 (FR), mainly released simulated gastric conditions (26.1 to 40.0%) but their passive diffusion was low. There is a synergistic effect of both fruit ripening and digestion phases on the bioaccesibility of mango phenolics associated to microstructural and chemical changes in its food matrix.

Keywords: *Mangifera indica* L. phenolic compounds, gastrointestinal digestion, bioaccessibility, ripening, food matrix

1. Introduction

Mexico is the leading mango (*Mangifera indica* L) exporting country worldwide, being its varietal 'Ataulfo' the most exported to United States [1]. 'Ataulfo' mango fruit is a popular and economically important tropical fruit consumed at different ripening stages throughout the world, due to its excellent sensorial (bright, color, sweet taste and luscious flavor), nutritional (vitamins, minerals, dietary fiber) and functional (phytochemicals) quality [2]. It is considered as a good source of dietary antioxidants, especially phenolic compounds that have shown different health-promoting properties, mainly due to their remarkable antioxidant capacity [3]. Several authors have reported that 'Ataulfo' mango presents the highest phenolics content and antioxidant capacity among several mango varieties [2]. In this sense, its popularity as a research model has been increased over the years.

Phenolic compounds must be released (bioaccessibility) from the fruit matrix, absorbed by the gastrointestinal epithelia and released to the bloodstream (bioavailability) and finally delivered to target tissues to perform their functional action [4]. Several factors affect the absorption efficiency of phenolic compounds including their type and content in food sources, degree of fruit ripeness and processing treatments and the chemical nature of food matrices , all having positive or negative effects on their further absorption [5,6]. Particularly, many physiological, chemical and biochemical changes occur during fruit ripening that strongly affect fruits' matrices and consequently the bioaccessibility/bioavailability of bounded phenolic compounds [7]. During ripening, plant cell walls modify their microstructure and composition resulting in the loss of firmness [8,9], mostly related to enzymatic modification of polysaccharides including starch and dietary [10]. In this sense, previous studies from our group have shown that 'Ataulfo' mango possesses a distinctive pattern of extractable phenols and antioxidant capacity during ripening, associated to several physicochemical (e.g. firmness, soluble solids and pH), physiological (e.g. respiration rate, ethylene production) and biochemical (e.g. enzyme expression and activity) changes [2,11]; such compositional changes surely modifies the bioaccessibility and antioxidant capacity of phenolic compounds from this mango during ripening, although this has not been evaluated yet. In support of this, Ornelas-Paz *et al*. [12] examined the influence of stage of ripening of 'Ataulfo' mango on micellarization during digestion and intestinal cell uptake of β-carotene.

They reported that the quantity of *β*-carotene transferred to the micelle fraction during a simulated digestion significantly increase as the fruit ripened. Therefore, the objective of this work was to evaluate the effect of ripening stage on *in vitro* bioaccessibility and antioxidant capacity of phenolic compounds in 'Ataulfo' mango.

2. Materials and methods

2.1 Fruit material

Fresh 'Ataulfo' mangoes (average weight of 210-250 g) were purchased from the local market (Hermosillo, Sonora, Mexico) and transported immediately to the laboratory for evaluation. Fruits were sanitized with chlorinated water (200 ppm sodium hypochlorite) for 5 min and left to dry at room temperature and subjectively classified according to their peel surface color into the following groups, "Slightly ripe" (SR), "moderately ripe" (MR) and "fully ripe" (FR). Thirty-five mangoes of each group were peeled and the fruit flesh was either processed immediately fresh (physicochemical analyses) or freeze-dried (phenolic compounds and *in vitro* digestion), as further explained.

2.2 Physiological and chemical characterization

The color of mango flesh and peel were longitudinally determined on four points of each flat side of fruits (n = 3), using a Minolta CR-300 colorimeter (Konica Minolta Sensing, Inc., USA). L* value represents the luminosity of the fruit, where 0 = black and 100 = white. a* value ranges from the negative (green) to the positive (red) scale while the b* value ranges from negative (blue) to positive (yellow) scale. To know the real color changes of the fruit, a* and b* values were used to calculate the Hue angle (°Hue) value ($°Hue = \tan^{-1}(b/a)$).

Flesh tissue firmness was measured by the puncture method, using a Chatillon Penetrometer, Model DFM50 (Ametek Inc, USA) with 8 mm diameter flat-head stainless-steel cylindrical prove. Tissue's opposition force against the penetration was registered on 3 points in the equatorial region of the whole piece of fruit with skin removed and results were reported in Newton (N).

pH, total soluble solids (TSS, ^{o}Brix) and titratable acidity (TA,g citric acid/L) contents were evaluated in a 10 g sample of fruit pulp homogenized in 50 mL of distilled water; the mixture was filtered and 50 mL of the filtered mixture were taken to quantify pH and TA, using a Triator Model DL28 (Mettler Toledo, USA). TA was

reported as citric acid percent. TSS was measured directly from the filtered residue, using an Abbe digital refractometer (E-Inginst Electron Corp., USA) and expressed as °Brix.

2.3 Total phenolic compounds content and antioxidant capacity evaluation

For the quantification of extractable phenolic compounds (free), organic-aqueous extraction was performed on freeze-dried 'Ataulfo' mango (1 g) samples with methanol-water (80:20 v/v) solution (30 mL), sonicating for 30 min. For non-extractable phenolic compounds (linked to food matrix) extraction, residues from the organic-aqueous extraction were dispersed in 20 mL of methanol and 2 mL of sulfuric acid were added. The extracts were incubated in a shaking water bath at 85 °C for 20 h. They were cooled at room temperature and centrifuged at 3000 rpm for 10 min. Then, the supernatants were recovered. Subsequently, the residue was washed twice with 10 mL of distilled water and the supernatants were mixed in a 50 mL volumetric flask. The final extracts were stored at -25 °C to be used in the determination of total phenolic compounds content and antioxidant capacity of raw samples (before *in vitro* digestion).

Total phenolic compounds content is given by the sum of the extractable and non-extractable phenolics. The extractable phenolic compounds and hydrolysable tannins (part of the non-extractable phenolics) were measured by the Folin-Ciocalteau procedure according to Singleton and Rossi *et al.* [13] with slight modifications, using a microplate reader. Results were expressed as mg of gallic acid equivalents (GAE)/g of dry weight.

Antioxidant capacity was determined by Ferric Reducing Antioxidant Power (FRAP) and Trolox Equivalent Antioxidant Capacity (TEAC) assays. FRAP was carried out according with Benzie and Strain [14]. The reagent (280 µL) and sample solutions (20 µL) were added to each well and mixed thoroughly. The mixture was kept in the dark for 30 min and absorbance was read at 593 nm. For TEAC assay, the method of Re *et al*. [15] as used, with slight modifications. The working solution of ABTS radical cation ($ABTS^{\bullet+}$) was generated by mixing 19.2 mg of ABTS, 5 mL of mili-Q water and 88 µL of potassium persulfate (0.0378 g mL^{-1}) at room temperature in the dark for 16 h. The solution of $ABTS^{\bullet+}$ was then diluted with pure ethanol to an absorbance of 0.7 ± 0.02 at 734 nm. The reaction was initiated adding 245 µL of $ABTS^{\bullet+}$ and 5 µL of sample, allowed to react for 5 min. Absorbance was measured at

734. For both assays, trolox (6-hydrozy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was used as a standard and results were expressed as μmol of trolox equivalents (TE)/g of dry weight.

2.4 Carbohydrate composition

Soluble sugars (glucose, fructose and sucrose) and organic acids (citric and malic acid) were extracted twice from 0.1 g of sample with 80% ethanol (v/v), centrifuged at 3,000 rpm for 10 min and supernatants were combined and dried at 40ºC [16]. Dry extracts were re-dispersed in 2 mL of distilled water and subjected to HPLC analysis as previously reported. Precipitates from 80% ethanol extraction were incubated with thermostable α-amylase (150 U) and amyloglucosidase (40 U) at pH 4.5 in a boiling water bath for 15 min at 100 °C. Starch was quantified as free D-glucose (510 nm) with a GODOP format assay kit (Megazyme, International Ireland Ltd Wicklow, Ireland).

Insoluble and soluble dietary fibers were determined according to AOAC (Method 991.43) [16], using a Megazyme kit assay. Neutral sugars composition of both soluble and insoluble fibers were determined by the method of alditol acetates [17]. Briefly, two mg of sample were hydrolysed with 500 μL of 2N trifluoroacetic acid containing 100 μL ml^{-1} myo-inositol, at 121 °C for 1 h, centrifuged and supernatants were dried and converted to alditol acetates [18]. Samples were injected into a gas chromatograph Varian CP-3800 coupled to a FID detector (250 °C), using a DB-23 capillary column 30 m x 0.25 mm (210 °C) and helium as a carrier (3 $ml \cdot min^{1}$). Results were calculated using external standards of rhamnose, fucose, arabinose, xylose, mannose, galactose, glucose and myo-inositol as internal standard (Sigma-Aldrich, >95 pure).

2.5 Scanning electron microscopy (SEM)

Microstructure differences between SR, MR and FR fresh samples (gold/palladium covered) were examined by SEM (250x and 1000x) at an accelerative voltage of 15 kV.

2.6 *In vitro* bioaccessibility

To analyze the effect of ripening stage on the release of phenolic compounds under simulated gastrointestinal conditions, the bioaccessibility of phenolics in SR, MR

and FR mango was determined according to the methodology of Saura-Calixto *et al.* [19] and Granfeldt *et al.* [20] with slight modifications. Freeze-dried samples (300 mg) with 10 mL of HCl-KCl solution (pH 1.5) were incubated with a 0.2 mL of pepsin solution (300 mg/mL) during 60 min at 40 °C in a water bath with constant shaking, simulating gastric conditions. Then, 4.5 mL of phosphate buffer (pH 7.5) was added to the samples and pH was adjusted to 7.5. Aditionally, to simulated intestinal conditions, 1 mL of pancreatin (5 mg/mL) was added and the samples were incubated 6 h at 37 °C. Finally, samples were centrifuged (2615 x g for 15 min) and supernatants separated. The precipitate corresponds to the indigestible part that reaches the colon; however, supernatants contain soluble phenolic compounds for potential uptake. Then, supernatants were immediately transferred into semipermeable cellulose dialysis bags (12000-14-000 Da), sealed with clips, completely immersed into tubes contained phosphate buffer (pH 7.5), and dialysed for 3 h at 37 °C. Phenolic compounds content was analyzed by Folin-Ciocalteau method. Antioxidant capacity was determined in each phase using FRAP and TEAC assays. Individual experiments were conducted to measure bioaccessible phenolic compounds at each of the different phases of digestion and not affect the sample volume. Bioaccessibility assessment was performed by calculating difference based on the content of total phenolic compounds in the non-digested samples and the phenolics released from the food matrix during the *in vitro* digestion phases (Equation (1)).

$$\text{Bioaccessibility (\%)} = \left(\frac{\text{Phenolic compounds digestion phase}}{\text{Phenolic compounds non} - \text{digested}}\right) x\ 100 \qquad (1)$$

where phenolic compounds digestion phase = phenolics concentration at each phase of the *in vitro* digestion. Phenolic compounds non-digested = total phenolics content determined by chemical extraction.

2.7 Statistical analysis

All measurements were made in triplicate (n = 3). Results are expressed as means ± standard deviation of the mean. Data were statistically analyzed by one-way ANOVA procedure and Tukey-Kramer multiple comparison test at 95 confidence level. Number Cruncher Statistical System version 6.0 software (NCSS, LLC) was

used. Lastly, Pearson's product moment correlations (*r*) between chemical, physical, phenolic compounds, neutral sugars and polysaccharide contents were performed.

3. Results and discussion

3.1 Physiological and chemical characterization

Table 1 shows the physiological and chemical results of SR, MR and FR 'Ataulfo' mangoes. As expected, pH (+2.4), TSS (+2.7 ^{o}Brix) and peels *L** value increased while citric (-5.6 g/100g) and malic (-0.06 g/100g) acids, titratable acidity (-0.36 g citric acid/L) and flesh's tone (97.4 vs. 82.7 ^{o}Hue) decreased during ripening (SR to FR, $p<0.05$). TA was higher in SR mango (1.1 g/L) compared to FR mango (0.3 g/L). Flesh firmness values decreased during mango ripening, from 17.9 N (SR) to 9.7 N (FR). Peel color of 'Ataulfo' mango fruit changed during ripening from green to yellow, reported as lightness (L^{*}) in peel. Also, mango flesh color changed during maturity from green to yellow, reported as °Hue.

Results are in agreement with our previous studies [2,21]. During the ripening of 'Ataulfo' mango, the decline in the level of organic acids was accompanied by an increment in soluble sugars ($r\geq-0.73$) and the sugar-to-acid ratio has been related to consumer's acceptance as sweet taste develops [22]. This change is associated to the metabolic conversion of acids into sugars by gluconeogenesis (mainly citric, ascorbic and malic), consuming organic acids and thus declining total acidity as observed in this study ($r\geq0.98$). Also, the increase in TSS content during ripening is attributed to the accumulation of free sugars ($r=0.57$, **Table 2**) from the hydrolysis of starch ($r=-0.60$) by the action of amylases that are normally ethylene-dependent [23]. The loss of mango firmness during ripening is related to the modification of cell walls of fruit cells and their degradation by cell wall enzymes (**Figure 1**) that commonly increase in activity during the late stages of ripening [24]. Moreover, it has been reported that the mango peel and flesh color changes from green to yellow during ripening and is correlated with carotenoids concentration [25].

3.2 Total phenolic compounds content and antioxidant capacity

Total phenolic compounds content (extractable and non-extractable) in the different 'Ataulfo' mango ripening stages are presented in **Figure 1**. Conventionally, non-extractable phenolic compounds fraction are formed by hydrolyzable (phenolic acids polymers) and condensed (flavonoids polymers) tannins [26], but 'Ataulfo' mango

seems to have only hydrolysable tannins [27]. In this study, total (-5.7 mg GAE/g DW) and non-extractable (-8.9 mg GAE/g DW) decreased but extractable (+3.2 mg EAG/g DW) phenolic compounds increased during ripening (p<0.05).

Total phenolics content of ‘Ataulfo’ mango was higher than reported by others studies [2,28], which could be attributed to various factors such as fruit cultivar, growing conditions, among others. However, this difference is mainly attributed to the fact that, contrary to this study, in most works only extractable phenolic compounds was reported. The reduction of the non-extractable phenolic compounds fraction (hydrolysable tannins) during ripening is associated with their de-polymerization to adjust the fruit taste for animal feed [29], and in case of ‘Ataulfo’ mango to the complementary genetic expression of key enzymes of the phenylpropanoid pathway [11]. As hydrolysable tannins get de-polymerized they produce free soluble phenols (gallic and ellagic acid), so extractable phenolics fraction increase. Similar results were reported for hydrolysable tannins content in ‘Tommy Atkins’ mango by Kim *et al.* [30]. Palafox-Carlos *et al*.[2] reported a same trend in the first three stages of ripening for extractable phenolic compounds content in ‘Ataulfo’ mango flesh; higher in unripe stage than moderately ripe stage. The decrease of total phenolic compounds in ‘Ataulfo’ mango during ripening could be related with fruit senescence [2], probably mediated by channeling carbohydrates from phase I to polymeric phenolic compounds (non-extractable) during phase II metabolism, as supported by the inverse correlation between TSS content and total and non-extractable phenolics (r=-0.73) during ripening.

Lastly, the evaluation of the antioxidant capacity is usually done comparing different methods in order to take into account the large number of factors that can influence the antioxidant action. According to **Figure 1**, total antioxidant capacity, as assayed by TEAC, did not vary among ripening stages (~225 µmol TE/g DW) with an almost equal contribution from its extractable (~113 µmol TE/g DW) and non-extractable (~112 µmol TE/g DW) phenolic components. However, FRAP did showed a statistical significant (p<0.05) reduction between SR and FR stages, particularly in its non-extractable phytochemicals component (-24.4 µmol TE/g DW). As previously reported by Palafox-Carlos *et al*. [2], main mango phenolics (and possibly other antioxidants such as ascorbic acid) fluctuate not much affecting its overall antioxidant capacity during ripening.

3.3 Dietary fiber, cell wall composition and morphological analysis

According to **Table 2**, the percentage of total (-10.4) and soluble (-7.38) dietary fibers, starch (-5.36) and free but not bounded glucose (-4.24) declined during ripening (p<0.05), while the total soluble sugars increased (+22). It is noteworthy that the content of total dietary fiber is higher than that obtained in a previous study for 'Ataulfo' mango in commercial ripening stage (6% dietary fiber content, personal communication), possibly attributed to differences in the analytical method used [31]; in this sense, the AOAC method (991.43) use filtration instead centrifugation to separate the soluble and insoluble fractions; also, this method precipitates the soluble fiber fraction with ethanol instead of use a dialysis membrane against water. Nevertheless, similar results were reported for 'Keiit' and 'Tommy Atkins' mangoes [32]. From a biochemical standpoint, enzyme-catalyzed structural changes in the main polysaccharides, that is pectin, hemicellulose and cellulose, seems to be responsible for the "softness" of the cell wall structure during ripening (Figure2). Particularly, solubilisation and de-polymerisation of pectin during fruit decreases the content of total soluble dietary fiber [10]. Lastly, starch content in 'Ataulfo' mango decreased in almost 95% as fruit ripped (5.7% to 0.3%, p<0.05). The starch content in 'Ataulfo' mango was lower than that reported for 'Alphonso' mango; however, came down similarly during ripening [8]. As the fruit ripens the taste development increase in sweetness, which is result of increased hydrolysis of polysaccharides, especially starch. Starch breakdown leads concomitantly to sugars accumulation.

Neutral sugars such as fucose (-0.25), arabinose (-0.76), xylose (-1.71) and galactose (-1.24) associated to insoluble (-5.17) but not soluble fiber declined during ripening while other neutral sugars were maintained at relatively low concentrations (**Table 2**). This pattern was also reported for 'Keiit' and 'Tommy Atkins' mangoes, where arabinosyl, galactosyl and xylosyl residues were the most abundant neutral sugars. They found considerable decreases in arabinosyl, galactosyl and rhamnosyl residues in mango fruit [32]. Bonded-neutral sugars in both dietary fibers were xylose (insoluble fibers)/mannose (soluble fibers) >galactose> glucose/arabinose which are key substrates for ascorbic acid synthesis [33]. The presence of xylose and galactose in insoluble dietary fiber suggest the presence of hemicellulose and the high concentration of specific neutral sugars could be the result of their bio-transformation from other sugars (e.g. sucrose) by enzymatic modification.[34]

Also, an almost perfect correlation between the loss of firmness and reduction in starch (r=0.83) and total-soluble-insoluble dietary fibers (r>0.91) contents was observed. Apparently, starch is being metabolized to sucrose during 'Ataulfo' mango ripening, as it has been demonstrated for bananas [35,36] and apples [37]. As a consequence of sucrose breakdown and metabolism, glucose and fructose are produced increasing total soluble sugars from 50% in SR to 72% in FR mango, as it has been observed during ripening for other mango varieties [38] and apples [39]. Glucose levels decreased, while fructose and sucrose increased. It has been reported that starch content in food affects texture, viscosity and gel formation, which consequently may affect the absorption/release of phenolic compounds during digestion [40]. Differences in the fiber content, cell wall constituents and morphological structure between samples, clearly indicates that the architecture of mango flesh differs during ripening. Scanning electron microscopy micrographs studies revealed notable morphological differences during the different stages of ripening of 'Ataulfo' mango (**Figure 2**). The images shown an amorphous structure in SR mango, which is changing to a more soft structure in the FR mango. The amorphous matrix presented in the SR mango could be related to the high content of soluble dietary fiber and starch since many granules (< 10 μm) are more present in SR stage disappearing during ripening. This coincides with the negligible presence of starch content in the ripe mango (**Table 2**), and the resulting microstructure rearrangement and metabolic adaptation of mango during ripening seems to be related to a higher non-bound (free) phenolic content. In support of this, a perfect positive (TSS) (r=0.79) and negative (firmness, citric acid, non-extractable phenols, starch and total, soluble and insoluble dietary fibers) (r≥-0.93) correlation explained the higher content of extractable phenolic compounds during ripening.

3.4 *In vitro* bioaccessibility

The impact of *in vitro* digestion phases on the bioaccessibility of extractable phenolic compounds at different ripening stages of mango 'Ataulfo' is shown in **Figure 3** and accumulative data in **Table 3.** When compared to ethanol-water extractable phenolic compounds (as described in 2.3), digestion efficiency follow a perfect trend during ripening from 30.8% to 43.4%. Most of phenolics were released at gastric level (26.1 to 40.0%, respectively) not being statistically different at later stages (MR and FR), as it also happens at simulated intestinal conditions (p>0.05). Results obtained for FR

mango are similar with data obtained in a previous work where the bioaccessibility of phenolic compounds of 'Ataulfo' mango flesh at commercial ripening was evaluated [27]. These results also agree with the later study as to most of mangoes phenolic compounds are released during gastric phase, with a minor contribution of the intestinal phase. This behavior is attributed to the acidic hydrolysis of phenol glycosides to their corresponding aglycons during simulated gastric digestion [41,42]. Acidic hydrolysis may also have an important role in breaking down the cell walls and releasing certain phenolic compounds [43]. Moreover, the increased bioaccessibility of phenolic compounds after intestinal phase is attributed to the additional time of extraction (plus 6 h) and the effect of small intestinal digestive enzymes on the complex food matrix (carbohydrates), facilitating the release of some phenolic compounds associated to the fruit matrix [44].

α-Glucosidase and α-amylase are the key enzymes involved in the digestion of carbohydrates. α-Amylase degrades complex dietary carbohydrates to oligosaccharides and disaccharides that are ultimately converted into monosaccharides by α-glusosidase [45]. This process takes places in the small intestine, where the enzymes are secreted [46]. In addition to this, recent studies have reported that microbiota compositions that are typically found in small intestine are involved for fast import and conversion of relatively simple carbohydrates, contributing to a rapid adaptation to the overall nutrient availability [47,48]. This can also contribute to the release of phenolic compounds trapped.

On the other hand, the total amount of dialysable phenolic compounds increased from SR (1.62 mg GAE/g of dry weight) to FR (2.45 mg GAE/g of dry weight) mango, being the SR ripening stage different ($p<0.05$) from the others. However, the amount of dialysable phenolics in the three ripening stages of 'Ataulfo' mango was lower to that observed at the intestinal phase (**Figure 3**), suggesting a possible interaction between digested food matrix components, which prevents the phenolic compounds transport. This is consistent with previous findings (personal communication, personal communication), where the diffusion behavior of the gastrointestinal released phenolics from 'Ataulfo' mango was evaluated by a kinetic model and the results showed an interaction between phenolic compounds with the food matrix components. Also, dialysable results of FR mango coincide with those obtained in a previous study of 'Ataulfo' mango flesh in a commercial ripening stage (2.7 mg GAE/g of dry weight). Although SR mango presented the highest phenolic

compounds content, it showed the lesser bioaccessibility of phenolics during gastric phase (26.1%). This could be attributed to the high concentration of non-extractable phenolic compounds, which are strongly linked to the food matrix and need extreme conditions to release (acidic conditions for 20 h) [49]. Inversely, higher release of phenolics during intestinal phase was presented in SR mango (4.7%), which could be by the action of the intestinal enzymes on the amorphous fruit matrix that releases embedded phenolic compounds [50]. Pearson's product moment correlations (*r*) between gastric and total bioaccesibility of extractable phenols during mango 'Ataulfo' ripening revealed a positive (TSS; $r \geq 0.79$) and negative (starch and all dietary fibers; $r \geq -0.88$) correlation with diverse carbohydrate fluctuations ($p < 0.01$).

Together, all these findings indicate a synergistic effect of both fruit ripening and digestion phase on the bioaccesibility of mango phenolic compounds. These results agree with those reported by Ornelas-Paz *et al.* [12] where the *in vitro* bioaccessibility of carotenoids in 'Ataulfo' mango is affected by different stages of ripening. Unripe 'Ataulfo' mango presented the lower phenolic compounds bioaccessibility which could be attributed to the food matrix characteristics presented in this stage of *r*ipening, such as firmness, amorphous structure, higher dietary fiber and starch content, as well as higher non-extractable phenolic compounds fraction. Saura-Calixto *et al.* [51] reported that the non-extractable phenolic compounds fraction are partially bioaccessible during gastrointestinal digestion; however, most of them (>95%) arrive nearly intact to the colon. Once in the colon, the non-extractable fraction become accessible by fermentation by colonic microbiota or by the action of some intestinal enzymes able to break covalent bonds, such as esterases [49]. Furthermore, softening of fruit increase the accessibility of some phytochemicals by facilitating the mechanical and enzymatic disruption of the flesh during digestion. This, ripening likely has a similar effect as homogenization and thermal processing that disrupt cell walls to provide digestive enzymes with access to macromolecules to facilitate release of phenolic compounds [12].

However, we hypothesized that the main factors affecting the release of phenolic compounds are the presence of dietary fiber and starch. SR 'Ataulfo' mango has higher starch and fiber content, particularly soluble dietary fiber. It has been reported that phenolic compounds may have influence on enzyme susceptibility and digestion of starch. Studies observed that some phenolics such as hydrolysable tannins decreased the starch digestion; which could affect the release of phenolic

compounds embedded in the matrix rich in starch [52]. Furthermore, starch and phenolic compounds interact to form either inclusion complex in the form of amylose single helices facilitated by hydrophobic effect, or complex with much weaker binding most thought hydrogen bonds. These interactions may impact on the phenolic compounds bioaccessibility and bioavailability [53]. By other hand, dietary fiber can act as a physical trapping that prevents the release of phenolics during gastrointestinal conditions. One physicochemical property of dietary fiber is the viscosity, which is recognized to affect physiological responses. Viscous fibers (soluble fiber) thicken when mixed with fluids, as a result, increasing the viscosity of the gastric fluids restricts the peristaltic mixing process that promotes transport of enzymes to their substrates, as well as affects the release and absorption of various nutrients to the intestinal wall, including phenolic compounds [54].

Lastly, the influence of *in vitro* digestion on antioxidant capacity in SR, MR and FR 'Ataulfo' mangoes determined by TEAC and FRAP assays is shown in **Figure 3**. Both assays show a significant decrease ($p<0.05$) in the antioxidant capacity after gastric digestion, compared to non-digested samples. By TEAC assay, there was observed a significant increase ($p<0.05$) after intestinal digestion, with a consequent significant decrease ($p<0.05$) during the dialysis phase. This behavior is related to the content of phenolic compounds during *in vitro* digestion. However, the FRAP test showed a significant ($p<0.05$) subsequent decrease after intestinal phase and a lower activity in dialysis phase. In addition to their concentration, pH could also play a role in the antioxidant activity of phenolic compounds. Some phenolics (quercetin and resveratrol in grape extracts) exhibited higher antioxidant activities during intestinal phase (neutral pH conditions) as measured by TEAC assay [44]. It is thought that the transition from acidic to alkaline environment enhances the antioxidant power of phenolic compounds by causing deprotonation of the hydroxyl moieties present on their aromatic rings. Despite this hypothesis, Bouayed *et al*. [44] suggested that due the chemical conditions of the FRAP assay (pH of 3.6), this test exert higher response in gastric phase and for that reason this assay could be more appropriate to evaluate the antioxidant capacity during gastric digestion than intestinal digestion.

4. Conclusions

This research demonstrates that the physiological and ripening processes of 'Ataulfo' mango modifies the nature of this fruit's matrix (mainly carbohydrate), the releasable portion of phenolic compounds, as well as the resulting antioxidant capacity in such way that non-ripe fruit has greater presence of dietary fiber and starch, which prevents the *in vitro* bioaccessibility and absorption phenolic compounds. Future studies should evaluate the effect of colonic fermentation of bounded phenols to dietary fiber as well as the possible physicochemical interactions between starch and phenolic compounds.

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Tables and Figures

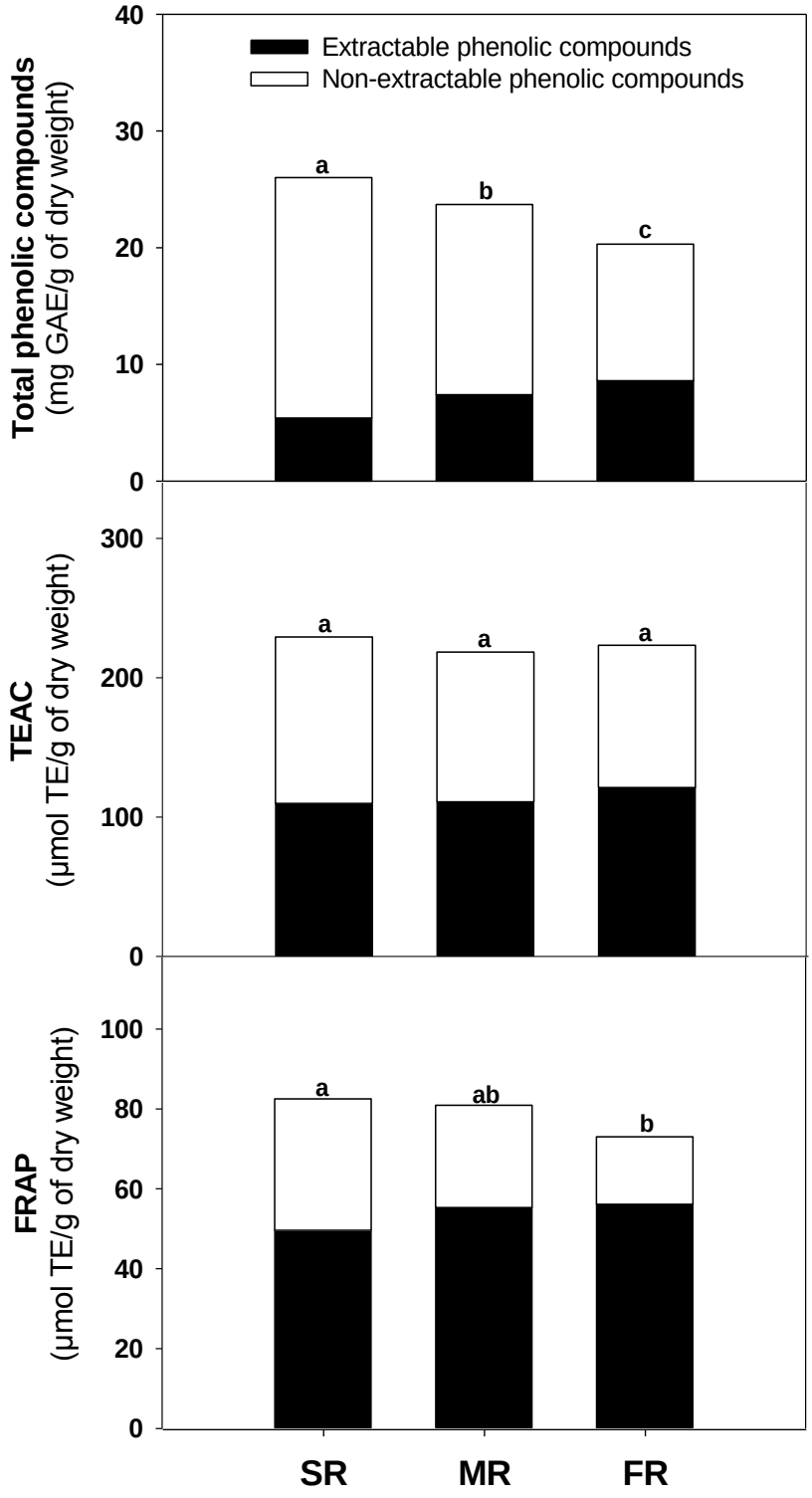

Figure 1. Total phenolic compounds and antioxidant capacity in "slightly ripe" (SR), "moderately ripe" (MR) and "fully ripe" (FR) 'Ataulfo' mangoes flesh. Means values. Different letter indicates significant difference (p<0.05) for total amount.

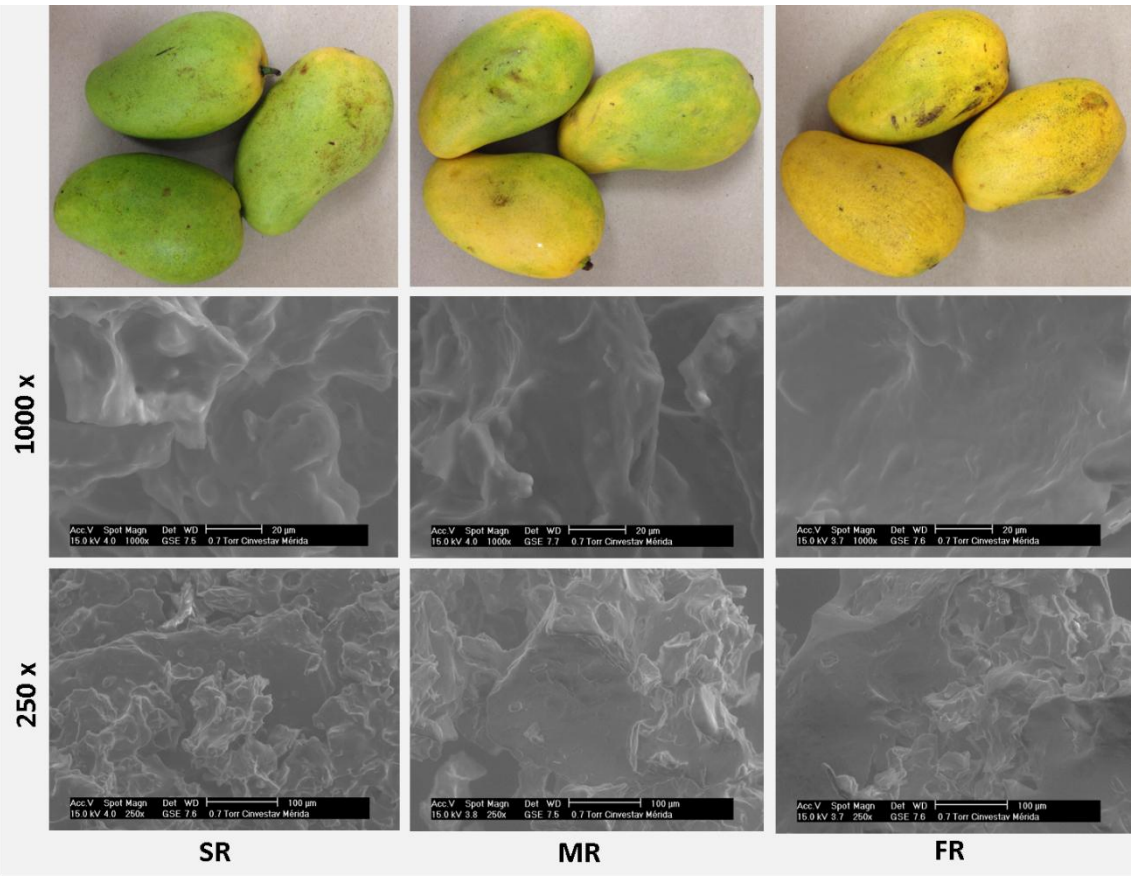

Figure 2. Microstructure of "slightly ripe" (SR), "moderately ripe" (MR) and "fully ripe" (FR) 'Ataulfo' mangoes flesh, by scanning electron microscopy.

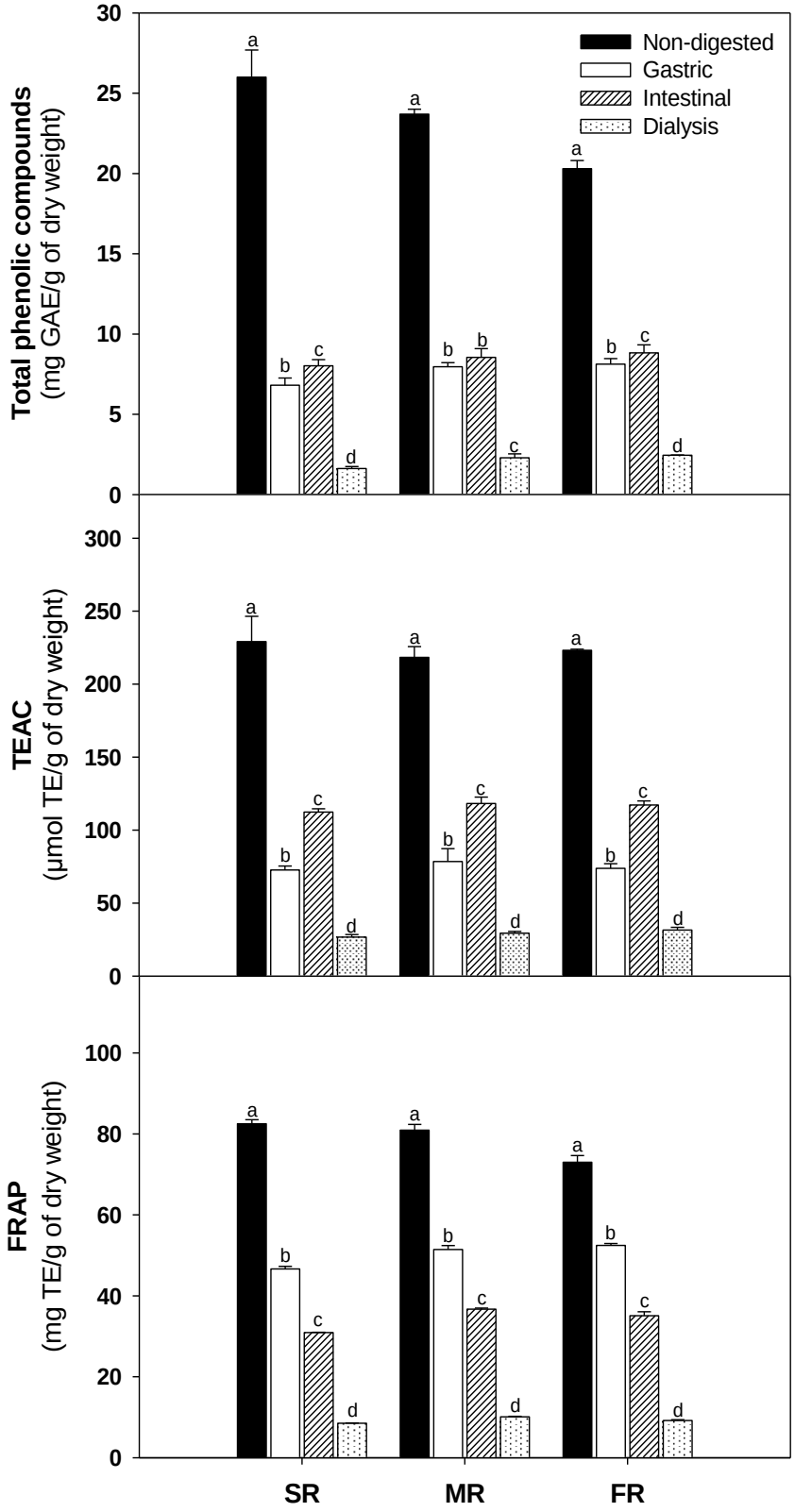

Figure 3. Total phenolic compounds and antioxidant capacity in "slightly ripe" (SR), "moderately ripe" (MR) and "fully ripe" (FR) 'Ataulfo' mangoes during *in vitro* digestion phases. Means values. Different letter at each ripeness stage indicates significant difference (p<0.05) between digestion phases.

Table 1. Changes in pH, total soluble solids (TSS), citric and malic acid, titratable acidity (TA), firmness and color parameters in "slightly ripe" (SR), "moderately ripe" (MR) and "fully ripe" (FR) 'Ataulfo' mangoes.

Ripening state	pH	TSS (°Brix)	Citric acid (g/100 g)	Malic acid (g/100 g)	TA (g/L)	Firmness (N)	Color	
							Flesh (°Hue)	Peel (L*)
SR	2.10[a]	12.5[a]	7.46[a]	0.08[a]	1.17[a]	17.9[a]	97.4[a]	60.5[a]
MR	3.21[b]	13.0[b]	3.62[b]	0.05[b]	0.74[b]	14.8[b]	88.9[b]	67.8[b]
FR	4.49[c]	15.2[c]	1.85[c]	0.02[c]	0.36[c]	9.73[c]	82.7[c]	72.2[c]

Mean values. Different letter between ripeness stages indicates significant difference (p<0.05).

Table 2. Dietary fiber, starch, total soluble sugars and neutral sugars content in "slightly ripe" (SR), "moderately ripe" (MR) and "fully ripe" (FR) 'Ataulfo' mangoes (%).

Parameter	SR	MR	FR
Total dietary fiber	**25.5^{a}**	**18.3^{b}**	**15.1^{c}**
Soluble fiber	15.2^{a}	9.89^{b}	7.82^{c}
Insoluble fiber	10.3^{a}	8.43^{b}	7.37^{b}
Starch	**5.70^{a}**	**0.96^{b}**	**0.34^{c}**
Total soluble sugars	**50.2^{a}**	**68.4^{b}**	**72.2^{b}**
Glucose	6.83^{a}	5.46^{b}	2.59^{c}
Fructose	12.3^{a}	18.1^{b}	18.1^{b}
Sucrose	31.1^{a}	44.8^{b}	51.5^{c}
Neutral sugars (Soluble fiber)	**12.4^{a}**	**13.7^{a}**	**12.2^{a}**
Rhamnose	0.45^{a}	0.42^{a}	0.37^{a}
Fucose	0.05^{a}	0.05^{a}	0.05^{a}
Arabinose	2.50^{a}	2.44^{a}	2.28^{a}
Xylose	0.12^{a}	0.09^{a}	0.12^{a}
Mannose	3.69^{a}	4.15^{a}	3.32^{a}
Galactose	3.41^{a}	3.31^{a}	3.07^{a}
Glucose	2.24^{a}	3.29^{b}	3.06^{b}
Neutral sugars (Insoluble fiber)	**12.0^{a}**	**8.53^{b}**	**6.83^{c}**
Rhamnose	0.26^{a}	0.14^{b}	0.11^{b}
Fucose	0.63^{a}	0.49^{b}	0.38^{c}
Arabinose	1.64^{a}	1.14^{b}	0.88^{c}
Xylose	3.61^{a}	2.63^{b}	1.90^{c}
Mannose	1.10^{a}	0.74^{b}	0.62^{b}
Galactose	3.15^{a}	2.26^{b}	1.91^{c}
Glucose	1.68^{a}	1.13^{a}	1.04^{a}

Means values. Different letter between ripening stages indicates significant difference (p<0.05).

Table 3. Bioaccessibility (%) of phenolic compounds in "slightly ripe" (SR), "moderately ripe" (MR) and "fully ripe" (FR) 'Ataulfo' mangoes during gastric and intestinal *in vitro* digestion.

Ripening state	Bioaccessibility (%)		Accumulated (%)
	Gastric	Intestinal	
SR	26.1[a]	4.7[a]	30.8[a]
MR	33.5[b]	2.4[b]	36.0[b]
FR	40.0[b]	3.4[b]	43.4[c]

Mean values. Different letter between ripeness stage indicates significant difference ($p<0.05$).

CAPÍTULO IV

Efecto del procesamiento de mango cv. 'Ataulfo' en jugo sobre la bioaccessibilidad y capacidad antioxidante de compuestos fenólicos

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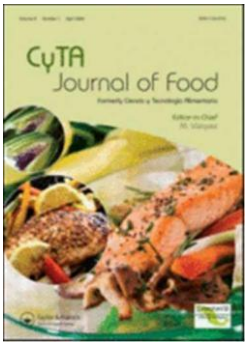

Influence of juice processing on the in vitro bioaccessibility of phenolic compounds and antioxidant capacity of the 'Ataulfo' mango

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Key Words:	'Ataulfo' mango juice, phenolic compounds, processing, bioaccessibility, in vitro digestion

Influence of juice processing on the antioxidant capacity and *in vitro* bioaccessibility of phenolic compounds from 'Ataulfo' mango

Efecto del procesamiento de mango cv. 'Ataulfo' en jugo sobre la bioaccessibilidad y capacidad antioxidante de compuestos fenólicos

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Abstract

The effect of juice processing on the phenolic compounds content, antioxidant capacity and *in vitro* bioaccessibility of phenolics of 'Ataulfo' mango was analysed. The carbohydrates composition of mango juice was also evaluated. Results demonstrate that juice processing decrease approximately 37% of 'Ataulfo' mango phenolic compounds, as well their antioxidant capacity. Total dietary fiber content was 12.71% with a low presence of starch (0.24%), which corresponds to an 16% and 30% decrease of dietary fiber and starch, respectively, compared to mango flesh. After *in vitro* simulated gastrointestinal digestion, phenolic compounds bioaccessibility was 53%. Gastric phase release the majority of phenolic compounds (52%), with a slight further release (1%) during intestinal digestion. The bioaccessibility of phenolic compounds of mango juice was 3% higher than non-processed fruit. This slight difference is attributed to the effect of processing on the composition of the food matrix.

Keywords: 'Ataulfo' mango juice, phenolic compounds, processing, bioaccessibility, *in vitro* digestion

1. Introduction

The Mexican variety of 'Ataulfo' mango has a best potential as functional food due to its high content of bioactive compounds, such as vitamins, dietary fiber and antioxidants including carotenoids and phenolic compounds (Bello-Pérez, Tovar, & Sáyago-Ayerdi, 2015; Palafox-Carlos *et al.*, 2012; M. Robles-Sánchez *et al.*, 2011). Many of these phytochemicals, especially phenolic acids and gallotannins, have anti-inflammatory, anti-proliferative and hypolipidemic effect (García-Solís, Yahia, & Aceves, 2008; Noratto *et al.*, 2010; M. Robles-Sánchez *et al.*, 2011), all of them useful to prevent non-communicable chronic diseases suchas cardiovascular diseases, type-2 diabetes and cancer (Wall-Medrano *et al.*, 2014).

Mango juice is produced by squeezing the flesh of edible mangoes (Bates, Morris, & Crandall, 2001). The goal in juice manufacture is to separate as much of the desirable components from the fruit as possible without extracting undesirable ones (e.g. insoluble compounds). Fruit juices retain their chemical profile but certain physicochemical (e.g. rheology) and organoleptic (e.g. texture, odor) features differ from fresh-cut flesh from which they are produced (Rodríguez-Roque *et al.*, 2016). Nevertheless, processed fruit are expected to have a lower health protecting capacity than fresh ones, due the consequences of fruit processing on food composition (Nicoli, Anese, & Parpinel, 1999). Some phenolics are associated to insoluble components of the fruit matrix and could be eliminated during juice extraction.

Juice processing involves changes in the microstructure of plant foods (*e.g.* the disruption of cell walls and membranes) which in turn help to release of phenolic compounds from phenolics-carbohydrates complexes, improving their solubility as free and ester derivates (Parada & Aguilera, 2007). Together, these changes modify the bioaccessibility of many bioactive compounds. Particularly, oranges, kiwis, pineapples, mangoes, apples and, grapes when processed as juices and subsequently processed with other treatments (e.g. pressure, thermal) negatively affects the content and bioaccessibility of carotenoids and phenolic compounds (He *et al.*, 2016; Lemmens *et al.*, 2014; Rodríguez-Roque *et al.*, 2016). However, opposite effects have been also reported on the intrinsic phenolic compounds content and their bioaccessibility in fruit juices can occur during processing (Nicoli *et al.*, 1999).

Despite the many reports on the chemical nature and physicochemical characteristics of mango "Ataulfo" by-products, there is very little information on its

juice, particularly on its phenolic profile, antioxidant characteristics and their bioaccesibility and bioavailability. That is why the aim of this study was to evaluate the effect of juice processing on the total phenolic compounds content, total antioxidant capacity and *in vitro* bioaccessibility of phenolic compounds from 'Ataulfo' mango.

2. Materials and Methods

2.1 Plant material

Mature 'Ataulfo' mango fruits, free from external defects, were purchased from a local supermarket (Hermosillo, Mexico) and transported to the laboratory. The fruits were rinsed with tap water, air dried and stored at 10-13 °C and 85-90% RH.

2.2 'Ataulfo' mango juice

Mature mangoes were sanitized, peeled, cut into pieces and the juice was extracted as suggested by Santhirasegaram *et al.* (2013) with some modifications. Mango flesh were macerate using a domestic juice extractor (Moulinex, Centri III, A75312V) and the supernatant was filtered through organza cloth. After that, the freshly-squeezed juice was freeze-dried and stored at room temperature in a desiccator until analysis. The mango juice moisture was determined by gravimetry, considering the percentage of fresh weight that the difference between fresh and dried weight represents (moisture = ((initial weight – dry weight)/ dry weight)*100).

2.3 Phenolic compounds content and antioxidant capacity

Determination of total phenolic compounds content (extractable and non-extractable fractions) and antioxidant capacity (TEAC and FRAP assays) were conducted as described by Quirós-Sauceda *et al*. 2016 (not published, CAPÍTULO III).

2.4 Carbohydrate and cell wall composition

Starch, insoluble and soluble dietary fiber and neutral sugars evaluation were determined as described by Quirós-Sauceda *et al*. 2016 (not published, CAPÍTULO III).

2.5 Simulated gastrointestinal digestion

A three-stage *in vitro* digestion model was performed based on the previously described procedure by Quirós-Sauceda *et al*. 2016 (not published, CAPÍTULO II y III). Farther, to evaluate the effect of the physicochemical conditions in each digestion phase, the same methodology was followed but in absence of enzymes.

2.6 Statistical analysis

All analysis were conducted in triplicate, with values reported as the mean ± standard deviation (SD). Data were statistically analyzed by one-way Analysis of variance (ANOVA) followed by a Tukey-Kramer multiple comparison test at 95 confidence level to determine significant differences (p<0.05). Number Cruncher Statistical System version 6.0 software (NCSS, LLC) was used.

3. Results and discussion

3.1 Phenolic compounds content

Results are presented in dry weight; however, the fresh 'Ataulfo' mango juice contained 80.2 % moisture. The content of phenolic compounds in mango juice is reported in **Table 1**. The total concentration of phenolic compounds (extractable + non-extractable fractions) was 11.79 mg GAE/g of dry weight (2.33 mg GAE/g of fresh weight). This results were higher than those reported for strawberry press-juice (0.61 mg GAE/g of fresh weight) and lower compared to black mulberry juice (18.66 mg GAE/g of dry weight) (Klopotek, Otto, & Böhm, 2005; Tomas *et al.*, 2015).

The mango juice extractable fraction content (8.88 mg GAE/g of dry weight = 1.74 mg GAE/g of fresh weight) was similar to that present in mango flesh obtained in Quirós-Sauceda *et al.* 2016 CAPÍTULO III (9.3 mg GAE/g of dry weight = 1.86 mg GAE/g of fresh weight, personal communication) and the reported by Velderrain-Rodríguez *et al.* (2016) (6.68 mg GAE/g of dry weight = 1.44 mg GAE/g of fresh weight). However, the non-extractable fraction decreased ~60% after processing mango juice, compared with the data previously obtained in the same studies. Furthermore, the results are consistent with previous reports that only showed presence of hydrolysable tannins in the fraction of non-extractable phenolic compounds in 'Ataulfo' mango flesh, paste and peels (Blancas-Benitez *et al.*, 2015; Sáyago-Ayerdi *et al.*, 2013; Velderrain-Rodríguez *et al.*, 2016).

According to the previous findings by Quirós-Sauceda *et al.* (2016) (personal communication, CAPÍTULO III) of the total phenolic compounds in 'Ataulfo'

mango flesh, approximately 36.59% (based on fresh weight) of the fruit phenolics were lost in the final mango press-juice. This result is similar to the reported for other fruits. Klopotek & Böhm (2005) reported the loss of 23% of phenolic compounds content during processing of strawberries to juice. Tomas *et al.* (2015) showed that 30-40% of anthocyanins are lost during industrial-scale juice production of black mulberry. These could all be linked to the release of native enzymes (polyphenols oxidase, peroxidase, etc.) from cells during the pressing process, as a result of cell wall or membrane disruption, acting in oxidation and degradation reactions (Renard *et al.*, 2011). In addition, during the extraction of juice the major part of insoluble material is removed, in which some fraction of phenolic compounds are linked (mostly non-extractable phenolic compounds). In this sense, the content of non-extractable phenolic compounds in 'Ataulfo' mango juice was reduced by 65.84% (based on fresh weight).

It is well known that food processing (pressing, filtration and fining) can have many effects on the naturally phytochemicals present in the whole fruit (Klopotek *et al.*, 2005). However, not all leads in a loss of quality and health properties. For instance, it has been found an improvement of phenolic compounds concentration during various food processing process, which is attributed to a release of the substances due to the cell wall damage during processing (Nicoli *et al.*, 1999).

3.2 Antioxidant capacity

The hydrophilic antioxidant capacity was analyzed by using two methods - TEAC and FRAP assays - showing different sensitivity and different reaction principles. The measured hydrophilic total antioxidant capacity (extractable + non-extractable fractions) values were 158.9 µmols TE/g of dry weight (31.46 9 µmols TE/g of fresh weight) (TEAC assay) and 62.68 µmols TE/g of dry weight (12.41 µmols TE/g of fresh weight) (FRAP assay) of fresh 'Ataulfo' mango juice (**Table 1**). The total antioxidant capacity of mature mango flesh were 223.2 µmols TE/g of dry weight (48.16 µmols TE/g of fresh weight) (TEAC assay) and 73.01 µmols TE/g of dry weight (48.16 µmols TE/g of fresh weight) (FRAP assay) (Quirós-Sauceda *et al*. 2016 CAPITULO III, personal communication), this indicates that the antioxidant capacity of mango juice determined as a TEAC value decreased by ~29% during the process. As compared with the results obtained with FRAP assay, there were less noticeable differences. The loss of the ferric reducing antioxidant potential was only

~14%. The decrease in antioxidant capacity could be related to the decrease of the non-extractable phenolic compounds fraction in the juice matrix that contributes to a reduced antioxidant activity. Analyzing results from both methods, it is possible to observe that the antioxidant capacity of juice showed a decrease within the processing procedure of 'Ataulfo' mango.

Similar results were expressed by others authors where the antioxidant capacity decreases through fruit juice processing (7-50% loss) (Klopotek *et al.*, 2005; Rodríguez-Roque *et al.*, 2016; Tomas *et al.*, 2015). Milling, mashing and pressing are among the main processing steps that causes the largest decrease of phytochemicals content and antioxidant capacity. This is due to the fact that most of the compounds are relatively unstable (Nayak, Liu, & Tang, 2015). Furthermore, operations such as peeling, cutting, slicing and pressing are expected to induce a rapid enzymatic depletion of several natural antioxidants (*i.e.* ascorbic acid, phenolic compounds) (R. M. Robles-Sánchez, Rojas-Graü, Odriozola-Serrano, González-Aguilar, & Martín-Belloso, 2009). The decrease of the contents of vitamin C, phenolic compounds and other antioxidants substances led to a reduction of the hydrophilic antioxidant capacity during the processing of 'Ataulfo' mango juice.

3.3 Compositional analysis

Dietary fiber, starch, citric acid, total soluble sugars and neutral sugars content in 'Ataulfo' mango juice is shown in **Table 2**. Total dietary fiber content was 12.71%, where soluble fiber represents the 6.46% and insoluble fiber the 6.25%. In general, the soluble and insoluble fractions showed very similar values, not presenting statistically difference ($p<0.05$) between them. These values were slightly lower than those present in 'Ataulfo' mango flesh (15.1% total dietary fiber, 7.82% soluble dietary fiber, 7.37% insoluble dietary fiber) (Quirós-Sauceda *et al*. 2016 CAPITULO III, personal communication). Decreasing insoluble dietary fiber is positively related ($r=0.83$) to the decrease of non-extractable phenolics fraction in 'Ataulfo' mango juice. In this regard, total dietary fiber content of 'Ataulfo' mango juice decreased by ~16% during the process. Neutral sugars content in soluble and insoluble mango juice dietary fiber were significantly different ($p<0.05$); however results not showed difference to those present in mango flesh. The juice is defined as the extractable fluid contents of cell or tissues. Although many fruit juices are the obvious result of expressing the liquid from the whole fruit, there are some fruits, such as mango,

where the distinction is not so apparent and yields a puree containing insoluble material (Bates *et al.*, 2001).

Starch content in 'Ataulfo' mango juice was 0.24% that means that decreased in almost 30% during the juice extraction process; although starch content decreased the mango juice presented haziness. The starch consists of glucose polymers, α-1,4 and α-1,6 linked and about 30 units in length. Although the granules tend to be present mainly in slightly ripe fruit, their small size in ripe fruit means that they may escape filtration procedures (Ashurst, 2013).The presence of starch in juice is a potential contributor to the haziness. Therefore, enzymes (amylase and amyloglucosidase) are used during juice production to remove starch (Singh & Singh, 2015). However, in this study were not used.

Citric acid is the predominant acid in mango (Medlicott & Thompson, 1985). Citric acid concentration in 'Ataulfo' mango juice increased (4.37%) when compared to mature mango flesh (1.85%). Total soluble sugars (glucose + fructose + sucrose) value was 67.68%. The amount of glucose, fructose and sucrose were different and presented the following trend: glucose (6.06%) < fructose (12.11%) < sucrose (49.49%). The total soluble sugars value decrease in ~6% during mango juice extraction, as well as fructose and sucrose (compared to data from Quirós-Sauceda *et al*. 2016 CAPITULO III, personal communication). Glucose in mango juice increase about twice compared to mango flesh (2.59%). The increase in the content of some compounds can be attributed to an efficiently extraction of acids, sugars and other water soluble in the pressing operation (Skrede, Wrolstad, & Durst, 2000). In addition, removing the insoluble fiber in the indigestible residue increases the content of other components.

3.4 Influence of simulated gastrointestinal digestion on bioaccessibility and antioxidant capacity

Bioaccessibility

The effect of a simulated gastrointestinal digestion on bioaccessibility of phenolic compounds present in 'Ataulfo' mango juice is shown in **Figure 2**. As can be seen, gastric digestion (using pepsin) released the majority of phenolic compounds (52%) from mango juice, with a poor increase during intestinal phase (~1%, using pancreatin) (**Figure 3**). This trend has been extensively reported by others authors and is mainly attributed to the effect of acidic hydrolysis on breaking down the cell

walls of food matrix and release some phenolics (Mandalari *et al.*, 2013; Mosele, Macià, Romero, Motilva, & Rubió, 2015; Velderrain-Rodríguez *et al.*, 2016). The physicochemical conditions of the digestion phases in the absence of enzymes were evaluated. As a result, the physicochemical conditions during gastric phase released the 45.29% of the phenolic compounds in mango juice, while intestinal conditions released a 6.19% increase. These bioaccessibility percentages were not statistically different (p>0.05) to the results in presence of enzymes. In this sense, it can been seen that pH, temperature, movement and time conditions are primarily responsible for the release of phenolic compounds from the food matrix.

Phenolic compounds in 'Ataulfo' mango juice had higher bioaccessibility after *in vitro* digestion than in mango flesh (~46%) (Quirós-Sauceda *et al.*, 2016 CAPÍTULO II y III, personal communication). However, bioaccessibility after intestinal phase was higher in mango flesh with ~6% than in juice (Quirós-Sauceda *et al.*, 2016 CAPÍTULO III, personal communication). This could be attributed to a higher presence of carbohydrates in flesh (including dietary fiber) that are being enzymatically hydrolyzed by the action of pancreatin resulting in the release of some associated/embedded phenolics. Moreover, the slight increase of released phenolics from 'Ataulfo' mango juice may be due to different factors. For example, (1) fruit processing rupture cells and leads to the release of food matrix constituents as well as increases surface are and reduces the crystallinity of cellulose, thereby exposing the phenolic compounds presumably trapped in the cell wall network and making the phenolics more accessible for diffusion into the juice (Soto, Acosta, Vaillant, & Pérez, 2015). (2) Mango juice has lower carbohydrate content, as a result there is less chance of the interaction between them and phenolic compounds. It has been well reported that dietary fiber and phenolic compounds are able to interact chemically in the food matrix and during gastrointestinal digestion, these interactions can decrease or delay the phenolic compounds release/absorption (A. Quirós-Sauceda *et al.*, 2014). (3) The starch content is even lower in juice than in flesh. Starch content in food affects texture, viscosity and gel formation, which consequently may affect the absorption/release of phenolic compounds during digestion (Zhu, 2015).

Rodríguez-Roque *et al.* (2014) reported that the bioaccessibility of phenolic constituents from a fruit blended juice (orange, kiwi, pineapple and mango) was 19.6% and in a fruit blended juice added with milk and sugar was 10.92%. These results were 64% and 80%, respectively, lower than the obtained for 'Ataulfo'

mango juice. This shows that the complexity of the food matrix in which bioactive compounds are contained can affect their degree of digestibility and therefore, their bioaccessibility. As well as carbohydrates, the presence of proteins leads to form complexes with phenolic substances that interfere in their bioaccessibility (Jakobek, 2015).

On the other hand, the dialysed fraction in 'Ataulfo' mango juice represented the 67.67% of the total bioaccessible phenolic compounds. These results were 41% higher than the obtained for mango flesh (Quirós-Sauceda *et al.*, 2016 CAPÍTULO II y III, personal communication). As mentioned before, during fruit processing into juice, the cell walls are broken and much of the insoluble material is removed, making the phenolics more accessible for diffusion, which is the main transport mechanism in the dialysis experiment. The presence of insoluble material in the food matrix can bound other food constituents, such as phenolics and therefore reduce their solubility (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004; A. E. Quirós-Sauceda *et al.*, 2014). Quirós-Sauceda *et al.* (2014) reported that after 2 h of contact between 'Haden' mango methanolic extract with mango dietary fiber, total phenols decreased 23.72%. Additionally, the incubation of a fiber insoluble nature (wheat-bran) and 'Haden' mango methanolic extract increase the depletion of phenolics to 38%.

In the absence of pancreatin, the percentage of the dialyzable fraction in mango juice was only the 33%. This corresponds to 17% less than in the presence of the enzyme. Results could be associated to a longer incubation time of the pancreatin (+ 3 h) during dialysis phase, in where the enzyme is incubated in ideal conditions for enzymatic activity, resulting in increased release and transport of phenolic compounds.

Antioxidant capacity

Health benefits of phenolic compounds are partly attributed to their antioxidant capacity. The antioxidant capacity of initial and digested sample was evaluated by TEAC and FRAP assays. Changes in antioxidant capacity during *in vitro* digestion of 'Ataulfo' mango juice are shown in **Figure 4**. The antioxidant capacity of sample after gastric and intestinal digestion phases were significant lower (p<0.05) than those determined in the non-digested mango juice sample. The $ABTS^{\bullet+}$ radical scavenging activity decrease by 42% during gastric digestion, with a subsequent

increase by 20% during intestinal phase. The dialysis fraction has a 50% lower radical activity compared to intestinal phase. On the other hand, ferric reducing activities decrease by 30.35%, 20.72 and 37.79, respectively. The reduction of these parameters could be presumably due to the decrease in the concentration of phenolic compounds by oxidation or chemical transformations that strongly influence their antioxidant capacity. In addition, the pH of the substance is known to affect racemization of molecules, possibly creating two chiral enantiomers with different reactivity in the respective reagents. In theory, this could alter their biological reactivity and may render the antioxidants more reactive early in the digestive process, particularly at acidic pH in the gastric phase and less reactive at pH 7.4 in the duodenal phase as racemization can increase with pH in other compounds (Jamali, Bjørnsdottir, Nordfang, & Hansen, 2008).

This results do not differ much from those obtained during *in vitro* digestion of 'Ataulfo' mango flesh (Quirós-Sauceda *et al.* 2016 CAPÍTULO III, personal communication). Antioxidant results from digestion of mango flesh were slightly higher because the concentrations of phenolic compounds during digestion phases were high (The bioaccessibility % changes as related to initial phenolic compounds content of the sample). The TEAC assay results for mango flesh and juice digestion were as follow: gastric<intestinal>dialysis. Moreover, FRAP assay results for mango flesh and juice digestion were as follow: gastric>intestinal>dialysis. It has been reported that by TEAC assay, some phenolic compounds show higher antioxidant capacities during intestinal phase due the transition from acidic to alkaline environment enhances the antioxidant power of phenolic compounds by causing deprotonation of the hydroxyl moieties present on their aromatic rings (Bouayed, Hoffmann, & Bohn, 2011).

4. Conclusions

In this study we examined the effect of juice processing of 'Ataulfo' mango on the phenolic compounds content, bioaccessibility and antioxidant capacity. The results demonstrate that juice processing decrease approximately 37% of 'Ataulfo' mango phenolic compounds, as well their antioxidant capacity. During *in vitro* simulated gastrointestinal digestion, phenolic compounds had a slight higher bioaccessibility than in mango flesh, attributed to the food matrix composition, a lower concentration of carbohydrates and that processing allow a greater solubility of phenolics in the

juice. Further *in vivo* investigations on the bioavailability of phenolic compounds of 'Ataulfo' mango juice must be performed in order to clarify whether the *in vitro* results coincides with *in vivo* trials.

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Tables and Figures

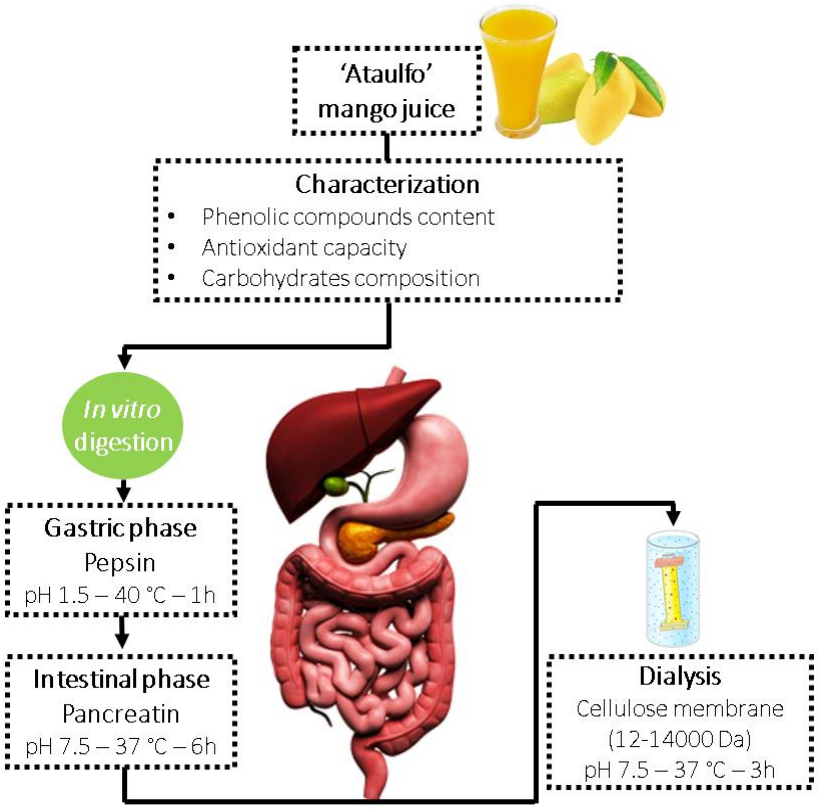

Figure 1. Sample determinations and *in vitro* digestion protocol.

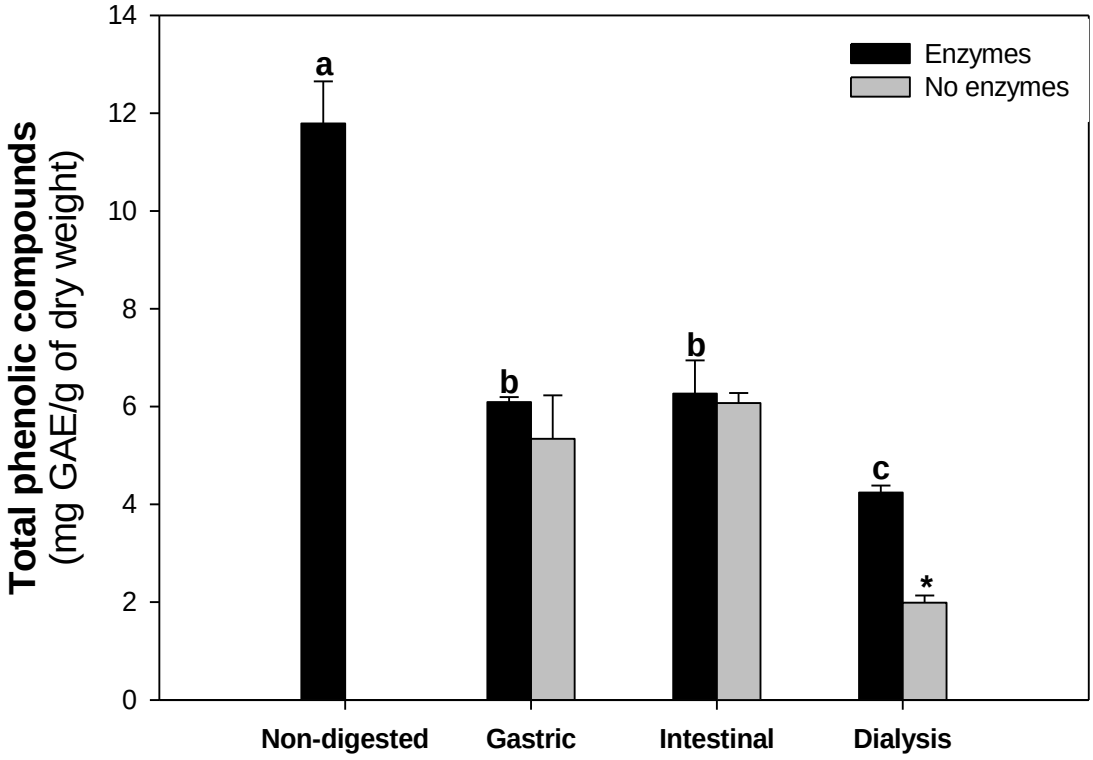

Figure 2. Changes in total phenolic compounds content during *in vitro* digestion of 'Ataulfo' mango juice. Different letters indicate significance differences (p<0.05) between digestion phases. *: indicate significance differences (p<0.05) in the presence or absence of enzymes.

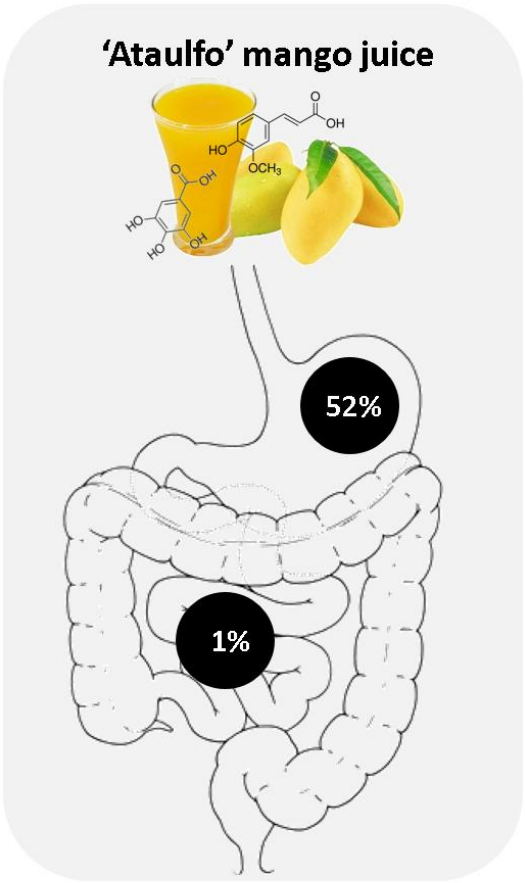

Figure 3. Bioaccessibility (%) of phenolic compounds in 'Ataulfo' mango juice during gastric and intestinal *in vitro* digestion.

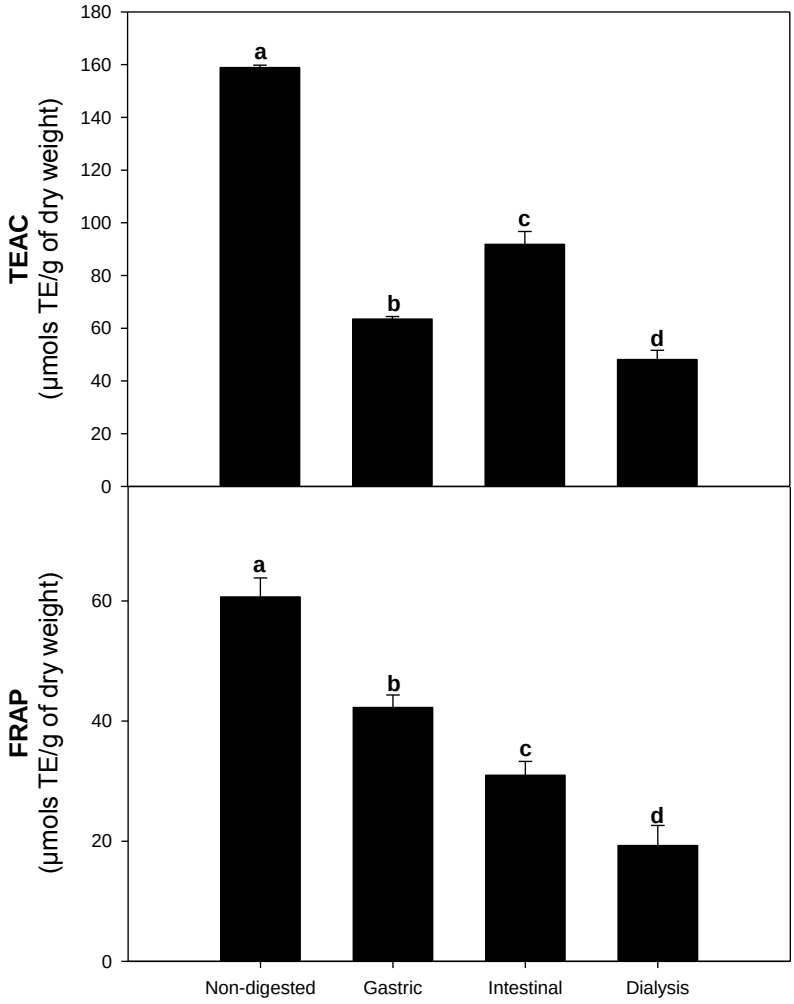

Figure 4. Changes in antioxidant capacity (TEAC and FRAP assays) during *in vitro* digestion of 'Ataulfo' mango juice. Different letters indicate significance differences (p<0.05) between digestion phases.

Table 1. Total phenolic compounds content and antioxidant capacity of 'Ataulfo' mango juice.

Parameter	**'Ataulfo' mango juice**
Total phenolic compounds (mg GAE/g of dry weight)	**11.79 ± 0.86**
Extractable PCs	8.88 ± 0.85
Non-extractable PCs	2.90 ± 0.08
Antioxidant capacity (µmols TE/g of dry weight)	
TEAC	**158.9 ± 0.13**
Extractable PCs	127.3 ± 0.34
Non-extractable PCs	31.62 ± 0.26
FRAP	**60.67 ± 3.15**
Extractable PCs	53.83 ± 1.22
Non-extractable PCs	6.84 ± 1.93

Mean ± standard deviation (n = 3).

Table 2. Dietary fiber, starch, citric acid and total soluble sugars content in 'Ataulfo' mango juice (%).

Parameter	'Ataulfo' mango juice
Total dietary fiber	**12.71± 0.63**
Soluble fiber	6.46 ± 0.17
Insoluble fiber	5.25 ± 0.46
Starch	**0.24 ± 0.05**
Citric acid	**4.37 ± 0.11**
Total soluble sugars	**67.68 ± 2.06**
Glucose	6.06 ± 0.32
Fructose	12.11 ± 0.46
Sucrose	49.49 ± 2.55
Neutral sugars (Soluble fiber)	**11.56 ± 1.96**
Rhamnose	0.39 ± 0.04
Fucose	0.04 ± 0.00
Arabinose	1.07 ± 0.14
Xylose	0.12 ± 0.01
Mannose	2.87 ± 0.87
Galactose	4.04 ± 0.34
Glucose	3.03 ± 0.59
Neutral sugars (Soluble fiber)	**6.27 ± 1.44**
Rhamnose	0.06 ± 0.01
Fucose	0.46 ± 0.05
Arabinose	0.71 ± 0.11
Xylose	1.78 ± 0.23
Mannose	0.69 ± 0.10
Galactose	2.04 ± 0.43
Glucose	0.53 ± 0.17

Mean ± standard deviation (n = 3).

CAPÍTULO V

La biodisponibilidad y capacidad antioxidante de compuestos fenólicos presentes en mango cv. 'Ataulfo' no se afecta por su consumo como pulpa o jugo

Preparado: ***Clinical Biochemistry***

Bioavailability and antioxidant capacity of phenolic compounds of 'Ataulfo' mango is not affected by consumption as flesh or juice

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Running title: Bioavailability of mango phenolic acids

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Abstract

The health-promoting effects attributable to dietary phenolic compounds strongly depend on their bioaccessibility from the food matrix and their consequent bioavailability. We carried out a pilot randomized crossover clinical trial to evaluate the matrix effect (flesh and juice) of 'Ataulfo' mango on the acute bioavailability of phenolic compounds. Twelve healthy male subjects consumed a single dose of mango flesh and mango juice. Blood was collected at zero time and at six intervals over the six hours after consumption; urine was collected at zero time and at four intervals for the following 24-h. Plasma and urine samples were subjected to a phenolics extraction, followed by HPLC-ECD analysis. Five phenolic compounds (gallic, protocatechuic, chlorogenic, ferulic and gentisic acid) were identified and quantified in plasma after the ingestion of the mango samples. Six phenolic compounds plus a microbial metabolite (pyrogallol) were also quantified in urine samples. Phenolic compounds detected in plasma reached maximum concentrations (C_{max}) 2 to 4 h after mango consumption. Moreover, excretion rates were maximum at 8 to 24 h. Consumption of flesh mango contributed to greater protocatechuic acid absorption (49%), while consumption of mango juice contributed to higher chlorogenic acid absorption (62%). Urine results showed that gallic acid is being metabolized by the microorganisms present in the colon to pyrogallol. In general, results indicate that there was no statistically significant difference of phenolics concentrations absorbed between the two 'Ataulfo' mango matrices consumption. 'Ataulfo' mango consumption has potential to increase the presence of phenolic acids in the human body, which have been shown to have bioactive properties.

Keywords: mango, pharmacokinetics, phenolic acids, human, food matrix, antioxidant

1. Introduction

Mango (*Mangifera indica* L) is one of the most consumed tropical fruits in the world due to its sensorial attractiveness and nutritional and phytochemical composition (Kim *et al.*, 2010; Hugo Palafox-Carlos *et al.*, 2012). Mexico is the leading mango-exporting country (41%) and among Mexican cultivars, 'Ataulfo' is one of the most consumed due to its amazing organoleptic and sensory characteristics (Robles-Sánchez *et al.*, 2009; Sistema de Información Agroalimentaria y Pesquera (SIAP), 2013). Moreover, compared to other varieties, 'Ataulfo' mango has the highest content of phenolic compounds and antioxidant capacity (Manthey & Perkins-Veazie, 2009; Hugo Palafox-Carlos *et al.*, 2012). Thus, 'Ataulfo' mango can be considered as a "natural functional food" for preventing several chronic diseases, including cardiovascular diseases and some types of cancer (Kris-Etherton *et al.*, 2002; Manach, Mazur, & Scalbert, 2005).

The ulterior health benefits of a functional food requires to know how much of its bioactive constituents are indeed bioaccessible and bioavailable to specific target organs after consumption. Bioaccessibility is defined as the fraction of a food compound that is readily available for absorption in the gastrointestinal (GI) tract (Benito & Miller, 1998), while bioavailability is defined as the rate and extent to which a compound is absorbed, transported and becomes available at the site of action (Schümann *et al.*, 1997). Bioaccessibility precedes bioavailability in nutrient/drug pharmacokinetics and plays a critical role in the final bioefficacy at target organs. The concept of "food matrix" encloses the fact that nutrients are contained in a large continuous medium, where they may interact physicochemically at different length scales with other components and structures, such as proteins, carbohydrates, dietary fiber, lipids, organic acids, and others (Parada & Aguilera, 2007). These interactions govern both the bioaccessibility and bioavailability of nutrients and xenobiotics present in foods. In this sense, phenolic compounds are generally bound tightly with food matrices (Quirós-Sauceda *et al.*, 2014) and so, most of the ingested phenolics (67-99%) are not absorbed in the upper GI tract because they are not accessible (Manach, Williamson, Morand, Scalbert, & Rémésy, 2005). Nevertheless, the impact of food matrix on this bioaccessibility phenomenon is stronglt influenced by certain food technologies such as homogenization, pressing, grinding, fermentation and heating (D'Archivio, Filesi, Varì, Scazzocchio, & Masella, 2010; Gouado, Schweigert, Ejeh, Tchouanguep, & Camp, 2007; Parada &

Aguilera, 2007; Roura *et al.*, 2008), as well as many intrinsical factors related to the digestive process.

Fruit juice is produced using food technologies such as pressing and squeezing. , As a result, fruits and their corresponding juices differ in nutrient density, sugar profile, pectin and dietary fiber contents (Vatai, 2010). Also, phenolic compounds from fruit juices are expected to be more bioaccessible and bioavailable that those from fruit flesh because of their differences in proximate fiber content (Kris-Etherton *et al.*, 2002). Dietary fiber content in 'Ataulfo' mango flesh and juice is ~18 and 14%, respectively, and our preliminary studies have shown that the bioaccessibility of phenolics from 'Ataulfo' mango flesh and juice is about 50 and 53%, respectively (personal communication). However, *in vivo* evidence on the bioavailability of phenolic compounds from this mango consumed as flesh or juice, has not been reported yet. This information is important to substantiate health benefits of phenolics in mangos. Thus, the objective of this pharmacokinetic study was to investigate the effect of food matrix (flesh and juice) on the bioavailability and antioxidant capacity of phenolic in 'Ataulfo' mangos in humans.

2. Experimental

2.1 Chemicals and solvents

All solvents, salts and acids were purchased from Fisher Scientific Co (Montreal, Canada). Phenolic standards, *β*-glucuronidase (containing sulfatase from *Helix pomatia*), and creatinine assay kit were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2 'Ataulfo' mangos and products

Three hundred (200-300 g) 'Ataulfo' mangoes at commercial ripeness stage (Stage 4) (Hugo Palafox-Carlos *et al.*, 2012) were obtained from the local market in Leon, Guanajuato, Mexico and transported to the laboratory. They were washed under tap water, sanitized, peeled, and processed to obtain fresh raw slices (flesh) and juice, which were further administered to participants during the cross-over intervention. Mango juice was juice was extracted as suggested by Santhirasegaram *et al.* (2013) with some modifications. First, flesh was blending using a domestic juice extractor (Moulinex, Centri III, A75312V) and filtered through organza cloth. The freshly-blended juice the day it will be administered and stored under refrigeration.

2.3 Phenolic profile of 'Ataulfo' mango flesh and juice

'Ataulfo' mango flesh and juice were first freeze-dried (Labconco Corporation, Kansas City, MO). One g of the resulting products was homogenized in 20 mL 80% methanol using an IKA® Works homogenizer, (Model T25, Willmington, NC) at room temperature. The homogenate was sonicated for 30 min (Bransonic Ultrasonic Co., Model 2210, Danbury, CT, USA) and spun at 14,000 rpm for 15 min at 4°C. The pellet was homogenized in 10 mL 80% methanol in the same condition. The supernatants were combined, filtered (Whatman No. 1, Springfield Mill, Maidstone Kent, UK), and made up to a final volume of 30 mL with 80% methanol to generate phenolic extract. For the acidic hydrolysis, 1 mL of 2.4 M HCl was added to 1 mL of the phenolic extract. After incubation for 2 h in the dark at 80°C, the mixture was filtered using a 0.22 µm Ultrafree Durapore Centrifugal filter device (Merck Millipore, Billerica, MA) and directly injected to a HPLC system equipped with an electrochemical detector (HPLC-ECD) for analysis of phenolic compounds.

2.4 Subjects and study design

A randomized, crossover clinical trial (**Figure 1**) was conducted to evaluate the postprandial plasma (0-6 h) bioavailability and urinary excretion (0-24 h) of phenolic compounds from mango flesh and juice. The protocol was approved by the Bioethics Committee of the Center for Food Research and Development, A.C (CIAD). Twelve middle-aged healthy men aged (22 to 34 y) were recruited from CIAD (**Table 2**). In order to avoid gender-related differences (e.g. body composition, hormonal status), just male participants were selected. In this small scale trial, the bioavailability of mango phenolics was tested in men only because anthropometric variables and the menstrual cycle phase-related variability in women may affect the absorption, metabolism and excretion of phenolics. After the study details were explained to subjects, written informed consent was obtained before any study conducts were performed. All subjects were free of diagnosed heart disease, homeostatic disorders, gastrointestinal disease, or other medical conditions and were not taking any medication or any vitamin/mineral supplement prior to the study enrollment.

Volunteers were recruited through local advertisement. The subjects were underwent a 3-d wash-out period to diminish the confounding effect of phenolic compounds in habitual diets, during which they were instructed to refrain from

consuming foods high in phenolic compounds, such as fruits, berries, vegetables, juices, nuts, tea, coffee, cocoa, olive oil, coffee, wine, and beer. Twenty four-hour dietary recall using a slightly modified food frequency questionnaire was performed to evaluate subjects compliance to the low-polyphenol protocol during the wash-out period (Macedo-Ojeda *et al.*, 2013). At the end of the wash-out period, subjects reported to the study site without eating foods for 12 hours. After baseline (time 0) blood and urine were collected, subjects were randomly assigned to consume either mango flesh slices (500 g) or juice (721 g) within ten min, in random order separated by a 3-day wash-out period. Six additional blood samples were collected at 1, 2, 3, 4, 5, and 6 h post consumption and 4 addition urine samples were collected 0-4, 4-8, 8-12, and 12-24 h. Blood was collected in a vacutainer containing EDTA as the anticoagulant. Plasma was isolated and 0.5-mL aliquots were acidified immediately using 20 µL HCl (4 M). Acidified plasma and urine samples were stored at -80 °C until analysis. During blood collection, subjects were not allowed to eat or drink except water. After blood collection, the volunteers eat a hamburger without vegetables and ketchup for lunch. For dinner, they were allowed to eat a meal, except food not allowed during wash-out. A graphical representation of the study is shown in **Figure 1**.

2.5 Determination of plasma phenolic compounds

Phenolics in plasma were quantified according to the method of Chen *et al.* (2005). Briefly, 20 µL vitamin C-EDTA (100 mg ascorbic acid plus 1 mg EDTA in 1.0 mL of 0.4 mol/L NaH_2PO_4, pH 3.6), 20 µL of 10 µg/mL internal standard (2', 3', 4'-trihydroxyacetophenone) and 20 µL *β*-glucuronidase/sulfatase (98,000 kU/L *β*-glucuronidase and 2400 kU/L sulfatase) were added to 500 µL plasma and the mixture was incubated at 37°C for 60 min. Subsequently, phenolics were extracted with acetonitrile. After centrifugation, supernatant was removed, dried under nitrogen gas, and reconstituted in the aqueous HPLC mobile phase, filtered using an Ultrafree Durapore Centrifugal filter devices (0.22, Merck Millipore, Billerica, MA), and then injected onto the HPLC-ECD system for analysis of phenolics. Concentration of individual phenolics were calculated based on calibration curves constructed using authentic phenolics ($R^2 >$ 0.999) with the adjustment with the internal standard. The intraday coefficient of variation (CV) for standards spiked into plasma was <8%.

2.6 Determination of urine phenolic compounds

Phenolics in urine were quantified according to the method of McKay *et al*. (2015). Urine (200 uL) was mixed with 800 µL buffer (0.1 mol/L sodium acetate, pH 5) and 30 µL *β*-glucuronidase/sulfatase (98,000 kU/L *β*-glucuronidase and 2400 kU/L sulfatase), followed by incubation at 37°C for 60 min. After incubation, 10 µL of 100 µg/mL internal standard (2', 3', 4'-trihydroxyacetophenone) and 200 µL glacial acetic acid was added and vortexed briefly. After the addition of ~1 g NaCl, phenolics were extracted twice with 3 mL ethyl acetate. The combined ethyl acetate fractions were dried under nitrogen gas and reconstituted in 1 mL of the aqueous HPLC mobile phase, filtered through a 0.22 µm Millex syringe driven Filter unit (Millipore Corporation, Bedford MA, USA), and then injected (50 µL) onto HPLC-ECD system. Concentration of individual phenolics were calculated based on calibration curves constructed using authentic phenolics (R^2 > 0.999) with the adjustment with the internal standard. The intraday CV for standards spiked into urine was <7%. Urinary phenolics were adjusted with urinary creatinine.

2.7 Antioxidant capacity

The Oxygen Radical Absorbance Capacity (ORAC) assay was performed according to the method of Prior *et al.* (2003), using a FLUOstar Optima Microplate reader (BMG Labtechnologies, Inc. Durham, NC). Frozen plasma samples were thawed slowly and vortexed briefly. Then, plasma samples were mixed with 0.5 M perchloric acid (1:1 v/v) to obtain the protein-free supernatant. The resulting supernatant samples were then used in the ORAC assay. Urine samples were measured without previous dilution (Torres, Galleguillos, Lissi, & López-Alarcón, 2008). The Ferric Reducing antioxidant Power (FRAP) assay was performed according to Benzie and Strain (Benzie & Strain, 1996). Plasma and urine samples were measured directly. All results are expressed as µmol trolox equivalent (TE)/mL.

2.8 Statistics

Results are expressed as mean ± SD. The maximum concentration (C_{max}) in plasma and the time to reach C_{max} (T_{max}) were visually identified. The area under the concentration-time curve (AUC) of plasma phenolics was calculated using the linear trapezoidal integration equation (Chiou, 1978). The differences in C_{max}, T_{max}, and

AUC of phenolics between mango flesh and juice interventions were examined using an analysis of covariance with time 0 values as the covariant, followed by the Tukey-Kramer multi-comparison test. The Number Cruncher Statistical System version 6.0 software (NCSS, LLC) was used to perform all statistical analyses.

3. Results

3.1 'Ataulfo' mango fruit phenolic compounds

The major phenolics identified in 'Ataulfo' mango flesh and juice included gallic, chlorogenic, vanillic, sinapic, protocatechuic, ferulic, gentisic, and caffeic acids **(Table 1)**. Of quantified phenolics, *p*-coumaric, gallic, and chlorogenic acids were predominate in both flesh and juice. Chlorogenic acid content in juice was 59% smaller than in flesh. The sum of quantified phenolics in flesh and juice was 47.60 mg/500 g fresh weight and 43.24 mg/721 g of fresh weight was comparable.

3.2 Plasma and urine pharmacokinetic analysis

Five phenolic acids present in 'Ataulfo' mango flesh and juice were detected in plasma (gallic, chlorogenic, protocatechuic, ferulic and gentisic acid) (**Fig. 2)**. The C_{max} of phenolic acids was larger in the subjects consuming mango juice than flesh, with the exception of gallic acid **(Table 2)**. The relative absorption was estimated from the AUC and dose-adjusted (AUC/dose) (**Table 2**); chlorogenic and ferulic acid showed statistically significant (p>0.05) difference between the 'Ataulfo' mango food matrices consumption. The five phenolic compounds detected in plasma reached maximum concentrations (C_{max}) 2-4 h after mango consumption. While the T_{max} of all detected phenolic acids did not statistically differ between flesh and juice, the T_{max} of the juice consumption was slightly shorter than that of the flesh. Consistent with mango juice having the highest phenolic compounds C_{max}, the AUC of chlorogenic, ferulic and gallic acid was larger 4 to 43% than that of mango flesh samples (**Table 2**). To equalize the dose of individual phenolic compounds differences, the mean of total AUD/dose was calculated. Protocatechuic and gentisic acids showed the highest AUC/dose plasma concentration (**Table 2**). Low doses had the higher AUC per dose.

Six major phenolic acids present in 'Ataulfo' mango flesh and juice were detected in urine, including chlorogenic, vanillic, ferulic, sinapic, gallic and *p*-coumaric acid (Fig. 3). Pyrogallol was not detected in mango but found in urine.

Mango matrices did not affect concentration of phenolic acids in urine of subjects consuming mango flesh or juice, except for pyrogallol and *p*-coumaric acid **(Table 3)**. The pyrogallol metabolite was the compound with C_{max} excreted; almost 10-fold higher than the compound excreted in a lesser C_{max} (*p*-coumaric acid). All phenolic compounds detected in urine were excreted 8-24 h after mango matrices consumption. Although there was no difference in the T_{max} following the 'Ataulfo' mango flesh and juice consumption, the T_{max} of mango juice consumption was shorter than that of the mango flesh. An interesting behavior was that the concentration of gallic acid excreted in urine decreased as time increased. In contrast, the concentration of pyrogallol present in urine increased. Pearson's correlation coefficients (*r*) were -0.42 for flesh and -0.47 for juice, indicating a linear negative correlation between the two compounds. Higher AUC/dose excretions were showed by vanillic and sinapic acids (**Table 3**), values were higher for mango juice.

3.3 Antioxidant capacity

Plasma and urine antioxidant capacity by ORAC and FRAP assays after 'Ataulfo' mango flesh and juice intake is show in (Fig. 4). No significant differences were found in the antioxidant capacity of plasma and urine between mango flesh and juice. No significant changes were found in FRAP and ORAC values of plasma and urine in all time points . Although not significant difference between food matrices was shown, the plasma antioxidant capacity values after juice consumption were slightly higher than in flesh, by both antioxidant methods. On the other hand, urine antioxidant capacity tended to increase with time. No significant difference were found in FRAP assay. The ORAC assay showed significant difference ($p<0.05$) between food matrices at baseline (time 0) and at the first bottle collection (0-4 h). The highest antioxidant capacity evaluated by FRAP and ORAC was shown at 8-24 h after mango and juice consumption. This is positively associated with the increased concentration of phenolic compounds in urine to these times (Correlation between FRAP and flesh phenolic compounds $r = 1$; correlation between ORAC and flesh phenolic compounds $r = 0.73$; correlation between FRAP and juice phenolic compounds $r = 1$; correlation between ORAC and juice phenolic compounds $r = 0.80$).

3.4 Discussion

Phenolics contribute to the inverse association between consumption of plant foods and chronic diseases, such as metabolic disorders and cancers through multiple bioactions, including antioxidant, anti-inflammation, and anti-proliferation (Hollman, 2001; Kris-Etherton *et al.,* 2002). 'A large body of preclinical and clinical data illustrate that phenolics in plant foods are bioavailable and capable of enhancing antioxidant capacity in humans (Manach, Williamson, *et al.*, 2005; Scalbert & Williamson, 2000). 'Ataulfo' mango fruit has the highest content of total phenols among others commercial varieties of mango (Hugo Palafox-Carlos *et al.*, 2012). In this study, we investigated bioavailability of phenolic acids in 'Ataulfo' in healthy adults. We also studied whether their bioavailability would differ when they were delivered in different mango forms (flesh vs. juice) because phenolics bound tightly to flesh fiber might be less bioaccessible and bioavailable.

Results coincides with reports by other authors (Palafox-Carlos, Yahia, & González-Aguilar, 2012; Sáyago-Ayerdi *et al.*, 2013), where phenolic acids are the major phenolic compounds in 'Ataulfo' mango fruit; being gallic acid and its derivatives the compounds present in higher concentration. Gallic acids (*m*-digallic acid and *m*-trigallic acids), gallotannins and mangiferin are among the mainly phenolic compounds already identified in the flesh of different cultivars of mango ('Ataulfo', 'Alphonso', 'kitchener', 'Abu Samaka', 'keitt') (Masibo & He, 2008; H Palafox-Carlos *et al.*, 2012; Sáyago-Ayerdi *et al.*, 2013; Schieber, Ullrich, & Carle, 2000). Gallic acid has been identified as the major phenolic compound present in mango fruits. It was found that 'Ataulfo' mango flesh contains 33.04 mg/kg of gallic acid. Kimberley *et al.* (2014) reported 1.74 mg/kg of gallic acid in 'Keitt' mango. Ramirez *et al.* (2013) reported 6 mg/kg of gallic acid in 'Pica' mango and 17 mg/kg of gallic acid in 'Tommy Atkins' mango. Moreover, 'Ataulfo' mango juice has 31.8 mg/kg of gallic acid; however this corresponds to 25-35 fold less than the gallic acid content in green tea (Zuo, Chen, & Deng, 2002).

Five phenolic compounds were detected in plasma after mango flesh and juice consumption; with not statistically significant difference between food matrices. Four phenolic compounds detected in mango matrices were not detected in the biological samples (*p*-coumaric, vanillic, sinapic and caffeic acids). This could be attributed to an oxidative degradation (low stability in the gastrointestinal-tract), low concentration in plasma/urine or quick absorption (<1 h) and tissue distribution (Bell,

2001). Phenolic compounds identified in plasma showed a T_{max} of 1-4 h after mango matrices consumption suggesting that the absorption of these phenolics occurred in the small intestine transit (Wilson, 2011). Even though some phenolic compounds such as gallic, chlorogenic, caffeic and *p*-coumaric, acids can be absorbed in the stomach via monocarboxylic acid transporters (MCT) within 5 min after gastric administration (Konishi, Zhao, & Shimizu, 2006), the small intestine has been known to be the major site for the absorption of phenolic acids. The *T*max of absorption of gallic acid from 'Ataulfo' juice was 3.5 ± 1.0 h, which is slower compared to the *T*max in tea (*T*max: 1.39 ± 0.21 h) and red wine (*T*max: <1.5 h) (Lafay & Gil-Izquierdo, 2008; Shahrzad, Aoyagi, Winter, Koyama, & Bitsch, 2001). Both mango food matrices contain similar amounts of the individual phenolic compounds, therefore AUC/dose showed no difference, except for chlorogenic and ferulic acids.

Six phenolic compounds were detected in urine, plus one microbial metabolite, no significant difference between matrices. Protocatechuic and gentisic acids detected in plasma were not detected in urine. Vanillic, sinapic and *p*-coumaric acids were detected in urine but were not detected in plasma. Excretion rates were maximum at 8-24 h. Free and polymeric gallic acid is the predominate phenolic acid in 'Ataulfo' mango. A certain percentage of this phenolic is absorbed in the small intestine. However, another percentage of this compound is not bioaccessible in the small intestine and reaches the colon, where apparently it is metabolized to pyrogallol via decarboxylation mediated by microbial gallic acid descarboxylase (Pimpão *et al.*, 2014; Soni, Sharma, & John, 2012; Tian, Giusti, Stoner, & Schwartz, 2006). Pyrogallol has been previously found as product of metabolism whit consumption of mango 'Keitt', berry fruits, Concord grape juice and black tea (Pimpão *et al.*, 2014; Stalmach, Edwards, Wightman, & Crozier, 2013; Tian *et al.*, 2006). Stalmach *et al.* (2013) reported urinary pyrogallol at later time points after ingestion of Concord grape juice, but this compound was not observed in the urine of ileostomy patients, which strongly suggest a colonic origin based on degradation of other phenolic compounds. This trend was expected as previous studies have identified increased concentrations of gallic acid microbial metabolites in plasma and urine at 6-8 h from baseline (Barnes, Krenek, Meibohm, Mertens-Talcott, & Talcott, 2016; Pimpão *et al.*, 2014).

As mentioned before, scientific data appear to demonstrate that in the case of many food components, including phenolic compounds, the state of the matrix favor

or hinder their bioaccessibility and bioactive response *in vivo* (Parada & Aguilera, 2007). In particular, it has been reported that due the physicochemical properties of dietary fiber, this compound can reduce the release and absorption of macronutrients, some minerals and trace elements, as well as phytochemicals such as phenolic compounds and carotenoids (Palafox-Carlos, Ayala-Zavala, & González-Aguilar, 2011). Food processing such as macerating, grinding, fermentations and/or mild heating may improve bioaccessibility and bioavailability, most likely as a result of disruption of the cell walls (dietary fiber) of plant tissues, dissociation the bioactive compound-matrix complexes, or transformation into more active molecular structures (Parada & Aguilera, 2007). Based on this, we evaluate the effect of the 'Ataulfo' mango food matrix on phenolic compounds bioavailability. Mango flesh represents the raw food, without modifying the structural matrix; mango juice is the soluble part as a result of the elimination of the dietary fiber. Results not showed significant difference between the bioavailability of phenolic compounds from both mango food matrices. This could be attributed to the 'Ataulfo' mango matrix that mainly consist of amylaceous carbohydrates, which can be digested by human digestive enzymes. The dietary fiber percentage is slightly lower in mango juice (~16%), but seems not have direct influence on the bioavailability of phenolic compounds. In addition, the starch (which can cause viscosity) content in these foods matrices is very low.

After eating, normal metabolic and oxidative processes produced reactive oxygen species. Expectedly, postprandial oxidative stress reduces plasma antioxidant capacity following a meal (Seymour *et al.*, 2014). This pattern could be related to the plasma antioxidant capacity values found in this study. Some of the absorbed antioxidants (phenolic compounds, vitamins) could exert their antioxidant power in plasma, as a result, thus can partially attenuate the oxidative stress, elevating plasma antioxidant capacity or even not increase and keep it constant. As mentioned before, higher antioxidant capacity showed in urine is attributed to the higher concentration of phenolic compounds excreted. Also, the increase in urinary pyrogallol may be contributing to this increase.

4. Conclusion

The consumption of 'Ataulfo' mango flesh and juice indicates absorption, metabolism and excretion of phenolic compounds, with no statistically significant difference

between both food matrices. In this regard, 'Ataulfo' mango consumption, as flesh or juice, has potential to increase the presence of phenolic acids in the human body, which have been shown to have anti-inflammatory and anti-carcinogenic properties.

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Tables and Figures

Table 1. Phenolic compounds content of 'Ataulfo' mango flesh and juice by HPLC-ECD analysis.

No.	Phenolic compound	Flesh mg/500 g of fresh weight	Juice mg/721 g of fresh weight
1	*p*-coumaric acid	19.36 ± 2.46	16.63 ± 1.24
2	Gallic acid	16.52 ± 0.95	15.90 ± 0.34
3	Chlorogenic acid	17.96 ± 0.50	7.32 ± 0.53
4	Ferulic acid	1.94 ± 0.28	1.59 ± 0.02
5	Vanillic acid	1.07 ± 0.06	0.87 ± 0.02
6	Protocatechuic acid	0.41 ± 0.02	0.53 ± 0.03
7	Gentisic acid	0.24 ± 0.01	0.18 ± 0.03
8	Sinapic acid	0.06 ± 0.00	0.06 ± 0.00
9	Caffeic acid	0.03 ± 0.00	0.03 ± 0.00
Total		**47.60 ± 3.72**	**43.24 ± 0.28**

Table 2. Pharmacokinetics parameters of the plasma phenolic compounds after acute intake of 'Ataulfo' mango flesh and juice. C_{max}: maximal concentration. T_{max}: maximal time. AUC: area under the curve from time 0 to 6 h.

Phenolic compound	Flesh				Juice			
	C_{max} (ng/mL)	T_{max} (h)	AUC (ng.h/mL)	AUC/dose ((ng.h/mL)/mg)	C_{max} (ng/mL)	T_{max} (h)	AUC (ng.h/mL)	AUC/dose ((ng.h/mL)/mg)
Chlorogenic acid	49.7 ± 7.3*	3.5 ± 1.4	208.7 ± 24.5*	11.5 ± 1.78*	109.7 ± 0.26*	2.5 ± 1.8	366.9 ± 130.7*	50.12 ± 15.5*
Protocatechuic acid	30.8 ± 13.3	3.5 ± 2.0	141.4 ± 73.9	344.8 ± 138.3	34.5 ± 18.0	3.7 ± 1.7	108.6 ± 5.4	204.90 ± 9.9
Ferulic acid	16.5 ± 3.9*	2.8 ± 2.1	60.2 ± 22.7*	31.0 ± 9.63*	32.7 ± 10.9*	2.3 ± 1.5	133.4 ± 47.7*	83.8 ± 30.2*
Gentisic acid	11.8 ± 2.1	4.0 ± 1.4	53.1 ± 8.3	221.2 ± 34.0	12.2 ± 0.2	2.8 ± 1.9	50.9 ± 11.0	282.7 ± 59.1
Gallic acid	8.7 ± 1.7	4.4 ± 1.1	36.9 ± 24.3	2.2 ± 0.91	7.9 ± 4.7	3.5 ± 1.0	38.5 ± 14.5	2.4 ± 0.94

Values are means ± SD of phenolic compounds of twelve volunteers. *Statistically significant differences (p<0.05) between mango matrices.

Table 3. Pharmacokinetics parameters of the urinary phenolic compounds after acute intake of 'Ataulfo' mango flesh and juice. C_{max}: maximal concentration. T_{max}: maximal time. AUC: area under the curve from time 0 to 24 h.

Phenolic compound	**Flesh**				**Juice**			
	C_{max} (ng/µg creatinine)	**T_{max} (h)**	**AUC (ng.h/mL)**	**AUC/dose ((ng.h/mL)/mg)**	**C_{max} (ng/ µg creatinine)**	**T_{max} (h)**	**AUC (ng.h/mL)**	**AUC/dose ((ng.h/mL)/mg)**
Pyrogallol	1535.6 ± 528.0	3.6 ± 0.7	1806.6 ± 371.6*	-	911.6 ± 297.8	3.6 ± 0.5	1006.2 ± 111.9*	-
Chlorogenic acid	1168.9 ± 688.3	3.2 ± 1.1	1513.1 ± 418.5	206.70 ± 51.4	627.4 ± 201.8	2.0 ± 1.2	1253.3 ± 610.0	171.21± 77.2
Vanillic acid	820.7 ± 461.3	3.0 ± 1.2	872.4 ± 206.8	815.32 ± 183.9	510.5 ± 134.5	2.0 ± 1.2	924.3 ± 175.2	1062.41 ± 191.3
Ferulic acid	258.2 ± 94.3	3.7 ± 0.4	351.9 ± 114.9	181.39 ± 51.0	356.7 ± 138.7	2.7 ± 1.2	447.6 ± 119.3	281.50 ± 77.6
Sinapic acid	65.5 ± 30.4	3.8 ± 0.4	78.1 ± 18.4	1301.6 ± 274.9	49.1 ± 22.6	2.8 ± 1.2	86.0 ± 32.8	1433.33 ± 539.5
Gallic acid	13.6 ± 4.7	2.0 ± 0.5	24.7 ± 11.9	1.49 ± 0.6	30.8 ± 19.5	1.4 ± 0.5	47.5 ± 20.0	2.98 ± 1.1
p-coumaric acid	9.1 ± 5.2*	3.8 ± 0.4	18.6 ± 6.1*	0.96 ± 0.2*	57.2 ± 19.2*	1.4 ± 0.7	62.6 ± 16.4*	3.76 ± 0.9*

Values are means ± SD of phenolic compounds of twelve volunteers. Times corresponds to urine collection bottles, 1: 0-4 h, 2: 4-8h, 3: 8-12 h, 4: 12-24 h. *Statistically significant differences (p<0.05) between mango matrices.

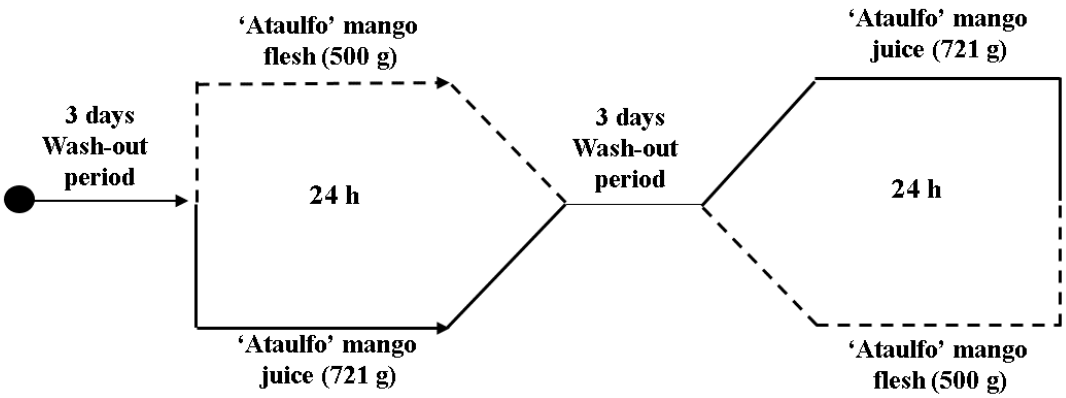

Figure 1. Graphical representation of the randomized crossover clinical trial.

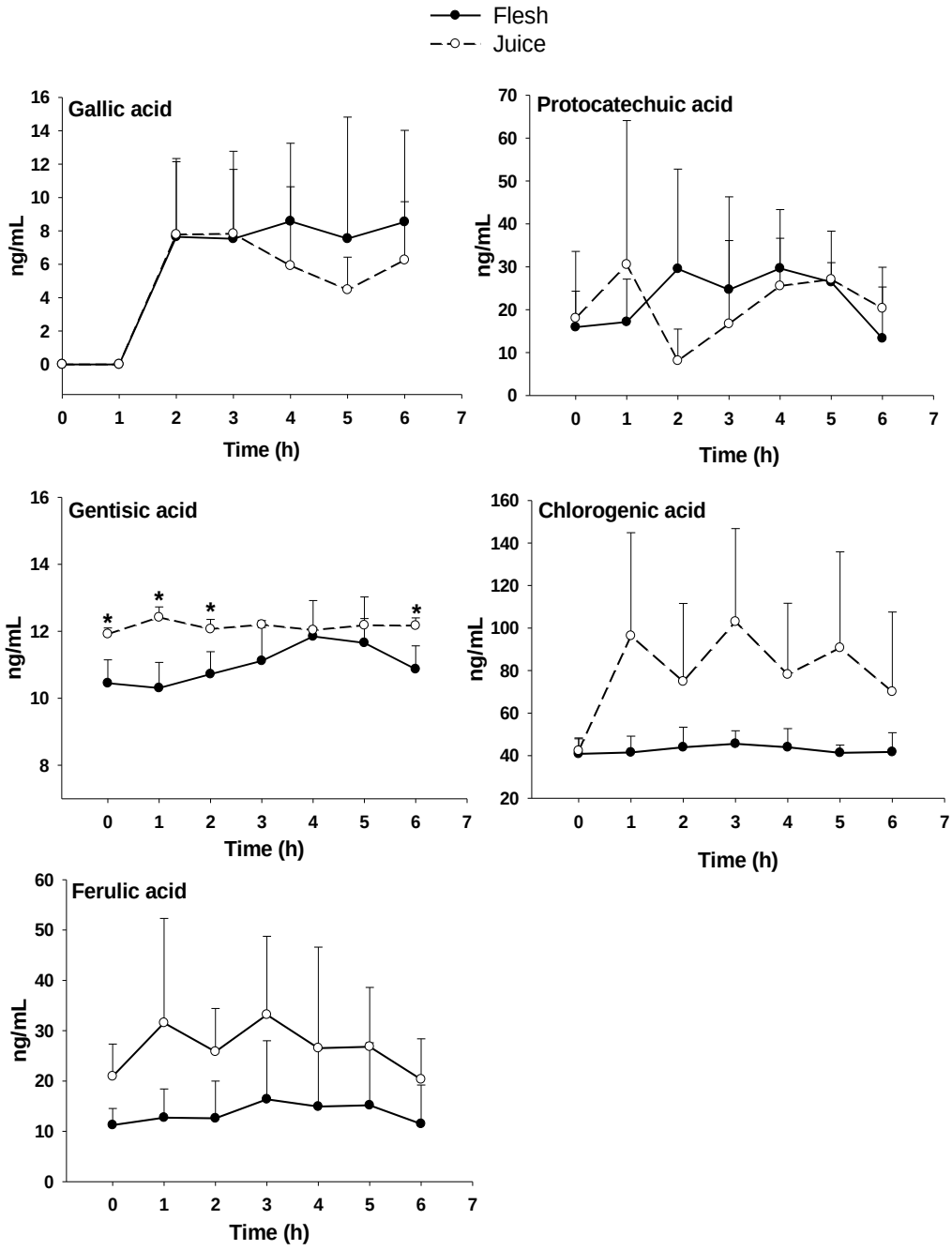

Figure 2. Average concentration of gallic, protocatechuic, gentisic, chlorogenic and ferulic acid in plasma (ng/mL) after be treated with *β*-glucuronidase. Blood samples were collected after consumption of 'Ataulfo' mango flesh (-•-) and juice (- ° -). *Statistically significant differences (p<0.05) between mango matrices.

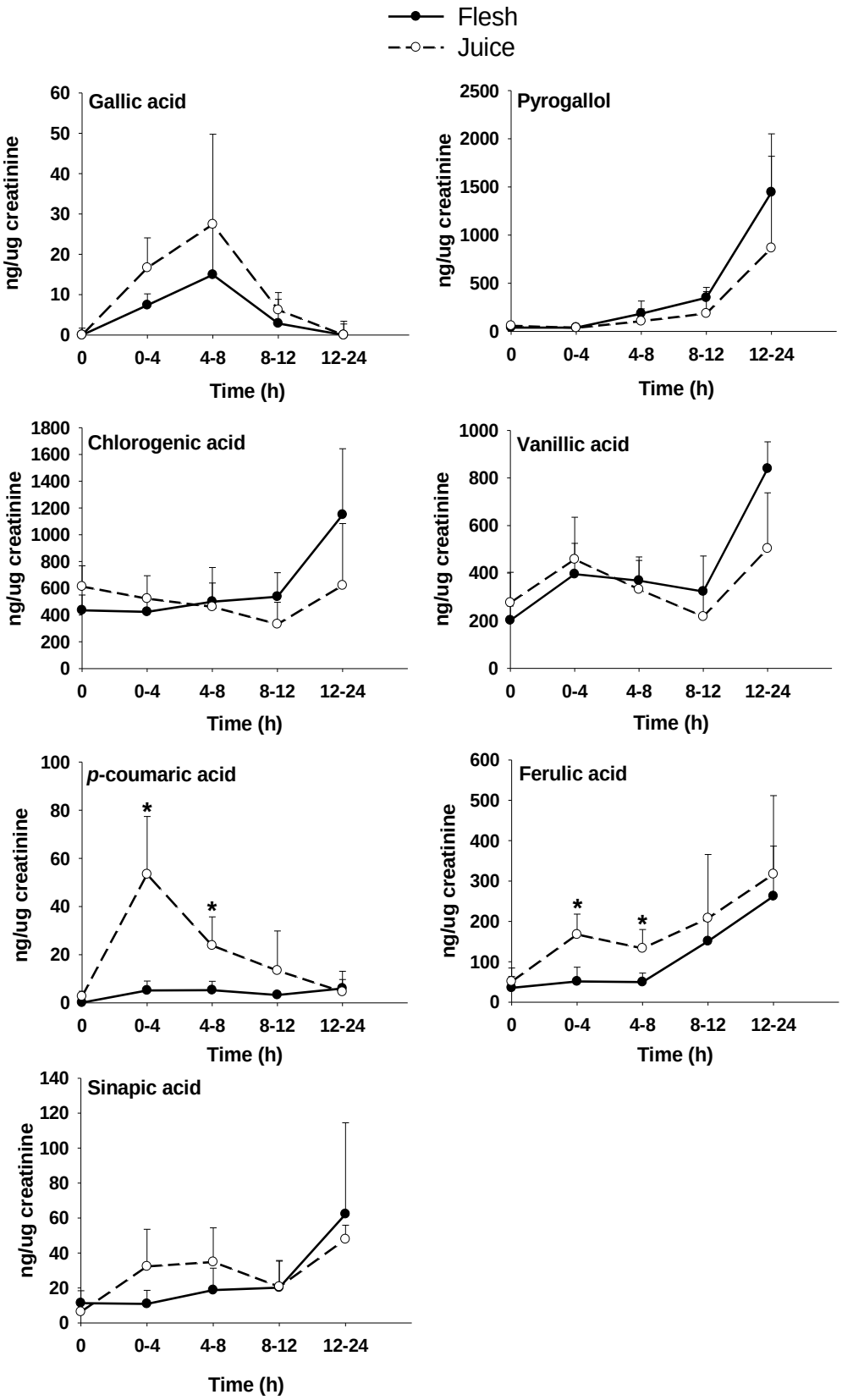

Figure 3. Average concentration of gallic, gentisic and chlorogenic acid in urine (ng/mL) after be treated with *β*-glucuronidase. Samples were collected after consumption of 'Ataulfo' mango flesh (-•-) and juice (-ᵒ-). *Statistically significant differences (p<0.05) between mango matrices.

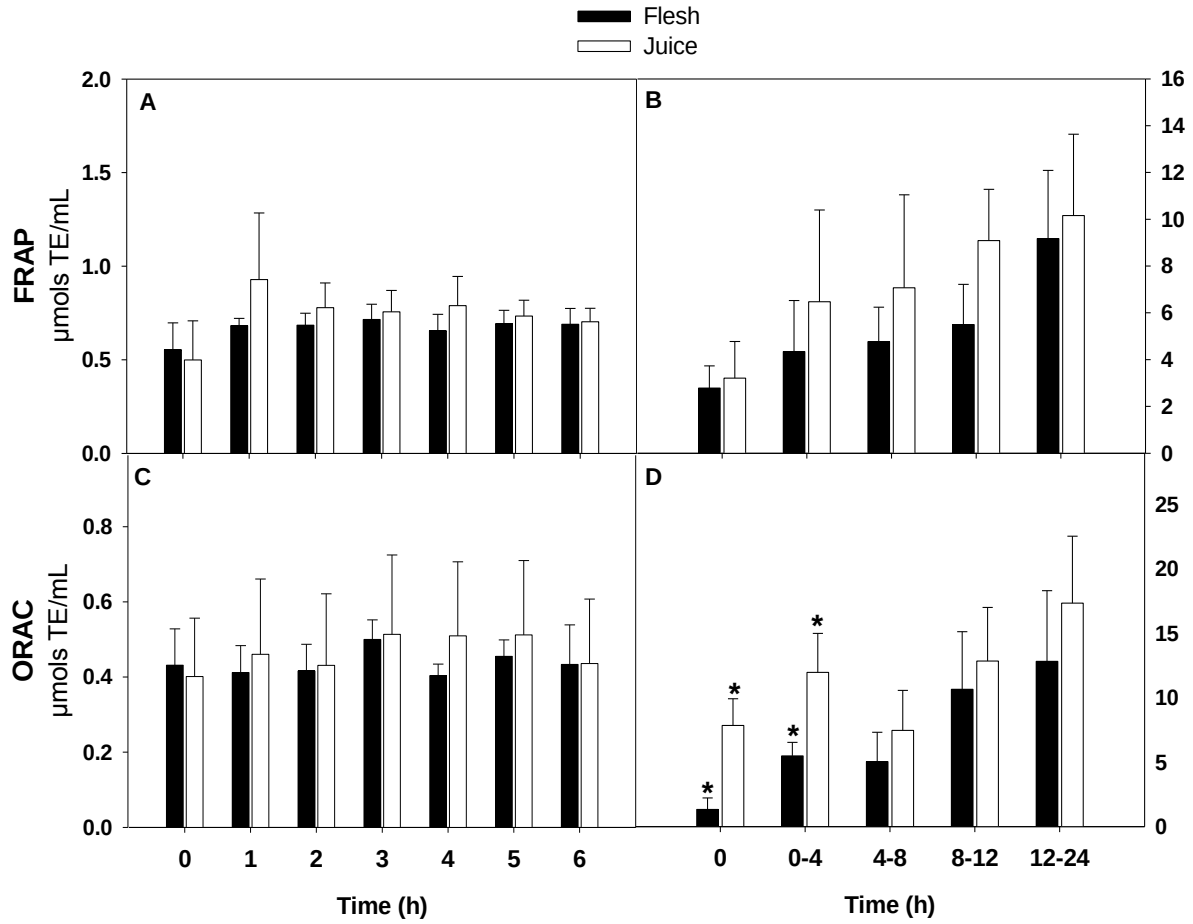

Figure 4. (A) Plasma antioxidant capacity by FRAP assay after 'Ataulfo' mango flesh and juice intake. (B) Urine antioxidant capacity by FRAP assay after 'Ataulfo' mango flesh and juice intake. (C) Plasma antioxidant capacity by ORAC assay after 'Ataulfo' mango flesh and juice intake. (D) Urine antioxidant capacity by ORAC assay after 'Ataulfo' mango flesh and juice intake. *Statistically significant differences (p<0.05) between mango matrices.