Centro de Investigación en Alimentación

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EFECTO DE LAS PROPIEDADES ANTIOXIDANTES DEL EXTRACTO DE CÁSCARA DE MANGO cv. ATAULFO COMO ADITIVO ALIMENTARIO E INGREDIENTE FUNCIONAL PARA PEZ CEBRA (*Danio rerio*).

Por:

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CONTENIDO

LISTA DE FIGURAS

LISTA DE TABLAS

RESUMEN

El uso de antioxidantes provenientes de subproductos vegetales como ingredientes y aditivos alimentarios se ha incrementado en los últimos años, debido principalmente a su efectividad para combatir el daño oxidativo *in vivo* y a su capacidad para preservar la calidad de alimentos con alto contenido de ácidos grasos poliinsaturados. Sin embargo, en acuicultura existe información limitada respecto al efecto de los antioxidantes naturales sobre indicadores de estrés oxidativo en peces de cultivo y sobre la prevención de la peroxidación lipídica de los ingredientes utilizados en su alimentación. Por lo tanto, en la presente investigación se plantearon los siguientes objetivos: 1) evaluar la capacidad de los polifenoles de la cáscara de mango para prevenir la peroxidación lipídica y mejorar el estado antioxidante en pez cebra (*Danio rerio*), y 2) evaluar los mecanismos de acción de antioxidantes hidrofílicos y lipofílicos de la cáscara de mango, así como su potencial antioxidante para prevenir la peroxidación del aceite de pescado. Se obtuvieron extractos hidrofílicos (polifenoles) y lipofílicos de cáscaras de mango secas. Se utilizó cromatografía líquida de ultraeficiencia acoplada a un detector de masas con cuadrupolo tiempo de vuelo equipado con ionización por electrospray (UPLC ESI-Q-TOF/MS/MS) y cromatografía liquida de alta eficiencia (HPLC) para determinar los perfiles de polifenoles y carotenoides, respectivamente. Se evaluó el efecto de los polifenoles sobre la peroxidación lipídica medida por formación de malonaldehido (MDA) y la actividad de las enzimas superóxido dismutasa (SOD), glutatión peroxidasa (GPx) y catalasa (CAT) en el tejido muscular y el hígado de peces cebra, respectivamente. Se determinó la capacidad de los polifenoles y carotenoides para inhibir la formación de MDA en el aceite de pescado y se comparó contra el antioxidante sintético butil hidroxitolueno (BHT). Se identificaron ocho compuestos fenólicos (ácidos fenólicos, flavonoides y polifenoles) y un carotenoide (β-caroteno) en los extractos hidrofílicos y lipofílicos de cáscara de mango. Los compuestos fenólicos redujeron los niveles de MDA en el músculo e incrementaron la actividad de CAT en el hígado. Asi mismo, retardaron la peroxidación lipídica en el aceite de pescado, en forma similar al BHT, mientras que los carotenoides mostraron un efecto prooxidante. Por lo tanto, se concluye que los polifenoles tienen efecto protector

contra la peroxidación lipídica *in vivo* y efectos modulatorios sobre la CAT. Además, los polifenoles representan una alternativa de uso al antioxidante sintético BHT, que comúnmente se utiliza para prevenir la peroxidación en los aceites de pescado.

ABSTRACT

The use of antioxidants from plants by-products as ingredients and food additives has increasing in recent years, mainly due to their effectiveness in combating oxidative damage *in vivo* and their ability to preserve the quality of foods with a high content of polyunsaturated fatty acids. However, in aquaculture there is limited information regarding the effect of natural antioxidants on indicators of oxidative stress in farmed fish, as well as on the prevention of lipid peroxidation of food ingredients used in their diet. Therefore, the aims of this research were 1) to evaluate the polyphenols capacity of the mango peel to prevent lipid peroxidation and enhance the antioxidant status in zebrafish (*Danio rerio*), and 2) to evaluate the mechanisms of action of hydrophilic and lipophilic antioxidants from mango peel, as well as its antioxidant potential to prevent the peroxidation of fish oil. Hydrophilic (polyphenols) and lipophilic extracts were obtained from dried mango peel. Ultra-Performance Liquid Chromatography coupled to a Quadrupole-Time-of-Flight mass spectrometer equipped with electrospray ionization. (UPLC ESI-Q-TOF / MS / MS) and High-Performance Liquid Chromatography (HPLC) was used to determine polyphenol profiles and carotenoids, respectively. The effect of polyphenols on lipid peroxidation by malonaldehyde formation (MDA) was measured and the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were evaluated in muscle tissue and liver of zebrafish, respectively. The ability of polyphenols and carotenoids to inhibit the formation of MDA in fish oil was determined and compared against the synthetic antioxidant butyl hydroxytoluene (BHT). We identified eight phenolic compounds (phenolic acids, flavonoids and polyphenols) and one carotenoid (β-carotene) in the hydrophilic and lipophilic extracts from mango peel. The polyphenols reduced MDA levels in the muscle and increased the CAT activity in the liver. Likewise, they delayed lipid peroxidation and showed a similar effect than that BHT, whereas carotenoids showed a prooxidant effect. Therefore, it is concluded that polyphenols have a protective effect against lipid peroxidation in vivo and modulatory effects on CAT. In addition, polyphenols represent an alternative to the synthetic antioxidant BHT, which is commonly used to prevent peroxidation in fish oils.

ESTRUCTURA DE LA TESIS

La presente tesis está conformada por 6 capítulos, entre los cuales se incluye introducción general, artículos científicos, discusión y conclusiones generales. Con el objeto de ofrecer un mejor panorama acerca de su contenido, a continuación, se describen de forma concisa los capítulos que integran el documento.

El Capítulo I presenta de forma general y precisa la relevancia del tema de investigación, la problemática por resolver, las hipótesis y los objetivos del estudio.

El capítulo II muestra una revisión detallada de los principales efectos antioxidantes e inmunoestimulantes de polifenoles de diferentes fuentes vegetales, sobre la respuesta inmune y antioxidante en peces de importancia comercial. Además, en este capítulo se promueve el aprovechamiento de los polifenoles de fuentes vegetales de bajo costo, como ingredientes funcionales para acuicultura. Esta revisión se encuentra publicada en la revista Ciencia UAT.

El capítulo III está basado en el estudio del uso de polifenoles de cáscara de mango cv. Ataulfo como ingrediente alimentario para el pez cebra. En este capítulo se evaluaron algunas propiedades antioxidantes de los polifenoles sobre la prevención de la pérdida de la calidad post-mortem y la capacidad de estos, para modular las principales enzimas antioxidantes (SOD, GPx y CAT) involucradas en la respuesta al estrés oxidativo como medida de bienestar animal. De este trabajo se derivó un artículo científico el cual se encuentra sometido en la revista Aquaculture Research.

El capítulo IV se enfoca en la extracción de antioxidantes hidrofílicos y lipofílicos presentes en la cáscara de mango cv. Ataulfo; en el estudio de sus mecanismos antioxidantes, en su aplicación como aditivos alimentarios en el aceite de pescado y como posibles alternativas al antioxidante sintético BHT que comúnmente es utilizado para prevenir el proceso de peroxidación en el aceite de pescado. De este trabajo se derivó un artículo científico el cual fue aprobado para su publicación en la revista CyTA Journal of Food.

El capítulo V aborda la discusión general de los resultados derivados del trabajo de investigación.

El capítulo VI presenta las conclusiones generales de la presente investigación.

1. INTRODUCCIÓN GENERAL

El crecimiento acelerado de la acuicultura ha dado lugar a la intensificación del cultivo de peces, situación que puede conducir eventos del del estrés oxidativo, debido particularmente a malas prácticas de manejo de los organismos bajo cultivo, aunado a las condiciones ambientales y factores nutricionales no adecuados para su desarrollo. Recientemente, el estudio del estrés oxidativo en peces ha recibido atención por el potencial de afectar la salud de los organismos y causar problemas relacionados con la calidad post-mortem del tejido muscular, a través de la generación de especies reactivas del oxígeno (EROS) las cuales causan daño directo a los lípidos (particularmente a los ácidos grasos poliinsaturados; PUFA, por sus siglas en inglés: polyunsaturated fatty acids) de los peces mediante el proceso conocido como peroxidación lipídica, el cual también está implicado en la pérdida de la homeostasis celular (Ayala et al., 2014; Lizárraga-Velázquez, Hernández, González-Aguilar, & Basilio-Heredia, 2018).

La célula activa sistemas de defensa antioxidante, para contrarrestar el estrés oxidativo, que incluyen a las enzimas superóxido dismutasa (SOD), glutatión peroxidasa (GPx) y catalasa (CAT) consideradas como el principal mecanismo de defensa, por lo que su análisis es fundamental en el estudio del estrés oxidativo (Li et al., 2015; Martínez-Álvarez, Morales, & Sanz, 2005).

La capacidad de defensa celular puede ser superada por el estrés oxidativo, a pesar de la eficiencia del sistema antioxidante, por lo que se ha recurrido al uso de compuestos bioactivos de origen vegetal como ingredientes alimentarios para prevenir el estrés oxidativo en peces. Entre los principales compuestos bioactivos provenientes de vegetales comúnmente utilizados en las dietas para peces, se encuentra el grupo de los polifenoles, que se caracterizan por exhibir propiedades antioxidantes e inmunoestimulantes, ambas están relacionadas directamente con beneficios a la salud del pez. Actualmente, existe vasta información dirigida al uso de polifenoles como inmunoestimulantes (Chakraborty & Hancz, 2011; Chiara, Donatella, & Marco, 2015; Reverter, Bontemps, Lecchini, Banaigs, & Sasal, 2014). Se requieren investigaciones que evalúen el efecto de los

antioxidantes en peces, en aras de contribuir al bienestar de los organismos y de prevenir la pérdida de la calidad del producto final, considerada como un factor determinante en términos comerciales, que como consecuencia del estrés oxidativo se puede afectar el contenido de PUFA y dar lugar a la formación de compuestos volátiles que afectan algunas características organolépticas y, por ende, su comercialización.

Los estudios sobre la propiedad antioxidante de los polifenoles en peces son limitados. Sin embargo, ha sido estudiada durante décadas debido a sus múltiples beneficios a la salud humana y por su alta capacidad para preservar alimentos con alta proporción de PUFA (Ganesan & Xu, 2017; Seneviratne, Prasadani, & Jayawardena, 2016; Sharma, 2014; Topuz, Gokoglu, Yerlikaya, Ucak, & Gumus, 2016). En acuicultura, uno de los ingredientes principales en la producción de alimentos es el aceite de pescado (Tacon, 2004), el cual contiene alta proporción de PUFA de la serie omega-3 como el ácido eicosapentaenoico y el ácido docosahexaenoico, que comúnmente se consideran deseables para la nutrición de peces. Sin embargo, su alto grado de insaturaciones los hace susceptibles a la peroxidación lipídica (Sekhon-Loodu, Warnakulasuriya, Rupasinghe, & Shahidi, 2013), proceso que conduce a la formación de productos químicos indeseables que afectan la calidad sensorial y nutricional (por ejemplo, disminución del contenido de PUFA de la serie omega-3) en el aceite de pescado. Por consiguiente, el antioxidante sintético butil hidroxitolueno (BHT), se ha utilizado durante mucho tiempo para retrasar y/o prevenir peroxidación de aceites (Taghvaei & Jafari, 2015). El uso de este antioxidante en aceites de pescado ha recibido considerable atención desde una perspectiva de inocuidad alimentaria, debido a que el aceite se incluye en la dieta para peces de cultivo, por lo que el BHT se transfiere al filete destinado para consumo humano (Lundebye, Hove, Måge, Bohne, & Hamre, 2010). Por consiguiente, los polifenoles representan una alternativa al BHT como aditivos alimentarios en el aceite de pescado.

El uso de polifenoles como ingredientes funcionales para peces y/o aditivos alimentarios en ingredientes para alimentos acuícolas implica la búsqueda e identificación de subproductos vegetales que comúnmente son descartados y considerados como basura. La industria del mango genera una gran cantidad de cáscaras, las cuales representan aproximadamente el 20% del peso total de la fruta. Con el propósito de aprovechar este

subproducto, se han llevado a cabo numerosas investigaciones para determinar su composición química y la funcionalidad de sus componentes. La cáscara de mango aporta sustancias con actividad antioxidante, debido a la presencia de compuestos como los polifenoles, cuyo tipo y cantidad depende de la variedad de mango.

En México, el mango variedad Ataulfo es la de mayor importancia agronómica en el país; presenta una diversidad de polifenoles en su cáscara, entre los que destacan los ácidos gálico, protocatéquico, siríngico y 2-hidroxicinámico, manguiferina y quercetina, que poseen actividad antioxidante en sistemas *in vivo* e *in vitro* (Pacheco-Ordaz, Antunes-Ricardo, Gutiérrez-Uribe, & González-Aguilar, 2018; Velderrain-Rodríguez et al., 2015).

El pez cebra (*Danio rerio*) ha sido propuesto como un modelo para estudios sobre la nutrición acuícola. El estudio de las propiedades antioxidantes de los polifenoles de cáscara de mango cv. Ataulfo como ingrediente funcional para esta especie permite la generación de conocimiento básico para su posterior aplicación en peces de importancia comercial con posibilidad de abordar problemas relacionados con la salud y la calidad post-mortem. Además, el uso de polifenoles del mango como aditivo alimentario en uno de los principales ingredientes del alimento para peces (ejem. aceite de pescado), es una propuesta relevante para la industria alimentaria.

Hipótesis

I. La administración dietaria de polifenoles de cáscara de mango cv. Ataulfo, modula la actividad de enzimas antioxidantes en el hígado y previene la peroxidación lipídica en el músculo del pez cebra.

II. Los antioxidantes presentes en la cáscara de mango cv. Ataulfo inhiben la peroxidación lipídica del aceite de pescado, en una medida similar al del antioxidante comercial butil hidroxitolueno.

Objetivos

Objetivo general

Evaluar el efecto de las propiedades antioxidantes de extractos de cáscara de mango cv. Ataulfo como aditivo alimentario e ingrediente funcional para el pez cebra *Danio rerio*.

Objetivos específicos

I. Caracterizar los polifenoles y los carotenoides presentes en la cáscara de mango cv. Ataulfo.

II. Evaluar el efecto de la administración de polifenoles de cáscara de mango cv. Ataulfo en las dietas para pez cebra: mediante los parámetros de productividad, estrés oxidativo y calidad post-mortem.

III. Evaluar la capacidad antioxidante de extractos hidrofílicos y lipofílicos de la cáscara de mango cv Ataulfo; en la prevención de la peroxidación lipídica del aceite de pescado.

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2. PROPIEDADES ANTIOXIDANTES E INMUNOESTIMULANTES DE POLIFENOLES EN PECES CARNÍVOROS DE CULTIVO

ANTIOXIDANT AND IMMUNOSTIMULANT PROPERTIES OF POLYPHENOLS IN CARNIVOROUS FARMED FISH

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Resumen

El cultivo intensivo de peces es una estrategia económicamente importante para producir alimento. Sin embargo, las prácticas de cultivo intensivo generan estrés oxidativo e inmunosupresión en los organismos, lo que ocasiona pérdidas de la calidad del espécimen y aumento en la mortalidad. Para contrarrestar estos efectos, se ha optado por la administración de vegetales como fuente de polifenoles con propiedades antioxidantes e inmunoestimulantes en peces carnívoros de cultivo. El objetivo de este trabajo fue describir los efectos de los polifenoles de origen vegetal como antioxidantes e inmunoestimulantes en peces carnívoros, y promover su uso como ingredientes funcionales en la acuicultura. Los vegetales como fuente de polifenoles tienen la capacidad de mejorar los sistemas de defensa inmune y antioxidante de las especies analizadas, con un tejido de mejor calidad nutricional y un mayor contenido endógeno de antioxidantes. No obstante, las propiedades biológicas de los polifenoles dependen del tipo y concentración en el vegetal, de la dosis y el tiempo de administración, así como de la matriz alimentaria, la cual determina la bioaccesibilidad y biodisponibilidad de los polifenoles en el organismo. Es escasa la información generada sobre el efecto de los polifenoles en la calidad post mortem, por lo que se deben realizar más estudios.

Palabras clave: acuicultura, estrés oxidativo, respuesta inmune innata, enzimas antioxidantes, alimento funcional.

Abstract

Fish production by intensive aquaculture, is an economically important strategy to produce food. However, intensive fish farming generates oxidative stress and suppress the immune system, causing loss of product quality and increasing fish mortality rates. To diminish these effects, plants as a source of polyphenols with antioxidants and immunostimulant properties were administered to carnivorous farmed fish. The aim of this study was to describe the effects of plant polyphenols as antioxidants and immunostimulants on carnivorous fish, and to promote their use as functional ingredients in aquaculture. Plants as a source of polyphenols showed the ability to improve the immune and antioxidant defense systems of the analyzed species, resulting in a tissue of better nutritional quality and a higher endogenous antioxidant content. However, the biological properties of polyphenols are dependent on the type of plant and their concentration within it, the dose and the time of administration, as well as the food matrix, which determines their bioaccessibility and bioavailability in the organism. There is little information on the effec of polyphenols in post mortem quality; therefore, further studies should be conducted.

Keywords: aquaculture, oxidative stress, innate immune system, antioxidant enzymes, functional food.

Introducción

La intensificación de la acuicultura y la globalización de la comercialización de los pescados y mariscos han producido un desarrollo muy importante en la industria de la acuicultura. Sin embargo, el cultivo de peces a altas densidades incrementa la probabilidad de exponer a los organismos a condiciones de estrés elevado. Estas condiciones pueden provocar la supresión del sistema inmune y por tanto, favorecer la incidencia de enfermedades infecciosas, que dan lugar a una elevación en la tasa de mortalidad y a pérdidas económicas considerables (Alexander y col., 2010). En décadas recientes, la prevención de las enfermedades y su control ha llevado a un sustancial aumento en el uso de aditivos químicos y medicina veterinaria, que pueden acumularse en el tejido, pero con la desventaja de promover, en el caso de los antibióticos, la resistencia de las bacterias (Magrone y col., 2016). De la misma forma, el uso de aditivos químicos puede provocar daños en la salud y al medio ambiente (Harikrishnan y col., 2012). Por otro lado, la especies carnívoras requieren de altos niveles de lípidos en su dieta, lo que puede incrementar la adiposidad en diferentes tejidos y la susceptibilidad a la peroxidación lipídica, proceso implicado en el desarrollo del estrés oxidativo y en la pérdida de la calidad post mortem, debido principalmente a la oxidación de ácidos grasos poliinsaturados (PUFA, por sus siglas en inglés: polyunsaturated fatty acids) de la serie omega 3 y a la formación de compuestos volátiles relacionados con la rancidez (Villasante y col., 2015).

En la búsqueda de promover el bienestar y preservar la calidad del animal, sin comprometer al medio ambiente y la salud de los consumidores, la investigación científica se ha enfocado en la evaluación de inmunoestimulantes y antioxidantes provenientes de fuentes naturales (Bulfon y col., 2013). En este sentido, los polifenoles presentes en frutas, verduras, legumbres, cereales y bebidas, como el té verde y el vino tinto, exhiben propiedades inmnunoestimulantes y antioxidantes (Bulfon y col., 2013; Reverter y col., 2014; Vaseeharan y Thaya, 2014; Afzal y col., 2015; Shahidi y Ambigaipalan, 2015); estas últimas relacionadas directamente con propiedades antiestrés (Chakraborty y Hancz, 2011). Los polifenoles son compuestos que se derivan del metabolismo secundario de las

plantas a través de la vía fenilpropanoide. La característica general de los polifenoles es que tienen anillos aromáticos con grados de hidroxilación variable y la mayoría se encuentran en forma conjugada con uno o más restos de azúcares unidos a grupos hidroxilo o directamente al anillo aromático, incluso pueden encontrarse asociados a otros compuestos (Kumar y Pandey, 2013). La diversidad estructural deriva en una amplia gama de polifenoles (Figura 1) (Bravo, 1998).

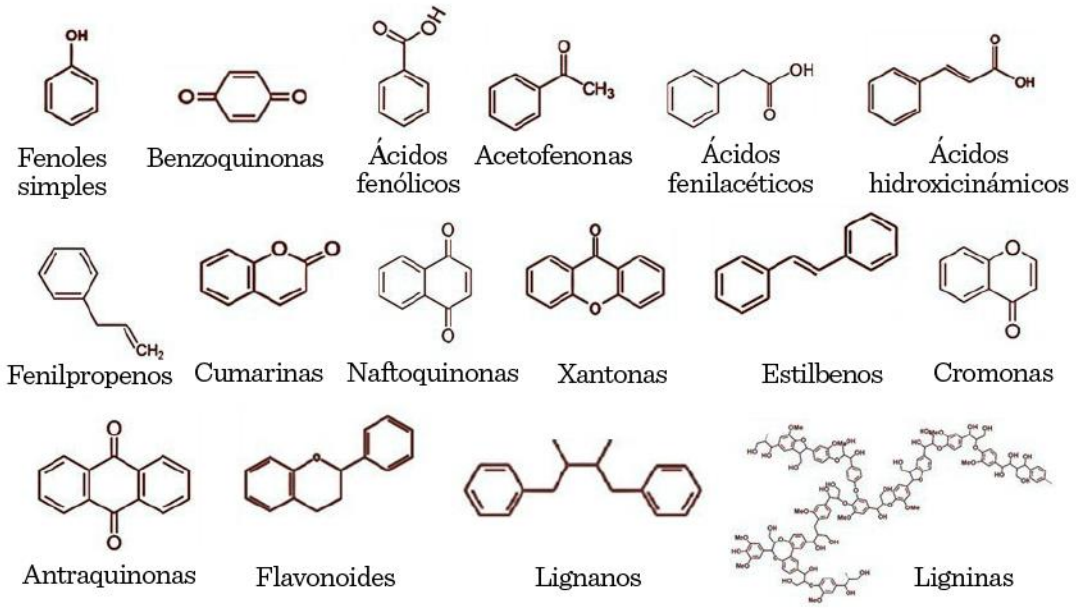

Figura 1. Estructura química básica de las principales clases de polifenoles.

La utilización de vegetales, como fuentes de polifenoles en la dieta, puede reducir el estrés y mejorar el sistema inmune innato tanto de peces omnívoros, herbívoros (Tabla 1) y carnívoros; en estos últimos, se ha empleado el té verde [*Camellia sinensis* (L.) Kuntze], la granada (*Punica granatum* L.) y la cebolla (*Allium cepa* L.), entre otros (Harikrishnan y col., 2011; Nootash y col., 2013).

El objetivo del presente trabajo fue describir los principales efectos antioxidantes e inmunoestimulantes de polifenoles presentes en diferentes vegetales, sobre la respuesta inmune y el estrés oxidativo en peces carnívoros, así como promover el uso de polifenoles de

fuentes vegetales y sus subproductos, como ingredientes, para el desarrollo de alimentos funcio-nales en la acuicultura.

Estrés oxidativo y peroxidación lipídica

El estrés oxidativo es descrito como un desequilibrio entre la generación de prooxidantes como son los radicales libres, las especies reactivas de oxígeno (ROS, por sus siglas en inglés: Reactive Oxygen Species) y especies reactivas de nitrógeno (RNS, por sus siglas en inglés: Reactive Nytrogen Species) y la generación de antioxidantes en favor de los prooxidantes, a nivel celular, de tejido y órganos (Ayala y col., 2014; Lushchak, 2014). Altos niveles de prooxidantes causan daño directo a los lípidos mediante el proceso conocido como peroxidación lipídica, en el cual los agentes prooxidantes atacan a los lípidos que contienen dobles enlaces carbono-carbono (ácidosgrasos insaturados), y generan radicales peróxidos e hidroperóxidos con capacidad de reaccionar y causar daño oxidativo a las proteínas y al ácido desoxirribonucleico (ADN) (Barrera, 2012). Los PUFA de la serie omega 3 como los ácidos eicosapentaenoico (EPA, 20:5 ω-3) y do-cosahexaenoico (DHA, 22:6 ω-3), conforman la estructura de los fosfolípidos de la bicapa-lipídica de la membrana celular. La peroxidación de los PUFA de la membrana favorece la pérdida de su integridad y en consecuencia, la inactivación de proteínas unidas a la membrana y la alteración de las vías de señalización intracelular (Ayala y col., 2014). En respuesta a la peroxidación lipídica en la membrana, la célula estimula su mantenimiento y supervivencia a través de los sistemas de defensa antioxidantes de naturaleza enzimática y no enzimática. El sistema de defensa enzimático incluye a la superóxido dismutasa (SOD), glutatión peroxidasa (GPx), catalasa (CAT), glutatión reductasa (GR), glutatión-S-transferasa (GST), glutarredoxin y tiorredoxina reductasa. La línea de defensa no enzimá-tica incluye al glutatión (GSH), las vitaminas E, C, el β-caroteno y el selenio (Shalaby y Shanab, 2013).

Tabla 1. Uso de polifenoles derivados de vegetales como antioxidantes e inmunoestimulantes en peces de cultivo.

Pez	**Vegetal**	**Administración (Dieta, p/p)**	**Compuesto bioactivo**	**Efecto**	**Referencia**
Tilapia del Nilo	Té verde	0.5 g/kg	Catequinas	Inmunoestimulante	Abdel-Tawwab y col. (2010)
Carpa hervíbora	Té verde	50 g/kg	Catequinas	Antioxidante e inmunoestimulante	Zhou y col. (2016)
Rohu	Mango	5 g/kg	Flavonoides	Inmunoestimulante	Sahu y col. (2007)
Tilapia	Maíz	2 g/kg	Antocianinas	Antioxidante e inmunoestimulante	Catap y col. (2015)

En los sistemas de cultivo existen diversos factores que pueden generar estrés oxidativo, sin embargo, entre los más importantes se encuentra el factor nutricional. Esto debido a que la dieta de peces carnívoros requiere de altos niveles de lípidos o PUFA altamente sus-ceptibles a la oxidación (Villasante y col., 2015). Estudios en salmón del Atlántico (*Salmo salar* Linnaeus, 1758) han reportado que dietas con alto contenido de lípidos conducen a la deposición de grasa, al desencadenamiento del estrés oxidativo y a la pérdida de la calidad nutricional del filete (Hamre y col., 2004; Todorcevic y col., 2009). Por lo tanto, para contrarrestar los efectos causados por el estrés oxidativo, se ha recurrido al uso de antioxidantes como aditivos alimentarios.

Sistema inmune innato

El sistema inmune protege a los organismos contra enfermedades, mediante la identificación y eliminación del patógeno, y se divide en sistema inmune innato y sistema inmune adquirido. En peces, el sistema inmune adquirido es poco eficiente por ser organismos poiquilotérmicos (de sangre fría), por lo que dependen fuertemente del sistema inmune innato, el cual se divide comúnmente en tres mecanismos de defensa: físicos, celulares y humorales. Los parámetros físicos comprenden la barrera epitelial, mucosa en piel, branquias y el tracto digestivo (Magnadóttir, 2010). Los principales componentes celulares son los granulocitos (neutrófilos), con actividad fagocítica. Cuando los fagocitos son estimulados se presentará la actividad denominada explosión respiratoria (liberación de ROS), la producción de citocinas y de moléculas de comunicación celular (Zou y Secombes, 2016). Los parámetros humorales incluyen, la actividad de lisozima y la actividad hemolítica del complemento. Ésta última se ha reconocido como un mecanismo clave de la resistencia bacteriana en teleósteos, como la trucha arcoíris (*Oncorhynchus mykiss* Walbaum, 1792), salmón del Atlántico y bagre (*Ictalurus punctatus* Rafinesque, 1818), entre otros (Sunyer y col., 2013; Buonocorea y col., 2014).

En sistemas de cultivo intensivo, la alta densidad, malas prácticas en el manejo, alteracines en las condiciones óptimas ambientales (temperatura, oxígeno, salinidad, pH, nitritos y carga orgánica) y factores nutricionales (deficiencia o exceso de nutrientes) generan un entorno fisiológico estresante que conduce a la reducción del crecimiento, supresión del sistema inmune y a la susceptibilidad para contraer enfermedades infecciosas que pueden generar altas tasas de mortalidad (Martínez-Álvarez y col., 2005; Lushchak, 2011; Reverter y col., 2014; Philip y col., 2015), que en consecuencia ocasionan pérdidas económicas considerables, que conllevan al uso de agentes quimioterapéuticos, que causan el desarrollo de bacterias resistentes y la contaminación del ambiente (Nootash y col., 2013; Done y col., 2015). Por esa razón, se ha optado por el uso de inmunoestimulantes que mejoran el estado de salud y confieran resistencia contra

patógenos, a través del fortalecimiento del sistema inmune innato (Vaseeharan y Thaya, 2014).

Propiedades biológicas de los polifenoles en peces carnívoros

Fuentes de origen vegetal como antioxidantes Se ha reportado que la inclusión dietaria de hojas de té verde (0.1 g/kg) disminuye el nivel de peroxidación lipídica e incrementa la actividad de la enzima SOD en suero de trucha arcoíris (Nootash y col., 2013). También se ha documentado que la inclusión de extracto de té verde (50 g/kg) a la dieta para black rockfish (*Sebastes schlegelii* Hilgen, 1880) disminuye el nivel de colesterol en plasma (Hwang y col., 2013). En ambos estudios, el efecto benéfico del té verde es atribuido a las catequinas, las cuales pueden inducir la actividad de enzimas antioxidantes y neutralizar las ROS (Wang y col., 2013). Estudios en humanos han demostrado que el té verde incrementa el potencial antioxidante en suero y disminuye los valores de lipoproteínas de baja densidad (LDL) y colesterol en plasma, así como la concentración de productos de la peroxidación lipídica (Onakpoya y col., 2014; Domanski y col., 2015). Los resultados de los estudios en trucha arcoíris y black rockfish, indicaron que las catequinas son antioxidantes efectivos para combatir la peroxidación lipídica y reducir los niveles de colesterol en plasma *in vivo*.

La inclusión dietaria de polvo de cebolla (10 g/kg) disminuye los niveles de colesterol y triglicéridos en el suero de esturión beluga (*Huso huso* Linnaeus, 1758) (Akrami y col., 2015). La cebolla es una fuente rica en quercetina (Aditya y col., 2017), la cual se ha demostrado que previene la oxidación de LDL en plasma, e impide la biosíntesis de colesterol mediante la inhibición de la actividad de síntesis de ácidos grasos (Moon y col., 2012). Por lo que, la disminución de colesterol en esturión beluga, se atribuye a la influencia de la quercetina sobre la biosíntesis de colesterol. Contrariamente, se ha reportado que la inclusión de cebolla a la dieta para fletán japonés (*Paralichtys olivaceus* Temminck Schleges, 1846) no afecta los niveles de colesterol y triglicéridos (Cho y Lee, 2012). La

diferencia de resultados en el esturión beluga y fletán japonés puede relacionarse con la fisiología de cada especie, ya que la acción de los polifenoles dependerá directamente de la biodisponibilidad de estos compuestos y por tanto de factores intrínsecos, como el pH gástrico, la actividad de enzimas digestivas y la microflora bacteriana, dado que pueden inducir la hidrólisis y/o transformación de los polifenoles a moléculas biológicamente activas y biodis-ponibles (Velderrain-Rodríguez y col., 2014).

La dorada (*Sparus aurata* Linneasus, 1758) alimentada con una dieta enriquecida con el subproducto de la refinación de aceite de oliva (10 g/kg y 50 g/kg), mostró un ligero retraso en el proceso de oxidación lipídica de su filete almacenado a 4 °C (Sicuro y col., 2010). El aceite de oliva contiene polifenoles como el hidroxitirosol, tirosol, oleuropeína, ácidos hidroxicinámicos y ácido cafeíco, los cuales poseen la capacidad de reducir los niveles de peroxidación lipídica, y mejorar el sistema de defensa antioxidante (Rafehi y col., 2012; Servili y col., 2013). Es por ello que el conjunto de polifenoles del aceite de oliva inhibe el proceso de peroxidación lipídica y contribuye en la preservación de la calidad del filete de la dorada.

En trucha arcoíris, se evaluó el efecto de la inclusión dietaria del extracto de maíz morado (50 g/kg), sobre la actividad antioxidante (expresión de los genes GPx1 y SOD1) en los eritrocitos, la concentración de biomarcadores del daño oxidativo (ADN, lípidos y proteínas) en el plasma y el perfil de PUFA omega 3 y 6 en el cuerpo (Villasante y col., 2015). Los autores registraron un aumento en la expresión del gen GPx1, que codifica para la GPx, una tendencia a disminuir los niveles de peroxidación lipídica y un incremento en la proporción de PUFA omega 3 y 6. El maíz morado es una fuente importante de antocianinas, como la cianidina-3-glucósido y pelargonidina-3-glucósido (Ramos-Escudero y col., 2012). Aboonabi y Singh (2015), reportaron que las antocianinas inducen la expresión de enzimas relacionadas con el glutatión (GR, GPx y GST), por la vía de activación del factor nuclear derivado de eritroide 2 (Nrf2). Debido a lo anterior, se sugiere que las antocianinas mejoran la protección antioxidante en plasma y eritrocitos de trucha arcoíris, a través de la modulación de la actividad de la enzima GPx y probablemente, a través de la quelación de hierro (Fe^{3+}) o donación de protones a especies

reactivas, las cuales están implicadas directamente en el proceso de peroxidación lipídica *in vivo*.

Fuentes de origen vegetal como inmunoestimulantes

Los vegetales contienen diversos tipos de polifenoles con actividad inmunoestimulatoria, por lo que se estudia el uso de diferentes fuentes para controlar enfermedades y fortalecer el sistema inmune innato. Nootash y col. (2013), reportaron que la administración dietaria de extracto de té verde (0.1 g/kg) disminuyó los niveles de transcritos codificantes para distintas citocinas (interleucina-1β e interleucina-8, en bazo e hígado, respectivamente) e incrementó los niveles de proteína total y la actividad bactericida en trucha arcoíris. Por su parte, Harikrishnan y col. (2011), registraron que la administración dietaria de extracto de té verde (0.1 g/kg y 1 g/kg) aumentó la producción de RNS, la actividad de lisozima y la actividad hemolítica del complemento sérico en mero diente largo (*Epinephelus bruneus* Bloch, 1793), infectado con *Vibrio carchariae*. Hwang y col. (2013), indicaron que la administración dietaria de extracto de té verde (10 g/kg), incrementó la actividad de lisozima y el porcentaje de supervivencia de black rockfish, cultivado en condiciones de estrés inducido. Esto se debe a que los flavonoides, una vez que son absorbidos, pueden influenciar la síntesis de proteínas (Carlo y col., 1999). El alto contenido de proteína en suero y la alta actividad bactericida se asocia con la síntesis de proteínas activas, lo que resulta en una fuerte respuesta innata. En particular, las catequinas pueden regular reacciones inmunológicas por modulación de citocinas proinflamatorias, o por influenciar la actividad de células del sistema inmune (Patel y Vajdy, 2015). De acuerdo con lo anterior, es evidente que las catequinas pueden mejorar el sistema inmune innato a través de la modulación de la respuesta humoral y celular.

Akrami y col. (2015), reportaron que la inclusión de cebolla en polvo (10 g/kg), en la dieta para juveniles de esturión beluga, elevó la actividad de lisozima y la actividad de explosión respiratoria. En la cebolla se han identificado glucósidos de flavonoides como la querce-

tina y fructooligosacáridos, a los cuales se les atribuye el efecto modulatorio (Kumar y col., 2015; Oliveira y col., 2015). No obstante, se desconoce el mecanismo de acción por el cual estos compuestos mejoran el sistema inmune innato. Por lo que, se requiere de estudios adicionales con los compuestos bioactivos purificados y probados por separado, en la misma especie, para poder esclarecer qué tipo de compuesto es el responsable de la modulación de la respuesta inmune innata.

La administración por vía intraperitoneal de extracto de granada (0.1 g/kg de peso corporal) en fletán japonés, infectado naturalmente con el virus de linfocistis, incrementó la tasa de supervivencia, la actividad de lisozima, la actividad fagocítica, la explosión respiratoria y la actividad del complemento sérico (Harikrishnan y col., 2010). Otro estudio, en la misma especie, infectada con el parásito *Philasterides dicentrarchi*, reportó que las dietas enriquecidas con extracto de granada (10 g/kg) mejoraron el sistema inmune innato celular, mediante el aumento de leucocitos, que incluyen los linfocitos, monocitos y neutrófilos (Harikrishnan y col., 2012). La granada es rica en polifenoles, tales como, ácido elágico, elagitaninos (punicalaginas), galotaninos y antocianinas (Galego y col., 2013). Ross y col. (2001), indicaron que la administración oral de granada estimuló la respuesta inmune innata humoral y celular de conejos. Sin embargo, aunque se desconocen los compuestos que desencadenan la respuesta inmune innata, estos podrían estar relacionados directamente con el conjunto de polifenoles que conforman el extracto de granada. Por lo tanto, se requiere de más investigación para una mejor comprensión del efecto inmunoestimulante de los compuestos polifenólicos que se encuentran en la granada.

Magrone y col. (2016), analizaron la influencia de la dieta suplementada con extracto de uva (1 g/kg y 2 g/kg), sobre la modulación de citocinas en el bazo e intestino de la lubina (*Dicentrarchus labrax* L.) y observaron una disminución en la concentración de las interleucinas intestinales (IL-1β) e IL-6; y un aumento en la producción de interferón-(IFN-) en el bazo. Así mismo, los autores identificaron proantocianidinas y catequinas como los polifenoles principales del extracto de uva, los cuales poseen propiedades antiinflamatorias e inmunomodulatorias (Zhou y Raffoul, 2012; Chu y col., 2016). Por lo

que, el efecto inmunoestimulante observado en los diferentes órganos analizados de la lubina, lo atribuyeron a los polifenoles del extracto de uva.

Aunque los alimentos para acuicultura proveen los nutrientes necesarios para el desarrollo de los organismos, la inclusión de los polifenoles como compuestos bioactivos, le confieren una funcionalidad dirigida a la salud animal, con un tejido de mejor calidad nutricional y un mayor contenido endógeno de antioxidantes, combatiendo el estrés oxidativo y mejorando el sistema inmune innato. Polifenoles como la epigalocatequina y la quercetina, tienen la capacidad tanto de combatir el estrés oxidativo como de modular la respuesta inmune innata en especies como la trucha arcoíris y la dorada (Shin y col., 2010; Thawon-suwan y col., 2010).

Debido al impacto positivo de las propiedades biológicas de los polifenoles, se ha incrementado el número de investigaciones enfocadas en la extracción e identificación de estos compuestos a partir de subproductos vegetales tales como la cáscara de mango (Blancas-Benítez y col., 2015), plátano (Aboul-Enein y col., 2016), manzana (Giomaro y col., 2014), uva (Makris y Kefalas, 2013), semilla de aguacate (Kosinska y col., 2012) y café (Murthy y Naidu, 2012). El té verde es el vegetal mayormente utilizado en los estudios con peces carnívoros (Hwang y col., 2013; Nootash y col., 2013; Hasanpour y col., 2017).

Conclusiones

Los vegetales, como fuente de polifenoles, tienen la capacidad de mejorar los sistemas de defensa inmune y antioxidante de especies de peces carnívoros, siendo el té verde el vegetal mayormente utilizado, por su alto contenido polifenólico. Sin embargo, es necesaria la evaluación de polifenoles purificados, extraídos de fuentes vegetales, con el propósito de demostrar cuáles son los componentes responsables de la modulación de la respuesta inmune y antioxidante en las diferentes especies estudiadas, y de este modo,

potenciar su aprovechamiento como ingredientes en el desarrollo de alimentos funcionales para acuicultura. Se requiere establecer las dosis óptimas que generen el efecto deseado por especie, analizando cómo influyen los componentes de la matriz del alimento sobre las variables de respuesta de interés. Por otro lado, es escasa la información generada sobre el efecto de los polifenoles en la calidad post mortem, factor fundamental en términos comerciales, ya que el estrés oxidativo puede afectar el contenido de ácidos grasos poliinsaturados de la serie omega 3 y dar lugar a la formación de compuestos volátiles que afectan algunas características sensoriales y, en consecuencia, su comercialización. Se requiere analizar parámetros relacionados con la calidad post mortem ocasionados por estrés oxidativo, aprovechando los conocimientos generados en el desarrollo de productos funcionales para humanos a partir de subproductos de vegetales, los cuales presentan alto contenido de polifenoles. Este conocimiento puede aprovecharse en la elaboración de alimentos funcionales para peces, dentro del marco de la acuicultura sostenible.

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3. DIETARY INTAKE OF PHENOLIC COMPOUNDS FROM MANGO PEEL EXTRACT REDUCES LIPID PEROXIDATION IN MUSCLE AND MODULATES ANTIOXIDANT ENZYME ACTIVITIES IN LIVER OF ZEBRAFISH (*Danio rerio*)

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Abstract

Four experimental diets were formulated with 50, 100, 150 and 200 mg of phenolic compounds (PCs), derived from mango peel, per kg of feed. The control diet did not contain PCs. A total of 120 male zebrafish (average weight: 166 mg) were fed for 8 weeks to assess the ability of PCs to prevent lipid peroxidation and enhance antioxidant status. Growth performance was calculated at the end of the experimental trial. Lipid peroxidation in muscle and antioxidant enzyme activity in liver were evaluated at the end of the experiment. There was no significant difference in growth performance among treatments. Malondialdehyde (MDA) levels in muscle were significantly lower in fish with diets containing 50 and 100 mg PCs/kg feed. Incorporation of PCs into zebrafish diet did not have any significant effects on glutathione peroxidase (GPX) and superoxide dismutase (SOD) activity. However, catalase (CAT) activity increased significantly in fish with diets containing 100, 150 and 200 mg PCs/kg feed. These results suggest a potential protective effect against *in vivo* lipid peroxidation, and CAT-modulating effects.

Keywords: mango peel, phenolic compounds, antioxidant enzymes, lipid peroxidation

Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ω3, omega 3; PUFAs, polyunsaturated fatty acids; ROS, reactive oxygen species; O_2•-, super oxide radical; OH•, hydroxyl radical; HO_2•-, hydroperoxyl radical; MDA, malondialdehyde; CAT, catalase; SOD, superoxide dismutase; GPX, glutathione peroxidase; TPCs, total phenolic content; PCs, phenolic compounds; MPE, mango peel extract; WG, weight gain; FI, feed intake; FCR, feed conversion ratio; SGR, specific growth rate; S, survival; PER, protein efficiency ratio; H_2O_2, hydrogen peroxide; O_2, molecular oxygen; Nrf2, nuclear factor-E2-related factor 2; AREs, antioxidant response elements.

Introduction

In general, fish contain high levels of long-chain omega-3 (ω3) polyunsaturated fatty acids (ω3 PUFAs) such as eicosapentaenoic acid (EPA; 20:5, ω3) and docosahexaenoic acid (DHA; 22:6, ω3), which make up the phospholipids of their cells' lipid membranes, providing structural and functional maintenance to cell membranes (Ardiansyah and Indrayani, 2007; Ayala et al., 2014). Paradoxically, ω3 PUFAs are highly susceptible to lipid peroxidation by reactive oxygen species (ROS) such as the superoxide anion (O_2•-), hydroxyl radical (OH•) and hydroperoxyl radical (HO_2•-). These molecules are produced during normal cellular metabolism, but during oxidative stress, which is provoked by nutritional factors, environmental factors (biotic and abiotic) and/or fish handling (Martínez-Álvarez et al., 2005). Lipid peroxidation products, such as lipid peroxyl radicals and malondialdehyde (MDA, a good indicator to analyse the oxidative damage of lipids), can affect the nutritional quality (e.g. decrease ω3 PUFAs content) of fish flesh intended for human consumption (Secci and Parisi, 2016). In addition, there is an increased interest to prevent peroxidation of ω3 PUFAs in fish flesh since their human consumption has a host of positive health effects related to the prevention of cardiovascular and inflammatory diseases (Delgado-Lista et al., 2012).

Studies of oxidative stress in farmed fish are becoming increasingly important especially as an index of fish welfare. In addition, analyses of oxidative stress in different tissues can be performed providing greater detail of underlying fish health (Poli, 2009); for example, the liver of fishes is an important detoxifying organ where oxidative stress can be analysed because it has an enzymatic antioxidant defense system that helps to maintain redox homeostasis (Martínez-Álvarez et al., 2005; Li et al., 2015). The main enzymes responsible for the antioxidant response measured in fish are catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) which act jointly to protect cells against oxidative damage (Martínez Álvarez et al., 2005; Karadag et al., 2014). Furthermore, they are used as biomarkers of oxidative stress and fish welfare, both of which have an impact on fish flesh quality (Chulayo and Muchenje, 2015).

In absence of adequate antioxidant enzyme activity, MDA concentration increases of in fish flesh, which diminishes its quality. Natural antioxidants such as dietary additives could, therefore, be used to protect farmed fish against oxidative damage to lipids and enhance fish welfare.

Mango (*Mangifera indica* L.) contains phenolic compounds (PCs) with high antioxidant activity (Velderrain-Rodríguez et al., 2016). Mango cv. Ataulfo" is a Mexican variety of high agronomic importance; it is used to produce juices, concentrates and snacks, generating large amounts of peel as industrial waste. Mango byproducts, such as peels, have a high PCs content which is capable of preventing lipid peroxidation of fish oil (data not yet published). There are multiple *in vitro* and *in vivo* studies reporting the anti-inflammatory, anti-cancer, anti-diabetic and anti-obesogenic potential of mango peel PCs (Shah et al., 2010); however, their antioxidant benefits have not been explored in fish. Therefore, the aim of this work was to evaluate the ability of mango peel PCs to delay lipid peroxidation in muscle, and enhance antioxidant status in the liver of zebrafish, which is considered a good aquaculture model organism.

Materials and methods

Preparation of phenolic compounds

Mango peel PCs were extracted according to Sekhon-Loodu et al. (2013) with minor modifications. Mango peel powder (10 g) was homogenized in 1 L of 70% ethanol, sonicated three times for 15 min in a 3510-model ultrasonic bath (Branson, Whetersfield, CT, USA). After sonication, samples were centrifuged (3000*g*,15 min, 4°C) in an Allegra X-30R model A99470 centrifuge (Beckman Coulter, Germany). Finally, a rotovapor R-114 (Büchi Labortechnik AG, Flawil, Switzerland) was used (37°C) to remove the ethanol and concentrate the supernatants. Total phenolic content (TPC) was determined with the

Folin-Ciocalteu reagent using gallic acid as standard. After knowing TPC, dilutions were prepared with concentrations of 50, 100, 150 and 200 mg of mango PCs.

Analysis of PCs by UPLC ESI-Q-TOF/MS/MS

Identification of PCs from mango peel was determined through Ultra-Performance Liquid Chromatography (UPLC) using ACQUITY UPLC; H-Class system (Waters, Milford, Mass., U.S.A.) coupled to a G2 XS Quadrupole-Time-of-Flight (Q-Tof) mass spectrometer (Agilent, Santa Clara, CA) equipped with electrospray ionization (ESI). Briefly, phenolic compounds were separed by UPLC to 40°C with a column ACQUITY BEH C18 (1.7 µm, 3.0 x 100 mm) using a mobile phase composed of 0.1% formic acid (A) and acetonitrile (B) at a flow rate of 0.3 mL/min. The gradient procedure was as follows: 0 min, 95% (A); 2.5 min, 75% (A); 3 min, 50% (A); 3.5 min, 35% (A); 5 min, 5% (A); 6 min, 95% (A); 7 min, 95% (A). An electrospray source in negative mode was used to collect mass spectra under the following conditions: nitrogen gas; desolvation temperature, 350°C; desolvation gas, 13.3 L/min; capillary voltage, 1500 V; and fragmentor voltage, 10 V.

Experimental diets

The preparation of experimental diets was carried out as follows: Fish meal, fish oil, dextrin, cellulose, soy lecithin, alginate, mineral premix, vitamin premix, vitamin C and synthetic antioxidant butylated hydroxytoluene (BHT), were used to prepare the control diet. Four additional diets were prepared and supplemented with PCs from the mango peel extract (MPE) at concentrations of 50, 100, 150 and 200 mg PCs/kg feed. These were denoted as MPE-5, MPE-10, MPE-15 and MPE-20, respectively (Table 2). Fish meal was ground in a hammer mill to a particle size of 250 µm. The macronutrients (fish meal,

dextrin, cellulose and alginate) were mixed in a model AT-200 Hobart mixer (Offenburg, Germany), and the micronutrients (mineral premix, vitamin premix, vitamin C and BHT) were then added. Fish oil and soy lecithin were added to the previously made mixture, finally PCs that had been homogenized in warm water were added until a homogeneous mixture was obtained. The resulting mash was passed through a model 22 meat grinder (Torrey®, IN, USA) to produce pellets, which were dried at 38 °C for 12 h, reduced to a 600 µm of diameter and stored at 4 °C until use.

Chemical Analysis

Ingredients and diets were analysed for moisture, crude protein crude fat, and ash (method 942.05; AOAC, 2000). Moisture was determined using a Craft stove (method 930.15; AOAC, 2000). Crude protein was determined with the Dumas combustion method (Ebeling, 1968) using a Flash 2000 Organic Elemental Analyser (Thermo Scientific, Italy). Crude fat content was analysed using a micro Soxhlet Foss Soxtec Avanti 2050 Automatic System (Foss Soxtec, Hoganäs, Sweden) using an official method (method 920.39; AOAC, 2000). Ash content was analysed by calcination of the samples in a muffle furnace at 550°C (Fisher Scientific International, Inc. Pittsburgh, PA) using an official method (method 942.05; AOAC, 2000).

The PCs content in the experimental diets was analysed to determine the final concentration in zebrafish feed. Briefly, diets (0.5 g) were homogenized in 20 mL of 70% ethanol, stirred for 24 h at 20 °C, and sonicated for 15 min. After sonication, samples were centrifuged (3000*g*, 15 min, 4°C) and supernatants were collected. PCs content was determined with the Folin–Ciocalteu reagent using gallic acid as standard.

Tabla 2. Ingredients, proximate composition and PCs content of the experimental diets administered to zebrafish *Danio rerio.*

Ingredients (%)	Diet				
	Control	MPE-5	MPE-10	MPE-15	MPE-20
Fish meal[a]	73	73	73	73	73
Fish oil[b]	8	8	8	8	8
Dextrin[b]	3	3	3	3	3
Celulose[b]	10.56	10.547	10.542	10.537	10.532
Soy lecithin[b]	0.6	0.6	0.6	0.6	0.6
Minerals premix[c]	0.23	0.23	0.23	0.23	0.23
Vitamin premix[c]	0.1	0.1	0.1	0.1	0.1
Vitamin C[d]	3	3	3	3	3
Alginate[e]	1.5	1.5	1.5	1.5	1.5
BHT[e]	0.01	0.01	0.01	0.01	0.01
MPE[f]	0	0.005	0.01	0.015	0.02
Proximate composition (% dry matter)[g]					
Crude protein	54.62±0.21	54.49±0.19	54.59±0.11	54.71±0.33	54.56±0.17
Crude lipid	12.39±0.33	12.04±0.30	12.17±0.18	12.39±0.54	12.33±0.27
Ash	15.83±0.25	15.89±0.04	15.72±0.25	15.73±0.09	15.85±0.04
NFE[h]	17.16±0.32	17.58±0.12	17.52±0.34	17.17±0.19	17.26±0.14
Gross energy (kJ/g)[i]	18.97	18.88	18.94	18.99	18.95
Phenolic content (% dry matter)					
Total phenolic compounds	0	0.005 ± 0.00	0.111 ± 0.00	0.165 ± 0.00	0.022 ± 0.00

[a] Selecta de Guaymas, S.A de C.V, Guaymas, Sonora México.

[b] Droguería Cosmopolita, S.A. de C.V. México, D.F., Mexico.

[c] Trout nutrition México S.A de C.V. (by courtesy). Vitamin premix composition: Vitamin A, 2400 IU or mg/g ; Vitamin D 3, 2250 IU; Vitamin E, 160 g; Vitamin K3 8.00 g; Vitamin B1, 20.00 g; Vitamin B2, 40.00 g; Vitamin B6, 16.00 g; Pantothenic acid, 60.00 g; folic acid, 4.00 g; vitamin B12, 80 mg; nicotinic acid, 160.00 g; biotin, 500 mg; vitamin C, 100 g; Choline, 300 g. Excipient c.b.p. 2000 g.

[c] Mineral premix composition: manganese, 100 g; Magnesium, 45 g; Zinc, 160g; Iron, 200g; Copper, 20g; Iodine, 5g; Selenium 400mg; Cobalt 600 mg.

[d] DSM Nutritional Products Mexico S.A. de C.V., El Salto, Jalisco, Mexico.

[e] Sigma-Aldrich Chemical, S.A. de C.V. Toluca, Mexico State, Mexico.

[f] Mango peel extract.

[g] Mean ± SD, number of replicates=3.

[h] Nitrogen-free extract (including fiber) =100−(% protein+% lipid+% ash).

[i] Gross energy (kJ/g) was calculated according to the physiological fuel values of protein, 20.93 kJ/g; lipids, 37.68 kJ/g; and nitrogen-free extract, 16.75 kJ/g.

Fish rearing and feeding

Adult zebrafish were obtained from the Bioassay Laboratory in aquatic organisms of the Research Center for Food and Development (CIAD, Mazatlán Unit), which come from a standardized genetic line (line AB, www.zfin.org). A completely randomized experimental design with three replicates per treatment was used. A total of 120 male zebrafish mean initial weight of 166 ± 1 mg were maintained under a 12 h light:dark cycle. Zebrafish were placed in fifteen aquarium tanks (6 L) in groups of eight fish, each tank had a static renewal system. The effect of PCs on growth performance and antioxidant response was evaluated using the following feeding protocol: zebrafish were fed manually twice a day (9:00 and 16:00 h) to apparent satiety for 8 weeks. Each morning, fecal matter and excess leftover feed were carefully syphoned out from the bottom of each tank, and 30% water volume was renewed daily. Water conditions were as follows: temperature 27 ± 0.5°C, pH 6.9 ± 0.5, dissolved oxygen 5.0 ± 0.3 mg/L and chlorine 0.0 mg/L.

Growth parameters and feed efficiency

Fish were weighed individually under anesthesia [0.005% (w/v) tricaine (Sigma-Aldrich, St. Louis, MO, USA)] every two weeks to calculate their mean body weight.

Growth and feed efficiency of the fish were monitored in terms of weight gain (WG), specific growth rate (SGR), survival (S), feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER) and hepatosomatic index (HSI). Biological indicators were calculated as follows: WG (mg) = [*final mean weight* - *initial mean weight*]; SGR (%/day) = [100 × (ln (*final mean body weight*) - ln (*initial mean body weight*))/*number of days*]; S (%) = (*final number*/*initial number*) x 100]; FI (mg/fish) = $\sum_i 60$[(*total feed consumption*)/(number of fish)]/*number of days*; FCR = *feed intake*/*weight gain*; PER= *weight gain*/*protein intake* and HSI (%) = [100 × (*liver weight*/*body weight*)].

Muscle lipid peroxidation

To quantify lipid peroxidation, the head, tail and viscera were removed from each fish. Four fishes (about 400 mg) from each tank were homogenized (Ultra-Turrax D25 basic, IKA©-Werke, Germany) with 1.2 mL of PBS buffer (pH 7.4). Homogenized samples were centrifuged at 3000*g* for 15 min at 4 °C, supernatants were recovered and used to quantify peroxidized lipids as described by Solé et al. (2004). Briefly, 200 µL of the homogenate were mixed with 1300 µL of 1-methyl-2-phenylindole (10.3 mM) in methanol: acetonitrile (1:3; v/v), 200 µL of water and 300 µL of 37% HCl. This mixture was incubated at 45 °C for 40 min, cooled on ice for 10 min and centrifuged at 3000*g* for 15 min at 4 °C. Absorbance was read at 586 nm, and the amount of peroxidized lipids (nmol MDA/g of tissue; w/w) was calculated using a standard solution of 1,1,3,3-tetraethoxypropane (10 mM).

Hepatic antioxidant-enzyme activities

On the last day of the experiment, zebrafish were euthanized under anesthesia with 0.0075% (w/v) tricaine (Sigma-Aldrich, MO, USA). The liver was removed and manually homogenized in 300 µL of PBS buffer (pH 7.4) and centrifuged at 3000*g* for 15 min at 4 °C. The supernatant was used to determine total protein content and antioxidant enzyme activities. Total protein content was determined using Bradford's reagent (Bradford, 1976) and bovine serum albumin as standard. CAT activity was determined using a Cayman Chemical kit (Ann Arbor, MI, USA); one unit of CAT was defined as the amount of enzyme that catalysed the formation of 1 nmol of formaldehyde per minute at 25°C. GPX activity was measured using a Cayman Chemical kit; one unit of GPX was defined as the amount of enzyme that catalysed the oxidation of 1 nmol of NADPH to NADP+ per minute at 25°C. SOD activity was assayed using a Sigma-Aldrich kit); one unit of SOD was defined as the amount of enzyme that inhibited the formation of WST-1 formazan by 50%. All enzyme activity results were expressed as specific enzyme activity in U/ mg protein.

Statistical Analysis

Data were evaluated for normality (Kolmogorov–Smirnov test) and homogeneity of variance (Levene´s test) prior to the statistical analysis. Dependent variables (growth performance, MDA content and antioxidant enzymes activities) were analysed using a one-way analysis of variance (ANOVA); a post hoc Tukey's test was conducted when significant differences were found ($P < 0.05$). Statistical analyses were run using Statistica v.7 (StatSoft, Inc., 2004).

Results

Mango peel extract (MPE) phenolic compound (PC) profile

Eight PCs were identified, five of which were phenolic acids, two flavonoids and a polyphenol, xanthone (Table 3). Gallic acid (m/z $[M-H]^-$, 169.01), 2-hydroxicinnamic acid (m/z $[M-H]^-$, 163.038), mangiferin (m/z $[M-H]^-$, 421.089) and quercetin (m/z $[M-H]^-$, 301.035) were identified by comparing the m/z $[M-H]^-$ and fragment ions with standards. Methyl gallate (m/z $[M-H]^-$, 183.027), ethyl gallate (m/z $[M-H]^-$, 197.041) and isoquercitrin (m/z $[M-H]^-$, 463.085) were compared with literature. While protocatechuic acid (m/z $[M-H]^-$, 153.014) was confirmed with database (MassBank of North America).

Tabla 3. PCs detected in mango peel cv. Ataulfo extract by UPLC-ESI Q-Tof-MS/MS.

Identification	Retention time (min)	m/z $[M-H]^-$	Fragment ions MS/MS (m/z) $[M-H]^-$	Molecular formula
Gallic acid[a]	1.521	169.01	125.017	$C_7H_6O_5$
2- Hydroxycinnamic acid[a]	4.18	163.0382	119.044, 136.922, 138.925	$C_7H_6O_5$
Mangiferin[a]	3.482	421.089	331.035, 331.045	$C_{19}H_{18}O_{11}$
Quercetin[a]	4.817	301.035	179.997, 150.999	$C_{15}H_{10}O_7$
Methyl gallate[b]	3.425	183.027	124.01	$C_8H_8O_5$
Ethyl gallate[b]	4.328	197.041	124, 125, 169.01	$C_9H_{10}O_5$
Isoquercitrin[b]	4.283	463.085	301.035	$C_{21}H_{20}O_{12}$
Protocatechuic acid[c]	2.585	153.014	108.02, 109.020	$C_7H_6O_4$

[a]Confirmed with standard.
[b] Confirmed with literature (Dorta et al., 2014).
[c] Confirmed with database (MassBank of North America).

Growth and feed efficiency parameters

The WG, SGR, S, FI, FCR, PER and HSI were not significantly affected by supplementation of PCs from MPE into the diet of zebrafish (Table 4). Zebrafish survival was greater than 90% and no significant difference (P > 0.05) among the different dietary treatments was observed.

Tabla 4. Growth parameters and feed efficiency of zebrafish fed experimental diets for 8 weeks.[a]

	Diet				
	Control	MPE-5	MPE-10	MPE-15	MPE-20
IW (mg)	162 ± 0.07	162 ± 0.06	162 ± 0.06	162 ± 0.06	162 ± 0.06
FW (mg)	194.82 ± 0.99	193.39 ± 2.53	193.87 ± 1.08	192.56 ± 1.16	193.51 ± 0.992
WG (mg)	32.32 ± 0.99	30.89 ± 2.53	31.37 ± 1.08	30.06± 1.16	31.01 ± 0.92
SGR (%/day)	0.30 ± 0.01	0.29 ± 0.03	0.29 ± 0.01	0.28 ± 0.01	0.29 ± 0.01
S (%)	91.6 ± 7.21	91.6 ± 14.43	91.6 ± 7.21	95.83 ± 7.21	95.83 ± 7.21
FI (mg/fish)	1.35 ± 0.06	1.40 ± 0.14	1.40 ± 0.12	1.16 ± 0.18	1.25 ± 0.09
FCR	2.56 ± 0.03	2.51 ± 0.01	2.51 ± 0.02	2.50 ± 0.02	2.53 ± 0.02
PER	0.79 ± 0.05	0.73 ± 0.07	0.75 ± 0.08	0.87 ± 0.07	0.83 ± 0.06
HSI (%)	1.47 ± 0.03	1.48 ± 0.03	1.47 ± 0.01	1.48 ± 0.02	1.49 ± 0.03

[a] Data is expressed as mean ± SD for three tanks per group, 8 fish each. IW = Initial weight; FW = Final weight; WG = Weight gain; SGR = Specific growth rate; S= Survival; FI = Feed intake; FCR = Factor conversion ratio; PER= Protein efficiency ratio; HSI = Hepatosomatic index.

Lipid peroxidation

Lipid peroxidation is presented in Figure 6. MDA levels were significantly lower ($P < 0.05$) in MPE-5 and MPE-10 groups, while the control group presented significantly higher MDA levels than PCs in all supplemented groups.

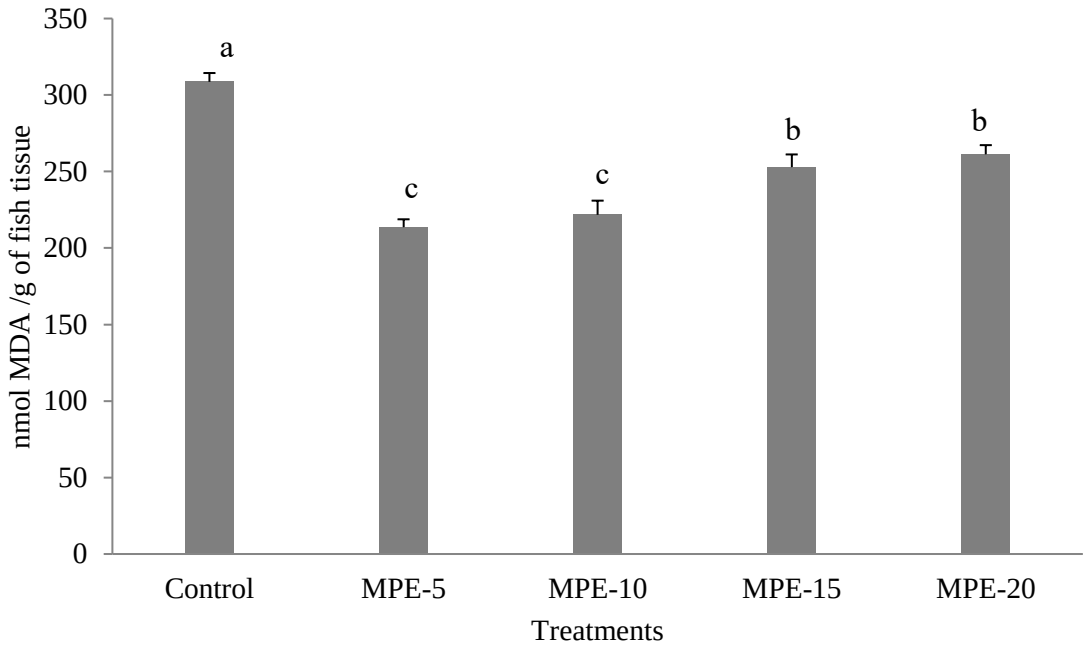

Figura 2. Formation of malondialdehyde (MDA) as a measurement of lipid peroxidation in zebrafish muscle of control, MPE-5, MPE-10, MPE-15 and MPE-20 groups, fed for 8 weeks. Data is expressed as mean ± SD (n=3). Different letters indicate significant differences ($P < 0.05$) among experimental diets.

Antioxidant enzymes activities

Figure 7 shows that the incorporation of MPE into zebrafish diets did not have a significant effect on GPX and SOD ($P > 0.05$) activities. However, CAT activity significantly increased in MPE-10, MPE-15 and MPE-20 groups, as compared to the control and MPE-5 groups. No significant difference ($P < 0.05$) was found between control and MPE-5 groups.

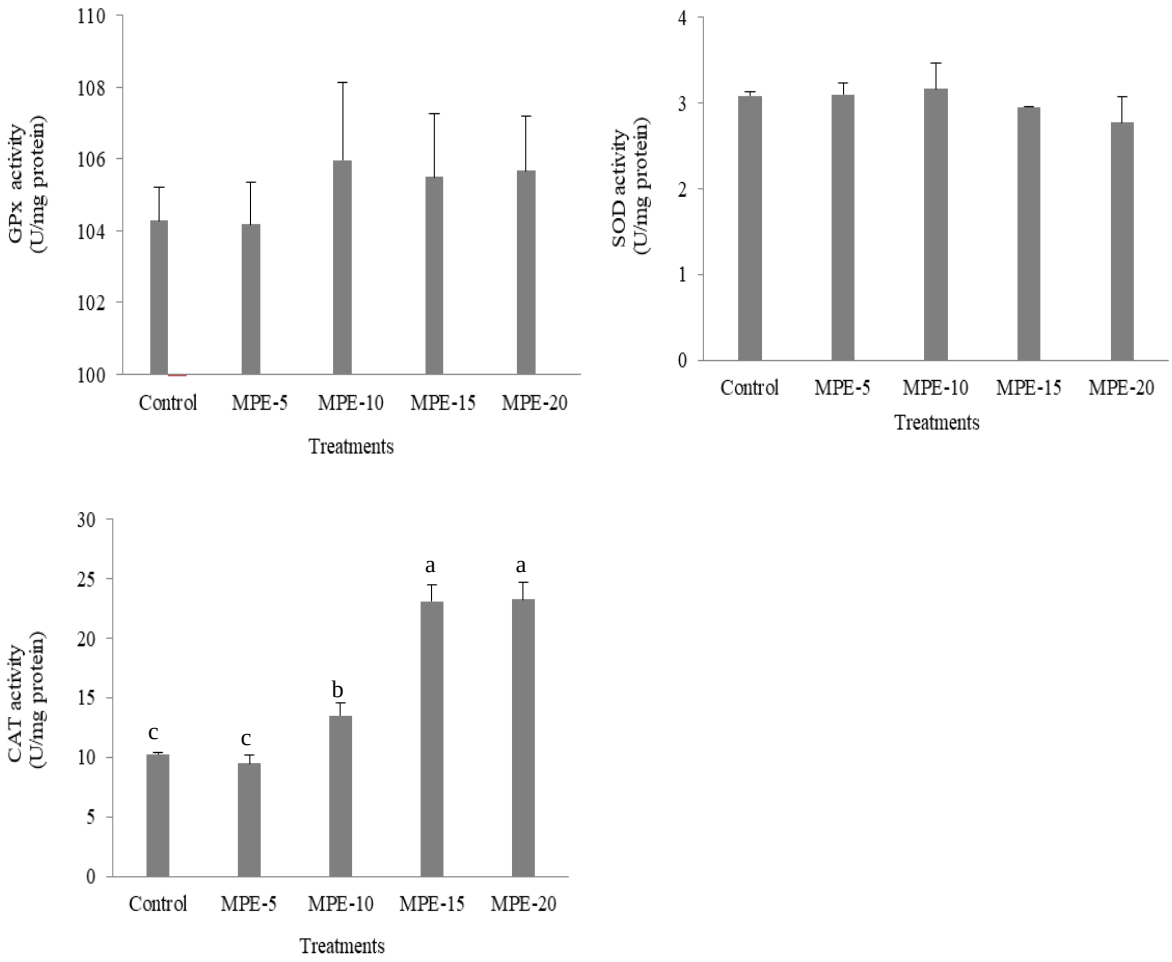

Figura 3. Antioxidant activities of GPX (a), SOD (b) and CAT (c) in zebrafish liver of control group, MPE-5, MPE-10, MPE-15 and MPE-20 groups, fed for 8 weeks. Data is expressed as mean ± SD (n = 3). Different letters indicate significant differences ($P < 0.05$) among experimental diets.

Discussion

Mango peel extract (MPE) phenolic compounds (PCs) profile

Mango peel extract is a rich source of PCs such as phenolic acids and flavonoids, which exhibit high antioxidant activity both *in vivo* and *in vitro*. The PCs identified in this study have also been reported by Velderrain-Rodríguez et al., 2015 in which gallic acid, protocatechuic acid, 2-hydroxycinnamic acid were observed as major PCs in mango peel

cv. Ataulfo extract. Additionally, Pacheco-Ordaz et al. (2018) indicated that mangiferin was the predominant PC found in mango peel extract cv. Ataulfo. In addition, other studies have reported the presence of methyl gallate, ethyl gallate, quercetin and isoquercitrin in mango peel (Dorta et al., 2014) which is in accordance with our results.

Growth performance and feed efficiency

In aquaculture, the inclusion of some feed additives such as PCs from plant extracts could negatively affect growth performance and feed efficiency. In the present study, the predominating compounds of the MPE PCs, which were incorporated into zebrafish feed, were phenolic acids (gallic, 2-hydroxycinnamic and-protocatechuic acids; gallic acid derivatives such as methyl and ethyl gallate), flavonoids (quercetin and isoquercitrin) and a polyphenol xanthone known as mangiferin, which did not show effects on growth performance and feed efficiency. Our results are in agreement with other studies conducted on gilthead sea bream (*Sparus aurata*) and rainbow trout (*Oncoryhnchus mykiss*), where PCs extracted from olive oil (hydroxytyrosol and hydroxycinnamic acids) and purple maize (anthocyanins) were supplemented in the diets of these species (Sicuro et al., 2010; Villasante et al., 2015). This may be attributed to the fact that the specific extracts used do not contain PCs of high molecular weight as condensed tannins (>5000 Da) which are considered as antinutrients for aquatic organisms since they can form stable complexes with proteins and could lead to reduced protein digestibility if they bind with digestive proteases, which is directly related to a reduction in growth performance and feed efficiency in farmed fish (Omnes et al., 2017). Therefore, the MPE-derived PCs used here are apparently safe to administer to zebrafish in concentrations of up to 200 mg/kg of feed.

Lipid peroxidation

Oxidative stress can promote the peroxidation of PUFAs in fish flesh. Lipid peroxidation is an important process that can lead to decreased quality of flesh, including a decreased shelf life, losses of nutritionally relevant compounds (e.g. PUFAs) and formation of off-flavors (Secci and Parisi, 2016). Our results suggest that MPE PCs were absorbed, distributed and metabolized by zebrafish, while acting as radical scavengers and/or metal-chelating agents. This was indicated by a decrease in MDA production in zebrafish tissue. The process of lipid peroxidation depends on the amount of ROS produced, concentrations of Fe^{2+} and the level of endogenous and exogenous antioxidants (Ayala et al., 2014; Charão et al., 2014). The major PCs found in MPE, such as mangiferin, quercetin, gallic acid and its derivatives, are characterized by high antioxidant capacity which can mitigate oxidative stress through various mechanisms. For example, PCs can directly scavenge ROS through electron or hydrogen atom donation, or they can prevent their formation by chelating Fe^{2+} (and other transition metals) which promote oxidative stress by catalyzing hydroxyl radical formation via the Fenton reaction. There is evidence confirming that mangiferin, quercetin, gallic acids and its derivatives have iron-chelating ability and can scavenge ROS which constitute their main antioxidant mechanisms preventing lipid peroxidation *in vitro* (Boadi et al., 2003; Pardo-Andreu et al., 2008; Badhani et al., 2015). Previous experiments in our laboratory (data not yet published) have shown that MPE PCs delay MDA formation in fish oil, in a comparable manner to that of synthetic antioxidant BHT. Other *in vitro* studies corroborate that the addition of grape PCs (catechin and procyanidins) and quince extracts (containing procyanidin B dimer and hydrocycinnamic acids) prevented lipid peroxidation in the muscle of different fish species during storage (Pazos et al., 2005; Fattouch et al., 2008). There are also studies that support the effectiveness of PCs from plant extracts such as feed additives which prevent lipid peroxidation in animal tissue (Surai, 2014). However, studies that report the use of PCs from plant extracts as a feed additive with antioxidant properties on the prevention of lipid peroxidation in muscle of farmed fish are limited. For example, Villasante et al. (2015) reported that dietary inclusion of an extract rich in anthocyanins increased ω3 and ω6 PUFAs levels in the body and muscle of rainbow trout

(*Oncorhynchus mykiss*), respectively. Another study indicated that dietary administration of an olive oil extract rich in tyrosol and hydroxytyrosol decreased MDA levels in muscle of gilthead sea bream (*Sparus aurata*) (Sicuro et al., 2010). The majority of the available reports on PCs in aquaculture have mainly focused on their use as immunostimulants (Nootash et al., 2013; Magrone et al., 2016). Our data shows that MPE PCs significantly mitigate lipid peroxidation in zebrafish, which merits further investigation in fish that are used for human consumption in order to conclusively validate their use as feed supplements.

Antioxidant enzymes activities

During oxidative stress tissues respond by inducing enzymatic and non-enzymatic antioxidant defense mechanisms. However, prolonged or enhanced oxidative stress may depress the endogenous antioxidant system by decreasing enzyme activities of SOD, CAT and GPX (Samarghandian et al., 2016). These enzymes work jointly; SOD catalyses the dismutation of $O_2^{\bullet-}$ to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), which is then reduced to water and O_2 by CAT and GPX. An inefficient endogenous antioxidant system can increase the production of $O_2^{\bullet-}$ and H_2O_2 and can consequently produce highly reactive free radicals like OH•, which is considered the main initiator of lipid peroxidation (Ayala et al., 2014). PCs may offer cellular protection as indirect antioxidants by modulating the expression or activity of the predominating endogenous antioxidant enzymes (SOD, CAT and GPX). Sellamuthu et al. (2013) have reported that mangiferin can protect the kidney and liver of rats with induced oxidative stress, through an increase in SOD, CAT and GPX activities by activation of Nrf2 (nuclear factor-E2-related factor 2). Nrf2 is a transcription factor involved in cellular antioxidant response, by modulating gene expression of various enzymes and proteins. In the present study, MPE PCs did not have any effect on SOD and GPX activities in liver of zebrafish which suggest that because oxidative stress was not induced, there was a lower production of substrates ($O_2^{\bullet-}$ and H_2O_2) available to the antioxidant enzymes.

In contrast, CAT activity dose-dependently increased with MPE PCs. The increase in CAT activity suggests higher H_2O_2 concentrations in peroxisomes (dos Santos Carvalho et al., 2012). H_2O_2 is the main cellular precursor of the hydroxyl radical which is considered as the most biologically active free radical, which is why the removal of H_2O_2 is a good strategy against oxidative stress (Karadag et al., 2014). Peroxisomal H_2O_2 is an important byproduct of the β-oxidation of PUFAs (Dansen and Wirtz, 2001); therefore, the biological importance of CAT is evident in aquatic organisms because PUFAs are their main dietary lipids. According to our results the intake of dietary MPE PCs at varying concentrations (100, 150 and 200 mg PCs/kg feed) enhances CAT activity, possibly due to high production of H_2O_2 in zebrafish liver.

Conclusions

Supplementation of MPE PCs did not impair growth performance and feed efficiency of zebrafish. Supplementation of 50 and 100 mg PCs/kg feed decreased lipid peroxidation end-products (MDA) in zebrafish muscle and could, therefore, be used to avoid the decrease of ω3 PUFAS in fish flesh destined for human consumption, potentially providing health benefits to consumers. Regarding antioxidant enzymes activity, MPE PCs significantly increased hepatic CAT activity, without having a significant effect on SOD and GPX activites. On the basis of these findings, MPE PCs could be used as feed additives in order to preserve PUFAs concentrated in muscle tissue of farmed fish. Further studies are necessary to support these findings.

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4. EFFECT OF HYDROPHILIC AND LIPOPHILIC ANTIOXIDANTS FROM MANGO PEEL (*MANGIFERA INDICA* L. CV. ATAULFO) ON LIPID PEROXIDATION IN FISH OIL

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Abstract

Antioxidant capacities of hydrophilic (phenolic compounds; H-MPE) and lipophilic (carotenoids; L-MPE) antioxidants from mango peel by ABTS and DPPH assays were determined. The ability of H-MPE and L-MPE to inhibit fish oil peroxidation by the formation of malondialdehyde (MDA) was measured and compared against synthetic antioxidant butylated hydroxytoluene (BHT) to study their antioxidant potential in the stabilization of omega-3 polyunsaturated fatty acids contained in the fish oil. H-MPE and L-MPE showed significantly higher antioxidant activity than BHT ($P<0.05$) by ABTS and DPPH assays. In addition, H-MPE significantly inhibited fish oil peroxidation compared with the control without antioxidant ($P<0.05$), while L-MPE showed a prooxidant effect. On the other hand, H-MPE at 200 mg/L showed a similar effect than that of BHT on the prevention of fish oil peroxidation. Therefore, H-MPE could be used as a new alternative to BHT to prevent fish oil peroxidation.

Keywords: Fish oil; lipid peroxidation; mango peel; antioxidant capacity; phenolics; carotenoids

Resumen

Las capacidades antioxidantes de compuestos hidrofílicos (compuestos fenólicos; H-MPE) y lipofílicos (carotenoides; L-MPE) de la cáscara de mango fue determinada mediante los ensayos ABTS y DPPH. La capacidad de H-MPE y L-MPE para inhibir la peroxidación en el aceite de pescado fue analizada por la formación de malonaldehído (MDA) y comparada contra el antioxidante sintético butil hidroxitolueno (BHT), para estudiar su potencial antioxidante en la estabilización de ácidos grasos poliinsaturados de la serie omega 3 presentes en el aceite de pescado. H-MPE y L-MPE mostraron mayor capacidad antioxidante que el BHT ($P<0.05$) mediante los ensayos ABTS y DPPH. Además, H-MPE inhibió significativamente la peroxidación del aceite de pescado en comparación con el control sin antioxidante ($P<0.05$), mientras que L-MPE mostró un efecto prooxidante. Contrariamente, la concentración de 200 mg/L de H-MPE mostró un efecto similar al BHT en la prevención de la peroxidación del aceite de pescado. Por lo tanto, H-MPE podría ser usado como una nueva alternativa del BHT para prevenir la peroxidación en el aceite de pescado.

Palabras clave: Aceite de pescado; peroxidación lipídica; cáscara de mango; capacidad antioxidante; compuestos fenólicos; carotenoides

Introduction

Fish oil is the main source of long-chain omega-3 polyunsaturated fatty acids (ω3 PUFAs) such as eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6) (Srigley and Rader, 2014), which are related with the prevention of the development of cardiovascular and inflammatory diseases (Delgado-Lista, Perez-Martinez, Lopez-Miranda, & Perez-Jimenez, 2012; Wall, Ross, Fitzgerald, & Stanton, 2010). Therefore, fish oil is commonly used as dietary supplement. Paradoxically, the chemical characteristics of the fatty acids composition of fish oil could pose a problem. Lipid quality may be reduced by a degenerative oxidation process which lipid undergoes. Particularly, ω3 PUFAs are highly susceptible to lipid peroxidation by the action of atmospheric oxygen, light and temperature, due to their high degree of unsaturation (Sekhon-Loodu, Warnakulasuriya, Rupasinghe, & Shahidi, 2013). Lipid peroxidation conduces to formation of hydroperoxides, aldehydes, ketones, alcohols, alkanes and alkenes, which are related to losses of nutritional values (e.g. decrease of ω3 PUFAs content), production of unpalatable flavor and odour, shortening of shelf life of fish oil. Likewise, lipid peroxidation can to cause formation of free radicals which may exert adverse effects on human health (Tao, 2015). In addition, due to the above, the synthetic antioxidant butylated hydroxytoluene (BHT) has been used for a long time to delay fish oil peroxidation rate by the oil industry. Even though, the use of this synthetic antioxidant as feed additive has received increasing attention from a food safety perspective. Therefore, the use of natural antioxidants as an alternative source is recommended. The food industry has focused its attention on the use of plant extracts as natural antioxidants in preventing fish oil peroxidation. These include extracts from potato peel (Habeebullah, Nielsen, & Jacobsen, 2010), apple peel (Sekhon-Loodu, et al., 2013), and pomegranate peel (Topuz et al., 2015), all of which have antioxidant activities comparable to that of BHT.

Mango has been reported to contain antioxidants such as, carotenoids, phenolic compounds (PCs), vitamin A and vitamin C with high antioxidant capacity (Manthey and Perkins-Veazie, 2009; Palafox-Carlos, Yahia, & González-Aguilar, 2012; Rymbai,

Srivastav, Sharma, Patel, & Singh, 2013). Mango (*Mangifera indica* L. cv. Ataulfo) is used by the juice industry, generating large amount of by-product such as peels and seed. Peels contain higher amounts of bioactive compounds such as PCs (hydrophilic antioxidants) and carotenoids (lipophilic antioxidants) (Sáyago-Ayerdi et al., 2013). Recently, we found that mango peels contain bound PCs that, after *in vitro* digestion they are released and its permeability in Caco-2 cells model have permeability similar to pure compounds and exert an important antioxidant capacity (Pacheco-Ordaz, Antunes-Ricardo, Gutiérrez-Uribe, & González-Aguilar, 2018). Nowadays, there are multiple *in vitro* and *in vivo* studies reporting the anti-inflammatory, anti-cancer and anti-diabetic potential of mango peel antioxidants (Kim, Banerjee, Ivanov, Talcott, & Mertens-Talcott, 2014; Shah, Patel, Patel, & Parmar, 2010). In conjunction, these finding can be applied in the development of nutraceuticals using this important by-product from the mango processing industry. Even different studies have been reported the PCs and carotenoids present in mango peel (Pacheco-Ordaz, et al., 2018; Palafox-Carlos, et al., 2012). However, the antioxidant effect and possible mechanisms of hydrophilic and lipophilic fraction against lipid peroxidation has not been elucidated completely and their possible effectiveness to reduce peroxidation in foods could be of great interest in the food industry. Therefore, the aim of this study was to analyze antioxidant mechanisms of hydrophilic and lipophilic antioxidants from mango peels and evaluate their antioxidant potential as food additive to prevent fish oil lipid peroxidation as compared to the synthetic antioxidant BHT.

Materials and methods

Materials

Fish oil was donated for Mazindustrial SA de CV (Mazatlán, Sinaloa, México), while mango peels (*Mangifera indica* L. cv. Ataulfo) were collected from Pure Mango SA de CV (Escuinapa, Sinaloa, México). Fresh mango peels were dried at 50°C during

15 h in oven and ground to a particle size of 0.25 mm using a 50703-model hammer mill (California Pellet Mill laboratory Mill Champion, Waterloo, IA, USA). Mango peel powder was stored at -20°C until analysis.

Extraction of lipophilic antioxidants from mango peel

Carotenoids were obtained following the methodology described by the AOAC (method 43.015). Mango peel powder (3 g) was homogenized in 100 mL of hexane: acetone (3:2, v/v) and 0.1 g MgCO3. The extract was filtered (Whatman No.1) under vacuum and the residue was mixed with acetone (25 mL, twice), and finally, with 25 mL of hexane until the residue was colorless. The extract was then transferred to a separating funnel and mixed with distilled water (100 mL, five times) to remove acetone. The upper layer containing carotenoids was placed in 100 mL volumetric flask containing 9 mL acetone and diluted to volume with hexane. Total carotenoids (TC) content were determined by spectrophotometry at 446 nm using β-carotene as standard, and the results were expressed as µg β-carotene equivalents (µg βE)/g of DW. Dilutions of these solutions with concentrations of 100 and 200 mg/L βE of the lipophilic antioxidants from mango peel (L-MPE) were prepared.

Extraction of hydrophilic antioxidants from mango peel

PCs were extracted according to Sekhon-Loodu, et al. (2013) with minor modifications. Mango peel powder (3 g) was homogenized in 100 mL of 70% ethanol, sonicated three times for 15 min in a 3510-model ultrasonic bath (Branson, Wethersfield, CT) and centrifuged (15000 rpm, 15 min, 4°C) in an Allegra X-30R model A99470 centrifuge (Beckman Coulter, Germany). The procedure was repeated two times and was done in triplicate. Finally, extracts were concentrated to 10 mL using a rotavapor R-114

(Büchi Labortechnik AG, Flawil, Switzerland). Total phenolic content (TPC) was measured using the Folin-Ciocalteu method described by Montreau (1972). Aliquots of 0.5 mL of the extract were mixed with 0.5 mL of Folin-Ciocalteu reagent for 3 min. Subsequently, the solution was mixed with 10 mL of Na_2CO_3 (7.5%, w/v) and 14 mL of water. The solution was placed in the dark at room temperature for 1 h. Absorbance was read at 734 in a UV-Vis spectrophotometer (Hewlett Packard, Texas, United States). A standard curve generated from gallic acid was used to calculate the TPC. Results were expressed as mg of gallic acid equivalents (mg GAE)/g of dry weight (DW). Dilutions of these solutions with concentrations of 100 and 200 mg/L GAE from mango peel (H-MPE) were prepared.

Analysis of PCs by UPLC ESI-Q-TOF/MS/MS

Identification of PCs was determined through Ultra-Performance Liquid Chromatography (UPLC) using ACQUITY UPLC; H-Class system (Waters, Milford, Mass., U.S.A.) coupled to a G2 XS Quadrupole-Time-of-Flight (Q-Tof) mass spectrometer (Agilent, Santa Clara, CA) equipped with electrospray ionization (ESI). Briefly, PCs were separed by UPLC to 40°C with a column ACQUITY BEH C18 (1.7 µm, 3.0 x 100 mm) using a mobile phase composed of 0.1% formic acid (A) and acetonitrile (B) at a flow rate of 0.3 mL/min. The gradient procedure was as follows: 0 min, 95% (A); 2.5 min, 75% (A); 3 min, 50% (A); 3.5 min, 35% (A); 5 min, 5% (A); 6 min, 95% (A); 7 min, 95% (A). An electrospray source in negative mode was used to collect mass spectra under the following conditions: nitrogen gas; desolvation temperature, 350°C; desolvation gas, 13.3 L/min; capillary voltage, 1500 V; and fragmentor voltage, 10 V.

Antioxidant capacity assay

ABTS radical cation scavenging capacity

ABTS radical cation ($ABTS^{\bullet+}$) scavenging capacity was measured according to Re et al. (1999). The generation of the radical cation $ABTS^{\bullet+}$ was prepared with 5 mL of 7 mM ABTS [2,2-azinobis(3-ethylbenzo-thiazoline-6-sulfonate)] and 88 µL of 2.45 mM of potassium persulfate, allowing the mixture to stand in the dark at 20 °C for 16 h. The reaction was adjusted to an absorbance of 0.7± 0.02 at 754 nm. Each sample (33 µL) was mixed with 967 µL of $ABTS^{\bullet+}$ and kept in the dark at room temperature for 6 min. Trolox was used as a standard to calculate the antioxidant capacity of different concentrations of BHT, H-MPE and L-MPE. Absorbance was read at 754 nm and results were expressed as mg Trolox equivalents (mg ET)/ L of extract.

DPPH radical scavenging capacity

DPPH radical (DPPH•) scavenging capacity was determined according to Brand-Williams, Cuvelier, & Berset (1995) modified by Gayosso-García, Yahia, & González-Aguilar (2013). The working solution was prepared freshly by mixing 2.5 mg of DPPH (2, 2-diphenyl-1-picrylhydrazyl) with 100 mL of absolute methanol. The solution was adjusted to an absorbance of 0.7± 0.02 at 516 nm. DPPH• solution (933 µL) was placed in a test tube and 67 µL of sample were added. The mixture was kept in the dark for 30 min. Absorbance was read at 516 nm and results were expressed as mg ET/L of extract. Trolox was used as a standard to calculate the antioxidant capacity of different concentrations of BHT, H-MPE and L-MPE.

Determination of lipid oxidation in fish oil

L-MPE solvents were removed completely using nitrogen flow. 100 and 200 mg/L L-MPE dried compounds were dissolved with 1 mL of fish oil and vortexed by 1 min. H-MPE were rotaevaporated at 37°C to removed the ethanol and then; they were freeze-dried. 100 and 200 mg/L H-MPE dried compounds were dissolved in 200 µL of ethanol and mixed with 1 mL of fish oil and vortexed by 1 min. The ethanol was removed completely under a nitrogen flow. Concentrations used were selected according to legal restrictions imposed to BHT (100 and 200 mg/L) by the CODEX Alimentarius. BHT and control (without antioxidant) were used to compare the efficacy of antioxidants from mango peel. 100 and 200 mg/L BHT were dissolved with 1 mL of fish oil and vortexed by 1 min. The mixtures were incubated in an oven at 30 °C and peroxidation of fish oil was measured every 2 days for 14 days. Each treatment was performed in triplicate.

The lipid peroxidation of fish oil was evaluated by the formation of malondialdehyde (MDA) using assay described by Solé, Potrykus, Fernández-Díaz, & Blasco (2004) with some modifications. Briefly, 200 µL of fish oil were mixed with 1300 µL of methyl-2-phenylindole (10.3 mM) in methanol:acetonitrile (1:3; v/v), 200 µL of water and 300 µL of 37% HCl. This mixture was incubated at 45 °C for 40 min, cooled and centrifuged at 13000 rpm for 15 min at 4 °C. Absorbance was read at 586 nm, and the amount of peroxidized lipids [mg malondialdehyde (MDA)/kg] was calculated using 1,1,3,3-tetraethoxypropane for the preparation of standard curve of MDA.

Statistical analyses

Date were evaluated for normality (Kolmogorov–Smirnov test) and homogeneity of variance (Levene´s test) prior to the statistical analysis. Results of antioxidant capacity were analyzed using two-way analysis of variance (ANOVA). MDA data of fish oil was

subjected to a two-way ANOVA with a block design. The post hoc Tukey's test was conducted when significant differences were found ($P < 0.05$). All statistical analyzes were conducted using the statistical software SAS version 9.0 (SAS Institute Inc. Cary, N.C. USA).

Results and discussion

Total PC and carotenoid contents from mango peel

The antioxidant properties of mango peel have been attributed to its bioactive compounds, being the carotenoids and PCs the most important (Pacheco-Ordaz, et al., 2018; Palafox-Carlos, et al., 2012). Mango peel had a TPC of 45.53±0.57 mg GAE/g DW. This result was lower to that reported by García-Magaña, García, Bello-Pérez, Sáyago-Ayerdi, & de Oca (2013). Probably such differences could be attributed to environmental conditions, pre and postharvest conditions, as well as use of different solvents and extraction methods (Kalt, 2005; Tomsone, Kruma, & Galoburda, 2012). Regarding CT, mango peel presented 132.14 µg βE/g DW. This result was low compared to those reported by Ajila, Bhat, & Rao (2007) (1400 - 3945 µg EC/g DW) and Ajila, Naidu, Bhat, & Rao (2007) (194 - 436 µg βE/g DW). These differences could be attributed to the drying method conditions used in this study (50 °C, 24 h) that influenced the carotenoids content. Previous studies indicated thermal drying methods can cause losses of carotenoids and PCs in fruits, due to cell wall destruction, exposure of bioactive compounds to heat, light and oxygen during drying (Chuyen, Roach, Golding, Parks, & Nguyen, 2017; İzli, Yıldız, Ünal, Işık, & Uylaşer, 2014). However, drying methods using ovens appears to be more convenient for the industry it reduce the production cost of mango peel powder as an antioxidant source to prevent lipid peroxidation in fish oil.

Identification of PCs of mango peel extract

Eight PCs were identified five of which were phenolic acids, two flavonoids and one polyphenol xanthone. Gallic acid (m/z 169.01), 2-hydroxicinnamic acid (m/z 163.038), mangiferin (m/z 421.089) and quercetin (m/z 301.035) were identified by comparing the [M-H]- ion and fragment ions with standards. Methyl gallate (m/z, 183.027), ethyl gallate (m/z, 197.041) and isoquercitrin (m/z, 463.085) were compared with literature. While protocatechuic acid (m/z, 153.014) was confirmed with datebase (MassBank of North America). Our results agree with those reported by Velderrain-Rodríguez et al., 2015 which previously identified gallic acid, protocatechuic acid and 2-hydroxycinnamic acid as major PCs in mango peel cv. Ataulfo. Additionally, Pacheco-Ordaz et al. (2018) and Dorta, González, Lobo, Sánchez-Moreno, & de Ancos, (2014) have reported the presence of methyl gallate, ethyl gallate, quercetin, isoquercitrin and mangiferin, which is according with results of this study.

ABTS and DPPH radical scavenging capacity of L-MPE and H-MPE

Antioxidant capacity of L-MPE and H-MPE from mango peel is highly relevant because it provides information about of their radical scavenging ability in a specific media and time of reaction. Using ABTS•+, the scavenging ability of synthetic antioxidant BHT, L-MPE and H-MPE were measured at different concentrations. Higher concentrations exhibited higher antioxidant capacity for all antioxidants (Figure 2). L-MPE exhibited the highest ABTS•+ scavenging ability (1216.26 ± 15.75 mg TE/L) ($P<0.05$) which was about 13-fold higher than BHT (95.86 ± 0.59 mg TE/L). H-MPE also presented significantly higher ABTS•+ scavenging ability (973.36 ± 17.75 mg TE/L) than BHT. Similarly, when DPPH• scavenging ability was evaluated, antioxidant capacity also increased with antioxidant concentration ($P < 0.05$) (Figure 3), and L-MPE and H-MPE

exhibited significantly higher DPPH• scavenging abilities 1131.51 ± 11.78 and 797.72 ± 7.63 mg TE/L, respectively) than BHT (74.47 ± 6.48 mg TE/L).

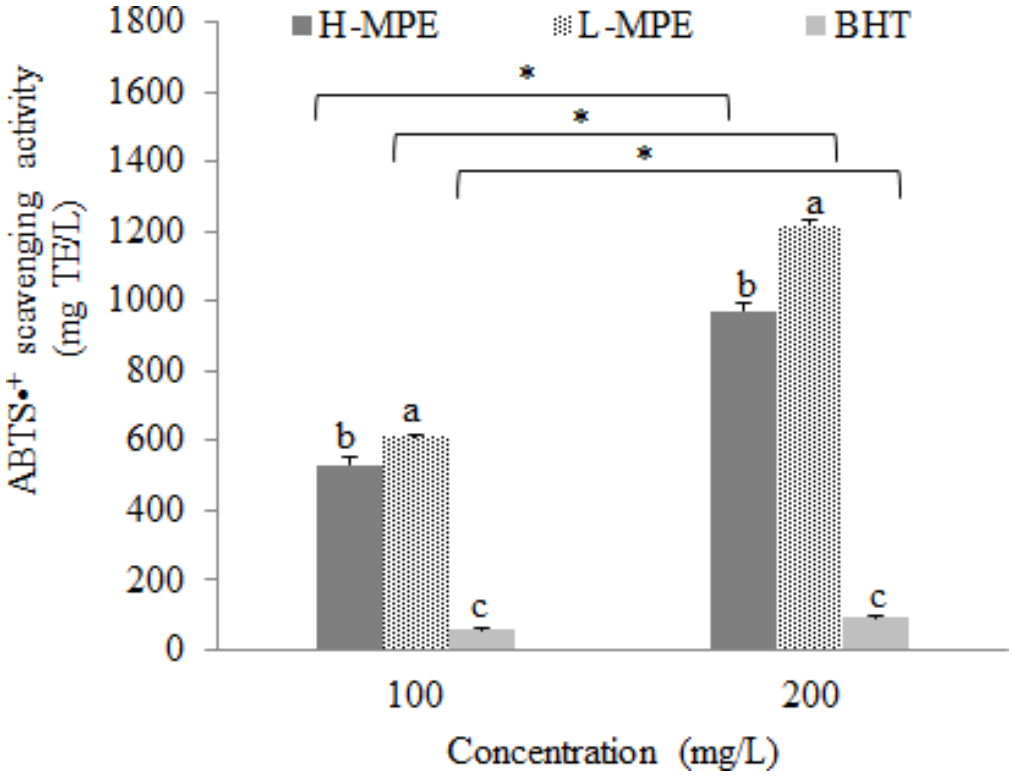

Figura 4. ABTS•+ scavenging capacity of phenolic compounds (H-MPE), carotenoids (L-MPE) and BHT at 100 and 200 mg/L. Error bars represent the standard deviation (n = 3). Letters indicate significant differences ($p < 0.05$) among types of antioxidants used at the same concentration. Asterisks indicate significant differences ($p < 0.05$) between concentrations used for each type of antioxidant.

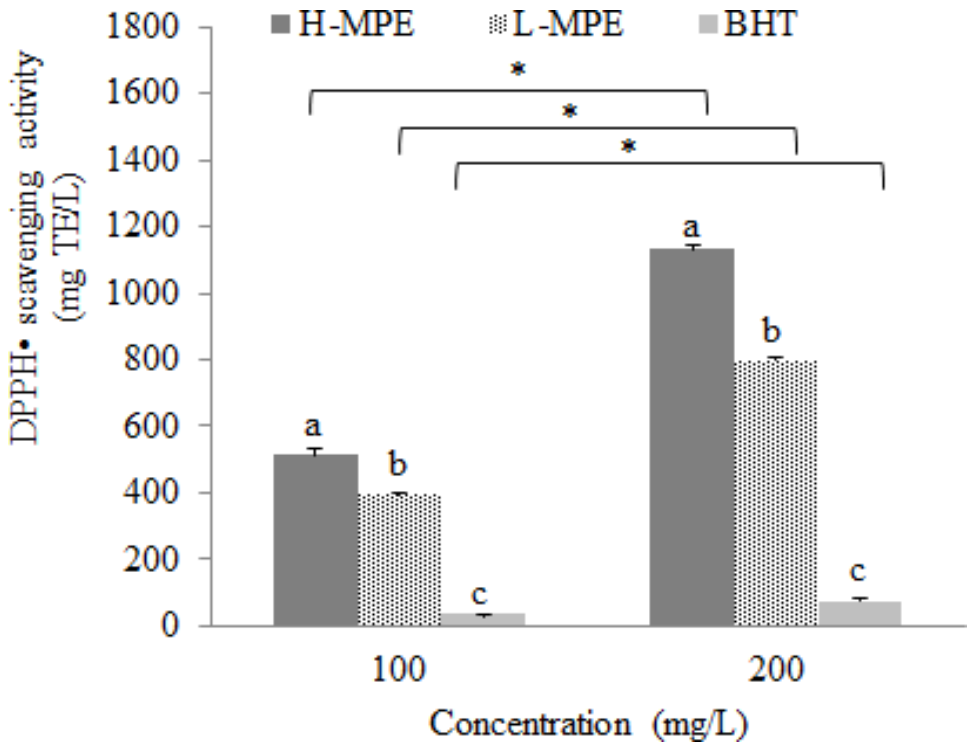

Figura 5. DPPH• scavenging capacity of phenolic compounds (H-MPE), carotenoids (L-MPE) and BHT at 100 and 200 mg/L. Error bars represent the standard deviation (n = 3). Letters indicate significant differences ($p < 0.05$) among types of antioxidants used at the same concentration. Asterisks indicate significant differences ($p < 0.05$) between concentrations used for each type of antioxidant.

The results of antioxidant capacity assays suggest that L-MPE and H-MPE react quickly with free radicals ABTS•+ or DPPH• through the donation of hydrogen atom compared to BHT. The high antioxidant capacity of L-MPE might be attributed to the presence of carotenoids which react quickly with free radicals to produce radical adducts with a resonance stabilised carbon-centered radical (Ornelas-Paz, Yahia, Gardea, & Failla, 2009; Terao, 1989) due to their conjugated double bonds and the presence of ring structures at the end of the polyenes (Stahl and Sies, 2003). All-trans-β-carotene, all-trans-violaxanthin dibutyrate and 9-cis-violaxanthin dibutyrate are the main carotenoids found in mango cv Ataulfo pulp (Ornelas-Paz et al., 2009), while that β-carotene, cis isomers of β-carotene, violaxanthin, lutein, neochrome and luteoxanthin are main carotenoids identified in mango peel (Chen, Tai, & Chen, 2004; Ajila, Rao, & Rao, 2010). Gayosso-García et al. (2013) reported that a mix of carotenoids (β-carotene, lycopene, and β-cryptoxanthin)

promotes a synergistic effect, increasing overall antioxidant capacity relative to the individual capacity of each carotenoid. Therefore, we suggest that enhanced scavenging ability of L-MPE likely be due to the presence of a mix carotenoids, to their high reactivity with free radicals and the possible synergy among carotenoids.

Additionally, H-MPE also presented higher $ABTS^{\bullet+}$ and DPPH• scavenging ability than BHT. This effect is attributed to the mix of PCs (phenolic acids: gallic acid, protocatechuic acid, syringic acid and methyl gallate; flavonoids: quercetin and isoquercitrin; mangiferin) identified in this study. Phenolic acids, flavonoids and mangiferin, are considered to be efficient hydrogen donors due to their characteristic carboxylic group, which is easily ionized to the number and position of the hydroxyl groups and the type of substituent on the aromatic rings (Balasundram, Sundram, & Samman, 2006). Other studies have reported that gallic acid reduces from three to six molecules of DPPH•, while BHT reduces around three molecules of DPPH•. Therefore, the scavenging ability of gallic acid is higher than that of BHT (Brand-Williams et al., 1995). Another possible reason of the higher antioxidant capacity observed of PCs from MPE compared with that of BHT might be due to its synergistic effect. Antioxidants can chemically reduce each other, acting in synergy to scavenging reactive oxygen species. For example, binary combination of PCs such as myricetin-kaempferol, quercetin-rutin, resveratrol-catechin, gallic-protocatechuic acids, increased their antioxidant capacity (hydrogen atoms transfer and electron transfer), compared with the theoretical sum of its individual antioxidant capacity (Ornelas-Paz, et al., 2009; Skroza, Mekinić, Svilović, Šimat, & Katalinić, 2015). About, we suggested that the mix of PCs present in MPE exerted a synergic effect which was reflected in its high antioxidant capacity compared to BHT.

Inhibition of fish oil peroxidation by H-MPE

The MDA is a secondary product of lipid peroxidation considered as the most abundant aldehyde resulting from hydroperoxides oxidation (primary lipid oxidation

product). The MDA formation is of great interest because this compound can adversely affect the PUFAs content (Yun and Surh, 2012). Mango peel extracts contain a mix of PCs with high antioxidant capacity, which could counter the effects previously mentioned. Because of that, H-MPE and BHT were added at 100 and 200 mg/L into bulk fish oil and its effect on lipids peroxidation was evaluated. According to the results, MDA content indicated that 100 and 200 mg/L H-MPE significantly inhibited fish oil peroxidation compared to that of control without antioxidant ($P<0.05$) (Figure 4). In general, it was observed MDA content increased after storage time up to 14 days ($P< 0.05$). Regarding BHT, no significant differences ($P<0.05$) between H-MPE and BHT at 200 mg/L was observed. Additionally, the ability of antioxidants to prevent peroxidation of fish oil varied with the type and concentration of antioxidant ($P<0.05$). Topuz et al. (2015) reported that PCs of pomegranate peel at a concentration of 1000 mg/L had a similar effect as BHT in controlling peroxidation of fish oil storaged to 60°C for 12 days. However, PCs from potato peel at concentrations lower than 1600 mg/L did not prevent lipid peroxidation in fish oil storaged 55°C with magnetic stirred for 3 days (Habeebullah, Nielsen, & Jacobsen, 2010). Sekhon-Loodu, et al. (2013) observed that the flavonol-rich fractions of apple peel were better able to inhibit fish oil peroxidation (experimental conditions: 50°C using shaker oven for 3 h) than PCs from crude apple peel extracts.

The effectiveness of PCs may be related to their localization in bulk fish oil as well. Yehye et al. (2015) reported that hydrophilic antioxidants (e.g. PCs) delay lipid peroxidation because these antioxidants concentrate at the oil-water interface in bulk oils, which is where most of the lipid peroxidation takes place due to the high concentration of oxygen and prooxidants. On the other hand, the ability of different PCs to prevent lipid oxidation also depends on their mechanism of action, which can include chelation of transition metals, free radicals and quenching of reactive oxygen species (Bravo, 1998). Therefore, we hypothesise that the efficacy of mango peel PCs could arise from a combined effect of their concentration, chemical structures, mechanisms of action and localization in the bulk oil. We recently reported the synergism of PCs present in mango “Ataulfo” to stabilize different radicals and its effectiveness was in function of its chemical structure and position and OH number (Palafox-Carlos, et al., 2012; Velderrain-Rodríguez et al., 2015).

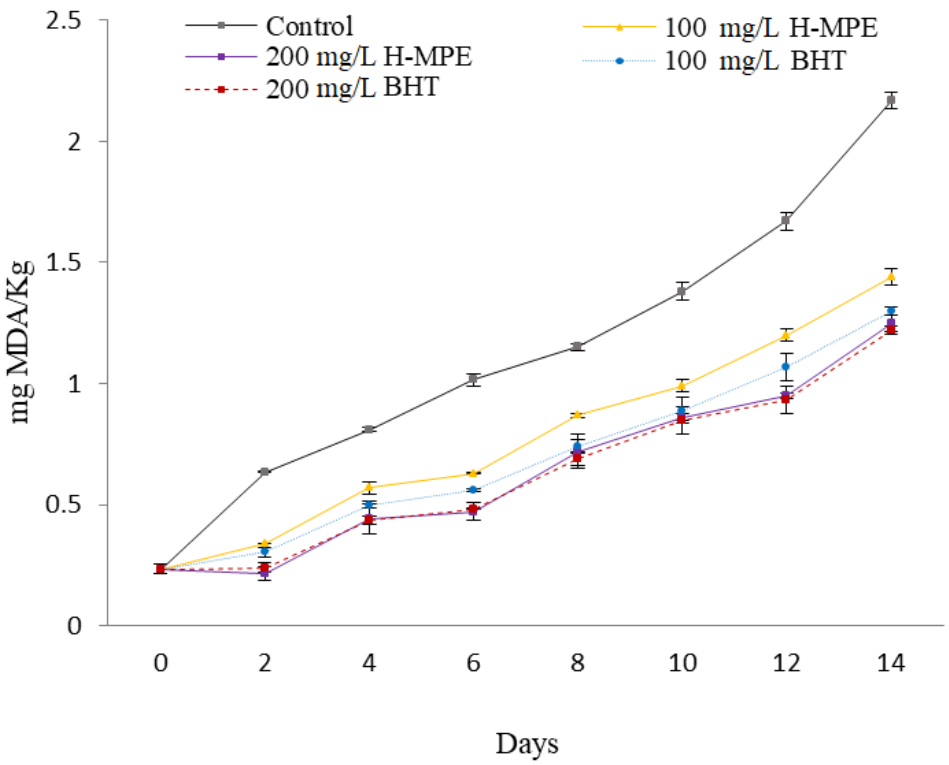

Figura 6. Effect of different concentrations of phenolic compounds from mango peel extract (H-MPE) and BHT on the malondialdehyde (MDA) content, a lipid peroxidation product in fish oil, when stored at 30 °C for 14 days. Error bars represent the standard deviation (n = 3).

Inhibition of fish oil peroxidation by L-MPE

L-MPE and BHT were incorporated at two concentrations (100 and 200 mg/L) into bulk fish oil to compare their effectiveness at preventing fish oil peroxidation. MDA content for all samples increased as the storage time increased over 14 days ($P < 0.05$) (Figure 5). The ability of antioxidants to prevent peroxidation in fish oil varied with the type of antioxidant and the concentration used ($P < 0.05$). At both concentrations tested, L-MPE exhibited higher MDA content ($P < 0.05$) than BHT or the control, indicating that L-MPE acted as a prooxidant rather than an antioxidant. Jacobsen (2010) reported that scavenging of peroxyl and alkoxyl radicals is the most important mechanism for lipid peroxidation prevention. In this sense, carotenoids inhibit autoxidation of lipid systems by its capacity to quench peroxyl and alkoxy radicals, through hydrogen donation and

delocalization of unpaired electrons in its conjugated polyene, at low oxygen concentration (Choe and Min, 2009). However, prooxidative behaviour of carotenoids has been reported as well. For example, purified carotenoids as β-carotene at 20 and 500 mg/L acts as a prooxidant in rapeseed oil and safflower seed oil, respectively (Haila and Heinonen, 1994; Henry, Catignani, & Schwartz, 1998). This effect results as a consequence of peroxyl and β-carotene reaction, where a β-carotene radical is formed and readily autoxidized which then produces lipid radicals that propagate the chain reaction of lipid peroxidation (Subagio and Morita, 2001). In this study, the prooxidant effect observed in fish oil could be due to the presence of β-carotene in MPE, being the major carotenoid identified in both pulp and mango peel (Chen et al., 2004; Ajila, Rao, & Rao, 2010), which could have led to the formation of highly reactive radicals.

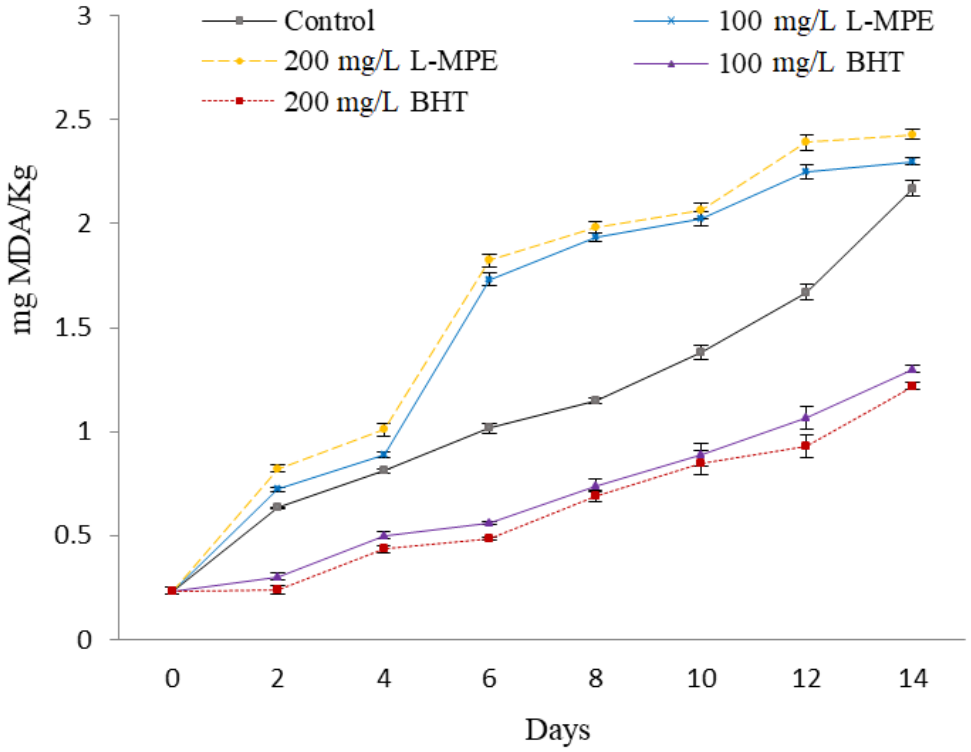

Figura 7. Effect of different concentrations of carotenoids from mango peel extract (L-MPE) and BHT on the malondialdehyde (MDA) content, a lipid peroxidation product in fish oil, when stored at 30 °C for 14 days. Error bars represent the standard deviation (n = 3).

Conclusions

H-MPE and L-MPE exhibited high ABTS•+ and DPPH• scavenging. 200 mg/L H-MPE had a strong inhibitory effect on fish oil peroxidation, which showed a similar effect than that of 200 mg/L BHT. However, carotenoids exhibited a prooxidant effect in the concentrations tested. Therefore, results of this study suggest that 200 mg/L H-MPE have the potential to be used as natural antioxidants and a good alternative to BHT in the prevention of fish oil peroxidation which is commonly used as dietary supplement.

Currently, there are lots of bioactive compounds with antioxidant properties, which are considered as safe and can be extracted from low cost sources such as agro industrial by-products. In this sense, further investigations are needed to study their application and subsequently the commercialization of these compounds in fish oil, even in foods with a high proportion of polyunsaturated fatty acids.

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Conflicts of Interest: The authors declare no conflict of interest.

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5. DISCUSIÓN GENERAL

En acuicultura, particularmente en el cultivo de peces existe información relacionada con el estudio de la propiedad inmunoestimulante de los polifenoles de origen vegetal, debido a que se ha demostrado que tienen la capacidad para sustituir a antibióticos comúnmente utilizados para reducir las mortalidades causadas por enfermedades infecciosas que generan pérdidas económicas. Entre los principales mecanismos inmunoestimulantes estudiados en peces destacan la expresión génica de citocinas (IL-1β, IL-8 e IL-6), la explosión respiratoria, la actividad de lisozima y la actividad hemolítica del complemento sérico, los cuales se han relacionado con un incremento en la supervivencia de peces expuestos a patógenos (Lizárraga-Velázquez et al., 2018). Sin duda, el estudio de la propiedad inmunoestimulante de los polifenoles, de alguna forma ha restado importancia al estudio de la propiedad antioxidante, que en términos generales es la propiedad que ha caracterizado a los polifenoles durante décadas, debido a que se relaciona con una menor incidencia de enfermedades vinculadas al estrés oxidativo (Mao, Gu, Chen, Yu, & He, 2017). El estudio estrés del oxidativo ha adquirido relevancia en el cultivo de peces porque éste afecta el estado de salud y la calidad post-mortem. Ello ha llevado a un incremento en el número de investigaciones dirigidas al estudio de la propiedad antioxidante de los polifenoles, basadas fundamentalmente en el análisis de la respuesta antioxidante enzimática (SOD, CAT y GPX) y en la medición de productos derivados de la peroxidación lipídica, proceso implicado en la pérdida de la post-mortem (Sicuro, Badino, et al., 2010; Sicuro, Barbera, et al., 2010; Villasante et al., 2015). No obstante, la información sigue siendo muy limitada, incluso en cuanto al tipo de vegetal explorado como fuente potencial de polifenoles, ya que el té verde sigue siendo el vegetal de mayor interés para este tipo de estudios (Abdel-Tawwab, Ahmad, Seden, & Sakr, 2010; Harikrishnan, Balasundaram, & Heo, 2011; Sheikhzadeh, Nofouzi, Delazar, & Oushani, 2011).

La falta de conocimiento básico acerca del efecto antioxidante de los polifenoles sobre indicadores de estrés oxidativo en peces, condujo a la realización de la investigación presentada en el capítulo 3, en la que se sugiere que el uso de polifenoles de cáscara de

mango como ingredientes alimentarios mejora la calidad post-mortem del tejido muscular del pez cebra, mediante la disminución de los niveles de lípidos peroxidados que indirectamente se relacionan con la preservación del contenido de ácidos grasos poliinsaturados esenciales, con la producción de productos tóxicos y con la conservación de características sensoriales que comúnmente presenta el filete de pescado (Secci & Parisi, 2016). Indudablemente, este resultado es de interés para la industria acuícola ya que el mejoramiento de la calidad post-mortem en peces de cultivo facilita su comercialización. No obstante, este estudio únicamente da la pauta para continuar con futuras investigaciones en peces de importancia comercial, en las cuales se recomienda que se incluya un estudio completo sobre el efecto de los polifenoles en la calidad post-mortem, por ejemplo, la determinación de lípidos peroxidados, el perfil de ácidos grasos poliinsaturados y la evaluación sensorial del filete

Por otra parte, en el Capítulo 3 también se reporta que los polifenoles modulan la actividad de la enzima CAT considerada como una de las enzimas antioxidantes más importantes en el combate del estrés oxidativo en peces (dos Santos Carvalho, Bernusso, de Araújo, Espíndola, & Fernandes, 2012). Contrariamente, estos compuestos no fueron capaces de modular la actividad de las enzimas SOD y GPx, posiblemente debido a que los organismos fueron alimentados y mantenidos en condiciones óptimas de cultivo, lo cual evitó la producción excesiva de ERO. Numerosos estudios sugieren que los polifenoles modulan las actividades de las enzimas antioxidantes SOD, CAT y GPx en organismos con estrés oxidativo inducido con etanol y peróxido de hidrógeno (Devipriya, Srinivasan, Sudheer, & Menon, 2007; Kasdallah-Grissa et al., 2007; Kim et al., 2011). Por lo tanto, es recomendable que se realicen estudios en los que se considere un factor estresante. Por ejemplo, inclusión de aceites oxidados en el alimento para peces, cambios de temperatura durante el cultivo o exposición a patógenos. Lo anterior, con el propósito de probar el efecto antioxidante de los polifenoles sobre las principales enzimas antioxidantes en diferentes especies de peces de interés acuícola.

La aplicación tecnológica de antioxidantes provenientes de subproductos vegetales en alimentos con alto contenido de ácidos grasos poliinsaturados ha ido en aumento en los

últimos años. Con ello se busca desplazar a los antioxidantes sintéticos del mercado (Maqsood, Benjakul, Abushelaibi, & Alam, 2014). Con la finalidad de contribuir a la generación de conocimiento básico y aplicado en el Capítulo 4 se probó el efecto de antioxidantes hidrofílicos (polifenoles) y lipofílicos (carotenoides) de la cáscara de mango sobre la prevención de la peroxidación del aceite de pescado utilizado como ingrediente en el alimento para peces. Los resultados confirman la efectividad de los antioxidantes hidrofílicos para retardar la peroxidación lipídica a través de distintos mecanismos de acción entre los que destacan su capacidad para neutralizar ERO y para quelar metales (Fe^{2+}) involucrados en la generación de ERO (Zhang & Tsao, 2016). Por el contrario, los antioxidantes lipofílicos aceleran el proceso de peroxidación lipídica; sin embargo, su uso como alternativa de los antioxidantes sintéticos no puede descartarse, a menos que se realicen estudios con concentraciones menores a las analizadas en este estudio y el efecto prooxidante prevalezca. Los resultados derivados de este estudio generan preguntas de investigación acerca del efecto de los polifenoles sobre el perfil de ácidos grasos poliinsaturados y la palatabilidad del aceite de pescado. Por lo que se requieren estudios adicionales para complementar la información presentada en el capítulo 4.

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6. CONCLUSIONES GENERALES

La cáscara de mango cv. Ataulfo es fuente de antioxidantes hidrofílicos y lipofílicos, tales como polifenoles y carotenoides, respectivamente.

Los principales polifenoles presentes en extractos hidrofílicos de cáscara de mango son ácido gálico, ácido 2- hidroxicinámico, ácido protocatéquico, metil galato, etil galato, quercetina, isoquercetina y manguiferina.

Los polifenoles presentes en los extractos hidrofílicos de cáscara de mango cv. Ataulfo exhiben actividad antioxidante y previenen la peroxidación lipídica en el aceite de pescado, a través de la transferencia de electrones y de átomos de hidrógeno a radicales libres producidos durante almacenamiento a 30°C por 14 días. En contraste, los carotenoides aceleran el proceso de peroxidación en el aceite de pescado.

La inclusión dietaria de polifenoles de la cáscara de mango cv. Ataulfo no tienen efecto sobre los índices biológicos y la eficiencia alimentaria en el pez cebra utilizado como modelo de estudio. Sin embargo, su inclusión reduce la peroxidación lipídica y modula la actividad de la catalasa en el músculo e hígado del pez cebra, respectivamente. Se sugieren más estudios para validar estos hallazgos en peces de importancia comercial en condiciones de cultivo.

Los resultados sugieren que los polifenoles de la cáscara de mango cv. Ataulfo tienen potencial para ser utilizados como antioxidantes en la prevención de la peroxidación de aceites con alta proporción de ácidos grasos poliinsaturados.