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“Biomarcadores de estrés oxidativo e inflamación en suero de individuos obesos en respuesta al consumo de pan adicionado con salvado de trigo bioprocesado”

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APROBACIÓN

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RESUMEN

La inflamación y el estrés oxidativo se han asociado a diferentes patologías relacionadas con la obesidad, como resistencia a la insulina, diabetes tipo 2, síndrome metabólico y enfermedad cardiovascular. Existe un creciente interés en el uso de antioxidantes naturales para aplicaciones terapéuticas. Los compuestos fenólicos son considerados como potenciales agentes terapéuticos contra un amplio rango de enfermedades. El ácido ferúlico (AF) ha sido identificado como el principal contribuyente de la capacidad antioxidante en los productos de la molienda del trigo. El AF representa hasta un 90% de los ácidos fenólicos totales y del cual el 99% se encuentra en forma ligada a los arabinoxilanos, dado que a nivel de intestino delgado no se cuenta con enzimas esterasas para liberar el AF, surge la necesidad de desarrollar tecnologías que permitan su liberación de tal manera que se mejore su bioaccesibilidad y por tanto su biodisponibilidad.

Se evaluó el efecto del bioprocesamiento del salvado de trigo en el contenido de fenoles totales, capacidad antioxidante y contenido de ácido ferúlico libre. En panes adicionados con salvado bioprocesado se incrementó significativamente el contenido de fenoles totales, capacidad antioxidante y contenido de ácido ferúlico libre comparado con pan elaborado con salvado sin bioprocesar. Los panes con salvado bioprocesado con la mezcla de enzimas Alphamalt H® SternIngredients, mostraron mayor contenido de ácido ferúlico libre (583.3 µg/g) que el pan adicionado con salvado nativo (14.7 µg/g). Los panes elaborados con salvados bioprocesados tuvieron un mayor volumen comparado con el pan elaborado con salvado nativo.

Se determinó la bioaccesibilidad y la absorción *in vitro* del ácido ferúlico de los panes elaborados a partir de salvado bioprocesado. Los resultados mostraron que los panes formulados con salvados bioprocesados con la enzima Alphamalt H a las concentraciones de 0.1% y 0.05% presentaron mayor cantidad de AF bioaccesible (con valores de 82.6±8.18 y 80.4±7.17 ug/g, respectivamente) que el pan con

salvado control (34.3±1.51 ug/g). La absorción del ácido ferúlico fue significativamente mayor en los panes con salvado bioprocesado con la enzima Alphamalt H a las concentraciones de 0.1% y 0.05% (11.8±1.3 y 11.4±0.23 ug/g, respectivamente) en comparación al pan con salvado control (ND, acido ferúlico no detectado).

Se evaluó el efecto del consumo regular de pan de barra adicionado con salvado de trigo bioprocesado sobre biomarcadores de estrés oxidativo e inflamación en suero de individuos obesos metabólicamente sanos. El estudio de intervención dietaria demostró que en individuos obesos el consumo de 50 g de pan bioprocesado/día mostró una tendencia de aumento la actividad antioxidante sérica después de siete días de consumo. El factor de necrosis tumoral alfa (TNF-α), mostró un incremento significativo después de siete días de consumo de pan nativo y bioprocesado. También se encontró una correlación positiva entre interleucina-6 (IL-6) y TNF-α con capacidad antioxidante sérica. Los mecanismos que explican este comportamiento deberán ser estudiados a mayor profundidad. Sin embargo, podemos suponer que un incremento en la capacidad antioxidante sérica después del consumo de pan adicionado con salvado de trigo bioprocesado, está relacionada con el incremento de la cantidad de AF bioaccesible.

ABSTRACT

Inflammation and oxidative stress have been associated with different pathologies related to obesity, such as insulin resistance, type 2 diabetes, metabolic syndrome and cardiovascular disease. There is a growing interest in the use of natural antioxidants for therapeutic applications. Phenolic compounds are considered as potential therapeutic agents against a wide range of diseases. Ferulic acid (FA) has been identified as the main contributor to antioxidant capacity in wheat milling products. The AF represents up to 90% of the total phenolic acids and of which 99% is in bound form to the arabinoxylans, since at the level of the small intestine no esterase enzymes are available to release the AF, the need arises to develop technologies that allow a greater release of this compound in such a way that its bioaccessibility is improved and therefore its bioavailability.

The effect of the bioprocessing of wheat bran on the content of total phenols, antioxidant capacity and free ferulic acid content was evaluated. In breads added with bioprocessed bran the content of total phenols, antioxidant capacity and free ferulic acid content was significantly increased compared with bread made with bran without bioprocessing. The loaves bioprocessed with the Alphamalt H® SternIngredients enzyme mixture showed the highest content of free ferulic acid (583.3 µg / g) compared to bread added with native bran (14.7 µg/g). The breads made with bioprocessed bran reached a higher volume compared to the bread made with native bran.

The bioaccessibility and absorption of ferulic acid in breads made from bioprocessed bran was determined by *in vitro* models. The results showed that the breads formulated with bioprocessed bran with the enzyme Alphamalt H at concentrations of 0.1% (82.6 ± 8.18 ug/g) and 0.05% (80.4 ± 7.17 ug / g) presented the highest bioaccessible amount (ug/g) of AF with respect to control bran (34.3 ± 1.51 ug/g). In terms of bioaccessibility percentage, it was found that for the same treatments this

was 7% and 4%, higher values compared to bran bread without bioprocessing (2%). The absorption of ferulic acid was significantly higher in the breads with bioprocessed bran compared to bread with native bran bread.

The effect of regular consumption of bar bread added with bioprocessed wheat bran on biomarkers of oxidative stress and inflammation in serum of obese individuals was evaluated. The study of dietary intervention showed that in obese individuals the consumption of bioprocessed bread showed a trend of greater increase in serum antioxidant activity after seven days of consumption of 50 g of bread / day. The tumor necrosis factor alpha (TNF-α) showed a significant increase after seven days of native bran bread consumption and bioprocessing. A positive correlation was also found between interleukin IL-6 and TNF-α with serum antioxidant capacity. The mechanisms that explain this behavior should be studied in greater depth. However, we can suppose that an increase in the serum antioxidant capacity after the consumption of bread added with bioprocessed wheat bran could be related to the increase in the bioaccessible amount of FA.

CONTENIDO

INTRODUCCIÓN

Actualmente, el sobrepeso y la obesidad son uno de los principales problemas de salud pública en todo el mundo, según la OMS, el 2016, más de 1,900 millones de adultos tenían sobrepeso y más de 550 millones eran obesos. En México, según la encuesta nacional de salud y nutrición (ENSANUT) del 2016, la prevalencia de sobrepeso y obesidad en adultos fue del 72.5%. El sobrepeso y la obesidad se definen como una acumulación excesiva y anormal de grasa que puede ser perjudicial para la salud. La inflamación y el estrés oxidativo se han asociado a diferentes patologías relacionadas con la obesidad, como resistencia a la insulina, diabetes tipo 2, síndrome metabólico y enfermedad cardiovascular. Las citocinas proinflamatorias estimulan la producción de las especies reactivas del oxígeno y nitrógeno por macrófagos y monocitos; por lo tanto, un aumento en la concentración de citocinas podría ser responsable del aumento del estrés oxidativo.

Existe un creciente interés en el uso de antioxidantes naturales para aplicaciones terapéuticas. Los compuestos fenólicos son considerados como potenciales agentes terapéuticos contra un amplio rango de enfermedades: cáncer, diabetes, disfunción cardiovascular, enfermedades inflamatorias y envejecimiento (Poutanen *et al.,* 2014). Los fenoles están ampliamente distribuidos en el reino vegetal y son por consiguiente parte integral de la dieta con cantidades significativas reportadas en frutas, verduras, y cereales. El consumo de fenoles varía considerablemente entre regiones geográficas, se ha estimado que el consumo diario está en el rango de 20 mg - 1.0 g, lo cual pudiera ser mayor que el consumo de vitamina E (Stalikas C.D., 2007). Los fenoles conforman un amplio grupo de compuestos con diversas estructuras químicas y por tanto diferentes propiedades biológicas tales como: antioxidante, antibacterial, antiinflamatoria, antitrombótica, anticarcinogénica, antialergénica, hepatoprotector, antiviral (Andersson *et al*., 2014). Los fenoles dietarios ejercen estos efectos a través de algunos mecanismos de acción como antioxidantes, agentes quelantes, modulación enzimática, entre otras (Vitaglione *et al.,* 2008). Los fenoles están

recibiendo especial atención a partir de la última década debido a su rol en la prevención de enfermedades humanas particularmente ateroesclerosis, cáncer y otras enfermedades crónicas no transmisibles. Los alimentos elaborados con productos y subproductos de la molienda de los cereales pudieran ser una fuente importante de estos compuestos y a través de un consumo regular pudieran brindar un efecto protector sobre estas enfermedades.

Por lo anterior el objetivo de la presente investigación fue evaluar el potencial biológico de pan de trigo adicionado con salvado bioprocesado y su efecto sobre biomarcadores de inflamación y estrés oxidativo en suero de individuos obesos, asociado a un incremento en la biodisponibilidad del ácido ferúlico.

REVISIÓN BIBLIOGRÁFICA

Generalidades del Trigo

Los cereales se definen como los granos o semillas comestibles de la familia *gramíneae.* El fruto de los cereales es denominado botánicamente cariópside y consta de tres partes fundamentales: pericarpio, endospermo y germen (Slavin J., 2004). Dentro de los granos de cereales en orden de mayor producción a nivel mundial se encuentran: maíz, arroz, trigo, cebada, sorgo, mijo, avena, centeno y triticale (FAOSTAT, 2016). Particularmente el maíz, el arroz y el trigo contribuyen con alrededor del 90% de la producción mundial de cereales (FAO, 2016). En lo que respecta a México, la producción anual de trigo para 2016 fue de alrededor de 3,863 toneladas y el consumo anual per cápita fue de 57 kg (SIAP, 2016).

Se estima que los cereales aportan 30-70% del total de energía diaria consumida por la población humana, lo que los hace la fuente más importante de calorías con respecto al aporte de otros alimentos (McKeown *et al.,* 2013; Poutanen *et al.,* 2014). Los cereales son también fuente primordial de antioxidantes y fitoquímicos los cuales pueden ejercer un efecto protector en la prevención de enfermedades crónico-degenerativas tales como las enfermedades cardiovasculares, diabetes, algunos tipos de cáncer entre otras (Hemery *et al.,* 2010, Andersson *et al.,* 2014). A pesar de que los cereales ocupan la base de la pirámide nutricional, todavía persiste en la población un bajo consumo de cereales menor a una porción diaria aun cuando la Organización Mundial de la Salud recomienda un consumo diario de 2-3 porciones. Es sustancial encaminar esfuerzos hacia un mayor consumo de este grupo de alimentos en particular cuando ya existen estudios sobre los efectos benéficos que aportan a la salud humana.

El procesamiento es un prerrequisito para la manufactura de productos de cereales, el objetivo es obtener un producto atractivo y de buena palatabilidad. Sin embargo, el procesamiento puede disminuir o incrementar los niveles de compuestos bioactivos en los granos y también modificar su biodisponibilidad (Zaupa *et al.,* 2014). Actualmente la Organización Mundial de la Salud recomienda el consumo de granos integrales esto debido a que estudios en la última década han encontrado un beneficio a la salud en comparación al consumo de harinas refinadas. Los granos integrales y la adición de subproductos de la molienda pueden proporcionar un valor agregado a los productos de los cereales, particularmente del trigo, y en especial, aquellos productos derivados del proceso de panificación.

En referencia al trigo, este cereal es el cultivo más importante en muchas partes del mundo, pertenece al género Triticum siendo T. *aestivum* y T. *durum* las especies más importantes desde el punto de vista comercial (McKevith B., 2004). De acuerdo con su uso, el trigo es clasificado en suave, duro y cristalino. Los dos primeros son generalmente transformados en harinas para la manufactura de pan fermentado y leudado con agentes químicos, productos de pastelería, galletas, botanas y cereales para desayuno. Los trigos cristalinos son molidos y purificados en una fracción más gruesa que la harina, llamada semolina, que se utiliza en la manufactura de pastas extrudidas o troqueladas (Ragaee *et al.,* 2012). El trigo (*Triticum aestivum*) es con mucho el cereal más importante en la elaboración de pan, dado su especial comportamiento durante el horneado en comparación con otros cereales. Los productos de pan han sido consumidos desde tiempos antiguos y considerados como parte de la dieta básica de muchas poblaciones de aquí su importancia global en la nutrición mundial.

Composición Química del Grano de Trigo

La mayoría de los granos de cereales comparten características anatómicas similares, todos poseen una cubierta externa (pericarpio), endospermo y germen existiendo variaciones en cuanto a proporción de cada una de ellas, pero en general constituyen

7-10, 82-85 y 3-10%, respectivamente (Serna-Saldívar, 1996). En referencia al grano de trigo, este posee una longitud promedio de 8 mm y su peso es cercano a los 35 mg. El tamaño varía ampliamente dependiendo de las características de cultivo. Los granos de trigo varían en textura y color y esta última característica está relacionada a la pigmentación de la cubierta del grano (Slavin J., 2004).

El endospermo junto con la capa aleurona ocupan alrededor del 80% del peso del grano. La harina blanca está constituida principalmente por el endospermo donde las células están empacadas en gránulos de almidón y embebidas dentro de una matriz proteica. Las proteínas del endospermo constituyen al gluten la cual es la proteína de almacenamiento del trigo (8-18%). El almidón posee propiedades muy particulares que determinan la funcionalidad de una gran variedad de alimentos principalmente en productos de panificación. Desde el punto de vista nutricional el almidón es la principal fuente de energía en la dieta humana (Goesaert *et al.,* 2005).

Entre el endospermo y la cubierta exterior del grano se encuentra la aleurona, la cual está constituida por una delgada capa de células. La capa aleurona representa cerca del 7% del peso del grano de trigo (peso seco), contiene la mayor parte de las vitaminas del complejo B y cerca de la mitad del contenido de minerales del grano de trigo. Comparado a las otras capas periféricas del grano la capa aleurona tiene un alto contenido de proteínas, con un mejor balance de aminoácidos (particularmente más alto en lisina) que las proteínas del endospermo. La aleurona también está constituida principalmente por polisacáridos no amiláceos (PNA) tales como arabinoxilanos (AX), celulosa, β-glucano y lignina. Los arabinoxilanos representan aproximadamente alrededor del 80% de los polisacáridos no amiláceos presentes en la capa aleurona y el 20% restante lo conforman lignina y β-glucanos principalmente (Ragaee *et al.,* 2012).

Los arabinoxilanos están formados por una cadena lineal de xilosas unidas por enlaces glucosídicos β-(1→4), a la cual se unen residuos de arabinosas mediante enlaces glucosídicos α-(1→3) o α-(1→2), o ambos (Morales-Ortega *et al.,* 2013). Los residuos

de arabinosa de los AX están generalmente sustituidos con residuos de ácidos fenólicos como el ácido ferúlico (AF), que es el principal ácido fenólico presente en la capa aleurona. Diversos estudios han demostrado que el ácido ferúlico desempeña un papel importante en relación con la prevención de algunas enfermedades, sin embargo, su compleja ubicación en la capa aleurona limita en gran parte su bioaccesibilidad por las enzimas del tracto digestivo humano limitando en gran medida su biodisponibilidad (Pekkinen *et al.,* 2014). En las últimas décadas se han venido proponiendo diversos procedimientos para modificar la estructura de los arabinoxilanos con la finalidad de liberar el ácido ferúlico y así potenciar su actividad biológica en circulación.

El pericarpio o salvado es la estructura más externa del grano de trigo que protege el grano de su entorno, incluyendo; clima, insectos, mohos, y bacterias. El pericarpio total comprende cerca del 5% del peso total del grano. Este consiste en aproximadamente 20% celulosa, 6% proteínas, 2% cenizas y 0.5 % grasa. El pericarpio también es rico en xilanos y fibra insoluble. Durante el proceso de molienda del grano el pericarpio y el germen son removidos. Las fracciones del salvado y germen derivados de la molienda convencional proporcionan la mayoría de los compuestos biológicamente activos que se encuentran en el grano (Slavin J., 2004; Blandino *et al.,* 2013).

Capacidad Antioxidante del Ácido Ferúlico

En cereales, los compuestos fenólicos más abundantes son los ácidos fenólicos de los cuales existen dos grupos: los derivados del ácido hidroxibenzoico y los derivados del ácido hidroxicinámico, estos últimos son los que se encuentran en mayor concentración en trigo (Hollman *et al.,* 2011). En el trigo, el ácido ferúlico se encuentra ligado por enlaces éster en la posición O-5 a las cadenas laterales de α-L-arabinofuranosas de los arabinoxilanos de la pared celular, representa hasta 90% de los ácidos fenólicos totales y el 99% de los cuales se encuentra en la forma ligada (Adom & Liu, 2002; Fardet *et al.,* 2008; Tao Wang *et al.,* 2014). El ácido ferúlico se encuentra principalmente en las partes exteriores del grano. La capa aleurona y el

pericarpio del grano de trigo contienen el 98% del total de ácido ferúlico (Vitaglione *et al.,* 2008; Ragaee *et al.,* 2012; Andersson *et al.,* 2014).

Liyana-Pathirana et al. (2006) encontraron que entre las diferentes fracciones de la molienda de trigo de dos variedades (*Triticum turgidum*, *Triticum aestivum*), el salvado presentó el mayor contenido de fenoles totales, mientras que el endospermo presentó el menor contenido. Los compuestos fenólicos se concentraron principalmente en las capas más externas del grano reflejándose en una mayor capacidad antioxidante comparada con endospermo. De la misma manera, Abozed *et al.* (2014) demostraron que el contenido de fenoles totales y la capacidad antioxidante eran más altos en el salvado en comparación con la harina integral de trigo. Yu *et al.* (2013) encontraron que la harina integral de trigo mostró una capacidad antioxidante significativamente mayor en comparación con la harina blanca. Adom & Liu (2002) demostró que el ácido ferúlico se encontraba predominantemente en el grano de trigo, principalmente en la forma unida. En otro estudio, Kim *et al.* (2006) demostraron que, en el salvado de trigo, la mayoría de los ácidos fenólicos están unidos, y mencionaron que los extractos después de la hidrólisis alcalina contenían la mayor capacidad antioxidante, el ácido ferúlico fue el ácido fenólico que se encontró en una cantidad significativamente mayor (1.359-1.934 µg/g).

El potencial antioxidante del ácido ferúlico se atribuye principalmente a un mecanismo de transferencia de un átomo de hidrógeno, en este caso el ácido ferúlico puede ceder más fácilmente el hidrógeno del grupo carboxilo el cual se encuentra lejano al anillo bencénico, al mismo tiempo que el ácido ferúlico estabiliza a un radical libre transfiriéndole su hidrógeno, este se convierte en un radical fenoxilo, el cual también puede ser estabilizado por resonancia (Mateo-Anson *et al.,* 2008). Bajo estas condiciones el ácido ferúlico puede inhibir un proceso de carcinogénesis inducida químicamente e inhibir la formación de aductos de ADN. Al ser un eficaz captador de radicales libres ya se ha probado en algunos países como un aditivo alimentario para prevenir la peroxidación lipídica. *In vitro*, también se observó que puede proteger al colesterol LDL del daño oxidativo inducido por metamioglobina (Adam *et al.,* 2002).

Estudios previos han establecido que la compleja ubicación del ácido ferúlico en el pericarpio y la capa aleurona restringe en gran parte su bioaccesibilidad por las enzimas del tracto digestivo humano limitando en gran medida su biodisponibilidad (Pekkinen *et al.,* 2014). En las últimas décadas, se han propuesto diversos procedimientos para modificar la estructura de los arabinoxilanos con la finalidad de liberar el ácido ferúlico y así potenciar su actividad biológica en circulación.

Tecnologías para Mejorar la Bioaccesibilidad y Biodisponibilidad del Ácido Ferúlico

El desarrollo de técnicas innovadoras de procesamiento parece un enfoque prometedor para mejorar la bioaccesibilidad de compuestos en los granos de cereales y de esta manera puedan ejercer su actividad biológica. La liberación de estos compuestos fenólicos a partir de matrices de salvado y/o el aumento de su accesibilidad han demostrado ser eficaces en la mejora de su biodisponibilidad. La bioaccesibilidad es un término que refleja la liberación de nutrientes y otros componentes, como compuestos fenólicos, de la matriz de alimentos. La bioaccesibilidad puede depender de la complejidad de la matriz alimentaria o de las condiciones en el tracto gastrointestinal, entre otros parámetros. Estos pueden limitar la liberación de nutrientes para su posterior absorción en el intestino delgado (Mateo-Anson *et al.,* 2009; Hemery *et al.,* 2010; Saura Calixto, 2011; Velderrain-Rodríguez *et al.,* 2016).

Es importante establecer la biodisponibilidad de los compuestos bioactivos, ya que esto representa la cantidad total que se libera y se absorbe principalmente en el intestino delgado, llegando al torrente sanguíneo, donde se envían a los diferentes tejidos del cuerpo (Velderrain-Rodríguez *et al.,* 2016). Los hallazgos basados en la simulación de la digestión y absorción que se han desarrollado nos brindan una herramienta útil para conocer la bioaccesibilidad y biodisponibilidad de compuestos,

sin embargo, una alta actividad *in vitro* no siempre se traduce en una actividad comparable *in vivo*, pero podría darnos una idea de lo que puede ocurrir en condiciones i*n vivo* (Swieca *et al.,* 2017). Para este propósito, se han desarrollado diversas tecnologías de procesamiento entre los cuales se pueden mencionar los tratamientos mecánicos (molienda ultrafina, clasificación por aire, y separación electrostática) y el bioprocesamiento (tratamientos enzimáticos, germinación y fermentación) (Nordlund *et al.,* 2013; Rosa *et al.,* 2013; Pekkinen *et al.,* 2014; Wang *et al.,* 2014; Poutanen *et al.,* 2014; Zaupa *et al.,* 2014; Coda *et al.,* 2014).

Los granos integrales y la adición de subproductos de la molienda pueden proporcionar un valor agregado a los productos de los cereales, particularmente del trigo, y en especial, aquellos productos derivados del proceso de panificación. El reemplazo de harina refinada con un porcentaje de fracciones de salvado obtenidos durante el proceso de molienda de rodillos es en realidad la principal forma de aumentar la concentración de compuestos bioactivos en la harina de trigo y los productos derivados (Noort *et al.,* 2010). Sin embargo, el uso de salvado de trigo en la harina de trigo para la panificación también da lugar a cambios en las propiedades de la masa, técnicas de procesamiento y las características de calidad del pan (menor volumen, corteza oscura y textura densa de la miga) (De Kock *et al.,* 1999; Noort *et al.,* 2010).

En un estudio realizado por Blandino *et al.* (2013) encontró un aumento lineal y de manera significativa de proteína, fibra dietaria, β-glucano, contenido de fenoles totales, alquilresorcinoles, contenido de cenizas y actividad antioxidante total en pan donde se adicionó a la harina refinada con las fracciones de salvado al 5%, 10%, 15%, 20% y 25%. El valor nutricional del pan mejoró significativamente a un nivel de adición del 10% de salvado y las propiedades reológicas y tecnológicas fueron similares a las del control. Por otra parte, el bioprocesamiento puede aumentar la biodisponibilidad de nutrientes y otros compuestos a través de reacciones químicas o enzimáticas que hidrolizan o liberan los nutrientes de la matriz alimentaria (Mateo-Anson *et al.,* 2011). Mateo-Anson *et al.* (2009) encontró que el bioprocesamiento del salvado de trigo aumentaba el contenido de ácidos fenólicos libres en los panes con salvado

bioprocesado en comparación con el pan control. La combinación de fermentación y tratamiento enzimático del salvado aumentó la cantidad de ácido ferúlico en el pan 8 veces más (12 a 100 µg/g de materia seca). Rosa *et al.* (2013) encontraron que en la capa de aleurona de trigo el tratamiento enzimático con xilanasa y feruloil esterasa libera hasta 86% de ácido ferúlico (formas conjugada y libre).

Hemery *et al.* (2010) informó que el porcentaje de bioaccesibilidad de ácido ferúlico va desde un rango de 2.5 - 5.1% para panes adicionados con salvado, y los valores más altos fueron para el pan elaborado con salvado fraccionado por separación electrostática. Mateo-Anson *et al*. (2009) encontraron que el porcentaje de bioaccesibilidad de ácido ferúlico para panes adicionados con salvado fueron de 1.1-5.5%, y los valores más altos correspondieron al pan elaborado con salvado bioprocesado (tratamiento enzimático y de fermentación).

Pérez-Vicente *et al.* (2002) evaluaron la absorción aparente de compuestos fenólicos, antocianinas y vitamina C de jugo de granada después de la digestión con pancreatina-sales biliares, encontraron que el 29%, 3% y 5%, respectivamente, de estos compuestos estaban presentes en la fracción dializada, mientras que el resto permaneció en la fracción no dializada. En otro estudio realizado por Swieca *et al.* (2017) en pan de trigo enriquecido con café verde indicaron que el ácido ferúlico absorbido fue menor en comparación con la fracción potencialmente bioaccesible. En relación con la absorción de los compuestos fenólicos mediante un modelo de membrana de celulosa semipermeable (transporte pasivo), pudieran predominar las interacciones de los compuestos fenólicos con otros componentes activos y/o matrices de alimentos; tales como hierro, otros minerales, fibra dietética o proteínas, por lo que pudiera reflejarse en una disminución en la absorción de los compuestos. Además, estas interacciones pueden disminuir significativamente la bioactividad de los compuestos fenólicos en las partes superiores del tracto digestivo, lo que reduce la digestibilidad de los nutrientes (Pérez-Vicente *et al.,* 2002). La capacidad antioxidante se encuentra relacionada con la estructura química de los ácidos fenólicos esta depende principalmente del número y la posición de los grupos hidroxilo donadores de

hidrógeno en los anillos aromáticos de las moléculas fenólicas. Se ha informado que la actividad antioxidante de los fenoles libres es más alta que los quelatos de hierro y fenol, pero, por otro lado, las agliconas exhiben un mayor poder antioxidante que sus glucósidos (Bouayed *et al.,* 2011).

Se supone que la modificación de la matriz alimentaria con enzimas mejora la biodisponibilidad y la bioaccesibilidad del ácido ferúlico libre en los panes, y esto pudiera reflejarse en su absorción *in vitro*. Sin embargo, la cantidad bioaccesible de ácido ferúlico libre que se encuentra en el intestino delgado podría no ser necesariamente absorbida en su totalidad, y parece que la cantidad biodisponible (absorbida) de ácido ferúlico es menor que la cantidad bioaccesible (libre de la matriz alimentaria). Esto no significa que no tenga ningún papel en la protección de la salud, ya que estos compuestos, si no se absorben en el intestino delgado, pueden pasar al intestino grueso, donde en presencia de la microflora del colon puede transformarlos y/o degradarlos. Los metabolitos obtenidos podrían tener un efecto beneficioso sobre las células y/o bacterias del intestino grueso y ser absorbidos, ejerciendo así un efecto benéfico a la salud (Pekkinen *et al.,* 2014).

Estrés Oxidativo e Inflamación en Individuos Obesos

Actualmente, el sobrepeso y la obesidad son uno de los principales problemas de salud pública en todo el mundo, según la OMS, en 2016, más de 1,900 millones de adultos tenían sobrepeso y más de 650 millones eran obesos. En México, según la encuesta nacional de salud y nutrición (ENSANUT) en 2016, la prevalencia de sobrepeso y obesidad fue del 72.5% en adultos. El sobrepeso y la obesidad se definen como una acumulación excesiva y anormal de grasa que puede ser perjudicial para la salud. El tejido adiposo está compuesto de adipocitos, preadipocitos, células endoteliales, pericitos, fibroblastos, mastocitos y células inmunes (macrófagos y linfocitos T) (Izaola *et al.,* 2015).

La función principal de los adipocitos es el almacenamiento de ácidos grasos en forma de triacilgliceroles. En sujetos obesos, el desequilibrio crónico entre las calorías consumidas y las calorías gastadas provoca un aumento en el almacenamiento en forma de triacilgliceroles en el tejido adiposo, que se manifiesta de las siguientes maneras: aumento de los lípidos intracelulares, aumento del tamaño de los adipocitos (hipertrofia), aumento en el número de adipocitos (hiperplasia). El aumento de los lípidos dentro del adipocito, junto con la hipertrofia y la hiperplasia del tejido adiposo, conduce a una disfunción celular que se manifiesta con anormalidades tales como la secreción de citocinas y moléculas proinflamatorias por el tejido adiposo como TNF-α, IL-6, la proteína quimiotáctica de monocitos 1 (MCP-1), los factores estimulantes de colonias (CSF) y la óxido nítrico sintetasa inducible (iNOS), que generan el estado proinflamatorio descrito y reconocido en pacientes obesos (Ramírez Alvarado & Sánchez Roitz, 2012; Choe et al. 2016).

La inflamación y el estrés oxidativo se han asociado a diferentes patologías relacionadas con la obesidad, como resistencia a la insulina, diabetes tipo 2, síndrome metabólico y enfermedad cardiovascular. Las citocinas proinflamatorias estimulan la generación de especies reactivas del oxígeno y el nitrógeno en los macrófagos y monocitos; por lo tanto, un aumento en la concentración de citocinas podría ser responsable del aumento del estrés oxidativo. IL-6 y TNF-α se encuentran entre los principales mediadores de la inflamación liberada por el tejido adiposo. Se han demostrado asociaciones positivas de IL-6 y suero TNF-α con índice de masa corporal (IMC), circunferencia de la cintura, relación cintura / cadera y síndrome metabólico. Por otro lado, se ha observado que la leptina, una hormona sintetizada por el tejido adiposo, se eleva en circulación sanguínea en sujetos obesos y estos aumentos son proporcionales a la masa de grasa corporal. Además, estos mediadores de la inflamación se han asociado con hipertensión, diabetes, dislipidemias, infecciones y cáncer (Zulet et al., 2007, Fernández-Sánchez et al., 2011).

Por lo tanto, una estrategia efectiva para promover la salud y reducir el riesgo de enfermedades en individuos obesos podría ser la prevención y el tratamiento del estrés

oxidativo y la inflamación asociados con la obesidad. Los antioxidantes de la dieta pueden ser una estrategia de costo-beneficio para reducir el estrés oxidativo y la inflamación en pacientes obesos. Varios estudios ya han demostrado el efecto protector de los cereales integrales contra enfermedades crónicas, como el cáncer y la enfermedad cardiovascular, y estos efectos se han atribuido a los antioxidantes presentes en estos alimentos. El objetivo del estudio fue evaluar el efecto de un pan adicionado con salvado de trigo bioprocesado, esto con la finalidad de liberar el ácido ferúlico ligado a la matriz alimentaria y poder observar un incremento en los ensayos *in vitro*; seguido de la formulación del pan, se planteó una intervención dietaria dirigida a individuos en condiciones de obesidad para observar cambios en las citocinas proinflamatorias y capacidad antioxidante en suero, atribuido principalmente a la liberación del ácido ferúlico.

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HIPÓTESIS

El consumo regular de pan de barra adicionado con salvado de trigo bioprocesado promueve cambios en biomarcadores de estrés oxidativo e inflamación en suero de individuos obesos siendo atribuidos a un incremento en la biodisponibilidad del ácido ferúlico.

OBJETIVO GENERAL

Evaluar el potencial biológico del pan de trigo adicionado con salvado bioprocesado y su efecto sobre biomarcadores de inflamación y estrés oxidativo en suero de individuos obesos, asociado a un incremento en la biodisponibilidad del ácido ferúlico.

Objetivos Específicos

1. Determinar el potencial biológico de pan de barra obtenido a partir de diferentes flujos de molienda de trigo.

2. Evaluar el efecto del bioprocesamiento del salvado de trigo en productos de la panificación sobre el contenido de fenoles totales, actividad antioxidante y contenido de ácido ferúlico.

3. Determinar la bioaccesibilidad del ácido ferúlico en panes elaborados a partir de salvado bioprocesado.

4. Evaluar el efecto del consumo regular del pan de barra adicionado con salvado de trigo bioprocesado sobre biomarcadores de estrés oxidativo e inflamación en suero de individuos obesos.

DESARROLLO DEL TRABAJO DE INVESTIGACIÓN

Para responder a la pregunta de investigación, previamente se evaluó la actividad antioxidante y el contenido de ácido ferúlico en salvado de trigo bioprocesado con enzimas xilanasas, posteriormente se elaboró un pan de barra adicionado con salvado de trigo bioprocesado y sin bioprocesar y se determinó la bioaccesibilidad del ácido ferúlico. Asimismo, se evaluó el efecto de la adición de salvado bioprocesado y sin bioprocesar en las propiedades farinográficas de la harina y en el volumen específico del pan. Además, se cuantificó el ácido ferúlico libre en un modelo de digestión in vitro, para finalmente llevar a cabo una intervención dietaria en individuos que presentaban condiciones de obesidad, esto con el objetivo de poder observar un efecto biomarcadores de estrés oxidativo e inflamación en suero relacionado con la liberación del ácido ferúlico de su matriz alimentaria.

El desarrollo de los estudios para cumplir con los objetivos propuestos está descrito en cuatro capítulos que conforman esta tesis. Cada uno de ellos representa lo siguiente: un capítulo de libro (**Capítulo I**), un artículo de investigación original publicado (**Capítulo II**), un artículo de investigación original enviado (**Capítulo III**) y un manuscrito preparado para envío (**Capítulo IV**). Los artículos de investigación original fueron preparados para su envió y publicación a revistas internacionales indizadas en el Journal Citation Reports (JCR) del Institute of Scientific Information de la base de datos Thomson-Reuters.

Descripción del Capítulo I

Está formado por un capítulo del libro titulado *"Tecnologías que aumentan la funcionalidad de los arabinoxilanos contenidos en los subproductos de la molienda de cereales*" del libro "*Aprovechamiento de subproductos para el desarrollo de alimentos funcionales y nutraceúticos: Validación de sus propiedades funcionales*".

El capítulo describe los subproductos de la molienda de cereales y su potencial biológico. Uno de los subproductos que en la actualidad se le han atribuido efectos benéficos a la salud es el salvado, él cual es utilizado como ingrediente funcional, en productos de la panificación, cereales para desayuno, entre otros. El salvado de cereales como el trigo, maíz, sorgo, arroz, cebada, centeno y avena proporcionan fibra dietaria soluble e insoluble, de estos últimos, los arabinoxilanos contienen en su estructura compuestos fenólicos los cuales se han reportado que presentan actividad antioxidante y anti-inflamatoria, pero estructuralmente tienen baja accesibilidad. Con ese enfoque, se analizan las tendencias de la tecnología para modificar el salvado y mejorar la disponibilidad de compuestos fenólicos en cereales, mediante tratamientos termo-mecánicos, térmicos y tratamientos biológicos, mejorando sus bioaccesibilidad y biodisponibilidad para ejercer efectos benéficos a la salud de los alimentos que los contengan.

Descripción del Capítulo II

Este capítulo corresponde a un artículo original publicado en la revista CyTa-Journal of Food (Factor de impacto de 1.180) que lleva por título: “Bioprocessing of wheat (Triticum aestivum cv. Kronstad) bran from Northwest Mexico: effects on ferulic acid bioaccessibility in breads”.

El articulo original muestra los resultados del efecto de la adición de salvado bioprocesado en la bioaccesibilidad de ácido ferúlico en panes control y experimentales mediante un modelo de digestión *in vitro*, utilizando una enzima *1-4-β*-endoxilanasa, una levadura comercial y la combinación de ambos para bioprocesar el salvado, también se describe el efecto del bioprocesamiento en el volumen especifico de los panes. Los resultados de esta investigación muestran que el salvado bioprocesado con enzima endoxilanasa, fermentación con levadura y la combinación de ambos tratamientos mostraron los valores más altos de contenido de fenoles totales y capacidad antioxidante que el salvado sin bioprocesar, en donde la fracción ligada

presentó la mayor proporción respecto a la fracción libre. El ácido ferúlico se encontró en proporciones más altas respecto a los otros ácidos hidroxicinámicos evaluados. El bioprocesamiento aumentó seis veces más el contenido de ácido ferúlico libre en comparación con el salvado sin bioprocesar. El % de bioaccesibilidad del ácido ferúlico en los diferentes panes se incrementó en el siguiente orden: pan adicionado con salvado bioprocesado con ambos tratamientos<pan adicionado con salvado sin bioprocesar< pan adicionado con salvado fermentado con levadura< pan adicionado con salvado bioprocesado con enzima endoxilanasa. La adición de salvado bioprocesado puede ser una manera sencilla de aumentar los compuestos bioactivos en un producto pan.

Descripción del Capítulo III

Este capítulo corresponde a un artículo original enviado a la revista Evidence-Based Complementary and Alternative Medicine (Factor de impacto de 1.740) que lleva por título: “Bioaccessible ferulic acid in breads with bioprocessed wheat bran added: Effect on apparent absorption”.

El capítulo antes citado muestra el efecto en la absorción *in vitro* del ácido ferúlico a partir de panes con salvados bioprocesados con una mezcla de enzimas con actividad xilanasas a diferentes concentraciones (0.05% y 0.1%), utilizando un modelo de digestión *in vitro* y un modelo de absorción *in vitro*. Los resultados de esta investigación muestran que los panes con salvado bioprocesado adicionado presentan un mayor contenido de fenoles totales y capacidad antioxidante (fracciones libres y conjugadas), la fracción ligada disminuyó significativamente en panes con salvado bioprocesado en comparación con los panes con salvado sin bioprocesar. La bioaccesibilidad del ácido ferúlico libre en panes con salvado bioprocesados aumentó 2.4 veces y 2.3 veces para H 0.1% y 0.05%, respectivamente, en comparación con el pan formulado con salvado sin bioprocesar. En la digestión *in vitro* (fracción dializable) en pan con salvado bioprocesado (H 0.1% y 0.05%) mostraron la mayor cantidad de ácido ferúlico, y para los otros panes, no hubo cantidad detectable de este ácido. Por otro lado, para la

fracción no dializable, no se encontraron diferencias significativas entre los panes de salvado bioprocesados. El % de absorción fue 0.9% y 0.6% para panes con salvado bioprocesados con H 0.1% y 0.05%, respectivamente. La capacidad antioxidante en la fracción dializable fue mayor en el pan de salvado bioprocesado (H 0.1%) en comparación con todos los demás panes. Los panes de salvado de trigo bioprocesados con enzimas tenían un mayor contenido de ácido ferúlico, que se reflejó en su mayor bioaccesibilidad y aparente absorción.

Descripción del Capítulo IV

Este capítulo corresponde a un artículo original que tiene como título tentativo: “Proi-inflammatory cytokines and antioxidant capacity in serum of obese young people after consumption of bioprocessed bread: Randomized controlled trial”, el cual será enviado a la revista Journal of Nutrition & Food Science (Factor de impacto de 1.49).

El artículo describe un ensayo clínico controlado aleatorizado con individuos jóvenes en condiciones de obesidad, se evaluaron citocinas pro-inflamatorias (IL-6 y TNF-α) y capacidad antioxidante en suero, seguido de las medidas antropométricas y análisis clínicos en respuesta al consumo de pan con salvado de trigo bioprocesado. En el estudio de intervención dietaria se encontró que en individuos en condiciones de obesidad que consumieron 50 g de pan/día con salvado de trigo bioprocesado, durante siete días de consumo mostraron una tendencia a incrementar la actividad antioxidante en suero medida como ORAC. El factor de necrosis tumoral alfa (TNF-α) mostró un aumento significativo después de siete días de consumo tanto de pan nativo como de pan con salvado de trigo bioprocesado.

Asimismo, se encontró una correlación positiva entre IL-6 y TNF-α con capacidad antioxidante sérica. Los mecanismos que expliquen este comportamiento deberán estudiarse con mayor profundidad, sin embargo, podemos suponer que un aumento en la capacidad antioxidante del suero después del consumo de pan con salvado de trigo bioprocesado podría estar relacionado con el incremento en la cantidad

bioaccesible de ácido ferúlico. De esta manera se estaría finalmente cumpliendo con el objetivo de poder observar un efecto biomarcadores de estrés oxidativo e inflamación en suero relacionado con la liberación del ácido ferúlico de su matriz alimentaria.

CAPITULO I

Tecnologías que aumentan la funcionalidad de los arabinoxilanos contenidos en los subproductos de la molienda de cereales

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Capítulo

22

Tecnologías que aumentan la funcionalidad biológica de los arabinoxilanos contenidos en los subproductos de la molienda de cereales

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Resumen

El subproducto de la molienda de algunos cereales, el salvado, es un ingrediente funcional que se incorpora a los productos de panificación para mejorar sus propiedades promotoras de la salud. Sin embargo, la incorporación de estos ingredientes también influye en las propiedades tecnológicas y sensoriales.

Los arabinoxilanos son fibras dietéticas importantes, presentes predominantemente, en el salvado y en la harina, que han captado gran atención debido a su capacidad antioxidante, atribuida a sus características moleculares y al tipo, disponibilidad y accesibilidad de los compuestos fenólicos que contienen. Este capítulo proporciona una visión general de los pre-tratamientos y métodos tecnológicos que se han investigado, desarrollado e informado para la modificación de estas características, así como los estudios *in vivo* e *in vitro* que evidencian, el efecto de esas tecnologías, en su bioaccesibilidad y biodisponibilidad.

Palabras clave: arabinoxilanos, compuestos fenólicos, funcionalidad biológica, subproductos, molienda de cereales.

Introducción

Los cereales son los granos o semillas comestibles de la familia *gramíneae*. El fruto de los cereales botánicamente se denomina cariópside y consta de tres partes fundamentales: pericarpio, endospermo y germen (Delcour & Hoseney, 2010). La producción mundial en orden descendente es maíz, arroz, trigo, cebada, sorgo, mijo, avena, centeno y triticale (FAOSTAT, 2018). Particularmente, el maíz, el arroz y el trigo contribuyen con alrededor del 90 % de la producción mundial de cereales.

En México, la producción de maíz para alimento humano es de 14 millones de toneladas, mientras que de trigo y de arroz es 4 millones y 1 millón de toneladas, respectivamente. La producción de sorgo no es para consumo humano y es de 7 millones de toneladas (FAOSTAT, 2018).

Se estima que los cereales aportan entre el 30 y el 70 % de la energía diaria total que consume la población humana, lo que los hace la fuente más importante de calorías con respecto al aporte de otros alimentos. También, son la fuente principal de nutrientes, especialmente cuando se usan como granos integrales. Sin embargo, la mayoría de los granos se procesan aún más después de la limpieza y de la clasificación para producir productos finales útiles para la industria de los alimentos.

Estas operaciones del procesamiento como descascarillado, molienda, refinado, pulido, entre otras, alteran en diversos grados, la composición nutricional del producto resultante. También, se pueden modificar las matrices, el entorno en el que los nutrientes están incrustados en el grano, lo que a su vez influye en su biodisponibilidad (Oghbaei & Prakash, 2016).

En tanto que algunos cereales como el arroz se consumen como granos integrales, la mayoría de los cereales se convierten en harina antes de su uso. En todos los cereales, las principales fuentes de energía son el almidón, las proteínas y, en menor medida, los lípidos. Los granos también contienen, en la pared celular, una variedad de polisacáridos distintos al almidón, no digeribles por el sistema digestivo del humano, nombrados fibra dietética, así como, minerales, vitaminas y fitoquímicos. Los tipos y cantidades relativas de estos nutrientes varían en los diferentes granos. Lo que es común a todos los granos es la necesidad de procesarlos antes del consumo. Para algunos granos, el descascarillado es un paso esencial, mientras que para otros, lo es la molienda del grano en harina.

En consecuencia, se obtienen grandes cantidades de desechos abundantes en fibra dietética, vitaminas, minerales y fitoquímicos, que se utilizan generalmente para la alimentación animal o como materia prima para procesos de bioenergía.

La molienda de los cereales da como resultado la alteración de la calidad nutricional del producto y de los subproductos, que puede ser la reducción de nutrientes, fitoquímicos y antinutrientes o una mejora en la digestibilidad o la disponibilidad de nutrientes. El grado de molienda y refinación puede producir harina muy fina que contiene diferente cantidad de nutrientes en comparación con sus fuentes originales.

Por lo general, la capa externa de los cereales es rica en antinutrientes que se pueden reducir descascarando. La principal diferencia compositiva entre los granos enteros y su forma molida es la disminución de todos los nutrientes que se almacenan en la capa externa: la fibra dietética y los componentes asociados con fibras que incluyen ácido fítico, taninos, polifenoles y algunos inhibidores enzimáticos como el inhibidor de la tripsina, así como algunos minerales y vitaminas (Oghbaei & Prakash, 2013).

Es importante entender los cambios que ocurren en la calidad nutricional del grano a causa de la molienda, para seleccionar los pretratamientos y procesos apropiados para obtener los máximos beneficios nutricionales y de salud. En la mayoría de los estudios, se informa que la reducción de los fitatos, los taninos y los compuestos fenólicos conduce a una mejor disponibilidad de minerales y digestibilidad de proteínas y carbohidratos, sin embargo, estos componentes también tienen propiedades antioxidantes que pueden detener la actividad de radicales libres y reducir el estrés oxidativo en el cuerpo humano (Harland & Morris, 1995).

La información acerca del uso potencial del salvado de cereales como ingrediente funcional, así como de las estrategias y propuestas para su incorporación en productos lácteos, barras de cereales, productos horneados y cereales para el desayuno, se tiene disponible en libros y revistas científicas publicados en las últimas dos décadas.

Sin embargo, se percibe la necesidad de proporcionar información sobre los diversos tipos de pretratamientos que se han aplicado a estos subproductos, antes de utilizarlos en la fabricación de productos de panadería, así como, sobre su eficacia para mejorar el contenido de nutrientes, para disminuir los antinutrientes, y para aumentar la digestibilidad y la disponibilidad de los compuestos bioactivos. Es el propósito de este capítulo. Para este fin, se cita una amplia variedad de referencias de investigación primaria y secundaria, que proporcionan una visión general del tema, así como, los resultados obtenidos en los estudios realizados por los integrantes del Cuerpo Académico de Fisicoquímica de Biomoléculas en Alimentos de la Universidad de Sonora.

22.1. Subproductos de la molienda de cereales

La molienda se define como una operación de aplastar, por presión o golpe, a un objeto para disminuir su grosor o espesor a partículas pequeñas o polvo, utilizada especialmente para obtener harina de los cereales (Bender, 2006). El objetivo básico del proceso de molienda es eliminar la cáscara y algunas veces las capas del pericarpio, para producir la harina con un tamaño de partícula variable y que esté libre de impurezas (Oghbaei & Prakash, 2016).

El endospermo comprende alrededor del 80 % del grano entero, mientras que, los porcentajes de germen y de pericarpio varían de acuerdo con el grano (Delcour & Hoseney, 2010). El proceso de molienda puede ser de dos tipos, **1**) en el que el grano entero se convierte en harina sin extraer ninguna parte o, **2**) en el que somete al grano a molienda diferencial para separarlo en diferentes partes (Bender, 2006).

Así, el trigo puede molerse como harina de trigo integral o someterse a molienda de rodillos para obtener, principalmente, la harina de trigo refinada y el salvado, aunque también se producen otros subproductos de diferente origen tisular, así, la capa de aleurona que es parte del endospermo, se separa como parte del salvadillo, que lo enriquece de xilanos, β-glucanos, vitaminas, minerales y fitoquímicos (Hemery et al., 2007). En los flujos de un molino experimental de trigo, la proporción de salvado y de salvadillo que sale dependió de la adhesión entre los tejidos del grano, que, entre el endospermo y la capa de aleurona, es más fuerte en el trigo *durum* que en el trigo panadero, lo que produjo un mayor rendimiento de salvadillo (**Figura 22.1.**) (Pavlovich-Abril, 2015).

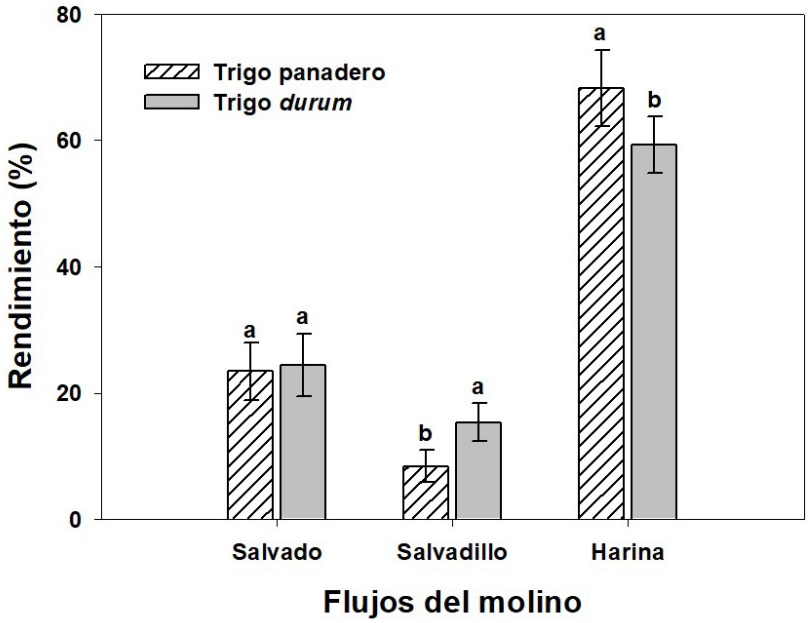


Figura 22.1. Rendimiento de los flujos de un molino harinero experimental de trigo.

A continuación, se describen los tipos de salvado que se obtienen en la industria molinera de los cereales, y que se incorporan en los productos de panadería y en otros productos de cereales, haciendo énfasis en su composición.

22.1.1. Salvado de cereales

El término "salvado" se aplica a una gama de productos derivados de la molienda de los granos de cereales, que, por lo general, se compone de las capas externas del grano, incluyendo a la capa aleurona, la testa y el pericarpio (Delcour & Hoseney, 2010). La mayoría de los salvados de cereales son buenas fuentes de fibra dietética, proteína, minerales y fitoquímicos (Anil, 2012). La composición del salvado de algunos cereales se resume en el **Cuadro 22.1.**, específicamente de fibra dietética total con sus fracciones soluble e insoluble, proteína y cenizas.

Numerosas investigaciones informan que la fracción insoluble de la fibra se ha relacionado con la regulación intestinal, mientras que, la fibra soluble se asocia con reducciones de los niveles de colesterol y de la absorción de la glucosa en el intestino. Los resultados de esas investigaciones, en las últimas tres décadas, respaldan los beneficios de la fibra dietética total en la salud y en la nutrición, mediante la reducción de enfermedades crónicas como las enfermedades cardiovasculares, ciertas formas de cáncer y del estreñimiento.

Cuadro 22.1. Composición química (g/100 g) del salvado en algunos cereales. Las cantidades de fibra dietética total, de fibra soluble y de fibra insoluble de los salvados de cereales son diferentes. Las cantidades de fibra dietética total y fibra dietética insoluble son más altas en el salvado de maíz, en el de sorgo, en el de arroz y en el de trigo, respectivamente.

Cereal	FDT	FDI	FDS	Proteína	Cenizas	Referencias
Trigo	36.08	34.20	1.88	18.17	4.16	Nedeljkovic et al., 2017
Trigo panadero	9.57-28.70	5.39-23.64	4.18-5.06	11.67-15.62	0.91-2.27	Pavlovich-Abril et al., 2015
Trigo *durum*	8.59-19.40	4.63-15.27	3.96-4.13	12.17-14.90	1.74-1.91	Pavlovich-Abril et al., 2015
Maíz	60.13	53.47	6.66	16.48		Liu et al., 2017
Arroz	38.40	34.56	3.84	14.8	9.80	Wen et al., 2017
Sorgo	41.38	40.21	1.17	14.59	4.25	Moraes et al., 2015
Cebada	10.2-48.5	4.1-40.2	6.0-16.6	12.9-22.8	1.0-4.6	Wiege et al., 2016
Avena	15.55	11.47	4.08	17.93	2.63	Nedeljkovic et al., 2017
Centeno	29.90	23.40	6.50	19.00	3.70	Nordlund et al., 2013

FDT, fibra dietética total; FDI, fibra dietética insoluble; FDS, fibra dietética soluble; NR, no reportado.

Aunque la fibra dietética total del salvado de avena es menor que la de algunas de las otras fuentes, la proporción de fibra dietética soluble es más alta que en muchos de los otros cereales. A continuación se presenta una información más amplia para cada salvado del **Cuadro 22.1.**

22.1.2. Salvado de trigo

El salvado de trigo se ha estudiado mucho por su contenido de fibra dietética total y por ser fuente potencial de compuestos bioactivos naturales. Según lo descrito por Onipe et al. (2015), este subproducto de la molienda de trigo está compuesto por 33.4-63.0 g/100 g de fibra dietética total y 9.6-21.9 g/100 g de proteína. Estos autores también informaron del contenido de algunos minerales, que incluyen Fe (19-340 mg/kg), Zn (83-140 mg/kg), Mn (9-101 g/kg), Mg (5300-10300 mg/kg) y P (9000-15000 mg/kg) (Onipe et al., 2015).

La composición química del salvadillo obtenido de un molino experimental de trigo, difiere con el tamaño de partícula. Pavlovich-Abril et al. (2015) fraccionaron con tamices el salvadillo de los dos genotipos de trigo de mayor importancia comercial, *Triticum aestivum* y *Triticum durum*, la fracción más fina (180 a 155 µm), estuvo compuesta con menos fibra y proteína, pero con más almidón, que la fracción de 265 a 180 µm, con más fibra y proteína y menos almidón, lo que repercutió en la calidad del pan.

22.1.3. Salvado de maíz

El salvado de maíz es un subproducto de la molienda seca del maíz, que es un proceso cuyo objetivo es obtener el endospermo para usarlo como sémola y harina, así como de recuperar el germen para obtener aceite (Rose et al., 2010). Este salvado de maíz consiste principalmente de fibra insoluble con ~280 g/kg de celulosa y 700 g/kg de hemicelulosa (Liu et al., 2017).

En la molienda húmeda del maíz, también se obtienen coproductos que incluyen a la fibra de maíz, que, al igual que el salvado, se compone principalmente del pericarpio, sin embargo, la fibra de maíz también contiene material de la pared celular del endospermo, con componentes bioactivos como gomas, geles, xilooligosacáridos, compuestos fenólicos y otros fitoquímicos (Rose et al., 2010; Liu et al., 2017).

La cáscara de maíz nixtamalizado es un subproducto del proceso de masa instantánea de maíz que se separa del endospermo por aspiración y es completamente diferente al subproducto que se obtiene de la molienda seca y de la molienda húmeda del maíz, que contiene 4.5 % de fibra soluble. Esta cáscara al agregarse como ingrediente en formulaciones de tortillas de maíz, galletas y panecillos de trigo, incrementaron significativamente la fibra dietética total y el contenido de fibra dietética soluble en estos productos (Soto-Mendívil & Vidal Quintanar, 2001).

22.1.4. Salvado de arroz

El salvado de arroz constituye aproximadamente el 10 % del peso del arroz integral. Debido a que la mayor parte del arroz se consume como arroz molido, aproximadamente se producen 74 millones de toneladas métricas de salvado de arroz al año, la mayoría de las cuales se subutilizan como alimento para animales o se descartan directamente.

El contenido de fibra dietética total en el salvado de arroz es aproximadamente del 20-30 %, pero casi el 90 % de ese contenido consiste en fibra dietética insoluble (Zhao et al., 2018). Wen et al. (2017) informaron que, en el salvado de arroz desgrasado, los valores de proteína cruda, almidón total, ceniza y humedad fueron 384 g/kg, 148 g/kg, 249 g/kg, 98 g/kg y 102 g/kg, respectivamente. El salvado de arroz superfino exhibió una extractabilidad más alta tanto en fenoles libres como en ligados, una mayor bioaccesibilidad fenólica y en propiedades antioxidantes que su homólogo grueso (Zhao et al., 2018).

22.1.5. Salvado de sorgo

El pericarpio, la testa y el germen del grano de sorgo se separan del endospermo por medio de un método abrasivo conocido como decorticación. Los compuestos fenólicos y la fibra dietética se concentran en el salvado, mientras que el almidón es el componente principal de la sémola (Moraes et al., 2015). El salvado de sorgo es fuente de minerales, fibra y lípidos, y contiene cantidades significativas de compuestos fenólicos, que varía con el grado de pulido del grano (Moraes et al., 2015; Buitimea-Cantúa et al., 2013).

Una característica particular del salvado de sorgo es que está constituido de polisacáridos no almidonados, los glucuronoarabinoxilanos (GAX), que tienen un alto grado de sustitución comparados con los del trigo y de la cebada (Taylor et al., 2006), lo que repercute en una menor disponibilidad de los fitoquímicos. Agregar salvado de sorgo a la harina de maíz nixtamalizado con extrusión, parece ser una buena estrategia para retener los compuestos fenólicos totales en la tortilla (Buitimea-Cantúa et al., 2017).

22.1.6. Salvado de cebada

Es creciente el interés por usar las fracciones de la molienda del grano de cebada, que son abundantes en beta-glucanos, el principal componente de la fibra soluble, y en arabinoxilanos, arabinogalactanos y galactomananos, como ingredientes funcionales para producir alimentos mejorados nutricionalmente ha ido en aumento (Izydorczyk et al., 2011). De las fracciones de la molienda comercial que Wiege et al. (2016) compararon, el contenido más alto de β-glucanos y de arabinoxilanos lo observaron en la harina integral, en la harina del salvadillo, en el salvado grueso y en el salvadillo; siendo el más alto en el salvadillo (14.2-11.3 %).

22.1.7. Salvado de avena

La molienda convencional de la avena incluye descascarillado, secado en horno, corte, cocción al vapor y división en trozos delgados (hojuelas) o molienda en harina de avena. El salvado de avena se separa de la harina en una o varias operaciones de molienda y tamizado (Girardet & Webster, 2011). En el salvado hay una mayor concentración de β-glucanos y arabinoxilanos que en la harina, pero la solubilidad de los arabinoxilanos de la harina es mayor que en los del salvado (Flander, 2012). La importancia nutricional del salvado de avena está en su contenido de vitaminas del complejo B, proteínas, grasas, minerales, β-glucano, arabinoxilano, oligosacáridos, tocoferoles y compuestos fenólicos (Patel, 2015).

22.1.8. Salvado de centeno

El fraccionamiento convencional del salvado de centeno se hace moliendo los granos con un molino de rodillos y separando el salvado del endospermo mediante tamizado. Este salvado contiene proteína, grasa cruda, carbohidratos, fibra dietética, arabinoxilano, β-glucano, celulosa, fructano, ácidos fenólicos, ácido fítico y lipooxigenasa (Nordlund et al., 2013). El alto contenido de compuestos fenólicos del salvado lo hace más estable que el endospermo, porque reducen la formación de los productos de la oxidación de los lípidos.

El alto contenido de arabinoxilanos en las capas externas de los cereales como el trigo, el maíz, el arroz, la cebada, la avena, el centeno y el sorgo, son objeto de estudio

para químicos y tecnólogos, ya que se ha encontrado que tienen una influencia significativa en la calidad de los productos alimenticios. En las últimas décadas, el interés se ha ampliado ante la evidencia de que poseen diversas actividades biológicas, como la disminución del colesterol sérico, la modificación del nivel de azúcar en la sangre, la actividad antioxidante, la reducción de la respuesta glicémica posprandial y la mejora de la inmunidad, así como la capacidad de reducir el riesgo de cardiopatía coronaria y aplicaciones en sistemas de control de peso. En este contexto, a continuación se presentan los avances de los estudios de estos compuestos.

22.2. Los arabinoxilanos de los subproductos de la molienda de cereales

El salvado de los cereales, al estar constituido por los tejidos externos del grano, contiene principalmente polisacáridos no amiláceos, tales como arabinoxilanos (AX), celulosa, β-glucanos y lignina. Los arabinoxilanos son polímeros lineales de xilosa sustituidos principalmente con arabinosa, aunque dependiendo de la fuente botánica, también puede estar la galactosa y el ácido glucurónico.

Los AX pueden tener algunos de los residuos de arabinosa unidos por enlace éster en C (O) -5 a ácido ferúlico (3-metoxi, 4-ácido hidroxicinámico), que forma uniones inter/intra-cadena, que al romperse producen residuos monos o di-ferulados con capacidad potencial de estabilizar radicales libres (Hoffstetter et al., 2018; Kale et al., 2018; Mendis & Simsek, 2014).

En el **Cuadro 22.2.**, se resume que el salvado de los cereales contiene AX en proporciones relativamente altas. Por lo tanto, este subproducto del procesamiento de los cereales es una fuente desiderable de AX. Como resultado de su alto peso molecular y alto contenido de ácido ferúlico, los AXs forman fácilmente enlaces covalentes y no covalentes entre sus cadenas y con otros componentes de la pared celular, tales como proteínas, β-glucanos, lignina y celulosa, de ahí que una gran proporción no puede ser extraída por agua.

Por lo anterior, se han desarrollado varios métodos para su extracción y purificación, incluida la extracción alcalina y ácida, la hidrólisis enzimática, la extracción asistida con microondas y con ultrasonido, extracción por explosión de vapor, extracción con compresión de agua caliente, extracción por expulsión con doble tornillo, la purificación con etanol y la precipitación con sulfato de amonio, entre otros (Zhang et al., 2014).

Cuadro 22.2. Contenido de arabinoxilanos (% b.s.) en el salvado de algunos cereales.

Cereal	Tejido	AXT	AXEA	Referencias
Trigo	Salvado	25	2.8	Hollmann & Lindhauer, 2005
Trigo	Salvado sin almidón	29.1	N.R.	Koegelenberg & Chimphango, 2017
Trigo panadero	Salvadillo	8.5	2.9	Pavlovich-Abril, 2015
Trigo durum	Salvadillo	4.9	3.1	Pavlovich-Abril, 2015
Maíz	Salvado	26	0.7	Zhang et al., 2016
Arroz	Salvado	8.5	0.2	Choct, 1997
Sorgo	Salvado	5.5	-	Qiu et al., 2017
Cebada	Salvado	3.7-9.7	-	Wiege et al., 2016
Cebada	Salvadillo	6.6-11.3	N.R.	Wiege et al., 2016
Avena	Salvado	3.0	0.1	Hashimoto et al., 1987
Centeno	Salvado	13	2.8–4.3	Sárossy et al., 2013

AXT, arabinoxilanos totales; AXEA, arabinoxilanos extraíbles en agua; NR, no reportado.

Las investigaciones señaladas en el **Cuadro 22.2.**, coinciden que los rendimientos de extracción, y las características macromoleculares de los AX varían en función de los métodos de extracción y modificación utilizados. Sin embargo, está claro que los AX de salvados de arroz, sorgo, mijo y maíz tienen cadenas laterales más complejas (incluyendo xilopiranosa, galactopiranosa y ácido α-D-glucurónico o residuos de 4-Ometil-α-D-glucurónico) que los del trigo, centeno y cebada. En general, los AX se entrecruzan con ácido ferúlico en las posiciones C (O) -5 a través de un enlace éster, pero también, las cadenas laterales de ácido ferúlico pueden formar enlaces con β-glucano, celulosa, glucosa y proteína.

Las diferencias más frecuentes entre los AXs de los cereales radican en la sustitución del residuo de arabinosa en la estructura del xilano, en las proporciones relativas y en la secuencia de los enlaces entre estos dos azúcares (xilosa y arabinosa), así como también, en la presencia de otros sustituyentes.

La proporción arabinosa/xilosa (A/X) del endospermo de trigo puede variar de 0.50 a 0.71 pero generalmente es más bajo que el encontrado en el salvado (1.02-1.07). En los AXEA, la A/X fue mayor en el salvadillo de trigo panadero (0.9) que en trigo cristalino (0.4), con algunos enlaces β-(1/3) en el xilano del trigo cristalino (Pavlovich-Abril et al., 2016).

Los AXs del endospermo de centeno están menos sustituidos (0.48-0.55) que en el tejido equivalente del trigo. En contraste, el salvado de maíz generalmente tiene A/X en el rango de 0.75 a 1.82 (Rose & Inglett, 2010). Los AX del salvado de sorgo también tienen un alto grado de sustitución (A/X, 0.9) y contienen ácidos urónicos y sustituyentes ferulados y acetilados (Verbruggen et al., 1993).

Algunos de esos estudios (**Cuadro 22.2.**) y otros (Yuwang et al., 2018; Malunga & Beta 2015; Lin et al., 2014; Veenashri & Muralikrishna, 2011), han demostrado que las bioactividades de los AX se pueden asociar con sus características moleculares, que son específicas del método de extracción.

El tratamiento alcalino es una forma eficiente de extraer los AX de la pared celular de los tejidos. Sin embargo, las propiedades funcionales cambian porque se hidrolizan algunos grupos funcionales, como el ácido ferúlico, y se obtienen fracciones de AX de alto peso molecular (100-200 kDa). Los AXs de salvado de trigo y de arroz con bajo peso molecular (< 100 kDa) tienen propiedades prebióticas potenciales *in vitro* y actividades inmunomoduladoras *in vitro* e *in vivo*, respectivamente.

La producción de fracciones con menor peso molecular que las producidas por la hidrólisis alcalina, se consigue con los tratamientos enzimáticos, pero con un menor rendimiento de extracción. También se han aplicado pretratamientos físicos que causan una variedad de reacciones químicas y transformaciones moleculares, como la extrusión, que aumentó la solubilidad de los AXs extraíbles con agua en la fibra de maíz (Jeon et al., 2014).

Diversos estudios han mostrado las posibles interacciones de los arabinoxilanos con algunos fitoquímicos, especialmente los ácidos fenólicos derivados del ácido cinámico (ácidos hidroxicinámicos). Estos compuestos, particularmente el ácido ferúlico y el ρ-coumárico, se encuentran unidos mediante enlaces éster a las arabinosas. Sin embargo, también se encuentran en forma libre y conjugada.

Otras interacciones son con la lignina por enlaces de éter (Pihlava et al., 2015). En el **Cuadro 22.3.**, se presentan los ácidos fenólicos predominantes en el salvado de los cereales y su actividad biológica, que depende del tipo de ácido del cereal que lo contiene.

Cuadro 22.3. Principales ácidos hidroxicinámicos contenidos en salvado de cereales y su potencial biológico.

Compuestos	Potencial biológico	Salvado	Referencia
Ácido cafeico	Capacidad antioxidante*#+ Inhibición de hemólisis de eritrocitos*	Sorgo*, trigo#, maíz+	Li et al., 2017; Salazar-López et al., 2016; Salazar-López et al., 2017; Vaher et al., 2010.
Ácido cumárico	Capacidad antioxidante*#& Capacidad antiinflamatoria+	Trigo#, sorgo*, maíz+, arroz&	Jun et al., 2015; Kim et al., 2012; Salazar-López et al., 2017; Vaher et al., 2010.
Ácido ferúlico	Capacidad antioxidante*+ & Capacidad antiglicémica* Capacidad antiobesogénica* Capacidad antiinflamatoria*+	Maíz+, sorgo*, arroz&	Jun et al., 2015; Kim et al., 2012; Lopez-Martinez et al., 2009; Salazar-López et al., 2017; Salazar-López et al., 2017.
Ácido gálico	Capacidad antioxidante	Arroz	Jun et al., 2015
Ácido protocateico	Capacidad antioxidante	Arroz, sorgo	Jun et al., 2015; Shelembe et al., 2014
Ácido sinápico	Capacidad antioxidante	Trigo, sorgo, arroz	Jun et al., 2015; Salazar-López et al., 2017; Vaher et al., 2010.
Ácido siríngico	Capacidad antioxidante	Trigo, arroz	Jun et al., 2015; Vaher et al., 2010.
Antocianinas	Capacidad antioxidante+&, capacidad antiproliferativa (MCF-7, cáncer de mama)*	Maíz+, arroz&, sorgo*	Devi et al., 2011; Gul et al., 2015; Lopez-Martinez et al., 2009.
Arabinoxilanos	Capacidad antioxidante*& Capacidad antiglicémica+ Capacidad antitumorogénica#	Sorgo*, maíz+, arroz&, trigo #	Ayala-Soto et al., 2015; Gul et al., 2015; Malunga et al., 2017; Onipe et al., 2015
Carotenoides	Capacidad antioxidante	Trigo	Brewer et al., 2014

Lo anterior sugiere que, en el futuro inmediato, los estudios se dirijan no sólo a optimizar los rendimientos de extracción, sino también, a producir los AX con características moleculares específicas, que se puedan utilizar como ingredientes en alimentos que beneficien la salud del consumidor.

Además de extraer y purificar los polisacáridos y los fitoquímicos que componen la fibra dietética, para añadirlos como aditivos, también se ha explorado la alternativa de agregar directamente las fracciones del grano con alto contenido de fibra dietética, tales como el salvado y el salvadillo, a las formulaciones de los alimentos (Amaya Villalva et al., 2018; Pavlovich et al., 2015; Kaprelyants et al., 2013). Esta última opción parece ser más práctica y simple de implementar. Además, tiene la ventaja económica de utilizar subproductos de la molienda, sin los costos adicionales de la extracción de los compuestos y posterior adición.

La finalidad de estas estrategias es proveer de ingredientes que formen parte de la formulación de un alimento que contribuya a la salud del consumidor. La fibra dietética es importante en la salud intestinal y parece estar significativamente asociada con un menor riesgo de desarrollar enfermedad coronaria, accidente cerebrovascular, hipertensión, diabetes y obesidad. Por otra parte, resulta relevante diseñar estrategias enfocadas a la liberación de compuestos fenólicos asociados a la fibra dietética, de tal manera, que sea posible su absorción en el intestino delgado. O bien, ser liberados en el colon por la acción de la microbiota bacteriana, produciendo metabolitos y un ambiente antioxidante.

22.3. Capacidad antioxidante de los arabinoxilanos y los efectos relacionados con la salud

En años recientes la investigación sobre la estructura y la funcionalidad de los arabinoxilanos, ha repuntado principalmente porque contienen compuestos fenólicos con actividad antioxidante y antiinflamatoria (Aguedo et al., 2014; Salazar-López et al., 2017). La función de los AX en la prevención de problemas crónicos de salud asociados con la producción excesiva de especies reactivas de oxígeno (EROS) y con el estrés oxidativo, está bien reconocida.

En un estudio realizado por Anson et al. (2011), se demostró que la actividad antioxidante del salvado de trigo se incrementó al usar enzimas esterasas, específicamente la feruloil esterasa. Salazar López et al. (2016), observó un incremento de la concentración de ácido ferúlico, al procesar salvado de sorgo con extrusión. Este incremento tuvo una relación directa con la actividad antioxidante *in vitro* y antiinflamatoria en cultivo celular. Chen et al. (2018), demostraron que la suplementación con AX en dietas altas en grasa mejoró el metabolismo de lípidos y daño hepático en modelo murino, además observaron una reducción de la peroxidación lipídica, aumento del superóxido dismutasa y de la glutatión peroxidasa en el hígado.

En la obesidad, la sobrecarga energética activa en el tejido adiposo el proceso de inflamación crónica de bajo grado, produciendo mediadores de inflamación que promueven efectos sistémicos importantes, que pueden resultar en resistencia a la insulina, disfunción metabólica y enfermedad cardiovascular. Existen diferentes rutas que desencadena el proceso inflamatorio a nivel celular.

Una de estas rutas es la activación del factor nuclear NF-kB, el cual puede ser activado por el acoplamiento de diferentes moléculas como la IL-1β o TNF-α con sus receptores celulares, así como también la sobreexpresión de óxido nítrico sintasa inducible (iNOS). Esta cascada de expresión de mediadores inflamatorios también puede activarse por un exceso de radicales libres (estrés oxidativo), donde la función de los antioxidantes no es removerlos totalmente, sino mantenerlos a niveles en los cuales no puedan desencadenar procesos inflamatorios.

Estos mecanismos de inflamación asociados a obesidad y estrés oxidativo se muestran en la **Figura 22.2.** (McArdle et al., 2013; Pashkow, 2011; Rocha & Libby, 2008).

La presencia de ácidos hidroxicinámicos proporciona capacidad antioxidante a los AX. La actividad general de reducción de radicales de xilooligosacáridos y xilanos del salvado de trigo también se ha atribuido a los ácidos hidroxicinámicos (Veenashri & Muralikrishna, 2011). Los estudios de Malunga & Beta (2015) y de Bagdi et al. (2016) informaron que la actividad de eliminación de radicales de los AX se asocia con la presencia de ácido ferúlico. El ácido ferúlico es más abundante en cereales como trigo, arroz, maíz y sorgo (Gallardo et al., 2006; Chiremba et al., 2012).

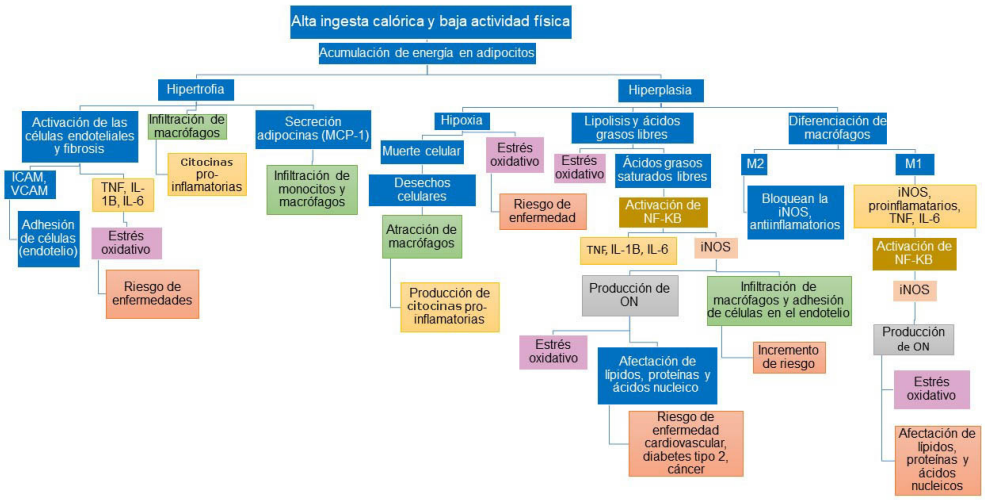


Figura 22.2. Mecanismos de inflamación asociados a obesidad y estrés oxidativo (Tomado de McArdle et al., 2013; Lynch et al., 2012; Maury & Brichard, 2010).

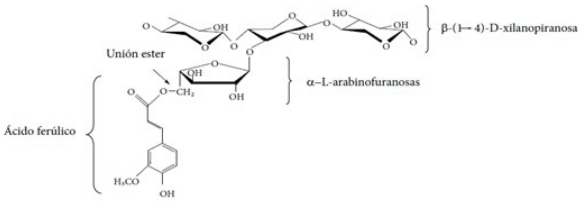
Los estudios presentados han dejado evidencia de la actividad biológica de los AX antioxidantes. No obstante, parte de esta actividad biológica se ve limitada por la disposición estructural de los fenoles asociados a los AX, así como también, por los métodos de separación de los subproductos de la molienda y de los procesos de extracción y purificación de los componentes bioactivos.

Además, el salvado de los cereales es una matriz bioquímicamente activa que compromete su estabilidad. Por lo que actualmente las investigaciones se están dirigiendo a estudiar la aplicación de diversos tipos de pretratamientos a estos subproductos, antes de seguir utilizándolos en la fabricación de productos de panadería.

22.4. Tecnologías para mejorar la funcionalidad biológica de los subproductos de la molienda de cereales

El desarrollo de tecnologías innovadoras de procesamiento parece ser prometedor para mejorar la bioaccesibilidad de compuestos fenólicos asociados a los AX del salvado de los cereales y ejerzan su actividad biológica. Entre las tecnologías de procesamiento que se han desarrollado e informado en la literatura científica, están los tratamientos mecánicos, tales como la molienda ultrafina, la clasificación por aire, y la separación electrostática; y los que utilizan un bioproceso, como los tratamientos enzimáticos, la germinación y la fermentación (**Figura 22.3.**), (Hoffstetter et al., 2018, Rosa et al., 2013; Pekkinen et al., 2014; Wang et al., 2014; Poutanen et al., 2014; Zaupa et al., 2014; Coda et al., 2014).

La germinación y el malteado de los granos enteros, así como la extrusión y la fermentación de los subproductos se asocian con una mejora en el contenido de nutrientes, así como en una disminución de los antinutrientes, lo que aumenta la digestibilidad y la disponibilidad (Gupta et al., 2015; Rasane et al., 2015).



Métodos Químicos	Métodos Termomecánicos	Métodos Biológicos
Hidrólisis ácida Hidrólisis alcalina	Reducción tamaño partícula Extrusión Cocción Microondas	Enzimas esterasas Germinación Fermentación
Ruptura del enlace éster ácido ferúlico-arabinosa	• Aumenta la porosidad de la matriz mejorando la solubilización de los compuestos. • Mayor contacto solvente-compuesto • Formación de oligómeros ferulados	• Ruptura del enlace ester ácido ferúlico-arabinosa • Liberación de ácido ferúlico por actividad bioquímica durante germinación • Liberación de ácido ferúlico por actividad bioquímica de levaduras y bacterias
• Alto grado de liberación de ácido ferúlico. • Puede presentarse degradación de compuestos fenólicos. • Utilizados comúnmente para fines de cuantificación	Su efectividad puede depender de las condiciones del método, tipo de cereal y de la disposición estructural de los compuestos fenólicos	Alto grado de liberación (enzimas esterasas). Su efectividad puede depender de las condiciones de germinación y/ fermentación. Tipo de cereal y de la disposición estructural de los compuestos fenólicos

Figura 22.3. Algunas tecnologías aplicadas para incrementar la disponibilidad de ácido ferúlico asociado a arabinoxilanos de cereales.

En nuestro grupo de trabajo hemos estudiado la aplicación de tratamientos termomecánicos (extrusión) y térmicos (cocción), en harina y en salvado de sorgo, con el fin de evaluar su efectividad para liberar los fenoles ligados a la pared celular. Los resultados indican que es posible tener un aumento de la actividad biológica (antioxidante, inhibición de oxidación de membrana eritrocitaria) y de la actividad antiinflamatoria.

No obstante, se ha documentado ampliamente que la actividad biológica de algunos componentes bioactivos presentes en alimentos no siempre es el reflejo de una activi-

dad biológica *in vivo,* dados los eventos bioquímicos y metabólicos que intervienen en los procesos de digestión, absorción y utilización (Salazar-López et al., 2016; 2017). También, hemos aplicado los tratamientos biológicos, fermentación y enzimáticos, en el salvado de trigo, con resultados de un aumento significativo de la liberación de ácido ferúlico (Amaya-Villalva et al., 2018). Estos estudios han marcado la pauta para continuar explorando otras formas de procesamiento y en otros cereales, como el maíz, con el fin de proponer alternativas dietarías saludables al consumidor.

Los métodos de extracción y purificación de AX de los cereales, que se han desarrollado para aumentar el rendimiento se presentan en la **Figura 22.4**. Entre estos métodos, los tratamientos con álcalis y los métodos asistidos mecánicamente han demostrado ser más eficientes que los otros métodos, cuando se usan a escala de laboratorio.

Sin embargo, se ha informado que la extracción alcalina afecta la estructura molecular de los AX debido a la alteración de los enlaces cruzados, lo que da como resultado AXEA con estructuras moleculares diferentes a las que tienen naturalmente, por lo que resultan con características funcionales diferentes.

Además, la acción de las soluciones alcalinas podría liberar el ácido ferúlico debido a la ruptura del enlace éster entre los AX y la cadena lateral de ácido ferúlico, lo que da como resultado la pérdida de la funcionalidad antioxidante. La extracción alcalina no es amigable con el medio ambiente ya que produce residuos peligrosos.

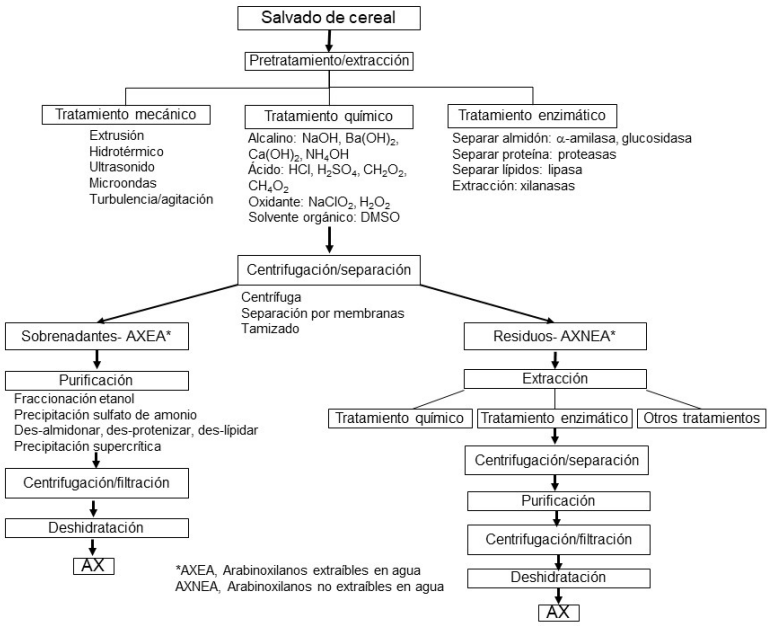


Figura 22.4. Diagrama de flujo del proceso de extracción general de AX del salvado de cereales (Tomado de Zhang et al., 2014).

El tratamiento enzimático proporciona menores rendimientos de extracción en comparación con el tratamiento alcalino, sin embargo, no produce desechos peligrosos como lo hace el tratamiento alcalino. Estos rendimientos pueden mejorarse incorporando un paso de pretratamiento mecánico en el proceso de extracción.

Por lo tanto, una combinación de pretratamiento mecánico o tratamiento con disolvente químico con enzimas puede ser una forma alternativa de aumentar los rendimientos de AX a partir de subproductos de cereales. Los métodos mecánicos, que se han utilizado para mejorar la eficiencia de extracción de AX, incluyen tratamiento asistido por ultrasonido, tratamiento asistido por microondas, reventamiento con vapor y extrusión de doble tornillo. De estas cuatro tecnologías, el reventamiento con vapor y extrusión de doble tornillo son más amigables con el medio ambiente cuando se aplican a escala piloto.

22.5. Perspectivas y conclusiones

El interés creciente en la investigación de polisacáridos contenidos en los subproductos de la molienda de los cereales, en particular, los arabinoxilanos, se dirige a explorar pretratamientos y métodos tecnológicos que modifiquen la disponibilidad y accesibilidad de los compuestos fenólicos que contienen. Así, con la implementación del proceso correcto, el salvado pretratado se podrá usar como ingrediente funcional en la industria de alimentos y aumentar su valor comercial.

En resumen, los resultados de los estudios que aquí se citan, muestran que el método de extracción y de purificación tiene una gran influencia en los rendimientos de extracción de los AX. Por lo tanto, los estudios futuros deberían centrarse en la optimización de los métodos de extracción de los AX, que después se pueden utilizar como ingredientes de alimentos. Además, se necesitan más estudios para delinear los mecanismos exactos que subyacen a los efectos terapéuticos beneficiosos de los AX.

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CAPITULO II

Bioprocessing of wheat (Triticum aestivum cv. Kronstad) bran from Northwest Mexico: effects on ferulic acid bioaccessibility in breads

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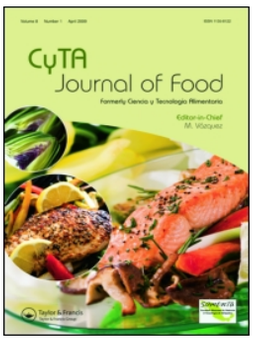

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Bioprocessing of wheat (*Triticum aestivum* cv. Kronstad) bran from Northwest Mexico: effects on ferulic acid bioaccessibility in breads

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ABSTRACT

Evaluating the biological potential of wheat grain cultivated in northwestern Mexico is important because it mainly varies according to the cultivar and production area. The aim of the present study was to evaluate ferulic acid (FA) bioaccessibility in breads supplemented with bioprocessed bran. Bran that was bioprocessed with xylanase enzyme (EB), yeast fermentation (FB), and a combination of both treatments (FEB) showed a higher total phenol content and antioxidant capacity compared with native bran (NB), and the majority of these components were found in a bound fraction. For NB and bioprocessed bran, FA was found at higher levels compared to the other hydroxycinnamic acids evaluated. Bioprocessing increased the free FA content six-fold compared to NB. The % bioaccessibility of FA in different breads increased in the following order: FEB<NB<FB<EB. The addition of bioprocessed bran is may be the simple way to increase the amount of bioactive compounds in a bread product.

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Wheat bran; antioxidant capacity; bioaccessibility; bioprocessing bran; ferulic acid

PALABRAS CLAVE
Salvado de trigo; capacidad antioxidante; bioaccesibilidad; bioprocesamiento del salvado; ácido ferúlico

Bioprocesamiento de salvado de trigo (*Triticum aestivum* cv. Kronstad) del Noroeste de México: Efecto sobre la bioaccesibilidad del ácido ferúlico en panes

RESUMEN

La evaluación del potencial biológico de trigo cultivado en el Noroeste de México es importante porque este varía principalmente según el cultivar y área de producción. El objetivo del presente estudio fue evaluar la bioaccesibilidad del ácido ferúlico (FA) en panes adicionados con salvado bioprocesado. El salvado bioprocesado con enzima xilanasa (EB), fermentación con levadura (FB) y la combinación de ambos tratamientos (FEB) mostraron los valores más altos de contenido de fenoles totales y capacidad antioxidante que el salvado nativo (NB), en donde la fracción ligada presentó la mayor proporción respecto a la fracción libre. Tanto para el NB como para los bioprocesados, el FA se encontró en proporciones más altas respecto a los otros ácidos hidroxicinámicos evaluados. El bioprocesamiento aumentó seis veces más el contenido de FA libre en comparación con el NB. El % de bioaccesibilidad del FA en los diferentes panes se incrementó en el siguiente orden: FEB<NB<FB <EB. La adición de salvado bioprocesado puede ser una manera sencilla de aumentar los compuestos bioactivos en un producto pan.

Introduction

It is widely accepted that whole grains have many healthful properties. Several epidemiological and clinical investigations have demonstrated a significant decrease in morbidity and mortality from cardiovascular and other diseases among cereal consumers (Guo & Beta, 2013; Kristensen et al., 2012; McKeown et al., 2013; Slavin, 2004; Vitaglione, Napolitano, & Fogliano, 2008; Ye, Chacko, Chou, Kugizaki, & Liu, 2012). The positive influence of these grains is attributed to their dietary fiber and antioxidants mainly phenolic compounds. Studies have indicated in mice with diet-induced obesity that enrichment of the diet with enzymatically treated aleurone they have a tendency toward lower body weight gain, visceral adipose tissue accumulation, fasting plasma insulin, and leptin levels (Rosa et al., 2014). On the other hand, studies carried out in healthy men where the bioavailability from ferulic acid (FA) from the bioprocessed bread was two- to three-fold higher that from the control bread. They also found that after *ex vivo* stimulation with LPS the pro-anti-inflammatory cytokines in blood were significantly lower (Mateo-Anson, Aura, et al., 2011). For other hand, it is important to note that free FA can be absorbed directly at the gastrointestinal level, transported, biotransformed and exert its biological action. Additionally, we have to consider that FA bound to food matrix not absorbed, can be released by bacterial enzymes of microbiota present in the large intestine, creating a local antioxidant environment with different health benefits (Pekkinen et al., 2014).

In cereal grains, the most abundant phenolic compounds are hydroxycinnamic acids (HCA); among them, FA is the major component, followed by diferulic, sinapic, ρ-coumaric, and caffeic acids. A high portion of the FA (98%) is found in the outer parts of the wheat grain (aleurone layer and pericarp) (Andersson, Dimberg, Åman, & Landberg, 2014;

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Brewer, Kubola, Siriamornpun, Herald, & Shi, 2014; Ragaee, Guzar, Abdel-Aal, & Seetharaman, 2012).

However, in wheat, FA is linked to the α-L-arabinofuranose side-chains of cell wall arabinoxylans by ester bonds at position O-5; in wheat, FA represents up to 90% of the total phenolic acids, and 99% of this is bound, thus reducing bioaccessibility and consequent bioavailability (Itagaki et al., 2009; Kroon, Faulds, Ryden, Robertson, & Williamson, 1997; Mateo-Anson, Van Den Berg, et al. 2008; Rosa, Barron, Gaiani, Dufour, & Micard, 2013; Acosta-Estrada, Gutierrez-Uribe, & Serna-Saldivar, 2014). It was reported that antioxidant capacity and total phenolic contents were significantly higher in whole meal bread compared to white bread (Yu, Nanguet, & Beta, 2013). However, the release of phenolic compounds from the matrices of bran or increasing their accessibility has proven to be effective in improving bioavailability.

Bioprocessing can increase the bioavailability of nutrients and other compounds through chemical or enzymatic reactions that hydrolyze or release the nutrients from the food matrix (Mateo et al., 2011). The bioprocessing of wheat bran with enzymes and microbes has been performed to improve the technological properties of bran in wheat dough and bread with subsequent improvements in the end product volume, crumb texture, and shelf life (Nordlund, Katina, Aura, & Poutanen, 2013). Bran bioprocessing with enzymes and yeast also increases the content of bioactive compounds in bread with subsequent beneficial physiological effects; after bioprocessing with enzyme addition, the concentration of total phenols increased 20% (Coda, Rizzello, Curiel, Poutanen, & Katina, 2014).

Mateo-Anson, Selinheimo, et al. (2009) found that the bioprocessing of wheat bran increased the content of free phenolic acids in breads compared to unprocessed control breads. The combination of fermentation and the enzymatic treatment of bran increased the amount of free FA in the bread eight-fold from 12 to 100 µg/g of dry matter. Rosa et al. (2013) found that a synergistic combination of xylanase and feruloyl esterase is the most efficient enzymatic treatment, which releases up to 86% of total FA in bioaccessible forms.

Most Mexican consumers are very conservative and generally consume white bread made of 100% white flour; a smaller percentage of the population consumes bread with added bran or whole grain. The low utilization of whole grains occurs primarily because the population is unaware of the benefits to health that can result from the consumption of these products. Bioprocessing techniques, such as use of the xylanases and microbes as dough improvers, have resulted in the generation of new products with potential benefits to health for consumers. Therefore, the objectives of this study were as follows: (1) evaluate the effects of bioprocessing wheat bran on the content of total phenols, HCA, and antioxidant activity and (2) determine the bioaccessibility of total phenols and HCA in breads made from bioprocessed bran by an *in vitro* digestion model.

Materials and methods

Materials

Folin–Ciocalteu reagent, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 6-hydroxy-2,5,7,8 tetramethychroman-2-carboxylic acid (Trolox), and ferulic, ρ-coumaric and sinapic acids were purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, U.S.A. Food-grade β-1,4-xylanase (Xyl) was obtained from Bioxylanase. ®Kerry and bakers' yeast were purchased in a local market. All chemicals were analytical grade.

Sample preparation

Kronstad F2004 variety wheat (*Triticum aestivum*) was kindly donated by "Molino La Fama" S.A. de C.V. Hermosillo, Sonora, México. This is hard wheat that is commonly used for industrial bread production. Wheat grain was cleaned by passing it over a 7/64" sieve to separate broken kernels, branches, and stones; it was subsequently preconditioned to 14% moisture prior to the milling process.

Wheat grain was milled using an experimental roller mill (Model Brabender Senior Quadrumat, Germany, Duisburg) wherein white flour was separated from milling by-products to save bran. To obtain whole flour, a blade mill and corrugated shell (Model 200, Pulvex, S.A. de C.V., Mexico, D. F.) were used together with a sieve for particle sizes less than 0.5 mm. The particle size of bran was reduced to the same size as flour.

Bioprocessing of wheat bran

The bioprocessing of bran was conducted according to a procedure proposed by Mateo-Anson, Selinheimo, et al. (2009) with some modifications. Table 1 includes the amounts of enzyme, yeast and water added to each mixture for the bioprocessing of bran. The following three treatments were performed: (1) fermented bran (FB); (2) enzymatically treated bran (EB); and (3) fermented and enzymatically treated bran (FEB). The three treatments were placed in a proofing cabinet at 30°C with 75–80% relative humidity for 6 h. The bioprocessed brans were freeze-dried and stored at −20°C. Native bran (NB) was used as control.

Bread making procedure

The following breads were prepared: (1) white flour plus 30% native bran bread (NBB); (2) white flour plus 30% fermented bran bread (FBB); (3) white flour plus 30% enzymatic-treated bran bread (EBB); and (4) white flour plus

Table 1. Preparation of mixtures for bran bioprocessing.[a,b]

Tabla 1. Preparación de las mezclas para el bioprocesamiento del salvado.[a,b]

Samples	Bran (g)	Enzyme (g)	Yeast (g)	Water (mL)
NB	110	–	–	390.00
EB	110	0.055	–	389.94
FB	110	–	0.297	389.70
FEB	110	0.055	0.297	389.64

[a]The mixtures contained 22% of bran.
[b]Bran treated with xylanase enzyme (EB) was prepared by mixing 0.05% (w/w) EB. Fermented bran (FB) was prepared by mixing 0.27% (w/w) baker's yeast. Fermented and enzymatically treated bran (FEB) was prepared by mixing 0.05% (w/w) EB and 0.27% (w/w) baker's yeast. Native bran (NB) was used as control.

[a]El salvado fue añadido en un 22%.
[b]El salvado tratado con ezima xilanasa (EB) fue preparado con 0.05% de la enzima xilanasa. El salvado fermentado (FB) fue preparado con 0.27% (w/w) de levadura de panficacion y el salvado fermentado y tratado con la enzima (FEB), fue preparado con un 0.05% (w/w) de la enzima xilanasa y un 0.27% (w/w) de levadura de panificación. El salvado nativo (NB) fue utilizado como control.

30% fermented and enzymatic-treated bran bread (FEBB). In order to compare with other breads traditionally consumed, additionally two breads were prepared: white flour bread (WFB); and whole-meal flour bread (WMB). The baking process was performed according to 10-10 (AACC, 1995)and farinographic measurements were obtained in a Brabender Farinograph/Resistograph using the constant flour weight procedure (54–21, AACC, 2000).

The breads were prepared by mixing all ingredients in a spiral mixer. The water absorption and dough development time were determined according to farinographic measurements. Subsequently, dough was fermented for 30 min at 30°C with 75–80% relative humidity. Next, a first dough degassing was performed using a dough sheeter roller (opening was 9/32 in.). The dough was fermented again for 30 min under the same conditions and was then divided in three parts before undergoing a second degassing with a 3/16-inch opening roller; then, it was molded. Molded dough pieces were fermented for 1 h and then baked for 10 min at 240°C. Loaf volume and weight were determined after 1 h of cooling. Specific volume was determined foreach experimental bread. Bread samples were freeze-dried for chemical analyses.

Free and bound phenolic compounds extraction

Free phenolic compounds from samples (bran and breads) were extracted. Briefly, one gram of each sample was mixed with methanol (80% v/v) and sonicated for 1 h, and samples were centrifuged at 100 × *g* for 15 min. The supernatants were filtered (Whatman No. 1 paper filter). The residues were used to repeat extraction twice. The supernatants were pooled and concentrated to dryness at 50°C on a rotatory evaporator and were then reconstituted to 5 ml with methanol (50% v/v) and stored at −20°C until analysis (Chiremba, Taylor, Rooney, & Beta, 2012).

Bound phenolic compounds were extracted according to the procedure of Guo & Beta (2013). Briefly, the residues obtained from free phenolic compound extraction were dried at 45°C, and 100 mg of dry residue was weighed and mixed with 5 mL 2 M NaOH (degassed). Air was displaced with N_2 for 30 s, and samples were sonicated for 3 h and further acidified to pH 1.5–2 with 6 M HCl. Then, liquid–liquid extractions were conducted using ethyl acetate (7 mL); this procedure was repeated three times, and the organic layer was recovered and evaporated under vacuum to dryness in a rotatory evaporator at 35°C. The residue in the bottom of the flask was reconstituted in 5 mL methanol (50% v/v) and kept at −20°C until analysis.

Total phenolic compounds (TPC) quantification

Free and bound phenolics were quantified using a method based on that of Singleton & Rossi (1965), which was adapted for use with a FLUOstar OPTIMA multidetection microplate reader (BMG LABTECH, Ortenberg, Germany). Briefly, in each well, 10 µL of sample extracts were mixed with 150 µL of Folin-Ciocalteu reagent (2 N diluted 1:10 with deionized water), and 120 µL of sodium carbonate solution (7 g/L) was added to each well and mixed. After incubation in the dark for 1 h, absorbance readings were taken at 765 nm. Total phenolic content was expressed as µg GAE/g d.m. using a standard solution of gallic acid at different concentrations (0–240 µg/mL).

Trolox equivalent antioxidant capacity (TEAC)

The TEAC assay is based on the ability of antioxidant molecules to decolorize the ABTS radical cation. A stable stock solution of ABTS was prepared by reacting 5 mL of an aqueous solution of 7 mM ABTS with 0.088 mL of 148 mM $K_2S_2O_8$. The mixture was allowed to stand in the dark at room temperature for 16–18 h. An ABTS working solution was prepared immediately before use by diluting the stock solution in ethanol (?1:88, v/v) to obtain an absorbance value at 734 nm of 0.7 ± 0.02. The assay for the scavenging capacity of antioxidants for the non-biological radical cation $ABTS^{+\bullet}$ was modified for use with a multidetection microplate reader. In the microplate wells, 280 µL of ABTS working solution was combined with 20 µL of sample (free or bound phenol extracts). The reduction of absorbance at 734 nm was monitored as the ABTS radical scavenging activity. The results were expressed as µmol TE/g d.m. using a Trolox solution for to create a calibration curve (0–120 µg/mL) (Re et al., 1999; Robles Sanchez et al., 2009).

Quantification of hydroxycinnamic acids

The phenolic acid content (free and bound extracts) was quantified using a UHPLC system (Agilent Technologies, 1260, Germany) equipped with a diode array detector (DAD). The separation was achieved with a Zorbax Eclipse Plus-C18 RRHD reversed phase column (1.8 µm particle size 2.1 × 50 mm); column temperature was set to 30°C. Binary gradient elution was employed with A (0.1% acetic acid/water) and B (0.1% acetic acid/methanol) at a flow rate of 0.7 mL/min. The solvent gradient was as follows: initial 91% A and 9% B; 0–11 min, 9% to 14% B; and 11–15 min, 15% B. Peak detection was performed at 280 nm, and quantitation was performed using commercial standards of ρ-coumaric, ferulic, and sinapic acids. The results were expressed as µg phenolic acid/g d.m. (Chiremba et al., 2012).

In vitro digestion

In order to evaluate the effect of bioprocessing wheat bran on bioaccessibility of FA in breads, we performed a gastrointestinal digestion *in vitro* assay, which consisted of a three-step procedure simulating the digestive processes in the mouth, stomach, and small intestine. The large intestinal tract was not taken into account, since *in vivo* food digestion and the absorption of compounds occur primarily in the small intestine (Dziki, Dziki, Baraniak, & Lin, 2009; Oomen et al., 2003).

The gastrointestinal digestion study was performed with the technique developed by Velderrain-Rodríguez et al. (2016), with slight modifications. Briefly, three healthy volunteers selected from the laboratory staff chewed 1 g of each bread for 15 s. After this time, the chewed bread was deposited in tubes, and the volunteers rinsed their mouths twice with 5 ml of water for 60 s and combined these rinses with initial chewed samples. The combined samples were centrifuged at 100 × g for 15 min at 4°C, and supernatants were separated and freeze-dried.

For stomach digestion, the indigestible fraction from mouth digestion was diluted with 5 mL of KCl–HCl buffer (0.2 M pH 1.5), and the solution was then mixed with 667 µL of a solution of pepsin (300 mg/mL) in HCl/KCl buffer. The mixture was stirred for 1 h at 37°C, and the supernatant was

separated and freeze-dried. Finally, the indigestible fraction from stomach digestion was used for intestinal digestion. This fraction was diluted with 9 mL phosphate buffer (pH 7.5), and the solution was then mixed with 1 mL pancreatin (17 mg/mL) in phosphate buffer (pH 7.5). For this stage, 80 mg of bile salts were added, and the mixture was stirred at 37°C for 6 h; the supernatant was separated and freeze-dried.

Calculations

The bioaccessibility (% B) of FA was calculated as the value in the intestinal digestion divided by the original value in the bread samples.

Statistical analysis

All data are reported as the means ± standard deviation for three replicates. The data were tested by ANOVA using statistical software JMP 5.0.1 (U.S.A, SAS institute, Inc.) followed by Tukey's test; differences of $p < 0.05$ were considered significant.

Results and discussion

Phenolic compounds and antioxidant capacity in bioprocessed bran

Wheat cv. Kronstad is largely grown in the Northwest of Mexico; it is classified as hard wheat because this cultivar possesses physical and chemical characteristics that allow bread production with good sensory and nutritive qualities. However, little information exists regarding its bioactive compounds, such as phenolics and antioxidants, which are considered to be contributors to the reduction of chronic degenerative disease risk. There are also few studies of the biological properties of bran, which is the main by-product obtained from industrial milling this cultivar.

In this study, we first evaluated the effect of bran bioprocessing on total phenolic compounds (TPC) (Table 2). A higher proportion of bound phenols relative to free phenols was observed for all bioprocessed brans and NB (73–78%). The EB, FB, and FEB treatments increased the total quantity of bound phenolics by 32%, 42%, and 38%, respectively, compared to NB. Free phenolic compounds also increased notably for all bioprocessed brans with respect to NB ($p < 0.05$). Therefore, the total of phenols (free + bound) was significantly increased. The increase in free phenols using xylanase enzyme (EB treatment) was due the fragmentation of arabinoxylans, which were more soluble during methanolic extraction. The application of yeast (FB treatment) promoted a similar effect of depolymerizing arabinoxylans; it is well known that yeast and other microorganisms produce compounds with depolymerizing properties.

With respect to the increase of bound phenols, it is relevant to mention that both enzyme and yeast bran treatment promoted food matrix degradation with the consequent release of compounds, such as proteins, amino acids, carbohydrates, and flavonoids, among other compounds, which may have undergone polymerization or conjugation reactions. Similar results have been shown by Messia et al. (2016), who found a nearly two-fold increase of soluble arabinoxylans in bran after pre-fermentation with lactic acid bacteria compared with control bran. Coda, Rizzello, Curiel, Poutanen, & Katina, (2014) reported an increase in protein content, peptides, and extractable arabynoxilans and a decrease in starch content in fermented or enzymatically treated bran. The production of these compounds was associated with an increase in total phenols (20%). This study clearly revealed that both native and bioprocessed bran both show higher total phenol content compared with white flour or whole flour.

A study of Liyana-Pathirana, Dexter, & Shahidi (2006) reported that among different fractions of wheat milling of two varieties (Canadian Western Amber Durum, *Triticum turgidum* L. var durum; and hard red spring Canadian Western, *Triticum aestivum* L), bran showed the highest phenols content, and endosperm had the lowest content. These results were reflected in their respective ability to eliminate reactive oxygen species. Additionally, Abozed, El-Kalyoubi, Abdelrashid, & Salama (2014) demonstrated that the phenolic content and antioxidant capacity was higher in bran compared with whole-wheat flour. Yu et al. (2013) found that whole-wheat flour showed a significantly higher antioxidant capacity compared to white flour.

It is to relevant to note that phenolic compounds include a large group of compounds that differ in their chemical and structural characteristics, but in addition to phenol compounds, several molecules, such as proteins, lipids, and carbohydrates, can be detected as phenols by the Folin-Ciocalteu assay. Therefore, we must consider that the enzymatic or fermentation processing of bran may have increased the levels of phenolic or non-phenolic molecules and the potential interactions between them.

These results were consistent with antioxidant capacity evaluated in bioprocessed brans (Table 3); higher antioxidant values corresponded to the bound phenolic fraction but not to the free phenolic fraction. Although the free phenolic fraction also increased, it was not reflected in increased antioxidant capacity values. Adom & Liu (2002) reported in wheat grain, that the highest proportion of phenolic acids was bound (75%), and phenols content of 7.99 ± 0.39 µmol GAE/g.

In our study, we analyzed specific phenolics that are primarily associated with antioxidant properties, such as HCA, which are predominant in wheat bran. Table 4 shows the

Table 2. Total phenolic compounds (TPC) and proportions of free and bound phenolics (%) in bioprocessed bran.

Tabla 2. Polifenoles totales (TPC) y proporciones (%) de extractos de fenoles libres y ligados en salvado bioprocesado.

	TPC		
	Free phenolics	Bound phenolics	Free + Bound phenolics
Samples	µg GAE/g dry matter		
WF*	431.4 ± 50.3 (65%)	234.6 ± 11.4 (35%)	666.0 ± 61.7
WM*	605.0 ± 40.3 (47%)	692.0 ± 60.0 (53%)	1297.0 ± 100.3
NB	1148.9 ± 13.0 (27%)^{b}	3124.9 ± 66.6 (73%)^{c}	4273.8 ± 79.6^{c}
EB	1441.0 ± 151.0 (24%)^{a}	4616.6 ± 403.8 (76%) ^{b}	6057.6 ± 554.8^{b}
FB	1499.6 ± 139.0 (22%)^{a}	5429.6 ± 492.4 (78%) ^{a}	6929.2 ± 631.4^{a}
FEB	1515.1 ± 144.1 (23%)^{a}	5034.2 ± 437.9 (77%) ^{ab}	6549.3 ± 582.0^{ab}

The values are expressed as mean±standard deviation. *Samples were not included in the statistical analysis. $^{a-c}$Different superscripts in a column indicate significant difference ($p < 0.05$).

Los valores se expresan como promedio ± desviación estándar.

*Muestras que no fueron incluidas en el análisis estadístico. $^{a-c}$Diferentes superíndices en una misma columna indican diferencia significativa ($p < 0.05$).

Table 3. Antioxidant capacity (TEAC) and proportions (%) of free and bound phenolic extracts in bioprocessed bran.

Tabla 3. Capacidad antioxidante (TEAC) y proporciones (%) de extractos de fenoles libres y ligados en salvado bioprocesado.

	TPC		
	Free phenolics	Bound phenolics	Free + Bound phenolics
Samples	µmol TE/g dry matter		
WF*	0.4 ± 0.03 (100%)	ND	0.4 ± 0.01
WM*	1.7 ± 0.01 (25%)	5.1 ± 0.23 (75%)	6.8 ± 0.24
NB	2.2 ± 0.01 (24%)^{a}	16.4 ± 0.45 (76%)^{c}	18.6 ± 0.4^{c}
EB	1.8 ± 0.05 (4%)^{c}	45.3 ± 3.64 (96%)^{b}	47.1 ± 3.6^{b}
FB	1.9 ± 0.11 (3%)^{c}	56.4 ± 4.29 (97%)^{a}	58.3 ± 4.4^{a}
FEB	2.0 ± 0.08 (4%)^{b}	49.4 ± 4.97 (96%)^{b}	51.4 ± 5.0^{b}

The values are expressed as mean±standard deviation. *Samples were not included in the statistical analysis. $^{a\text{-}c}$Different superscripts in a column indicate significant difference ($p < 0.05$). ND (antioxidant capacity not detectable).

Los valores se expresan como promedio ± desviación estándar. *Muestras que no fueron incluidas en el análisis estadístico. $^{a\text{-}c}$Diferentes superíndices en una misma columna indican diferencia significativa ($p < .05$). ND (actividad antioxidante no detectable).

effects of bran bioprocessing on ρ-coumaric, ferulic, and sinapic acids. These HCA were measured both in methanolic (free phenolics) and alkaline (bound phenolics) extracts. A larger proportion of HCA was found in bound phenolics compared to free phenolics for both native and bioprocessed bran. These results confirm the data of other authors, who found a higher content of bound HCA in wheat bran (Hemery et al., 2010).

Alkaline hydrolysis is used experimentally because it breaks ester bonds linking phenolic acids in the cell wall and is therefore an effective way to release phenolic compounds from non-starch polysaccharides (Acosta-Estrada et al., 2014). Ragaee et al. (2012) performed a study on 21 varieties of hard and soft wheat obtained from different locations in Ontario, Canada and found that most of the phenolic compounds are present in a bound form (90%).

All of the bran treatments were effective in releasing ρ-coumaric and sinapic acids because a lower quantity of these acids was found in the bound form compared to NB. In the specific case of FA, the enzymatic treatment (EB) was followed by the combined treatment (FEB) as the most effective treatments for releasing this acid; the bound fraction was reduced by 25% and 16%, respectively. Adom & Liu (2002) reported that FA was found predominantly in wheat grain and that it was found mainly in the bound form. Another study by Kim, Tsao, Yang, & Cui (2006) showed that in hard red and soft white wheat bran, most of the phenolic acids are bound, and they mentioned that extracts after alkaline hydrolysis contained the highest antioxidant capacity; only FA was released in a significantly higher amount (1,359–1,934 µg/g).

A reduction in phenolic acid content in the bound phenolic fraction associated with an increase in the free phenolic fraction could indicate that bioprocessing with enzymes, fermentation, or the combination had an effect on the release of these acids. In our study, this result was more evident for FA (five-fold change relative to NB). However, the reduction in the content of bound HCA by EB, FB, and FEB was not proportional to the increase of free phenolics. Apparently, the reduction of bound HCA could be attributed to the possible interaction and/or conjugation with other compounds as well as the polymerization of these phenolics.

Table 4. Hydroxycinnamic acids content (free and bound phenolic extracts) in bioprocessed bran.

Tabla 4. Contenido de ácidos hidroxicinámicos (extractos de fenoles libres y ligados) en salvado bioprocesado.

	Hydroxicinnamic acids		
	Free phenolic	Bound phenolic	Free + Bound phenolic acids
Samples	µg/g dry matter		
ρ-Coumaric acid			
WF*	4.8 ± 0.01	ND	4.8 ± 0.01
WM*	5.0 ± 0.03	55.2 ± 0.07	60.2 ± 0.1
NB	4.9 ± 0.07^{c}	89.2 ± 0.35^{a}	94.1 ± 0.42^{a}
EB	5.5 ± 0.04^{b}	69.2 ± 2.32^{b}	74.7 ± 2.36^{b}
FB	6.3 ± 0.27^{a}	72.4 ± 1.36^{b}	78.7 ± 1.63^{b}
FEB	6.0 ± 0.03^{a}	71.8 ± 2.56^{b}	77.8 ± 2.59^{b}
Ferulic acid			
WF*	3.3 ± 0.07	32.5 ± 0.04	35.8 ± 0.11
WM*	4.8 ± 0.05	298.2 ± 1.3	303.0 ± 1.35
NB	12.6 ± 1.02^{b}	1341.9 ± 2.63^{a}	1354.5 ± 3.65^{a}
EB	66.3 ± 1.16^{a}	997.6 ± 41.5^{c}	1063.9 ± 42.66^{c}
FB	68.0 ± 3.66^{a}	1272.4 ± 19.85^{a}	1340.4 ± 23.51^{a}
FEB	70.1 ± 1.23^{a}	1121.7 ± 25.36^{b}	1191.8 ± 26.59^{b}
Sinapic acid			
WF*	9.4 ± 0.07	ND	9.4 ± 0.07
WM*	10.7 ± 0.10^{d}	202.1 ± 8.82	212.8 ± 8.92
NB	12.2 ± 0.33^{b}	942.9 ± 14.75^{a}	955.1 ± 15.08^{a}
EB	35.4 ± 0.29^{a}	106.7 ± 0.58^{b}	142.1 ± 0.87^{b}
FB	37.3 ± 0.90^{a}	113.9 ± 4.13^{b}	151.2 ± 5.03^{b}
FEB	18.8 ± 14.3^{b}	114.5 ± 1.33^{b}	133.3 ± 15.63^{b}

The values are expressed as mean±standard deviation.

*Samples were not included in the statistical analysis. $^{a\text{-}c}$Different superscripts in a column indicate significant difference ($p < 0.05$). ND (hydroxycinnamic acid not detectable).

Los valores se expresan como promedio ± desviación estándar.

*Muestras que no fueron incluidas en el análisis estadístico. $^{a\text{-}c}$Diferentes superíndices en una misma columna indican diferencia significativa ($p < 0.05$). ND (ácido hidroxicinámico no detectable).

Physical and biological characteristics of breads with bioprocessed bran added

Wheat bran is often used as an ingredient for several bakery products primarily to improve fiber dietary content, but in the last few years, the effects of adding bran on the sensory, nutritive, and biological properties of bread have been studied. In particular, bran can increase the levels of bioactive compounds, such as phenolics. However, the addition of bran has negative effects on the rheological properties of both the dough and the final product. Therefore, several technological procedures have been developed for bran to ensure an increase of the bioactive compounds without affecting the sensory properties of the product.

As part of this study, we evaluated the effects of adding bioprocessed bran to white flour on the physical and biological characteristics of breads. Figure 1 shows the specific volumes of experimental breads including WFB, whole meal bread (WMB), bioprocessed bran breads (EBB, FBB, and FEBB), and native bran bread (NBB). The highest specific volume was for WFB (6 mL/g). The addition of bran to flours had a negative effect on the specific volume of breads compared with WFB. When bran was treated with enzyme, fermented, or the combination was applied, the specific volume was higher than that of NB bread. However, the EBB had a higher specific volume than that of EBB or FEBB. In a study by Coda, Kärki, Nordlund, Heiniö, Poutanen & Katina (2014), breads supplemented with bioprocessed bran showed a 10–40% improvement in the specific volume depending on the particle size and the fermentation time compared to breads containing control bran.

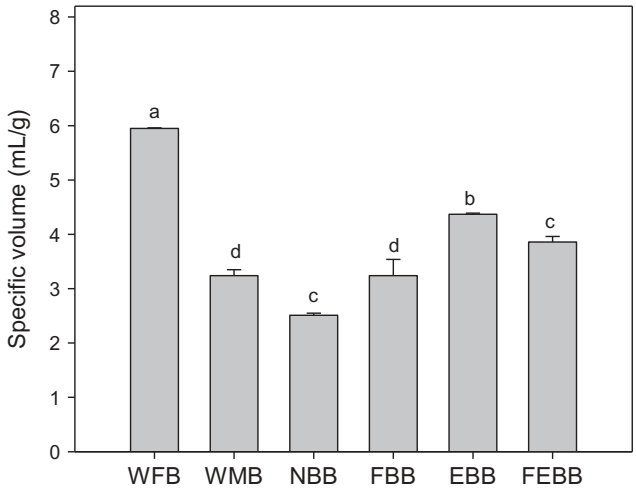

Figure 1. Specific volume of breads.[1] The bars represent the means±standard deviation (n = 3). Different letters on the bars indicate significant difference (p < 0.05). Abbreviations: WFB (white flour bread); WMB (whole meal bread); NBB (native bran bread); FBB (fermented bran bread); EBB (enzymatically treated bran bread); FEBB (fermented and enzymatically treated bran bread).

Figura 1. Volumen específico de los panes.[1] Las barras representan las medias ± desviación estándar (n = 3). Diferentes letras en las barras indican diferencia significativa (p < 0.05). Abreviaturas: WFB (pan de harina blanca); WMB (pan integral); NBB (pan de salvado nativo); FBB (pan de salvado fermentado); EBB (pan de salvado tratado enzimáticamente); FEBB (pan de salvado fermentado y tratado enzimáticamente).

It was known that enzyme treatment with xylanase in flours destined for bread making has a positive influence on the rheological properties and final bread characteristics; the EB modifies the structures of the wall cell and promotes changes that impact water absorption and other farinograph parameters (Blandino et al., 2013). In our study, the addition of enzyme to the bran did not have a great influence on the absorption of water, but the time of development for the dough was greater compared to bread made with NB (data not shown). This result could be related to the higher volume obtained with EBB.

With respect to the biological characteristics of breads, we found a decrease of phenolic compounds (free and bound) compared to that found in their respective bran, these results were mainly due to the dilution effect when the bran (30%) was added to flours. Nevertheless, it was possible to observe that breads showed a similar effect to that found in bioprocessed bran. As a consequence it was observed an increase of phenolics for all breads supplemented with bioprocessed brans compared to bread with NB; both the free and bound phenolic fractions significantly increased compared to NBB (Table 5). The antioxidant capacity in bioprocessed bran breads showed consistent results with total phenolic contents; the antioxidant capacity was higher in bioprocessed bran breads compared to NBB. In addition, the major proportion of antioxidant capacity was found in the bound phenolic fraction (Table 6).

It was evident that both native and bioprocessed bran addition (30%) to flours improved the biological properties of breads compared with WFB and WMB. Similar results were shown by Blandino et al. (2013), who used different wheat bran levels to supplement white flours and found that the nutritional value of the breads improved with the addition of 10% wheat bran. The bread making process may had contributed to these results, particularly because it is well known that during mixing, fermentation, and baking, several reactions can occur to enable the production of phenolic compounds (Dziki, Rozylo, Gawlik-Dziki, & Swieca, 2014; Han & Koh, 2011; Moore, Luther, Cheng, & Yu, 2009).

Bran bioprocessing with enzymes and yeast has also been shown to increase the content of bioactive compounds in bread with potentially positive physiological effects. After bioprocessing with enzyme addition, the concentration of total phenols increased 20% (Coda, Rizzello, Curiel, Poutanen, & Katina, 2014). The effect of adding bioprocessed bran to breads on HCA content was evaluated (Table 7). A decreased bound fraction was observed for all HCA for any bioprocessed bran breads compared to NBB, which was consistent with an increase of the free fraction with the exception of ρ-coumaric acid. This result could indicate that the addition of bioprocessed bran to white flour can contribute to the free phenolic acid content in bread. For all bioprocessed bran breads, the free FA content as increased to a greater extent compared with sinapic acid (24.8–28.4 µg/g vs. 15.1–16.7 µg/g); these values correspond to increases of 40–48% and 13–21%, respectively, compared to NBB.

Table 5. Total phenolic compounds (TPC) and proportions (%) of free and bound phenolic extracts in different breads.

Tabla 5. Polifenoles totales (TPC) y proporciones (%) de fenoles libres y ligados en los diferentes panes.

	TPC		
	Free phenolics	Bound phenolics	Free + Bound phenolics
Samples	µg GAE/g dry matter		
WFB*	744.2 ± 70.6 (100%)	ND	744.2 ± 70.6
WMB*	790.0 ± 60.4 (52%)	725.6 ± 60.9 (48%)	1515.7 ± 121.3
NBB	823.9 ± 24.4 (53%)^{b}	735.5 ± 57.3 (47%)^{c}	1559.5 ± 81.7^{c}
EBB	974.9 ± 75.5 (50%)^{a}	995.7 ± 107.4 (50%)^{b}	1970.6 ± 182.9^{b}
FBB	935.3 ± 48.2 (41%)^{a}	1338.1 ± 143.2 (59%)^{a}	2273.4 ± 191.4^{a}
FEBB	970.2 ± 61.7 (43%)^{a}	1294.5 ± 128.3 (57%)^{a}	2264.7 ± 190.0^{a}

The values are expressed as mean±standard deviation. *Samples were not included in the statistical analysis. $^{a\text{-}c}$Different superscripts in a column indicate significant difference (p < 0.05).

Los valores se expresan como promedio ± desviación estándar. * Muestras que no fueron incluidas en el análisis estadístico. $^{a\text{-}c}$Diferentes superíndices en una misma columna indican diferencia significativa (p < 0.05).

Table 6. Antioxidant capacity (TEAC) and proportions (%) of free and bound phenolic extracts in breads.

Tabla 6. Capacidad antioxidante (TEAC) y proporciones (%) de extractos de fenoles libres y ligados en panes.

	TEAC		
	Free phenolics	Bound phenolics	Free + Bound phenolics
Samples	µmolTE/g dry matter		
WFB*	2.0 ± 0.01 (100%)	ND	2.0 ± 0.01
WMB*	2.1 ± 0.01 (25%)	6.2 ± 0.1 (75%)	8.3 ± 0.1
NBB	2.2 ± 0.01 (24%)^{a}	6.9 ± 0.1 (76%)^{c}	9.1 ± 0.1^{c}
EBB	2.1 ± 0.09 (14%)^{a}	13.3 ± 1.06 (86%)^{b}	15.4 ± 1.1^{b}
FBB	2.1 ± 0.09 (11%)^{a}	17.1 ± 1.87 (89%) ^{a}	19.2 ± 1.9^{a}
FEBB	2.1 ± 0.03 (11%)^{a}	17.9 ± 1.25 (89%)^{a}	20.0 ± 1.2^{a}

The values are expressed as mean±standard deviation.

*Samples were not included in the statistical analysis. $^{a\text{-}c}$Different superscripts in a column indicate significant difference (p < 0.05). ND (antioxidant capacity not detectable).

Los valores se expresan como promedio ± desviación estándar. *Muestras que no fueron incluidas en el análisis estadístico. $^{a\text{-}c}$Diferentes superíndices en una misma columna indican diferencia significativa (p < 0.05). ND (actividad antioxidante no detectable).

Table 7. Hydroxycinnamic acids content (free and bound phenolic extracts) in breads.

Tabla 7. Contenido de ácidos hidroxicinámicos en extractos de fenoles libres y ligados) en panes.

	Hydroxycinnamic acids		
Samples	Free phenolic	Bound phenolic	Free + Bound phenolic acids
	µg/g dry matter		
***ρ*-Coumaric acid**			
WFB*	4.7 ± 0.01	ND	4.7 ± 0.01
WMB*	5.5 ± 0.02	53.4 ± 0.04	58.9 ± 0.06
NBB	5.6 ± 0.01^{a}	57.5 ± 0.06^{a}	63.1 ± 0.07^{a}
EBB	4.7 ± 0.01^{c}	50.4 ± 1.33^{b}	55.1 ± 1.34^{b}
FBB	4.9 ± 0.03^{b}	51.3 ± 1.34^{b}	56.2 ± 1.37^{b}
FEBB	4.9 ± 0.01^{b}	51.9 ± 0.99^{b}	56.8 ± 1.0^{b}
Ferulic acid			
WFB*	3.9 ± 0.01	34.5 ± 0.04	38.4 ± 0.05
WMB*	11.1 ± 0.03	315.1 ± 0.58	326.2 ± 0.6
NBB	14.7 ± 0.01^{c}	440.7 ± 0.47^{a}	455.4 ± 0.48^{a}
EBB	24.8 ± 0.69^{b}	278.5 ± 27.64^{c}	303.3 ± 28.33^{c}
FBB	24.9 ± 0.68^{b}	357.5 ± 26.0^{bc}	382.4 ± 26.68^{bc}
FEBB	28.4 ± 0.19^{a}	366.8 ± 11.28^{ab}	395.2 ± 11.47^{ab}
Sinapic acid			
WFB*	9.9 ± 0.12	ND	9.9 ± 0.12
WMB*	13.6 ± 0.07	244.5 ± 0.7	258.1 ± 0.77
NBB	13.1 ± 0.05^{b}	240.0 ± 0.4^{a}	253.1 ± 0.45^{a}
EBB	15.1 ± 0.01^{ab}	ND	15.1 ± 0.01^{b}
FBB	15.9 ± 1.20^{a}	ND	15.9 ± 1.20^{b}
FEBB	16.7 ± 0.22^{a}	ND	16.7 ± 0.22^{b}

The values are expressed as mean±standard deviation. *Samples were not included in the statistical analysis. $^{a\text{-}c}$Different superscripts in a column indicate significant difference ($p < 0.05$). ND (hydroxycinnamic acid not detectable).

Los valores se expresan como promedio ± desviación estándar. *Muestras que no fueron incluidas en el análisis estadístico. $^{a\text{-}c}$Diferentes superíndices en una misma columna indican diferencia significativa ($p < 0.05$). ND (ácido hidroxicinámico no detectable).

Mateo et al. (2009) found that bioprocessing wheat bran increased the content of free phenolic acids in breads compared to bread controls. The combination of the fermentation and enzymatic treatment (enzyme mixture) of bran increased the amount of free FA in the bread eight-fold, from 12 to 100 µg/g of dry matter. Other studies have been performed with the bioprocessed bran of other cereals, such as rye (Nordlund et al., 2013) and barley (Ktenioudaki et al., 2015). The findings of these studies were similar to ours.

The previous results indicate that the addition of bioprocessed bran may improve the bioactive compound content in bread products; however, it is also important to determine whether these bioactive compounds that are available in the bioprocessed bran breads are truly bioaccessible and consequently bioavailable for utilization such that they can exert any beneficial effects on health, such as protecting against chronic degenerative diseases.

Ferulic acid bioaccessibility in bioprocessed bran breads

Bioaccessibility is a term that reflects the release of nutrients and other components, such as phenolic compounds, from the food matrix. Bioaccessibility can depend not only on the phenolic compounds availability in the food matrix but also on other parameters, such as food matrix complexity, conditions in the gastrointestinal tract, among others. These parameters may limit nutrient release for subsequent absorption (Hemery et al., 2010; Mateo et al., 2009; Saura-Calixto, 2011; Velderrain-Rodríguez, 2016). In this matter, it is very important to considered that digestion process acts as a biological extraction system in which the efficiency of extraction apparently depend of aforementionated.

In our study, we evaluated the behavior of free FA in both native and bioprocessed bran breads during an *in vitro* digestion model, and the results are shown in Figure 2. Compared with the initial values of free FA (Table 7), the mouth digestion process promoted the release free FA from NBB (14.7 vs. 26.3 µg/g d.m + 44%), FBB (24.9 vs. 73.83 µg/g d.m. +66%), EBB (24.8 vs. 58.06 µg/g d.m. +58%), and FEBB (28.4 vs. 52.15 µg/g d.m. +45%). The free FA values were increased to a greater extent under gastric digestion conditions (stomach) for NBB (14.7 vs. 64.4 µg/g d.m. +77%), FBB

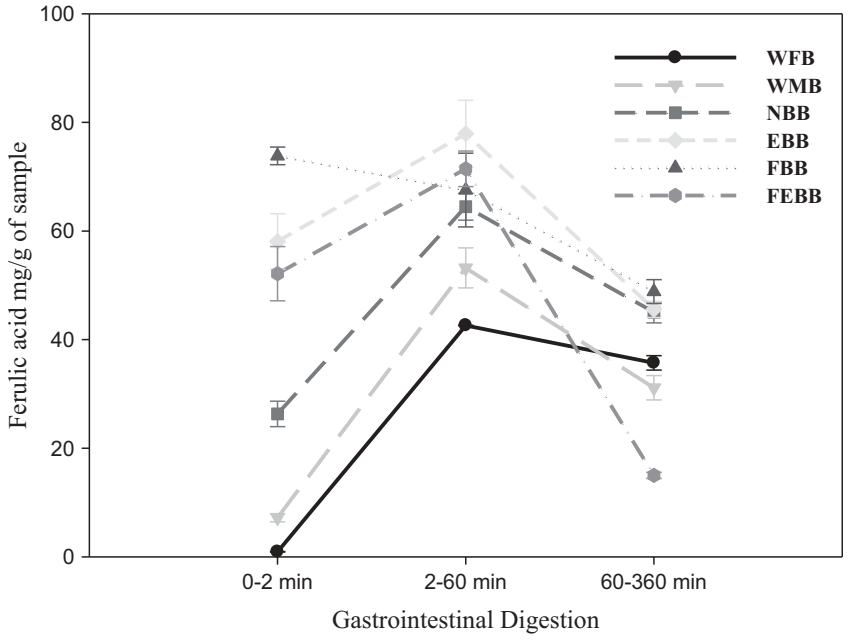

Figure 2. *In vitro* digestion-mediated release of ferulic acid from breads.

Figura 2. Liberación de ácido ferúlico mediada por digestión *in vitro* de panes.

(24.9 vs. 67.5 µg/g d.m. +63%), EBB (24.8 vs. 77.9 µg/g d.m. +68%), and FEBB (28.4 vs. 71.4 µg/g d.m. +60%).

Finally, in intestinal digestion, the free FA values decreased with respect to mouth and gastric digestions but with higher levels than those initials of free ferulic for NBB (14.7 vs. 45.0 µg/g d.m. +67%), FBB (24.9 vs. 48.8 µg/g d.m. +45%), and EBB (24.8 vs. 45.5 µg/g d.m. +48%). The exception was the FEBB sample, which showed a reduction compared with initial values (28.4 vs. 15.0 µg/g d.m. −47%). The free FA levels in WFB and WMB samples showed similar behavior during the digestion process, the values were always less than that obtained with bioprocessed bran bread.

Several studies on wheat bran have been performed to improve the biological potential that is attributed to its phenolic acids; the results have been consistent with the behavior of these acids during *in vitro* digestion. Hemery et al. (2010) studied the effects of dry-fractionated wheat bran added to white flours for bread elaboration on the FA content during *in vitro* digestion and reported a maximal amount of this acid during the gastric phase; they observed decreased levels when the samples were digested under intestinal conditions. Similar results were obtained by Mateo et al. (2009), who made breads with wheat bran (16%) treated with commercial enzymes including xylanases and ferulic esterase; they evaluated the effect of these treatments on *in vitro* digestion, and the results were similar to those obtained in our study.

During the bread *in vitro* digestion, we assume that the mouth digestion promoted a release of FA of food matrix higher to that of initial values due to break of food matrix by chewing effect which increase the surface of contact for salival enzyme (amylase) which catalyze the starch hydrolysis improving the solubilization of FA. The gastric digestion conditions played an important role in the release of FA independently of the technological procedure applied to wheat bran. The acidic conditions and enzymatic action presents during gastric digestion promoted the release of free ferulic additional to mouth digestion.

However, it is important to highlight that the release of FA during intestinal digestion although it was lower than that found in mouth and gastric digestion, is a pivotal event because absorption occurs in this phase. The above results can be understood better when the FA bioaccessibility percentage is calculated.

Table 8 shows the FA (%) bioaccessibility for each bread sample. The WFB shows the highest FA bioaccessibility value (93%); the bioaccessibility values decreased notably in all bread containing bran. WMB and NBB samples showed the same bioaccessibility value (10%), whereas in bioprocessed bran breads, the bioaccessibility was higher for EBB samples (15%), followed by FBB (13%) and FEBB samples (4%).

The FA in the WFB sample was mainly in the bound form, but when this sample was digested, practically all of this acid was released. For this reason, we obtained a high % *B* value. We assumed that the simplicity of the food matrix contributed to this result because this sample consists of starchy endosperm (~90%) that can be easily digested by gastrointestinal enzymes to release FA. In contrast, the bran added to white flour modified the food matrix composition and made it more complex and more difficult to degrade by digestive enzymes, which was reflected in a lower % B compared with WFB. Hemery et al. (2010) and Mateo et al. (2009) found a 10.2 and 4.9% B, respectively, for WFBs. Compared with whole meal breads, they found a reduction of 3.5 – and 4.4-fold, respectively, whereas we found a 9.3-fold reduction in our study.

In our study, bran addition to flours resulted in bioaccessibility values ranging from 4% to 15% B; the higher values were for bread samples with enzymatically treated bran. Hemery et al. (2010) reported FA bioaccessibility values in the range from 2.5% to 5.1% B for breads with bran added, and the higher values were for bread prepared with bran fractionated by electrostatic separation. Mateo et al. (2009) found FA bioaccessibility values for breads with bran added in the range of 1.1–5.5% *B*, and the higher values corresponded to bread prepared with bioprocessed bran (combined fermentation and enzymatic treatment). The use of bioprocessing techniques has been shown to also be a good approach for improving the bioaccessibility of health-promoting compounds in bran (Coda, Rizzello, Curiel, Poutanen, & Katina, 2014). Therefore, the incorporation of wheat bran into cereal-based products is an interesting and low-cost strategy to improve nutritional properties and physiological effects (Hemdane et al., 2015).

Table 8. Amount of bioaccesible ferulic acid (µg/g bread intake) recovered after intestinal digestion of the different breads and % bioaccessibility of ferulic acid.

Tabla 8. Cantidad de ácido ferúlico bioaccesible (µg/g de pan consumido) recuperada después de la digestión intestinal de los diferentes panes y % de bioaccesibilidad de ácido ferúlico.

	Ferulic acid	
Samples	(µg/g)	(%) *B*
WFB*	35.7 ± 1.3	93
WMB*	31.1 ± 2.2	10
NBB	45.0 ± 1.9[a]	10
EBB	45.5 ± 1.5[a]	15
FBB	48.8 ± 2.2[a]	13
FEBB	15.0 ± 0.5[b]	4

The values are expressed as mean±standard deviation; Bioaccessibility (%B) of ferulic acid was calculated related to their initial content (free + bound) in breads. [a-b]Different superscripts in a column indicate significant difference ($p < 0.05$). *Samples were not included in the statistical analysis.

Los valores se expresan como promedio ± desviación estándar; Bioaccesibilidad (%B) de ácido ferúlico se calculó en relación a su contenido inicial en los panes. [a-b]Diferentes superíndices en una misma columna indican diferencia significativa ($p < 0.05$). *Muestras que no fueron incluidas en el análisis estadístico.

Conclusion

The addition of wheat milling by-products, such as bran, is a feasible method to increase the content of bioactive compounds in products, such as bread, while adding functional value due to the increased antioxidant phenolic acid capacity. Bran bioprocessing with EB improved the specific volume of breads and the FA content, which was reflected in increased bioaccessibility. Further studies could focus on human interventions to determine whether the intake of bread prepared with bioprocessed bran has positive effects on biomarkers of chronic diseases, such as obesity, diabetes, and cardiovascular disease.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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CAPITULO III

Bioaccessible ferulic acid in breads with bioprocessed wheat bran added: Effect on apparent absorption

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Dear Dr. Robles-Sanchez,

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Bioaccessible ferulic acid in breads with bioprocessed wheat bran added: Effect on apparent absorption

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Abstract

The potential bioaccessibility and bioavailability of ferulic acid in wheat breads with bioprocessed bran were studied. The aim of the present study was to evaluate apparent absorption under simulated-digestion release of ferulic acid (FA) in breads supplemented with bioprocessed bran. Bran was treated with a mixture of enzymes with xylanase activity (Alphamalt H 19480 0.1 % and 0.05 %). Breads with bioprocessed bran added showed a higher total phenol content and antioxidant capacity (free and conjugated fractions), and the bound fraction of bran with bioprocessed bran decreased significantly compared with breads with native bran added. The bioaccessibility of free FA in bioprocessed bran breads increased by 2.4-fold and 2.3-fold for H 0.1 % and 0.05 %, respectively, compared to bread formulated with native bran. Apparent digestion (dialyzable fraction) in bioprocessed bran bread (H 0.1 % and 0.05 %) showed the highest amount of FA, and for the other breads, there was no detectable amount of this acid. On the other hand, for the non-dialyzable fraction, no significant differences were found between bioprocessed bran breads. The % absorption was 0.9 % and 0.6 % for bioprocessed bran breads with H 0.1 % and 0.05 %, respectively. The antioxidant capacity in the dialyzable fraction was higher in bioprocessed bran bread (H 0.1%) compared to all other breads. Wheat bran breads bioprocessed with enzymes had an improved FA content, which was reflected by their increased bioaccessibility and apparent absorption.

Introduction

The bioprocessing of wheat bran with enzymes and microbes has been performed to improve the technological properties of bran in wheat dough and bread with subsequent improvements in the product volume, crumb texture, and shelf-life [1]. Bioprocessing can increase the bioavailability of nutrients and other compounds through chemical or enzymatic reactions that hydrolyze or release the nutrients from the food matrix [2]. The bioaccessibility is a term that reflects the release of nutrients and other components, such as phenolic compounds, from

the food matrix. Bioaccessibility can depend not only on the availability in the food matrix but also on other parameters, such as the food matrix complexity and conditions in the gastrointestinal tract, among others. These parameters may limit nutrient release for subsequent absorption [3,4,5,6].

Additionally, it is important to establish the bioavailability of bioactive compounds, as this represents the total amount that is released and absorbed mainly in the small intestine, thus reaching the bloodstream, where they are delivered to various body tissues [3]. Recently, findings have been based on developed simulated digestion and absorption methods that have been successfully introduced. However, a high activity *in vitro* does not always translate into a comparable activity *in vivo*, but it could give us an idea of what can occur under *in vivo* conditions [7]. Mateo Anson *et al.* [5] found that the bioprocessing of wheat bran increased the content of free phenolic acids in breads compared to unprocessed control breads. The combination of fermentation and the enzymatic treatment of bran increased the amount of free ferulic acid in the bread 8-fold from 12 to 100 μg/g of dry matter. Rosa *et al.* [8] found that in the wheat aleurone layer, enzymatic treatment with xylanase and feruloyl esterase releases up to 86 % of ferulic acid (conjugated and free forms). Hemery *et al.* [4] reported ferulic acid bioaccessibility percentages in the range from 2.5-5.1 % for breads with bran added, and the higher values were for bread prepared with bran fractionated by electrostatic separation. Mateo Anson *et al.* [5] found ferulic acid bioaccessibility percentages for breads with bran added in the range of 1.1-5.5 %, and the higher values corresponded to bread prepared with bioprocessed bran (combined fermentation and enzymatic treatment).

Pérez-Vicente *et al.* [9], who studied the apparent absorption of phenolic compounds, anthocyanins and vitamin C from pomegranate juice after pancreatin-bile salt digestion, found that 29 %, 3 % and 5 % of these compounds, respectively, were present in the dialyzed fraction, whereas the rest remained in the non-dialyzed fraction. Another study by Swieca *et al.* [7] on wheat bread enriched with green coffee indicated that the adsorption of phenolics, such as ferulic acid, was lower compared to the potentially bioaccessible fraction. The diminished potential bioavailability of phenolics may be partially explained by the use of model systems (passive transport). In addition, an important role has been proven for the interactions of phenolics with other active components and/or food matrices. Such relationships may significantly diminish the bioactivity of phenolics in the upper parts of the digestive tract and may lower the digestibility of nutrients. Therefore, the objectives of this study were as follows: 1) to evaluate the content of total phenols, antioxidant capacity and ferulic acid in bioprocessed wheat bran breads; 2) to determine the bioaccessibility of ferulic acid by an *in vitro* digestion model; and 3) to evaluate the apparent absorption of ferulic acid after simulated *in vitro* digestion.

Materials and Methods

Materials

Folin–Ciocalteu reagent, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (Trolox), and ferulic acid were purchased from the Sigma-Aldrich Chemical Co., St. Louis, MO, USA. Alphamalt H 19480® (H) was kindly donated by Stern Ingredients S.A. de C.V., México. All chemicals were of analytical grade.

Sample Preparation

Kronstad F2004 variety wheat (*Triticum aestivum*) was kindly donated by "Molino La Fama" S.A. de C.V. Hermosillo, Sonora, México. This hard wheat is frequently used for industrial bread production. Wheat grain was cleaned by passing it over a 7/64" sieve to separate broken kernels, branches, and stones; it was subsequently preconditioned to 14 % moisture prior to the milling process.

Wheat grain was milled using an experimental roller mill (Model Brabender Senior Quadrumat, Germany, Duisburg), wherein the white flour was separated from milling by-products to save the bran. To obtain whole flour, a blade mill and corrugated shell (Model 200, Pulvex, S.A. de C.V., Mexico, D. F.) were used together with a sieve for particle sizes less than 0.5 mm. The particle size of bran was reduced to the same size as that of the whole flour.

Bioprocessing of Wheat Bran

The bioprocessing of bran was carry out according to the method proposed by Nordlund *et al.* [1] with some modifications. The enzymatic treatment of bran was applied using Alphmalt H 19480, which is a mixture of standardized enzymes for baking with xylanase activity. Two treatments were performed as follows: 250 g of bran/500 mL water was mixed with 0.1 and 0.05 % (w/w) of Alphamalt H 19480. Both treatments were placed in a proofing cabinet at 40 ° C and 75-80% relative humidity for 4 h. The bioprocessed bran samples were freeze-drying and stored at -20 °C until the time of bread preparation. Native bran was used as a control.

Bread-Making Procedure

The following experimental breads were prepared: (1) white flour bread (WFB); (2) whole meal flour bread (WMB); (3) white flour plus 30 % native bran bread (NBB); (4) white flour plus 30 % Alphamalt H 19480 0.1 % bran bread (H 0.1% BB) and (5) white flour plus 30 % Alphamalt H 19480 0.05 % bran bread (H 0.05 % BB). The baking process was performed according to the AACC [10], and farinographic measurements were obtained in a Brabender Farinograph/Resistograph using the constant flour weight procedure [11].

The breads were prepared by mixing all ingredients in a spiral mixer. The water absorption and dough development time were determined according to farinographic measurements. Subsequently, the dough was fermented for 30 min at 30 ° C with 75-80 % relative humidity. Next, a first dough degassing was performed using a dough sheeter roller (opening of 9/32 inches). The dough was fermented again for 30 min under the same conditions and was then divided in 3 parts before undergoing a second degassing with a 3/16-inch opening roller; then, it was molded. Molded dough pieces were fermented for 1 hour and then baked for 10 min at 240 ° C. The loaf volume and weight were determined after 1 h of cooling. The specific volume was determined for each experimental bread. Bread samples were freeze-dried for chemical analyses.

Free, Conjugated and Bound Phenolic Extraction

Free phenolic compounds from sample breads were extracted. Briefly, one gram of each sample was mixed with methanol (80 % v/v) and sonicated for 1 h, and the samples were

centrifuged at 100 x g for 15 min. The supernatants were filtered (Whatman No. 1 paper filter). The residues were used to repeat the extraction twice. The supernatants were pooled and concentrated to dryness at 50 ° C on a rotatory evaporator and were then reconstituted to 5 mL with methanol (50 % v/v), followed by storage at -20 ° C until analysis [12].
Conjugated phenolic compounds were extracted as proposed by Adom and Liu [13] from the mixing of the free phenolic extract (1 mL) of each sample with 5 mL of 2 M NaOH (degassed). Air was displaced with N_2 for 30 secs, and the samples were sonicated for 1 h, followed by further acidification to a pH of 1.5-2 with 6 M HCl. Then, liquid-liquid extractions were conducted using ethyl acetate (6 mL); this procedure was repeated three times, and the organic layer was recovered and evaporated under vacuum in a rotary evaporator at 35 ° C. The residue in the bottom of the flask was reconstituted in 2 mL of methanol (50 % v/v) and kept at -20 ° C until analysis.

Bound phenolic compounds were extracted according to the procedure of Guo and Beta [14]. Briefly, the residues obtained from the free phenolic compound extraction were dried at 45 ° C, and 100 mg of dry residue was weighed and mixed with 5 mL of 2 M NaOH (degassed). Air was displaced with N_2 for 30 secs, and samples were sonicated for 3 h, followed by further acidification to a pH of 1.5-2 with 6 M HCl. Then, liquid-liquid extractions were conducted using ethyl acetate (7 mL); this procedure was repeated three times, and the organic layer was recovered and evaporated under vacuum in a rotary evaporator at 35 ° C. The residue in the bottom of the flask was reconstituted in 5 mL of methanol (50 % v/v) and kept at -20 ° C until analysis.

Total Phenolic Content (TPC) Quantification

Free, conjugated and bound phenolics were quantified according to the method of Singleton and Rossi [15], which was adapted for use with a FLUOstar OPTIMA multidetection microplate reader (BMG LABTECH, Ortenberg, Germany). Briefly, in each well, 10 μL of sample extract was mixed with 150 μL of Folin-Ciocalteu reagent (2 N, diluted 1:10 with deionized water), and 120 μL of sodium carbonate solution (7 g/L) was added to each well, followed by mixing. After incubation in the dark for 1 h, absorbance readings were taken at 765 nm. The total phenolic content was expressed as μg GAE/g dry sample using a standard solution of gallic acid (0-240 μg/mL).

Trolox Equivalent Antioxidant Capacity (TEAC)

The TEAC assay is based on the ability of antioxidant molecules to decolorize the ABTS [2, 2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation [16]. A stable stock solution of ABTS was prepared by reacting 5 mL of an aqueous solution of 7 mM ABTS with 0.088 mL of 148 mM $K_2S_2O_8$. The mixture was allowed to stand in the dark at room temperature for 16–18 h. An ABTS working solution was prepared immediately before use by diluting the stock solution in ethanol (~1:88, v/v) to obtain an absorbance value at 734 nm of 0.7±0.02. The assay for the scavenging capacity of antioxidants for the non-biological radical cation ABTS+• was modified for use with a multidetection microplate reader. In the microplate wells, 280 μL of ABTS working solution was combined with 20 μL of sample (free or bound phenolic extracts). The reduction of the absorbance at 734 nm was monitored as the ABTS radical scavenging activity. The results were expressed as μmol TE/g dry sample using a Trolox solution to create a calibration curve (0-120 μg/mL) [16, 17].

Quantification of Hydroxycinnamic Acids (HCAs)

The phenolic acid content (free, conjugated and bound extracts) was quantified using a UHPLC system (Agilent Technologies, 1260, Germany) equipped with a diode array detector (DAD). The separation was achieved with a Zorbax Eclipse Plus-C18 RRHD reversed phase column (1.8 µm particle size 2.1x50 mm); the column temperature was set to 30 ° C. Binary gradient elution was employed with solvents A (0.1 % acetic acid/water) and B (0.1 % acetic acid/methanol) at a flow rate of 0.7 mL/min. The solvent gradient was as follows: initial 91 % A and 9 % B; 0–11 min, 9 % to 14 % B; and 11–15 min, 15 % B. Peak detection was performed at 280 nm, and quantitation was performed using commercial standards of ferulic acid. The results were expressed as μg phenolic acid per gram of dry sample [12].

Digestion *in vitro*-Bioaccessibility

To determine the effect of bioprocessing wheat bran on the bioaccessibility from breads, it was necessary to perform an *in vitro* gastrointestinal digestion assay, which consisted of a three-step procedure simulating the digestive processes in the mouth, stomach, and small intestine. The large intestinal tract was not considered since *in vivo* food digestion and the absorption of compounds occur primarily in the small intestine [18, 19].

The gastrointestinal digestion study was performed with the technique developed by Velderrain-Rodríguez *et al.* [3] with slight modifications. Briefly, 1 g of each bread was chewed for 15 secs by three healthy volunteers selected from the laboratory staff. After this time, the chewed bread was deposited in tubes, and the volunteers rinsed their mouths twice with 5 mL of water for 60 secs. These rinses along with the initial chewed samples were transferred into a 50 mL screw top polypropylene tube that contains mouth digest. Blanks were prepared without the food matrix following the same simulated digestion conditions as the samples.

For stomach digestion, the mouth digest was diluted with 5 mL of KCl-HCl buffer (0.2 M, pH = 1.5), and the solution was then mixed with 667 µL of a solution of pepsin (300 mg/mL) in HCl/KCl buffer. The mixture was incubated for 1 h in a shaking water bath (Precision Scientific Mod. 66800, Winchester, VA, USA) at 37 ° C and 100 rpm to obtain the gastric digest. The digestion product from the gastric phase was used for intestinal digestion. This fraction was diluted with 9 mL of phosphate buffer (pH = 7.5), and the solution was then mixed with 1 mL of pancreatin (17 mg/mL) in phosphate buffer (pH = 7.5). For this stage, 80 mg of bile salts was added, and the mixture was incubated for 6 h in a shaking water bath at 37 ° C and 100 rpm to obtain the intestinal digest.

Finally, the products of the three digestion phases (mouth, gastric and intestinal) and the blanks were centrifuged for 10 min at 3000 rpm and 4 ° C; then, the supernatants were recovered and lyophilized. The lyophilized samples were dissolved in 5 mL of 50% methanol, filtered (Econofltr Nyln 0.25 mm 0.45 µm, Santa Clara, CA, United States) and stored at -20 ° C until analysis.

Absorption *in vitro*-Bioavailability

The intestinal phase with dialysis was performed with the technique developed by Bouayed *et al.* [20] with slight modifications. Segments of dialysis bags (cellulose membrane with a molecular weight cutoff of 12000 Da; Sigma, Steinheim, Germany) were cut to a specified

length (10.5 cm), rinsed (outer and inner surfaces) with 0.9 % NaCl solution and then one end of each segment was sealed with clips. The bags were filled bubble-free with 5.5 mL of NaCl (0.9 %), and 5.5 mL of $NaHCO_3$ (0.5 M) was added to neutralize the gastric samples. The bags were sealed with clips and completely immersed into the gastric digest immediately after digestion. The samples were then incubated for 45 min in a shaking water bath at 37 ° C and 100 rpm. Afterwards, 18 mL of a mixture of pancreatin and porcine bile extract (2 mg/mL pancreatin and 12 mg/mL bile extract dissolved in 0.1 M $NaHCO_3$) was added to the digest, which was further incubated in a shaking water bath for an additional 2 h at 37 ° C. The contents of each dialysis bag were then transferred into a Falcon tube (dialyzable fraction), freeze-dried and re-suspended with 5 mL of MeOH (50 %). The non-dialyzable fraction was freeze-dried, and afterwards, from the residues obtained, bound ferulic acid was extracted, followed by storage at -20 ° C until analysis. The apparent absorption (A %) was calculated from the results obtained in the dialyzable fraction in relation to the total amount of ferulic acid in the bread extract.

Statistical analysis

All data are reported as the mean ± standard deviation for three replicates. The data were tested by ANOVA using the statistical software JMP 5.0.1 (USA, SAS institute, Inc.), followed by Tukey's test; differences of $P \leq 0.05$ were considered significant.

Results and Discussion

It is currently well known that many bioactive compounds are concentrated in wheat bran, and given the intense search for foods with healthy properties, this by-product of wheat milling could function as an effective strategy to increase the content of phenolic compounds in bakery products [7]. In wheat bran, the most abundant phenolic compounds are hydroxycinnamic acids, mainly ferulic acid that is found primarily in the bound form, thus reducing its bioaccessibility and consequently its bioavailability [8, 21, 22, 23, 24]. Bran bioprocessing with hydrolytic enzymes has also been shown to increase the content of bioactive compounds in bread with potentially positive physiological effects. It has been reported that after bioprocessing with the addition of enzymes, the concentration of total phenols increased by 20 % [25].

Table 1 shows the total phenolic contents of the experimental breads, and we found an increase in the free and conjugated phenolic fractions for all breads enriched with bioprocessed bran compared to bread with native bran added, followed by whole meal bread and white flour bread. With regards to the bound phenolic fraction in bioprocessed breads, it was decreased significantly compared to native bran bread and white flour bread but did not exhibit significant differences from whole meal bread. The antioxidant capacity of bioprocessed bran breads showed consistent results with the total phenolic content; the antioxidant capacity was higher in bioprocessed bran breads (free and conjugated fractions) compared to native bran bread (Table 2).

Table 1. Total phenolic content (TPC) and proportions (%) from the free, conjugated and bound fractions in breads with bioprocessed bran.

	Free	Conjugated	Bound	Total
Breads				
WFB	334.0±17.9^{d} (29%)	145.4±4.4^{c} (13%)	669.5±61.5^{b} (58%)	1148.9±83.8^{d}
WMB	584.2±29.0^{c} (26%)	246.8±24.0^{b} (11%)	1436.0±139.7^{a} (63%)	2267.0±192.7^{b}
NBB	666.3±29.7^{b} (27%)	258.0±21.1^{b} (10%)	1575.7±160.4^{a} (63%)	2500.0±211.2^{a}
H 0.1% BB	742.1±70.9^{a} (46%)	293.0±8.1^{a} (18%)	586.9±40.2^{b} (36%)	1622.0±119.2^{c}
H 0.05% BB	775.2±44.8^{a} (49%)	306.6±27.6^{a} (19%)	510.1±46.2^{b} (32%)	1591.9±118.6^{c}

Values are the mean of three determinations ± standard deviation. Values in each column for each sample with different superscripts are statistically significant (P≤0.05).

Table 2. Antioxidant capacity measured as the TEAC (μmol TE/g of sample) from the free, conjugated and bound fractions in breads with bioprocessed bran.

	Free	Conjugated	Bound	Total
Breads				
WFB	1.4±0.15^{c} (45%)	0.4±0.03^{c} (13%)	1.3±0.11^{e} (42%)	3.1±0.29^{d}
WMB	2.6±0.20^{b} (17%)	1.3±0.04^{b} (8%)	11.5±0.09^{b} (75%)	15.4±0.33^{b}
NBB	2.6±0.12^{b} (14%)	1.3±0.05^{b} (7%)	14.2±0.35^{a} (79%)	18.1±0.52^{a}
H 0.1% BB	3.5±0.35^{a} (31%)	1.8±0.13^{a} (16%)	6.1±0.80^{d} (53%)	11.4±1.28^{c}
H 0.05% BB	3.6±0.16^{a} (24%)	1.7±0.15^{a} (12%)	9.4±0.86^{c} (64%)	14.7±1.17^{b}

Values are the mean of three determinations ± standard deviation. Values in each column with different superscripts are statistically significant (P≤0.05).

The contents of ferulic acid in breads with bioprocessed bran were evaluated, and the results are shown in Table 3. Increases in the free and conjugated ferulic acid were observed for any bioprocessed bran breads compared to native bran bread, whole meal bread and white flour bread. The addition of 30 % bioprocessed bran to white flour increased the free ferulic acid by 4.4-fold (H 0.1 % BB) and 6.6-fold (H 0.05 % bb), respectively. In the same way, the conjugated ferulic acid content also increased by 1.1-fold (H 0.1 % BB) and 2.6-fold (H 0.05 % BB), respectively, compared to native bran bread. Conversely, the bound ferulic acid of bioprocessed bran breads decreased significantly compared to native bran bread.

Table 3. Ferulic acid (μg/g) content in breads[1].

Breads	Free	Conjugated	Bound	Total
WFB	12.5±0.68^{e}	9.9±0.79^{e}	149.6±6.14^{e}	172.0±7.61^{e}
WMB	26.6±1.43^{d}	93.9±9.18^{d}	1293.7±4.72^{b}	1414.2±15.33^{c}
NBB	39.0±1.23^{c}	121.4±9.45^{c}	1641.9±0.26^{a}	1802.3±10.94^{a}
H 0.1% BB	173.4±2.68^{b}	144.2±8.61^{b}	872.5±12.6^{d}	1190.1±23.89^{d}
H 0.05% BB	259.7±9.01^{a}	323.6±6.28^{a}	1247.8±0.64^{c}	1831.1±15.93^{b}

[1]The results are expressed as μg ferulic acid per gram of sample. Values are the mean of three determinations ± standard deviation. Values in each column with different superscripts are statistically significant (P≤0.05).

Several studies carried out in bread have shown an increase in solubilization of arabinoxylans by addition of enzymes with xylanase activity (Messia *et al* [26]; Nordulund *et al* [1]. In addition, others studies has been focused at effect of xylanases on structural changes in arabinoxylans and their influence on ferulic acid Mateo Anson *et al* [5] found that bioprocessing wheat bran increased the content of free free ferulic acid in breads compared to the control bread. The combination of the fermentation and enzymatic treatment of bran increased the amount of free FA in the bread 8-fold, from 12 to 100 μg/g of dry matter. Similarly, Rosa *et al* [8] found that in the wheat aleurone layer, enzymatic treatment with xylanase and feruloyl esterase releases up to 86 % of ferulic acid (conjugated and free forms).

However, the effects of adding different doses of enzyme on the release of ferulic acid have been barely studied. In our study, we found a higher amount of free ferulic acid when xylanase was added at low concentrations (0.05%), and it would be expected that this effect is also produced when the enzyme is added in larger quantities. To understand this effect, we conducted a brief review of the possible mechanisms that could explain this behavior.

Arabinoxylan (AX) is one of the most important dietary fibers in whole-wheat flour. AX content reaches up to 4.79 – 6.12% in whole grains, but only about 2.5 – 3.0% in refined wheat flour. AX can be divided into water-extractable arabinoxylan (WEAX) and water-unextractable arabinoxylan (WUAX) [27, 28]. The water-unextractable nature of the latter is due to the presence of a combination of non-covalent and covalent interactions between neighboring AX molecules and other cell wall components. Native WE-AX has a high molecular weight and produces a highly viscous solution when introduced into an aqueous media [29, 30]. In both cases, the basic structure of AX is a xylan chain with substitutions of arabinose, which can be found esterified with ferulic acid [27]. It is known that about 99% of the ferulic acid present in wheat grains is present in this way, mainly in bran [2]. As long as the grains do not suffer any kind modification, AX may remain unchanged; however, processing is inevitable if one is to eat this cereal. The baking process begins with the grinding of grains, during which the anatomical parts of the grains (bran, endosperm and germ) are separated; then comes the formation of dough, which is subjected to consecutive fermentations before and during baking to finally produce bread. Grinding, dough formation and fermentation cause significant changes in the structure of AX, which manifest in the rheological properties of dough and, therefore, in the final characteristics of bread [31, 28]. These processes activate endogenous enzymes, particularly endoxylanases, which may be naturally present in whole grains [28, 32]. These enzymes are able to hydrolyze the xylan backbone of AX, transforming WU-AX into WE-AX [33, 34]; depending on the process conditions, the activity of the enzyme can vary, resulting in monomeric products such as xylose, xylobiose, and feruloylated oligomers; the latter ones are associated with the apparent inability of the endoxylanase enzyme to act on xylose units substituted with arabinose [35]. With this degree of degradation of AX, the intervention of other enzymes such as esterases and arabinofuranosidases can hydrolyze the ester linkage between arabinose and ferulic acid, resulting in a higher content of free ferulic acid [36, 37].

The addition of enzymes with xylanase activity (endoxylanases) can substantially change this processes; it is a common practice in the bread making process, not only because it improves the rheological characteristics of the dough but also because it improves the sensory characteristics of the final product, and increases the release of ferulic acid [5].

There are several types of endoxilanase that can be added to flours, each of which has a different effect. For example, the source of endoxylanase (bacterial or fungal) can change its specificity towards the substrate. The amount of enzyme added can cause significant changes in the structure of AX [34], with higher amounts of enzyme contributing to a more complete degradation of AX, even to its constitutive monomers, and possibly to an increase in the release of ferulic acid. However, a series of events could prevent the increased release of ferulic acid. One of these events is related to oxidative gelation, a peculiar property of WE-AX. The phenomenon begins with the combined action of hydrogen peroxide and oxidase peroxide, which catalyze the formation of a radical, which in turn oxidizes the esterified ferulic acid, resulting in the formation of dimers or other forms of isomeric ferulic acid [38, 39]. These dimers can interact with other WE-AX chains through crosslinking and gelation of WE-AX. This effect manifests as an increase in the viscosity of the dough [40]. In this case, it might be possible that endogenous esterases do not have easy access to the ester bonds between ferulic acid and arabinose that hinder the release of ferulic acid, which may not happen if the added concentration of endoxylanase is relatively low and, as consequence, less WU-AX is transformed into WE-AX.

Another possible mechanism that could affect the release of ferulic acid when adding a relatively high amount of endoxylanase is related to the presence of inhibitors of enzyme activity; some of them may be metals (Mn, Mg Ca, Cu etc), or xylose, galactose and xylobiose monomers, or a new group of recently studied inhibitors called TAXI I and TAXI II (*Triticum aestivum* L. endoxylanase inhibitors) [41]. Xylose is produced during the extreme degradation of AX and can act as inhibitor of enzyme activity, reducing the degree of depolymerization and thus the release of ferulic acid. It is worth noting that these mechanisms are not yet well understood, and it may even be possible that they occur simultaneously.

There are other events that could result in a lower concentration of free ferulic acid in the food matrix, such as the transformation of ferulic acid into caffeic acid, which is associated with the oxidation process mentioned before. Or the degradation of ferulic acid into other structural shapes that have not been methodologically detected [40].

Our results indicate that the addition of bioprocessed bran may improve the contents of bioactive compounds in bread products. However, it is also important to determine whether the bioactive compounds that are available in the bioprocessed bran breads are truly bioaccessible and consequently bioavailable for utilization such that they can exert any beneficial effects on health, such as protecting against chronic degenerative diseases.

In vitro Bioaccessibility, Bioavailability and Antioxidant Capacity of Ferulic Acid in Bioprocessed Bran Breads

Bioaccessibility is a term that reflects the release of nutrients and other components, such as phenolic compounds, from the food matrix. The bioaccessibility can depend not only on the availability in the food matrix but also on other parameters, such as the food matrix complexity, and conditions in the gastrointestinal tract, among others. These parameters may limit nutrient release for subsequent absorption [3, 4, 5, 6].

It is important establish the bioavailability of bioactive compounds, as this represents the total amount that is released and absorbed mainly in the small intestine and reaches the bloodstream, where they are delivered to different body tissues [3]. Many factors can be related to the bioavailability and bioaccessibility of bioactive compounds, such as the chemical structure, hydrophobicity, total amount, composition of the food matrix, particle size, enzyme activity, ionic composition, applied mechanical stresses and digestion times of the *in vitro* digestion model for each step (mouth, stomach, and small intestine). *In vivo*, the digestion time depends upon individual characteristics (age, sex, health status, mental state, and time of day) [7, 42].

In our study, we evaluated the behavior of free ferulic acid in both native and bioprocessed bran breads during mouth, stomach and small intestine digestion, and the results are shown in Figure 1. Compared with white flour bread, whole meal bread and native bran bread, all bioprocessed bran breads (H 0.1 % BB and 0.05 % BB) presented higher contents of free ferulic acid during mouth, stomach and small intestine digestion. At the stage of stomach digestion, breads with bioprocessed bran added showed the highest amount of free ferulic acid, and no significant differences were found between treatments. Finally, for small intestine digestion, the amount bioaccessible of free ferulic acid was higher in bioprocessed bran breads, and the ferulic acid amount available increased by 2.4-fold (H 0.1 % BB) and 2.3-fold (H 0.05 % BB) compared with native bran bread, with no significant differences found between treatments.

There are great differences between chemical extraction of ferulic acid and *in vitro* digestion. This because chemical extraction is designed to extract the maximal amount of this compound from the food matrix, including its free, conjugated, and bound forms, while in the simulation of digestion, the conditions, such as the use enzymes, temperature and different pH conditions, might not be favorable for an efficient ferulic acid extraction.

Hemery *et al.* [4] studied the effects of the addition of wheat bran (different particle size) to white flours (16 %) on the ferulic acid content during *in vitro* digestion and reported a maximal amount of this acid during the gastric phase; they observed decreased levels when the samples were digested under intestinal conditions. Similar results were obtained by Mateo Anson *et al.* [5], who made breads with wheat bran (16 %) treated with commercial enzymes, including xylanases and ferulic esterase; they evaluated the effect of these treatments on *in vitro* digestion, and the results were like those obtained in our study. It is important to highlight that the bioprocessing of the bran added to the white flour for the making of the bread increased the free ferulic acid content, and therefore, it is expected that this available amount can be bioaccessible during intestinal digestion

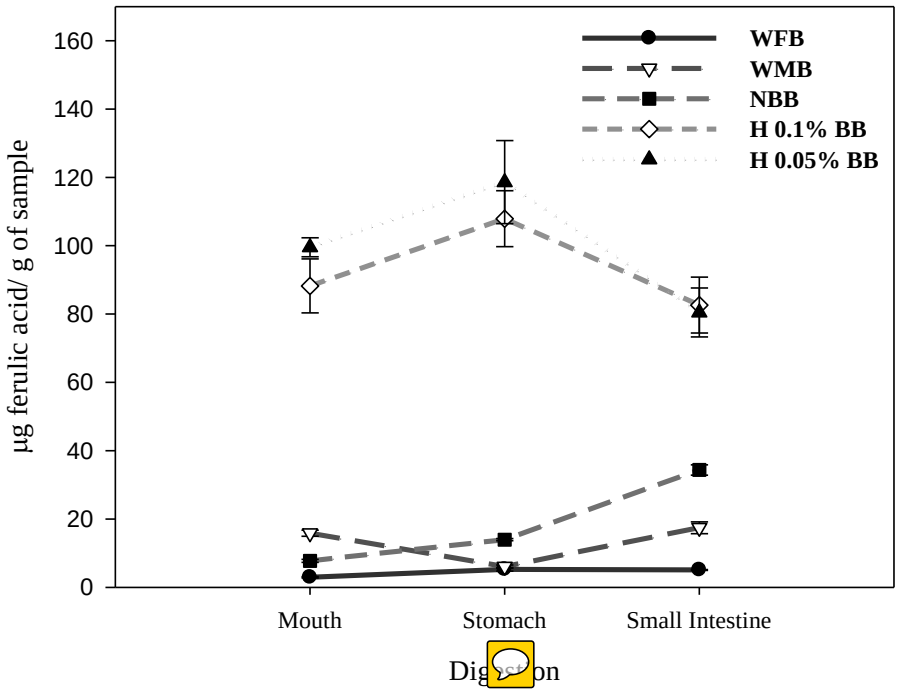


Figure 1. Amount of free ferulic acid, expressed as µg phenolic acid, as determined from the bread matrix (1 g) after simulated *in vitro* digestion.

. However, the digestion conditions were not enough to release the ferulic acid from the food matrix in the same amount that was found before that digestion process was carried out. Recently, findings based on simulated digestion and absorption have been developed and successfully introduced. However, a high activity *in vitro* does not always translate into comparable activity *in vivo*, but it could give us an idea of what can occur under *in vivo* conditions [6].

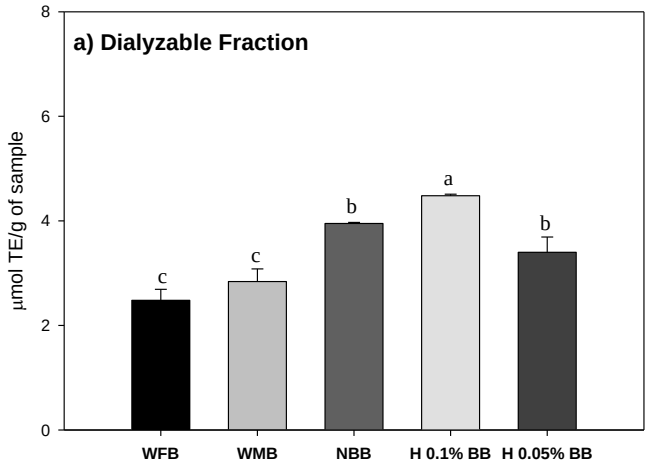
Table 4 shows the amount of ferulic acid recovered in the dialyzable and non-dialyzable fractions. Free ferulic acid was detected in the dialyzable fractions from bioprocessed bran breads, and there were no significant differences between both treatments (H 0.1 % and H 0.05 % BB). Ferulic acid was not detected in the dialyzable fractions of native bran bread, white flour bread and whole meal bread. With respect to the non-dialyzable fraction, it was observed that native bran bread, H 0.1 % and H 0.05 % BB showed the highest amounts of bound ferulic acid, followed by whole meal bread and white flour bread. The apparent absorption (A %) was calculated taking account the initial ferulic acid content in samples before the intestinal digestion process and relating it with the free ferulic acid found in the dialyzable fraction. The ferulic acid released during the intestinal digestion of breads with bioprocessed bran was found in the dialysate fraction, corresponding with a 0.9 % and 0.6 % increase of apparent absorption for the H 0.1 % and H 0.05 % BB samples, respectively. Meanwhile, the ferulic acid released during the intestinal digestion of WFB, WMB, and NBB was not found in this fraction

Table 4. Ferulic acid amount (μg/g) recovered in the dialyzable fraction, non-dialyzable fraction, total (sum of fractions) and apparent absorption (A %).

	Ferulic Acid[1]			
Breads	Dialyzable fraction	Non-dialyzable fraction	Total	A %[2]
WFB	ND	8.2 ± 0.77^{c}	8.2 ± 0.77^{c}	-
WMB	ND	113.7 ± 11.5^{b}	113.7 ± 11.5^{b}	-
NBB	ND	159.8 ± 13.4^{a}	159.8 ± 13.4^{a}	-
H 0.1% BB	11.8 ± 1.3^{a}	146.7 ± 13.5^{a}	158.5 ± 14.8^{a}	0.9
H 0.05% BB	11.4 ± 0.23^{a}	147.4 ± 13.7^{a}	158.8 ± 13.9^{a}	0.6

[1]The results are expressed as μg ferulic acid per gram of sample recovered from the dialyzable fraction, non-dialyzable fraction and total. Values are the mean of three determinations ± standard deviation. [2] (%) the apparent absorption of ferulic acid was calculated in relation to the total amount of ferulic acid in the bread extract. Values in each column with different superscripts are statistically significant ($P\leq0.05$).

It was important to determine the antioxidant capacity of the dialysates to corroborate whether the release of ferulic acid may impact the antioxidant capacity. In Figure 2, the antioxidant capacities measured by TEAC (μmol TE/ g of sample) in both the dialyzable and non-dialyzable fractions are shown. In the dialyzable fraction, it was found that bioprocessed bran bread with H 0.01% showed the highest antioxidant capacity, followed by bioprocessed bran bread with H 0.05% and native bran bread ($P\geq0.05$), while whole meal bread and white flour bread had no significant differences between them. For the non-dialyzable fraction, native bran bread and bioprocessed bran with H 0.05% showed the highest antioxidant capacity, compared with bioprocessed bran with H 0.1%, whole meal bread and white flour bread.



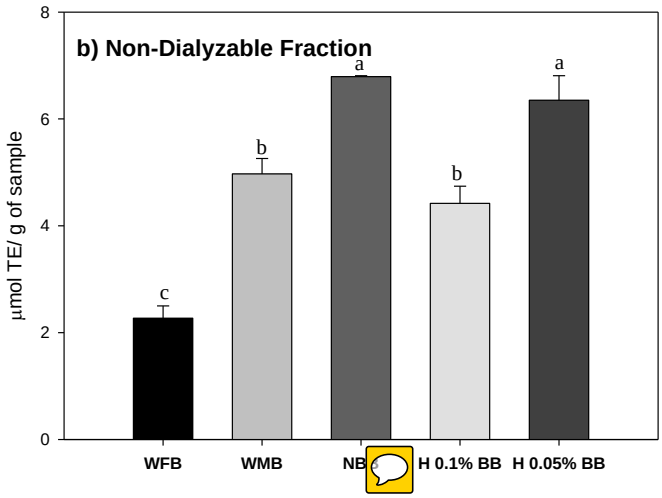


Figure 2. Antioxidant capacity in breads measured by TEAC (µmol TE/ g of sample) for the a) dialyzable fraction and b) non-dialyzable fraction.

According to results in this study, we assume than the modification of the food matrix with enzymes improves the bioavailability and bioaccessibility of free ferulic acid in breads, and this can be reflected in their *in vitro* absorption and antioxidant capacity. Nevertheless, the bioaccessible amount of free ferulic acid found in the small intestine cannot necessarily be absorbed in its totality, and it seems that the bioavailable amount of ferulic acid is less than the bioaccessible amount. This does not mean that it had no role in health protection, as these compounds, if they are not absorbed in the small intestine, can reach the large intestine, where they can be transformed and/or degraded by the colon microflora. The metabolites obtained might have a beneficial effect on the large intestine cells and/or bacteria and be absorbed, thus exerting a biological action away from the large intestine [43].

The study by Gil-Izquierdo *et al.* [43] in orange juice showed that the concentration of flavonoids in the dialyzable fraction is less than that in the non-dialyzable fraction, and they mentioned than in the pancreatin digestion, which takes place in a mild alkaline medium (pH = 7.5), flavanones are partly transformed into chalcones (50-60 %). These compounds are even less soluble than the flavones at this pH, and therefore, they are not available for absorption under the *in vitro* conditions. In pomegranate juice, Pérez-Vicente *et al.* [9] found phenolic compounds, anthocyanins and vitamin C, and their concentrations after the pancreatin-bile salt digestion were analyzed; 29 %, 3 % and 5 %, of these compounds, respectively, were present in the dialyzed fraction, whereas the rest remained in the non-dialyzed fraction. Even if these results show the concentration of phenolic compounds in a soluble form available for absorption, under the conditions of the small intestine, the concentration is lower than in the fresh juice.

Another study by Swieca *et al.* [7] in wheat bread enriched with green coffee indicated that the phenolics, such as ferulic acid, absorbed were lower compared to the potentially bioaccessible fraction and exhibited a significantly higher antioxidant activity (compared to the control). The diminished bioavailability of the phenolics may be partially explained using a semipermeable cellulose membrane model (passive transport). In addition, an important role has been proven for the interactions of phenolics with other active components and/or food matrices, such iron, other minerals, dietary fiber or proteins, and these interactions may significantly diminish the bioactivity of phenolics in the upper parts of the digestive tract,

thus lowering the digestibility of nutrients. The release of free ferulic acid could impact the antioxidant capacity related to the chemical structure of phenolics that also play a role in the free radical-scavenging activity, which is mainly dependent on the number and position of hydrogen-donating hydroxyl groups on the aromatic rings of the phenolic molecules. It has been reported that the antioxidant activity of free phenols is higher than iron-phenol chelates, but on the other hand, aglycones display a higher antioxidant power than their glycosides [20].

Specific volume of breads with the addition of bioprocessed bran

Wheat bran is often used as an ingredient for several bakery products, primarily to improve the dietary fiber content, but in the last few years, the effects of adding bran on the sensory, nutritive, and biological properties of bread have been studied. Several studies have shown that bran can increase the levels of bioactive compounds, such as phenolics [18, 25]. However, the addition of bran could have negative effects on the rheological properties of both the dough and the final product. Therefore, several technological procedures have been improved for bran to ensure an increase in the bioactive compounds without affecting the sensory properties of the product.

As part of this study, we evaluated the effects of adding bioprocessed bran to white flour on the specific volume of breads. Figure 3 shows the specific volume values of experimental breads, including white flour bread, whole meal bread, bioprocessed bran breads (H 0.1 % BB and H 0.05 % BB) and native bran bread. The highest specific volume value was for WFB (4.54 ± 0.09 mL/g). The addition of bran to flours had a negative effect on the specific volume of breads compared with white flour bread. When bran was treated with the enzyme mixture, the specific volume was higher than that of native bran bread. However, the H 0.1 % BB had higher specific volume than the H 0.05 % BB. In a study by Coda *et al.* [25], breads supplemented with bioprocessed bran showed a 10-40 % improvement in the specific volume depending on the particle size and fermentation time compared to breads containing control bran.

It was known that a variety of hydrolytic enzymes in flours destined for bread making have a positive influence on the rheological properties and final bread characteristics; the enzyme mixture modifies the structures of the cell wall and promotes changes that affect the water absorption and other farinograph parameters [44]. In our study, the addition of enzyme to the bran did not have a great influence on the absorption of water, but the time of development for the dough was greater compared to bread made with native bran (data not shown). This result could be related to the higher volume obtained with H 0.1 % BB.

Conclusions

Wheat breads with bioprocessed bran added had improved bioaccessibility of ferulic acid, which was reflected in the increased bioavailability and antioxidant capacity. In addition, the specific volume of bioprocessed bran breads was enhanced. Further studies could focus on human interventions to determine whether the intake of bread elaborated with bioprocessed bran has positive effects on biomarkers of chronic diseases, such as obesity, diabetes, and cardiovascular disease.

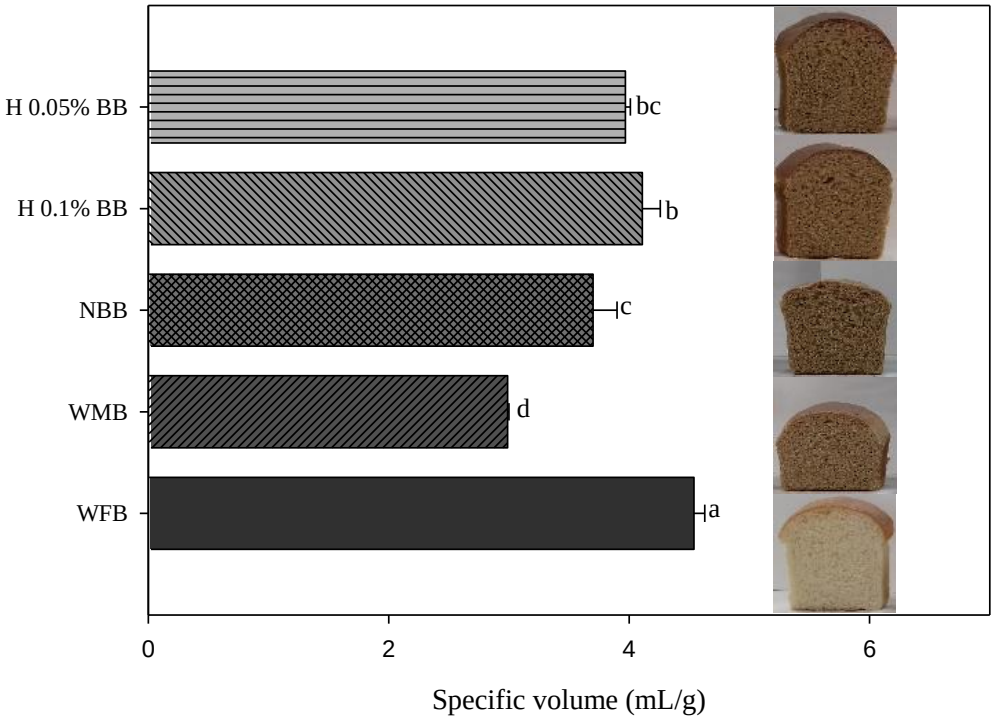


Figure 3. Specific volume of breads.

[1] The bars represent the mean ± standard deviation (n = 3). Different letters on the bars indicate significance (P≤0.05). Abbreviations: WFB (white flour bread); WMB (whole meal bread); NBB (native bran bread); H 0.1 % BB (H 0.1 % bran bread); H 0.05 % (H 0.05 % bran bread).

Conflicts of Interest

No potential conflicts of interest are reported by the authors.

Acknowledgments

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CAPITULO IV

Pro-inflammatory cytokines and antioxidant capacity in serum of obese young people after consumption of bioprocessed bread: Randomized controlled trial

Manuscrito preparado para: Journal of Nutrition & Food Science

Pro-inflammatory cytokines and antioxidant capacity in serum of obese young people after consumption of bioprocessed bread: Randomized controlled trial

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Abstract

Inflammation and oxidative stress have been associated with different pathologies related to obesity, such as insulin resistance, type 2 diabetes, metabolic syndrome and cardiovascular disease. There is a growing interest in the use of natural antioxidants for therapeutic applications. Phenolic compounds are considered as potential therapeutic agents against a wide range of diseases. Ferulic acid (FA) has been identified as the main contributor to antioxidant capacity in wheat milling products. The effect of regular consumption of bread added with bioprocessed wheat bran on biomarkers of oxidative stress and inflammation in serum of obese individuals was evaluated. The study of dietary intervention showed that in obese individuals the consumption of bioprocessed bread showed a trend of greater increase in serum antioxidant activity after seven days of consumption of 50 g of bread / day. The tumor necrosis factor alpha (TNF-α) showed a significant increase after seven days of native bread consumption and bioprocessing. A positive correlation was also found between IL-6 and TNF-α with serum antioxidant capacity. The mechanisms that explain this behavior should be studied in greater depth. However, we can suppose that an increase in the serum antioxidant capacity after the consumption of bread added with bioprocessed wheat bran could be related to the increase in the bioaccessible amount of FA.

Introduction

Currently, overweight and obesity are one of the main public health problems worldwide, according to WHO, in 2016, more than 1,900 million adults were overweight and more than 550 million were obese. In México, according to the national health and nutrition survey (ENSANUT) in 2016, the prevalence of overweight and obesity was 72.5% in adults. Overweight and obesity are defined as an excessive and abnormal accumulation of fat that can be harmful to health. Adipose tissue is composed of adipocytes, preadipocytes, endothelial cells, pericytes, fibroblasts, mast cells, and immune cells (macrophages and T lymphocytes). It exerts its autocrine, endocrine and paracrine functions on the rest of organs through the secretion of a great variety of enzymes, growth factors, hormones and cytokines or interleukins, proteins responsible for the intercellular communication, which induces the activation of specific membrane receptors, cell proliferation and differentiation functions, chemotaxis, growth and modulation of immunoglobulin secretion (Ramírez Alvarado & Sánchez Roitz, 2012; Izaola et al., 2015).

The main function of adipocytes is the storage of fatty acids in the form of triacylglycerols. In obese subjects, the chronic imbalance between calories consumed and spent calories causes an increase in storage in the form of triacylglycerols in adipose tissue, which manifests itself in the following ways: increase in intracellular lipids, increase in size of adipocytes (hypertrophy), increase in the number of adipocytes (hyperplasia). The increase of lipids within the adipocyte, together with hypertrophy and hyperplasia of the adipose tissue, leads to a cellular dysfunction that manifests itself with abnormalities such as the secretion of cytokines and proinflammatory molecules by adipose tissue such as TNF-α, IL-6, the monocyte chemotactic protein-1 (MCP-1), Colony Stimulating Factors (CSF) and inducible nitric oxide synthetase (iNOS), generating the proinflammatory state described and recognized in obese patients (Ramírez Alvarado & Sánchez Roitz, 2012; Choe et al, 2016).

Inflammation and oxidative stress have been associated to different pathologies related to obesity such as insulin resistance, type 2 diabetes, metabolic syndrome and cardiovascular disease. Pro-inflammatory cytokines are potent stimulators of reactive oxygen and nitrogen production by macrophages and monocytes; therefore, an increase in the concentration of cytokines could be responsible for the increase in oxidative stress. IL-6 and TNF-α are among the main mediators of inflammation released by adipose tissue and most studied. Positive associations of IL-6 and serum TNF-α with body mass index (BMI), waist circumference, waist / hip ratio and metabolic syndrome have been demonstrated. On the other hand, leptin, a hormone synthesized by adipose tissue, has been observed to increase in circulation in obese subjects and these increases are proportional to body fat mass. In addition, these mediators of inflammation have been associated with hypertension, diabetes, dyslipidemias, infections and cancer (Zulet and Col., 2007, Fernández-Sánchez and Col., 2011).

Therefore, an effective strategy to promote health and reduce the risk of diseases in obese individuals could be the prevention and treatment of oxidative stress and inflammation associated with obesity. Dietary antioxidants may be a cost-benefit strategy to reduce oxidative stress and inflammation in obese patients. Several studies have already shown the protective effect of whole grains against chronic diseases including cancer and cardiovascular disease and these effects have been attributed to the antioxidants present in these foods. The objective of the study was to evaluate the effect of a bread added with bioprocessed wheat bran in individuals in conditions of obesity on proinflammatory cytokines and antioxidant capacity in serum.

Materials and Methods

Recruitment of participants

Twenty-five volunteers (nine females and sixteen males) were recruited in the Department of Food Research & Graduate Program (DIPA), University of Sonora (México).

Inclusion criteria were as follow: a) age >20years; b) body mass index (BMI) ≥24.99 kg/who were overweight or obese; c) habitual moderate exercise level or sedentary and stable body weights for >1 year; d) not having consumed vitamin supplements in the last three months prior to the study. Before participation in the study, the volunteers signed an informed consent approved by ethics committee in research of the CIAD (Food and Development Research Center, Sonora, México).

Design

This study was a simple randomized controlled clinical trial. A randomization in balanced blocks was carried out, three treatments were blocked according to level of serum total antioxidant activity (TEAC mmolTE/L) from each participant previously measured. Each block (group A, B and C) was given a type of bread respectively (bread A, B and C) that will be described later.

Sample size

The sample size was calculated with paired data by the following formula (Friedman et al., 2015):

$$2N = 4\left(Z_\alpha + Z_\beta\right)^2 \sigma_\Delta^2 / \delta^2$$

Zα= at a level of significance of 0.05 the value is 1.96

Zβ= at a statistical power of 0.90 the value is 1.282

σ_{Δ}^2= The variance is the squared standard deviation, the data was taken from the standard deviation found in the serum total antioxidant activity measured as TEAC of individuals in conditions of obesity according to Amaya-Villalva et al. (2015), the data was 0.07.

δ^2= The delta is the accuracy of the data, the minimum difference to be detected based on previous results. The data was a difference of 0.1.

2N= 4 (1.96 + 1.282)2 (0.07)2/ (0.1)2= 20.60

A sample size of 21 subjects was estimated with a statistical power of 90% and a two-sided alpha error of 0.05.

Intervention

For seven days the participants consumed approx. 50 g of bread daily. They followed specific dietary advices, they should not have consumed bread products, such as: cakes, cookies, muffins, bread sticks or any kind of bread, among others during the intervention. They should not have performed heavy physical activity and avoiding sleep deprivation.

The participants answered a questionnaire of risk factors to detect if they had positive family history of non-transmissible chronic disease (PHF)
(diabetes, cancer and cardiovascular disease), smoking and sedentary lifestyle. Initial clinical analyzes were performed to detect hyperglycemia, hypertriglyceridemia, hypercholesterolemia, low HDL, high LDL, hyperuricemia, insulin and $HOMA_{IR}$, also anthropometric measures to detect overweight, obesity, abdominal (WC) and intra-abdominal obesity (WHR). In total sixteen risk factors were evaluated in all participants.

The intervention was performed in the morning at 8:00 am until half day, the participant they must have consume the portion of the bread during this time lapse. Blood samples were collected in the day 0 before consumption of bread and after the consumption of bread for 7 days.

Bread preparation

The following experimental breads were prepared: (bread A) white flour bread (100 % white flour), (bread B) native bran bread (70 % white flour: 30 % native bran) and (bread C) H 0.05 % bran bread (70 % white flour: 30 % Alphamalt H 0.05 % bioprocessed bran) previously described by Amaya-Villalva et al. (data not published). The baking process was performed according to the Method 10-10, AACC (2000), and farinographic measurements were obtained in a Brabender Farinograph/Resistograph using the constant flour weight procedure (data not shown) (Method 54-21, AACC, 2000).

The breads were prepared by mixing all ingredients in a spiral mixer. The water absorption and dough development time were determined according to farinographic measurements. Subsequently, the dough was fermented for 30 min at 30 ° C with 75-80 % relative humidity. Next, a first dough degassing was performed using a dough sheeter roller (opening of 9/32 inches). The dough was fermented again for 30 min under the same conditions and was then divided in 3 parts before undergoing a second degassing with a 3/16-inch opening roller; then, it was molded. Molded dough pieces were fermented for 1 hour and then baked for 10 min at 240 ° C. The loaf volume and weight were determined after 1 h of cooling. The specific volume was determined for each experimental bread. Bread samples were freeze-dried for chemical analyses.

Biomarker measurements

Blood samples (5 mL) were taken from each study subjects voluntarily and collected in vacutainer tubes, after fasting for 12-14 h on days 0 and 7 of the intervention. Samples were centrifuged at 130 g for 15 min and serum was separated from whole blood. Total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL), tryglicerides (TG), uric acid (UA) and glucose (GLU) in serum were measured by enzymatic methods using commercial test kits (Randox Lab. Ltd., UK). The values of

lipids profile considered as obesity related-indicators were defined as TC (>200 mg/dl), LDL (>130 mg/dl), HDL (male <40 mg/dl, female <50 mg/dl) and TG (≥150 mg/dl) according to National Cholesterol Education Program Adult Treatment Panel III (2001). Hyperuricemia was considered as UA (men >7.0 mg/dl, women >5.7 mg/dl) according to the standard established by the supplier. Hyperglycemia was considered as GLU (≥115 mg/dl) according to the standard established by the supplier. Serum insulin concentration was determined with an ELISA kit (Industrial MexLab SA de CV, Zapopan, Jalisco, México). The homeostatic model assessment index of insulin resistance (HOMA-IR) for each group assayed was calculated by multiplying insulin plasma (μUI/mL) by glucose plasma (mmol/L) and dividing by 405. A high rate of HOMAIR means a low sensitivity to insulin. IL-6 and TNF-α concentrations were measured by commercial ELISA kits from SIGMA (St Louis MO, USA).

Total Antioxidant Capacity ($ORAC_T$)

The total antioxidant capacity in serum was determined on day 0 and 7 of the intervention using oxygen absorbance capacity ($ORAC_T$) assay according to the technique proposed by (Robles et al., 2009). with some modifications. It is a fluorometric technique that measures the ability of an antioxidant to inhibit the loss of fluorescence of a test protein by the hydroxyl radical. The fluorescence monitoring is carried out at the following lengths λ Em = 515 nm and λ Ex = 484 nm. The results were expressed as mmol of Trolox equivalents / L of sample

Anthropometric Measures

Height (h) and weight (W) were measured following internationally accepted techniques using a stadiometer (model 202, Seca Ltd, Birmingham, UK) and a digital scale (1631 solar scale, Tanita Corp, Tokyo, Japan), respectively. Obesity was determined by BMI and using the following equation: $BMI=W/h^2$ according to the World Health Organization (WHO). The cut-off points were overweight (24.99-29.9 kg/m^2) and obesity (≥30 kg/m^2).

Waist circumference (WC) was measured at the mid-point between the highest part of the iliac crest and the lowest part of the ribs margin of the median axial line. If WC was ≥90 cm in men or ≥80cm in women, the subject was classified as having central obesity based on the International Diabetes Federation (Alberti et al, 2006). Intra-abdominal obesity (WHR) was calculated from the relationship between waist and hip, the normal values for women and men are 0.8 and 1 respectively.

Statistical analyses were performed using a SAS version 8 software (SAS Institute Inc, Cary, NC). Chi-square test was used for the distribution of frequencies of the risk factors from subjects. A T-student test was performed to see significant differences between the population of men and women. Wilcoxon rank sum test was also used to observe significant differences between the treatments at the beginning and at the end of the consumption of bread. Spearman's correlation coefficients were used to assess the relationship between biomarker measurements, antioxidant capacity levels and obesity related indicators. In all analyses, $P \leq 0.05$ was considered statistically significant.

Results and Discussion

Regarding the composition of the sample studied, there was a voluntary participation of 25 individuals. The age range of the participants was 20-39 years and their body mass index were ranged 27-39 kg / m^2, classified as overweight and obese by the WHO. The final sample was 24 individuals, one of them did not finish the trial, finally the sample was 37.5% women and 62.5% men.

Table 1 shows the frequency of the distribution of risk factors at the beginning of the study in women and men, it was found that approximately 60% of women and men had obesity, the rest presented overweight (40%), followed by 80% with abdominal fat above normal values and the 50% intra-abdominal obesity. In the clinical analyzes all the participants presented low HDL values (100%), 21% showed hypertriglyceridemia, 17% had high LDL values and 17% hyperuricemia, lower percentages were found for hyperglycemia (8%), hypercholesterolemia (13%), insulin resistance (4%). On the other hand, positive family history (cancer, diabetes and cardiovascular disease) had the high percentage 96%, more than half of the participants were sedentary (54%) and only 4% presented smoking. No significant differences were found between women and men according to the Chi-square test ($P \leq 0.05$) for the frequency of risk factors.

Figure 1 shows the accumulation of risk factors for the subjects of the study, it was found that around 38% presented the accumulation of up to 5 risk factors, followed by 20% that showed the accumulation of up to 6 risk factors and 17% accumulate 4 risk factors. A lower percentage accumulated 12, 9, 8, 7 and 3 risk factors respectively (4%, 4%, 8%, 4% and 4%) was found in study subjects. The most prevalent risk factors were low HDL (100%) followed by PHF (98%) WC (83%), sedentarism (54%) and obesity (58%). These results showed that most of the biochemical indicators classified as obesity risk factors were not above 20% prevalence in the study population.

Table 1. Frequency distribution of risk factors at the beginning of the study according to gender. [1]

	Female (N= 9) Nº (%)	Male (N= 15) Nº (%)	Total (N=24) Nº (%)	P[2]
Overweight (25-29.9 kg/m^2)	4 (44.4)	6 (40.0)	10 (41.67)	0.830
Obesity (≥30 kg/m^2)	5 (55.5)	9 (60.0)	14 (58.33)	0.830
WC (cm)	9 (100.0)	11 (73.3)	20 (83.3)	0.897
WHR	6 (66.6)	6 (40.0)	12 (50.0)	0.677
GLU (mg/dL)	1 (11.11)	1 (11.11)	2 (8.33)	0.7029
TC (mg/dL)	1 (11.1)	2 (13.33)	3 (12.5)	0.8734
HDL (mg/dL)	9 (100.0)	15 (100)	24 (100)	0.8790
LDL (mg/dL)	2 (22.22)	2 (13.33)	4 (16.67)	0.5716
TG (mg/dL)	2 (22.22)	3 (20.0)	5 (20.83)	0.8967
UA (mg/dL)	1 (11.11)	3 (20.0)	4 (16.67)	0.5716
INS (µIU/mL)	0	1 (6.67)	1 (4.17)	0.4288
$HOMA_{IR}$	0	1 (6.67)	1 (4.17)	0.4288
PFH	9 (100.0)	14 (93.3)	23 (95.83)	0.4288
Smoking	1 (11.11)	0	1 (4.17)	0.1872
Sedentarism	7 (77.78)	6 (25.0)	13 (54.17)	0.0721

Significant differences between female and male (p≤0.05) according to the Chi-square test.

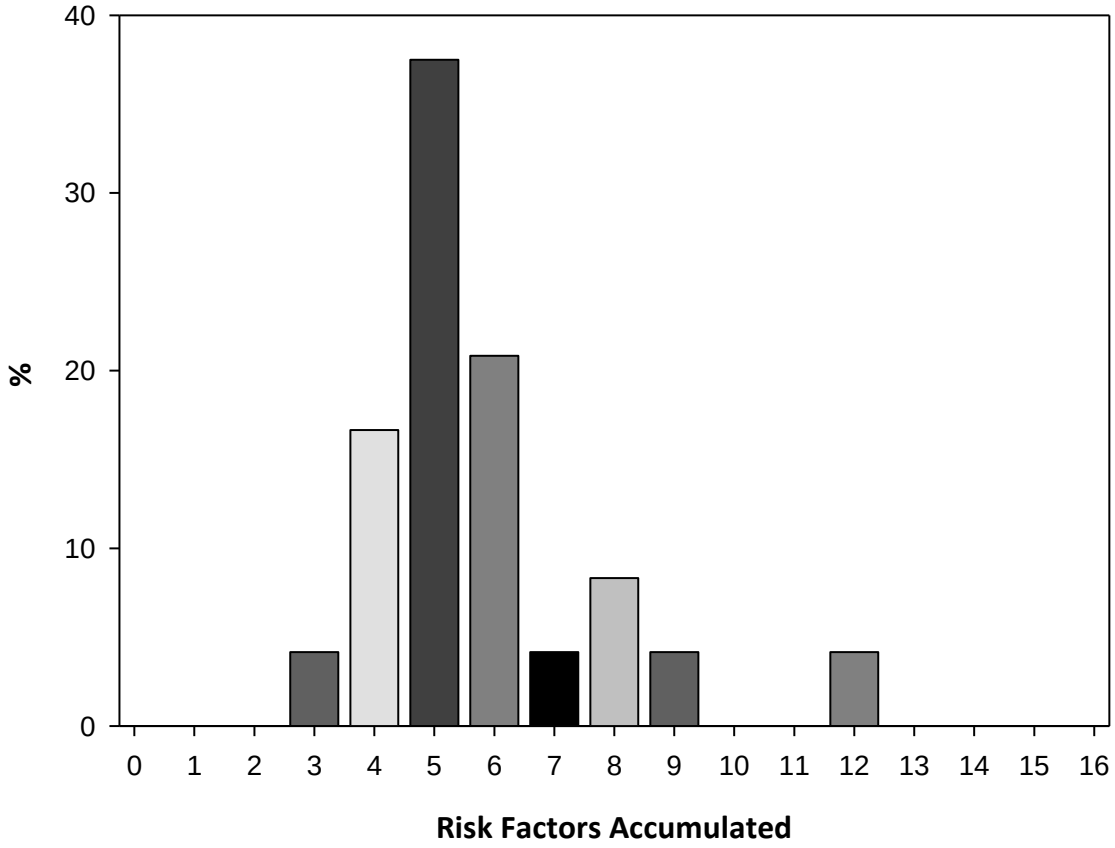

Figure 1. Risk factors accumulated in the subjects at the beginning of the study.

[1]The bars correspond to the frequencies of the factors accumulated by participants.

Table 2 shows the anthropometric measures and the values of the clinical biomarkers of the subjects at the beginning of the study, the means values of the anthropometric measures for women and men corresponded to a body mass index classified as obesity, similar result was found for waist circumference and waist hip ratio. On the other hand, the means values for clinical analysis between women and men was considered as normal values to exception body weight and uric acid values, which were higher in men that women. In Table 3 shows the results obtained for the serum cytokines and the antioxidant capacity in the subjects at the beginning of the study. No significant differences were found between women and men for IL-6 and TNF-α, both

Table 2. Anthropometric measures and clinical analysis of the subjects at the beginning of the study.[1,2]

	Women (n= 9)	Men (n= 15)
Age (years)	26.8 ±1.50	28.0 ±1.16
Weight (kg)	85.0 ±3.54	96.2 ±2.74*
BMI (kg/m^2)	32.1 ±1.0	30.6 ±0.77
CC (cm)	100.1 ±3.45	100.8 ±2.67
Waist/ hip ratio	0.86 ±0.02	0.93 ±0.02
GLU (mg/dL)	85.5 ±12.88	97.9 ±9.97
TC (mg/dL)	174.2 ±12.4	170.1 ±9.6
HDL (mg/dL)	46.8 ±2.12	42.4 ±1.65
LDL (mg/dL)	126.3 ±2.31	124.4 ±1.79
TG (mg/dL)	124.8 ±25.0	126.8 ±19.33
UA (mg/dL)	4.5 ±0.40	6.4 ±0.31*
INS (µlU/mL)	3.7 ±1.21	3.6 ±0.94
$HOMA_{IR}$	0.8 ±0.33	0.9 ±0.25

[1]The values correspond to the mean± standard deviation of the determinations by gender. [2] * Statistical difference (P≤0.05) according to T-student test.

did not represented values that indicated a state of acute inflammation (Park et al., 2005). In the same way, the antioxidant capacity in serum was not significantly different according to the Wilcoxon rank sum test for women and men.

Despite the marked accumulation of risk factors of the study subjects, they do not present an alteration in the metabolism and apparently did not present an acute inflammation. The obese individuals who do not present signs of lipo-inflammation they are known by the term "metabolically healthy obese" to refer to that they do not present any of the metabolic alterations typical of obese individuals, although the risk of morbidity and mortality in relation to diabetes mellitus type 2 (DM 2) and cardiovascular disease (CVD) is the same as in the rest of obese patients (Izaola et al., 2015).

Table 3. Pro-inflammatory cytokines and serum antioxidant capacity (ORAC) of the subjects at the start of the study.[1,2]

	Women (n= 9)	Men (n= 15)
Cytokines (pg/mL)		
IL-6	2.98 (2.75-3.74)	2.98 (2.95-3.24)
TNF-α	4.40 (4.30-4.74)	4.39 (4.35-4.77)
ORAC (mmol ET/ L)		
Serum	9.67 (8.53-10.93)	9.42 (8.11-10.71)

[1]The values correspond to the median of each of the variables by men and women. Values in parentheses correspond to the confidence interval of the median at 95%. *Statistical difference between the medians were established according to the Wilcoxon rank sum test.

From the results obtained at the beginning of study, 15 overweight participants were excluded from the study, this with the purpose of being able to observe a greater effect of the dietary intervention in the study subjects. Dietary intervention was directed only to individuals who presented a body mass index classified as obesity (5 women and 9 men), in Figure 2 shows the flow chart of the clinical trial. The results of the anthropometric measurements for each treatment group are shown in table 4, no significant differences were found between day 0 and 7, for body mass index, waist circumference and waist-hip ratio in the dietary groups. Despite the restriction of bread products in the subjects during the study, the consumption of white bread, bread added with native bran and bioprocessed with H 0.05% for seven days do not seem to modify the weight not even waist circumference.

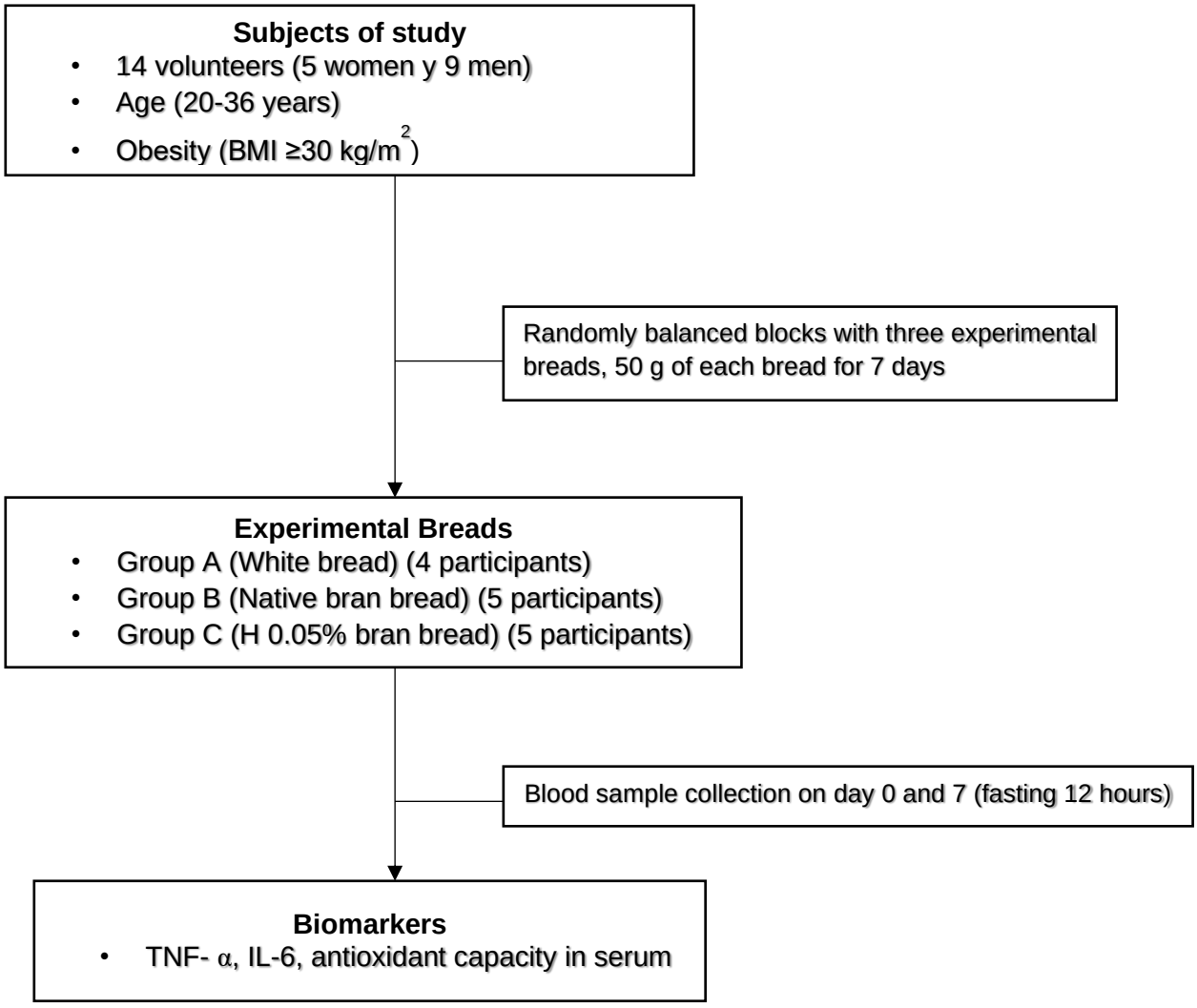

Figure 2. Flow chart of the clinical trial.

Table 4. Anthropometric measurements of obese subjects at the beginning and at the end of the study.[1,2]

	White Bread (n=4)		Native Bran Bread (n=5)		H 0.05% Bran Bread (n=5)	
	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
BMI (kg/m^2)	33.3 ±2.68	33.0 ±2.84	34.26 ±3.29	34.22 ±3.33	31.38 ±1.42	31.28 ±1.48
CC (cm)	98.0 ±9.7	108.7 ±7.63	90.7 ±4.89	101.4 ±12.09	97.2 ±8.7	99.4 ±7.63
Waist hip ratio	0.89 ±0.09	0.96 ±0.9	0.84 ±0.05	0.87 ±0.15	0.91 ±0.08	0.90 ±0.06

[1]The values correspond to the mean± standard deviation of the anthropometric measures by intervention group. [2] * Statistical difference (P≤0.05) according to T-student test.

Figure 3 shows the serum concentration of IL-6 before and after of dietary intervention. It was not observed any effect on intervention time neither between dietary groups. on the other hand, in Figure 4 it was found that the concentrations of TNF-α increased slightly but significantly ($p<0.05$) after 7 days of consumption of bread added with native bran or bran bioprocessed with H 0.05%. In a study conducted by Giannopoulou et al. (2005), it was observed that in women with type II diabetes (postmenopausal) after diet and exercise did not modify the concentrations of IL-6 and TNF-α. Another study by Zhao et al. (2007) in individuals with hypercholesterolemia who consumed a diet rich in linolenic acid significantly decreased the concentrations of IL-6 and TNF-α.

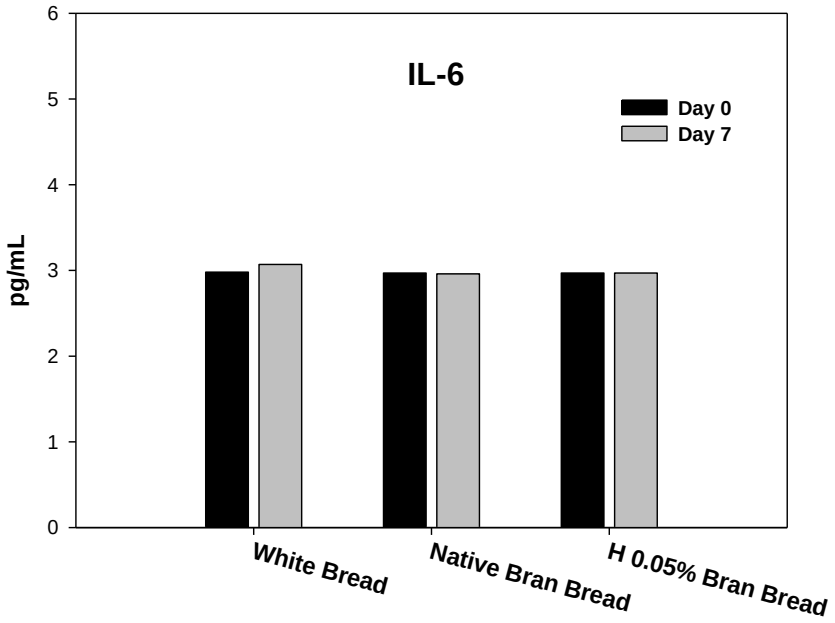

Figure 3. Concentrations of IL-6 from obese subjects before and after of the study.[1]

[1]The values correspond to the median of each of the variables by men and women. Values correspond to the confidence interval of the median at 95%. * Statistical difference between the medians were established according to the Wilcoxon rank sum test.

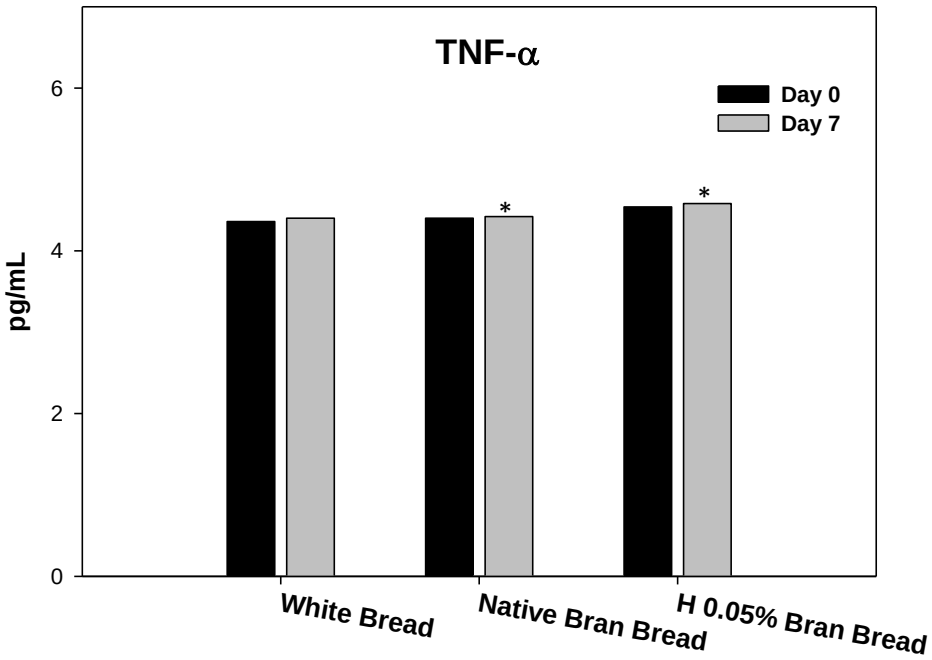

Figure 4. Concentrations of TNF-α from obese subjects at the beginning and end of the study.[1]

[1]The values correspond to the median of each of the variables by men and women. Values correspond to the confidence interval of the median at 95%. * Statistical difference between the medians were established according to the Wilcoxon rank sum test.

The antioxidant capacity in serum measured as ORAC shown in figure 5, no significant differences were found in the medians, however, an increased trend was observed in the group that consumed bread added with bioprocessed bran H 0.05% for seven days. It is important to note that the obese subjects did not presented a markedely lipo-inflammation, it was documented that in individuals non-affected by this condition or apparently healthy, the effects of dietary intervention on serum inflammation biomarkers could be not observed. On the other hand, consuming a bread added with bioprocessed bran could increase the serum antioxidant capacity, this could be attributed to bioactive compounds mainly phenolic compounds that could be released of food matrix after a digestion gastrointestinal process, being able to exert a potential beneficial effect on health.

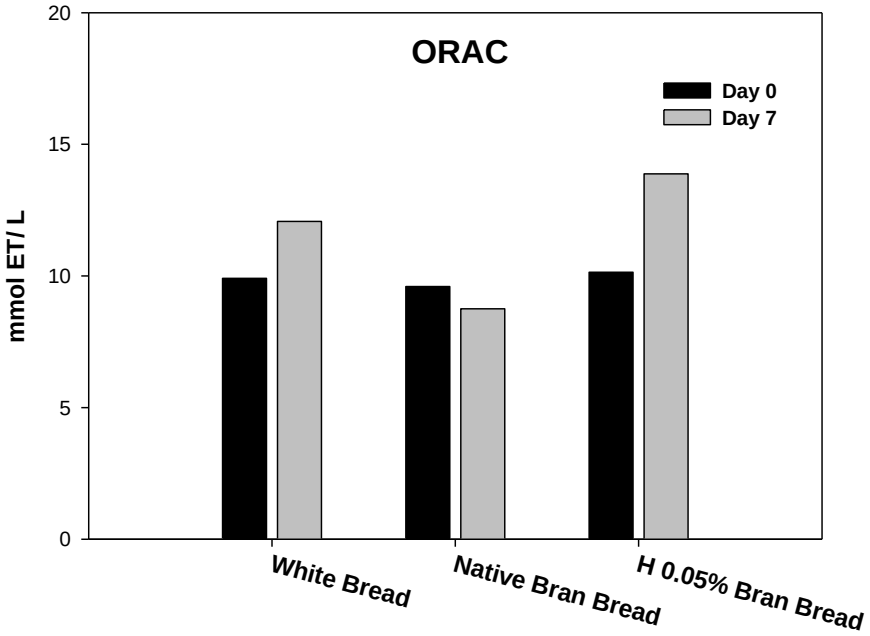

Figure 5. Antioxidant capacity in serum measured as ORAC from obese subjects at the beginning and end of the study.[1]

[1]The values correspond to the median of each of the variables by men and women. Values correspond to the confidence interval of the median at 95%. * Statistical difference between the medians were established according to the Wilcoxon rank sum test.

Finally, the correlations between IL-6 and TNF-α and BMI, waist circumference, waist-hip ratio and serum antioxidant capacity are shown in Table 5, no correlations were found between IL-6 and TNF with BMI, waist circumference and the waist hip ratio, but a positive correlation was found between IL-6 and TNF with antioxidant capacity. It was possible to assume that in individuals under obesity condition the serum antioxidant capacity could be increased by either by endogenous mechanisms or dietary antioxidants to counteract the pro inflammatory interleukines production despite the individuals did not show a markedly metabolic syndrome and independently of dietary group.

Table 5. Correlation between the concentrations of pro-inflammatory cytokines and BMI, CC, waist / hip ratio and serum antioxidant capacity.[1,2]

	TNF-α	**IL-6**
BMI	NS	NS
WC	NS	NS
WHR	NS	NS
$ORAC_T$	0.393*	0.405*

[1]Spearman correlation. * P≤0.05. NS, not significant.

In our study it was concluded that the consumption of a bread added with bioprocessed bran tends to increase the antioxidant capacity in serum, together with a modification in proinflammatory cytokines, positive correlations between these biomarkers confirm the close relationship between obesity, proinflammatory cytokines and antioxidant capacity. Subsequent studies should be focused on obese individuals with metabolic syndrome and lipoinflammation in order to observe a significant modification in biomarkers.

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CONCLUSIONES

El potencial biológico medido como contenido de compuestos fenólicos, ácido ferúlico y actividad antioxidante de pan de trigo se mejora en respuesta a la adición de salvado bioprocesado.

La bioaccesibilidad de ácido ferúlico evaluada mediante simulación de digestión *in vitro* se incrementa en panes adicionados con salvado de trigo bioprocesado.

La absorción aparente de ácido ferúlico presente en panes con salvado bioprocesado es mayor con respecto a panes formulados con salvado nativo y pan blanco.

El consumo de un pan adicionado con salvado bioprocesado tiende a aumentar la capacidad antioxidante en suero, junto con una modificación en citocinas proinflamatorias, las correlaciones positivas entre estos biomarcadores confirman la estrecha relación entre obesidad, citoquinas proinflamatorias y capacidad antioxidante.

RECOMENDACIONES

Estudios posteriores podrán encaminarse hacia intervenciones dietarias a largo plazo y en individuos obesos con lipo-inflamación establecida, con el fin de observar de manera significativa los efectos del consumo de pan adicionado con salvado bioprocesado.

Llevar a cabo estudios enfocados hacia las posibles interacciones que se presentan entre los compuestos fenólicos presentes en salvado bioprocesado y componentes de la matriz alimentaria, con el fin de profundizar en los aspectos de bioaccesibilidad y biodisponibilidad de estos compuestos.