UNIVERSIDAD DE SONORA

DIVISIÓN DE CIENCIAS BIOLÓGICAS Y DE LA SALUD

DEPARTAMENTO DE INVESTIGACIÓN Y POSGRADO EN ALIMENTOS

Programa de Posgrado en Ciencias y Tecnología de Alimentos

Optimización del proceso de nixtamalización por extrusión para la obtención de tortillas de maíz azul (*Zea mays* L.) de calidad con alto contenido de antocianinas bioaccesibles.

TESIS

Como requisito parcial para obtener el grado de:

DOCTOR EN CIENCIAS DE LOS ALIMENTOS

Presenta:

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Hermosillo, Sonora Noviembre de 2020

APROBACIÓN

Optimización del proceso de nixtamalización por extrusión para la obtención de tortillas de maíz azul (*Zea mays* L.) de calidad con alto contenido de antocianinas bioaccesibles

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Noviembre de 2020

Hermosillo, Sonora a 12 de noviembre de 2020.

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AGRADECIMIENTOS

Este es un momento muy especial y al concluir esta maravillosa etapa de mi vida, quiero extender un profundo agradecimiento a quienes me inspiraron y me ayudaron a llegar a donde he llegado.

A **Dios** por darme fortaleza y sabiduría.

A la **Universidad de Sonora** que me permitió formarme en ella y por las oportunidades incomparables que me brindó.

Al **Consejo Nacional de Ciencia y Tecnología** por la beca otorgada durante el periodo de mis estudios.

Un especial, y muy sincero agradecimiento a mi director de tesis el **Dr. Benjamín Ramírez Wong**. Las palabras no pueden expresar mis sentimientos pero me gustaría que supiera que siempre tendrá mi infinita y profunda gratitud, respeto y admiración hacia su persona y su trabajo. No puedo imaginar mejor persona para haber dirigido mi proyecto de investigación. Gracias por creer en mi, por su paciencia, motivación, orientaciones y lineamientos para llevar a cabo este trabajo de tesis. Fue un gran honor poder guiarme de su mano, gracias por toda la confianza que depositó en mi.

A mi co-director de tesis, el **Dr. Armando Quintero Ramos** quien amablemente compartió conmigo ideas que ayudaron a impulsar mi investigación. Gracias por el carisma y profesionalismo que demostró en cada reunión de avances.

A los miembros de mi comité de tesis, **Dra. Patricia Isabel Torres Chávez**, **Dra. Ana Irene Ledesma Osuna**, y **Dra. Olga Nydia Campas Baypoli**, todos ellos expertos en su campo de estudio, y además personas con un gran corazón que tuvieron a bien guiarme en todo mi trabajo de investigación con sus siempre acertados comentarios y sugerencias.

Al **Dr. Roberto Gutiérrez Dorado** de la Universidad Autónoma de Sinaloa quien hizo valiosos aportes en el desarrollo del trabajo.

Al personal administrativo de la División de Ciencias Biológicas y de la Salud, **Dr. Juan Carlos Gálvez Ruíz**, **Dr. Eduardo Ruiz Bustos**, **Ing. Karlha Flores**, y **Lic. María Isabel Meneses**

Tarazón por su amabilidad y compromiso en la gestión y seguimiento de los diversos apoyos que se me otorgaron a lo largo de los cuatro años de estudio.

Al personal académico del laboratorio de reología y panificación, **Dra. Guadalupe Salazar**, **Dra. Concepción Lorenia Medina**, **Dra. Guadalupe López**, **Dra. María Irene**, **M.C. Ignacio Morales**, **M.C. Refugio Ortega**, y a la **Dra. Carmen Lizette Del Toro Sánchez** del laboratorio de Biotecnología, por su apoyo y amistad brindados desde el inicio hasta el final de mi estancia en la universidad.

Al personal administrativo del Departamento de Investigación y Posgrado en Alimentos (DIPA), **Coty Favela**, **Gloria Pavlovich**, **Thelma**, **Juanita**, **Consuelo**, por siempre recibirme con una sonrisa, por brindarme su tiempo para escuchar mis historias, por sus consejos interminables, y por nuestras pláticas tan divertidas.

A los coordinadores del programa, **Dr. Enrique Márquez Ríos**, **Dr. Francisco Rodríguez Félix**, y **Dra. Carmen María López Saiz**, por el apoyo otorgado durante su gestión.

A la **tortillería Pimentel**, por las facilidades brindadas para llevar a cabo parte de mi trabajo experimental en sus instalaciones.

Al **Dr. Francisco Javier Wong Corral,** el decir solo gracias nunca podrá pagar de regreso el hecho de que mediante su apoyo se me permitió concretar planes con los que tan solo soñaba. Gracias por su cordialidad y gestiones realizadas en pro de impulsar mi formación académica y profesional.

A la **Universidad Miguel Hernández (UMH**) por aceptarme como alumna de doble doctorado internacional.

Al **Dr. Ángel Antonio Carbonell Barrachina**, a la **Dra. María José Frutos Fernández**, **Dra. Estefanía Valero Cases,** y a la **M.C. Raquel Muelas Domingo,** todos parte importante del desarrollo del proyecto de investigación, gracias por sus atenciones, por el compromiso, apoyo y creatividad durante el trabajo experimental realizado en la UMH.

A mis compañeros de generación, **Daniel**, **Ivan**, **Encinas**, **Betza**, **Uriel**, gracias por su compañía, apoyo moral y buenos momentos que compartimos, aunque algunos nos separamos en el camino, siempre los recordaré con cariño.

A quienes considero ahora **mis amigos**, Raquel Ortíz (**la more**), Jesús Gerardo (**el chapito**), Daniela Denisse (**Dani**), gracias por hacer que los momentos difíciles fueran superables y los felices fueran aún mas gratificantes.

A **Carlos Enríquez**, mi buen Charly, me diste tu confianza, tu tiempo, y tus consejos y creo que esos son regalos invaluables que atesoraré por siempre.

A **Fátima Méndez,** gracias mi dulce, noble, y tierna Fati por brindarme el regalo mas valioso de todos, tu amistad. Gracias por todo lo que hiciste por mi a lo largo de nuestros cuatro años de amistad, gracias por ser quien eres, deseo que nuestra unión perdure en el tiempo. En mi siempre tendrás a una amiga.

A **Liliana Pérez**, mi querida Maribel, gracias por tanto, gracias por ser una parte muy importante en la historia de mi vida. Mi amiga incondicional, te ves tan delicada y frágil y en el fondo eres una guerrera que me enseñó tanto, gracias por confiar en mi para ser tu roomie, por tus consejos de belleza y cuidado de la piel, por prepararme tus micheladas especiales, por compartir conmigo tus tardes de telenovelas, por ser mi compañera de eventos (despedidas de soltera, bodas, baby showers, cumpleaños, etc). Nunca olvidaré que siempre estuviste ahí para mi en cada momento. Espero algún día poder corresponder con algo a todo tu cariño.

A **Raquel Bourne**, mi baby, si te diera un millón de gracias aún así me quedaría corta. Fuiste para mi un angelito guardián que me cuidó como si me conociera desde siempre. Tenías la respuesta correcta a todas mis preguntas. Gracias por impulsarme a conocer el mundo, a viajar, a probar nuevas cosas. Me abriste las puertas de tu hogar y me recibiste como un miembro mas ofreciendome el cariño y la calidéz de una familia, y por ello te estaré eternamente agradecida.

A **Jésus Enrique**, el **Chan,** te comencé a hablar por casualidad y al final te robaste un cachito de mi corazón con tus ocurrencias y locuras que hacían mas divertidos los días en la universidad y fuera de ella. Gracias por todo tu apoyo cuando planeaba mi viaje a España, por no cansarte

en explicarme una y otra vez los requisitos que tenía que cumplir, y por estar ahí cuando regresé para escuchar mis aventuras. Por nuestras salidas los fines de semana en los que no sabíamos que pasaría, por las pijamadas que siempre terminaban en terapia, pero sobre todo por tu amistad.

Gracias a todos ustedes por darme una familia en Hermosillo.

A mis queridos **Fariborz Habibi**, **Ángeles de la Rosa**, y **Marlene Aguilar** por los momentos tan entrañables que compartimos durante mi estancia en España.

A **Francesca Gargiulo**, **Sthefany Otero**, **Carmen Vidal**, **Natalia Noguera**, **Luis Noriega**, **Paola**, **Raquel Lucas**, **Sonia Arronis**, **Noeli Sirini**, **Leotina Lipan**, **Tonpalm Pannuan**, **Encarna**, **Jose**, por permitirme llegar a conocerlos y compartir vivencias que recordaré por siempre.

A mi **mamá**, la mujer con el alma mas pura que conozco, en ella tengo el espejo en el cual me quiero reflejar, que por sus sus virtudes y su gran corazón me llevan a admirarla cada día mas. Eres mi heroína. Te amo.

A mi **hermano**, por ser mi compañero de alma y de vida. Por ser el mayor tesoro que me pudieron regalar, pues nuestra unión permanecerá inmutable por siempre. Podemos estar sentados el uno al lado del otro y calmarnos sin decir media palabra. Y a pesar que estamos lejos, sabemos que no hay distancias insalvables entre dos personas que se quieren.

A aquellos seres queridos que siempre aguardo en mi alma, y que se hacen presente en mi vida en muchas formas. A mi tía **Sandra,** que fue el principal cimiento para la construcción de mi vida profesional, y sentó en mi las bases de la responsabilidad y deseos de superación.

A los demás miembros de **mi familia y amigos**, que junto a mi caminaron en todo momento y fueron una fuente de inspiración, confianza y fortaleza. Muchas gracias a ustedes por demostrarme que el verdadero amor es incondicional.

Gracias infinitas a todos ustedes.

DEDICATORIA

A mi yo del pasado, a quien le diría que confie mas en si misma, que merece lo que sueña, que no tenga miedo, que los anhelos si se hacen realidad.

A mi yo del presente, disfruta este logro personal tan grande, sientete honrada, satisfecha y orgullosa.

A mi yo del futuro, le diría sé humilde y agradecida. No pierdas de vista quien eres y en lo que no te quieres convertir. Conserva tu educación y buenos principios siempre.

RESUMEN

Las propiedades nutracéuticas del maíz azul derivan de sus metabolitos secundarios. La tortilla de maíz es el alimento básico de la población mexicana y algunos países de Centro América. El proceso de extrusión actualmente se emplea como una alternativa al proceso de nixtamalización tradicional para elaborar tortillas. El objetivo de esta investigación fue optimizar las condiciones del proceso de nixtamalización por extrusión para la obtención de tortillas con alto contenido de antocianinas, textura adecuada y evaluar la estabilidad y capacidad antioxidante de los fitoquímicos de la tortilla bajo un sistema de digestión gastrointestinal simulado. La investigación se dividió en tres etapas. Durante la primera etapa, se evaluó el efecto de los factores del proceso de extrusión sobre diferentes características de las harinas nixtamalizadas para encontrar el área óptima de procesamiento.Se utilizó maíz azul molido (malla 2 mm) acondicionado con 0.3 % de $Ca(OH)_2$ y se elaboraron las harinas con las condiciones obtenidas bajo un arreglo experimental de un diseño central compuesto, donde los factores fueron: humedad de alimentación (HA, 15-30%), temperatura de la cuarta zona del extrusor (TE, 70-110 °C) y velocidad de tornillo (VT, 50-145 rpm). Los extrudidos se secaron a 50 °C durante 1 h, y se molieron (2 mm) para obtener las harinas. Las variables respuesta fueron químicas, funcionales y amilográficas. Se utilizó la metodología de superficie de respuesta para evaluar los datos experimentales y se optimizó en función del máximo contenido de antocianinas y máximo pico de viscosidad. Con la harina de las condiciones óptimas se obtuvo la tortilla la cual fue caracterizada física, química y texturalmente. Los resultados de la primera etapa indican que la HA fue el factor que tuvo un gran efecto sobre las propiedades evaluadas en las harinas. El área óptima se determinó a una HA de 18.17%, una TE de 92.03 °C y una VT de 76.61 rpm.

Las tortillas obtenidas mostraron una textura adecuada y un alto contenido de antocianinas, con una retención del 56.7 %, con respecto al maíz crudo. En la segunda etapa se realizó el estudio cinético y termodinámico de la degradación de antocianinas presentes en el maíz azul, la harina óptima y su tortilla. Las antocianinas extraídas se resuspendieron en una solución tampón a pH (2.5) y fueron tratadas térmicamente a 3 temperaturas diferentes (60, 75 o 90 °C) durante 2 h. El cambio de concentración respecto al tiempo fue medido y con estos datos se estimaron los parámetros cinéticos y termodinámicos. Se utilizó un diseño bifactorial completamente al azar. Los datos se analizaron mediante el análisis de varianza (ANDEVA), con un nivel de significancia <0.05 %. Los resultados de la segunda etapa demostraron que la transformación de antocianinas tiene alta dependencia con la temperatura. El mecanismo de degradación de las antocianinas siguió una cinética de reacción de primer orden, fue una reacción endotérmica y no espontánea. En la tercera etapa se realizó la digestión gastrointestinal *in vitro* del maíz azul, la tortilla azul extrudida y una tortilla blanca tradicional. Las condiciones fisiológicas de la boca, estómago e intestino delgado fueron simuladas. La estabilidad y capacidad antioxidante de las antocianinas y compuestos fenólicos antes y después de la digestión fueron evaluados. Se utilizó un diseño bifactorial completamente al azar. Los datos se analizaron mediante el ANDEVA, con un nivel de significancia <0.05. Los resultados de la tercera etapa indicaron que la digestión contribuyó a la liberación de los compuestos fenólicos y que, a pesar de la evidencia de reducciones en las antocianinas, las tortillas exhibieron una capacidad antioxidante efectiva después de la digestión. Se concluye que el enfoque de este estudio puede proporcionar una guía útil para desarrollar y optimizar productos innovadores a base de maíz pigmentado con pérdidas mínimas en compuestos biológicamente activos y predecir sus efectos potenciales *in vivo* una vez que estos son consumidos.

ABSTRACT

The nutraceutical properties of blue corn are derived from its secondary metabolites. Corn tortilla is the staple food of the Mexican population and some countries of Central America. The extrusion process is currently used as an alternative to the traditional nixtamalization process to make tortillas. The objective of this research was to optimize the conditions of the nixtamalization extrusion process to obtain tortillas with high anthocyanin content, adequate texture, and to evaluate the stability and antioxidant capacity of the phytochemicals of corn tortilla under simulated gastrointestinal digestion. The investigation was divided into three stages. During the first stage, the effect of the extrusion factors on different characteristics of the nixtamalized flours was evaluated in order to find the optimal processing conditions. Ground corn (2 mm mesh) was conditioned with 0.3% $Ca(OH)_2$ and flours were produced under the conditions obtained from a central composite design matrix, where the factors were: feed moisture (FM, 15-30%), temperature of the fourth zone of the extruder (TE, 70-110 ° C) and screw speed (SS, 50-145 rpm). The extrudates were dried at 50 °C for 1 hr, and milled (2 mm) to obtain the flours. The response variables were chemical, functional and amylographic properties. The response surface methodology was used to evaluate the experimental data and the optimizing was according to the maximum anthocyanin content and maximum peak viscosity. Tortilla was obtained with the optimal flour, which was characterized physically, chemically and texturally. Results of the first stage indicate that FM was the factor that had a great effect on the properties evaluated in the flours. The optimal process area was determined at FM of 18.17%, TE of 92.03 °C and SS of 76.61 rpm. Tortillas obtained showed adequate texture characteristics and a high anthocyanin content with a retention of 56.7%, with respect to raw corn. In the second stage, a kinetic and thermodynamic study of the anthocyanins degradation present in blue corn, optimal flour and their tortilla was carried out. The extracted

anthocyanins were resuspended in a buffer solution at pH (2.5) and were heat treated at 3 different temperatures (60, 75 or 90 °C) for 2 h. The change in concentration with respect to time was measured and with these data, the kinetic and thermodynamic parameters were estimated. A completely randomized two-factor design was used. The data were analyzed using the analysis of variance (ANOVA), with a level of significance <0.05%. Results of the second stage showed that the transformation of anthocyanins has a high dependence on temperature. The anthocyanin degradation mechanism followed a first order reaction kinetics, it was an endothermic and not spontaneous reaction. In the third stage, the *in vitro* gastrointestinal digestion of of the blue corn, the extruded blue tortilla and a traditional white tortilla was performed. The physiological conditions of the mouth, stomach and small intestine were simulated. The stability and antioxidant capacity of anthocyanins and phenolic compounds before and after digestion were evaluated. A completely randomized two-factor design was used. The data were analyzed using ANOVA, with a level of significance <0.05. Results of the third stage indicated that digestion contributed to the release of phenolic compounds and that despite evidence of reductions in anthocyanins, tortillas exhibited effective antioxidant capacity after digestion. It is concluded that the approach of this study can provide a useful guide to develop and optimize innovative pigmented corn-based products with minimal losses in biologically active compounds and predict their potential effects *in vivo* once they are consumed.

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INTRODUCCIÓN

El maíz azul se caracteriza por su amplia gama de fitoquímicos como los ácidos fenólicos y los flavonoides, especialmente las antocianinas (Liu, 2007). Estos compuestos han sido reconocidos como promotores de la salud debido a sus diversas propiedades biológicas, como propiedades antioxidantes y antiinflamatorias, que pueden ayudar a reducir los riesgos de diversas enfermedades relacionadas con el estrés oxidativo (Sui *et al*., 2014).

Los efectos *in vivo* de los fitoquímicos dependen no solo de su concentración, sino también de su bioaccesibilidad y biodisponibilidad después de la ingestión (Palafox-Carlos *et al*., 2011). Dado que los alimentos se consumen en forma integral, los fitoquímicos se mezclan comúnmente con diferentes macromoléculas como carbohidratos, lípidos y proteínas para formar la matriz alimentaria (Parada y Aguilera 2007). Estas interacciones podrían interferir con la bioaccesibilidad de los compuestos fenólicos y antocianinas durante la digestión gastrointestinal. Modelos de digestión *in vitro* se han desarrollado para imitar las complejas condiciones fisiológicas del tracto gastrointestinal humano y predecir la liberación de fitoquímicos de la matriz alimentaria (Alminger *et al*., 2014). Se ha reportado una buena correlación entre los resultados obtenidos usando sistemas *in vitro* e *in vivo* (Carbonell-Capella *et al*., 2014).

Actualmente, el principal problema en el uso de materiales alimenticios ricos en antocianinas es su susceptibilidad al deterioro durante el procesamiento (Nayak *et al*., 2015). Entre los factores que pueden influir en la estabilidad de las antocianinas, los más importantes son el pH y la temperatura (Fracassetti *et al*., 2013). El mecanismo de degradación de las antocianinas es bastante complejo y el procesamiento térmico podría inducir algunas reacciones químicas inesperadas y no deseadas. El conocimiento de los parámetros cinéticos y termodinámicos es necesario para predecir y minimizar los cambios no deseados. Esto podría permitir el diseño, mejora y optimización de procesos para preservar la calidad de alimentos específicos ricos en antocianinas.

El maíz azul se procesa mediante cocción térmica alcalina antes de su consumo. Las tortillas son el alimento más consumido en México, Centroamérica, Estados Unidos y algunos países de

Europa y Asia. Durante el proceso de nixtamalización, el pH altamente alcalino y el largo tiempo de cocción a temperatura elevada, así como el descarte de algunas partes anatómicas del grano, como el pericarpio, conducen a una mayor degradación de los fitoquímicos en el producto final (Mora-Rochin *et al*., 2010). Debido a estas desventajas, se han utilizado procesos alternativos para la producción de harinas y tortillas, como el proceso de nixtamalización por extrusión. Esta tecnología permite el procesamiento de materiales a alta temperatura y corto tiempo, evita el daño térmico excesivo a las antocianinas lábiles y muestra características prometedoras, como la producción de tortillas de maíz con el uso de una pequeña cantidad de agua y sin la generación de efluentes contaminantes (Mora-Rochín *et al*., 2010).

El almidón es el componente principal del maíz y la conversión de este en un material termoplástico conduce a la pérdida de la organización molecular natural. El nivel de daño del almidón puede ser seguido por el valor del pico viscosidad, medido con técnicas analíticas como la viscoamilografía que cuantifican los cambios en el almidón de la harina de maíz. Adicionalmente, la textura evaluada como la firmeza de una tortilla de maíz se ha correlacionado con el daño del almidón en la harina (Campas-Baypoli *et al*., 2002).

Considerando que la estabilidad de las antocianinas, los cambios en el almidón, y las propiedades antioxidantes y bioaccesibilidad de los fitoquímicos presentes en el maíz azul pueden verse afectadas por efecto del procesamiento y el proceso de digestión una vez que el maíz y los productos derivados (tortilla) son consumidos, es de importancia evaluar el impacto del procesamiento del maíz azul para la obtención de harinas y tortilla y su relación con el proceso de digestión.

Los resultados obtenidos en esta investigación podrían ser útiles para la industria de la tortilla, desarrollando harinas de maíz nixtamalizadas con características deseables para hacer tortillas saludables utilizando el proceso de extrusión, con pérdidas mínimas en compuestos biológicamente activos como las antocianinas (promotores de la salud) sin afectar negativamente la calidad del producto (buena textura), y a su vez proporcionar información científica que ayude en la predicción de los cambios en los fitoquímicos y capacidad antioxidante de la tortilla durante la digestión gastrointestinal a través de modelos *in vitro*.

ANTECEDENTES

Producción y Consumo del Maíz

El maíz (*Zea mays* L.) es uno de los cultivos más importantes del mundo; su producción mundial supera los 1,000 millones de toneladas (FAO, 2018). En México representa el sector más importante de la producción agrícola por ser la principal fuente de alimentación (Sierra-Macías *et al*., 2010). Desde el punto de vista económico contribuye con el 9.9 % del producto bruto interno de la agricultura nacional, y se siembra en más de 7 millones de hectáreas, que representa el 25% de la superficie agrícola nacional (INEGI, 2018). El grano de maíz puede ser empleado en diversos tipos de industrias, de las cuales la industria alimentaria es la más importante, debido a que existen diversas maneras de elaborar productos como la variabilidad genética lo permita (Rooney *et al*., 2003). La cocina tradicional mexicana, que tiene como base al maíz, es considerada Patrimonio Cultural Inmaterial de la Humanidad por la Organización de Naciones Unidas para la Educación, la Ciencia y la Cultura (UNESCO, 2010). El maíz se utiliza para la obtención de botanas, atoles, pinoles, y en general en una amplia variedad de productos, cuyos usos están asociados con los cultivares, características físicas y su adaptación a las diversas regiones agrícolas (Mauricio *et al*., 2004). Entre las propiedades importantes para la clasificación del uso alimentario del maíz están su color (Mauricio *et al*., 2004). En este sentido el maíz azul, como fuente de pigmentos (antocianinas) y antioxidantes naturales es muy apreciado para la elaboración de tortillas y otros productos (De la Parra *et al*., 2007; Salinas *et al*., 2007).

Importancia de la Tortilla de Maíz

La tortilla de maíz es uno de los alimentos tradicionales más importantes en México, es considerada como la base de la supervivencia del pueblo mexicano desde hace más de 3500 años (Paredes-López *et al*., 2009). Alrededor de 82 % de los hogares mexicanos incluyen a las tortillas en su dieta, y representa el 6.4 % del gasto total en alimentos, aunque la población de menores ingresos puede destinar más de 25 % de su presupuesto alimentario en este producto (INEGI, 2010). De acuerdo con cálculos del Consejo Nacional de Evaluación de la Política de Desarrollo Social, el consumo anual per capita de tortilla es de 56.7 kg en las zonas urbanas

hasta 79.5. kg en las zonas rurales (CONEVAL, 2018). La importancia de la tortilla en la dieta no es menor, pues aporta el 45 % de calorías, 39 % de proteínas y 49 % de calcio, además que puede proporcionar del 32 a 62 % de los requerimientos mínimos de hierro (Cruz y Guzmán, 2007; Paredes-López *et al*., 2009). La producción de tortilla de maíz además se clasifica como una de las actividades agroindustriales más importantes y su consumo ha penetrado ampliamente en el mercado de los Estados Unidos y en algunos países de Asia y Europa (Cortés-Gómez *et al*., 2005).

Maíz Azul (*Zea mays* L.)

El maíz azul pertenece al reino Plantae, a la clase angiosperma, a la subclase monocotiledónea, al orden de los cereales y a la familia de las gramíneas (Galarza, 2011). En México existe una gran diversidad de variedades de maíz azul las cuales varian en el tamaño, densidad, dureza del grano, así como en su composición química. Estas variables están definidas por el factor genético, prácticas de cultivo, condiciones climáticas y tipo de suelo (Agama, 2011). La mayoría de los maíces azules son típicamente de grano harinoso, el endospermo es de textura suave y el color azul se encuentra en la capa de células llamada aleurona, donde la mayor concentración de pigmentos antociánicos hacen que los granos parezcan negros (Betrán *et al*., 2001). El maíz azul ofrece algunas características nutricionales muy interesantes destacando una menor cantidad de almidón, un índice glucémico inferior al maíz blanco y una carga proteica superior en un 20% al del maíz blanco (Méndez *et al*., 2005). Debido a estas caraccterísticas representa una gran oportunidad para el desarrollo de nuevos productos, con nuevas o mejores características funcionales y nutricionales (Bello-Perez *et al*., 2016).

Estructura del Grano y Composición Química

El grano de maíz consiste en cuatro estructuras físicas principales: la capa externa o pericarpio endospermo, germen o embrión, y pedicelo como se muestra en la Figura 1. El pericarpio, cáscara o salvado constituye el 5-6% del peso seco del grano de maíz, se caracteriza por un elevado contenido de fibra cruda, la cual está constituida fundamentalmente por hemicelulosa,

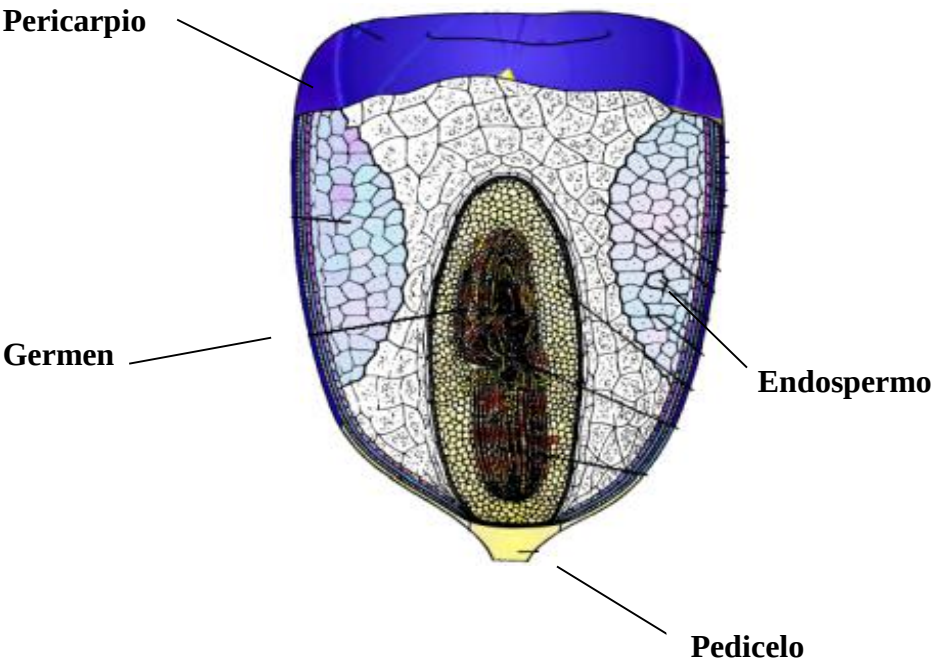


Figura 1. Morfología del grano de maíz azul.

Fuente: Zazueta-Morales, (2014).

celulosa y lignina. El resto de la composición química del pericarpio son cenizas, proteínas y azúcares (Singh *et al*., 2014). El endospermo es el componente mayoritario del grano, constituye el 80-85% del peso seco del grano. Contiene alrededor del 70 al 75% de almidón, 2 % de carbohidratos simples en forma de azúcares en estructuras sencillas como monosacáridos (D-fructosa y D-glucosa), 8-10% de proteínas y un bajo contenido de lípidos (1%) (Lawton y Wilson, 1987; Prasanna *et al*., 2001). El endospermo está compuesto por una gran cantidad de células, cada una empacada con gránulos de almidón incrustados en una matriz continua de proteína. La pared celular consiste en polisacáridos no amiláceos (β-glucano y arabinoxilanos), proteínas y ácidos fenólicos. Las proteínas de almacenamiento del endospermo se encuentran dentro de cuerpos subcelulares conocidos como cuerpos proteicos los cuales están compuestos casi en su totalidad por una fracción rica en prolamina (zeínas) (Singh *et al*., 2014). Los lípidos representan el 5% del maíz azul y se encuentran en mayor proporción en el germen. El germen constituye del 10 al 12% del peso seco del grano, la mayoría de los lípidos encontrados en esta estructura son triglicéridos compuestos por ácidos grasos poliinsaturados como ácido linoleico (50 %), ácido oleico (35 %), ácido palmítico (13%), ácido esteárico (4 %) y ácido linolénico (3 %) (Paredes-López *et al*., 2000). Finalmente, el pedicelo es la estructura cónica de tejido inerte que une al grano con el olote. Al igual que el pericarpio está compuesto principalmente de celulosa y hemicelulosa, entre otros carbohidratos complejos.

Fitoquímicos del Maíz Azul

Los fitoquímicos son metabolitos secundarios de las plantas, los cuales en los tejidos vegetales actúan como defensa contra factores bióticos y abióticos como hongos patógenos, luz UV, clima seco y estrés hídrico; y que una vez ingeridos en la dieta exhiben una actividad biológica dentro del organismo, cumpliendo una función en el cuerpo que se traduce en beneficios a la salud (Liu, 2004). Los principales fitoquímicos en los cereales se clasifican como compuestos fenólicos de los cuales las antocianinas y ácidos fenólicos son los mas representativos en el maíz azul (Liu, 2007). Los ácidos fenólicos y antocianinas mayormente reportados en maíz azul se describen en la Tabla 1.

Tabla 1. Principales antocianinas y ácidos fenólicos reportados en granos de maíz azul

Antocianinas	Ácidos fenólicos	Referencias
Cianidina-3-glucósido	Ácido gálico	Mora-Rochín *et al*., (2016), Lao y Giusti, (2016), Urías-Lugo *et al*., (2015), Yang y Zhai, (2010), Castañeda-Ovando *et al*., (2010), Žilić *et al*., (2012), Sánchez-Madrigal et al., (2015), Pedreschi y Zevallos, (2007)
Pelargonidina-3-glucósido	Ácido vanílico	Mora-Rochín *et al*. (2016), Urías-Lugo *et al*., (2015), Castañeda-Ovando *et al*., (2010), Sánchez-Madrigal *et al*., (2015), Escalante-Aburto *et al*., (2016), Pedreschi y Zevallos, (2007)
Peonidina-3-glucósido	Ácido siríngico	Zhao *et al*., (2009), Salinas-Moreno *et al*., (2012), Castañeda-Ovando *et al*., (2010)
Cianidina-3-(6″-malonilglucósido)	Ácido *p*-hidroxibenzóico	Žilić *et al*. (2012), Salinas-Moreno *et al*., (2012), Cuevas-Montilla *et al*., (2011), Yang y Zhai, (2010)
Pelargonidina-3-(6″-maolonilglucósido)	Ácido protocatéquico	Lao y Giusti, (2016), Zhao *et al*., (2009), Žilić *et al*., (2012), Cuevas-Montilla *et al*., (2011)
Peonidina-3-(6″-malonilglucósido)	Ácido ferúlico	Cuevas-Montilla *et al*., (2011), Yang y Zhai, (2010), Urías-Peraldi *et al*., (2013)
Cianidina-3-(succinilglucósido)	Ácido cafeico	Lao y Giusti, (2016), Urías-Peraldi *et al*., (2013)
Cianidina-3,5-diglucósido	Ácido sinápico	Escalante aburto *et al*., (2016), Žilić *et al*., (2012)
Cianidina- succinil-glucósido	Ácido *p*-cumárico	Mora-Rochín *et al*., (2016), Abdel Aal *et al*., (2006), De la Parra *et al*., (2007)
Cianidina- 3- rutinósido	Ácido *di*-ferúlico	Abdel-Aal *et al*., (2006), Žilić *et al*., (2012), Urías-Lugo *et al*., (2015), Del Pozo-Insfran *et al*., (2006)

Antocianinas

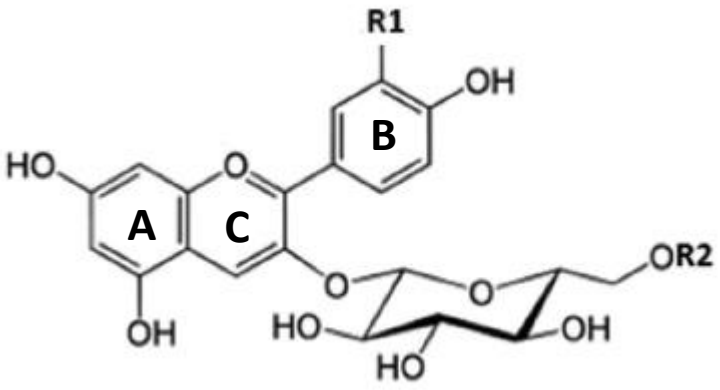
Las antocianinas son pigmentos polares que se encuentran en las vacuolas de los tejidos vegetales responsables de otorgar la coloración azul al maíz. La estructura general de las antocianinas consiste en dos anillos aromáticos (A y B), unidos por un anillo heterocíclico de tres carbonos (C) que contiene oxígeno (Figura 2). En su forma natural, esta estructura se encuentra esterificada a uno o varios azúcares, y se denominan antocianinas simples. La glicosilación en la posición 3 de la estructura es la más común. Los azúcares más comúnmente unidos son la glucosa, ramnosa, galactosa, xilosa y arabinosa. Si además del azúcar en la molécula hay un radical acilo, entonces se denominan antocianinas aciladas, es decir, que presentan enlaces del tipo éster entre el azúcar y ácidos orgánicos alifáticos como el ácido malónico y oxálico, y/o con ácidos orgánicos aromáticos como el ácido ferúlico y cafeico (Francis, 1989). Las principales antocianinas reportadas en maíz azul incluyen la cianidina-3-glucósido, pelargonidina-3-glucósido, peonidina-3-glucósido y sus derivados de ácido malónico unidos a la posición C-6 del resto de la glucosa (Tabla 1) (Žilić *et al*., 2012).

Ácidos fenólicos

Los ácidos fenólicos son otro grupo de compuestos encontrados en alta concentración en el grano de maíz (Liu, 2007). Poseen en su estructura un anillo aromático con uno o más grupos hidroxilos (Xiao *et al*., 2015). En el maíz azul la mayoría de los ácidos fenólicos están en formas conjugadas o unidas a componentes de la pared celular como la celulosa, proteínas y hemicelulosa através de enlaces éster y solo una pequeña proporción está en forma soluble libre que se puede extraer fácilmente sin tratamiento de hidrólisis (Montilla *et al*., 2011; Žilić *et al*., 2012). Más de 9 ácidos fenólicos diferentes se ha informado que se encuentran en el maíz azul (Tabla 1) (Montilla *et al*., 2011; Žilić *et al*., 2012). Dentro de los cuales el ácido el ácido ferúlico es el que se encuentra en mayor abundancia (90%) seguido del ácido diférulico y cumarico (Urías-Lugo *et al*., 2015).

Interacciones entre los Fitoquímicos y los Componentes del Grano de Maíz

La matrix alimentaria tiene una estructura porosa y muy compleja. Los macronutrientes que la conforman interactúan y atrapan a los fitoquímicos, lo que conduce a cambios en las propiedades



Antocianinas	**R1**	**R2**
Cianidina-3-glucósido	OH	H
Pelargonidina-3-glucósido	H	H
Peonidina-3-glucósido	OCH_3	H
Cianidina-3-(6¨-malonilglucósido)	OH	$COCH_2COOH$
Pelargonidina-3-(6¨-malonilglucósido)	H	$COCH_2COOH$
Peonidina-3-(6¨-malonilglucósido)	OCH_3	$COCH_2COOH$

Figura 2. Estructura química de 6 antocianinas en el maíz azul.

Fuente: Lao *et al.* (2017)

estructurales, funcionales y nutricionales de ambos componentes. Los compuestos fenólicos pueden estar asociados con carbohidratos (azúcares y almidón), lípidos, proteínas, así como también pueden estar unidos a componentes de la pared celular (Palafox-Carlos *et al*., 2011). De acuerdo con Bello-Pérez *et al*.(2016), la posible interacción de antocianinas con el almidón ocurre mediante enlaces no covalentes (puentes de hidrógeno) lo cual provoca cambios estructurales en el almidón. Las moléculas de antocianinas a través de sus grupos hidroxilo establecen enlaces puente de hidrógeno con las cadenas de amilosa del almidón, evitando así que las dobles hélices de amilosa se empaqueten en estructuras ordenadas y cristalinas (Bordenave *et al*., 2014) mostrando una correlación positiva con la formación de almidón resistente, reduciendo su digestibilidad en el tracto intestinal (Barros *et al*., 2012; Camelo-Mendez *et al*., 2016; Hanhineva *et al*., 2010).

Así mismo, existe evidencia científica sugiriendo que los componentes no digeribles de la pared celular o fibra dietaria (celulosa, hemicelulosas, pectinas, fructanos y arabinoxilanos) pueden asociarse e interactuar químicamente con los compuestos fenólicos (Saura-Calixto *et al*., 2011). Los compuestos fenólicos tienen anillos aromáticos hidrofóbicos y grupos hidroxilo hidrofílicos con la capacidad de unirse a polisacáridos en varios sitios en la superficie de la pared celular. Estos se unen por puentes de hidrógeno (entre el grupo hidroxilo de los compuestos fenólico y átomos de oxígeno de los enlaces glicosídicos de los polisacáridos), interacciones hidrofóbicas y/o enlaces covalentes tales como enlaces éster (Figura 3) (Quirós-Sauceda *et al*., 2014). En el grano de maíz se ha reportado que el 95% de los compuestos fenólicos vinculados a los polisacáridos, principalmente a arabinoxilanos, están unidos covalentemente a través de enlaces éster (Quirós-Sauceda *et al*., 2014).

A su vez, el grupo fenólico es un excelente donador de atómos de hidrógeno que forma enlaces de hidrógeno con el grupo carboxilo de las proteínas (Mulaudzi *et al*., 2012). En las interacciones entre los compuestos fenólicos con proteínas generalmente están involucradas uniones no covalentes del tipo de interacciones hidrófobas, fuerzas de van der Waals, de puente de hidrógeno e iónicas (Nagy *et al*., 2012). Como efecto, la formación de este complejo compuesto fenólico-proteína conduce a la agregación y eventual pérdida de solubilidad y precipitación de las proteínas.

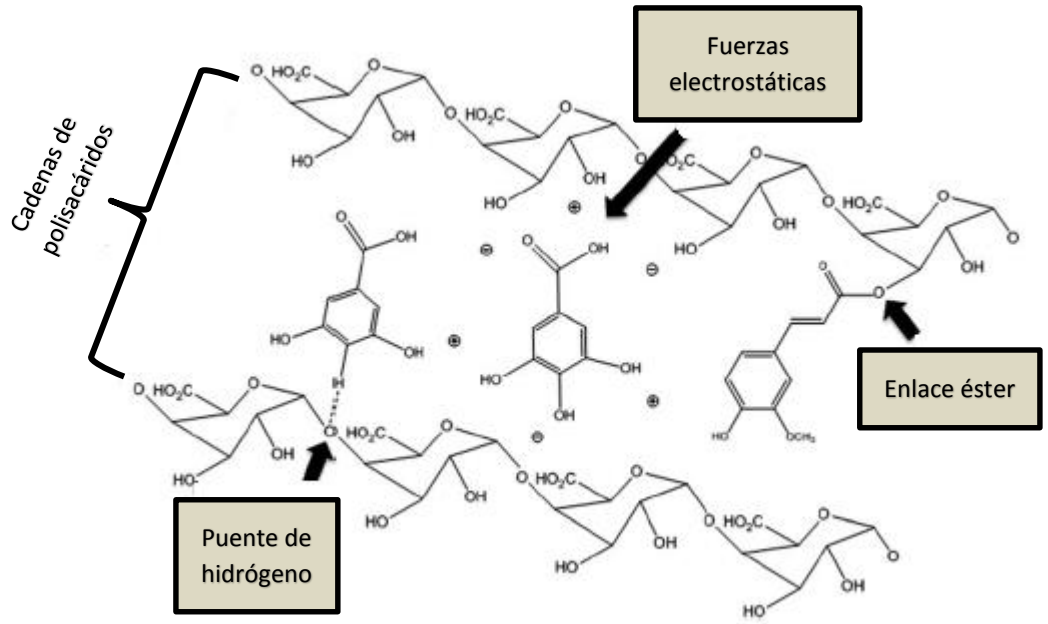


Figura 3. Tipos de interacciones entre los compuestos fenólicos y polisacáridos de la matriz celular.

Fuente: Quirós-Sauceda *et al*. (2014)

Las diferentes interacciones entre los fitoquímicos con los macronutrientes del maíz pueden ocurrir durante la fase de maduración, procesamiento del alimento o durante el proceso de digestión gastrointestinal y puede atribuirse a la capacidad de los diferentes componentes del maíz para unirse y atrapar compuestos fenólicos en varios sitios (Saura-Calixto *et al*., 2011).

Los estudios realizados en los últimos años han mostrado la importancia de estas interacciones. Se ha vuelto cada vez más claro que los polifenoles tienen diversas bioactividades potenciales en el cuerpo humano que se ven afectadas por las interacciones de los polifenoles con otras macromoléculas (Le Bourvellec y Renard, 2012). Las propiedades biológicas y los efectos en la salud de los compuestos fenólicos dependen de su ingesta y biodisponibilidad, que pueden verse afectadas por diferentes factores, incluida las interacciones químicas entre los constituyentes de la matriz alimentaria (Quirós-Sauceda *et al*., 2014). Estas interacciones podrían proteger a los fitoquímicos de la oxidación durante su paso por el tracto gastrointestinal, llegando al colón en donde pueden ser metabolizados bajo la influencia de la microflora bacteriana (Jakobek *et al*., 2015). Sin embargo, estas interacciones también pueden conducir a la pérdida de valor nutricional, actividad enzimática y otros efectos biológicos.

Propiedades Nutracéuticas de los Fitoquímicos del Maíz Azul

Se define como nutracéutico a cualquier alimento o ingrediente de los alimentos que ejerce acción benéfica en la salud humana (Biruete-Guzmán *et al*., 2009). Existe una gran cantidad de evidencia científica que sugiere que los fitoquímicos del maíz azul pueden ayudar a reducir la incidencia de una gran variedad de enfermedades crónicas (Bello-Perez *et al*., 2016). Las propiedades nutracéuticas del maíz azul se han relacionado con la actividad biológica (antioxidante) derivada del contenido de sus metabolitos secundarios (antocianinas y ácidos fenólicos) (Visioli *et al*., 2000). Estos compuestos ejercen efectos antidiabéticos y antiobesidad y actúan como agentes neuroprotectores (Prior y Wu, 2006; Tsuda,2012), reducen la inflamación, la mutagénesis (Zhao *et al*., 2009; Zhu *et al*., 2013), y la proliferación del crecimiento de células cancerosas (Urias-Lugo *et al*., 2015), ejercen protección cardiovascular (Mazza, 2007; He y Giusti 2010), además de tener acción protectora hacia las nefropatías que se desarrollan en pacientes con diabetes tipo 2 (Li *et al*., 2012).

Capacidad Antioxidante

La reacción en cadena inducida por los radicales libres es el mecanismo generalmente aceptado para la oxidación degenerativa en el tejido vivo (Wang y Stoner, 2008). La capacidad antioxidante se refiere a la capacidad de eliminar radicales reactivos de oxígeno: superóxidooxígeno singlete, peróxido, peróxido de hidrógeno y radical hidroxilo (Wang y Stoner, 2008). Por lo tanto, los antioxidantes pueden retrasar o prevenir el daño oxidativo en los sistemas biológicos (Halliwell *et al*., 1992). La propiedad antioxidante del maíz azul se ha evaluado exhaustivamente en ensayos celulares *in vitro* y estudios en animales *in vivo*. Los métodos *in vitro* incluyen el poder antioxidante reductor férrico, la actividad quelante de metales, así como la capacidad de eliminación de los radicales DPPH y ABTS. Por otro lado, los modelos *in vivo* incluyen estudios con ratas y ratones. Un estudio realizado por Zhang *et al*., (2014) demostró que la dieta de maíz azul sumistrada a ratas con daño oxidativo en hígado y riñon elevó la capacidad antioxidante y redujo el daño oxidativo en estos órganos.

Está bien establecido que los fitoquímicos, incluídos los ácidos fenólicos y las antocianinas, tienen excelente capacidad antioxidante que depende de su estructura (Cai *et al*., 2006). El potencial antioxidante de las antocianinas está influenciado por: (i) el número de grupos hidroxilo; (ii) el resto catecol en el Anillo B; (iii) el ion oxonio en el anillo C; (iv) la hidroxilación y patrón de metilación; (v) la acilación; y (vi) la glucosilación (Yang et al., 2011). La glucosilación de antocianinas disminuye la actividad captadora de radicales en comparación con la aglicona, ya que reduce la capacidad para deslocalizar electrones (Wang y Stoner, 2008). La contribución de los sustituyentes del anillo B a la eficiencia de la capacidad antioxidante es $-OH > -OCH_3 >> -H$, y por lo tanto el potencial antioxidante está en el orden de delfinidina> petunidina> malvidina>cianidina> peonidina> pelargonidina (Rossetto *et al*., 2007; Rahman *et al*., 2006). Además, la carga positiva del átomo de oxígeno en la molécula de antocianina hace que sea un potente donador de atomos de hidrógeno (Kong *et al*., 2003). En este sentido, se ha reportado que algunas antocianinas y sus agliconas asemejan la actividad de conocidos antioxidantes como el α-tocoferol, trolox y superan el poder antioxidante del ácido ascórbico (Kähkönen y Heinonen, 2003).

La capacidad antioxidante de los compuestos fenólicos es debida a sus propiedades redox, las cuales juegan un papel muy importante en la neutralización de radicales libres esto se debe a que las estructuras fenólicas tienen la capacidad de recuperar su estado reducido mediante un equilibrio redox a través de la donación de átomos de hidrógeno o donación de electrones (Shahidi y Wanasundara, 1992). Además, los compuestos fenólicos a menudo eliminan otras especies reactivas como $OH^{\bullet}$, $NO_2^{\bullet}$, N_2O_3, ONOOH y HOCl (Lee *et al*., 2010; Rent, 2007). El otro mecanismo por el cual los compuestos fenólicos funcionan para contrarrestar el estrés oxidativo es estimulando la síntesis y/o reposición del estado antioxidante celular induciendo la respuesta de las enzimas antioxidantes celulares en el cuerpo a través de los sistemas de superóxido dismutasa (SOD) y catalasa (CAT) (Shetty, 2004).

La acción antioxidante de los compuestos fenólicos y antocianinas incluye también la supresión de enzimas y oligoelementos que participan en la producción de radicales libres y especies reactivas de oxígeno y nitrógeno y en la protección de las defensas naturales de los antioxidantes. Los compuestos fenólicos y antocianinas inhiben las enzimas responsables que generan la producción de radicales, incluyendo xantina oxidasa, ciclooxigenasa y NADH oxidasa, así mismo son eficientes quelantes de metales traza que tienen una función importante en la generación de especies reactivas de oxígeno (Cos *et al*., 2003).

Factores que Intervienen en la Estabilidad de las Antocianinas

pH

La estabilidad química de las antocianinas es de considerable interés dados sus beneficios para la salud (Castañeda-Ovando *et al*., 2009). Su estabilidad puede verse afectada por factores como el pH y la temperatura. Dependiendo del pH las antocianinas pueden existir en cuatro especies diferentes: catión flavilio, pseudobase carbinol, chalcona y base quinoidal (Figura 4). En soluciones ácidas a pH=1-3 el catión flavilio (color rojo) es la estructura más estable. En medios acuosos, a medida que el pH se eleva a 4-5, reacciones de hidratación generan la pseudobase carbinol incolora, que puede además someterse a la apertura del anillo y dar lugar a las chalcones de color amarillo claro, ambas estructuras bastante inestables, mientras que a pH 7 a 8 se forma la base quinoidal azul-púrpura (Castañeda-Ovando *et al*., 2009).

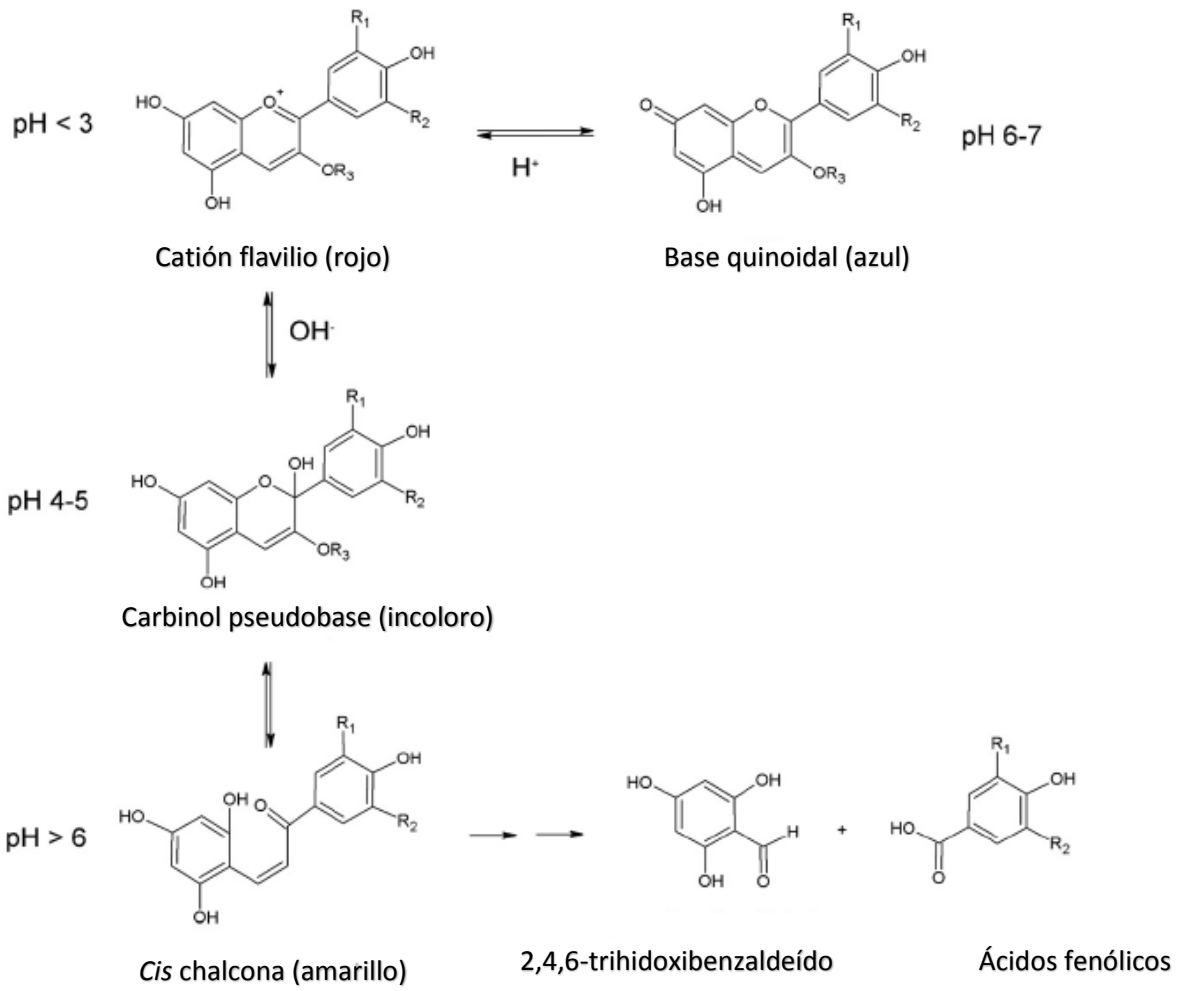


Figura 4. Formas químicas de las antocianinas dependientes del pH y reacciones de degradación.

Fuente: Castañeda-Ovando *et al*. (2009)

Los cambios en el color de estos compuestos son más significativos en la región alcalina debido a su inestabilidad. Esto sugiere que las antocianinas exhiben mayores tasas de degradación a pH superior, lo cual impacta negativamente en la concentración, y actividad biológica de estos compuestos (Cabrita *et al*., 2000).

Temperatura

La temperatura es otro de los factores críticos que influyen en la degradación de antocianinas. Un número variado de posibles reacciones de degradación de las antocianinas en las matrices de alimentos ocurren durante el procesamiento térmico, lo que puede involucrar varios mecanismos de reacción. Markaris *et al*., (1957) plantearon la hipótesis de que la apertura del anillo heterocíclico y la formación de la chalcona es el primer paso de la degradación de antocianinas. Adams, (1973) propuso que la hidrólisis del azúcar y la formación de agliconas son las etapas iniciales de la degradación térmica de las antocianinas posiblemente debido a la formación de aductos cíclicos. Adicionalmente, Tanchev y Ioncheva, (1976) identificaron como productos de degradación de las antocianinas a la quercetina, floroglucinaldehído, y ácido protocatéquico. Se ha informado en varios estudios que las antocianinas resisten bien procesos térmicos a altas temperaturas durante cortos periodos de tiempo. Por otro lado, un largo tiempo de exposición a elevada temperatura puede dar lugar a una degradación bastante rápida del catión flavilio (Nakay *et al*., 2015). En la Figura 5 se muestra el mecanismo propuesto de la degradación térmica de dos antocianinas. En general, la degradación térmica de las antocianinas es causada principalmente por oxidación, escisión de enlaces covalentes o reacciones de oxidación que dan como resultado una variedad de especies y compuestos intermedios dependiendo de la severidad y la naturaleza del tratamiento térmico (Patras *et al*., 2010).

Parámetros Cinéticos y Termodinámicos

Uno de los factores importantes a considerar en el procesamiento de alimentos es la pérdida de nutrientes (Patras *et al*., 2010). La retención de los fitoquímicos con capacidad antioxidante en el maíz después de su procesamiento parece complicada debido a que están expuestos a varias condiciones de proceso incluyendo la temperatura. Por lo tanto, estudios cinéticos son necesarios para minimizar el cambio no deseado y optimizar la calidad de alimentos específicos.

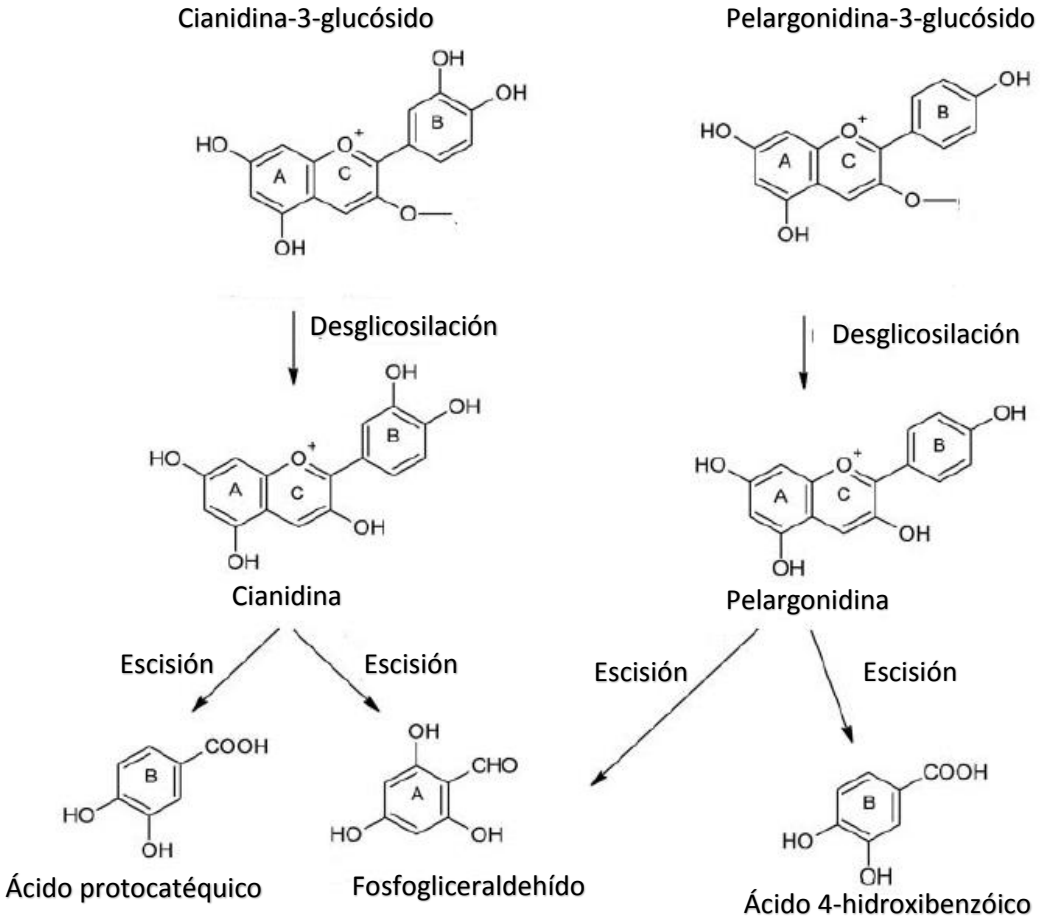


Figura 5. Posible degradación térmica de dos antocianinas.

Fuente: Sadilova *et al*. (2006).

El conocimiento de la cinética de degradación térmica incluido el orden de reacción, la constante de velocidad y la energía de activación, es vital para predecir influencia del procesamiento en los parámetros críticos de calidad y prevenir la pérdida de compuestos de interés (Patras *et al*., 2010).

Varios estudios han reportado un curso logarítmico de destrucción de compuestos termolábiles como las antocianinas con el aumento aritmético de temperatura, es decir que la degradación de las antocianinas en condiciones isotérmicas sigue una cinética de primer orden (Bolea *et al*., 2016; Turturică *et al*., 2016; Hernández-Herero y Frutos, 2011). Esto sugiere que los factores inestables a la temperatura pueden acelerar la destrucción de las antocianinas. La velocidad de degradación de estos compuestos se refleja en los valores numéricos de las estimaciones de los parámetros cinéticos (Nayak *et al*., 2015). Es importante para la cinética de degradación de primer orden la estimación del valor de la constante de velocidad de degradación (*k*) y el tiempo de vida media (t ½). A partir de la pendiente de la curva que describe la variación de la concentración de antocianinas en función del tiempo de calentamiento es posible obtener el valor de la constante de velocidad de degradación de las antocianinas. La constante *k* es una función del número de moléculas que reaccionan en el sistema, cuanto más bajo es su valor, mejor es la estabilidad de las antocianinas. El tiempo de vida media, a su vez, indica el tiempo necesario para que la concentración de las antocianinas disminuya hasta la mitad de su concentración inicial (Peron *et al*., 2017).

La relación existente entre la temperatura y la velocidad de deterioro de las antocianinas en un alimento se puede expresar matemáticamente de diversas maneras (Labuza, 1984). La forma más clásica de representar la velocidad de deterioro en función de la temperatura, es por medio de la ecuación de Arrhenius. A través del modelo de Arrhenius se puede calcular el parámetro de energía de activación (Ea) (Fracassetti *et al*., 2013). El valor numérico de la energía de activación de una reacción química indica la barrera energética que las moléculas deben superar para reaccionar. Adicionalmente, existen otros parámetros para estimar la influencia de la temperatura en la velocidad de degradación, tal como el valor *z*, valor D y el coeficiente Q_{10}, los cuales son empleados para estimar el intervalo de temperatura que ocasiona una variación de diez veces en la velocidad de transformación, calcular el tiempo de calentamiento requerido

para reducir la concentración de antocianinas en un 90%, y determinar el aumento en la velocidad de reacción cuando la temperatura se eleva 10 °C, respectivamente (Mercali *et al*., 2013; Fracassetti *et al*., 2013; Toledo, 1991).

La estimación de los parámetros termodinámicos tales como el cambio de entalpía (ΔH), entropía (ΔS) y energía libre de Gibbs (ΔG) aporta mayor información sobre las características de la reacción. El valor de ΔH indica si el calor es liberado o absorbido durante la reacción (Peron *et al*, 2017). El ΔS puede interpretarse como una medida del desorden del sistema. El cambio de energía libre de Gibbs (ΔG) permite determinar si se puede pasar de un estado de transición a otro por medio de un cambio espontáneo (Mercali *et al*., 2013). Cuanto más pequeña es la energía libre de Gibbs más rápidamente avanza la reacción de degradación (Brown *et al*., 2004), mientras que a mayor ΔG, menor es la constante de velocidad de reacción y por lo tanto más lenta es la reacción (Sykes, 1982).

Procesos de Nixtamalización

Nixtamalización Tradicional

La nixtamalización es el proceso mediante el cual se realiza la cocción del maíz con agua y cal para obtener el nixtamal que, después de molido da origen a la masa nixtamalizada utilizada para la elaboración de tortillas. La palabra nixtamalización deriva del Náhuatl: nixtli =cenizas y tamalli = masa de maíz cocido. Durante el proceso tradicional, el maíz es sometido a condiciones de alto contenido de humedad, temperatura (80 a 105 °C) y pH elevado (11 a 12) (De la Parra *et al*., 2007). El proceso de nixtamalización requiere la cocción de los granos de maíz en una solución de agua con hidróxido de calcio ($Ca(OH)_2$) a temperatura de ebullición, seguido de un período de reposo del orden de 12 horas. Después del periodo de reposo, la solución alcalina conocida como nejayote es drenada y en este punto al maíz se le llama nixtamal. El nixtamal es molido y como resultado se obtiene la masa para elaborar las tortillas de maíz (Serna-Saldívar *et al*., 1990).

La nixtamalización provoca cambios en la estructura, composición química y valor nutricional del maíz. Promueve el aumento significativo en el contenido de calcio, incrementa la fibra dietaria soluble y la biodisponibilidad de aminoácidos esenciales, lo que aumenta el valor

biológico de la proteína (Paredes-López *et al*., 2009). También favorece la formación de almidón resistente, el cual al no ser digerido se comporta de forma similar a la fibra soluble, con los beneficios para la salud que esto conlleva. Adicionalmente, se ha reportado la degradación de aflatoxinas durante la nixtamalización y elaboración de tortillas (Méndez-Albores *et al*., 2004).

Por otro lado, la nixtamalización también induce cambios negativos como la eliminación parcial del pericarpio o salvado debido al tratamiento con álcali y el lavado nixtamal, de modo que los productos terminados se consideran alimentos de grano semi-integral. Esto es importante porque el consumo de granos integrales se ha asociado con la prevención de enfermedades cardiovasculares.

Respecto al efecto de la nixtamalización en el contenido de fitoquímicos, diversos autores han reportado una reducción significativa en el contenido de antocianinas y compuestos fenólicos (Del Pozo-Insfran *et al*., 2006; Lopez-Martínez *et al*., 2011). En general, estas pérdidas significativas pueden atribuirse al efecto combinado del ambiente alcalino y procesamiento térmico durante la nixtamalización, así como a pérdidas físicas del pericarpio y lixiviación de compuestos en el licor de cocción (nejayote).

Adicional a los cambios químicos, estructurales y nutricionales que provoca la nixtamalización tradicional en el maíz, la generación de altas cantidades de descargas de líquido de desecho alcalino que tiene una gran demanda de oxígeno (3-10 litros de efluentes contaminantes / kg de maíz) y los altos costos energéticos debido a la baja eficiencia de transferencia de calor hacen necesario la búsqueda y utilización de tecnologías alternativas más ecológicas y rentables (Cortés-Gómez, 2005).

Nixtamalización por Extrusión

Una de las tecnologías alternativas a la nixtamalización tradicional es la extrusión, la cual es un proceso que combina operaciones unitarias como transporte, mezclado, cocimiento y formado (Alam *et al*., 2016). La tecnología de extrusión se define como un proceso continuo de alta temperatura y corto tiempo que combina el corte mecánico y el calor para la gelatinización del almidón y la desnaturalización de las proteínas en los alimentos, obteniéndose un producto

plastificado y reestructurado (El-Dash, 1981; Harper, 1989). El extrusor se puede dividir en tres regiones: transporte, compresión y fusión/plastificación en términos de la transición del almidón (Figura 6) (Alam *et al*., 2016).

El proceso de cocción por extrusión ofrece la ventaja principal de la nula generación de aguas residuales (nejayote); la retención de nutrientes asociados con los tejidos del pericarpio y aleurona y la producción de alimentos integrales (Serna-Saldivar *et al*., 1988). La nixtamalización por extrusión se emplea en la fabricación de harinas pre-gelatinizadas adecuadas para tortillas (Milán-Carillo *et al*., 2006). El maíz en este proceso se usa molido integralmente, es acondicionado con cal y agua y la mezcla es calentada, el cocimiento por extrusión se realiza en condiciones de temperatura alta (90–120°C), baja humedad y cortos tiempos de proceso (Harper, 1990). Debido a estas características el proceso de extrusión está ganando popularidad, ya que además es un proceso versátil, los costos de procesamiento son relativamente bajos, presenta una alta tasa de rendimiento, producción automatizada, y los productos obtenidos se consideran de alta calidad.

No obstante, durante este proceso, se producen cambios químicos y estucturales que afectan las propiedades funcionales de las harinas nixtamalizadas incluyendo la capacidad de absorción de agua, la densidad aparente y la viscosidad, así como la presencia y concentración de fitoquímicos (Ruíz-Gutiérrez *et al*., 2014).

Efecto de las condiciones del proceso de extrusión sobre las propiedades funcionales y nutracéuticas

Los factores empleados en la extrusión con mayor efecto en los fitoquímicos y en los componentes de la matriz alimenticia, principalmente el almidón el cual es el componente mayoritario del maíz reponsable del desarrollo de las propiedades reológicas, amilográficas y texturales de sus productos son: el tamaño de partícula, el contenido de humedad, la temperatura y la velocidad de tornillo. En la literatura hay diversos reportes que relacionan estas variables con cambios estructurales importantes en el almidón, así como en la degradación, retención o incremento de los compuestos fenólicos en las matrices alimentarias lo cual impacta

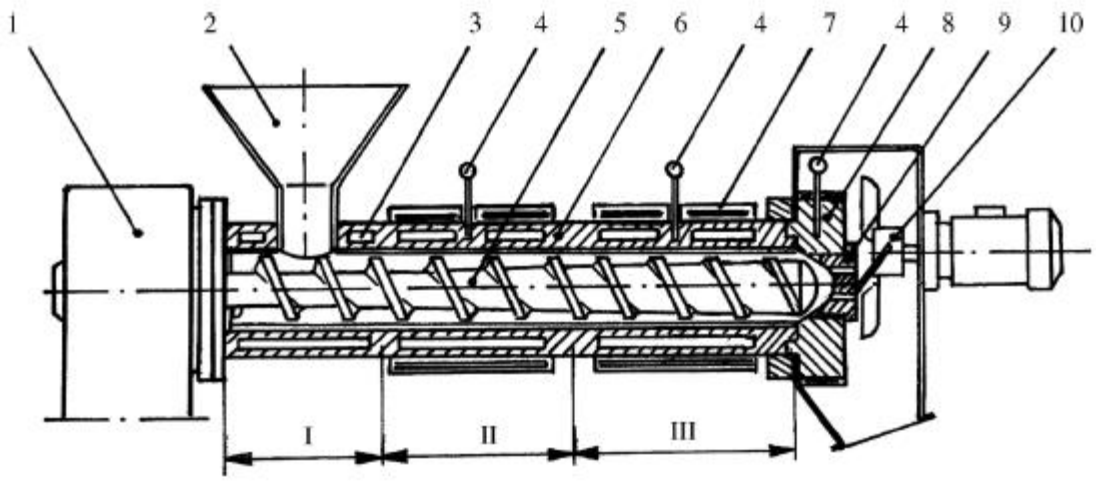


Figura 6. Sección transversal de un extrusor de tornillo simple. 1-motor, 2 – tolva de alimentación, 3-chaqueta de enfriamiento, 4-termopar, 5-tornillo, 6-barril, 7-chaqueta de calentamiento, 8-cabezal, 9-dado, 10-cortador, I -sección de transporte, II - sección de compresión, III sección de fusión y plastificación.

Fuente: Moscicki *et al*. (2013).

directamente en la calidad del producto final (Mora-Rochín *et al*., 2010; Escalante- Aburto *et al*., 2016; Li *et al*., 2014).

El tamaño físico de las partículas alimentadas al extrusor es determinante, partículas más pequeñas tienen distancias más cortas y el calor viaja más rápido, la temperatura se eleva, volviéndose más fluidas en el barril del extrusor, por otro lado, si son de mayor tamaño pueden tardar más tiempo en fundirse debido al requisito de transferencia de calor (Guy, 2001).

El contenido de humedad del material alimentado al extrusor es otra variable con impacto en el contenido de los fitoquímicos y la integridad del almidón. El contenido de humedad del material alimentado influye en propiedades tales como la viscosidad del fluido, el tiempo de residencia del material en el extrusor y el esfuerzo cortante aplicado, lo que afecta las características físicas de los extrudidos y el consumo de energía (Ruíz-Gutiérrez *et al*., 2014). A bajas humedades, el material se encontrará menos hidratado por lo tanto la severidad del proceso se acenturá en un mayor grado. A moderada humedad se provoca un efecto de lubricación, la fluidez del material aumenta y menos energía mecánica es gastada, el agua entonces actúa como plastificante protegiendo de la degradación a éstos compuestos. A medida que la cantidad de agua aumenta influye en la propiedad de viscosidad, y se genera la polimerización de los compuestos fenólicos, lo cual decrementa su capacidad antioxidante (Guy, 2001).

El incremento de la temperatura puede afectar negativamente a los compuestos termolábiles como ejemplo las antocianinas. Las altas temperaturas > 80 °C, pueden descomponerse o alterar la estructura molecular de estos compuestos, con la consecuente disminución de la capacidad antioxidante debido a sus cambios estructurales (Dlamini *et al*., 2007; Altan *et al*., 2009), específicamente debido a su capacidad para donar átomos de hidrógeno de los grupos hidroxilo a los radicales libres (Devi *et al*., 2014). Por otro lado, se ha reportado que el aumento de la temperatura puede ejercer efectos positivos en la retención de compuestos termolábiles, ya que al elevarse la temperatura se crea una pasta que fluye con mayor rapidez a través del extrusor lo que conlleva a un menor tiempo de residencia y un menor daño térmico de estos compuestos por el poco tiempo de exposición (Guy, 2001).

La configuración y velocidad del tornillo es otro parámetro que afecta el grado de mezclado, el tiempo de residencia y el daño mecánico, afectando el grado de cocimiento y la fragmentación del almidón, así como la estabilidad de los fitoquímicos. A baja velocidad de tornillo se presenta un menor daño mecánico y un aumento en el tiempo de residencia en el extrusor. A alta velocidad de tornillo ocurre lo contrario, aumenta el daño mecánico causado a los componentes del material alimentado. El principal cambio en los compuestos fenólicos debido a la velocidad de tornillo es la descarboxilación, rompimiento de estructuras celular y liberación de compuestos. Las grandes fuerzas de corte además causan deterioro de la estructura cuaternaria y terciaria del almidón. Esta degradación macromolecular se refleja como cambios en la reología, las propiedades funcionales del producto, como el grado de solubilidad en agua, la capacidad de absorción agua y el desarrollo de la viscosidad (Fitton, 1986; Vergnes y Villemaire, 1987; Doublier *et al*., 1986; Colonna y Mercier, 1983).

La combinación de los factores del proceso de extrusión producirá harinas con un menor o mayor daño al almidón, que repercutirá en su funcionalidad, como la absorción de agua, y posterior rendimiento y dureza de la masa y la tortilla, así como en las propiedades nutraceúticas de la tortilla debido a una mayor conservación de compuestos con capacidad antioxidante. Por lo que en este sentido se destaca la relevancia de realizar una optimización del proceso para evaluar el efecto de las condiciones empleadas y sus interacciones con los parámetros estudiados, y así encontrar la región óptima con la mayor retención de compuestos de interés sin afectar las características de calidad propias del alimento, en este caso la tortilla azul.

Digestión y Bioaccesibilidad de los Fitoquímicos

Para ejercer efectos dentro del organismo humano, los compuestos fenólicos deben liberarse de la matriz alimenticia durante la digestión y luego absorberse en el intestino en cierta cantidad (Figura 7) (Parada y Aguilera, 2007). Debido a ello, su bioaccesibilidad y biodisponibilidad ha sido sujeto de varios estudios y revisiones (Mosele *et al*., 2016). La bioaccesibilidad se define como la cantidad de un compuesto ingerido que está disponible para su absorción en el intestino (Palafox-Carlos *et al*., 2011).

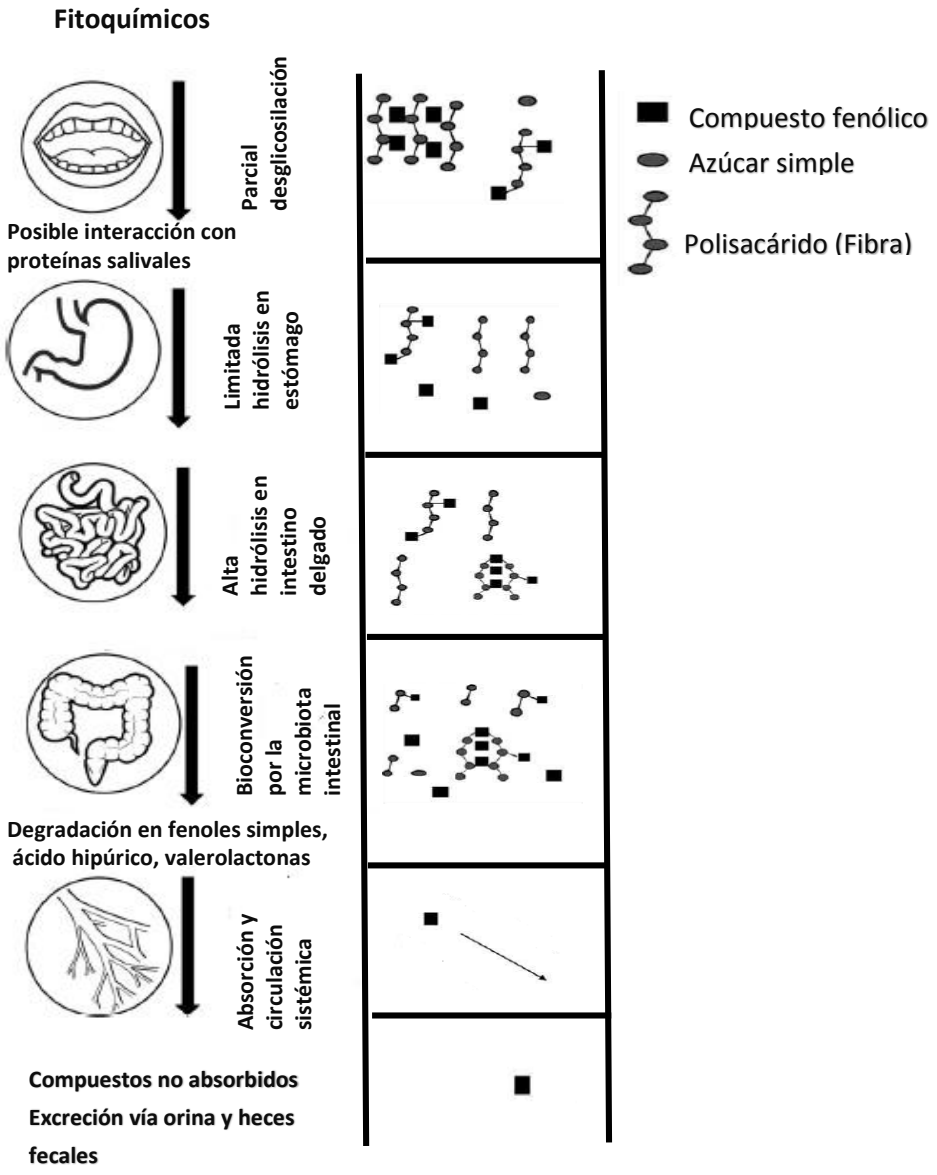


Figura 7. Bioaccesibilidad gastrointestinal general de los compuestos fenólicos.

Fuente: Adaptado de Karás *et al*. (2017) y Palafox-Carlos *et al*. (2010)

Métodos de digestión *in vitro* e *in vivo* se han utilizado para estudiar la liberación de compuestos fenólicos de la matriz alimentaria (Alminger *et al*., 2014). Los métodos *in vivo*, que usan animales o humanos, generalmente son más confiables, pero son complejos, muy variables, requieren mucho tiempo, son costosos y están restringidos por preocupaciones éticas (Parada y Aguilera, 2007). Por ello, modelos *in vitro* se han desarrollado y empleado para predecir la bioaccesibilidad de los fitoquímicos. El enfoque *in vitro* incluye el uso de modelos de simulación de la digestión humana, que se han desarrollado porque son técnicamente simples, seguros, relativamente económicos, rápidos y sin restricciones éticas (Gil-Izquierdo *et al*., 2002; Ahmad-Qasem *et al*., 2014). Los modelos de digestión *in vitro* imitan las condiciones fisicoquímicas y fisiológicas del tracto gastrointestinal humano (Carbonell-Capella *et al*., 2014), éstos se realizan básicamente mediante una hidrólisis enzimática que involucra a las enzimas α -amilasa, pepsina y pancreatina, las muestras de estudios son homogeneizadas durante un periodo de tiempo a dierentes pH´s, dependiendo de la fase de la digestión, y a una temperatura constante de 37 °C imitando la temperatura interna del cuerpo (Alminger *et al*., 2014). En general, se ha considerado que la digestión gastrointestinal *in vitro* es una herramienta útil para evaluar la bioaccesibilidad de compuestos con actividad biológica, esto respaldado por varios estudios que han demostrado una buena correlación entre los resultados obtenidos utilizando sistemas *in vitro* e *in vivo* (Carbonell-Capella *et al*., 2014).

La bioaccesibilidad de los fitoquímicos inicia desde que el alimento es consumido vía oral. En la boca, la masticación provoca la ruptura de algunas células, lo que permite que los compuestos fenólicos y otros nutrientes se liberen (Padayachee *et al*., 2012). La trituración mecánica además disminuye el tamaño de partícula y aumenta la superficie de contacto para la interacción de los micronutrientes del alimento con las proteínas salivares como la α -amilasa formándose así el bolo alimenticio. En esta etapa las antocianinas y compuestos fenólicos se ingieren en su forma nativa, sin embargo, la biotransformación inicia, aunque debido a que generalmente la fase oral es de corta duración (2-5 minutos) la influencia de la amilasa salival con el bolo alimenticio es limitada (Kamonpatana *et al*., 2014; Mosele *et al*., 2016).

Posterior a la fase oral, el bolo alimenticio pasa al estómago, en donde una vez mezclado con los jugos gástricos forma el quimo. En la fase gástrica el HCl activa la pepsina, enzima

responsable de romper enlaces peptídicos de las proteínas dando lugar a la presencia de aminoácidos y péptidos en los fluidos gástricos (Alminger *et al*., 2014). Las condiciones ácidas (pH=2-3) favorecen la estabilidad de los fitoquímicos, especialmente a las antocianinas, donde la forma nativa como catión flavilio predomina (Cavalcante-Braga *et al*., 2018).

Después de la desintegración del alimento en la boca y el estómago, la mayor digestión enzimática y principal zona de absorción de nutrientes tienen lugar en el intestino delgado. Después de la fase gástrica, el quimo ácido se neutraliza con bicarbonato de sodio para proporcionar un pH apropiado para la actividad proteolítica, lipolítica y amilolítica de la pancreatina que junto con las sales biliares son las reponsables de la digestión del quimo en el intestino delgado (Alminger *et al*., 2014). En esta fase de la digestión las antocianinas son convertidas a su forma carbinol y chalcona debido al mayor pH de esta fase (7-7.5) (Figura 8) (Cavalcante Braga *et al*., 2018). Por otro lado, una gran proporción de los compuestos de mayor peso molecular como las proantocianidinas, antocianinas aciladas o ácidos fenólicos que permanecían ligados a los componentes de la pared celular como lignina, celulosa, pectinas y algunos otros ligados a proteínas, son liberados y solo una pequeña fracción sigue su curso hasta llegar al intestino grueso o colon en donde por acción de la microbiota (enzimas del tipo esterasas y xilanasas) son liberados (Pérez-Jiménez *et al*., 2013).

En el colon (pH>7), se da la biotransformación por completo de los ácidos fenólicos y antocianinas; estos son metabolizados por la microbiota hasta formar compuestos como el ácido hipúrico, valerolactonas y catecol generando un ambiente antioxidante con efectos pre-bióticos, intensificando el desarrollo de lactobacilos y bifidobacterias y reduciendo la prevalencia de *E. coli* y Clostridium (Pérez-Jiménez *et al*., 2013; Saura-Calixto, 2011). Los metabolitos generados por la microbiota, asi como otros que no fueron metabolizados, son transportados a través del sistema circulatorio llegando al hígado, en donde las enzimas de la fase II convierten a estos compuestos en sus derivados metilados, sulfo-conjugados y/o glucoronidados (Pérez-Jiménez *et al*., 2013). De esta forma es como se distribuyen a los tejidos como el cerebro, riñones, ojos entre otros en donde llevan acabo su bioactividad. Algunos otros compuestos son excretados vía urinaria o en las heces fecales dando fin al proceso de digestión gastrointestinal (Pérez-Jiménez *et al*., 2013; Passamonti *et al*., 2005; Talavera *et al*., 2005).

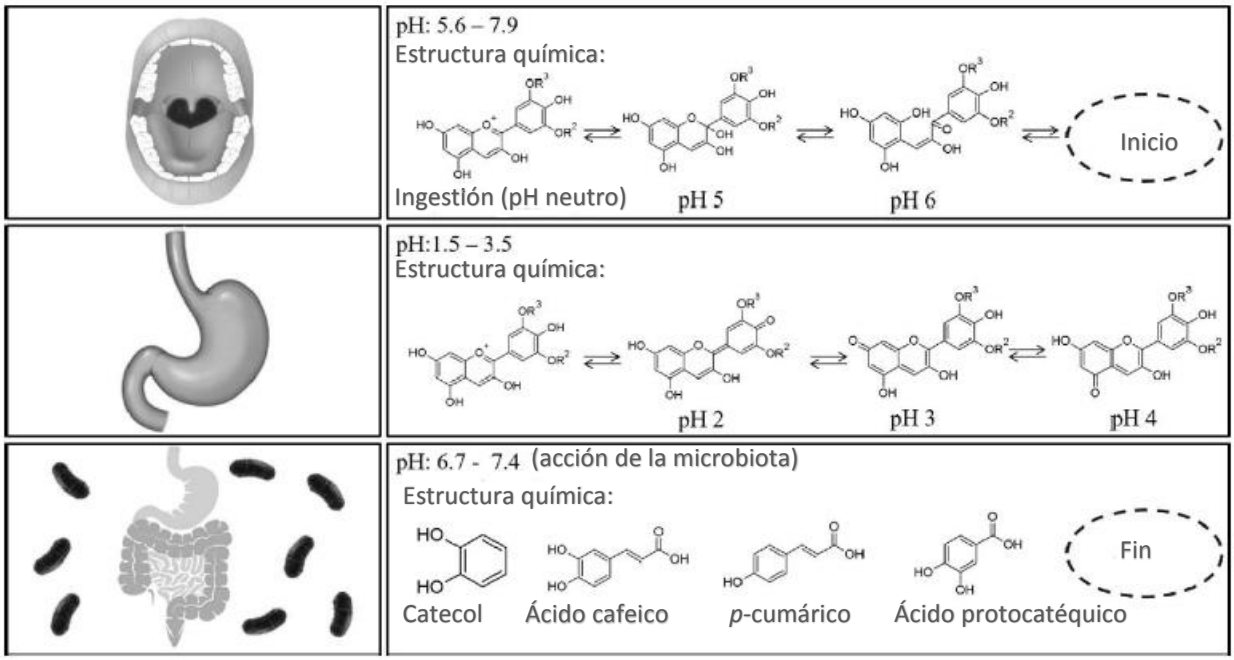


Figura 8. Estructuras químicas de las antocianinas influenciadas por las fases del proceso de digestión, pH y bioconversión en compuestos fenólicos Donde R_2=H y R_3 = Metilo.

Fuente: Cavalcante Braga *et al*. (2018)

HIPÓTESIS

La optimización de las condiciones del proceso de extrusión impactará positivamente en la retención de antocianinas en harinas nixtamalizadas de maíz azul y propiedades estructurales en harinas y tortillas, contribuyendo favorablemente en una mayor bioaccesibillidad de los fitoquímicos con capacidad antioxidante en la tortilla azul comparado con una tortilla blanca tradicional.

OBJETIVO GENERAL

Optimizar las condiciones del proceso de nixtamalización por extrusión de maíz azul para la obtención de tortillas con alto contenido de antocianinas y textura adecuada, y evaluar la estabilidad y capacidad antioxidante de los fitoquímicos de la tortilla bajo un sistema de digestión gastrointestinal simulado.

Objetivos Particulares

1. Determinar el contenido de fitoquímicos (antocianinas, compuestos fenólicos), capacidad antioxidante y características físicoquímicas del maíz azul (*Zea mays* L.).

2. Evaluar el efecto de las condiciones del proceso de extrusión sobre el contenido de antocianinas, propiedades funcionales y de pasta de harinas nixtamalizadas y optimizar el proceso para la obtención de una harina de maíz con un máximo contenido de antocianinas y máxima viscosidad.

3. Determinar las propiedades texturales y estimar los parámetros cinéticos y termodinámicos de la degradación térmica de las antocianinas presentes en las tortillas obtenidas a partir de la harina de maíz optimizada.

4. Evaluar la estabilidad y capacidad antioxidante de los compuestos fenólicos y antocianinas presentes en la tortilla extrudida sometida a un proceso de digestión gastrontestinal *in vitro.*

DESARROLLO DEL TRABAJO DE INVESTIGACIÓN

La presente investigación se dividió en 3 etapas (Figura 9), que corresponden al desarrollo de los objetivos planteados. En la Figura 10 se describe el diagrama general de la investigación en donde se incluye la metodología empleada para llevar a cabo las 3 etapas experimentales. El trabajo realizado en cada una de las etapas derivó en la escritura y publicación o en vías de publicarse de artículos cientifícos en revistas indizadas.

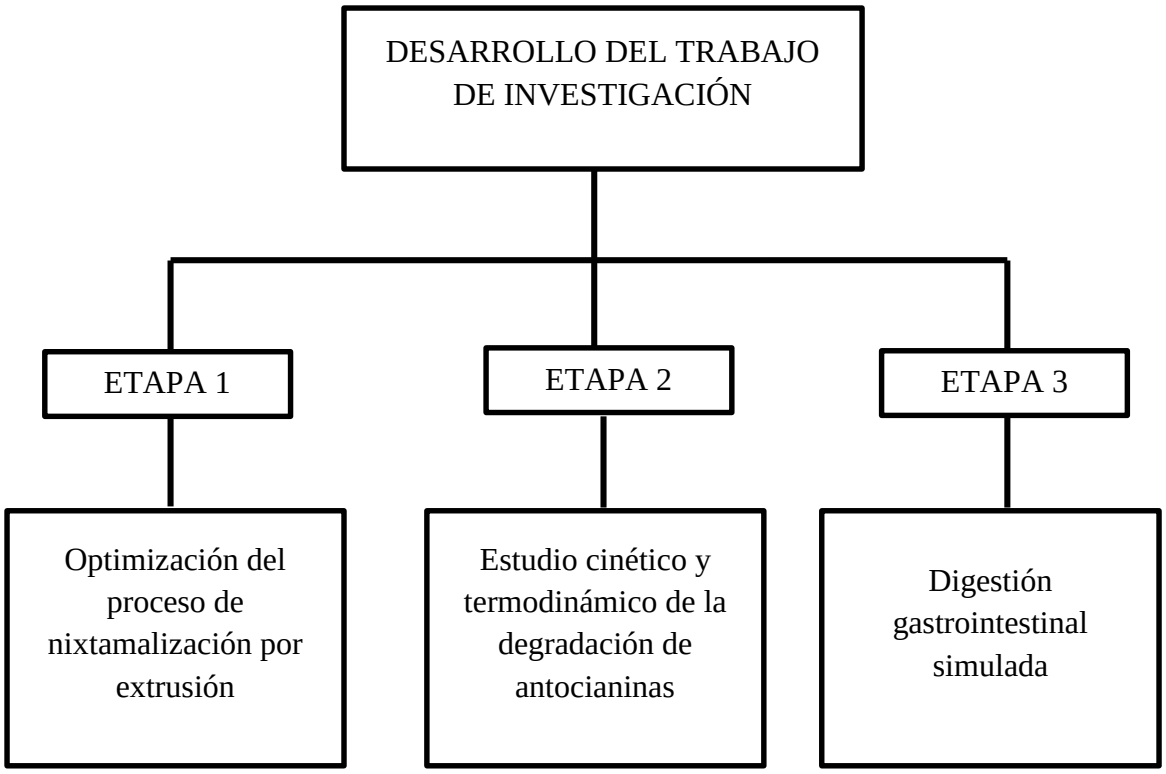


Figura 9. Representación esquemática del desarrollo del trabajo de investigación.

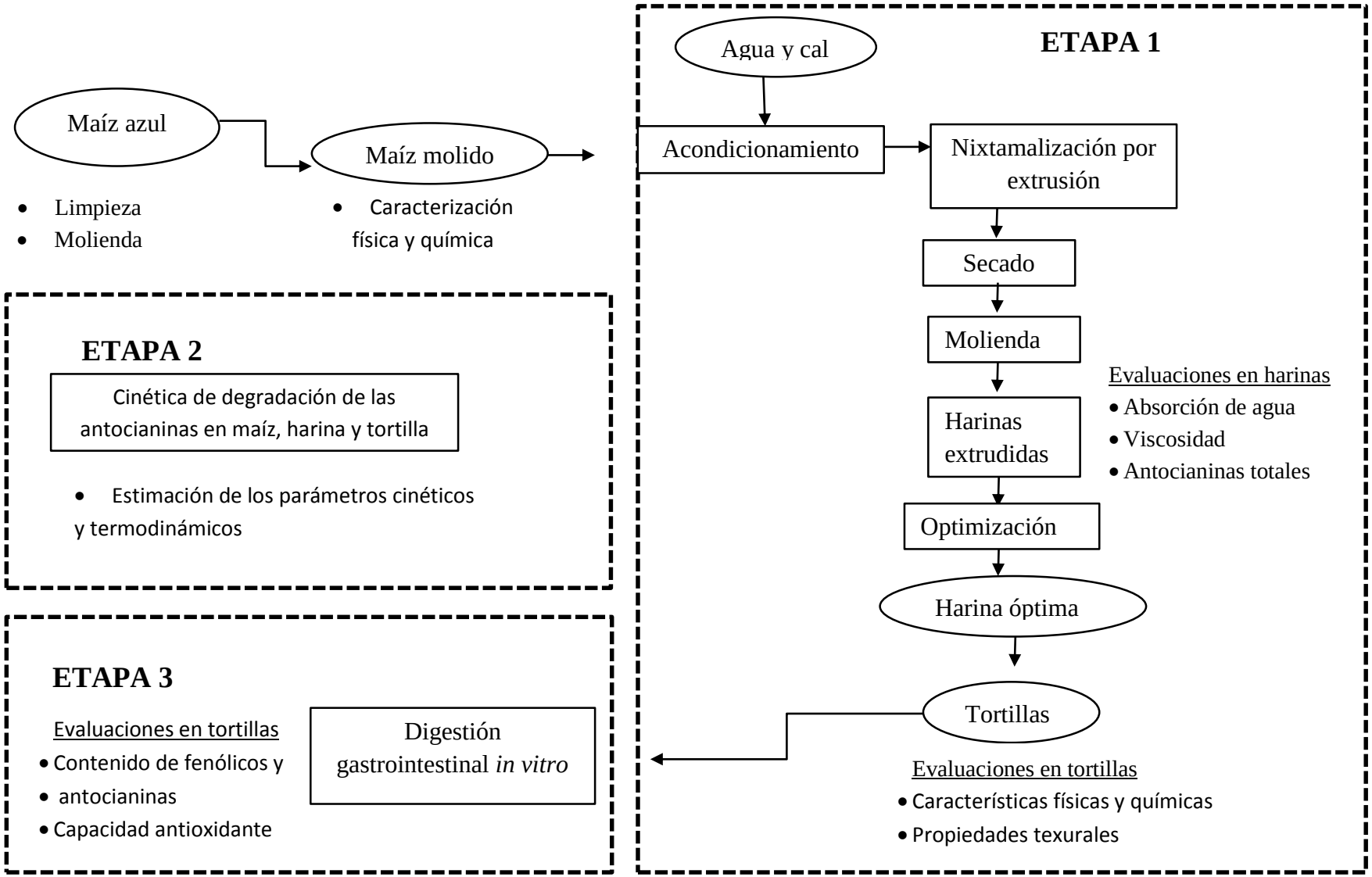


Figura 10. Diagrama general de la investigación.

CAPÍTULO 1. OPTIMIZACIÓN DEL PROCESO DE NIXTAMALIZACIÓN POR EXTRUSIÓN

Artículo Científico Derivado de la Etapa 1: Effect of Extrusion Conditions on The Anthocyanin Content, Functionality, and Pasting Properties of Obtained Nixtamalized Blue Corn Flour (*Zea mays* L.) and Process Optimization

Este artículo fue publicado en la revista Journal of Food Science (Editorial Wiley, by John Wiley & Sons, Inc.). El objetivo fue evaluar el efecto de los factores de extrusión sobre las propiedades de las harinas de maíz nixtamalizadas extrudidas, determinar las condiciones óptimas y producir una tortilla con textura y características nutracéuticas aceptables para los consumidores. Se utilizó maíz azul molido (malla 2 mm) acondicionado con 0.3 % de hidróxido de calcio y se elaboraron las harinas bajo las condiciones obtenidas de una matriz de un diseño central compuesto, donde los factores fueron: humedad de alimentación (HA, 15-30%), temperatura de la cuarta zona del extrusor (TE, 70-110 °C) y velocidad de tornillo (VT, 50-145 rpm). Los extrudidos se secaron a 50 °C durante 1 h, y se molieron (2 mm) para obtener las harinas. Las variables respuesta fueron químicas, funcionales y de pasta. Se utilizó la metodología de superficie de respuesta (MSR) para evaluar los datos experimentales y se optimizó en función del máximo contenido de antocianinas y máximo pico de viscosidad utilizando el método numérico de deseabilidad global. Con la harina óptima se obtuvo la tortilla la cual fue caracterizada física, química y texturalmente. Los resultados indican que la HA fue el factor que tuvo un gran efecto sobre las propiedades evaluadas en las harinas. El área óptima de proceso se determinó a una HA de 18.17%, una TE de 92.03 °C y una VT de 76.61 rpm. Las tortillas obtenidas mostraron características de textura adecuadas y un valor nutracéutico prometedor a través de un alto contenido de antocianinas. El enfoque presente en este estudio puede proporcionar una guía útil para desarrollar y optimizar productos innovadores a base de maíz pigmentado. En el artículo se detalla el planteamiento, metodología, resultados y discusión de esta etapa de la investigación.

Effect of extrusion conditions on the anthocyanin content, functionality, and pasting properties of obtained nixtamalized blue corn flour (*Zea mays* L.) and process optimization

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Abstract: The aim of this study was to evaluate the effect of extrusion factors on the properties of extruded nixtamalized corn flours (ENCFs), determine the optimal conditions, and produce a tortilla with texture and nutraceutical characteristics acceptable for consumers. The processing factors used were feed moisture (FM, 15 to 30%), extruder temperature (T, 70 to 110 °C), and screw speed (SS, 50 to 145 rpm). The properties evaluated in the flours were total anthocyanins (TA), subjective water absorption capacity, and peak viscosity (PV). Response surface methodology and analysis of variance were used in the evaluation. The linear and quadratic terms of FM had a greater effect on all evaluated parameters. The optimization was performed using the numerical method of global desirability. The response variables that were optimized in the ENCF were TAs (maximize) and PV (maximize). The optimal region was the following: FM (18.17%), T (92.03 °C), and SS (76.61 rpm). The experimental value for the TA in the optimized ENCF was 226.07 mg/kg, and the PV was 1063.9 cP. The results of this study could help develop nixtamalized corn flours with desirable characteristics to make tortillas using the extrusion process.

Keywords: bioactive compounds, pigmented corn, starch, Tortilla texture, viscosity

Practical Application: The results obtained would be useful for the tortilla industry, developing nixtamalized corn flours with desirable characteristics to make healthy tortillas using the extrusion process, with minimum losses in biologically active compounds such as anthocyanins (health promoters) without affect negatively the eating quality of the product (good texture).

1. INTRODUCTION

Corn tortillas obtained by the nixtamalization process are a staple food of the Mexican population. They are the most important source of protein, calcium, fiber, and energy. Corn tortilla production is now classified as one of the most important agro-industrial activities. The consumption of corn tortillas has penetrated widely into the United States market and that of some countries in Asia and Europe (Cortés-Gómez, San Martín-Martínez, Martínez-Bustos, & Vázquez-Carrillo, 2005).

The traditional nixtamalization process (TNP) for making tortillas consists of cooking corn grains in an alkaline solution, followed by a steeping period (10–14 hr). After this, the cooked grains (nixtamal) are washed and ground to obtain masa. The high cost and requirements of space and sanitation, as well as the generation of large amounts of alkaline waste (pH 11 to 13), are some of the technological and environmental disadvantages of this process. Recently, alternatives to traditional nixtamalization have been studied. The extrusion nixtamalization process (ENP) presents promising characteristics, such as the production of corn tortillas with the use of a small amount of water and without the generation of polluting effluents, as well as the use of the whole grain (Aguayo-Rojas et al., 2012).

Currently, the use of pigmented maize, such as blue corn (BC), is of interest due to its health-promoting effect. There is an inverse relationship between the consumption of certain natural compounds found in this type of corn and the incidence of degenerative diseases (Bello-Pérez, Camelo-Méndez, Agama-Acevedo, & Utrilla-Coello, 2016). The nutraceutical properties of BC derive from secondary metabolites (anthocyanins and phenolic acids); these chemical compounds contribute to the antioxidant activity of BC, which protects cells from oxidative damage (Bello-Pérez et al., 2016).

Anthocyanins have a direct free-scavenging capacity due to their hydrogen (electron) donation ability (Bello-Pérez et al., 2016). The consumption of anthocyanins does not represent adverse health effects due to their high level of safety, which has been

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doi: 10.1111/1750-3841.15312

supported by extensive scientific research and pharmacovigilance (Pojer, Mattivi, Johnson, & Stockley, 2013). Depending on the diet, the daily estimated consumption by men is 19.8 to 64.9 mg/day and by women is 18.4 to 44.1 mg/day (Pojer et al., 2013). The positive effect of anthocyanins supports the current exploitation of this type of pigmented corn as a healthy food alternative for humans.

During the TNP, chemical changes occur in the corn components due to the nixtamalization process conditions. The combination of the high temperature, water content, and calcium hydroxide concentration facilitate the release of the compounds of interest from the cell wall, as well as protein denaturation and starch gelatinization (Almeida & Rooney, 1996). On the other hand, the main process factors in the ENP that affect the corn components are feed moisture (FM), temperature (T), and screw speed (SS). Losses in anthocyanins are caused by high temperatures, which causes the loss of glycosylating sugars with the consequent opening of the ring and production of colorless chalcones; high FM promotes polymerization, and the SS produces decarboxylation (Ruíz-Gutiérrez, Sánchez-Madrigal, & Quintero-Ramos, 2018). The ENP retains more anthocyanins (47 to 59%) than the TNP (0 to 39%) due to the low exposure time to high temperatures. In contrast, during the TNP, corn kernels are exposed to high temperature for a long period of time under more alkaline conditions than those of the ENP. In addition, there are losses of anthocyanins during the TNP by leaching into the steep solution.

Over the years, several authors reported more anthocyanins retention during the extrusion process than the traditional process (Aguayo-Rojas et al., 2012; Escalante-Aburto et al., 2013). These works indicated that half of the anthocyanins present in grain were retained during the ENP. Aguayo-Rojas et al. (2012) reported a 46.5% retention in anthocyanin content when the kernel was processed into tortillas. Other researchers observed good retention (17.7 to 38.5%) of these compounds in nixtamalized BC expanded extrudates when compared with the content in raw corn (Escalante-Aburto et al., 2013). These results indicated that the extrusion process retained high levels of anthocyanins. However, evaluation the effects of the extrusion process factors, when finding the best and optimized conditions of the ENP to produce extruded nixtamalized corn flour (ENCF) with a high anthocyanin content and tortillas with good texture have not been reported.

Starch is the major component of corn, and the conversion of starch to a thermoplastic material leads to a loss of the natural molecular organization. Texture evaluated as the firmness of a corn tortilla has been correlated with starch damage in the nixtamalized corn flour (Campas-Baypoli, Rosas-Burgos, Torres-Chávez, Ramírez-Wong, & Serna-Saldívar, 2002). The level of starch damage can be followed by the value of peak viscosity (PV), measured with analytical techniques such as viscoamylography that quantify changes in starch of the corn flour. Flours with a low viscosity indicate that the starch has been completely gelatinized and the granules have collapsed. The tortilla obtained from this flour will have undesirable texture characteristics (Weber, 2008).

The aim of this study was to evaluate the effect of the nixtamalization extrusion optimized process factors on the chemical, functional, and pasting properties of ENCF, determine the optimal conditions for obtaining a flour with a maximum anthocyanin content and maximum viscosity using response surface methodology (RSM), and to obtain a tortilla from the optimized flour with quality characteristics acceptable to consumers.

2. MATERIALS AND METHODS

2.1 Raw material

BC was acquired in the state of Chihuahua, México, native to the Tarahumara Sierra. Materials were cultivated and harvested in 2016. The kernels were cleaned in a vibrating cleaner (Clipper, Model V230, Clipper Products, Bluffton, IN, USA), to eliminate impurities and stored at −20 °C until use. The chemical composition of BC was as follows: moisture content, 10.35%; protein content, 8.63% (DW); hectoliter weight, 76.4 kg/hL; and Hunter color parameters, an L^* of 45.5, an a^* of 0.28, and a b^* of 7.68. All chemical composition assays were performed following the AOAC official methods (AOAC, 1995). Commercial lime (Nixtacal Calhidra, S.A. de C.V.; Hermosillo, Sonora, México) was used as the alkali for the ENP.

2.2 Corn grinding

BC was ground (retaining the germ and pericarp) with a hammer mill (Model 8; Christy Turner Ltd., England, UK) using a 2 mm mesh size., and stored in polyethylene bags at 5 °C in the dark.

2.3 Extrusion nixtamalization process

2.3.1 Conditioning. The ground corn (GC) was divided into 20 batches of 1000 g each and conditioned with lime (0.3%, w/w) and distilled water in a mixer (Model MK45SSWH; Kitchen Aid, St. Joseph, MI, USA) at low speed (600 rpm) for 3 min until the moisture content was in a range of 15% to 30% (according to the experimental design, Table 1). To achieve better hydration of the starch granules, conditioned samples were placed in plastic bags and rested for 12 hr at 4 °C in a commercial refrigerator (Model 2155060-A; Whirlpool, Benton Harbor, MI, USA).

2.3.2 Extrusion process. Each batch of conditioned GC was fed at 45 rpm into a single screw extruder (Model E 19/25 D; Brabender Instruments, OHG Duisburg, Germany). The equipment consists of a separate barrel with four independent heating/cooling zones, with a screw number 1 (compression ratio 1:1) and a die opening of 4 mm. The temperatures of the first, second, and third zones were held constant at 60, 70, and 80 °C, respectively. The temperature in the fourth zone varied from 70 to 110°C (according to the experimental design, Table 1), and the SS ranged from 50 to 145 rpm (according to the experimental design, Table 1). BC extrudates were obtained.

2.4 Obtaining ENCFs

2.4.1 Drying. The extrudates obtained were divided into two batches. The first batch was dried in a tunnel dryer (no brand) at 50°C for 1 hr, and the other batch was freeze-dried (Model 7753020; Labconco, Kansas City, MO, USA) in a vacuum at −52 °C for 24 hr prior to carrying out the chemical analyses.

2.4.2 Grinding. Both batches of dried extrudates were ground to obtain ENCF (Model 8; Christy Turner Ltd.) with a 2 mm mesh size. ENCFs were obtained and stored at 5 °C in polyethylene bags protected from light until further analysis.

2.5 ENCF evaluations

2.5.1 Subjective water absorption capacity (SWAC). The methodology described by Flores-Farías, Martínez-Bustos, Salinas-Moreno, and Ríos (2002) was used. One-hundred grams of ENCF were weighed and water was added to make a manual kneading until masa showed good consistency to make tortillas.

Table 1–Central composite design arrangement for the process optimization and main values for each response variable.[a].

	Process factors			Response variables		
Tr	FM (%)	T (°C)	SS (rpm)	SWAC (mL of water/100 g of flour)	TA (mg ECG/kg)	PV (cP)
1	18.04 (−1)	78.11 (−1)	69.26 (−1)	117.3	214.5	1045
2	26.96 (+1)	78.11 (−1)	69.26 (−1)	96.0	200.5	364.05
3	18.04 (−1)	101.89 (+1)	69.26 (−1)	121.3	227.7	1037
4	26.96 (+1)	101.89 (+1)	69.26 (−1)	105.3	202.9	409.75
5	18.04 (−1)	78.11 (−1)	125.74 (+1)	126.7	199.3	884.3
6	26.96 (+1)	78.11 (−1)	125.74 (+1)	109.3	188.1	443.65
7	18.04 (−1)	101.89 (+1)	125.74 (+1)	114.7	213.3	1016.7
8	26.96 (+1)	101.89 (+1)	125.74 (+1)	94.7	205.6	384.55
9	15 (−1.682)	90 (0)	97.5 (0)	106.7	218.3	1137.5
10	30 (+1.682)	90 (0)	97.5 (0)	91.7	188.4	364.15
11	22.5 (0)	70 (−1.682)	97.5 (0)	117.3	201.6	787.05
12	22.5 (0)	110 (+1.682)	97.5 (0)	121.3	204.3	801
13	22.5 (0)	90 (0)	50 (−1.682)	112.7	232.0	742.35
14	22.5 (0)	90 (0)	145 (+1.682)	126.3	213.9	845.45
15	22.5 (0)	90 (0)	97.5 (0)	116.0	227.9	840.8
16	22.5 (0)	90 (0)	97.5 (0)	121.3	223.8	927.45
17	22.5 (0)	90 (0)	97.5 (0)	124.0	228.2	975.4
18	22.5 (0)	90 (0)	97.5 (0)	125.3	222.2	1007.6
19	22.5 (0)	90 (0)	97.5 (0)	125.3	216.2	950.8
20	22.5 (0)	90 (0)	97.5 (0)	125.3	219.4	872

[a]Values in the parentheses denote coded level of independent variables.
Tr, standard treatment; FM, feed moisture; T, temperature; SS, screw speed; SWAC, subjective water absorption capacity; TA, total anthocyanins expressed as equivalents of cyanidin-3-glucoside (ECG); PV, peak viscosity.

The amount of water added was registered as the water absorption rate of the flour in ml of water/100 g of flour.

2.5.2 Total anthocyanins (TA). The anthocyanin content was determined by using the method described by Abdel-Aal and Huel (1999). The anthocyanin extracts were prepared with 0.1 g of freeze-dried sample and acidified cold ethanol (95% methanol and 1 N HCl, 85:15, v/v). After that, the sample was centrifuged (Model 5415D; Eppendorf AG, Hamburg, Germany) at 3000 × *g* for 10 min, and the supernatant was collected. The absorbance of the samples was measured immediately at 520 nm in a microplate reader (Model xMark TM; Bio-Rad, CA, USA).

2.5.3 Pasting property. The pasting behaviors of the ENCF suspensions were analyzed under conditions of continuous shear. Analysis was carried out by using a starch cell of an Anton Paar rheometer model MCR 102 (Graz, Austria). A suspension of extruded flour (3 g) in 25 mL of distilled water with a final weight of 28 g was used. The suspension was then manually homogenized using a plastic paddle to avoid lump formation before the run. Paddle rotation (193 rpm) was performed at a temperature of 50 °C for 1 min for temperature stabilization and uniform dispersion of particle samples. The solution was then heated from 50 to 95 °C for 8 min and held constant at 95 °C for 5 min. Then, the suspension was cooled to 50 °C over 7.5 min and maintained at that temperature for 2 min (Rincón-Londoño, Millan-Malo, & Rodríguez-García, 2016). The PV was reported in centipoises (cP). All analyses were conducted in duplicate, and average values were reported.

2.6 Tortilla production

Tortillas were prepared according to Platt-Lucero et al. (2010) using the ENCF obtained from the best combination of the extrusion process factors. To obtain corn masa, 4 kg of the optimized ENCF was mixed (Model AS200; Hobart Mfg. Co., Troy, OH, USA) for 3 minutes with the amount of distilled water determined by the SWAC test. After 20 minutes of resting, the obtained corn masa (moisture: 50.77 ± 0.22%) was processed in a commercial tortillería (Tortillería Pimentel, Hermosillo, Sonora, México). The corn masa was placed in the tortilla-forming machine (Model MLR 30; Lenin manufactures, San Luis Potosí, México) to form a masa disk of 25 g. Disks were baked on a three-zone oven, and the first, second and third zones were heated at the following temperatures: 258 ± 10 °C, 308 ± 10 °C, and 257 ± 10 °C. Baked tortillas were subsequently cooled and packed in polyethylene bags to avoid moisture loss. Finished tortillas were then transported to the laboratory and stored at room temperature (25 °C) for texture evaluation or lyophilized (Model 7753020; Labconco) for chemical analyses. The resultant tortillas were flat disks with a weight of 24.5 ± 1.38 g, a moisture content of 32.07 ± 0.01%, a diameter of 13.57 ± 0.11 cm, a thickness of 1.05 ± 0.09 mm, and a TA content of 180 ± 15.4 mg/kg.

2.7 Tortilla firmness and rollability

Tortilla firmness and rollability were measured at 2, 24, and 48 hr of storage at room temperature (25 °C) after baking. Tortilla firmness was determined using the procedure reported by Ramírez-Wong et al. (2007). The peak stress required to break the tortilla was recorded in units of kg-f, and the firmness value was corrected for tortilla thickness. Ten tortillas were measured, and the results were expressed in maximum stress (kPa/mm thickness). Tortilla rollability was evaluated with the Waniska (1976) procedure. Ten tortillas were tested, and the average was reported.

2.8 Experimental design and statistical analysis

The independent variables considered for this investigation were FM (15 to 30%), T at the fourth zone of the extruder (70 to 110 °C), and SS (5 to 145 rpm). The levels of each variable were established based on values obtained in earlier experiments. The five levels of process variables were coded as −1.682, −1, 0, +1, and +1.682, indicating a minimum axial point, minimum factorial point, center point, maximum factorial point, and maximum axial point, respectively (Table 1). Three responses were

Table 2–Values of calculated regression coefficients, ANOVA results of the second-order polynomial models, and the effects of the process factors on response variables[a].

	Response variables		
Process factors	**SWAC (mL of water/100 g of flour)**	**TA (mg ECG/kg)**	**PV (cP)**
Intercept			
β	122.93	223.03	931.35
Lineal			
β1 (FM)	−12.30 ($P = 0.0002$)	−7.90 ($P = 0.0002$)	−269.58 ($P < 0.001$)
β2 (T)	−0.81 ($P = 0.7154$)	3.77 ($P = 0.0198$)	9.85 ($P = 0.6656$)
β3 (SS)	3.49 ($P = 0.1389$)	−5.11($P = 0.0038$)	3.43 ($P = 0.8799$)
Quadratic			
β11 (FM)^2	−24.55 ($P < 0.0001$)	−7.45 ($P = 0.0002$)	−78.27 ($P = 0.0046$)
β22 (T)^2	−4.38 ($P = 0.2457$)	−7.59 ($P = 0.0002$)	−63.00 ($P = 0.0151$)
β33 (SS)^2	−4.21 ($P = 0.2629$)	−0.52 ($P = 0.7016$)	−63.04 ($P = 0.0151$)
Interaction			
β12 (FM*T)	0.94 ($P = 0.8471$)	−0.90 (p = 0.6222)	−17.23 ($P = 0.5642$)
β13 (FM*SS)	0.000 ($P = 1.0000$)	2.47 ($P = 0.1946$)	29.42 ($P = 0.3324$)
β23 (T*SS)	−14.15 (0.0141)	2.00 ($P = 0.2878$)	4.45 ($P = 0.8806$)
Model (F value)	29.95	23.49	56.10
Model (P-value)	<0.0001	< 0.0001	<0.0001
R^2	0.8488	0.893	0.937
Adjusted R^2	0.8205	0.855	0.920
Lack of fit (P-value)	0.2573	0.4603	0.3548
CV (%)	4.07	2.34	9.09

Note: P values in the parentheses denote the statistical significance to the terms of the quadratic regression models; model terms with P values ≤ 0.1 are significant, whereas model and lack of fit with P values ≤0.05 are significant.
[a]β1, feed moisture; β2, temperature, β3, screw speed; SWAC, subjective water absorption capacity; TA, total anthocyanins expressed as equivalents of cyanidin-3-glucoside (ECG); PV, peak viscosity; CV, coefficient of variation.

measured in the ENCFs: SWAC, TA, and PV. The experimental design was applied after selection of the variables and ranges. Twenty experiments were performed according to a second-order central composite rotatable design (CCRD) with three variables and five levels of each variable (Table 1). This design was chosen assuming that a quadratic polynomial would provide a reasonably good approximation to the true relationship between the response variables and the process factors. Experiments were randomized to minimize the effects of unexplained variability in the observed responses due to extraneous factors. RSM was employed to evaluate the experimental data using a commercial statistical package, Design Expert V.7.0 software (State-Ease, Minneapolis, MN, USA). The statistical significance of the terms in the regression equation was examined by analysis of variance (ANOVA) for each response (Table 2). Data were modeled by multiple regression analysis adopting backward stepwise analysis, and only the variables significant at $P \leq 0.1$ level were selected for model construction. The adequacy of the model was determined based on the lack of fit, adjusted R^2 value, and coefficient of variation (CV).

2.9 Optimization and validation

The numerical optimization technique was also performed with Design expert software used for simultaneous optimization of the multiple responses. The desired goals for each variable and response were chosen. All the independent variables were kept within their respective ranges while the responses were maximized. The response variables that were optimized in the ENCF were TAs (maximize) and PV (maximize). To find a solution that maximizes multiple responses, the goals were combined into an overall composite function called the desirability function. Then, the numerical optimization found a point that maximized the desirability function. The validation of the nixtamalization extrusion process conditions estimated by the model was evaluated experimentally. An ENCF was processed according to the optimized treatment. The values of the anthocyanin content and PV were determined, and the data were compared with the values predicted by the model.

3. RESULTS AND DISCUSSION

3.1 Fitted model checking

Response surface analysis was applied to the experimental data. Table 1 shows the different combinations of extrusion process factors (treatments) used to obtain ENCFs and the average experimental values of the three responses obtained for each combination. Regression analysis and ANOVA were performed for each of the response variables. Table 2 presents the regression coefficients and ANOVA results of the second order prediction model, showing the relationships between the response variables and process factors (FM, T, and SS).

The predictive models in terms of coded factors were:

$$\text{SWAC} = 122.93 - 12.30(\text{FM}) - 24.55(\text{FM})^2 - 14.15(\text{T})(\text{SS})$$
$$(P\text{−value of model} < 0.0001; \text{adjusted } R^2 = 0.8205)$$

$$\text{TA} = 223.03 - 7.90(\text{FM}) + 3.77(\text{T}) - 5.11(\text{SS}) - 7.45(\text{FM})^2 - 7.59(\text{T})^2 (P - \text{value of model} < 0.0001; \text{adjusted } R^2 = 0.855)$$

$$\text{PV} = 931.35 - 269.58(\text{FM}) - 78.27(\text{FM})^2 - 63.00(\text{T})^2 - 63.04(\text{SS})^2$$
$$(P\text{−value of model} < 0.0001; \text{adjusted} R^2 = 0.920)$$

To verify the model adequacy, the estimated regression coefficients of the quadratic polynomial models for the response

variables were checked. ANOVA showed that the models were highly significant for all responses ($P < 0.0001$; Table 2). The F values for the three responses were significant. The predictive capability of the model is commonly explained by the coefficient of determination (R^2), the values of which for all responses were high (>0.8), indicating that a high proportion of variability was explained by the data (Table 2). The lack of fit did not result in a significant P value (Table 2), indicating that these models should be used for predicting those responses. As a general rule, the coefficients of variation (CV) should not be greater than 10%. In this study, the CV were less than 10% for all responses (Table 2). The estimated regression coefficient values indicate that the models were adequate for describing the behavior of the variables.

3.2 Effect of FM, T, and SS on the functional properties

The functional property of the ENCF was measured as the SWAC. From the regression coefficients and P-value, the linear and quadratic terms of FM had significant effects ($P < 0.0001$) on the ENCFs (Table 2). Only the interaction of T and SS (T*SS) had a significant effect within the model ($P < 0.01$). The multiple regression model for predicting the SWAC could explain 84% of the observed variations (Table 2).

The values for SWAC varied between 91.7 and 126.7 mL of water/100 g of flour (Table 1). Figure 1 shows the relationship between the processing factors on the water absorption of the ENCFs. The response surface graphs indicate that increasing the T and SS resulted in an increase in the SWAC (Figure 1A). In Figure 1B, the FM*SS interaction shows that the SWAC increases as a function of increasing SS at low-intermediate values of feed moisture (15–22.5%). Similar behavior occurs in the interaction of T*FM, where at a low-intermediate level of FM and a high T, the corn flours reached maximum SWAC values (Figure 1C).

During the extrusion process, changes in the starch molecular size and degradation occur due to independent or combined effects of process factors (T, FM, and SS) that increase the water absorption capacity of the flour. Thermal energy contributes mainly to starch gelatinization, and FM acts as a plasticizer or lubricant, while SS causes shear degradation (Robin, 2001). Rodis, Wen, and Wasserman (1993) reported that shear and thermal fields affect the fragmentation of starch at high temperatures (>100 °C) and low moisture levels (<30%), similar to the behavior found in this study. In another work, the authors reported that the large shearing forces due to the movement of the screw, as well as high temperatures, cause structural changes at molecular, crystalline, and granular levels (level 2, 3, and 5, respectively; Li, Hasjim, Xie, Halley, & Gilbert, 2014). According to Alam, Kaur, Khaira, and Gupta (2015), higher temperatures increased the activation energy for starch conversion with the consequent loss in the original structure of the granules. However, increasing the SS increased the water absorption capacity, which may be due to the mechanical energy reducing the starch molecular size and the degree of starch crystallinity. Changes in the structure could lead to the exposure of functional groups, such as hydrophilic groups, from inside the structure, thereby improving the hydration properties. Li et al. (2014) suggested that the rigid crystallites of amylopectin in starch granules are more susceptible to shear degradation than the flexible amorphous amylose; the use of high levels of mechanical shear during extrusion can reduce the average molecular weight through the cleavage of the glycosidic bonds near or at the branching point. The shear degradation is more pronounced at lower FM contents. Under low moisture conditions, the initial physical interactions cause frictional and mechanical energy dissipation; this energy source serves to increase the temperature. As the water level decreases, the fluidity of the paste decreases, and a large amount of mechanical energy is expended, causing a change in the physical form of the starch and increasing the SWAC (Robin, 2001). The ENCF produced under the conditions of 18.07% FM, a T of 78.11 °C, and a SS of 125.74 rpm had the highest water absorption capacity.

3.3 Effect of FM, T, and SS on the TAs

The concentration of TAs has been related to color and antioxidant activity in the final product. Statistical analysis revealed that linear effects of FM ($P < 0.0001$), T ($P < 0.05$), and SS ($P < 0.01$), as well as the quadratic terms of $(FM)^2$ ($P < 0.0001$) and $(T)^2$ ($P < 0.0001$), were found to significantly influence the TA. The regression model explained 89% of the total variability (Table 2).

TA values ranged from 188.1 to 232 mg ECG/kg (DW; Table 1). Figure 2 illustrates the effects of processing variables on the anthocyanin content in ENCFs as three-dimensional graphs. Figure 2A shows the anthocyanin content in flour as a function of T and SS. Higher levels of anthocyanin content were observed at intermediate-high temperatures (85–105 °C) and low-intermediate screw revolutions in the extruder (50–97 rpm). In Figure 2B, the interaction of FM*SS indicates that at low-intermediate levels of FM (15–22.5%) and SS, the ENCFs reached a high content of TA. The interaction effect of FM*T is presented in Figure 2C, where the highest TA were presented at low-intermediate levels of FM and intermediate-high temperatures.

The maximum TA of the ENCF corresponded to 15% (FM), 90 °C (T), and 97.5 rpm (SS). The stability of bioactive compounds in the extruded products is reported as a loss (Aguayo-Rojas et al., 2012) or increase (Escalante-Aburto et al., 2013) after extrusion cooking. During the ENP, chemical changes occur, affecting the concentration of bioactive compounds, such as anthocyanins. The main changes are the breaking of covalent bonds, decomposition of heat-labile compounds, and disruption of cell wall matrices, improving compound accessibility (Ruíz-Gutiérrez et al., 2018). The net effect of the ENP conditions on anthocyanin content depends on which of these phenomena are predominant. According to the results obtained, TA increases with increasing T. Escalante-Aburto et al. (2013) attribute the retention or increase in the anthocyanin content (cyanidin-3-glucoside, a major anthocyanin in BC) to the presence of thermoresistant anthocyanins and to the protective effect of some anatomical parts of the kernel, such as the pericarp. Depending on the chemical structure and the type of substitutions in the molecule, the anthocyanin stability will be greater (Habibi, Ramezanian, Guillén, Serrano, & Valero, 2020). Substitution of hydroxyl and methoxyl groups has an influence on the stability. Anthocyanin stability increases with the increasing number of methoxyl groups in the B-ring and decreases as the number of free hydroxyl groups in the B-ring increases, which is due to the methoxyl groups being less reactive than the hydroxyl groups. In addition, glycosylation and acylation increase anthocyanin stability. Diglucoside derivatives are more stable than monoglucosides due to the protective effect of the bound sugars, through inhibiting the formation of unstable intermediates, that will further degrade into phenolic acids and aldehydes (Sadilova, Stintzing, & Carle, 2006). According to White, Howard, and Prior (2010), anthocyanins that are more affected by high temperature are cyanidin-3-arabinoside and peonidin-3-arabinoside in contrast to cyanidin-3-galactoside,

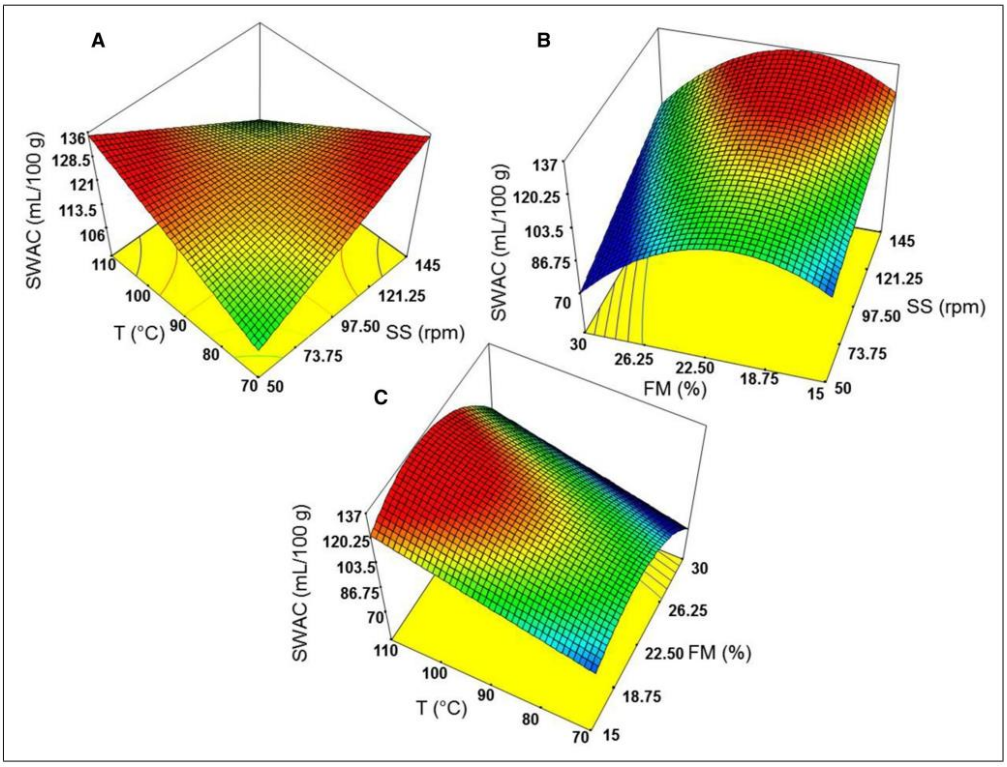

Figure 1–Response surface plots for subjective water absorption capacity (SWAC) of ENCFs as a function of: (A) temperature (T) and screw speed (SS); (B) feed moisture (FM) and screw speed (SS); and (C) temperature (T) and feed moisture (FM).

cyanidin-3-glucoside and peonidin-3-glucoside. TAs increase at low-intermediate FM contents. According to Brennan, Brennan, Derbyshire, and Tiwari (2011), at this FM, anthocyanins were most likely released from the cell matrix of the aleurone layer, forming monomers and dimers. On the other hand, increasing FM causes TA to decrease. Two reasons may cause this behavior. First, the relatively high FM results in starch gelatinization, causing paste formation and inducing a reduction in flow rate, and longer exposure to the high temperature and mechanical shear is conducive to TA degradation. Second, high FM causes self-associations of anthocyanin molecules, increasing the molecular weight of these compounds, thus decreasing their extractability and quantification (Balunkeswar, Liu, & Tang, 2015). Finally, the increased values of TA at low-intermediate SSs may be due to the mechanical shear causing disruption in the cell wall matrix, with the consequent release of compounds (Aguayo-Rojas et al., 2012). Reports suggest that breakage of ester bonds of the acyl group from the acylated anthocyanins causes the release of monoglycosylated anthocyanins, mainly in the form of cyanidin-3-glucoside (Escalante-Aburto et al., 2013).

3.4 Effect of FM, T, and SS on the pasting property

The pasting property measured as PV of the ENCFs has been used as an indirect estimation to infer tortilla texture. Flours with a high viscosity indicate that the starch has not been completely gelatinized, then the tortilla obtained from this flour will have desirable texture characteristics (Weber, 2008). According to the ANOVA, the FM was the only variable that exhibited a linear effect on the PV (Table 2, $P < 0.0001$). The quadratic effects of $(FM)^2$, $(T)^2$, and $(SS)^2$ were found to significantly influence the pasting parameter ($P < 0.001$, $P < 0.01$, and $P < 0.01$, respectively). The regression model explained 93% of the total variability (Table 2).

The maximum PV value was 1137.5 cP (Table 1). Figure 3 presents the effects of processing factors on PV. The viscosity plot (Figure 3A) indicates that at intermediate T and SS, the values of PV were maintained. On the other hand, decreasing FM at intermediate SSs (73 to 121 rpm) caused an increase in PV level (Figure 3B). In Figure 3C, the interaction of T*FM shows that at low-intermediate FM levels (15 to 22.5%) and an intermediate T (80 to 100 °C), a noticeable increase in the PV was observed.

The present findings revealed that both T and SS were equally important parameters responsible for viscosity development. ENCF exposed to intermediate temperature and intermediate shear resulted in relatively high PV values, probably due to moderate thermomechanical damage. According to Lillford (1997), high shear causes a large decrease in the molecular weight of amylopectin in starch granules, leading to a subsequent

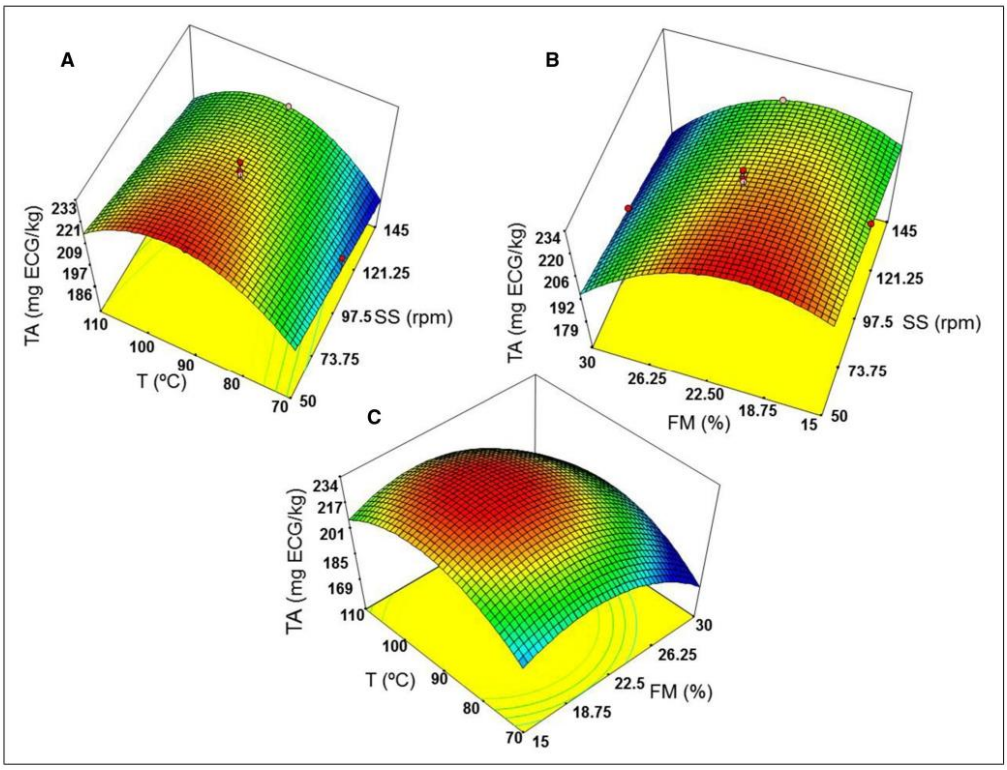

Figure 2–Response surface plots for total anthocyanins (TA) of ENCFs as a function of: (A) temperature (T) and screw speed (SS); (B) feed moisture (FM) and screw speed (SS); and (C) temperature (T) and feed moisture (FM).

decrease in viscosity. Similar behavior occurs at high temperatures. At a high temperature, a decrease in viscosity occurs due to the granules breaking down. At a low-intermediate moisture content, the starch in the flour was not completely gelatinized. These results indicate the presence of intact amylopectin molecules in the whole starch. Consequently, the integral granules in the ENCF will be available for starch swelling. The maximum PV level then occurred when the majority of the granules were fully swollen during the test. The maximum viscosity level in the ENCF (1137.5 cP) was found under the following conditions: an FM of 15%, a T of 90 °C, and an SS of 97.5 rpm. The maximum viscosity value was below that reported for a nixtamal flour (2000-4000 cP), which may be due to the differences in the raw material and process conditions (Castillo et al., 2009). According to the results obtained in our study, the ENCF that presented the highest values in the viscosity profile were those that underwent a lower degree of gelatinization during extrusion. This is because the ENCF conserved a greater percentage of non-hydrated starch granules, which were available for swelling, increasing size and, consequently, the viscosity when suspended in the aqueous system.

3.5 Tortilla texture properties

The major factors in consumer acceptance of tortillas are the texture and handling properties of the product. Tortillas produced using the ENCF obtained from the best combination of the extrusion process factors were stored at room temperature (25 °C) for 48 hr to measure changes in texture properties (flexibility and firmness; Figure 4). Figure 4A shows that the tortilla firmness after 2 hr elaboration was smooth and soft, requiring minimum force for breakage (21.25 ± 1.94 kpa/mm). At 24 hr of storage, tortilla firmness increased (46.45 ± 2.54 kpa/mm). After 24 hr, more force to break the tortilla was required. At 48 hr, the tortilla texture was 16% more firm than when stored for 24 hr (53.97 ± 4.74 kpa/mm). The results observed in this study agree with previous investigations (Platt-Lucero, Ramírez-Wong, Torres-Chávez, & Morales-Rosas, 2012). Losses of the texture during storage indicate that the molecules in the corn tortilla (amylose and amylopectin) were realigning themselves into a more ordered crystalline structure due to starch retrogradation, which results in a harder crumb (Campas-Baypoli et al., 2002). In addition, some authors have reported that in the first 2 to 24 hr after tortilla baking, there was a rapid increase in resistant starch content (Campas-Baypoli et al., 2002).

Rollability testing is another technique to evaluate changes in tortilla texture, which allows for the complementation of the results obtained through the instrumental method. There is an inverse relationship between the rollability capacity and tortilla firmness. While the firmness value increased during storage, the

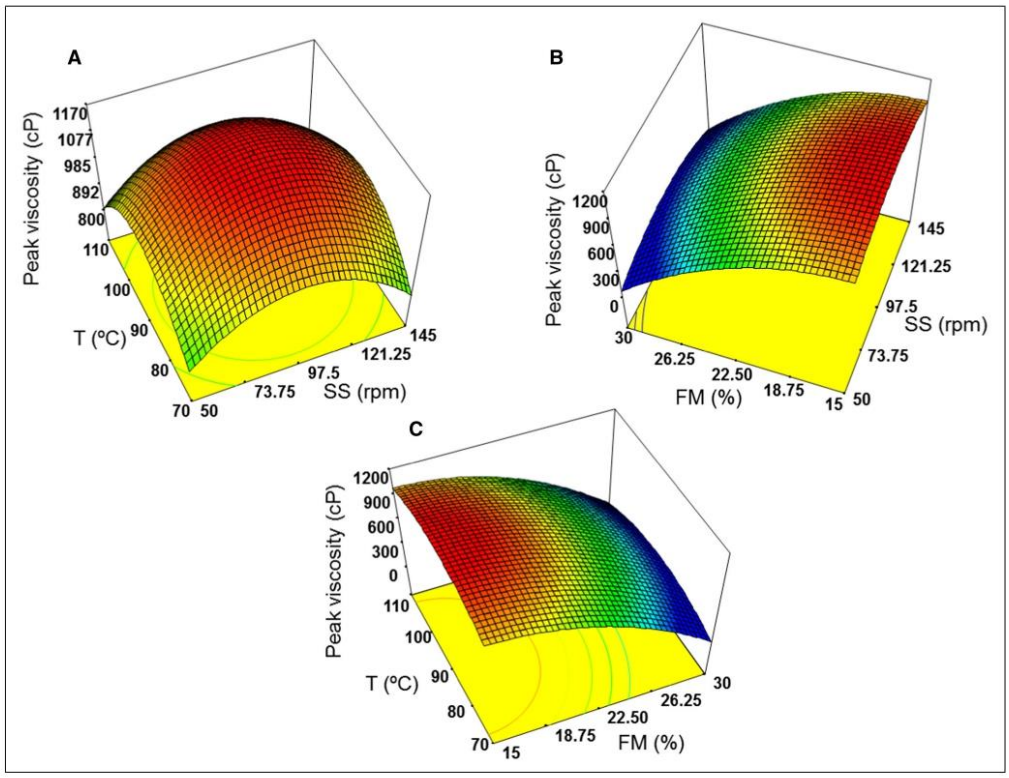

Figure 3–Response surface plots for peak viscosity (PV) of ENCFs as a function of: (A) temperature (T) and screw speed (SS); (B) feed moisture (FM) and screw speed (SS); and (C) temperature (T) and feed moisture (FM).

rollability score decreased. The dowel flexibility scores of the tortillas during storage are shown in Figure 4B. As expected, in their fresh state (2 hr), the tortillas were easily rolled without cracking, which indicates high flexibility (a value of 5). After 24 hr, loss of texture occurred, and the rollability score was lower (3.7). Rollability values reported by Enríquez-Castro et al. (2020) behaved in a similar manner. At 48 hr, a significantly ($P < 0.05$) lower value of rollability (1.2) was obtained, indicating hardening of the product.

Although more force was needed to break the tortilla, and the scores of the rollability parameter were lower during storage, with ENP optimization, it was possible to obtain a tortilla with values close to those reported for tortillas made via the TNP (Enríquez-Castro et al., 2020). Additionally, these results are similar to those found for tortillas with certain types of additives (gums) that attempt to inhibit the retrogradation phenomenon and improve the textural characteristics (Platt-Lucero et al., 2010).

3.6 Optimization

The response variables that were optimized in the ENCF were TA (maximize) and PV (maximize). Numerical optimization was carried out for selecting the optimal area with the best combination of processing conditions (FM, T, and SS) for obtaining optimized ENCF. This region corresponded to where the maximum value of desirability (D) was obtained. The desirability value obtained during the optimization of the ENP was $D = 0.913$ (Figure 5). Desirability values in the range of 0.7 to 1.0 provide a good and acceptable product according to a subjective scale reported by Fabila-Carrera (1998). Figure 5 shows the optimal area or region that corresponded to the process variables of FM (18.17%), T (92.03 °C), and SS (76.61 rpm). This combination of process factors was applied to obtain an optimized ENCF, which was then used for preparing tortillas. The optimum conditions estimated the production of ENCFs with an anthocyanin content of 227.5 mg ECG/kg and a PV value of 1082.8 cP.

3.7 Verification of the model

Extrusion nixtamalization experiments were conducted at the optimum process conditions, and the content values of TAs and PV were determined. The observed experimental values were then compared with the values predicted by the model. Experimentally, an anthocyanin content value of 226.07 mg ECG/kg was obtained, indicating a 99.3% adjusted value from that of the model. The anthocyanin content in the optimized ENCF found in this work is higher than that reported by Cortés, Salinas, San Martín-Martínez, and Martínez-Bustos (2006), who reported a

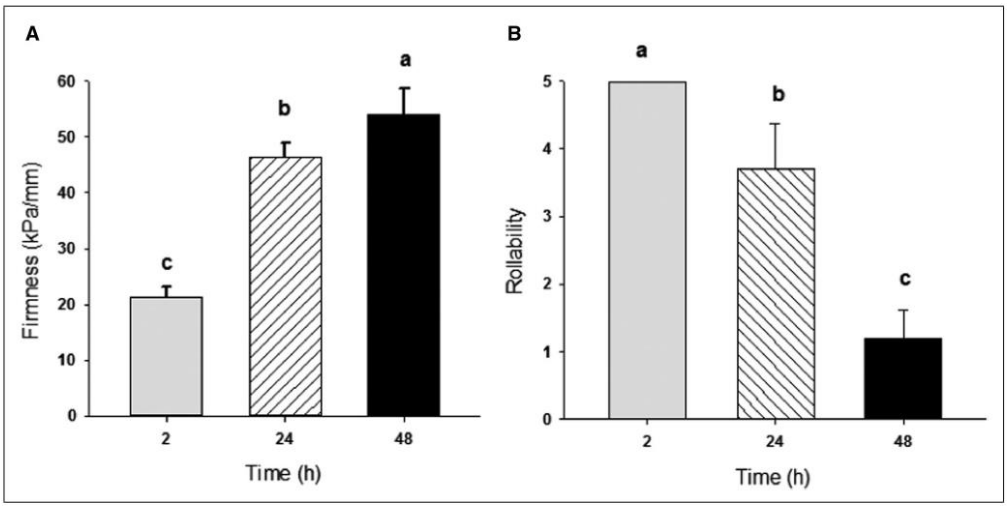

Figure 4–Texture of tortillas produced from the optimized ENCF at 2, 24, and 48 hr of storage after baking: (A) tortilla firmness and (B) tortilla rollability. Error bars are standard deviations. Significant differences of values are indicated by different letters ($P < 0.05$).

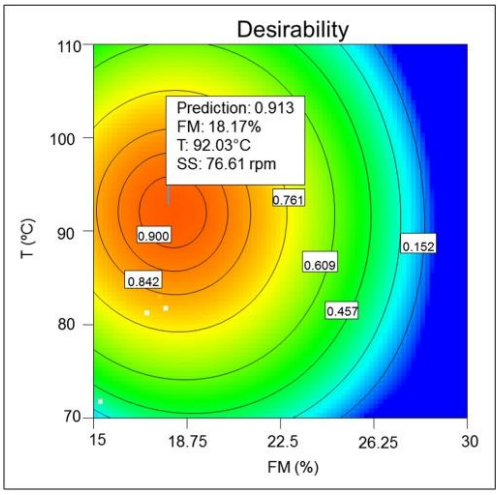

Figure 5–Contour plot of optimum conditions to produce ENCF.

TA value in nixtamalized BC flour obtained by the traditional process of 49.36 mg/kg, corresponding to a loss of 81.8% with respect to the TA content in raw corn. On the other hand, the value obtained for the viscosity was 1063.9 cP, which means that 98.2% of the model was adequate. These values were within the range of the results of Cornejo-Villegas et al. (2013), who obtained PV values from 665.8 to 2403 cP in extruded corn flour processed at low temperatures. Closeness between the experimental and predicted values of the response variables indicated the suitability of the models.

4. CONCLUSIONS

RSM was successfully applied to assess and model effects of three factors (FM, T, and SS) on the functional and pasting properties and bioactive compounds of an ENCF. The nixtamalization extrusion process factors (FM, T, and SS) affected all parameters evaluated in the ENCF. FM was the main factor that had a great effect on the SWAC, PV, and TA, in their linear and quadratic term. The optimum ENCF qualities in terms of maximum anthocyanin content (226.07 mg/kg) and PV (1063.9 cP) were found at FM of 18.17%, a T of 92.03 °C, and a SS of 76.61 rpm. PV was used to inferred the tortilla texture. Texture is one of the most important factors in the tortilla acceptance, finding optimum textural properties based on the corn flour PV value could be a goal for researches about the effect of processing factors on the textural parameters. Tortillas obtained from the optimized flour showed suitable textural characteristics (firmness, rollability) for consumer acceptance and promising nutraceutical value through high anthocyanin content. The modelling of experimental data allowed the generation of useful equations in predicting the behavior of the material under different combinations of factor process. The approach present in this study can provide a useful guideline to develop and optimize innovative pigmented corn-based products.

ACKNOWLEDGMENTS

Mariela Menchaca Armenta is grateful to CONACyT for the provided doctoral scholarship and Sonora and Miguel Hernández University for providing laboratories and facilities for conducting the experimental analyses. In addition, the authors would like to thank Tortillería Pimentel for the facilities given to make the experimental tortillas, and Dr. Jesús Enrique Gerardo Rodríguez and Dr. Carlos Martín Enríquez Castro for the laboratory assistance and Dra. Maria Guadalupe Salazar García and Dra. Concepción Lorenia Medina for all technical support.

AUTHORS' CONTRIBUTIONS

Mariela Menchaca-Armenta performed the experiments and drafted the manuscript. Benjamín Ramírez-Wong conceived and planning the investigation. Armando Quintero-Ramos and Roberto Gutiérrez-Dorado designed the experiments and statistical analysis. Patricia I. Torres-Chávez, Ana I. Ledesma-Osuna and Olga N. Campas-Baypoli were responsible for the interpretation of the data and reviewing and correcting the manuscript. María J. Frutos-Fernández co-directed the experiments carried out at the Miguel Hernández University. Ignacio Morales-Rosas helped with the execution of the extrusion runs.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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CAPÍTULO 2. ESTUDIO CINÉTICO Y TERMODINÁMICO DE LA DEGRADACIÓN DE ANTOCIANINAS

Artículo Científico Derivado de la Etapa 2: Estimation of the Thermodynamic and Kinetic Parameters of Anthocyanins Thermal Degradation in Blue Corn (*Zea mays* L.), Extruded Nixtamalized Flour and Tortillas

El presente manuscrito se encuentra en el formato de la revista Journal of Food Engineering (Editorial Elsevier), el cual se encuentra actualmente bajo revisión. El objetivo fue investigar la estabilidad de extractos de antocianinas del maíz azul, harina nixtamalizada extrudida optimizada y tortilla, y estimar los parámetros cinéticos y termodinámicos asociados a su degradación térmica bajo diferentes temperaturas. Las antocianinas se extrajeron con metanol acidificado y se concentraron hasta sequedad. Los extractos se resuspendieron en una solución tampón de acetato a pH (2.5). La solución tampón de cada muestra fue tratada térmicamente a 3 temperaturas (60, 75 o 90 °C) durante 2 h. El cambio de concentración respecto al tiempo fue medido y con estos datos se estimaron los parámetros cinéticos y termodinámicos. Se utilizó un diseño bifactorial completamente al azar. Los datos se analizaron mediante el análisis de varianza (ANDEVA), con un nivel de significancia <0.05 %. Los resultados demostraron que la transformación de antocianinas tiene alta dependencia con la temperatura. El mecanismo de degradación de las antocianinas siguió una cinética de reacción de primer orden, fue una reacción endotérmica y no espontánea. Los hallazgos mostraron que el uso de modelos cinéticos y termodinámicos como herramientas para predecir la degradación de compuestos biológicamente activos podría ser útil para optimizar las condiciones de procesamiento industrial de alimentos y establecer pautas adecuadas de procesamiento térmico para minimizar las pérdidas de antocianinas en productos pigmentados a base de maíz. En el manuscrito se detalla el planteamiento, metodología, resultados y discusión de esta etapa de la investigación.

Estimation of the thermodynamic and kinetic parameters of anthocyanin thermal degradation in blue corn (*Zea mays* L.), extruded nixtamalized flour and tortillas

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Highlights

- Thermal degradation of anthocyanins was shown to follow first-order kinetics.
- Temperature dependence was expressed according to the Arrhenius model.
- Anthocyanins in extruded corn flour and tortillas showed high stability.
- Heat stability of TAC increased in the following order: tortilla < flour < corn.

Abstract

Blue corn is rich in anthocyanins. Kinetic and thermodynamic parameters are essential for determining anthocyanin stability and predicting quality changes that occur during thermal processing. The aim of this study was to estimate the kinetic and thermodynamic parameters of the thermal degradation of anthocyanins from raw corn, extruded nixtamalized flour and tortillas. A comparative study of anthocyanin thermal stability in these matrices in a buffer solution (pH 2.5) was investigated at different temperatures (60, 75 or 90 °C). The mechanism of anthocyanin degradation followed first-order reaction kinetics. The degradation rate constants increased as temperature increased, demonstrating the greater temperature dependence of anthocyanin transformation in processed corn-based products. Thermodynamic parameters showed that the anthocyanin degradation in extruded nixtamalized corn products was an endothermic and a nonspontaneous reaction. This study provides valuable technological and scientific information for the improvement of the functional quality of the blue corn and tortilla manufacturing industry.

***Keywords**:* anthocyanin stability, half-life, reaction rate constant, activation energy, first-order reaction kinetics**,** phenolic compounds.

Abbreviations: ENCF, Extruded nixtamalized corn flour; TAC, Total anthocyanin content; ECG, Equivalents of cyanidin-3-glucoside; MW, molecular weight; ε, molar extinction coefficient; DF, dilution factor; C, anthocyanin content at any time; C_0, initial anthocyanin content; *k*, first-order rate constant (h^{-1}); t ½, half-life (h); D-value, decimal reduction time (h); Ea, activation energy (kJ/mol); R, universal gas constant (8.314 J/mol K); T, absolute temperature (K); A, frequency factor; $Q_{10,}$ temperature coefficient; *z*-value, temperature interval that causes 10-fold variation in the degradation rate (°C); ΔH, activation enthalpy (kJ/mol); ΔG, Gibbs free energy (kJ/mol); ΔS, activation entropy (J/mol K); *k*B, Boltzmann´s constant (1.3806×10^{-23} J K^{-1}); *h*, Planck´s constant (6.6262×10^{-34} J s).

1. **Introduction**

Blue corn is an important dietary source of anthocyanins (Bolea *et al*., 2016). These compounds have been recognized as health-enhancing substances due to their diverse biological properties, such as antioxidant and anti-inflammatory properties, which may help reduce the risks of various oxidative stress-related diseases (Sui *et al*., 2014).

However, the major current problem in the use of anthocyanin-rich food materials is their susceptibility to deterioration during processing under different conditions, depending on several factors such as the pH, light, and temperature of thermal treatment, as well as oxygen (Nayak *et al*., 2015).

Among the many factors that can influence anthocyanin stability, the most significant are pH and temperature (Fracassetti *et al*., 2013). Blue corn is processed by alkaline thermal cooking before consumption. The products obtained, such as flours, corn chips, tamales and tortillas, are considered functional foods due to their high concentration of bioactive compounds.

Tortillas are the most commonly consumed food in México, Central America, the USA and some countries in Europe and Asia; thus, it is of considerable interest to study this product to improve its value as a health-promoting food (Cortés-Gómez *et al*., 2005). During the nixtamalization process, the highly alkaline pH and the long cooking time at an elevated temperature, as well as the discarding of some anatomical parts of the kernel, such as the pericarp, lead to further degradation of these bioactive compounds in the final product (Mora-Rochin *et al*., 2010).

Due to these technological disadvantages, alternative processes have been used to produce flours and tortillas, such as the nixtamalization extrusion process. This technology allows high-temperature short-term processing of materials, avoids excessive thermal damage to labile anthocyanins, and shows promising characteristics, such as the production of corn tortillas with the use of a small amount of water and without the generation of polluting effluents (Mora-Rochín *et al*., 2010).

Some studies on the thermal degradation of anthocyanins have been reported (Bolea *et al*., 2016; Turturică *et al*., 2016). These works revealed a significantly faster decrease in anthocyanin content as temperature increased with an asymptotic tendency toward high temperature values. Despite these findings, little is known about the mechanisms of thermal degradation (Nayak *et al*., 2015), and no data are available concerning the degradation kinetics of the anthocyanins found in nixtamalized products.

The mechanism of degradation of anthocyanins is quite complex, and thermal processing could induce some unexpected and unwanted chemical reactions. Knowledge of the kinetic and thermodynamic parameters is necessary to predict and minimize the undesired changes that could occur during various conditions of thermal processing. This could allow the design, improvement, and/or optimization of processes to preserve the quality of specific anthocyanin-rich foods.

Anthocyanins in unprocessed blue corn are probably more preserved when exposed to high temperature, due to the integrity of their chemical structure. During the nixtamalization extrusion process, a combination of unit operations involving heat and a highly alkaline medium can affect the stability and quantity of these compounds. This supports the hypothesis that anthocyanins in processed blue corn-based products (flour and tortillas) will

be more damaged and susceptible to accelerated destruction at elevated temperatures than those in raw corn. The aim of this study was to investigate the stability of anthocyanin extracts from blue corn, extruded nixtamalized flour and tortillas. In addition, the kinetic and thermodynamic parameters of anthocyanin degradation under different temperatures were estimated.

2. Materials and methods

2.1 Chemicals

Methanol (HPLC grade) and sodium acetate 3-hydrate (analytical grade) were purchased from Panreac AppliChem (Barcelona, Spain). Formic acid (HPLC grade) was obtained from LiChropur (Darmstadt, Germany). Deionized water was produced by a Milli-Q unit (Millipore, Quantum TEX, Darmstadt, Germany), and commercial lime used for alkali treatment was purchased from Nixtacal Calhidra, SA de CV, Hermosillo, Sonora, México.

2.2 Blue corn sample

Blue corn was cultivated and harvested in 2016 in the state of Chihuahua, México. The kernels were cleaned in a vibrating cleaner to eliminate impurities (Model V230, Clipper, USA) and ground in a laboratory mill (Model 8, Christy Turner Ltd., England, United Kingdom) using a 2 mm mesh size to obtain ground whole corn. The ground corn was stored at -20 °C in the dark until analysis.

2.3 Nixtamalization extrusion process

The nixtamalization process was performed in a single-screw laboratory cooking extruder (19 mm screw diameter; length-to-diameter ratio of 20:1; nominal compression ratio of 1:1;

and die opening of 4 mm) with four independent heating/cooling zones (Brabender Instruments, model E 19/25 D, OHG Duisburg, Germany). The extruder was operated at a screw speed of 76.6 rpm, and the temperatures of the four zones remained constant at 60, 70, 80 and 92 °C, previously established conditions for obtaining the maximum anthocyanin content during the extrusion of blue corn (Menchaca-Armenta *et al*., 2020). Before extrusion, the ground corn was conditioned with 0.3% w/w lime and mixed for 3 min at low speed (600 rpm) (Hobart model AS200, Troy, OH). Afterward, the hydrated ground corn was kept under refrigeration at 4°C for 12 h and achieved an 18.17% moisture content. Then, the conditioned ground corn was brought to approximately room temperature (25 °C) and fed at a rate of 45 rpm. The extrusion process was performed in duplicate and brought to a steady state as indicated by constant torque and melt temperatures before sampling and data collection. The extrudates obtained were dried at 50 °C for 60 min in a tunnel dryer and cooled. Afterward, they were ground to obtain ENCF using a hammer mill (Christy Turner Ltd., England) with a mesh size of 2 mm. Then, the extruded nixtamalized corn flour (ENCF) was stored in polyethylene bags at -20 °C in the absence of light until analysis.

2.4 Corn tortilla preparation

Corn tortillas were prepared according to the procedure reported by Platt-Lucero *et al*., (2010) using the ENCF optimized obtained previously. Four kilograms of the ENCF weremixed (Model AS200, Hobart MFG. CO., Troy, Ohio, USA) for 3 min with an amount of distilled water determined by a subjective water absorption capacity test (Flores-Farías *et al*., 2002) to obtain corn dough (masa). After 20 min of resting, the obtained corn masa was processed in a commercial tortillería (Tortillería Pimentel, Hermosillo, Sonora, México). Corn masa was placed in a tortilla-forming machine (Model MLR 30, Lenin Manufactures,

San Luis Potosí, México) to form a masa disk of 25 g. Disks were baked in a three-zone oven, where the first, second and third zones of the oven were heated to the following temperatures: 258 ± 10 °C, 308 ± 10 °C and 257 ± 10 °C, respectively. The residence time in the oven was of 56 s. The baked tortillas were cooled and subsequently lyophilized (Model 7753020, Labconco, Kansas City, Missouri, USA) and packed in polyethylene bags to avoid moisture loss.

2.5 Anthocyanin extraction

Anthocyanin extraction was carried out using the lyophilized samples of raw corn, ENCF and tortillas and performed as follows: four grams of each sample was dissolved in 30 mL of acidified cold methanol (60% methanol, 37% water, 3% formic acid v/v/v) to prepare a concentrated anthocyanin solution. The suspension was homogenized and placed on a digital magnetic stirrer (OVAN, Multimix Heat, Model MMH30E, Badalona, Spain) at room temperature (25 °C) for 30 min. After extraction, samples were immediately centrifuged (C30P, B. Braun Biotech International) at 9500 rpm for 30 min at 4 °C. Next, the resulting supernatant was concentrated to dryness under reduced pressure using a rotary vacuum evaporator at 40 °C. All the steps were carried out under dark conditions to avoid anthocyanin degradation.

2.6 Anthocyanin buffer solution preparation

The buffer solution was prepared using 0.4 M sodium acetate-3-hydrate. The pH was adjusted to 2.5 by the addition of concentrated formic acid to ensure the stability of the anthocyanins. The pH was verified by a pH meter (Crison, Model GLP 21, Barcelona, Spain). The resulting residue from the anthocyanin extraction (raw corn, ENCF and tortillas) was

resuspended in 16 mL of buffer solution. Each sample was passed through a 0.45 µm nylon syringe filter (Filter-Lab, Barcelona, Spain) to remove impurities (Bolea *et al*., 2016).

2.7 Anthocyanin thermal treatment

The effect of temperature on the stability of anthocyanins of each sample (raw corn, ENCF and tortilla) was evaluated in buffer solution (pH 2.5) at three different temperatures (60, 75 or 90 °C). In earlier experiments, when the temperature was elevated to 100 °C, we observed that the quantification of anthocyanins was complicated by faster degradation due to the physical conditions of the buffer solution (boiling point). For this reason, lower temperatures (<100 °C) were selected to perform the thermal treatments. Aliquots of 5 ml of buffer solution were placed into 20 mL brown glass tubes and covered with plastic caps to avoid evaporation of the thermally sensitive compounds. Thermal treatment was conducted for 2 h in a previously equilibrated thermostatic water bath (Unitronic OR, P Selecta, Barcelona, España). All anthocyanin stability kinetics at each temperature were measured in triplicate. At regular time intervals (0, 0.5, 1, 1.5 and 2 h), samples were randomly removed from the bath and quickly cooled in ice water to prevent further thermal degradation, and the anthocyanin analyses were carried out immediately.

2.8 Quantification of total anthocyanins

After the thermal treatment, the total anthocyanin content (TAC) of the anthocyanin buffer solution was analyzed according to Abdel-Aal and Huel (1999). Briefly, the absorbance of the samples was measured immediately at 520 nm in a UV-visible spectrometer (UV/vis T80, PG Instruments Ltd.). The total anthocyanin content of the samples was calculated using Eq. (1):

$$\text{Anthocyanins (mg/kg)} = \left(\frac{\text{A } X \text{ MW } X \text{ DF } X \text{ 1000}}{\varepsilon}\right).\left(\frac{V}{m}\right).1000 \quad (1)$$

where A is the absorbance at a wavelength of 520 nm, MW is the molecular weight of cyanidin-3-glucoside (449.2 $gmol^{-1}$), ε is the molar extinction coefficient (26,900 L mol^{-1} cm^{-1}), V is the volume of the extract (L), DF is the dilution factor, and m is the weight of the sample (g). The results were expressed as mg of cyanidin-3-glucoside equivalents per kg of dry weight.

2.9 Kinetic data analysis

Based on previous studies (Hernández-Herrero and Frutos, 2011; Bolea *et al*., 2017), it was assumed that the thermal degradation of the total anthocyanins in this experiment followed a first-order reaction as described by Eq. (2):

$$C = C_0\, exp^{-kt} \quad (2)$$

Where C is the total anthocyanin content after time t at a given temperature (mg/kg), C_0 is the initial anthocyanin content (mg/kg), t is the heating time (h) and *k* is the first-order rate constant (h^{-1}).

In practice, Eq. (2) is frequently expressed in logarithmic form, as shown in Eq. (3). The kinetic rate constant (*k*) of thermal degradation was obtained from the slope of ln (C/C_0) versus t. The adequacy of the model was verified by examining the linearity of the graph, regression coefficients and residuals (Qiu *et al*., 2018):

$$ln\frac{C}{C0} = -kt \quad (3)$$

The half-life of the reaction (t ½), which is the time needed to achieve 50% anthocyanin degradation, was calculated assuming first-order kinetics according to Eq. (4) (Peron *et al*., 2017):

$$t_{1/2} = \left(\frac{\ln 2}{k}\right) \quad (4)$$

The decimal reduction time (D-value), which is the time needed for a tenfold reduction in the initial anthocyanin concentration at a given temperature, is related to the *k*-value according to Eq. (5) (Mercali *et al*., 2013):

$$D = \left(\frac{\ln 10}{k}\right) \quad (5)$$

2.10 Determination of thermodynamic parameters

The temperature dependence of the degradation rate constant was determined by applying the Arrhenius model, as reported by several studies (Fracassetti *et al*., 2013; Bolea *et al*., 2016; Peron *et al*., 2017), according to Eq. (6):

$$\ln k = \frac{Ea}{R}\left(\frac{1}{T}\right) \ln(A) \quad (6)$$

where *k* is the rate constant (h^{-1}), Ea is the activation energy (kJ/mol), R is the universal gas constant (8.314 J/mol K), T is the absolute temperature (Kelvin, K) and A is the frequency factor. The values of Ea were estimated by linear regression of the natural logarithm of the degradation rate constant [ln (*k*)] against the reciprocal of the absolute temperature (1/T), where the slope of the linear graph is equivalent to –Ea/RT.

The temperature coefficient Q_{10} expresses anthocyanin degradation when the temperature is increased by 10 °C, and it was calculated according to Eq. (7) (Fracassetti *et al*., 2013):

$$Q_{10} = \left(\frac{k2}{k1}\right)^{(10/(T2-T1)} \quad (7)$$

where k_1 is the kinetic rate constant relative to temperature T_1, k_2 is the kinetic rate constant relative to temperature T_2, and T_1 and T_2 are the temperatures in degrees Celsius.

The *z*-value represents the temperature interval that causes 10-fold variation in the degradation rate and was determined using Eq. (8) (Toledo, 1991):

$$z = \left(\frac{10\ln(10)}{\ln(Q10)}\right) \quad (8)$$

The activation enthalpy (ΔH, kJ/mol) and the Gibbs free energy (ΔG, kJ/mol) at each temperature studied were calculated using Eqs. (9) and (10), respectively (Mercali *et al*., 2013; Peron *et al*., 2017):

$$\Delta H = Ea - (R \times T) \quad (9)$$

$$\Delta G = -R \times T \ln\left(\frac{kd\ x\ h}{kB\ x\ T}\right) \quad (10)$$

where Ea is the activation energy (J mol $^{-1}$), R is the ideal gas constant (8.314 J/mol K), T is the absolute temperature (K), *k*d is the anthocyanin loss rate (s $^{-1}$), *k*B is Boltzmann´s constant (1.3806 x 10 $^{-23}$ J K $^{-1}$), and *h* is Planck´s constant (6.6262 x 10 $^{-34}$ J s).

From Eqs. (9) and (10), it was possible to calculate the activation entropy (ΔS, J/mol K) using Eq. (11):

$$\Delta S = \left(\frac{\Delta H - \Delta G}{T}\right) \quad (11)$$

3. Statistical analysis

A completely randomized bifactorial design was used. The factors were the type of product with three levels (blue corn, corn flour, and tortilla) and the heat treatment with three levels (60, 75 and 90 °C). All experiments were conducted in triplicate (n=3) and presented as the mean ± SD. All data collected were analyzed using analysis of variance (ANOVA), which was performed using the Statistical Analytical Systems package (SAS Institute, Cary, North Carolina). Significant differences were determined by Tukey's test according to $p \leq 0.05$. The coefficient of determination (R^2) and mean square error (MSE) were used as criteria for the adequacy of data fitting.

3. Results and discussion

3.1 Anthocyanin thermal degradation

Since the anthocyanins in corn are related to the pigmentation of processed products and their antioxidant capacity, it is very important to evaluate the total content of anthocyanins in corn-based products. The average concentrations of total anthocyanins in raw corn, flour and tortillas were 109.7 ± 1.5, 96.6 ± 2.6 and 91.1 ± 3.0 mg ECG/kg, respectively.

The relationship between the total anthocyanin concentration and heating time is presented in Figure 1. A linear decrease can be seen in the anthocyanin content with respect to temperature and time for all the treatments. The anthocyanin degradation percentage for raw corn after 2 h of heating increased as temperature increased, reaching 5.0%, 20.8% and 50.2% at 60, 75 and 90 °C, respectively. For corn flour, the degradation percentages were 5.9% at 60 °C, 19.4% at 75 °C and 49.1% at 90 °C. For tortillas, the anthocyanin degradation percentages at 60, 75 and 90 °C were 5.4%, 19.8% and 50.8%, respectively. The results show

that exposure time can determine the transformation of the flavylium cation into the colorless pseudobase and, at least to some extent, the formation of chalcones (Marin *et al*., 2002).

3.2 Kinetic parameter estimates

The rate of retention/degradation of anthocyanins during heating is reflected by the numerical values of the kinetic parameters (Nayak *et al*., 2015). For all the samples, it was observed that the thermal degradation of the TAC clearly followed the first-order reaction kinetic model with high regression coefficients ($0.914 < R^2 < 0.997$) (Figure 1). Our results are in agreement with those from previous studies reporting a first-order reaction model for the degradation of anthocyanins from various sources (Hernández-Herero and Frutos, 2011; Bolea *et al*., 2016). In the first-order plot of Figure 1a, it can be observed that the degradation kinetics of anthocyanins at 60 °C was similar for extruded corn flour and tortillas and higher than that of raw corn. The difference increased with the time of treatment. At higher temperatures, the differences in the degradation kinetics of ENCF and tortillas compared to raw blue corn were smaller (75 °C) (Figure 1b) or very similar (90 °C) (Figure 1c).

The reaction rate constant (*k*) is an indicator that enables the prediction of the thermal degradation of anthocyanins, and it is a function of the number of molecules reacting in the system. The lower the *k* value is, the better the anthocyanin stability is. In Table 1, it can be observed that the degradation rate constants (k) of anthocyanins at 60 °C were similar for extruded corn flour and tortillas and higher than that of raw blue corn ($p<0.05$). However, at higher temperatures (> 60 °C), values of the degradation rate constant (*k*) of ENCF and tortillas were found to be similar to that of raw corn ($p>0.05$) (Table 1). This indicates that the reaction rate constants (*k*) of the anthocyanin extracts from raw corn, flour and tortillas followed the same pattern of increasing with temperature. This can be related to the fact that

although anthocyanins in blue corn are probably more preserved than those in processed corn-based products (which are believed to be more damaged due to the effects of the extrusion conditions), the isolation of these compounds from the cell matrix possibly exposes the total anthocyanins from raw corn, ENCF and tortillas equally to the effects of heat, resulting in quite rapid degradation (Nayak *et al*., 2015).

Higher temperatures led to faster degradation of the anthocyanins for all evaluated matrices. This behavior has already been observed by other researchers, who suggested that at higher temperatures, a larger fraction of molecules have the energy necessary to react. Therefore, there will be more effective collisions, and the reaction will take place at a higher speed (Fracassetti *et al*., 2013; Bolea *et al*., 2016). An increase in temperature from 60 to 75 °C increased the degradation rate constant by 3–4 times for all the samples. In the same way, an increase from 75 to 90 °C roughly doubled the degradation rate constant. These observations agreed with the Q_{10} law, which states that a reaction rate approximately doubles with every 10 °C temperature increase (Sui *et al*., 2014).

Table 1 also shows the half-life times (t ½) and the decimal reduction times (D-values) for each experiment. The half-life times were determined from the *k* values of the first-order reaction. The higher the *k* value was, the shorter the t ½ was. For all samples, a more pronounced effect of temperature on anthocyanin degradation was observed at 90°C. Increases in temperature led to a significant decrease in anthocyanin half-life times, reaching values 3-fold lower at 75 °C and 8 to 12-fold lower at 90 °C than the t ½ values found at 60 °C. The greatest time to reach 50% degradation of anthocyanins was presented in the blue corn extract at 60 °C, compared with nixtamalized flour and tortillas. The lower t ½ values for anthocyanin extracts from ENCF and tortillas can be attributed to the impact of the

combination of unit operations and the highly alkaline medium involved in the nixtamalization extrusion process, as well as the baking conditions that markedly affected anthocyanin stability.

The time needed for 90% degradation of anthocyanins (D-value) at 90 °C was approximately 8–11 times shorter than that at 60 °C. This behavior suggests that the stability of anthocyanins is strongly influenced by the magnitude and duration of heating. The greater amount of energy applied in the form of heat at elevated temperature causes faster anthocyanin degradation, probably due to the closeness of the reacting molecules in the aqueous buffer solution. These molecules readily decompose to form colorless or undesirable brown compounds, possibly through the hydrolysis of sugar moieties and the formation of aglycone, which in turn oxidize easily. This ultimately results in shorter decimal reduction times (D-values) at higher temperatures (Nayak *et al*., 2015).

3.3 Thermodynamic parameters

The effect of temperature on the degradation rate constants was expressed by the linearized Arrhenius equation by plotting -ln *k* against 1/T (1/*k*) (Figure 2). The Arrhenius model adjusts adequately for the treatments evaluated ($0.998 < R^2 < 0.999$), showing strong dependence on temperature for all the samples, which means that the reaction of anthocyanin degradation runs very slowly at low temperatures but relatively fast at high temperatures (Mercali *et al.*, 2013).

The estimated values of Ea were as follows: 89.2 kJ/mol for raw corn, 75.1 kJ/mol for ENCF and 75.5 kJ/mol for tortillas (Table 2). The differences in Ea values could be due to different changes occurring in the extracts of the different samples during heating (Turturică

et al., 2016). Our results showed that anthocyanins from raw corn required greater energy to activate the thermal degradation reaction ($p<0.05$), indicating a slower degradation process, probably due to the higher thermostability of anthocyanins. In contrast, the anthocyanins in flour and tortillas were more sensitive to temperature changes. The lowest Ea values estimated for ENCF and tortillas suggest that the energy barrier that anthocyanin molecules must overcome to react is lower due to the high energy applied previously in the extrusion process, which resulted in a greater number of molecules reaching the transition state. Most of the reported Ea values fell into the range of 20–200 kJ/mol (Sui *et al*., 2014). The Ea values in our work were also within this range and in good agreement with the reported values.

The Q_{10} values for the TAC ranged from 2.12 to 2.57 when the temperature increased from 60 to 75 ° C and from 1.94 to 2.22 when the temperature increased from 75 to 90 °C (Table 2). The Q_{10} coefficient, as reported in the literature, decreased as the temperature increased (Fracassetti *et al*., 2013). In general, the Q_{10} values were approximately 2.0. The results are similar to those obtained by Fracassetti *et al*. (2013), who indicated that an increase in temperature of 10 °C approximately doubled the degradation rate.

The *z*-values obtained for raw corn, ENCF and tortillas were 28.8, 35.1 and 33.8, respectively. The rates of anthocyanin destruction in flour and tortillas confirmed that the anthocyanins were more sensitive to high temperatures than those in raw corn. The destruction rates of TAC obtained in this research were similar to the values reported by Peron *et al.* (2017), who determined the z-value in crude grape anthocyanin extracts (*z*=23.2-24.4).

ΔH is a measure of the energy barrier that must be overcome by the reacting molecules and is related to the strength of the bonds, which are broken and made during the transition

state from the reactants (Mercali *et al*., 2013). ΔH values calculated at different temperatures ranged from 71.5 to 86.5 kJ mol^{-1} (Table 3). The observed decrease in ΔH in flour and tortillas indicated that the energy barrier to break the bonds of the anthocyanin molecules was lower than that of raw corn. The positive values of ΔH revealed that anthocyanin degradation was an endothermic reaction.

ΔG, the fundamental criterion for the spontaneity of chemical reactions, represents the difference between the activated state and reactants (Peron *et al*., 2017). The values of ΔG were similar for all conditions evaluated in this study, varying between 67.91 and 69.2 kJ mol^{-1}. The positive sign observed for all temperatures demonstrated that anthocyanin degradation is a nonspontaneous reaction.

ΔS measures the disorder change of the anthocyanin molecules in the system (Mercali *et al*., 2013). The ΔS ranged from 10.6 to 51.5 $Jmol^{-1}$ K^{-1}. Positive values for ΔS for all the samples suggest that the molecules in the transition state are more disorganized than those in the initiation reaction, possibly indicating that the heavier molecules or monomeric anthocyanins were divided into several smaller ones through the oxidation reactions and cleavage of covalent bonds due to thermal processing. During heat treatment, anthocyanins or their conjugated sugars are probably broken down into small molecules such as aldehydes and benzoic acid derivatives or their corresponding anthocyanidins (Turturică *et al*., 2016).

4. Conclusions

This is the first comparative study that focuses on kinetic thermal degradation of anthocyanin extracts from raw corn, extruded nixtamalized flour and tortillas to assess the potential impact of industrial pretreatments or manufacturing on the kinetic behavior of

anthocyanins. Our results suggest that the first-order model is suitable for predicting anthocyanin thermal degradation of extruded nixtamalized corn products at temperatures ranging from 60 to 90 °C. The kinetic parameters (D-value, t ½ and *k*) were affected by temperature. The higher the temperature was, the higher the degradation rate constant was and the shorter the half-life and the decimal reduction time of all the samples were. In the same way, the higher the energy activation and Q_{10} were, the greater the temperature dependence of the anthocyanin transformation was. The results showed that the anthocyanins in flour and tortillas required less energy to activate the thermal degradation reaction than those in raw corn, indicating a faster degradation process due to the impact of extrusion processing and baking on the anthocyanins. The ΔH, ΔG and ΔS indicated that anthocyanin degradation in the extruded nixtamalized corn products at any temperature was an endothermic and a nonspontaneous reaction, requiring the application of an external heating source to carry out the transition of the molecules. The thermodynamic parameters estimated in ENCF and tortillas also indicated that anthocyanins followed a similar degradation pattern during the thermal treatments. The findings in this work showed that using kinetic models and thermodynamic parameters as tools for predicting the degradation of biologically active compounds could be useful for optimizing industrial food processing conditions and establish appropriate thermal processing guidelines to minimize anthocyanin losses in pigmented corn-based products.

Author contributions

Mariela Menchaca-Armenta: Investigation, Writing - Original Draft preparation. Benjamín Ramírez-Wong: Conceptualization, Methodology. María José Frutos: Conceptualization, Resources. Armando Quintero-Ramos: Formal analysis. Raquel Muelas-

Domingo: Supervision. Estefanía Valero-Cases: Investigation. Patricia I. Torres-Chávez: Writing - Review & Editing. Ana I. Ledesma-Osuna, and Olga N. Campas-Baypoli: Validation.

Declaration of competing interests

None.

Acknowledgments

Mariela Menchaca-Armenta is grateful to CONACyT for the provided doctoral scholarship.

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Table 1

Estimated kinetic parameters of anthocyanin thermal degradation in blue corn, extruded nixtamalized flour and tortillas. [1,2,3,4,5,6]

T (°C)	$t_{1/2}$ (h)	D-value (h)	k (h^{-1})
Raw corn			
60	25.3 ± 0.06 ^{aA}	83.9 ± 0.21 ^{aA}	0.027 ± 7.07 x 10^{-05} ^{bA}
75	6.4 ± 0.11 ^{aB}	21.4 ± 0.38 ^{aB}	0.107 ± 1.91 x 10^{-03} ^{aB}
90	1.9 ± 0.01 ^{aC}	6.5 ± 0.05 ^{aC}	0.354 ± 2.76 x 10^{-03} ^{aC}
ENCF			
60	19.1 ± 0.15 ^{bA}	63.6 ± 0.49 ^{bA}	0.036 ± 2.83 x 10^{-04} ^{aA}
75	6.1 ± 0.14 ^{aB}	20.3 ± 0.49 ^{aB}	0.113 ± 2.76 x 10^{-03} ^{aB}
90	2.0 ± 0.03 ^{aC}	6.8 ± 0.12 ^{aC}	0.340 ± 6.2 x 10^{-03} ^{aC}
Tortillas			
60	18.7 ± 0.67 ^{bA}	62.0 ± 2.24 ^{bA}	0.037 ± 1.34 x 10^{-03} ^{aA}
75	6.1 ± 0.03 ^{aB}	20.4 ± 0.10 ^{aB}	0.112 ± 5.7 x 10^{-04} ^{aB}
90	1.9 ± 0.02 ^{aC}	6.6 ± 0.09 ^{aC}	0.347 ± 5.0 x 10^{-03} ^{aC}

[1] Results are means ± standard deviations (n = 3). [2] Means were separated by rows, applying Tukey's test. [3] Means with the same letter are not statistically significant ($p > 0.05$). [4] Lowercase letters correspond to the processing stage. [5] Capital letters correspond to thermal treatments. [6] ENCF: Extruded nixtamalized corn flour.

Table 2

Degradation rate constants (Ea, Q_{10} and Z) corresponding to anthocyanin degradation in blue corn, extruded nixtamalized flour and tortillas under different thermal treatments [1,2,3,4]

Product		Q_{10}		
	Ea (kJ/mol)	**60-75 °C**	**75-90 °C**	***z*-value (°C)**
Raw corn	89.2 ± 0.27 [a]	2.57 ± 0.09 [a]	2.22 ± 6 x10^{-04} [a]	28.8 ± 0.009 [b]
ENCF	75.1 ± 0.25 [b]	2.12 ± 0.06 [b]	1.96 ± 0.10 [ab]	35.1 ± 1.31 [a]
Tortillas	75.5 ± 0.11 [b]	2.18 ± 0.05 [b]	1.94 ± 0.4 [b]	33.8 ± 0.007 [a]

[1]Results are means ± standard deviations (n = 3).[2] Means were separated by rows, applying Tukey's test. [3]Significant differences between values within the same column are indicated by different letters ($p<0.05$). [4]ENCF: Extruded nixtamalized corn flour.

Table 3

Thermodynamic parameters calculated for anthocyanin degradation in blue corn, extruded nixtamalized flour and tortillas [1,2,3,4,5,6]

T (°C)	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (J/mol K)
Raw corn			
60	86.5 ± 0.27 [aA]	69.2 ± 0.007 [aA]	51.5 ± 1.41 [aA]
75	86.3 ± 0.27 [aA]	68.4 ± 0.05 [aB]	51.3 ± 0.92 [aA]
90	86.2 ± 0.27 [aA]	67.9 ± 0.02 [aC]	50.3 ± 0.80 [aA]
ENCF			
60	72.2 ± 0.04 [bA]	68.4 ± 0.02 [bA]	10.6 ± 0.71 [bA]
75	72.0 ± 0.04 [bA]	68.4 ± 0.04 [aA]	10.7 ± 0.06 [bA]
90	71.5 ± 0.04 [bA]	68.1 ± 0.05 [aB]	11.1 ± 0.99 [bA]
Tortillas			
60	72.8 ± 0.11 [bA]	68.3 ± 0.10 [bA]	12.5 ± 0.48 [bA]
75	72.6 ± 0.11 [bA]	68.3 ± 0.01 [aA]	12.4 ± 0.29 [bA]
90	72.5± 0.11 [bA]	68.0 ± 0.04 [aB]	11.8 ± 0.51 [bA]

[1] Results are means ± standard deviations (n = 3). [2] Means were separated by rows, applying Tukey's test. [3] Means with the same letter are not statistically significant ($p > 0.05$). [4] Lowercase letters correspond to the processing stages. [5] Capital letters correspond to thermal treatments. [6] ENCF: Extruded nixtamalized corn flour.

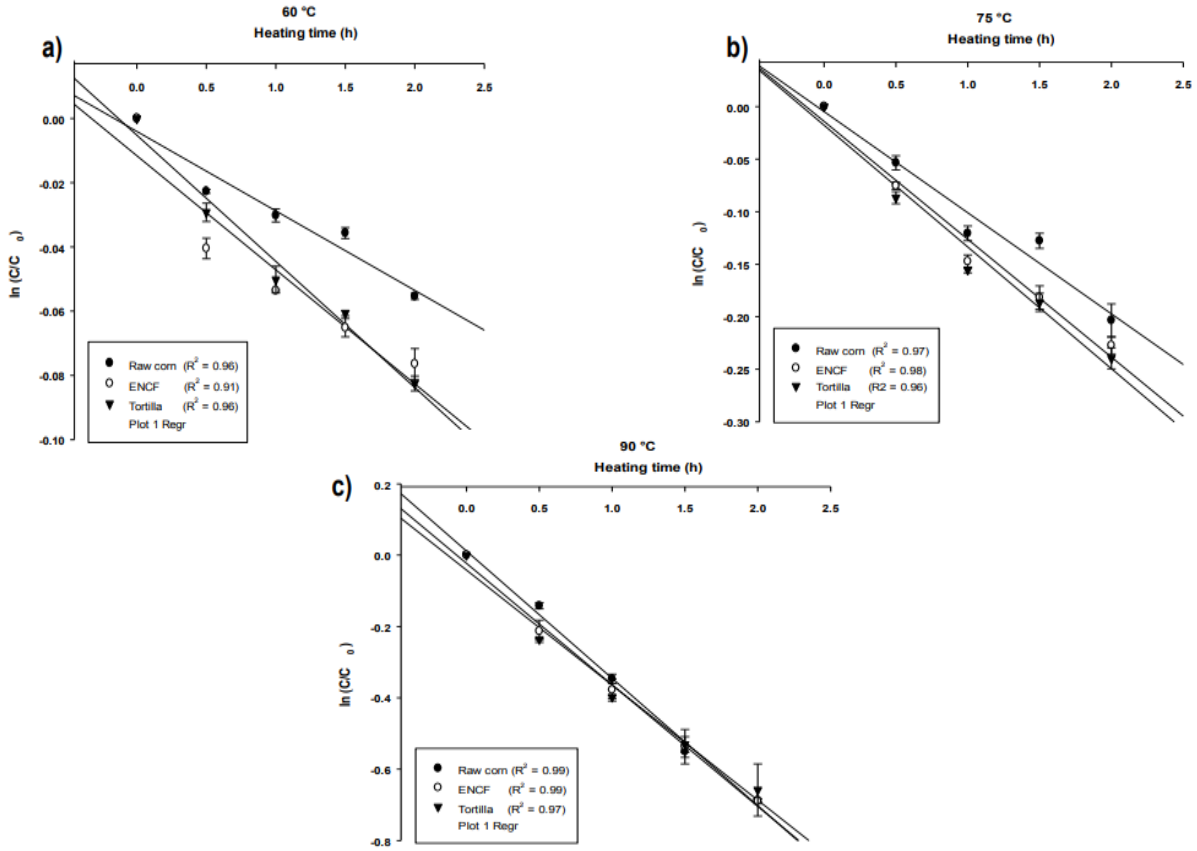

Fig. 1. First-order plot for the degradation of total anthocyanins in blue corn, extruded nixtamalized flour and tortillas at a) 60 °C, b) 75 °C and c) 90 °C during 2 h. Each point represents the mean ± standard deviation (n =3). Numbers in parentheses are the determination coefficients (R^2). Co, initial concentration.

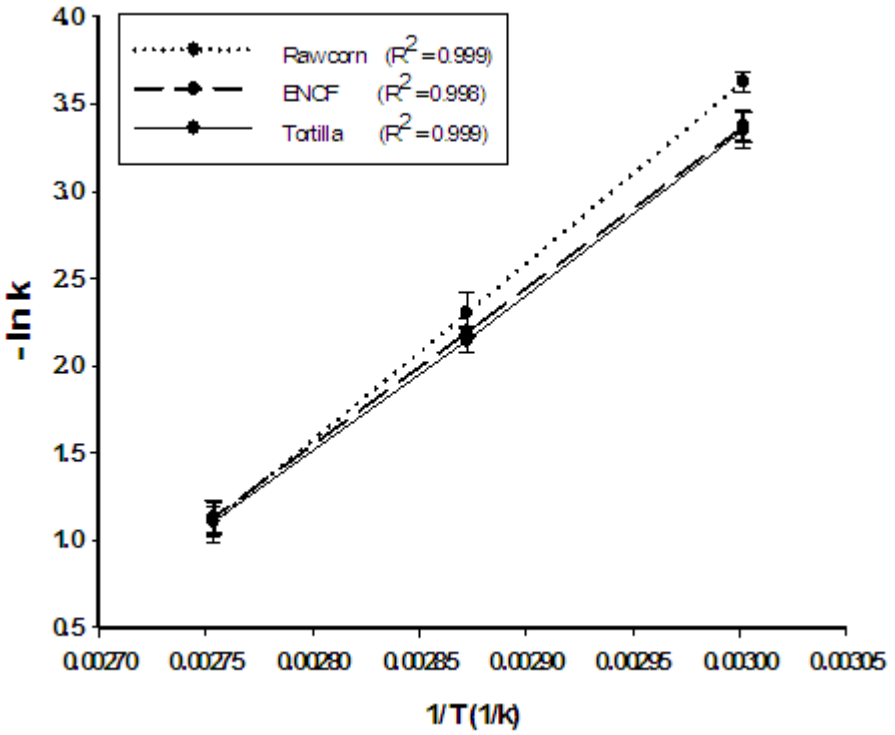

Fig. 2. Arrhenius plot for anthocyanin degradation in raw blue corn, extruded nixtamalized flour and tortillas during thermal treatment. Each point represents the average ± standard deviation of 3 replicates. Numbers in parentheses are the determination coefficients (R^2).

CAPÍTULO 3. DIGESTIÓN GASTROINTESTINAL SIMULADA

Artículo Científico Derivado de la Etapa 3: Stability and Antioxidant Capacity of Phenolic Compounds and Anthocyanins of Corn Tortillas (*Zea mays* L.) Under Simulated *in vitro* Gastrointestinal Digestion

El presente manuscrito se encuentra en el formato de la revista Journal of Functional Foods (Editorial Elsevier). El objetivo de esta parte de la investigación fue evaluar el efecto de cada fase de la digestión gastrointestinal simulada (fase oral, gástrica e intestinal) sobre la liberación, estabilidad y capacidad antioxidante de los fitoquímicos (compuestos fenólicos y antocianinas) de tortillas de maíz. Tortillas de maíz azul y blancas fueron sometidas al proceso de digestión *in vitro* simulando las condiciones fisiológicas de la boca, estómago e intestino delgado con el fin de determinar la liberación y estabilidad de sus fitoquímicos. Para la evaluación de la capacidad antioxidante y los cambios inducidos por la digestión se emplearon los extractos recuperados como fracción soluble y no soluble de la digestión enzimática y se realizaron tres ensayos antioxidantes. Se utilizó un diseño bifactorial completamente al azar. Los datos se analizaron mediante el ANDEVA, con un nivel de significancia de $p<0.05$. Los resultados indicaron que la digestión contribuyó fuertemente a la liberación de los compuestos fenólicos y que, a pesar de la evidencia de reducciones en las antocianinas, las tortillas exhibieron una capacidad antioxidante efectiva después de la digestión. La información generada es fundamental para la industria involucrada en la producción de tortilla debido a que los modelos *in vitro* pueden ayudar en la predicción de cambios en los fitoquímicos durante la digestión gastrointestinal y contribuir al desarrollo de nuevos productos funcionales y nutracéuticos. También podría ser importante para los consumidores, ya que los compuestos fenólicos y antocianinas liberados de las matrices de cereales son potencialmente bioaccesibles y pueden ejercer capacidad antioxidante en el tracto gastrointestinal con posibles efectos beneficiosos para la salud de los seres humanos. En el manuscrito se detalla el planteamiento, metodología, resultados y discusión de esta etapa de la investigación.

Stability and antioxidant capacity of phenolic compounds and anthocyanins of corn tortillas (*Zea mays* L.) under simulated *in vitro* gastrointestinal digestion

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Highligths

- Gastrointestinal digestion contributed strongly to the release of phenolic content.
- Food matrix played an important role in the prevention of losses in anthocyanins.
- The highest content of phenolic compounds was found in the intestinal phase.
- Corn tortillas exhibited antioxidant capacity after the gastrointestinal digestion.

Abstract

Corn tortillas from extruded blue corn and white commercial flour were subjected to *in vitro* digestion, simulating the physiological conditions of the mouth, stomach and small intestine in order to determine the stability of their phytochemicals. For evaluation of the antioxidant capacity and the changes induced by the simulated digestion, extracts recovered as soluble and non-extractable fraction from enzymatic digestion were employed and three different antioxidant assays were carried out. Digestion contributed to the released of the phenolic compounds into the digested fluids. Despite the evidence of reductions in anthocyanins after intestinal digestion, the food matrix protected these labile compounds from a total degradation. Corn tortillas exhibited effective antioxidant capacity, differences in trends reflected the different mechanisms of antioxidant action between the methods. This study provides a new contribution in the bioaccesibility of the phytochemicals in corn based products after the digestion and their potential beneficial health effects in humans associated with the antioxidant properties.

Keywords: bioaccesibility, non-extractable phenolics, extrusion, dietary antioxidants, gut health, nixtamalization

1. Introduction

Blue corn (*Zea mays* L.) is noted for its wide array of phytochemicals such as phenolic acids, and flavonoids especially anthocyanins (Liu, 2007). These compounds are located mainly in the pigmented testa layer and pericarp and exist in the free, soluble conjugate and insoluble bound forms associated with cell wall polysaccharides (Adom & Liu, 2002).

In Mexico, corn is primarily consumed in the form of nixtamalized products, most notably tortilla. The processing method employed for obtaining these product is important due that could improve specific characteristics from the nutritional point of view. The traditional nixtamalization method constists in alkaline cooking of corn followed by a resting time. The alkaline cooking of corn facilitate hydrolysis of the pericarp, allows release of niacin making it available for the organism, and improves the digestibility of the protein (Paredes-López *et al*., 2009). However, the large amounts of high solids liquid waste and the losses in nutrients such as, riboflavin, fat and fiber are important disadvantages. On the other hand, extrusion is an alternative method to produce corn tortillas with little amount of water used, and no environmentally deleterious effluents (Martínez-Bustos *et al*., 1996).

The anthocyanins and phenolic compounds in blue corn and their based products (tortillas) have been shown to possess antioxidant properties; therefore, these products have potential health-promoting properties (Mora-Rochín *et al*., 2010). Epidemiological studies have strongly suggested that the daily consumption of these compounds could play a role in the reduction or prevention of chronic disease through their antioxidant capacity (Adom & Liu, 2002).

The *in vivo* effects of antioxidants depend not only on their concentrations, but also on their bioaccessibility and bioavailability after ingestion (Palafox-Carlos *et al*., 2011). Bioaccessibility is defined as the amount of a food constituent that is solubilised in the gastro-

intestinal fluids as a consequence of their release from the solid matrix by the digestive enzymes activity and that may be able to absorbed in the gut in a certain amount (Pérez-Jiménez *et al*., 2013). Since foodstuffs are consumed as a whole, the phytochemicals are commonly mixed with different macromolecules such as carbohydrates, lipids, and proteins to form the food matrix (Parada & Aguilera 2007). These interactions could interfere with the bioaccesibility of the phenolics and anthocyanins in corn tortilla after ingestion.

As human and animal studies are time consuming, complex, highly variables, expensive, and restricted by ethical concerns (Parada & Aguilera, 2007); *in vitro* digestion models have been developed to mimic the complex physicochemical and physiological conditions of the human gastrointestinal tract and predict the release of the phytochemicals from the food matrix (Alminger *et al*., 2014). A good correlation between the results obtained using *in vitro* and *in vivo* systems have been reported (Carbonell-Capella *et al*., 2014).

Simulated digestion typically includes the oral, gastric and small intestinal steps, and occasionally large intestinal fermentation, taking into account the presence of digestive enzymes, pH, body temperature (37 °C), digestion time, and salt concentrations (Minekus *et al*., 2014). Due to digestion process comprises several steps which promotes an intense variation in pH, the recovery of anthocyanins after digestion is low due to degradation into new compounds. Anthocyanins are distinguished by their re-arrangements in response to pH changes. In the oral cavity, where salivary amylase begins to digest the food at an optimal pH of 5.6–7.9, the anthocyanins biotransformation reactions have already been initiated. When they reach the stomach, the acid pH (1.5-3.5) maintains the natural structural form of anthocyanins, flavylium cation (red color). Under basic conditions (pH > 7) that occur in the small intestine anthocyanins are present as colorless carbinol structure, restrained their identification, quantification, and thus

underestimating the detected values (Cavalcante-Braga *et al*., 2018). In the case of phenolic acids, stability may differ greatly from its total concentration, they are largely released and solubilized exhibiting effective antioxidant capacity after digestion (Carbonell-Capella *et al*., 2014).

During the last decades, although much has been reported on this topic, most studies on antioxidant bioaccesibillity are focused on foods and beverages from which antioxidants are easily released (Pérez-Jiménez *et al*., 2013), however, the bioaccesibility from whole foods may be substantially different (Palafox-Carlos *et al*., 2011) and no data are available on the bioaccesibility, stability and antioxidant capacity of phytochemicals in nixtamalized corn products after digestion. The wide free and bound phenolic distribution observed in pigmented corn varieties should be considered when evaluating the beneficial effects of phytochemicals during the different stages of gastrointestinal digestion (Rocchetti *et al*., 2018).

Considering the continuous developments of new "functional foods¨ products by the food industry; determination the bioaccesibility of antioxidants directly from the food matrix for the prediction of their potential *in vivo* effects is vital. The aim of this work was evaluate the impacts of each phase of the simulated gastrointestinal digestion (oral, gastric and intestinal phase) on the release, stability and antioxidant capacity of the phytochemicals (phenolic compounds and anthocyanins) of whole raw blue corn, extruded nixtamalized blue corn tortilla and compare the results whit a traditional nixtamalized white corn tortilla.

2. Materials and methods

2.1 Chemicals and reagents

Ultrapure water was obtained from a Milli-Q water purification system (Millipore Corp., Darmstadt, Alemania). Methanol, ethanol, hexane, formic acid (all HPLC-grade), NaOH, $NaHCO_3$, Na_2CO_3, $CH_3COONa.3H_2O$, ethyl acetate hydrochloric acid (37%), and Folin-Ciocalteu reagent, were purchased from Pancreac Quimica S.A. (Barcelona, Spain). α-amylase (A3176; EC 3.2.1.1), pepsin (P700; EC 3.4.23.1), pancreatin (P3292; EC 232-468-9), bile salts (B8756), gallic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl radical), ABTS (2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxilic acid), $K_2S_2O_8$, TPTZ (2,4,6-tri(2-pyridyl)1,3,5-triazine), $FeCl_3.6H_2O$, and phosphate-buffered saline were obtained from Sigma Aldrich (St. Louis, MO, USA).

2.2 Materials

Blue corn was cultivated and harvested in 2016 in the state of Chihuahua, México. Nixtamalized commercial white flour (Maseca®, Gruma, S.A.B. de C.V., Nuevo León, México) was purchased from the local supermarket and commercial lime used for alkali treatment was purchased from Nixtacal Calhidra, S.A de C.V, Hermosillo, Sonora, México.

2.3 Blue corn sample

The blue corn (BC) was cleaned in a vibrating cleaner to eliminate impurities (Model V230, Clipper, USA) and ground in a laboratory mill (Model 8, Christy Turner Ltd., England, United Kingdom) using a 2 mm mesh size to obtain ground whole corn. The ground corn was stored at -20 °C in the dark until analysis.

2.4 Nixtamalization extrusion process

The nixtamalization extrusion process was performed according to Menchaca-Armenta *et al*. (2020) who established the optimal extrusion conditions for obtaining an extruded nixtamalized

blue corn flour (ENCF). Before extrusion, the blue ground corn was conditioned with 0.3% w/w lime and water, then mixed for 3 min at low speed (600 rpm) (Hobart model AS200, Troy, OH). Afterward, the hydrated ground corn was kept under refrigeration at 4°C for 12 h and achieved an 18.17% moisture content. Then, the conditioned ground corn was brought to approximately room temperature (25 °C) and fed at a rate of 45 rpm. A single-screw laboratory cooking extruder (19 mm screw diameter; length-to-diameter ratio of 20:1; nominal compression ratio of 1:1; and die opening of 4 mm) with four independent heating/cooling zones was used (Brabender Instruments, model E 19/25 D, OHG Duisburg, Germany). The extruder was operated at a screw speed of 76.6 rpm, and the temperatures of the four zones remained constant at 60, 70, 80 and 92 °C. The extrudates obtained were dried at 50 °C for 60 min in a tunnel dryer and cooled at room temperature. Afterward, they were ground to obtain an extruded nixtamalized corn flour (ENCF) using a hammer mill (Christy Turner Ltd., England) with a mesh size of 2 mm. Then, the ENCF was stored in polyethylene bags at -20 °C in the absence of light until analysis.

2.5 Extruded nixtamalized blue tortilla preparation

Extruded nixtamalized blue tortillas (ENBT) were prepared according to the procedure reported by Platt-Lucero *et al*. (2010) using the ENCF obtained previously. Four kilograms of the ENCF was mixed (Model AS200, Hobart MFG. CO., Troy, Ohio, USA) for 3 min with an amount of distilled water. After 20 min of resting, the obtained corn masa was processed in a commercial tortillería (Tortillería Pimentel, Hermosillo, Sonora, México). The corn masa was placed in a tortilla-forming machine (Model MLR 30, Lenin Manufactures, San Luis Potosí, México) to form a masa disk of 25 g. Disks were baked in a three-zone oven, where the first, second and third zones of the oven were heated to the following temperatures: 258 ± 10 °C, 308 ± 10 °C

and 257 ± 10 °C, respectively. The residence time in the oven was of 56 s. The baked tortillas were cooled and subsequently lyophilized (Model 7753020, Labconco, Kansas City, Missouri, USA), milled a mesh size of 2 mm, packed and stored at -20°C in polyethylene bags to avoid moisture loss.

2.6 Traditional nixtamalized white tortilla preparation

Traditional nixtamalized white tortillas (TNWT) were prepared by mixing 225 g of commercial flour with 315 ml of water to obtain corn dough (masa). Masa was wrapped in a plastic bag and allowed to rest for 20 min before processing. Next, the masa was molded into a flat disk, using a manual machine. The masa disks (25 g) were cooked on a hot griddle at 255 ± 10 ° C for 20 s on one side, followed by 35 s on the other side, then flipped again during 15 s until expansion (swelling). Once the tortillas were obtained, they were cooled, frozen (-80°C), and freeze-dried. The tortillas were homogenized in a food processor and stored at -20°C in polyethylene bags until analysis. The procedure was run in three replications.

2.7 Phenolic compounds extraction before *in vitro* digestion

Free and bound phenolic compounds were extracted in triplicate from each lyophilized sample and according to the method described by Adom and Liu (2002). A ground sample (0.5 g) was mixed with 10 ml hydro-alcoholic solution consisting of 80% ethanol (v/v) for 10 min in a shaker at 500 rpm. Next, the extracts were centrifuged at 3000 ×g for 10 min at 4 °C. The corresponding supernatants, representative of soluble phenolics fraction (SF), were filtered using 0.45 μm nylon filters and collected in amber vials for further analysis. The extracts were concentrated at 35 °C using a vacuum evaporator, and the residue was reconstituted in 2 ml of methanol-water (50:50 v/v) and stored at −20 °C until use. After extraction of soluble phenolic

compounds, the pellets were further hydrolysed with 10 mL of 2 M NaOH and kept at room temperature for 1 h. After alkaline hydrolysis, the hydrolyzed was neutralized with 2 ml of HCl before removing lipids with 10 ml of hexane. Bound phenolics were extracted five times with 10 ml of ethyl acetate and centrifuged for 10 min at 3000×g at 4 °C. Next, the ethyl acetate supernatant was dried under vacuum at 35 °C and the residue was dissolved in 2 ml of 50 % methanol. The resulting solution, representative of non-extractable phenolic fraction (NEF) was filtered using 0.45 μm nylon filters and stored at −20 °C until use.

2.8 Anthocyanins extraction before *in vitro* digestion

The anthocyanins extraction for test matrix (BC and ENBT) was obtained using an acid methanol, and these values were assumed as 100% of anthocyanin content of samples. Anthocyanin extraction was carried out using the lyophilized samples and performed as follows: four grams of each sample was dissolved in 30 mL of acidified cold methanol (60% methanol, 37% water, 3% formic acid v/v/v) to prepare a concentrated anthocyanin solution. The suspension was homogenized and placed on a digital magnetic stirrer (OVAN, Multimix Heat, Model MMH30E, Badalona, Spain) at room temperature (25 °C) for 30 min. After extraction, samples were immediately centrifuged (C30P, B. Braun Biotech International) at 9500 rpm for 30 min at 4 °C. Next, the resulting supernatants were filtered using 0.45 μm nylon filters and collected in amber vials and stored at −20 °C for further analysis. All the steps were carried out under dark conditions to avoid anthocyanin degradation.

2.9 Simulated *in vitro* gastrointestinal digestion

The procedure described by Valero-Cases *et al.* (2017) was used to perform the simulated gastrointestinal digestion. It consisted of a three-step procedure to mimic the digestive process

in the mouth (oral phase), stomach (gastric phase) and small intestine (intestinal phase). Representation of the simulated gastrointestinal digestion procedure carried out in the blue corn and both tortillas is presented in Figure 1. Briefly, the digestion starts by adding 10 g/L of α-amylase in 250 ml of a phosphate buffer (PBS) solution (pH 7) to 25 g of the of freeze-dried sample, which is incubated at 37 °C for 2 min in a water bath shaker at 200 rpm. Samples were placed in a 500 ml screw capped conical flask equipped in three inlets allowing the introduction of pH electrode, thermometer, and dosage of biochemical agents. Magnetic marbles were added to each flask for uniform mixing during incubation. After the oral step, the sample was incubated under gastric conditions. For this, the pH of the digested sample was decreasing to 2.5 with 1 M HCl and adding 3 g/L of pepsin, and incubated for 2 h under the same conditions. Then, to imitate intestinal digestion, the pH was adjusting to 7 with 1 M $NaHCO_3$, followed by the addition of 4.5 g/L of bile salts and 1 g/L of pancreatin and the incubation was continued for another 2 h to complete the intestinal phase. At the end of each phase of digestion, aliquots of 5 ml of digested samples were removed and placed immediately on ice to deactivate enzymes. Digested samples were centrifuged at 9500 × rpm for 10 minutes at 4 °C and supernatants and pellets were separated and stored at −80 °C in dark conditions until analysis. The supernatant represents the soluble fraction (SF) of phenolic compounds and anthocyanins, while the pellet is considered as the non-extractable fraction (NEF) available to uptake by gut microbiome. The *in vitro* digestion was performed in triplicate in absence of light.

2.10 Determination of phenolic content

Determination of phenolic content in the soluble and non-extractable fractions of undigested and digested samples were conducted according to the previous method (Singleton & Rossi, 1965). An aliquot (0.5 ml) of sample dissolved in 50% of methanol was mixed with 2.5 ml of

Folin–Ciocalteu reagent and 2 ml of 7.5% Na_2CO_3, followed by incubation for 1.5 h at room temperature. After incubation the absorbance was recorded at 750 nm. The content of phenolic compounds was calculated using standard curve for gallic acid. Results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of sample (dw). Total phenolic content in each sample was determined by the sum of the soluble and non-extractable fractions. Phenolic content of the samples was assessed before and after the *in vitro* gastrointestinal digestion.

2.11 Quantification of anthocyanin content

The anthocyanin content in soluble and non-extractable fractions of undigested and digested samples was analyzed according to Abdel-Aal and Huel (1999). Briefly, the absorbance of the samples was measured at 520 nm in a UV-visible spectrophotometer (UV/vis T80, PG Instruments Ltd.). The anthocyanin content of samples was calculated using the following equation:

Anthocyanins (mg/kg) = ((A X MW X DF X 1000)/ε).(V/m) .1000

where A is the absorbance at a wavelength of 520 nm, MW is the molecular weight of cyanidin-3-glucoside (449.2 gmol-1), ε is the molar extinction coefficient (26,900 L mol-1 cm-1), V is the volume of the extract (L), DF is the dilution factor, and m is the weight of the sample (g). The results were expressed as mg of cyanidin-3-glucoside equivalents (CGE) per kg of dry weight. The total anthocyanin content in each sample was determined by the sum of the soluble and non-extractable fractions.

2.12 Determination of the *in vitro* antioxidant capacity

2.12.1 DPPH radical scavenging assay

The free radical scavenging capacity was determined according to the methodology described by Brand-Williams *et al*., (1995) using the stable radical DPPH. The absorbance was measured at 515 nm. Results were expressed as μmoles of Trolox equivalents (TE)/100 g of sample (dw).

2.12.2 ABTS radical cation scavenging capacity assay

The ABTS scavenging capacity assay was measured as described by Re *et al*., (1999). The absorbance of the samples was measured on a spectrophotometer (UV/vis T80, PG Instruments Ltd.) at 734 nm. Results were expressed as μmoles of Trolox equivalents (TE)/100 g of sample (dw).

2.12.3 Ferric reducing antioxidant power assay

The ferric reducing antioxidant power **(**FRAP) was determined using the methodology described by Benzie and Strain (1996). The FRAP values were measured on a spectrophotometer (UV/vis T80, PG Instruments Ltd.) at 593 nm and the results estimated in μmoles of Trolox equivalents (TE)/ 100 g of sample (dw).

2.13 Statistical analysis

A completely randomized bifactorial design was used. The factors were the type of product with three levels (BC, ENBT and TNWT) and the gastrointestinal digestion phases with four levels (Undigested, oral, gastric and intestinal). All experiments were conducted in triplicate (n=3) and presented as the mean ± SD. All data collected were analyzed using analysis of variance (ANOVA), which was performed using the Statistical Analytical Systems package (SAS Institute, Cary, North Carolina). Significant differences were determined by Tukey's test according to $p \leq 0.05$.

3. Results and discussion

3.1 Phenolic content

In order to test the stability of phenolic compounds in samples during gastrointestinal digestion, their concentration was measured before and after each phase of this process.

3.1.1 Phenolic content in undigested samples

The higher and lower concentration of phenolic compounds in undigested samples were found in ENBT and TNWT (192.3 and 63.1mg GAE/ 100 g, respectively) ($p<0.05$) (Table 1). The prevalence of insoluble-bound phenolic compounds (NEF) in different corn samples when compared to the free fraction (SF) is in agreement with Rocchetti *et al.* (2018). The insoluble fraction represents around 80%, 82 % and 61% of total phenolic content of BC, ENBT and TNWT, respectively.

3.1.2 Phenolic compounds stability during oral phase

In the oral phase, results showed an increased by 300 to 400 % for phenolic content in soluble fraction (SF) for all the samples with respect to the undigested values ($p < 0.05$) (Table 1). It is estimated that nearly 5% of the consumed starch is already degraded in the oral step by salivary α-amylase, helping on the release of phenolic compounds which initially may be insoluble (Alminger *et al*., 2014). In addition, the agitation conditions (simulated mechanical action during mastication in the mouth) could facilitate breakage of large molecules. As a consequence, the solubilisation an amount of the bound phenolic compounds into the oral digested fluid occur (Palafox-Carlos *et al*., 2011).

3.1.3 Phenolic compounds stability during gastric phase

In gastric phase, there was an increased in the concentration of phenolic compounds in the soluble fraction for BC (84 %), ENBT (97 %) and TNWT (258 %) with respect to the values obtained in the oral digestion, and their content was still higher than undigested values for all samples ($p<0.05$) (Table 1). The high presence of phenolic compounds in SF indicates that the phenolic compounds initially insoluble were released from the food matrix as a result of enzymatic digestion. Results showed the TNWT presented a higher total phenolic concentration than BC and ENBT. It could be related to the difference in matrix composition, particle sizes and processing treatment that could affect the potentially digestion. The ENBT used the whole grain (retaining the germ and pericarp), as a consequence, the presence of a higher level of dietary fiber from the pericarp could interfere with the bioaccesibility of phenolic compounds. Dietary fiber presents quickly hydration properties that generated increase in the viscosity of the bolus entraping an amount of phenolic compounds (Palafox-Carlos *et al*., 2011). In the opposite side, the alkaline cooking and soaking steps used in the traditional nixtamalization process for obtaining the TNWT causes the pericarp become brittle, facilitating its partial removal, which decreases the content of dietary fiber (Paredes-López *et al*., 2006), thus, increasing the accessibility of the enzyme, increased in this way the amount of the phenolic compounds realeased. The possible formation of links between some phenolic compounds structures and the pectin-gel formed under the acidic conditions of the gastric phase (pH 2.0), could explain the lower recovery of the main phenolic compounds in ENBT.

3. 1.4 Phenolic compounds stability during intestinal phase

In the intestinal digestion there was an increased about 15-30 % of phenolic compounds in the soluble fraction with respect to the gastric values for all samples, and their contents were still higher than oral, and undigested values (Table 1). The higher phenolic concentration of the SF

in the intestinal digested samples compared to the gastric samples could be due to the additional digestion time (2 h), the alkaline environment and the use of more enzymes (pancreatin) (Adarkwah-Yiadom & Kwaku Duodu, 2017). This behavior is very important because the intestinal tract is where most nutrients are absorbed. Thus, the mere presence of liberated phenolic compounds in the simulated intestine may exert a local effect (Chandrasekara & Shahidi, 2012). As unexpected, the higher phenolic concentration post intestinal step was found in TNWT, despite the pigmented corn (BC) and its tortilla (ENBT) had higher phenolic contents in the undigested samples. According to Camelo-Méndez *et al*., (2017) polyphenol-rich extracts from blue maize reduced the activity of enzymes in the *in vitro* gastrointestinal digestion more than did the white maize extract, due to the higher levels and different compositions of polyphenol compounds. In addition, in TNWT starch granules are presented in a more disrupted form than BC and ENBT due to the long cooking time, resulting in gelatinised starch that is easily available to pancreatic amylase activity (Colonna *et al*., 1992). The pancreatic amylase activity produces the release of reducing sugars from the hydrolysis of starch that may have reacted with the Folin Ciocalteu reagent, contributing to a greater quantification of phenolic compounds.

Despite of the liberation of an important amount of phenolic content in the intestinal phase, in the insoluble residue remained the non-extractable compounds. These trapped compounds have gained interest due that they can reach the colon (Rochetti *et al*., 2018). Once in the colon, those compounds that are not solubilised in the small intestine become accessible either by fermentation by colonic microbiota of the molecules to which they are associated (carbohydrates or proteins), or the action of some intestinal enzymes able to break covalent bonds, such as esterases (Pérez-Jiménez *et al*., 2013). The metabolites and/or catabolites produced by the

colonic bacteria can encourage the growth of beneficial bacteria and inhibit the growth of pathogenic bacteria (Saura-Calixto, 2011). Additionaly, they may be exerting a local health effect promoting an antioxidant environment (Pérez-Jiménez *et al*., 2013).

3.2 Anthocyanin content

3.2.1 Anthocyanin content in undigested samples

Anthocyanin content before simulated digestion was significantly higher in BC (152.8 mg CGE/kg dw) than ENBT (134.5 mg CGE/kg dw) (Table 2). The extrusion process, as well the baking conditions of obtaining the blue tortilla modify the anthocyanins due to the high temperature, screw speed, and alkaline conditions employed, provoking that a certain amount of these compounds to degrade. Due that white corn does not contain anthocyanins, these compounds were not detected in TNWT.

3.2.2 Anthocyanins stability during oral phase

In the oral phase the salivary amylase begins to digest the blue corn and tortilla impregnating the small pieces of samples to form a bolus and biotransformation reactions of anthocyanins have already been initiated (Palafox-Carlos *et al*., 2011). After the simulated oral digestion, anthocyanins concentration of BC and ENBT recovered in the digested soluble fraction were 41% and 70 % lower than the undigested values ($p < 0.05$) (Table 2). The low results does not necessarily indicate a complete reduction in the amount of these compounds. Despite the alkaline environment in the oral step causing a decreased in the anthocyanin content, it is important to notice that in the NEF, certain amount of anthocyanins remaining in the insoluble form after the oral digestion: 18.2 and 25.5 mg CGE/kg for BC and ENBT respectively. These values suggest that during oral phase, anthocyanins were incomplete release from the matrix

due possibly to the interactions with other components of corn such as fibre, carbohydrates, and lipids (Peixoto *et al*., 2016). The formation of strong non-covalent anthocyanin/starch interactions lead to the formation of insoluble networks structures, these phenomena may lead to reduce the complete release of anthocyanins from the matrix into the digested fluid, limiting the anthocyanin destruction. Anthocyanins also can produces α-amylase inhibition by binding to their active sites with limited availability for amylolytic attack by physical shielding. (Bello-Perez *et al*., 2015). In addition, in the oral phase, moderated effects in anthocyanin losses were expected due to the short exposure time (2 min), low contact with the pH (7.0) and marginal effects of α-amylase (Lucas-González *et al*., 2016).

3.2.3 Anthocyanins stability during gastric phase

After gastric step (pepsin/HCl digestion), anthocyanins found in the soluble fraction were increased by 30 % of their concentration in both samples with reference to oral phase, although their contents were still lower than undigested values ($p < 0.05$) (Table 2). This is according with previous studies reported that anthocyanins from different fruits and vegetables were stable under acidic gastric conditions (Podsezdek *et al*., 2014; Mosele *et al.*, 2015). Mosele et al. (2015), reported a slightly increased, 1.75 to 4.39 %, in anthocyanins released of pomegranate products after the gastric digestion. The low pH (1.5–3.5) in the gastric phase would favour the stability of these molecules, maintaining the natural structural form of anthocyanins, the flavylium cation (red color) (Cavalcante-Braga *et al*., 2018). Another possible explanation is that that acidic conditions of gastric phase could improve the release of anthocyanins from the solid food matrix (Podsezdek *et al*., 2014). The acid medium (HCl) might promotes the partial breakage of high molecular compounds such as proanthocyanin oligomers increasing the amount of the monomeric compounds quantified in the soluble fraction (Stanisavljević *et al*.,

2015). According with several works, anthocyanins in the soluble gastric digested may be quickly absorbed in their intact form into the stomach wall (Fernandes *et al*., 2013; Manach *et al*., 2004; Passamontia *et al*., 2002).

3.2.4 Anthocyanins stability during intestinal phase

The transition of BC and ENBT from the acidic gastric conditions to the intestinal environment (pancreatin-bile salts digestion) caused 64 % and 78 % decrease in total anthocyanins, respectively (Table 2). The decreasing trend in the anthocyanin concentration after the intestinal step was in accordance with Vallejo *et al*., (2004), who also detected important losses of these compounds after simulated *in vitro* intestinal digestion of broccoli inflorescence. Under intestinal pH (7.4), anthocyanins change their structure to quinoids, hemiketal and chalcone forms due to anthocyanin chromophore destruction, leading to their colourless form. This structure modification restrained its identification, quantification, and thus underestimating the detected values (Peixoto *et al*., 2016).

Despite the evidence of significant reductions in monomeric anthocyanins, the stability of these compounds after intestinal digestion could be explained by the food matrix composition. The physical and chemical interactions between food components such as soluble and insoluble fibre and anthocyanins, could modulate their release in the gastrointestinal tract, protecting in this form these labile compounds (Podsezdek *et al*., 2014). In the previous digestion step (gastric phase) the presence of dietary fiber in BC and ENBT increasing the tortuosity and viscosity of the environment (food matrix). The viscosity of fluids in the intestine phase restricted the mixing process that promotes transport of enzymes to their substrates, bile salts to unmicellized fat, serving as a barrier to bile salts and enzyme digestive action (Palafox-Carlos *et al*., 2011). In a previous study, a higher recovery of anthocyanins was observed in solid-whole foods in relation

to liquid products, indicating the importance of the food matrix in their stability during digestion (Parada & Aguilera, 2007). Correa-Betanzo *et al.*, (2014) reports that approximately 50% of delphinidin and malvidin-6-acetoyl 3-glucoside remained intact after the *in vitro* digestion. Furthermore, He *et al*. (2009) found in the small intestine tissue 7.5% of ingested anthocyanins in their native form after 2 hours of administration.

Anthocyanins that after ingestion were not released from the food matrix either by mastication, an acidic pH in the stomach or the action of digestive enzymes could reach the large intestine, then be subjected to extensive transformation by colonic microflora (Pérez-Jiménez *et al*., 2013). The microbial metabolites could be absorbed though the portal vein, reaching the liver giving rise to phase II metabolites. Once formed these metabolites, may return to the digestive tube through the bile, or pass into the bloodstream as a first step and delivered to the appropriate location within the body to exert pharmacological activity in different tissues and organs (Pérez-Jiménez *et al*., 2013). Several studies have found that anthocyanins can reach to the brain, eye, and other organs with a maximum concentration of nanomolar levels (Fernandes *et al*., 2013).

3.3 Antioxidant capacity

3.3.1 Antioxidant capacity of phenolic compounds

Antioxidant capacity related to phenolics compounds of soluble as well non-soluble fraction in BC, ENBT and TNWT digested samples are presented in Figure 2. In DPPH assay, undigested samples had values of 687.361 µmol ET/100 g for BC, 863.72 µmol ET/100 g for ENBT and 355.749 µmol ET/100 g for TNWT (Figure 2a), where the highest value of antioxidant capacity was found in blue tortilla. This may associate with higher phenolic content in this sample. It is interesting to note that the greatest contribution to antioxidant capacity in all undigested samples

is associated with the non-extractable fraction. It is well known that ferulic acid is the most abundant phenolic acid in this fraction and promoter of antioxidant capacity in corn products (Mora-Rochín *et al*., 2010). After oral and gastric phases, despite the increase in phenolic content in the intestinal phase, the antioxidant capacity of digested extracts decreased by 75 %, 63 % and 51 % for BC, ENBT and TNWT, respectively, with respect to undigested values (Figure 2a). In intestinal phase, again ENBT had higher antioxidant values ($p < 0.05$) than BC and TNWT. Intestinal phase had a deep impact on antioxidant capacity of blue corn and tortillas determined with DPPH assay. The same effect was observed by Lucas-Gonzalez *et al*. (2016) in digested maqui berries, with a reduction by 75.4% in DPPH values after the intestinal step with reference to non-digested sample. In the same way, the digestion of Chilean white strawberry showed a significant loss of DPPH activity after these phase (Thomas-Valdés *et al*., 2018).

Regarding to ABTS assay, as occurs with DPPH, in the undigested samples, the highest value of antioxidant capacity was found in ENBT (3759.1 µmol ET/100 g dw) (Figure 2b). After oral phase, increased in the antioxidant capacity of BC and TNWT was achieved (53 and 72 % respectively), while in ENBT this capacity decreased by 45% ($p < 0.05$). Gastric digestion caused a decrease of 74 %, 83 % and 38% in the antioxidant capacity of BC, ENBT and TNWT respectively, with respect to undigested values ($p<0.05$). This behavior was in concordance with Tagliazucchi *et al*. (2010) who reported that ABTS radical scavenging capacity of grape polyphenols decreased during gastric digestion. The intestinal digestion caused a deep increased in the ABTS scavenging capacity for all samples with respect to the corresponding undigested samples (Figure 2b), with higher ABTS values of ENBT and TNWT than BC ($p <0.05$). The increment in antioxidant capacity of the intestinal digested samples might be related with a

higher released of phenolic compounds with scavenging properties, probably due to more effective digestion in the intestinal phase compared to the oral and gastric steps, resulting in a gradual release of phenolic compounds from the food matrix during the digestion process (Gullon *et al*., 2015). These hypothesis is supported by observing that the antioxidant capacity is mainly provided by the soluble fraction in all samples. Results obtained are in agreement with Chandrasekara and Shahidi (2012), who informed an increase in antioxidant capacity of millet grains as measured by ABTS radical scavenging assay for neutral to a slightly alkaline condition in the intestinal digestion.

Regarding to ferric reducing power (FRAP), Figure 2c shows the FRAP values of undigested and digested samples. In FRAP assay, undigested samples had values of 4293.9 µmol ET/100 g, 3260.9 µmol ET/100 g, 1300.5 µmol ET/100 g for BC, ENBT and TNWT, respectively (Figure 2c), where the highest values of antioxidant capacity were found in BC, followed by ENBT ($p < 0.05$). In oral phase, the FRAP values in BC and ENBT decreased approximately 60 to 70 % with respect to undigested samples, while the FRAP value for TNWT remained constant. In gastric phase, the FRAP values obtained in ENBT and TNWT digested samples increased about 20 % with respect to oral phase, while the FRAP value for BC was similar with those obtained in the oral step, but lower that in the undigested sample. In the intestinal digestion, a deep reduction ($p < 0.05$) of reducing power was obtained for BC (67 %) and ENBT (62 %) with respect to undigested samples, while the FRAP values of TNWT remained constant. A similar decrease in FRAP values of phenolic compounds of different varieties of apples after the gastrointestinal digestion was reported by Bouayed *et al*. (2011). These results suggest that variations in the pH conditions and/or enzymes employed during the gastrointestinal digestion

exerted a considerable effect on phenolic compounds and decreased their reducing power (Gullon *et al*., 2015; Lucas *et al*., 2016).

Results support the need to carry out several antioxidant assays with diverse chemical mechanisms that evaluate different aspects of the reactivity of the samples. *In vitro* antioxidant studies cannot be directly compared, since different antioxidant tests rely on different mechanisms, so they should be considered as complementary rather than alternative (Rocchetti *et al*., 2018).

3.3.2 Antioxidant capacity of anthocyanins

Figure 3 descriptively presents the *in vitro* antioxidant capacity of anthocyanins of BC and ENBT samples, and their changes induced by the simulated gastrointestinal digestion. Undigested anthocyanins extracts showed a significant difference in DPPH scavenging capacity with values of 368.64 and 506.08 µmol ET/100 g (dw), for BC and ENBT respectively. After the oral phase, the antioxidant capacity in BC increased 95 % with respect to undigested values, while in ENBT a slight decreased (13 %) was observed (Figure 3a). In gastric and intestinal phases, DPPH scavenging capacity in BC digested samples were similar with those obtained in the oral step, but higher than the undigested samples ($p<0.05$), while in ENBT the DPPH scavenging capacity in gastric phase decreased 33 % compared with the undigested values, and remained without changes after the intestinal step.

Regarding to the ABTS scavenging capacity, the highest value of antioxidant capacity in undigested samples was found in ENBT (523.51µmol ET/100 g, dw) ($p<0.05$) (Figure 3b). In oral phase, as occurs with the DPPH assay, similar trend was observed in the digested samples, the antioxidant capacity in BC increased 71 %, while in ENBT a deep decreased (86 %) was

observed with respect to undigested values. In gastric step, the ABTS scavenging capacity increased in BC (165 %) and decreased in blue tortilla (94 %) compared with undigested samples. In the intestinal digestion, the BC showed 36 % higher ABTS scavenging capacity, while the antioxidant capacity of intestinal digested samples of ENBT was 65 % lower than the undigested values ($p<0.05$).

With respect to FRAP assay, Figure 3c shows the reducing power of anthoyanins before and after the digestion. In the undigested samples, not significant difference was found in both BC and ENBT samples ($p>0.05$). In oral digested samples, the FRAP value increased 124 % in BC and decreased 39 % in ENBT. In gastric step, the reducing power values were the same ($p > 0.05$) than those obtained in the oral phase for both samples. In the intestinal phase, the FRAP values remained without significant changes in ENBT, while in BC these values decreased 23 % with respect to gastric digested extracts.

The differences observed in trends may reflect the relative differences in the stability of the anthocyanins compounds in the samples during simulated gastrointestinal digestion. The antioxidant capacity in digested samples can be affected by the variation of anthocyanins composition in an array. The antioxidant capacity of anthocyanins compounds is related to its redox properties. Anthocyanins structures probably were altered in the digestion process especially due to the changes in pH, and a proportion of these compounds may have transformed into new different structural forms, which altered their reactivity and the ability to transfer electrons, impacting their antioxidant capacity (Yildirim *et al*., 2001). In summary, the antioxidant capacity of anthocyanins from ENBT was partially lost after the gastrointestinal process, and showed that anthocyanins of BC without processing had the higher antioxidant capacity mainly in oral and gastric phases. According to Wootton-Beard *et al*. (2011),

anthocyanins compounds would be more reactive particularly at acidic pH (as occurs in gastric digestion) and less reactive at pH close to neutrality (as occurs in intestinal digestion).

4. **Conclusions**

Gastrointestinal digestion contributed strongly to the release of the bound phenolic compounds of the food matrix in blue corn and both types of tortillas. The highest phenolic concentration was found in the intestinal phase for all samples. Although the different phases of digestion affected the anthocyanin content due to changes in pH, food matrix played an important role in the prevention of total losses of these compounds, their greatest stability was observed in the gastric phase. Results suggest that the phenolic compounds present in blue corn and tortillas have the potential to exert physiological effects once they are consumed, since they can have the potential to protect against oxidative damage due to their free radical scavenger capacity. Blue tortillas also showed antioxidant capacity of their anthocyanins in the different phases of digestion, which represents an added value as a functional food compared to the white tortilla. The information generated is essential to the industry involved in tortilla production due to the *in vitro* models can help in the prediction of phytochemical changes during gastointestinal digestion and contribute to the development of new functional and nutraceutical products.

Declaration of competing interests

None.

Acknowledgments

Mariela Menchaca-Armenta is grateful to CONACyT for the provided doctoral scholarship.

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Table 1. Phenolic compounds of soluble (SF) and non-extractable fraction (NEF) of blue corn (BC), extruded nixtamalized blue tortilla (ENBT) and traditional nixtamalized white tortilla (TNWT) before and after the simulated gastrointestinal digestion.

Digestion phase	Phenolic compounds (mg GAE[1]/ 100 g, dw)		
	SF	**NEF**	**Total**
	Blue corn (BC)		
Undigested	34.9 ± 4.03[2] d[3]A[4]	140.9 ± 7.22 aB	175.9 ± 3.19 bB
Oral	168.4 ± 3.42 cA	14.8 ± 0.45 cB	183.2 ± 2.96 bA
Gastric	310.1 ± 13.13 bB	38.6 ± 3.92 bB	348.8 ± 17.06 aB
Intestinal	359.7 ± 17.79 aB	13.1 ± 1.58 cB	372.8 ± 16.21 aB
	Extruded nixtamalized blue tortilla (ENBT)		
Undigested	33.0 ± 0.91 dA	159.3 ± 0.71 aA	192.3 ± 1.62 bA
Oral	132.6 ± 5.54 cB	26.0 ± 4.30 cB	158.7 ± 9.84 cA
Gastric	261.1 ± 2.07 bB	45.0 ± 2.98 bB	306.2± 0.90 aB
Intestinal	311.4 ± 5.89 aB	15.3 ± 1.80 cB	326.8 ± 7.69 aC
	Traditional nixtamalized white tortilla (TNWT)		
Undigested	24.6 ± 3.42 dA	38.5 ± 0.01 cC	63.1 ± 3.42 dC
Oral	125.8 ± 2.30 cB	57.1 ± 2.57 bA	182.9± 4.87 cA
Gastric	448.1 ± 3.79 bA	82.7 ± 3.46 aA	530.9 ± 20.46 bA
Intestinal	583.2 ± 8.70 aA	45.5 ± 5.49 bcA	628.7 ± 3.21 aA

[1]GAE, gallic acid equivalents.
[2]Values are means of three replications (n = 3 ± SD).
[3]Different lowercase letters denote significant difference on phenolic compounds between different phases of *in vitro* digestion for the same type of product ($p < 0.05$).
[4]Different capital letters denote significant difference on phenolic content between the type of product for the same phase of *in vitro* digestion ($p < 0.05$).

Table 2. Anthocyanins content of soluble (SF) and non-extractable fraction (NEF) of blue corn (BC) and extruded nixtamalized blue tortilla (ENBT) before and after the simulated gastrointestinal digestion.

Digestion phase	Anthocyanins (mg CGE [1]/kg dw)		
	SF	**NEF**	**Total**
	Blue corn (BC)		
Undigested	152.8 ± 4.03[2] a[3]A[4]	N.D.	152.8 ± 4.03 aA
Oral	89.1 ± 9.33 cA	18.2 ± 0.13 bB	107.3 ± 9.47 bA
Gastric	118.0 ± 1.69 bA	46.8 ± 0.38 aB	164.9± 1.30 aA
Intestinal	46.6 ± 1.04 dA	7.3 ± 0.65 cA	53.6 ± 2.09 cA
	Extruded nixtamalized blue tortilla (ENBT)		
Undigested	134.5 ± 0.64 aB	N.D.[5]	134.5 ± 0.64 aB
Oral	39.5 ± 2.30 cB	25.1 ± 1.33 bA	77.8 ± 1.21 cB
Gastric	52.7 ± 0.12 bB	61.4 ± 1.69 aA	100.9 ± 3.99 bB
Intestinal	23.9 ± 0.48 dB	5.3 ± 0.96 cA	29.3 ± 1.45 dB

[1]CGE, cyanidin 3-glucoside equivalents.
[2]Data are means ± SD (n = 3).
[3]Different lowercase letters denote significant difference on anthocyanin content between different phases of *in vitro* digestion for the same type of product ($p < 0.05$).
[4]Different capital letters denote significant difference on anthocyanin content between the type of product for the same phase of *in vitro* digestion ($p < 0.05$).
[5]N.D. not detected.

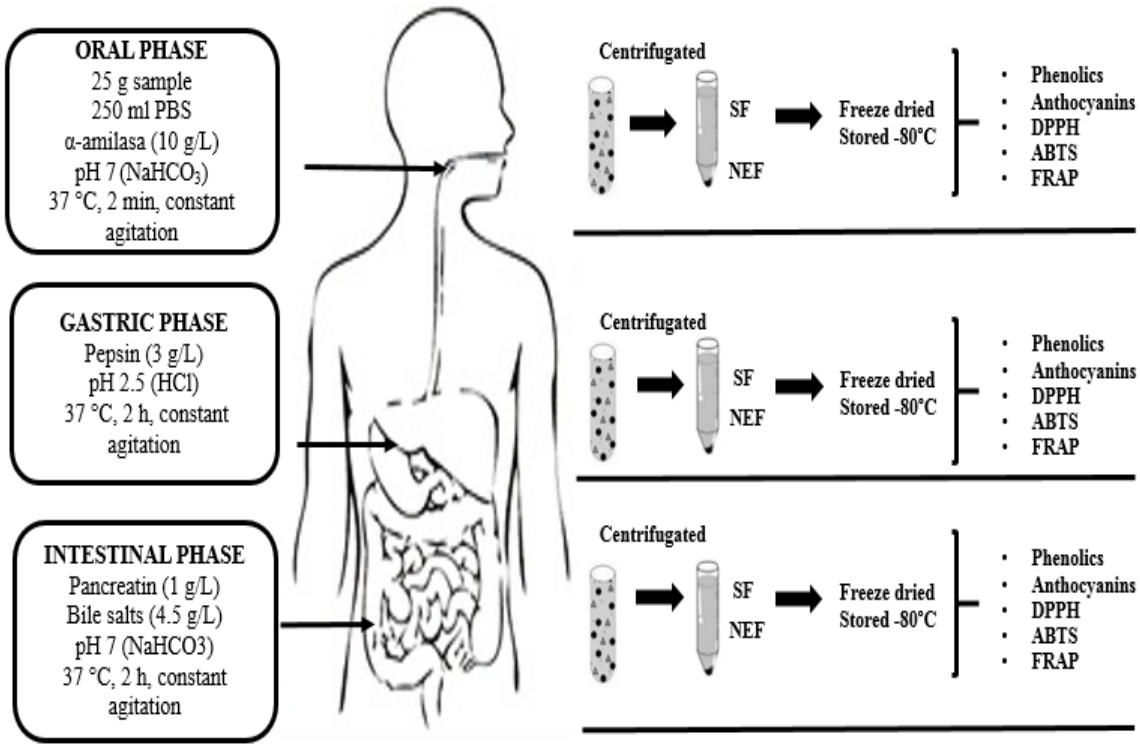

Figure 1. Graphic representation of the simulated gastrointestinal digestion procedure.

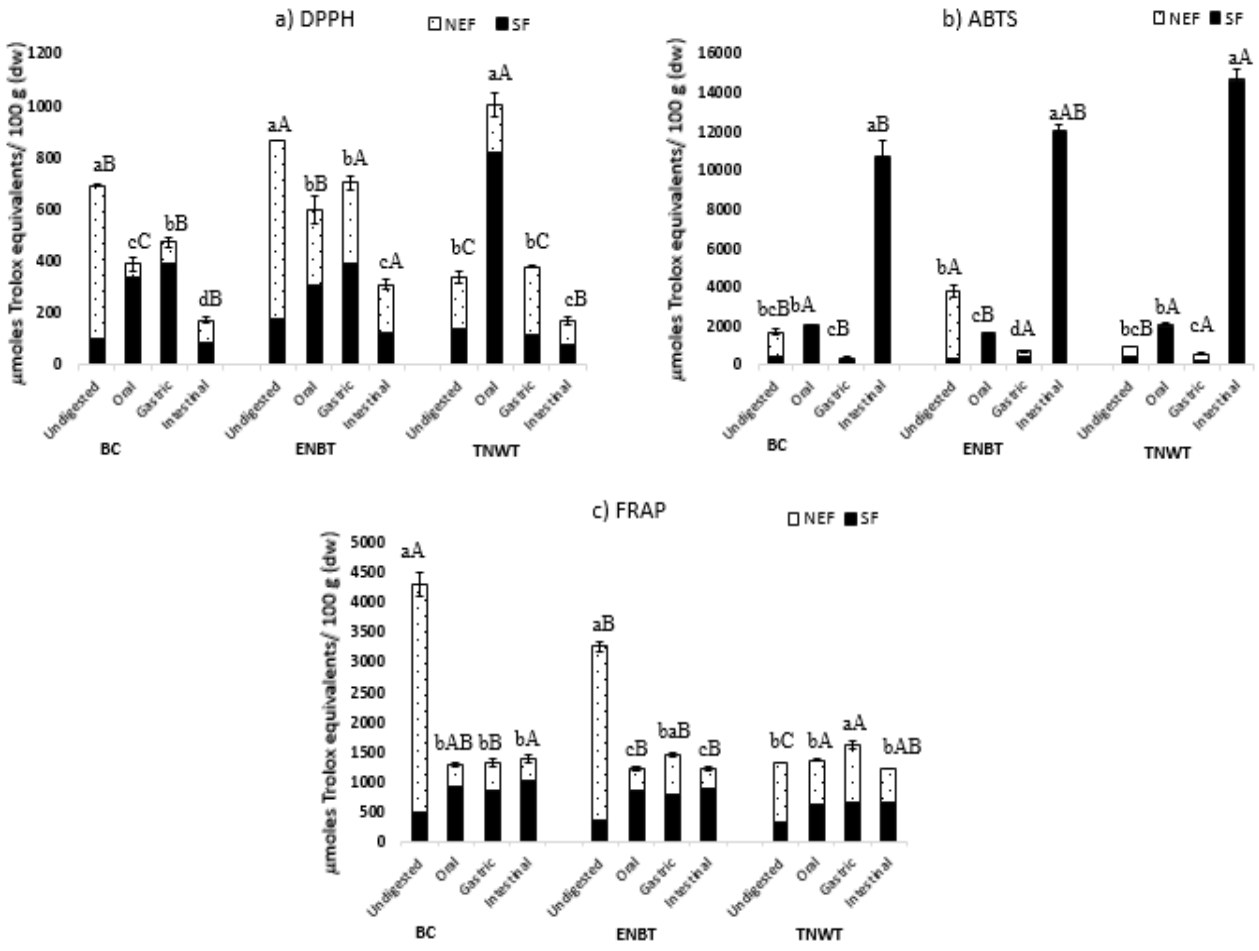

Figure 2. Antioxidant capacity of phenolic compounds (μmol ET/ 100 g dw) of initial (undigested sample), oral, gastric and intestinal digested samples of blue corn and tortillas measured by three different methods **a)** DPPH, **b)** ABTS and **c)** FRAP. Data are means ± SD (n = 3). Different lowercase letters denote significant difference on antioxidant capacity between different phases of *in vitro* digestion for the same type of product ($p < 0.05$). Different capital letters denote significant difference on antioxidant capacity between the type of product for the same phase of *in vitro* digestion ($p < 0.05$).

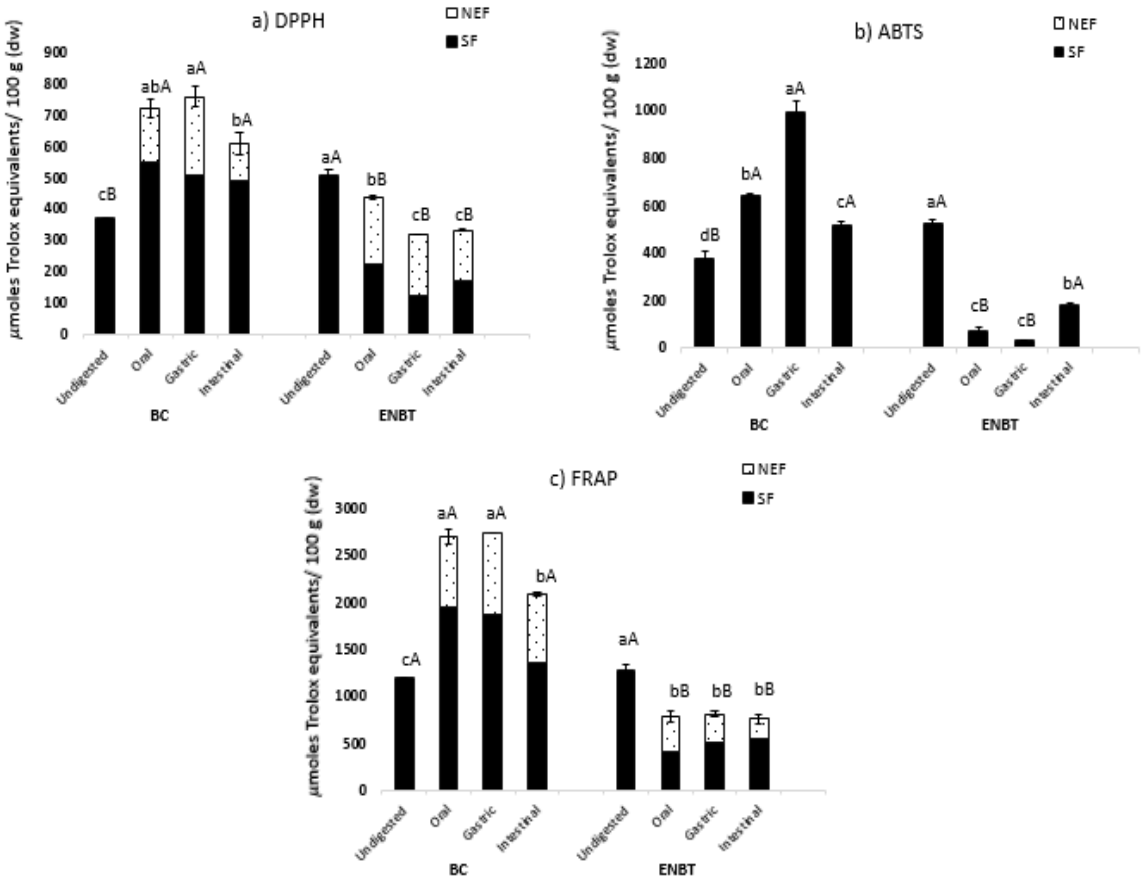

Figure 3. Antioxidant capacity of anthocyanins (μmol ET/ 100 g dw) of initial (undigested sample), oral, gastric and intestinal digested samples of blue corn and extruded nixtamalized blue tortilla measured by three different methods **a)** DPPH, **b)** ABTS and **c)** FRAP. Data are means ± SD (n = 3). Different lowercase letters denote significant difference on antioxidant capacity between different phases of *in vitro* digestion for the same type of product ($p < 0.05$) according to Tukey's Multiple Range Test. Different capital letters denote significant difference on antioxidant capacity between the type of product for the same phase of *in vitro* digestion ($p < 0.05$) according to Tukey's Multiple Range Test.

CONSIDERACIONES FINALES

El enfoque presente en esta investigación puede proporcionar una guía útil para desarrollar y optimizar productos innovadores a base de maíces pigmentados

Encontrar propiedades de textura óptimas basadas en el valor máximo de viscosidad de la harina de maíz podría ser un objetivo para las investigaciones futuras en las que se estudie el efecto de diferentes factores de procesamiento en la obtención de harina nixtamalizada por extrusión.

El uso de modelos cinéticos y parámetros termodinámicos puede ser empleado como herramientas para predecir la degradación de los compuestos biológicamente activos y establecer pautas apropiadas de procesamiento térmico para minimizar las pérdidas de antocianinas en productos a base de maíz pigmentado.

La información generada es de importancia para los consumidores, ya que los compuestos fenólicos y antocianinas liberados de las matrices de cereales son potencialmente bioaccesibles y pueden ejercer capacidad antioxidante en el tracto gastrointestinal con posibles efectos beneficiosos para la salud humana.

CONCLUSIONES

1. Fue posible realizar la optimización del proceso de nixtamalización por extrusión obteniendo una harina con pérdidas mínimas en compuestos biológicamente activos como las antocianinas (promotores de la salud) sin afectar negativamente la calidad de la tortilla obtenida evaluada como textura.

2. Los factores del proceso de nixtamalización del maíz por extrusión afectaron todos los parámetros evaluados en las harinas. La humedad de alimentación fue el principal factor que tuvo un gran efecto sobre la absorción de agua, el pico de viscosidad y el contenido de antocianinas, en su término lineal y cuadrático.

3. Las cualidades óptimas de la harina en términos de contenido máximo de antocianinas (226.07 mg/kg) y viscosidad máxima (1063.9 cP) se encontraron con una humedad de alimentación del 18.17%, una tempertura de la cuarta zona del extrusor de 92.03 °C y una velocidad de tornillo de 76.61 rpm.

4. Los resultados sugieren que el modelo de primer orden fue adecuado para predecir la cinética de degradación térmica de las antocianinas de los productos de maíz nixtamalizados extrudidos.

5. Los parámetros cinéticos se vieron afectados por la temperatura. Cuanto mayor la temperatura, mayor la velocidad de degradación (*k*), menor el tiempo de vida media ($t_{½}$) y menor tiempo de reducción decimal de todas las muestras (D). Por otro lado, cuanto mayor el coeficiente Q_{10} y la energía de activación (Ea), mayor la dependencia de la transformación de las antocianinas a la temperatura.

6. Las antocianinas en la harina y la tortilla requirieron menor energía para activar la reacción de degradación térmica que las del maíz crudo, lo que indica un proceso de degradación más rápido debido al impacto del procesamiento de extrusión y el horneado en estos compuestos.

7. El ΔH, ΔG y ΔS indicaron que la degradación de las antocianinas en los productos de maíz nixtamalizado extrudidos a cualquier temperatura fue una reacción endotérmica y no espontánea, requiriendo la aplicación de una fuente de calor externa para llevar a cabo la transición de estas moléculas.

8. La digestión gastrointestinal contribuyó fuertemente a la liberación de los compuestos fenólicos ligados de la matriz alimentaria del maíz azul y ambos tipos de tortilla. La concentración fenólica más alta se encontró en la fase intestinal para todas las muestras.

9. Si bien, las diferentes fases de la digestión afectaron el contenido de antocianinas debido a los cambios en el pH, la matriz alimentaria jugó un papel importante en la prevención de la pérdida total de estos compuestos. La mayor estabilidad se observó en la fase gástrica.

10. Los resultados sugieren que los compuestos fenólicos presentes en el maíz azul y las tortillas podrían ejercer efectos antioxidantes en el tracto gastrointestinal después de ser sometidos al proceso de digestión, ya que son potencialmente bioaccesibles y muestran capacidad de eliminación de radicales libres.

11. Las antocianinas de la tortilla de maíz azul adicionalmente mostraron capacidad antioxidante en las diferentes fases de la digestión, lo que representa un valor agregado como alimento funcional frente a la tortilla blanca tradicional.

RECOMENDACIONES

1. Continuar con el estudio del efecto de las variables del proceso de extrusión en otros tipos de maíces pigmentados para la obtención de harinas instantáneas con características adecuadas para el mercado de los consumidores.
2. Realizar estudios de digestión *in vivo* de las tortillas azules para la evaluación del impacto de las condiciones fisiológicas en la absorción y metabolismo de los compuestos fenólicos y evaluar su bioactividad.

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ANEXO 1

Asistencia a Congresos, Cursos, Talleres, Seminarios y Jornadas

Congresos Nacionales

- XXI Reunión Universitaria de Investigación en Materiales (RUIM). Trabajo titulado ¨Efecto del proceso de extrusión cocción alcalina en el perfil de antocianinas de tortillas elaboradas a partir de maíz azul. Hermosillo, Sonora, México, 30 de noviembre al 2 de diciembre del 2016.

- XXII Reunión Universitaria de Investigación en Materiales. Trabajo titulado ¨Efecto de la molienda en las propiedades fisicoquímicas y texturales de una botana expandida saludable de maíz azul. Hermosillo, Sonora, México, 22-24 de noviembre del 2017.

- XXIV Reunión universitaria de investigación en materiales. Trabajo titulado ¨Effect of extrusión conditions on anthocyanin content, functional, and pasting properties to obtain nixtamalized blue corn flour (*Zea mays* L.), and process optimization using response Surface methodology¨. Hermosillo, Sonora, México,6-8 de noviembre del 2019.

Congresos Internacionales

- 10 th International Workshop on Anthocyanins. Trabajo titulado ¨Thermal degradation kinetics of blue maize anthocyanins from tortillas produced using the extrusión nixtamalization process. San Michele all'Adige, Italia, 9-11 de septiembre del 2019.

- 8 vo Congreso Internacional de Nixtamalización. Trabajo titulado ¨Effect of extrusión conditions on anthocyanin content, functional, and pasting properties to obtain nixtamalized blue corn flour (*Zea mays* L.), and process optimization using response Surface methodology¨. Universidad Nacional Autónoma de México (UNAM), campus

Juriquilla, Querétaro, 21-23 de octubre del 2019. Segundo lugar obtenido en la modalidad de mejor trabajo presentado en póster.

- Congreso Internacional en Ciencias Alimentarias y Biotecnología. Trabajo titulado ¨Efecto del proceso de extrusión cocción alcalina en el perfil de antocianinas de tortillas elaboradas a partir de maíz azul. Hermosillo, Sonora, México,14-18 de noviembre del 2016.

Cursos, Talleres, Seminarios y Jornadas

- Eighth Annual Student Conference on Renewable Energy Science, Technology, and Policy at the Energy-Water-Food Nexus. Universidad de Arizona, Tucson, Estados Unidos, 11-15 de noviembre del 2019.

- Seminario sobre Estadística. Impartido por el Dr. Joaquín Sánchez Soriano. Universidad Miguel Hernández de Elche, Orihuela, España, 6 de mayo de 2019.

- Seminario sobre "Escritura y publicación de trabajos científicos". Impartido por el Dr. Ángel Carbonell Universidad Miguel Hernández de Elche, Orihuela, España 13 de marzo de 2019.

- Asistencia a la 1ª Jornada UMH-Aspanias, Interés sensorial de la alimentación en discapacidad intelectual y funcional. Elche, España 9 de febrero de 2019.

- Avances en el estudio de compuestos bioactivos: Aislamiento, Caracterización y Aplicaciones. Duración 20 horas. Hermosillo, Sonora, México, 25-27 de octubre del 2017.

- Curso antioxidantes en alimentos: Generalidades y mecanismos de inhibición a los radicales libres. Impartido por la Dra. Carmen Lizette Del Toro Sánchez. Duración 40 horas. Hermosillo, Sonora, México, 18 de abril al 30 de mayo del 2017.

- Curso alimentos funcionales. Principales beneficios en la salud. Impartido por el Dr. Gustavo González Aguilar. Duración 25 horas. Hermosillo, Sonora, México,14-18 de noviembre del 2016.

- Taller análisis y evaluación de riesgos en laboratorios de investigación. Impartido por la Dra. Clara Rosalía Álvarez Chávez. Duración 20 horas. Hermosillo, Sonora, México, 19-23 de septiembre del 2016.

ANEXO 2

Estancias de Investigación y Programas de Radio

- Estancia académica de 10 meses en la universidad Miguel Hernández (UMH), relacionada al convenio de doble doctorado internacional del programa de Doctorado en Recursos y Tecnologías Agrarias Agroambientales y Alimentarias, bajo la co-dirección de la Dra. María José Frutos Fernández. Realizada del día 1 de septiembre de 2018 al 30 de junio de 2019 en la ciudad de Orihuela, Alicante, España.

- Participación en entrevista de radio en la Universidad de Sonora (UNISON) en el programa Alimentación: problema de nuestro tiempo con el tema ¨Estancia doctoral Universidad Miguel Hernández¨. Dirigido por el Q.B. Franciso J. Parra Vergara y realizado el 30 de agosto del 2018 en la ciudad de Hermosillo, Sonora, México.

- Participación en entrevista de radio en la Universidad Miguel Hernández en el programa ¨Retos en las ondas¨ con el tema ¨Obtención de un doble doctorado¨. Dirigido por el Dr. José Ángel Pérez y realizado el 2 de octubre del 2019.