UNIVERSIDAD MIGUEL HERNÁNDEZ DE ELCHE

PROGRAMA DE DOCTORADO EN RECURSOS Y TECNOLOGÍAS AGRARIAS, AGROAMBIENTALES Y ALIMENTARIAS

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 DOCTORAL
Presentada por:
Mariela Menchaca Armenta
Director:
María José Frutos Fernández
Codirector:
Benjamín Ramírez Wong

Orihuela, Sonora **2022**

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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Dr. Dña. Juana Fernández López, Catedrática de Universidad y Coordinadora del Programa de Doctorado en Recursos y Tecnologías Agrarias, Agroambientales y Alimentarias (ReTos-AAA) de la Universidad Miguel Hernández de Elche (UMH),

CERTIFICA:

Que la Tesis Doctoral titulada "**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** " de la que es autora la Licenciada en Ingeniería Bioquímica y Master en Ciencia y Tecnología de Alimentos **Dña. Mariela Menchaca Armenta**, ha sido realizada bajo la dirección de la **Dra. María José Frutos Fernández (UMH)** y la codirección del **Dr. Benjamín Ramírez Wong (UNISON)**, actuando como tutor/a de la misma el **Dr.Ángel Antonio Carbonell Barrachina (UMH)**. Considero que la Tesis es conforme, en cuanto a forma y contenido, a los requerimientos del Programa de Doctorado ReTos-AAA, siendo por tanto apta para su exposición y defensa pública.

Y para que conste a los efectos oportunos firmo el presente certificado en Orihuela a 14 de junio de 2022.

Dra. Dña. Juana Fernández López

Coordinadora del Programa Doctorado ReTos-AAA

Dña. María José Frutos Fernández, Dra. en Ciencias Biológicas, Catedrática de Universidad de la Universidad Miguel Hernández de Elche.

D. Benjamín Ramírez Wong, Dr. en Ingeniería de Alimentos, Profesor-Investigador Titular C de Universidad de Sonora.

CERTIFICAN:

Que la Tesis Doctoral Titulada "**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**" de la que es autora la Licenciada en Ingeniería Bioquímica y Master en Ciencia y Tecnología de Alimentos **Dña. Mariela Menchaca Armenta**, ha sido realizada bajo nuestra dirección y autorizamos a que sea presentada para optar a la obtención del grado de Doctor por la Universidad Miguel Hernández.

Y para que conste a los efectos oportunos firmamos el presente certificado en Orihuela a 14 de junio de 2022.

Fdo.: Dra. María José Frutos Fernández **Fdo.: Dr. Benjamín Ramírez Wong**

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DEDICATORIA

A Dios y a mi familia

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

CALIDAD DEL COMPENDIO DE CADA PUBLICACIÓN

Esta Tesis se presenta como compendio de las siguientes publicaciones:

Mariela Menchaca-Armenta, Benjamín Ramírez-Wong, Patricia I. Torres-Chávez, Armando Quintero-Ramos, Ana I. Ledesma-Osuna, María J. Frutos, Roberto Gutiérrez-Dorado, Olga N. Campas-Baypoli, and Ignacio Morales-Rosas. (2020). Effect of extrusion conditions on the anthocyanin content, functionality, and pasting properties of obtained nixtamalized blue corn flour (*Zea mays* L.) and process optimization. Journal of Food Science, 85 (7): 2143-2152. https://doi.org/10.1111/1750-3841.15312

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ESTRUCTURA DE LA TESIS

El contenido de la presente Tesis Doctoral ha sido preparado de acuerdo a la normativa vigente de la Universidad Miguel Hernández de Elche para su presentación como compendio de artículos.

La Tesis Doctoral se estructura en los siguientes apartados:

- **Abstract:** donde se presente un breve resumen de los antecedentes, metodología, objetivos y principales resultados y conclusiones de la investigación realizada.
- **Introducción**: contiene información sobre la composición del maíz azul, los procesos de elaboración de la tortilla de maíz (extrusión y nixtamalización), parámetros cinéticos y termodinámicos de la degradación térmica de las antocianinas, así como el efecto del proceso de extrusión en la bioaccesibilidad y propiedad antioxidante de los principales compuestos bioactivos de la tortilla.
- **Objetivos:** el objetivo general y específicos se detallan en este apartado.
- **Publicaciones Científicas**: donde tres artículos científicos se presentan, dos artículos publicados en revistas indizadas, y uno que se encuentra bajo revisión.
    - Mariela Menchaca-Armenta, Benjamín Ramírez-Wong, Patricia I. Torres-Chávez, Armando Quintero-Ramos, Ana I. Ledesma-Osuna, María J. Frutos, Roberto Gutiérrez-Dorado, Olga N. Campas-Baypoli, and Ignacio Morales-Rosas. (2020). Effect of extrusion conditions on the anthocyanin content, functionality, and pasting properties of obtained nixtamalized blue corn flour (*Zea mays* L.) and process optimization. Journal of Food Science, 85 (7): 2143-2152. https://doi.org/10.1111/1750-3841.15312
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- **Resumen de Resultados y Discusión:** donde se presenta un resumen global de los resultados más relevantes obtenidos.
- **Conclusiones Generales:** en esta sección se enlistan las conclusiones generales mas importantes de todo el trabajo realizado.
- **Referencias Bibliográficas**: en este apartado se enlistan las referencias consultadas en las secciones complementarias (Introducción, Resultados y Discusión), sin incluir las referencias utilizadas en las publicaciones científicas.

Nota: Este trabajo no incluye la sección de ¨Materiaes y Métodos¨ debido a que estos se describen en las publicaciones correspondientes.

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LISTADO DE ABREVIATURAS Y SIMBOLOS

HA: Humedad de alimentación

TE: Temperatura de la cuarta zona del extrusor

VT: Velocidad del tornillo

ANDEVA: Análisis de varianza

pH: Potencial de hidrógeno

FAO: Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO por sus siglas en inglés)

INEGI: Instituto Nacional de Estadística y Geografía

UNESCO: Organización de las Naciones Unidas para la Educación, la Ciencia y la Cultura

CONEVAL: consejo Nacional de Evaluacion de la Politica de Desarrollo Social

UV: radiación ultravioleta

SOD: Superóxido dismutasa

CAT: Catalasa

NADH: Nicotinamida adenina dinucleótido

$Ca(OH)_2$: hidróxido de calcio

MRS: Metodología de superficie de respuesta

cP:centipoise

ENCF, Extruded nixtamalized corn flour

FM: feed moisture

T: temperatura

SS: screw speed

Pv: peak viscosity

TA: total anthocyanins

ENP: 20ethanol20 nixtamalization process

TNP: traditional nixtamalization process

AOAC: Association of Official Analytical Chemists

GC: ground corn

BC: Blue corn

SWAC: Subjective 20etha absorption capacity

Tr: standard treatment

CCRD: central composite rotatable design

RSM: Response Surface methodoly

ANOVA: 21ethanol of variance

CV: coefficient of variation

R2: coefficient of determination

ECG: Equivalents of cyanidine-3-glucoside

MW: molecular weight

ε: molar extinction coefficient

DF: dilution factor

C: anthocyanin content at any time

C0: 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 21ethanol2121e (K)

A: frequency factor

Q_{10}: 21ethanol2121e coefficient

z-value: 21ethanol2121e 21ethanol that causes a 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)

kd:anthocyanin 21et rate (s -1)

kB: Boltzmann´s constant (1.3806 x 10 -23 J K -1)

h: Planck´s constant (6.6262 x 10 -34 J s)

ENBT: Extruded nixtamalized blue tortilla

TNWT: Traditional nixtamalized 21etha tortillas

PBS: Phosphate buffer solution

SF: Soluble fraction

NEF: Non-extractable fraction

GAE: Gallic acid equivalents

M: Weight of the sample

dw: Dry weight

TE: Trolox equivalents

FRAP: Ferric reducing antioxidant power

DPPH: 2,2-diphenyl-1-picrylhydrazyl radical

ABTS: 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

TPTZ: 2,4,6-tri(2-pyridyl)1,3,5-triazine)

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RESUMEN

Las propiedades nutracéuticas del maíz azul derivan de sus 24ethanol2424e secundarios. La tortilla de maíz es el alimento básico de la población 24ethanol y algunos países de Centro América. El proceso de 24ethanol24 actualmente se emplea como una alternativa al proceso de nixtamalización tradicional para elaborar tortillas. El objetivo de esta investigación fue 24ethanol24 las condiciones del proceso de nixtamalización por 24ethanol24 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 24ethano de 24ethanol24 gastrointestinal simulado. La investigación se dividió en tres etapas. Durante la primera etapa, se evaluó el efecto de los factores del proceso de 24ethanol24 sobre diferentes características de las harinas nixtamalizadas para encontrar el área óptima de procesamiento.Se 24ethano 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%), 24ethanol2424e 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 24ethano la metodología de superficie de respuesta para evaluar los datos experimentales y se 24ethanol 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 24ethanol que la HA 24ethan factor que tuvo un gran efecto sobre las propiedades evaluadas en las harinas. El área óptima se 24ethanol24 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 24ethano al maíz crudo. En la segunda etapa se 24ethanol24 el efecto del proceso de 24ethanol24 de nixtamalización y elaboración de tortillas sobre la estabilidad de las antocianinas del maíz azul a través de los parámetros cinéticos y termodinámicos. Las antocianinas se extrajeron con 24ethanol acidificado, se concentraron y se resuspendieron en una solución 24ethan a pH (2.5) y fueron tratadas térmicamente a 3 temperaturas diferentes (60, 75 o 90 °C) durante 2 h. El cambio de concentración en el tiempo fue medido espectofotométricmente, y

con estos datos se estimaron los parámetros cinéticos y termodinámicos. Se 25ethano un diseño bifactorial completamente al azar. Los datos se analizaron 25ethanol2525e análisis de varianza (ANDEVA), con un nivel de significancia <0.05 %. Los resultados de la segunda etapa indicaron que la degradación de las antocianinas en productos nixtamalizados extruidos a base de maíz azul siguió un modelo de primer orden con parámetros termodinámicos estimados que muestran que el proceso fue endotérmico y no espontáneo. La cinética de degradación de las antocianinas de la harina de maíz extruida y de la tortilla fue similar, aunque la elaboración de la tortilla involucró temperaturas mucho más altas (300 °C) que las aplicadas en el proceso de 25ethanol25. En la tercera etapa se evaluaron los cambios en el contenido de los fitoquímicos, el índice de bioaccesibilidad y capacidad antioxidante durante la 25ethanol25 gastrointestinal simulada in vitro del maíz azul, la tortilla azul nixtamalizada extrudida y una tortilla blanca tradicional. Las condiciones fisiológicas de la boca, estómago e 25ethanol25 25ethano fueron simuladas. La estabilidad, bioaccesibilidad y capacidad antioxidante de las antocianinas y compuestos fenólicos antes y después de la 25ethanol25 fueron evaluados en la fracción soluble e insoluble de los extractos recuperados. Se 25ethano un diseño bifactorial completamente al azar. Los datos se analizaron 25ethanol2525e ANDEVA, con un nivel de significancia <0.05. Los resultados de la tercera etapa indicaron que la 25ethanol25 contribuyó a incrementar la bioaccesibilidad de los compuestos fenólicos, especialmente en la fase intestinal, lugar donde la mayoría de los 25ethanol25 son absorbidos, y que a pesar de la evidencia de reducciones en el contenido de las antocianinas debido a los cambios de pH, las tortillas exhibieron una capacidad antioxidante efectiva en el tracto gastrointestinal simulado con posibles efectos beneficiosos para la salud de los seres humanos. Se concluye que el enfoque de este 25ethan puede proporcionar una guía útil para desarrollar y 25ethanol25 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, The effect of the nixtamalization extrusion process and the elaboration of tortillas on the stability of anthocyanins in blue corn was determined through kinetic and thermodynamic parameters. The anthocyanins were extracted with acidified methanol, concentrated and 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 over time was measured spectrophotometrically, and with these data the kinetic and thermodynamic parameters were estimated. A completely randomized bifactorial design was used. The data were analyzed using the analysis of variance (ANDEVA), with a level of significance

<0.05%.Results of the second stage showed that the degradation of anthocyanins in blue corn-based extruded nixtamalized products followed a first-order model with the determined thermodynamic parameters showing that the process was endothermic and non-spontaneous. The anthocyanin degradation kinetics of extruded corn flour and of prepared tortilla were similar, although tortilla making involved much higher temperatures (300 °C) than those applied in the extrusion process, probably in relation to the matrix protective effects, which caused a slower heat flow to the center of the tortilla due to some anatomical parts of the kernel. In the third stage, the changes in the content of phytochemicals, the bioaccessibility index and antioxidant capacity were evaluated during the *in vitro* simulated gastrointestinal digestion of blue corn, the extruded nixtamalized blue tortilla and a traditional white tortilla. The physiological conditions of the mouth, stomach and small intestine were simulated. The stability, bioaccessibility and antioxidant capacity of anthocyanins and phenolic compounds before and after digestion were evaluated in the soluble and insoluble fraction of the recovered extracts. A completely randomized two-factor design was used. The data were analyzed using ANOVA, with a level of significance <0.05. The results of the third stage indicated that digestion contributed to increase the bioaccessibility of phenolic compounds, especially in the intestinal phase, where most of the nutrients are absorbed, and that despite the evidence of reductions in the content of the anthocyanins due to pH changes, tortillas exhibited effective antioxidant capacity in simulated gastrointestinal tract with potential beneficial effects on human health. 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.

CAPÍTULO 1. INTRODUCCIÓN

1.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 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). Una buena correlación entre los resultados obtenidos usando sistemas *in vitro* e *in vivo* se ha reportado (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 muchos 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 compuestos bioactivos en el producto final (Mora-Rochin et al., 2010). Debido a estas desventajas tecnológicas, se han utilizado procesos alternativos para la producción de harinas y tortillas, como el proceso de extrusión por nixtamalización. Esta tecnología permite el procesamiento de materiales a alta temperatura a corto plazo, 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, el estudio de los factores de procesamiento, así como las condiciones de la digestión es de importancia.

Los resultados obtenidos en esta investigación podían 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 actividad antioxidante de la tortilla durante la digestión gastrointestinal a través de modelos *in vitro*.

1.2. 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).

1.2 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).

1.3 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 32ethanol3232e32cas representa una gran oportunidad para el desarrollo de nuevos productos, con nuevas o mejores características funcionales y nutricionales (Bello-Perez *et al*., 2016).

1.3.1 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,

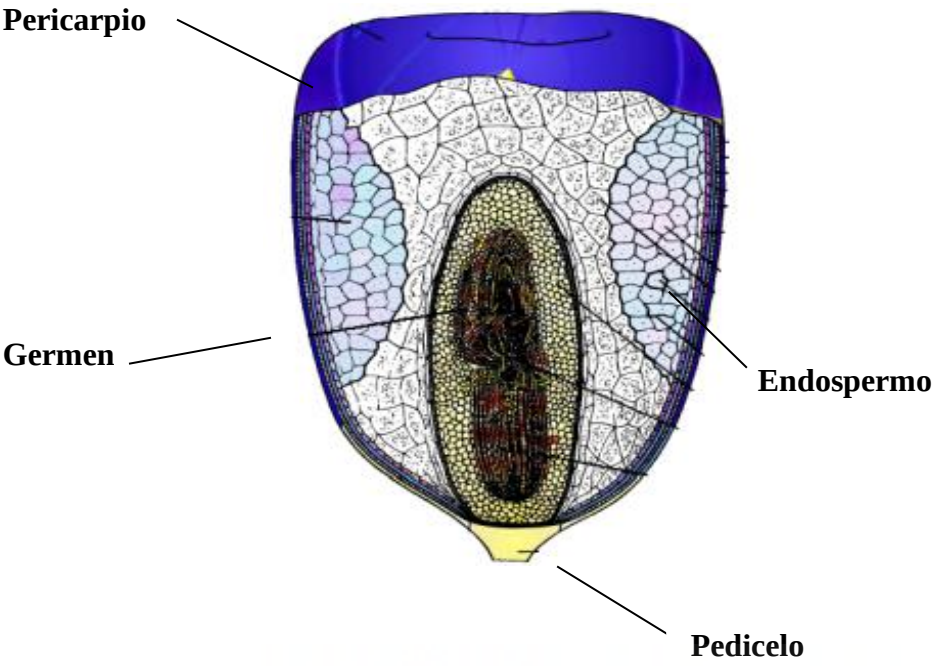


Figura 1. Morfología del grano de maíz azul.

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.

1.3.2 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)

1.3.2.1 Antocianinas

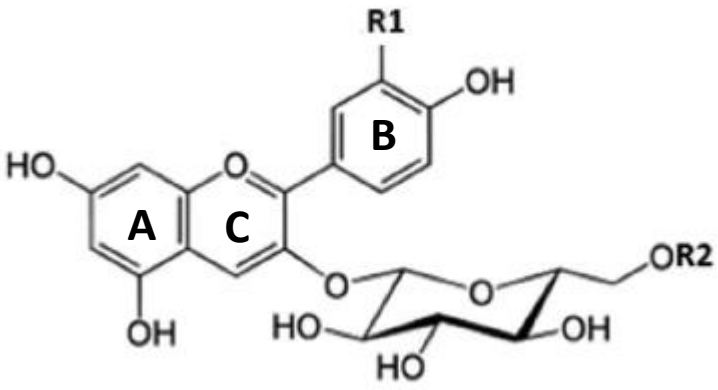
Las antocianinas son pigmentos polares que se encuentran en las vacuolas de los tejidos vegetales responsables de otorgar la coloración azul al maíz. La estructura general de las antocianinas consiste en dos anillos aromáticos (A y B), unidos por un anillo heterocíclico de tres carbonos fue que contiene 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).

1.3.2.2. 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).

1.4 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



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)

propiedades 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.

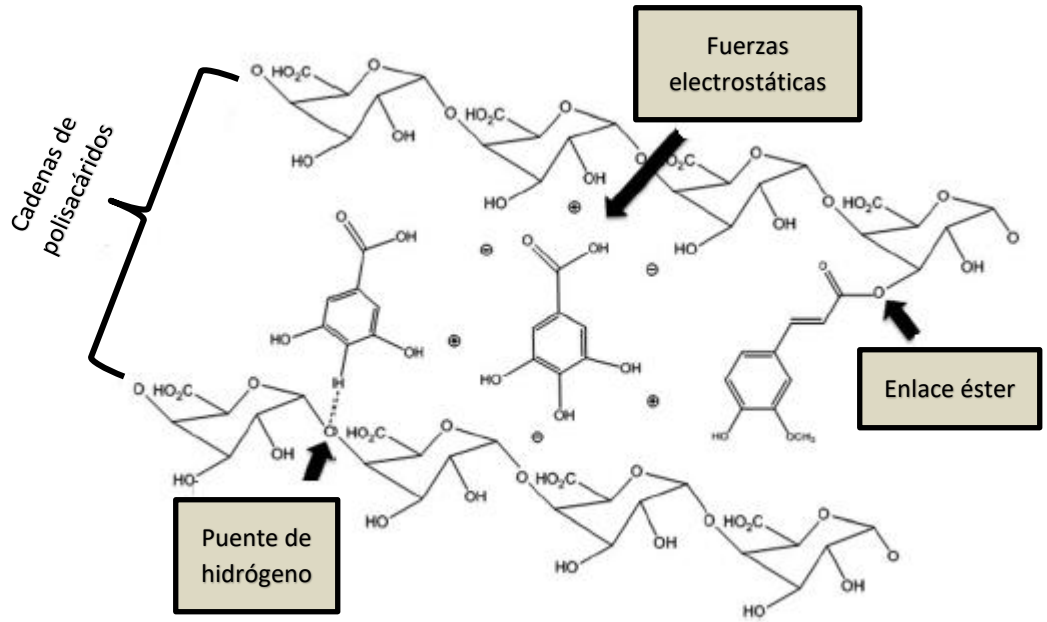


Figura 3. Tipos de interacciones entre los compuestos fenólicos y polisacáridos de la matriz celular.

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.

1.5 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).

1.5.1 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).

1.6 Factores que Intervienen en la Estabilidad de las Antocianinas

1.6.1 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).

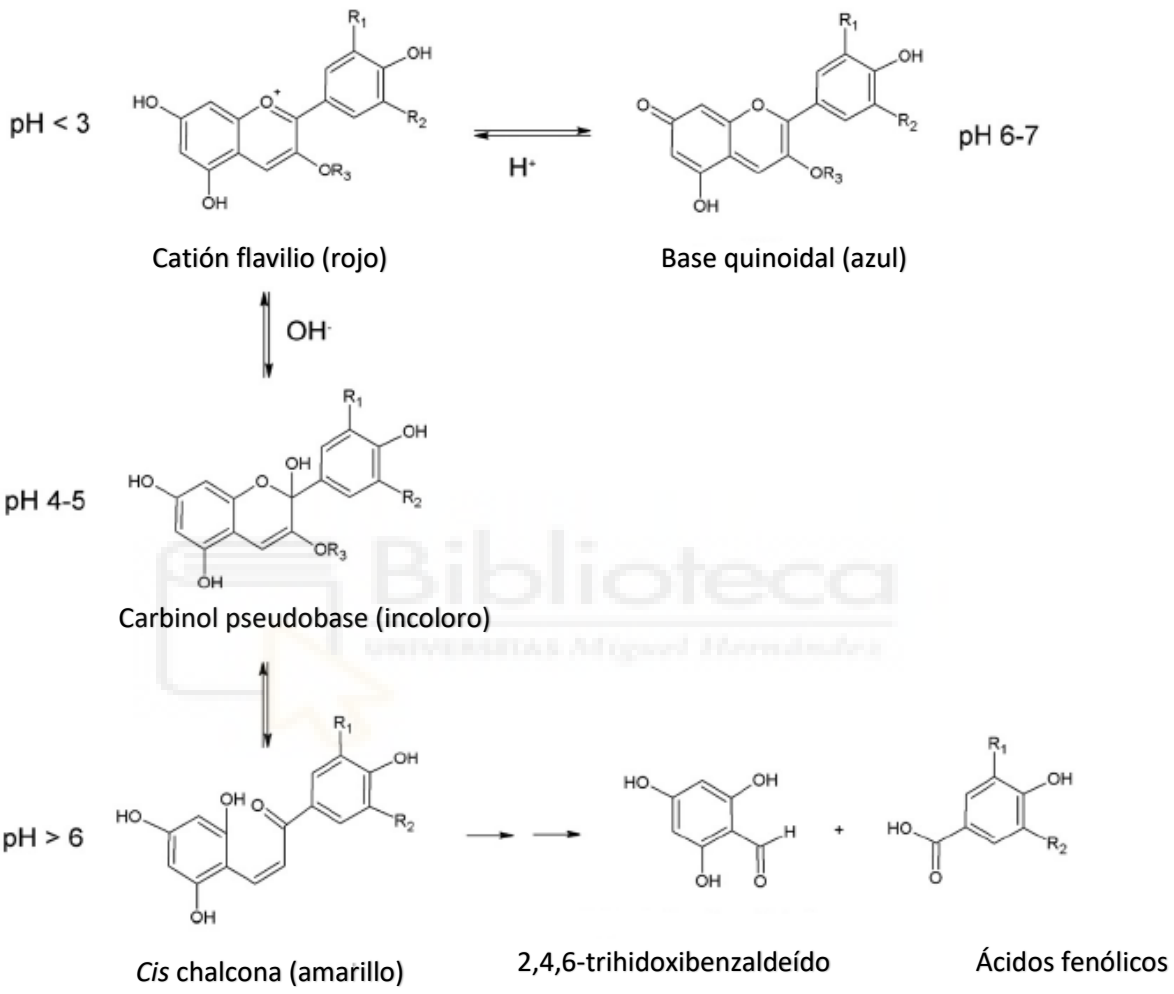


Figura 4. Formas químicas de las antocianinas dependientes del pH y reacciones de degradación.

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).

1.6.2 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).

1.7 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.

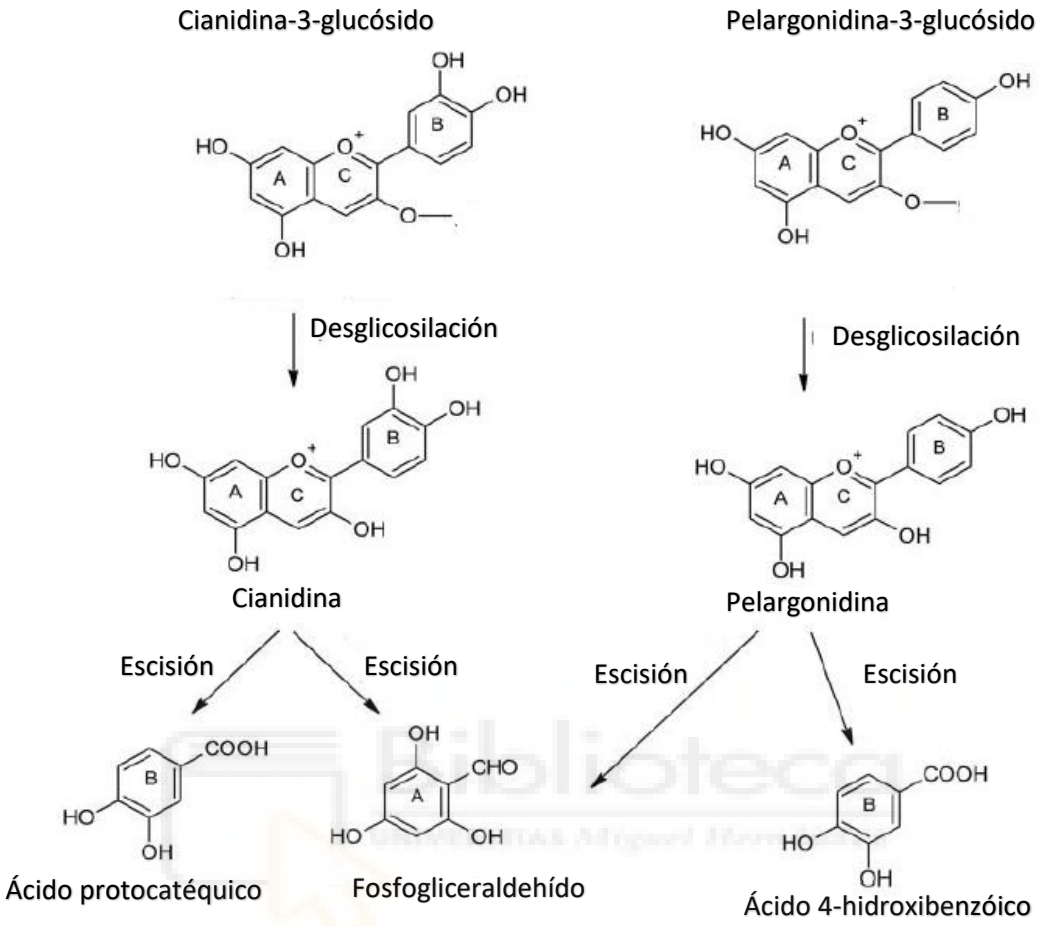


Figura 5. Posible degradación térmica de dos antocianinas.

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

cinéticos son necesarios para minimizar el cambio no deseado y optimizar la calidad de alimentos específicos.

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).

1.8 Procesos de Nixtamalización

1.8.1 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).

1.8.2 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).

1.9 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 ma49ethanol4949eble 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

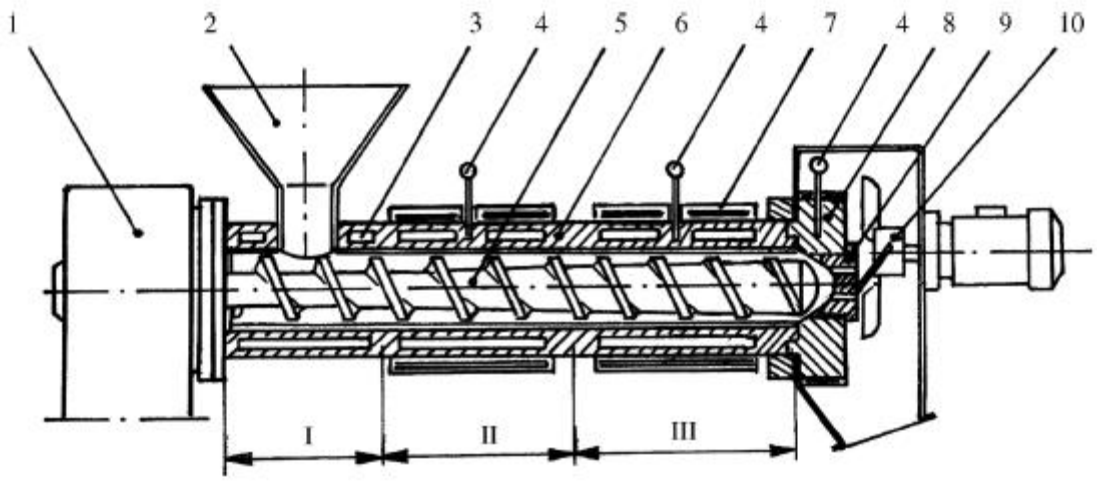


Figura 6. Sección transversal de un extrusor de tornillo simple. 1-motor, 2 – tolva de alimentación, 3-chaqueta de enfriamiento, 4-termopar, 5-tornillo, 6-barril, 7-chaqueta de calentamiento, 8-cabezal, 9-dado, 10-cortador, I -sección de transporte, –I - sección de compresión, III sección de fusión y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

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

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 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.

1.10 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).

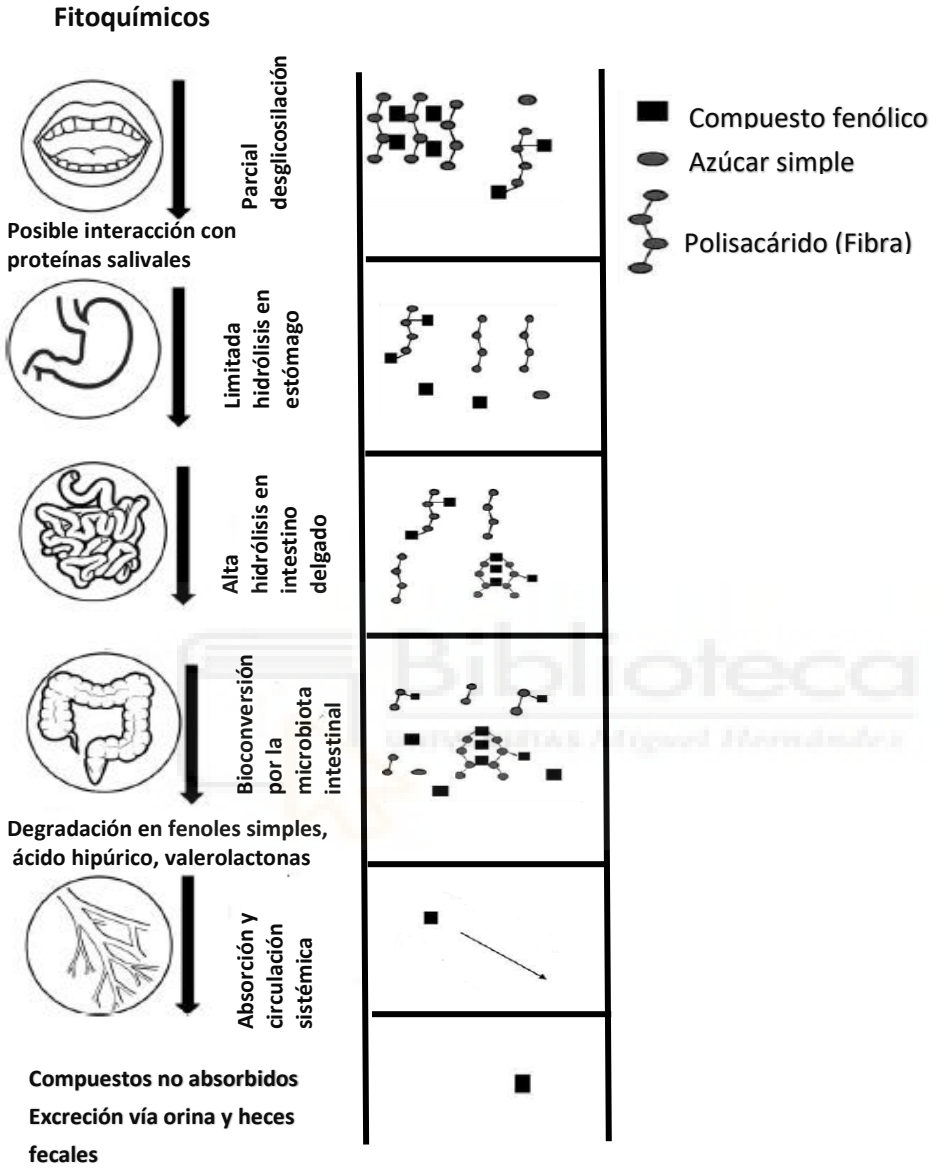


Figura 7. Bioaccesibilidad gastrointestinal general de los compuestos fenólicos.

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).

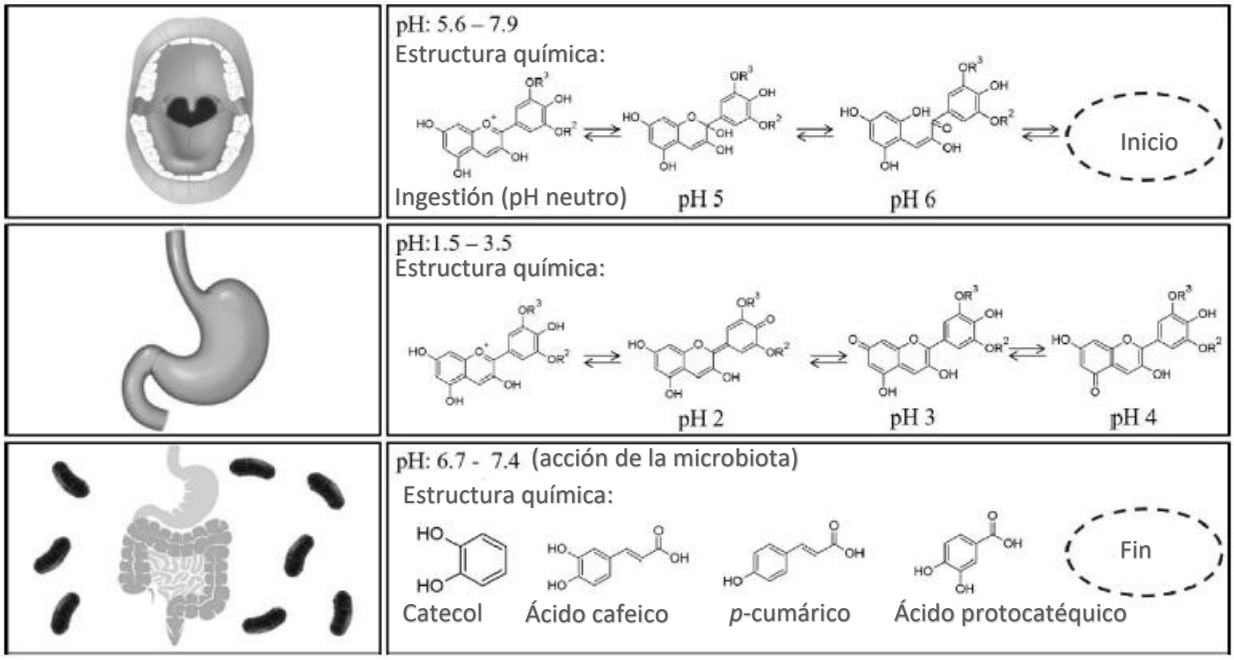


Figura 8. Estructuras químicas de las antocianinas influenciadas por las fases del proceso de digestión, pH y bioconversión en compuestos fenólicos Donde R_2=H y R_3 = Metilo.

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

CAPÍTULO 2. OBJETIVOS

2.1 Objetivos

2.1 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.

2.2 Objetivos Particulares

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

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 viscosida

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 optimizad

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 vitr*

CAPÍTULO 3. PUBLICACIONES

3.1 PUBLICACIÓN 1

Título del artículo: Effect 61ethanol61ion 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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Agricultural and Biological Sciences Food Science	Q1	57/144	3.167

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, 15-30%), extruder temperature (T, 70-110 °C) and screw speed (SS, 50-145 rpm). The properties evaluated in the flours were total anthocyanins (TA), subjective water absorption capacity (SWAC) and peak viscosity (PV). Response surface methodology and ANOVA 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 extruded nixtamalized corn flour were total anthocyanins (maximize) and peak viscosity (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**:* Tortilla texture, starch, viscosity, bioactive compounds, pigmented corn

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 minimun losses in biologically active compounds such as anthocyanins (healh promoters) wihout 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 h). 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 =11-13), 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, 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 blue corn derive from secondary metabolites (anthocyanins and phenolic acids); these chemical

compounds contribute to the antioxidant activity of blue corn, 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 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/d and by women is 18.4 to 44.1 mg/d (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, temperature, and screw speed. 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 feed moisture promotes polymerization, and the screw speed produces decarboxylation (Ruíz-Gutiérrez, Sánchez-Madrigal, & Quintero-Ramos, 2018). The ENP retains more anthocyanins (47-59%) than the TNP (0-39%) 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 (17.7-38.5%) of these compounds in nixtamalized blue corn 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 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, 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 extruded nixtamalized corn

flour, determine the optimal conditions for obtaining a flour with a maximum anthocyanin content and maximum viscosity using response surface methodology, and to obtain a tortilla from the optimized flour with quality characteristics acceptable to consumers.

2. MATERIALS AND METHODS

2.1. Raw material

Blue corn (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 (Model V230, Clipper, USA), to eliminate impurities and stored at -20 °C until use. The chemical composition of blue corn 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 extrusion nixtamalization process.

2.2. Corn grinding

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

2.3. Extrusion nixtamalization process (ENP)

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 15-30% (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 h at 4 °C in a commercial refrigerator (Model 2155060-A, Whirlpool, Benton Harbor, Michigan, USA).

2.3.2. Extrusion process

Each batch of conditioned ground corn 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 70-110 °C (according to the experimental design, Table 1), and the screw speed ranged from 50 to 145 rpm (according to the experimental design, Table 1). Blue corn extrudates were obtained.

2.4. Obtaining extruded nixtamalized corn flours (ENCFs)

2.4.1. Drying

1.2.1. The extrudates obtained were divided into two batches. The first batch was dried in a tunnel dryer (no brand) at 50 °C for 1 h, and the other batch was freeze-dried (Model 7753020, Labconco, Kansas City, Missouri, USA) in a vacuum at -52 °C for 24 h prior to carrying out the chemical analyse*2. Grinding*

Both batches of dried extrudates were ground to obtain extruded nixtamalized corn flour (Model 8, Christy Turner Ltd., England, United Kingdom) with a 2 mm mesh size. Extruded

nixtamalized corn flours (ENCFs) were obtained and stored at 5 °C in polyethylene bags protected from light until further analysis.

2.5. Extruded nixtamalized corn flour evaluations

2.5.1. Subjective water absorption capacity (SWAC)

The methodology described by Flores-Farías., Martínez-Bustos, Salinas-Moreno, & Ríos (2002) was used. One-hundred g of ENCF were weighed and water was added to make a manual kneading until masa showed good consistency to make tortillas. 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, California, 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 minute 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 peak viscosity 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 extruded nixtamalized corn flour 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, Ohio, 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, Kansas City, Missouri, USA) 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 total anthocyanin content of 180 ± 15.4 mg/kg.

2.7. Tortilla firmness and rollability

Tortilla firmness and rollability were measured at 2, 24 and 48 h 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 feed moisture (FM: 15-30%), temperature at the fourth zone of the extruder (T: 70-110 °C) and screw speed (SS: 50-145 rpm). The levels of each variable were established based on values obtained in earlier experiments. Three responses were measured in the ENCFs: subjective water absorption capacity (SWAC), total anthocyanins (TA) and peak viscosity (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. Response

surface methodology (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 extruded nixtamalized corn flour were total anthocyanins (maximize) and peak viscosity (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 peak viscosity 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 extruded nixtamalized corn flours (ENCFs) and the average experimental values of the three responses obtained for each combination. Regression analysis and analysis of variance (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 (feed moisture, temperature and screw speed).

The predictive models in terms of coded factors were:

$SWAC = 122.93 - 12.30(FM) - 24.55(FM)^2 - 14.15(T)(SS)$ (p-value of model <0.0001; adjusted $R^2 = 0.8205$)

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

$YPV = 931.35 - 269.58(FM) - 78.27(FM)^2 - 63.00(T)^2 - 63.04(SS)^2$ (p-value of model <0.0001; 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 coefficients of variation 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 feed moisture (FM), temperature (T) and screw speed (SS) on the functional properties

The functional property of the extruded nixtamalized corn flour was measured as the subjective water absorption capacity (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 temperature and screw speed (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 temperature and screw speed 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 screw speed 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 (temperature, feed moisture and screw speed) that increase the water absorption capacity of the flour. Thermal energy contributes

mainly to starch gelatinization, and feed moisture acts as a plasticizer or lubricant, while screw speed 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, 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. On the other hand, increasing the screw speed 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 feed moisture 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 feed moisture (FM), temperature (T) and screw speed (SS) on the total anthocyanins (TA)

The concentration of total anthocyanins has been related to color and antioxidant activity in the final product. Statistical analysis revealed that linear effects of feed moisture ($p < 0.0001$), temperature ($p < 0.05$) and screw speed ($p < 0.01$), 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 temperature and screw speed. 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 feed moisture (15-22.5%) and screw speed, 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 feed moisture and intermediate-high temperatures.

The maximum TA of the extruded nixtamalized corn flour 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 blue corn) 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 78ethanolcule, 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, cyanidin-3-glucoside and peonidin-3-glucoside. Total anthocyanins increase at low-intermediate feed moisture 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 feed moisture 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 screw speeds 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 feed moisture (FM), temperature (T) and screw speed (SS) on the pasting property

The pasting property measured as peak viscosity (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 feed moisture 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 feed moisture at intermediate screw speeds (73-121 rpm) caused an increase in PV level (Figure 3b). In Figure 3c, the interaction of

T*FM shows that at low-intermediate feed moisture levels (15-22.5%) and an intermediate temperature (80-100 °C), a noticeable increase in the PV was observed.

The present findings revealed that both temperature and screw speed were equally important parameters responsible for viscosity development. Extruded nixtamalized corn flour 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 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 h to measure changes in texture properties (flexibility and firmness) (Figure 4). Figure 4a shows that the tortilla firmness after 2 h elaboration was smooth and soft, requiring minimum force for breakage (21.25 ± 1.94 kpa/mm). At 24 h of storage, tortilla firmness increased (46.45 ± 2.54 kpa/mm). After 24 h, more force to break the tortilla was required. At 48 h, the tortilla texture was 16% more firm than when stored for 24 h (53.97 ± 4.74 kpa/mm). The results observed in this study agree with previous investigations (Platt-Lucero et al. 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 2-24 h 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 rollability score decreased. The dowel flexibility scores of the tortillas during storage are shown in Figure 4b. As expected, in their fresh state (2 h), the tortillas were easily rolled without cracking, which indicates high flexibility (a value of 5). After 24 h, 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 h, 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 extruded nixtamalized corn flour. 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 extruded nixtamalized corn flours 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 total anthocyanins 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 total anthocyanin value in nixtamalized blue corn 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. CONCLUSION

Response surface methodology (RSM) was successfully applied to assess and model effects of three factors (feed moisture, temperature and screw speed) on the functional and pasting properties and bioactive compounds of an extruded nixtamalized corn flour (ENCF). The nixtamalization extrusion process factors (FM, T and SS) affected all parameters evaluated in the ENCF. Feed moisture was the main factor that had a great effect on the subjective water absorption capacity (SWAC), peak viscosity (PV) and total anthocyanins (TA), in their linear and quadratic term. The optimum extruded nixtamalized corn flour qualities in terms of maximum anthocyanin content (226.07 mg/kg) and peak viscosity (1063.9 cP) were found at feed moisture of 18.17%, a temperature of 92.03 °C and a screw speed of 76.61 rpm. Peak

viscosity 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 peak viscosity 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.

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AUTHOR 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.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abdel-Aal, E.S.M., & Huel, P. (1999). A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheats. *Cereal Chemistry*, 76(3), 350-354. https://doi.org/10.1094/cchem.1999.76.3.350

Aguayo-Rojas, J., Mora-Rochín, S., Cuevas-Rodríguez, E.O., Serna-Saldívar, S.O., Gutiérrez-Uribe, J. A., Reyes-Moreno, C., & Milán-Carrillo, J. (2012). Phytochemicals and antioxidant capacity of tortillas obtained after lime-cooking extrusion process of whole pigmented Mexican maize. *Plant Foods for Human Nutrition*, 67(2), 178-185. https://doi.org/10.1007/s11130-012-0288-y.

Alam, M.S., Kaur, J., Khaira H., & Gupta, K. (2015). Extrusion and extruded products: changes in quality attributes as affected by extrusion process parameters: a review. *Critical Reviews in Food Science and Nutrition*, 56(3), 445-473. https://doi.org/10.1080/10408398.2013.779568.

Almeida, H., & Rooney, L.W. (1996). Avances en la manufactura y calidad de productos de maíz nixtamalizado. Seminario de la Asociación Americana de Soya. México DF Industria Alimentaria 6, 4-13.

AOAC (1995). Official methods of analysis of AOAC. 16º edn. Arlington, VA, USA.

Balunkeswar, N., Liu, R.H., & Tang, J. (2015). Effect of processing on phenolic antioxidants of fruits, vegetables, and grains—a review. *Critical Reviews in Food Science and Nutrition*, 55(7), 887-918. https://doi.org/10.1080/10408398.2011.654142.

Bello-Pérez, L.A., Camelo-Méndez, G.A., Agama-Acevedo, E., & Utrilla-Coello, R.G. (2016). Nutraceutic aspects of pigmented maize: digestibility of carbohydrates and anthocyanins. *Agrociencia*, 50, 1041-1063.

Brennan, C., Brennan, M., Derbyshire, E., & Tiwari, B.K. (2011). Effects of extrusion on polyphenols, vitamins and antioxidant activity of foods. *Trends in Food Science and Tech*nology, 22(10), 570-575. https://doi.org/10.1016/j.tifs.2011.05.007.

Campas-Baypoli, O.N., Rosas-Burgos, E.C., Torres-Chávez, P.I., Ramírez-Wong, B., Serna-Saldívar, S.O. (2002). Physicochemical changes of starch in maize tortillas during storage at room and refrigeration temperatures. *Starch/Stärke,* 54(8), 358-363. https://doi.org/10.1002/1521-379x(200208)54:8<358::aid-star358>3.0.co;2-w.

Castillo, V.K.C., Ochoa, M.L., Figueroa, C.J.D., Delgado, L.E., Gallegos, I.J.A., & Morales, C.J. (2009). Efecto de la concentración de hidróxido de calcio y tiempo de cocción del grano de maíz (*Zea mays* L.) nixtamalizado, sobre las características fisicoquímicas y reológicas del nixtamal. *Archivos Latinoamericanos De Nutrición*, 59, 425-432.

Cornejo-Villegas, M.A., Gutiérrez-Cortez, E., Rojas-Molina, I., Del Real-López, A., Zambrano-Zaragoza, M.L., Martínez-Vega, V., & Rodríguez-García, M.E. (2013). Physicochemical, morphological, and pasting properties of nixtamalized flours from quality protein maize and its particle distribution. *LWT-Food Science and Technol*ogy,53(1), 81-87. https://doi.org/10.1016/j.lwt.2013.01.023.

Cortés, G.A., Salinas, M.Y., San Martín-Martínez, E., & Martínez-Bustos, F. (2006). Stability of anthocyanins of blue maize (*Zea mays* L.) after nixtamalization 87ethanol87ted pericarp-germ tip cap and endosperm fractions. *Journal of Cereal Science,* 43(1), 57-62. https://doi.org/10.1016/j.jcs.2005.05.003.

Cortés-Gómez, A., San Martín-Martínez, E., Martínez-Bustos, F., & Vázquez-Carrillo, G.M. (2005). Tortillas of blue maize (*Zea mays* L.) prepared by a fractionated process of nixtamalization: analysis using response surface methodology. *Journal of Food Engineering*, 66(3), 273-281. https://doi.org/10.1016/j.jfoodeng.2004.03.018.

Enríquez-Castro, C.M, Torres-Chávez, P.I., Ramírez-Wong, B., Quintero-Ramos, A., Ledesma-Osuna, A.I., López-Cervantes, J., & Gerardo-Rodríguez, J.E. (2020). Physicochemical, rheological, and morphological characteristics of products from traditional and extrusion nixtamalization processes and their relation to starch. *International Journal of Food Science*. https://doi.org/10.1155/2020/5927670

Escalante-Aburto, A., Ramírez-Wong, B., Torres-Chávez, P.I., Figueroa Cárdenas, J.D., López-Cervantes, J., Barrón-Hoyos, J.M., & Morales-Rosas, I. (2013). Effect of extrusion processing parameters on anthocyanin content, physicochemical properties of nixtamalized blue corn expanded extrudates. *CYTA-Journal of Food*, 11(sup1), 29-37. https://doi.org/10.1080/19476337.2013.764929.

Fabila-Carrera, G. (1998). Diseño y análisis de experimentos industriales. Universidad Iberoamericana: México pp 19-29.

Flores-Farías, R., Martínez-Bustos, F., Salinas-Moreno, Y., & Ríos, E. (2002). Characterization of commercial nixtamalized maize flours. *Agrociencia*, 36, 557-567.

Habibi, F., Ramezanian, A., Guillén, F., Serrano, M., & Valero, D. (2020). Blood oranges maintain bioactive compounds and nutritional quality bypostharvest treatments with γ-aminobutyric acid, methyl jasmonate or methyl salicylate during cold storage. *Food Chemistry*, 306, 125634. https://doi.org/10.1016/j.foodchem.2019.125634.

Li, M., Hasjim, L., Xie, F., Halley, P.J., & Gilbert, R.G. (2014). Shear degradation of molecular, crystalline, and granular structures of starch during extrusion. *Starch/Stärke*, 66(7-8), 595-605. https://doi.org/10.1002/star.201300201.

Lillford, P.J.M. (1997). Structure/function relationship of starches in food. In: Frazier, P.J, Donald, A.M., Richmond, P., (Eds.), Starch: Structure and Functionality, Cambridge, United Kingdom, pp. 1-8.

Platt-Lucero, L.C., Ramírez-Wong, B., Torres-Chávez, P.I., López-Cervantes, J., Sánchez-Machado, D.I., Reyes-Moreno, C., … Morales-Rosas, I. (2010). Improving textural characteristics of tortillas by adding gums during extrusion to obtain nixtamalized corn flour. *Journal of Texture Studies*, 41(5), 736-755. https://doi.org/10.1111/j.1745-4603.2010.00252.x.

Platt-Lucero, L.C., Ramírez-Wong, B., Torres-Chávez, P.I., & Morales-Rosas, I. (2012). Viscoelastic and textural characteristics of masa and tortilla from extruded corn flours with xanthan gum, Viscoelasticity-From Theory to Biological Applications. Intech Open, 237-258. http://dx.doi.org/10.5772/49975.

Pojer, E., Mattivi, F., Johnson, D., & Stockley C.S. (2013). The case for anthocyanin consumption to promote human health: a review. *Comprehensive Reviews in Food Science and Food Safety*, 12(5), 483-508. https://doi.org/10.1111/1541-4337.12024.

Ramírez-Wong, B., Walker, C.E., Ledesma-Osuna, A.I., Torres-Chávez, P.I., Medina-Rodríguez, C.L., López-Ahumada, G.A., & Flores, R.A. (2007). Effect of flour extraction rate on white and red winter wheat flour compositions and tortilla texture. *Cereal Chemistry*, 84(3), 207-213. https://doi.org/10.1094/cchem-84-3-0207.

Rincón-Londoño, N., Millan-Malo, B., & Rodríguez-García, M. E. (2016). Analysis of thermal pasting profile in corn starch rich in amylose and amylopectin: physicochemical transformations, Part II. *International Journal of Biological Macromolecules*, 89, 43–53. http://dx.doi.org/10.1016/j.ijbiomac.2016.04.057

Robin, G. (2001). Extrusion cooking technologies and applications. CRC Press, Boca Ratón, Florida.

Rodis, P., Wen, L.F. & Wasserman, B.P. (1993). Assessment of extrusion-induced starch fragmentation by gel permeation chromatography and methylation analysis. *Cereal Chemistry*, 70, 152-157.

Ruíz-Gutiérrez, M.G., Sánchez-Madrigal, M.A., & Quintero-Ramos, A. (2018). The extrusion cooking process for the development of functional foods, extrusion of metals, polymers and food products. Intech Open, 83-102. http://dx.doi.org/10.5772/intechopen.68741.

Sadilova, E., Stintzing, F.C., & Carle, R. (2006). Thermal degradation of acylated and nonacylated anthocyanins. *Journal of Food Science,* 71(8), C504-C512. https://doi.org/10.1111/j.1750-3841.2006.00148.x.

Waniska, R.D. (1976). Methods to asses quality of boiled sorghum, gruel and chapaties from sorghum with different characteristics. M.S. Dissertation. Texas A&M University, College Station, TX.

Weber, J.R. (2008). Shelf life extension of corn tortillas. Dissertation, Kansas State University

White, B.L., Howard, L.R., & Prior, R.L. (2010). Polyphenolic composition and antioxidant capacity of extruded cranberry pomace. *Journal of Agricultural and Food Chemistry*, 58(7), 4037-4042. https://doi.org/10.1021/jf902838b.

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

Tr	Process factors			Response variables		
	FM (%)	T (ºC)	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 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.

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]

Process factors	Response Variables		
	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

[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; 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, while model and lack of fit with p values ≤ 0.05 are significant.

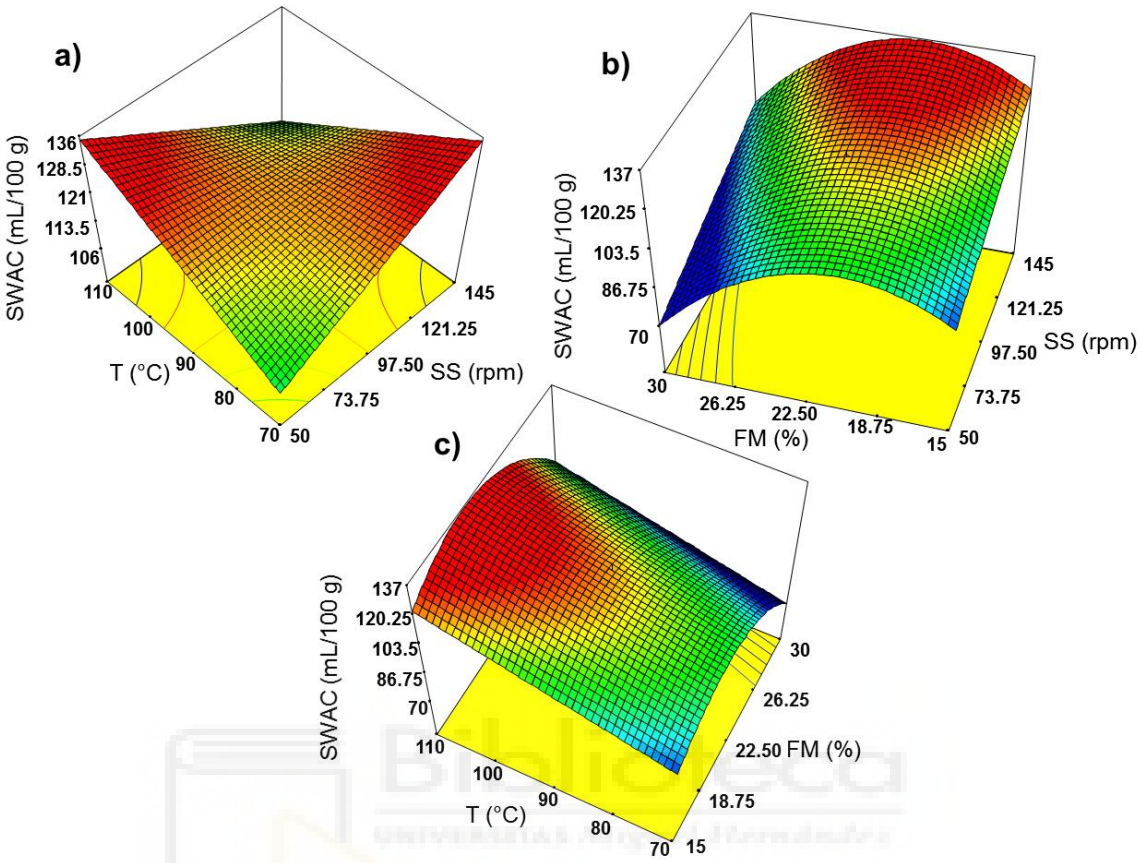

Figure 1. Response surface plots for subjective water absorption capacity (SWAC) of ENCFs as a function of: (a) temperature (T) and screw speed (SS); (b) feed moisture (FM) and screw speed (SS); and (c) temperature (T) and feed moisture (FM).

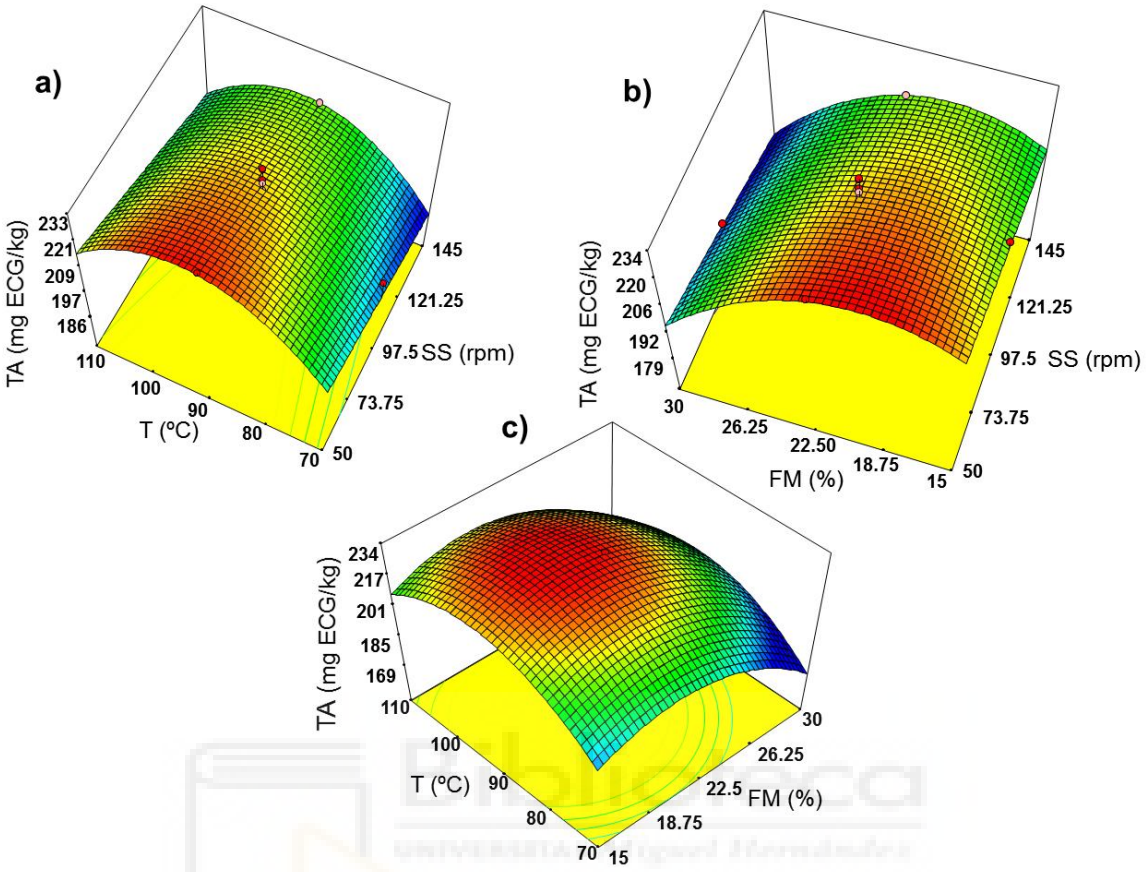

Figure 2. Response surface plots for total anthocyanins (TA) of ENCFs as a function of: (a) temperature (T) and screw speed (SS); (b) feed moisture (FM) and screw speed (SS); and (c) temperature (T) and feed moisture (FM).

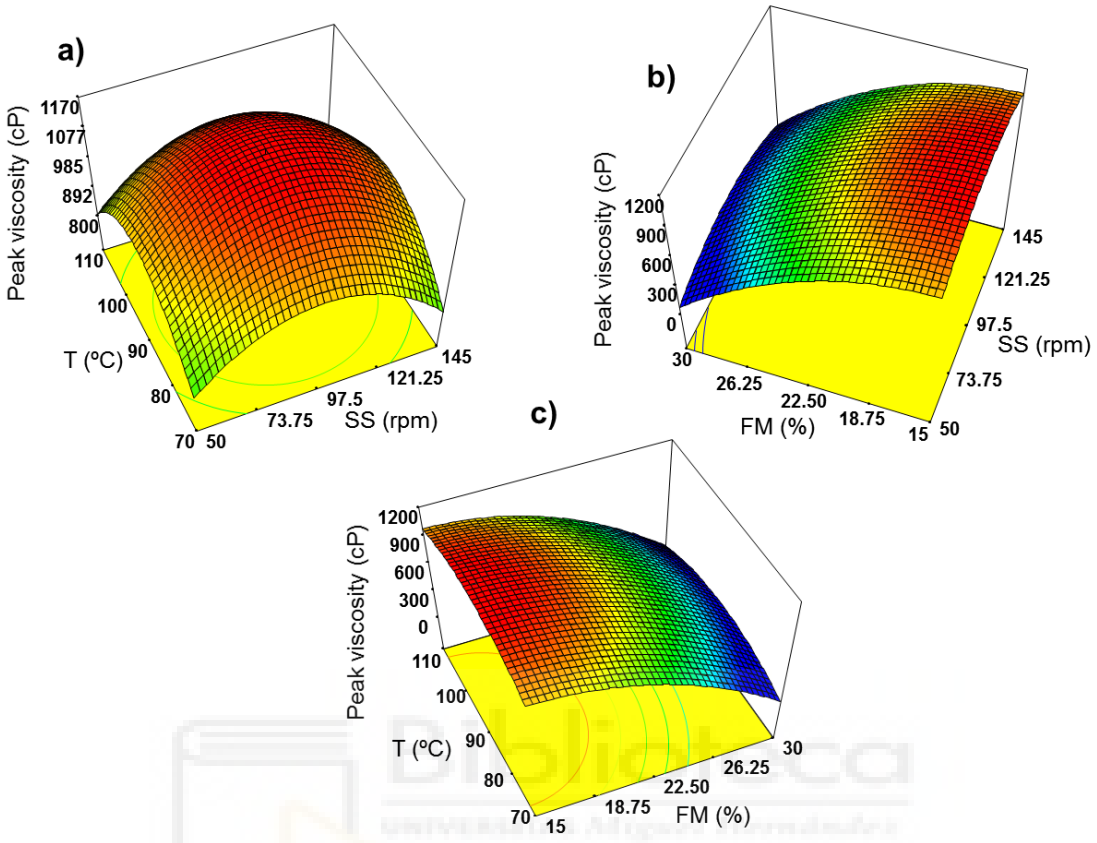

Figure 3. Response surface plots for peak viscosity (PV) of ENCFs as a function of: (a) temperature (T) and screw speed (SS); (b) feed moisture (FM) and screw speed (SS); and (c) temperature (T) and feed moisture (FM).

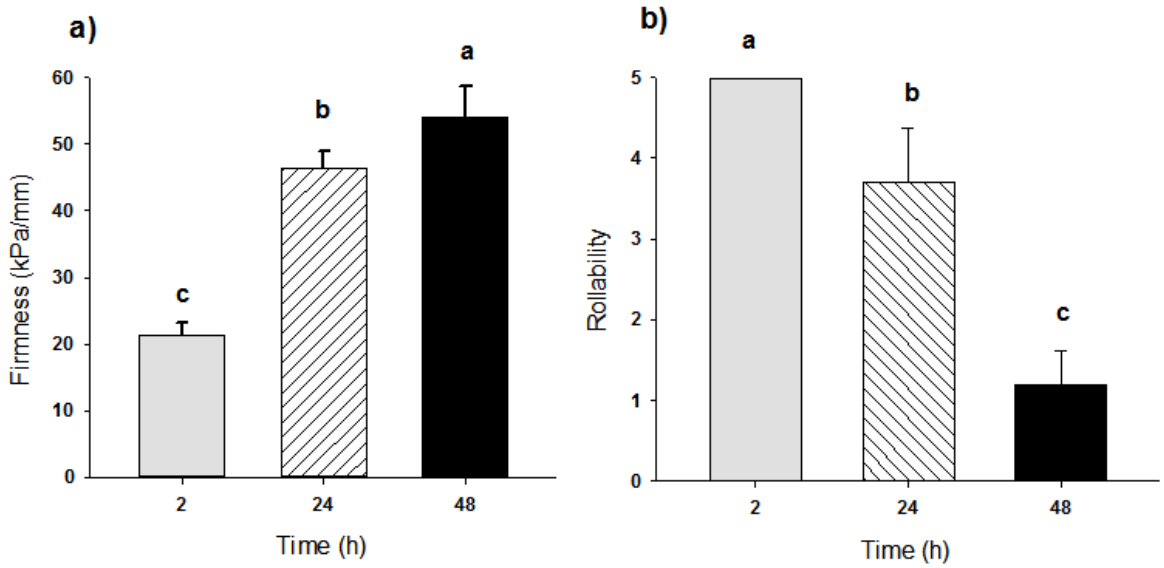

Figure 4. Texture of tortillas produced from the optimized ENCF at 2, 24 and 48 hours of storage after baking: (a) tortilla firmness and (b) tortilla rollability. Error bars are standard deviations. Significant differences of values are indicated by different letters ($p<0.05$).

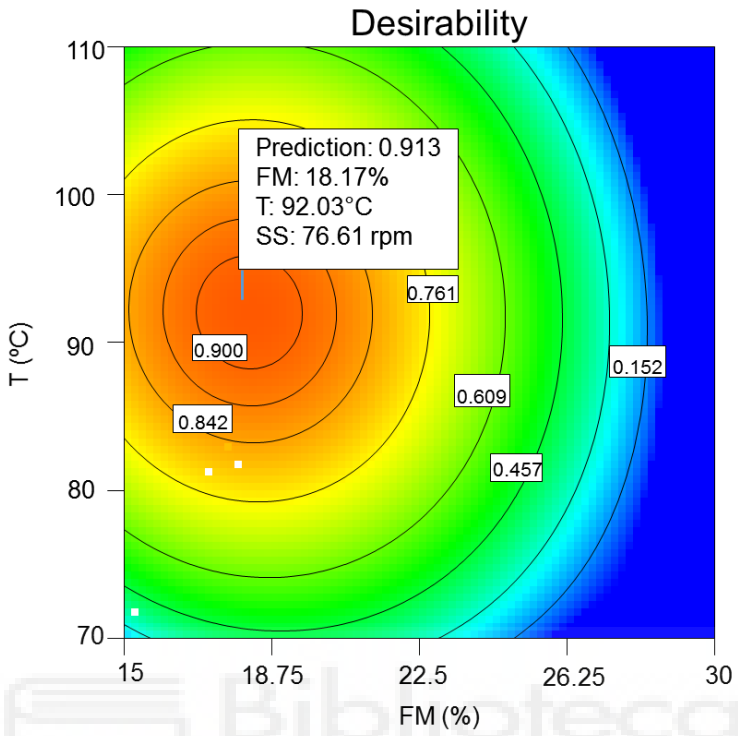

Figure 5. Contour plot of optimum conditions to produce ENCF.

3.2 PUBLICACIÓN 2

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The Effect of Nixtamalization Extrusion Process and Tortillas Making on the Stability of Anthocyanins from Blue Corn through the Kinetic and Thermodynamic Parameters

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Abstract

The blue corn-based products are considered functional foods due to their high concentration of anthocyanins. The aim of this study was to estimate the kinetic and thermodynamic parameters of the thermal degradation of anthocyanins from extruded nixtamalized corn products. A comparative study of anthocyanins thermal stability in these matrices in a buffer solution (pH 2.5) was investigated at different temperatures (60, 75 or 90 °C). Results showed the mechanism of anthocyanins degradation followed first-order reaction kinetics. The values of the reaction rate constant (*k*) were found to be in a range of 0.027-0.037 h^{-1} at 60 °C, 0.107-0.113 h^{-1} at 75 °C and 0.340-0.354 h^{-1} at 90 °C. The higher the *k* value was, the shorter the half-life time and D-value. The activation energy (Ea) and *z*-values were in the range of 75.1-89.2 kJ/mol and 28.8-35.1 °C, respectively. The coefficient Q_{10} indicated the reaction rate approximately doubles with every 10 °C temperature increase. ΔH, ΔS and ΔG indicated the degradation of anthocyanins was an endothermic and nonspontaneous reaction. Even the major susceptibility of the anthocyanins in extruded nixtamalized corn products at the time–temperature combination applied, there was not difference between flour and tortilla, this imply that most of the anthocyanins were degraded during the nixtamalization extrusion process and no significative further degradation occur in the cooking step. This study provides and advance in the knowledge on the effect of nixtamalization extrusion process and tortillas making on the stability of anthocyanins from blue corn. However, further studies are needed.

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

Introduction

Blue corn is an important source of bioactive compounds such as phenolic acids and anthocyanins [1]. It is an excellent vehicle to improve the nutritional quality of the populations in which it is included in the diet [2]. Their based products are considered functional foods due to their high concentration of these compounds that have been recognized as health-enhancing substances [3].

The main consumption form of corn is the tortilla. Tortillas constitute the staple food for most people in Mexico and Central America. In the last decades their consumption has penetrated widely into the United States market and some countries in Asia and Europe [4].

Hence, when considering the use of anthocyanins as health promoting substances, their thermal treatment and the pH stability should be considered. The type of process and the combination of factors involved, might induce changes in the concentration, bioaccesibility and bioactivity of the anthocyanin compounds, which further affects the consumer acceptance [5].

The tortilla is obtained through the traditional nixtamalization process. This alkaline thermal process induces chemical and enzymatic reactions that may degrade the anthocyanins to colorless products or transform them into new structures [6]. Due to this technological disadvantage, 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 [3].

A recent study relates the thermal degradation kinetics of anthocyanins extracted from purple maize flour and the effect of heating on biological functionality. Results showed that the extracts heated at lower temperatures showed a high stability under *in vitro* gastric environment, whereas

after heating at higher temperatures, the digestion ended quickly [5]. Thermal degradation of anthocyanins was positively correlated with the *in vitro* decrease of antioxidant activity, indicating the importance of the study of kinetic and thermodynamic parameters to evaluate the effect of the process on the anthocyanins stability as a previous nutritional control prior to food consumption [5].

Ensuring the chemical stability of anthocyanins has become a focal point in recent studies, some of them revealed a significantly faster decrease in anthocyanin content as temperature increased with an asymptotic tendency toward high temperature values [7,8]. However, there is few information about the mechanisms of thermal degradation, and no data are available concerning the degradation kinetics of the anthocyanins found in nixtamalized products.

The aim of this study was to advance the knowledge on the effect of nixtamalization extrusion process and tortillas making on the stability of anthocyanins from blue corn through the kinetic and thermodynamic parameters at different temperatures based on the total monomeric anthocyanins of their crude extracts.

Materials and methods

The section of materials and methods is shown in the Supplementary Material.

Results and discussion

Anthocyanins thermal degradation

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. 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. A linear decrease can be seen in the anthocyanin content with respect to temperature-time combination for all the treatments.

Kinetic parameter estimates

The rate of retention/degradation of anthocyanins during heating is reflected by the numerical values of the kinetic parameters [9]. For all the samples, it was observed that the thermal degradation of the anthocyanins content 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 [10,11]. 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.

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, the differences in the degradation rate constant (*k*) of extruded nixtamalized corn flour (ENCF) and tortillas compared to raw blue corn were smaller (75 °C) or very similar (90 °C) (Table 1). The lower the *k* value is, the better the anthocyanin stability is.

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 [7,8]. 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.

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 anthocyanins degradation was observed at 90°C (Table 1). Increases in temperature led to a significant decrease in anthocyanins 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 different susceptibilities of anthocyanins to heat treatment might be attributed to the anthocyanins chemical structure, processing condition, and interactions among matrix components [12].

The time needed for 90% degradation of anthocyanins (D-value) at 90 °C was approximately 8–11 times shorter than that at 60 °C (Table 1). This behavior suggests that the stability of anthocyanins is strongly influenced by the magnitude and duration of heating [9].

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. The coefficients of determination (R^2) values were more than 0.996 for all cases (Table 2). Figure 2 show a strong dependence on temperature for all the samples, which means that the reaction of anthocyanins degradation runs very slowly at low temperatures but relatively fast at high temperatures [13].

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). Most of the reported Ea values fell into the range of 20–200 kJ/mol [14]. 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 anthocyanin content 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). In general, the Q_{10} values were approximately 2.0. The results are similar to those obtained by Fracassetti et al. [8], 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 destruction rates of anthocyanins obtained in this research were similar to the values reported by Peron et al. [15], who determined the z-value in crude grape anthocyanin extracts (*z*=23.2-24.4).

ΔH values calculated at different temperatures ranged from 71.5 to 86.5 kJ mol^{-1} (Table S1, provided as a supplementary material). 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 anthocyanins degradation was an endothermic reaction.

The values of ΔG were similar for all conditions evaluated in this study, varying between 67.91 and 69.2 kJ mol^{-1} (Table S1). The positive sign observed for all temperatures demonstrated that anthocyanins degradation is a nonspontaneous reaction.

The ΔS ranged from 10.6 to 51.5 $Jmol^{-1}$ K^{-1} (Table S1). 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 [10].

The determined parameters indicated the anthocyanins present in raw corn were less susceptible to degradation at elevated temperatures required greater energy to activate the thermal reaction than those in flour and tortilla, due to the cell matrix in raw corn is unaltered preserving the integrity of this compounds.

Even the major susceptibility of the anthocyanins in extruded nixtamalized corn products compared with the raw corn at the time–temperature combination applied, there was not difference between flour and tortilla, this imply that most of the anthocyanins were degraded during the nixtamalization extrusion process and no significative further degradation occur in the cooking step. This can be related to the fact that although the main changes caused by the extrusion process to obtain the flour are the disruption of cell wall matrices, breaking of covalent bonds, improving compound accessibility and the decomposition of heat-labile substances; the conditions used in our study allowed to retain a high concentration of anthocyanins even after the cooking process. In addition, anthocyanins stability is related to their structures and copigmentation capacity. The methoxyl groups on the B-ring of anthocyanins confer a higher

thermoresistance to the molecule. Stability of anthocyanins can increase also with intermolecular copigmentation. Crude extracts, with high anthocyanin content, contain mixtures of different compounds that may serve as copigments for intermolecular association with anthocyanins [9,7].

Conclusions

This is the first comparative study that focuses on the kinetic thermal degradation of anthocyanins found in extruded nixtamalized corn products. Results suggest that the first-order model was suitable for predicting their degradation at temperatures ranging from 60 to 95 °C. Interestingly, degradation kinetics revealed that the flour and tortilla followed a similar pattern of degradation although the tortilla preparation used temperatures around 300 °C, higher than that used in the extrusion process. This behavior probably depends on the anthocyanins chemical structure, the tortilla physical characteristic and the protective effect of some anatomical parts of the kernel allow a slower heat flow to the center of the tortilla that preventing the anthocyanins easily degraded during cooking. The knowledge and evaluation of the kinetic and thermodynamic parameters of extruded corn-based foods could allow the industry to adapt the process conditions based on the desired characteristics of the food offered to consumers, minimizing the loss of compounds with potential bioactive. However, further studies should be conducted to achieve a better understanding.

Author´s contributions

All authors contributed to the study conceptualization and design. Project administration and resources were performed by Benjamín Ramírez-Wong and María José Frutos. Investigation and the first draft of the manuscript was carried out by Mariela Menchaca-Armenta and Review

& Editing the original draft by Patricia I. Torres-Chávez. Material preparation and data collection were in charge of Raquel Muelas-Domingo and Estefanía Valero-Cases. Formal analysis and validation were performed by Armando Quintero-Ramos, Ana I. Ledesma-Osuna and Olga N. Campas-Baypoli. All authors read and approved the final manuscript.

Data Availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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Compliance with Ethical Standards

Conflict of Interest: The authors declare that they have no conflict of interest.

References

1. Escalante-Aburto A, Ramírez-Wong B, Torres-Chávez PI, Figueroa Cárdenas JD, López-Cervantes J, Barrón-Hoyos JM, Morales-Rosas I (2013) Effect of extrusion processing parameters on anthocyanin content, physicochemical properties of nixtamalized blue corn expanded extrudates. CYTA-J Food 11:29-37. https://doi.org/10.1080/19476337.2013.764929
2. Rivera-Castro VM, Muy-Rangel MD, Gutiérrez-Dorado R, Escobar-Álvarez JL, Hernández-Castro E, Valenzuela-Lagarda JL (2020) Nutritional, physicochemical and anatomical

evaluation of creole corn varieties from t111ethanion of the Costa Chica Guerrero. Food Sci Technol 40:938-944. https://doi.org/10.1590/fst.2019

3. Mora-Rochín S, Gutiérrez-Uribe JA, Serna-Saldívar SO, Sánchez-Peña P, Reyes-Moreno C, Milán-Carrillo J (2010) Phenolic content and antioxidant activity of tortillas produced from pigmented maize processed by conventional nixtamalization or extrusion cooking. J Cereal Sci 52:502- 508. https://doi.org/10.1016/j.jcs.2010.08.010
4. Cortés-Gómez A, San Martín-Martínez E, Martínez-Bustos F, Vázquez-Carrillo GM, (2005) Tortillas of blue maize (*Zea mays* L.) prepared by a fractionated process of nixtamalization: analysis using response surface methodology. J Food Eng 66:273-281. https://doi.org/10.1016/j.jfoodeng.2004.03.018
5. Slavu M, Aprodu I, Milea SA, Enachi E, Râpeanu G, Bahrim GE, Stănciuc N (2020) Thermal degradation kinetics of anthocyanins extracted from purple maize flour extract and the effect of heating on selected biological functionality. Foods 9:1593. https://doi.org/10.3390/foods9111593
6. Escalante-Aburto A, Ramírez-Wong B, Torres-Chávez PI , Barrón-Hoyos JM, Figueroa-Cárdenas JD, López-Cervantes J (2013) The nixtamalization process and its effect on anthocyanin content of pigmented maize, a review. Rev Fitotec Mex 36: 429 – 437.
7. Bolea C, Turturică M, Stănciuc N, Vizireanu C (2016) Thermal degradation kinetics of bioactive compounds from black rice flour (*Oryza sativa* L.) extracts. J Cereal Sci 71:160-166. https://doi.org/10.1016/j.jcs.2016.08.010
8. Fracassetti D, Del Bo' C, Simonetti P, Gardana C, Klimis-Zacas D, Ciappellano S (2013) Effect of time and storage temperature on anthocyanin decay and antioxidant activity in wild

blueberry (*Vaccinium angustifolium*) powder. J Agric Food Chem 61:2999−3005. https://doi.org/10.1021/jf3048884

9. Nayak B, Liu RH, Tang J (2015) Effect of processing on phenolic antioxidants of fruits, vegetables, and grains- A review. Crit Rev Food Sci 55:887-918. https://doi.org/10.1080/10408398.2011.654142

10. Turturică M, Stănciuc N, Bahrim G, Râpeanu G (2016) Effect of thermal treatment on phenolic compounds from plum (*prunus domestica*) extracts – A kinetic study. J Food Eng 171:200-207. https://doi.org/10.1016/j.jfoodeng.2015.10.024

11. Hernández-Herrero JA, Frutos MJ (2011) Degradation kinetics of pigment, colour and stability of the antioxidant capacity in juice model systems from six anthocyanin sources. Int J of Food Sci Tech 46:2550–2557. https://doi.org/10.1111/j.1365-2621.2011.02780.x

12. Qiu G, Wang D, Song X, Deng Y, Zhao Y (2018) Degradation kinetics and antioxidant capacity of anthocyanins in air-impingement jet dried purple potato slices. Food Res Int 105:121-128 https://doi.org/10.1016/j.foodres.2017.10.050

13. Mercali GD, Jaeschke DP, Tessaro IC, Ferreira-Marczak LD (2013) Degradation kinetics of anthocyanins in acerola pulp: Comparison between ohmic and conventional heat treatment. Food Chem 136:853–857. https://doi.org/10.1016/j.foodchem.2012.08.024

14. Sui X, Dong X, Zhou W (2014) Combined effect of pH and high temperature on the stability and antioxidant capacity of two anthocyanins in aqueous solution. Food Chem 15:163-70. https://doi.org/10.1016/j.foodchem.2014.04.075.

15. Peron DV, Fraga S, Antelo F (2017) Thermal degradation kinetics of anthocyanins extracted from juçara (*Euterpe edulis Martius*) and ‘‘Italia” grapes (*Vitis vinifera* L.), and the

effect of heating on the antioxidant capacity. Food Chem 232:836–840. https://doi.org/10.1016/j.foodchem.2017.04.088

Table 1

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

T (°C)	$t_{1/2}$ (h)	D-value (h)	k (h^{-1})	R^2
Raw corn				
60	25.3 ± 0.06 aA	83.9 ± 0.21 aA	0.027 ± 7.07 x 10 -05 bA	0.96
75	6.4 ± 0.11 aB	21.4 ± 0.38 aB	0.107 ± 1.91 x 10 -03 aB	0.97
90	1.9 ± 0.01 aC	6.5 ± 0.05 aC	0.354 ± 2.76 x 10 -03 aC	0.99
ENCF				
60	19.1 ± 0.15 bA	63.6 ± 0.49 bA	0.036 ± 2.83 x 10 -04 aA	0.91
75	6.1 ± 0.14 aB	20.3 ± 0.49 aB	0.113 ± 2.76 x 10 -03 aB	0.98
90	2.0 ± 0.03 aC	6.8 ± 0.12 aC	0.340 ± 6.2 x 10 -03 aC	0.99
Tortillas				
60	18.7 ± 0.67 bA	62.0 ± 2.24 bA	0.037 ± 1.34 x 10 -03 aA	0.96
75	6.1 ± 0.03 aB	20.4 ± 0.10 aB	0.112 ± 5.7 x 10 -04 aB	0.96
90	1.9 ± 0.02 aC	6.6 ± 0.09 aC	0.347 ± 5.0 x 10 -03 aC	0.97

[1] Results are means ± standard deviations (n = 3). [2] Means were separated by rows, applying Tu'ey'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-value) corresponding to anthocyanins degradation in blue corn, extruded nixtamalized flour and tortillas under different thermal treatments [1,2,3,4]

Product	Ea (kJ/mol)	R^2	Q_{10} 60-75 °C	Q_{10} 75-90 °C	*z*-value (°C)
Raw corn	89.2 ± 0.27 [a]	0.999	2.57 ± 0.09 [a]	2.22 ± 6 x10^{-04} [a]	28.8 ± 0.009 [b]
ENCF	75.1 ± 0.25 [b]	0.996	2.12 ± 0.06 [b]	1.96 ± 0.10 [ab]	35.1 ± 1.31 [a]
Tortillas	75.5 ± 0.11 [b]	0.999	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 Tu'ey'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.

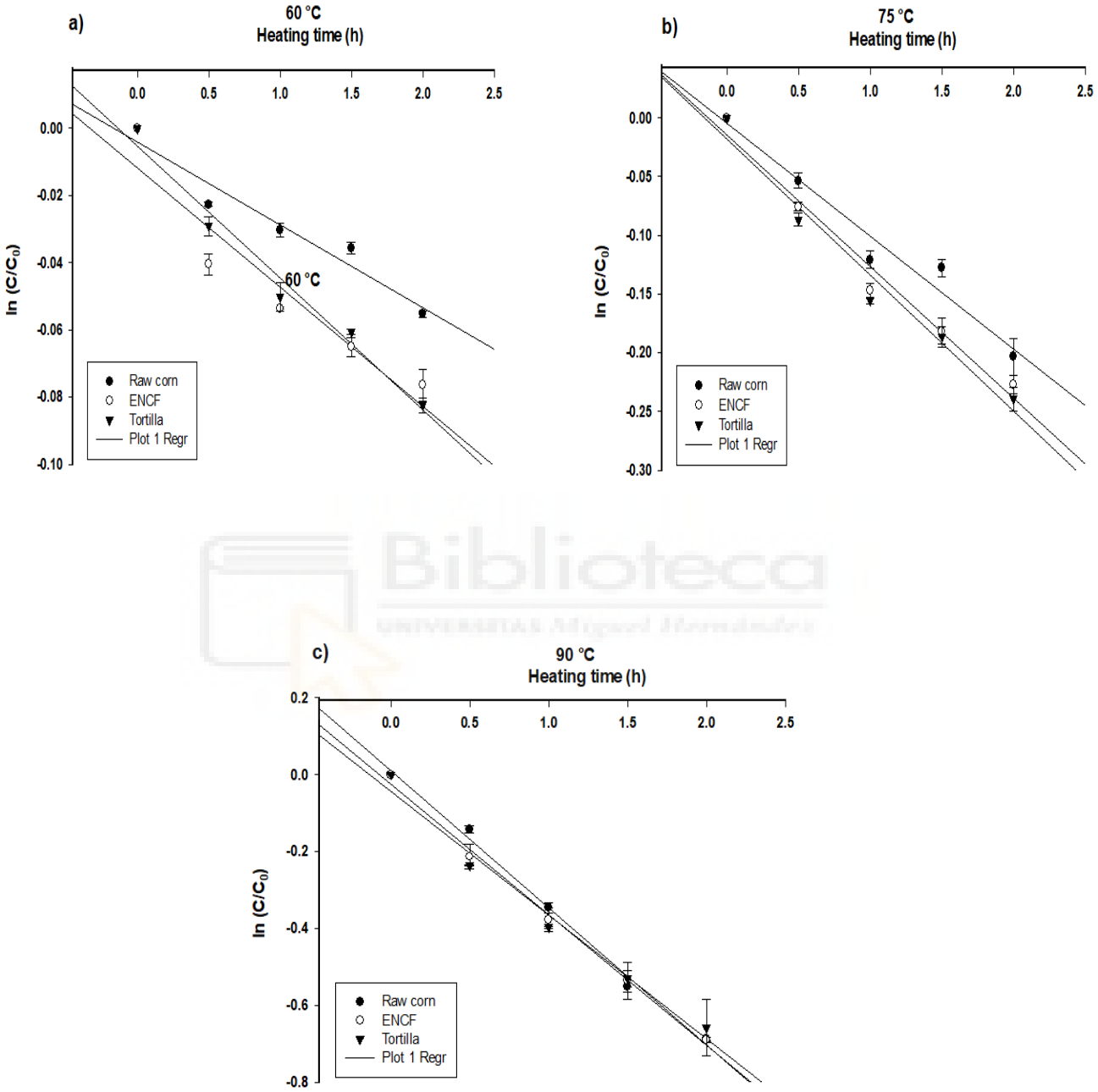

Fig. 1. First-order plot for the degradation of total anthocyanins in blue corn, extruded nixtamalized flour and tortillas at a) 60 °C, b) 75 °C and c) 90 °C during 2 h. Each point represents the mean ± standard deviation (n =3). Numbers in parentheses are the determination coefficients (R^2). C_0, initial concentration.

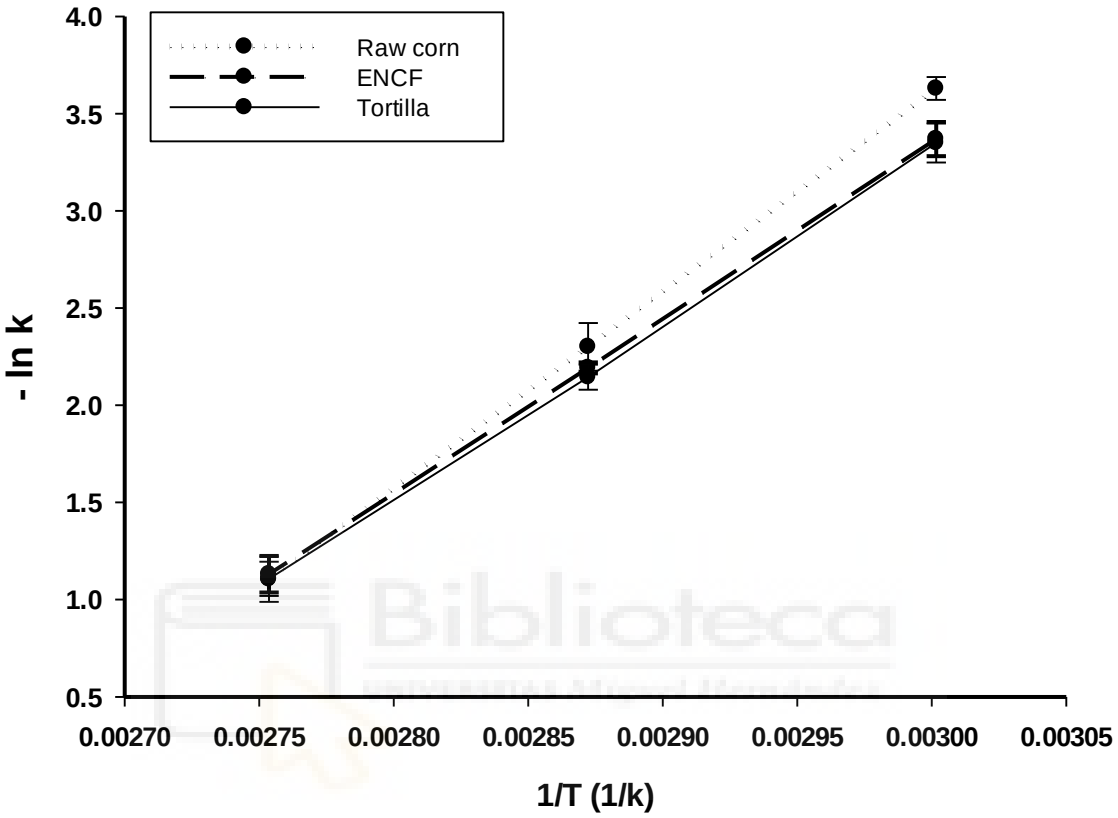

Fig. 2. Arrhenius plot for anthocyanins degradation in raw blue corn, extruded nixtamalized flour and tortillas during thermal treatment. Each point represents the average ± standard deviation of 3 replicates. Numbers in parentheses are the determination coefficients (R^2).

CAPÍTULO 4. RESUMEN DE RESULTADOS Y DISCUSIÓN

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

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4.1.1. Objetivo: El objetivo de este estudio fue evaluar el efecto de los factores del proceso de nixtamalización por extrusión sobre las propiedades químicas, funcionales y amilográficas de la harina de maíz azul obtenida, y determinar las condiciones óptimas para la obtención de una harina con un máximo contenido de antocianinas y máxima viscosidad utilizando la metodología de superficie de respuesta (MSR), obteniendo una tortilla a partir de la harina optimizada con características de calidad aceptables para los consumidores.

4.1.2.Resumen de Materiales y Métodos: 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 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 (Contenido total de antocianinas, AT), funcionales (Capacidad de absorción de agua subjetiva, CAAS) y amilográficas (Pico de viscosidad, PV). 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 optima se obtuvo la tortilla la cual fue caracterizada física, química y texturalmente.

4.2.3. Resumen de Resultados y Discusión: Los factores del proceso de nixtamalización por extrusión (HA, TE y VT) afectaron todos los parámetros evaluados en las harinas. Los resultados indican que la HA fue el factor que tuvo un mayor efecto sobre las propiedades evaluadas en las harinas (CAAS, PV, AT) en su término lineal y cuadrático. Las características óptimas de la

harina nixtamalizada extruida en términos de máximo contenido de antocianinas (226.07 mg / kg) y máxima viscosidad (1063.9 cP) se encontraron a una humedad de alimentación de 18.17%, una temperatura de la cuarta zona del extrusor de 92.03 ° C y una velocidad de tornillo de 76.61 rpm.

4.1.4 Conclusiones: La metodología de superficie de respuesta (MSR) se aplicó con éxito para evaluar y modelar los efectos de tres factores del proceso de nixtamalización por extrusión (HA, TE y VT) sobre las propiedades químicas, funcionales y amilográficas de las harinas nixtamalizadas extruidas. El pico de viscosidad de las harinas (PV) fue utilizado para inferir la textura de la tortilla obtenida a partir de esta. La textura es una de los factores mas importantes en la aceptación de la tortilla, encontrar propiedades de textura óptimas basadas en el valor de PV de la harina de maíz podría ser un objetivo de las investigaciones futuras sobre el efecto de los factores de procesamiento en el parámetros de textura.Las tortillas obtenidas de la harina optimizada mostraron características de textura adecuadas (firmeza, y rollabilidad) para aceptación del consumidor y un valor nutracéutico prometedor a través del 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.

4.2 The Effect of Nixtamalization Extrusion Process and Tortillas Making on the Stability of Anthocyanins from Blue Corn through the Kinetic and Thermodynamic Parameters

Menchaca-Armenta, Mariela, Frutos, María José, Ramírez-Wong, Benjamín, Quintero-Ramos, Armando, Torres-Chávez, Patricia Isabel, Valero-Cases, Estefanía, Muelas-Domingo, Raquel, Ledesma-Osuna, Ana Irene, Campas-Baypoli, Olga Nydia. (2021).

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4.2.1. Objetivo: El objetivo de este estudio fue avanzar en el conocimiento sobre el efecto del proceso de extrusión de nixtamalización y elaboración de tortillas sobre la estabilidad térmica de las antocianinas del maíz azul a través de los parámetros cinéticos y termodinámicos a diferentes temperaturas.

4.2.2. Resumen de Materiales y Métodos: Las antocianinas del maíz azul crudo, harina nixtamalizada extruida y tortilla, se extrajeron con metanol acidificado (6124ethanolnol, 37% agua, 3% ácido fórmico v/v/v) y se concentraron a 40 °C hasta sequedad. Los extractos se resuspendieron en una solución tampón de acetato de sodio a pH (2.5) y se filtraron para remover impurezas. La solución tampón de cada muestra fue tratada térmicamente a 3 temperaturas diferentes (60, 75 y 90 °C) durante 2 h. A intervalos de tiempo regulares (0.5, 1, 1.5, 2 h), las muestras se retiraron aleatoriamente del baño maría y se enfriaron rápidamente en agua con hielo para evitar una mayor degradación térmica. El cambio de concentración respecto al tiempo fue medido espectofotométricamente, y con estos datos se estimaron los parámetros cinéticos (t ½, *k*, D) y termodinámicos (Ea, Q_{10}, Z, ΔH, ΔG, ΔS) empleando la ecuación de primer orden y la ecuación de Arrhenius para ello. Se utilizó un diseño bifactorial completamente al azar. Los factores fueron el tipo de producto con tres niveles (maíz azul, harina de maíz y tortilla) y el tratamiento térmico con tres niveles (60, 75 y 90 ° C). Todos los experimentos se realizaron por triplicado (n = 3) y se presentaron como la media ± desviación estándar. Los datos se analizaron mediante el análisis de varianza (ANDEVA), con un nivel de significancia <0.05 %, empleando la prueba de Tukey.

4.2.3. Resumen de Resultados y Discusión: La degradación térmica de las antocianinas en productos nixtamalizados extruidos a base de maíz azul siguió un modelo cinético de primer

orden con altos coeficientes de regresión (0.914 <R^2 <0.997). La constante de velocidad de degradación (*k*) confirmó que las temperaturas más altas condujeron a una degradación más rápida de las antocianinas para todas las matrices evaluadas. El tiempo necesario para la degradación del 90% de las antocianinas (Valor D) a 90 °C fue aproximadamente 8-11 veces más corto que a 60 °C. El mayor tiempo para alcanzar el 50% de degradación de las antocianinas se presentó en el extracto de maíz azul a 60 °C, en comparación con la harina nixtamalizada y las tortillas. Se observó que cuanto mayor fue el valor de *k*, más corta la t ½. El modelo de Arrhenius se ajustó adecuadamente a los tratamientos evaluados. Los valores de los coeficientes de determinación (R^2) fueron superiores a 0.996 para todos los casos. Los valores estimados de Ea estuvieron en un rango de 75.1 a 89.2 kJ / mol. Los valores obtenidos de Z para maíz crudo, harina y tortillas fueron 28.8, 35.1 y 33.8, respectivamente. El parámetro Q_{10} indicó que un aumento en temperatura de 10 °C aproximadamente duplicó la velocidad de degradación. Y finalmente, de acuerdo a los cambios de entalpía, entropía y energía libre de Gibss (ΔH, ΔG, ΔS), se determinó que el mecanismo de degradación de las antocianinas fue una reacción endotérmica y no espontánea. Los parámetros estimados indicaron que las antocianinas presentes en el maíz crudo fueron menos susceptibles a la degradación en las temperaturas elevadas, requiriendo mayor energía para activar la reacción térmica que las antocianinas presentes en la harina y la tortilla, debido probablemente a que la matriz celular en el maíz crudo no se altera conservando la integridad de estos compuestos. Interesantemente, aunque se observó una mayor susceptibilidad de las antocianinas en los productos nixtamalizados extruidos en la combinación de tiempo y temperatura aplicada, no se presentaron diferencias entre la harina y tortilla, esto implica que la mayoría de las antocianinas se degradaron durante el proceso de nixtamalización por extrusión y no mas cambios significativos ocurrieron durante la cocción.

4.2.4. Conclusiones: Los resultados sugieren que el modelo de primer ordfuefué adecuado para predecir la degradación de las antocianinas de productos nixtamalizados a temperaturas que oscilan entre 60 y 95 °C. Curiosamente, la cinética de degradación reveló que la harina y la tortilla siguieron un patrón similar de degradación, aunque la preparación de tortilla usa temperaturas alrededor de 300 °C, superior a la utilizada en el proceso de extrusión. Este comportamiento probablemente depende de la estructura química de las antocianinas, las

características físicas de la tortilla y el efecto protector de algunas partes anatómicas del grano como el pericarpio, que permiten un flujo mas lento de calor al centro de la tortilla que evita que las antocianinas se degradan fácilmente durante la cocción. 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.

CAPÍTULO 5. OTRAS PUBLICACIONES DERIVADAS DE LA TESIS DOCTORAL

Food Chemistry

Changes in phytochemical content, bioaccesibility and antioxidant capacity of corn tortillas during simulated in vitro gastrointestinal digestion

--Manuscript Draft--

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Abstract:	Corn tortillas were subjected to in vitro digestion simulating the physiological conditions of the mouth, stomach and small intestine in order to determine the changes in phytochemicals, antioxidant capacity and bioaccesibility. For evaluation of the antioxidant capacity and changes induced by the 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 improving their bioaccessibility. The food matrix has a protective role against the total losses of anthocyanins in blue tortilla. Corn tortillas exhibited effective antioxidant capacity even at the end of digestion. 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.
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5.1 Changes in phytochemical content, bioaccesibility and antioxidant capacity of corn tortillas during simulated *in vitro* gastrointestinal digestion

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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 changes in phytochemicals, antioxidant capacity and bioaccesibility. For evaluation of the antioxidant capacity and 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 improving their bioaccessibility. The food matrix have a protective role against the total losses of anthocyanins in blue tortilla. Corn tortillas exhibited effective antioxidant capacity even at the end of digestion. 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: *in vitro* digestion, non-extractable phenolics, extrusion, gut health, nixtamalization

Abbreviations

BC, Blue corn

ENCF, Extruded nixtamalized corn flour

ENBT, Extruded nixtamalized blue tortilla

TNWT, Traditional nixtamalized white tortillas

PBS, Phosphate buffer solution

SF, Soluble fraction

NEF, Non-extractable fraction

GAE, Gallic acid equivalents

A, Absorbance at 520 nm

MW, Molecular weight

ε, Molar extinction coefficient

DF, Dilution factor

M, Weight of the sample

CGE, Cyanidin-3-glucoside equivalents

dw, Dry weight

TE, Trolox equivalents

FRAP, Ferric reducing antioxidant power

DPPH, 2,2-diphenyl-1-picrylhydrazyl radical

ABTS, 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

1. Introduction

In Mexico, corn is primarily consumed in the form of tortilla. The traditional nixtamalization is the main processing method employed for obtaining these product (Paredes-López, Guevara-Lara & Bello-Pérez , 2009). However, the large amounts of high solids liquid waste and the losses in nutrients are important disadvantages. 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).

Blue corn is noted for its wide array of phytochemicals such as phenolic acids, and flavonoids especially anthocyanins (Liu, 2007). Epidemiological studies have strongly suggested that the daily consumption the phytochemicals in blue corn and their based products (tortillas) could play a role in the reduction or prevention of chronic disease through their antioxidant capacity (Adom & Liu, 2002). These compounds have been shown to possess a potential antiinflammatory, antitumor, anticancer, antidiabetic properties (Mora-Rochín et al., 2010).

The *in vivo* effects of antioxidants depend not only on their concentrations, but also on their bioaccessibility and bioavailability after ingestion (Palafox-Carlos, Ayala-Zavala & González-Aguilar, 2011). 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 in the digestive tract.

To predict the release of the phytochemicals from the food matrix, *in vitro* digestion models have been developed to mimic the complex physicochemical and physiological conditions of the human gastrointestinal tract (Alminger et al., 2014). A good correlation between the results

obtained using *in vitro* and *in vivo* systems have been reported (Carbonell-Capella, Buniowska, Barba, Esteve & Frígola, 2014).

Although during the last decades much has been reported on this topic, no data are available on the bioaccesibility, stability and antioxidant capacity of phytochemicals in nixtamalized corn products after digestion.

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 (BC) was cultivated and harvested in 2016 in the state of Chihuahua, México.

2.3 Blue corn sample

Blue corn 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

135 at 60, 70, 80 and 92 °C. The extrudates obtained were dried at 50 °C for 60 min in a tunnel dryer
136 and cooled at room temperature. Afterward, they were ground to obtain an extruded
137 nixtamalized corn flour (ENCF) using a hammer mill (Christy Turner Ltd., England) with a
138 mesh size of 2 mm. Then, the ENCF was stored in polyethylene bags at -20 °C in the absence
139 of light until analysis.

140 **2.5 Extruded nixtamalized blue tortilla preparation**

141 Extruded nixtamalized blue tortillas (ENBT) were prepared according to the procedure reported
142 by Platt-Lucero et al., (2010) using the ENCF obtained previously. Four kilograms of the ENCF
143 was mixed (Model AS200, Hobart MFG. CO., Troy, Ohio, USA) for 3 min with an amount of
144 distilled water. After 20 min of resting, the obtained corn masa was processed in a commercial
145 tortillería (Tortillería Pimentel, Hermosillo, Sonora, México). The corn masa was placed in a
146 tortilla-forming machine (Model MLR 30, Lenin Manufactures, San Luis Potosí, México) to
147 form a masa disk of 25 g. Disks were baked in a three-zone oven, where the first, second and
148 third zones of the oven were heated to the following temperatures: 258 ± 10 °C, 308 ± 10 °C
149 and 257 ± 10 °C, respectively. The residence time in the oven was of 56 s. The baked tortillas
150 were cooled and subsequently lyophilized (Model 7753020, Labconco, Kansas City, Missouri,
151 USA), milled a mesh size of 2 mm, packed and stored at -20°C in polyethylene bags to avoid
152 moisture loss.

153 **2.6 Traditional nixtamalized white tortilla preparation**

154 Traditional nixtamalized white tortillas (TNWT) were prepared by mixing 225 g of commercial
155 flour with 315 ml of water to obtain corn dough (masa). Masa was wrapped in a plastic bag and
156 allowed to rest for 20 min before processing. Next, the masa was molded into a flat disk, using

157 a manual machine. The masa disks (25 g) were cooked on a hot griddle at 255 ± 10 ° C for 20 s
158 on one side, followed by 35 s on the other side, then flipped again during 15 s until expansion
159 (swelling). Once the tortillas were obtained, they were cooled, frozen (-80°C), and freeze-dried.
160 The tortillas were homogenized in a food processor and stored at -20°C in polyethylene bags
161 until analysis. The procedure was run in three replications.

162 **2.7 Simulated *in vitro* gastrointestinal digestion**

163 The procedure described by Valero-Cases, Nuncio-Jauregui and Frutos, (2017) and Valero-
164 Cases and Frutos, (2017), was used to perform the simulated gastrointestinal digestion. It
165 consisted of a three-step procedure to mimic the digestive process in the mouth (oral phase),
166 stomach (gastric phase) and small intestine (intestinal phase). Representation of the simulated
167 gastrointestinal digestion procedure carried out is presented in **Fig. 1.**

168 **2.7.1 Mouth digestion**

169 The digestion starts by adding artificial saliva: 10 g/L of α-amylase in 250 ml of a phosphate
170 buffer solution (PBS, pH 7) to 25 g of the of freeze-dried sample, which was incubated at 37 °C
171 for 2 min in a water bath shaker at low speed (200 rpm).

172 **2.7.2 Stomach digestion**

173 After the oral step, the sample was incubated under gastric conditions. For this, the pH of the
174 digested sample was decreasing to 2.5 with 1 M HCl and adding 3 g/L of pepsin, incubated for
175 2 h under the same conditions.

176 **2.7.3 Intestinal digestion**

To imitate the 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. 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), while the pellet is considered as the non-extractable fraction (NEF) available to uptake by gut microbiome. In both fractions SF and NEF, the phenolic and anthocyanins content was determinate. The *in vitro* gastrointestinal digestion was performed in triplicate in absence of light.

2.8 Bioaccessibility index

The bioaccessibility was calculated according Sánchez-Rodríguez, Cano-Lamadrid, Carbonell-Barrachina, Hernández & Sendra, (2020), equation [1]:

$$\text{Bioaccessibility index (\%)} = (PC_{SF} / PC_{TM}) \times 100 \quad (1)$$

Where, PC_{SF} is the phytochemical content (mg) in the soluble fraction of the digested sample after oral, gastric and intestinal digestion, and PC_{TM} is the total phytochemical content (SF + NEF) in test matrix (undigested sample).

2.9 Phytochemicals extraction before *in vitro* digestion

2.9.1 Phenolic compounds extraction

Free and bound phenolic compounds were extracted from each lyophilized sample 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. Supernatants were filtered using 0.45 μm nylon filters and concentrated at 35 °C using a vacuum evaporator. 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. The residue was then dissolved with 2 ml of 50 % methanol and filtered using 0.45 μm nylon filters and stored at −20 °C until use.

2.9.2 Anthocyanin's extraction

Anthocyanin extraction was carried out using the lyophilized samples and performed as follows: 4 grams of each sample was dissolved in 30 mL of acidified cold methanol (60% methanol, 37% water, 3% formic acid v/v/v). 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 steps were carried out under dark conditions to avoid anthocyanin degradation.

2.10 Quantification of phenolic content

Determination of phenolic content 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 (SF + NEF).

2.11 Quantification of anthocyanin content

The anthocyanin content of undigested and digested samples were analyzed according to Abdel-Aal and Huel (1999). Total anthocyanin content in each sample was determined by the sum of the two fractions (SF + NEF). 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 [2]:

$$\text{Anthocyanins (mg/kg)} = ((A * MW * DF * 1000) / \varepsilon)* (V/m) * 1000 \quad (2)$$

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.

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, Cuvelier & Berset, (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 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 at 593 nm, and 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 Bioaccessibility index

The bioaccesibility index of phenolic and anthocyanins of blue corn and both tortillas obtained after the *in vitro* gastrointestinal digestion are shown in **Fig. 2**.

In oral phase, the bioaccessibility of phenolic compounds present in BC, ENBT, and TNWT were 95, 69 and 207 %, respectively (**Fig. 2a**). All samples increased the bioaccesibility with respect to the initial values. 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).

The bioaccesibility values for anthocyanins in the undigested samples were obtained prepared extracts directly from the samples using an acidified methanol extraction and these values were assumed as the 100 %. When compared these values with the obtained in the oral phase, the bioaccesibility of the anthocyanins were reduced, reaching values of 58 and 39 % for BC and ENBT, respectively (**Fig. 2b**). Although, it has been reported that anthocyanins are degraded after the incubation with saliva under a neutral/weak basic pH condition, the low values of bioaccesibility found in this work does not necessarily indicate the complete reduction. According with Bello-Perez, Flores-Silva, Camelo-Méndez, Paredes-López & Figueroa-Cárdenas, (2015), anthocyanins can produce α-amylase inhibition by binding to their active sites with limited availability for amylolytic attack. In addition, the formation of strong non-covalent anthocyanin/starch interactions lead to the formation of insoluble networks structures. Those phenomena may lead to reduce the complete release of anthocyanins from the matrix into the oral fluid.

In gastric phase, a significant increase in the bioaccesibility of phenolic compounds in all samples was observed, showing TNWT higher values (738%) than BC (174%) and ENBT (134 %). The differences could be related to the composition of the matrix, the particles size and the processing treatment to which each food was subjected, which could affect its potential digestion. 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 released in traditional tortilla.

In the opposite side, 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 major bioaccesibility of phenolic compounds. Dietary fiber presents quickly hydration properties that generated increase in the tortuosity and viscosity of the environment. The viscosity in the intestine phase restricted the mixing process that promotes transport of enzymes to their substrates, serving as a barrier to bile salts and enzyme digestive action (Palafox-Carlos et al., 2011), explained in this form the lower recovery of the main phenolic compounds in ENBT than the TNWT.

Additionally, the bioaccesibility index for anthocyanins in gastric phase increased in blue corn (76%), and decreased in blue tortilla (29 %), when compared with the oral phase. The acidic environmental (HCl, pH 2) and digestive enzymes action could improve the release of anthocyanins bound to solid food matrix, and also could bring about some hydrolysis of high molecular compounds such as proanthocyanin oligomers increasing the amount of the

306 monomeric compounds quantified in the soluble fraction leading to a significant increase in
307 their concentrations after gastric digestion (Podsezdek, Redzynia, Klewicka & Koziołkiewicz,
308 2014; Stanisavljević et al., 2015). Our results confirm the data cited, that the conditions
309 occurring in gastric step cause a significant anthocyanins stability.
310 In intestinal phase, found the highest bioaccessibility values for phenolics in all the samples
311 evaluated (**Fig. 2a**). The bioaccesibility index for ENBT (163 %) was similar to the BC (202
312 %), and lowest than the TNWT (961%). These results were a higher value than that found in
313 other foods. He, Wallace, Keatley, Failla & Giusti, (2016) informed that the bioaccessibility
314 after intestinal digestion of phenolic compounds in five wild and two domesticated cereal grains
315 found in Zimbabwe in the small intestine ranges between 16.76 and 33.06 %.
316 Despite the BC and its tortilla (ENBT) had higher phenolic contents in the undigested samples,
317 the higher phenolic bioaccesibility post intestinal step was found in TNWT. In the TNWT due
318 to the long alkaline-cooking time, starch granules are presented in a more disrupted form,
319 resulting in gelatinised starch that is easily available to pancreatic amylase activity. It might be
320 possible that the pancreatic amylase activity produced the release of reducing sugars from the
321 hydrolysis of starch and peptides from the hydrolysis of proteins provoked the presence of non-
322 phenolic reducing substances during the *in vitro* digestion that may have reacted with the Folin
323 Ciocalteu reagent (Stanisavljević et al., 2013). As a consequence the quantification of phenolic
324 content in TNWT digested extracts was increased, obtaining a higher bioaccesibility values of
325 the oral, gastric and intestinal phase.
326 On contrary, the less damage starch present in BC and ENBT, as well, the presence of the
327 aleurone and pericarp fractions could explain the lower phenolic bioaccessibility values during
328 the *in vitro* digestion when compared to the traditional tortilla. In addition, according to

329 Camelo-Méndez, Agama-Acevedo, Tovar & Bello-Pérez, (2017) polyphenol-rich extracts from
330 blue maize reduced the activity of enzymes in the *in vitro* gastrointestinal digestion more than
331 the white maize extract, due to the higher levels and different compositions of polyphenol
332 compounds, which could produce a reduction in their bioaccessibility.
333 Respect to the anthocyanins bioaccesibility at the end of intestinal phase, these compounds were
334 profoundly affected, obtaining values of 30% for BC and 18% for ENBT (**Fig. 2b**). This
335 observation is in agreement with the work carried out by Rodríguez-Roque, Rojas-Graü, Elez-
336 Martínez, & Martín-Belloso, (2013) who reported that the total flavonoids present in soymilk
337 after the intestinal step showed a bioaccessibility of 16%. According with the literature, under
338 intestinal pH (7.4), anthocyanins change their structure to quinoids, hemiketal and chalcone
339 forms due to anthocyanin chromophore destruction, leading to their colourless form (Peixoto et
340 al., 2016).
341 Anthocyanins and phenolic compounds that after digestion were not released from the food
342 matrix could reach to the large intestine, then be subjected to extensive transformation by
343 colonic microflora which could result in metabolites more biologically active than the original
344 compounds (Pérez-Jiménez, Díaz-Rubio & Saura-Calixto, 2013). These metabolites could be
345 absorbed though the portal vein, reaching the liver giving rise to phase II, induce their sulfation,
346 glucuronidation, and methylation (Han et al., 2019). Once formed these metabolites, may return
347 to the digestive tube through the bile, or pass into the bloodstream as a first step and delivered
348 to the appropriate location within the body to exert pharmacological activity in different tissues
349 and organs (Pérez-Jiménez et al., 2013). In addition, the metabolites and/or catabolites produced
350 by the colonic bacteria can encourage the growth of beneficial bacteria and inhibit the growth

of pathogenic bacteria, exerting a local health effect promoting an antioxidant environment (Saura-Calixto, 2011).

3.2 Changes on phytochemical content during *in vitro* digestion

3.2.1 Changes on phytochemical during oral phase

The phenolic content in soluble fraction of oral digested extracts ranged from 125.8 to 168.4, whereas that of insoluble fraction ranged from 14.8 to 57.1 mg GAE/100 g (dw) (**Table 1**). Results showed higher solubilisation of these compounds in SF with respect to the undigested values. The α-amylase activity, and 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 bound phenolic compounds into the oral digested fluid occur (Palafox-Carlos et al., 2011).

After the simulated oral digestion, anthocyanins content of BC and ENBT recovered in the soluble fraction were lower (89.1 and 39.5 mg CGE/kg (dw)) than the undigested values (**Table 2**). Despite it, the remained antothocyanins in the insoluble fraction (18.2 mg CGE/kg for BC and 25.5 mg CGE/kg ENBT), could indicated a moderated effects in anthocyanin degradation in oral phase 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). Although, a certain amount of these compounds can be degraded in the oral phase, anthocyanins could improve oral cavity health through modulating the oral microbiota concentration (Han et al., 2019).

3.2.2 Changes on phytochemical during gastric phase

371 The total phenolic content in extracts obtained following gastric phase were 348.8, 306.2 and
372 530.9 mg GAE/100 g (dw), for BC, ENBT and TNWT, respectively. Their content was
373 increased with respect to the total values obtained in oral digestion, and was still higher than
374 non-digested values (**Table 1**). The high presence of phenolic compounds in SF indicates that a
375 greater amount of insoluble phenolic compounds were released from the food matrix as a result
376 of enzymatic digestion.
377 After gastric step (pepsin/HCl digestion), anthocyanins content found in SF were increased in
378 both samples with reference to oral phase, although their contents were still lower than
379 undigested values (**Table 2**). This is according with previous studies reported that anthocyanins
380 from different fruits and vegetables were stable under acidic gastric conditions (Podsezdek et
381 al., 2014). Mosele, Macià, Romero, Motilva & Rubió, (2015) reported a slightly increased, 1.75
382 to 4.39 %, in anthocyanins released of pomegranate products after the gastric digestion. The low
383 pH (1.5–3.5) in gastric phase would favour the high stability of these molecules, maintaining
384 the natural structural form, the flavylium cation (red color) (Cavalcante-Braga, Murador,
385 Mendes de Souza Mesquita, & Vera de Rosso, 2018). According with the literature, anthocyanin
386 absorption can begin in the stomach and appear in the blood almost immediately after ingestion
387 of food (Han et al., 2019).
388 **3. 2.3 Changes on phytochemical during intestinal phase**
389 In the intestinal digestion there was an increased of phenolic compounds in the soluble fraction
390 with respect to the gastric values, and their contents were still higher than oral, and undigested
391 values (**Table 1**). The higher phenolic concentration of the SF in the intestinal digested samples
392 compared to the gastric samples could be due to the additional digestion time (2 h), the alkaline
393 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.

The total anthocyanins content in extracts obtained following intestinal phase were 53.6 and 29.3 mg CGE /kg dw for BC and ENBT, respectively (**Table 2**). The decreasing trend observed in anthocyanin concentration after the intestinal step was in accordance with other works (Correa-Betanzo et al., 2014; He et al., 2009).

The low recovery of anthocyanins during pancreatic digestion may be mainly due to transformation of the flavylium cation into a new structural forms. In the small intestine under basic conditions anthocyanins are present in their carbinol and chalcone forms. Chalcone, are not stable at the physiological conditions of the intestine and are rapidly metabolized into phenolic acids. The main phenolic acids generated from the degradation of anthocyanins are protocatechuic, gallic, syringic, vanillic and *p*-hydroxybenzoic acids. Anthocyanins that conserved their native glycosylated form can be absorbed through intestinal epithelial cells via a Na + dependent glucose transporter (GLUT 2), reaching to the brain, eye, and other organs with a maximum concentration of nanomolar levels (Fernandes, Faria, Calhau, de Freitas & Mateus, 2013; Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004; Passamontia, Vrhovsek & Mattivi, 2002).

3.3 Changes on antioxidant capacity during *in vitro* digestion

In DPPH assay, after oral and gastric phases, despite the increase in phenolic content in the intestinal phase, we did not observe increase of the antioxidant capacity (**Fig. 3a**). The antioxidant capacity of digested extracts decreased by 75 %, 63 % and 51 % for BC, ENBT and TNWT, with respect to undigested values. 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.

While in anthocyanins extracts, the DPPH scavenging capacity after oral phase increased 95 % in BC, while in ENBT a slight decreased (13 %) was observed with respect to undigested values (**Fig. 4a**). In gastric and intestinal phases, DPPH scavenging capacity in BC was similar with those obtained in the oral step, while the ENBT conserved the 67 % of the DPPH scavenging capacity in gastric phase compared with the undigested values, and remained without changes after the intestinal step.

Regarding to ABTS assay, after oral phase, increased in the antioxidant capacity of phenolic compounds of BC and TNWT was achieved (53 and 72 % respectively), while in ENBT this capacity decreased by 45% (**Fig. 3b**). The intestinal digestion caused a deep increased in the ABTS scavenging capacity for all samples with respect to the corresponding undigested samples, with higher ABTS values of ENBT and TNWT than BC. This trend are not surprising, considering that an increase in antioxidant activity could be associated with the major release of phenolic compounds, probably due to the more effective digestion during intestinal phase compared to the oral and gastric steps, resulting in a gradual release of phenolic compounds with scavenging properties (Gullon, Pintado, Fernández-López, Pérez-Álvarez, &Viuda-Martos, 2015). 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.

In other hand, the ABTS scavenging capacity of anthocyanins in oral phase increased 71 % in BC, while in ENBT a deep decreased (86 %) was observed with respect to undigested values

(**Fig. 4b**). In gastric step, the ABTS scavenging capacity increased 165 % in BC and remained constant in blue tortilla when compared with the undigested samples. In the intestinal phase, BC showed a significative decreased in ABTS scavenging capacity compared with the gastric phase, while the antioxidant capacity of ENBT was 65 % lower than the undigested values. According to Wootton-Beard, Moran & Ryan, (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).

Regarding to ferric reducing power (FRAP) of phenolic compounds, **Fig. 3c** shows that after oral phase, the reducing power decreased with respect to undigested samples and remained without significant changes at the end of the digestion. A similar decrease in FRAP values of phenolic compounds of different varieties of apples after the gastrointestinal digestion was reported by Bouayed, Hoffmann & Bohn, (2011).

Additionally, the reducing power of anthoyanins in oral phase was increased 124 % in BC and decreased 39 % in ENBT (**Fig. 4c**). After the oral phase, the gastric and intestinal conditions do not cause a negative impact on the reducing capacity of anthocyanins, preserving this capacity even at the end of digestion, as was observed with phenolic compounds.

The differences in trends observed in the antioxidant assays may reflect that a proportion of the compounds may have transformed into new different structural forms due to the digestion process, which altered their reactivity and the ability to transfer electrons (Yildirim et al., 2001).

4. **Conclusions**

Gastrointestinal digestion contributed strongly to improve the bioaccesibility of the bound phenolic compounds in corn tortillas, mainly in the intestinal phase. Although the pH changes

during the digestion process affected the anthocyanin content, we hypothesize that the solid-whole food matrix could play a protective role in the prevention on the 100% losses, increasing their stability in the gastric phase. Phytochemicals present in corn tortillas have the potential to protect against oxidative damage, due to their free radical scavenger capacity even at the end of digestion, which is essentially important since corn is consumed as processed. The compounds that were not solubilized during digestion, and remained into the non-extractable fraction could be metabolized in the colon exerting local antioxidant effects. The information generated allows to obtain preliminary results before advancing to *in vivo* studies and provide valuable information to the industry involved in tortilla production that can help in the development of new functional and nutraceutical products. However, further studies are needed.

Author´s contributions

Mariela Menchaca-Armenta: Investigation, Writing - Original Draft preparation. Benjamín Ramírez-Wong and María José Frutos: Conceptualization, Resources. Estefanía Valero-Cases and Ángel A. Carbonel Barrachina: Methodology. Armando Quintero-Ramos: Formal analysis. Raquel Muelas-Domingo: Supervision. Patricia I. Torres-Chávez: Writing - Review & Editing. Ana I. Ledesma-Osuna, and Olga N. Campas-Baypoli: Validation.

Declaration of competing interests

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References

1. Abdel-Aal, E.S.M., & Huel, P. (1999). A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheats. Cereal Chemistry, 76(3), 350-354. https://doi.org/10.1094/cchem.1999.76.3.350
2. Adarkwah-Yiadom, M., & Duodu, K.G.(2017). Effect of extrusion cooking and simulated in vitro gastrointestinal digestion on condensed tannins and radical scavenging activity of type II and type III whole grain sorghum. International Journal of Food Science & Technology, 52 (10), 2282-2294. https://doi.org/10.1111/ijfs.13510
3. Adom, K.K., & Liu, R.H. (2002). Antioxidant activity of grains. Journal of Agricultural and Food Chemistry, 50(21), 6182–6187. https://doi.org/10.1021/jf0205099
4. Alminger, M., Aura, A.-M., Bohn, T., Dufour, C., El, S.N., Gomes, A., Karakaya, S., Martínez-Cuesta, M.C., McDougall, G.J., Requena, T., & Santos, C.N. (2014). In vitro models for studying secondary plant metabolite digestion and bioaccessibility. Comprehensive Reviews in Food Science and Food Safety, 13(4), 413–436. https://doi.org/10.1111/1541-4337.12081
5. Bello-Pérez, L.A., Flores-Silva, P.C., Camelo-Méndez, G.A., Paredes-López, O., & Figueroa-Cárdenas, J.D. (2015). Effect of nixtamalization process on dietary fiber content, starch digestibility, and antioxidant capacity of blue maize tortilla. Cereal Chemistry, 92 (3), 265-270. https://doi.org/10.1094/cchem-06-14-0139-r

6. Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. Analytical Biochemistry, 239(1), 70−76. https://doi.org/10.1006/abio.1996.0292
7. Bouayed, J., Hoffmann, L., & Bohn, T. (2011). Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: Bioaccessibility and potential uptake. Food Chemistry, 128, 14–21. https://doi.org/10.1016/j.foodchem.2011.02.052
8. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology, 28(1), 25−30. https://doi.org/10.1016/s0023-6438(95)80008-5
9. Camelo-Méndez, G.A., Agama-Acevedo, E., Tovar, J., & Bello-Pérez, L. A. (2017). Functional study of raw and cooked blue maize flour: Starch digestibility, total phenolic content and antioxidant capacity. Journal of Cereal Science, 76, 179–185. https://doi.org/10.1016/j.jcs.2017.06.009
10. Carbonell-Capella, J.M., Buniowska, M., Barba, F.J., Esteve, M.J., & Frígola, A. (2014). Analytical Methods for Determining. (2014). Bioavailability and bioaccessibility of bioactive compounds from fruits and vegetables: a review. Comprehensive Reviews in Food Science and Food Safety, 13(2),155-171. https://doi.org/10.1111/1541-4337.12049
11. Cavalcante Braga, A.R., Murador, D.C., Mendes de Souza Mesquita, L., & Vera de Rosso, V.(2018). Bioavailability of anthocyanins: Gaps in knowledge, challenges and future research. Journal of Food Composition and Analysis,68, 31–40. https://doi.org/10.1016/j.jfca.2017.07.031

12. Chandrasekara, A., & Shahidi, F. (2012). Bioaccessibility and antioxidant potential of millet grain phenolics as affected by simulated in vitro digestion and microbial fermentation. Journal of Functional Foods, 4(1), 226–237. https://doi.org/10.1016/j.jff.2011.11.001
13. Correa-Betanzo, J., Allen-Vercoe, E., McDonald, J., Schroeter, K., Corredig, M., & Paliyath, G. (2014). Stability and biological activity of wild blueberry (Vaccinium angustifolium) polyphenols during simulated in vitro gastrointestinal digestion. Food Chemistry, 165, 522–531. http://dx.doi.org/10.1016/j.foodchem.2014.05.135
14. Fernandes, I., Faria, A., Calhau, C., de Freitas, V., & Mateus, N. (2013). Bioavailability of anthocyanins and derivatives. Journal of Functional Foods,7 54 -66. https://doi.org/10.1016/j.jff.2013.05.010
15. Gullon, B., Pintado M.E., Fernández-López J., Pérez-Álvarez J.A., &Viuda-Martos M. (2015). In vitro gastrointestinal digestion of pomegranate peel (Punica granatum) flour obtained from co-products: Changes in the antioxidant potential and bioactive compounds stability. Journal of Functional Foods, 19, 617–628. https://doi.org/10.1016/j.jff.2015.09.056
16. Han, F., Yang, P., Wang, H., Fernandes, I., Mateus, N., & Liu, Y. (2019). Digestion and absorption of red grape and wine anthocyanins through the gastrointestinal tract. Trends in Food Science & TechnologyVolume, 83, 211-224. https://doi.org/10.1016/j.tifs.2018.11.025
17. He, J., Wallace, T.C., Keatley, K.E., Mark L. Failla, M. Giusti, M. (2009). Stability of black raspberry anthocyanins in the digestive tract lumen and transport efficiency into

548 gastric and small intestinal tissues in the rat. Journal of Agricultural and Food Chemistry,
549 57(8), 3141–3148. https://doi.org/10.1021/jf900567t
550 18. Liu, R. H. (2007). Whole grain phytochemicals and health. Journal of Cereal Science,
551 46(3), 207–219. https://doi.org/10.1016/j.jcs.2007.06.010
552 19. Lucas-González, R., Navarro-Coves, S., Pérez-Álvarez, J. A., Fernández-López, J.,
553 Muñoz, L. A., & Viuda-Martos, M. (2016). Assessment of polyphenolic profile stability
554 and changes in the antioxidant potential of maqui berry (Aristotelia chilensis (Molina)
555 Stuntz) during in vitro gastrointestinal digestion. Industrial Crops and Products, 94, 774–
556 782. https://doi.org/10.1016/j.indcrop.2016.09.057
557 20. Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols:
558 food sources and bioavailability. The American Journal of Clinical Nutrition, 79(5),727–
559 47. https://doi.org/10.1093/ajcn/79.5.727
560 21. Martínez-Bustos, F., Figueroa, J.D.C., Sánchez-Sinencio, F., González-Hernandez, J.,
561 Martínez, J.D.L., & Ruiz, M.T. (1996). Extrusion apparatus for the preparation of instant
562 fresh corn dough or masa. Patent U.S., number 5,558,886.
563 22. Menchaca-Armenta, M., Ramírez-Wong, B., Torres-Chávez, P.I., Quintero-Ramos, A.,
564 Ledesma-Osuna, A.I., Frutos-Fernández, M.J., Gutiérrez-Dorado, R. Campas-Baypoli,
565 O. N., & Morales-Rosas, I. (2020). Effect of extrusion conditions on the anthocyanin
566 content, functionality, and pasting properties of obtained nixtamalized blue corn flour
567 (Zea mays L.) and process optimization. Journal of Food Science, 85(7), 2143-2152
568 https://doi.org/10.1111/1750-3841.15312
569 23. Mora-Rochín, S., Gutiérrez-Uribe, J.A., Serna-Saldívar, S.O., Sánchez-Peña, P., Reyes-
570 Moreno, C., & Milán-Carrillo, J. (2010). Phenolic content and antioxidant activity of

571 tortillas produced from pigmented corns processed by conventional nixtamalization of
572 extrusion cooking. Journal of Cereal Science, 52(3), 502-508.
573 https://doi.org/10.1016/j.jcs.2010.08.010
574 24. Mosele, J.I., Macià, A., Romero, M.P., Motilva, M.J., & Rubió, L. (2015) Application
575 of in vitro gastrointestinal digestion and colonic fermentation models to pomegranate
576 products (juice, pulp and peel extract) to study the stability and catabolism of phenolic
577 compounds. Journal of functional foods, 14, 529–540.
578 https://doi.org/10.1016/j.jff.2015.02.026
579 25. Palafox-Carlos, H., Ayala-Zavala, J.F., & González-Aguilar, G.A. (2011). The role of
580 dietary fiber in the bioaccessibility and bioavailability of fruit and vegetable
581 antioxidants. Journal of Food Science, 76(1), R6-R15. https://doi.org/10.1111/j.1750-
582 3841.2010.01957.x
583 26. Parada, J., & Aguilera, J. (2007). Food microstructure affects the bioavailability of
584 several nutrients. Journal of Food Science,72(2), R21-R32.
585 https://doi.org/10.1111/j.1750-3841.2007.00274.x
586 27. Paredes-López, O., Guevara-Lara, F., & Bello-Pérez, L. A. (2009). La nixtamalización
587 y el valor nutritivo del maíz. Ciencias, 92, 60-70.
588 28. Passamonti, S., Vrhovsek, U., & Mattivi, F. (2002). The interaction of anthocyanins with
589 bilitranslocase. Biochemical and Biophysical Research Communications, 296 (3), 631–
590 6. https://doi.org/10.1016/s0006-291x(02)00927-0
591 29. Peixoto, F.M., Fernandes, I., Gouvêa, A.C.M.S., Santiago, M.C.P.A., Galhardo
592 Borguini, R., Mateus, N., Freitas, V., Godoy, R.L.O., & Ferreira, I. M.P.L.V.O. (2 0
593 1 6). Simulation of in vitro digestion coupled to gastric and intestinal transport models

to estimate absorption of anthocyanins from peel powder of jabuticaba, jamelão and jambo fruits. Journal of Functional Foods, 24, 373–381. https://doi.org/10.1016/j.jff.2016.04.021

30. Pérez-Jiménez J., Díaz-Rubio, M.E., & Saura-Calixto, F. (2013). Non-extractable polyphenols, a major dietary antioxidant: occurrence, metabolic fate and health effects. Nutrition Research Reviews, 26(2), 118–129. https://doi.org/10.1017/s0954422413000097
31. Platt-Lucero, L.C., Ramírez-Wong, B., Torres-Chávez, P.I., López-Cervantes, J., Sánchez-Machado, D.I., Reyes-Moreno, C., Milán-Carrillo, J., & Morales-Rosas, I. (2010). Improving textural characteristics of tortillas by adding gums during extrusion to obtain nixtamalized corn flour. Journal of Texture Studies, 41(5), 736-755. https://doi.org/10.1111/j.17454603.2010.00252.x
32. Podsezdek, A., Redzynia, M., Klewicka, E. & Koziołkiewicz, M. (2014). Matrix effects on the stability and antioxidant activity of red cabbage anthocyanins under simulated gastrointestinal digestion. BioMed Research International, 1–11. https://doi.org/10.1155/2014/365738
33. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26(9-10), 1231−1237. https://doi.org/10.1016/s0891-5849(98)00315-3
34. Rodríguez-Roque, M. J., Rojas-Graü, M. A., Elez-Martínez, P., & Martín-Belloso, O. (2013). Soymilk phenolic compounds, isoflavones and antioxidant activity as affected by *in vitro* gastrointestinal digestion. Food Chemistry, 136, 206–212.

35. Sánchez-Rodríguez, L., Cano-Lamadrid, M., Carbonell-Barrachina, A.A., Hernández, F., & Sendra, E. (2020). Impact of gastrointestinal *In Vitro* digestion and deficit irrigation on antioxidant activity and phenolic content bioaccessibility of "Manzanilla" table olives. Journal of Food Quality. https://doi.org/10.1155/2020/6348194
36. Saura-Calixto, F. (2011) Dietary fibre as a carrier of dietary antioxidants: an essential physiological function. Journal of Agricultural and Food Chemistry,59(1), 43–49. https://doi.org/10.1021/jf1036596
37. Singleton, V.L., & Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American Journal of Enology and Viticulture,16,144–158.
38. Stanisavljević, N., Jovanović, Ž. Čupić, T., Lukić, J., Miljuš Đukić, J., Radović, S., Mikić, A. (2013). Extractability of antioxidants from legume seed flour after cooking and in vitro gastrointestinal digestion in comparison with methanolic extraction of the unprocessed flour. International journal of food science & technology, 48 (10): 2096-2104. https://doi.org/10.1111/ijfs.12191
39. Stanisavljević, N., Samardžić, J., Janković, T., Šavikin, K., Mojsin, M., Topalović, V., & Stevanović, M. (2015). Antioxidant and antiproliferative activity of chokeberry juice phenolics during in vitro simulated digestion in the presence of food matrix. Food Chemistry, 175, 516–522. https://doi.org/10.1016/j.foodchem.2014.12.009
40. Valero-Cases, E., Frutos, M.J. (2017). Effect of inulin on the viability of L. plantarum during storage and *in vitro* digestion and on composition parameters of vegetable fermented juices. Plant Foods for Human Nutrition, 72, 161–167. https://doi.org/10.1007/s11130-017-0601-x

41. Valero-Cases, E., Nuncio-Jauregui, N., & Frutos, M. J. (2017). Influence of fermentation with different lactic acid bacteria and in vitro digestion on the biotransformation of phenolic compounds in fermented pomegranate juices. Journal of Agriculture and Food Chemistry, 65(31), 6488- 6496. https://doi.org/10.1021/acs.jafc.6b04854

42. Wootton-Beard, P. C., Moran, A., & Ryan, L. (2011). Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after in vitro digestion measured by FRAP, DPPH, ABTS and Folin–Ciocalteau methods. Food Research International, 44, 217–224. https://doi.org/10.1016/j.foodres.2010.10.033

43. Yildirim, A., Mavi, A., & Kara, A. (2001). Determination of antioxidant and antimicrobial activities of Rumex crispus L. extracts. Journal of Agricultural and Food Chemistry, 49(8),4083–4089. https://doi.org/10.1021/jf0103572

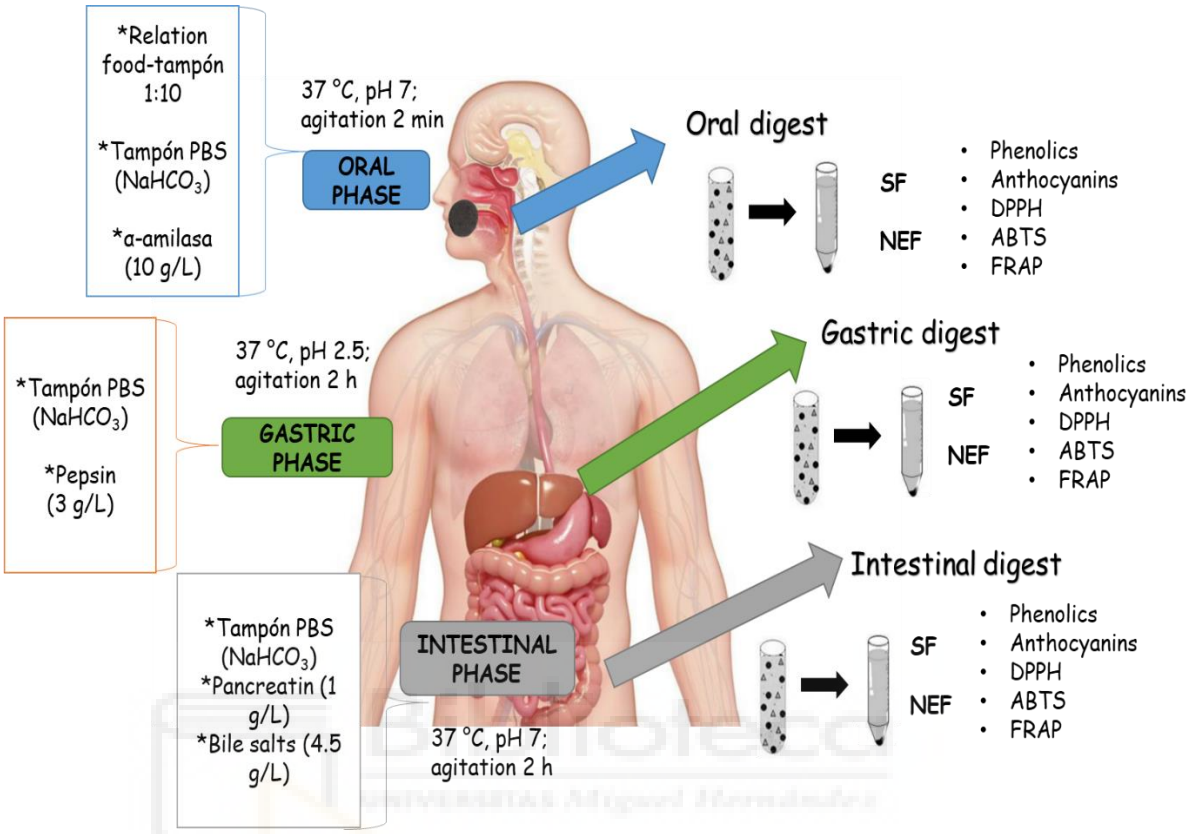

Fig. 1. Graphic representation of the simulated gastrointestinal digestion procedure.

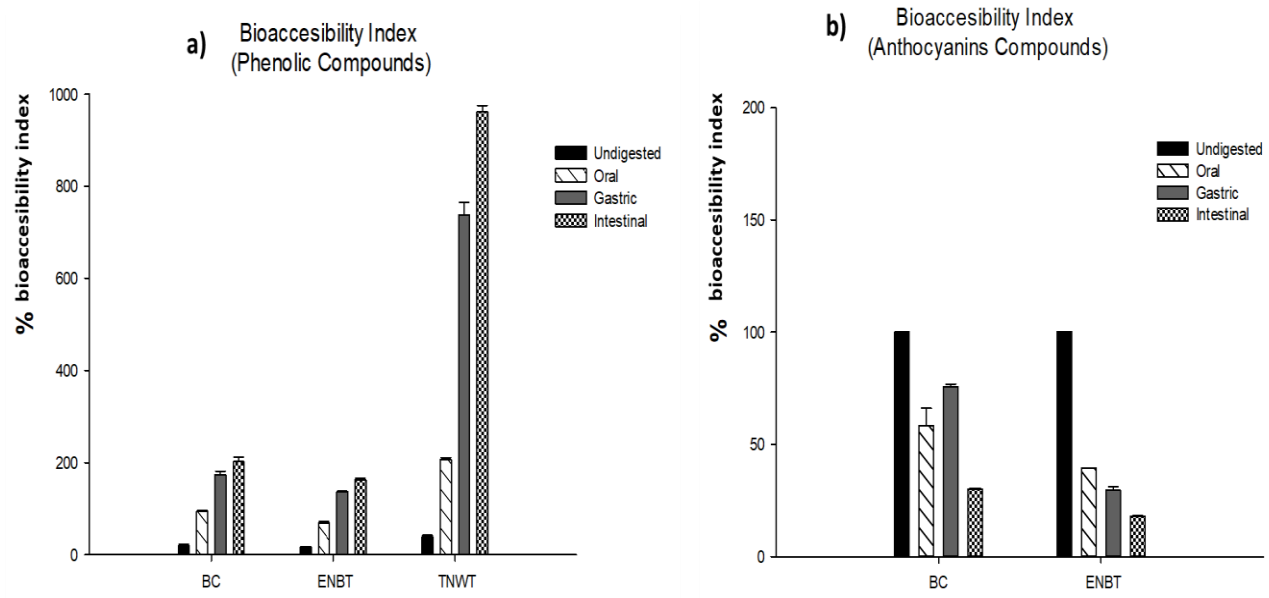

Fig. 2. Bioaccesibility index of a) phenolic and b) anthocyanins compounds obtained after each step of *in vitro* gastrointestinal digestion (oral, gastric and intestinal) of raw corn and tortillas. Data are means ± SD (n = 3).

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 ($p < 0.05$).

720 **Table 2**. Anthocyanins content of soluble (SF) and non-extractable fraction (NEF) of blue corn
721 (BC) and extruded nixtamalized blue tortilla (ENBT) before and after the simulated
722 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

723 [1]CGE, cyanidin 3-glucoside equivalents.
724 [2]Data are means ± SD (n = 3).
725 [3]Different lowercase letters denote significant difference on anthocyanin content between different phases of *in*
726 *vitro* digestion for the same type of product ($p < 0.05$).
727 [4]Different capital letters denote significant difference on anthocyanin content between the type of product for the
728 same phase of *in vitro* digestion ($p < 0.05$).
729 [5]N.D. not detected.
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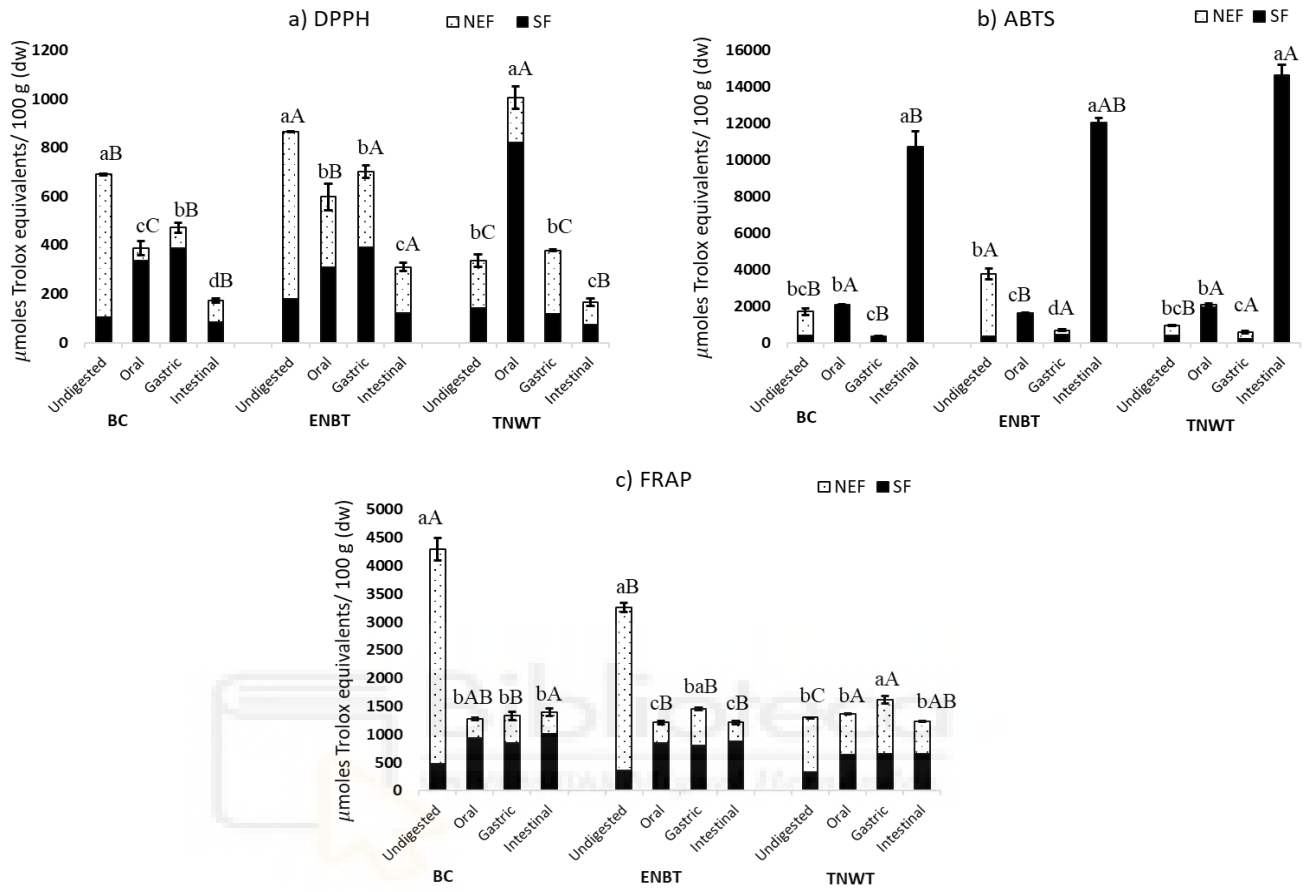

Fig. 3. Antioxidant capacity of phenolic compounds (μmol ET/ 100 g dw) of initial (undigested sample), oral, gastric and intestinal digested samples of blue corn and tortillas measured by three different methods **a)** DPPH, **b)** ABTS and **c)** FRAP. Data are means ± SD (n = 3). Different lowercase 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 ($p < 0.05$).

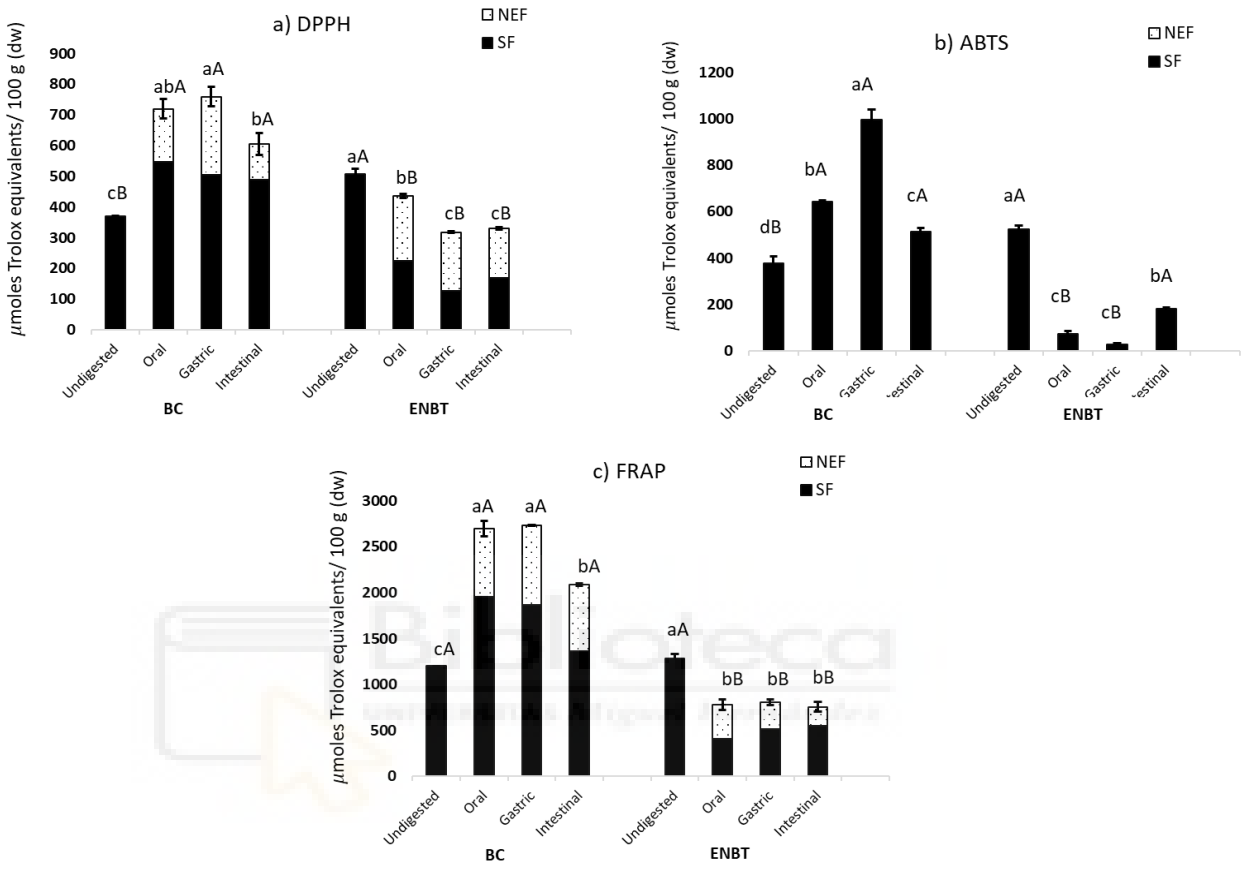

Fig. 4. Antioxidant capacity of anthocyanins (μmol ET/ 100 g dw) of initial (undigested sample), oral, gastric and intestinal digested samples of blue corn and extruded nixtamalized blue tortilla measured by three different methods **a)** DPPH, **b)** ABTS and **c)** FRAP. Data are means ± SD (n = 3). 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.

CAPÍTULO 6. CONCLUSIONES GENERALES

6.1 CONCLUSIONES

1. La metodología de superficie de respuesta (MSR) se aplicó con éxito para evaluar y modelar los efectos de los tres factores del proceso de nixtamalización por extrusión (HA, TE y VT) sobre las propiedades químicas, funcionales y amilográficas de las harinas nixtamalizadas extruidas.

2. 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.

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

4. 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.

5. Los resultados sugieren que el modelo de primer orden fue adecuado para predecir la cinética de degradación térmica de las antocianinas de los productos nixtamalizados extrudidos a base de maíz azul a temperaturas que oscilan entre 60 y 95 °C. Y que el modelo de Arrhenius se ajustó adecuadamente a los tratamientos evaluados, con coeficientes de determinación (R^2) superiores a 0.996.

6. Los parámetros cinéticos se vieron afectados por la temperatura. Cuanto mayor la temperatura, mayor la constante de velocidad de degradación (*k*), menor el tiempo de vida media (t ½) y un menor tiempo de reducción decimal (D) de todas las muestras. 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.

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 cinética de degradación reveló que las antocianinas presentes en el maíz crudo fueron menos susceptibles a la degradación en las temperaturas elevadas, mientras que la harina y la tortilla siguieron un patrón similar de degradación, aunque la preparación de tortilla usa temperaturas superiores a la utilizada en el proceso de extrusión.

9. 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 y bioaccesibilidad fenólica más alta se encontró en la fase intestinal para todas las muestras.

10. 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. En la fase intestinal fue posible lograr valores de bioaccesibilidad de las antocianinas de un 30 % en el maíz azul y un 18 % la tortilla extrudida, sin embargo, la mayor estabilidad se observó en la fase gástrica.

11. Los resultados sugieren que los fitoquímicos presentes en el maíz azul y las tortillas podrían ejercer efectos antioxidantes en el tracto gastrointestinal después de ser sometidos al proceso de digestión con posibles efectos beneficiosos para la salud de los seres humanos, ya que son potencialmente bioaccesibles y muestran capacidad de eliminación de radicales libres de acuerdo a los tres metódos evaluados.

12. Se concluye que el enfoque de este estudio puede brindar información valiosa que sirva como guía para desarrollar y optimizar productos innovadores, funcionales y nutraceúticos a base de maíz pigmentado con pérdidas mínimas en compuestos biológicamente activos y predecir sus efectos antioxidantes potenciales *in vivo* una vez que estos son consumidos.

6.2 CONCLUSIONS

1. The response surface methodology (RSM) was successfully applied to evaluate and model the effects of the three factors of the extrusion nixtamalization process (FM, T and SS) on the chemical, functional and amylographic properties of the extruded nixtamalized corn flours.

2. It was possible to optimize the extrusion nixtamalization process obtaining a flour with minimal losses in biologically active compounds such as anthocyanins (health promoters) without negatively affecting the quality of the tortilla obtained evaluated as texture.

3. The factors of the process of nixtamalization of corn by extrusion (FM, T and SS) affected all the parameters evaluated in the flours. Feed moisture (FM) was the factor that had the greatest effect on water absorption, peak viscosity and anthocyanin content, in linear and quadratic terms.

4. The optimal qualities of the flour in terms of maximum anthocyanin content (226.07 mg / kg) and maximum viscosity (1063.9 cP) were found with a feed moisture of 18.17%, a temperature of the fourth zone of the extruder of 92.03 ° C and a screw speed of 76.61 rpm.

5. The results suggest that the first order model was adequate to predict the kinetics of thermal degradation of anthocyanins in blue corn extruded nixtamalized products at temperatures ranging between 60 and 95 ° C. And that the Arrhenius model was adequately adjusted to the evaluated treatments, with determination coefficients (R2) higher than 0.996.

6. Kinetic parameters were affected by temperature. The higher the temperature, the higher the degradation rate constant (k), the shorter the half-life time (t ½) and the shorter the

decimal reduction time (D) of all samples. On the other hand, the higher the Q10 coefficient and the activation energy (Ea), the greater the dependence of the transformation of anthocyanins on temperature.

7. The ΔH, ΔG and ΔS indicated that the degradation of anthocyanins in extruded nixtamalized corn products at any temperature was an endothermic and not spontaneous reaction, requiring the application of an external heat source to carry out the transition of these molecules.

8. The degradation kinetics revealed that the anthocyanins present in raw corn were less susceptible to degradation at elevated temperaturas, while the flour and tortilla followed a similar pattern of degradation, although the tortilla preparation uses temperatures higher than that used in the extrusion process.

9. Gastrointestinal digestion strongly contributed to the release of bound phenolic compounds from the food matrix of blue corn and both tortillas. The highest phenolic concentration and bioaccessibility was found in the intestinal phase for all samples.

10. Although the different phases of digestion affected the anthocyanin content due to changes in pH, the food matrix played an important role in preventing the total loss of these compounds. In the intestinal phase it was possible to achieve bioaccessibility values of anthocyanins of 30% in blue corn and 18% in extruded tortilla, however, the greatest stability was observed in the gastric phase.

11. The results suggest that the phytochemicals present in blue corn and tortillas could exert antioxidant effects on the gastrointestinal tract after being subjected to the digestion process with possible beneficial effects on human health, since they are potentially bioaccessible and show the ability to eliminate free radicals according to the three evaluated methods.

12. It is concluded that the approach of this study can provide valuable information that serves as a guide to develop and optimize innovative, functional and nutraceutical products based on pigmented corn with minimal losses in biologically active compounds and predict their potential antioxidant effects *in vivo*.

CAPÍTULO 7. CONSIDERACIONES FINALES Y RECOMENDACIONES

7.1 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.

7.2 RECOMENDACIONES

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.

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.

CAPÍTULO 8. REFERENCIAS

8. REFERENCIAS

Abdel-Aal El-Sayed M., Young J. C., Rabalski, I. (2006). Anthocyanin composition in black, blue, pink, purple, and red cereal grains. *Journal of Agricultural and Food Chemistry*, 54,4696-4704.

Adams, J.B. (1973). Thermal degradation of anthocyanins with particular reference to the 3-glycosides of cyanidin in acidified aqueous solution at 100 °C. *Journal of Science and Food Agriculture*, 24,747–762.

Agama, E. (2011). Características físicas y químicas de dos razas de maíz azul. *Revista Mexicana de Ciencias Agrícolas*, 3, 317-329.

Ahmad-Qasem, M.H., Canovas, J., Barrajon-Catalan, E., Carreres, J.E., Micol, V. & Garcia-Perez, J.V. (2014). Influence of olive leaf processing on the bioaccessibility of bioactive polyphenols. *Journal of Agricultural and Food Chemistry*, 62, 6190–6198.

Alam, M. S., Kaur, J., Khaira, H., & Gupta, K. (2016). Extrusion and extruded products: changes in quality attributes as affected by extrusion process parameters: a review. *Critical Reviews in Food Science and Nutrition*, 56 445-473.

Alminger, M., Aura, A.-M., Bohn, T., Dufour, C., El, S.N., Gomes, A., Karakaya, S., Martínez-Cuesta, M.C., McDougall, G.J., Requena, T., & Santos, C.N. (2014). In vitro models for studying secondary plant metabolite digestion and bioaccessibility. *Comprehensive Reviews in Food Science and Food Safety*, 13, 413–436.

Altan, A., McCarthy, K.L., & Maskan, M. (2009). Effect of extrusion process on antioxidant activity, total phenolics and β-glucan content of extrudates developed from barley-fruit and vegetable by-products. *International Journal of Food Science and Technology*,44,1263-

Barros, F., Awika, J.M. & Rooney, L.W. (2012). Interaction of tannins and other sorghum phenolic compounds with starch and effects on in vitro starch digestibility. *Journal of Agricultural and Food Chemistry*, 60,11609-11617.

Bello-Pérez, L.A., Camelo-Mendez, G.A., Agama-Acevedo, E., & Utrilla-Coello, R.G. (2016). nutraceutic aspects of pigmented maize: digestibility of carbohydrates and anthocyanins. *Agrociencia* ,50, 1041-1063.

Betrán, F., Bockholt, A. & Rooney, L. (2001). Blue corn in specialy corns. *Food Hydrocolloids*, 5,455-467.

Biruete-Guzmán, B., Juárez-Hernández, E., Siero-Ortega, E., Romero-Viruegas, R., & Silencio-Barrita, J.L. (2009). Nutraceutics. What is convenient to know. *Revista Mexicana de Pediatría*, 76, 136-145.

Bolea, C., Turturică, M., Stănciuc, N., & Vizireanu, C. (2016). Thermal degradation kinetics of bioactive compounds from black rice flour (Oryza sativa L.) extracts. *Journal of Cereal Science*,71, 160-166.

Bordenave, N., Hamaker, B. R., & Ferruzzi, M. G. (2014). Nature and consequences of non-covalent interactions between flavonoids and macronutrients in foods. *Food & Function*, 5, 18–34.

Brown, T.E., Le-May, H.E., Bursten, B.E., Burdge, J.E. (2004). Química. La ciencia central. 9a ed. México: Ed Pearson

Cabrita, L., Fossen, T., & Andersen, O. M. (2000). Colour and stability of the six common anthocyanidin 3-glucosides in aqueous solutions. *Food Chemistry*, 68,101–107.

Cai, Y. Z., Sun, M., Xing, J., Luo, Q., & Corke, H. (2006). Structure–radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. *Life Sciences*, 78, 2872−2888.

Camelo-Méndez, G. A., & Bello-Pérez, L. A. (2014). Antioxidant capacity of extractable and non-extractable polyphenols of pigmented maize. *Journal of Chemical, Biological and Physical Sciences*, 4, 6–13.

Campas-Baypoli, O.N., Rosas-Burgos, E.C., Torres-Chávez, P.I., Ramírez-Wong, B., Serna-Saldívar, S.O. (2002). Physicochemical changes of starch in maize tortillas during storage at room and refrigeration temperatures. *Starch/Stärke*, 54(8), 358-363.

Carbonell-Capella, J.M., Buniowska, M., Barba, F.J., Esteve, M.J., & Frígola, A. (2014). Analytical Methods for Determining. (2014). Bioavailability and bioaccessibility of bioactive compounds from fruits and vegetables: a review. *Comprehensive Reviews in Food Science and Food Safety*, 13.

Castañeda-Ovando, A., Galán-Vidal, C.A., Pacheco, L., Rodriguez, J.A., Paez-Hernandez, M.E. (2010). Characterization of main anthocyanins extracted from pericarp blue corn by MALDI-ToF MS. *Food Analytical Methods*,3,12-16.

Castañeda-Ovando, A., Pacheco-Hernández, M.D., & Páez-Hernández, M.E., (2009). Chemical studies of anthocyanins: A review. *Food Chemistry*, 113,859–871.

Cavalcante Braga, A.R., Murador, D.C., Mendes de Souza Mesquita, L., & Vera de Rosso, V. (2018). Bioavailability of anthocyanins: Gaps in knowledge, challenges and future research. *Journal of Food Composition and Analysis*,68, 31–40.

Colonna, P., & Mercier, C. (1983). Macromolecular modifications of manioc starch components by extrusion-cooking with and without lipids. *Carbohydrate Polymers*, 3, 87– 108.

CONEVAL. (2018). Evolución de la canasta alimentaria. Consulta del valor de las líneas de bienestar. Disponible en: https://www.coneval.org.mx/Medicion/MP/Paginas/Lineas-de-bienestar-y-canasta-basica.aspx. Fecha de consulta: Marzo 2019.

Cortés-Gómez, A., San Martín-Martínez, E., Martínez-Bustos, F., & Vázquez-Carrillo, G.M. (2005). Tortillas of blue maize (Zea mays L.) prepared by a fractionated process of nixtamalization: analysis using response surface methodology. *Journal of Food Engineering*, 66, 273-281.

Cortés-Gómez, A., San Martín-Martínez, E., Martínez-Bustos, F., & Vázquez-Carrillo, G.M. (2005). Tortillas of blue maize (Zea mays L.) prepared by a fractionated process of nixtamalization: analysis using response surface methodology. *Journal of Food Engineering*, 66, 273-281.

Cos, P., De-Bruyne, T., Hemans, N., Apers, S., Vanden-Berghe, D., & Vlietnick, A. (2003). Proanthocyanidins in health care: current and new trends. *Current Medicinal Chemistry*, 10, 1345-1359.

Cruz, E. & Guzmán, V. (2007). Tortillas de maíz: una tradición muy nutritiva. La ciencia y el hombre. Universidad Veracruzana. México. Disponible en: http://www.uv.mx/cienciahombre/revistae/vol20num3/articulos/tradicion/ Fecha de consulta: Agosto 2009.

Cuevas, Montilla, E., Hillebrand, S., Antezana A., & Winterhalter, P. (2011). Soluble and bound phenolic compounds in different bolivian purple corn (*Zea mays* L.) cultivars. *Journal of Agricultural and Food Chemistry*, 59, 7068–7074.

De la Parra, C., Serna-Saldívar, S. O. y Lui, R. H. (2007). Effect of processing on the phytochemical profiles and antioxidant activity of corn production of masa, tortillas and tortilla chip. *Journal of Agricultural and Food Chemistry*, 55, 4177-4183.

Del Pozo-Insfran, D., Serna-Saldivar, S.O., Brenes, C.H. & Talcott, S.T. (2006). Polyphenolics and antioxidant capacity of White and blue corns processed into tortillas and chips. *Cereal Chemistry*, 84, 162-168.

Devi, P.B., Vijayabharathi, R., Sathyabama, S., Malleshi, N.G., & Priyadarisini, V.B. (2014). Health benefits of finger millet (*Eleusine coracana* L.) polyphenols and dietary fiber: A review. *Journal of Food Science and Technology*,51,1021-1040.

Dlamini, N.R., Taylor, J.R.N., & Rooney, L.W. (2007). The effect of sorghum type and processing on the antioxidant properties of African sorghum-based foods. *Food Chemistry*,105,1412-1419.

Doublier, J. L., Colonna, P., & Mercier, C. (1986). Extrusion cooking and drum drying of wheat starch. II Rheological characterization of starch pastes. *Cereal Chemistry*. 63, 240–246.

El-Dash, A.A. (1981). Application and control of thermoplastic extrusion of cereals: a renewable resource-theory and practice. American Association of Cereal Chemists, St. Paul, MN, EUA, Pp, 165-216.

Escalante-Aburto, A., Ponce-García, N., Ramírez-Wong, B., Torres-Chávez, P.I., Figueroa-Cárdenas, J.D., & Gutiérrez-Dorado, R. (2016). Specific anthocyanin contents of whole blue maize second-generation snacks: an evaluation using response surface methodology and lime cooking extrusion. *Journal of Chemistry*, 2016,1-8

FAO. (2018). http://otca.gob.do/fao-cosecha-mundial-de-cereales-2018-2019/#:~:text=La%20producci%C3%B3n%20mundial%20de%20ma%C3%ADz,el%20Brasil%2C%20donde%20se%20prev%C3%A9. Fecha de consulta: 10 Julio 2020.

Fitton, M. G. (1986). In Gums and Stabilisers for the Food Industry, 3, eds G.O. Phillips, D.J. Wedlock & P.A. Williams, pp. 213-220. Elsevier Applied Science Publishers, London.

Fracassetti, D., Del Bo', C., Simonetti, P., Gardana, C., Klimis-Zacas, D., & Ciappellano, S. (2013). Effect of time and storage temperature on anthocyanin decay and antioxidant activity in wild blueberry (*Vaccinium angustifolium*) powder. *Journal of Agriculture and Food Chemistry*, 61, 2999−3005.

Francis, F. J. (1989). Food colorants-anthocyanins. *Critical Reviews in Food Science*, 28, 273-314.

Galarza, J.M. (2011). Situación actual y perspectivas del maíz en México. Disponible en: http://siap.gob.mx.

Gil-Izquierdo, A., Zafrilla, P. & Tomas-Barberan, F.A. (2002). An in vitro method to simulate phenolic compound release from the food matrix in the gastrointestinal tract. *European Food Research and Technology*, 214, 155–159.

Guy, R. (2001). Extrusion cooking. DOI: https://doi.org/10.1533/9781855736313

Halliwell, B., Gutteridge, J.M., & Cross, C.E. (1992). Free radicals, antioxidants, and human disease: *where* are we now?. *Journal of Laboratory and Clinical Medicine*,119, 598–620.

Hanhineva, K., Törrönen, R., Bondia-Pons, I., Pekkinen, J., Kolehmainen, M., Mykkänen, H., & Poutanen, K. (2010). Impact of dietary polyphenols on carbohydrate metabolism. *International Journal of Molecular Sciences*,11, 1365-1402.

Harper, J. M. (1990). Extrusion of foods. Biotechnology and food process engineering. In C. Schwartzberg & A. Rao (Eds.) Institute of Food Technologists (pp. 295–308). Boca Raton, FL: CRC Press LLC

Harper, J.M. (1989). Foods extruders and their applications. Extrusion cooking. American association of cereal chemists. St. Paul, MN. EUA, Pp, 1-15.

He, J., & Giusti, M.M. (2010). Anthocyanins: natural colorants with health-promoting properties. *Annual Review of Food Science and Technology*, 1,163–87.

Hernández-Herrero J.A, & Frutos, M.J. (2011). Degradation kinetics of pigment, colour and stability of the antioxidant capacity in juice model systems from six anthocyanin sources. *International Journal of Food Science and Technology*, 46, 2550–2557.

INEGI. (2010). Encuesta Nacional de Ingresos y Gastos de los Hogares (ENIGH). Disponible en: http://www.inegi.org.mx/est/contenidos/Proyectos/Encuestas/Hogares/regulares/Enigh/ Fecha de consulta: Mayo 2013.

INEGI. (2018). https://www.contralinea.com.mx/archivo-revista/2018/07/30/inegi-solo-16-de-hectareas-del-pais-se-destinan-a-produccion-agricola/ Fecha de consulta: 20 Julio 2020.

Jakobek, L. (2015). Interactions of polyphenols with carbohydrates, lipids and proteins. *Food Chemistry*,175, 556–567.

Kähkönen, M.P., & Heinonen, M. (2003).Antioxidant activity of anthocyanins and their aglycons. *Journal of Agricultural and Food Chemistry*, 51, 628-633

Kamonpatana, K., Failla, M.L., Kumar, P.S., & Giusti, M.M. (2014). Anthocyanin structure determines susceptibility to microbial degradation and bioavailability to the buccal mucosa. *Journal of Agricultural and Food Chemistry*, 62, 6903–6910.

Karaś, M., Jakubczyk, A., Szymanowska, U., Złotek, U., & Zielińska, E. (2017). Digestion and bioavailability of bioactive phytochemicals. *International Journal of Food Science and Technology*, 52, 291–305.

Kong, J.M., Chia, L., Goh, N., Chia, T. (2003). Analysis and biological activities of anthocyanins. *Phytochemicals*, 69,1939–40.

Labuza, T.F. (1984). Aplication of chemical kinetics to deterioration of foods. *Journal of Chemical Education*, 6, 348.

Lao, F., & Giusti, M.M. (2016). Quantification of Purple Corn (*Zea mays* L.) Anthocyanins using spectrophotometric and HPLC approaches: method comparison and correlation. *Food Analytical Methods*, 9,1367- 1380.

Lao, F., Sigurdson, G.T., & Giusti, M.M (2017). Health benefits of purple corn (*Zea mays* L.) phenolic compounds. *Comprehensive Reviews in Food Science and Food Safety*, 16, 234-246.

Lawton, J.W., & Wilson, C.M. (1987) Proteins of the kernel. In: Johnson LA, White PJ (eds) Corn chemistry and technology. American Association of Cereal Chemists, St. Paul, pp 313–354.

Le Bourvellec, C., & Renard, C. M. G. C. (2012). Interactions between polyphenols and macromolecules: Quantification methods and mechanisms. *Critical Reviews in Food Science and Nutrition*, 52, 213–248.

Lee, C.H., Garcia, H.S., & Parkin, K.L. (2010). Bioactivities of kernel extracts of 18 strains of maize (*Zea mays* L.). *Journal of Food Science*,75, C667–C672.

Li, J., Lim, S. S., Lee, J. Y., Kim, J. K., Kang, S. W., Kim, J. L., & Kang, Y. H. (2012). Purple corn anthocyanins dampened high-glucose-induced mesangial fibrosis and inflammation: possible renoprotective role in diabetic nephropathy. *Journal of Nutritional Biochemistry*, 23,320-331.

Li, M., Hasjim, L., Xie, F., Halley, P.J., & Gilbert, R.G. (2014). Shear degradation of molecular, crystalline, and granular structures of starch during extrusion. *Starch/Stärke*, 66, 595-605.

Liu, R. H. (2004). Potential synergy of phytochemicals in cancer prevention:Mechanism of action. *Journal of Nutrition*, 134,3479s–3485s.

Liu, R. H. (2007). Whole grain phytochemicals and health. *Journal of Cereal Science*, 46, 207–219.

López-Martinez, L. X., Parkin, K. L., & Garcia, H. S. (2011). Phase II-inducing, polyphenols content and antioxidant capacity of corn (*Zea mays* L.) from phenotypes of white, blue, red and purple colors processed into masa and tortillas. *Plant Foods for Human Nutrition*, 66, 41–47.

Markaris, P., Livingston, G. E., & Fellers, C. R. (1957). Quantitative aspects of strawberry pigment degradation. *Journal of Food Science*, 22,117–130.

Mauricio, S.R.A., Figueroa C. J.D., Taba, S., Reyes, V.M.L., Rincón, S. F. & Mendoza, G. A. (2004). Caracterización de accesiones de maíz por calidad de grano y tortilla. *Revista Fitotecnia Mexicana*, 27, 213-222.

Mazza, G. (2007). Anthocyanins and heart health. Ann Ist Super Sanitá, 43,369–74.

Méndez, G., Solorza, J. & Paredes, O. (2005). Composición química y colorimétrica de híbridos y variedades de maíz. *Agrociencia*, 39,267-274.

Méndez-Albores, J. A., Arámbula-Villa, M. G., Loarca-Piña, J., González-Hernández, E., & Moreno-Martínez, E. (2004). Aflatoxins fate during the nixtamalization of contaminated maize by two tortilla-making processes. *Journal of Stored Products Research*, 40, 87-94.

Mercali, G. D., Jaeschke, D. P., Tessaro, I. C., & Ferreira-Marczak, L. D. (2013). Degradation kinetics of anthocyanins in acerola pulp: Comparison between ohmic and conventional heat treatment. *Food Chemistry*, 136,853–857.

Milán-Carrillo, J., Gutiérrez-Dorado, R., Perales-Sánchez, J.X.K., Cuevas-Rodríguez, E. O., Ramírez-Wong, B., & Reyes-Moreno, C. (2006). The optimization of the extrusion process when using maize flour with a modified amino acid profile for making tortillas. *International Journal of Food Science and Technology*, 41, 727-736.

Minekus, M., Alminger, M., Alvito, P., Balance, S., Bohn, T., Bourlieu, C., Carriére, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, D. J., Ménard, O., Recio, I., Santos, C. N., Singh, R. P., Vegarud, G. E., Wickham, M. S. J., Weitschies, W., & Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food —An international consensus. *Food & Function*, 5, 1113–1124.

Montilla, E.C., Hillebrand, S., Antezana, A., & Winterhalter, P. (2011). Soluble and bound phenolic compounds in different Bolivian purple corn (*Zea mays* L.) cultivars. *Journal of Agricultural and Food Chemistry*, 59:7068–74.

Mora-Rochin, S., Gutierrez-Uribe, J.A., Serna-Saldívar, S.O., Sánchez-Peña, P., Reyes-Moreno, C., & Milán-Carrillo, J. (2010). Phenolic content and antioxidant activity of tortillas produced from pigmented corns processed by conventional nixtamalization of extrusion cooking. *Journal of Cereal Science*, 52, 502-508.

Moscicki, L., Mitrus, M., Wojtowicz, A., On- iszczuk, T., & Rejak, A. (2013). Advances in agrophysical research, intech. Extrusion- Cooking of Starch. In Prof. Stanis law Grundas.

Mosele, J.I., Macià, A., Romero, M.P., Motilva, M.J. (2016). Stability and metabolism of Arbutus unedo bioactive compounds (phenolics and antioxidants) under in vitro digestion and colonic fermentation. *Food Chemistry,* 201, 120–130.

Mulaudzi, R. B., Ndhlala, A. R., Kulkarni, M. G., & Staden, J. V. (2012). Pharmacological properties and protein binding capacity of phenolic extracts of some Venda medicinal plants used against cough and fever. *Journal of Ethnopharmacology*,143, 185–193.

Nagy, K., Courtet-Compondu, M. C., Williamson, G., Rezzi, S., Kussmann, M., & Rytz, A. (2012). Non-covalent binding of proteins to polyphenols correlates with their amino acid sequence. *Food Chemistry*, 132, 1333–1339.

Nayak, B., Liu, R.H., & Tang, J., (2015). Effect of processing on phenolic antioxidants of fruits, vegetables, and grains- A review. *Critical Reviews in Food Science*, 55, 887-918.

Padayachee, A., Netzel, G., Netzel, M., Day, L., Zabaras, D., Mikkelsen, D. (2012). Binding of polyphenols to plant cell wall analogues – Part 1:Anthocyanins. *Food Chemistry*, 134, 155–161.

Palafox-Carlos, H., Ayala-Zavala, J.F., & González-Aguilar, G.A. (2011).The role of dietary fiber in the bioaccessibility and bioavailability of fruit and vegetable antioxidants. *Journal of Food Science*, 76, 1.

Parada, J., & Aguilera, J. (2007). Food microstructure affects the bioavailability of several nutrients. *Journal of Food Science*,72,21-32.

Paredes-López, O., Guevara-Lara, F., & Bello-Pérez, L. A. (2009). La nixtamalización y el valor nutritivo del maíz. *Ciencias*, 92, 60-70.

Passamonti, S., Vrhovsek, U., Vanzo, A., & Mattivi, F. (2005). Fast access of some grape pigments to the brain. *Journal of Agricultural and Food Chemistry*, 53,7029–7034.

Patras, A., Brunton, N. P., O'Donnell, C., & Tiwari, B. K. (2010). Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends Food Science and Technology*, 21, 3–11.

Pedreschi, R., & Cisneros-Zevallos, L. (2007). Phenolic profiles of Andean purple corn (*Zea mays* L.). *Food Chemistry*,100, 956–963.

Pérez-Jiménez J., Díaz-Rubio, M.E., & Saura-Calixto, F. (2013). Non-extractable polyphenols, a major dietary antioxidant: occurrence, metabolic fate and health effects. *Nutrition Research Reviews*, 26, 118–129.

Peron, D.V., Fraga, S., & Antelo, F. (2017). Thermal degradation kinetics of anthocyanins extracted from juçara (Euterpe edulis Martius) and ‘‘Italia” grapes (Vitis vinifera L.), and the effect of heating on the antioxidant capacity. *Food Chemistry*, 232, 836–840.

Prasanna, B.M., Vasal, S.K., Kassahun, B., & Singh, N.N. (2001). Quality protein maize. *Current science*, 81, 1308–1319.

Prior, R.L., & Wu, X. (2006). Anthocyanins: structural characteristics that result in unique metabolic patterns and biological activities. *Free Radical Research*, 40,1014–28.

Quirós-Sauceda, A. E., Palafox-Carlos, H., Sáyago-Ayerdi, S. G., Ayala-Zavala, J. F., Bello-Pérez, L. A., Álvarez-Parrilla, E., de la Rosa, L. A., González-Córdova A. F., & González-Aguilar, G. A. (2014). Dietary fiber and phenolic compounds as functional ingredients: interaction and possible effect after ingestion. *Food & Function*, 5, 1063–1072.

Rahman, M. M., Ichiyanagi, T., Komiyama, T., Hatano, Y., & Konishi, T. (2006). Superoxide radical- and peroxynitrite-scavenging activity of anthocyanins; structure activity relationship and their synergism. *Free Radical Research*, 40, 993−1002.

Rent, M. (2007). Antioxidant defenses: endogenous and diet derived. In: Halliwell B, Gutteridge J, eds. Free Radicals in Biology and Medicine. Oxford University Press, New York, pp. 79–185.

Rooney, L., & S. O. Serna-Saldívar. (2003). Food use of whole corn and dry-milled fractions. In: Corn chemistry and technology. American association of cereal chemists, Inc. 2nd Ed. St. Paul, MN. USA. Pp 495-535.

Rossetto, M., Vanzani, P., Lunelli, M., Scarpa, M., Mattivi, F., & Rigo, A. (2007). Peroxyl radical trapping activity of anthocyanins and generation of free radical intermediates. *Free Radical Research*, 41,854–9.

Ruíz-Gutiérrez, M.G., Sánchez-Madrigal, M.A., & Quintero-Ramos, A. (2018). The extrusion cooking process for the development of functional foods, extrusion of metals, polymers and food products. Intech Open, 83-102.

Sadilova, E., Stintzing, F. C., & Carle, R. (2006). Thermal degradation of acylated and nonacylated anthocyanins. *Journal of Food Science*, 71, C504–C512.

Salinas, M. Y., López-Reynoso, J. J., González-Flores, G. B., & Vázquez-Carrillo, G. (2007). Compuestos fenólicos del grano de maíz y su relación con el oscurecimiento de masa y tortilla. *Agrociencia*, 41, 295-305.

Salinas-Moreno, Y., Pérez-Alonso, J. J., Vázquez-Carrillo, G., Aragón-Cuevas, F., & Velázquez-Cardenas, G. A. (2012). Antocianinas y actividad antioxidante en maíces (Zea mays L.) de las razas Chalqueño, Elotes cónicos y Bolita. *Agrociencia*, 46,693-706.

Sánchez-Madrigal, M.A., Quintero-Ramos, A., Martínez-Bustos, F., Meléndez-Pizarro, C. O., Ruiz-Gutiérrez, M. G., Camacho-Dávila, A., Torres-Chávez, P. I., Ramírez-Wong, B. (2015). Effect of different calcium sources on the bioactive compounds stability of extruded and nixtamalized blue maize flours. *Journal of Food Science and Technology*, 52,2701-2710.

Saura-Calixto, F. (2011) Dietary fibre as a carrier of dietary antioxidants: an essential physiological function. *Journal of Agricultural and Food Chemistry*,59, 43–49.

Saura-Calixto, F. (2011). Dietary fiber as a carrier of dietary antioxidants: an essential physiological function. *Journal of Agricultural and Food Chemistry*,59, 43–49.

Serna-Saldívar, S.O., Cannet, R., Vargas, J., González, M., Bedolla, S., & Medina, C. (1988). Effect of soybean and sesame addition on the nutritional value of maize and decorticated sorghum tortillas produced by extrusion cooking. *Cereal Chemistry*, 65, 44-48.

Serna-Saldívar, S.O., Gómez, M.H. & Rooney, L.W. (1990). Technology, chemistry and nutritional value of alcaline-cooked corn products. En: Advances in cereal science and technology. American Association of Cereal Chemists, St. Paul, MN, EUA, Pp, 243-307.

Shahidi, F., & Wanasundara, P. (1992). Phenolic antioxidants. Critical reviews. *Food Science and Nutrition*, 32, 67-103.

Shetty, K. (2004). Role of proline-linked pentose phosphate pathway in biosynthesis of plant phenolics for functional food and environmental applications: a review. *Process Biochemistry*, 39, 789–803.

Singh, N., Kaur, A., & Shevkan, K. (2014). Maize: grain structure, composition, milling, and starch characteristics. Maize: Nutrition Dynamics and Novel Uses. DOI: 10.1007/978-81-322-1623-0_5.

Sui, X., Dong, X., Zhou, W., 2014. Combined effect of pH and high temperature on the stability and antioxidant capacity of two anthocyanins in aqueous solution. *Food Chemistry* 163, 163–170.

Sykes, P. (1982). Investigación de mecanismos de reacción en química orgánica. España: Reverté S.A.

Talavera, S., Felgines, C., Texier, O., Besson, C., Gil-Izquierdo, A., Lamaison J.L., & Rémésy, C. (2005). Anthocyanin metabolism in rats and their distribution to digestive area, kidney, and brain. *Journal of Agricultural and Food Chemistry*, 53, 3902–3908.

Tanchev, S., & Ioncheva, N. (1976). Products of thermal degradation of the anthocyanins cyanidin-3-glucoside, cyanidin-3-rutinoside and cyanidin-3-sophoroside.*Food/Nahrung*, 20,889–893.

Toledo, R.T. (1991). Fundamentals of food process engineering, 2nd edition.Van Nostrand Reinhold: New York.

Tsuda, T. (2012). Dietary anthocyanin-rich plants: biochemical basis and recent progress in health benefits studies. *Molecular Nutrition & Food Research*, 56,159–70.

Turturică, M., Stănciuc, N., Bahrim, G., & Râpeanu, G. (2016). Effect of thermal treatment on phenolic compounds from plum (prunus domestica) extracts -A kinetic study. *Journal of Food Engeeniering*, 171, 200-207.

UNESCO. (2010). La Lista Representativa del Patrimonio Cultural Inmaterial de la UNESCO se enriquece con 46 nuevos elementos. Disponible en: http://www.unesco.org/new/es/media-services/singleview/news/forty_six_new_elements_added_to_representative_list_of_the_intngible_cultural_heritage/#.UmVtGnCP8rw. Fecha de consulta: Mayo 2013.

Urías-Lugo, D. A., Heredia, J. B., Muy-Rangel, M. D., Valdez- Torres, J. B., Serna-Saldívar, S. O. & Gutiérrez-Uribe, A. (2015). Anthocyanins and phenolic acids of hybrid and native blue maize (Zea mays L.) extracts and their antiproliferative activity in mammary (MCF7), liver (HepG2), colon (Caco2 and HT29) and prostate (PC3) Cancer Cells. *Plant Foods for Human Nutrition*, 70,193–9.

Urías-Peraldi, M., Gutiérrez-Uribe, J.A., Preciado-Ortiz, R. E., Cruz-Morales, A. S., Serna Saldívar, S. O., & García-Lara, S. (2013). Nutraceutical profiles of improved blue maize (Zea mays L.) hybrids for subtropical regions. *Field Crops Research*, 141, 69–76

Vergnes, B., & Villemaire J. P. (1987). Rheological behaviour of low moisture molten maize starch. *Rheologica Acta*, 26,570-576.

Visioli, F., Borsani, L., & Gali, C. (2000). Diet and prevention of coronary heart disease: the potential role of phytochemicals. *Cardiovascular Research*, 47, 419-425.

Wang, L. S., & Stoner, G.D. (2008). Anthocyanins and their role in cancer prevention. *Cancer Letters*, 269:281–90.

Xiao, J., Ni, X., Kai, G., & Chen, X. (2015). Advance in dietary polyphenols as aldose reductases inhibitors: structure-activity relationship aspect. *Food Science and Nutrition*, 55, 16-31.

Yang, M., Koo, S.I., Song, W.O., Chun, O.K. (2011). Food matrix affecting anthocyanin bioavailability: review. *Current Medicinal Chemistry*,18, 291–300.

Yang, Z., & Zhai, W. (2010). Identification and antioxidant activity of anthocyanins extracted from the seed and cob of purple corn (Zea mays L.). *Innovative Food Science & Emerging Technologies*,11, 169-176.

Zazueta-Morales, J.J. (2014). Comunicación personal, 16 de abril de 2014.

Zhang, Z., Zhou, B., Wang, H., Wang, F., Song, Y., Liu, S., & Xi, S. (2014). Maize purple plant pigment protects against fluoride-induced oxidative damage of liver and kidney in rats. *International Journal of Environmental Research and Public Health*, 11,1020–33.

Zhao, X., Zhang, C., Guigas, C., Ma, Y., Corrales, M., Tauscher, B., & Hu, X. (2009). Composition, antimicrobial activity an antiproliferative capacity of anthocyanin extracts of purple corn (Zea mays L.) from China. *European Food Research and Technology*, 228,759-765.

Zhu, Y., Ling, W., Guo, H., Song, F., Ye, Q., Zou, T., Li, D., Zhang, Y., Li, G., Xiao, Y., Liu, F., Li, Z., Shi, Z., & Zhang, Y. (2013). Anti-inflammatory effect of purified dietary anthocyanins in adult with hypercholesterolemic: A randomized controlled trial. *Nutrition, Metabolism and Cardiovascular Diseases*, 23,842-849.

Žilić, S., Serpen, A., Akıllıoğlu, G., Gökmen, V., & Vančetović, J. (2012). Phenolic compounds, carotenoids, anthocyanins, and antioxidant capacity of colored maize (Zea mays L.) kernels. *Journal of Agricultural and Food Chemistry*, 60,1224–31.

CAPÍTULO 9. ANEXOS

9.1 Supplementary material

The Effect of Nixtamalization Extrusion Process and Tortillas Making on the Stability of Anthocyanins from Blue Corn through the Kinetic and Thermodynamic Parameters

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Materials and methods

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.

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.

Nixtamalization extrusion process

The term nixtamalization extrusion process is used to define the combination of both processes indicating that nixtamalization was done using an extruder. 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 [1]. 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 the 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.

Corn tortilla preparation

Corn tortillas were prepared according to the procedure reported by Platt-Lucero et al. [2] using the ENCF optimized obtained previously. Four kilograms of the ENCF were mixed (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 to obtain corn dough (masa) [3]. 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.

Anthocyanin's extraction

Anthocyanin's 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 anthocyanins degradation.

Anthocyanins 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 selected for the treatments simulated the gastric fluid environment in the human body, where the anthocyanins stability is high under these physiological conditions and they have the possibility of being absorbed or exerting local antioxidant effects. The pH was verified by a pH meter (Crison, Model GLP 21, Barcelona, Spain). The resulting concentrated anthocyanins extracts (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 [4].

Anthocyanins thermal treatment

According to literature, in extrusion process, cereals used to make flours are extruded for short time at temperatures ranging from 60, 70, 80 and 110 °C, which correspond to the first, second, third and fourth zones of the extruder [1]. So, the thermal degradation kinetics of anthocyanins were studied by isothermal heating at selected temperatures (60, 75, and 90 °C). The selected treatment time do not represent the industrial extrusion process time, which is shorter than our study. In order to determine the time required for anthocyanins to reach their half-life, heat treatment was applied up to 2 h. 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 treatments were conducted in a previously equilibrated thermostatic water bath (Unitronic OR, P Selecta, Barcelona, España). At regular time intervals (0.5, 1, 1.5, 2 h), samples were randomly removed from the bath and quickly cooled in ice water to prevent further thermal degradation. Anthocyanins degradation of the extracts was followed by spectrophotometric measurements. All assays were performed in triplicate [8].

Quantification of total anthocyanins

After the thermal treatment, the total anthocyanin content of the buffer solution was analyzed according to Abdel-Aal and Huel [5]. 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.

Kinetic data analysis

Based on previous studies [6,4], 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. (3) 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 [7].

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

The half-life of the reaction (t ½), which is the time needed to achieve 50% anthocyanins degradation, was calculated assuming first-order kinetics according to Eq. (4), [8]:

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

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), [9].

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

Determination of thermodynamic parameters

The temperature dependence of the degradation rate constant was determined by applying the Arrhenius model, as reported by several studies [10,4,8], 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 (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 anthocyanins degradation when the temperature is increased by 10 °C, and it was calculated according to Eq. (7), [10]:

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

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), [11]:

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

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 [9,8]:

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

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

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 anthocyanins 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):

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

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.

Results and discussion

Table S1

Thermodynamic parameters calculated for anthocyanins degradation in blue corn, extruded nixtamalized flour and tortillas

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}

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

References

1. Menchaca-Armenta M, Ramírez-Wong B, Torres-Chávez PI, Quintero-Ramos A, Ledesma-Osuna AI, Frutos-Fernández MJ, Gutiérrez-Dorado R, Campas-Baypoli ON, Morales-Rosas I (2020) Effect of extrusion conditions on the anthocyanin content, functionality, and pasting properties of obtained nixtamalized blue corn flour (*Zea mays* L.) and process optimization. J Food Sci. https://doi.org/10.1111/1750-3841.15312
2. Platt-Lucero LC, Ramírez-Wong B. Torres-Chávez PI, López-Cervantes J, Sánchez-Machado DI, Reyes-Moreno C, Milán-Carrillo J, Morales-Rosas I (2010) Improving textural characteristics of tortillas by adding gums during extrusion to obtain nixtamalized corn flour. J. Texture Stud 41:736-755. https://doi.org/10.1111/j.1745-4603.2010.00252.x.
3. Flores-Farías R, Martínez-Bustos F, Salinas-Moreno Y, Ríos E (2002) Characterization of commercial nixtamalized maize flours. Agrociencia 36:557-567.
4. Bolea C, Turturică M, Stănciuc N, Vizireanu C (2016) Thermal degradation kinetics of bioactive compounds from black rice flour (*Oryza sativa* L.) extracts. J Cereal Sci 71:160-166. https://doi.org/10.1016/j.jcs.2016.08.010
5. Abdel-Aal ESM, Huel P (1999) A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheats. Cereal Chem 76:350-354.
6. Hernández-Herrero JA, Frutos MJ (2011) Degradation kinetics of pigment, colour and stability of the antioxidant capacity in juice model systems from six anthocyanin sources. Int. J of Food Sci Tech 46: 2550–2557. https://doi.org/10.1111/j.1365-2621.2011.02780.x

7. Qiu G, Wang D, Song X, Deng Y, Zhao Y (2018) Degradation kinetics and antioxidant capacity of anthocyanins in air-impingement jet dried purple potato slices. Food Res Int 105:121-128. https://doi.org/10.1016/j.foodres.2017.10.050
8. Peron DV, Fraga S, Antelo F (2017) Thermal degradation kinetics of anthocyanins extracted from juçara (*Euterpe edulis Martius*) and ‘‘Italia” grapes (*Vitis vinifera* L.), and the effect of heating on the antioxidant capacity. Food Chem 232:836–840. https://doi.org/10.1016/j.foodchem.2017.04.088
9. Mercali GD, Jaeschke DP, Tessaro IC, Ferreira-Marczak LD (2013) Degradation kinetics of anthocyanins in acerola pulp: Comparison between ohmic and conventional heat treatment. Food Chem 136:853–857. https://doi.org/10.1016/j.foodchem.2012.08.024
10. Fracassetti D, Del Bo’ C, Simonetti P, Gardana C, Klimis-Zacas D, Ciappellano S, (2013) Effect of time and storage temperature on anthocyanin decay and antioxidant activity in wild blueberry (*Vaccinium angustifolium*) powder. J Agric Food Chem 61:2999−3005. https://doi.org/10.1021/jf3048884
11. Toledo RT (1991) Fundamentals of food process engineering, 2nd edition.Van Nostrand Reinhold: New York.