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Instituto de Ciencias Agropecuarias
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Instituto de Ciencias de la Salud

DOCTORADO EN CIENCIAS DE LOS ALIMENTOS Y SALUD HUMANA

TESIS DOCTORAL

"Efecto antihipertensivo de extractos de cebada germinada en un sistema *in vitro* e *in vivo*"

Para obtener el grado de
Doctora en Ciencias de los Alimentos y Salud Humana

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Por este medio se informa que el comité tutorial asignado al **Mtra. Abigail García Castro** con número de cuenta **185930**, estudiante del Doctorado en Ciencias de los Alimentos y Salud Humana ha terminado el trabajo de tesis titulado "**Efecto antihipertensivo de extractos de cebada germinada en un sistema *in vitro* e *in vivo***", y por lo tanto se autoriza la impresión del documento en extenso propuesto por el/la estudiante después de haber sido revisado, analizado y evaluado de acuerdo a lo estipulado en el Artículo 73, VI del Reglamento General de Estudios de Posgrado. Lo anterior, en función de que, el/la estudiante realizó todas las correcciones, adiciones y/o modificaciones sugeridas por el comité en la revisión previa con fecha 03 de mayo 2024. Por tal motivo, solicitamos a usted tenga a bien permitir al doctorando dar continuidad al proceso necesario que conlleve a la obtención del grado de Doctor en Ciencias de los Alimentos y Salud Humana.

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Debido a los anterior, la estudiante cumple con los requerimientos de egreso establecidos por el programa de posgrado, al contar con tres artículos aceptados en una revista indizada. Por lo que solicitamos a usted tenga a bien permitir al doctorando dar continuidad al proceso necesario que conlleve a la obtención del grado de Doctor en Ciencias de los Alimentos y Salud Humana

ATENTAMENTE
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Dedicatorias y agradecimientos

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ÍNDICE GENERAL

ÍNDICE DE TABLAS

ÍNDICE DE FIGURAS

ABREVIATURAS

ANOVA Análisis de varianza

BRA Bloqueador de los receptores de angiotensina II

BSE Extracto de germinado de cebada

EBSE Extracto de germinado de cebada Esmeralda

ECA Enzima convertidora de angiotensina

g Gramo

GAE Equivalentes de ácido gálico

H-E Hematoxilina-Eosina

HPLC Cromatografía líquida de alta resolución

iACE Inhibidor de la enzima convertidora de angiotensina

kDa Kilodaltons

L-NAME N(ω)-Nitro-L-Arginine Methyl Ester

mL Mililitro(s)

mM Milimolar

PBSE Extracto de germinado de cebada Perla

QE Equivalentes de quercetina

SRAA Sistema Renina-Angiotensina-Aldosterona

µg Microgramo

µL Microlitro

µM Micromolar

RESUMEN

La hipertensión arterial es un problema de salud pública a nivel mundial, en donde la prevención y tratamiento son el principal objetivo para la disminución de enfermedades cardiovasculares. Además de los fármacos, existen alimentos que debido a sus compuestos bioactivos regulan la presión sanguínea a través de la inhibición de la enzima convertidora de angiotensina (ACE). Existen pocos estudios sobre las propiedades bioactivas de extractos de germinado de cereales sobre la hipertensión. El objetivo principal de este estudio fue estandarizar y caracterizar extractos de cebada variedad Esmeralda y Perla sin germinar y germinada y evaluar su efecto sobre actividad de la ACE en un modelo *in vitro* e *in vivo*. Los resultados mostraron que la germinación mejoró la biodisponibilidad de aminoácidos, carbohidratos y compuestos fenólicos en ambas variedades. Los extractos de cebada Esmeralda germinada por 7 días pueden inhibir la actividad de la ACE *in vitro* hasta 83%. Los extractos de germinados de 7 días de ambas variedades de cebada disminuyeron los efectos de la presión arterial mejorando la funcionalidad renal y endotelial, niveles de ACE I y II y los efectos en órganos blanco. Estos resultados muestran que el consumo de extractos de cebada germinada podría influir en la mejora de los efectos negativos de la presión arterial.

ABSTRACT

High blood pressure is a global public health issue and preventing and treating it is crucial for reducing cardiovascular diseases. In addition to medication, certain foods containing bioactive compounds can help regulate blood pressure by inhibiting angiotensin-converting enzyme (ACE). There is limited research on the bioactive properties of cereal sprout extracts in relation to hypertension. The main objective of this study was to standardize extracts of ungerminated and sprouted Emerald and Pearl barley and to evaluate their effect on ACE activity in an *in vitro* and *in vivo model.* The results showed that germination improved the bioavailability of amino acids, carbohydrates and phenolic compounds in both varieties. Extracts of Emerald barley sprouted for 7 days can inhibit ACE activity *in vitro* up to 83%. Moreover, the sprout extracts from the two 7-day-old barley varieties mitigated the effects of high blood pressure by improving renal and endothelial function, ACE I and II levels, and impacts on target organs. These results suggest that consuming sprouted barley extracts may help ameliorate the adverse effects of high blood pressure.

CAPÍTULO I. INTRODUCCIÓN A LA INVESTIGACIÓN

1.1 Introducción

La hipertensión es un problema de salud pública que a nivel mundial afecta al 31.1% de adultos, con una mayor prevalencia en los países de bajos ingresos (31.5%) en comparación con los países de altos ingresos (28.5%) (Campos-Nonato et al., 2021). Esta condición se caracteriza por la presión continua en los vasos sanguíneos y es regulada por el Sistema Renina-Angiotensina-Aldosterona (SRAA). Cuando hay una hiperfusión renal debido al aumento de sales en el aparato yuxtaglomerular del riñón, la renina estimula la producción de angiotensina I, la cual se convierte en angiotensina II bajo la acción de la ACE (Oparil et al., 2018).

Existen inhibidores que actúan sobre la ACE que evitan la producción de angiotensina II y disminuyen la presión en pacientes hipertensos (Vaduganathan et al., 2020). Sin embargo, debido a los efectos adversos como tos seca, daño renal y reacciones alérgicas ha aumentado la investigación sobre el desarrollo de alimentos funcionales que podrían participar sobre la actividad de la ACE.

Los principios bioactivos provenientes de distintos grupos de alimentos como frutas, verduras, granos y cereales pueden actuar como inhibidores de la ACE , entre estos se encuentran péptidos, proteínas y compuestos fenólicos (Huang et al., 2013). Diversos estudios destacan el consumo de alimentos ricos en compuestos bioactivos, como compuestos fenólicos o antocianinas, que podrían participar sobre la actividad de la ACE (Ojeh et al., 2020; Wang et al., 2021).

En cereales, como la cebada, se ha mostrado que algunos péptidos, proteínas y compuestos fenólicos pueden actuar como inhibidores de la ACE y disminuir la presión arterial (Gangopadhyay et al., 2016; Hokazono et al., 2010; Ra et al., 2020). Además, se ha descrito una sinergia entre fitoquímicos de cebada que actúan sobre las vías de ciclooxigenasa y lipoxigenasa para la prevención de enfermedades inflamatorias y cardiovasculares (Gul et al., 2014). La cebada se utiliza para la elaboración de malta, la calidad de esta depende de la capacidad que tiene la semilla en el metabolismo y producción de compuestos durante la germinación (Pérez-Ruiz et al., 2015). Se ha reportado la relevancia en el uso de mostos de malta en la alimentación, debido a su calidad nutricional, proporcionando aminoácidos y fenoles bioactivos generados durante las primeras etapas de germinación (Cortese et al., 2020; Szwajgier, 2009; Yalçınçıray et al., 2020). Sin embargo, hasta el momento no se han encontrado reportes sobre la funcionalidad de extractos de cebada de larga germinación. Por lo tanto, el presente proyecto tiene como objetivo estandarizar y caracterizar extractos elaborados a partir de dos variedades de cebada (Perla y Esmeralda), sin germinar y durante 3, 5 y 7 días, para evaluar su capacidad inhibitoria de la enzima convertidora de angiotensina (ACE) en modelos *in vitro* e *in vivo.*

En este contexto, el presente trabajo está dividido en seis capítulos principales. El capítulo uno muestra una introducción general al trabajo de investigación para comprender la importancia y finalidad del proyecto. Dicho capítulo comprende la justificación, objetivos y el diagrama metodológico del proyecto de investigación.

El segundo capítulo abarca un artículo de revisión que destaca la importancia de los compuestos bioactivos presentes en distintos cereales, los cuales influyen en la actividad de la ACE y que podrían estar relacionados con la prevención del COVID-19. Dicho artículo se encuentra publicado en la revista de *Foods.*

El tercer capítulo presenta una investigación sobre la participación de la germinación y el uso de distintos solventes para la obtención de compuestos fenólicos en dos variedades de cebada.

Este artículo fue el preámbulo para el diseño de los extractos de germinados de cebada, este trabajo se encuentra publicado en la revista de *Chemistry and Biodiversity.*

Más adelante, en el cuarto capítulo se encuentra la primera parte experimental del proyecto. En este capítulo se muestra un artículo original publicado en la revista de *Food Measurement and Characterization,* donde se muestra el análisis de compuestos bioactivos y la actividad antioxidante de extractos liofilizados de cebada de dos variedades (sin germinar y con diferentes días de germinación). Dichos resultados nos encaminaron hacia la evaluación antihipertensiva *in vitro* e *in vivo.*

En el quinto capítulo, se muestra la evaluación de extractos de cebada sobre la actividad de la ACE. El modelo *in vitro* proporcionó la información necesaria para la selección del extracto del día 7 de germinación para utilizarlo en el modelo *in vivo.* Posteriormente, se evaluó el efecto de la variedad con mayor efecto inhibidor de la ACE, en un modelo de hipertensión inducido en ratas Wistar. Se determinaron los cambios en la funcionalidad renal y endotelial (NOx), niveles de ACE I y ACE II y los efectos histopatológicos. Estos resultados han sido sometidos a revisión en la revista *Food Chemistry.*

Por último, el sexto capítulo comprende las conclusiones generales de la investigación y se incluyen las perspectivas a futuro.

1.2 Justificación

La Organización Mundial de la Salud (OMS), menciona que la hipertensión es el factor número uno de riesgo de muerte a nivel mundial (OMS, 2020). En México, una de cada cuatro personas padece hipertensión arterial. En los hombres la prevalencia es de 24.9% y 25.1% en mujeres, lo que representa un total de 30 millones de personas (Piña-Pozas & Araujo-Pulido, 2020). En Hidalgo, se han reportado 34 mil 499 casos (SSH, 2020). La mayoría de los tratamientos para esta enfermedad incluyen fármacos inhibidores de la enzima convertidora de angiotensina, los cuales producen una disminución en la vasoconstricción y en la retención de sodio y agua (Messerli et al., 2018). Sin embargo, existen otras alternativas que actúan como inhibidores de la ACE, entre ellos están algunos compuestos bioactivos presentes en diversas fuentes de alimentos, incluidos los cereales, que han sido identificados para su uso potencial contra la hipertensión (Taylor et al., 2013). En distintos estudios se ha sugerido que algunos polifenoles de la cebada presentan actividad inhibitoria de la enzima convertidora de angiotensina (Ra et al., 2020), sin embargo, este campo aún es poco estudiado. En el presente estudio se evaluará el efecto de los compuestos bioactivos presentes en la cebada a partir de la elaboración de extractos de germinados, dicho proceso aumentará la concentración de fitoquímicos derivados de la cebada germinada, los cuales podrán ser utilizados para medir la inhibición de la ACE en modelos *in vitro* e *in vivo* y de obtener resultados positivos podrán ser utilizados como coadyuvantes en el tratamiento contra la hipertensión.

1.3 Objetivos

1.3.1 Objetivo general

Estandarizar y caracterizar extractos de cebada germinada mediante técnicas analíticas y modelos *in vivo* para analizar el efecto sobre la hipertensión, funcional renal y endotelial.

1.3.2 Objetivos específicos

1. Identificar el impacto de los compuestos bioactivos de cereales en la salud a través de una metabúsqueda para su divulgación científica.
2. Obtener extractos de cebada germinada (0, 3, 5 y 7 días) variedad Perla y Esmeralda con el uso de distintos solventes para determinar la concentración de compuestos fenólicos y flavonoides totales.
3. Caracterizar el perfil de compuestos bioactivos de extractos de cebada germinada mediante técnicas analíticas para evaluar sus beneficios a la salud.
4. Determinar la inhibición de la enzima convertidora de angiotensina de extractos de cebada germinada a través de un ensayo enzimático para valorar su capacidad antihipertensiva.
5. Evaluar la actividad de los extractos de cebada germinada sobre la enzima convertidora de angiotensina en un modelo *in vivo* a partir de la medición de la presión arterial de cola, análisis bioquímico de suero y tejidos para analizar su potencial antihipertensivo.

1.4 Diagrama metodológico

En la Figura 1 se muestra el diagrama metodológico que resume el trabajo en 4 objetivos principales.

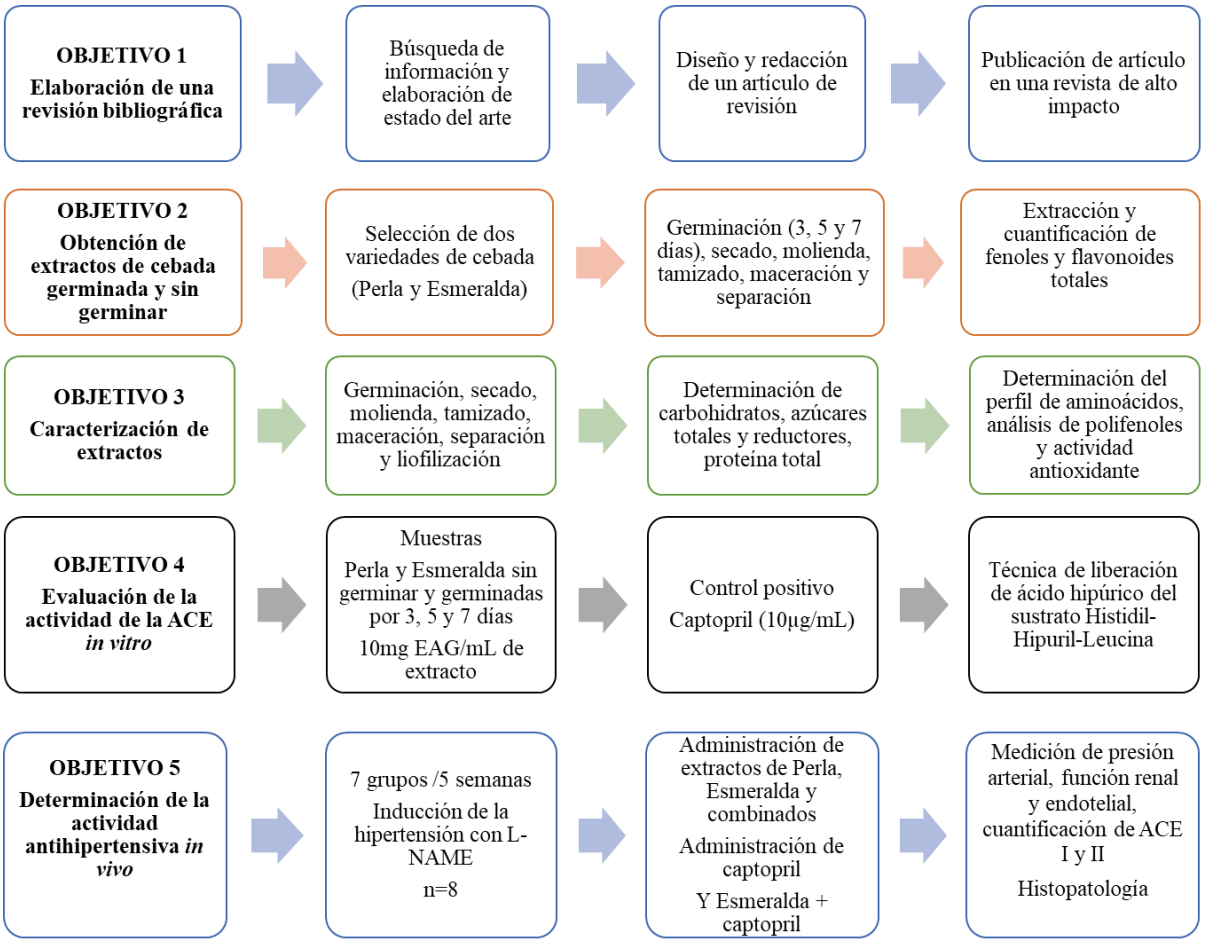

Figura 1. *Diagrama metodológico del proyecto.*

1.5 Referencias

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CAPITULO II. CEREALS AS A SOURCE OF BIOACTIVE COMPOUNDS WITH ANTI-HYPERTENSIVE ACTIVITY AND THEIR INTAKE IN TIMES OF COVID-19

2.1 Introducción

El presente capítulo muestra una revisión bibliográfica publicada en la revista *Foods*. En este artículo se destaca la importancia del consumo de cereales, sobre todo durante la pandemia del COVID-19. Dentro del trabajo se describe la fisiopatología de la hipertensión arterial, además se menciona como este padecimiento es la principal comorbilidad en pacientes con COVID-19. Se describen los fármacos antihipertensivos como, los inhibidores de la enzima convertidora de angiotensina, los bloqueadores de los receptores de angiotensina II (iACE y BRAs) y su uso complementario en el tratamiento contra COVID-19. De igual manera, se destaca como algunos compuestos bioactivos presentes en cereales podrían tener actividad similar a los fármacos antihipertensivos y como podrían interactuar en los receptores de SARS-CoV-2. En este contexto, se destaca principalmente la presencia de compuestos fenólicos y péptidos presentes en cereales como arroz, cebada, maíz, trigo, avena, mijo, centeno y sorgo. Además, se mencionan fitoquímicos, proteínas, fibras y otros componentes de estos cereales, con actividad antihipertensiva, antiviral, antioxidante y antiinflamatoria. Se destacan estudios *in vivo*, *in vitro*, *in silico* y clínicos sobre los mecanismos que presentan algunos compuestos presentes en cereales para disminuir la presión arterial, destacando la importancia de su consumo durante y después de la pandemia del COVID-19.

foods

MDPI

Review

Cereals as a Source of Bioactive Compounds with Anti-Hypertensive Activity and Their Intake in Times of COVID-19

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Abstract: Cereals have phytochemical compounds that can diminish the incidence of chronic diseases such as hypertension. The angiotensin-converting enzyme 2 (ACE2) participates in the modulation of blood pressure and is the principal receptor of the virus SARS-CoV-2. The inhibitors of the angiotensin-converting enzyme (ACE) and the block receptors of angiotensin II regulate the expression of ACE2; thus, they could be useful in the treatment of patients infected with SARS-CoV-2. The inferior peptides from 1 to 3 kDa and the hydrophobic amino acids are the best candidates to inhibit ACE, and these compounds are present in rice, corn, wheat, oats, sorghum, and barley. In addition, the vitamins C and E, phenolic acids, and flavonoids present in cereals show a reduction in the oxidative stress involved in the pathogenesis of hypertension. The influence of ACE on hypertension and COVID-19 has turned into a primary point of control and treatment from the nutritional perspective. The objective of this work was to describe the inhibitory effect of the angiotensin-converting enzyme that the bioactive compounds present in cereals possess in order to lower blood pressure and how their consumption could be associated with reducing the virulence of COVID-19.

Keywords: cereals; COVID-19; diet therapy; drug therapy; hypertension; phytochemicals

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1. Introduction

Cereals constitute an important part of the daily diet due to their high content of proteins, dietary fiber, and bioactive compounds with antioxidant and anti-inflammatory activities, which help prevent diseases related to metabolic syndromes such as obesity, cardiovascular diseases, and type 2 diabetes [1,2]. Wheat, oats, barley, and rice have been reported to have antihypertensive and antioxidant activities due to their content of phytochemical compounds that participate in hormonal regulation mechanisms that help lower blood pressure and other non-transmissible diseases [3–6].

Peptides derived from food have a high potential regarding the development of nutraceuticals and functional foods due to their specificity and molecular weight [7]. According to Cavazos and Mejia [8], the anti-hypertensive activity of the bioactive peptides presents in cereals with hypotensive effects contribute to preventing cardiovascular diseases. Likewise, it has been discovered that the hydrolyzed proteins and phenolic compounds promote the regulation of oxidative stress and decrease the appearance of associated chronic diseases [9,10].

Hypertension has been one of the most important comorbidities that contribute to the development of cardiovascular diseases. Recently, during the pandemic caused by the coronavirus SARS-CoV-2, the most common comorbidities in patients with COVID-19 have been reported, of which hypertension (30%), diabetes (19%), and coronary diseases (8%) stand out [11]. Some recent findings showed an important role of the Renin–angiotensin–aldosterone system (RAAS) in hypertensive patients diagnosed with COVID-19. This is because SARS-CoV-2 utilizes the angiotensin-converting enzyme 2 (ACE2) to unite in the surface of epithelial cells. Thus, controlling the production of ACE2 can mediate the entry of SARS-CoV-2 in the cells [12].

Some hypertensive drugs, such as the blocking receptors of angiotensin II (BRA), can modify the expression of ACE2 [13,14], which could decrease the virulence of SARS-CoV-2. The objective of this review is to describe the anti-hypertensive activity present in some bioactive compounds in cereals, wherein activities such as the inhibition of ACE, its participation in oxidative stress, and its consumption could be associated with the prevention of COVID-19. Furthermore, some angiotensin-converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARBs) used in the treatment of hypertensive patients diagnosed with COVID-19 are described.

2. Physiopathology of Hypertension

High blood pressure, also known as hypertension, is a public health problem suffered by around 1.3 million adults worldwide. This condition occurs when an elevation in the systolic and diastolic pressure occurs above 140/90 mmHg, respectively [15]. Studies relate the hyperactivation of the renin angiotensin system [16,17], oxidative stress [18], and chronic inflammation [19] as the principal causes in the development of hypertension. Other factors related to hypertension include biochemical processes, such as the increase in the sympathetic activity of the nervous system, the inadequate intake of calcium and potassium, and alterations in the secretion of renin, a hormone related to the elevation in the activity of the angiotensin renin system [20]. In addition, the increased activity of ACE causes a high production of the hormone angiotensin II, as well as deficiencies in vasodilators including vascular inflammatory factors, which promote an alteration in cellular ion channels [20].

The RAAS is the principal mechanism that affects the regulation of blood pressure. An increase in the renin hormone caused by an increment in the intake of sodium provokes the stimulation of the production of the physiologically inactive hormone called angiotensin-I (Ang-I), which is converted into angiotensin II (Ang-II) due to the angiotensin-converter enzyme (ACE-I). Ang II is a vasoconstrictor that stimulates the production of aldosterone, which causes an increase in blood pressure through the retention of sodium and water. This induces the activation of the epithelial sodium channel stimulating the reabsorption of the Na+ in the cortical duct (Figure 1) [17,21].

Although the potential of antihypertensive drugs to lower blood pressure in individuals with hypertension has been shown, lifestyle habits, such as regular exercise and healthy eating, have also been reported to have a positive effect on blood pressure control [22]. Some mechanisms used by the bioactive compounds present in food, mainly polyphenolic compounds to reduce hypertension, include the reduction in the levels of the vasoconstrictor molecule I and the increase in the antioxidant glutathione [23], which improve the production of vasodilator factors such as oxide nitric [24] and inhibit the expression of proangiogenic factors such as vascular endothelial growth factor [25].

Therefore, a better understanding of the hormonal mechanisms that control high blood pressure could clarify the causes and effects that a drug treatment combined with a diet rich in cereals could have on the control of hypertension, effectively reducing inflammation and oxidative stress and strengthening the immune system during the COVID-19 crisis.

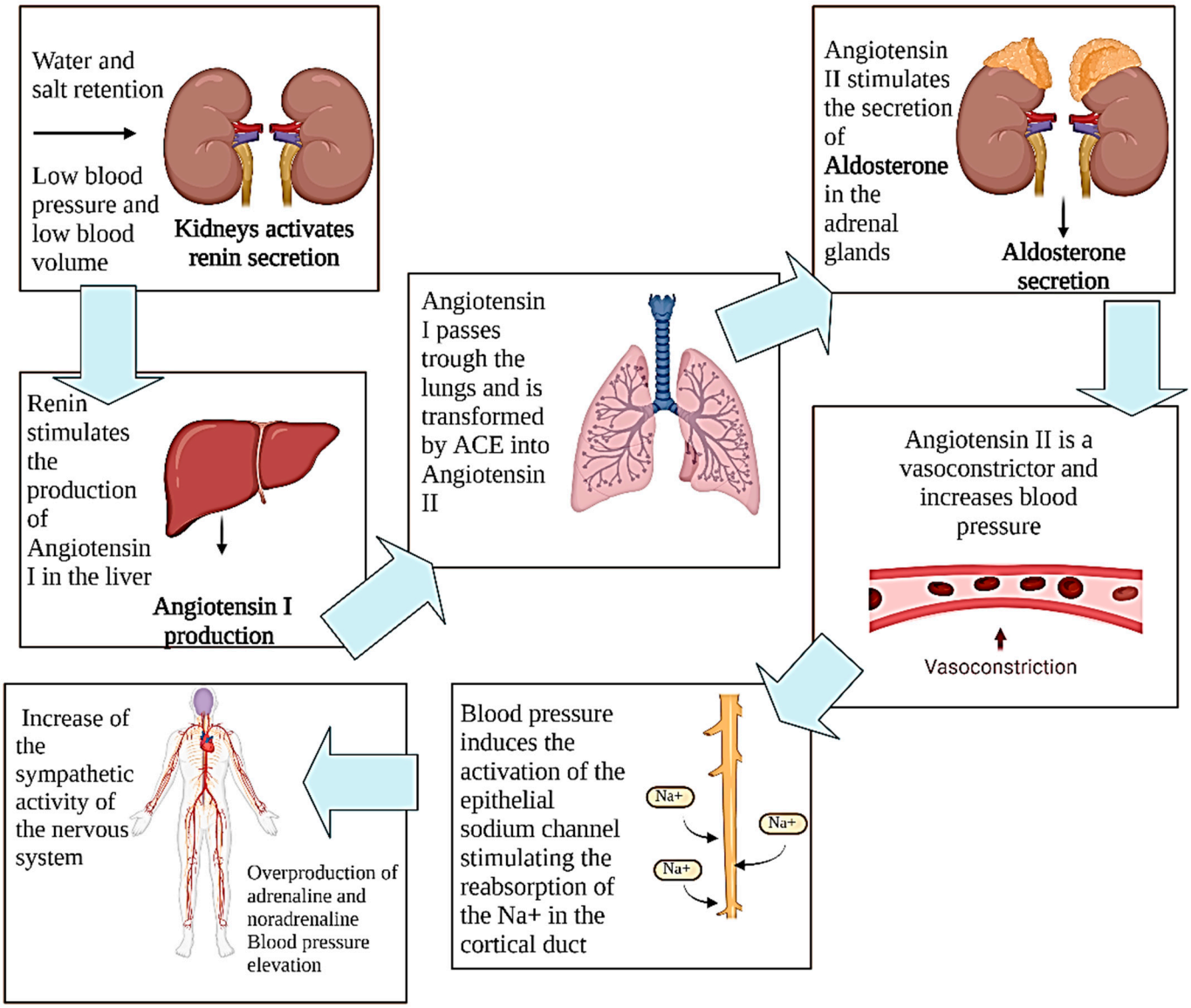

Figure 1. Renin–angiotensin–aldosterone system. Created with BioRender.com (accessed on 5 October 2022).

3. Hypertension: Main Comorbidity in Patients with COVID-19

COVID-19, caused by the SARS-CoV-2 virus, is an infectious disease that has provoked a sanitary crisis worldwide. The pathogenesis of the SARS-CoV-2 virus starts by means of the union of the protein of the viral peak with the target receptor of ACE2, which facilitates the internalization of the virus within the host cells. It was reported that SARS-Cov-2 is a virus whose tropism is based on the use of ACE2 to unite the epithelial cells of the organism [26,27]. The ACE balances blood pressure and converts angiotensin I into angiotensin II with a vasoconstrictive function and at the same time facilitates the degradation of a vasodilator termed bradykinin [28]. The control over these hormonal processes balances the health of hypertensive patients. However, a combination of other diseases makes it difficult to control and, in many cases, can worsen the evolution of each illness. Therefore, the initial reports suggest that hypertension, diabetes, and cardiovascular diseases are the most frequent comorbidities in COVID-19 [29].

The ACE2 can change the balance of the RAAS by means of the conversion of Ang II to Ang (1-7). Therefore, hypertension and COVID-19 have developed into a recent concern

over the susceptibility of patients with hypertension to develop COVID-19, as it increases the severity of the illness and the use of ACEi and ARBs [30].

The inhibitors utilized in the treatment of hypertension increase the expression of ACE2 on the cellular surface and can increase the expression of the intestinal messenger ribonucleic acid (ARNm) of ACE2. Although there are few data concerning the effects of these drugs regarding the expression of the ARNm of ACE2 in the pulmonary epithelial cells, there exists the concern that the patients who take these treatments can encourage the contraction of the virus [31].

An optimum immune response is the key to maintaining control over infectious and non-infectious diseases. An increase in the intake of whole cereals rich in fiber and polysaccharides is associated with a reduction in PCR-hs (a marker used to predict cardiovascular events in patients with atherosclerosis via Polymerase Chain Reaction) [32]; decreased interleukin-6 (IL-6) [33], which is produced in response to infections and tissue damage; and tumor necrosis factor alpha (TNF-α), an inflammatory cytokine produced by macrophages/monocytes during acute inflammation [2]; therefore, cereals reduce the risk of suffering illnesses predicted by inflammation such as cardiovascular diseases [34] and diabetes type II [35].

Since blood pressure is difficult to control, the most widely used resources involve identifying drug targets to effectively control and manage blood pressure in hypertensive patients.

4. Anti-Hypertensive Drugs and Their Use in the Treatment of COVID-19

The use of ACEi and ARBs have been associated with a decrease in the mortality of a hospital population diagnosed with COVID-19 and with a reduction in the hospital in-patient stay observed with a greater effect in patients with hypertension [36].

However, it has been shown that ACEi and ARBs could facilitate the entry of the virus into the host cell and increase the chances of infection or its severity, although there are no conclusive studies [37]. In a study of 187 patients with COVID-19 (the mean age was 58.5 years), it was observed that the mortality of those treated with ACEi/ARBs did not show a significant difference with those who were not treated with ACEi/ARBs [38].

Martínez-del Río et al. [39] reported that the use of ACEi and antagonists of the angiotensin receptor 2 (ARA2) in elderly patients does not increase the risk of death or the use of assisted ventilation, but the use of these drugs overexpress ACE2 and increases the risk of infection. This enzyme acts by inhibiting angiotensin 2 and increasing the production of angiotensin 1–7 with anti-inflammatory and vasodilator effects [40], which have been found in greater levels in persons that have survived respiratory stress than in persons who have perished [41].

Braude et al. [36] reported the influence of ACEi and ARBs on mortality in 1371 patients with a mean age of 74 years diagnosed with COVID-19. The results showed a significant reduction in hospital stay. This was because ACEi decreases the production of ACE2, as it blocks the conversion of ACE1 to ACE2, and the ARBs block the receptor of angiotensin II type I impeding the actions of ACE2 concerning pulmonary vasoconstriction and endothelial permeability, thereby diminishing the injury at the pulmonary level. Therefore, the use of ACEi could decrease the progression and mortality of patients with COVID-19.

One strategy to treat infection with COVID-19 is to inhibit the entry of SARS-CoV-2 in the host cell through the receptors of ACE2 [42]. Consequently, the positive regulation of ACE2 in infected patients with SARS-CoV-2 could be clinically useful due to the vascular protection provided by the activity of angiotensin 1–7, thereby diminishing the effects of angiotensin II on vasoconstriction and the retention of sodium [43].

Bioactive compounds are valuable for drug development and adjunctive therapies for the related infection. These compounds can act as preventive agents or as treatment accelerators. Flavanones, flavones, and saponins are some natural ACE2 inhibitors [44,45]. Saponins can inhibit the binding of COVID-19 protein S to ACE2 receptors [46] (Figure 2).

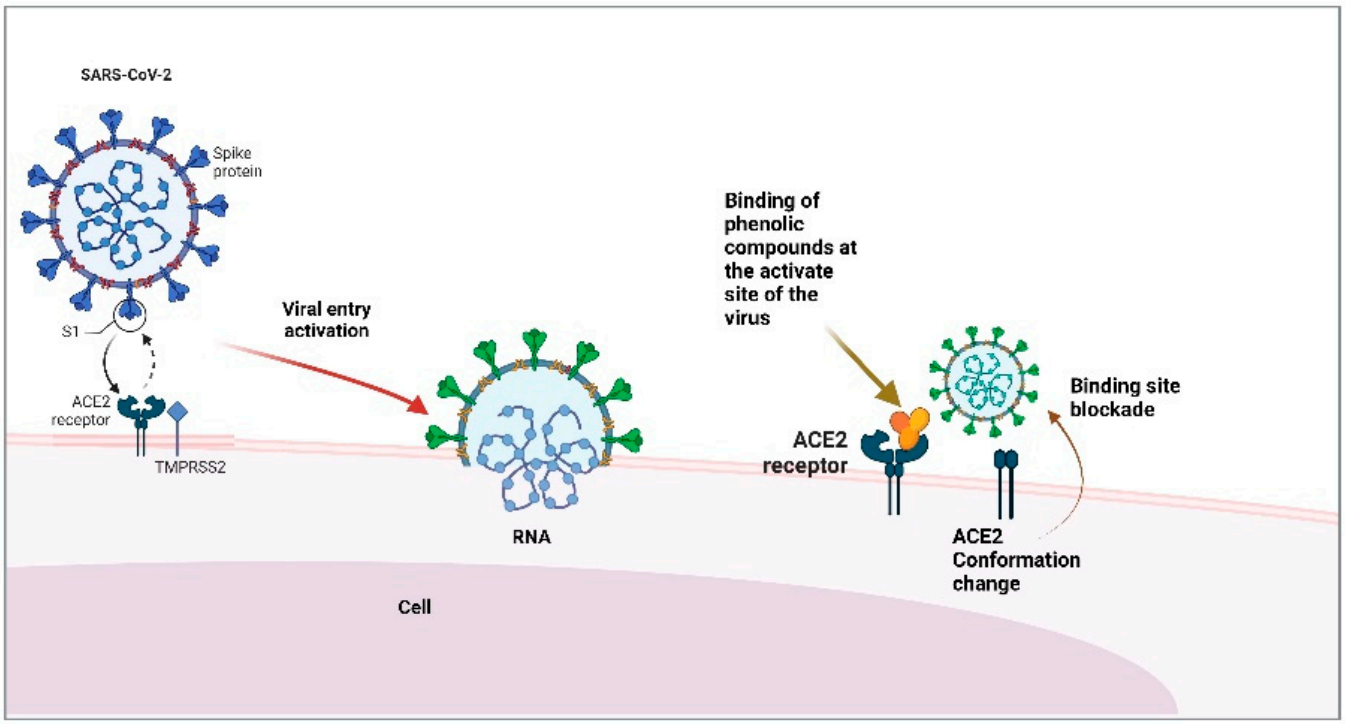

Figure 2. Phenol binding to the ACE2 receptor and protein S blockade of SARS-CoV-2. Created with BioRender.com (accessed on 28 August 2022).

The peptide inhibitors that are used in the treatment of diverse diseases could also be potential agents against COVID-19. The bioactive peptides with unique sequences of amino acids can mitigate the inhibition of the transmembrane proteases and serine type II (TMPRSS2), a gene regulated by androgens, for the priming of the viral protein peak, furin split, and the members of the renin–angiotensin–aldosterone system (RAAS). On the other hand, it has been shown that the inhibition of virus replication could be mediated by hydrogen bonding through the binding of amino acid residues [47,48] (Figure 3).

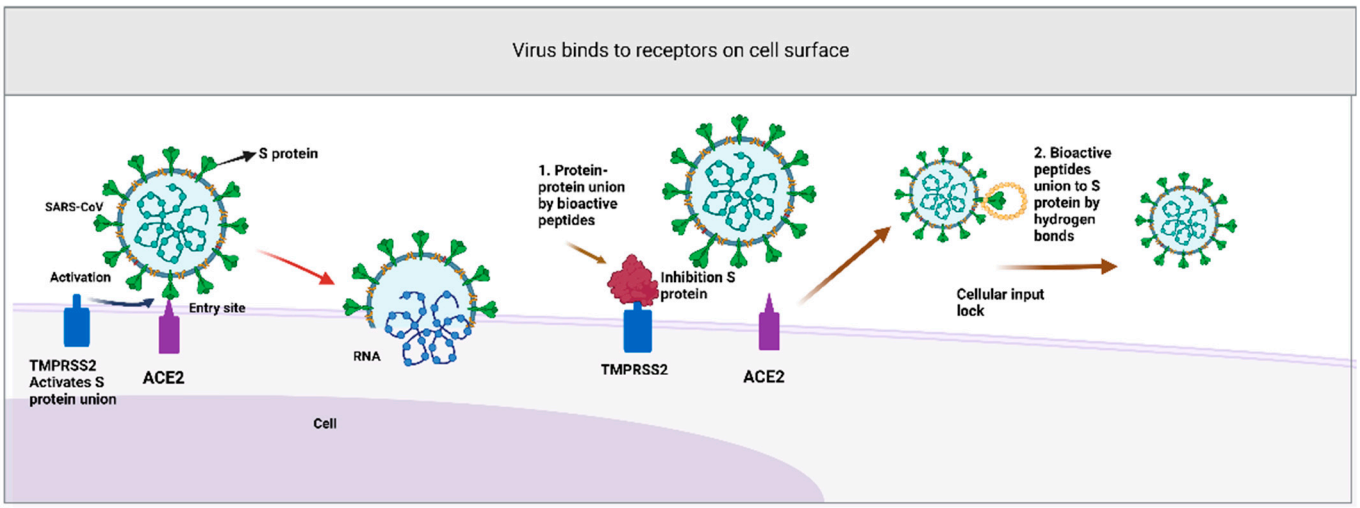

Figure 3. Peptide activity on SARS-CoV-2: (1) Inhibition of TMRPSS2 by bioactive peptides blocks priming of virus S proteins. (2) Inhibition of protein S by amino acid residues through hydrogen bonds prevents SARS-CoV-2 virus' entry. Created with BioRender.com (accessed on 28 August 2022).

The peptides of a food origin can perform diverse bioactivities, including antiviral activities, depending on their characteristics and sequencing [49]. Therefore, the peptides

derived from cereals could serve as inhibitors of multiple processes regarding the entry into the host cell and the viral replication of SARS-CoV-2. Diverse epidemiological studies highlight the importance of the consumption of diets rich in cereals and products of a natural origin that help to protect against hypertension and viral diseases such as COVID-19 [33,50,51].

5. Cereals as a Source of Compounds with Anti-Hypertensive Activity

The flavonoids and phenolic acids present in cereals have an ACE-inhibitory capacity mainly associated with blood pressure-lowering effects due to their antioxidant capacity [52]. The regulation of reactive oxygen species, the reduction in oxidative stress, and the formation of zinc chelates are factors that promote the lowering of blood pressure [53,54]. In Table 1, the in vivo or in vitro antihypertensive mechanisms of phenolic compounds present in some cereals are described.

Table 1. Phenolic compounds derived from cereals with antihypertensive activity.

Food	Main Phenolic Compound	Test	IC_{50} or % IECA	Decrease BP	Main Mechanism	Reference
Virgin rice bran oil	Sterols, tocopherols, and tocotrienols	in vivo	ND	25.5%	Regulation of NOS and reduction in oxidative stress	[54]
Raw rice	Phenol acids Flavonoids	in vitro	97%	ND	Competitive inhibition of ECA	[55]
Rice bran hydrolysate	Phenolic compounds	in vivo	ND	31.5%	Endothelium-derived hyperpolarizing factor-mediated vasorelaxation and L-type Ca 2+ channel-mediated vasoconstriction	[56]
Barley seedlings	Polyphenols	in vitro	66.5%	ND	Non-competitive inhibitors of ECA and formation of chelates with ions of zinc	[57]
Barley whole grain Barley bran	Anthocyanins	in vitro	8770 µg/mL 4540 µg/mL	ND	Natural competitive inhibitors of ECA	[58]
Solid-state fermented wheat	Phenolic compounds	in vitro	53.8%	ND	Inhibition of ECA by proteolysis	[59]
Bioprocessed wheat middlings	Phenolic compounds	in vitro	94.9%	ND	The hydrolysis of short chain peptides increases ECA- inhibitory capacity	[60]
Sorghum roasted grain	Phenolic acids and flavonoids	in vitro	20.99 µg/mL	ND	Hydrogen and the hydrophobic union caused by the denaturation of enzymes	[61]
Sorghum grains	Phenolic compounds	in vitro	46.3%	ND	Production of peptides and free amino acids before germination increases ECA-inhibitory activity	[62]
Extruded maize products added with a red seaweed	Phenolic compounds	in vitro	41%	ND	ECA inhibition trough sequestration of enzyme metal factor Zn^{2+}	[53]
Water extracts of maize	Soluble phenols	in vitro	50%	ND	Small peptide compounds may represent the bioactive factors contributing to the total ECA-inhibitory activity	[63]

BP: Blood Pressure; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; % IECA: Percent inhibition of ECA; ND: Not determined.

In addition to phenolic compounds, studies have been conducted on multiple candidates for antihypertensive peptides, which, because of their biological activity, can be generated or incorporated into functional foods. Table 2 summarizes studies highlighting cereal peptides and proteins with antihypertensive activity. Proteins with a molecular weight lower than 1 kDa favor their entry through cell membranes enabling their absorption and circulation [64]. Hydrolyzed proteins with high levels of proline and other

amino acids contribute to enzyme inhibition by chelation with zinc at the active center of the enzyme and its interaction with hydrophobic sites. Therefore, the ionic interaction between amino acids and zinc enhances the competitive activity for the catalytic sites of ACE [65,66]. Since there are antihypertensive peptides from cereals rich in proline and other hydrophilic amino acids related to the S protein of SARS-CoV-2, they could serve as multi-target inhibitors against host cell entry. The antihypertensive rice bran tripeptide Tyr-Ser-Lys, reported by Wang et al. [67], has two aliphatic amino acids in its chain with a hydroxyl in the C-terminal chain; thus, it could have antiviral effects. Similarly, the peptide Gly-Phe-Pro-Thr-Leu-Lys-Ile-Phe—reported by Gangopadhyay et al. [68]—in barley flour presents four hydrophilic amino acids, increasing the chances that it will be coupled to the S protein of the virus that causes COVID-19. An in silico study showed that some oligopeptides from barley, oats, wheat, and soybeans (PISCR, VQVVN, PQQQF, and EQQQR) were identified as potential binders of the SARS-CoV-2 spike protein receptor-binding domain (RBD) [69]. This feature is also observed in short-chain peptides isolated from cereals [70]. Antihypertensive peptides generally contain amino acid residues at the C-terminus or N-terminus. The presence of tyrosine, phenylalanine, tryptophan, proline, lysine, isoleucine, valine, leucine, and arginine present in the peptides influence the binding of the ACE substrate or inhibitor [71]. According to the reported studies, an association has been established between the presence of bioactive compounds and the ACE-inhibitory mechanism and this could have a significant impact on the active sites of SARS-CoV-2.

Table 2. Peptides derived from cereals with antihypertensive activity.

Food	Bioactive Compound	MW	Test	IC_{50} or % IECA	Decrease BP	Main Mechanism	Reference
Bran of rice	Peptide	<4 kDa	in vitro	30 µg/mL	ND	Reducer and inhibitor of ECA	[67]
Rice protein hydrolysates	Dipeptides	ND	in vitro	76.58-µg/mL	ND	Blocker of ECA due to the presence of aromatic amino acids	[72]
Barley flour	Peptide	<3 kDa	in vitro	70.3%	ND	Inhibitors of ECA via the presence of hydrophobic amino acids	[68]
Corn germ flour	Peptide	<3 kDa	in vivo	830 µg/mL	15.7%	Regulation of vasoconstrictors increases in NO and prostacyclin decreases in Ang II	[73]
Corn germ	Peptides	<6 kDa	in vitro	1389 µg/mL	ND	Inhibitory effect on ECA	[74]
Corn gluten flour	Peptides	<3 kDa	in vivo/in vitro	290 µg/mL	>30 mmHg SBP	Persistent inhibition of the ECA in tissues	[75]
Corn gluten flour	Dipeptide	ND	in vivo-in vitro	37 µg/mL	35–45 mmHg SBP	Inhibitor of ECA by possible synergy between peptides	[76]
Hydrolyzed wheat gluten	Peptides	<1 kDa	in vitro	2 µg/mL	ND	Inhibition of ECA by electrostatic interactions and interactions with hydrogen bonds	[66]
Hydrolyzed wheat gluten	Peptides	<1 kDa	in vitro	4 µg/mL	ND	Competitive and non-competitive inhibitors of ECA	[77]
Defatted wheat germ	Peptides	<5 kDa	in vitro	452 µg/mL	ND	Inhibition of ECA by enzymolysis and ionization of proteins	[78]
Defatted wheat germ	Hydrolyzed proteins	ND	in vitro	220 µg/mL	ND	Inhibition of ECA by hydrophobic amino acids	[79]
Wheat flour	Phenolics from peptide fractions	ND	in vitro	84.52%	ND	Inhibition of ECA by bound phenols after acid hydrolysis	[80]

Table 2. *Cont.*

Food	Bioactive Compound	MW	Test	IC_{50} or % IECA	Decrease BP	Main Mechanism	Reference
Oat-isolated protein	Peptides	<3 kDa	in vitro	60%	ND	Ultrasonic pretreated enzymolisis increased ECA-inhibitory activities of the oat peptides	[81]
Oat protein hydrolysate	Peptides	ND	in silico	96.5%	ND	Inhibition of ECA-I by aromatic, small acids with low lipophilicity and high electronic properties	[82]
Oat protein hydrolysate	Peptides	<3 kDa	in vitro e in silico	35 µg/mL	ND	Competitive inhibitors of ECA	[83]
Sweet sorghum grain	Peptides fractions	<1 kDa	in vitro	31.6 µg/mL	ND	Binding of the C-terminal of Serine with the active sites of ECA	[84]
Sorghum protein hydrolysate	Tripeptides	ND	in vitro	1.3 µg/mL	ND	Competitive inhibitor of ECA	[85]
Bread produced with addition of 6% rye-malt gluten	Peptides	ND	in vitro	0.002 µM/mL	ND	ECA binding at the N-terminal and proline or aromatic amino acids at the C-terminus	[86]
Extruded and fermented millet	Peptides	ND	in vivo	ND	14.6%	Reduction in the indexes of RAAS	[87]
Bread or sandwiches with pure millet grains	Protein	ND	Clinical	ND	3%	Inhibition of vasoconstrictors and induction of vasodilators	[88]

BP: Blood Pressure; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MW: Molecular weight; % IECA: Percent inhibition of ECA; Ala: Alanine; Arg: Arginine; Cys: Cysteine; Gln: Glutamine; Glu: Glutamic acid; Ile: Isoleucine; Leu: Leucine; Lys: Lysine; Phe: Phenylalanine; Pro: Proline; Ser: Serine; Thr: Threonine; Trp: Tryptophan; Tyr: Tyrosine; Val: Valine; NOS: Nitric oxide synthase; ND: Not determined.

5.1. Rice

Wild rice (*Zizania* spp.) is one of the cereals that has presented anti-hypertensive, antiallergic, and immunomodulating activities, which are associated with the phenolic acids, flavonoids, and other phytochemicals with antioxidant properties that aid in the prevention of chronic illnesses [89,90]. Okarter and Liu [91] report that the low incidence of chronic diseases in regions where rice is consumed is related to the presence of phytochemical antioxidants in this cereal. Consequentially, these studies suggest the potential use of rice and its by-products in the prevention or contributory treatment of non-transmissible diseases such as hypertension.

Gong et al. [89] quantified the total phenolic content and flavonoids in different varieties of rice, such as black rice, red rice, whole rice, and plain rice. They reported concentrations of 1159, 669, 108.7, and 58.88 mg of Gallic Acid Equivalents (GAE)/100 g. With respect to the total content of flavonoids, the authors reported 1503, 598.2, 77.94, and 26.52 mg Quercetin Equivalents (QE)/100 g in black rice, red rice, whole rice, and plain rice. Deng et al. [92] demonstrated the antihypertensive effects of wild rice (*Zizania latifolia)* in spontaneously hypertensive rats, attributing these effects to the influence of the polyphenol content, principally quercetin, due to previous studies that have demonstrated that this compound reduces blood pressure and, moreover, since it presents protective effects against cardiovascular diseases. Table 1 shows the main mechanism used in the ACE inhibition of some phenolic compounds derived from cereals such as rice. The phytochemical composition of wild rice is so complex that the decrease in hypertension could be related to the synergic effects of bioactive compounds such as polyphenols and bioactive tripeptides [92,93].

Michelke et al. [72] evaluated possible ACE inhibitor peptides found in hydrolyzed whey, soy, and rice protein. The evaluation of ACE inhibition was performed in different ACE systems such as human plasma, venous endothelial cells from human umbilical cord, rabbit lungs, and rat aortic rings. The IC50 values observed in the soybean and rice peptide mixtures were approximately 2 to 2.5 times higher than the IC50 value of the

serum-derived peptides. Therefore, the best ACE-inhibitory activity was from the serum peptides consisting of isoleucine and tryptophan.

Some studies have shown the effectiveness of dipeptides made up of isoleucine and tryptophan (IW) in decreasing ACE, showing anti-inflammatory and antioxidant activities in endothelial cells [94,95]. Lunow et al. [96] mention that the IW dipeptide acts as a competitive and selective inhibitor for the C-Terminal of ACE in plasma.

Jan-on et al. [54] demonstrated that virgin rice bran oil prevents hypertension induced by the L-NG-nitroarginine methyl ester (L-NAME) in rats, improving the hemodynamic alterations, as well as the reduction in oxidative stress and vascular inflammation. This suggests that these activities could be mediated by the content of unsaturated fat, antioxidants, phytochemicals such as g-oryzanol, phytosterols, and tocopherols, which possess antioxidant activities and provide vascular and inflammatory protection.

On the other hand, rice bran presents a high concentration of biologically active compounds that are important for human health, of which are found cellulose, hemicellulose, pectin, arabinoxylan, lignin, β-glucan, polyphenols, γ-oryzanol, β-sitosterol, vitamin B9, vitamin E, tocopherols, micronutrients (such as calcium and magnesium), and essential amino acids (such as arginine, cysteine, histidine, and tryptophan) [97].

Due to the high content of nutrients, a diet rich in rice increases immunological, antioxidant, anticancer, and antidiabetic activities, protecting the organism against multiple diseases [98]. Therefore, the use of these compounds and their different functions as collectors of free radicals, antiallergy agents, antiatherosclerosis agents, anti-influenza agents, anti-obesity agents, and antitumor agents offer protection against numerous chronic diseases and degenerative diseases in humans, including hypertension and some cases that could interfere with the infection of COVID-19.

5.2. Barley

In barley (*Hordeum vulgare* L), phytochemical concentrations have been reported in relation to a reduction in heart disease, colon cancer, gallstones, and cardiovascular illnesses [99]. The Food and Drug Administration reported that the intake of barley is related to a decrease in cardiovascular diseases [100], such as chronic coronary diseases, due to the decrease in plasma cholesterol promoted by β-glucans from hulled barley, which promote the excretion of fecal lipids [101].

Some of the properties that are attributed to barley for reducing the risk of cardiovascular diseases such as hypertension are related to their different bioactive components, which include peptides and ACE-inhibitory proteins [102]. However, some studies mention that the high inhibition of ACE is principally stimulated by the combination of components that come from antioxidants [103], peptides [68], or phenolic compounds [104].

Different authors have also demonstrated the great variety of bioactive compounds originating from barley [57,68], among which the most utilized are inhibitors of ACE. The total concentration of phenolic acids ranges between 604 and 1346 mg/g [105]. Kim et al. [106] studied the content of 127 varieties of barley with and without husks and they found that the flavonoid content ranged from 62–300.8 mg/g. Andersson et al. [107] studied 10 varieties of barley and found that the concentration of phytosterols ranges between 820 and 1153 mg/g. With respect to anthocyanins, the most common found in barley is the cyanidin 3-glucosidic type (214.8 mg/g) [108]. On the other hand, the lignans are the most studied polyphenols in barley, whose concentration ranges between 6.6 and 541 mg/100 g [109].

The peptides obtained from barley also present inhibitory effects towards ACE. The effects of the peptides occur principally because of the presence of hydrophobic peptides in the C-terminal chain of the peptide that are united in the active sites of ACE [68]. The presence of anthocyanins and polyphenols extracted from whole grains and seedlings of barley, respectively, have also been studied as potential inhibitors of ACE, presenting competitive and non-competitive inhibitory mechanisms. Some polyphenols show a non-competitive inhibition of ACE when a structural difference with the natural substrate of ACE is produced (Table 1) [57,58].

In addition to phenolic compounds and inhibitory peptides, the soluble fiber in barley and other cereals has an important role in human health. A study by Behall et al. [110] observed a reduction in systolic and diastolic blood pressure in middle-aged men and women after a 5-week integral diet. Fiber has anti-inflammatory effects, and in adults with asthma, an average fiber intake of 5 g/day plus a controlled mineral-rich diet is inversely associated with the eosinophilic inflammation of the respiratory tract and pulmonary function [111,112]. Epidemiological studies in humans have demonstrated that fiber can promote health and prevent chronic diseases, especially those related with inflammation [113], which could improve the cognitive function of people infected with COVID-19 [113]. Therefore, the intake of dietary fiber can support antiviral and immunosuppressive therapeutic treatments, thereby ameliorating the suffering of COVID-19 [114].

5.3. Corn

Across the globe, there are different varieties of corn, which is rich in fiber, vitamins, minerals, phenolic acids, flavonoids, sterols, and a great variety of phytochemicals [115]. There are reports that indicate that corn is one of the cereals with the highest availability of nutrients, mainly β-carotene and α-tocopherol, which suggests that it may be the most suitable for biofortification [116]. However, this may depend on the pigments in the grain. Blue, red, and purple corn have a higher concentration of anthocyanidins; in Chinese purple corn, approximate concentrations of 256.5 mg of cyanidin 3-glucoside/100 g at a dry weight have been reported, while in American corn, the anthokinin content ranges from 54 to 115 mg/100 g per sample [117,118]. Yellow corn is rich in carotenoids with a concentration of 0.823 mg/100 g per dry weight of corn [119]. Violeta et al. [116] have reported concentrations of 26.9 μg/g of β-carotene and 27.2 μg/g of α-tocopherol in dark orange corn grains, while in dark red corn, they were 2.51 and 4.95 μg/g, respectively, and in red corn, they only reported a concentration of α-tocopherol of 4.87 μg/g. Pigmented genotypes have shown a strong antioxidant capacity using DPPH and TEAC techniques [120]. In black corn, a higher antioxidant activity has been reported than in yellow and white corn [121]. According to reports, the type of phenolic compound and/or flavonoids are associated with grain pigmentation. The bioactive compounds have been related to antioxidant [122], anticancer [123], antimicrobial, and anti-viral activities [124]. The anthocyanin content differs by the variety of corn; in pink corn, it is approximately 12.74 mg of cyanidin 3-glucoside/100 g at a dry weight, while in black corn, the anthocyanin content is 304.5 mg of cyanidin 3-glucoside/100 g at a dry weight [118]. Corn has the highest antioxidant activity with 181.4 μmol equivalents of vitamin C/g per grain compared to cereals such as rice that have 55.77 μmol equivalents of vitamin C/g per grain, wheat with 76.70 μmol equivalents of vitamin C/g per grain, and oats with 74.67 μmol equivalents of vitamin C/g per grain [125].

Mellen et al. [126] carried out a metanalysis regarding the intake of whole grains and clinical cardiovascular events. According to their estimates, the consumption of whole cereals reduces the risk of suffering cardiovascular diseases by 21%. Similarly, this has been related to a decrease in the risks of suffering chronic diseases such as diabetes type 2 [127], obesity, some cancers [128,129], and cardiovascular diseases [130]. Wu et al. [74] evaluated the antihypertensive activity of ACE inhibitor peptides from corn germ using the hydroenzymatic lysis method with alkaline protease that allows for the production of a high concentration of inhibitor peptides. They carried out an ultrafiltration that allowed them to obtain smaller peptides of 6 kDa, increasing the IC50 of the inhibitory activity of ACE and demonstrating that the smaller the size the better absorption, according to the authors [7,75].

It has been reported that the peptides extracted from corn germ flour promote the balance between vasoconstrictor factors, vascular endurance, and the reduction in the level of renin and Angiotensin II, thus controlling blood pressure [73,74]. Huang et al. [75] demonstrated the antihypertensive effect of the peptides of corn in spontaneously hypertensive rats. They reported that two types of mechanisms of action exist in the peptide inhibitors

of ACE: those that compete with the availability of the substrate of ACE and those that combine the bioactivity of ACE to inhibit its enzymatic activity. These are normally made up of more than four amino acids and from two to three amino acids. It is shown in this study that the molecular size of the inhibitory peptide of ACE plays an important role in its inhibitory activity because the peptides less than 3 kDa had an inhibition four times greater than the peptides of 5 kDa. In a dipeptide (Ala-Tyr) isolated from a hydrolyzed corn gluten flour, an IC50 of 82.92% was observed; therefore, due to its size, it is a potential ACE inhibitor [131]. Some peptides and proteins derived from cereals with antihypertensive activity are shown in the Table 2.

Duru [132] showed that the minerals and phytochemical content present in corn husks contribute to multiple health benefits. Among the most abundant minerals that can be found are calcium, sulfur, and potassium, which contribute to nerve and muscle regulation. This is the case for calcium; sulfur is present in different amino acids and potassium plays a part in the acid–base balance and osmotic regulation. As a consequence, a modification of the diet that includes the consumption of corn could be a strategy to prevent cardiovascular diseases and infectious diseases such as COVID-19.

5.4. Wheat

Wheat (*Triticum* spp.) has been used for the elaboration of basic foods since time immemorial and is highly essential in human nutrition, providing 55% of starch and more than 20% of food calories. Clinical studies have demonstrated that the regular consumption of wheat is associated with a reduction in chronic diseases, specifically the intake of dietetic fiber and other bioactive compounds [4].

Wheat is a rich source of diverse phytochemicals, among which are phenolic acids, terpenoids, tocopherols, and sterols [133]. The concentration of phenolic acids in whole wheat ranges between 200 to 1200 mg/g in dry weight [134]. The type of milling and the use given to this cereal has a great impact on the composition of the bioactive compounds and, thus, the health benefits as well as the improvement of the functions of the colon, those against cancer, those that protect against obesity, those that promote weight loss, and those that mitigate cardiovascular diseases [4,135].

Zhang et al. [66] isolated peptides from wheat gluten for their potential use as ACE inhibitors, showing the importance of generating gluten hydrolysates to increase their benefits, especially for the celiac population.

Besides gluten, wheat germ has widely been studied because of its high protein content. Diverse studies have demonstrated that the peptides isolated from wheat germ and some isolated from wheat gluten, such as VPL (Val-Pro-Leu), WL (Trp-Leu), WP (Trp-Pro), and IAP (Ile-Ala-Pro), present antihypertensive effects principally for ACE inhibition, which is caused principally by the high presence of hydrophobic amino acids such as proline and tryptophan (Table 2) [8,78,79].

Asoodeh et al. [77] performed a characterization of ACE-inhibitory peptides from wheat gluten protein hydrolysates through the use of trypsin. The sequences with the highest inhibitory activity were Ile-Pro-Ala-Leu-Leu-Lys-Arg and Ala-Gln-Gln-Leu-Ala-Ala-Gln-Leu-Pro-Arg-Met-Cys-Arg; as in most inhibitory peptides, this activity is influenced by the peptides' structure, since some peptides that have tryptophan, tyrosine, phenylalanine, and proline residues and hydrophobic amino acids in the C-terminal sequence show greater inhibitory activity towards ACE [136].

Besides the extraction and evaluation of inhibitory peptides, Gammoh et al. [80] demonstrated that the isolation of phenols from protein fractions in wheat flour increased antihypertensive activity in an in an vitro model, alongside increasing antioxidant properties and decreasing allergenicity.

Recently, studies have demonstrated the capacity of polysaccharides to increase the immune response to infectious diseases. In cells such as macrophages, the polysaccharides activate the protein tracts, stimulating the control processes of the immune response [137]. Therefore, the polysaccharides of wheat induce the expression of cytokines, activating

macrophages and increasing the phagocytotic activity [138,139]. Thus, the polysaccharides activate the important immunosuppression tracts for the treatment of persons infected with COVID-19 because they stimulate the production of anti-inflammatory substances, which could apply to the treatment of grave cases [137].

5.5. Oats

Oats (*Avena sativa*) are a whole cereal that provide proteins, unsaturated fatty acids, vitamins, minerals, dietetic fiber, and phenols such as the avenanthramides [140]. Soycan et al. [141] determined the concentration of phenolic acids and avenanthramides in commercial products of oats and showed that there was a greater concentration of these compounds (1518.6 μg/g) compared to oat bran (626.3 μg/g). Different bioactive compounds have been reported in oats, such as phenolic compounds, with a concentration between 180 and 576 mg Routine Equivalents (RE)/100 g. As to the phytosterols, oats present a concentration between approximately 35 and 68.2 mg/100 g. On the other hand, the tocopherol content (vitamin E) ranges between 0.5 and 3.61 mg/100 g [142].

Diverse studies mention that a regular consumption of oats reduces cholesterol [5,143], improves the sensitivity of insulin [144], and controls blood pressure [145]. Soyca et al. [141] reported a concentration of total phenolic acids of 39.5-62.75mg/100 g per sample. In this study, it is mentioned that ferulic acid is the principal component present in commercial oats, consisting of 58-78.1% of the total compounds. Ferulic acid presents antioxidant activities that can prevent chronic diseases [146]. It has been demonstrated that avenanthramides offer health benefits such as antioxidative properties that can help protect against cardiovascular diseases [147].

Few studies have investigated the benefits offered by oats in hypertension. However, their positive effects on cardiovascular diseases have not been discarded. Wang et al. [81] evaluated the ultrasonic pre-treatment of the protein in oats and its activity as a protein inhibitor of ACE, utilizing the enzymatic pre-treatment with ultrasound for the improvement of the hydrolysis of proteins and the process of enzymolysis for the liberation of peptides less than 3kDa. The results showed that the ultrasonic energy, the duration of treatment, and the time of enzymolysis greatly influenced the hydrolysis grade and inhibitory activities of the ACE of the peptides. They showed that the inhibition of ACE provoked by the peptides had an increase of 32.1 to 53.8% compared to samples without ultrasonic treatment. According to the authors, the rate of enzymatic hydrolysis after ultrasonic pre-treatment was due to the increase in the affinity between the alcalase and the isolated protein. Alcalase is a specific endonuclease enzyme that combines exposed hydrophobic sides, which could have brought about an increase in the production of inhibitor peptides of ACE, provoked by the high grade of the hydrolysis it promoted [148].

Besides the protein inhibitors of ACE, the soluble fibers such as the β-glucans of oats have been widely studied, demonstrating prebiotic effects and improving glycemic control and regulating blood pressure [149,150]. Maki et al. [151] evaluated the effect of the consumption of foods that contain the β-glucan from oats in blood pressure. The study consisted of a controlled randomized clinical trial, which was double blinded, where 97 men and women, with a mean age of 63 years, systolic blood pressure of 130–179 mmHg, and/or a diastolic blood pressure of 85–109 mm Hg were assigned to consume foods containing oat β-glucan or control foods for 12 weeks. Although the results did not show a significant difference in terms of the decrease in blood pressure between the groups, the decrease in blood pressure significantly decreased both the systolic (8.3 mm Hg, $p = 0.008$) and diastolic (3, 9 mm Hg, $p = 0.018$) pressure in the subjects with a body mass index above the mean (31.5 kg/m^2) compared to the control groups.

The extracts of β-glucans produce immunomodulatory effects and pulmonary cryoprotections, which could have therapeutic implications in patients with COVID-19. In the same way, these could reduce oxidative stress and activate macrophages [33].

McCarty and DiNicolantonio [152] recently described the potential role of β-glucan as a natural nutraceutical to boost the response of interferon type 1 to RNA viruses such as the

influenza and the coronavirus. Therefore, the intake of oat products provides a rich source of phytochemicals that provides health benefits such as decreasing high blood pressure and influencing the immunotherapies against infections such as COVID-19 due to the presence of inhibitory peptides of ACE and of β-glucans.

5.6. Millet

Millet includes numerous species that are not related genetically. However, it contains various phytochemicals, phenolic compounds, phytosterols, policosanols, and bioactive peptides [153]. Chandrasekara and Shahidi [154] evaluated different varieties of this cereal that presented approximate concentrations of hydroxybenzoic and hydroxycinnamic acids and their by-products from 9.3 to 62.2 μg/g and 9.1 to 173 μg/g of defatted flour, respectively, both in their free forms. As to flavonoids, this cereal contains from 2 to 100 mg/g, which differs because of the variety of the species [153]

The protein of foxtail millet (*Setaria italica Beauv*) can have physicochemical and physiological properties. Some studies have found that foxtail millet presents antioxidant activities, reduces the levels of cholesterol, and can present anticancer effects [155,156].

Furthermore, foxtail millet presents antihypertensive effects. Studies reported the inhibitory capacity of the ACE of hydrolyzed proteins derived from this cereal [87]. The consumption of whole grains can reduce blood pressure. Hou et al. [88] reported that the consumption of 50 g of whole grains of pulverized foxtail millet extruded in the form of bread or millet pancakes for 12 weeks showed a significant reduction in SBP of 133.61 and 129.48 mmHg, as well as a reduction in the mass index and body fat in 45 middle-aged hypertensive patients. However, Chen et al. [87] showed the best results with respect to decreasing blood pressure. In this study, they used spontaneously hypertensive rats. They showed that a diet of 200 mg of peptides per kg of body weight for four weeks reduces blood pressure via the intake of raw samples and in extruded and fermented samples with *Rhizopus oryzae*. Compared to the extruded and fermented samples, the raw samples caused a greater decrease in blood pressure with a reduction of 28.3 mmHg in PAS. As to the extruded and fermented hydrolyzed proteins, there was a reduction of 24.8 and 13.6 mmHg, respectively. A controlled group treated with captopril had a reduction of 23.6 mmHg.

Therefore, the consumption of foxtail millet protein, specifically hydrolyzed, raw, and extruded millet protein, improves hypertension due to the antioxidant and anti-inflammatory properties whereby vascular conditions can be regulated gradually (Table 2) [157]. In both studies, the levels of ACE and Ang II decreased, which could indicate that the antihypertensive mechanism of foxtail millet consists of inhibiting the activity of the ACE in the serum of subjects with slight hypertension. The antihypertensive effects produced by cereals are related to the improvement in the endothelial function that is achieved by inhibiting the effects of vasoconstrictors such as Ang II, inducing vasodilatation through nitric oxide, and affecting the vasorelaxation tracts involved. Along with the previously mentioned cereal, the consumption of millet can aid the modulation of immune functions, which helps to protect against the COVID-19 ailment [158].

5.7. Rye

Among cereals, rye (*Secale cereale* L.) contains the highest concentration of dietetic fiber, which is composed of arabinoxylan, cellulose, β-glucan, fructans, and lignin. Arabinoxylan is the most abundant fiber in rye (7.6–12.1% of the dry grain weight) [159]. Pihlava et al. [160] reported 0.5, 4.6, and 20.5 mg/100 g of dry weight of total flavonoids present in the fine flour of rye, whole rye flour, and rye bran, respectively. As to the quantity of anthocyanins, the authors reported 0.15 mg/100 g in rye bran, 0.18 mg/100 g in whole rye flour, and 0.026 mg/100 g in fine rye flour. They also reported 66.3, 15.5, and 291.6 mg/100 g in the dry weight of alkylresorcinols in whole rye flour, fine rye flour, and rye bran, respectively.

There is important evidence within the studies of the physiological effects of rye foods with possible health benefits, such as the positive effects on tumors in prostate cancer [2], antihyperglycemic properties, and antihypertensive activities [86]. Zhao et al. [86] evaluated the concentration of inhibitors of the ACE of different bakery products starting with rye sourdough. They reported eight ACE-inhibitory tripeptides. The dominant tripeptide was IPP (Ile-Pro-Pro) with 58 to 73 mmol/kg. Moreover, the peptide that showed the greatest inhibition of ACE was LPP (Leu-Pro-Pro) (57 mmol/L), which is characterized by the presence of leucine, an amino acid with a greater hydrophobicity, which is a principal characteristic of the inhibitors of ACE.

Rye grains are a source of diverse phytochemicals such as phenolic acids, lignans, and alkylresorcinols [160]. Multiple studies have demonstrated the capacity of the secondary metabolites of plants to generate antiviral activities besides the importance of phytochemicals against SARS-CoV [161,162]. There are studies that link the effectiveness of dietary fiber to the prevention of diseases related to lifestyle such as hypertension [163,164]. Dietary fibers reach the colon and produce short-chain fatty acids, which are released into the circulation to reach the organs involved in the regulation of hypertension [165]. Due to the high content of dietary fiber, proteins, and various bioactive compounds, rye can enhance immunomodulatory and antihypertensive activities.

5.8. Sorghum

Sorghum (*Sorghum* spp.) contains tannins, phenolic acids, anthocyanins, and phytosterols. These phytochemicals have the potential to provide a significant impact on human health, promoting cardiovascular health by reducing the plasma levels of lipoproteins of a low density and hepatic cholesterol [166]. Sorghum contains benzoic acids and cinnamic acids, which range from 16 to 131 mg/g and from 41 to 444 mg/g, respectively [167].

Anthocyanins are the most studied flavonoids in sorghum; Awika et al. [168] reported that the anthocyanin content in black sorghum bran is three to four times higher than in whole grain and had at least twice the anthocyanin levels (10.1 mg/g) in comparison with red sorghum (3.6 mg/g). The quantitative data of the phytosterols present in sorghum are limited, although approximate contents of 44 to 72 mg/100 g have been reported [169,170].

The generation of ACE-inhibitory peptides has been carried out in different forms. Most of these techniques were based on the production of peptides from food proteins via enzymatic hydrolysis [66]. Wu et al. [84] developed a kinetic method that describes the enzymatic hydrolysis of the protein of sweet sorghum grain utilizing alcalase to purify ACE-inhibitory peptides (Table 2). The authors demonstrated that 19% hydrolysis exhibited the strongest inhibitory activity of ACE. On the other hand, they obtained a tripeptide inhibitor composed of Threonina (Thr)-Leucine (Leu)-Serine (Ser), which, due to the serine union at the C-terminal of the chain, manages to interact in the peak protein subunits (S1 and S2) of ACE, thereby achieving its inhibition. Some studies explained the relationship between the structure and the activity of the inhibitory peptides of ACE, which are influenced by the C-terminal and the presence of hydrophobic amino acids or aromatic residues such as Tryptophan (Trp), Tyrosine (Tyr), Proline (Pro), and Phenylalanine (Phe). However, this structure-activity relationship has not been completely established [148].

The polyphenols have an ample antiviral activity against diverse groups of viruses such as influenza A (H1N1), hepatitis B and C (VHB/VHC), herpes simplex 1 (VHS-1), human immunodeficiency virus (HIV) and, recently, the virus that caused the COVID-19 disease (SARS-CoV-2) [171].

Besides their antiviral capacity, the phenolic compounds can also present antihypertensive activity. Irondi et al. [61] analyzed raw and toasted red sorghum grain flour (150 and 180 °C) to determine the inhibitory activities of different enzymes including ACE. They found that the raw grains showed high inhibitory activities (19.64 μg/mL) because of the high presence of phenolic acids (gallic, chlorogenic, caffeic, ellagic, and p-coumaric) and flavonoids (quercetin, luteolin, and apigenin), as increasing the temperature when toasting decreases the presence of phenolic compounds and, consequentially, causes a decrease in

inhibitory activity, with an IC50 in the grains roasted at 150 °C of 20.99 μg/mL and in the grains roasted at 180 °C of 22.81 μg/mL. Therefore, the parallel decrease in the inhibitory activity of the enzymes and the phenolic composition of the grains with the increase in the toasting temperature suggests that the phenolic acids and the flavonoids could be the principal inhibitors of the enzymes of the grain.

In this way, sorghum is a cereal with high potential to control hypertension and, in some cases, its consumption could reduce the probability of viral infection by SARS-CoV-2 due to its high phytochemical content. In general, this cereal seems to have a great potential to form part of a healthy diet and its consumption as grains or as food products could reinforce the bioavailability of nutrients to prevent chronic diseases and infections.

6. Conclusions

Different components of cereals have been characterized, such as anthocyanins, flavonoids, phenolic acids, proteins, and fibers, which have biological activities that help prevent or control hypertension acting on the RAAS, inflammation, and oxidative stress. According to the studies reported in this review, pigmented raw rice exhibits the greatest ACE inhibition. In an in vitro study, raw rice was shown to inhibit up to 97% of ACE. This activity is related to the reduction in oxidative stress and the reduction in NOS, caused by the presence of phenolic compounds such as proanthocyanidins. In silico studies showed that peptides derived from oats, made up mainly of aromatic amino acids, can inhibit up to 96.5% of ACE. The presence of this type of amino acid is also related to the ability to inhibit the TMPRSS protease of the host to prevent the entry of the SARS-CoV-2 virus. ACE inhibitor drugs (ACEi) and angiotensin II receptor blockers (ARBs) participate in processes that regulate the expression of ACE2, thus being useful in the treatment of patients who developed SARS-CoV-2. Ultimately, this review highlighted the mechanisms used by bioactive compounds in cereals to lower blood pressure and how these processes could be involved in reducing the degree of COVID-19 infection.

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CAPÍTULO III. TOTAL PHENOLS AND FLAVONOIDS IN GERMINATED BARLEY USING DIFFERENT SOLVENTS

3.1 Introducción

Este capítulo muestra un artículo original de investigación publicado en la revista *Chemistry and Biodiversity.* Se seleccionaron dos variedades: Perla y Esmeralda. Estas variedades fueron seleccionadas debido a que son las cultivadas en el estado de Hidalgo y se diferencian por la presencia de cascarilla (Esmeralda) y ausencia de cascarilla (Perla). Se realizó la extracción y cuantificación de fenoles y flavonoides totales en semillas sin germinar y con diferentes días de germinación (3, 5 y 7), utilizando solventes con diferente polaridad. Se utilizaron agua, acetona 50%, metanol y etanol al 80% como solventes para la extracción de fenoles y flavonoides. Los resultados mostraron que además de la germinación, la selección de un disolvente repercute en la eficacia para extraer fenoles y flavonoides totales. La germinación de 7 días fue la mejor opción para incrementar la concentración de fenoles con 27.34 y 21.85 mg EAG/g y para flavonoides con 8.24 y 5.50 mg EQ/g en Esmeralda y Perla, respectivamente. Se observó que existe una alta correlación entre la concentración de fenoles y flavonoides totales con el tamaño de radícula (r= 0.780-0.995). Por otro lado, los resultados mostraron que el metanol al 80% extrajo la mayor concentración de fenoles para Esmeralda (40.55 mg EAG/g) y flavonoides en Perla (6.97 mg EQ/g). A partir de este estudio se estandarizaron los extractos que serían utilizados en los estudios posteriores.

Total Phenols and Flavonoids in Germinated Barley Using Different Solvents

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Sprouts are a source of secondary metabolites as phenolic compounds. Germination and the use of solvents can affect their content. The aim of this work was to identify the total content of phenols and flavonoids in ungerminated and germinated (3, 5, and 7 days) Esmeralda and Perla barley. Different solvents (water, 50% acetone, 80% methanol, 80% ethanol) were used to recover total phenols and flavonoids. The 7-day germination proved to be ideal for total phenol and flavonoid obtention from Esmeralda barley and the highest total phenol and flavonoid content in Perla variety was observed at 5 and 7 days of germination, respectively. Methanol and ethanol (80%) yielded the highest extraction percentage of total phenols; 50% acetone recovered the highest flavonoid concentrations in Esmeralda barley and 80% methanol in Perla barley. The highest total phenol concentration was obtained from Perla samples at 13.60 mg GAE/g, and the highest total flavonoids were observed in Esmeralda barley at 1.73 mg QE/g. A high correlation was found between the concentration of phenols (0.995) and total flavonoids (0.780) with the radicle size in the Esmeralda samples.

Introduction

Barley (Hordeum vulgare L.) is a cereal widely consumed worldwide and is used in food, beverages, and some health products such as barley-based fibers and probiotics.[1–5] The consumption of germinated grains, including barley, has been reported to be increasing due to their nutritional quality and improved functional properties.[3,6]

The importance of barley lies in the presence of various metabolites, such as phenolic compounds, which have been shown to be involved in various biological activities related to different diseases.[7–9]

Germination is a simple and cost-effective process that has led to an increase in secondary metabolites in cereals and legumes.[10,11] This allows for enhancing the functionality of the human diet by using sprouts for direct consumption or incorporating them into the development of new foods that enhance their phenolic content and antioxidant activity.[6]

During the early stages of germination, limited resources are available to meet physiological processes, and when growth is restricted, carbon diversion towards the production of secondary metabolites is activated.[12] Additionally, the generation of bioactive compounds occurs through the mobilization of reserve compounds and the phenylpropanoid pathway.[13]

As a result, the level of phenols varies throughout germination, and this process can increase the nutritional quality of sprouts. The content of phenolic compounds and other nutrients may vary according to genetic or morphological characteristics, such as the presence or absence of hulls and/or the radicle size in cereals like rice and barley.[14–16] On the other hand, in maize and rice, roots have been reported to be associated with hormonal activities that allow for better nutrient absorption. A larger root has a greater capacity for nutrient storage and absorption.[17] However, some vegetables may store most of their bioactive compounds in leaves, fruits, or flowers.

Furthermore, the concentration of phenolic compounds is also related to the choice of solvent for extraction, impacting the efficiency of the process. In research and the food industry, various extraction techniques and solvents are used to obtain and concentrate phenolic compounds of interest, which can then be applied in the formulation of supplements, functional foods, or pharmaceutical products.[18,19]

The use of solvents with different polarities allows for the extraction of a wide range of phenolic compounds with varying solubility affinities. Polar solvents such as ethanol and methanol are efficient in extracting water-soluble phenolic compounds like flavonoids and anthocyanins.[14,20]

However, despite the existence of various studies on the effect of solvents in phenolic compound extraction, there is limited information on the extraction of these compounds in different barley sprouts using solvents with different polarities.[8,21]

Therefore, the objective of this article was to obtain an extract with the highest concentration of phenolic compounds from non-germinated and germinated barley for 3, 5, and 7 days, using methanol, ethanol, water, and acetone as extractants, and to examine the relationship between the content of phenolic compounds and radicle size.

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Results and Discussion

Total phenol concentration in germinated barley

Changes in total phenol contents in the two germinated barley varieties (Esmeralda and Perla) are shown in Table 1. The first column of the table presents the total phenol content using a high-polarity solvent (water) for barley sprout extract (BSE) resuspension.

Ungerminated extracts from Esmeralda variety (EBSE0) and Perla variety (PBSE0) showed concentrations of 0.64 and 0.91 mg GAE/g, respectively, and both presented significant differences ($p < 0.05$). In contrast, total phenols in Esmeralda and Perla barley sprout extract from 3 days of germination (EBSE3, PBSE3) were 7.8 and 7.9 times higher, respectively (Table 1).

These results represent a significant increase when compared against ungerminated EBSE0 and PBSE0 ($p < 0.05$).

Furthermore, germination for 5 days increased total phenols to 19 and 42% in Esmeralda and Perla barley sprout extract from 5 days of germination (EBSE5, PBSE5), respectively, as compared with germination for 3 days. Then, PBSE5 showed higher total phenols (10.36 mg GAE/g) *vs.* EBSE5. Germination for 7 days produced a 19% increase in total phenols (EBSE7) when compared to EBSE5.

Contrastingly, total phenols in Perla barley sprout extract from 7 days of germination (PBSE7) were reduced by 31.2% *vs.* PBSE5; however, the concentration was increased up to 11-fold. Similarly, PBSE0 presented an 11-fold increase after germination for 5 days (Table 1).

These results are higher than those reported by Lu *et al.* and Lee *et al.* in barley grains germinated for 5 and 6 days, respectively. The concentrations of total phenols they obtained increased 1.2–2.4 times when compared to ungerminated samples.[22,23]

Tomé-Sánchez *et al.* reported that total phenols in wheat increased 3.4 times after germination for 7 days and up to 2.3 times in corn after 6 days of germination.[10,24] According to previous works, the differences found are related to the type of seed and germination time.

Additionally, the increase in phenolic contents during germination is affected by enzymatic activities that promote the degradation of the cell wall.[25,26] This process is a consequence of chorismate biosynthesis and shikimate pathway, key metabolites to the synthesis of primary products, leading to the release of precursor amino acids for certain phenolic compounds (tryptophan, tyrosine, cysteine).[27]

The increase is related to biosynthesis and/or hydrolysis of phenolic compounds bound to cell walls, as a direct result of the enzymatic action induced by the embryo during its development.[28,29]

Therefore, it has been proven that germination mobilizes stored nutrients through enzymatic activity to significantly increase the concentration of total phenols.[30] As in the studies reported, the results in this work indicate that germination is essential to increasing total phenols. Still, extraction efficiency depends on the solvent used.[31]

Use of solvents to recover total phenols in extracts of germinated barley

Table 1 shows the concentrations obtained when using different solvents to extract phenolic compounds from EBSEs and PBSE.

We used two aqueous solutions of polar protic solvents (80% methanol and 80% ethanol), which show interactions between hydrogen bonds due to hydroxy groups (water, ethanol, and methanol).[32] An aqueous solution of a polar aprotic solvent (50% acetone), with a dipole due to a carbonyl group, was also employed.

The concentration of total phenols in non-germinated barley sprout extracts (EBSE0) ranged from 0.12 to 1.19 mg GAE/g (Table 1). Compared to water, there was an 85% increase in total phenols in EBSE0 when 50% acetone was used as the solvent. In

Table 1. Total phenolic content in barley extracts of the Esmeralda and Perla varieties (mg GAE/g of sample*).

Sample	Water	Methanol 80%	Ethanol 80%	Acetone 50%
Esmeralda				
EBSE0	0.64 ± 0.05^{Db}	0.12 ± 0.11^{Dc}	0.38 ± 0.06^{Dbc}	1.19 ± 0.18^{Ba}
EBSE3	5.02 ± 0.28^{Ca}	2.02 ± 0.18^{Cb}	1.78 ± 0.03^{Cb}	5.63 ± 0.49^{Aa}
EBSE5	6.00 ± 0.16^{Ba}	5.62 ± 0.25^{Ba}	4.91 ± 0.16^{Ba}	5.11 ± 0.45^{Aa}
EBSE7	7.15 ± 0.46^{Ac}	$12.29 \pm 0.51^{A\alpha}$	9.94 ± 0.19^{Ab}	6.33 ± 0.58^{Ac}
Perla				
PBSE0	0.91 ± 0.06^{Cb}	0.11 ± 0.01^{Cc}	0.10 ± 0.00^{Cc}	1.94 ± 0.13^{Ca}
PBSE3	7.26 ± 0.43^{Ba}	1.61 ± 0.00^{Cb}	1.10 ± 0.08^{Cb}	6.45 ± 0.02^{Ba}
PBSE5	10.36 ± 0.86^{Aa}	6.00 ± 0.13^{Bbc}	4.90 ± 0.45^{Bc}	8.40 ± 0.71^{Abab}
PBSE7	7.23 ± 0.36^{Bc}	12.44 ± 1.14^{Aa}	13.60 ± 0.35^{Aa}	10.04 ± 0.98^{Ab}

EBSE0: Ungerminated extracts from Esmeralda variety; EBSE3: Esmeralda barley sprout extract from 3 days of germination; EBSE5: Esmeralda barley sprout extract from 5 days of germination; EBSE7: Esmeralda barley sprout extract from 7 days of germination; PBSE0: Ungerminated extracts from Perla variety; PBSE3: Perla barley sprout extract from 3 days of germination; PBSE5: Perla barley sprout extract from 5 days of germination; PBSE7: Perla barley sprout extract from 7 days of germination; A–D: Capital letters indicate statistically significant differences ($p < 0.05$) between germination days with the same solvent; a–c: Lowercase letters show statistically significant differences ($p < 0.05$) between the same day of germination with different solvents. *Dry basis.

the germinated barley sprout extracts at 3 days (EBSE3), the concentration of total phenols in Esmeralda ranged from 1.78 to 5.63 mg GAE/g, following the same trend as in EBSE0, although no significant differences were observed compared to water ($p > 0.05$). In the non-germinated rice bran sprout extracts (PBSE0), concentrations of total phenols ranged from 0.10 to 1.94 mg GAE/g. Like EBSE0, acetone was the most efficient solvent. Compared to water, PBSE0 showed a 113% increase in total phenols when 50% acetone was used. In the 3-day germinated rice bran sprout extracts (PBSE3), the concentration of total phenols obtained ranged from 1.10 to 7.26 mg GAE/g (Table 1). The solvent that extracted the highest concentration of total phenols was a combination of water and acetone.

Meneses et al. evaluated different solvents to extract phenolic compounds from Brewer's spent grain. They obtained the highest content of total phenols (9.9 mg/g) using 60% acetone, which represented a 175% increase *vs.* water. The use of acetone improved the extraction of total phenols, as compared to other solvents, in samples from corn and wheat.[31,33,34] These results suggest that the cell walls of these grains, including barley, are non-polar, allowing for less polar solvents (water, acetone) to promote phenol extraction.[35]

On the other hand, in the 5-day germinated barley sprout extracts (EBSE5) and 5-day germinated rice bran sprout extracts (PBSE5), an increase in the concentration of total phenols was observed compared to the 0-day and 3-day BSE of both varieties (Table 1). The use of different solvents resulted in the extraction of varying concentrations of total phenols, indicating an increase in these compounds with germination time.

In EBSE5, concentrations ranged from 4.91 to 6.00 mg GAE/g. In PBSE5, the results showed a content of 4.90 to 10.36 mg GAE/g. In both Perla and Esmeralda, the highest concentration was obtained when water was used for the extraction of total phenols. This suggests that the polarity of the extracted compounds may change during germination. Other studies that have used water for obtaining phenolic compounds have been carried out on purple corn grains, chia seeds, and walnut green husk, however, the concentrations obtained have been lower compared to other solvents such as methanol and acetone.[33,36,37]

In the 7-day germinated barley sprout extracts (EBSE7), concentrations of total phenols ranged from 6.33 to 12.29 mg GAE/g (Table 1). Compared to water, EBSE7 values showed a 71% increase when 80% methanol was used. On the other hand, in PBSE7, concentrations of total phenols ranged from 7.23 to 13.60 mg GAE/g. In these BSE, 80% ethanol was the solvent that extracted the highest content of total phenols. Compared to water, PBSE7 showed an increase of up to 88%. Phenolic compounds are generally polar; therefore, they can be recovered with highly polar solvents such as methanol, ethanol, and acetone.[20]

The choice of solvent concentration has been a topic of controversy, as there is no single solvent that can recover the highest phenolic content. The addition of ethanol modifies the dielectric constant and polarity of the extraction solvent, which favors the extraction of a greater variety of biological compounds.[38] The polarity of the solvent plays a key role in increasing the solubility of phenolic compounds, allowing them to migrate from the matrix to the solvent system, thereby improving the extraction yield.[39]

Total flavonoid concentration in germinated barley

Table 2 shows the total flavonoid contents in EBSE and PBSE, both ungerminated and germinated for 3, 5, and 7 days. The results were expressed a mg QE/g dry sample.

The total flavonoids at different germination times are presented in the first column of Table 2. In ungerminated BSEs, 0.23 and 0.19 mg QE/g were obtained from EBSE0 and PBSE0, respectively.

After 3-day germination, both varieties presented a significant increase ($p < 0.05$), 0.91 and 0.83 mg QE/g in EBSE3 and PBSE3,

Table 2. Total flavonoid content in barley extracts of the Esmeralda and Perla varieties (mg QE/g of sample*).

Sample	Water	Methanol 80%	Ethanol 80%	Acetone 50%
Esmeralda				
EBSE0	0.23 ± 0.005^{Db}	0.05 ± 0.002^{Cc}	0.26 ± 0.05^{Cb}	0.84 ± 0.03^{Ca}
EBSE3	0.91 ± 0.07^{Cb}	0.50 ± 0.02^{Bc}	0.57 ± 0.05^{Bc}	1.22 ± 0.05^{Ba}
EBSE5	0.59 ± 0.03^{Bb}	0.57 ± 0.05^{Bb}	0.67 ± 0.02^{Abb}	1.56 ± 0.03^{Aa}
EBSE7	1.66 ± 0.14^{Aa}	0.98 ± 0.06^{Ab}	0.78 ± 0.02^{Ab}	1.73 ± 0.14^{Aa}
Perla				
PBSE0	0.19 ± 0.007^{Cb}	0.04 ± 0.002^{Cc}	0.07 ± 0.006^{Cc}	0.51 ± 0.03^{Ca}
PBSE3	0.83 ± 0.07^{Ba}	0.39 ± 0.02^{Bba}	0.54 ± 0.05^{Bb}	0.58 ± 0.05^{Cb}
PBSE5	0.74 ± 0.06^{Bab}	0.55 ± 0.05^{Bbc}	0.47 ± 0.009^{Bc}	0.82 ± 0.08^{Ba}
PBSE7	1.14 ± 0.11^{Ab}	1.51 ± 0.03^{Aa}	0.74 ± 0.06^{Ac}	1.19 ± 0.10^{Ab}

EBSE0: Ungerminated extracts from Esmeralda variety; EBSE3: Esmeralda barley sprout extract from 3 days of germination; EBSE5: Esmeralda barley sprout extract from 5 days of germination; EBSE7: Esmeralda barley sprout extract from 7 days of germination; PBSE0: Ungerminated extracts from Perla variety; PBSE3: Perla barley sprout extract from 3 days of germination; PBSE5: Perla barley sprout extract from 5 days of germination; PBSE7: Perla barley sprout extract from 7 days of germination; A–D: Capital letters indicate statistically significant differences ($p < 0.05$) between germination days with the same solvent; a–c: Lowercase letters show statistically significant differences ($p < 0.05$) between the same day of germination with different solvents. *Dry basis.

respectively. In contrast, the concentration was reduced by 35% and 10% in PBSE5 and EBSE5, respectively.

Nevertheless, when compared to the concentrations obtained at day 5, BSEs germinated for 7 days produced a higher amount of flavonoids. Total flavonoids increased by 181% in EBSE7 and 54% in PBSE7.

These results show that 7-day germination yields larger amounts of total flavonoids. When compared to ungerminated BSEs, Esmeralda barley presented 7.2 times more total flavonoids while the increase was 6-fold in Perla variety.

This increment is higher than what Lee et al. reported (2.1 times) in barley germinated for 6 days. Although few studies evaluate the content of total flavonoids, it has been observed that, as total phenols, flavonoids are increased along with germination, a fact that has been identified in samples from other grains as millet and quinoa.[23,40,41]

The increase in flavonoid concentration in germinated seeds can be attributed to the synthesis of the polymer-bound form resulting from plant germination. In addition, this process, along with maceration, is related to the activation of catalytic enzymes associated to the phenylpropanoid pathway, promoting the formation of secondary metabolites.[42] The capacity of germination to release bioactive compounds leads to condensed tannin solubilization and the migration of phenolic compounds to the outer layer of germinated seeds. This can produce an increase in these metabolites.[11,43]

Sokrab et al. reported higher polyphenol concentrations (up to 138%) in two corn samples germinated for 2, 4, and 6 days. Then, germination is a key factor to the increase in flavonoid concentrations in BSEs.[10]

Total flavonoid concentration with solvents in germinated barley

Table 2 presents the concentrations obtained in EBSE and PBSE when using different solvents (water, 80% methanol, 80% ethanol, and 50% acetone). Comparisons were carried out to observe significant differences ($p < 0.05$) between the same germination day and different solvents. The results were expressed as mg QE/g dry sample.

The EBSE0 showed a concentration of total flavonoids ranging from 0.05 to 0.84 mg QE/g (Table 2). Meanwhile, the PBSE0 presented contents of 0.04 to 0.51 mg QE/g. The results showed that 50% acetone was the solvent that extracted the highest content of total flavonoids in both varieties.

In the EBSE3, the concentration of total flavonoids was from 0.50 to 1.22 mg QE/g, and these results followed the same trend as in non-germinated BSEs, where the highest concentration was obtained with 50% acetone. Likewise, the EBSE5 and EBSE7 showed the highest content of total flavonoids with 50% acetone, with a total of 0.57 to 1.56 mg QE/g and 0.78 to 1.73 mg QE/g, respectively (Table 2).

On the other hand, the PBSE3 presented the highest concentration when water was used for extraction, with values of up to 0.83 mg QE/g. After 5 days of germination, the concentration of total flavonoids in PBSE5 was from 0.47 to 0.82 mg QE/g, where the highest content was obtained with 50% acetone. Compared to the most polar solvent, the flavonoid content in PBSE7 increased by 32% when 80% methanol was used. These results are in agreement with Meneses *et al.*, who reported an increase in flavonoid concentration when 80% methanol was used in brewer's spent grain samples. These results indicate how the flavonoid content in barley samples could be related to their polarity.[31]

Furthermore, it has been reported that the use of water as an extraction solvent is not common, as a significant portion of flavonoids are poorly soluble in water. The double bond between positions 2 and 3 of flavones and flavonoids is susceptible to forming flatter structures, making it difficult for solvent molecules to penetrate.[44] Therefore, 50% acetone managed to extract the highest content of total flavonoids in the BSE. This could be because the solubility of flavonoids may be lower in methanol, ethanol, and/or water due to their original structure, and they can form strong hydrogen bonds between their own molecules. Moreover, not only hydrogen bond donation but also the capacity for accepting hydrogen bonds is strong.[45]

Comparison of total phenol and flavonoid contents between barley varieties

Figure 1 shows the phenol concentrations in the two varieties using solvents with different polarities.

In Figure 1A, the comparisons between varieties and the same germination day using water are shown. The Perla BSEs displayed a significantly higher concentration than the Esmeralda BSEs ($p < 0.05$). However, at 3 and 7 days of germination, no significant differences were observed in the concentration ($p > 0.05$).

In the comparison with 80% methanol (Figure 1B), no change in the content of total phenols was observed between varieties. The extracts with 80% ethanol showed no significant difference between varieties on days 0, 3, and 5 of germination ($p > 0.05$) (Figure 1C). However, in the 7-day germinated BSEs, the PBSEs statistically showed a higher content of total phenols compared to the EBSEs ($p < 0.05$).

Regarding the use of 50% acetone (Figure 1D), the Perla variety presented the highest content of total phenols on all germination days compared to Esmeralda. These differences may be related to the absence of hulls in the Perla barley. Zilic et al. reported that hull-less barley had the highest content of phenols compared to other cereals such as rye, wheat, and oats. Additionally, the absence of hulls in some grains allows for greater synthesis of phenolic compounds as a protective response, and these types of grains are richer in bound phenolic acids.[46,47]

The concentration of flavonoids between varieties and the same germination day using different solvents (80% methanol, 80% ethanol, and 50% acetone) is shown in Figure 2.

The content of total flavonoids when extracted with water was statistically similar ($p > 0.05$) between varieties on days 0, 3, and 5 of germination. However, in the 7-day germinated BSEs, the content of total flavonoids was statistically higher in the EBSEs compared to the PBSEs ($p < 0.05$).

With the use of 80% methanol (Figure 2B), no significant difference was observed between the two barley varieties on days

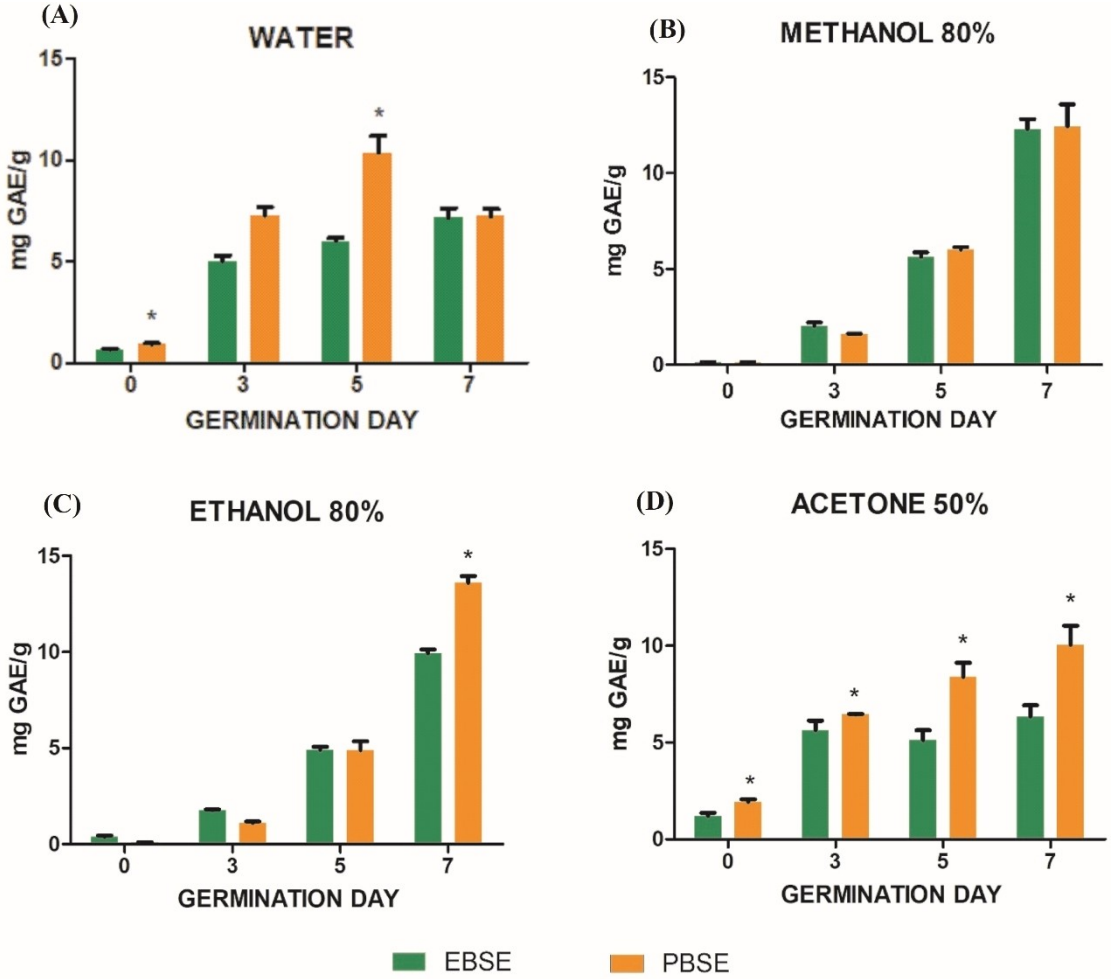

Figure 1. Total phenol content in extracts of two barley varieties. EBSE: Emerald germinated barley extracts; PBSE: Perla germinated barley extracts. *: Indicates significant differences ($p < 0.05$) between varieties and the same day of germination.

0, 3, and 5 ($p > 0.05$). However, at 7 days of germination, the Perla variety showed a higher concentration. When using 80% ethanol (Figure 2C), both varieties presented a statistically similar concentration of total flavonoids on days 3 and 7. On the other hand, when 50% acetone was used (Figure 2D), the content of total flavonoids in the EBSEs was statistically higher compared to the PBSEs on all germination days. These results may be due to Esmeralda barley showing greater morphological changes in starch throughout germination, suggesting greater enzymatic activity during this process compared to Perla barley.[48]

Table 3. Correlation between root size and total phenol and flavonoid content.

Esmeralda	Root size (cm)
Total phenols mg GAE/g	0.995
Total flavonoids mg QE/g	0.780
Perla	Root size (cm)
Total phenols mg GAE/g	−0.201
Total flavonoids mg QE/g	0.855

Relationship between radicle size and phenolic compound concentration in germinated barley extracts

Figure 3 shows the average radicle size in the two barley varieties. Perla exhibited more elongation as compared to Esmeralda across germination days.

Table 3 presents the relationship between radicle size and total phenols. High correlation was observed between radicle size and the content of total phenols and flavonoids in Esmeralda. However, in Perla, correlation was only observed in the content of flavonoids. Probably because the phenolic compounds are acting as protection against pathogens during the growth of the seedling.[49] Bakhouche, et al. reported a similar behavior in *L. delicatulum*, found a higher concentration of phenolic compounds in the radicle than in the leaves, but lower concentration of flavonoids.[50]

Besides germination, the presence of hull in Esmeralda likely affects the differences in total phenols as compared to Perla barley.

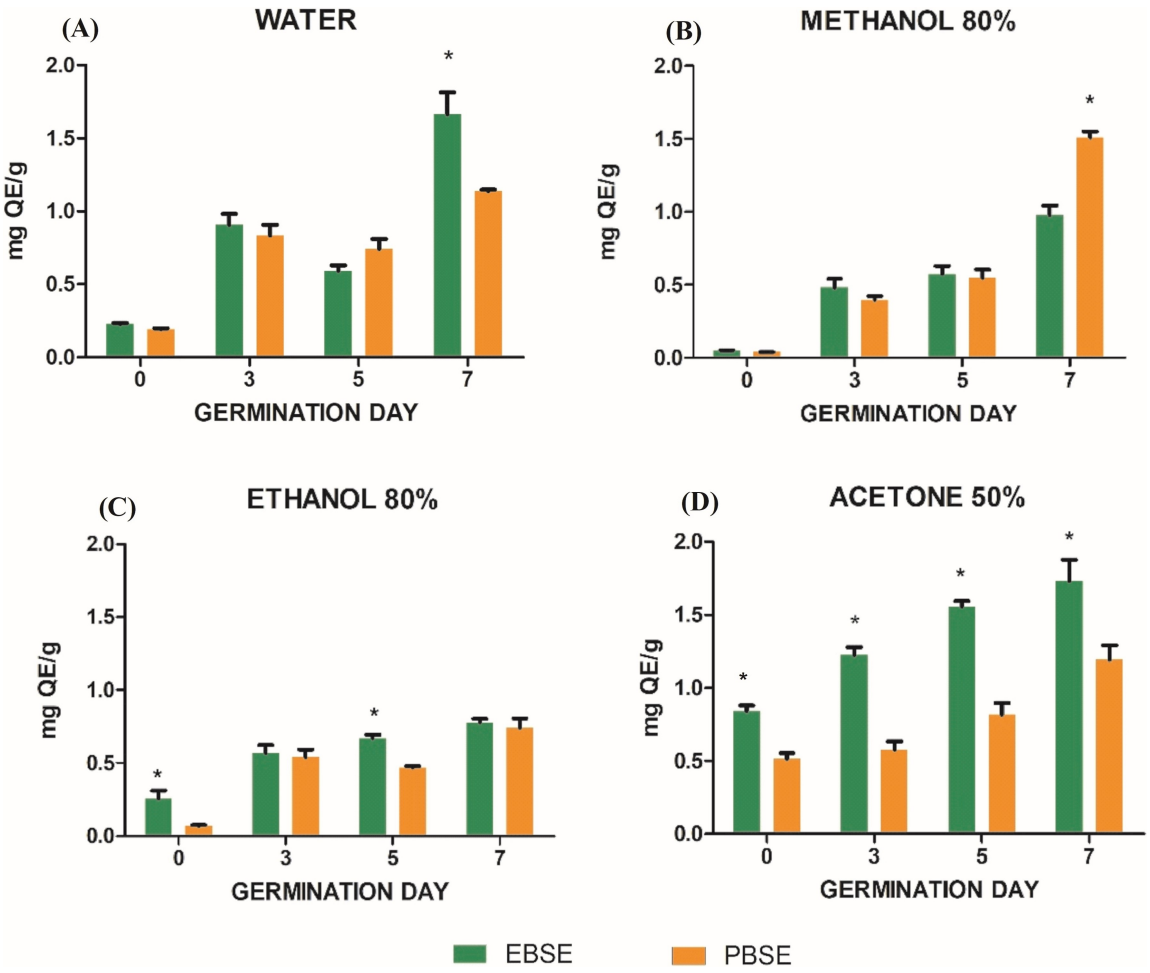

Figure 2. Total flavonoids content in extracts of two barley varieties. EBSE: Emerald germinated barley extracts; PBSE: Perla germinated barley extracts. *: Indicates significant differences ($p < 0.05$) between varieties and the same day of germination.

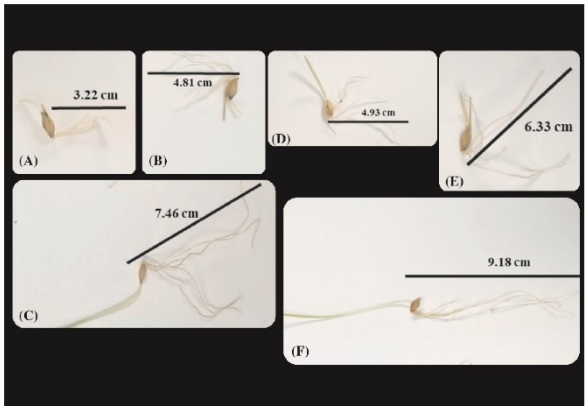

Figure 3. Radicle size of two varieties of barley. Sprouts of two varieties of barley. Esmeralda (A) 3 days, (B) 5 days, (C) 7 days; Perla (D) 3 days, (E) 5 days and (F) 7 days.

Similarly, some authors demonstrated that germination improved the concentration of bioactive compounds in soy samples. As well as the presence of husk in cereals such as rice.[51,52]

López-Perea *et al.* identified the phenolic composition in barley hull using different solvents. Their results concluded that the hull contains 0.82–3.4 mg GAE/g total phenols. Additionally, the biosynthesis processes of phenols in Esmeralda barley grains might be different since the hemicellulose fractions in the hull are mainly constituted by arabinoxylans.[14,53–55]

Arabinoxylans are a heterogeneous polysaccharide group formed by a xylose chain bound by β-(1-4) links to which arabinose and ferulic acid residues are bound by ester linkages.[54] This characteristic could affect the increase in phenolic compounds bound to gallates or ferulates and thus the content of total phenols is different between Esmeralda and Perla varieties.

On the other hand, Esmeralda is a smaller sprout than Perla (Figure 3), pointing to a stress process that leads to a greater metabolite synthesis, as reported by Cornejo et al. in germinated rice. Therefore, there is no correlation between radicle size and phenolic compound concentration, yet the evaluation of the two barley varieties allowed for the identification of morphological differences between them.[12]

Conclusions

The results showed that germination for 5 and 7 days constituted the best options to obtain total phenols and flavonoids, respectively, from barley samples. The best solvent to extract total phenols from barley samples was 80% ethanol, while the best one to extract total flavonoids was 50% acetone. The concentration of phenolic compounds depended on the germination time. The results of this study reflect the importance of germination to improve the total phenolic content and the use of solvents to increase the concentration in different barley varieties.

Experimental Section

Samples

Barley grains (Hordeum vulgare L.) were collected in the city of Apan, Hidalgo state, Mexico. These samples belong to the Esmeralda and Perla varieties, characterized by the presence and absence of shell, respectively. Esmeralda barley shows six-row spikes and Perla barley has two-row spikes. Both samples were cultivated with rains in summer of 2019, the average precipitation of the surface of the region was 105 to 110 mm. Grains were donated by producers.

Barley germination

Germination was carried out according to the method described by Gutiérrez-Osnaya *et al.* Viable barley grains were selected and disinfected with 0.05% sodium hypochlorite.[48] The seeds were soaked in water for 24 h and placed in a plastic tray for moistening to saturation. The trays were kept at room temperature (21–24 °C) and 65% relative humidity for 3, 5, and 7 days. Radicle size was measured on each germination day by choosing 10 random grains. Ungerminated barley samples and those obtained at different germination times (3, 5, and 7 days) were dried in an oven at 50 °C to obtain moisture lower than 5%. The sprouts were ground and sieved (0.2 mm particle size), and the flours obtained were stored at 4 °C for later analysis.

Preparation of extracts from germinated barley

Water (25 mL) was added to 2 g flour from different samples and the mix was macerated on a heating plate at 60–70 °C for 2 h under constant stirring. The mix was centrifuged at 3000 rpm for 10 min, and each supernatant was labeled as BSE (barley sprout extract), indicating whether the variety was E or P (Esmeralda or Perla) along with the germination time (0, 3, 5, and 7 days) (Table 4).

Phenolic compound extraction

Each BSE was dried at 50 °C for 20 h (10–12 mL), resuspended in 4 mL solvent (water, 50% acetone, 80% methanol, or 80% ethanol), and kept under constant stirring at 200 rpm for 16 h. The mixtures were centrifuged at 3000 rpm for 10 min, and supernatants were labeled EBSE (Esmeralda barley sprout extract) or PBSE (Perla barley sprout extract), indicating the corresponding germination time (0, 3, 5, and 7 days).

Table 4. Sample nomenclature

Barley variety	Germination day	Abbreviation
Esmeralda	0	EBSE0
	3	EBSE3
	5	EBSE5
	7	EBSE7
Perla	0	PBSE0
	3	PBSE3
	5	PBSE5
	7	PBSE7

Quantification of total phenols and flavonoids

Total phenols

Total phenols were determined following the Folin-Ciocalteu method.[56] Distilled water (1580 µL) was added to 300 µL 10% Na_2CO_3 aqueous solution, 20 µL BSE, and 100 µL Folin-Ciocalteu reagent (1:2); the mix was kept at room temperature for 120 min, and absorbance was read at 765 nm in triplicate for each BSE. Total phenols were expressed as mg of gallic acid equivalents (GAE)/g barley flour dry base (dry base).

Flavonoids

The quantification of total flavonoids was carried out following the method described by Žilić et al. Briefly BSE (500 µL) was mixed with 75 µL 5% NaNO2 in test tubes away from light.[46] Then, 150 µL 10% AlCl3 was added and the mix was left to rest for 5 min. Finally, 500 µL 1 M NaOH was added and the volume was adjusted to 2.5 mL with distilled water. Absorbance was immediately read at 510 nm. The procedure was done in triplicate for each BSE, and results were expressed as mg of quercetin equivalents (QE)/g of barley flour db.

Statistical analysis

Data were analyzed through ANOVA, and means were compared using Tukey's test with a confidence interval of 95%. In addition, Student's t test was used to compare the two barley varieties, and Pearson's correlation coefficient indicated the relationship between total phenol and flavonoid contents and radicle size. All analyses were carried out using Minitab v19.

Author Contributions

Conceptualization, A.D.R.-G. and F.A.G.-O.; Methodology, A.G.-C. and F.A.G.-O; Validation, A.C.-O.; Formal Analysis, A.D.R.-G.; Investigation, A.D.R.-G. and F.A.G.-O.; Data Curation, A.C. O.; Writing – Original Draft Preparation, A.G.-C., A.D.R.-G.; Writing – Review & Editing, A.D.R.-G., F.A.G.-O. and A.C.-O.; Visualization, A.C.-O; Supervision, A.D.R.-G.; Project Administration, A.D.R.-G. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: flavonoids · germination · phenols · phenolic extraction · solvents

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CAPÍTULO IV. ANALYSIS OF BIOACTIVE COMPOUNDS IN LYOPHILIZED AQUEOUS EXTRACTS OF BARLEY SPROUTS

4.1 Introducción

En este capítulo se presenta un artículo original publicado en la revista *Food Measurement and Characterization.* En este estudio se muestra la caracterización de extractos acuosos de cebada sin germinar y germinada (3, 5 y 7 días). Se utilizaron las variedades Perla y Esmeralda. Se determinó el perfil de oligosacáridos y se calculó el contenido de azúcares totales y reductores. Además, se determinó el contenido total de proteína y perfil de aminoácidos. Se realizó la cuantificación e identificación de fenoles y flavonoides totales, y se realizaron distintos ensayos para determinar la actividad antioxidante (DPPH, ABTS y FRAP). Los resultados mostraron el efecto de la germinación de ambas variedades sobre el contenido de compuestos bioactivos y su actividad antioxidante. Se observó un aumento gradual en el contenido de carbohidratos, aminoácidos, proteínas y compuestos fenólicos a medida que avanzaba el proceso de germinación. Los oligosacáridos más abundantes fueron la glucosa y la maltosa, mientras que los aminoácidos principales fueron el ácido glutámico y el aspártico. En cuanto a los compuestos fenólicos, el ácido ferúlico y la catequina fueron los más destacados. Además, se observó un aumento en la actividad antioxidante, especialmente a los 5 y 7 días de germinación, según los ensayos de ABTS, FRAP y DPPH. Los resultados obtenidos sugieren una alternativa prometedora para obtener compuestos bioactivos, aunque se requieren ensayos clínicos adicionales para respaldar su eficacia en el tratamiento de diversas condiciones relacionadas con la oxidación.

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ORIGINAL PAPER

Analysis of bioactive compounds in lyophilized aqueous extracts of barley sprouts

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Abstract

The objective of this study was to characterize the lyophilized aqueous extracts of barley variety Esmeralda and Perla ungerminated and germinated for 3, 5, and 7 days. Significant changes were observed in both barley varieties, particularly in the freeze-dried extracts of 7 days (P7, E7) compared to ungerminated barley extracts (P0, E0). There was an increase in the total content of carbohydrates, proteins, amino acids, and phenols. The freeze-dried extract P7 exhibited the highest content of total sugars, reducing sugars, and oligosaccharides, with values of 269.52, 23.95, and 853.8 mg/g dry weight (dw) respectively. The increase in protein and amino acid content was similar in P7 and E7. Additionally, a concentration of total phenols and flavonoids was observed to be 18.17 mg GAE/g and 2.54 mg QE/g in P7, and 21.73 mg and 2.8 mg QE/g in E7. Ferulic acid and catechin were the predominant compounds in all extracts. E7 showed a 327% increase in DPPH radical scavenging activity compared to ungerminated barley. On the other hand, in the ABTS assay, a 624% increase in antioxidant activity was observed in P5 extracts, and the capacity for Fe (II) ion oxidation increased by 134% in P7 extracts. These findings suggest that barley germination can enhance its nutrient profile and antioxidant capacity. The obtaining of aqueous extracts indicates the relevance for easy consumption, which has significant implications for human health, as barley-derived foods could provide additional health benefits or even the consumption of aqueous extracts as a base for a potential beverage.

Keywords Barley extracts · Germination · Bioactive compounds · Antioxidant activity

Introduction

Barley contributes greatly to improving health, mainly due to its anti-inflammatory, antioxidant and antidiabetic properties [1, 2]. Research conducted by Donkor et al. [3] revealed that sprouted grains of barley, rye, and sorghum have higher concentrations of total phenols compared to unsprouted grains. These differences are closely related to the quality of the hypocotyl, including its thickness, color, texture, and length, as well as water storage and germination conditions. During metabolism, the chemical composition of sprouted grains is modified by enzymatic activity, which can result in the increase of the concentration of ascorbic acid, thiamine, riboflavin, minerals, phenolic compounds, proteins, sugars, and other nutrients of interest for health [4–6].

During the germination process of barley, starch, fats, and proteins are hydrolyzed to form new cellular components. Amino acid metabolism plays an essential role in protein synthesis and hormone metabolism such as insulin [7, 8]. Additionally, this process is associated with phenolic

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metabolism activation, whose compounds play a crucial role in various aspects of plant growth, including metabolism, growth, reproduction, and defense against pathogens, adverse environmental conditions, stress, and ultraviolet radiation [9, 10]. The release of phenolic compounds also contributes to increasing properties such as antioxidant, antidiabetic, anticancer, anti-inflammatory, analgesic, antidepressant, and antihypertensive activity, and it has even been reported to be effective in neutralizing snake venoms [11–14]. Furthermore, phenolic compounds, along with sterols and tocopherols, prevent neurological diseases and reduce cholesterol levels [15]. On the other hand, flavonoids are also associated with moderating cancer and coronary diseases [11].

Barley is a significant source of these beneficial compounds for health. Carbohydrates also undergo modifications during germination, with some oligosaccharides regulating the immune system and improving intestinal microbiota [12]. Thus, detailed characterization of germinated barley is promising for its application in food.

The available information on barley varieties, such as Esmeralda and Perla, is limited. Previous studies on germinated barley have focused mainly on extracting phenolic compounds and studying antioxidant activity, mostly analyzing grain extracts and germinated flours, excluding lyophilized samples and broader compound characterization. Similar research on other germinated grains and seeds (corn, chia, quinoa, beans, lentils, soybeans, and lupine) has primarily focused on antioxidant compounds [16–18]. Generating lyophilized aqueous barley extracts can ensure food safety, as they do not leave chemical residues and can be incorporated into functional foods, enhancing their nutritional profile and promoting health beyond basic nutrition.

The design of plant extracts for inclusion in functional foods is gaining attention due to their potential health benefits [19]. The bioactive compounds present in barley extracts have the potential to improve the nutritional profile of foods, following the trend of offering functional foods that promote health beyond basic nutrition [20, 21]. This practice also reflects the growing preference for using bioactive compounds of plant origin in the search for healthy foods with biological functions.

In addition to its health benefits, barley stands out for its wide application in the food industry and beverage production. For example, malted barley is a fundamental ingredient in beer manufacturing, providing essential nutrients [22]. Additionally, barley is used in the production of various baked goods, such as bread and cookies, due to its starch and protein content, which contribute to the texture and nutritional value of these foods [23]. Processes like germination allow for an increase in the nutritional value and functionality of grains [24]. Therefore, the objective of this study was to characterize the lyophilized aqueous extracts of barley variety Esmeralda and Perla ungerminated and germinated for 3, 5, and 7 days that allow us to demonstrate their potential as functional ingredients in the formulation of foods and beverages with improved antioxidant properties and greater nutritional content, in order to promote health and human well-being.

Materials and methods

Sample preparation

Barley grains (*Hordeum vulgare* L.) were collected in the municipality of Apan in the state of Hidalgo. The Esmeralda variety is characterized by the presence of husk and the presence of 6 rows in its spike, and the Perla variety without husk and with 4 rows in its spike.

Germination

Germination was carried out according to the methodology of Gutiérrez-Osnaya et al. [25] with some modifications. The seeds were washed with water and subsequently placed in a solution with 0.05% sodium hypochlorite for 30 min and rinsed with running water. The grains were soaked in drinking water for 24 h and placed in a plastic tray, moistening until saturation. The trays were placed in a humidity chamber (LabTech, Mod, LHT-0250E, Korea) at a temperature of 24 °C and 60–70% relative humidity. To prepare the extracts, non-sprouted and sprouted grains of 3, 5, and 7 days were taken.

Preparation of barley sprout extracts

Barley extracts were obtained from 3, 5, and 7 days of germinated and ungerminated barley. It was carried out following the maceration method described by the European Brewery Convention (EBC) [26] with modifications. The germinated barley was dried in an oven (Barnstead Lab-Line, Mod. 3478, USA) at 50–55 °C, and until a humidity of less than 5% was obtained. Subsequently, grinding was carried out using a Nutri-bullet (Corning Mexicana, Mod. 1368 – 160, Mexico), and the flour was sieved to obtain a particle size of 0.2 mm. Subsequently, 50 g of flour was taken and 200 mL of water at 45 °C was added. Then, this temperature was maintained in an oven (Thermo Scientific, Mod. SP131325Q, China) for 30 min and raised 1 °C for 30 min until reaching 70 °C. Finally, 100 mL of water at 70 °C was added and that temperature was maintained for one hour. Subsequently, it was allowed to cool for 10 min. Afterward, the weight was adjusted to 450 g with water and centrifuged (Solbat, Mod.

J-12, Mexico) at 5000 rpm for 10 min. Finally, the supernatants were lyophilized (Labconco, Mod. 816-333-8811, USA) at a temperature of -62 °C and 0.040 mBar pressure. The lyophilized extracts were weighed and stored frozen and under vacuum until use. On average, for every 400 mL of aqueous extract, 20 g of lyophilized extract was obtained, that is, a proportion of 0.05 g/mL. For the corresponding analyses, each lyophilizate was resuspended in water until the corresponding original volume was obtained.

Extract characterization

Oligosaccharide profile

For the determination of oligosaccharides, the procedure followed was Shaw [27], using high-performance liquid chromatography (HPLC) (Knauer Azura, Germany). The separation was carried out on an Agilen Technologies Intersil 5µ ODS-2 column (150×4.6 mm). Water at 30 °C was used as the mobile phase at a flow rate of 0.8 mL/min. The results were expressed as mg/g of sample.

Reducing sugars

Reducing sugar determination was carried out using the 3.5-dinitrosalicylic acid (DNS) technique Miller [28]. In a tube, 180 µL of DNS reagent and 30 µL of the lyophilized sample (0.05 g/mL) were combined. Subsequently, the mixture was homogenized and boiled for 15 min. Afterward, 1230 µL of distilled water was added, and the solution was allowed to stand for 10 min. Finally, the absorbance was measured at 540 nm (UV/VIS Genesys 10 UV, Thermo Electron, Mexico). The results were expressed as mg of glucose per g of sample, on a dry weight basis (dw).

Total sugars

The total sugar content was determined using the method Dubois et al. [29]. Briefly, 100 µL of sample, 900 µL of distilled water and 200 µL of 5% w/v phenol were added to a glass tube and vortexed (Barnsteard Thermolyne, type 37,600 mixer, Malaysia). Then 1 mL of concentrated H_2SO_4 was added and mixed briefly. Finally, the samples were read in a spectrophotometer (UV/-VIS Genesys 10 UV, Thermo Electron, Mexico) at 490 nm. The sugars obtained were calculated and expressed as mg of glucose per gram of sample (dw).

Protein

The percentage of protein was determined using the Kjeldahl method, described in AOAC method 955.04 (2003). To calculate the protein, 6.25 was used as the conversion factor.

Determination of free amino acids (AA)

The AA profile was determined according to the methodology of Vázquez-Ortiz et al. [30] using HPLC (Thermo Scientific Accela) equipped with a fluorescence detector (Thermo Scientific Dionex Ultimate 3000). The separation of AA was carried out on a Microsorb column (100- 3 C18 100×4.6 mm). Methanol was used as the mobile phase, and the buffer solution was sodium acetate (0.5 M) at a flow rate of 1.2 mL/min. Identification was performed using a wavelength of 330 mm and an emission of 455 mm. Peak identification was performed by retention time comparison with amino acid standard solution. Calibration curves with standard concentrations were used for the amino acid quantification. Results were expressed as mg/g dw.

Analysis of polyphenols

Total phenolic compounds

Total phenols were determined according to the Folin-Ciocalteu spectrophotometric method [31]. To 1580 µL of distilled water, 300 µL of aqueous sodium carbonate solution (Na2CO3) was added, then 20 µL of the extract and 100 µL of the Folin-Ciocalteu reagent (diluted 1:2) were added and kept at room temperature for 120 min. Subsequently, the absorbance was measured (Thermo Electron Corporation, Mod. Genesys 10 UV, USA) at 765 nm. Total phenols were expressed as mg Gallic Acid Equivalents (GAE)/g dw.

Total flavonoids

The flavonoid content was determined using the technique of Žilić et al. [32] and Eberhardt et al. [33]. Each extract obtained was diluted with 10% water. Then, 500 µL of the extract was taken and mixed with 75 µL of 5% $NaNO_2$ in glass tubes protected from light. Then 150 µL of 10% $AlCl_3$ was added and allowed to stand for 5 min. Finally, 500 µL of 1 M NaOH were added and the volume was completed to 2.5 mL with distilled water. The absorbance was immediately measured at 510 nm (Thermo Electron Corporation, Mod. Genesys 10 UV, USA). Quercetin was used as a standard and total flavonoid were expressed as mg Quercetin Equivalents (EQ)/g dw.

Identification and quantification of phenolic compounds

The quantification of phenolic compounds by HPLC was described by Ramamurthy et al. [34] with slight modifications. It was performed in reverse phase using a Zorbax (ODS)-C18 column (5 µm particle size, 15 cm x 4.6 mm i.d). The mobile phase ran at 1.5 mL/min and consisted of solvent A (acetic acid/water, 2:98 v/v) and solvent B (acetic acid/acetonitrile/water, 2:30:68 v/v). During the analysis, the solvent gradient was programmed from 10 to 100% B in A in 30 min. A diode array detector was used and programmed at four different wavelengths: 260, 280, 320, and 360 nm. The injection volume was 20 µL. All solvents used were filtered through 0.45 µm membranes. The identification of phenolic acids was carried out by comparison with the retention time and absorption spectra of standards of commercial phenolic compounds, and their quantification was carried out using their calibration curves. The results were expressed as µg/g of dry sample [35].

Antioxidant activity

DPPH assay

The DPPH assay of each barley extract was determined using the method of Brand-Williams et al. [36]. 2.5 mg of the radical (DPPH) was dissolved in 40 mL of methanol. The solution was diluted to 50 mL with methanol and protected from light. Its absorbance was read at 520 nm and adjusted to 0.5. Each extract (100 µL) was mixed with 2000 µL of the DPPH. A reading of each sample was taken at 60 min of reaction. The results were calculated from a standard curve of 0 to 25 µM Trolox and were expressed as µmol Trolox equivalents (TE)/g of sample on dw.

ABTS assay

The ABTS assay method was used as described by Pastrana-Bonilla et al. [37] with slight modifications. ABTS has dissolved in water to 7 mM and allowed to react with a 2.45 mM potassium persulfate solution for 16 h in darkness. The volumes used in this analysis to generate the colorimetric reaction were: 990 µL ABTS (adjusted to an absorbance of 0.7) and 20 µL of each extract. The absorbance was measured at 734 nm each minute for 5 min. The results were calculated from a standard curve of 0 to 25 µM Trolox and expressed as µmol Trolox equivalents (TE)/g of sample on dw.

FRAP assay

The antioxidant capacity to measure ferric reduction was determined by the FRAP according to the method described by Abdel-Aal and Hucl [38]. The FRAP reagent was prepared with 10 mM TPTZ solution in 40 mM hydrochloric acid, 20 mM FeCl3 solution, and 0.3 M acetate buffer (pH 3.6). The test solution consisted of a mixture of 30 µL of the extract, 90 µL of water, and 900 µL of the FRAP reagent. The absorbance was measured after 30 min of reaction at 593 nm. The results were calculated from a standard curve (0-100 µM $FeSO_4$) and expressed as µM $FeSO_4$/g on dw.

Statistical analysis

The results were expressed as the average of three determinations ± their standard deviation. Data were analyzed using one-way analysis of variance (ANOVA). A comparison of means was performed using the Tukey test with a confidence level of 95%. All analyses were performed with Minitab statistical software version 19.2, USA.

Results and discussion

Carbohydrate content

Freeze-dried aqueous extracts were utilized, derived from two barley varieties, Perla and Esmeralda, ungerminated (P0 and E0) and after germination for 3, 5, and 7 days (P3, P5, P7, E3, E5, and E7). Table 1 shows the levels of total and reducing sugars in freeze-dried aqueous extracts of barley. In the Perla variety, the total sugar content ranged from 112.23 to 269.52 mg/g dw. Extract P7 exhibited a 2.4-fold increase in total sugar content compared to sample P0, showing statistical significance ($p < 0.05$). Conversely, in the Esmeralda variety, the increment in total sugars on the seventh day of germination was comparatively lower than that in the Perla variety, with concentrations ranging from 81.69 to 155.73 mg/g dw. The rise in E7 was 1.9 times that of the E0 sample, also statistically significant ($p < 0.05$). In both varieties, the total sugar content in the aqueous extracts increased with germination time. In the Perla variety, all the extracts from the different days of germination showed a significant difference ($p < 0.05$). However, in the Esmeralda variety, the total sugar content in the extracts from days 5 and 7 of germination was not significant. ($p > 0.05$). The increase in the Esmeralda variety was less than in the Perla variety. These results were similar to those reported by Qin et al. [6] where they observed that the total sugar content increased after 5 days of germination in Chinese domestic barley musts.

The content of reducing sugars ranged between 3.42 and 23.95 mg/g dw in Perla and between 3.08 and 12.97 mg/g dw in Esmeralda (Table 1). The trend in the increase in germination time was similar to that of total sugars. The results showed that the highest content of reducing sugars was observed in extracts from 5 days of germination in both varieties. These values increased significantly 7 and 4.2 times in P5 and E5, in relation to P0 and E0, respectively ($p < 0.05$). The results were lower compared to what was reported by Zhang et al. [39] and Kumari et al. [40], who observed an increase in reducing sugars in buckwheat of up to 18.9 and 21.9 times in 3 and 4 of germination, respectively.

The increase of sugars observed in the aqueous extracts during germination may stem from the dynamic alterations in sugar content over the germination period, attributed to the activation of enzymatic hydrolysis. This process allows for the release and enhanced bioavailability of sugars within the seed [41]. Germination initiates with an increase in moisture content, increasing from 14 to 40% during seed soaking, thereby softening the grain cover and enhancing its permeability. Consequently, seeds activate and commence the production of enzymes, such as α-amylase, which catalyze the breakdown of amylose and amylopectin into simple sugars [42]. Barley sprouts have been found to contain up to 7% glucose, while extracts derived from barley sprouts can reach levels as high as 12.3% [6, 35]. Notably, maceration temperature can influence sugar content by promoting starch gelatinization and augmenting enzymatic activity, thereby facilitating the conversion of sugars and dextrins [43].

During germination, starch reserves in the grain endosperm are mobilized to provide energy to the growing plant. Amylases act on starch, breaking it down into shorter chains of maltose and, ultimately, into glucose, resulting in an increase of reducing sugars [44]. On the other hand, glucanases are enzymes that break down polysaccharides in the cell walls of barley into glucose units. This process also contributes to the increase in levels of reducing sugars during germination. However, it has been reported that after 36 h of germination, metabolic reactions increase, causing a greater energy demand and, therefore available carbohydrates such as reducing sugars, which could be causing the decrease in reducing sugars after 5 days of germination [45].

The total oligosaccharide content exhibited an increase in the extracts derived from 7-day sprouts (Table 1). In P7 it was 853.8 mg/g dw and in E7 it was 755.4 mg/g dw. Glucose, maltose, and maltotriose had a similar behavior in the extracts. The concentration increases in extracts from 3 days of germination afterward, decreases after 5 days and increases again after 7 days. Glucose concentration exhibited variability among samples of the Perla variety, with the P7 extract displaying the highest concentration (266.80 mg/g), while the P0 extract showed the lowest (38.2 mg/g). The E7 extract presented the highest glucose concentration

Table 1 Carbohydrate content in lyophilized aqueous extracts of Perla and Esmeralda barley

Carbohydrate (mg/g) / Germination day	0	3	5	7
PERLA				
Glucose	38.20 ± 0.60^{D}	113.80 ± 3.72^{B}	100.70 ± 3.01^{C}	266.80 ± 2.37^{A}
Maltose	179.40 ± 0.82^{C}	482.70 ± 18.2^{A}	403.10 ± 0.70^{B}	459.00 ± 5.00^{A}
Maltotriose	15.90 ± 0.68^{C}	110.40 ± 0.35^{A}	101.60 ± 4.25^{B}	100.90 ± 2.88^{B}
Maltoetraose	4.30 ± 0.12^{D}	11.30 ± 0.28^{C}	21.80 ± 0.31^{A}	15.60 ± 0.40^{B}
Maltopentose	4.00 ± 0.17^{B}	3.90 ± 0.14^{B}	4.60 ± 0.03^{A}	3.70 ± 0.11^{B}
Maltohexaose	1.50 ± 0.02^{D}	2.20 ± 0.03^{C}	4.50 ± 0.22^{A}	3.70 ± 0.03^{B}
Maltoheptaose	12.60 ± 0.28^{A}	10.50 ± 0.08^{B}	6.50 ± 0.25^{C}	4.10 ± 0.11^{D}
Maltooctaose	5.60 ± 0.07^{A}	4.00 ± 0.03^{B}	4.00 ± 0.06^{B}	ND
Reducing sugars	3.42 ± 0.33^{Ca}	8.55 ± 0.26^{Bb}	23.95 ± 0.33^{Aa}	3.70 ± 0.11^{Ca}
Total sugars	112.23 ± 3.20^{Da}	161.04 ± 5.08^{Ca}	236.87 ± 0.99^{Ba}	269.52 ± 0.55^{Aa}
% Soluble solids [1]	86.6	93.51	94.44	98.76
ESMERALDA				
Glucose	40.70 ± 0.06^{C}	133.10 ± 1.80^{B}	125.40 ± 0.73^{B}	281.50 ± 10.17^{A}
Maltose	282.80 ± 0.20^{D}	450.40 ± 0.80^{A}	411.90 ± 2.96^{B}	340.70 ± 13.6^{C}
Maltotriose	15.00 ± 0.37^{C}	114.40 ± 5.72^{A}	101.10 ± 1.20^{B}	112.20 ± 2.15^{A}
Maltoetraose	8.70 ± 0.07^{A}	7.00 ± 0.06^{B}	3.40 ± 0.05^{D}	6.22 ± 0.14^{C}
Maltopentose	ND	3.20 ± 0.09^{C}	4.20 ± 0.21^{B}	5.10 ± 0.04^{A}
Maltohexaose	ND	4.00 ± 0.13^{B}	4.30 ± 0.18^{B}	5.60 ± 0.03^{A}
Maltoheptaose	ND	8.90 ± 0.21^{A}	2.90 ± 0.07^{C}	4.10 ± 0.20^{B}
Reducing sugars	3.08 ± 0.28^{Ba}	2.96 ± 0.22^{Ba}	12.97 ± 0.47^{Ab}	4.14 ± 0.40^{Ba}
Total sugars	81.69 ± 1.10^{Cb}	145.97 ± 2.76^{Bb}	151.90 ± 3.64^{ABb}	155.73 ± 0.88^{ABb}
% Soluble solids [1]	82.69	88.32	91.12	93.85

ND: Not detected; [1]:°Brix; Capital letters indicate significant differences between germination days and the same variety ($p < 0.05$); Lowercase letters indicate significant differences between varieties ($p < 0.05$)

compared to E0, where 281.5 and 40.7 mg/g of dw samples were obtained, respectively. Maltose levels varied between samples: the highest were in P3 extracts (482.7 mg/g) and the lowest in P0 extracts (179.4 mg/g). It has been previously demonstrated that maltose is the primary product of starch hydrolysis during mashing [43].

Maltoetraose presented a different behavior; its highest concentration was observed in extracts from 5 days of germination in Perla and Esmeralda at 3 days of germination. The oligosaccharides maltotriose, maltopentose, malthexose, maltoheptaose, and maltooctaose were identified at low concentrations. Barley shows a higher content of oligosaccharides and a lower proportion of monosaccharides (glucose), indicating that the action of starch hydrolysis may change between varieties, possibly related to enzymatic activity which breaks down stored polysaccharides, mobilization of carbohydrate reserves, and biosynthesis of new compounds [6].

Protein and free amino acids content

A heat map showing the change in protein and amino acid content over germination time in aqueous extracts of barley is presented in Fig. 1.

The lyophilized aqueous extracts of the Perla variety presented protein percentages of 4.36 to 8.19% dw. In the P7 extracts, a significant increase in protein content was observed, being 1.8 times higher than that in the P0 extracts ($p < 0.05$). The extracts of the Esmeralda variety presented percentages of 4.33 and 8.91%. The protein content in the E7 extracts was 2 times higher than in the E0 extracts. The results showed a significant increase in protein content throughout the days of germination in both varieties ($p < 0.05$). A higher protein content was observed in the E7 extracts compared to P7 ($p < 0.05$). P7 extracts come from naked barley, which can interfere with the yield

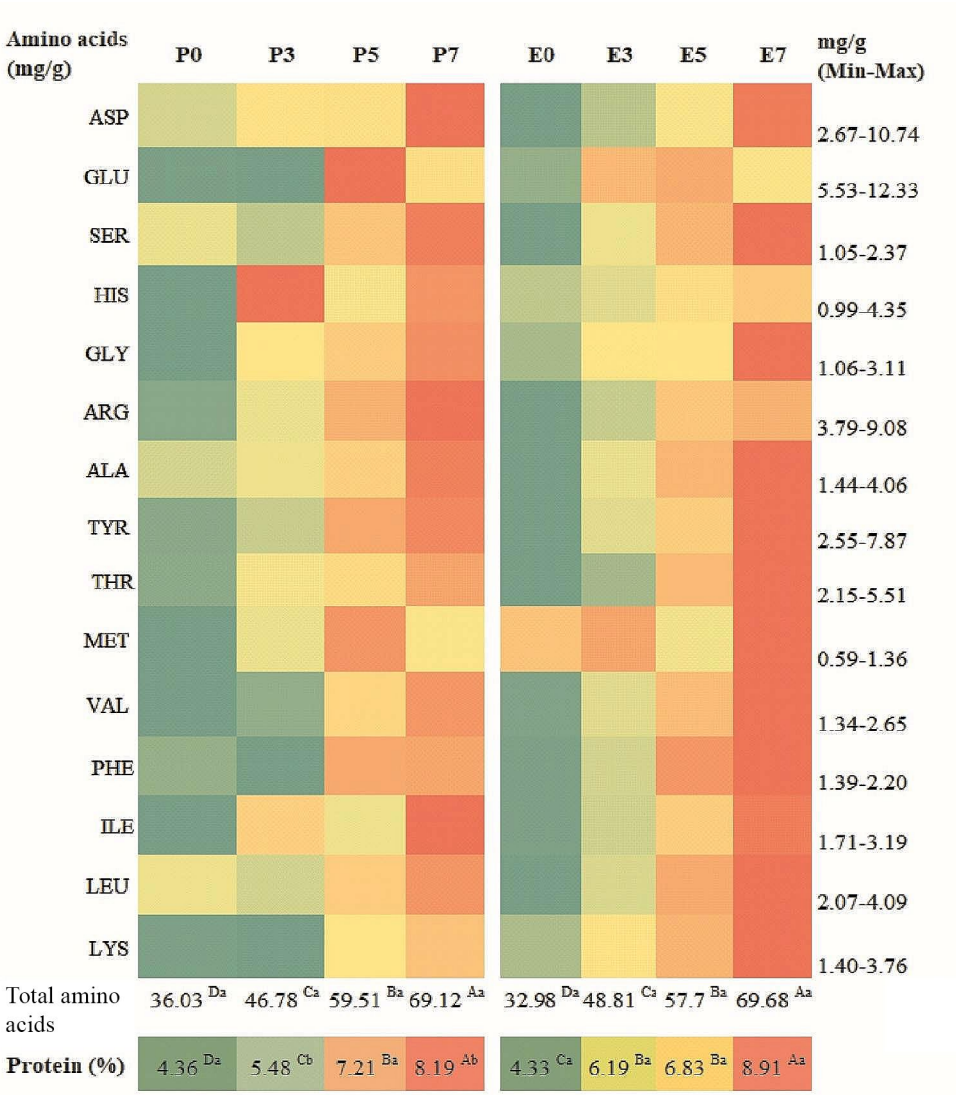

Fig. 1 Heat map showing the content of biomolecules in barley extracts with different germination times. Capital letters indicate significant differences between germination days and the same variety ($p < 0.05$); Lowercase letters indicate significant differences between varieties and the same germination day ($p < 0.05$)

for obtaining protein because barley husk can contain up to 24% of the total protein [46].

Other studies have demonstrated the correlation between the increase in protein content and the duration of germination [5, 47]. Anaemene and Fadupin [48] reported that after 3-day germination, a protein percentage increase in pigeon pea grains was observed, from 21.2% in ungerminated samples to 22.3% in germinated grains. Although the germination time is shorter (3 days) compared to the present study (7 days), both studies demonstrate how germination significantly influences the increase in protein content. Furthermore, during grain imbibition, protein synthesis begins, which contributes to the protein increase in sprouted grains [49].

The content of amino acids in the extracts of sprouts of the two varieties of barley (P0, P3, P5, P7, E0, E3, E5, and E7) are shown in Fig. 1. The total amino acid content increased with the duration of germination. In the Perla variety, the total content ranged from 36.03 to 69.19 mg/g dw. Notably, P7 exhibited the highest concentration, being 1.9 times greater than P0 and demonstrating statistical significance ($p < 0.05$). Esmeralda variety showed concentrations ranging from 32.98 to 69.68 mg/g dw, with E7 displaying a 2.11-fold increase compared to E0, also significant ($p < 0.05$). No significant differences in protein content were observed between varieties and the same day of germination ($p > 0.05$).

Among the main components of barley extracts or musts are amino acids, being the main source of nitrogen for metabolic routes necessary for processes such as fermentation, increase of nutrients and alcohol precursors [50, 51]. Germination enhances the nutritional quality of cereals through the hydrolysis of prolamins and amino acids, such as glutamic acid, which acts as the main precursor for amino acids like arginine [52].

An increase in aromatic amino acids such as phenylalanine and tyrosine was observed; and essential amino acids such as histidine and arginine that may participate in antioxidant activities [53]. Furthermore, the findings indicate that a significant portion of essential amino acids, including threonine, methionine, lysine, valine, leucine, isoleucine, phenylalanine, and histidine, experienced an increase in their content as germination progressed. These results are similar to those reported by Gorzolka et al. [54], who demonstrated a gradual rise in amino acid content from 3 to 8 days of germination in barley samples. The presence of essential amino acids such as leucine is key for protein synthesis, because they can play a fundamental role in insulin and glucose metabolism [7].

Various studies have reported that germination activates proteolytic enzymes, leading to the release of peptides and amino acids in cereals such as wheat, rice, and corn [55–57]. The presence of amino acids, particularly aromatic and essential ones, contributes significantly to the increase in antioxidant activity. This is due to the ability of these amino acids to act as hydrogen donors and maintain the molecular stability of radicals, which in turn promotes greater antioxidant activity in grains and sprouted foods [58]. This highlights the importance of considering germination as a strategy to improve the nutritional quality and health benefits of foods.

Total phenolic and flavonoid content in germinated barley extracts

The results of total phenolic and flavonoid content in germinated barley extracts are shown in Fig. 2. The results showed concentrations of total phenols from 4.71 to 18.17 and from 8.36 to 21.73 mg GAE/g in Perla and Esmeralda, respectively. The concentration of flavonoids was from 0.61 to 2.54 and from 2.24 to 2.80 mg QE/g in Perla and Esmeralda.

The Perla and Esmeralda extracts presented a significant increase ($p < 0.05$) in total phenols throughout the days of germination. In the Perla variety, P5 and P7 presented the highest concentration, being statistically different from P0 and P3 ($p < 0.05$). The increase in P5 and P7 compared to P0 was 3.3 and 1.91 times, respectively. A similar behavior was observed in the Esmeralda variety. E5 and E7 presented the highest concentration of total phenols, which increased 2.3 and 2.5 times in relation to E0.

Research has demonstrated a correlation between the germination of grains and cereals and the total phenolic content [59, 60]. During this process, there is an increase in concentration attributed to the synthesis of phenolic compounds from glucose and/or aromatic amino acids released, which also elevate with germination time in both barley varieties (Table 1; Fig. 1) [61]. It can be hypothesized that the phenolic compound synthesis was favored by the greater availability of these compounds. In general, changes in polyphenol content could be due to the hydrolysis of polymeric polyphenols due to the enzyme glucosidase or esterases that can produce free or extractable polyphenolic compounds during germination and malting processes [62]. Additionally, apart from germination, the maceration process utilized in the production of worts or extracts could influence the release of total phenols, as hydrolytic enzymes are activated, leading to the liberation and solubilization of phenolic compounds in water within the temperature range of 45 to 78 °C [63].

The flavonoid content exhibited a similar pattern, increasing during germination in both barley varieties. Specifically, P3, P5, and P7 extracts demonstrated a significant increase ($p < 0.05$), with concentrations reaching 2.8, 4.1,

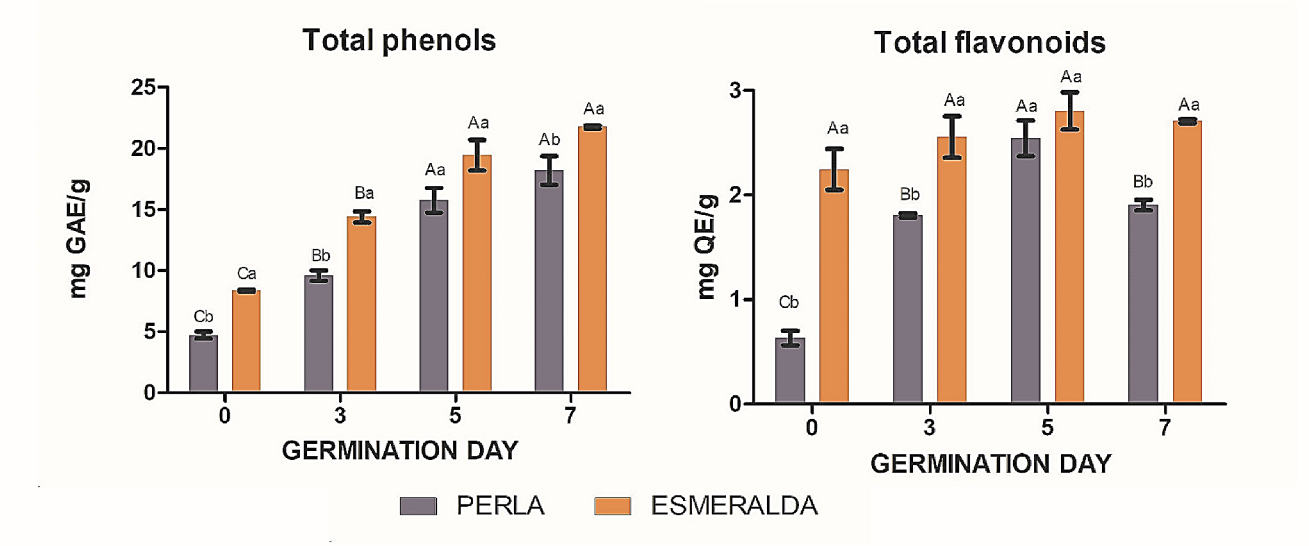

Fig. 2 Total concentration of phenols in barley extracts. Capital letters indicate significant differences between germination days and the same variety ($p < 0.05$); Lowercase letters indicate significant differences between varieties and the same germination day ($p < 0.05$)

and 3 times higher than P0, respectively. While Esmeralda extracts displayed an upward trend throughout germination, the differences were not statistically significant ($p > 0.05$). Conversely, E3, E5, and E7 extracts exhibited increases in total flavonoid content by up to 1.13, 1.27, and 1.22 times, respectively, compared to E0. Additionally, both varieties showed a decline in flavonoid content in P7 and E7 extracts compared to P5 and E5, with reductions of 3.6% in P7 and 24% in E7, respectively.

Heat treatment has been demonstrated to induce the degradation of free phenolic compounds, particularly flavonoids, which have been reported to be more susceptible to heat-induced degradation, with reductions of up to 51% [23]. Likewise, prolonged germination can lead to this decrease [59, 64]. This decrease in flavonoid content could be related to the action of oxidants such as Reactive Oxygen Species (ROS) that are generated in cells, especially in mitochondria, during the electron transfer chain [65]. Furthermore, the degradation of phenolic compounds could be related to enzymes such as phenoloxidase and peroxidase, which promote the oxidation of phenolic substrates [66].

On the other hand, UV radiation has been shown to enhance the production of free phenols by affecting the proline-linked pentose phosphate pathway [67]. Therefore, Perla barley, since it does not have hulls, could significantly increase the content of flavonoids as protection against UV rays, unlike Esmeralda barley. Since flavonoids represent most phenolic compounds in the human diet, it is important to understand how germination can influence their content and presence in foods.

Phenolic profile

The quantitative analysis of phenolic compounds from germinated barley extracts was performed using HPLC equipment. In the extracts of Perla and Esmeralda sprouts, a gradual increase in the concentration of different phenolic compounds was observed concerning the ungerminated samples (Table 2). Ferulic acid was the most abundant, the concentration of ferulic acid in Perla was from 6.57 to 30.67 µg/g. The increase was 4.6 times more in P7 compared to P0 (ungerminated sample). The same was observed in Esmeralda barley. The concentration of ferulic acid ranged from 16.03 to 77.57 µg/g, with E7 being 4.8 times greater than E0. The next major phenolic compound was p-coumaric, later syringic and gallic acid, it was evident that these phenolic acids are synthesized during germination because they were not identified in the ungerminated samples of both varieties. These results coincide with Tomková-Drábková et al. [68]; they describe how the maceration and germination phases are responsible for an increase in ferulic and p-coumaric acid.

Swajgier [69] demonstrated that the concentration of ferulic acid, as well as other phenolic acids, increases during the initial stages of maceration, due to hot water extraction and the release of ester-bound forms assisted by cinnamoyl esterases. Cortese et al. [70] evaluated the phenolic composition of different extracts from six varieties of beer and showed that trans-p-coumaric acid, ferulic acid and catechin were the main components in the extracts. Ham et al. [71] obtained different phenolic compounds in waxy barley extracts, among the most abundant flavonoids and

Table 2 Phenolic profile of lyophilized aqueous extracts of Perla and Esmeralda barley (µg/g)

Sample	Gallic acid	*p*-cumaric acid	Syringic acid	Ferulic acid	4-hydroxybenzoic acid	Chlorogenic acid	Catechin
PERLA							
P0	< LOD	< LOD	< LOD	6.57 ± 2.6^{g}	< LOD	< LOD	22.03 ± 5.7^{g}
P3	< LOD	$16.14 \pm 7.1b^{c}$	19.55 ± 1.8^{a}	21.22 ± 3.1^{f}	< LOD	9.64 ± 0.8^{b}	358.32 ± 9.2^{c}
P5	< LOD	36.80 ± 0.1^{a}	15.87 ± 0.1^{ab}	59.71 ± 0.5^{c}	< LOD	< LOD	256.93 ± 0.3^{d}
P7	< LOD	27.53 ± 0.0^{ab}	21.35 ± 0.3^{a}	30.67 ± 0.3^{e}	< LOD	7.96 ± 0.1^{a}	432.88 ± 5.2^{b}
ESMERALDA							
E0	< LOD	< LOD	< LOD	16.03 ± 0.1^{f}	5.87 ± 0.76	< LOD	< LOD
E3	2.5 ± 0.4^{b}	7.54 ± 0.07^{c}	11.95 ± 3.0^{b}	43.34 ± 0.7^{d}	< LOD	< LOD	136.78 ± 21.8^{f}
E5	8.3 ± 0.8^{a}	21.77 ± 0.6^{b}	19.19 ± 1.3^{a}	69.25 ± 1.0^{b}	< LOD	< LOD	193.76 ± 7.5^{e}
E7	10.2 ± 1.2^{a}	24.46 ± 2.4^{b}	21.14 ± 1.8^{a}	77.57 ± 3.5^{a}	< LOD	< LOD	517.84 ± 5.8^{a}

Freeze-dried aqueous extracts of ungerminated barley (P0 and E0) and germinated for 3, 5 and 7 days (P3, P5, P7, E3, E5 and E7) were used; The results are shown as the means of three repetitions ± their standard deviation; LOD: Limit Of Detection; Lowercase letters indicate significant differences between germination days and the same variety ($p < 0.05$); Extracts with the same letter did not present significant differences ($p > 0.05$)

phenolic acids were naringin, prunin, catechin, ferulic acid, and vanillic acid. These compounds are associated with the prevention of cancer, cardiovascular diseases and diabetes [71, 72].

On the other hand, catechin was the only flavonoid identified in both extracts. This flavonoid was synthesized during germination and its concentration increased as germination time increased. In Pearl barley the concentration of catechin was from 22.03 to 432.88 µg/g, P7 was 19.6 times more than P0. However, in the ungerminated sample of Esmeralda barley, catechin was not identified; from day 3 of germination, a concentration of 136.78 µg/g was observed, and it increased to 517.84 µg/g in E7. Catechin was the most abundant compound in barley extracts, mainly in E7 with up to 517 µg/g. Catechin is a natural polyphenolic flavonoid with antioxidant activities, such as lipid peroxidation and free radical scavenging [73].

The nutritional value of sprouted seeds as well as higher phenolic content and antioxidant activity compared to dried seeds have been previously reported [74]. Germination increases the content of phenolic compounds several times and diversifies the phenolic profile [75].

In addition to germination, the boiling caused during maceration could influence the increase in catechin compared to other phenolic compounds. Different works have mentioned that the contents of catechin, gallic acid, p-coumaric acid, and ferulic acid increase significantly during boiling, representing almost 50% of the total individual phenolic compounds identified in musts [70, 76]. The differences in the phenolic profile presented in this study when compared with other profiles from barley could be related to the type of cereal and the extraction conditions [77, 78].

Antioxidant activity

The antioxidant capacity of barley extracts is shown in Fig. 3A-C.

The DPPH radical uptake increased 4.2 and 3.02 times in extracts P7 and E7, concerning P0 and E0, respectively (Fig. 3-A). The highest activity was observed in extracts from P7 and E7. The Esmeralda variety presented the highest activity compared to Perla ($p<0.05$). Like the DPPH method, the ABTS method increased its antioxidant capacity concerning germination time (Fig. 3-B). The values increased 5 times in E7 and 7.9 times in P7 extracts, concerning the extracts E0 and P0. The highest ABTS radical scavenging activity was observed in P5 extracts. On the other hand, the oxidation capacity of the Fe (II) ion increased 1.83 and 2.3 times in extracts of E7 and P7, respectively, concerning E0 and P0 (Fig. 3-C). The highest activity was noted in E5 extracts, with these results being statistically significant compared to P5 extracts ($p < 0.05$).

The findings revealed that germination periods of 5 and 7 days resulted in increased antioxidant activities across all assays (DPPH, ABTS, and FRAP). These results are similar to those reported by García-Castro et al. [79]. In contrast to DPPH, both ABTS and FRAP methods exhibited enhanced antioxidant activity from the initial day of germination in both varieties. This observation is consistent with studies conducted by Zhou et al. [80] and Rico et al. [81], where improvements in antioxidant activity were noted in barley after 4 and 6 days of germination. It has been reported that the increase in antioxidant activity is related to the release of phenolic compounds during the germination process in different grains [82, 83]. This behavior may be related to the affinity of each method for the compounds with which it can react. For instance, in the DPPH method, aromatic and essential amino acids, such as tryptophan, tyrosine, phenylalanine, histidine, and arginine, may significantly contribute

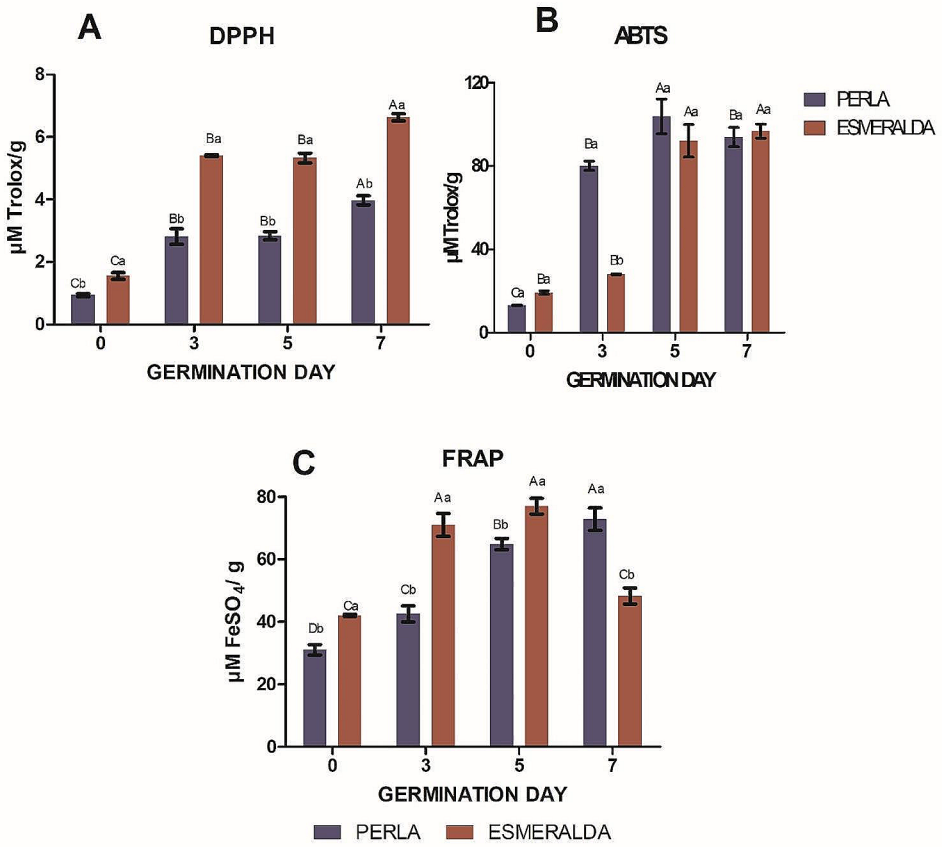

Fig. 3 (**A**) Capture capacity of the DPPH^{+} radical; (**B**) ABTS^{+}; and (**C**) Oxidation capacity of the Fe^{+z} ion in germinated barley extracts. Capital letters indicate significant differences between the same variety ($p < 0.05$); Lowercase letters indicate significant differences between varieties ($p < 0.05$)

to the observed biological activity [53], which is due to the ability of phenolic, indolic, and imidazole groups to function as hydrogen donors and maintain the molecular stability of the radicals. The ABTS method generally has greater affinity with polyphenols, while the FRAP method can bind to a wide range of antioxidant compounds (mainly polyphenols) and other compounds that have some reducing power [84]. The results of this study indicated that an extended germination period leads to higher antioxidant activity due to the release of significantly contributing compounds.

Conclusions

The results revealed an increase in the content of carbohydrates, amino acids, protein, and phenolic compounds in freeze-dried aqueous extracts of Perla and Esmeralda barley as the germination time was prolonged. The most abundant oligosaccharides were glucose and maltose, while the predominant amino acids were glutamic and aspartic acid. Regarding phenolic compounds, ferulic acid and catechin were the most abundant. In addition, an increase in antioxidant activity was observed, especially at 5 and 7 days of germination, according to the ABTS, FRAP, and DPPH tests. These findings highlight how germination can be an effective process for improving the nutritional quality and antioxidant properties of barley, which could have beneficial implications for human health and food formulation in the future.

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Author contributions AGC investigation and statistical analysis; FAGO writing—review and editing; ADRG writing—review and formatting; GHH supervision and editing. All authors have read and agreed to the published version of the manuscript.

Declarations

Conflict of interest The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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CAPÍTULO V. EFFECT OF BARLEY SPROUT EXTRACTS VAR. PERLA AND ESMERALDA IN A WISTAR RAT-INDUCED HYPERTENSION MODEL

5.1 Introducción

En este capítulo se aborda la parte central del proyecto el cual ha sido sometido a revisión en la revista *Food Chemistry*. Este estudio muestra los resultados de la evaluación de los extractos de cebada (Perla y Esmeralda) sin germinar y germinada (3, 5 y 7) sobre su participación en la actividad de la ACE tanto *in vitro* como *in vivo.* La primera etapa consistió en la evaluación de los extractos de cebada sobre la actividad de la ACE *in vitro*. La actividad inhibidora de ACE se ensayó midiendo la liberación de ácido hipúrico (HA) del sustrato Hipuril-Histidil-Leucina (HHL). La actividad de los extractos fue comparada con captopril, un ACEi de uso común. Los resultados mostraron un porcentaje de inhibición del 53 al 83%, mientras que el captopril inhibió la actividad de la ACE en un 95%. Los extractos de germinados de 7 días (Perla y Esmeralda) mostraron el mayor porcentaje de inhibición de la ACE. Por lo tanto, estos extractos fueron seleccionados para los ensayos *in vivo.* En la segunda etapa, se utilizó un modelo de inducción a la hipertensión con L-NAME y se administraron extractos de cebada durante 5 semanas para evaluar el efecto antihipertensivo. Se evaluó la función renal y endotelial, la actividad de la ACE I y II, y se midió la presión arterial de cola semanalmente. Además, se observaron los cambios histopatológicos en corazón, riñón e hígado. El tratamiento logró una disminución de la actividad de la ACE I y ACE II en un 81 y 76.5%, respectivamente. Los extractos pudieron mejorar la función renal y endotelial en ratas hipertensas. El estudio histopatológico mostró un efecto protector de los extractos contra el daño en el tejido cardíaco, renal y hepático. En esta etapa de la investigación se permitió cumplir con dos objetivos específicos del trabajo doctoral, además los resultados mostraron un alcance en el tratamiento coadyuvante contra la hipertensión.

Food Chemistry

Effect of barley sprout extracts var. Perla and Esmeralda in a Wistar Rat-Induced Hypertension Model

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Abstract:	Hypertension increases the risk of heart attacks and strokes, especially in younger individuals. To control or prevent it, dietary and lifestyle changes are recommended. In a recent study, researchers evaluated the impact of sprouted barley extracts (SBE) on Angiotensin-Converting Enzyme (ACE) activity in vitro. They then tested the most effective variety on Wistar rats with induced hypertension, measuring renal and endothelial function (NOx), ACE I and ACE II levels, and histopathological effects. Esmeralda barley extracts reduced ACE activity by 83% in vitro. Combined with BSE and captopril, they decreased blood pressure and ACE I and II activity by 22%, 81%, and 76% respectively. There was also a decrease in protein, creatinine, uric acid, and urea by 3%, 38%, 41%, and 47%, respectively, and a 66% increase in plasma NO levels. These findings suggest that sprouted barley extracts could be a useful strategy for improving blood pressure.
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Effect of barley sprout extracts var. Perla and Esmeralda in a Wistar Rat-Induced Hypertension Model

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ABSTRACT

Hypertension increases the risk of heart attacks and strokes, especially in younger individuals. To control or prevent it, dietary and lifestyle changes are recommended. In a recent study, researchers evaluated the impact of sprouted barley extracts (SBE) on Angiotensin-Converting Enzyme (ACE) activity in vitro. They then tested the most effective variety on Wistar rats with induced hypertension, measuring renal and endothelial function (NOx), ACE I and ACE II levels, and histopathological effects. Esmeralda barley extracts reduced ACE activity by 83% in vitro. Combined with BSE and captopril, they decreased blood pressure and ACE I and II activity by 22%, 81%, and 76% respectively. There was also a decrease in protein, creatinine, uric acid, and urea by 3%, 38%, 41%, and 47%, respectively, and a 66% increase in plasma NO levels. These findings suggest that sprouted barley extracts could be a useful strategy for improving blood pressure.

Keywords: Hordeum vulgare L., L-NAME, renal profile, Angiotensin Converting Enzyme, Nitric oxide, blood pressure.

1. Introduction

Hypertension is a major public health globally, which affects 31.1% of adults, with a higher prevalence in low-income countries (31.5%) compared to high-income countries (28.5%) (Campos-Nonato et al., 2021). Hypertension is a condition closely linked to several diseases, which makes it a significant risk factor for health. Cardiovascular diseases, such as coronary heart disease, ischemic heart disease, cerebrovascular disease, as well as heart and kidney failure, are some of the diseases associated with hypertension. (Aguilera-Méndez et al., 2020; López-López et al., 2017; Toala & Peñaherrera, 2023). In addition, hypertension can lead to a decrease in quality of life and a reduction in the life expectancy of patients (Markowicz & Grzeszczak, 2017). On the other hand, treating hypertension can generate significant expenses, including the cost of medication and medical appointments (Osibogun & Okwor, 2014).

Hypertension is characterized by a continuous tension of the blood vessels, normally regulated by the Renin-Angiotensin-Aldosterone System (RAAS). RAAS is a hormonal system crucial in regulating blood pressure and balancing fluids and electrolytes in the body (Ojeh et al., 2020). Renin, released by the kidneys in response to low blood pressure or low blood volume, converts angiotensinogen to angiotensin I. Angiotensin I is then converted to angiotensin II by -converting enzyme (ACE), leading to vasoconstriction and the release of aldosterone, which increases sodium and water reabsorption in the kidneys (Vaduganathan et al., 2020; Volpe & Battistoni, 2020). Reduced bioavailability of nitric oxide (NO) is linked to increased production of reactive oxygen species (ROS), a key factor in hypertension pathogenesis (Jordan et al., 2018). The increase in ROS generation results in impaired endothelial function, inadequate vasodilation, and increased blood pressure. Therefore, NO, released by endothelial cells, is a crucial regulator of vascular function. (Pérez et al., 2021).

The entire system that regulates the production of ACE II plays a crucial role in maintaining cardiovascular homeostasis. This system is the target of various medications and treatments that aim to reduce the risk of disease and related conditions. By controlling the production of ACE II through the consumption of bioactive compounds found in different food sources, such as barley, we can help prevent various diseases (García-Castro et al., 2022; Rizwan et al., 2023; Walls et al., 2020)

Drug therapies include ACE inhibitors, such as captopril or lisinopril, which prevent ACE from producing angiotensin II, a vasoconstrictor hormone that raises blood pressure. Inhibiting ACE reduces the formation of angiotensin II, which can result in dilation of blood vessels and lowering blood pressure (Bernátová et al., 1999; Vaduganathan et al., 2020).

In addition to ACE inhibitors, angiotensin II receptor blockers may be involved in increasing nitric oxide levels by promoting blood vessel dilation and improving blood circulation (Martínez-del Río et al., 2020). Due to the negative side effects of drug consumption such as

dizziness, dry cough, kidney problems, and hypotension, there has been an increase in research on lifestyle changes (Maniero et al., 2023; Messerli et al., 2018). Maniero et al., (2023) show the non-pharmacological approaches commonly used for the prevention and treatment of hypertension include reducing salt consumption and weight, increasing physical activity, quitting smoking, limiting alcohol consumption, and changing diet. Several studies highlight the consumption of foods rich in bioactive compounds, such as phenolic compounds or anthocyanins, which could participate in the activity of ACE (Ojeda et al., 2010; Wang et al., 2021). In cereals, such as barley, peptides, proteins, and phenolic compounds have been shown to act as inhibitors of ACE and show antihypertensive effects in different study models (Gangopadhyay et al., 2016; Hokazono et al., 2010; Ra et al., 2020). In addition, barley phytochemicals that act on the cyclooxygenase and lipoxygenase pathways have been described to synergistically prevent inflammatory and cardiovascular diseases (Gul et al., 2014).

Barley plays a critical role in the brewing and food industry. In beer production, barley is used for malt brewing, where the quality of the malt obtained is closely related to the germination capacity of the barley seeds (Pérez-Ruiz et al., 2015). In addition, barley malt bagasse, a nutrient-rich by-product, is used in the food and industrial industry (Barberan & Muñoz, 2022). However, no reports on the functionality of extracts from sprouted barley have been found so far. Therefore, the present project is aimed at the evaluation of extracts made from two varieties of barley germinated for 3, 5, and 7 days to determine the mechanisms of regulation of blood pressure in *vitro* and *in vivo models.*

2. Materials and methods

2.1. Reagents

L-NAME, ACE, and Hypuril-Histidyl-Leucine were acquired from Sigma-Aldrich, USA. Captopril was purified to 95-98% purity. All other chemicals used in this study were of analytical quality obtained from Merck and SPINREACT ®.

2.2. Obtaining barley extracts.

Barley extracts were obtained using the maceration method with modifications (EBC, 2003; García-Castro 2024). Briefly, non-sprouted and sprouted 3, 5- and 7-day grains from two barley varieties were used (Perla y Esmeralda). Sprouted barley was dried in a stove (Barnstead Lab-Line, Mod. 3478, USA) at 50-55°C for 24 h or until a humidity of less than 5% was obtained. After the initial milling process, the flour was sifted to obtain a particle size ≤ 0.2 mm. Next, 50 g of the sifted flour was measured and mixed with 200 mL of water at 45°C. The mixture was kept at this temperature for 30 minutes and then increased by 1°C every 30 minutes until it reached 70°C. Once it reached 70°C, an additional 100 mL of water was added, and the temperature was maintained for one hour. The mixture was then allowed to cool for 10 minutes. The weight was adjusted to 450 g using water and then centrifuged at 5000 rpm for 10 minutes. After centrifugation, the supernatants were freeze-dried at a temperature of -62°C and 0.040 mBar pressure, using a Labconco freeze dryer (Model 816-333-8811, USA). The freeze-dried extracts were then weighed and stored in a freezing and vacuum-sealed container until use. On average, for every 400 mL of aqueous extract, 20 g of lyophilized extract was obtained, which is a ratio of 0.05g/mL.

2.2. Evaluation of barley extracts on ACE activity

The antihypertensive activity of barley extracts was determined using the modified method of Cushman & Cheung, (1971) using a Hypuryl-Histidyl-Leucine (HHL) substrate. The test was conducted by measuring the release of hippuric acid (HA) from the HHL substrate. Initially, 20 µL of ACE solution (2.5 mU/mL) was mixed with 40 µL of different EBS (sprouted barley extracts) (10 mg/g) or 0.01 mg/mL of captopril and incubated at 37°C for 5 minutes. Afterward, 100 µL of HHL (5 mM) was added and incubated at 37°C for 30 minutes. The reaction was stopped by adding 150 µL of HCl (1 M). Hippuric acid was extracted by adding 1000 µL of ethyl acetate, which was then mixed using a vortex for 20 seconds and centrifuged at 4000 rpm for 12 minutes. Afterward, 750 µL of the organic phase was taken and boiled (92°C) to remove ethyl acetate and concentrate HA. Finally, 800 µL of sterile double-distilled water was added and mixed using a vortex for 20 seconds.

The absorbance was measured at 228 nm using a UV-Vis spectrophotometer. The percentage of ACE inhibition was calculated using the following equation.

ACE inhibition (%) =

$$\left[\frac{(A_{sample} - A_{blank})}{A_{control} - A_{blank\ sample}}\right] \times 100$$

A sample = Absorbance of the analyzed sample of the extract
A blank = HHL absorbance (added buffer instead of ACE and sample)
A control = ACE absorbance (Added buffer instead of sample)
A blank Sample = Sample absorbance without ACE

2.3. Experimental animals

Male Wistar rats weighing 200-220 g and 8-10 weeks old were used. All experimental animals were provided by the Livery Center of the Autonomous University of the State of Hidalgo. The animal procedures in the present study were approved by the Institutional Ethics Committee for the Care and Use of Laboratory Animals of the Autonomous University of the State of Hidalgo, Mexico (Approval No. CICUAL/004/2022). The animals were kept under standard conditions (22±2°C with 12:12 h light-dark cycles, with free access to water and feed). The handling of experimental animals was carried out following the guidelines described in the NOM-062-ZOO-1999 Standard (Oboh et al., 2021; SEMARNAT, 1999).

2.3.1. Experimental design

After one week of acclimatization, 56 Wistar rats were randomly assigned to 7 groups (n = 8). The first group (G1) was given a standard diet, the second group (G2) was treated with L-NAME (N(ω)-Nitro-L-Arginine Methyl Ester) at a dose of 40 mg/kg/day via intragastric administration, and the third group (G3) was treated with both L-NAME (40 mg/kg/day) and Captopril (5 mg/kg/day), an ACE inhibitor. L-NAME was mixed into drinking water to induce hypertension. Four groups were given barley sprout extracts as follows: G4. L-NAME+ Esmeralda SBE (500 mg/kg/day); G5. L-NAME + SBE Perla (500 mg/kg/day); G6. L-NAME + a combination of Esmeralda and Perla SBE (250+250 mg/kg/day); and G7. L-NAME + Esmeralda SBE + Captopril (250 mg/kg/day + 2.5 mg/kg/day). All groups were administered via intragastric cannula for 35 days.

2.3.2. Blood pressure measurement

Blood pressure levels (systolic and diastolic) of all animals involved in the study were measured weekly using the CODA™ blood pressure monitor (Kent Scientific Corp., Torrington, CT, USA) with a tail-cuff method. The animals were pre-trained to minimize stress that could interfere with blood pressure measurements. The conscious rats were kept in a temperature-controlled room (30-32°C) and placed in plastic cylinders to immobilize them, leaving the tail outside. Then, the pressure reading was taken. The measurements included 5 acclimatization cycles and 10 measurement cycles, following the recommendations of the CODA™ team. The results were expressed in mm/Hg.

2.4. Biochemical tests

2.4.1 Sample collection and tissue preparation

After 35 days of treatment, the rats were euthanized by cervical dislocation, and blood samples were collected via cardiac puncture for further analysis. The blood samples were centrifuged at 5000 rpm for 10 minutes to separate the plasma for biochemical determinations. Additionally, 500 mg of kidney tissue was homogenized in TRIS-HCl 0.1 M pH 7.4 and centrifuged at 10,000 × g for 10 minutes. The supernatants were used for the RCT II activity trial. A portion of the kidney, heart, and liver were washed with a 0.9% saline solution and preserved in 10% formalin for further processing.

2.4.2. NOx quantification

Nitrite/nitrate (stable metabolites of NO) in the plasma samples were measured based on the Griess reaction (Green et al., 1982). Nitrate was reduced to nitrite by 30-minute incubation with nitrate reductase in the presence of nicotinamide adenine dinucleotide phosphate oxidase in its reduced form (NADPH). The colorimetric reaction is obtained by adding 100 µL of Griess reagent to 50 µL of previously diluted sample (1:10) and completing a final volume of 200 µL with deionized water. The nitrite is then detected as an azole dye resulting from the Griess reaction visible at 540nm. The amount of nitrite/nitrate present in the plasma sample was estimated from the standard curve obtained from 3-200 µMol/l nitrite/nitrate levels were expressed as µMol/l.

2.4.3. Quantification of markers of renal function

Plasma urea, uric acid and creatinine were estimated according to the manual provided by commercial diagnostic kits (SPINREACT,® Mexico) based on the Fawcett & J. E. Scott, (1960), Caraway, (1955) and Jaffe M, (1886), respectively. The plasma glucose level was determined from an enzymatic reaction using the method described by Trinder, (1969) in which

glucose oxidase catalyzes the oxidation of glucose to gluconic acid and was measured at 492 nm. The concentration of total protein in plasma was determined from the Biuret colorimetric method at an absorbance of 540nm. Commercial diagnostic kits (SPINREACT®, Mexico) were used. All results were expressed as mg/dL.

2.4.4. Quantification of ACE I and ACE II

The concentration of ACE I in plasma was determined using a fluorometric assay kit (Sigma-Aldrich, St. Lois, MO, USA), based on the cleavage of a synthetic fluorogenic peptide, where the fluorescence emitted is directly proportional to the activity of the ACE present (Ex/Em= 320/405 nm), the results were expressed in mU/mL. On the other hand, the concentration of ACE II in the kidney using a fluorometric assay kit (Sigma-Aldrich, St. Lois, MO, USA), where 500 mg of kidney was homogenized to measure the ability of ACE II to cleave a synthetic peptide substrate based on 7-metaxicomarin-4-acetic acid (MCA) for the release of a free fluorophore (Ex/Em= 320/420 nm), results were expressed in mU/mg tissue.

2.5. Histopathological analysis

Samples fixed in 10% formalin were dehydrated with ethanol, clarified with xylene, and enclosed with paraffin. 5 μm slices were made and stained with hematoxylin-eosin and examined with a high-resolution (40x) light microscope.

2.6. Statistical analysis

The results were expressed as the average of six determinations ± SD. Data were analyzed using a unidirectional analysis of variance (ANOVA). A comparison of means was performed using Tukey's test with a 95% confidence level. The nonparametric Spearman correlation coefficient was calculated to identify correlations between bioactive compounds and the percentage of inhibitory activity of ACE. All of these analyses were performed using Minitab statistical software version 19.2 USA.

3. Results

3.1. Effect of barley extracts on ACE inhibition *in vitro*

Figure 1 shows the reduction in ACE activity percentages of captopril (positive control) and various EBS of Esmeralda and Perla varieties. At 0.01 mg/mL, captopril exhibited 95% ACE inhibition. Meanwhile, the SBEs of Perla and Esmeralda varieties inhibited ACE activity from 56 to 63% and from 59 to 83% (10 mg/g extract), respectively. All samples showed statistically significant differences compared to captopril activity ($p < 0.05$). The percentage of ACE inhibition increased in the 7-day BSE of Perla and Esmeralda. Therefore, the BSE of P7 and E7 were selected for administration in the in vivo model (Section 3.2). Earlier studies have reported

captopril's 90% ACE inhibition, which was used to compare its activity with another compound (Gangopadhyay et al., 2016; Jain et al., 2021).

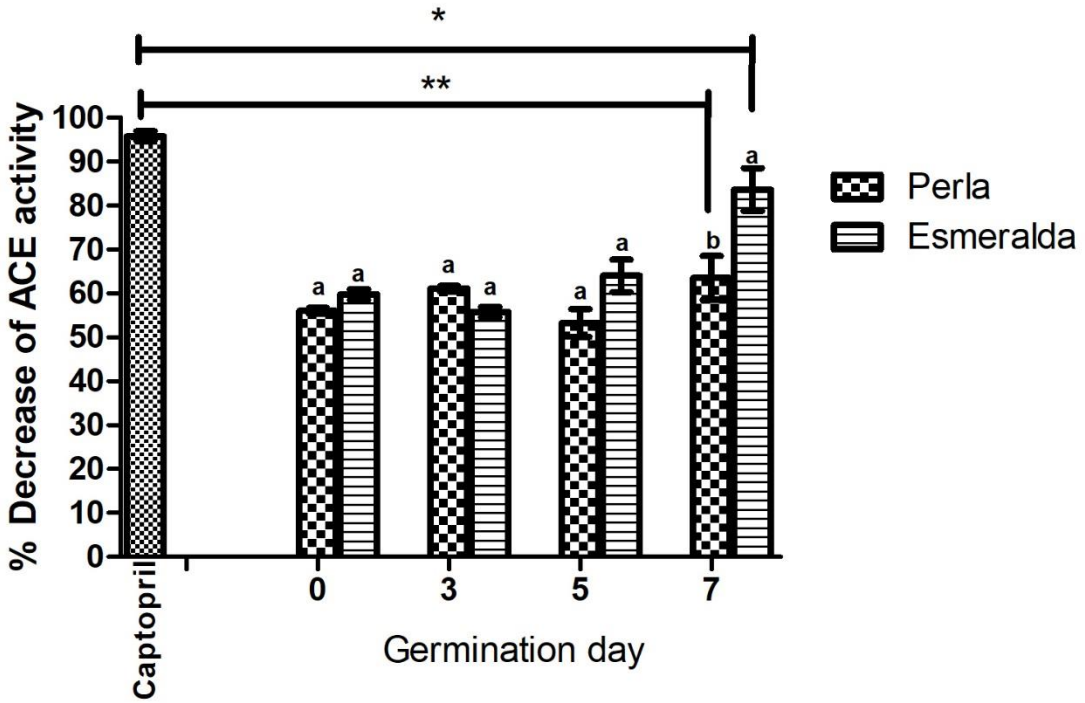

Fig. 1. Percentage decrease in ECA activity. Each bar represents the mean ± SD. The significance levels are represented by the value of $p < 0.05$ (* < 0.05 and ** < 0.0001) compared to the control group (Captopril). Different letters indicate significant differences between varieties and the same day of germination. And ANOVA followed by a Tukey test was performed.

The germination of grains and seeds is an important factor that involves various metabolic activities and physicochemical changes to produce different secondary metabolites (Hübner & Arendt, 2013). Likewise, this process increases the content of proteins, carbohydrates, and phenolic compounds that promote antioxidant activity and in some cases activity on ACE (Chinma et al., 2015; Mamilla & Mishra, 2017). This activity could be related to the increase of bioactive compounds during germination (Cornejo et al., 2015; Mamilla & Mishra, 2017). Foods rich in polyphenols may reduce elevated blood pressure through inhibition of ACE (Jain et al., 2021; Santos et al., 2020). Quercetin, orientin, isovitexin, and some isoorientin and isovitexin glycosides are among the phenolic compounds in barley that exhibit ACE inhibitory activity. (Ra et al., 2020).

Previously, the total content of phenols and flavonoids, as well as the phenolic and amino acid profile of the extracts used in the present study (García Castro et al., 2024) and which are involved in the activity of ACE have been reported. Phenolic acids such as gallic, syringic, and ferulic acids; Flavonoids such as catechin and amino acids such as valine and leucine showed a linear correlation (r= 0.388 to 0.925) with the inhibition of ACE (Appendix A. Supplementary

Material). Ra et al., (2020) were evaluated and obtained a percentage of inhibition of ACE activity from 32 to 66.5%, attributing this activity to the polyphenols present in barley. Other antihypertensive mechanisms reported in phenolic compounds are the prevention of NADPH oxidase expression and the production of reactive oxygen species (ROS) (Yousefian et al., 2019). García-Castro et al (2024), showed how 7-day germination can increase the content of phenols and total flavonoids by 285% and 316%, respectively. Therefore, the concentration of phenolic compounds and flavonoids could influence the activity on ACE.

On the other hand, it has been shown that some peptides and amino acids may interfere with the active site of ACE or its ability to bind to substrates (Mirzaei et al., 2015). Alu'Datt et al., (2012) y Gangopadhyay et al., (2016) obtained barley protein hydrolysates that can inhibit ACE by 60 to 70%. Some naturally occurring amino acids can compete with ACE's natural substrates for the enzyme's active site. By binding to the active site, they prevent the ACE from performing its normal function of converting angiotensin I to angiotensin II (Wang et al., 2021).

3.2. Effect of BSE on renal function and nitric oxide concentration

Table 1 shows the measurements of glucose, markers of renal function, and plasma NOx expression in the different groups.

No changes in glucose levels were observed between the standard diet group (G1) and the L-NAME-induced group (G2) ($p > 0.05$). However, an increase in glucose of 21% and 29% was observed in the G3 and G6 groups, respectively, compared to the (G1) group ($p < 0.05$).

In terms of renal function, there were no significant differences observed in total protein content between the treatment groups ($p > 0.05$). However, animals treated with L-NAME (G1) showed a 50% increase in serum uric acid and a 70% increase in urea levels ($p < 0.05$), while creatinine levels increased by 20%, although no significant differences were observed ($p > 0.05$). On the other hand, the results indicated that treatment with the Esmeralda variety (G4) significantly improved ($p < 0.05$) creatinine levels by up to 55%, while uric acid and urea levels were reduced by 41% and 47% from treatments G6 and G7, respectively.

The study found that endothelial function (NOx) decreased by 37% ($p < 0.05$) in the G2 group when compared to the G1 group (Table 1). However, the administration of barley extracts for 5 weeks significantly increased ($p < 0.05$) the level of plasma NOx metabolites, leading to a 66% increase by the G7. The groups administered with captopril and SBE (G3, G4, G5, G6, and G7) showed improvements in renal function and a significant increase in NOx ($p < 0.05$), compared to rats induced with L-NAME (G2). Kumar et al., (2011) reported that induction with L-NAME in rats reduced nitric oxide concentration by up to 60%.

On the other hand, it was observed that the combination of extracts of both barley varieties (G6) significantly increased ($p < 0.05$) the serum glucose concentration. Barley germination has

been reported to elevate the content of oligosaccharides such as glucose and maltose in BSE (García-Castro et al., 2024). This could have caused the increase in glucose with this treatment, due to changes in the absorption, metabolism, or use of sugars by the body (Qin et al., 2021). However, captopril decreased the development of L-NAME-induced hypertension, indicating the involvement of ACE inhibitors in the nitric oxide (NOx) pathway (Bernátová et al., 1999). In contrast to captopril, all extracts evaluated and even in combination with captopril increased the concentration of serum NOx. Certain phenolic acids and flavonoids, such as caffeic acid, chlorogenic acid, and catechin, attenuate the development of hypertension by modulating the production of NOx (Kim et al., 2013; Zibadi et al., 2007). On the matter, Kumar et al. (2011) and Anikina et al., (2020), showed that the administration of vanillic acid and catechin improved the bioavailability of nitric oxide, renal markers, and decreased the activity of ACE in rats. In addition, quercetin was observed to prevent cardiac and renal hypertrophy and reduce blood pressure in animal models (Duarte et al., 2001; Jalili et al., 2006). Similarly, it has been suggested that quercetin may modulate the permeability of the proximal tubular epithelium of the kidney to maintain the water content in the urinary lumen and promote the solubilization of calcium and sodium in this segment, relieving renal function (Gamero-Estevez et al., 2019).

According to Pisoschi et al., (2024), the presence of phenolic compounds like chlorogenic and/or gallic acid, and flavonoids like catechin in barley can help regulate nitric oxide expression and protect the kidney's functional capacity from the harmful effects of L-NAME. This is due to the antioxidant and anti-inflammatory properties of these compounds that promote nitric oxide modulation.

Table 1

Effect of barley extracts on glucose content, renal function (mg/dL) and NOx production in plasma of rats induced with L-NAME.

Group	Glucose	Total protein	Creatinine	Uric acid	Urea	NOx µMol/L
G1. Standard diet	108.72±10.85^{b}	6.71±0.91^{a}	1.11±0.15^{ab}	1.13±0.15^{b}	18.73±1.89^{b}	83.88±8.50^{a}
G2. L-NAME (40 mg/kg)	106.69±6.02^{b}	6.20±0.72^{a}	1.34±0.19^{a}	1.70±0.02^{a}	31.25±2.19^{a}	52.66±4.03^{b}
G3. L-NAME + Captopril (5 mg/kg)	131.65±4.73^{a}	6.33±0.66^{a}	0.71±0.07^{cd}	0.97±0.07^{b}	19.82±1.93^{b}	86.11±7.53^{a}
G4. L-NAME + E7 (500 mg/kg)	104.81±4.70^{b}	7.21±0.78^{a}	0.60±0.08^{d}	1.09±0.11^{b}	16.35±0.89^{b}	80.00±8.09^{a}
G5. L-NAME + P7 (500 mg/kg)	98.11±10.36^{b}	6.97±0.67^{a}	0.94±0.11^{bc}	1.05±0.12^{b}	18.26±1.89^{b}	79.16±6.18^{a}
G6. L-NAME + E7+P7 (250+250 mg/kg)	140.95±11.01^{a}	6.00±0.67^{a}	0.80±0.09^{bcd}	0.98±0.1^{b}	17.71±1.91^{b}	78.75±7.88^{a}
G7. L-NAME + E7+ Captopril (250mg/kg+2.5 mg/kg)	108.78±10.85^{b}	6.02±0.98^{a}	0.83±0.10^{bcd}	1.00±0.08^{b}	16.30±1.64^{b}	87.91±6.85^{a}

Each data represents the mean ± SD (n=6 rats); E7: BSE Esmeralda variety 7 days of germination; P7: BSE Esmeralda variety 7 days of germination; Different lowercase letters indicate significant differences ($p < 0.05$) when compared into groups (ANOVA followed by the Tukey test).

3.3. Administration of extracts of barley sprouts and measurement of blood pressure in rats

Table 2 shows changes in systolic and diastolic blood pressure in different experimental groups over 5 weeks of treatment. G2 showed a significant increase in systolic and diastolic blood pressure, up to 30%, compared to G1, demonstrating the hypertensive effect of L-NAME. While the administration of captopril (G3) decreased the effects of L-NAME in the following weeks, decreasing BP by up to 18%, after 5 weeks. On the other hand, BSE treatments resulted in a 9% and 12% decrease in BP by G4 and G5, respectively, compared to G2 after 5 weeks of treatment (Table 2). Similarly, a significant reduction of up to 12% in BP was observed concerning G2 when the extracts (G6) were combined and greater stability in pressure over time was observed. In the third week of treatment, G7 showed an improvement in BP, due to a significant reduction of 22% compared to G2 ($p<0.05$). These results suggest that treatment combined with half a dose of the drug plus administration with BSE achieves an antihypertensive effect like that of a dose of captopril (Table 2).

Jan-on et al., (2020) showed that the presence of fatty acids and phytochemical antioxidants from rice bran are involved in improving BP in rats, reducing it by 21%. Previously, it has been shown how the use of chronic treatment with L-NAME induces high blood pressure, providing an experimental model to study this condition (Silva-Herdade, 2011). One of its main effects is the decrease of plasma nitric oxide synthase that causes arteriolar vasoconstriction in rats induced with L-NAME (Chia et al., 2021). The continuous application of ACE inhibitors, such as captopril, is effective in preventing the onset of hypertension caused by L-NAME. This suggests the involvement of ACE inhibitors in the nitric oxide pathway (Bernátová et al., 1999; Efosa et al., 2023; Pecháňová et al., 1997).

Previous studies have shown that a diet rich in cereals and/or sprouts could prevent the development of hypertension due to the antihypertensive activity of some peptides, phenolic acids and flavonoids (Deng et al., 2014; Duarte et al., 2001; Mamilla & Mishra, 2017). Research has demonstrated the efficacy of specific phenolic compounds, such as catechin, in binding to the active sites of ACE, and their ability to bind to the zinc ion at the ACE's active site, thereby promoting antihypertensive effects (Wang et al., 2021). Similarly, the presence of one or more hydroxyl groups that have a strong antioxidant capacity, inhibits the production of ROS and leads to increased bioavailability of NO or through the binding of amino acids to the ACE substrate (Lunow et al., 2015; Ra et al., 2020). Therefore, the decrease in blood pressure in the treatment groups can be attributed to the presence of phenolics and/or bioactive amino acids, which could be due to their inhibitory and antioxidant activities of ACE.

Table 2

Hemodynamic effects of the administration of germinated barley extracts in rats.

	Systolic blood pressure (mm/Hg)			Diastolic blood pressure (mm/Hg)		
Groups /week	**1st**	**3rd**	**5th**	**1st**	**3rd**	**5th**
G1. Standard diet	167.15±6.41^{bc}	166.58±6.39^{c}	161.36±13.10^{d}	134.46±2.18^{abcd}	127.73±11.27^{c}	115.82±13.74^{c}
G2. L-NAME (40 mg/kg)	193.32±12.17^{a}	214.72±9.69^{a}	218.62±9.46^{a}	154.52±10.96^{a}	177.33±14.30^{a}	182.92±17.71^{a}
G3. L-NAME + Captopril (5 mg/kg)	167.03±10.05^{bc}	178.11±9.56^{bc}	178.28±12.72^{cd}	127.81±10.38^{bcd}	145.22±9.96^{bc}	148.35±14.37^{b}
G4. L-NAME + E7 (500 mg/kg)	165.86±10.14^{bc}	192.17±6.44^{b}	198.97±9.97^{b}	125.72±12.72^{cd}	151.18±10.19^{b}	159.94±13.07^{b}
G5. L-NAME + P7 (500 mg/kg)	161.41±14.44^{bc}	189.30±10.29^{b}	190.02±15.97^{bc}	120.98±18.60^{d}	152.52±11.14^{b}	157.85±11.56^{b}
G6. L-NAME + E7+P7 (250+250 mg/kg)	187.08±9.11^{a}	189.93±11.80^{b}	190.92±11.68^{bc}	149.39±15.29^{ab}	158.12±12.86^{b}	156.12±11.31^{b}
G7. L-NAME + E7+ Captopril (250mg/kg+2.5 mg/kg)	176.56±9.52^{bc}	167.37±12.81^{c}	179.92±11.56^{bc}	142.32±17.12^{abcd}	132.27±10.97^{cd}	142.4±12.37^{bc}

All experiments represent the average of the measurements, followed by the standard deviation (n=6-8); E7: BSE Esmeralda variety 7 days of germination; P7: BSE Esmeralda variety 7 days of germination; Different lowercase letters indicate significant differences ($p<0.05$) between groups in each time period.

3.4. Effects of BSE on ACE I and ACE II activity in L-NAME-induced rats

Figure 2a shows changes in the activity levels of ACE I in plasma (Figure 2a) and ACE II in kidney homogenate (Figure 2b).

The basal activity of ACE I in plasma was 54.4 mU/mL (G1), which increased to 233% due to the administration of L-NAME (G2) (Figure 2a). The administration of captopril G3 significantly reduced the activity of ACE I by 80.8% compared to G2 ($p<0.0005$). Groups G4 and G5 also showed decreased ACE I activity by 63.3% and 29.3%, respectively. However, Esmeralda (G4) was selected for combined administration with captopril (G7) as it showed the most significant reduction in enzyme activity. All other treatment groups showed a significant reduction ($p < 0.05$) in the enzymatic activity of ACE I, except for the combination of both BSE (G6), where no significant difference was observed ($p > 0.05$). The G7 group exhibited an 81.6% reduction in ACE I activity, similar to G3 ($p > 0.05$).

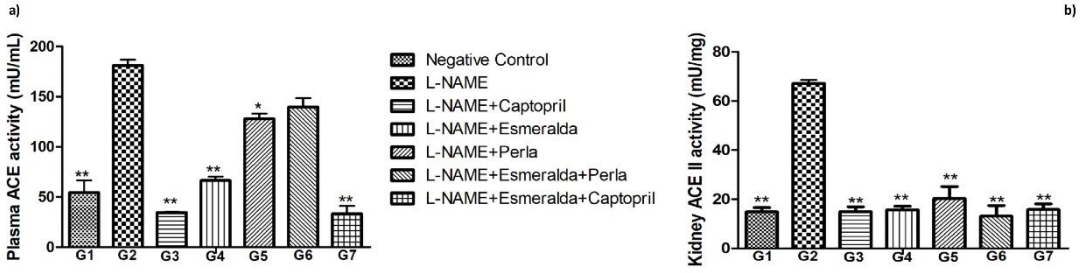

Fig. 2. A) Effects of barley extracts and captopril supplementation on serum ACE I activity. **B)** Effects of barley extracts and captopril supplementation on kidney ACE II activity. Each bar represents the mean ± SD Significance levels are indicated by $p < 0.05$ (* < 0.05 and ** < 0.0001) when compared with the L-NAME group (ANOVA followed by the Tukey test).

In the study, Figure 2b illustrates the changes in ACE II activity in the kidney. All groups were compared to the G2 group, and it was observed that there was a significant decrease ($p<0.0005$) in the activity of ACE II in the kidney for all the groups. The results depicted that the baseline activity of ACE II in the kidney was 14.8 mU/mg in G1, while the G2 group showed an increase of 353%. The groups administered with BSE (G4-G6) and its combination with captopril (G7) showed a decrease in ACE II activity between 76.5 and 80% compared to the group induced with L-NAME. This reduction in ACE II activity was achieved while maintaining an activity level similar to the standard diet group (G1) ($p<0.05$). The BSEs, particularly the Esmeralda variety, significantly reduced the activity of ACE I and II in the plasma and kidney of rats hypertensive with L-NAME, as shown in Figures 2a and 2b. Maneesai et al., (2017) reported that the increase of ACE in hypertensive rats is one of the main signs of activation of the RAAS system, with inhibition being the main target for the treatment of hypertension. Treatment for ACE inhibition

may be associated with relieving oxidative stress and increasing nitric oxide formation, conferring endothelial protection in hypertensive rats with L-NAME (Jan-on et al., 2020). The data indicate important antioxidant properties in barley extracts and the differences between the Perla and Esmeralda varieties (García-Castro et al., 2024).

3.5. Target organs histopathology

Figures 3 and 4 show the histopathologic changes of the heart and kidney in normotensive and hypertensive rats induced by L-NAME (G2). Figure 3A shows normal heart tissue, with no inflammation. On the other hand, the heart of hypertensive rats showed myocardial fibrosis, infiltration of mononuclear inflammatory cells indicating chronic inflammation (Figure 3B). On the other hand, the treatment groups (G4, G5, G6 and G7) caused a reduction in muscle fibrosis and moderate myocardial degeneration (Figure 3C-3G).

Figure 4A shows normal histological features of the kidney and the renal glomerulus with spaces and Bowman's capsules. Administration of L-NAME caused necrosis of the renal glomerulus, protein leakage into space, and dilation of Bowman's spaces, which decreases fluid leakage (Figure 4B). Similarly, after the use of L-NAME, liver tissue showed an increase in the number of Kupffer cells caused by oxidative stress and inflammatory response (Appendix B. Supplementary Material).

Blood pressure is commonly linked to liver and kidney damage. When microcirculation is repeatedly exposed to high blood pressure, it can cause damage at the cellular level in the liver and kidneys (Ibarrola et al., 2022; Santos Neto et al., 2020). For example, hypertension can lead to damage to the blood vessels of the liver and kidneys, resulting in a decrease in blood supply and therefore oxygen and nutrients. This effect is increased due to oxidative stress and alteration of cellular redox balance, which are closely related to various types of liver and kidney injuries, both acute and chronic (Chen et al., 2022). In L-NAME induction models, there is an association between high blood pressure and oxido-inflammatory processes (Gonzalez et al., 2000). Therefore, it is important to investigate the anti-inflammatory and antioxidant properties of natural antihypertensive therapies.

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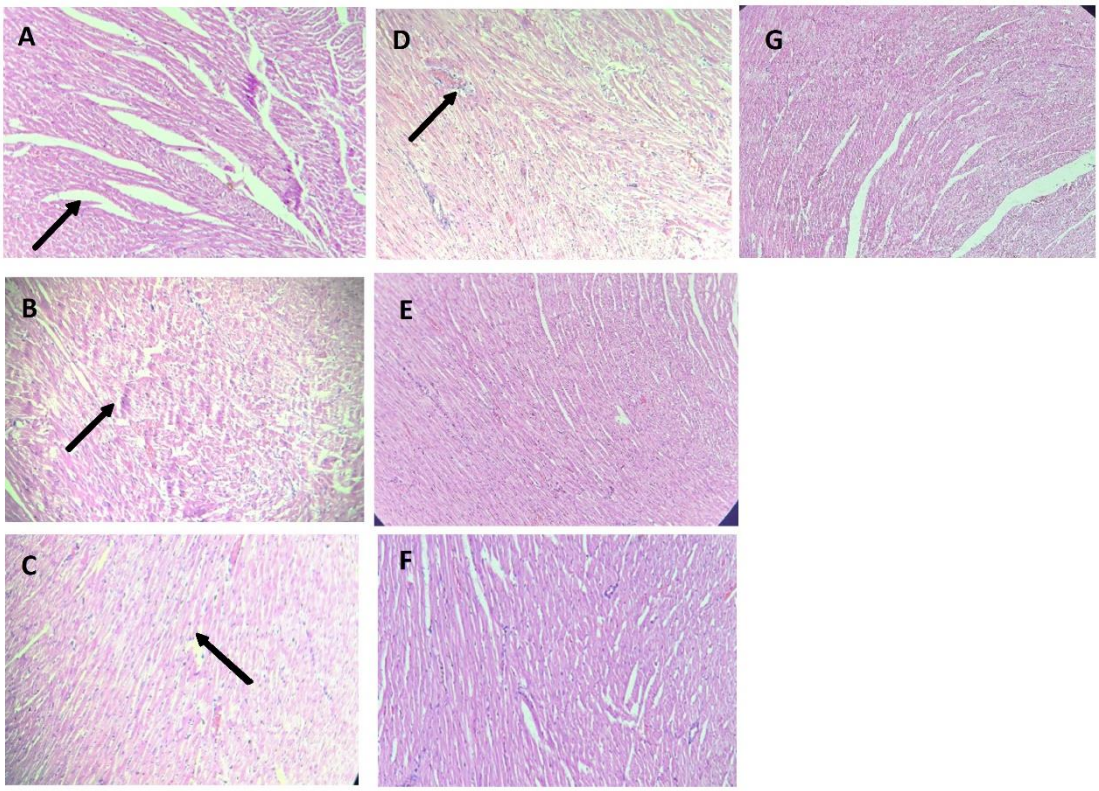

415

416 **Fig. 3.** Histopathological changes in the cardiac muscle (H-E stain). A) Normotensive group, normal
417 cardiac muscles; B) L-NAME Group, chronic inflammation; C) L-NAME + Captopril Group, inflammatory
418 cells decreased inflammation; D) L-NAME + Esmeralda Group, inflammatory cells are observed, moderate
419 inflammation; E) L-NAME + Perla Group, reduction of inflammation; F) L-NAME + Perla + Esmeralda
420 Group, without histopathological changes; G) Group L-NAME Esmeralda + Captopril, inflammation relief.

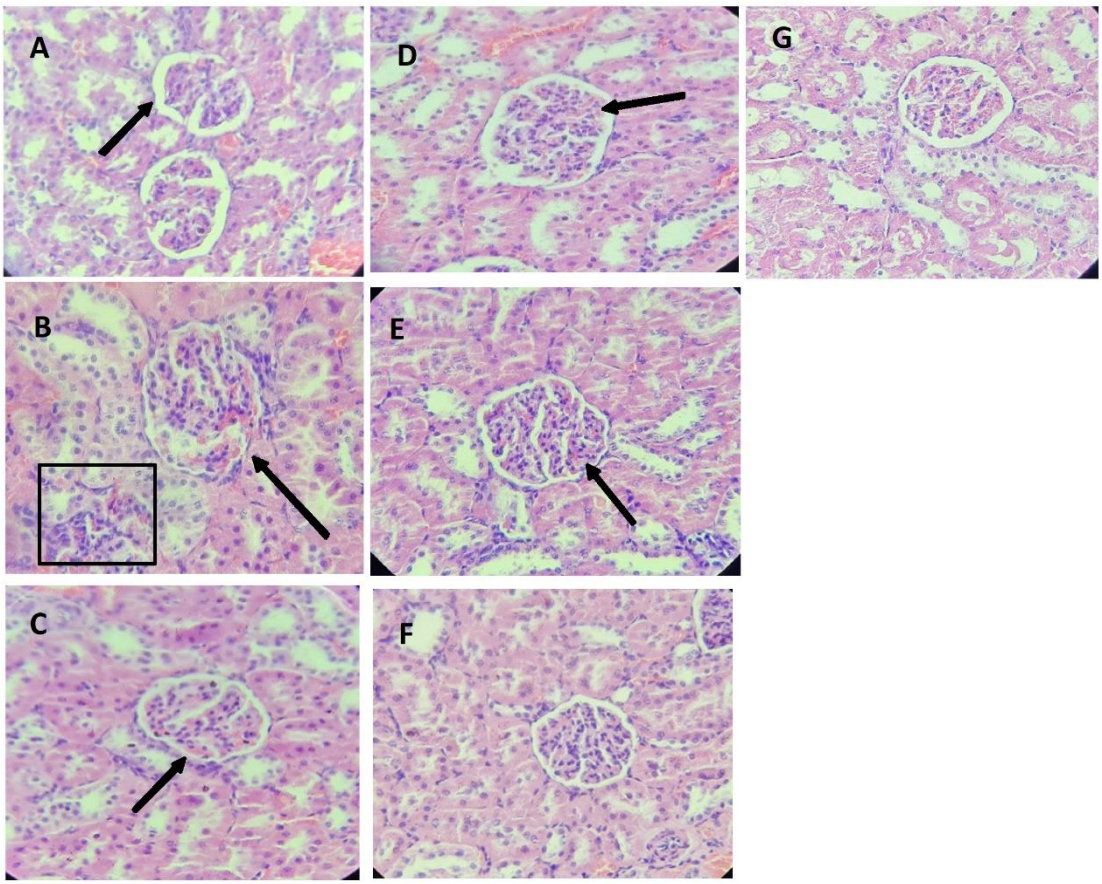

Fig. 4. Histopathological changes in the renal glomerulus. A) Normotensive group, without observable changes; B) L-NAME Group, degeneration and necrosis of renal glomerulus; C) L-NAME + Captopril Group, partial adhesion of the glomerulus to Bowman's capsules; D) L-NAME + Esmeralda Group, slight glomerular necrosis; E) Group L-NAME + Perla, Bowman space dilation; F) L-NAME + Perla + Esmeralda Group, decreased inflammation of the renal glomerulus; G) L-NAME Esmeralda + Captopril Group, without observable histological changes.

4. Conclusions

The research findings indicate that the use of Perla and Esmeralda SBE can reduce ACE activity in vitro by 83%. When BSE is administered along with captopril, it can lead to a reduction in systolic blood pressure by 22%. Additionally, the treatment is effective in decreasing the activity of ACE I and ACE II by 81% and 76.5%, respectively. BSEs may also have a positive impact on renal function and increase plasma NOx levels in L-NAME-induced hypertensive rats. The histopathological study revealed that BSE has a protective effect against damage to heart, kidney, and liver tissue. Overall, the combination of BSE with captopril can decrease the activity of ACE, which provides a promising alternative for obtaining bioactive compounds. However, further mechanistic and clinical studies are needed to better understand the potential benefits of this treatment.

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CAPITULO VI. CONCLUSIONES

Este estudio ha demostrado que la germinación de la cebada Esmeralda y Perla, en particular durante 5 y 7 días, influye significativamente en el contenido de compuestos fenólicos, macronutrientes y en la actividad antioxidante.

La variedad con cascarilla (Esmeralda), mostró un incremento notable en el contenido de flavonoides y fenoles totales. Se identificaron la catequina y el ácido ferúlico como los compuestos más abundantes, teniendo una correlación positiva entre la actividad antioxidante y el contenido de compuestos fenólicos en los extractos de cebada germinada.

Los extractos de cebada, especialmente de la variedad Esmeralda, presentaron una fuerte inhibición de la ACE, lo que podría ser beneficioso para reducir la presión arterial. Además, se observó que la administración de extractos de cebada resultó en mejoras para la función renal y cardiovascular, incluida una reducción en la inflamación, hipertrofia cardíaca, necrosis del glomérulo renal y presencia de células Kupffer en el hígado.

Estos resultados sugieren que los extractos de cebada germinada tienen el potencial para disminuir los efectos adversos en diversos sistemas del organismo, lo que podría ser un preámbulo en el tratamiento coadyuvante al tratamiento farmacológico de enfermedades relacionadas con la oxidación, inflamación y función cardiovascular. Sin embargo, se necesitan más estudios clínicos para confirmar estos hallazgos y comprender mejor los mecanismos involucrados para el diseño de nuevas terapias que disminuyan el consumo de fármacos.

ANEXOS

ANEXO 1

Productos obtenidos Objetivo 1

Artículo de divulgación

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Compuestos bioactivos presentes en alimentos con actividad antihipertensiva y su efecto en COVID-19

Bioactive compounds present in foods with antihypertensive activity and their effect on COVID-19

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Resumen

Los alimentos de origen natural presentan compuestos bioactivos que pueden disminuir la incidencia de enfermedades crónicas, como la hipertensión. La presión arterial alta es una de las enfermedades cardiovasculares con mayor prevalencia, la cual está regulada por el Sistema Renina Angiotensina Aldosterona. La enzima convertidora de angiotensina 2 participa en la modulación de la presión arterial, la homeostasis de la presión arterial y es el principal receptor del virus SARS-CoV-2. Entre estos compuestos se encuentran los péptidos bioactivos y los compuestos fenólicos que han sido unos de los más estudiados. Los péptidos inferiores de 1 kDa y la presencia de aminoácidos hidrofóbicos son los mejores candidatos para inhibir la enzima convertidora de angiotensina (ECA). Por su parte, los compuestos fenólicos como los ácidos fenólicos y flavonoides son capaces de inhibir la ECA al reducir el estrés oxidativo implicado en la patogenia de la hipertensión. Este trabajo presenta una síntesis crítica sobre el efecto de los compuestos bioactivos sobre la ECA, la hipertensión y su relación con el COVID-19.

Palabras Clave: Péptidos, Fenólicos, Hipertensión, COVID-19, Enzima Convertidora de Angiotensina.

Abstract

Foods of natural origin have bioactive compounds that can decrease the incidence of chronic diseases, such as hypertension. High blood pressure is one of the most prevalent cardiovascular diseases, which is regulated by the Renin Angiotensin Aldosterone System. Angiotensin-converting enzyme 2 participates in the modulation of blood pressure, blood pressure homeostasis, and is the main receptor for the SARS-CoV-2 virus. Among these compounds are bioactive peptides and phenolic compounds that have been some of the most studied. Lower 1 kDa peptides and the presence of hydrophobic amino acids are the best candidates for inhibiting angiotensin converting enzyme (ACE). For their part, phenolic compounds such as phenolic acids and flavonoids are capable of inhibiting ACE by reducing oxidative stress implicated in the pathogenesis of hypertension. This work presents a critical synthesis on the effect of bioactive compounds on ACE, hypertension and its relationship with COVID-19.

Keywords: Peptides, Phenolics, Hypertension, COVID-19, Angiotensin Converting Enzyme.

1. Introducción

Los compuestos bioactivos son sustancias que presentan actividades biológicas. Se encuentran en diversas plantas y alimentos como verduras, frutas, cereales, frutos secos y aceites, estos ofrecen propiedades antiinflamatorias, antioxidantes, antidiabéticas y anticancerígenas, debido a su participación en la modulación de funciones enzimáticas como procesos de inhibición, inducción o recepción (Shrinet et al., 2021), mismos que pueden ser utilizados como coadyuvantes para el tratamiento de distintas enfermedades, como la hipertensión (Kris-Etherton et al., 2002).

La hipertensión ha sido una de las comorbilidades más importantes que contribuyen al desarrollo de enfermedades

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cardiovasculares. Recientemente, durante la pandemia causada por el coronavirus SARS-CoV-2, se han informado las comorbilidades más comunes en pacientes con COVID-19, de las cuales destacan la hipertensión (30%), diabetes (19%) y enfermedad coronaria (8%) (Huang et al., 2020). Hallazgos recientes mostraron un papel importante del Sistema Renina-Angiotensina-Aldosterona (RAAS) en pacientes hipertensos diagnosticados con COVID-19, debido a que SARS-CoV-2 utiliza la enzima convertidora de angiotensina 2 (ECA2) para unirse a la superficie de células epiteliales. Por lo que, controlar la producción de ECA2 podría mediar la entrada de SARS-CoV-2 en las células (Walls et al., 2020).

Entre los compuestos con actividad inhibitoria de la ECA más estudiados, se encuentran los péptidos bioactivos y los compuestos fenólicos aislados de diversas fuentes de alimentos, los cuales son producidos por acciones enzimáticas específicas (Ganguly et al., 2019). Los péptidos derivados de los alimentos presentan un alto potencial para el desarrollo de nutracéuticos y alimentos funcionales, debido a su especificidad y su peso molecular (Chai et al., 2017). Se ha descubierto que las proteínas hidrolizadas y péptidos bioactivos promueven la regulación del estrés oxidativo y disminuyen la aparición de enfermedades crónicas asociadas (Esfandi et al., 2019).

De igual manera, los compuestos fenólicos se han relacionado con actividades antioxidantes, anticancerígenas, antimicrobianas, antiinflamatorias y antivirales (Brglez Mojzer et al., 2016; Van Hung, 2016). Los esteroles y tocoles previenen enfermedades neurológicas y disminuyen los niveles de colesterol (Bartłomiej et al., 2012); los flavonoides son responsables de la moderación del cáncer y enfermedades coronarias del corazón (Gani et al., 2012); los lignanos presentan actividad antioxidante, antitumorales, antivirales y antibacterianos (Idehen et al., 2016); los folatos son capaces de realizar la misma actividad biológica que el ácido fólico, participando en muchas vías metabólicas (Romano et al.,1995).

El consumo de estos compuestos impacta positivamente en la función del organismo. En este trabajo se demuestra la importancia del consumo de compuestos bioactivos como péptidos y compuestos fenólicos, los cuales al presentar actividades antihipertensivas podrían impactar positivamente sobre pacientes con COVID-19.

2. Péptidos bioactivos

Los péptidos se definen como pequeños fragmentos aislados de proteínas, los cuales están conformados de 2 a 20 aminoácidos unidos por enlaces peptídicos, los cuales pueden proporcionar los nutrientes necesarios para el crecimiento y desarrollo humano, y también tienen características de actividad fisiológica únicas en relación con las proteínas. (Yang et al., 2021). Entre los péptidos bioactivos más estudiados se encuentran los aislados a partir de fermentación microbiana e hidrólisis enzimática. Se ha descrito una amplia variedad de funciones peptídicas como actividades antioxidantes, inmunomoduladoras y antihipertensivas, capaces de controlar diversas enfermedades asociadas. Los péptidos antioxidantes ejercen efectos citoprotectores a través de la eliminación de radicales libres, incluidas las especies reactivas de oxígeno (ROS) (Chen et al., 2019). Por otro lado, los péptidos inmunomoduladores pueden regular la función inmunológica del cuerpo evitando la aparición de enfermedades (Chalamaiah et al., 2014). Con respecto a los péptidos antihipertensivos, estos se destacan por la inhibición de la ECA, causada principalmente por la presencia de aminoácidos hidrofóbicos en la cadena C-terminal, los cuales se unen a los sitios activos de la ECA (Gangopadhayay et al., 2016).

Algunos estudios han demostrado la efectividad que presentan dipéptidos conformados por isoleucina y triptófano para disminuir la ECA, mostrando actividades antiinflamatorias y antioxidantes (Gu et al., 2019; Kaiser et al., 2016; Kopaliani 2016). Lunow et al., (2015) reportaron dipéptidos conformados por triptófano como IW (Ile-Trp) y VW (Val-Trp,), liberados selectivamente de alfa-lactoalbúmina bovina o lisozima de clara de huevo de gallina, actúan como inhibidores competitivos y selectivos para el C-terminal de ECA en plasma. Por otro lado, Lunow et al., (2013), mencionaron que IW es un dipéptido estable y con rápida absorción, lo que podría facilitar su unión con la ECA.

Guo et al., (2020) y Wu et al., (2014) reportaron péptidos <3 kDa extraídos de harina de germen de maíz con efecto en la regulación del equilibrio entre factores vasoconstrictores, resistencia vascular y reducción en los niveles de renina y angiotensina II controlando la presión arterial. Huang et al., (2011) evaluaron el efecto antihipertensivo de los péptidos menores a 1 kDa extraídos de maíz, en ratas espontáneamente hipertensas, reportaron que existen dos tipos de mecanismo de acción de los péptidos inhibidores de la ECA, los que compiten con la disponibilidad del sustrato de ECA y los que combinan la bioactividad de ECA para inhibir su actividad enzimática, normalmente conformados por más de cuatro aminoácidos y de dos a tres aminoácidos respectivamente. Los competidores de sustrato de ECA regularmente están compuestos por más de cuatro aminoácidos, mientras que los inhibidores del sitio activo de la ECA están conformados de dos a tres (Huang et al., 2011). Por lo tanto, el tamaño molecular del péptido inhibidor de la ECA juega un papel importante en su actividad inhibitoria ya que los péptidos menores a 3 kDa presentan una inhibición cuatro veces mayor a los de 5 kDa, sin embargo, hay otros factores como la secuenciación del péptido que influyen en la eficiencia del mismo.

Los efectos antihipertensivos producidos por péptidos están relacionados con una mejoría en la función endotelial que se logra inhibiendo los efectos de los vasoconstrictores como Ang II, induciendo vasodilatación a través de óxido nítrico y afectando vías de vasorrelajación involucradas, por lo tanto, el consumo de alimentos ricos en péptidos bioactivos puede participar en la modulación de funciones inmunes lo que coadyuva el padecimiento de COVID-19 (Baksi et al., 2009).

Los péptidos de origen alimentario pueden tener diversas bioactividades, incluida la actividad antiviral (Agarwal & Gabrani, 2020). Al respecto, los péptidos inhibidores que se utilizan en el tratamiento de diversas enfermedades, también podrían ser agentes potenciales contra COVID-19. En este sentido, los péptidos bioactivos con secuencias únicas de aminoácidos pueden mitigar la inhibición de serina y proteasas transmembrana de tipo II (TMPRSS2), un gen regulado por andrógenos, para el cebado de la proteína pico viral, la escisión de furina y miembros del sistema renina-angiotensina-aldosterona (RAAS). Según el análisis de estructura-función,

algunos péptidos bioactivos podrían presentar potencial para neutralizar el virus (Bhullar, Drews, & Wu, 2021).

Por lo tanto, los péptidos derivados de alimentos al presentar un efecto inhibitorio de la ECA así como actividades inhibidoras de múltiples objetivos contra la entrada de la célula huésped y la replicación viral del SARS-CoV-2, podrían ser utilizados para el diseño de un tratamiento coadyuvante alternativo para la mejora de pacientes hipertensos y en algunos casos pacientes con COVID-19. Los péptidos no son los únicos compuestos que poseen beneficios potenciales en la salud, compuestos bioactivos como los fenólicos, también presentan efectos importantes en la salud del consumidor.

3. Compuestos fenólicos

Los compuestos fenólicos son moléculas que presentan uno o más grupos hidroxilo unido a un anillo aromático (fenil), estos se pueden encontrar en diferentes órganos vegetales como, frutas, verduras, frutos secos, semillas, flores y cortezas (Peñarrieta et al., 2014). Estos se clasifican de acuerdo al número de anillos fenólicos y los elementos que los unen, por lo tanto, se clasifican en fenoles simples, ácidos fenólicos, flavonoides, xantonas, estilbenos y lignanos (Vuolo et al., 2019).

Los compuestos fenólicos se han convertido en una fuente de interés debido a sus propiedades antioxidantes y su capacidad de neutralizar radicales libres (Tsao, 2010). Diversos estudios epidemiológicos han demostrado los beneficios del consumo de compuestos fenólicos, que incluyen actividades inmonumoduladoras antiinflamatorias, antihipertensivas, antivirales y antioxidantes (Dykes et al., 2006; Talhaoui et al., 2016; Rho et al., 2020; Khan et al., 2018; Zhang et al., 2014)

La capacidad antioxidante de los compuestos fenólicos está regulada por eliminación de radicales libres, reduciendo la tasa de oxidación inhibiendo la formación o desactivando las especies activas y precursores de radicales libres (Tsao, 2010). Además de la captación de radicales, algunos polifenoles participan en la quelación de metales, evitando así la oxidación causada por radicales hidroxilos altamente reactivos (Kim et al., 2021). Este proceso antioxidante es la clave para la prevención de varias enfermedades, incluidos los trastornos asociados con la inflamación crónica. Los flavonoides y compuestos fenólicos pueden tener un efecto antiinflamatorio al regular la actividad celular en las células inflamatorias y al modular las actividades de las enzimas implicadas en el metabolismo de ácido araquidónico, lipoxigenasa, metabolismo de arginina y modulando otras moléculas proinflamatorias (Hussain et al., 2016), las cuales pueden disminuir el riesgo de padecer enfermedades crónicas como la hipertensión.

Los alimentos ricos en polifenoles, como es el caso de algunas herbáceas y frutos, pueden reducir la presión arterial elevada a través de la inhibición de la ECA (Santos et al., 2020). Algunos mecanismos utilizados por distintos compuestos fenólicos para la disminución de la presión arterial pueden estar relacionados con la inhibición competitiva de la ECA y efectos diuréticos, como las antocianinas de Jamaica (delfinidin-3-O-sambubiósido y cianidin-3-O-sambubiósido), las cuales ejercen estos mecanismos a través de procesos antagonistas de la aldosterona (Ojeda et al., 2010). Por otro lado, la quercetina un flavonoide presente en frutas y verduras, ha demostrado la capacidad de reducir la presión arterial en modelos animales con enfermedades cardiovasculares, atenúa la hipertrofia cardiaca y disminuye el engrosamiento medial aórtico, a través de la regulación de la expresión del canal de Na+ epitelial del riñón (Duarte et al., 2001; Jalili et al., 2006).

Se ha comprobado que los compuestos fenólicos presentan actividad antihipertensiva previniendo la expresión de nicotinamida adenina dinucleótido fosfato oxidasa (NADPH oxidasa) y producción de especies reactivas de oxígeno (ROS) (Yousefian et al., 2019), mientras que los flavonoides de maracuyá, atenúan el desarrollo de la hipertensión modulando la producción de óxido nítrico (NO) y están relacionados con la capacidad de unirse al ion zinc en el sitio activo de la ECA promoviendo efectos antihipertensivos (Zibadi et al., 2007). Algunos estudios han demostrado la eficacia de extractos de cereales ricos en fenoles que funcionan como inhibidores de enzimas implicadas en enfermedades metabólicas, así como su uso potencial en un tratamiento coadyuvante en pacientes con COVID-19 (Costamagna et al., 2016; Paraiso et al., 2020).

Los polifenoles tienen una amplia actividad antiviral contra diversos grupos de virus como el de la influenza A (H1N1), los de la hepatitis B y C (VHB/VHC), el del herpes simple 1 (VHS-1), el de la inmunodeficiencia humana (VIH), entre otros (Utomo et al., 2020) y recientemente en el virus que causa la enfermedad del COVID-19 (SARS-CoV-2) (Paraiso et al., 2020).)

El galato de epigalocatequina (EGCG) es una de las catequinas polifenólicas más abundantes que se encuentran en Camellia sinensis (planta del té), principalmente en el té verde. El EGCG ha sido probado para determinar su actividad antiviral contra varios virus y se encontró que es una opción de tratamiento potencial de origen natural frente a fármacos utilizados para el tratamiento de infecciones virales (Chacko et al., 2010). EGCG, presenta diversos mecanismos de acción en una gran variedad de virus como el de la influenza A, H1N1, H3N2 y B, VIH (virus de la inmunodeficiencia humana), calcivirus, VHC (virus de la hepatitis C) y dengue, entre otros (Liu et al., 2005; Song, Lee, & Seong, 2005)

Por otro lado, las teaflavinas (TF) del té negro, son otra clase de polifenoles conocidos por sus propiedades antitumorales, antivirales, antiinflamatorias, antioxidantes y antibacterianas. Se ha demostrado que los TF muestran actividad directa sobre partículas virales en infecciones como el VHC, ayudando a inhibir la unión con la superficie del receptor (Chowdhury et al., 2018). De igual manera, la luteolina y quercetina presentan mecanismos que promueven la inhibición de la entrada del virus SARS-CoV en células Vero E6 (Yi et al., 2004).

Ya que los compuestos fenólicos son de origen natural y la mayoría de la población los consume en diversos alimentos, el indagar en las propiedades que ofrecen a la salud, así como sus mecanismos, podría ser un avance en la búsqueda de un tratamiento que coadyuve al padecimiento de enfermedades prevalentes como la hipertensión y recientemente de COVID-19.

4. Hipertensión y COVID-19

La infección de COVID-19 causada por el virus del SARS-CoV-2 es una enfermedad infecciosa que ha provocado una crisis sanitaria en todo el mundo. La patogenia del SARS-CoV-2 se inicia mediante la unión de la proteína de pico viral

con el receptor diana de la ACE2, lo que facilita la internalización del virus dentro de las células huésped. Recientemente, se reportó que el SARS-Cov-2 es un virus cuyo tropismo se basa en el uso de la ACE2 para unirse a las células epiteliales del organismo (Wang & Cheng, 2020; Zhao et al., 2020). La ACE equilibra la presión arterial y convierte la angiotensina I en angiotensina II con función vasoconstrictora y al mismo tiempo facilita la degradación del vasodilatador, bradicinina. El control sobre estos procesos hormonales equilibra la salud de pacientes hipertensos, sin embargo, la combinación con otros padecimientos dificulta su control y en muchos casos puede empeorar la evolución de cada enfermedad. Por lo tanto, los informes iniciales sugieren que la hipertensión, la diabetes y las enfermedades cardiovasculares son las comorbilidades más frecuentes en la enfermedad COVID-19 (Bavishi et al., 2020).

La ACE2 puede cambiar el equilibrio del RAAS mediante la conversión de Ang II en Ang (1-7). Por lo tanto, la hipertensión y el COVID-19 se han convertido en una preocupación reciente sobre la susceptibilidad de los pacientes con hipertensión a contraer COVID-19, ya que aumenta la gravedad de la enfermedad y el consumo de fármacos como ACEi y ARBs (Devaux et al., 2020). Los inhibidores utilizados en el tratamiento contra la hipertensión aumentan la expresión de ACE2 en la superficie celular y pueden aumentar la expresión de ARN mensajero (ARNm) de ACE2 intestinal. Aunque faltan datos sobre los efectos de estos fármacos sobre la expresión del ARNm de ACE2 en las células epiteliales pulmonares, existe la preocupación de que los pacientes que toman estos tratamientos puedan favorecer la captura del virus (Furuhashi et al., 2015).

Una respuesta inmune óptima es la clave para mantener un control sobre enfermedades infecciosas y no infecciosas, un aumento de la ingesta de cereales integrales ricos en fibra se asocia a la disminución del marcador utilizado para predecir eventos cardiovasculares en pacientes con ateroesclerosis (PCR-hs); disminución de interleucina-6 (IL-6), producida en respuesta a infecciones y lesiones tisulares; y el factor de necrosis tumoral alfa (TNF-α), citoquina inflamatoria producida por macrófagos/monocitos durante la inflamación aguda, por lo tanto, los alimentos con fuente de ciertos péptidos y compuestos fenólicos pueden reducir el riesgo de padecer enfermedades mediadas por inflamación como enfermedades cardiovasculares, diabetes tipo II y obesidad (Herder et al., 2009; Gaskins et al., 2010; Gogebakan et al., 2011; Goletzke et al., 2014).

5. Conclusiones

Los compuestos bioactivos como los péptidos y los compuestos fenólicos se han destacado por su papel inmunomodulador, antioxidante, antiviral y antihipertensivo, contribuyendo en la prevención y el tratamiento de enfermedades crónicas incluyendo hipertensión y recientemente la enfermedad de COVID-19. No existe un modelo específico a seguir para mejorar el sistema inmunológico contra COVID-19. Sin embargo, un mayor consumo de alimentos variados y ricos en compuestos bioactivos podría mejorar el padecimiento de las infecciones virales y enfermedades inflamatorias. Por todo esto, es necesario estudiar puntualmente los mecanismos que intervienen y así relacionar con mayor certeza la capacidad de los inhibidores de la ECA en el control de la expresión de enzimas que podrían disminuir la unión de SARS-Cov-2 en los receptores diana del organismo.

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Congreso 1

UAEH | ICBI

Otorgan el presente

RECONOCIMIENTO

a

Abigail García Castro

Por la presentación del trabajo "**Compuestos bioactivos presentes en cereales con actividad antihipertensiva y su efecto en COVID-19**", cuya autoría se comparte con: *A. D. Román Gutiérrez, A. Castañeda Ovando, F. A. Guzmán Ortiz y R. Cariño Cortés*, misma que se efectuó de manera virtual, durante el **V Seminario Regional de Materiales Avanzados**, organizado por el Cuerpo Académico de Materiales Avanzados perteneciente al Área Académica de Ciencias de la Tierra y Materiales del 6 al 8 de octubre de 2021.

Mineral de la Reforma, Hgo., 7 de octubre de 2021.

Atentamente

"Amor, Orden y Progreso"

Dr. Otilio Arturo Acevedo Sandoval
Director del ICBI

Dr. Ventura Rodríguez Lugo
Líder del Cuerpo Académico de Materiales Avanzados

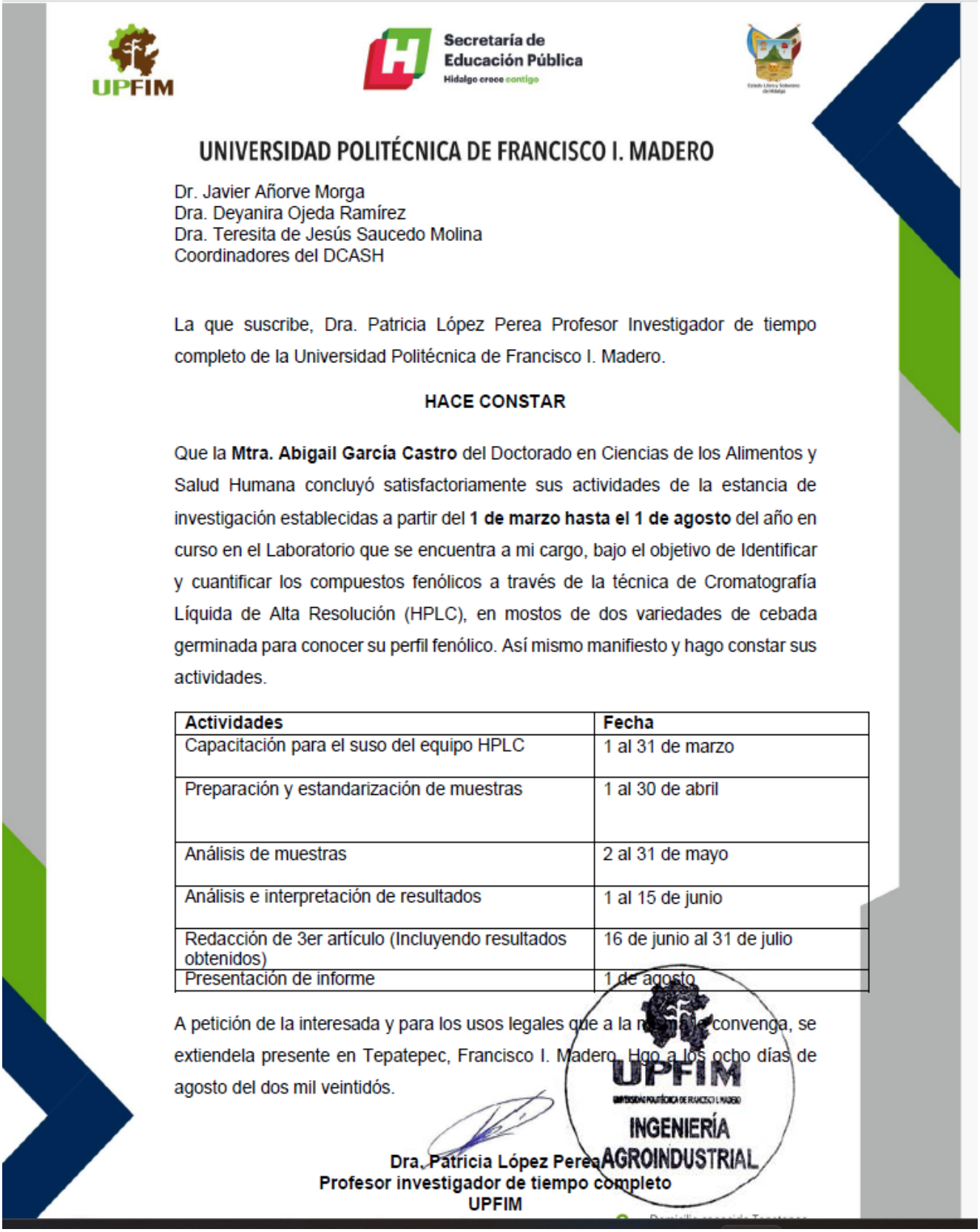
Dr. Félix Sánchez De Jesús
Jefe de AACTyM

ICBI Número de control: ICBI-AACTyM/2055

ANEXO 2

Productos obtenidos Objetivo 2

Estancia de investigación 1



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Secretaría de Educación Pública
Hidalgo crece contigo

UNIVERSIDAD POLITÉCNICA DE FRANCISCO I. MADERO

Dr. Javier Añorve Morga
Dra. Deyanira Ojeda Ramírez
Dra. Teresita de Jesús Saucedo Molina
Coordinadores del DCASH

La que suscribe, Dra. Patricia López Perea Profesor Investigador de tiempo completo de la Universidad Politécnica de Francisco I. Madero.

HACE CONSTAR

Que la **Mtra. Abigail García Castro** del Doctorado en Ciencias de los Alimentos y Salud Humana concluyó satisfactoriamente sus actividades de la estancia de investigación establecidas a partir del **1 de marzo hasta el 1 de agosto** del año en curso en el Laboratorio que se encuentra a mi cargo, bajo el objetivo de Identificar y cuantificar los compuestos fenólicos a través de la técnica de Cromatografía Líquida de Alta Resolución (HPLC), en mostos de dos variedades de cebada germinada para conocer su perfil fenólico. Así mismo manifiesto y hago constar sus actividades.

Actividades	Fecha
Capacitación para el suso del equipo HPLC	1 al 31 de marzo
Preparación y estandarización de muestras	1 al 30 de abril
Análisis de muestras	2 al 31 de mayo
Análisis e interpretación de resultados	1 al 15 de junio
Redacción de 3er artículo (Incluyendo resultados obtenidos)	16 de junio al 31 de julio
Presentación de informe	1 de agosto

A petición de la interesada y para los usos legales que a la misma convenga, se extiendela presente en Tepatepec, Francisco I. Madero, Hgo a los ocho días de agosto del dos mil veintidós.

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Dra. Patricia López Perea
Profesor investigador de tiempo completo
UPFIM

ANEXO 3

Productos Objetivo 3

Congreso Internacional I

CONAHCYT | Centro de Investigación en Alimentación y Desarrollo CIAD | Tecnológico Nacional de México | CIATEJ

La Red de Alimentos Funcionales y Nutracéuticos

otorga el presente

RECONOCIMIENTO

a:

García-Castro, A., Román-Gutiérrez, A.D., Guzmán-Ortiz, F.A., Pardo-Santos O.

Por su valiosa participación en la **presentación oral** del trabajo titulado "Caracterización de extractos de germinados de dos variedades de cebada"

Nuevo Vallarta, Nayarit; 12 al 14 de noviembre de 2023

Gustavo A. González Aguilar
Líder de la Red Alfanutra

Aarón F. González Córdova
Coordinador de la Red Alfanutra

Janet Alejandra Gutiérrez Uribe
Comité técnico académico de la Red Alfanutra

Sonia Guadalupe Sáyago Ayerdi
Comité organizador del 6° Congreso

Adrián Hernández Mendoza
Comité científico del 6° Congreso

CYTED | BiQuesArte | CIAT | Tecnológico de Monterrey | UACJ | Universidad Autónoma de Nayarit

ALIMENTOS FUNCIONALES Y NUTRACÉUTICOS

6to Congreso Internacional de Alimentos Funcionales y Nutracéuticos

Sostenibilidad como eje estratégico para el desarrollo de alimentos funcionales y nutracéuticos

Congreso Internacional II

ANEXO 4

Productos Objetivo 4 y 5

Estancia de investigación 2

DIF HIDALGO

HNDIF-D.-CEI. OF. NO. 2154/VI/2023

Pachuca de Soto, Hgo., a 07 de junio de 2023.

DR. JAVIER AÑORVE MORGA
COORDINADOR DEL DCASH
ICBI-ICAP-ICSA UAEH

Sirva la presente para enviarle un afectuoso saludo y en respuesta a su similar de fecha 2 de junio de 2023, y derivado del proceso de la Revisión del **Protocolo para el desarrollo de técnicas Histológicas como herramientas en la Investigación,** que realizará la estudiante **Abigail García Castro**, quien cursa en el programa de **Doctorado en Ciencias de los Alimentos y Salud Humana de la Universidad Autónoma del Estado de Hidalgo,** hago de su conocimiento que es te Hospital, autoriza a la **C. García Castro,** realizar una estancia de Investigación en el **Servicio de Patología** bajo la Supervisión del **Biólogo Álvaro Rubén Hernández Cruz**, cuya duración es de 2 meses a partir de **5 de junio al 4 de agosto de 2023**.

Sin más por el momento, reciba un saludo afectuoso.

ATENTAMENTE

DR. RUBÉN GENARO HURTADO DELO ÁNGEL
DIRECTOR DEL HOSPITAL DEL NIÑO DIF HIDALGO

C.c.p. Expediente

RGHDA/RGG/AJHG/JRHM/kgp

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