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Effect of microwave cooking and solid-state fermentation on nutritional, antioxidant, and antiparasitic properties of blue maize

Efecto de la cocción por microondas y fermentación en estado sólido en propiedades nutricionales, antioxidantes y antiparasitarias de maíz azul

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ABSTRACT

Parasitic infections are a public health issue in Mexico. It has recently been reported that native maize has antimicrobial properties. Microwave cooking reduces food preparation time without affecting its nutritional and sensory quality. Several authors have reported that solid-state fermentation (SSF) improves fermented grains' nutritional and nutraceutical properties. This work aimed to evaluate the effect of microwave cooking and SSF on phenolic compounds and antioxidant and antiparasitic properties of blue maize. Maize grains were cooked in a microwave and then fermented with *Rhizopus oligosporus* for 108 h. Free and bound extracts of raw, microwave-cooked, and fermented grains were obtained to evaluate their phenolic content antioxidant and antiparasitic potentials against *Giardia duodenalis*. SSF increased protein, total phenolic content, and antioxidant activity by 31 %, 28 %, and 19 %, respectively. About 70 % of parasitic inhibition was found at 50 mg/mL polyphenols for all three samples. Microwave cooking and fermentation of blue maize show beneficial potential for health, increasing its bioactive components and allowing the inhibition of the *Giardia duodenalis* parasite.

KEY WORDS: Blue maize, Microwave, Fermentation, Tempeh, Antiparasitic, Antioxidant.

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RESUMEN

Las parasitosis son consideradas como un problema de salud pública en México. Recientemente se ha reportado que el maíz criollo tiene propiedades antimicrobianas. La cocción por microondas reduce el tiempo de preparación de los alimentos sin afectar su calidad nutricional y sensorial. Diversos autores han informado que la fermentación en estado sólido (SSF) mejora propiedades nutricionales y nutracéuticas de los granos fermentados. El objetivo del presente trabajo fue evaluar el efecto de la cocción por microondas y de la SSF sobre compuestos fenólicos, propiedades antioxidantes y antiparasitarias de maíz azul. El maíz fue cocido por microondas y posteriormente fermentado con *Rhizopus oligosporus* durante 108 h. Se obtuvieron los extractos libres y ligados del grano crudo, cocido por microondas y del grano fermentado, para evaluar su contenido fenólico, potencial antioxidante y antiparasitario contra Giardia duodenalis. La SSF incrementó 31 %, 28 % y 19 % el contenido de proteínas, fenólicos totales, y actividad antioxidante, respectivamente. La inhibición del 70 % del parásito se encontró a 50 mg/mL de polifenoles para las tres muestras. La cocción por microondas y la fermentación de maíz azul presentan un potencial benéfico a la salud, incrementando sus compuestos bioactivos y permitiendo la inhibición del parásito *Giardia duodenalis*.

PALABRAS CLAVE: Maíz azul, Microondas, Fermentación, Tempeh, Antiparasitario, Antioxidante.

Introduction

Gastrointestinal diseases are a major public health issue in Mexico. These diseases are transmitted through the fecal-oral route by consuming contaminated food and water (Zavala *et al*., 2020). Giardiasis is a disease caused by *Giardia duodenalis* (also known as *G. lamblia* and *G. intestinalis*), a ubiquitous enteropathogenic protozoan that causes acute diarrheal disease and gastroenteritis in humans, especially in children (Vargas *et al*., 2018). Due to the adverse effects of antiparasitic drugs, the ingestion of phytochemicals with similar impacts to metronidazole has been proposed, including some flavonoids such as chalcones and polyphenols, which showed *in vitro* antiparasitic activity against *Giardia lamblia* (Montes-Ávila *et al*., 2009; Anthony *et al*., 2011). Blue maize has high contents of anthocyanins and polyphenols, so it can be considered a functional food of great value for combating chronic diseases and intestinal parasitosis (Mora-Rochín *et al*., 2016; Chen *et al*., 2017; Domínguez-Hernández *et al*., 2022).

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Some processing methods increase the phenolic content of cereals and legumes; among these are solid-state fermentation (SSF) and microwave cooking (Sánchez-Magaña *et al*., 2019; Teoh *et al*., 2024). SSF is defined as the growth of microorganisms on the surface of a moist, porous solid substratum. The process occurs between the particles in the absence or near absence of surface water. This humidity is sufficient to maintain microbial growth and metabolism, especially the development of filamentous fungi, due to their peculiar capacity to colonize the spaces between the particles of solid matrices. Tempeh is a grayish-white cake that ferments cooked soybeans in a solid-state using *Rhizopus* spp. fungi. This fermented food originated in Indonesia and has been consumed as a low-cost source of protein for more than 300 years. The general elaboration process consists of soaking, removing water, dehulling, cooking in acetic acid, removing cooking liquid, inoculation, packaging, and incubation. Due to its meat-like consistency, high protein content, and vitamin B12 supply, tempeh has gained acceptance in the Western vegan diet as meat for burgers, sausages, nuggets, and other food preparations. In addition, other legumes and cereals have been used to produce this fermented product (Ahnan-Winarno *et al*., 2021; Teoh *et al*., 2024). Sánchez-Magaña *et al*. (2019) and Ramírez-Esparza *et al*. (2024) performed SSF on maize grains using *R. oligosporus* and *R. oryzae*, respectively. Both authors reported that this process significantly increased the content of phenolic compounds.

Another technology that has gained interest is microwave heat treatment. One of its main advantages is that it favors the release of phenolics bound to cell wall materials (Deng *et al*., 2022). Microwave energy can easily penetrate the wet food matrix, promoting the dissolution of polyphenols in the cell tissue. For heat-sensitive polyphenols such as proanthocyanidins, however, a higher temperature accelerates their decomposition and destruction (Hu *et al*., 2021).

SSF was modified by replacing the traditional cooking stage with a microwave heat treatment, allowing the cooking liquid to evaporate and preventing grain component leaching. These changes were made to improve the release and retention of bioactive compounds with potential nutraceutical benefits. This scientific study aimed to assess the effects of SSF applied to microwave-cooked blue maize and the individual impact of this cooking method on the levels of phenolic compounds and the processed grain's nutritional, antioxidant, and antiparasitic properties.

Materials and Methods

Materials

The creole blue maize (*Zea mays* L.) of the Elotero de Sinaloa race was used. After the harvest, the grains were cleaned and stored in refrigeration (4 °C) until use. The ATCC strain 25922 of *G. duodenalis* was used to evaluate the antiparasitic activity, and the microorganism *R. oligosporus* NRRL2710 was used to ferment the maize grain.

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Methods

Chemical composition

AOAC (2000) methods 925.10, 920.39, and 934.01 were used to determine corn samples' moisture, lipid, and ash, respectively. In addition, the total nitrogen micro-Kjeldahl method was used to analyze the crude protein, applying a factor of 6.25. Finally, the percentage of carbohydrates was calculated by a difference of 100, considering the percentages of moisture, crude protein, lipids, and ashes. All tests were performed in triplicate, and the mean of the results were reported.

Microwave cooking

With slight modifications, the conditions reported by Martínez-Bustos *et al*. (2000) were used to obtain microwave-processed blue maize flour. The maize grain was mixed with 0.1 % acetic acid (1:3 ratio). The mixture was then stirred for 2 min and placed inside a commercial domestic microwave oven (voltage/frequency 120 V / 60 Hz, 1350 W, 2450 MHz frequency) for 30 min at medium power (level 5). Every 10 min, the contents were manually mixed to ensure uniform heating. The cooking liquid was almost completely evaporated at the end of this treatment. This grain was cooled and used to produce fermented blue maize, and another batch was brought to dryness (50 °C/16 h) and then milled to 80 mesh. The flour obtained was sealed in a polyethylene bag and stored (-20 °C) until analysis.

Solid-state fermentation of blue maize

For fermentation, the fungus *R. oligosporus* was propagated on potato dextrose agar in slant tubes at 35 °C until the culture reached adequate sporulation. Spores were harvested using sterile distilled water to create a suspension, which was then adjusted to a concentration of 1 x 10^6 spores/mL. This suspension was used to inoculate microwaved maize kernels at a ratio of 3 mL of suspension per 100 g of the initial dry substrate. After inoculation, the kernels were placed in polyethylene bags measuring 15 x 25 cm, with perforations made every 4 cm. Solid-state fermentation (SSF) conditions were established following the methodology of Sánchez-Magaña *et al*. (2019), with a fermentation temperature of 35 °C and a fermentation time of 108 h.

Production of raw and processed blue maize flour

Raw, microwaved, and fermented maize kernels were subjected to freeze-drying (UD Cyclone Sample Mill, UD Corp, Boulder, CO, USA) and milled to pass through 80 mesh (0.180 mm). The flours obtained were stored in hermetically sealed plastic bags at -20 °C until use.

Extraction of free and bound phenolics

The extraction of free and bound phenolics was performed, as reported by Gámez-Valdez *et al*. (2021), with some modifications. The starting point was 0.050 g flour + 1 mL of 80 % (v/v)

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ethanol. This suspension was vortexed for 10 min and centrifuged at 3000 x g/ 10 min. The supernatant was placed in a new microtube and concentrated at 35 °C at low pressures (Speed Vac Concentrator, Thermo Electron Corporation) to dryness. The compounds were reconstituted with 200 µL of 50 % methanol to obtain the free phenolics extract, which was stored at -20 °C until further use. The bound phenolics were obtained from the precipitate of the free extract, to which 1 mL of 2 M NaOH was added, and which was subjected to heat treatment for 30 min at 95 °C and subsequently stirred for one hour at room temperature. The mixture was neutralized with 200 µL of concentrated HCl, vortexed for 2 min, and 500 µL of hexane was added to remove lipids. The resulting mixture was washed four times with 500 µL ethyl acetate each, vortexed for 10 min, and centrifuged at 3000 x g/ 10 min. The ethyl acetate fraction was evaporated to dryness in a concentrator using low pressures (Speed Vac Concentrator, Thermo Electron Concentrator). The extracted compounds were reconstituted with 200 µL of 50 % methanol and stored at -20 °C for later use. Extractions were performed in quadruplicate.

Determination of total phenolic compounds

The Folin-Ciocalteu colorimetric method described by Gámez-Valdez *et al*. (2021) was used to determine the concentration of total phenolics in the fractions. In a 96-cell plate, 20 µL of a standard solution of gallic acid was added. In the following cells, 20 µL of the free and bound phenolic extracts were added. The standard and samples were mixed with 180 µL of Folin's reagent. The reaction was neutralized with 50 µL of 7 % Na_2CO_3 and then incubated in the spectrum. After 90 min, the absorbance was recorded at 750 nm in a microplate reader (Synergy HT, Biotek Instrument), using methanol as a blank. A calibration curve was constructed with gallic acid. The results were expressed as mg gallic acid equivalents (GAE)/100 g dry sample. The content of total phenolic compounds was calculated by adding the fractions of phenolics present in the free and bound extracts. The determination was made in quadruplicate.

Determination of total anthocyanins

Total anthocyanins were determined using a method outlined by Abdel-Aal & Hucl (1999) and Mora-Rochín *et al*. (2016), with minor modifications. We began by weighing 0.05 g of the sample and added 1 mL of acidified cold methanol (composed of 95 % methanol and 1 N HCl in a ratio of 85:15, v/v). The mixture was then centrifuged at 3000 x g for 10 min, after which the supernatant was collected. The absorbance of the samples was recorded immediately at wavelengths of 535 nm and 700 nm (for turbidity correction) using a microplate reader (Synergy HT, Biotek Instruments). The anthocyanin content was calculated using the following equation:

$$C = \left[\left(\frac{A_{535\,nm} - A_{700\,nm}}{\varepsilon}\right) x(total\ volume\ extract\ ) x\ MW\right] /(sample\ weight)$$

where: C is the concentration (mg cyanidin 3-glucoside equivalents g^{-1} sample), A is absorbance reading, ε is molar absorptivity of cyanidin 3-glucoside (25965 cm^{-1} M^{-1}), MW is the molecular weight of cyaniding 3-glucoside (449.2 g mol^{-1}).

Determination of antioxidant capacity

Antioxidant activity by ABTS

The antioxidant activity of free and bound extracts was determined by free radical scavenging, which was estimated in terms of the free radical scavenging activity of the ABTS radical cation decolorization assay proposed by Gámez-Valdez *et al*. (2021), which is based on the reduction of the ABTS-+ radical by the antioxidants present in the extracts evaluated. The radical cation ABTS (ABTS-+) at a concentration of 7 mM was generated by reacting ABTS with 2.45 mM potassium persulfate for 16 h before use. The radical was diluted in PBS to an absorbance of 0.7 ± 0.02 at 765 nm. The blank and extracts (20 µL) were read and transferred to a plastic cuvette; the assay was started by adding 1980 µL of ABTS-+ solution. The absorbance was recorded at 735 nm, 15 min after initial mixing; the absorbance loss of ABTS-+ relative to a blank was calculated. A calibration curve was created using Trolox, and the data were expressed in µmol Trolox equivalent (TE) per 100 grams of sample on a dry weight basis (µmol TE/100 g, dw).

Antioxidant activity by ORAC

The antioxidant capacity was also determined using the oxygen radical absorbance capacity (ORAC) method developed for hydrophilic compounds (Gámez-Valdez *et al*., 2021). Thermal degradation of the compound 2-2'-Azobis-aminopropane (AAPH) produces peroxyl radicals (ROO-), which oxidize the fluorescent compound dichlorofluorescein (DCFH) to the non-fluorescent compound dichlorofluorescein (DCF). The degree of inhibition of antioxidants (phytochemicals) traps free radicals. The free radical generating solution (2-2'-Azobis-aminopropane) AAPH was prepared by adding 0.207 g of the reagent and volumetrized in a 5 mL flask. The fluorescein was prepared at a concentration of 0.1 µM. Then, 150 µL of the fluorescein was added to 25 µL of the sample and mixed by stirring at 1200 rpm for 20 s. The reaction starts when 25 µL of AAPH is added. Fluorescence was recorded at 485 nm excitation and 583 nm emission. The effect of an antioxidant is integrated by the net area under the curve on the loss of fluorescence (AUC) (AUCAOX - AUCno AOX). The results were expressed as µmol Trolox equivalent per 100 g dry weight basis (µmol TE/100g, dw).

Determination of antiparasitic activity

Samples were extracted with ethanol (80 %) by sonication (10 min) and shaking at room temperature (5 h, 27 °C). The supernatant was recovered by centrifugation (10 min at 10,000 rpm and 10 °C). The sample was subjected to a second wash with ethanol and agitation (3 h, 27 °C). Recovery of the supernatant was brought to dryness and resuspended in sterile deionized water. The solvent was removed in a rotary evaporator; the residue was lyophilized and stored at -20 °C for later use. The antiparasitic activity was determined according to the method described by Montes-Ávila *et al*. (2009) with some modifications. The extracts were resuspended in sterile deionized water to contact *G. duodenalis* trophozoites, which were cultured in TYI-S-33 medium and 10 % fetal bovine serum, one g/L bile, pH 7.0, and 50 µg/mL gentamicin. Trophozoites in a

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mid-log phase were resuspended in the medium after cooling on ice for 20 min and counted in a hemocytometer. The count determined mortality by cell staining (trypan blue) and the absence of motility. These cells were used as inoculum for the antigiardial evaluation of the compounds. In the test procedure, 24-well cell culture plates were used. Trophozoites were incubated (300 µL of inoculum, $1.5x10^5$ cells) (24 h/ 37 °C) with 1.2 mL of the extracts to obtain a final volume of 1.5 mL. Concentrations of 50, 75, 100, 150, and 180 mg/mL of soluble polyphenols from the samples studied were used for the assay. Control experiments were performed under similar conditions without extracts: positive with metronidazole (1, 2, 3, 4, and 5 µg/mL) and negative with the extraction solvent. After 24 h of incubation, the plates were cooled on ice (20 min).

Statistical analysis

The results were analyzed using a one-factor ANOVA with the Minitab 16 statistical package. The Tukey test was applied for mean comparisons, maintaining a significance level of 95 % ($p \leq 0.05$).

Results

Chemical composition

The results of the chemical composition of blue maize kernels (Table 1) found in this research are similar to those reported by Montoya-Rodríguez *et al*. (2020), who obtained average values of 8.9, 5.0, 1.4 and 84.6 % in blue maize kernels for protein, lipids, ash, and carbohydrates, respectively. Microwave cooking of blue maize kernels had no significant effect on the chemical composition of raw kernels (Table 1). These observations agree with those reported by other investigations (Khatoon & Prakash, 2004; Alajaji & El-Adawy, 2006) that applied microwave cooking to different legumes without affecting their proximate chemical composition. Incidentally, solid-state fermentation (SSF) of blue maize grain caused a significant ($p \leq 0.05$) increase of 31 % in protein content (12.22 % *vs.* 9.32 %), 33 % in lipids (5.05 *vs.* 3.8 %), and 23 % in ash (1.74 *vs.* 1.41 %) and a slight decrease ($p \leq 0.05$) in carbohydrate (< 5 %, 80.99 *vs.* 85.47 %), concerning raw and microwaved. Protein increase by SSF action has been reported previously (Mora-Uzeta *et al*., 2019; Sánchez-Magaña *et al*., 2019). This increase is attributed to the growth of the fungal biomass and the decrease of other components that could be lost by leaching during the initial steps (soaking, cooking) of the SSF or could be consumed by the fungus for growth (Mora-Uzeta *et al*., 2019; Sánchez-Magaña *et al*., 2019). Unlike what was reported by Reyes-Bastidas *et al*. (2010) in common beans where SSF caused a decrease in ash content, in this study, an increase of 23 % was observed, which could be attributed to the fact that the blue maize was fermented without removing the soaking water and microwave cooking water, allowing the retention of these nutrients from the raw and cooked grain. Incidentally, increased lipid content during SSF has also been reported in rice bran fermented with *R. oryzae* (Dos Santos-Oliveira *et al*., 2011) and *Canavalia* fermented with *R. oligosporus* (Niveditha *et al*., 2012). The increase in total lipids by fungal fermentation has been attributed to the dissociation of lipoprotein complexes and the synthesis of endogenous lipids during the growth of the microorganism on the substratum

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or to the possibility that the fermenting fungi transform the carbohydrate content of the seeds into lipids (Dos Santos-Oliveira *et al*., 2011; Niveditha *et al*., 2012).

Table 1. Chemical composition of raw and processed blue maize

COMPONENT (g/100g)	BLUE MAIZE [1]		
	Raw	Microwave cooked	Fermented
Proteins	9.32 ± 0.35[b]	9.43 ± 0.38[b]	12.22 ± 0.36[a]
Lipids	3.8 ± 0.05[b]	3.8 ± 0.2[b]	5.05 ± 0.17[a]
Ash	1.41 ± 0.03[b]	1.53 ± 0.05[b]	1.74 ± 0.09[a]
Carbohydrates	85.47 ± 0.42[a]	85.24 ± 0.6[a]	80.99 ± 0.42[b]

[1] Mean ± standard deviation (n=3). [a-b]Values between columns with letters in common show no statistical differences (Tukey, $p \leq 0.05$).

Phytochemical content of raw and processed blue maize

Total anthocyanins

Total anthocyanin content was expressed as milligram cyanidin 3-glucoside equivalents dryly (mg CGE/100 g, dw) in raw, cooked, and fermented blue maize kernels (Table 2). The maize grain used in this study showed a total anthocyanin content that is within the range reported by Mora-Rochín *et al*. (2016), who conducted a study with 15 genotypes of blue maize in the state of Sinaloa and reported values ranging from 14.1 to 34.3 mg CGE/100 g, dw. Blue maize subjected to microwave cooking and solid-state fermentation showed 52 and 57 % decrease in total anthocyanins concerning the unprocessed kernel, respectively (Table 2). Several authors have reported that heat treatments for long periods and the increase in pH due to the use of lime affect the total anthocyanin content in blue maize kernels, causing decreases more significant than 50 % concerning unprocessed blue maize kernels (Sánchez-Madrigal *et al*., 2015; Mora-Rochín *et al*., 2016; Parra-Aguilar, 2018). The reduction in anthocyanin content observed in this study was lower than the more than 60 % decrease reported by Parra-Aguilar (2018) during the microwave nixtamalization of blue maize. This discrepancy can be attributed to Parra-Aguilar's use of lime in the cooking process, which was not included in the microwave cooking method used in this study. Additionally, prolonged heat treatment can cause anthocyanins in blue maize to form complexes and precipitate with denatured proteins after cooking at 100 °C, ultimately decreasing total anthocyanins (Jing & Giusti, 2007). On the other hand, it has been reported that increasing the fermentation time of legumes of *Cajanus* spp. with *Rhizopus* spp. leads to a decrease in the anthocyanin content of the beans (Mushollaeni & Tantalu, 2020). Anthocyanins are highly sensitive

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to variations in temperature and pH. During fermentation, both the substrate temperature and pH levels rise due to the metabolic activity of the fungus, which may be linked to the reduction of these compounds (Mushollaeni & Tantalu, 2020; Ahnan-Winarno *et al*., 2021). Additionally, it has been observed that anthocyanins can undergo hydrolysis to form anthocyanidins during fermentation, which may then polymerize into other types of polyphenols (Mushollaeni & Tantalu, 2020).

Phenolic compounds

The total phenolic content was calculated as the sum of free and bound phenolics, expressed as milligrams of gallic acid equivalents per 100 g of dry weight (mg GAE/100 g, dw) (Table 2). In this study, the free phenolic content was higher than that reported by Gaxiola-Cuevas *et al*. (2017) for creole blue maize from the Elotero de Sinaloa race (41.2 mg GAE/100 g, dw), but lower than the bound phenolic content reported by these authors (202.8 mg GAE/100 g, bs). However, the free phenolic fraction was similar to the findings of Aguayo-Rojas *et al*. (2012), which reported 113.2 mg GAE/100 g, dw. The bound phenolic fraction accounted for the largest portion of the total phenolic content in raw (60 %), microwave-processed (60 %), and fermented (54 %) maize kernels (Table 2). This finding is consistent with reports from other researchers studying various types of raw and processed maize, where bound phytochemicals formed the majority fraction (Aguayo-Rojas *et al*., 2012; Gaxiola-Cuevas *et al*., 2017; Sánchez-Magaña *et al*., 2019).

Microwave cooking did not significantly change the levels of free, bound, or total phenolics in comparison to raw blue maize kernels. These findings suggest that microwave cooking is more efficient than traditional cooking methods. For instance, Sánchez-Magaña *et al*. (2019) found that traditional cooking prior to fermentation decreased the content of free phenolics by 49 % compared to raw grains. Additionally, Liazid *et al*. (2007) investigated the impact of microwave-assisted extraction on the stability of 22 phenolic compounds, concluding that maintaining a temperature of 100 °C for 20 min is effective without causing degradation of these compounds.

Table 2. Phytochemical content of raw and processed blue maize

	BLUE MAIZE[1]		
PHYTOCHEMICALS	**Raw**	**Microwave cooked**	**Fermented**
Total anthocyanins[2]	25.7 ± 1.5[a]	12.4 ± 1.3[b]	11.1 ± 0.1[b]
Phenolics[3]			
Free	73.5 ± 5.4[b]	70.9 ± 1.4[b]	110.0 ± 4.2[a]
Bound	111.8 ± 6.7[b]	104.5 ± 7.6[b]	127.2 ± 10.1[a]
Total	185.3 ± 5.6[b]	175.4 ± 6.6[b]	237.3 ± 5.1[a]

[1] Mean ± standard deviation (n=3); [2] mg cyanidin-3-glucoside equivalents (CGE)/ 100 g sample on dry weight basis (dw); [3] mg gallic acid equivalents (mg GAE) / 100 g sample on dry weight basis (dw); [a-b] Values within columns without letters in common are statistically different (Tukey, $p < 0.05$).

The SSF treatment of blue maize grain significantly increased ($p \leq 0.05$) the total phenolic content by 28 % compared to the unprocessed grain, bringing the values to 237.3 mg GAE/100 g from 185.3 mg GAE/100 g. Additionally, increases of 49.65 % and 14 % were observed in the free and bound phenolic fractions, respectively (Table 2). This bioprocess also increased ($p \leq 0.05$) the free and bound phenolic content by 55 and 22 %, respectively, regarding the microwave-cooked grain. The aforementioned trends were similar to those reported by Sánchez-Magaña *et al*. (2019), who studied the influence of solid-state fermentation on the phenolic acid profile of commercial white maize at different fermentation times. Biotransformation of the grain by solid-state fermentation facilitates access to the substratum, causing an increased release of free-conjugated phenolic acids bound to cell wall materials in the maize kernel (Sánchez-Magaña *et al*., 2019). The increase in phenolic compounds could be linked to the prior stage of microwave cooking. This technology is known to affect complex cell wall materials, such as lignin, which enhances the availability of phenolic acids during the extraction process. Additionally, solid-state fermentation is a complex biochemical process that produces various enzymes, including α-amylase, xylanase, β-glucosidase, and esterases. These enzymes have been associated with the release of water-soluble phenolic compounds and improved bioavailability of phenolic compounds bound in an insoluble form. In addition to the enzymatic release of phenolic compounds, other unidentified biochemical pathways may also play a role in increasing phenolic content during the SSF process (Mora-Uzeta *et al*., 2019; Sánchez-Magaña *et al*., 2019).

Antioxidant activity of raw and processed blue maize

Antioxidants react with free radicals through different mechanisms, primarily hydrogen atom transfer (HAT), single electron transfer (SET), or a combination of both mechanisms. Consequently, it is essential that, for antioxidant property studies, at least three evaluation methods are selected: one to evaluate exclusively HAT, another SET, and a combined method, HAT/SET. The ABTS method can act through both mechanisms, and the oxygen radical absorbance capacity (ORAC) assay involves hydrogen atom transfer (Shukla & Sathyanarayana, 2021). In this research, the ABTS and ORAC methods were used to study the antioxidant properties of the analyzed samples. The results were expressed in μmol Trolox equivalent/100 g of sample on a dry weight basis (μM TE/100 g, dw) (Table 3).

The fraction of bound phenolics in raw and microwaved blue maize exhibited the highest antioxidant activity as measured by the ABTS and ORAC assays. In contrast, the free fraction of fermented blue maize was the primary contributor to the ORAC antioxidant activity, while the bound fraction was the major contributor to the total activity measured by the ABTS assay (Table 3). In this study, the total antioxidant activity of raw blue maize, evaluated using the ABTS and ORAC methods, was found to be higher than previously reported by Gaxiola-Cuevas *et al*. (2017), with values of 7,661.1 and 10,878.6 μmol TE/100 g, respectively. Additionally, the total antioxidant activity results from this research surpassed those reported by Sánchez-Magaña *et al*. (2019) for cooked and fermented white maize. For the ABTS assay, their reported values were 3,904 and 10,041 μmol TE/100 g, compared to our findings of 28,551 and 29,614 μmol TE/100 g. Similarly, for the ORAC assay, their values were 12,058 and 20,422 μmol TE/100 g, while our results were 19,692 and 30,630 μmol TE/100 g. These differences could be attributed to

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factors such as the chemical structure of the compounds in the samples, the nature of the solvent used, temperature, pH, as well as the reactivity and chemical structure of the free radicals involved (Shukla & Sathyanarayana, 2021).

Table 3. Antioxidant activity of raw and processed blue maize

ANTIOXIDANT ACTIVITY[2]	BLUE MAIZE[1]		
	Raw	Microwave cooked	Fermented
ABTS			
Free	10,243.9 ± 954.8[b]	9,146.1 ± 824.8[b]	16,072.2 ± 413.4[a]
Bound	18,295.3 ± 608.8[a]	19,405.6 ± 1951[a]	13,542.4 ± 1311b
Total	28,539.2 ± 1087.6[a]	28,551.7 ± 1989.7[a]	29,614.6 ± 985.1a
ORAC			
Free	7,933.0 ± 219.9[b]	6,032.2 ± 439.7c	11,340.0 ± 110.3a
Bound	17,751.91 ± 276.6[b]	13,659.5 ± 553.2c	19,289.9 ± 697.1a
Total	25,684.9 ± 121.1b	19,691.7 ± 274.8c	30,629.9 ± 588.9a

[1] Mean ± standard deviation (n=3); [2]µmol Trolox equivalents (TE)/ 100 g sample on dry weight (dw); [a-b] Values within columns without letters in common are statistically different (Tukey, $p < 0.05$).

The microwave process did not cause significant changes in the antioxidant activity measured by the ABTS method in both the free and bound fractions compared to raw grain. However, this thermal treatment did lead to a notable decrease ($p < 0.05$) in ORAC antioxidant activity, with reductions of -32 % in the free fraction and -30 % in the bound fraction relative to the raw grain (Table 3). In contrast, fermented maize exhibited considerable increases in total antioxidant activity, with increases of 16 % and 35 %, respectively, when compared to unprocessed and microwaved maize (Table 2). This behavior in antioxidant capacity has been documented in white maize, where a positive correlation was observed between antioxidant activity and total phenolic content across different fermentation times (Sánchez-Magaña *et al*., 2019). Ethanolic solutions extract phenolics while removing high-polarity compounds such as sugars and proteins (Tai *et al*., 2020). Consequently, these substances primarily contribute to antioxidant activity in aqueous extracts. However, their impact should be considered at low concentrations in ethanolic or ethyl acetate extracts, as utilized in this research to evaluate both antioxidant and antiparasitic activities. Unlike the results from the ORAC method, solid-state fermentation (SSF) of blue maize did not result in significant changes in total ABTS antioxidant activity compared to raw and microwave-processed grain (Table 3). The antioxidant properties of the phenolic compounds examined in this study may have influenced the varying effects of SSF on ABTS and ORAC antioxidant activities in both raw and microwaved grains (Table 3). It is essential to highlight that phenolic acids, especially

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ferulic acid—the most abundant phenolic compound in maize—along with anthocyanins, are the primary contributors to the antioxidant activity found in blue maize kernels (Mora-Rochín *et al*., 2016; Gaxiola-Cuevas *et al*., 2017). Research indicates that gallic acid, caffeic acid, and epicatechin are the phenolic compounds most likely to undergo hydrogen atom transfer (HAT), while flavonoids such as kaempferol and resveratrol exhibit better single-electron transfer (SET) capacity. This suggests that phenolic compounds can engage in both mechanisms, and their effectiveness largely depends on their chemical structure (Shukla & Sathyanarayana, 2021).

Antiparasitic activity of raw and processed blue maize

Table 4 presents the inhibition percentages obtained from the antiparasitic activity assay conducted with *Giardia duodenalis* trophozoites. The study evaluated hydroalcoholic extracts at concentrations ranging from 50 to 180 mg/mL. Notably, all extracts demonstrated greater than 70 % inhibition of *Giardia duodenalis* at a concentration of 50 mg/mL. Among these, the microwaved maize extract exhibited the highest inhibitory effect, achieving 92.1 % ($p \leq 0.05$) inhibition of the parasite. At a concentration of 180 mg/mL, the extracts of raw and fermented blue maize achieved complete (100 %) inhibition of *Giardia*, while microwaved maize achieved total inhibition at a lower concentration of 100 mg/mL (see Table 4). Lower IC_{50} values indicate higher antiparasitic effectiveness. In this regard, the IC_{50} value calculated for fermented blue maize was higher ($p \leq 0.05$) than that for cooked maize. However, there was no significant difference when comparing it to the raw maize IC_{50} value (29 mg/mL for fermented and 21.8 mg/mL for raw). Despite these differences, all extracts demonstrate similar effects, as they exhibit the same level of inhibitory activity at concentrations of 100 mg/mL and 180 mg/mL (refer to Table 4).

Table 4. Antiparasitic activity of raw and processed blue maize

Concentration (mg/mL)	Inhibition of Giardia duodenalis (%)[1]		
	Raw	Microwave cooked	Fermented
50	79.9 ± 3.8^{Bb}	92.1 ± 0.4^{aB}	70.1 ± 2.3^{Bc}
100	95.4 ± 1.9^{abA}	100.0 ± 0.0^{aA}	86.9 ± 4.6^{bB}
150	97.8 ± 0.0^{Aa}	100.0 ± 0.0^{aA}	89.4 ± 3.5^{aAB}
180	100.0 ± 0.0^{Aa}	100.0 ± 0.0^{aA}	100.0 ± 0.0^{aA}
IC_{50}	29.0 ± 1.7^{a}	21.8 ± 0.1^{b}	32.9 ± 1.1^{a}

[1]Mean ± standard deviation (n=3). $^{a-c}$ Values within a row with equal lowercase letters are not statistically different (Tukey, $p \leq 0.05$); $^{A-B}$ Values within a column with capital letters in common are not statistically different (Tukey, $p \leq 0.05$). IC_{50} = maximum Inhibitory concentration mean.

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The IC_{50} values obtained in this research were lower than those reported by Parra-Aguilar (2018), who found an IC_{50} of 58.2 mg/mL for microwave nixtamalized blue maize tortilla extracts. Parra-Aguilar also observed 100 % inhibition of the parasite at a concentration of 180 mg/mL, which is very close to the 100 mg/mL needed to achieve the same inhibitory effect in the present study. The differences in these results may be attributed to the type of blue maize used, the nixtamalization process employed, and the extraction method applied. Additionally, the values obtained in this work were comparable to those reported by Delgado-Vargas *et al*. (2008), who found that methanolic extracts of guamúchil (*Pithecellobium dulce*) achieved 100 % mortality of *Giardia duodenalis* at a concentration of 50 mg/mL.

Some authors have reported that the main bioactive compounds found in blue maize include phenolic acids such as ferulic, *p*-coumaric, sinapic, *p*-hydroxybenzoic, and vanillic acids (Gaxiola-Cuevas *et al*., 2017) as well as anthocyanins like cyanidin 3-glucoside (Mora-Rochín *et al*., 2016). Notably, it has been observed that the concentration of phenolic acids (ferulic, *p*-coumaric, sinapic, and *p*-hydroxybenzoic) increases due to traditional cooking and solid-state fermentation of white maize (Sánchez-Magaña *et al*., 2019). However, in this study, blue maize was cooked using a different processing method, which may involve other compounds. Additionally, blue maize is rich in phenolic polymers known as condensed tannins or proanthocyanidins (PA) (Chen *et al*., 2017). These polyphenols have demonstrated inhibitory effects on the growth of *Giardia duodenalis* (Anthony *et al*., 2011). The effectiveness of these compounds, along with their functional properties, largely depends on the chemical structure of their monomeric flavan-3-ol units, the types of bonds between these units, and their degree of polymerization (Chen *et al*., 2017). Moreover, other phenolic compounds may enhance antiparasitic activity. For example, extracts from blackberries that exhibit high inhibitory activity contain unconjugated ellagitannins, *p*-coumaric acid, and benzoic acid (Anthony *et al*., 2011). Researchers have also investigated the effects of flavonoids (Najumudin *et al*., 2018; Montes-Ávila *et al*., 2009; Hernández-Bolio *et al*., 2015) and saponins (González *et al*., 2010) on the *in vitro* growth of *Giardia duodenalis*.

Although microwave treatment significantly reduced the content of phenolic compounds and antioxidant activity, it exhibited the highest activity level against *G. duodenalis* (see Tables 2 and 3). This data suggests that heat-resistant compounds may contribute to this inhibitory effect, which is altered during solid-state fermentation (SSF). As mentioned in previous sections, SSF increases the content of phenolic acids in maize, resulting in more significant antioxidant activity in the fermented grain (Sánchez-Magaña *et al*., 2019). However, studies have also indicated that SSF can decrease the levels of condensed tannins (Teoh *et al*., 2024), which are known to have antiparasitic properties (Anthony *et al*., 2011). The complex interplay of these processes and the chemical nature of the compounds involved could explain the observed lack of correlation between the phenolic content (Table 2) in fermented and cooked blue maize and their respective inhibition percentages of *G. duodenalis* (Table 4). Further identification of the specific compounds released during microwave cooking is needed to determine whether they are responsible for the enhanced antiparasitic effect of this extract.

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Conclusions

Microwave cooking of blue maize kernels did not change their chemical composition, phenolic content, or antioxidant activity compared to raw kernels. However, it did lead to a significant reduction in total anthocyanin content. Interestingly, microwave-cooked blue maize exhibited the highest antiparasitic activity against *Giardia duodenalis*. In contrast, the solid-state fermentation (SSF) of blue maize increased protein, lipid, ash, total phenolics, and antioxidant activity, as measured by the ORAC method. The findings of this study show that microwave cooking and solid-state fermentation (SSF) enhance the bioactive components of blue maize grain and inhibit the growth of the parasite *Giardia duodenalis*. These results suggest that these processing methods provide alternatives for producing products with potential health benefits.

Authors' contribution

Authorship should be limited to individuals who significantly contributed to the reported work. Dr. Mora-Rochín and Dr. Sánchez-Magaña were responsible for the project's conceptualization. Dr. León-Sicairos and undergraduate student Meza-López conducted the development of the antiparasitic and antioxidant activities. Dr. Mora-Rochín managed the microwave technique, while Dr. Sánchez-Magaña oversaw the solid state fermentation (SSF). Software management and statistical analysis were carried out by Dr. León-López, who also performed experimental validation and result analysis. Both Dr. Sánchez-Magaña and Dr. Mora-Rochín contributed to experimental validation and result analysis. Additionally, they jointly developed the antiparasitic and antioxidant activities. Data management was handled by student Meza-López. Dr. Mora-Rochín and Dr. Sánchez-Magaña took charge of writing and manuscript preparation, while Dr. Reyes-Moreno and Dr. León-López were responsible for writing, revision, and editing. Dr. Mora-Rochín served as the project administrator, and Dr. Sánchez-Magaña participated in securing funding for the project.

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Conflict of interest

"The authors declare that they have no conflict of interest."

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