Bioactive compounds and microbiological analysis of hydroalcoholic extracts of Peruvian purple corn cob var. Canteño obtained by ultrasound.

Compuestos bioactivos y análisis microbiológico de extractos hidroalcohólicos de coronta de maíz morado peruano var. Canteño obtenidos por ultrasonido.

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Highlights

- Sono-processed extracts showed higher phenolic content than maceration for 2.5 h.
- Ultrasound processing at 480 W / 40 KHz / 45°C / 90 min yielded 1327 uM g-1 of Trolox antioxidant activity
- The purple corn powder showed a higher presence of molds and yeasts than the hydroalcoholic extract processed by ultrasound.

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ABSTRACT

Introduction. The cob is a byproduct generated from the marketing of purple corn grains. Currently, bioactive compounds are extracted from purple corn using conventional techniques that require substantial volumes of solvents and extended extraction periods. **Objective.** To determine the content of phenolic compounds and perform a microbiological analysis of purple corn cob extracts obtained by ultrasound. **Materials and methods.** The extract was prepared with 80% ethanol (pH 2.3) at a sample-to-solvent ratio of 1:12.5 (g/mL). The extraction process was performed using an ultrasonic bath (480 W, 40 KHz, 45°C for 90 minutes). Physicochemical and microbiological analyses were conducted on the purple corn cob powder and the hydroalcoholic extract, focusing on anthocyanin and phenolic compound content, as well as antioxidant activity. **Results and discussion.** The results indicated that the powdered cob had a high mineral and carbohydrate content, while the extract exhibited significant levels of anthocyanins (34.52 mg cyanidin-3-glucoside/g dry sample), phenolic compounds (324.59 mg gallic acid/g dry sample), and antioxidant activity (1327 µmol Trolox/g dry sample) compared to the control sample. Notably, the hydroalcoholic extract was free from molds and yeasts, unlike the purple corn cob powder. **Conclusion.** The ultrasound-processed extract of purple corn cob has potential applications as a food additive to retard oxidation and microbiological spoilage in food products.

RESUMEN

Introducción. La mazorca es un subproducto generado en la comercialización de granos de maíz morado. Actualmente, los compuestos bioactivos se extraen del maíz morado utilizando técnicas convencionales que requieren grandes volúmenes de solventes y largos periodos de extracción. **Objetivo.** Determinar el contenido de compuestos fenólicos y realizar un análisis microbiológico de extractos de coronta de maíz morado obtenidos por ultrasonido. **Materiales y métodos**. El extracto se preparó con etanol al 80% (pH 2.3) en una relación muestra-solvente de 1:12.5 (g/mL). El proceso de extracción se realizó utilizando un baño ultrasónico (480 W, 40 KHz, 45°C durante 90 minutos). Se realizaron análisis fisicoquímicos y microbiológicos en el polvo de mazorca de maíz morado y en el extracto hidroalcohólico, centrándose en el contenido de antocianinas y compuestos fenólicos, así como en la actividad antioxidante. **Resultados y discusión**. Los resultados indicaron que el polvo de mazorca tenía un alto contenido de minerales y carbohidratos, mientras que el extracto presentaba niveles significativos de antocianinas (34.52 mg cianidina-3-glucósido/g muestra seca), compuestos fenólicos (324.59 mg ácido gálico/g muestra seca) y actividad antioxidante (1327 µmol Trolox/g muestra seca) en comparación con la muestra de control. Cabe destacar que el extracto hidroalcohólico estaba libre de mohos y levaduras, a diferencia del polvo de mazorca de maíz morado. **Conclusión.** El extracto de mazorca de maíz morado procesado por ultrasonido tiene aplicaciones potenciales como aditivo alimentario para retardar la oxidación y el deterioro microbiológico en productos alimenticios.

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INTRODUCTION

The byproducts of purple corn mainly include the cob and the kernel, which contain bioactive compounds that can help prevent chronic diseases such as obesity and diabetes [1,2]. Purple corn is marketed and exported both fresh and as a dye to countries such as the United States, Spain, Germany, and China[3]. During the marketing of one ton of fresh grain, approximately 150 kg of cobs are generated in a native variety, and up to 100 kg of cobs and 40 kg of kernels with anthocyanin contents of 6.42% and 4.12%, respectively[4], are produced in an improved variety such as INIA-609. Therefore, there is a significant interest in utilizing the agro-industrial byproducts of corn to obtain phytochemicals for use in the food industry.

The extraction of phytochemicals from plant samples has traditionally been performed using conventional techniques such as aqueous, alcoholic, or hydroalcoholic maceration, which depend on the polar or non-polar affinity of the compounds to the solvent. However, these conventional techniques require large amounts of solvents and energy due to lower mass transfer rates, and they necessitate longer extraction times and higher temperatures. Consequently, the use of green technologies like ultrasound has been considered to improve the yield and purity of the product, making it economically attractive and efficient for industrial applications, as it can be performed at lower temperatures and shorter times[5]. The ultrasound extraction technique generates a cavitation phenomenon, forming small bubbles that increase the mass transfer rate between the plant matrix and the solvent, causing cellular disruption and particle decomposition, which increases the surface area of the solid sample[6]. Ultrasound has been employed for extracting tannins, anthocyanins, and total phenolic compounds (Folin-Ciocalteu method) from plant samples such as Lagenaria siceraria, Jamaica flower, and safflower seeds. For instance, Devi et al.,[7] verified that by applying ultrasound at an amplitude of 47.76% for 27 minutes, a yield of 75.81% dietary fiber and a high phytochemical content were obtained from Lagenaria siceraria seeds, revealing its potential for developing functional foods. Similarly, Zhang et al., [8] and Yuniati et al.,[9] confirmed that ultrasound extraction at 150W/66°C/36 min and 24KHz /40°C/5 min increased the extraction yield of pigments in safflower and anthocyanins in Jamaica flower by approximately 1.28% and 7%, respectively. The use of ultrasound in plant extracts promotes cell viability greater than 80% compared to extracts obtained by conventional methods[10]. Considering these advantages, the objective of this study is to obtain hydroalcoholic extracts from the Peruvian purple corn cob variety Canteño using ultrasound-assisted extraction. Subsequently, the bioactive compounds in these extracts will be characterized, and a microbiological analysis will be conducted.

MATERIALS AND METHODS

Physicochemical analysis of purple corn cob powder

A total of 2100 grams of purple corn cobs were purchased from the Makro commercial store in the La Libertad region. The ears were washed and disinfected with sodium hypochlorite at 10 ppm for 5 minutes. After manual shelling, 312.23 grams of cob were obtained and dried in a MERMMERT® model SN-N30 incubator at 40°C for 24 hours. After drying, the cob was crushed using an OSTER® blender model BLSTBPST (700 W) and sieved through an ADVANTECH® mesh with an opening size of 212 µm (65 mesh) until purple corn cob powder was obtained. The powder was characterized according to the protocols established by the Peruvian Technical Standards for flours, analyzing moisture[11], ash[12], lipids[13], proteins [14] and carbohydrates (by difference). **(Figure 1)**, shows the samples obtained from the purple corn cob: the chopped cob, the powdered cob, and the cob extract obtained by ultrasound.

An 80% hydroalcoholic solution was prepared by mixing 160 mL of ethanol (99.6% grade) and 40 mL of distilled water. To counteract the decrease in alcoholic content due to esterification reactions, 40 mL of ethanol (99.6%) was added to the acidified solution[15]. The final alcoholic strength of the acidified solution

was 50, as measured with an alcoholmeter. Twenty grams of corn cob powder was diluted with 250 mL of the hydroalcoholic solution[16], maintaining a solid-to-solvent ratio of 1:12.5 (w/v).

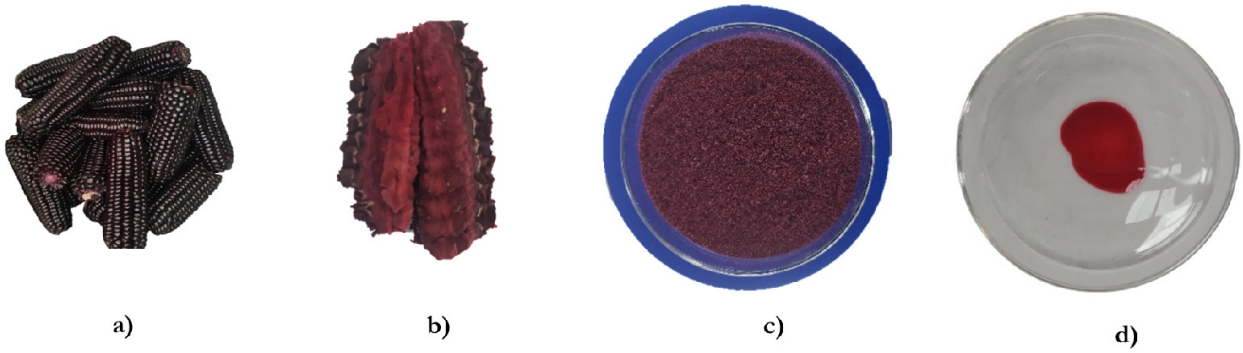

Figure 1. Obtaining Peruvian purple corn cob hydroalcoholic extracts by ultrasound. (a) Purple corn; (b) Purple corn cob; (c) Purple corn cob powder; (d) Ultrasonically processed purple corn cob extract.

The hydroalcoholic extract of purple corn cob was placed in a beaker lined with aluminum foil and subjected to ultrasonic processing (BOECO® ultrasound) at a frequency of 40 KHz and a power of 480 watts for 90 minutes at 45°C[17,18,19,20]. The obtained extract was filtered under vacuum (room temperature) using Whatman No. 1 paper and concentrated in an ISOLAB® rotary evaporator model 605.01.001 at a pressure of 508 mmHg, 50 rpm, and a temperature below 50°C. The concentrated extract was stored in amber bottles lined with aluminum foil and refrigerated until use.

Determination of monomeric anthocyanins (TAC)

The total anthocyanin content (TAC) was determined using the differential pH method following the AOAC (Association of Analytical Communities) methodology[21]. This method is based on the color change that anthocyanins undergo with a change in pH, with the colored oxonium form present at pH 1.0 and the colorless hemiacetal form present at pH 4.5. The difference in absorbance of the pigments at 520 nm is proportional to the pigment concentration. Measurements were taken at 520 nm and 700 nm using a Jenway™ 6305 spectrophotometer. The maximum absorbance is recorded at 520 nm, corresponding to cyanidin-3-O-glucoside with a molecular weight of 449.2 and a molar absorptivity of 26900. Absorbance is calculated according to equation (1):

$$\text{xz}A = (A520\ nm - A700)\ pH\ 1 - (A520\ nm - A700)\ pH\ 4.5 \qquad (1)$$

The concentration (C) of anthocyanins (in milligrams of cyanidin-3-O-glucoside/L or Cy3G/L) was calculated from equation (2):

$$C\ (Cy3G/L) = A\ x\ \text{MW}\ x\ FD\ x\ 1000\ /\varepsilon\ x\ l \qquad (2)$$

where MW is the molecular weight of cyanidin (MW 449.2), ε its molar absorptivity (ε= 26,900), FD is the dilution factor and A is the absorbance.

The results were expressed in milligrams of cyanidin-3-O-glucoside per gram of dry weight because the purple corn cob has more content of this type of anthocyanin. Three replicates were carried out for each sample.

Determination of phenolic compounds from the extract

Total phenolic compounds (TPC) were determined using the Folin-Ciocalteu colorimetric method with some modifications[22]. Gallic acid was used as a standard. This method is based on the reduction of the Folin-Ciocalteu reagent, which contains phosphomolybdic acid and phosphotungstic acid. In the presence of phenolic compounds, this reagent is reduced, forming molybdenum and tungsten compounds that produce a blue coloration. A calibration curve was prepared with 1 mg/mL gallic acid (GAE) in solutions of 0.1 mg/mL, 0.075 mg/mL, 0.050 mg/mL, 0.025 mg/mL, and 0.016 mg/mL. Aliquots of 0.5 mL from this solution were transferred to test tubes, followed by the addition of 0.8 mL of water and 0.05 mL of the Folin-Ciocalteu reagent. The samples were allowed to stand in the dark for 3 minutes. Subsequently, 0.1 mL of a saturated sodium carbonate solution was added, and the samples were placed back in the dark for 2 hours. Absorbance readings were taken at 725 nm using a UV/Vis spectrophotometer. All assays were performed in triplicate, and results were expressed in mg of gallic acid equivalent per gram of sample (mg GAE/g).

Determination of *in vitro* antioxidant activity

The ORAC procedure was conducted following the methodology of Prior et al.,[23]. Fluorescence readings were taken using a microplate reader, with sodium fluorescence recorded every minute for 80 minutes. Results were expressed in µmol of Trolox (TE) per gram of dry extract.

Microbiological analysis of sonoprocessed purple corn cob extract

Under sterile conditions in a biological biosafety cabinet, 10 g of each hydroalcoholic and powder extract were weighed and separately added to Stomacher bags with Side Filter. Subsequently, 90 mL of buffered dilution water (ADT) was added, constituting the initial sample for inoculation and equivalent to a 10-1 dilution. Three consecutive dilutions were prepared using a graduated micropipette, with each dilution containing 9 mL of ADT. The initial dilution and its subsequent dilutions were used to determine coliforms, *Escherichia coli* (*E. coli*), molds, and yeasts. For Salmonella spp. detection, a sterile wide-mouth bottle was used to which 25 g of the sample was added with 225 mL of trypticase soy broth (TSB)[24].

Detection of *Salmonella* spp.

The bottle containing the initial suspension (25 g of sample with 225 mL of TSB) was incubated at 35°C for 24 ± 2 hours. The mixture was then transferred to two tubes: one containing 10 mL of Rappaport-Vassiliadis (RV) broth with the addition of 0.1 mL of the mixture, and the other containing 10 mL of tetrathionate (TT) broth with the addition of 1 mL of the sample. The RV broth tubes were incubated at 42 ± 0.2°C for 24 ± 2 hours, and the TT broth tubes were incubated at 35 ± 2.0°C for 24 ± 2 hours. Following incubation, the contents of each tube were inoculated onto plates with bismuth sulfite agar (BS) and xylose lysine deoxycholate agar (XLD) using a bacteriological loop. The inoculated plates were incubated at 35°C for 24 ± 2 hours, and growth was verified to confirm the presence of *Salmonella* spp. through biochemical tests on Triple Sugar Iron Agar (TSI) and Lysine Iron Agar (LIA), and identification through serological tests[25].

Determination of coliforms and *Escherichia coli*

One milliliter of the initial dilution and each of the serial dilutions was transferred to sterile Petri dishes. Violet red bile agar (VRBA) was poured into each dish (18-20 mL), and the dishes were left to solidify for

a few minutes. The Petri dishes were then incubated at 35°C for 18-24 hours. Following incubation, colony growth on the plates was subjected to confirmation tests. Coliforms were confirmed by inoculating the colonies in brilliant green lactose bile broth, and *Escherichia coli* was confirmed using Lauryl Tryptose Broth - MUG (LST-MUG)[26].

Determination of molds and yeasts

One milliliter of the initial dilution and each of the serial dilutions was transferred to sterile Petri dishes. Potato dextrose agar (PDA) was poured into each dish (18-20 mL), and the dishes were left to solidify for a few minutes. The Petri dishes were then incubated at 25°C for 5-7 days. Following incubation, colony growth on the plates was observed[27].

RESULTS

The proximate analysis of the powdered purple corn is shown in **(Table 1)**. Carbohydrates constitute a significant fraction of the chemical composition of the purple corn cob, with insoluble carbohydrates being associated with phenolic compounds that influence antioxidant capacity. Moisture content is also crucial, as it can affect the development of *Aspergillus flavus*, a fungus that produces aflatoxins harmful to consumer health. The cob powder's moisture content of less than 8% ensures inadequate conditions for fungus growth.

Table 1. Characterization of purple corn cob powder

Components	Content (g/100g)
Moisture	7.070.002
Ashes	2.320.001
Lipids	0.740.001
Proteins	3.090.001*
Carbohydrates	86.780.001

***Note:** Nitrogen conversion factor 6.25

The content of monomeric anthocyanins (TMA) and phenolic compounds (TPC) in the sonoprocessed purple corn cob extract (480 W/40 KHz/45°C/90 min) was 34.52 ± 0.218 mg cyanidin-3-glucoside/g dry sample and 324.59 ± 0.475 mg gallic acid/g dry sample, respectively. These values were higher than those of the control sample (maceration at 45°C for 2.5 hours), which presented values of 25.15 mg cyanidin-3-glucoside/g dry sample and 96.31 mg gallic acid/g dry sample, respectively. To calculate the latter, the equation of the gallic acid standard curve was used, which was: $y=6.008\ x-0.02$ ($R^2 = 0.995$). The antioxidant activity was 1327 ± 0.367 µmol Trolox (TE) per gram of dry extract, 25% higher than the control sample.

Microbiological tests were carried out to ensure that there is no contamination in the handling of the sonicated hydroalcoholic extracts. The microbiological results obtained in the detection of *Salmonella* spp., coliforms and *Escherichia coli*, as well as molds and yeasts **(Table 2)**. The purple corn cob powder presented 50 CFU/g and 100 CFU/g of molds and yeasts, respectively, being within the permissible limit established by the Ministry of Health according to resolution RM 591-2008/MINSA. These microorganisms can develop due to poor storage of raw materials during harvest and distribution, mainly due to temperature and humidity factors. The most common microorganisms found in purple corn are fungi, which can form mycotoxins, which are difficult to eliminate during corn processing. The film exhibited an inhibition zone of 8.99 nm for *E. coli*, 8.81 nm for *Salmonella* spp. and 8.63 for *Staphylococcus aureus*, unlike the chitosan film that showed values of 1.30 nm, 1.67 nm and 1.69 nm ($p < 0.05$). respectively.

Table 2. Microbiological results of the hydroalcoholic and powder extract

Determinations	Unit	Result		PML according to standard RM591-2008/ MINSA
		Purple corn cob powder	Hydroalcoholic extract	
Coliform Count	UFC/g.	100	< 10	No reference
Escherichia coli Count	UFC/g.	< 10	< 10	10 a 500
Detection of *Salmonella* spp.	Presence or Absence in 25 g	Absence	Absence	Absence/25 g
Mold Count	UFC/g.	50	< 10	100 - 1000
Yeast Count	UFC/g.	100	< 10	100 - 1000

UFC – Colony forming units. The result < 10 is an indicator that there was no plaque growth. MPL: Maximum permissible limit.

DISCUSSION

The carbohydrate content of the powdered cob was higher than that reported in the study by Urquizo y Sánchez[28] for whole purple corn cobs (61.7 g/100g) and from Hernández-Santos et al.,[29] for purple corn and sweet potato flour mixture (82.84 g/100 g). In the study carried out by Ranilla et al.,[30] purple corn cobs grown in the lowlands and highlands of Peru do not present differences (p>0.05) in the content of lipids (4.6 g/100g and 4.1 g/100g, respectively) and proteins (8.2 g/100g and 8.9 g/100g, respectively), however, both contents were significantly higher than those obtained in the cob powder sample studied. On the other hand, the ash content for the lowland and highland cobs were 1.4 g/100g and 1.8 g/100g, respectively, differing from the values obtained in the study, which presented a higher ash content in 92% and 90%, respectively. It is inferred that the difference in these contents may be because the study cob was analyzed in powder form and not as a whole material. According to Jordan-Meille et al.,[31], the mineral composition of the cob can vary due to the pH of the crop soil which influences the mineral solubility, which is absorbed by the purple corn plant and redistributed in grains and cobs.

The value obtained for monomeric anthocyanins (TMA) was lower than the extract obtained in the study by Díaz-García et al.,[32]; however, this difference is based on the different varieties used in each study. The authors used improved purple corn varieties such as INIA 609, while the present study used a native variety (Canteño variety) widely commercialized on the Peruvian coast. Medina et al.,[33] mention that these improved cultivars have been developed by the National Institute of Agrarian Innovation to increase the productivity and anthocyanin content of purple corn, as well as guarantee greater resistance to pests.

There are currently several varieties of purple corn worldwide, differing in their physical and chemical characteristics. For example, the purple corn cob extracts produced in Lebanon and reported in the study by Rajha et al.,[17] have 68% less anthocyanin content than those obtained in this research. When comparing the anthocyanin content of the cob and kernel of purple corn, studies such as Charmongkolpradit et al.,[34] report an anthocyanin content in the methanolic extract of dry grain of Thai variety corn (13% moisture) of 35.73 mg anthocyanins/g of sample, a content calculated using the method of Fuleki y Francis[35], which includes the exclusion coefficient of cyanidin-3-glucoside. Thus, the measurement would reflect the peonidin content, considering that methanol promotes the extraction of peonidin derivatives, unlike hydroalcoholic solvents (ethanol and water) that favor the dissolution of cyanidin derivatives and formic and hydrochloric acid that favor the extraction of malonyl anthocyanin and succinyl anthocyanin, respectively[36-37].

Regarding phenolics, the TPC was significantly higher than the values reported in the studies by Barba et al.,[18] and Rajha et al.,[17] at 37 mg gallic acid/g dry sample and 13.79 mg gallic acid/g dry sample, respectively, but relatively lower, approximately 19% less than that reported by Juthamat et al.,[38]. However, it is worth noting that the content of these compounds may vary depending on the extraction process. In the research by Muangrat et al.,[6], it was confirmed that in the ultrasound extraction process (500 W / 20 KHz / 50% ethanol solvent / 60°C / 30 min) at a sonication amplitude greater than 25% but less than 50%, the release of phytochemicals from Thai purple corn was favored, ranging between 219.33 and 238.40 µg of cyanidin-3-glucoside/g of dry sample for anthocyanins and between 26.57 and 27.973 mg gallic acid/g dry sample for phenolics. Additionally, with a higher content of phytochemicals, the antioxidant power increases.

According to Ruilin et al.,[37], the TMA content determines the antioxidant capacity of the extracts, especially if they have a greater presence of cyanidin and pelargonidin. The anthocyanin and phenolic results from Muangrat et al.,[6] were well below those obtained in the ultrasonic extraction carried out at 480 W / 40 KHz / 40°C / 90 min using 80% ethanol (pH 2.3) as a solvent. These differences are influenced by the most significant extraction factors during sonoprocessing, such as solvent concentration, extraction temperature, and ultrasonic amplitude. Ethanol-water solvent mixtures appear more favorable in ultrasound extraction compared to solvents such as 100% water and 100% pure ethanol[39].

According to Zhu et al.,[19], increases in ultrasound power, temperature, and solvent pH up to 273 W, 72°C, and 3, respectively, enhance the yield in the extraction of bioactive compounds in black sesame seeds enriched with selenium by 1% to 2% compared to samples not processed by ultrasound. Acidification of the solvent is beneficial in the extraction of phenolic compounds because the flavylium ion in its chemical structure remains stable; however, at pH > 3, this ion hydrates to form a colorless pseudo base and degrades in the presence of oxygen in the water molecule. Likewise, the extraction temperature in ultrasound should not exceed 70°C as phytochemicals degrade since they are thermolabile compounds, as demonstrated in the studies by Yuniati et al.,[9] and Zhang et al.,[40] by optimizing phytochemical extraction processes in hibiscus and safflower samples, respectively. The optimal conditions for ultrasound extraction of both samples were a temperature of 66°C with a solvent-material ratio of 16 mL/g, time of 36 min, and ultrasonic power of 150 W for pigment extraction (hydroxy saffron yellow and anhydro saffron yellow) and a temperature of 40°C with a solvent-material ratio of 25 mL/g, time of 5 min, and ultrasonic frequency of 24 KHz for anthocyanin extraction, respectively. The effectiveness of ultrasound is highly attributable to the cavitation phenomenon formed by ultrasonic waves that allow the diffusion of solvents through cell walls and the release of phytochemicals, unlike conventional extraction, where the heated solvent acts on the surface of the cell by convection[9].

The mold and yeast count in the hydroalcoholic extract was lower than that reported by Velez[41] in an aqueous extract of purple corn also processed by ultrasound but with a shorter extraction time (40 KHz/10 min - 101 CFU/mL and 40 KHz/20 min - 84 CFU/mL). This suggests that the longer the sonoprocessing time, the better the extracts can retain their physicochemical characteristics, dependent on the microbial load, because the collapse of microbubbles raises the temperature and local pressure, and the shock waves can break some cell walls of microorganisms. Temperature plays an important role during processing; for example, the combination of temperature and sonication has been proven to reduce aerobic mesophiles and enterobacteria by 36% and 45%, respectively, in purple corn beverages[42-43]. In this study, the coliform count in the powdered purple corn cob was lower than that in the sonoprocessed hydroalcoholic extract.

Aqueous purple corn extracts with anthocyanin content between 0.62 and 2.5 mg cyanidin-3-glucoside/mL may have fungicidal activity, inhibiting the growth of fungi such as *Candida* spp. Qin et al.,[44] used the hydroalcoholic extract of purple corn kernels to mix it with silver nanoparticles and a chitosan film-forming

solution to evaluate its antimicrobial properties. The enhanced antimicrobial property of the film was due to the presence of anthocyanins, especially 24.69% cyanidin-3-glucoside, indicating that this film could be used as packaging material in the food industry. This context highlights the potential of purple corn extracts for various applications in foods as additives due to their positive effects on preservation.

CONCLUSIONS

Purple corn cobs are a significant source of carbohydrates and minerals. The microbiological analysis indicated that the purple corn cob powder had higher mold and yeast counts compared to the sonoprocessed hydroalcoholic extract. The bioactive compounds, reflected in the determination of anthocyanins and total phenolics in the sonicated hydroalcoholic extracts, were higher than those obtained via conventional extraction, demonstrating the efficiency of ultrasound extraction in terms of time. Due to the high content of phenolic compounds, its antioxidant power is suggested.

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