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RESEARCH ARTICLE

Phenolic extract from nejayote flour: Bioactive properties and its potential use as an antimicrobial agent of alginate-based edible coatings

Gloria M. Castañeda-Ruelas[1] | R. Karely Ibarra-Medina[1] | Guillermo Niño-Medina[2] | Saraid Mora-Rochín[1] | Julio Montes-Ávila[1] | Edith O. Cuevas-Rodríguez[1] | Maribel Jiménez-Edeza[1]

[1]Programa de Posgrado Integral en Biotecnología, Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Sinaloa, Culiacán, México

[2]Facultad de Agronomía, Universidad Autónoma de Nuevo Leon, San Nicolas de los Garza, Mexico

Correspondence
Maribel Jiménez-Edeza, Programa de Posgrado Integral en Biotecnología, Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Sinaloa, Calz. de las Americas S/N, Cd. Universitaria, Culiacán, Sinaloa, México. C.P. 80013.
Email: mjimeneze@uas.edu.mx

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Abstract

Background and objectives: The search for safe and effective natural sources of antioxidants and antimicrobials is a current trend. The corn nixtamalization industry involves the production of nejayote, a waste that offers a substrate for making value-added products. This study evaluated the phytochemical content and certain bioactive properties of a hydroalcoholic extract from nejayote flour and its potential use as an antimicrobial agent of edible coating.

Findings: Proximal composition of nejayote flour was 10.34% protein, 6.47% fat, and 76.72% carbohydrates. The total phenolic content of the hydroalcoholic extract was 68.62 mgGAE/100 g; ferulic acid, coumarins, tannins, triterpenes, and flavonoids were identified as major compounds. Antioxidant activity of the extract varied between the methods ($p < .05$): ORAC (1865.13 μmol TE/100 g) > ABTS•+ (1,670.02 μmol TE/100 g). Nejayote phenolic extract showed a moderate–high antimicrobial power depending on the bacteria ($p < .05$). The adding of nejayote phenolic extract (2.6%) in alginate coating improves physiochemical properties and exhibits a selective antimicrobial effect.

Conclusions: This research provides bases for the nutrimental and phytochemical content of nejayote flour and its bioactive properties.

Significance and novelty: The confinement and extraction of phytochemical compounds with biological activity from nejayote promote the technological innovation use and reduction of the environmental impact generated by this by-product.

KEYWORDS

antimicrobial, antioxidant, corn, nejayote, nixtamalization

1 | INTRODUCTION

The management of agri-food by-products or waste is considered a priority global industrial, economic, and environmental problem, and its solution requires a comprehensive and sustainable approach (García-García et al., 2017). The chemical composition of the residues that emerge is diverse and depends on the product nature and its processing. Inadequate disposal causes environmental problems, including greenhouse gas emissions, because of their high biodegradability, moisture, and microbial load (Fritsch et al., 2017). Certain wastes have a wide variety of components with a nutritional,

nutraceutical, and bioactive perspective that is beneficial for human health (Helkar et al., 2016). Due to the valuable potential of these by-products, they have become low-cost raw materials for the food, agriculture, pharmaceutical, and biotechnology industries, providing economic advantage, environmental benefit, and promotion of industrial symbiosis (Sadh et al., 2018).

The demand for nixtamalized products has increased corn production (5.7 million tons/year) in Mexico (SIAP, 2019). But it has also led to the generation of a polluting water by-product called nejayote (50 million m^3/year) (Acosta-Estrada, Lazo-Vélez, et al., 2015; Acosta-Estrada, Serna-Saldivar, et al., 2015). Nejayote is the residual alkaline liquid rich in grain endocarp and pericarp (Díaz-Montes et al., 2016), and it has been considered a pollutant of water due to certain physicochemical parameters (BDO, COD, alkaline pH, and $\%CaCO_3$) in addition to organic matter present (López-Maldonado et al., 2017).

Otherwise, some authors have characterized the organic matter that makes up the nejayote to validate its potential use. Heteroxylans (HX) made of xylose, galactose, arabinose, and glucuronic acid are the main polysaccharides of the nejayote, which can be used as adhesives, thickeners, emulsifiers, and film former (Martínez-Bustos et al., 2001). Acosta-Estrada, Lazo-Vélez, et al., (2015); Acosta-Estrada, Serna-Saldivar, et al., (2015) identified the nejayote as a rich source of dietary fiber (HX), Ca^{+2}, and phenolic compounds that can be added to foods to improve nutraceutical and antioxidant properties such as anti-inflammatory, antioxidant, anticancer, detoxifying, immunity booster, and antimicrobial activity against pathogens and environmental damage (Altemimi et al., 2017; Njeru et al., 2013). A previous phytochemical screen from nejayote identifies ferulic acid as a representative compound (Gutiérrez-Uribe et al., 2010). Although the antioxidant power of nejayote has been exposed (Gutiérrez-Uribe et al., 2010), its antimicrobial properties have not been widely explored.

One of the current trends in food technology is the search for safe, effective, and environmentally friendly natural sources of antioxidants and antimicrobials for the elaboration of edible coatings. Coatings are innocuous layers that protect food from mechanical damage, and increase shelf life and quality. Hydrocolloids (polysaccharides and proteins), lipids, and composites have been mainly used as base materials for coatings (Raghav et al., 2016). Moreover, some natural extracts and essential oils also have been added into coatings to provide antioxidant and antimicrobial capacity (Alkan & Yemenicioglu, 2015; Campos & Gerschenson, 2011).

The corn nixtamalization industry involves the production and disposal of 1.2 million m^3 of nejayote per month in Mexico (Valderrama-Bravo et al., 2012), which offers a rich substrate in phytochemical compounds for making value-added products (Gutiérrez-Uribe et al., 2010). Based on the chemical composition and volume of waste generated, there is an opportunity to use nejayote to promote sustainable development objectives. Therefore, this research evaluated the phytochemical content and certain bioactive properties of a phenolic extract from nejayote flour and its potential use as an antimicrobial agent for the formulation of edible coatings.

2 | MATERIALS AND METHODS

2.1 | Plant material

Commercial corn kernels (*Zea mays* L.) ideal for making tortillas were used as plant material in this study. The grains were inspected and stored in refrigeration (4°C) until they were transformed into nixtamal and nejayote.

2.2 | Nixtamalization and nejayote recovery

The white corn kernels (*Zea mays* L.) were subjected to the nixtamalization process described by Milán-Carrillo et al., (2004). Briefly, the kernels were cooked (1:3 corn kernels/distilled water) with a 5.4% w/v of a $Ca(OH)_2$ solution for 31 min at 85°C. The nixtamal (cooked corn) was left to stand for 8.1 hr, followed by the draining of the nejayote. The solid fraction of the nejayote was recovered by two periods of sedimentation (24 hr), dried in an oven (50°C for 24 hr), and processed with a manual mill (0.180 mm mesh) to obtain a homogeneous flour. The nejayote flour was stored at 4°C until use.

2.3 | Proximal chemical analysis

The proximal chemical analysis of nejayote flour was performed following the methodologies described by the AOAC International (1990), including the determination of humidity by the oven-drying method (AOAC 930.15), fat by the Soxhlet method (AOAC 920.39), nonprotein nitrogen by the Kjeldahl method (AOAC 984.13), ashes by the calcination method (AOAC 942.05), and carbohydrates by weight difference. All obtained values were expressed as a percentage (%).

2.4 | Extraction of phytochemical compounds

The extraction of soluble phytochemical compounds was performed according to the methodology proposed by Ovando-Martínez et al., (2009) with certain modifications. A 20 g sample of nejayote flour was extracted with 300 ml

of methanol/water solution (85:15) acidified with HCl (1 M) on a shaker at room temperature for 1 hr. Subsequently, the extracts were centrifuged at 11,200 *g* for 10 min. The supernatant was collected, and the pellet was subjected to a second extraction with 300 ml of acetone/water solution (70:30). The recovered supernatants were mixed, filtered (Whatman filter No. 2), and concentrated in a rotoevaporator at 40°C. The dry extract was dissolved with 5 ml of sterile deionized water and stored at 4°C until use.

2.5 | Determination of total phenolic content and phytochemical screening

The concentration of total phenolic compounds was determined by the Folin–Ciocalteu colorimetric method (Singleton et al., 1999). The quantification of phenolic content was expressed as mg of gallic acid equivalent (GAE) per 100 g on a dry base sample (mg GAE/g). On the other hand, the phytochemical tests of the nejayote extract were carried out through tube identification tests and their confirmation by thin-layer chromatography (silica gel matrix with 254 nm fluorescent indicator) as follows: the Salkowski reaction for terpenes/sterols; the Shinoda test for flavonoids; reaction with 1% gelatin solution and quinine sulfate solution with $FeCl_3$ for tannins; foaming for saponins; yellow fluorescence by reaction with NaOH for coumarins; and the Dragendorff and Mayer, and Wagner reagents for alkaloids (Harborne, 1998; Yawalikar et al., 2014). Results were reported as mild (+), moderate (++), abundant (+++), and undetected (-) presence for each chemical class.

2.6 | High-performance liquid chromatography analysis

The chromatographic analyses were carried out according to what was reported by Santos et al., (2014) in an Agilent Technologies 1,260 Infinity high-performance liquid chromatography (HPLC) equipment, equipped with an autosampler (G1329B), an Agilent quaternary pump (G1311B), a thermostatted column compartment (G1316A), a detector with diode array (G4212B), and a Zorbax Eclipse Plus C-18 analytical column (100 mm × 3 mm, 5 μm). The mobile phases used were mobile phase A (water at 0.1% of acetic acid) and mobile phase B (methanol at 0.1% of acetic acid), with a flow of 0.6 ml/min at 25°C, a running time of 60 min, and a sample injection of 10 μl. The gradient used was the following: 0 min of 95% mobile phase A; 4 min of 95% mobile phase A; 20 min of 73% mobile phase A; 50 min of 5% mobile phase A; 57 min of 99% mobile phase A; 58 min of 99% mobile phase A; and 60 min of 95% mobile phase A. Detection was carried out at 280 nm, and the UV spectrum (200–400 nm) of each detected compound was obtained. Individual phenolic compounds were quantified using a calibration curve with gallic acid, catechin, chlorogenic acid, caffeic acid, coumaric acid, ferulic acid, synapic acid, and quercetin, and the results were expressed in milligrams equivalent of each identified compound per kilogram of sample (mg/100 g).

2.7 | Determination of antioxidant activity by ORAC and ABTS•+ method

The antioxidant capacity of the soluble phenolic extract was determined using two methods. First, the oxygen radical absorbance capacity (ORAC) assay described by Ou et al., (2001) and Prior et al., (2005) was performed. Second, the ABTS•+ assay (2,2'-azino-bis (3-ethylbenzthiazoline -6-sulfonic acid)) was estimated according to the method described by Re et al., (1999). All data were expressed as μmol Trolox equivalent per 100 g of dry base sample (μmol TE/100 g).

2.8 | Agar well diffusion method

Mueller–Hinton agar (MHA) plates were lawn-cultured with standardized microbial culture broth (1×10^8 CFU/ml). Subsequently, wells (6 mm) were made in the culture medium using a sterile punch and filled with 50 μl of the extract. Additionally, a sulfamethoxazole–trimethoprim (STX) sensidisk (25 μg/ml) and sterile water were included as a control of the susceptibility and resistant phenotypes, respectively. The extracts could diffuse for 30 min at room temperature and further incubated at 37°C for 24 hr. The antimicrobial activity was interpreted by measuring the zone of inhibition (ZOI) expressed in mm and calculated using the following formula:

$$ZOI = complete\ zone\ of\ inhibition\ (mm) - well\ diameter\ (mm) \quad (1)$$

The classification of the degree of inhibition of nejayote extract was interpreted by the ZOI limits proposed by Mohd et al., (2020) (Figure 1).

2.9 | Determination of minimum inhibitory concentration and minimum bactericidal concentration of the nejayote extract

The broth microdilution method was used to determine the minimum inhibitory concentration (MIC). Twofold serial dilutions of extracts were prepared directly in a microtiter plate containing Mueller–Hinton broth to obtain 13 concentrations (512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.05, 0.025,

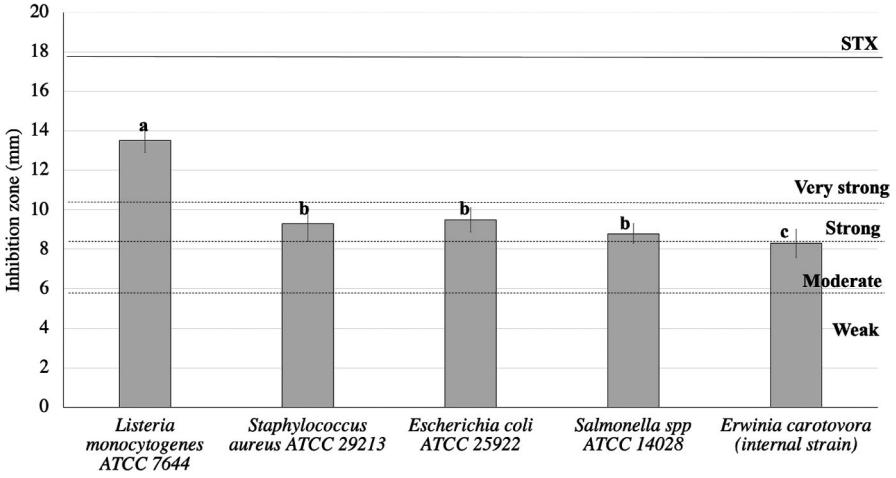

FIGURE 1 Inhibition zone of nejayote extract against pathogenic bacteria. The results are the means of three replicates each with two technical replicates ($n = 6$), and the bars represent the standard error. Means ± *SD* followed by different letters are significantly different ($p < .05$). The degree of inhibition is indicated with dashed lines: 6.5–9 mm (moderate inhibitory activity); 9–11 mm (strong inhibitory activity); and >11 mm (very strong inhibitory activity). The continuous line means the ZOI of STX (25 μg/ml)

and 0.0125 μg/ml) in a final volume of 250 μl. Subsequently, each tube was inoculated (1:1,000) with the adjusted bacterial suspension (1×10^8 CFU/ml) and incubated at 37°C for 18 hr. For the quality of the test, a positive control (culture medium with the inoculum) and a negative control (sterile culture medium) were included. The turbidity of the cultures was verified by direct exploration. MIC was defined as the minimum concentration of the extract that inhibits the visible growth of the microorganism. From the MIC point, the dilutions were plated on MacConkey agar, Hektoen agar, Modified Oxford agar, salt and mannitol agar, and King agar B, for *Escherichia coli* ATCC 25,922, *Staphylococcus aureus* ATCC 29,213, *Listeria monocytogenes* ATCC 7,644, *Salmonella* Typhimurium ATCC 14,028, and *Erwinia carotovora* (environmental strain), respectively (Bagul & Sivakumar, 2016). The microbial concentration was expressed according to the count dilution (CFU/ml). Minimum bactericidal concentration (MBC) was defined as the minimum concentration of the extract that inhibits 99.9% of microbial growth. The ratio MBC/MIC was also calculated to determine the susceptibility (≤4) of tolerant (≥4) phenotype of the strains tested (Mohd et al., 2020).

2.10 | Formulation of edible coating with phenolic extract of nejayote

The edible coatings were made based on alginate using the casting methodology, and the concentration of the nejayote phenolic extract added in the alginate film-forming solution was fixed according to the previously determined MIC/MBC values. A mixture of alginate (1.3% w/v) and glycerol (1.2% v/v) in distilled water was prepared and subjected to constant stirring at 70°C for 10 min. Once the film-forming solution was homogenized, 1 ml was poured onto a sterile spherical object to simulate the covering of a fruit. Then, the object was dipped into a volume of the nejayote phenolic extract (experimental coatings) or a solution (2.0% w/v) of $CaCl_2$ (control solution). The objects were retrieved from the experimental and control coatings and placed in a laminar flow hood to keep at room temperature for 24 hr. The overlays were peeled off the round surface for further evaluation. The application of nejayote phenolic extract to the film-forming solution by dipping and the lack of addition of $CaCl_2$ solution to the experimental coatings are due to the ability of the nejayote phenolic extract to favor the crosslinking of the alginate, presumably due to the residual content of Ca^{+2}.

2.11 | Evaluation of physicochemical properties of edible coating

The physicochemical analysis of alginate-based coatings with nejayote phenolic extract was performed following the methodologies described by Oregel-Zamudio et al., (2016). The characterization included the determination of thickness (mm), water vapor permeability (g/(s·Pa·m)), transparency (%), density (g/cm^3), humidity (%), and solubility (%).

2.12 | Evaluation of the antimicrobial activity of edible coating

The antimicrobial activity of alginate-based coatings with nejayote phenolic extract was tested against the previous panel of bacterial strains following the methodology described by Liao et al., (2010), with minor modifications. Squares (20 mm^2) of experimental alginate-based coatings

were deposited with 0.5 ml of a standard inoculum of each bacterium (1×10^6 CFU/ml). The cultures were incubated for 24 hr with gentle shaking. Subsequently, an aliquot of 100 µl was taken from each culture and serial decimal dilutions were made for their quantification by spotting (10 µl) in a selective medium for each bacterium. Plates for *E. coli*, *S. aureus*, *L. monocytogenes*, and *Salmonella* Typhimurium were incubated at 37°C for 18 hr and plates for *E. carotovora* at 30°C for 18 hr. The microbial concentration was expressed according to the dilution of the count (CFU/ml). The antibacterial effect of each treatment was calculated as the percentage of inhibition according to the following equation:

$$\%\ inhibition = \frac{CFU_{initial} - CFU_{final}}{CFU_{initial}}\, x\, 100\% \qquad (2)$$

2.13 | Statistical analysis

All analyses were performed in triplicate. An analysis of variance (ANOVA) followed by a Tukey–Kramer test was used to assess the difference between the means of the measured parameters. A $p \leq .05$ value was considered statistically significant. The MINITAB version 18 statistical package was used for data analysis.

3 | RESULTS AND DISCUSSION

3.1 | Proximal chemical analysis

Table 1 shows the proximal chemical analysis of the nejayote flour obtained from white corn (*Zea mays* L.). The carbohydrates were the major component followed by the content of proteins. The percentage of fat, humidity, and ashes were <7%.

TABLE 1 Proximal analysis of nejayote flour obtained from white corn

Parameters	Content %
Humidity	4.54 ± 0.03
Ash	1.93 ± 0.11
Fat	6.47 ± 0.04
Protein	10.34 ± 0.07
Carbohydrate	76.72 ± 0.17

Note: The results are the means ± standard deviation of three replicates each with two technical replicates ($n = 6$).

The nejayote contains hydrolyzed parts of the grain such as pericarp, tissue germ, and endosperm. For this reason, this waste obtained from nixtamalization is rich in carbohydrates, calcium, phenolic compounds, and proteins such as albumins, globulins, and prolamins (Acosta-Estrada, Lazo-Vélez, et al., 2015; Acosta-Estrada, Serna-Saldivar, et al., 2015). The values of carbohydrates (76.72 ± 0.17%), protein (10.34 ± 0.07%), and fats (6.47 ± 0.04%) observed are within the limits reported in previous works (Rosentrater, 2006). Carbohydrates are the representative component of the nejayote solid phase (71.93%–75.41%), and its main constituents correspond to polysaccharides and simple sugars (Ramírez-Romero et al., 2013; Rosentrater, 2006). Ramírez-Romero et al., (2013) based on the protein and carbohydrate composition of nejayote justified its suitability as a culture medium to produce probiotic bacteria and bacteriocins. Therefore, the transformation of the solid fraction of nejayote into flour offers a raw material with macronutrients that can be used for the food industry.

3.2 | Quantification, phytochemical analysis, and total phenolic content by HPLC

The total phenolic content of the nejayote extract (methanol/acetone) was 68.62 ± 1.53 mg GAE/100 g (Table 2); the ferulic acid (27.55 ± 0.43 mg GAE/100 g) was identified as one of the representative compounds in the nejayote extract by the HPLC analysis. Other compounds were not able to be detected and quantified by HPLC due to their low/absent concentration. However, the phytochemical screening performed on the nejayote phenolic extract discloses the presence of tannins, coumarins, triterpenes, and flavonoids to moderate-to-abundant amounts (Table 3).

This study provides preliminary evidence of the soluble phenolic content of nejayote flour (68.62 mg GAE/100 g) from white corn with a methanol/acetone extraction. Previously, Acosta-Estrada, Lazo-Vélez, et al., (2015); Acosta-Estrada, Serna-Saldivar, et al., (2015) and Gutiérrez-Uribe et al., (2010) determined a concentration of free phenolic (28–90 mg GAE/100 g and 18 mg GAE/100 g, respectively) in ethanolic extracts, depending on the use of lime

TABLE 2 Content and antioxidant activity of the total phenol content of nejayote extract

		Antioxidant activity (µmol TE/100 g)	
Total phenolics (mg GAE/100 g)	Ferulic acid (mg GAE/100 g)	ORAC	ABTS
68.62 ± 1.53	27.55 ± 0.43	1,865.13 ± 26.49[a]	1,670.02 ± 70.30[b]

Note: The results are the means ± standard deviation (*SD*) of three replicates each with two technical replicates ($n = 6$).

Means ± *SD* followed by different letters are significantly different ($p < .05$).

(concentration), the corn grain (type and quality of the grain), and its processing conditions (time and temperature). It has been demonstrated that the phenolic content in cereal grains is presented as a soluble and bound fraction, this last portion being the one with the high concentration of phenolic compounds (Tian et al., 2019), showing higher concentration after the nixtamalization process (Gutiérrez-Uribe et al., 2010).

Cereals, especially grains, are a great source of phytochemical compounds, and its biological activity has been little explored compared with fruits and vegetables (Tian et al., 2019). In this research, it was sought for phytochemicals on nejayote, and our findings support it as an agent with bioactive compounds, such as ferulic acid. The presence of ferulic acids and/or flavonoids in the nejayote methanolic extract corresponds to the reports made by Acosta-Estrada, Lazo-Vélez, et al., (2015); Acosta-Estrada, Serna-Saldivar, et al., (2015), Gutiérrez-Uribe et al., (2010), Rojas-García et al., (2012), and Serna-Saldivar et al., (2013).

3.3 | Antioxidant activity of nejayote extract

Based on the antioxidant values obtained by two methods (ORAC and ABTS•+), an analysis of the antioxidant capacity of the nejayote extract was performed. The comparison of the antioxidant activity values of the nejayote phenolic extract obtained with the ORAC and ABTS•+ methods varied significantly ($p < .05$), revealing a classification order as follows: ORAC (1865.1 ± 10.3 μmol TE/100 g) > ABTS•+ (1,670.0 ± 40.6 μmol TE/100 g) (Table 2).

Antioxidant activity is intrinsically related to the content of various phytochemical compounds such as phenolic compounds, anthocyanins, among others due to their chemical structure and its capacity of inhibiting the generation of free radicals responsible for diseases and cellular aging (Altemimi et al., 2017; Njeru et al., 2013; Shahidi & Ambigaipalan, 2015). In this sense, the antioxidant activity of white corn has been determined to be 19,312 μmol TE/100 g (Mora-Rochin et al., 2010). Comparing this value with the observed antioxidant activity of the nejayote phenolic extract, retention of 9.7% of antioxidant activity is inferred. It is therefore that this by-product (nejayote) is highly proposed as a substrate with a relevant phytochemical content to perform an antioxidant and protective function.

TABLE 3 Phytochemical analysis of the extract of nejayote flour

Phytochemical	Result
Alkaloids	-
Saponins	+
Volatile coumarins	++
Flavonoids	++
Tannins	++
Triterpenes /steroids	+++

Note: + = mild reaction; ++ = moderate reaction; +++ = abundant reaction; - = undetected reaction.

3.4 | Antimicrobial activity of nejayote extract

Figure 1 and Table 4 show the antimicrobial activity of the nejayote phenolic extract against the different bacteria evaluated. Nejayote phenolic extract shows an antimicrobial effect depending on the type of bacteria ($p < .05$). The zone of inhibition generated by the extract was expressed with different levels (8.3–14.5 mm) among the bacteria tested ($p < .05$), and with respect to the concentration of the control drug (mm) ($p < .05$). The ZOI classified the microbicidal activity of nejayote phenolic extract with a high–very high power for pathogenic bacteria, and moderate power for phytopathogenic bacteria (Figure 1). *L. monocytogenes* strain (ATCC 7,644) is mainly vulnerable to the biocidal activity of the extract in both methods. MIC and MBC for all bacteria were observed with values from 32 and 64 μg/ml, respectively. The ratio MBC/MIC for the tested bacteria ranged from 1 to 2, which means the susceptibility (MBC/MIC <4) of the bacteria tested against nejayote phenolic extract (Table 4).

This research exhibits the premise of the functionality of the phytochemical content of nejayote as an antimicrobial agent of important pathogenic bacteria in public health and agriculture. Our results showed that ferulic acid is one of the main bioactive compounds of nejayote, which may be responsible for the antimicrobial potential observed. However, the antimicrobial activity of phytochemical compounds may also be due to the combination of various chemical classes of metabolites present (Koche et al., 2016). The antimicrobial property of phytochemical content has been described from

TABLE 4 MIC and MBC of nejayote extract on the panel of pathogenic bacteria

Bacteria	MIC (mg/ml)	MBC (mg/ml)	Ratio
Listeria monocytogenes ATCC 7,644	32.0	64.0	2
Staphylococcus aureus ATCC 29,213	64.0	64.0	1
Escherichia coli ATCC 25,922	64.0	64.0	1
Salmonella Typhimurium ATCC 14,028	64.0	64.0	1
Erwinia carotovora (internal strain)	64.0	64.0	1

various plant sources in in vitro tests (Chang et al., 2013; Mohamed et al., 2010). Recently, Ramírez et al., (2020) demonstrated the antimicrobial activity of a nejayote phenolic extract against *Salmonella* and *Enterococcus* strains, but this power is improved only when nejayote has been subjected to fermentation process with *Bacillus claussi*. In this study, the nejayote hydroalcoholic extract has an efficient phytochemical content at a concentration ≥64 μg/ml to inhibit both pathogenic and phytopathogenic bacteria with high biocidal power. Given the MIC/MBC values, it was chosen for the elaboration of the edible alginate coatings to use higher concentrations of nejayote as: 1.3% (128 μg/ml) and 2.6% (256 μg/ml).

3.5 | Evaluation of physicochemical properties of edible coating

Table 5 describes the physicochemical characteristics of alginate-based coating with nejayote phenolic extract (0%, 1.3%, and 2.6%). The physicochemical properties were conditioned by the coating type ($p < .05$). The thickness, opacity, and water vapor permeability increased with the addition of nejayote phenolic extract, while the density and solubility gradually decrease with the increment of nejayote phenolic extract concentration.

Our results propose the extract of soluble phenols obtained from nejayote as a functional agent for the preparation of edible coatings (Table 5 and Table 6). The physicochemical properties of alginate-based coatings have been widely reported, whose attributes have been improved with the addition of organic acids and essential oils (Parreidt et al., 2018; Siracusa et al., 2018). The coating thickness is a key attribute since it determines other mechanical and structural properties. The thickness obtained in this study (0.112–0.198 mm) differs slightly from that previously published for alginate coatings without (0.127 mm) or with (0.104–0.111 mm) additives (Siracusa et al., 2018), presumably due to the coating procedure. Regarding density, this is an attribute that confers stability to the coating for protection of the product (Oregel-Zamudio et al., 2016). The coatings proposed in this study show that the density decreases with the addition of the phenolic extract (Table 5), which could indicate the interaction of the extract in the state of polymerization of the alginate. The alginate coatings (78.138%) decreased their solubility with the increase in the concentration of phenols (up to 34.218%) obtained from nejayote. Solubility and water vapor permeability are related parameters: When the solubility of the coating increases, its water vapor permeability decreases (Sánchez-Aldana et al., 2015). Water vapor permeability is considered an important property for regulating gas exchange and regulating deteriorative reactions in food (Charles-Rodríguez et al., 2020). In this sense, nejayote-enriched alginate coatings (with lower solubility) tend to slightly increase the speed of water evaporation.

3.6 | Evaluation of the antimicrobial activity of edible coating

The antimicrobial effect of the experimental coatings varied significantly ($p < .05$) with the addition of nejayote phenolic extract (Table 6). Only the alginate-based coating with 2.3% of nejayote phenolic extract showed total and low growth inhibition for bacteria Gram (+) and Gram (-), respectively.

Currently, the emergence of antimicrobial resistance is a priority for environmental and public health problems. Under this argument, efforts have been added to design effective treatments for microbiological control. Phenolic compounds have received special interest because they come from natural sources that can minimize the risk of microbe resistance (Godstime et al., 2014). As seen in this study and previously described by Ramírez et al., (2020), the nejayote can offer phytochemical compounds that act as efficient and eco-friendly antimicrobial agents.

There are two proposed mechanisms of the biocidal effect of phenolic compounds. One is related to the interaction with the cell wall, receptors, and ion channels of the cell membrane, and bacterial metabolites, which leads to death (Álvarez-Martínez et al., 2020; Martins et al., 2014). The second one refers to the intercalation of the acid in the phospholipid layers of the membrane of the microorganism, inhibiting the transport of the substrates used by the key enzymes of the microorganism (Pernin et al., 2019). Surprisingly, the antimicrobial activity was efficient for Gram (+) bacteria but limited against Gram (-) bacteria. However, its incorporation into the coating showed limited activity. The degradation of the active compounds or their interaction with the other components of the coating is factors that could influence their availability and antimicrobial capacity (Fabra et al., 2018). Also, the antioxidant and antimicrobial activity of nejayote phenolic extract could be enhanced by fermentation process as recently described (Ramírez et al., 2020). On the other hand, Koushki et al., (2015) have pointed out that alginate-based coatings constitute a barrier to reduce the growth of deteriorating microorganisms and consequently increase the shelf life. In this context, the proposed coatings could prevent microbial spoilage of food and minimize the growth of certain pathogenic bacteria.

4 | CONCLUSIONS

This research reports nutrimental, phytochemical content and bioactive functionality of nejayote for use as a promising

TABLE 5 Characterization physicochemical of alginate-based coating with nejayote extract (0%, 1.3%, and 2.6%)

		Alginate-based coating with nejayote extract		
Parameter	**Units**	**0%**	**1.3%**	**2.6%**
Thickness	mm	0.112 ± 0.004^{c}	0.162 ± 0.011^{b}	0.198 ± 0.004^{a}
Transparency	%	0.131 ± 0.026^{b}	0.170 ± 0.000^{a}	0.173 ± 0.029^{a}
Density	g/cm^3	0.232 ± 0.040^{a}	0.155 ± 0.010^{b}	0.117 ± 0.017^{b}
Water vapor permeability	$\times 10^{-3}$ g/(s·Pa·m)	1.135 ± 0.054^{b}	1.296 ± 0.058^{b}	8.851 ± 0.077^{a}
Humidity	%	45.83 ± 13.16^{a}	45.14 ± 8.88^{a}	49.26 ± 6.15^{a}
Solubility	%	78.14 ± 0.36^{a}	57.58 ± 0.59^{b}	34.22 ± 0.19^{c}

Note: The results are the means ± standard deviation (*SD*) of three replicates each with two technical replicates ($n = 6$).
Means ± *SD* followed by different letters are significantly different ($p < .05$).

TABLE 6 Antimicrobial effect of alginate-based coating with nejayote extract (0%, 1.3%, and 2.6%)

	Alginate-based coating with nejayote extract		
Bacteria	**0%**	**1.3%**	**2.6%**
Staphylococcus aureus	0.0^{e}	3.7^{d}	100.0^{a}
Listeria monocytogenes	0.0^{e}	0.0^{e}	100.0^{a}
Escherichia coli	0.0^{e}	0.0^{e}	13.7^{b}
Erwinia carotovora	0.0^{e}	0.0^{e}	10.7^{c}
Salmonella Typhimurium	0.0^{e}	0.0^{e}	10.0^{c}

Note: The mean values are expressed in % of inhibition. The results are the means ± standard deviation (*SD*) of three replicates each with two technical replicates ($n = 6$). Means ± *SD* followed by different letters are significantly different ($p < .05$).

substrate in the elaboration of value-added products in the food, agriculture, pharmaceutical, and biotechnology industries. Ferulic acid is proposed as one of the main bioactive agents of nejayote. The antioxidant and antimicrobial power of nejayote extract offers an alternative for the development of biodegradable edible coatings improving the quality and safety of food. Therefore, the confinement and processing of nejayote offers an adherent proposal with the global objective of sustainable development that favors technological innovation and social, economic, and environmental well-being.

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CONFLICT OF INTEREST

No potential competing interest was reported by the authors.

AUTHOR CONTRIBUTIONS

Gloria M. Castañeda-Ruelas performed formal analysis and wrote the original draft. R. Karely Ibarra-Medina collected data and provided technical support. Guillermo Niño-Medina performed analysis and conducted HPLC experiments. Saraid Mora-Rochín performed analysis and analyzed characterization of nejayote flour. Julio Montes-Ávila performed analysis and conducted phytochemical experiments. Edith O. Cuevas-Rodríguez contributed to methodology and revised the manuscript. Maribel Jiménez-Edeza contributed to conceptualization, funding acquisition, methodology, and manuscript approbation.

ORCID

Maribel Jiménez-Edeza https://orcid.org/0000-0002-9835-9665

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