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Effect of Storage Time on the Hydroxycinnamic Acids Profile, Cellular Antioxidant Activity, and Anti-Inflammatory Potential of Roasted Maize-Based Beverages Supplemented with Nejayote Solids from Different Maize Genotypes

Efecto del Tiempo de Almacenamiento Sobre el Perfil de Ácidos Hidroxicinámicos, la Actividad Antioxidante Celular y el Potencial Antiinflamatorio de Bebidas a Base de Maíz Tostado Suplementadas con Sólidos de Nejayote de Diferentes Genotipos de Maíz

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Resumen

Este estudio tuvo como objetivo evaluar el efecto del tiempo de almacenamiento en el contenido de calcio, parámetros de color, contenido de ácidos fenólicos, actividad antiinflamatoria y antioxidante celular *in vitro* de bebidas instantáneas a base de maíz tostado suplementadas con sólidos de nejayote (BENS) de diferentes genotipos de maíz. BENS_R (BENS suplementado con sólidos de nejayote de maíz rojo) presentó menor luminosidad (L> 66.22) y mayor coloración rojiza (a< 3.51). El BENS suplementado con sólidos de nejayote de maíz amarillo (BENS_Y) mostró el mayor contenido de calcio con 680 mg/100 g, actividad antioxidante celular (55.57 %) y ácidos ferúlico total (232 mg/100g), 8-5′benzofurano diferulico (3.90 mg/100 g) y 5-5′diferúlicos (2.85 mg/100 g) en comparación con el control. En promedio el 55 % de los fenólicos totales se pierden en el almacenamiento, siendo BenS Y la bebida con mayor cantidad de compuestos fenólicos ligados. El tiempo de almacenamiento disminuyó drásticamente el potencial antiinflamatorio de BENS, aumentando en promedio 30 puntos porcentuales la producción de óxido nítrico. El BENS suplementado con sólidos de nejayote de maíz rojo (BENS_R) y con alto contenido de carotenoides (BENS_HC) mostró la mejor actividad antiinflamatoria, mientras que BENS_Y y BENS_HC mostraron mayor actividad antioxidante celular, incluso después de 12 meses de almacenamiento. Los sólidos de nejayote de diferentes genotipos de maíz se pueden utilizar como ingrediente funcional para la producción de bebidas a base de maíz enriquecidas en calcio, ácido ferúlico, potencial antiinflamatorio y antioxidante.

Palabras clave: actividad anti-inflamatoria, actividad antioxidante celular, ácido ferúlico, nejayote.

Abstract

This study aimed to evaluate the effect of storage time on the calcium content, color parameters, phenolic acids content, *in vitro* anti-inflammatory and cellular antioxidant activity of instant roasted maize-based beverages supplemented with nejayote solids (BENS) of different maize genotypes. BENS_R (BENS supplemented with red maize nejayote solids) had less luminosity (L> 66.22) and higher reddish (a< 3.51) colorations. The BENS supplemented with yellow maize nejayote solids (BENS_Y) showed a higher calcium content with 680 mg/100 g, cellular antioxidant activity (55.57 %) and total ferulic (232 mg/100g), 8-5′benzofuran diferulic (3.90 mg/100g) and 5-5′diferulic acids (2.85 mg/100g) compared to control. On average, 55 % of the total phenolics are lost in storage, with BENS_Y being the beverage with the highest amount of bound phenolic compounds. Storage time drastically decreased the anti-inflammatory potential of BENS, increasing on average 30 percentage points the production of nitric oxide. The BENS supplemented with red (BENS_R) and high carotenoid (BENS_HC) maize nejayote solids showed better anti-inflammatory activity, while BENS_Y and BENS_HC showed the stronger cellular antioxidant activity, even after 12 months of storage. Nejayote solids of different maize genotypes can be used as a functional ingredient to produce maize-based beverages rich in calcium, ferulic acid, anti-inflammatory and antioxidant potential.

Keywords: Anti-inflammatory activity, cellular antioxidant activity, ferulic acid, nejayote.

INTRODUCTION

Maize (*Zea mays L.*) is the most domesticated plant around the world. In Mexico there are 59 different varieties of maize, within these varieties are the maize pigmented genotypes namely yellow, red, black, purple, yellow high carotenoids, and blue (FAOSTAT, 2023). In the last decade, a great interest has been generated by the study of pigmented maize due to the nutraceutical potential and correlation with health benefits, generating a line of research of great scientific interest (Colín-Chavez et al. 2020; Salinas-Moreno et al. 2017). Most of the phenolic compounds associated with several types of Mexican maize genotypes are ferulic and p-coumaric acids, anthocyanins type-flavonoids, and carotenoids among others that exhibited antioxidant properties (Mora-Rochin et al., 2010; Gutiérrez-Uribe et al., 2010; Mora-Rochin et al., 2016). The interest in antioxidants and bioactive properties has increased due to their potential health benefits such as the capacity to induce quinone reductase, anti-carcinogenic effects, high chemopreventive index, protection against oxidative stress, increased antimutagenic activity, and inhibition of colorectal carcinogenesis (Herrera-Sotero et al., 2017; Zhang et al. 2019). Furthermore, pigmented maize is utilized to manufacture nixtamalized food products (e.g. tortillas, tortilla chips) enhanced in phenolic compounds that exert antioxidant potential (Mora-Rochin et al., 2016; Sánchez-Madrigal et al., 2015).

The nixtamalization process or alkaline cooking is the first step to obtain maize-based products in Mexico; however, after the nixtamalization process, an alkaline effluent (nejayote) with the presence of anatomic parts (pericarp, endosperm, and germ) of maize grain is generated (Villela-Castrejón et al., 2017a). The nejayote annually generated in Mexico is around 14.4 million m3, 422 thousand tons of nejayote solids and is considered highly polluting (Acosta-Estrada et al., 2015). Nejayote soluble and insoluble solids are rich in calcium, phenolic acids (mainly ferulic acid) that leached during the nixtamalization process. These compounds exhibited chemopreventive, anti-inflammatory, and anticancer potential (Acosta-Estrada et al., 2014; Acosta-Estrada et al., 2015; Villela-Castrejón et al., 2017a; Villela-Castrejón et al., 2017b; Díaz-Montes et al., 2018; Buitimea-Cantúa et al., 2019). Nejayote solids can be re-incorporated into food products (e.g. bakery products) and beverages without affecting organoleptic properties (Acosta-Estrada et al., 2014; Buitimea-Cantúa et al., 2019). However, these studies have only been focused on white nejayote and there is limited information about the supplementation of food products with nejayote solids from pigmented maize genotypes. Salinity is the most common abiotic stress encountered during tomato production around the globe. High salt stress provokes osmotic imbalances, cell membrane disorganization, reactive oxygen species (ROS) overproduction, and photosynthesis inhibition, among other problematic effects (Parihar et al., 2015). According to the FAO, there are approximately 110 million hectares affected by high salinity, of which around 20–30 million are considered severely damaged (FAO, 2009). The use of iodine as an inducer of salt stress tolerance could be a viable option for dealing with this type of global problem. The present study was carried out to explore that possibility, focusing on determining if the foliar application of KIO_3 (100 μM) on tomato seedlings modify their tolerance to the adverse effects generated by the presence of high salt stress during growth.

Maize beverages such as pozol, tesguino, atole, and pinole are made with ground-roasted maize and have been prepared by different Mexican ethnic groups through centuries (Rooney et al., 2003). Previous studies have reported that roasting conditions such as temperature and time produces important changes decreasing phytochemicals content (phenolic acids and anthocyanins type-flavonoid), and therefore bioactive properties (Youn et al., 2012). Buitimea-Cantúa et al. (2019) reported that in maize-based beverages added with 9 % of nejayote solids, the anti-inflammatory and antioxidant activity were less affected after 12-months of storage. Pigmented maize has attracted the interest of the food industry, not only for its nutritional properties but also for its nutraceutical properties that will allow the development of new functional foods that satisfy the demands of current consumers, therefore in this work the effect of storage time in the color parameters, calcium content, phenolic acids content, *in vitro* anti-inflammatory, and the cellular antioxidant activity of instant roasted maize-based beverages supplemented with pigmented nejayote solids was evaluated.

MATERIALS AND METHODS

Nejayote solids

Four different maize genotypes (white, yellow, red, and high carotenoids) (Fig. 1.) were used to obtain nejayote solids. Maize was donated by CIMMYT’s Global Maize Program (Batan Experimental Station, Mexico). Maize samples were stored at -20 °C until use. Maize was lime-cooked at 93 °C, 1 % calcium hydroxide and optimal cooking time (time to increase nixtamal moisture to 48 % after 14 h steeping) (Serna-Saldivar et al., 1993). The cooking times for the white (W), yellow (Y), red (R), and high carotenoid (HC) maize were 42, 30, 21, and 34 min, respectively. The resulting nejayote was frozen at -80 °C, lyophilized, and milled to pass through a 0.8 mm mesh sieve in a mortar mill (RM 200, Retsch, Haan, Germany). The nejayote solids were packed in a laminated aluminum foil bag.

Figure 1. Maize genotypes used to obtain nejayote solids. A) white, B) Yellow, c) Red, D) High carotenoids.

Maize roasting process and productions of maize-based beverages (BENS)

White whole maize kernels (1000 g) were roasted manually in a round pan for 20 min at 270 ± 2 °C and cooled down for one hour at 25 °C according to Buitimea-Cantúa et al. (2019). The roasted maize kernels presented an average moisture content of 5.8 %. Roasted maize, cinnamon (0.1 %, w/w) and clove (0.1 %, w/w) were ground in a dry mill (Samap Ecosysteme SARL, model F 100, Andolsheim, France) and the resulting powders were sieved through a US-60 mesh (0.250 mm) screen. The maize-based beverages (BENS) supplemented with nejayote solids were produced blending for 5 min the BENS with nejayote solids from white maize (BENS_W), yellow (BENS_Y), red (BENS_R), and high-carotenoid (BENS-HC) maize at concentration 9 % (w/w) in a mixer (AS200, Hobart Corporation, Troy, OH) according to the procedure of Buitimea-Cantúa et al. (2019). The BENS supplemented with nejayote solids of different maize genotypes were subsequently packed in sealed aluminum bags and half of them were stored for up to 12 months at 25°C.

Solubility and Moisture content

Solubility was determined according to Villela-Castrejón et al. (2017a). The moisture content of samples was determined gravimetrically by drying in an oven (Thelco Model 28, Precision Scientific, Chicago, IL) at 105 °C until reaching a constant weight. Samples hygroscopicity was tested using the method proposed by Villela-Castrejón et al. (2017a).

Calcium content and color measurements

Calcium concentration in samples was determined using a complexometric titration by ethylene diamine tetra acetic acid (EDTA) (Acosta-Estrada et al., 2014). Data were expressed as milligrams per 100 g of dry weight (mg/100 g, DW). The color measurements of BENS were analyzed fresh and after 12 months of storage at 25 °C (L = lightness; +a = red, -a = green, +b = yellow, -b= blue) and were carried out with a Minolta (CR 300, Minolta, Japan) colorimeter according to Buitimea-Cantúa et al., (2017).

Extraction of free and bound phenolics

Free and bound phenolic compounds were extracted using the method reported by Acosta-Estrada et al. (2023). Briefly, 1 g of dried ground sample was mixed with 20 ml of ethanol (80 %) for 10 min in a shaker (Incubator with orbital shaker, Mrc Laboratories, Israel) at 250 rpm and 25 °C and then centrifuged (Thermofisher Scientific SL 16R, Waltham, MA) at 3000 g (10 min and 4 °C). The supernatant (free extract) was collected. Alkaline hydrolysis (10 ml NaOH 2 M) was performed in the resulting pellet for 1 h, the samples were then acidified with 2 M HCl to pH 2. The acidified samples were extracted with 50 ml of ethyl acetate and the fractions were evaporated to dryness (85 mbar, 25 ºC) (Genevac EZ-2 Series, SP Scientific, Warminster, PA, USA). The bound phenolics were resuspended in 50 % methanol. Free and bound phenolics were stored at -20 °C until analysis.

Detection and quantification of phenolic compounds

Free and bound extracts were quantified by HPLC-DAD (1200 Series, Agilent Technologies, Santa Clara, CA) according to the reported by Acosta-Estrada et al. (2019). Chromatograms were acquired at 320 nm. Identification and quantification of ferulic and p-coumaric acids were based on the retention time of their corresponding standards. The identification and quantification of dehydrodimers of ferulic acid was based on a previous research study (Acosta-Estrada et al., 2019). All compounds were quantified as ferulic acid equivalents (mg FAE/100 g sample).

Quantification of total carotenoids and total monomeric anthocyanins

The total carotenoids content was measured according to Sogi et al. (2015) and the results were expressed as beta-Apo-8'-carotenal equivalent using a standard curve. Total monomeric anthocyanin content was determined using the pH differential assay reported by Madiwale et al. (2011) and results expressed as cyanidin-3-O-glucoside equivalents (C-3-G Eq).

Cellular antioxidant activity and anti-inflammatory potential

Cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Cellular antioxidant activity

(CAA) of free phenolics extracted from maize-based beverages, was performed as reported by López-Barrios et al. (2016) using a cellular model of human colon cancer (Caco-2) cells. Regarding the anti-inflammatory potential, it was tested using the murine macrophage cell line (RAW 264.7) according to Buitimea-Cantúa et al. (2019) and the results were expressed as the percentage nitric oxide production (% NOX).

Statistical analysis

Each experiment was performed in triplicate and data were reported as mean ± standard deviation. Results were subjected to analysis of variance (ANOVA) and differences among means were compared by Tukey's tests (P-value < 0.05) and Pearson's correlation analyses were performed using JMP 11.11 statistical software (SAS Institute, Inc).

RESULTS

Physical properties of beverages

Maize-based beverages (BENS) supplemented with nejayote solids of different maize genotypes showed no significant changes in solubility, moisture, and hygroscopicity among themselves but were significantly different compared to control samples of BENS without supplementation (Table 1). Supplemented BENS exhibited the highest solubility (2.4-fold), moisture (1.34-fold), and hygroscopicity (1.07-fold) in contrast with the BENS_control. The higher solubility, moisture, and hygroscopicity in the supplemented BENS were attributed to nejayote solids that have high concentration of carbohydrates (dietary fiber) (Acosta-Estrada et al., 2014: Ayala-Soto et al., 2014). All the maize-based beverages presented lower water activity (aw) below 0.30 that prevents microbiological proliferation (Hamed et al., 2015) without observing statistical differences between them. Roasting and milling had a significant effect on water activity (aw) showing a lower aw than the reported (0.48-0.58) for nejayote powders obtained by spray-drying (Villela-Castrejón et al., 2017a).

Table 1. Physical properties of corn-based beverages (BENS) supplemented with different genotypes of maize nejayotes.*

BENS	**Solubility (%)**	**Moisture (%)**	**Hygroscopicity (g/100g)**	a_w
Control	0.58 ± 0.01^B	4.09 ± 0.01^B	7.79 ± 0.04^B	0.28 ± 0.01^A
White	1.40 ± 0.09^A	5.50 ± 0.01^A	8.42 ± 0.10^A	0.26 ± 0.01^A
Yellow	1.39 ± 0.06^A	5.50 ± 0.01^A	8.41 ± 0.05^A	0.27 ± 0.02^A
High-carotenoid	1.40 ± 0.07^A	5.49 ± 0.01^A	8.40 ± 0.02^A	0.27 ± 0.02^A
Red	1.40 ± 0.01^A	5.50 ± 0.01^A	8.39 ± 0.03^A	0.29 ± 0.01^A

*Different capital letter within each column indicates significant differences (P<0.05). Mean value ± SD (n-3). Water activity (aw).

Calcium content and color measurements

Supplemented BENS showed an increase in the calcium content (6.22 to 6.78-fold) compared to BENS_control (Fig. 2). BENS_Y contained 680 mg/100g of calcium content, whereas the control beverage contained 100.20 mg calcium/100g. One serving (225 mL) of BENS_Y provides 34 % of the recommended daily calcium intake for adults in many Latin American countries (Ross et al., 2011). Similarly, Acosta-Estrada et al. (2014) reported that enriched bread with nejayote solids 9 % of white maize presented 650 mg calcium/100 g providing 29 % of the recommended daily calcium intake. Buitimea-Cantúa et al. (2019) demonstrated that calcium content in maize-based beverages, added with 9 % of nejayote solids from white maize, was 342.84 mg calcium/100 g, about 1.9-fold lower than the calcium content determined for BENS_Y.

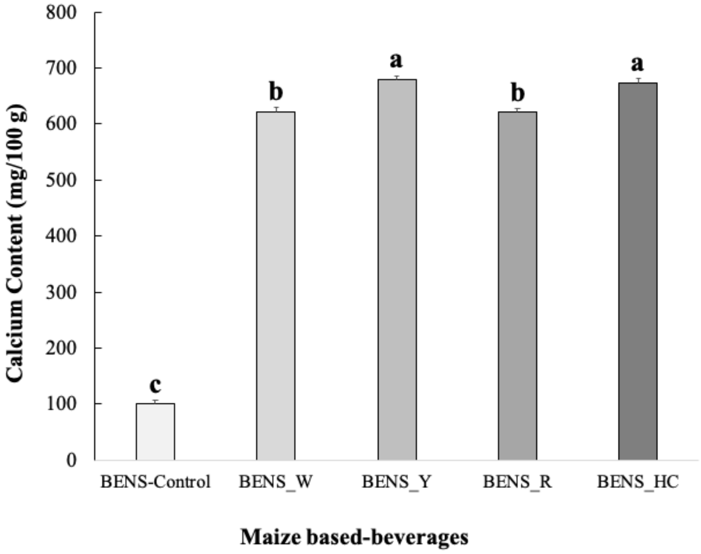

Figure 2. Calcium content in maize-based beverages supplemented with different genotypes of maize nejayote. Data expressed as mean value ± SD (n= 3).

Regarding color measurements, it was observed that among all BENS, BENS_R had less luminosity (L> 66.22) and higher reddish (a< 3.51) colorations (Table 2). There were no significant changes of yellowish intensity (b> 16.00) among samples (Table 2). The changes in a parameter might be directly correlated to the Maillard product formation (browning reactions) that occurred during the roasting of maize (Chung et al., 2014; Wani et al., 2016). The storage time significantly affected (P<0.05) the L, a, H°, Chroma and ΔE colors parameters stability in the maize-based beverages supplemented with nejayote solids of different maize genotypes (Table 2). The highest value for luminosity (L) was observed in BENS_W samples (L>77.52). After 12 months of storage, BENS_HC, BENS_R and BENS_control beverage presented the best reddish color stability derived from the roasting of maize.

Table 2. Effect of storage time on the *L*, *a*, *b*, Hue, Chroma and Δ*E* value of color parameters of Maize-based beverages (BENS) supplemented with different genotypes of maize nejayotes measured at one day and twelve months of storage time by tristimulus colorimetry*.

	Color					
BENS	**L**	**a**	**b**	**Hue**	**Chroma**	**Δ*E***
Storage time (1 day)						
Control	$66.79 \pm 3.04^{D,E}$	$2.76 \pm 1.39^{A,B}$	15.71 ± 3.36^{A}	1.37 ± 0.01^{D}	$16.86 \pm 0.02^{C,D}$	
White	$73.60 \pm 0.46^{A,B,C}$	$2.21 \pm 0.01^{A,B}$	15.74 ± 0.08^{A}	1.42 ± 0.01^{D}	$17.34 \pm 0.01^{A,B}$	
Yellow	$74.64 \pm 0.07^{A,B}$	$2.16 \pm 0.04^{A,B}$	15.59 ± 0.06^{A}	1.35 ± 0.01^{D}	16.68 ± 0.01^{D}	
High-carotenoid	$73.63 \pm 0.07^{A,B,C}$	$2.38 \pm 0.02^{A,B}$	16.07 ± 0.01^{A}	1.44 ± 0.02^{D}	$17.16 \pm 0.01^{B,C}$	
Red	66.22 ± 3.63^{E}	3.51 ± 0.37^{A}	16.00 ± 1.39^{A}	1.44 ± 0.01^{D}	16.67 ± 0.02^{D}	
Storage time (12 months)						
Control	$71.56 \pm 1.92^{B,C,D}$	$2.41 \pm 0.31^{A,B}$	16.51 ± 0.20^{A}	$26.53 \pm 1.94^{A,B}$	16.69 ± 0.24^{D}	4.36 ± 0.64^{A}
White	77.52 ± 0.23^{A}	0.64 ± 0.08^{C}	16.59 ± 0.19^{A}	20.48 ± 0.23^{C}	16.60 ± 0.19^{D}	4.30 ± 0.28^{A}
Yellow	$73.39 \pm 0.20^{A,B,C}$	2.02 ± 0.08^{B}	17.23 ± 0.15^{A}	25.01 ± 1.32^{B}	$17.35 \pm 0.14^{A,B}$	2.07 ± 0.18^{B}
High-carotenoid	$72.74 \pm 0.73^{A,B,C}$	$2.20 \pm 0.13^{A,B}$	17.58 ± 0.29^{A}	25.34 ± 0.74^{B}	17.72 ± 0.30^{A}	1.83 ± 0.53^{B}
Red	$69.34 \pm 0.58^{C,D,E}$	$2.40 \pm 0.10^{A,B}$	14.70 ± 0.05^{A}	28.75 ± 0.59^{A}	14.90 ± 0.04^{E}	2.53 ± 1.10^{B}

$^{A,\ B,\ C}$ Different capital letter within each column indicate significant differences ($P<0.05$). Mean value ± SD (n-3). L = color intensity, L = 100 for lightness, and 0 for darkness; +a = increasing red, -a = increasing green; +b = increasing yellow, -b = increasing blue; Hue = $\tan^{-1}(b/a)$; Chroma = $(a^2 + b^2)^{1/2}$; $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$.

Similarly, all supplemented BENS presented stability of yellowish colorations. The "L" color parameter was negatively correlated to the "a" (r=-0.79, P<0.001) and "a" color parameter was correlated with the ΔE values (r=-0.48, P<0.01) present in supplemented BENS (Table 3). The BENS_W and BENS_control presented the higher ΔE parameter (ΔE >4.36) indicating that storage time affected more to these maize-based beverages while the supplementation of pigmented NS (enriched in phenolic acids and flavonoids) to maize-based beverages stabilized the BENS colors (luminosity, yellowish, and reddish) after 12 months of storage.

Effect of the storage in free and bound phenolic bounds

The supplementation of BENS with nejayote solids had a highly significant (P<0.05) effect on the phenolic acids content (Table 4). BENS_Y had the highest amount of free and bound ferulic acid (236.74 mg/100 g). Ferulic acid (FA) was the most abundant phenolic acid in BENS. The lowest total ferulic acid content was observed in BENS_control (20.63 mg/100 g) followed by BENS_W (173.30 mg/100 g). In contrast, total ferulic acid in breads supplemented with white maize nejayote soluble solids at 9 % was lower (36.16 µg/100 g) than all supplemented BENS (Acosta-Estrada et al., 2014). Maize is reported to contain high concentrations of ferulic acid in bound form (84 to 94 %) (Chavez-Arias et al., 2022).

BENS_Y contained 37 % more free and bound ferulic acid than BENS_W. Diferulic acids such as 8-O-4 diferulic acid, 8-5′ benzofuran diferulic acid, and 5-5′ diferulic acid, were also present in the maize-based beverages. BENS_Y had the highest amount of bound and free 8-5′benzofuran diferulic and 5-5′diferulic acids (P<0.05) (Table 4). The 8-5′ benzofuran diferulic and 5-5′ diferulic acids increased 3.46-fold and 1.95-fold, respectively compared to BENS_control. While BENS_W showed the higher concentration of 8-O-4 diferulic acid, the 8-5′benzofuran diferulic acid in bound forms was the most abundant phenolic acid in all maize-based beverages, except control beverage (Table 4). The presence of these compounds has already been reported in nejayote solids and digested spray-dried nejayote powder from white maize; moreover, they have been closely related to chemopreventive and antioxidant activities exerted by these raw materials (Acosta-Estrada et al., 2015; Villela-Castrejón et al., 2017b). The differences in phenolic acids content in BENS supplemented with nejayote solids could be attributed to the genetic variations of maize grains as well as the grain physical properties mainly the ratio of the anatomical parts of the kernel since pericarp and endosperm´s aleurone layers are the structures richer in phenolic compounds (Mora-Rochin et al., 2010).

The storage time significantly decreased (P<0.05) the bound and free phenolic acids in the BENS after 12 months of storage, supplemented BENS lost from 35.43 to 58.62 % of total ferulic acid, 44.05-83.73 % of p-coumaric acid, 51.06-64.35 % of 8-O-4 diferulic acid, 64.08-72.82 % of 8-5′ benzofuran diferulic acid, and 30.27-65.80 % of 5-5′ diferulic acid.

Table 3. Correlations Pearson (r) analysis of variables tested.

	a	b	Hue	Chroma	ΔE	Bound ferulic	Free ferulic	Bound coumaric	Free coumaric	Bound 8-O-4 diFA	Free 8-O-4 diFA	Bound 8-5´ diFa	Free 8-5´diFa	Bound 5-5´diFa	Free 5-5´diFa	CAA	NOX	Viability
L	-0.79***	0.03	0.17	0.24	0.30	0.26	0.40	-0.04	-0.22	-0.04	-0.09	-0.14	-0.21	-0.04	-0.14	-0.06	0.13	-0.20
a		0.19	-0.32	-0.03	-0.48	0.06	-0.48	0.23	0.37	0.20	0.22	0.42	0.37	0.31	0.29	0.33	-0.36	-0.15
b			0.23	0.48	0.21	-0.03	0.35	-0.28	-0.23	-0.29	-0.34	-0.21	-0.26	-0.21	-0.21	-0.23	0.26	0.27
Hue				-0.25	0.83***	-0.52	0.65***	-0.53	-0.36	-0.85***	-0.80***	-0.72***	-0.50	-0.84***	-0.83***	-0.80***	0.61	0.66***
Chroma					-0.23	0.23	0.08	-0.18	-0.25	0.15	0.05	0.03	-0.12	0.12	0.07	0.09	0.05	-0.09
ΔE						-0.58	0.43	-0.52	-0.50	-0.73***	-0.69***	-0.72***	-0.69***	-0.68***	-0.67***	-0.79***	0.64***	0.61
Bound ferulic							0.04	0.80***	0.72***	0.58	0.58	0.81***	0.73***	0.76***	0.53	0.86***	-0.78***	-0.69***
Free ferulic								-0.19	-0.01	-0.51	-0.47	-0.41	-0.13	-0.53	-0.57	-0.38	0.31	0.41
Bound coumaric									0.86***	0.67***	0.71***	0.80***	0.82***	0.73***	0.70***	0.86***	-0.84***	-0.64***
Free coumaric										0.38	0.43	0.77***	0.82***	0.62	0.54	0.77***	-0.68***	-0.52
Bound 8-O-4 diFa											0.96***	0.58	0.55	0.68***	0.78***	0.77***	-0.68***	-0.64***
Free 8-O-4 diFa												0.59	0.56	0.67***	0.71***	0.74***	-0.74***	-0.57
Bound 8-5´diFa													0.79***	0.94***	0.69***	0.93***	-0.85***	-0.74***
Free 8-5´diFa														0.63	0.55	0.82***	-0.77***	-0.58
Bound 5-5´diFa															0.78***	0.91***	-0.78***	-0.76***
Free 5-5´diFa																0.82***	-0.52	-0.69***
CAA																	-0.81***	-0.81***
NOX																		0.56
Viability																		1.00

Significant at ***P<0.001; 8-O-4 diFA: 8-O-4 diferulic acid; 8-5´ diFA; 8-5′ benzofuran diferulic acid; 5-5´ diFa: 5-5′ diferulic acid; CAA: cellular antioxidant activity; NOX: nitric oxide production.

BENS_R lost the major concentration of ferulic and 5-5′ diferulic acids. This decrease in the concentration of the phenolic could be explained by the degradation through decarboxilation during prolonged storage time. Also, during the storage it could occur a certain degree of polymerization and interaction with proteins, therefore, reducing their extractability and subsequent quantification (Buitimea-Cantúa et al., 2019; Buitimea-Cantúa et al., 2018a; Buitimea-Cantúa et al., 2018b).

Effect of storage on nitric oxide assay in RAW 264.7 macrophages

All supplemented BENS with nejayote solids of different maize genotypes showed a reduction in the nitric oxide production. The BENS_R and BENS_HC beverages exhibited the highest reduction in the production of nitric oxide on RAW 264.7 cells without affecting their viability (>70 %) (Fig. 3). The production of nitric oxide in cells treated with maize-based beverages was reduced in the following order: BENS_R (54.56 %) > BENS_HC (48.95 %) > BENS_W (32.60 %) > BENS_Y (28.0 %) (Fig. 3A).

Table 4. Ferulic acid, coumaric acid and conjugated phenolics of free and bound phenolic compounds of maize-based beverages (BENS) with nejayote solids measured for one day and twelve months of storage time at 25°C.

	Phenolic acids									
	Ferulic Acid (mg FA/100 g dw)		***p*-Coumaric Acid (mg CA/100 g dw)**		**8-*O*-4 diferulic acid (mg FAE/100 g dw)**		**8-5′benzofuran diferulic acid (mg FAE/100 g dw)**		**5-5′diferulic acid (mg FAE/100 dw)**	
BENS	**Bound**	**Free**	**Bound**	**Free**	**Bound**	**Free**	**Bound**	**Free**	**Bound**	**Free**
Storage time (1 day)										
Control	17.71 ± 1.56^{I}	$2.92 \pm 0.04^{E,F}$	1.40 ± 0.15^{H}	0.10 ± 0.01^{F}	1.58 ± 0.16^{E}	$0.21 \pm 0.10^{D,E}$	1.06 ± 0.02^{E}	$0.15 \pm 0.01^{C,D}$	1.32 ± 2.40^{D}	$0.20 \pm 2.40^{C,D}$
White	163.66 ± 1.51^{D}	$9.64 \pm 0.06^{B,C}$	8.51 ± 0.19^{C}	0.16 ± 0.01^{E}	3.13 ± 0.19^{A}	0.36 ± 0.10^{A}	1.57 ± 0.01^{C}	$0.19 \pm 0.01^{B,C}$	1.62 ± 2.61^{C}	$0.23 \pm 2.61^{B,C}$
Yellow	224.33 ± 0.96^{A}	$12.41 \pm 0.08^{A,B}$	10.41 ± 0.18^{B}	0.32 ± 0.01^{B}	1.97 ± 0.30^{C}	$0.23 \pm 0.10^{C,D}$	3.67 ± 0.02^{A}	0.23 ± 0.01^{A}	2.58 ± 0.44^{A}	0.27 ± 0.44^{A}
High-carotenoid	214.66 ± 1.57^{B}	14.66 ± 0.05^{A}	6.58 ± 0.42^{D}	0.20 ± 0.02^{D}	1.90 ± 0.05^{D}	$0.26 \pm 0.10^{B,C}$	3.36 ± 0.01^{B}	$0.19 \pm 0.01^{B,C}$	2.51 ± 1.45^{B}	$0.17 \pm 1.45^{D,E}$
Red	189.04 ± 1.57^{C}	10.53 ± 0.05^{B}	12.25 ± 0.42^{A}	0.37 ± 0.42^{A}	2.05 ± 0.42^{B}	0.30 ± 0.42^{B}	3.64 ± 0.42^{A}	0.23 ± 0.42^{A}	2.47 ± 0.42^{B}	$0.25 \pm 0.42^{A,B}$
Storage time (12 months)										
Control	12.7 ± 0.52^{I}	0.26 ± 0.01^{F}	0.96 ± 0.07^{H}	0.05 ± 0.01^{G}	0.43 ± 0.07^{J}	0.10 ± 0.10^{G}	0.39 ± 0.01^{G}	0.10 ± 0.01^{E}	0.94 ± 0.71^{F}	$0.15 \pm 0.71^{E,F}$
White	98.61 ± 0.81^{G}	$3.45 \pm 0.01^{E,F}$	$1.30 \pm 0.19^{D,E}$	0.11 ± 0.01^{F}	1.12 ± 0.15^{F}	0.18 ± 0.10^{E}	0.45 ± 0.01^{G}	$0.13 \pm 0.01^{D,E}$	1.13 ± 1.12^{E}	$0.16 \pm 1.12^{E,F}$
Yellow	146.00 ± 0.97^{E}	$6.86 \pm 0.02^{C,D}$	4.03 ± 0.26^{F}	0.24 ± 0.01^{C}	0.72 ± 0.25^{H}	$0.14 \pm 0.01^{F,G}$	0.90 ± 0.01^{F}	$0.16 \pm 0.01^{C,D}$	0.89 ± 2.19^{F}	$0.14 \pm 2.19^{E,F}$
High-carotenoid	107.33 ± 1.51^{F}	$4.80 \pm 0.03^{D,E}$	2.93 ± 0.16^{G}	0.10 ± 0.02^{F}	0.66 ± 0.19^{I}	0.11 ± 0.01^{G}	1.00 ± 0.01^{E}	$0.18 \pm 0.01^{B,C}$	0.80 ± 1.01^{G}	0.13 ± 1.01^{F}
Red	79.07 ± 1.51^{H}	$3.50 \pm 0.05^{E,F}$	6.81 ± 0.42^{D}	0.25 ± 0.42^{C}	0.98 ± 0.42^{G}	$0.17 \pm 0.42^{E,F}$	1.19 ± 0.42^{D}	$0.20 \pm 0.42^{A,B}$	0.79 ± 0.42^{G}	$0.14 \pm 0.42^{E,F}$

*A, B, C Different capital letter within each column indicate significant differences (P<0.05). Data were expressed as mean ± standard deviation (n= 3).

The anti-inflammatory potential of nejayote powder has been reported previously by Villela-Castrejón et al. (2017b) and it was associated with the content of p-coumaric and ferulic acids in the nejayote preparations. Similarly, Buitimea-Cantúa et al. (2019) showed that maize-based beverages produced by roasting and supplemented with nejayote solids (9 % w/w), inhibited by 95.79 % the production of the nitric oxide, as an inflammatory biomarker.

The storage time significantly decreased (P<0.05) the production of nitric oxide on cells treated with BENS supplemented with nejayote solids of different maize genotypes. BENS_W and BENS_Y were the most affected by storage showing an increase in the production of nitric oxide by 26.6 % and 28 %, respectively. The production of nitric oxide was negatively correlated with the amount of bound 8-5´diFa acid (r=-0.85, P<0.001) and bound coumaric acid (r=-0.84, P<0.001) presented in maize-based beverages supplemented with nejayote solids from the pigmented maize, as well as, with the cellular antioxidant activity (Table 3). The storage time did not have a significant effect (P>0.05) on the viability of RAW 264.7 cells treated with the BENS supplemented with nejayote solids of different maize genotypes (Fig. 3B).

Effect of storage in cellular antioxidant activity (CAA) in Caco-2 cells

The supplementation of BENS with nejayote solids of different maize genotypes have a significant effect (P<0.05) on cellular antioxidant activity of beverages (Fig. 3C). At the concentration evaluated (1.0 ng/mL FA), the BENS_Y showed the highest cellular antioxidant activity (55.57 %) followed by the BENS_R (49.33 %), and BENS_HC (43.39 %) whereas the control beverage showed the lowest antioxidant activity (25.52 %).

These results agree with previous reports which indicated that alkaline hydrolysis generated during the nixtamalization process allows the leaching of important antioxidant compounds, into the nejayote and the release of bound phytochemicals associated to corn cell walls (Mora-Rochin et al., 2016; Acosta-Estrada et al., 2015; Villela-Castrejón et al., 2017b; Buitimea-Cantúa et al., 2019; Acosta-Estrada et al., 2019). The cellular antioxidant activity was mainly correlated to the content of bound 8-5´diFa acid (r=0.93, P<0.001), bound 5-5´diFa acid (r=0.91, P<0.001), bound ferulic acid (r=0.86, P<0.001) and bound coumaric acid (r=0.86, P<0.001) present in maize-based beverages supplemented with nejayote solids of different maize genotypes (Table 3).

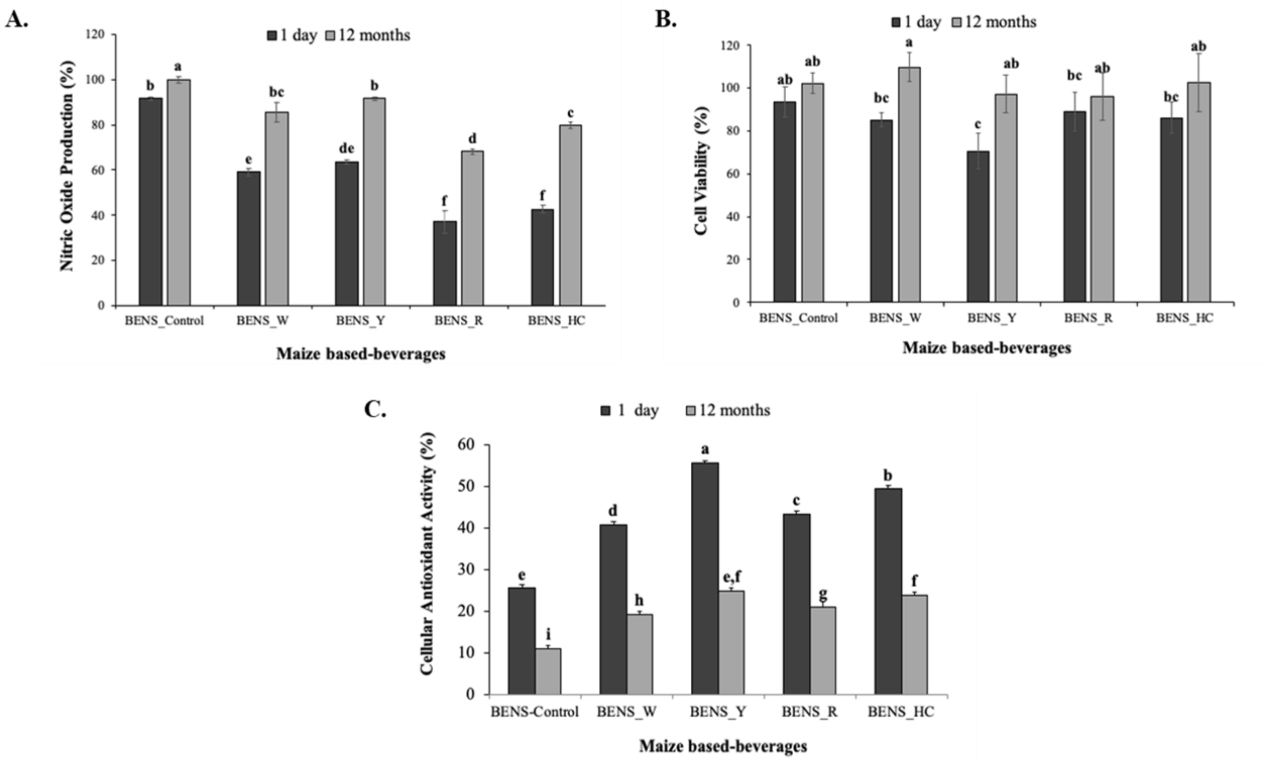

Fig. 3. Anti-inflammatory activity (macrophages cell line; RAW 264.7) of extracts of maize-based beverages supplemented with different genotypes of maize nejayote solids (1.0 ng/mL of ferulic acid concentration) measured during initial (1 day) and 12 months of storage (25°C). Nitric oxide inhibition (**A**) Viability (%) of Caco-2 cells (**B**). Cellular antioxidant activity of free phenolic compounds extracted from maize-based beverages with different levels of nejayote solids before and after twelve months of storage time at 25°C tested at 1.0 ng/mL of ferulic acid (**C**). Different letters denote statistical difference (*P*<0.05). Mean value ± SD (n-3).

Rojas-Garcia et al. (2012) reported that the antioxidant activity of nejayote obtained from pigmented maize showed a higher correlation with bound ferulic acid. Recently, Buitimea-Cantúa et al. (2012) demonstrated that the cellular antioxidant activity was directly correlated to the bound ferulic, dehydro-diferulic, and dehydro-triferulic acids present in maize-based beverages supplemented with nejayote solids of white maize.

The cellular antioxidant activity decreased between 55 to 60 % in the BENS supplemented with nejayote solids of different maize genotypes after 12 months of storage time. The storage time affected the content of hydroxycinnamic acids, and maybe other bioactive compounds present in maize, which have been previously identified as the compounds responsible for the antioxidant activity observed in maize and its derivatives (Villela-Castrejón et al., 2017b; Buitimea-Cantúa et al., 2019).

CONCLUSIONS

The maize-based beverages supplemented with nejayote solids of different maize genotypes presented higher calcium content, diverse phytochemicals profiles, as well as interesting anti-inflammatory and antioxidant activities. Among all maize-based beverages, the supplementation of the nejayote solids from red (54.6 %) and high carotenoid (48.9 %) maize presented a higher reduction in the production of nitric oxide compared to maize-beverage control. Likewise, the content of bound ferulic acid, bound coumaric acid, bound 8-5´diFa acid, and bound 5-5´diFa acid in the based-maize beverages were highly correlated to cellular antioxidant activity, as well as the production the nitric oxide, and therefore with the anti-inflammatory potential.

On the other hand, the storage time affected not only the concentration of the main phytochemicals present in the maize-based beverages but also the antioxidant and anti-inflammatory activities exhibited by these beverages. After twelve months of storage time, the based-maize beverages supplemented with nejayote solids from red and high carotenoid maize showed the best potential anti-inflammatory, whereas those based-maize beverages supplemented with nejayote solids from yellow and high carotenoid maize showed the highest cellular antioxidant activity.

These results show that storage stability is a critical parameter when designing new products, in this specific case, in the development of functional beverages, since the concentration of active ingredients and consequently the expected biological activities will be impacted by the degradation of the compounds responsible for these activities. From there arises the need to continue researching effective and scalable strategies to stabilize these compounds during storage. On the other hand, these findings also provide information on strategies to take advantage of agro-industrial waste for its re-incorporation into new value-added food products in line with the concept of circular economy and sustainability on the way to compliance with the Sustainable Development Goals (SDG).

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