Artículo

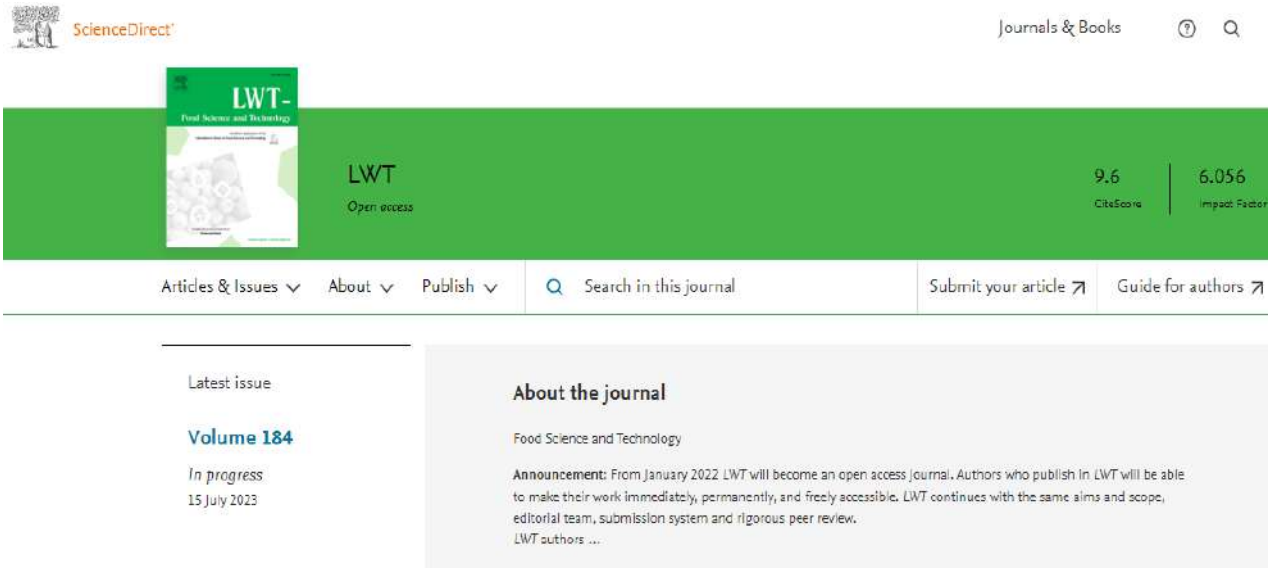
“Nutritional, antioxidant and phytochemical characterization of healthy ready-to-eat expanded snack produced from maize/common bean mixture by extrusion”

1. Contribución al fortalecimiento de la línea de investigación y su relación con los pronaces

En esta investigación es consecutiva de mi tesis doctoral (2da publicación), se elaboró un snack expandido saludable listo para comer utilizando una mezcla de maíz y frijol común (70/30%) y se caracterizó por su valor nutricional, potencial antioxidante y composición fitoquímica, fortaleciendo la línea de investigación de ciencia básica en el desarrollo de alimentos funcionales. Además relacionados con los pronaces “Salud” y “Soberanía alimentaria”, ya que obtuvo un snack expandido a partir de una mezcla de harinas de maíz y frijol común con buenas propiedades nutricionales, un interesante pérfil de fitoquímicos, relacionados a ciertas actividades biológicas como actividad antioxidante, por ello, podría ser utilizado para la promoción de la salud y la prevención de enfermedades crónicas (obesidad, diabétes, etc) que actualmente se padecen en nuestro país. Sumando la revalorización de los granos (maíz y frijol) altamente producidos en México, dando un valor agregado en la potencialización de otros alimentos novesodos, saludables que compitan con los existentes en el mercado.

2. Índices donde se encuentra incorporado

Revista **LWT - *Food Science and Technology***



3. Dictámenes

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Dear Dr. Julio Montes-Avila,

I am pleased to inform you that your paper "Nutritional, antioxidant and phytochemical characterization of healthy ready-to-eat expanded snack produced from maize/common bean mixture by extrusion" has been accepted for publication in LWT.

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Matteo Bordiga, Ph.D.
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Reviewer #1: The revised version of the manuscript LWT-D-20-07501R1 has been modified according the reviewers suggestions and substantially strengthened. So, I would recommend its publication in LWT - Food Science and Technology

Reviewer #3: As the authors have addressed all my previous remarks I do not have any additional comments.

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Nutritional, antioxidant and phytochemical characterization of healthy ready-to-eat expanded snack produced from maize/common bean mixture by extrusion

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ABSTRACT

In this research, a healthy ready-to-eat expanded snack was produced using a maize/common bean (70/30%) mixture and characterized for its nutritional value, antioxidant potential, and phytochemical composition. Free and bound extracts were obtained and analyzed for phenolic profiles by ultra-high performance liquid chromatography with diode-array detector–tandem mass spectrometry (UPLC-DAD-MS^n) and antioxidant activity (IC_{50}) by ABTS and DPPH methods. Fatty acids and amino acid profiles were obtained by gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC), respectively. Fourteen phenolic compounds were identified and quantified (mg/100 g, dw); the main compounds included three phenolic acids (ferulic, diferulic, *p*-coumaric) and three flavonoids (naringenin, kaempferol, methyl isoflavone). The IC_{50} (mg/mL) values obtained by ABTS (4.17 and 0.12) were smaller than those measured by DPPH (5.93 and 0.33). Seven fatty acids were also identified and the two most abundant were unsaturated (oleic, linoleic). The snack also showed an acceptable balance of amino acids according to the FAO, 2013 requirements, as well as a chemical score = 74.09 *in vitro* protein digestibility = 77.21%, C-PER = 1.53 and PDCAAS = 57.20%. The expanded snack could be source of bioactive, nutritional and antioxidant compounds for the improvement of the consumer's health.

1. Introduction

The demand of expanded snacks has increased worldwide in recent years; however, these foods are mostly made of maize and have low protein quality due to deficiencies in essential amino acids (lysine and tryptophan) based on the FAO (2013) recommendations. In addition, these snacks are high energy density foods because maize is rich in starch. However, it has been suggested that maize snacks can be improved by the incorporation of legumes such as beans (Félix-Medina et al., 2020); the incorporation of legume starch as a food ingredient may contribute to decrease the glycemic index due to its slow digestibility (Simons, Hall, & Biswas, 2017).

The combination of maize and beans has been associated with a reduction in the risk of developing certain diseases (e.g., diabetes, obesity, colon cancer, and cardiovascular diseases) when they are consumed on a regular basis (Chen et al., 2014; Das & Singh, 2016; Fan & Beta, 2016; Zillic et al., 2012). This has been attributed to the fact that maize and beans whole grains have bioactive compounds (e.g., phenolic acids, flavonoids, polyunsaturated fatty acids), proteins, minerals and vitamins that have some biological properties such as antioxidant and anti-inflammatory activities, anticancer effects, among others. Despite the potential opportunities of maize/bean mixtures, the commercial

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application of this type of food has been limited. Hence, it is necessary to develop some technological alternatives that allow the production of healthy snacks from mixtures of maize/bean whole grains with the health benefits mentioned above (Simons et al., 2017). Extrusion processing is considered an efficient process to produce expanded foods; it offers great versatility and is capable of causing structural changes of raw materials with an intense mixing for dispersion and homogenization of ingredients (Anton, Fulcher & Arnfield, 2009; Félix-Medina et al., 2020; Korus, Gumul, & Czechowska, 2007).

Different functional properties and applications of expanded snacks produced from maize/bean mixes by the extrusion technology have been reported (Espinoza-Moreno et al., 2016; Rodríguez-Miranda et al., 2014). Most of these studies have focused mainly on the evaluation of physicochemical and sensory properties. Some researchers have studied the influence of extrusion conditions (temperature, screw speed, cutting force) on the properties (expansion index, apparent density, hardness, colour, flavor) of certain expanded snacks prepared with maize/bean mixtures (Estrada-Girón, Martínez-Preciado, Michel, & Soltero, 2015; Pérez-Navarrete, González, Chel-Guerrero, & Betancur-Ancona, 2006); These authors obtained expanded snacks with acceptable physicochemical and sensory characteristics. However, there are few studies about the nutritional and antioxidant value of this type of snacks (Delgado et al., 2012; Delgado-Licon et al., 2009; Félix-Medina et al., 2020) and there are not reports about the analysis of phenolics, amino acids and fatty acids profiles in these food products.

Therefore, the objective of this study was to characterize the amino acids, fatty acids and phenolic profiles, as well as the nutritional value and antioxidant potential of an expanded snack prepared from a maize and common bean (70/30%) mixture by extrusion.

2. Materials and methods

2.1. Materials

White maize (*Zea mays* L.) and common bean (*Phaseolus vulgaris* L., var Azufrado Higuera) grains were purchased at a local market in Culiacan, Sinaloa, Mexico.

2.2. Methods

2.2.1. Expanded snacks (ES) preparation

White maize or common bean kernels (1 kg lots) were grinded to obtain grits that passed through a 40-US mesh (0.425 mm) screen. White maize (WMG) and common bean (CBG) grits were mixed to obtain lots of 250 g (175 g WMG + 75 g CBG). These lots were conditioned with purified water until they reached moisture contents of 18 g H_2O/100 g; each lot was packed in a polyethylene bag and stored at 4 °C for 12 h. Extrusion cooking was carried out in a single screw laboratory extruder Model 20 DN (CW Brabender Instruments, Inc., NJ, USA) equipped with a 19 mm diameter screw, 20:1 length/diameter, 3:1 nominal compression ratio, and 3 mm die opening. Expanded snacks (ES) were produced using optimized extrusion conditions, and they were obtained in a previous study by Félix-Medina et al. (2020): barrel temperature (BT = 164 °C) and screw speed (SS = 187 rpm). ES were cooled, equilibrated (25 °C, RH = 65%, 1 h) and packed in hermetic plastic bags until use.

2.2.2. Extraction of free and bound phenolic compounds

Free and bound phenolics extracts (FPE - BPE) were obtained according to Espinoza-Moreno et al. (2016). Flour samples (0.5 g) of unprocessed and expanded snack from maize/common mixture were mixed with 10 mL of 80% (v/v) chilled ethanol, stirred (10 min, 300 rpm), centrifuged (2500 x *g*/15 min); this extraction process was repeated two times more from some 0.5 g of sample. After, the supernatant of each steps were mixed, concentrated to dryness and stored until evaluation. The residues were used to obtain the extracts of bound phenolic compounds; they were hydrolyzed for 30 min with 10 mL of 2 M NaOH at 95 °C, acidified with 2 mL of 2 M HCl, and then defatted using hexane. The final mixture was extracted five times with 10 mL of ethyl acetate; the supernatant was recovered and concentrated at 35 °C. The extracts of free and bound phenolic compounds were stored and reconstituted in 2 mL of methanol before use. The extractions were made by triplicate.

2.2.3. Phenolic profile by UPLC-DAD-ESI-MS^n

A 500 μL aliquot of FPE and BPE was analyzed by ultra-performance liquid chromatography coupled to diode array detection and mass spectrometry (UPLC-DAD-ESI-MS^n) according to Quintero-Soto et al. (2018). The separation was performed using a C18 column (3 μm, 50 × 2.1 mm) (Fortis Technologies Ltd., Cheshire, UK) with mobile phases consisting of 60:40 ratio of formic acid 1% (v/v) (A) and acetonitrile (B). The samples and solvents were filtered using a PVDF membrane (17 mm × 0.45 μm, TITAN) (Thermo Scientific Inc., USA). The elution gradient started with 95% of A and 0.5% of B until it reached 40% of A and 60% of B at 40 min. The flow rate was 0.2 mL/min and injection volume was 10 μL. The compounds were detected at 280, 320 and 350 nm. The identification was based on the retention time, UV-spectra, mass spectrometry data (fragmentation patterns) and literature reports. Gallic acid and kaempferol were used as internal standards. The system was connected to a mass spectrometer with an electrospray ionization source (ESI) (LTQ XL, Thermo Scientific, USA) operating in positive/negative mode (35 V, 300 °C). The full scan spectra were obtained in negative mode over the range *m/z* 110 to 2000. Data were acquired and analyzed by the Xcalibur 2.2 software (Thermo Scientific, USA). Finally, for the spectrometry analysis (MS^n) the main ions were selected and fragmented by collision-induced dissociation adjusting 10–45 V. Helium and nitrogen were used for the collision and drying, respectively.

The compounds were quantified using calibration curves prepared with commercial standards (Sigma Aldrich, St. Louis, MO, USA), and the results were expressed as micrograms per gram of sample on a dry weight basis (μg/g dw).

2.2.4. Antioxidant activity (AoxA)

The antioxidant activity (AoxA) of FPE and BPE was evaluated by the ABTS [2,2′-azino-*bis*(3-ethylbenzothiazolin)-6-sulfonic acid and DPPH (2,2-diphenyl-1-picrylhydrazyl) methods (Félix-Medina et al., 2020). The two assays were performed using 96-well microplates and the AoxA values were expressed as IC_{50}. The inhibitory activity (inhibition of the color of free radical) of the FPE and BPE was calculated by the following equation:

$$\text{Inhibitory activity (\%)} = \{(Abs_{blank} - Abs_{sample}) / Abs_{blank}\} \times 100$$

Where: Abs_{sample} = Absorbance of free radical + phenolic extract; Abs_{blank} = Absorbance of free radical without phenolic extract.

Different concentrations of the phenolic extracts (g/mL) were plotted versus the corresponding inhibitory activity values (%), and the dose response curves were obtained by nonlinear sigmoid regression with Prism v5 (GraphPad Prism). The IC_{50} value was calculated as the concentration of phenolic extract that caused an inhibition of 50% in the color of the free radical. All determinations were made by triplicate.

2.2.5. Fatty acids profile by GC-MS analysis

Fatty acids were analyzed as described by López-Angulo et al. (2016) with different operating conditions. Hexanic extracts (HE) of the expanded snack (ES) and unprocessed grains mixture (UGM) were obtained by sonication. The flours (5 g) were mixed with 20 μL of the internal standard *n*-decanoic acid (3 mg/mL) and extracted with 15 mL of hexane. The mixtures were agitated, sonicated (15 min) and centrifuged (15.000 x *g*/15 min); the supernatants were concentrated in a vacuum oven at 40 °C and stored until use. The extractions were made by triplicate. For derivatization, HE sample (5 mg) was dissolved in 50 μL of pyridine and 50 μL of *N,O*-bis(trimethylsilyl)trifluoroacetamide + 1%

trimethylsilyl chloride and heated at 70 °C for 4 h. The solvent was eliminated using a N_2(g) stream, the residue was re-dissolved in 1 mL of hexane and filtered through a PVDF syringe filter (17 mm × 0.45 μm, TITAN) (Thermo Scientific Inc., USA) prior to GC-MS analysis. A 5 μL aliquot of the hexanic extract was injected without flow division into a Gas Chromatography Mass Spectrometry (GC-MS) system (HP 6890 GC Instrument, 5973 Network, Agilent Technologies, USA) and separated using a capillary column QUADREX 007 CARBOWAX 20M (30 m × 0.25 mm i.d., film thickness 0.25 μm) (Quadrex Corporation, USA). Helium was used as carrier gas (0.5 mL/min). The operation temperatures were: injector, 250 °C; oven, initial 70 °C, kept for 1 min, 5 °C/min to 200 °C, 10 °C/min to 280 °C, and held at 280 °C to the end of the analysis; ion source, 250 °C; and quadrupole, 150 °C. MS detection was performed in Electron Impact mode (EI) at 70 eV ionization energy, and operating in full-scan mode in the 50–800 amu range. Decanoic acid was used as internal standard.

The sample components were identified by comparison with the mass spectra in the National Institute of Standards and Technology Library (NIST08.LIB). The compounds were quantified using calibration curves constructed with commercial standards (Sigma Aldrich, St. Louis, MO, USA) and the results were expressed as milligrams per 100 g of sample on a dry weight basis (mg/100 g, dw).

2.2.6. Nutritional characterization: essential amino acids (EAA), in vitro protein digestibility (IVPD), chemical score (CS), calculated protein efficiency ratio (C-PER) and protein digestibility corrected amino acid score (PDCAAS)

The EAA content, IVPD, CS and PDCAAS in the unprocessed grains mixture (UGM) and the ES was determined using the methods described by Salas-López et al. (2018) using an HPLC-LC 5100 equipped with a fluorescence detector (GBC, Dandenong, Australia) set at 270 and 316 nm for excitation and emission, respectively; the separation was performed using an analytical column (4.6 × 250 mm) SGE Hypersil ODS C18 (SGE, Dandenong, Australia). The mobile phases used were: 30 mM ammonium phosphate (pH 6.5) in 15:85 (v/v) methanol:water (A); methanol:water 15:85 (v/v) (B) and acetonitrile:water 90:10 (v/v) (C). The initial eluent phase composition was 16.5/69/14.5 (A/B/C) followed by a gradual change to 11/44/45 at 26 min. From 26.1 to 30 min the eluent was 100% of B. Then, the eluent returned to its initial composition at a final time of 43 min. Tryptophan was detected at 280 nm with an ultraviolet detector. The identification was based on the retention time, UV-spectra and by comparison with commercial standards and literature reports. The compounds were quantified using calibration curves of commercial standards (Sigma Aldrich, St. Louis, MO, USA), and the results were expressed as milligrams per 100 g of sample on a dry weight basis (mg/100 g, dw).

The *in vitro* protein digestibility (IVPD) was evaluated as described using a multi-enzyme system. The chemical score (CS) was calculated based on the limiting EAA according to the recommendations of the FAO (2013) for children of 3 years-old and older. It was calculated as follows: CS = (Most limiting EAA content/Recommended EAA requirement) × 100. The protein efficiency ratio (C-PER) of the UGM and ES were calculate considering the values of IVPD and EAA composition obtained for the same samples. Protein digestibility corrected amino acid score (PDCAAS) was determined in the UGM and ES; it was calculated considering the total EAA content and the limiting EAA (g/100 g protein) of the sample, in relation to the same EAA of a reference protein (FAO, 2013) and multiplied by the IVPD value. All determinations were made in triplicate.

2.2.7. Statistical analysis

The results of phenolic compounds content and antioxidant activity were analyzed by two-way analysis of variance (ANOVA) and multiple comparison of means using the Tukey test ($p \leq 0.05$) by the general linear method (GLM) of MINITAB 17.0 software. The t-student test ($p \leq 0.05$) was used for analysis of fatty acids and essential amino acids data. All evaluations were done by triplicate.

3. Results and discussion

3.1. Phenolic profile

A total of 14 phenolic compounds were identified and quantified in the UGM and the ES by UPLC-DAD-MS (Table 1). The FPE of the unprocessed sample showed eight compounds (3 phenolic acids and 5 flavonoids) and that of the processed sample only six compounds (2 phenolic acids and 4 flavonoids) (Fig. 1). The BPE of both UGM and ES samples showed 14 compounds (7 phenolic acids and 7 flavonoids) (Fig. 2).

The chromatographic peaks 1, 3, 9, and 11 (Figs. 1 and 2) showed $[M\text{-}H]^-$ ions at m/z = 325, 359, 447 and 433, that corresponded to glycosides of *p*-coumaric acid, syringic acid, kaempferol and naringenin, respectively. These compounds were previously identified and quantified in other bean varieties in native form (Chen et al., 2014; Shahidi & Yeo, 2016) and after thermal processing (Korus et al., 2007; Xu & Chang, 2009; Žilić et al., 2013). After the MS^2 analysis of these ions, a loss was observed at m/z = 162 corresponding to a hexose, generating the aglycones at m/z = 163, 197, 285 and 271, respectively (Abu-Reidah, Arráez-Román, Lozano-Sánchez, Segura-Carretero, & Fernández-Gutiérrez, 2012; Aguilera et al., 2011; Mekky et al., 2015). The peaks 4, 5, 6 and 7 showed molecular ions at m/z = 163, 193, 223 and 163 corresponding to the acids *p*-coumaric, ferulic, sinapic and 2-hydroxycinnamic, respectively. Žilić et al. (2013) and Korus et al. (2007) identified and quantified some of these compounds in extrudates of soybean and dry beans, respectively, although they identified the compounds only by comparison with standards using HPLC. The peaks 2, 8, 10, 13 and 14 correspond to isoflavones. The peak 2 showed an ion at m/z = 577 and the MS^2 generated an ion at m/z = 253, corresponding to the isoflavone daidzein; this compound was identified as daidzein dihexoside. The peaks 8 and 10 have an ion at m/z = 283 and the MS^2 generated a loss at m/z = 15 corresponding to a methyl group; this compound was previously identified by Mekky et al. (2015) as methyl isoflavone isomer I and II. The peak 13 showed an ion at m/z = 283 and the MS^2 showed a fragmentation pattern of m/z = 268, 250 and 239; these fragments match those reported by Quintero-Soto et al. (2018) for the isoflavone Biochanin A. There are no reports showing the phenolic profiles of expanded snacks from maize/bean mixtures. Nonetheless, the analysis by UPLC-DAD-MS used in the present study could be a useful tool to provide insights into the extrusion process-induced changes in the composition of the mixtures used for ES preparation.

Table 2 shows the content of phenolic compounds present in the UGM and ES samples. The levels of these compounds individually were affected significantly ($p < 0.05$) by the extrusion process in both FPE and BPE. However, the content of total phenolics was significantly ($p < 0.05$) affected by the extrusion process only in the FPE. A similar behavior was observed by other authors (Delgado-Licon et al., 2009; Espinoza-Moreno et al., 2016; Félix-Medina et al., 2020) in the total phenolic content of free and bound extracts obtained from expanded snacks prepared with maize and beans mixtures. The extrusion processing conditions (humidity, temperature, screw speed) have a crucial impact on the levels of bioactive compounds (*e.g.* phenolic acids, flavonoids) by releasing and/or degrading them (Korus et al., 2007). The most abundant compound in the FPE was kaempferol hexoside with values of 12.15 and 3.93 mg/100 g in UMG and ES samples, respectively. These values are higher than those reported by Amarowicz et al. (2009, 2010) for red and green lentils. In the case of the BPE, the major compound was ferulic acid with values of 110.45 and 96.19 mg/100 g in UMG and ES samples, respectively. Ferulic acid has been reported as the main compound in bound extracts and is very characteristic in cereals such as maize (Kasprzak et al., 2018; López-Martínez et al., 2009; Shahidi & Yeo, 2016). The levels of ferulic acid found in this study were similar to those reported by López-Martínez et al. (2009) for 18 maize genotypes.

Table 1
Phenolic compounds identified by UPLC–ESI-MS in free and bound extracts of unprocessed grains mixture (UGM) and expanded snack (ES) (70% maize + 30% bean).

Peak #	RT (min)	λ_{max} (nm)	Experimental *m/z* [M-H]¯	Theorical Mass (g/mol)	Fragments Ions	Proposed compound	FPE		BPE		Reference
							UGM	ES	UGM	ES	
1	12.36	274	325	326	163 (58), 119 (46)	*p*-Coumaric acid hexoside	–	–	+	+	(Yasir, Sultana, & Amicucci, 2016)
2	14.21	287	577	578	415 (56), 253 (40), 197 (35), 135 (48), 133 (40)	Daidzein dihexoside	+	–	+	+	PubChem CID 44257218
3	17.49	288, 320	359	360	197 (9), 182 (28), 153 (22)	Syringic acid hexoside	+	–	+	+	PubChem CID 117927266
4	20.79	308	163	164	146 (90), 119 (20)	*p*-Coumaric acid	–	–	+	+	Mekky et al., 2015, Yasir et al., 2016
5	24.12	320	193	194	175 (100), 115 (42), 143 (49)	Ferulic acid	+	+	+	+	Chen et al., 2014, Mekky et al.,2015
6	25.84	322	223	224	208 (51), 193 (51), 164 (20)	Sinapic acid	–	–	+	+	Mekky et al., 2015, Yasir et al., 2016
7	27.01	276, 323	163	164	146 (41), 122 (23), 102 (3)	2-Hydroxycinnamic Acid	–	–	+	+	PubChem CID 637540
8	28.93	321	283	284	268 (28), 239 (20), 164 (20), 132 (84)	Methyl isoflavone isomer I	+	+	+	+	Mekky et al. (2015)
9	30.18	265, 344	447	448	285 (100)	Kaempferol hexoside	+	+	+	+	Aguilera et al., 2011, Abu-Reidah et al., 2012
10	31.52	318	283	284	268(31), 239(7), 151 (13), 132(10), 117(9)	Methyl isoflavone isomer II	+	+	+	+	Mekky et al. (2015)
11	31.89	320	433	434	271(22), 146(27), 119 (16)	Naringenin hexoside	+	+	+	+	Abu-Reidah et al. (2012)
12	32.55	322	385	386	192(100), 145(43.50)	Diferulic acid	+	+	+	+	PubChem CID 10475220
13	33.72	319	283	284	268(30.17), 250(27.42), 239(40.22)	Biochanin A	–	–	+	+	Mekkyy et al., 2015, Quintero-Soto et al., 2018
14	35.66	322	281	282	251(62.0, 17940.41), 101(10.71)	Dimethoxy isoflavone	–	–	+	+	PubChem CID 6710704

FPE, free phenolics extract. BPE, bound phenolics extract.
RT: retention time (min).
– Not Detected; + Detected.

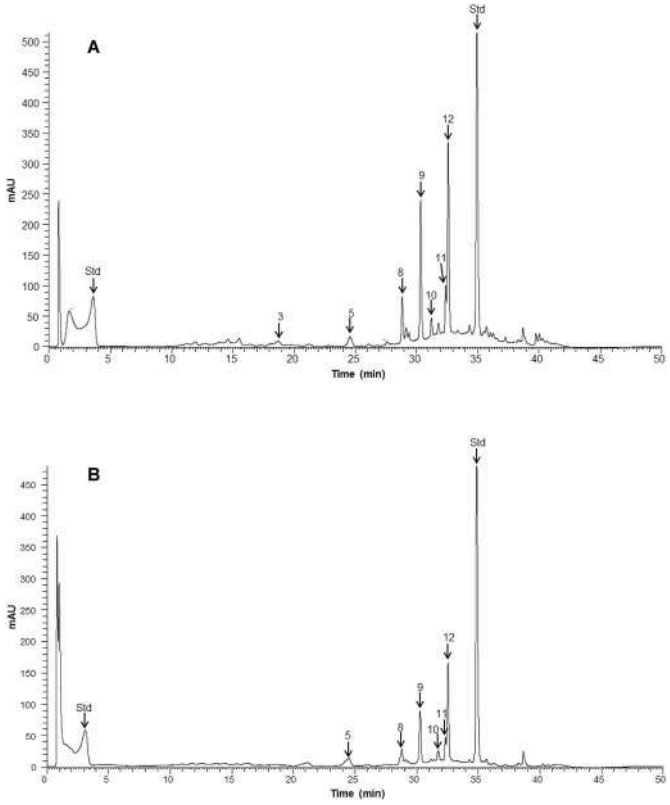

Fig. 1. Representative UPLC-DAD chromatograms of the free phenolic extracts (FPE) from the mix of unprocessed grains (A) and expanded snack (B). Gallic acid and kaempferol were used as internal standards (Std), respectively.

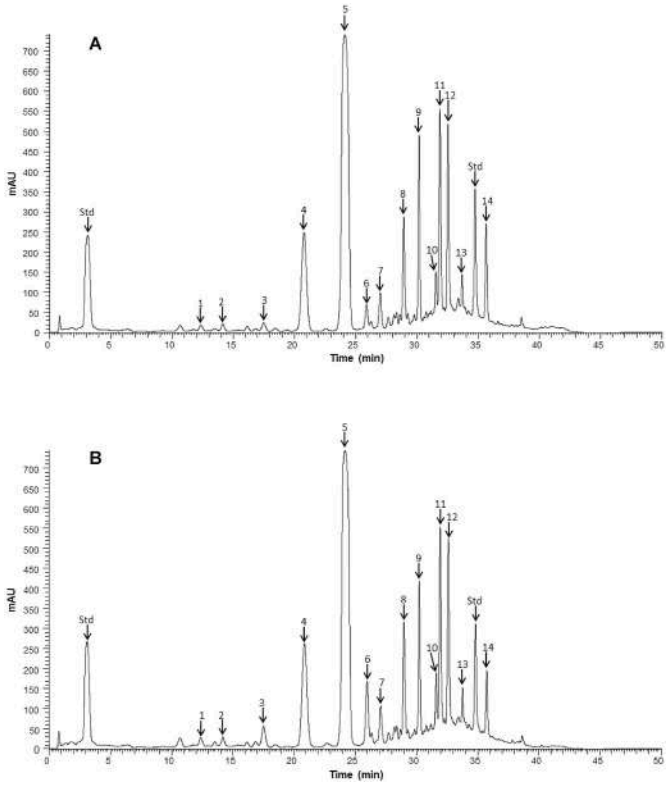

Fig. 2. Representative UPLC-DAD chromatograms of the bound phenolic extracts (BPE) from the mix of unprocessed grains (A) and expanded snack (B). Gallic acid and kaempferol were used as internal standards (Std), respectively.

Table 2
Concentration of phenolic compounds and antioxidant activity (IC_{50}) in free and bound extracts of unprocessed grains mixture (UGM) and expanded snack (ES) (70% maize + 30% bean).

Phenolic compounds (mg/100 g, dw)	Unprocessed grains mixture		Expanded snack	
	FPE	BPE	FBE	BPE
Phenolic acids				
p-Coumaric hexoside	ND	2.27 ± 0.03[b]	ND	2.56 ± 0.07[a]
Sinapic	ND	9.50 ± 0.28[b]	ND	16.01 ± 0.37[a]
2-Hydroxycinnamic	ND	5.59 ± 0.23[a]	ND	5.56 ± 0.28[a]
p-Coumaric	ND	21.29 ± 0.29[a]	ND	20.75 ± 0.27[b]
Syringic hexoside	2.83 ± 0.10[c]	5.22 ± 0.19[b]	ND	8.30 ± 0.19[a]
Ferulic	1.22 ± 0.04[c]	110.45 ± 3.19[a]	1.50 ± 0.04[c]	96.19 ± 3.21[b]
Diferulic	10.58 ± 0.21[c]	19.13 ± 0.27[b]	3.31 ± 0.11[d]	21.31 ± 0.29[a]
Flavonoids				
Biochanin A	ND	3.63 ± 0.13[b]	ND	4.75 ± 0.20[a]
Dimethoxy isoflavone	ND	8.28 ± 0.28[a]	ND	7.69 ± 0.24[b]
Daidzein dihexoside	1.22 ± 0.03[b]	1.89 ± 0.04[a]	ND	1.87 ± 0.06[a]
Methyl isoflavone isomer I	2.73 ± 0.08[c]	10.77 ± 0.24[a]	1.42 ± 0.01[d]	8.99 ± 0.29[b]
Kaempferol hexoside	12.15 ± 0.26[b]	20.25 ± 0.39[a]	3.93 ± 0.11[c]	20.10 ± 0.47[a]
Methyl isoflavone isomer II	1.60 ± 0.05[c]	5.48 ± 0.05[b]	1.29 ± 0.01[d]	6.00 ± 0.05[a]
Naringenin hexoside	4.31 ± 0.17[b]	37.07 ± 0.99[a]	0.76 ± 0.02[c]	36.83 ± 0.88[a]
Total phenolic	36.64 ± 0.94[b]	260.83 ± 3.60[a]	12.21 ± 0.30[c]	256.69 ± 3.89[a]
Antioxidant activity IC_{50} (mg/mL)				
ABTS	4.78 ± 0.151[a]	0.09 ± 0.007[c]	4.17 ± 0.069[b]	0.12 ± 0.001[c]
DPPH	7.56 ± 0.318[a]	0.19 ± 0.011[c]	5.93 ± 0.007[b]	0.33 ± 0.016[c]

Data are the mean ± SD (n = 3). [a-b]Values with different superscript letters in the same row indicate significant differences ($p \leq 0.05$) based on the Tukey's multiple range test.
FPE, free phenolics extract. BPE, bound phenolics extract.
ND, not detected.

Syringic acid and its derivative syringic acid hexoside are also commonly found in maize. Syringic acid hexoside was not detected in the FPE of the ES sample, although its content in the BPE of the same sample (8.30 mg/100 g) was higher than the sum of the two extracts in the UGM sample [(2.88, FPE) + (5.22, BPE) mg/100 g]. These values correspond to those reported by Quintero-Soto et al. (2018) for 18 chickpea genotypes. It has been reported that legumes such as beans are good sources of phenolic compounds (Abu-Reidah et al., 2012; Mekky et al., 2015; Mojica, Meyer, Berhow, & Mejia, 2015). Besides, five isoflavones were identified in the present study, including Biochanin A that was found only in the bound extracts at a concentration similar to that reported by Aguilera et al. (2011) in processed chickpea. In general, all compounds showed higher contents in the BPE fraction than the FPE fraction in both samples. This result could be due to the degradation and/or production of new compounds as a result of the extrusion processing used to prepare the expanded snack and/or to the extraction technique used to obtain the FPE and BPE.

3.2. Relationship between phenolics content and antioxidant activity (AoxA)

The extrusion processing improved the AoxA of the FPE as evidenced by the significant ($p < 0.05$) reduction in IC_{50} (mg/mL) values obtained by ABTS (4.78–4.17) and DPPH (7.56–5.93) (Table 2); however, extrusion did not affect the AoxA of BPE. The effect of the extrusion processing on the AoxA of the FPE is consistent with previous reports showing an improvement in the AoxA of optimized expanded snacks (Anton, Gary Fulcher, & Arntfield, 2009; Espinoza-Moreno et al., 2016). The low IC_{50} values obtained for both ABTS and DPPH in the BPE (Table 2) indicate the antioxidant potential of this fraction. The higher AoxA observed in the BPE than the FPE in both UGM and ES could be associated to the phenolic profiles where a higher number of compounds were identified in the bound fraction of both samples. Bound phenolics have been reported to contain primarily highly reactive antioxidant substances leading to greater free radical scavenging than free phenolics (Xu & Chang, 2009). This could be attributed to the fact that the bound compounds are removed from the cell walls during extraction, besides the fact that during the extrusion processing the less exposed part is found in that fraction (Das & Singh, 2016; Korus et al., 2007).

Fan and Beta (2016) reported that phenolic acids present in both free and bound fractions showed antioxidant activity in three different varieties of common bean (*Phaseolus Vulgaris* L.), where ferulic, synapic and *p*-coumaric acids contributed most of the AoxA. In the present investigation, these compounds were the most abundant in BPE. Similarly, Zilic et al. (2012) analyzed the antioxidant potential of phenolic acids present in white maize (*Zea mays* L.) and reported that ferulic acid had a higher contribution in AoxA, which is consistent with the fact that this compound was the most abundant in the present study. Several authors have suggested that the consumption of these grains provide health benefits associated with phenolic compounds that exhibit high antioxidant capacity and reduce oxidative stress (Chen et al., 2014; Das & Singh, 2016; Gao, Ma, Wang, & Feng, 2017; Mojica et al., 2015). In this study, the AoxA of the ES appears to be associated mostly with the content of phenolics; hence the ES could be considered a good source of antioxidant compounds.

3.3. Fatty acids profile

Seven fatty acids were identified by GC-MS in the UGM and ES samples (Table 3); they included three saturated (myristic, palmitic, and stearic), one unsaturated (oleic) and three polyunsaturated (linoleic, linolenic, and arachidonic). The most abundant fatty acids in UGM and ES were linoleic acid (22.0 mg/100 g) and oleic acid (3.02 mg/100 g). Simons, Hall & Biswas. (2017) also found these fatty acids were the most abundant in bean extrudates. The extrusion process used to prepare the

Table 3
Fatty acids profile identified by GC-MS in unprocessed grains mixture (UGM) and expanded snack (ES) (70% maize + 30% bean).

Peak	Compound[A]	Rt[B]	Unprocessed grains mixture mg/100 g	Expanded snack mg/100 g
1	Myristic	26.08	9.69 ± 0.20[a]	1.84 ± 0.01[b]
2	Palmitic	31.46	5.39 ± 0.92[a]	0.31 ± 0.07[b]
3	Linoleic	38.78	22.04 ± 5.19[a]	1.50 ± 0.09[b]
4	Linolenic	38.89	3.79 ± 0.79[a]	0.17 ± 0.04[b]
5	Oleic	39.29	13.39 ± 3.20[a]	3.02 ± 0.81[b]
6	Stearic	40.96	0.63 ± 0.18[a]	0.05 ± 0.03[b]
7	Arachidic	46.65	0.40 ± 0.07[a]	0.19 ± 0.08[b]

Data are the mean ± SD (n = 3).
[a-b]Values with different superscript letters in the same row indicate significant differences ($p \leq 0.05$) based on the Tukey's multiple range test.
[a] Compounds are listed in order of elution.
[b] RT, retention time (min).

ES caused a significant ($p < 0.05$) decrease (up to 95.5%) in all the fatty acids identified. The decrease in lipids content has been already reported by other researchers in extruded snacks (Espinoza-Moreno et al., 2016; Félix-Medina et al., 2020; Simons et al., 2017). This could be attributed to the effect of the extrusion conditions (temperature, screw speed, pressure, shear force) that can cause the formation of lipid complexes (amylose-lipids and proteins-lipids) that hinders the extractability and quantification of lipids (Bhatnagar & Hanna, 1994; Izzo & Ho, 1989), as well as to the oxidation and/or thermal degradation of certain lipids (Estrada-Girón et al., 2015). However, the reduction of available lipids can decrease the oxidation potential of these compounds and, therefore, favor the useful life of the final product (Simons et al., 2014). Thus, the ES could be a good alternative to the high-caloric snacks (cookies, fries and others) currently consumed.

3.4. Essential amino acids (EAA) profile, in vitro protein digestibility (IVPD), chemical score (CS), calculated protein efficiency ratio (C-PER) and protein digestibility corrected amino acid score (PDCAAS)

The essential amino acid profiles of the UGM and ES are shown in Table 4. The extrusion process caused a non-significant ($p > 0.05$) decrease in most essential amino acids (EAA) during the production of ES from UGM. However, the reduction (−2.17 to −15.99%) of certain EAA such as lysine could be due to the Maillard reaction, where lysine reacts with reducing sugars via its ϵ position amino group (Simons et al., 2017). These results are similar to those reported by Ruiz-Ruiz et al. (2008), who obtained reductions of up to −15.5% in EAA in extruded snacks made from quality protein maize (QPM) and common beans. Simons et al. (2017) and Ilo and Berghofer (2003) reported between −10 and −46% decreases of EAA in from bean and corn extruded products, respectively. However, it has been reported that the decrease and/or loss of certain EAA is directly proportional to the intensity of the thermo-mechanical process, where temperature, humidity content and residence time in the extruder (screw speed) have the greatest impact (Ilo & Berghofer, 2003; Ruiz-Ruiz et al., 2008).

On other hand, *in vitro* protein digestibility (IVPD) values in ES were significantly higher ($p < 0.05$) than UGM (77.21 vs 75.17%, respectively) (Table 4). Similar results have been reported for extruded snacks from maize/bean mixtures. Pérez-Navarrete et al. (2006) reported 82% digestibility in corn and lime bean extrudates (50/50, w/w); Ruiz-Ruiz et al. (2008) extruded QPM and common bean (60/40, w/w) at 170 °C and the product showed 80% IVPD. Chau and Cheung (1997) indicated that the digestibility is limited by the globular structure of legume proteins and the presence of anti-nutritional factors (protease inhibitors, polyphenols, phytates, etc.). The improvement in protein digestibility observed in the present study could be due to two phenomena caused during the extrusion process (temperature and cutting force): 1) denaturation of the protein, which can increase the exposure of sites susceptible to enzyme activity (Alonso, Aguirre, & Marzo, 2000); and 2) inactivation of trypsin and chymotrypsin inhibitors, promoting better digestibility (Pérez-Navarrete et al., 2006). Because some antinutritional compounds present in bean may produce adverse effects in the human health, trypsin inhibitors activity (TIA) and lectins (LA) were measured following the procedure by Anton, Fulcher & Arnfield (2009) and Espinoza-Moreno et al. (2016), respectively. In this regard, the extrusion cooking inactivated completely the TIA [2.57 trypsin inhibitory units (TIU)/mg to UMG vs 0 TIU/mg to ES] and reduced −98% the LA [320 hemagglutinin activity units (HAU)/g to UMG vs 5 HAU/g to ES] present in the UMG. These results are conclusive and agree with what has already been reported in the literature by other researchers (Anton, Fulcher & Arnfield, 2009; Delgado et al., 2012; Espinoza-Moreno et al., 2016) and denote the potential use of extrusion processing for this type of snack.

The extrusion process used to produce the ES did not cause a significant ($p > 0.05$) increase in the calculated protein efficiency (C-PER) with respect to the UGM (Table 4). This behavior in the C-PER could be related to the fact that extrusion decreased slightly the content of essential amino acids, but it improved the nutritional or digestibility value of the protein. In the same sense, protein digestibility corrected amino acids score (PDCAAS) in ES did not show significant differences with respect to UGM. Regarding the nutritional value, the extrusion processing allowed the generation of potentially nutritious products since the ES meets the minimum requirements of essential amino acids for children over 3 years and adults as recommended by the FAO/WHO (2013), except for Lys, which was the limiting amino acid with a chemical score of 74.09. In general, the extrusion process improved the *in vitro* digestibility of the ES protein and did not affect significantly its amino acid content (except Lys, Leu and Val), C-PER and PDCAAS. Based on these results, the ES could be an alternative food of high nutritional value.

Table 4
Nutritional characterization of the unprocessed grains mixture (UGM) and expanded snack (ES) (70% maize + 30% bean)[a].

EAA[c] content (g/100 g protein)	Unprocessed grains mixture	Expanded Snack	EAA requirements (3 years and older)[c]
His	2.53 ± 0.05[a]	2.50 ± 0.02[a]	1.60
Ile	3.07 ± 0.03[a]	3.04 ± 0.04[a]	3.00
Leu	13.44 ± 0.08[a]	11.29 ± 0.05[b]	6.10
Lys	3.67 ± 0.03[a]	3.56 ± 0.03[b]	4.80
Met + Cys	3.07 ± 0.02[a]	3.04 ± 0.03[a]	2.30
Phe + Tyr	7.98 ± 0.07[a]	7.92 ± 0.05[a]	4.10
Thr	3.03 ± 0.02[a]	3.01 ± 0.03[a]	2.50
Trp	0.69 ± 0.03[a]	0.67 ± 0.01[a]	0.66
Val	4.10 ± 0.04[a]	4.01 ± 0.02[b]	4.00
Chemical score[d]	76.45 ± 0.21[a]	74.09 ± 0.19[b]	
Limiting EAA	Lys	Lys	
In vitro protein digestibility (%)	75.17 ± 0.13[a]	77.21 ± 0.15[b]	
C-PER[e]	1.51	1.53	
PADCAAS[f] (%)	57.47	57.20	

Data are the mean ± SD (n = 3).
[a-b] Values with different superscript letters in the same row indicate significant differences at $p \leq 0.05$ using Tukey's multiple range test.
[c]EAA = Essential amino acid.
[d]Based on amino acid scoring pattern for 3 year-old children and older (FAO, 2013).
[e]CPER = Calculated protein efficiency ratio.
[f]Protein digestibility corrected amino acid score; based on amino acid scoring pattern for 3 year-old children and older (FAO, 2013).

4. Conclusions

The results of this research showed the utility of the extrusion technology to produce an expanded snack from a maize-bean mixture (70/30) with an acceptable nutritional balance, good antioxidant properties, and important bioactive compounds (phenolics, flavonoids and polyunsaturated fatty acids). This is the first study describing the phenolic profiles of expanded snacks from a maize-bean mixture. These compounds have important functions in the human body and help to reduce chronic-degenerative diseases due to their antioxidant potential. Therefore, the extrusion processing is an option for making expanded snacks that could be an ideal vehicle to improve the health of consumers of these type of foods.

CRediT authorship contribution statement

Jennifer V. Félix-Medina: Methodology, Investigation, Software, Formal analysis, Data curation, Writing - original draft, Visualization. **Roberto Gutiérrez-Dorado:** Conceptualization, Supervision, Validation, Resources, Data curation, Writing - review & editing, Visualization. **José A. López-Valenzuela:** Writing - review & editing. **Gabriela**

López-Ángulo: Validation, Resources, Formal analysis, Data curation, Visualization. **María F. Quintero-Soto:** Software, Formal analysis, Data curation, Visualization. **J. Xiomara K. Perales-Sánchez:** Validation, Resources, Data curation, Writing - review & editing. **Julio Montes-Ávila:** Conceptualization, Supervision, Software, Validation, Formal analysis, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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