



## Bioactive ingredients of huitlacoche (*Ustilago maydis*), a potential food raw material

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### ABSTRACT

Huitlacoche (*Ustilago maydis*) is a staple food traditionally consumed in Mexico. On one hand, it is considered a pathogen and a devastating disease for maize (*Zea mays*), and, conversely, some reports highlight it as a culinary delight and a food alternative for its nutritional and functional characteristics. The present work aimed to compare two different methods: maceration and ultrasound-assisted extraction (UAE), in order to achieve the highest amount of total phenolic compounds (TPC), followed by the antioxidant capacity (AC) measurement, as well as the effect of the *in vitro* digestion. UAE produced the highest TPC content (13.44 mg GAE/mg ds) for a 1:5 huitlacoche:solvent ratio using ethanol at 75%. The maximum values for AC were 22.5 mg TE/mL (for DPPH assay) and 45.26 mg TE/mL (for ABTS assay). The *in vitro* digestion showed that AC is maintained and increased through the digestion process. Thus, huitlacoche can be revalorised as a potential food raw material for human nutrition and health.

### 1. Introduction

Mexico is characterised by a vast and wide richness in plant species. Our ancient civilizations have consumed different crops that are still preserved; however, such indigenous edible plants are of low consumption, and, nowadays only consumed for producers (SADER, 2020a). Mushrooms are also included, in particular a member of the Ustilaginales order (SADER, 2020b). Huitlacoche (*Ustilago maydis*) is a fungus (basidiomycete) that infects maize (*Zea mays*), mainly the young plant, which develops malformations, initially pale grey in colour, but as it matures it obtains the black colour that characterises it (SADER, 2015). Huitlacoche is known as the pre-Hispanic delicacy of Mexico, since its consumption is endemic to our country. The diverse cultures established in the Mexican highlands have valued and consumed the mushroom as a traditional food for centuries (SADER, 2020b). In addition to its exquisite flavour, huitlacoche contains: a) essential fatty acids (oleic and linoleic which are sources of  $\Omega$ -3 and  $\Omega$ -6, respectively), b) essential amino acids, c) easily digestible sugars d) fibre, and, e) vitamin C and minerals. Further this important nutritional value, some

functional properties have been demonstrated for huitlacoche, namely, antioxidant, hypocholesterolaemia, immunomodulatory, anticancer, anti-inflammatory, antimicrobial, antidiabetic and antihypertensive. (Juárez-Montiel, Ruiloba de León, Chávez-Camarillo, Hernández-Rodríguez, & Villa-Tanaca, 2011; Lizárraga-Guerra & López, 1996; Valdez-Morales et al., 2010; Venegas, Valverde, Paredes-López, & Pataky, 1995; SADER, 2018; SADER, 2020b; SADER, 2020a). It has been reported and demonstrated that the chemical composition of huitlacoche depends on the type of maize, climatic and geographic conditions, stage of development of galls, and the cooking effect (Venegas, Valverde, Paredes-López, & Pataky, 1995; Valdez-Morales et al., 2010). In consequence, the release of the phytochemical content is also affected. The functionality of phenolic compounds depends on how they are metabolized, and research on bioaccessibility are crucial to validate functional foods health claims (Rein & da Silva Pinto, 2017). *In vitro* gastrointestinal models may be a useful approach to estimate the capacity of phenolic compounds to produce a beneficial effect, mimicking *in vivo* conditions.

Considering that Mexican society faces remarkable public health

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problems, the availability of products such as huitlacoche, constitutes a potential food raw material because it provides nutritional, functional and psychological components for a complete, sufficient and balanced diet. Therefore, it is important to deepen the knowledge of the nutraceutical properties of huitlacoche to generate information that guarantees it as a functional food with a potential for the development of new food products with relevant health promoting effects and sensory characteristics. The aim of the present work was to compare two different methods, maceration (M) and ultrasound-assisted extraction (UAE), to recover the highest amount of total phenolic compounds (TPC), followed by the measurement of antioxidant capacity (AC), and the *in vitro* digestion. Similarly, a chemical characterisation was obtained by proximate analysis. The extraction efficiency was determined by the effects of different huitlacoche:solvent ratios (1:5, 0.25 g/5 mL; 1:10, 0.5 g/ 5 mL and 1:20, 1 g/5 mL) and solvent concentration (ethanol at 50, 75 and 99%, in distilled water), using a full factorial experimental design  $3^2$  with two central points and two replicas. Because huitlacoche is important in local diets, our research intends to promote its consumption in an attempt to revalorise its nutritional and functional properties.

## 2. Materials and methods

### 2.1. Plant material and reagents

Huitlacoche (*Ustilago maydis*) was collected from infected maize plants located at the community of San Francisco Cheje, Jocotitlán, State of Mexico (19°39'33.4" N, 99°43'42.2" W) on August 2019. Huitlacoche was manually removed from the different plants and was homogenised to prepare a single sample. The resulting sample was manually washed twice, first with tap water and secondly, with distilled water, for dirt removal. After that, the sample was dried overnight at 60 °C in an incubator (Felisa model FE-133D; Felisa®, Jalisco, MX). Finally, the sample was ground to a powder (fine flour) in IKA all basic mill (IKA-Works, Inc., Wilmington, NC) for the obtention of the extracts. All the solvents employed were of reagent grade from J.T. Baker (New Jersey, USA).

### 2.2. Proximate analysis

The nutritional content of huitlacoche was determined according to official methods: moisture (NMX-F-083-1986), ash (NMX-F-607-NORMEX-2013), fat (NMX-F-089-S-1978), protein (NMX-F-608-NORMEX-2002), and crude fibre (NMX-F-090-S-1978). Carbohydrates were calculated by difference:  $100 - (\% \text{moisture} + \% \text{ash} + \% \text{fat} + \% \text{protein})$ .

### 2.3. Obtention of the extracts

Two different extraction methods were employed: maceration (M) and ultrasound-assisted extraction (UAE). Extraction process variables included different huitlacoche:solvent ratios (1:5, 0.25 g/5 mL; 1:10, 0.5 g/5mL and 1:20, 1 g/5 mL) and solvent concentration (ethanol at 50, 75 and 99%, in distilled water).

In the case of M, the prepared Falcon tubes were set in a Roto Mix 50,800 (Barnstead Thermolyne, USA) for 30 min at 45 °C and 250 rpm. With respect to UAE, amber vials containing the sample and the hydroethanolic solution were set in a sonicator bath (Sonicator VWR model 150 D; VWR International., West Chester, PA), for 30 min at 45 °C.

The resulting mixtures obtained from both extraction methods were filtered through filter paper #2 (Whatman, Maidstone, UK) and then centrifugated (Eppendorf centrifuge 5804R) at 8 000 rpm during 15 min. The supernatants were collected and stored in amber tubes at 4 °C until used.

### 2.4. Total phenolic compounds (TPC)

TPC were determined for the hydroethanolic extracts adapting the method of Alvarado-López, Gómez-Oliván, Heredia, Baeza-Jiménez, García-Galindo, & Lopez-Martínez, (2019). 20 µL of the extracts were combined with 20 µL of Folin-Ciocalteu reagent and the mixture was incubated for 5 min. Then, 20 µL of 0.01 M Na<sub>2</sub>CO<sub>3</sub> were added and the resulting mixture was incubated for 5 more min. After that, 125 µL of distilled water were added and the absorbance was measured at 790 nm in a microplate reader (Multiskan Go, Thermo Scientific). A standard calibration curve was prepared using gallic acid (0 – 6 mg/mL), and the amount of TPC in each extract was calculated and expressed as mg of gallic acid equivalent per mg of dried sample (mg GAE/mg ds).

### 2.5. Antioxidant capacity (AC) assays

The evaluation of the AC of the hydroethanolic extracts of huitlacoche was measured by the following assays:

**DPPH.** This assay was carried out by adapting the technique of López-Martínez, Oliart-Ros, Valerio-Alfaro, Lee, Parkin, & Garcia, (2009). 7 µL of the hydroethanolic extract were allowed to react with 193 µL of DPPH solution. The mixture was incubated in the dark for 30 min at room temperature, and the decrease in absorbance at 517 nm was determined with a microplate reader (Multiskan Go, Thermo Scientific). A standard calibration curve was prepared using Trolox (0 – 0.4 µmol/mL) to express the results as mg of Trolox equivalents per millilitre (mg TE/mL).

**ABTS.** This assay was performed adapting the protocol reported by López-Martínez et al. (2009). 10 µL of the hydroethanolic extract were allowed to react with 190 µL of ABTS radical. The mixture was incubated for 1 min and after that, the absorbance was measured at 734 nm in a microplate reader (Multiskan Go, Thermo Scientific). A standard calibration curve was prepared using Trolox (0 – 0.7 µmol/mL) to express the results as mg of Trolox equivalents per millilitre (mg TE/mL).

### 2.6. *In vitro* gastrointestinal digestion

Under this model, it was simulated the digestion processes in order to quantify the release (bioaccessibility) of phenolic compounds present in huitlacoche, adapting the procedure developed by Melquiades-Silva de Andrade et al. (2020). Then, the 3-step process carried out for the simulation of the human physiology of the gastrointestinal tract after eating, is detailed in Fig. 1. The digestion began by adding 2 mL of artificial saliva (oral phase) to 3 mL of huitlacoche extracts in digestion tubes, followed by stirring (200 rpm, 2 min, 37 °C, pH 6.0 ± 0.02). For the gastric phase, the resulting mixture was supplemented with 4 mL of gastric juice and then incubated (130 rpm, 120 min, 37 °C, pH 2.0 ± 0.02). Afterwards, for the intestinal phase, 4 mL of intestinal juice were added and incubated (45 rpm, 120 min, 37 °C, pH 6.0 ± 0.02). At the end of the *in vitro* digestion process, the digestion tubes were centrifuged at 10 000 rpm for 10 min at 4 °C, and the resulting supernatant was kept at -20 °C for further analysis. Samples without the addition of huitlacoche extracts were used as blanks. Each sample was processed in triplicate (n = 3) for a total of 27 independent digestions. At the end of each digestive phase, the whole digested sample in the tube was centrifuged under the aforementioned conditions, and the supernatants were kept frozen until further use (-20 °C).

### 2.7. Experimental design and statistical analysis

A set of 33 treatments in a full factorial experimental design  $3^2$  with two central points and two replicas (see Table 1) was employed to determine the best extraction conditions to reach the maximal amount of TPC and the highest values of AC. The factors were: mass/volume ratio (1:5, 1:10, 1:20) and ethanol concentration (50, 75, 99%). All the experiments were carried out by triplicate. Differences among treatment

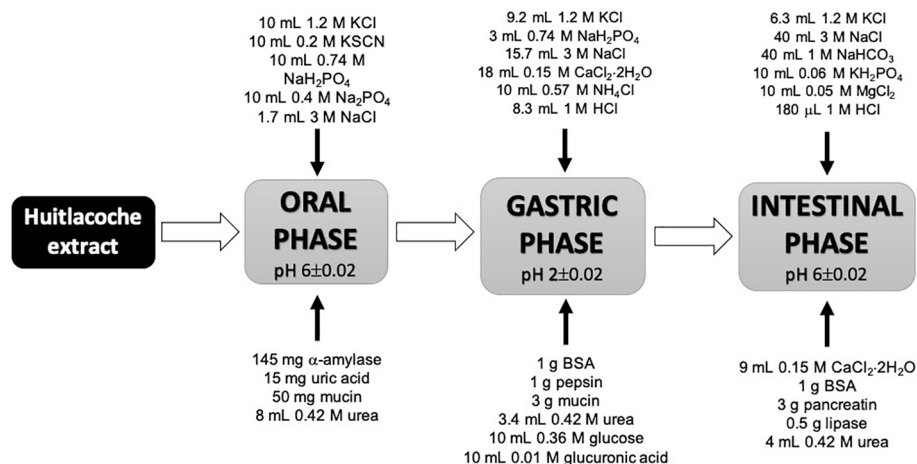


Fig. 1. *In vitro* gastrointestinal digestion simulation.

Table 1

Experimental setup: full factorial experimental design 3<sup>2</sup> with two central points and two replicas.

Experiment	Replica	Ethanol	Ratio (m/V)
1	1	-1	-1
2	1	-1	0
3	1	-1	1
4	1	0	-1
5	1	0	0
6	1	0	1
7	1	1	-1
8	1	1	0
9	1	1	1
10	1	0	0
11	1	0	0
12	2	-1	-1
13	2	-1	0
14	2	-1	1
15	2	0	-1
16	2	0	0
17	2	0	1
18	2	1	-1
19	2	1	0
20	2	1	1
21	2	0	0
22	2	0	0
23	3	-1	-1
24	3	-1	0
25	3	-1	1
26	3	0	-1
27	3	0	0
28	3	0	1
29	3	1	-1
30	3	1	0
31	3	1	1
32	3	0	0
33	3	0	0
Levels	-1	0	1
Ethanol	50%	75%	99%
Ratio (m/V)	1:5	1:10	1:20

mean values were evaluated through analysis of variance (ANOVA) and Tukey test using the SAS university edition software (SAS Institute Inc., Cary, NC), and statistical significance was set at  $\alpha = 0.05$ .

### 3. Results and discussion

Traditional Mexican gastronomy includes many vegetables and fruits that were the basis of good nutrition in Mesoamerica, and nowadays, they are still part of our culture, identity, and of utmost economic

importance. The present study was undertaken to promote the consumption and preservation of huitlacoche for its attractive sensory attributes as well as its nutritional and functional properties.

#### 3.1. Proximate analysis

The nutritional value of huitlacoche has a great importance for the diet, because of the high prevalence of obesity and diabetes in Mexico. This mushroom has been reported as a high-quality functional food for its selected nutrients, valuable compounds, and nutraceutical potential (Valverde, Hernandez-Perez, & Paredes-Lopez, 2012).

For that reason, the plant material collected for this study was submitted to a proximate analysis and its composition includes: moisture (26.81%), ash (3.37%), fat (0.73%), protein (3.27%), crude fibre (8.61%), and digestible carbohydrates (57.2%), as the main component. The energy supply was calculated, reaching a value of 248.53 cal/100 g. In comparison to other studies related to chemical characterization of huitlacoche, Beas, Loarca, Guzmán, Rodríguez, Vasco, & Guevara (2011), reported an average protein content of 12.4%, 2.9% of crude fat, moisture of 80–86%, total dietary fibre of 54–65%, insoluble dietary fibre of 47–49% and soluble dietary fibre of 8.6–12.5%. From their findings, Aydoğdu & Gölükçü (2017) cited 90% of moisture, 12% of crude protein, 1.8% of total fat and 45% of carbohydrate. The differences observed for the chemical composition of huitlacoche are attributed to the variety of maize and the state of development in which the fungus is harvested, as well as agronomic conditions.

#### 3.2. Total phenolic compounds (TPC) and antioxidant capacity (AC) assays

In addition to its important nutritional properties, huitlacoche has also been characterised as a nutraceutical food and a potential component to supplement other food because of its exquisite flavour and exceptional culinary quality (Valverde, Hernandez-Perez, & Paredes-Lopez, 2015).

To determine the functional properties of our plant material, first, two different extraction methods, M and UAE, were evaluated for the recovery of TPC from huitlacoche extracts. The columns M – TPC and UAE – TPC in Table 2, are the results obtained for TPC by M and UAE, respectively. As it can be noted, there are significant differences among the experiments for both methods. When M was explored, the highest amount of TPC was 13.94 mg GAE/mg ds, under the conditions of experiment 4: 75% of ethanol and a 1:5 ratio. In the case of UAE, the maximal amount of TPC was 7.68 mg GAE/mg ds with experiment 5: 75% of ethanol and a 1:10 ratio.

The results obtained were influenced by the extraction method, plant

Table 2

TPC content and AC of huitlacoche extracts.

Experiment	Ethanol	Ratio (m/V)	M – TPC (mg GAE/mg ds)	UAE – TPC (mg GAE/mg ds)	M – DPPH (mg TE/mL)	UAE – DPPH (mg TE/mL)	M – ABTS (mg TE/mL)	UAE – ABTS (mg TE/mL)
1	–1	–1	3.11 ± 0.07 <sup>g</sup>	0.044 ± 0.04 <sup>d</sup>	7.48 ± 0.13 <sup>d</sup>	22.50 ± 0.42 <sup>a</sup>	15.77 ± 0.04 <sup>c</sup>	23.19 ± 0.11 <sup>b</sup>
2	–1	0	4.21 ± 0.09 <sup>f</sup>	3.33 ± 0.02 <sup>g</sup>	10.70 ± 0.50 <sup>b</sup>	22.31 ± 0.40 <sup>a</sup>	4.78 ± 0.09 <sup>d</sup>	11.74 ± 0.04 <sup>d</sup>
3	–1	1	2.49 ± 0.03 <sup>h</sup>	3.49 <sup>f</sup>	9.45 ± 0.06 <sup>c</sup>	18.54 ± 0.06 <sup>b</sup>	5.48 ± 0.04 <sup>d</sup>	6.12 ± 0.034 <sup>e</sup>
4	0	–1	13.94 ± 0.04 <sup>a</sup>	7.28 ± 0.08 <sup>b</sup>	7.94 ± 0.12 <sup>d</sup>	12.71 ± 0.08 <sup>c</sup>	26.75 ± 0.74 <sup>b</sup>	10.75 ± 0.02 <sup>d</sup>
5	0	0	7.63 ± 0.02 <sup>c</sup>	7.68 ± 0.03 <sup>a</sup>	7.43 ± 0.10 <sup>d</sup>	12.29 ± 0.08 <sup>c</sup>	3.08 ± 0.11 <sup>d</sup>	5.82 ± 0.03 <sup>e</sup>
6	0	1	4.9 ± 0.03 <sup>e</sup>	6.79 ± 0.02 <sup>d</sup>	6.41 ± 0.52 <sup>e</sup>	8.90 ± 0.03 <sup>d</sup>	2.27 ± 0.03 <sup>d</sup>	4.65 ± 0.04 <sup>ef</sup>
7	1	–1	9.21 ± 0.08 <sup>b</sup>	2.52 ± 0.07 <sup>h</sup>	13.16 ± 0.05 <sup>a</sup>	2.50 ± 0.04 <sup>f</sup>	45.26 ± 0.11 <sup>a</sup>	26.45 ± 0.02 <sup>a</sup>
8	1	0	7.08 ± 0.03 <sup>d</sup>	6.91 ± 0.02 <sup>c</sup>	2.75 ± 0.09 <sup>g</sup>	9.47 ± 0.02 <sup>d</sup>	14.56 ± 0.10 <sup>c</sup>	15.28 ± 0.02 <sup>c</sup>
9	1	1	6.95 ± 0.02 <sup>d</sup>	6.07 ± 0.03 <sup>e</sup>	4.12 ± 0.04 <sup>f</sup>	8.19 ± 0.02 <sup>e</sup>	4.93 ± 0.07 <sup>d</sup>	3.68 ± 0.03 <sup>f</sup>
10	0	0	7.52 ± 0.03 <sup>c</sup>	7.64 ± 0.05 <sup>a</sup>	7.37 ± 0.07 <sup>d</sup>	12.48 ± 0.07 <sup>c</sup>	3.08 ± 0.04 <sup>d</sup>	5.79 ± 0.02 <sup>e</sup>
11	0	0	7.63 ± 0.04 <sup>c</sup>	7.59 <sup>a</sup>	7.38 ± 0.08 <sup>d</sup>	12.15 ± 0.08 <sup>c</sup>	3.11 ± 0.21 <sup>d</sup>	5.81 ± 0.02 <sup>e</sup>

**M – TPC:** total phenolic compounds obtained by maceration, **UAE – TPC:** total phenolic compounds obtained by ultrasound-assisted extraction; **M – DPPH:** antioxidant capacity under DPPH assay for maceration, **UAE – DPPH:** antioxidant capacity under DPPH assay for ultrasound-assisted extraction; **M – ABTS:** antioxidant capacity under ABTS assay for maceration, **UAE – ABTS:** antioxidant capacity under ABTS assay for ultrasound-assisted extraction.

**mg GAE/mg ds:** mg of gallic acid equivalent per mg of dried sample; **mg TE/mL:** mg of Trolox equivalents per millilitre.

Data is expressed as mean ± sd of 3 independent experiments.

Different letters indicate significant differences ( $p < 0.05$ ).

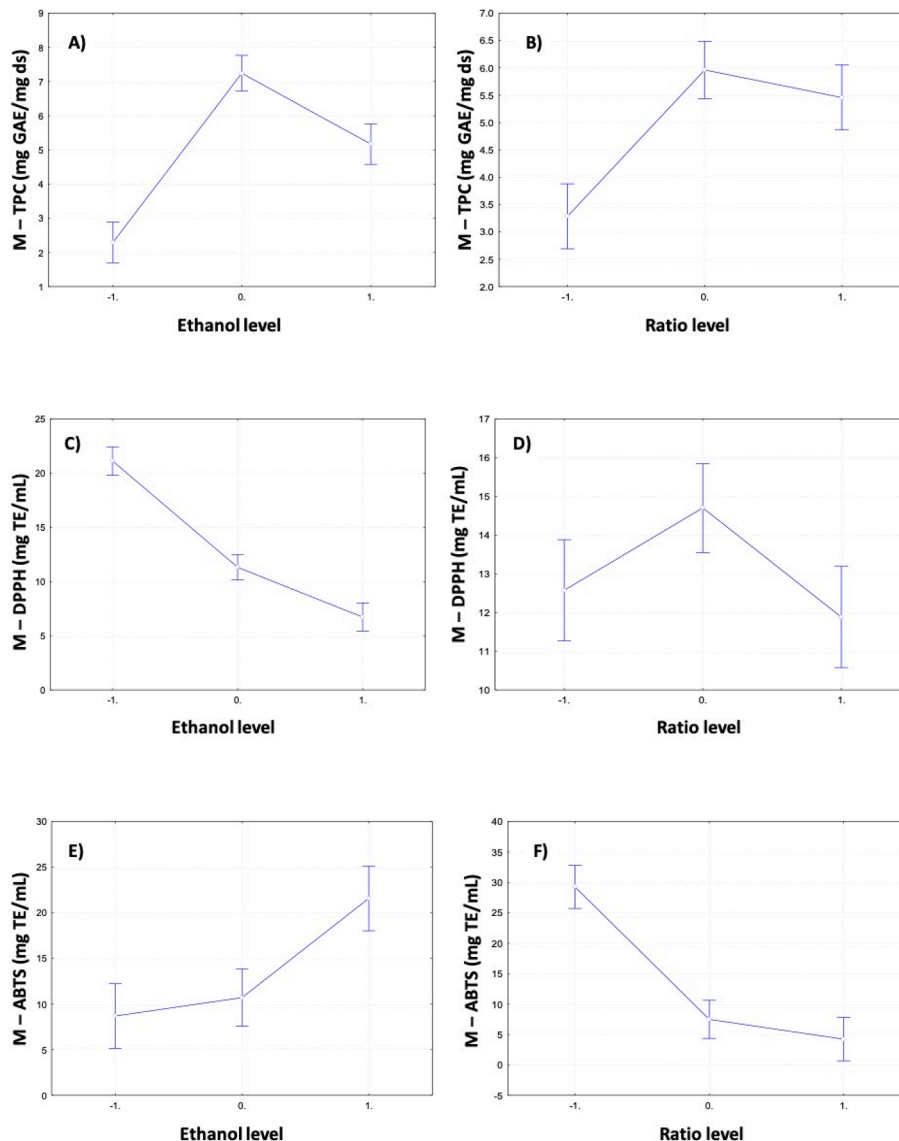


Fig. 2. Effect of extraction variables on maceration for TPC content (A and B), DPPH assay (C and D) and ABTS assay (E and F).

material and the solvent used. Concerning the extraction method, we employed a conventional, M, and a non-conventional, UAE. In M, agitation is important for two reasons: it increases the diffusion and removes the concentrated solution from the surface of the sample (Azmir, Zaidul, Rahman, Sharif, Mohamed, Sahena, & Omar, 2013). The relevance of agitation may explain why the highest TPC was reached under M.

In the case of the plant material, it constitutes the main variable due to its chemical composition defines the number of bioactive compounds to be further recovered. It is also important to mention that sample processing can exert a positive or negative effect, depending on the conditions explored.

Regarding the solvent, polarity is very important for identification and isolation. Properties such as low toxicity, a low boiling point, quick mass transfer, preservative action, and the inability to make the complex extract dissociate, are desirable for a good solvent (Silva, Rocha-Santos, & Duarte, 2016). In this study, a mixture containing water and ethanol was used.

Secondly, AC was evaluated in the huitlacoche extracts. For these determinations, significant effects were also observed among treatments. In the DPPH assay (see columns M – DPPH and UAE – DPPH in Table 2), the highest AC was attained with UAE for experiment 1 (50%

of ethanol and a 1:5 ratio): 22.5 mg TE/mL; whereas for M the maximal AC was 13.16 mg TE/mL (experiment 7: 99% of ethanol and a 1:5 ratio). During UAE cavitation occurs, meaning growth and collapse of bubbles, which implies diffusion through the cell wall, and rinsing the cell content after breaking the walls (Azmir, Zaidul, Rahman, Sharif, Mohamed, Sahena, & Omar, 2013). Besides the occurrence of this physical phenomenon, variables such as temperature, pressure, frequency, and sonication time, also affect the extraction efficacy (Vinatoru, 2001; Rajha, Boussetta, Louka, Maroun, & Vorobiev, 2015). All of these parameters allowed us to measure a higher DPPH value under UAE. In the case of the ABTS assay (see columns M – ABTS and UAE – ABTS in Table 2), the maximal values were attained with experiment 7 (99% of ethanol and a 1:5 ratio); however, the highest AC was measured for M: 45.26 mg TE/mL, compared to the 26.45 mg TE/mL with UAE.

Both TPC and AC were affected by the extraction parameters. The three more relevant experiments are 1, 4 and 7. In all of them, the lowest ratio permitted to achieved the highest DPPH, TPC and ABTS values, respectively. This is explained by the fact that larger amounts of ground huitlacoche sample required a larger volume of solvent. According to Fig. 2A, it can be noted that the central level for ethanol is the best for TPC recovery, which is in accordance to our findings in experiment 4. Regarding AC, the values are opposite: the lowest for DPPH (Fig. 2C) and

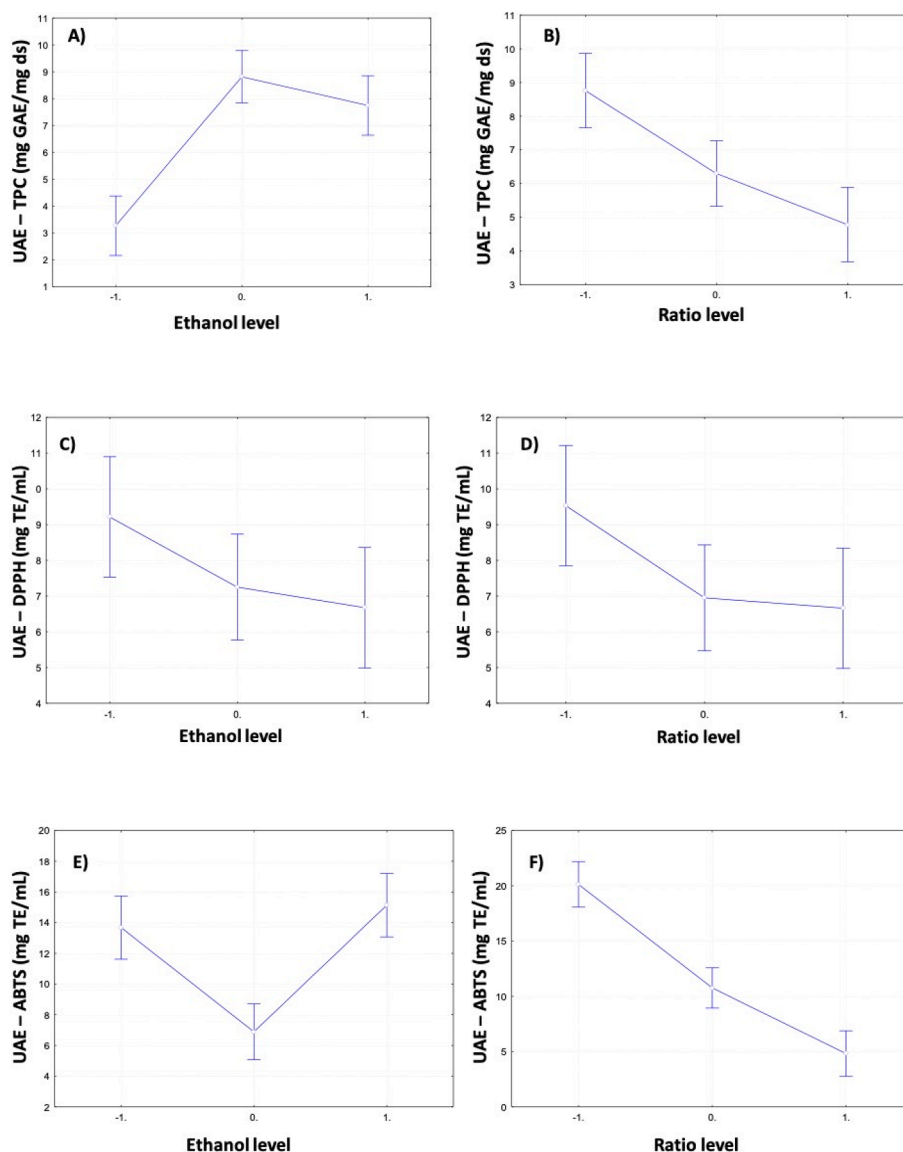


Fig. 3. Effect of extraction variables on ultrasound-assisted extraction for TPC content (A and B), DPPH assay (C and D) and ABTS assay (E and F).



the highest for ABTS (Fig. 2E). In the case of ratio, the central value was the best for TPC (Fig. 2B) and DPPH (Fig. 2D), whereas for ABTS (Fig. 2F) was the lowest level. Additionally, it can be observed in Fig. 3B, 3D and 3F, that the lowest ratios conduct to the highest values of TPC, DPPH and ABTS, respectively. However, for ethanol concentration there is not an apparent correlation of the data obtained (see Fig. 3A, 3C and 3E). This can be explained by the polarity of the resulting mixture, water and ethanol, as well as the chemical nature of the phenolic compounds extracted, which is also affected by the method *per se*.

In comparison to other studies, *Beas, Loarca, Guzmán, Rodríguez, Vasco, & Guevara, (2011)*, reported soluble phenols at a range of 390–640 mg GAE/100 g. When their methanolic extracts were compared to the commercial antioxidant BHT, the authors referred a higher antiradical activity. *Aydoğdu & Göllükçü (2017)* quantified a total phenolic matter of 113.11 mg GAE/kg dry sample, antioxidant activity, IC50 of 186.44 mg/mg DPPH, for 10 mL methanol:water (80:20) extracts after 1 h at 180 rpm. *Salazar-López, Martínez-Saldaña, Reynoso-Camacho, Chávez-Morales, Sandoval-Cardoso, & Guevara-Lara, (2017)*, reported two groups of ethanolic extracts: non-concentrated and concentrated. In both groups were raw and cooked samples with the following total soluble phenols: 49.6, 70.9, 182.7 and 161.7 µg GAE/mL extract, respectively. With respect to antioxidant activity: 200.1, 279.1, 247.5 and 312.8 nmol TE/mL extract, respectively for DPPH assay and 30.6, 41.1, 182.9 and 165 nmol TE/mL extract, respectively for ABTS assay.

According to our findings, we achieved similar results to the ones obtained by *Salazar-López, Martínez-Saldaña, Reynoso-Camacho, Chávez-Morales, Sandoval-Cardoso, & Guevara-Lara, (2017)*, for their raw samples, because when they evaluated the cooked samples higher values for both TPC and AC were attained. In the works of *Beas, Loarca, Guzmán, Rodríguez, Vasco, & Guevara (2011)*, and *Aydoğdu & Göllükçü (2017)*, the differences are explained by the extraction conditions as well as for the use of methanol instead of ethanol, causing different polarities in the extraction medium.

Other functional property evaluated for methanolic extracts of huitlacoche was conducted by *Valdez-Morales et al. (2010)*. These authors found that the antimutagenic activity of such extracts ranged from 41 to 76% according to their treatments, which are dependent on maize genotype, stage of development and cooking process. The authors also mentioned that their findings were comparable to similar assays that were previously reported.

### 3.3. *In vitro* bioaccessibility of phenolic compounds

When a phenolic extract shows certain AC, this should not to be considered as a predictor of its possible beneficial effect on human health (*Abderrahim, Huanatico, Segura, Arribas, Gonzalez, & Condezo-Hoyos, 2015*), mainly due to the complicated process of digestion and absorption. Evaluation of the bioaccessibility of these compounds is necessary since the bioactive compounds can only exert their beneficial effect if they are available for intestinal absorption after the various phases of gastrointestinal digestion had elapsed (*Teng & Chen, 2019*).

The bioaccessibility of phenolic compounds and AC during *in vitro* gastrointestinal digestion (oral, gastric and intestinal phases) of huitlacoche extracts are shown in Table 3. In all the stages of the *in vitro* digestion, the changes in the total phenolic content were significant ( $P < 0.05$ ). During the gastric phase, TPC increased 2.2-fold; some authors reported an increase in TPC at this stage, this behaviour could be attributable to the acidic environment during this phase, where phenolic compounds are more stable (*Chen et al., 2016; Gutiérrez-Grijalva, Angulo-Escalante, León-Félix, & Heredia, 2017*). In contrast, at the end of the intestinal phase, TPC decreased with respect to both the oral and gastric phases (2.9 and 4.4 times, respectively). Regarding the ABTS scavenging capacity, the non-digested sample showed a value of 9.52 mg TE/g (Table 3). Following the oral phase of the *in vitro* digestion, the ABTS scavenging capacity increased approximately four times and also

**Table 3**

Bioaccessibility of phenolic compounds and AC during *in vitro* digestion of huitlacoche extracts.

Digestion phase	TPC	DPPH	ABTS
Undigested	13.94 ± 1.1 <sup>c</sup>	12.51 ± 1.1 <sup>d</sup>	9.58 ± 0.5 <sup>d</sup>
Oral	19.76 ± 1.4 <sup>b</sup>	61.30 ± 4.1 <sup>a</sup>	32.73 ± 1.7 <sup>c</sup>
Gastric	30.22 ± 1.1 <sup>a</sup>	31.29 ± 2.2 <sup>b</sup>	64.71 ± 3.4 <sup>a</sup>
Intestinal	6.79 ± 0.5 <sup>d</sup>	25.51 ± 1.4 <sup>c</sup>	40.54 ± 2.1 <sup>b</sup>

TPC is expressed as mg GAE/g of dried sample. AC assays, DPPH and ABTS are expressed as mg TE/g of dried sample.

Data is expressed as mean ± sd of 3 independents experiments.

Different letters indicate significant differences ( $p < 0.05$ ).

increased after the digestive phase, with 1.97-fold changes. At the end of the intestinal phase, the sample had the lowest scavenging capacity. Despite the observed decrease in ABTS values during the intestinal phase, AC can be maintained through the digestion process.

In general, the DPPH and ABTS scavenging capacity was maintained and even increased during the phases of *in vitro* digestion. During the gastric phase, a higher TPC and AC values were observed, compared to the other phases. Some authors mentioned that this is explained by pH changes in the gastrointestinal environment, which causes that phenolic compounds enhance their ability to donate protons from their hydroxyl moieties on their aromatic rings (*Wootton-Beard, Moran, & Ryan, 2011; Chen et al., 2016; Gutiérrez-Grijalva et al., 2017*). Therefore, the preparation of huitlacoche-based foods or the consumption of huitlacoche can exert benefits in human nutrition due to its bioactive ingredients.

## 4. Conclusions

Many of the Mexican plant species are wasted and underutilized, and in the particular case of huitlacoche, it is still considered a plant pathogen and a devastating disease for maize. In an effort to promote its consumption and contribute to generate new technical data, the present study detailed the proximate analysis, antioxidant capacity (AC) and *in vitro* digestion of huitlacoche. It is important to highlight that the AC of the extracts was preserved and increased after the *in vitro* digestion, suggesting that huitlacoche can lead to the design of novel functional foods that can increase the bioaccessibility of its bioactive compounds. Also, this is a starting point for the development of new analytical protocols for following the assays and highlight that both extraction and digestion conditions play an important role in the release of huitlacoche components.

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## 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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