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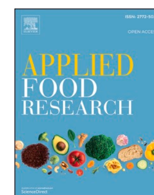

How to cook *Yucca* spp. flowers? An analysis of their chemical composition, microstructure, and bioactive compound bioaccessibility

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ABSTRACT

Yucca flowers are of great importance in traditional Mexican cuisine for their flavor and nutritional properties; however, they are rarely consumed raw. This study aimed to evaluate the effect of different cooking methods (stir-frying, boiling, and steaming) on *Yucca* spp. flowers on their chemical composition (moisture, protein, and glucose-derived carbohydrate content), microstructure, total bioactive compounds (carotenoids: CAR, chlorophyll: CHLO, and phenolic compounds: TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, and ferric reducing antioxidant power (FRAP), as well as their bioaccessibility. In general, cooking methods modified the chemical composition of flowers and decreased the concentration of their bioactive compounds and antioxidant capacity. However, steaming was the method that best preserved bioactive compounds of the flowers. Stir-frying and boiling improved the bioaccessibility of fat-soluble compounds (CHLO and CAR), but also caused great loss of flower microstructure. The importance of these results lies in finding processing conditions that generate the least impact on the nutritional and bioactive compounds of the flowers, in order to take full advantage of their initial concentrations in the raw flowers. In this sense, steaming the flowers for a short time (3 min) seems to be a viable alternative to preserve their chemical composition and bioactive compounds.

1. Introduction

Yucca is a genus belonging to the Asparagaceae family that groups together several species native to Central and North America, which produce yellowish-white flowers. Within these species, *Yucca elephantipes, Yucca filifera*, and *Yucca whipplei* are some of the best-known ones. In Mexico, when yucca plants bloom, which is once a year, their flowers are collected to be sold in local markets and are consumed mainly by people in rural communities (Sotelo et al., 2007). Fresh yucca flowers possess proteins, amino acids, carbohydrates, vitamins, and minerals (Pinedo-Espinoza et al., 2020; Sotelo et al., 2007), which makes them a food with high nutritional value. In addition, they contain bioactive compounds, especially phenolic compounds and pigments (Barth et al., 2015; Juárez-Trujillo et al., 2018), that are favorable for human health due to their chemoprotective effect against oxidative stress reactions caused by free radicals (Blumfield et al., 2022; Kritsi et al., 2022). On the other hand, fresh yucca flowers contain certain antinutritional compounds such as steroidal saponins, which have been related to decreased absorption of minerals (Sotelo et al., 2007). However, it has been shown that the concentration of these compounds can be decreased through cooking (Mulík & Ozuna et al., 2020).

The physicochemical, nutritional, and bioactive properties of edible flowers are being increasingly studied in the flowers' raw form (Mulík & Ozuna et al., 2020; Pensamiento-Niño et al., 2021; Pinedo-Espinoza et al., 2020; Sandoval-Gallegos et al., 2021). However, a recently published article showed that Mexican edible flowers, including yucca flowers, are mainly consumed cooked (Mulík et al., 2022). These flowers are prepared through recipes that involve long cooking periods (>15 min) (Mulík et al., 2022). This is interesting since it is well documented that cooking methods induce changes in plants. In these treatments, the energy distributed by the cooking medium (oil, water, or air) is transferred to the food, causing an alteration of its organoleptic, physical, and chemical properties (Castañeda-Rodríguez et al., 2023; Cattivelli et al., 2021; de Carvalho et al., 2014; Monalisa et al., 2020, 2020; Moorthy

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et al., 2020). Crucially, cooking time is usually an important factor that modulates the changes that occur in the food matrix, since an increase in cooking time represents a longer span of contact between the food and the cooking medium. In plant matrices, cooking has been shown to cause softening of the plant structure due to loss of turgor (Sandoval-Gallegos et al., 2021). Therefore, mass transfer phenomena are usually facilitated, whereby different food molecules can migrate into the cooking medium according to their solubility. In this regard, it has been shown that phenolic compounds, organic acids, and soluble carbohydrates resulting from the cooking process, are mostly lost in methods that incorporate water (Castañeda-Rodríguez et al., 2023; Monalisa et al., 2020). On the other hand, compounds such as chlorophylls, carotenoids, and some vitamins, being fat-soluble, tend to be preserved in these same methods (Fratianni et al., 2021), but are lost in those that use oil (Mao et al., 2015; Yang et al., 2019). This valuable knowledge has made it possible to choose from different cooking methods in order to select the one that allows the preservation of beneficial compounds of interest; however, even if the component is preserved after cooking, its structure and concentration may be modified during digestion. Human digestion is both a physical and chemical process, involving digestive enzymes as well as pH changes. During human digestion, macronutrients in food react with digestive enzymes through enzymatic hydrolysis reactions (Santos-Hernández et al., 2020). On the other hand, some bioactive compounds can be degraded or chemically modified due to pH transition during the digestive process (Bouayed et al., 2011; de Morais et al., 2020).

Information on how the main variables of cooking methods affect the properties of edible flowers is still scarce. Moreover, the effect of digestion on cooked flowers is still unknown. Therefore, the objective of this research was to study the effect of cooking time and cooking method on the nutritional and bioactive properties of yucca flowers. Also, to explore the structural changes caused by cooking methods in the flowers, a staining microstructure and autofluorescence analysis was performed. Finally, using an *in vitro* digestion model, we simulated human digestion to study the effect of cooking on the bioactive properties in the digested flowers. Overall, this allowed us to suggest preparation conditions so that processing would have the least impact on the nutritional and bioactive compound content of the flowers, and thus take full advantage of the existing properties of the raw flowers.

2. Materials and methods

2.1. Flower collection and cooking experiments

Yucca flowers (Fig. 1A, *Yucca* spp.) were collected in the municipality of Tierra Blanca, Guanajuato, Mexico, between March and April 2022. The petals (Fig. 1B) were separated and stored at 10 °C until being cooked by stir-frying, boiling, or steaming. To evaluate changes in chemical composition, bioactive compound content, and antioxidant capacity, flowers were cooked for 0 (control), 3, 10, and 15 min. In addition, raw flowers and those cooked for 15 min were used to determine their microstructure and bioactive compound bioaccessibility.

Stir-frying was performed with 100 g of petals, cooked in a stainless-steel pan (195 °C) with 5 mL of corn oil. At the end of the cooking time, the flowers were washed for 5 s with 50 mL of hexane to remove excess oil (Sultana et al., 2008). As for boiling, 40 g of petals were cooked in a 2 L beaker with 800 mL of purified water at 100 °C (Monalisa et al., 2020). Finally, steaming was performed with 80 g of petals, which were placed on a stainless-steel cylindrical rack attached to a 2 L beaker with 600 mL of purified water at 100 °C (Yang et al., 2019). Vapor content was validated by measuring dry and wet bulb temperatures. At the end of the cooking time, the petals were cooled with cold water for 3 min and drained on absorbent paper. Finally, both raw and cooked samples were dried to constant weight in a hot air oven (60 °C) (MMM Group, Munich,

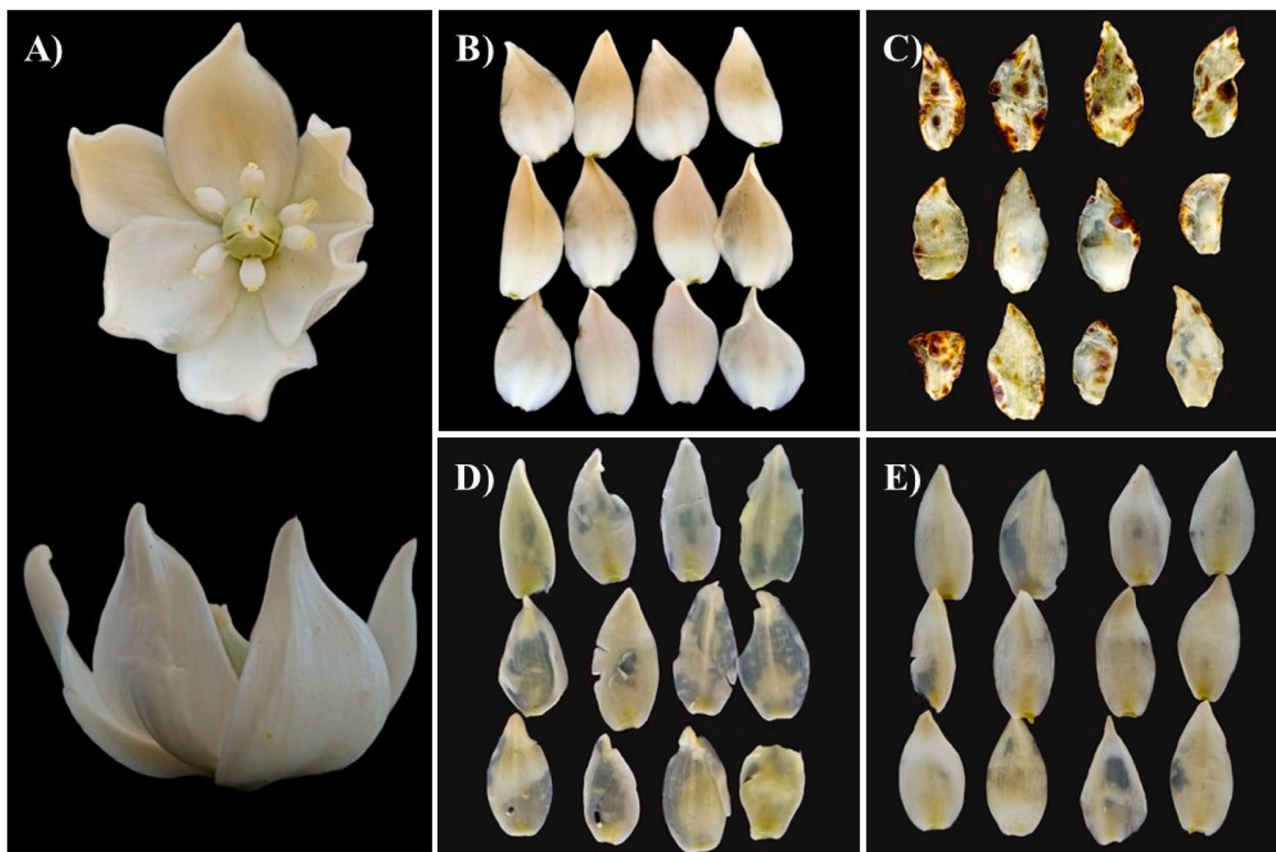

Fig. 1. *Yucca* spp. flower (A) and raw (B), stir-fried (C), boiled (D), and steamed (E) petals.

Germany) (Kaisoon et al., 2012) and were ground and sieved (particle size ≤ 0.8 mm). All cooking experiments were performed in duplicate.

2.2. Nutritional composition

Moisture content was estimated by the oven drying method until the sample reached a constant weight, according to the AOAC official method of analysis 930.15 (AOAC, 1997). Protein content was estimated by the spectrophotometric method according to Lowry et al. (1951). Proteins from 200 mg of dried petals were extracted with 20 mL of phosphate buffer (pH 7.2). The mixture was filtered and centrifuged at 6500 rpm for 10 min. Subsequently, 0.5 mL of extract was added to 5 mL of reaction mixture (50 mL of 2 % sodium carbonate in 0.1 N NaOH and 1 mL of 0.5 % copper sulfate in 1 s% sodium potassium tartrate). The samples were incubated for 10 min. Then, 0.5 mL Folin-Ciocalteau reagent (1 N) was added, and finally, the reaction mixtures were incubated for 30 min. The absorbance was read at 660 nm using a UV–Vis spectrophotometer Genesys 10S (Thermo Scientific, Waltham, USA). Protein concentration was expressed as milligrams of bovine serum albumin equivalents per gram of dry weight (mg BSAE/g DW).

The glucose-derived carbohydrate content was quantified following the methodology used by Chandran et al., and Parimelazhagan (2013). Preceded by a hydrolysis-extraction step (100 mg of dried petals with 5 mL HCl at 2.5 N), the reaction mixture was prepared with 250 μL of the extract and 2 mL of anthrone reagent (200 mg in 100 mL of 95 % (v/v) H_2SO_4) at 100 °C for 8 min. The absorbance was read at 630 nm using a UV–Vis spectrophotometer Genesys 10S. Carbohydrate content was reported as milligrams of glucose equivalents per gram of dry weight (mg GE/g DW).

2.3. Bioactive compounds and antioxidant capacity

2.3.1. Extract preparation

The extracts used for the determination of bioactive compound content and antioxidant capacity were obtained from the extraction (150 rpm, for 1 h at 25 °C) of 1 g of dried petals with 30 mL of 80 % (v/v) acetone or 80 % (v/v) methanol. The obtained mixture was filtered and centrifuged in a MiniSpin centrifuge (Eppendorf, Hamburg, Germany) at 14,000 rpm for 5 min and stored at −4 °C for future analyses. Hydroacetonic extracts were used for the determinations of total content of chlorophyll (CHLO), pheophytes (PHEO), and carotenoids (CAR). Hydromethanolic extracts were used for determinations of total phenolic compounds (TPC) and antioxidant capacity.

2.3.2. Determination of the content of chlorophyll, pheophytins, and total carotenoids

CHLO (646 and 663 nm), PHEO (653 and 665 nm), and CAR (480 nm) were determined by extract absorbance measurements. The measurements were made on a UV–Vis spectrophotometer Genesys 10S. Concentrations were calculated by Eqs. (1), 2 (Lichtenthaler & Wellburn, 1987), and 3 (Chrysargyris et al., 2018). Results were expressed as micrograms per gram of dry weight (μg/g DW).

$$CHLO = (7.15)(A_{663\ nm}) + (18.71)(A_{646\ nm}) \tag{Eq. 1}$$

$$PHEO = (3.84)(A_{665\ nm}) + (33.36)(A_{653\ nm}) \tag{Eq. 2}$$

$$CAR = (4)\ (A_{480\ nm})(v_{mL}) \tag{Eq. 3}$$

Where: constant values in the equations were calculated from the absorption coefficients of CHLO, PHEO, and CAR (80% acetone) and V_{mL} represents the sample volume (mL).

2.3.3. Determination of the total phenolic compound content

The method described by Chen et al., and Fu (2018) was used with modifications. For this, the reaction mixture was prepared by adding 250 μL of the flower extract to 250 μL of Folin-Ciocalteu's reagent with dilution factor 2 (Sigma Aldrich, Saint Louis, USA) and 2 mL of 2 % (w/v) sodium carbonate. Samples were allowed to rest for 60 min in the dark and absorbance was read at 765 nm using a UV–Vis spectrophotometer Genesys 10S. TPC was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

2.3.4. Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

An aliquot of 2.5 mL of methanolic DPPH reagent (6×10^{-5} M) and 65 μL of flower extract were mixed and allowed to react for 15 min to subsequently measure the final absorbance at 515 nm. The results of DPPH assay were expressed as micromol of Trolox equivalents per gram of dry weight (μmol TE/mg DW) following the method described by Pinedo-Espinoza et al. (2020).

2.3.5. Determination of ferric reducing antioxidant power

The determination of ferric reducing antioxidant power (FRAP) was carried out according to the method described by Benzie and Strain (1996). For this purpose, 65 μL of flower extract was mixed with 1950 mL of FRAP reagent and 195 mL of distilled water and allowed to react for 30 min; then, the final absorbance was measured at 593 nm. FRAP was expressed as micromol of Trolox equivalents per gram of dry weight (μmol TE/ mg DW).

2.4. Microstructure

The microstructure of the samples was studied using optical microscopy and scanning confocal laser microscopy. For the study by optical microscopy, flower petals were sectioned and prepared according to González et al. (2021) and a Nikon Eclipse 80i® optical microscope (Nikon Co. Ltd., Tokyo, Japan) was used, to which a camera Exwave HAD DXC-19 (Sony Electronics Inc., Park Ridge, USA) was coupled. The sections obtained were visualized via the brightfield and fluorescence techniques, using a mercury lamp with a FITC filter. Images were captured at 10, 20, and 60X magnifications and stored at 1280×1024 pixels using the microscope software NIS-Element M version 4.0 (Nikon, Tokyo, Japan) (González et al., 2022).

To study the digested samples by scanning confocal laser microscopy, a ZEISS 780 microscope was used, coupled to an Axio Observer Z1 inverted microscope (ZEISS, Oberkochen, Germany). Nile Red, (λ_{ex} = 561 nm, λ_{em} = 576/620 nm) was used for lipid detection. Calcofluor White was used to stain polysaccharides that were excited with the diode line (λ_{ex} = 405 nm, λ_{em} = 410/77 nm) (Hernández-Carrión et al., 2015).

2.5. Simulated human digestion and bioaccessibility

To evaluate the changes caused by digestion in the concentrations of CHLO, CAR, TPC, and antioxidant capacity (DPPH assay) of raw flowers and those cooked for 15 min, simulated human digestion experiments were performed, using an *in vitro* digestion model according to Diez-Sánchez et al., and Hernando (2021). The selected model used three stages: oral, gastric, and intestinal. The simulated solutions used for each stage (SSF: simulated salivary fluid, SGF: simulated gastric fluid, and SIF: simulated intestinal fluid) were prepared according to Minekus et al. (2014). First, a quantity of dried flowers was reconstituted with the addition of distilled water to complete 5 g, according to the moisture of each sample. The reconstituted samples were reacted with 4 mL of SSF + α-amylase, 19 μL of $CaCl_2$ and 981 μL of distilled water (pH 7, 37 °C, 2 min). Subsequently, gastric digestion started with the addition of 16 mL of SGF + pepsin, followed by 8 μL of $CaCl_2$ at 0.3 mM; the pH was adjusted to 3 with 0.1 M HCl and the volume was brought up to 20 mL using distilled water. To mimic conditions in the human intestine, the digests were incubated (1 h, 95 rpm, 37 °C) in a reaction station (Carousel 6 Plus, Radleys, UK) with N_2 circulation. The intestinal stage began with adjusting the pH of the gastric digesta to 7 with 0.1 M NaOH. Then, 12 mL of SIF + pancreatin were added, followed by 48 μL of $CaCl_2$ at 0.3 mM and 12 mL of SIF + bile extract. The pH was adjusted to 7 and

the volume was made up to 30 mL with distilled water. The digesta obtained was incubated (2 h, 95 rpm, 37 °C) and subsequently centrifuged (14,000 rpm, 20 min, 4 °C) and filtered (Whatman® grade 4). The centrifugation residue and the filtered solution were considered the soluble and insoluble fraction, respectively. Finally, both fractions were lyophilized. The extracts of the lyophilized digestions were obtained as described in Section 2.2.1. The *in vitro* digestion experiments were performed in duplicate and the percentage bioaccessibility index (BI) was calculated using Eq. (4).

$$BI(\%) = \frac{Compounds\ of\ soluble\ fraction}{Compounds\ of\ non-digested\ flowers} x\ 100 \quad \text{(Eq. 4)}$$

2.6. Statistical analyses

Different separate one-way ANOVAs were performed for each variable, using either *cooking time* (levels: 0, 3, 10, and 15 min), *cooking method* (levels: stir-frying, boiling, and steaming), or *digestion* (levels: before and after) as factors. A Tukey's *post-hoc* test ($p < 0.05$) was performed for each analysis. All experimental data were collected in triplicate and analyzed using the statistical program Statgraphics Centurion XVI (Statgraphics Technologies, Inc., Warrenton, USA).

3. Results and discussion

3.1. Nutritional properties

The effect of cooking time and cooking method on the content of moisture, glucose-derived carbohydrates, and proteins is shown in Table 1. The moisture content in the raw flower was 83.98 ± 0.97 %, this value being similar to that reported by Sotelo et al. (2007) for *Yucca filifera* flowers. Stir-frying caused a reduction of this value during the cooking time, which was not significant ($p > 0.05$) until 15 min of cooking. Contrary to this, in both boiling and steaming, flower moisture content increased ($p < 0.05$) with time; at the completion of 15 min of cooking this increase was 9.65 % and 3.13 %, respectively. Regarding the effect of the cooking method, for all times, the moisture content values obtained in stir-frying were significantly lower ($p < 0.05$) compared to the rest of the methods. High temperatures cause a softening of the plant structure, which could facilitate water diffusion from the inside of the cells to their exterior (in stir-frying) or from the cooking medium to the interior of the cells (in boiling and steaming) (Sandoval-Gallegos et al., 2021).

Regarding the content of glucose-derived carbohydrates (carbohydrate content) and proteins, the values found in the raw flowers were 559.91 ± 99.09 mg GE/g DW and 249.14 ± 10.16 mg BSAE/g DW, respectively. Neither carbohydrate nor protein contents have been previously measured in yucca flowers using the same methods as those employed in the present research; however, the concentrations we found are similar to those reported in other Mexican edible flowers using proximate chemical analysis (carbohydrates as nitrogen free extract = 538 mg/g and proteins as crude protein = 259 mg/g) (Sotelo et al., 2007). In general terms, stir-frying caused an increase in the carbohydrate content in the flowers, mainly in minutes 10 and 15 (up to 17.36 %). On the other hand, boiling the flowers for 10 and 15 min decreased ($p < 0.05$) their carbohydrate content. In the case of steaming, the same effect occurred at 3 and 10 min of cooking. Regarding the effect of the cooking method, higher carbohydrate contents ($p < 0.05$) were found in the stir-fried flowers both at 10 and 15 min with respect to the boiled and steamed ones. The comparison between cooking methods also showed that, for longer cooking times (10 and 15 min), carbohydrate content decreased more during boiling compared to steaming. The higher amounts of carbohydrates found in stir-frying could have been caused by a breakdown of the glycosidic bonds in the carbohydrates, resulting from the increase of the thermal energy released by the cooking medium over time (Moorthy et al., 2020). As for boiling and steaming, the produced monosaccharides could have leached into the cooking water.

Finally, the protein content originally present in the flower decreased in all cooking methods and for all cooking times (Table 1). At cooking times of 3 and 15 min, the protein content found was similar for stir-frying and steaming. However, regardless of time, boiling reduced ($p < 0.05$) the amount of protein in the flowers more than the other cooking methods did. There is contradictory information in the literature regarding the effect of cooking on food proteins. Although an increase in protein content (due to the breakage of protein peptide bonds) has been reported in boiled *Hibiscus surratensis* L. leaves (Moorthy et al., 2020), a 2.87 % reduction has also been found in *Myrtillocactus geometrizans* flowers (Sandoval-Gallegos et al., 2021). The latter was due to the solubilization of water-soluble proteins, so this same phenomenon could have occurred in our experiment.

3.2. Bioactive compounds and antioxidant capacity

Table 2 shows the effect of cooking time and cooking method on the content of total chlorophyll (CHLO), total pheophytins (PHEO), total carotenoids (CAR), total phenolic compounds (TPC), DPPH assay, and ferric reducing antioxidant power (FRAP) of yucca flowers. The CHLO value of the raw flower was 17.78 ± 5.38 μg/g DW, which is congruent with that reported in other Mexican edible flowers (Pensamiento-Niño et al., 2021). Cooking time only had an effect ($p < 0.05$) on CHLO in the stir-frying method. In this method, all times caused a reduction of CHLO in comparison to the raw flowers; however, no significant differences were found between CHLO values of the cooked flowers regardless of cooking time. Regarding the effect of the cooking method, this factor

Table 1
The effect of cooking time and cooking method on chemical composition of yucca flowers.

	Time (min)	Moisture (mg/g flower)	Total carbohydrates (mg GE/g DW)	Total protein (mg BSAE/g DW)
Stir-frying	0	842.31 ± 10.27^{A}	559.91 ± 99.09^{BC}	249.14 ± 10.16^{A}
	3	842.09 ± 9.39^{A,c}	431.90 ± 19.75^{C,b}	188.77 ± 10.36^{B,a}
	10	825.24 ± 2.93^{A,c}	727.69 ± 8.84^{A,a}	183.55 ± 6.52^{B,a}
	15	719.11 ± 14.35^{B,c}	657.16 ± 3.14^{AB,a}	140.66 ± 12.04^{C,a}
Boiling	0	842.31 ± 10.27^{D}	559.91 ± 99.09^{A}	249.14 ± 10.16^{A}
	3	884.56 ± 6.61^{C,a}	512.31 ± 10.21^{AB,a}	69.83 ± 2.36^{B,c}
	10	914.44 ± 3.47^{B,a}	240.03 ± 4.35^{C,c}	42.98 ± 2.44^{C,c}
	15	936.27 ± 2.22^{A,a}	356.72 ± 12.07^{BC,c}	19.55 ± 2.16^{D,b}
Steaming	0	842.31 ± 10.27^{B}	559.91 ± 99.09^{A}	249.14 ± 10.16^{A}
	3	859.13 ± 2.28^{A,b}	363.69 ± 7.13^{B,c}	153.81 ± 3.30^{B,b}
	10	875.82 ± 6.36^{A,b}	453.67 ± 5.80^{AB,b}	123.54 ± 6.72^{C,b}
	15	871.10 ± 5.08^{A,b}	462.67 ± 8.04^{AB,b}	125.88 ± 2.18^{C,a}

Average ± standard deviation is shown, and different letters show significant differences between cooking times (upper case) and cooking methods (lower case).

Table 2
The effect of cooking time and cooking method on bioactive properties and antioxidant capacity of yucca flowers.

	Time (min)	CHLO (μg)	PHEO (μg)	CAR (μg)	TPC (mg GAE)	DPPH (μmol ET)	FRAP (μmol ET)
Stir-frying	0	17.78 ± 5.38^{A}	21.09 ± 6.50^{A}	25.20 ± 2.54^{A}	14.54 ± 3.28^{A}	33.11 ± 0.42^{A}	49.56 ± 9.78^{A}
	3	$11.61 \pm 1.00^{B,b}$	$14.55 \pm 1.42^{B,b}$	$26.35 \pm 1.05^{A,b}$	$8.98 \pm 1.15^{B,b}$	$27.96 \pm 1.66^{B,b}$	$23.40 \pm 2.49^{B,b}$
	10	$9.46 \pm 1.21^{B,b}$	$10.48 \pm 1.87^{B,b}$	$20.70 \pm 1.34^{B,b}$	$7.48 \pm 1.10^{B,b}$	$24.81 \pm 3.92^{BC,b}$	$20.19 \pm 4.68^{B,b}$
	15	$9.22 \pm 2.02^{B,b}$	$9.47 \pm 3.37^{B,b}$	$24.10 \pm 2.46^{A,b}$	$7.49 \pm 0.88^{B,a}$	$23.24 \pm 1.81^{C,b}$	$19.10 \pm 4.26^{B,b}$
Boiling	0	17.78 ± 5.38^{A}	21.09 ± 6.50^{A}	25.20 ± 2.54^{A}	14.54 ± 3.28^{A}	33.11 ± 0.42^{A}	49.56 ± 9.78^{A}
	3	$15.95 \pm 2.00^{A,a}$	$20.66 \pm 4.59^{A,a}$	$24.60 \pm 2.27^{A,b}$	$6.83 \pm 3.28^{B,c}$	$27.84 \pm 1.11^{B,b}$	$23.50 \pm 0.69^{B,b}$
	10	$17.09 \pm 3.10^{A,a}$	$20.03 \pm 6.08^{A,a}$	$19.65 \pm 5.86^{AB,b}$	$3.35 \pm 0.03^{C,c}$	$12.23 \pm 0.42^{C,c}$	$8.46 \pm 0.38^{C,c}$
	15	$16.68 \pm 5.77^{A,a}$	$25.10 \pm 13.90^{A,a}$	$17.80 \pm 2.83^{B,c}$	$2.25 \pm 0.14^{C,b}$	$6.72 \pm 0.37^{D,c}$	$3.59 \pm 0.13^{C,c}$
Steaming	0	17.78 ± 5.38^{A}	21.09 ± 6.50^{A}	25.20 ± 2.54^{B}	14.54 ± 3.28^{A}	33.11 ± 0.42^{A}	49.56 ± 9.78^{A}
	3	$19.18 \pm 3.20^{A,a}$	$19.51 \pm 2.58^{AB,a}$	$33.75 \pm 1.17^{A,a}$	$10.55 \pm 3.28^{B,a}$	$32.80 \pm 0.47^{A,a}$	$42.22 \pm 0.57^{AB,a}$
	10	$15.91 \pm 4.57^{A,a}$	$25.26 \pm 7.67^{A,a}$	$27.60 \pm 3.25^{B,a}$	$9.50 \pm 0.41^{B,a}$	$31.95 \pm 0.30^{A,a}$	$35.51 \pm 1.88^{BC,a}$
	15	$14.07 \pm 3.08^{A,ab}$	$11.18 \pm 4.11^{B,b}$	$32.75 \pm 1.91^{A,a}$	$8.57 \pm 0.87^{B,a}$	$30.13 \pm 1.81^{B,a}$	$33.33 \pm 2.43^{C,a}$

Average ± standard deviation is shown, and different letters show significant differences between cooking times (upper case) and cooking methods (lower case). CHLO: total chlorophyll content, PHEO: total pheophytin content, CAR: total carotenoids content, TPC: total phenolic content, and DPPH: 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Values were expressed per gram of dry weight.

was significant at the three times analyzed (3, 10, and 15 min). A lower value ($p < 0.05$) of CHLO was found in the stir-frying method for all cooking times. Therefore, an increase in cooking time (greater than 3 min) only had an effect when flowers were cooked by stir-frying and not by boiling or steaming. This can be explained by the lipophilic nature of these compounds, due to which they could have been leached into the cooking oil. However, the heat provided by the cooking methods could also influence this response, as it has been reported that heat treatments can cause CHLO degradation reactions and production of derivatives such as PHEOs (Hayes & Ferruzzi, 2020).

Regarding the PHEO determination, cooking time had a significant effect in the stir-frying and steaming methods. In stir-frying, all cooking times caused a similar reduction of the initial PHEO value (21.09 ± 6.50 μg/g DW) compared to CHLO. On the other hand, steaming only reduced ($p < 0.05$) this concentration when flowers were cooked for 15 min. As for the effect of the cooking method, this factor was significant ($p < 0.05$) at all three times analyzed. Similar to CHLO, a significantly lower PHEO value ($p < 0.05$) was found in the stir-frying method for all cooking times.

As for CAR, the value found in the raw flowers was 34.40 ± 1.91 μg/g DW. This concentration is about 10 times lower than that reported by Pinedo-Espinoza et al. (2020) in other Mexican edible flowers (*Agave salmiana* and *Erythrina americana*), which could be related to their yellow and red color which may be correlated with the presence of CAR. In all three methods, a significant effect of cooking time ($p < 0.05$) was found, where in general terms a longer cooking time meant a greater CAR reduction. Cooking method also had a significant effect ($p < 0.05$) on CAR in all three cooking methods. For 3 and 10 min, no differences were found between the CAR values of flowers cooked by boiling and stir-frying, indicating that for these cooking times there was no difference between stir-frying and boiling the yucca flowers. On the other hand, the steaming method better preserved the CAR of the flowers, regardless of the cooking time.

Regarding TPC, the initial value found in this study for raw flowers was 14.54 ± 3.28 mg GAE/g DW, which is higher than that reported for flowers of *Myrtillocactus geometrizans*: 7.51 mg GAE/g DW (reported as 750.89 mg GAE/100 g DW), a Mexican edible flower (Sandoval-Gallegos et al., 2021). The time factor was significant in all three cooking methods ($p < 0.05$). For stir-frying and steaming, a significant reduction in the TPC of the raw flower was found at all cooking times. However, no differences were found between the times of cooked flowers, so cooking yucca flowers for more than 3 min did not cause an additional effect on TPC. Regarding the cooking method, this factor had a significant effect at all times analyzed. For any time (3, 10, or 15 min), boiling yielded the lowest TPC values, which means that regardless of the time this method reduced TPC concentrations more compared to stir-frying and steaming.

As for antioxidant capacity determinations (DPPH assay and FRAP), an effect ($p < 0.05$) of both cooking time and cooking method was found in both determinations. In the boiling and stir-frying methods, the initial values of both determinations (33.1 ± 0.42 μmol TE/g DW and 49.56 ± 9.78 μmol TE/g DW for DPPH and FRAP, respectively) decreased at all cooking times. As in TPC, the cooking method factor also had a significant effect ($p < 0.05$) on DPPH and FRAP at all times analyzed. At all three times, boiling yielded the lowest values of both DPPH and FRAP, which means that regardless of the time this method decreased the antioxidant capacity of the flowers more compared to stir-frying and steaming. These results obtained may be due to two factors, the cooking temperature and the solubility of the bioactive compounds. Thus, although stir-frying reaches higher temperatures (195 °C) compared to the boiling and steam methods (100 °C), which would more easily degrade the bioactive compound; the leaching phenomenon that occurs during boiling seems to have a greater effect on the loss of the antioxidant capacity of the yucca flowers.

In summary, cooking time was only a determinant factor of bioactive properties when yucca flowers were cooked by stir-frying and boiling. In this sense, an increase in cooking time led to significant reductions in CAR concentration in stir-frying. The same occurred with TPC when the flowers were cooked by boiling. Furthermore, comparisons between different cooking methods revealed that changes in CHLO concentrations are similar between boiling and steaming methods regardless of cooking time. This finding is relevant since, in a recently published study by Castañeda-Rodríguez et al. (2023), it was found that both CAR and CHLO of *Agave salmiana* flowers are mostly lost in the stir-frying method, while TPC and antioxidant capacity are lost in boiling, but the role of time as a factor had not yet been determined then.

Due to contact between the heating water and the flowers not being direct in the steaming method, this method preserved the bioactive and antioxidant properties of yucca flowers in a better way. Therefore, it is a suitable alternative to other cooking methods for these flowers. In addition, a short cooking time (3 min) practically does not change the concentrations of bioactive compounds in the steamed flowers. Therefore, cooking yucca flowers by steaming for 3 min is an appropriate way of preparation to cause the least deterioration to the bioactive compounds that yucca flowers have in their raw form.

3.3. Microstructural analysis

The microstructural analysis of raw yucca flowers and the microstructural changes caused by cooking the flowers for 15 min are shown in Fig. 2. In the staining microscopies (Fig. 2A and B), the cellular matrix of the raw flowers was uniform and with great physical integrity. In addition, the constituent cells appeared ordered, tightly bound to each

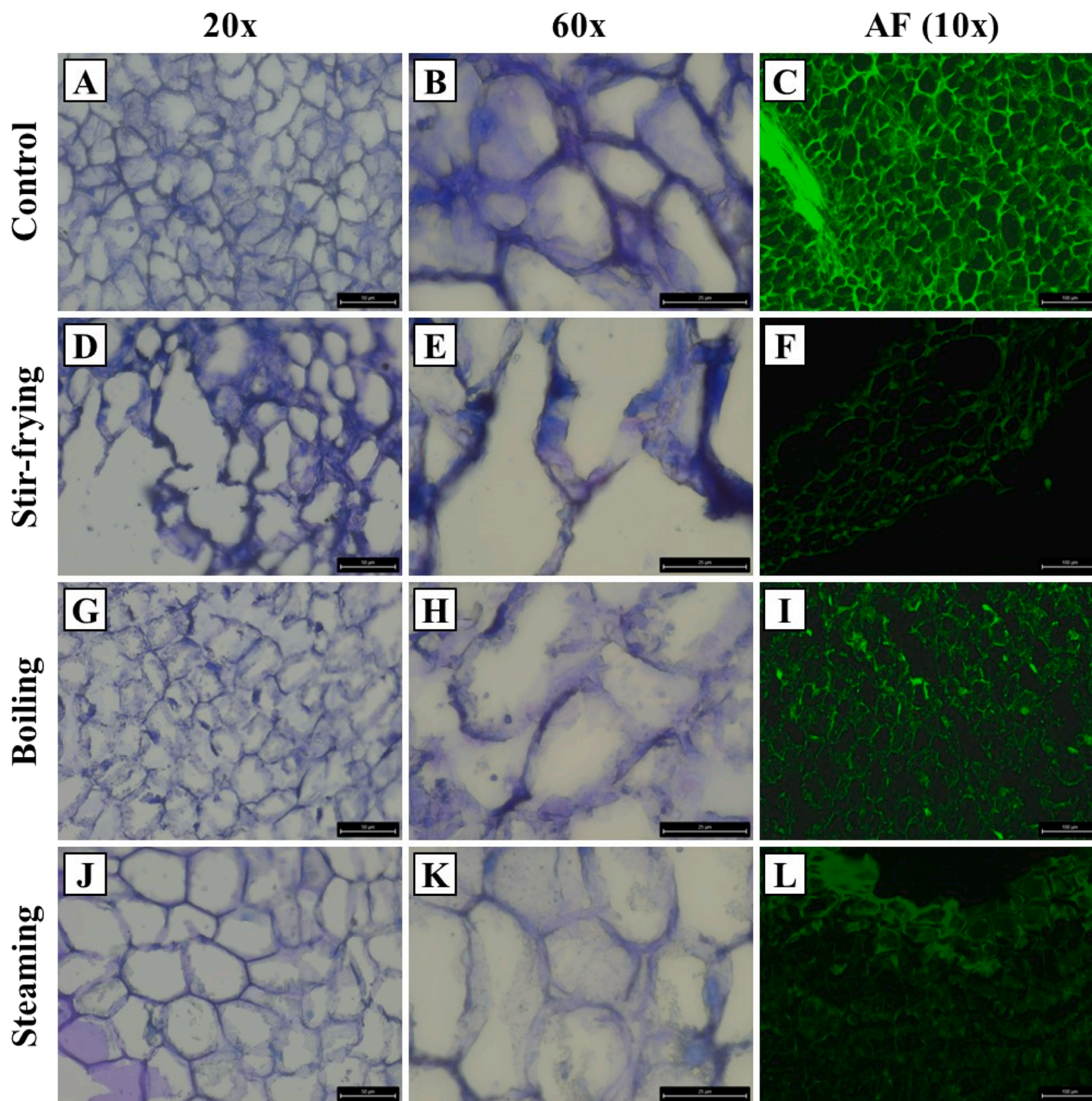

Fig. 2. Microstructure changes of raw and cooked yucca flowers observed at 20X (left column; A: raw, D: stir-frying, G: boiling, and J: steaming) and 60X (middle column; B: raw, E: stir-frying, H: boiling, and K: steaming) and autofluorescence (AF) (Right column; C: raw, F: stir-frying, I: boiling, and L: steaming). Blue and green colors represent vegetal tissue and carotenoid autofluorescence, respectively.

other, and with compact cell walls. In all cases, the cooking methods altered the physical integrity of the cellular structures, which resulted in a reduction of the thickness and definition of the cell walls; also, the cellular cohesion was reduced causing a greater size of the cellular median lamina which originates from the union of two adjacent cells. The stir-frying and boiling methods (Fig. 2D, E, and 2 G, H, respectively) caused the most notable deteriorations in the plant structure; this result is congruent with what is observed in Fig. 1C and D on the macroscopic scale. In stir-frying, this could be due to thermal damage (caused by the high temperature used) (Mao et al., 2015) and mechanical damage (caused by the characteristic movement during stir-frying) (Mao et al., 2015). On the other hand, the damage observed in boiling may be due to solubilization of cell wall components in the cooking water (Sandoval-Gallegos et al., 2021). In contrast, steamed flowers (Fig. 2J and K) showed a lower level of degradation and maintained their structural integrity to a greater extent, as also observed in Fig. 1E on the macroscopic scale.

In the case of boiling and steaming, the cells were swollen, which could be caused by the softening of the plant tissue provoked by the high temperatures and the subsequent absorption of water from the cooking medium. This observation is congruent with the increases found in moisture (Table 1).

On the other hand, autofluorescence emission (Fig. 2C, F, I and L) of the flower tissue could be seen in all the microscopic images obtained. This emission is attributed to the presence of CAR and, due to the higher presence of CAR, the emission detected in the raw flower (Fig. 2C) was the most intense. In addition, CAR were mainly distributed in the cell wall. In this sense, compared to the raw flower, the damage caused by all cooking methods at the microstructural level caused affectations both in the autofluorescence emission and in the distribution of CAR. Thus, in

the cooking methods that use water (Fig. 2I and L), CAR diffused through the cell tissue due to their low solubility in the cooking medium. In the stir-frying (Fig. F), on the other hand, CAR could have leached into the cooking oil. In summary, microstructural changes in the cooked flowers suggest that these methods may have an effect on their bioactive compound content and antioxidant capacity.

Microstructure results show that cooking yucca flowers for 15 min can modify their microstructure and alter both the presence and distribution of their CAR; however, the steaming method seems to preserve these attributes more adequately compared to the other cooking methods.

3.4. Bioaccessible compounds

As mentioned above, yucca flowers are usually cooked for longer times (Mulík et al., 2022). Therefore, raw flowers as well as those cooked for the longest studied cooking time (15 min) were subjected to *in vitro* digestion analysis. Fig. 3 shows the effect of both cooking method and digestion on CHLO, CAR, TPC, and DPPH, as well as their bioaccessibility index (BI). In the determination of CHLO (Fig. 3A), there was no difference ($p > 0.05$) between the value found before digestion (17.78 ± 5.38 μg/g DW) in the raw flower and its digested counterpart (14.61 ± 4.89 μg/g DW), so CHLO was almost completely bioaccessible (82.14 %). When the flowers were steamed, digestion did not cause changes in CHLO concentration (before: 14.07 ± 3.08 μg/g DW, after: 13.96 ± 1.91 μg/g DW), so the compounds were bioaccessible practically in their entirety as well (99.27 %). Interestingly, for the stir-frying and boiling methods, digestion had a positive effect on CHLO values, as the values found after digestion (17.66 ± 1.04 μg/g DW and 27.00 ± 0.72 μg/g DW, respectively) were higher ($p < 0.05$) than those obtained before digestion (9.22 ± 2.02 μg/g DW and 16.68 ± 5.77 μg/g DW, respectively). This represented a BI of 191.44 % for stir-frying and 161.83 % for boiling.

Regarding CAR determination (Fig. 3B), digestion had an effect ($p < 0.05$) on the raw flower as well as on the cooked ones. For stir-fried flowers and those cooked by steaming, CAR values decreased ($p < 0.05$) after digestion. In addition, the BI obtained for these two cases of cooked flowers was lower than that obtained for the raw flower. Conversely, in the boiled flowers CAR increased ($p < 0.05$) after digestion from 17.80 ± 2.83 μg/g DW to 63.30 ± 1.05 μg/g DW, which corresponded to a BI of 355.62%. The addition of oil has been shown to be a favorable step to increase the BI of fat-soluble compounds (Castañeda-Rodríguez et al., 2023). However, it is not clear why in our experiment this did not occur. It is known that the presence of fiber,

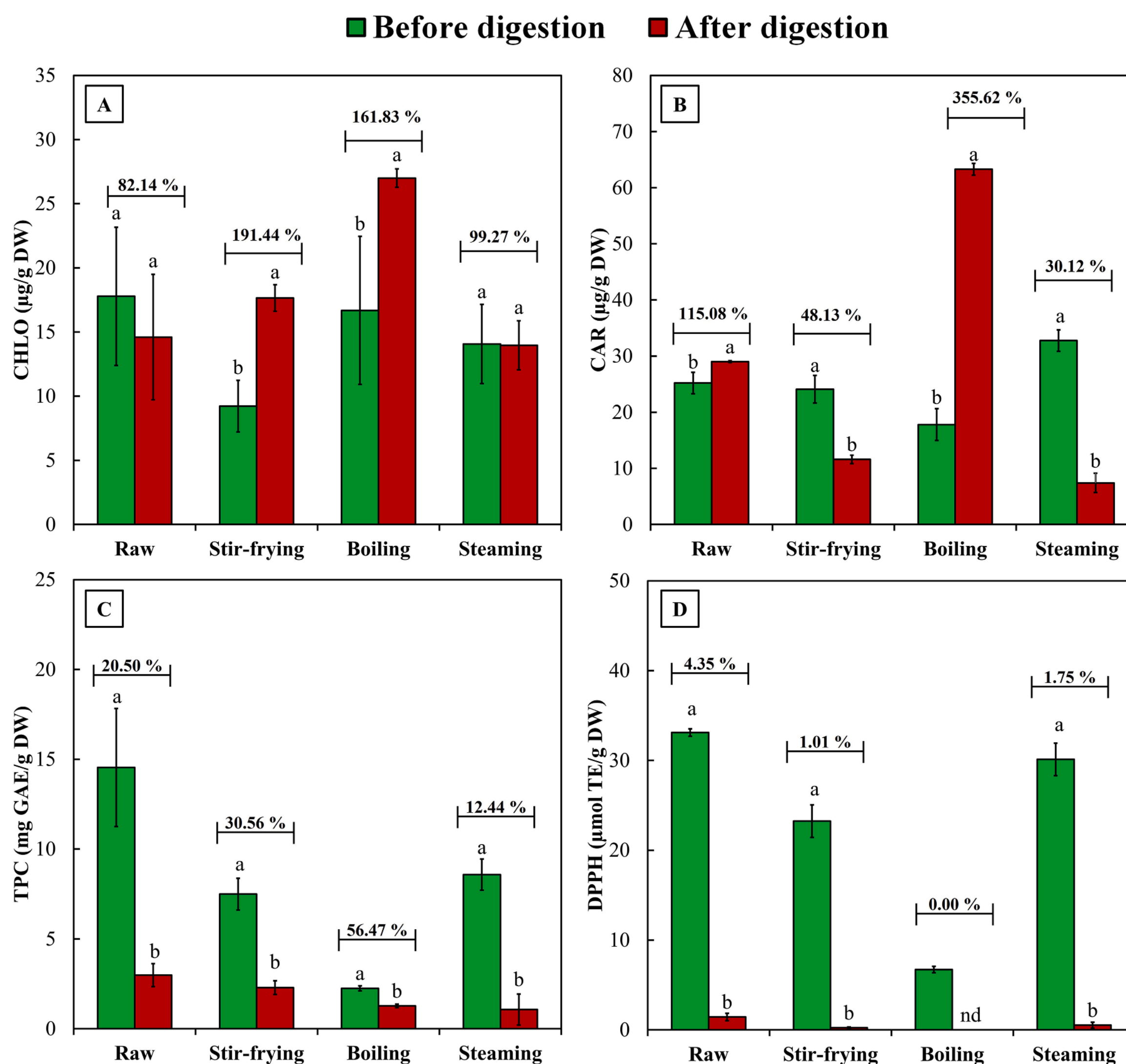

Fig. 3. The effect of *in vitro* digestion on bioaccesible bioactive compounds and antioxidant capacity of raw and cooked yucca flowers. A: total chlorophyll content (CHLO), B: total carotenoid content (CAR), C: total phenolic content (TPC), and D: DPPH assay (DPPH). nd: not detected. Bioaccesibility index is shown above the bars for each cooking method. Different letters show significant differences ($p < 0.05$) between flowers before and after digestion.

lignins, and polysaccharides can affect the digestion of some bioactive compounds (Holland et al., 2020; Veda et al., 2010). Therefore, in the present experiment, this could have affected the BI of the studied compounds.

Regarding TPC, the effect of digestion on the raw flowers and those cooked is shown in Fig. 3C. Digestion had an effect ($p < 0.05$) on TPC in all cases studied. In the raw and cooked flowers, digestion decreased the concentration of TPC compared to the flowers before digestion. However, the BI of TPC in stir-fried and boiled flowers was higher compared to its counterpart in the raw flower. The decrease in TPC may be due to their chemical degradation caused by the changes between alkaline and acidic pH occurring in the gastric and intestinal stages, respectively (de Morais et al., 2020).

Similar to TPC, digestion had an effect ($p < 0.05$) on DPPH in both raw and cooked flowers. The amounts found in the raw, stir-fried, boiled, and steamed samples (33.11 ± 0.42 μmol TE/g DW, 23.24 ± 1.81 μmol TE/g DW, 6.72 ± 0.37 μmol TE/g DW, and 30.13 ± 1.81 μmol TE/g DW, respectively) decreased almost completely after digestion; in fact, for this determination, BI values were between 1.01 % and 4.35 %. These low DPPH values may be related to the low TPC concentrations that were also found in the digested flowers, since TPC possess antioxidant capacity.

The bioactive molecules analyzed (CHLO, CAR, and TPC) differ in size, weight and chemical structure, which could modulate the effect caused by digestion. In general terms, digestion affected CAR and CHLO less, even so, the TPC values found in the digested samples ranged from 1.07 ± 0.86 to 2.98 ± 0.64 mg GAE/g DW, which is higher than those reported in other digested foods, such as onions (Cattivelli et al., 2021).

In summary, the effect of digestion was more evident in the determinations related to lipophilic compounds (CHLO and CAR) than in those related to hydrophilic compounds (TPC and DPPH). In this sense, digestion affected CHLO and CAR to a lesser extent compared to TPC and DPPH. In addition, the results show that the oil used in the stir-frying originated a positive effect on fat-soluble compounds bioaccessibility; this response has been further elaborated in another work (Castañeda-Rodríguez et al., 2023), which suggests that the addition of fat improves the digestion of fat-soluble compounds, mainly CAR, due to the formation of micelles that are more bioaccessible.

3.5. Non-accessible compounds

Fig. 4 shows the microscopies obtained from the non-soluble fraction of the digested flowers. Here, green color indicates the presence of autofluorescent CAR and blue color indicates the presence of carbohydrates. In the raw flowers (Fig. 4A), microscopy showed that the digested flowers are practically intact; a large presence of carbohydrates that make up the cell wall can be observed (blue color). Moreover, since the flowers are not cooked, they maintain their physical integrity to a large extent (as shown in the microstructure experiments: Fig. 2A-C) and their CAR remain in their native state and, therefore, are less accessible compared to the cooked flowers. These results explain the low CAR BI values found in the raw flower (Fig. 3B). As for the cooked flowers, although the three cooking methods resulted in similar CAR content (Table 2), no non-accessible CAR were found to be present in the microscopies. This result is unexpected considering the low BI values found in the stir-frying and steaming methods, so the CAR could have been degraded by pH action. Also, in all microscopies of the cooked flowers, a slight blue color is visible (Fig. 4B-D), indicating the presence of small amounts of carbohydrates that were not accessible and were retained in the non-soluble fraction.

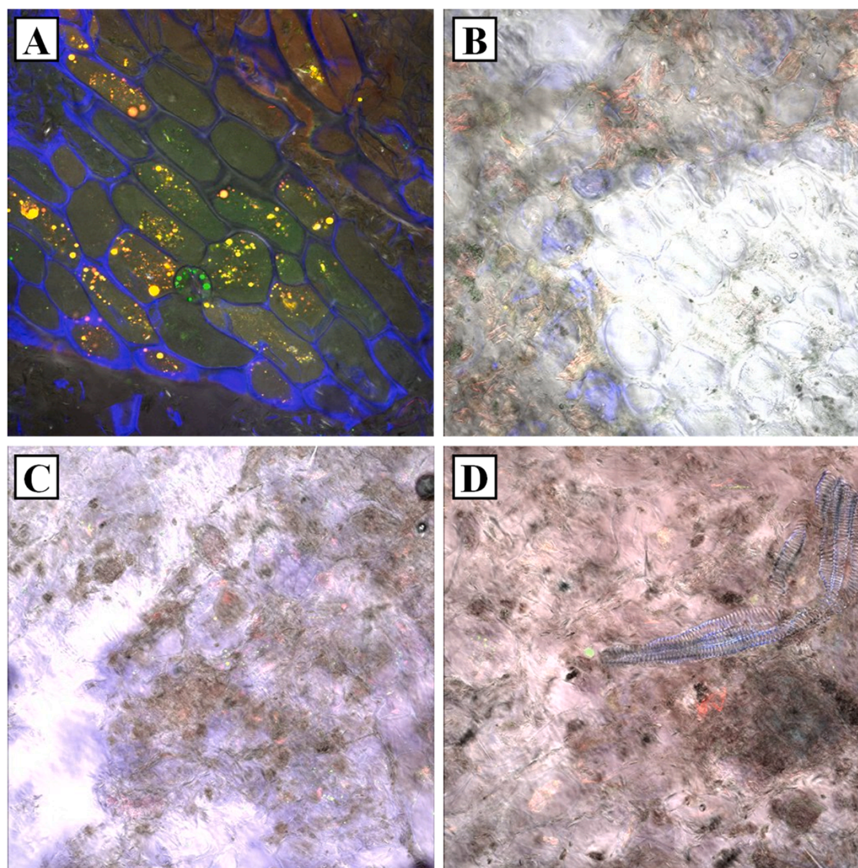

Fig. 4. The effect of cooking methods on non-bioaccesible fraction of digested flowers observed at 40X. A: raw flower, B: stir-fried flower, C: boiled flower, and D: steamed flower. The red and blue fluorescent colors stain lipids and carbohydrates, respectively, and green color are autofluorescence carotenoids.
Note: Colors should be used for all figures in the printed version.

4. Conclusions

The results found in this research contribute to understand how various household preparation techniques can enhance the value of Mexican edible flowers, particularly by improving the bioavailability of some of their beneficial components. Although raw yucca flowers are highly nutritious, they are often cooked for extended periods of time before being consumed. The results indicate that these culinary practices could be detrimental to the properties of these flowers, and therefore, also reducing their value as an ingredient for food-to-food fortification. In this regard, steaming and short-time cooking (3 min) would be a viable option for developing new preparations in which yucca flowers could provide greater fortification to the foods in which they are incorporated, in terms of increasing the macronutrient and bioactive content of these new recipes.

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Ethical statement - Studies in humans and animals

This investigation did not involve studies in humans and animals.

CRediT authorship contribution statement

Rey Castañeda-Rodríguez: Writing – original draft, Methodology, Formal analysis, Data curation. **Amparo Quiles:** Writing – review & editing, Supervision, Methodology, Funding acquisition. **Empar Llorca:** Writing – review & editing, Supervision, Methodology. **César Ozuna:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no potential conflicts of interest.

Data availability

Data will be made available on request.

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