



Article

Bioaccessibility and Antioxidant Capacity of Alkaloids from Microencapsulated Extract of Eggplant (*Solanum melongena* L.) Biomass

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Abstract: Eggplant is a vegetable grown worldwide, and due to quality standards, large amounts of biomass are generated after harvest. Biomass is considered a source of bioactive compounds with antioxidant properties. Therefore, this research aimed to evaluate the bioaccessibility (BA) and antioxidant capacity of microencapsulated alkaloids from eggplant fruit biomass. Eggplant biomass was collected, and the total alkaloid content, antioxidant capacity (TEAC, FRAP, and ORAC), and alkaloid profile (UPLC/MS) were determined before and after the *in vitro* digestion of encapsulated and non-encapsulated alkaloids. *In vitro* digestion significantly reduced the total alkaloid content and antioxidant capacity of alkaloid-rich extracts. Microencapsulation increased the bioaccessibility of alkaloid-rich extracts threefold, and the antioxidant capacity increased by up to 50%. The antioxidant capacity of digested microcapsules increased, and their bioaccessibility was higher than that of non-encapsulated alkaloids. Solamargine and solasonine decreased by 17 and 15% BA, respectively, during *in vitro* digestion; however, microencapsulation protected these alkaloids during *in vitro* digestion and enhanced their content. This study demonstrates that microencapsulation is a feasible option to protect alkaloids and preserve their antioxidant capacity during gastrointestinal digestion, as well as to give added value to eggplant plant biomass.

Keywords: eggplant; alkaloids; microparticles; bioaccessibility; antioxidant capacity



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1. Introduction

Eggplant (*Solanum melongena* L.) is a vegetable that belongs to the genus *Solanum*, considered the largest of the Solanaceae family, to which the potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum* L.) also belong [1]. In 2021, Mexico produced 125,531 t of eggplant, and the state of Sinaloa contributed 95.6% of the national production [2]. However, due to quality standards, an excess of agricultural biomass is produced in the fields [3,4]. Annually, according to the International Society of Horticultural Sciences (ISHS), 140 billion t of biomass from horticultural products is generated in the world, representing losses of 40% in post-harvest, and the main biomass found includes seeds, peels, leaves, roots, stems, and fruits [5,6]. Much of the biomass is not used, and to reduce the large amounts of biomass, it is returned to the soil, burned, or used as livestock feed [4,7]. These methods do not always turn out to be profitable and can have a negative effect on climate change; for this reason, biomass disposal is considered an issue of social, economic, and environmental concern [5,6].

In eggplant cultivation, biomass represents an important source of secondary metabolites that are present throughout the entire plant; one of the groups of compounds with important biological activity is alkaloids, which are compounds that contain at least one basic nitrogen atom in their structure; they are classified according to their origin, structure (nitrogen position), or biosynthetic route. They have been identified in eggplant fruit, stems, leaves, roots, and flowers [8–11]. A previous study identified the alkaloids khasianine, solamargine, solasonine, and solasodine in eggplant biomass [8]. Likewise, another study in over-mature eggplant pulp reported solasonine and solamargine as the alkaloids synthesized in the highest proportion [3]. In another investigation on the pulp of two eggplant varieties, 19 alkaloids were identified: solanidenetriol chacotriose, solanidenediol chacotriose, solanandaine isomer I, dehydrosolamargine, solasonine, robenoside B, solanandaine, malonyl-solanidenediol chacotriose, solamargine, solanidatetraenol chacotriose, malonyl-solanidatetraenol chacotriose, solanandaine isomer II, malonyl-solanandaine, arudonine, robenoside B isomer, malonyl-malonyl-solamargine, solamargine isomer, and solanandaine isomer III. In addition, the authors reported an inhibition of the enzyme acetylcholinesterase by the alkaloids in eggplant pulp [12]. Some studies have found that alkaloids are associated with various properties, such as antioxidant, anticancer, and antiproliferative effects [13–17], stroke prevention [18], and anti-inflammatory, antiepileptic, analgesic, hypolipidemic, and hypotensive properties, as well as functioning as nervous system depressants [14,19–21]. In this context, Chen, et al. [22] evaluated the effect of glycosylated alkaloids, finding that these had an antimalarial effect in an in vivo assay, achieving a 50% reduction in the disease with a dose of 7.5 mg/kg. Likewise, Al Sinani et al. [23] observed a cytotoxic effect of solamargine on melanoma cell lines (WM115 and WM239). Also, steroidal alkaloids from *Sarcococca saligna* presented an effect on glucose control in diabetic rats [24]. Structurally, alkaloids are varied, and their biological activity is dependent on their structure and bioaccessibility [25].

Bioaccessibility is defined as the percentage of compounds released from a food matrix that are accessible for absorption by the epithelial cells of the small intestine [26,27]. It has been shown that bioactive compounds have low bioaccessibility, which can hinder their biological activity. In this sense, the evaluation of piperine alkaloids from *Piper nigrum* mixed in food preparation showed a bioaccessibility of 60%. In terms of content, after digestion, the piperine alkaloid decreased from 6.5 to 3.9 µg/g [28]. On the other hand, Pasli et al. [29] reported that simulated digestion decreased the total phenolic and flavonoid content of eggplant extracts and reduced their antioxidant capacity. Therefore, there is a need to protect these compounds from degradation during the digestive process. One of the most used strategies to enhance the bioaccessibility of bioactive compounds such as alkaloids is the microencapsulation process; this technique also allows these to be released at specific sites in a controlled manner and under certain conditions [30]. Spray drying has become a widely used method for encapsulating compounds. The leading encapsulating agent is maltodextrin (a water-soluble biopolymer), which is used to protect different bioactive compounds, as described by Srinivasan and Shanmughasundaram [31], who microencapsulated the alkaloid vasicine, derived from *Adhatoda vasica* Nees., by spray drying using maltodextrin in various proportions and obtained an encapsulation efficiency in the range from 69 to 84%, as well as an alkaloid retention rate of 69%.

In addition to the above information, it is necessary to highlight that alkaloids have various uses in different areas, such as the food and pharmaceutical industries. These compounds are low or insoluble in aqueous media, and their activity has been observed to decrease or be lost after the digestive process, so it is essential to provide adequate protection for them to increase their solubility in aqueous media and increase their bioaccessibility. Therefore, this study aimed to evaluate the bioaccessibility and antioxidant capacity of alkaloids derived from the biomass of the eggplant plant (*Solanum melongena* L.) before and after microencapsulation.

2. Materials and Methods

2.1. Chemical and Biomass Collection

Methanol (Fermont, Monterrey, NL, México), chloroform, and mass spectrometry-grade acetonitrile (J.T Baker, Phillipsburg, NJ, USA). Chemical reagents, 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (Sigma Aldrich, St. Louis, MO, USA), potassium persulfate (Sigma Aldrich, St. Louis, MO, USA), 2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH) (Sigma Aldrich, St. Louis, MO, USA), fluorescein (Sigma Aldrich, St. Louis, MO, USA), α -solasonine (Sigma Aldrich, St. Louis, MO, USA), α -solamargine (Sigma Aldrich, St. Louis, MO, USA), solasodine $\geq 95\%$ (Sigma Aldrich St. Louis, MO, USA), and 6-hydroxy-2,5,7,8-tetramethylchroman-2 carboxylic acid (Trolox) (Sigma Aldrich, St. Louis, MO, USA), 2,4,6-Tri-(2-pyridyl)-s-triazine (TPTZ) (Sigma Aldrich, St. Louis, MO, USA), iron(III) chloride hexahydrate($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) (Sigma Aldrich, St. Louis, MO, USA).

Eggplant plants (*Solanum melongena* L.) were collected in June 2022, 15 days after the last harvest (open field), from a farm in Villa Juárez, Navolato, Sinaloa, Mexico (coordinates: 24°46'00.3" N 107°41'51.6" W; soil type: vertisol; climatic conditions: minimum and maximum temperature of 23 °C and 34 °C, respectively). The eggplant is a classic type, Barcelona variety, obtained from the seed company Fitó, based in Mexico. Species identification was provided by the Mexican Herbaria Network; the catalog number is v0272200WIS. The eggplant fruit was washed with water and rinsed in a chlorinated solution of 50 ppm; finally, samples were dried at 20 °C and then freeze-dried (−49 °C and 0.079 bars) in a freeze-dryer FreeZone 18 (Labconco, Kansas City, MO, USA), ground in an IKA Werke M20 (Wilmington, NC, USA), and stored in sealed acetate bags at −20 °C.

2.2. Alkaloid Extraction

An extract rich in alkaloids was obtained using the QuEChERS method with some modifications, as reported by Lehotay [32]. For this, 3 g of dried sample was homogenized with 12 mL of distilled water and 15 mL of 1% acidified acetonitrile and sonicated for 10 min; then, 4 g of magnesium sulfate and 1 g of sodium acetate were added and centrifuged at 4000 rpm for 5 min at 4 °C. The supernatant was purified with a C18 cartridge (Waters Corp., Milford, MA, USA), dried in a SyncorePlus evaporator (Buchi, New Castle, DE, USA), and stored at −20 °C.

2.3. Total Alkaloid Content

The method based on alkaloid reaction with bromocresol green (BCG) was used, with some modifications [33]. To quantify the alkaloid content, we weighted 63 mg of the dry extract obtained by the QuEChERS method and a 0.5 g microcapsule (1 mL of water was added to release the alkaloids); then, 2.5 mL of chloroform was added to each sample, the mixture was placed in a separation funnel, and 2.5 mL of phosphate buffer at pH 7.4 and 2.5 mL of BCG were added. The organic phase was collected and quantified using a spectrophotometer at 470 nm. The results were calculated as mg solasodine equivalent per g dry extract (mg ESS/g DE) and as mg solasodine equivalent per g microencapsulated powder (mg ESS/g powder).

2.4. Antioxidant Capacity Assay

The antioxidant capacity of the alkaloid-rich extract without microencapsulation and with microencapsulation was evaluated before and after in vitro gastrointestinal digestion through the Trolox equivalent antioxidant capacity (TEAC), ion reduction capacity by ferric ion-reducing antioxidant power (FRAP), and the oxygen radical absorbance capacity (ORAC) assays.

2.4.1. Trolox Equivalent Antioxidant Capacity (TEAC)

This assay is based on the absorbance inhibition of the ABTS•+ radical caused by the reaction with antioxidants, following the methodology of Karadag et al. [34]. The reaction solution was prepared by homogenizing 1 mL of 2.6 mM potassium persulfate and 1 mL of 7.4 mM ABTS•+. The mixture was left at room temperature for 16 h. For the assay, 10 µL of the sample was added to 190 µL of the reaction solution (prepared one day before with 1 mL of 7.4 mM ABTS•+ and 1 mL of 2.6 mM potassium persulfate). This was incubated in the dark for 2 h. The absorption was read at 734 nm on a Synergy HT microplate reader (BioTek, Inc., Winooski, VT, USA). The results were expressed as micromoles of Trolox per g of dry extract (µmol TE/g DE) and µmol TE/g powder.

2.4.2. Ferric Reducing Antioxidant Power Assay (FRAP)

This antioxidant assay was determined following the methodology of Benzie and Strain [35]. In this assay, 120 µL of the FRAP reagent (1 mL 30 mM TPTZ, 1 mL 60 mM FeCl₃·6H₂O, and 10 mL acetate buffer) was added to 30 µL of the sample. The mixture was incubated in the dark for 4 min, and the absorbance was read at 590 nm in a Synergy HT microplate reader (BioTek, Inc., Winooski, VT, USA). The results were expressed as µmol TE/g DE and µmol TE/g powder.

2.4.3. The Oxygen Radical Absorbance Capacity (ORAC)

The assay was determined according to Huang et al. [36]. For this assay, a 96-well microplate with a transparent bottom and black walls was used. To it, 25 µL of the sample and 75 µL of the phosphate buffer was added as a blank. This was placed in a Synergy HT microplate reader (BioTek, Inc., Winooski, VT, USA), which dispensed 75 µL of 95.8 µM AAPH (radical generator) and 200 µL of 0.96 µM fluorescein and started a kinetic of fluorescence loss at 37 °C for 70 min at 485 nm excitation and 580 nm emission wavelengths. The results were expressed as equivalent micromoles of Trolox per g of dry extract (µmol TE/g DE) and µmol TE/g powder.

2.5. Identification and Quantification of Alkaloids by Ultra-High-Resolution Liquid Chromatography/Mass Spectrometry (UPLC/MS)

An UPLC chromatographic system coupled to a Waters Xevo TQ-S mass spectrometer (Waters Corp., Milford, MA, USA) was used to identify and quantify the alkaloids and glycoalkaloids in the dry extract. Samples were automatically injected through a Waters Sample Manager–FTN Acquity system to an Acquity H series UPLC equipped with an Acquity UPLC BEH Phenyl 1.7 µm, 2.1 × 100 mm column. The conditions were mobile phase A (5 mM ammonium formate, pH 3.0) and phase B (acetonitrile + 0.1% formic acid). At time 0, it started with 90% A and 10% B with low flow that gradually increased 0.3 mL/min and was maintained in these conditions for 5 min, then 10% A and 90% B; at 5.1 min, it was raised to 90% A and 10% B and was maintained until 8 min. Likewise, the conditions for the Waters Xevo TQ-S mass spectrometer were established through the MassLynx software. The conditions were as follows: positive electrospray ionization (ESI+), source temperature 150 °C, cone voltages 60 to 100 V, and capillary voltages 3.21 kV. The desolvation temperature was 400 °C, the desolvation gas flow was 650 L/h, and the collision gas flow was 0.15 mL/min. The run time was 10 min with an injection volume of 5 µL, and the MRM (multiple reaction monitoring) mode was used for analyte analysis. Retention time and transitions using MRM were used for identification, and calibration curves (solamargine and solasonine) were used for quantification to compare the area under the curve of the obtained peaks.

2.6. Alkaloid Microencapsulation

An aliquot of 50 mL of an alkaloid-rich extract (concentration of 84 mg of dry extract) stock was mixed with 8 g maltodextrin (MD) with ten dextrose equivalents (DEs) as wall material. The mixture was homogenized on a stir plate at 600 rpm until completely dissolved. Subsequently, the mixture was fed to a Spray Dryer Yamato ADL311S (Yamato Scientific Co., Santa Clara, CA, USA). The conditions were as follows: the inlet temperature was 145 °C and the outlet temperature was 80 °C; the atomization pressure was 0.1 MPa; the feed flow was 5 mL/min; and the airflow was 0.32 m³/min. The recovered powder (microencapsulated alkaloids) was weighed to obtain the yield of the process and stored at 20 °C [37].

2.7. Physical Characterization of the Microcapsules Loaded with Alkaloids

2.7.1. Morphology

The morphology of the microcapsules was analyzed using an EVO-50 scanning electron microscope (SEM) (Carl Zeiss Oberkochen, Germany). The powder was coated with gold in a DESK II model ionizer, Denton Vacuum brand, operating with a voltage of 10 kV and under high vacuum conditions. The size was determined using ImageJ Software <https://imagej.net/ij/>.

2.7.2. Moisture

The moisture percentage in the microcapsules was determined using the gravimetric method AOAC 925.09 [38]. For this method, ~2 g of the microcapsules was weighed and dried in a Yamato IC103CW ventilated oven (Yamato Scientific Co., Santa Clara, CA, USA) at 110 °C for 24 h. After the time had elapsed, the microcapsules were removed from the oven, put on a desiccator, and cooled to room temperature before measuring the final mass.

2.7.3. Process Yield

The encapsulation yield of the process was calculated using a gravimetric technique, which measures the relationship between the weight of powder after drying and the total solids at the beginning of the feed. It was reported as a percentage [39].

$$\% = \frac{(\text{powder weight after drying})}{(\text{total solids at initial feeding})} \times 100 \quad (1)$$

2.7.4. Encapsulation Efficiency (EE)

This was calculated by the content of total alkaloids in the encapsulated powder using the value of the encapsulated extract obtained divided by the theoretical encapsulated extract (mg solasodine equivalents) and reported as a percentage.

$$EE\% = \frac{(\text{encapsulated extract obtained})}{(\text{theoretical encapsulated extract})} \times 100 \quad (2)$$

2.8. Bioaccessibility Assay

The percentage of bioaccessibility (% BA) of the alkaloids in the extract and the microparticles was determined following the INFOGEST method by Brodkorb et al. [40] with some modifications. This in vitro model simulates the digestion of products when they pass through the mouth, stomach, and small intestine, imitating the chemical composition, pH of the digestive fluids, temperatures, and transit times. To start the process, 8 mg of the dry extract rich in alkaloids and 0.5 g of the microencapsulated extract were used, and 2 mL of the simulated oral phase was added and incubated for 2 min at 37 °C. After that, 2 mL of the gastric phase was added, the pH was adjusted to 3, and the mixture was incubated at 37 °C for 2 h. Finally, 4 mL of the intestinal phase was added proportionately to the total liquid until the gastric phase. The pH was adjusted to 7 and incubated for 2 h at 37 °C. After this process, absolute methanol was added in a 1:1 ratio. The samples were centrifuged at

10,000 rpm for 15 min at a temperature of 4 °C. The supernatant was recovered and stored at −20 °C. The in vitro bioaccessibility of the dry extract and microencapsulated extract was calculated with the following equation:

$$%BA = \frac{(\text{final concentration})}{(\text{initial concentration})} \times 100 \quad (3)$$

The final concentration is the result obtained through an assay used to evaluate the end of the intestinal phase, and the initial concentration is the result obtained from undigested samples.

2.9. Statistical Analysis

This study followed a completely randomized experimental design with one factor and three replications. The results obtained were analyzed using an analysis of variance (ANOVA), using Tukey's mean comparison test ($p \leq 0.05$), with a significance value of 5% in case of significant differences. The statistical package used to analyze the results was Minitab version 2019.

3. Results

3.1. Total Alkaloid Content of Eggplant Biomass Extract

The content of total alkaloids in the fruit biomass extract was quantified before and after the in vitro gastrointestinal simulation. The total content of alkaloids (Figure 1A) was higher in the undigested extract (173 mg ESS/g), but after the digestion process, a significant reduction was seen (7 mg ESS/g); these changes resulted in a bioaccessibility of 4% (Figure 1C).

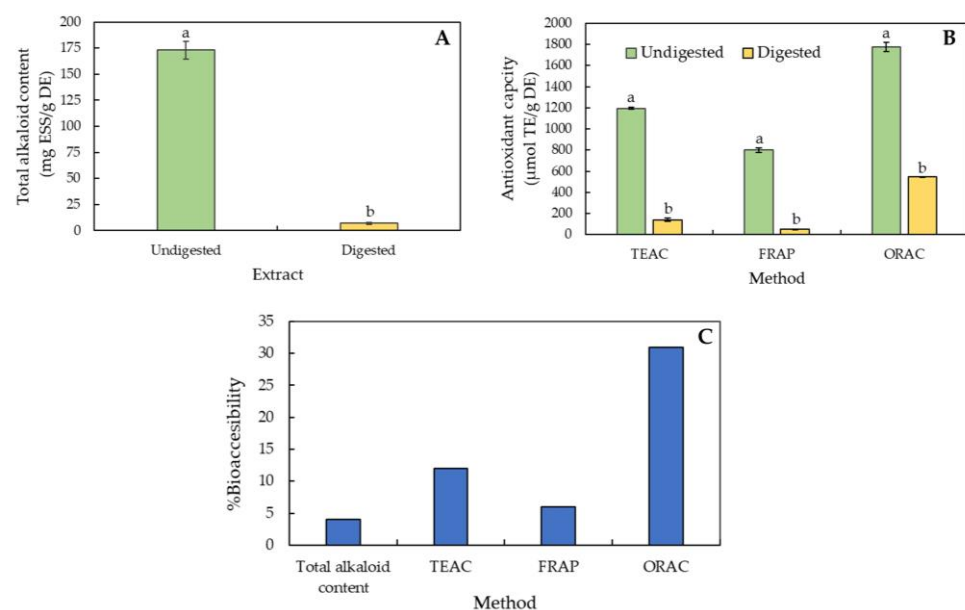


Figure 1. Total alkaloid content (A), antioxidant capacity (B), and bioaccessibility percentage (C) of alkaloid-rich extract of eggplant biomass fruit. mg ESS/g DE: mg solasodine equivalent per g dry extract; μmol TE/g DE: equivalent micromoles of Trolox per g of dry extract. Data are shown as means. Bars indicate standard deviation (n = 3). Different letters in (A,B) indicate statistically significant differences as assessed by the Tukey test ($p < 0.05$). Comparisons in (B) are by method.

3.2. Antioxidant Capacity of Alkaloid-Rich Eggplant Fruit Extract

The antioxidant capacity values of the alkaloid-rich extract from the fruit before and after the *in vitro* digestive simulation, measured by ORAC, TEAC, and FRAP, ranged from 50 to 1778 $\mu\text{mol TE/g DE}$ (Figure 1B). Our results showed a higher antioxidant capacity in the undigested extract measured by the ORAC and TEAC assays. *In vitro* digestion significantly reduced the antioxidant capacity of the alkaloids in each method evaluated. In addition, the percentage of bioaccessibility (%BA), determined by TEAC, FRAP, and ORAC, ranged from 6 to 31%, with the highest bioaccessibility being determined by the ORAC method, at 31% (Figure 1C).

3.3. Identification and Quantification of Alkaloids by Ultra-High-Resolution Liquid Chromatography Coupled to Mass Spectrometry (UPLC/MS) in Extract

The alkaloids and glycoalkaloids of eggplant fruit were extracted using the QuEChERS method. The alkaloid extract was quantitatively evaluated before and after the *in vitro* digestive simulation using commercial standards (solamargine and solasonine) previously reported in the genus *Solanum*. The extract from the eggplant biomass showed a higher concentration of solamargine, with values of 2485 ng/g, while solasonine had a value of 1724 ng/g before the *in vitro* digestion process. After digestion, the concentrations of these glycoalkaloids were reduced by up to 80% (Table 1).

Table 1. Alkaloids identified and quantified by UPLC-MS in the alkaloid-rich extract of eggplant fruit biomass.

Compound	Molecular Mass [M + H] ⁺	Retention Time (min)	Undigested (ng/g)	Digested (ng/g)	% BA
Solamargine	867.49	3.86	2485 ± 6 ^a	431 ± 11 ^b	17.34
Solasonine	883.49	3.81	1724 ± 35 ^a	263 ± 9 ^b	15.26

Data are shown as mean ± standard deviation (n = 3). Equal letters indicate no statistically significant difference as assessed by the Tukey test ($p < 0.05$). Comparisons are by row and treatment.

3.4. Physical Characterization of Microcapsules Loaded with Alkaloids

3.4.1. Morphology

One of the essential characteristics of the encapsulation process is size and shape. In this sense, the particles obtained had a diameter that varied from 1 to 14 μm ; the shapes were spherical and irregular, with depressions on the surface (Figure 2).

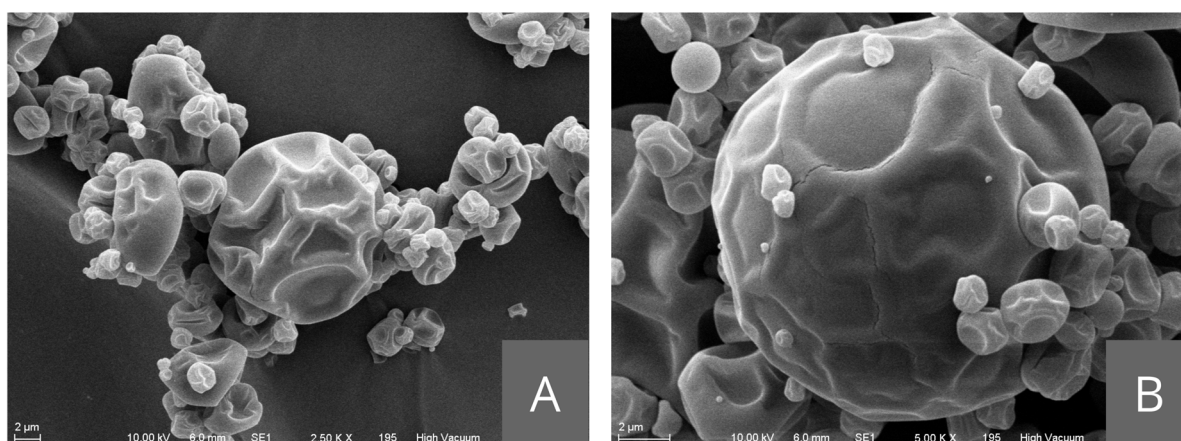


Figure 2. Scanning electron microscopy of the microcapsules of the alkaloid-rich extract of eggplant fruit. Specifications: (A), magnification: 2.5 K X; beam intensity (HV): 10.00 kV; sample to lens distance (WD): 6.0 mm. (B), magnification: 5.0 K X; beam intensity (HV): 10.00 kV; sample to lens distance (WD): 6.0 mm.

3.4.2. Moisture and Yield

The moisture of the encapsulated products was low (3.4%); according to NOM-183-SCFI-2012, powdered dairy products must have a maximum of 4% moisture to maintain their stability. The yield in this research was low (45%), which was considered favorable (>50%) [41].

3.4.3. Encapsulation Efficiency

This variable is defined as the amount of alkaloids that managed to be trapped within the wall material (maltodextrin) [42]. In this research, the encapsulation efficiency was 80%.

3.5. Total Alkaloid Content of Encapsulated Product from Eggplant Fruit

The results of the total alkaloid content of the digested and undigested microcapsules are shown in Table 2, where a decrease from 1.59 to 0.185 mg ESS/g of powder can be observed. The alkaloid content decreased after in vitro digestive simulation; however, regarding bioaccessibility percentage, this was higher (12%) than in the non-encapsulated extract (4% BA), as shown in Figure 1C.

Table 2. Total alkaloids and antioxidant capacity of eggplant fruit microcapsules.

Method	Undigested	Digested	%BA
Total alkaloid content *	1.59 ± 0.06 ^a	0.185 ± 0.04 ^b	12
TEAC **	3.90 ± 0.05 ^b	8 ± 0 ^a	>100
FRAP **	3.46 ± 0.22 ^a	2.75 ± 0 ^b	79
ORAC **	16 ± 0 ^b	30 ± 1 ^a	>100

* mg ESS/g powder; ** μmol TE/g powder. Data are shown as mean ± standard deviation (n = 3). Equal letters indicate no statistically significant difference as assessed by the Tukey test ($p < 0.05$).

3.6. Antioxidant Capacity of Encapsulated Product from Eggplant Fruit

The antioxidant capacity as determined using the ORAC assay presented the highest value in undigested microcapsules (Table 2). Meanwhile, in TEAC and FRAP, the values were lower. After the in vitro digestive simulation, the antioxidant capacity of the microcapsules increased almost twice, as determined using the ORAC (30 μmol TE/g) and TEAC (8 μmol TE/g) assays; FRAP, on the contrary, decreased. The bioaccessibility of the antioxidant capacity of the microcapsules loaded with the alkaloid-rich extract evaluated by the three methods ranged from 70% to values greater than 100%, which were higher than those presented by the unencapsulated extract (6 to 31% BA).

3.7. Identification and Quantification of Alkaloids by Ultra-High-Resolution Liquid Chromatography Coupled to Mass Spectrometry (UPLC/MS) of Encapsulated Product from Eggplant Fruit

The compounds that were quantified in the undigested and digested encapsulated eggplant alkaloid extract were solamargine and solasonine. Undigested microcapsules had a higher solamargine than solasonine content (Table 3). After the in vitro digestive simulation, an increase in the content of digested microencapsulated glycoalkaloids was observed compared to the undigested microcapsule; the values ranged between 9.743 and 12.74 ng/g.

Table 3. Alkaloids identified and quantified by UPLC-MS in eggplant fruit microcapsules.

Compound	Molecular Mass [M + H] ⁺	Retention Time (min)	Undigested (ng/g)	Digested (ng/g)
Solamargine	867.49	3.86	6.111 ± 1 ^b	12.74 ± 0 ^a
Solasonine	883.49	3.81	5.169 ± 1 ^b	9.743 ± 0 ^a

Data are shown as mean ± standard deviation (n = 3). Equal letters indicate no statistically significant difference as assessed by the Tukey test ($p < 0.05$). Comparisons are by row and treatment. ND: not detected.

4. Discussion

In plants, alkaloids act as a defense mechanism against biotic and abiotic stress, such as attacks by pests, herbivores, pathogens, UV radiation, drought, etc. Different types can be found, such as tropane alkaloids, pyrrolizidine alkaloids, indolic alkaloids, and steroidal alkaloids. In this context, research has indicated that the *solanum* species has many biologically active alkaloids [43].

According to the literature, total alkaloids can be expressed as equivalent of the alkaloid present in the species. In this context, Păltinean et al. [44] reported 8.6 mg of chelidonine equivalents per gram in an alkaloid-rich extract of *Fumaria schleicheri*. Likewise, 26 mg of atropine equivalents per gram was reported in eggplant biomass [8]. Our results were expressed as mg of solasodine per gram DE with a value of 173. These differences can mainly be attributed to how the results are described, as well as to the species, type of extract, and extraction method. On the other hand, after the in vitro digestion process, the total alkaloid content in extracts obtained from eggplant fruit biomass is almost entirely reduced, with a bioaccessibility of 4%; this behavior was previously reported regarding the content of piperine mixed in food, which after in vitro digestive simulation indicated a decrease due to pH variations [28]. This effect could be due to the conditions of the different digestion phases, mainly pH [45], which could be causing physicochemical transformations by oxidation or interactions with other groups of compounds [46].

The TEAC assay measures the antioxidant capacity of hydrophilic and lipophilic compounds, offering a perspective on the group of compounds able to interact with the radical [47]. In this research, a decrease in the digested extract was shown. However, it has greater antioxidant capacity than what was reported in eggplant fruits in the commercial stage (7 $\mu\text{mol TE/g}$) by Elizalde-Romero et al. [48]; these differences are attributed to the stage of maturity and the type of compounds evaluated because the extraction method that was carried out removes the majority of secondary metabolites, such as phenols, saponins, flavonoids, and some alkaloids. This allows us to show that the alkaloid-rich extract of eggplant biomass has antioxidant properties, as do the alkaloids of other species of the *Solanum* genus, such as *Solanum macrocarpon* L. and *Solanum nigrum* L. [49]. Regarding the effect of in vitro digestion on the TEAC assay, our results showed a lower antioxidant capacity in the digested alkaloid extract compared to the undigested extract, which indicates that it is less bioaccessible (12%); this behavior was similar to that of *Solanum nigrum* evaluated by Moyo et al. [50], who reported 650 $\mu\text{mol TE/g}$ of undigested extract and 379 $\mu\text{mol TE/g}$ of digested extract. Similarly, the undigested extract of the fruit of *Solanum lycopersicum* showed a greater antioxidant capacity than the digested extract, at 713 $\mu\text{mol TE/g}$ and 430 $\mu\text{mol TE/g}$, respectively [51]. These results are superior to those reported in this research; however, the behavior of the extracts during the assay was similar, demonstrating the negative impact of in vitro digestion caused by the gastrointestinal environment.

Regarding the results of FRAP, which consist of the reduction of ferric ions, a greater antioxidant capacity was obtained in the undigested extract of the eggplant fruit biomass; these results were higher than those of the eggplant fruit collected after harvest, with values of 107 $\mu\text{mol TE/g}$ [8]; likewise, in different varieties of eggplant, values of 0.82 to 8.11 $\mu\text{mol TE/g}$ were reported [52]. These differences are attributed to the group of compounds that were extracted and evaluated, as well as to the eggplant's variety, region, and stage of maturity. As for the in vitro digestive simulation, it was found that the digested fruit extract decreased its antioxidant capacity just like extract from the fruit of *Solanum lycopersicum* reported 0.477 $\mu\text{mol TE/g}$ of undigested extract to 0.276 $\mu\text{mol TE/g}$ of digested [51]. These behaviors are attributable to the physicochemical and structural characteristics of the compounds affected by the digestive simulation. On the other hand, the antioxidant capacity was higher in this research and is related to the ability of alkaloids to reduce metal ions [53].

The ORAC method uses a radical generator to analyze the antioxidant capacity of compounds based on the transfer of hydrogen atoms [54,55]. The inhibition of the peroxy radical in this study was more significant in the undigested extract (1778 $\mu\text{mol TE/g}$). Compared to that reported in eggplant fruit biomass, its antioxidant capacity is almost 3 times higher (547 $\mu\text{mol TE/g}$) [8]. The differences could be due to the type of extract used for the assay. Similarly, our data were higher than the benzyloisoquinoline alkaloids in *Plumula nelumbinis*, with a value of 0.00553 $\mu\text{mol TE/g}$ [56], the same as the alkaloids present in *Catharanthus roseus*, with values of 185 $\mu\text{mol TE/g}$ [57] and 56 $\mu\text{mol TE/g}$ [58]. The previously reported data were lower than those of this research, possibly due to the biosynthetic origin and the type of alkaloids specific to each species and genus [59]. After in vitro digestive simulation, a lower capacity to transfer hydrogen atoms was observed; however, our data were superior to the hydrophilic compounds of *Solanum lycopersicum*, with values of 310 $\mu\text{mol TE/g}$ of undigested extract and 270 $\mu\text{mol TE/g}$ of digested extract [60]. *Solanum nigrum* leaves obtained values of 299 $\mu\text{mol TE/g}$ of undigested extract and 620 $\mu\text{mol TE/g}$ of digested extract [50]. The authors maintain that these differences could be related to the availability of hydroxyl groups of the compounds in the extracts and their physicochemical properties.

In general, a reduction in the antioxidant capacity of the alkaloid extract from eggplant biomass was observed in the three assays after in vitro digestion. In addition, a low bioaccessibility was obtained; it varied from 6 to 31% depending on the assay.

In eggplant fruits, the main glycoalkaloids reported in *S. melongena* are solasonine and solamargine, both glycosides of solasodine [59]. In previous research, the content of solasonine and solamargine in eggplant fruit was 0.062 ng/g and 0.373 ng/g, respectively [12]; these values were lower than what was found in this study, which may have been caused by the collection time, type of species, and crop conditions [9]. Currently, there are few reports on the bioaccessibility of alkaloids of the *Solanum* genus, so this research study is one of the few to report the effect of simulated gastrointestinal digestion on solamargine and solasonine content. In previous studies, it was reported that the alkaloid piperine (*Piper nigrum*) decreased by 60% after the digestion process [28]. Bioactive compounds tend to undergo structural changes such as isomerization, attributed to digestion conditions, intestinal enzymes' action, chemical elements such as transition metals, and oxygen [45]. Regarding the decrease in glycoalkaloids, the possible hydrolysis of glycosylated molecules is considered, caused by the enzymes and the pH of the different digestive phases [61]. Moreover, the differences observed between the assays could be explained by the ability of the compounds to transfer electrons [53], reduce metal ions [44], and transfer hydrogen atoms [55]. Various investigations have deduced that the antioxidant capacity of bioactive compounds depends on the content of OH groups in their structure [62–65]. In this sense, solasodine is an alkaloid that can present a branched side chain of solatriose and/or chacotriose, composed of hydroxyl groups, to which the antioxidant effect is attributed [15].

In the next stage of this study, the alkaloid-rich extract of the fruit of the eggplant biomass was microencapsulated with maltodextrin. Hydrophilic polymers such as maltodextrin have charged and non-ionic functional groups capable of forming hydrogen bonds with mucosal surfaces, besides having GRAS status [66,67]. In this sense, the encapsulation was carried out with the objective of protecting and improving the stability and bioaccessibility of the alkaloids, reducing the possible environmental interactions (temperature, light, humidity, and oxygen) [42]. In this context, the size of the microcapsules obtained was considered relatively homogeneous and uniform and not very narrow, which is regarded as favorable to maintaining the consistency of the microencapsulation [68]. The observed roughness is a common characteristic of microcapsules made with maltodextrin and spray drying due to the rapid evaporation of moisture and cooling [69]. In a study by Febriyenti et al. [70], the authors reported that the spray dryer method, compared with the lyophilization method, was the best for drying haruan extract, because the microparticles had a small size and uniform distribution, in addition to a spherical, dimpled, and wrinkled shape, while lyophilization resulted in voluminous flakes. On the other

hand, maltodextrin microcapsules with anthocyanins from the peel of *Solanum melongena* were made with an inlet temperature of 180 °C, forming smooth, dented, and irregular microcapsules due to the rapid loss of moisture due to high inlet temperature [71]. On the other hand, agglomerations were reported in maltodextrin microcapsules with the extract of the alkaloid vasicine from *Adhatoda vasica* Nees prepared with an inlet temperature of 110 °C [31]. Under the same conditions as in our study, sizes of 12 µm and a spherical morphology with depressions were obtained in maltodextrin microcapsules loaded with oregano phenolic compounds [37]. The differences between our study and what was found in the literature are due to the variations in inlet temperature [41].

Moisture is an indicator of quality and stability, which is why the spray drying technique is used in the industry to reduce the water content and guarantee the microbiological stability of products [72]. Low moisture percentages prevent powder hardening, guarantee a long shelf life, and protect it from microbiological contamination during storage [73]. Our data are similar to those of Arrazola, Herazo, and Alvis [71], who reported 3.4% moisture in anthocyanin microparticles from eggplant peel using maltodextrin as an encapsulating agent. In addition, maltodextrin microcapsules with alkaloids from the *Adhatoda vasica* plant made with an inlet temperature of 110 °C had a moisture content of 5.1% [31].

The yield of the powder obtained indicates the efficiency of the spray drying process. In this research, the yield was lower than that obtained in maltodextrin microcapsules (20 DE) with purple potato compounds (*Solanum tuberosum* L.); the process was made at an inlet temperature of 130 °C with a feed of 100 g, generating a yield of 58% [74]. Our data were slightly lower than those reported by Sarabandi, Jafari, Mahoonak, and Mohammadi [61], who worked with eggplant peel metabolites microencapsulated with maltodextrin (18–20 DE) with an air inlet of 140 °C and a feed of 300 mL, resulting in a yield of 52%. This result could be due to the quantity and low viscosity of the solution fed, as it is related to greater water elimination and less adhesion of the encapsulated microparticles on the walls of the dryer. In this sense, the value considered favorable is greater than 50%, and the main factors that influence this percentage are the viscosity of the wall material and the content of fed solids, so the differences found are attributed to the content of the feeding solution.

Encapsulation efficiency (EE) is considered an essential characteristic during the encapsulation process, as it is defined as the amount of material that was encapsulated within a wall material [42]. This research was similar to the study of microcapsules of the alkaloid-rich extract from *Adhatoda vasica* Nees leaves prepared with maltodextrin at an inlet temperature of 80 °C, which obtained 84% EE [31]. Contrary to this, phenolic compounds from the fruit of *Malpighia emarginata* DC were microencapsulated with maltodextrin, using an inlet temperature of 170 °C, obtaining 69% EE [75]. These differences may be due to high temperatures during the spray drying process, which can cause the loss of volatile active compounds, resulting in a low encapsulation efficiency [76]. Therefore, the development of a successful encapsulation system depends on knowledge about the chosen bioactive compounds or products to be encapsulated, the properties of the wall material, and the suitability of the delivery system [72].

The total content of undigested and digested alkaloids was evaluated at the same stage as the microencapsulated alkaloids.

After *in vitro* digestion simulation, the total content of microencapsulated alkaloids decreased; however, bioaccessibility was higher in the encapsulated extract than in the non-encapsulated extract. These results agree with those previously reported, which claim that the microencapsulation of bioactive compounds increases the percentage of bioaccessibility concerning the non-encapsulated alkaloid extract when they pass through the three phases of the digestive system because the encapsulating agent manages to protect them from the conditions of the gastrointestinal simulation phases, namely, enzymatic and pH variation [46,77,78]. The protection of microencapsulated alkaloids with maltodextrin may be due to the possible formation of polysaccharide–alkaloid complexes through non-covalent interactions, as reported by [79], who attributes this to interactions between

the structural units of the encapsulating agent (glucose in MD) and the encapsulated compound (alkaloids), so that hydrogen bonds are generated with the hydroxyl groups of the polysaccharides and the hydroxyl groups of the glycoalkaloids (ionic and/or Van der Waals forces).

Antioxidant compounds are sensitive to high temperatures, light, and pH; for that reason, encapsulation techniques have been carried out to protect them and improve their functionality [80]. In this sense, the microcapsules were evaluated using the TEAC test, where a greater antioxidant capacity was found in the digested microencapsulate (8 $\mu\text{mol TE/g}$ powder), coinciding with what was reported in MD microcapsules from the fruit of *Eugenia stipitate*, with values of 136 $\mu\text{mol TE/g}$ and 253 $\mu\text{mol TE/g}$ for undigested and digested powder, respectively [81]. This may be due to the deprotonation of the hydroxyl groups of the bioactive compounds at high pH [29].

The reduction of metal ions was more significant in the undigested microcapsules (3.4 $\mu\text{mol TE/g}$ powder), similar to the MD microcapsules of bioactive compounds from the fruit of *Eugenia stipitate*; the authors reported that after in vitro digestion simulation, the antioxidant capacity decreases slightly [81]. This is attributed to the ability to chelate metals due to the sample's pH and the method's optimal pH and structural modifications due to enzymatic hydrolysis, which causes the breakdown of glycosidic bonds [46].

The inhibition of peroxy radicals by microencapsulated alkaloids was more significant after in vitro digestion simulation, consistent with Tomé-Sánchez et al. [82], who reported that after the digestion process, a two-fold increase in antioxidant capacity occurred, which they attributed to the possible degradation of the polymer during its passage through the different digestive phases, achieving the total release of the compounds in the intestinal phase and causing chemical transformations in the structures of the metabolites due to the effect of digestive enzymes, which cause deprotonation [46].

The antioxidant capacity of the alkaloids microencapsulated by the different assays after digestion in vitro was increased, obtaining a bioaccessibility above 50%.

Previous studies have indicated that solamargine and solasonine are the main chemical compounds in *Solanum* species and that they also have beneficial health properties [83]. In this context, we can observe the identification and quantification of these compounds in both the undigested and digested microcapsules. In the digested microcapsules, the content of solamargine and solasonine increased by more than 100%. The increase observed after in vitro digestive simulation is consistent with previous investigations into ergot alkaloids, where some compounds' increases were attributed to bidirectional epimerization caused by intestinal enzymes [84]. Likewise, other compounds' isomerization has been described, and the authors speculate that it is due to temperature and prolonged exposure to the small intestine [85]. In the same way, Vronen [86] described the chemical hydrolysis of glycoalkaloids in potatoes. They mentioned that it is caused by time, temperature, and acid concentration, allowing for the formation of compounds β and γ as new hydrolysis products.

5. Conclusions

Eggplants are an important source of alkaloids; however, these compounds have some disadvantages, such as their instability after the digestion process. In this context, this study showed that, indeed, after simulated in vitro digestion, total alkaloids were reduced, as was their antioxidant capacity and the content of the glycoalkaloids solamargine and solasonine in the non-encapsulated alkaloid extract. However, after the microencapsulation process with maltodextrin using the spray drying method, 80% of the alkaloids in the eggplant biomass extract was retained, which allowed them to be protected during in vitro gastrointestinal digestion, tripling their bioaccessibility up to 12%, while the antioxidant capacity increased by more than 50% in its bioaccessibility; likewise, alkaloid-type glycoalkaloids such as solamargine and solasonine in microcapsules increased during in vitro digestion. This suggests that microencapsulation with maltodextrin is an excellent alternative and that it is able to protect these bioactive compounds. However, due to the solubility of

maltodextrin, its combination with other polymers is recommended to obtain encapsulates with greater resistance to digestion, bioaccessibility, and antioxidant capacity. Likewise, it is recommended that the cytotoxic, anticancer, anti-inflammatory, and anticholinergic effects be determined. Thus, the microencapsulation of alkaloids from eggplant biomass demonstrated its potential for use as a possible functional food and nutraceutical.

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