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Microencapsulation of *Annona crassiflora* extract using maltodextrin: Material design and evaluation of antioxidant, antiglycation, and antiaging properties *in vitro* and *in silico*

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ABSTRACT

Annona crassiflora, a native Brazilian Cerrado fruit tree, is rich in bioactive compounds, particularly phenolic compounds and flavonoids, known for their antioxidant capabilities. The research addresses the limitations of seasonal fruit production by exploring the bioactive potential of leaves, aiming for sustainable harvesting practices. In view of this, the study aimed to investigate the antioxidant, antiglycation, and antiaging properties of *A. crassiflora* leaf extract (AcHE), exploring its potential for microencapsulation using maltodextrin. The methodology included the preparation of a hydroethanolic extract (AcHE) from *A. crassiflora* leaves, phytochemical analysis to determine total polyphenol, flavonoid, and tannin content using spectrophotometric techniques and GC–MS analysis. Antioxidant activity was assessed through DPPH radical scavenging, ferric ion reducing power (FRAP), inhibition of lipid peroxidation (TBARS assay), nitric oxide radical scavenging activity, and oxidative hemolysis tests. Antiglycation activity was evaluated by quantifying free amino groups using the OPA method in a glycation reaction mixture of bovine serum albumin (BSA) and ribose. The study showed the efficacy of AcHE in preventing oxidative stress, glycation, and premature aging, which are linked to chronic diseases. It also demonstrated the optimization of the microencapsulation process with maltodextrin, showing enhanced stability, bioavailability, and efficacy of the bioactive compounds present in *A. crassiflora* leaves for potential applications in the food, pharmaceutical, and cosmetic industries.

1. Introduction

Annona crassiflora is a fruit tree native and endemic to Brazilian Cerrado. It is popularly known as marolo, ariticum, araticum-do-cerrado, or pinha-do-cerrado and it grows 4–8 m tall with a canopy spread of around 4 m.[1,2] Its flowers are either solitary or clustered to form inflorescences and it presents greenish-yellow rounded fruits with a thick rind and yellow pulp surrounding seeds[3]. A. crassiflora is highlighted by its nutritional properties and wide variety of phytoconstituents including alkaloids, acerogenins, carotenoids, phytosterols, and especially phenolic compounds such as catechin, epicatechin, rutin, quercetin, naringenin, protocatechuic acid, 4-hydroxybenzoic acid, vanillic acid, chlorogenic acid, caffeic acid, p-coumaric acid, and ferulic acid.[3,4]

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In current scientific literature, numerous studies have focused on the potential of bioactive and nutritional compounds found in A. crassiflora fruits, particularly pulp, where several compounds have been identified and demonstrated their potential to promote benefits in preventing and treating different diseases, and contribute to overall health of the human body.[5] However, the use of fruits is limited by their seasonal production, while other plant parts could represent a source of active compounds throughout the year.[6] In addition, a sustainable harvest might increase the maintenance of the availability of active compounds from *A. crassiflora* during both vegetative and reproductive stages.[7]

A. crassiflora leaves are highlighted as the plant parts rich in various bioactive compounds with important multifunctional properties; including different phenolic compounds especially flavonoids, which play an important role as antioxidant agents.[8] These natural antioxidants have a high capacity to neutralize reactive oxygen species (ROS), which are byproducts of cellular respiration that can cause oxidative damage to biomolecules, such as DNA, lipids, and proteins, potentially leading to or aggravating chronic diseases namely diabetes, hypertension, cancer, and neurodegenerative disorders.[9] In addition to reducing inflammatory processes, the antioxidant compounds from this species exhibit great potential for pharmacological, nutritional, and industrial applications, thereby serving as a preferred alternative to synthetic antioxidants.[10]

Another process contributing to oxidative damage is glycation, a non-enzymatic chemical reaction between reducing sugars, such as glucose, and free amino groups of proteins, lipids, or nucleic acids. This interaction results on the formation of unstable early products, which, through a series of reactions, lead to the production of advanced glycation end products (AGEs). [11] AGEs play a significant role in increasing oxidative stress by interacting with specific receptors, such as the receptor of AGEs (RAGE), triggering intracellular signaling pathways that generate reactive oxygen species. Excess of AGEs result in increased oxidative damage to biomolecules and, similarly, oxidative stress intensifies glycation process. ROS can modify sugar and protein molecules, making them more reactive accelerating AGEs formation.[12] This vicious cycle between glycation and oxidative stress contributes to the development and progression of various chronic diseases, such as diabetes mellitus, cardiovascular diseases, Alzheimer's disease, and premature aging.[13,14] Antioxidant compounds, particularly from natural sources, can interrupt and mitigate this cycle, minimizing the deleterious effects of oxidative stress and glycation.[15]

Premature aging results from processes such as degradation of extracellular matrix promoted by enzymes including collagenase, elastase and tyrosinase, which destroys collagen, elastin, and interfere in melanin production, respectively.[16] This phenomenon is exacerbated by both oxidative stress and glycation due to the increase of enzymes activity and alteration of structure and function of biomolecules, intensifying oxidative damage.[17] These processes interact synergistically, forming a cycle of degradation and inflammation that accelerates the signs of aging, such as wrinkles, sagging, and loss of elasticity. Prevention involves sun protection, an antioxidant-rich diet, and products that inhibit enzymatic activity and the formation of AGEs, promoting better health and longevity.[18,19]

Currently, the demand for products with high concentrations of bioactive compounds has significantly increased and it has demonstrated the need for modifications and development of new processing techniques to maintain natural active compounds with quality and quantity.[20,21] Among these techniques, encapsulation (micro or nano scale) is widely used in order to preserve, enhance the stability, and potentiate the activity of bioactive compounds in various applications in food, pharmaceutical, and cosmetic industries. The process involves encapsulation of bioactive compounds such as antioxidants, essential oils, and vitamins, forming particles that protect compounds from adverse factors including light, oxygen, and pH variations.[22] In addition to prolonging the shelf life of bioactive compounds, it allows a controlled release, optimizing bioavailability and efficacy of these compounds in biological and industrial systems.[23]

Considering the above and the importance of evaluating the bioactive effects of leaves of species from Cerrado, which represents a significant contribution to the sustainable use of its biodiversity, the present study aimed to evaluate antioxidant, antiglycation, and anti-aging potential of A. crassiflora leaves extract. Besides, it aimed to optimize microencapsulation process using maltodextrin as an encapsulating agent.

2. Material and methods

2.1. Plant collection and identification

The leaves of *Annona crassiflora* were collected in Assis, São Paulo, Brazil (latitude: 22°39 '42''S, longitude: 50°24′44''W, altitude: 546 m). A specimen was taxonomically identified and deposited in the Herbarium of the Instituto Florestal de Assis (Assis, São Paulo, Brazil) under voucher number 1170. The collected leaves were dried in an air-forced oven set to approximately 40 °C, which was then grounded into a fine powder using a knife mill.

2.2. Preparation of Annona crassiflora hidroetanolic extract (AcHE)

AcHE was prepared at a 1:10 (w/v) ratio under mechanical stirring for 24 h at room temperature. The plant residue was vacuum filtered and re-extracted twice. The resulting filtrate was concentrated to dryness using a rotary evaporator (Marconi – MA-120, Brasil) at 60 °C. The final extract was then stored in amber glass vials.

2.3. Determination of phytochemical compounds of AcHE

2.3.1. Total polyphenols content

Total polyphenol content in AcHE and AgNPs was evaluated using the method of Singleton and Rossi,[24] employing Folin-Ciocalteu reagent. AcHE (100, 250, or 500 μL^{-1}) and AgNPs were analyzed in triplicate and the absorbance was measured at 765 nm with a spectrophotometer (Bel Photonics, Brazil). The results were expressed as mg gallic acid equivalent per gram of dry weight (mg GAE g^{-1} DW) based on a calibration curve plotted with gallic acid.

2.3.2. Total flavonoids content

Total flavonoids content was determined using the method of Christ and Müller [25], employing $AlCl_3$ as main reagent. AcHE (100, 250, or 500 μL^{-1}) and AgNPs were analyzed in triplicate and the absorbance was measured at 510 nm. The results were expressed as mg rutin equivalent per gram of dry weight (mg RE g^{-1} DW) based on a calibration curve plotted with rutin.

2.3.3. Total tannins content

Total tannins were quantified using the method of Hagerman and Butler [26], which involves a colorimetric reaction with vanillin at varying extract concentrations. AcHE (100, 250, or 500 μL^{-1}) and AgNPs were evaluated in triplicate and absorbance was measured at 500 nm using a spectrophotometer. The tannin concentration was expressed as mg tannic acid equivalents per gram dry weight (mg TAE g^{-1} DW) based on a calibration curve plotted with catechin.

2.3.4. Gas chromatography-Mass spectroscopy (GC–MS) analysis

GC–MS analysis was performed using a Shimadzu Model GCMS-QP2020 system, equipped with a quadrupole mass detector and a Shimadzu-Rtx-5MS column (30 mm × 0.25 mm × 0.25 μm). For silylation, 10 mg of the extract was diluted in 200 μL of pyridine in a 4 mL flask. To this, 200 μL of N-methyl-N(trimethylsilyl)trifluoroacetamide (MSTFA) was added, and the mixture was homogenized and heated at 37 °C for 30 min. Afterwards, it was maintained at 5 °C for 24 h, filtered through a 0.22 μm microfilter, and transferred to a 2 mL vial with a 350

μL insert. The GC/MS analysis was carried out with an injector temperature of 290 °C; an injected sample volume of 1.0 μL and a split ratio of 1:50 (v/v). The ionization energy was set to 70 eV and the mass spectra was acquired within a range of *m/z* 45 to 500. The temperature gradient started at 100 °C until it reached 320 °C, with a heating rate of 3 °C min^{-1}. Data was analyzed using the National Institute of Standard and Technology (NIST) database for mass spectrum interpretation where retention time, molecular weight, molecular formula and composition percentages were used to identify the components.

2.4. Determination of antioxidant activity of AcHE

2.4.1. DPPH radical scavenging activity assay

For DPPH assay, AcHE was diluted in methanol at varying concentrations (250, 500, and 1,000 μg mL^{-1}). A 100 μL was mixed with 3.9 mL of DPPH solution (0.1 mM diluted in methanol) and incubated in dark at room temperature for 30 min. Absorbance was measured at 517 nm using a spectrophotometer. Antioxidant activity (%AA) was calculated using the equation: %AA = [(Acontrol − Asample)/Acontrol] × 100, where Acontrol represents the absorbance of the solution without extract, and Asample represents the absorbance of the solution with extract. The results were expressed as percentage of antioxidant activity Scherer and Godoy.[27]

2.4.2. Ferric ion reducing power (FRAP) assay

FRAP assay as described by Benzie and Strain[28] was conducted to evaluate antioxidant activity. This method relies on the ability of antioxidant compounds from extracts to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), producing an intense blue complex with tripyridyltriazine (TPTZ). A 30 μL of AcHE (250, 500, and 1,000 μg mL^{-1}) were mixed with 270 μL reagent solution (solution of 10 mM TPTZ diluted in 40 mM HCl, 20 mM $FeCl_3{\cdot}6H_2O$, and 300 mM acetate buffer at pH 3.6). The resulting mixture was incubated at 37 °C for 30 min, and absorbance was measured at 593 nm. The antioxidant activity was expressed as μM Trolox equivalent per g of extract (μM TE g^{-1} extract).

2.4.3. Inhibition of lipid peroxidation assay

Thiobarbituric acid reactive substances (TBARS) assay was used to evaluate the inhibition of malondialdehyde (MDA) formation, which is a marker of lipid peroxidation. Dried egg yolk was mixed with AcHE at different concentrations (250, 500, and 1,000 μg mL^{-1}). Lipid peroxidation was induced with AAPH, and mixture was incubated with trichloroacetic acid and thiobarbituric acid. Absorbance sample was measured at 532 nm. The results were expressed as the percentage of inhibition of TBARS formation. The antioxidant capacity was determined by measuring the decrease in TBARS formation compared to control, which indicates the ability of the extract to prevent lipid peroxidation.[29]

2.4.4. Nitric oxide radical scavenging activity

Nitric oxide (NO) was produced by decomposition of sodium nitroprusside (SNP) in a 20 mM phosphate buffer (PBS, pH 7.4). NO reacted with oxygen to form nitrite ions (NO_2^-). The concentration of NO_2^- was quantified using Griess assay described by Marcocci et al. [30]. For the preparation of the reaction mixtures, AcHE (250, 500, and 1,000 μg mL^{-1}) and SNP were incubated at 37 °C for 1 h. After incubation, 0.5 mL Griess reagent was added to the mixture and absorbance was measured at 540 nm using a spectrophotometer. Results were expressed as percentage of inhibition of NO_2^- formation (μM nitrite formed mL^{-1}).

2.4.5. Oxidative hemolysis test

The antioxidant evaluation was carried out using oxidative hemolysis assay described by Magalhães et al. [31]. A 10 % erythrocyte suspension was incubated with 50 mM AAPH, with or without samples (AcHE at 100, 250, or 500 μg mL^{-1}) for 6 h at 37 °C in a water bath. At each hour, 400 μL were collected and the solution was centrifuged at 2500 rpm for 10 min, and the resulting supernatant was read at 540 nm using a UV–Vis spectrophotometer. Hemolysis percentage was calculated using the formula: Hemolysis (%) = [(Sample − Negative Control) / (Positive Control − Negative Control)] × 100, where Sample represents the absorbance of test sample, Positive Control represents 100 % hemolysis (erythrocyte suspension + AAPH), and Negative Control represents 0 % hemolysis (erythrocyte suspension + PBS buffer).

2.5. Determination of antiglycation activity in AcHE

2.5.1. Preparation of glycation reaction mixture

For the antiglycation analysis, glycation reaction mixtures were prepared by combining bovine serum albumin (BSA, 2 mg mL^{-1}) in PBS (10 mM, pH 8) with ribose (1 M) and aminoguanidine (AMG, 40 mM), or samples (AcHE at 100, 250, or 500 μg mL^{-1}).

2.5.2. Determination of free amino groups

The quantification of free amino groups was performed using the *ortho*-phthaldialdehyde (OPA) method, as described by Fayle et al. [32]. The glycation mixture (prepared as described in section 2.5.1) was incubated at 60 °C for 30 min in a water bath and then dialyzed for 24 h prior to analysis. The OPA reagent was prepared by combining 25 mL of 0.1 M sodium borate, 2.5 mL of sodium dodecyl sulfate (SDS, 20 %), 100 μL of 2-mercaptoethanol, and 40 mg of OPA (dissolved in 1 mL methanol), with the total volume adjusted to 50 mL with distilled water. For the assay, 200 μL of OPA reagent was added to each glycation reaction mixture, incubated at room temperature for 2 min. Absorbance was measured at 340 nm and results were expressed as percentage of free amino groups in comparison to the glycation mixture containing only BSA.

2.5.3. Inhibition of AGEs formation

The inhibition of advanced glycation end products (AGEs) formation was evaluated using the method of Rahbar and Figarola.[33] A sample from each glycation reaction mixtures were combined with 1 mL of OPA reagent previously prepared, incubated for 2 min at 37 °C, and analyzed using a spectrofluorometer. Fluorescence was measured with an excitation at 360 nm and emission at 460 nm. Results were expressed as percentage of inhibition of AGEs formation.

2.5.4. Relative electrophoretic mobility (REM)

The antiglycation activity was determined using REM assay method described by Perera and Handuwalage.[34] The glycation reaction mixture (prepared as described in section 2.5.1) was incubated at 37 °C for 7 days. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was carried out with a 4 % stacking gel and a 12 % separating gel. Prior to loading samples into the gel, glycation reaction mixtures were heated with sample buffer at 95 °C for 3 min. 10 μL was applied to the stacking gel, and electrophoresis was conducted at a constant current of 30 mA and 80 V for about 3 h. After electrophoresis, the gel was fixed with acetic acid and stained with Coomassie Blue (R-250). The intensity and appearance of bands in the presence of extract were compared to those of BSA and positive and negative controls.

2.6. Evaluation of anti-aging activity of AcHE by inhibitory of collagenase, elastase and tyrosinase enzyme

2.6.1. Collagenase inhibition test

Collagenase inhibitory was evaluated using EnzCheck® Gelatinase/Collagenase Kit (Molecular Probes, Eugene OR). 1 mg mL^{-1} DQ™ gelatin (pig skin conjugated with fluorescein) solution was prepared in distilled water. The 1X reaction buffer was made by diluting 10X buffer (pH 7.6, containing 0.5 M Tris-HCl, 1.5 M NaCl, 50 mM $CaCl_2$, and 2 mM sodium azide) in distilled water. The positive control (PC) was prepared by diluting 1,10-phenanthroline monohydrate solution in 1X buffer. Collagenase (type IV, *Clostridium histolyticum*) was diluted to 1 U mL^{-1}.

AcHE was prepared at concentrations of 100, 250, or 500 μg mL^{-1} in 1X buffer. The assay was performed in 96-well fluorescence microplates with 10 μL substrate solution, 90 μL AcHE or PC, and 100 μL collagenase solution per well. Blank contained 10 μL substrate and 190 μL buffer; and negative control (NC) contained 10 μL substrate, 90 μL buffer, and 100 μL collagenase. The plates were incubated at 37 °C and fluorescence was measured with excitation wavelength at 495 nm and emission at 515 nm over 35 min.

2.6.2. Elastase inhibition test

Elastase inhibitory activity was assessed using EnzCheck® Elastase Kit (Molecular Probes, Eugene OR). The substrate was prepared by dissolving 1 mg of DQ™ elastin (bovine neck ligaments conjugated with fluorescein) in 1 mL distilled water. A 1X reaction buffer was made by diluting 6 mL of 10X buffer (pH 8.0, 1 M Tris-HCl and 2 mM sodium azide) in 54 mL distilled water. Positive control (PC) was prepared by diluting 10 μL of N-methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone inhibitor solution (500 μg in 50 μL dimethylsulfoxide) in 2 mL 1X buffer. AcHE (100, 250, or 500 μg mL^{-1} in 1X buffer) was used for the assay in 96-well fluorescence microplates, adding 50 μL of substrate solution, 50 μL of AcHE or PC, and 100 μL of elastase solution to each well. The blank contained 50 μL substrate and 150 μL buffer, and the negative control (NC) contained 50 μL substrate, 50 μL buffer, and 100 μL elastase. After incubation at 37 °C and fluorescence was read at excitation (505 nm) and emission (515 nm) for 35 min.

2.6.3. Tyrosinase inhibition test

Tyrosinase inhibitory activity was evaluated using a UV–vis spectrophotometry method.[35] In a centrifuge tube, 500 μL phosphate buffer (pH 6.8), 2 mL tyrosinase (200 U mL^{-1}), and 50 μL of AcHE (100, 250, or 500 μg mL^{-1}) or kojic acid (70 μg mL^{-1}) were mixed in the dark. After 5 min, 2 mL of L-tyrosine (2 mM) was added and incubated for 20 min. Absorbance was measured at 475 nm. Inhibition was assessed by comparing tyrosinase inhibition with and without AcHE.

2.6.4. Molecular docking analysis

The major compound from AcHE (Catechin) were subjected for molecular docking. Enzyme-ligand docking was conducted for type IV collagenase (PDBid: 1QIB), elastase (PDBid: 1ELE), and tyrosinase (PDBid: 5M8P) using GOLD v. 2020.[36,37] Binding interactions were analyzed using CHIMERA 1.8 and BIOVIA (Dassault Systèmes, Discovery Studio Visualizer, version 20, San Diego: Dassault Systèmes, 2019), which were selected to generate 3D and 2D visualization of interactions.

2.7. Microencapsulation of AcHE

2.7.1. Microencapsulation by spray dryer

AcHE was spray-dried using a Mini Büchi B-290 (BÜCHI Labortechnik AG, Flawil, Switzerland). The extract was mixed with Maltodextrin at 10, 30 or 50 % and the equipment was operated on experimental design parameters. Between each experiment, the equipment was cleaned with distilled water for 10 min. The resulting microcapsules were stored in dark containers and closed during the process.

2.7.2. Experimental design

Box-Behnken experimental design was applied and resulted in 15 experiments. Variables included inlet temperature (IT), feed flow rate (FFR), and maltodextrin concentration (MD) were selected and exhibited responses including yield of drying (YD), capsule moisture (CM), water solubility index (WSI), water absorption index (WAI) and water activity (WA) given as Atmospheric Water Generator (AWG), encapsulated total polyphenols (TP) and antioxidant activity (AA). Inlet temperatures of 120, 150 and 180 °C, maltodextrin concentration of 10, 30 and 50 %, and FFR values of 8, 25 and 42 were tested. YD was calculated as ratio of powder weight after spray drying (WP) to initial weight (WI), expressed as a percentage YD = WP/WI*100.

WSI and WAI were determined by dissolving 1 g extract in 12 mL water and then centrifuging as described by Ahmed et al. [38]. WSI and WAI were calculated as: WSI=(WSOBR/WI)*100, where WSOBR is supernatant weight removed after centrifugation and WAI = WR/WI, where WR is microcapsules weight after centrifugation.

2.7.3. Determination of total polyphenols of the microcapsules

Microcapsules content was extracted according to Robert et al. [39] with a 50 % methanol, 8 % acetic acid and 42 % water (v/v/v) solution. Phenolic content was determined according to methodology described in section 2.3.1 and results were expressed as mg GAE per gram of dry microcapsules (DM).

2.7.4. Antioxidant activity of microcapsules by ABTS test

Antioxidant activity was assessed using the ABTS method described by Re et al. [40], with modifications. The ABTS radical solution was prepared by combining 7 mM ABTS with 2.45 mM potassium persulfate and incubating the mixture in the dark at 23 °C for 12–16 h. The solution was then diluted with 80 % ethanol to achieve an absorbance of 0.700 ± 0.002 at 734 nm. For the assay, 3.9 mL of the ABTS solution was mixed with 0.1 mL of the sample at concentrations of 100 μg mL^{-1}. The reaction was allowed to proceed at 23 °C for 6 min, and absorbance was measured at 734 nm. Results were expressed as μM Trolox Equivalent (TE) per gram of dry microcapsules (DM).

2.8. Statistical analysis

The data are expressed as the mean ± standard deviation by measuring three independent replicates. Analysis of variance using one-way ANOVA followed by Tukey's test was performed to test the significance of differences between means obtained among the treatments at the significance level of $p < 0.05$ using the software Bioestat, version 5.033.

3. Results and discussion

3.1. Determination of phytochemical compounds in AcHE

3.1.1. Quantification of total polyphenols, flavonoids, and tannins content

The phytochemical analysis of AcHE revealed high total polyphenols, flavonoids, and tannins content, demonstrating a dose-dependent increase. At the highest concentration (500 μg mL^{-1}), AcHE exhibited the highest values of total polyphenols (2,870 mg GAE g^{-1} DW), flavonoids (1,494 mg RE g^{-1} DW), and tannins (837 mg TAE g^{-1} DW) (Table 1).

Total phenolic content identified in AcHE is in accordance with findings from previous studies even though they evaluated different fruit components (peel, pulp, and seeds). Arruda and Pastore[4] and Ramos et al. [41] highlighted phenolic content in all three fruit parts. Meanwhile, Demir et al. [42] and Malunga et al. [43] suggested that variations in phenolic

Table 1
Determination of total polyphenols, flavonoids and tannins content of *A. crassiflora* hydroethanolic extract (AcHE).

Treatment	Concentration (μg mL^{-1})	Total polyphenols[1]	Total flavonoids[2]	Total tannins[3]
AcHE	100	714 ± 3.67a	563 ± 5.12a	527 ± 2.49a
	250	1,240 ± 4.11b	734 ± 3.25b	646 ± 5.01b
	500	2,870 ± 6.81c	1,494 ± 9.48c	837 ± 3.44c

Values are expressed as mean ± standard deviation. [1]mg gallic acid equivalent (GAE) g^{-1} dry weight (DW); [2]mg rutin equivalent (RE) g^{-1} DW and [3]mg tannic acid equivalent (TAE) g^{-1} DW. Same letters within the same column indicate no significant differences between samples by Tukey test ($p \leq 0.05$).

compound levels might be associated with differences in extraction methodologies, such as sample particle size, solvent ratio, and pH. Additionally, environmental factors including temperature, light exposure, soil composition, and harvest timing play a crucial role in the production and concentration of secondary metabolites, affecting both their quality and quantity.

3.1.2. GC–MS analysis

GC–MS analysis of AcHE exhibited approximately 17 compounds identified using NIST library (Table 2). Four of these compounds present the highest percentage of peak areas and they are of great interest due to their biological activity. Catechin in the form of Catechine (2R-cis)-, 5TMS derivative and [(2-{3,4-Bis[(trimethylsilyl)oxy]phenyl}-3,5-bis [(trimethylsilyl)oxy]-3,4-dihydro-2H-chromen-7-yl) oxy] (trimethyl) silane) exhibited 13.56 % and 31.46 % peak area, respectively; Quercetin in the form of Quercetin (5TMS) that demonstrated 11.8 % peak area; and Chlorogenic Acid in the form of Chlorogenic Acid (6TMS) that exhibited 1.94 % peak area. Moreover, Table 3 shows these main compounds, and their biological activities reported in the current literature.

Quercetin was identified in hydroalcoholic fractions from *A. crassiflora* leaves extract and reported in studies carried out by Costa Oliveira et al. [44], which also demonstrated anti-inflammatory and antinociceptive potential of the fractions. Machado et al. [45] prepared fractions of dichloromethane and ethyl acetate from hydroalcoholic extract of leaves and identified quercetin as well as different aminoacids.

3.2. Determination of antioxidant activity in AcHE

3.2.1. DPPH, FRAP, TBARS and NO tests

Table 4 summarizes the findings from various *in vitro* antioxidant assays (DPPH, FRAP, TBARS, and NO). DPPH assay demonstrated the greatest antioxidant activity at 1,000 μg mL^{-1} (78.39 %) and data indicated a dose-dependent effect with significant statistical difference between all concentrations and positive control. In FRAP assay, the highest reducing power (1,425.10 μM TE g^{-1} DW) was observed at the highest concentration and values differed between concentrations. In TBARS assay, lipid peroxidation inhibition (63.14 %) was also observed at the highest concentration and results were significant difference between concentrations; however, it did not differ from control. For the NO scavenging test, the greatest activity was recorded at 1,000 μg mL^{-1} (9.35 μM mL^{-1} of nitrite formed), which did not differ from control.

Table 3
Activity of the main phytoconstituents identified in *A. crassiflora* hydroethanolic extract (AcHE).

Suggested compound	Biological activities	Reference
Catechine, (2R-cis)-, 5TMS derivative	anti-microbial, anti-viral, antiinflammatory, anti-allergenic, and anti-cancer, antihyperpigmentation, antiaging, antioxidant	Mita et al.[46] Kim et al.[47]
[(2-{3,4-Bis[(trimethylsilyl) oxy]phenyl}-3,5-bis [(trimethylsilyl)oxy]-3,4-dihydro-2H-chromen-7-yl)oxy](trimethyl)silane (Catechine)	anti-microbial, anti-viral, anti-inflammatory, anti-allergenic, and anti-cancer, antihyperpigmentation, antiaging, antioxidant	Mita et al.[46] Kim et al. [47]
Chlorogenic acid	antioxidant, hepatoprotection, anti-bacterial, anti-tumor, regulation of sugar metabolism and lipid metabolism, anti-inflammatory, and protection of the cardiovascular and nervous system	Nguyen et al.[48] Wang et al.[49]
Quercetin	antioxidant, anti-inflammatory, antibacterial, antiviral, radical-scavenging, gastroprotective, and immune-modulatory, vasodilator, and anticancer effects	Silva et al.[50] Kim et al.[51]

3.2.2. Oxidative hemolysis test

Fig. 1 illustrates the inhibitory effects of AcHE on AAPH-induced hemolysis in human erythrocytes over time. AcHE exhibited consistent hemolysis suppression throughout the assessment period. However, after 4 h, no significant differences were observed between the tested concentrations of AcHE. At the 6th hour of incubation, an average hemolysis reduction of approximately 90 % was achieved for all concentrations evaluated.

The antioxidant potential of the hydroethanolic extract from the leaves of *A. crassiflora* has been demonstrated through various evaluation methods and mechanisms of action. This potential is likely associated with the extract's phytochemical composition, particularly its phenolic compounds, including flavonoids and tannins, which are known for their capacity to neutralize and eliminate free radicals. Research performed by Ramos et al. [41] on fruit pulp extracts of *A. crassiflora* demonstrated a correlation between total phenolic content and antioxidant activity. While earlier studies, such as those by Luzia

Table 2
Phytoconstituents detected in *A. crassiflora* hydroethanolic extract (AcHE) using gas chromatography-mass spectrometer (GC–MS).

Suggested compound	Retention time (Rt)	Molecular formula	Molecular weight	Peak area (%)	Class
Lactic Acid, 2TMS derivative	4.157	$C_9H_{22}O_3Si_2$	234.4402	0.24	Acid
2-Hexenoic acid, (E)-, TMS derivative	5.334	$C_9H_{20}O_2Si$	188.3394	0.07	Fatty acid
Oxalic acid, 2TMS derivative	5.924	$C_8H_{18}O_4Si_2$	234.3971	0.36	Acid
2-Hydroxyisocaproic acid, 2TMS derivative	8.712	$C_{12}H_{28}O_3Si_2$	276.5199	0.13	Fatty acid
Gulonic acid, γ-lactone, 4TMS derivative	21.669	$C_{18}H_{42}O_6Si_4$	466.8645	0.22	Acid
L-5-Oxoproline, 2TMS derivative	23.32	$C_{11}H_{23}NO_3Si_2$	273.4762	0.24	Amino acid
Methyl 3,4-dihydroxybenzoate, 2TMS derivative	26.546	$C_{14}H_{24}O_4Si_2$	312.5100	0.11	Phenolic acid
Protocatechoic acid, 3TMS derivative	30.103	$C_{16}H_{30}O_4Si_3$	370.6635	0.63	Phenolic acid
Shikimic acid (4TMS)	30.299	$C_{19}H_{42}O_5Si_4$	462.8758	0.18	Phenolic acid
Catechine, (2R-cis)-, 5TMS derivative	30.887 / 32.216	$C_{30}H_{54}O_6Si_5$	651.1737	13.56	Phenols
Hexadecanoic acid, methyl ester	33.14	$C_{17}H_{34}O_2$	270.4507	0.2	Ester
Palmitic Acid, TMS derivative	36.974	$C_{19}H_{40}O_2Si$	328.6052	0.43	Fatty acid
9,12-Octadecadienoic acid (Z,Z)-, TMS derivative	42.02	$C_{21}H_{40}O_2Si$	352.6266	0.57	Fatty acid
Oleamide, TMS derivative	47.344	$C_{18}H_{35}NO$	281.5000	1.51	Fatty acid
[(2-{3,4-Bis[(trimethylsilyl)oxy]phenyl}-3,5-bis[(trimethylsilyl)oxy]-3,4-dihydro-2H-chromen-7-yl)oxy](trimethyl)silane (Catechine)	59.105 / 59.672	$C_{30}H_{54}O_6Si_5$	651.1860	31.46	Phenols
Chlorogenic acid (6TMS)	64.81	$C_{34}H_{66}O_9Si_6$	787.3954	1.94	Phenolic acid
Quercetin (5TMS)	65.987 / 76.223 / 77.418	$C_{30}H_{50}O_7Si_5$	663.1413	11.8	Phenols

Table 4
Antioxidant activity of leaves *A. crassiflora* hydroethanolic extract (AcHE) and positive controls (Gallic Acid for DPPH test and Trolox for TBARS and NO tests).

Treatment	Concentration (μg mL^{-1})	DPPH[1]	FRAP[2]	TBARS[3]	NO[4]
AcHE	250	30.69 ± 1.23a	899.47 ± 2.98a	27.48 ± 2.77a	18.20 ± 1.98a
	500	48.25 ± 2.03b	1,102.17 ± 6.12b	51.17 ± 2.01b	14.11 ± 1.52b
	1,000	78.39 ± 1.89c	1,425.10 ± 2.47c	63.14 ± 2.41c	9.35 ± 1.77c
Gallic acid	100	89.47 ± 1.32d	ND	ND	ND
Trolox	140	ND	ND	63.49 ± 2.98c	9.73 ± 1.23c

Values expressed as mean ± standard deviation. ND: not determined. [1]% Antioxidant Activity; [2]μM Trolox Equivalent (TE) g^{-1} sample; [3]% TBARS inhibition; and [4]μM mL^{-1} nitrite formed. ND = not determined. Same letters within the same column indicate no significant differences between samples by Tukey test ($p \leq 0.05$).

and Jorge [52], Prado et al. [53], and Santos et al. [5], have confirmed the antioxidant activity of *A. crassiflora* extracts, these investigations primarily focused on fruit pulp and seeds, given their nutritional value, as highlighted by Arruda and Pastore [4] and Almeida et al. [3]. In contrast, the findings of the present study are novel and innovative, as they involve extensive trials to assess antioxidant activity using the hydroethanolic extract derived specifically from the leaves of this species. Despite their abundance, *A. crassiflora* leaves are not traditionally utilized for food or as a source of bioactive compounds, making this research a significant contribution to the exploration of their potential applications. Notably, the evaluation of antioxidant potential using the oxidative hemolysis method in human blood highlights the practical relevance of these findings. This research underscores the importance of exploring *A. crassiflora* leaves as a promising source of active compounds.

3.3. Determination of antiglycation activity in AcHE

3.3.1. Determination of free amino groups and inhibition of AGEs formation tests

The antiglycation activity of AcHE on BSA incubated with ribose is illustrated in Fig. 2. and 2A highlights the percentage of free amino groups, with the highest value observed in BSA treated with 500 μg mL^{-1} of AcHE (71.16 %). However, this value was significantly lower than that recorded for BSA treated with AMG (positive control). Similarly, as depicted in Fig. 2B, BSA exposed to ribose and treated with 500 μg mL^{-1} of AcHE showed the greatest inhibition of AGEs formation (68.94 %), yet this value also differed significantly from the positive control treated with AMG.

The results demonstrate that AcHE exhibits significant antiglycation activity, as evidenced by its ability to preserve free amino groups and inhibit the formation of advanced glycation end products (AGEs). However, the maximum values obtained in both tests were significantly lower than those observed with the positive control AMG, indicating that although AcHE shows promise, its antiglycation potential may not yet match that of more established inhibitors. The preservation of free amino groups by AcHE is crucial as it indicates its capacity to mitigate glycation-related modifications in proteins. Studies conducted by Najjar and Calvert [12] and Wei et al. [54] have shown that glycation leads to structural and functional impairments in proteins, which are implicated in various diseases, including diabetes complications and neurodegenerative disorders. AcHE's ability to inhibit AGE formation aligns with findings from other plant-derived bioactive that act by scavenging reactive carbonyl species or capturing free radicals generated during glycation, as demonstrated in the study by Pannucci et al. [55]. The observed difference between AcHE and AMG may be attributed to their mechanisms of action. AMG is a well-documented glycation inhibitor that primarily functions by capturing reactive intermediates such as methylglyoxal, effectively preventing AGE formation, as shown by Grzebyk and Piwowar [56]. On the other hand, the antiglycation results evaluated by the ability to preserve free amino groups in the presence of glycating agents are corroborated by previous studies conducted by Souza et al. [57], who found that *A. crassiflora* extract exhibits this activity. However, this potential may depend on polyphenolic or flavonoid compounds, which act through antioxidant mechanisms.

3.3.2. Relative electrophoretic mobility (REM) test

Fig. 3 shows the electrophoretic profile for evaluated samples: BSA alone (1), BSA combined with ribose (2), BSA with ribose and AMG (3), BSA with ribose and AcHE at concentrations of 100 μg mL^{-1} (4), 250 μg mL^{-1} (5), and 500 μg mL^{-1} (6). Band 2 illustrates the protein

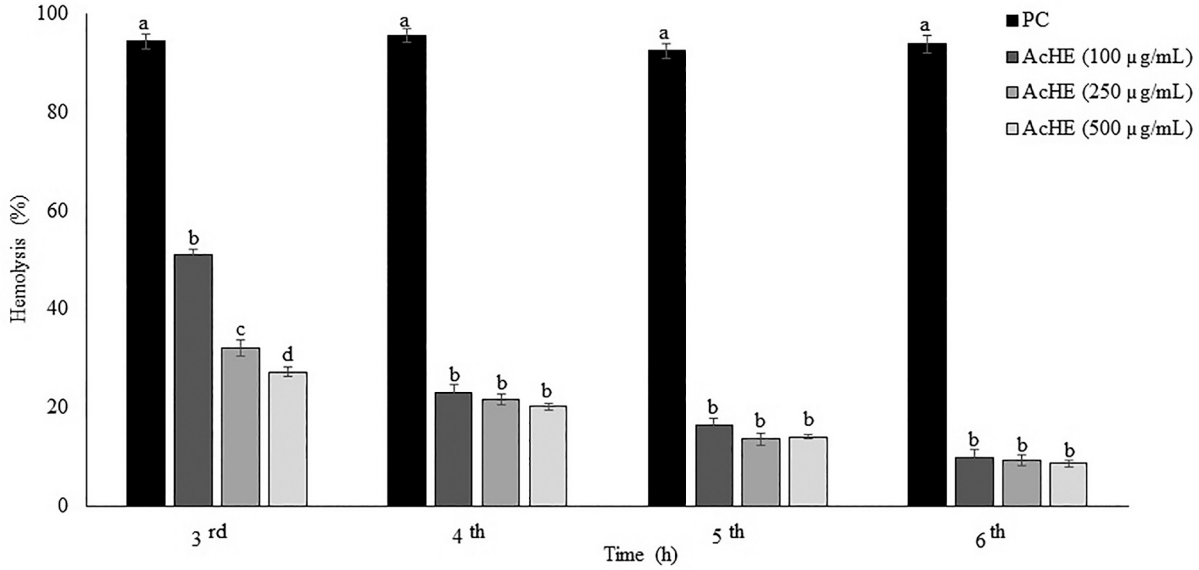

Fig. 1. Inhibitory effects of *A. crassiflora* hydroetanolic extract (AcHE) on the hemolysis of human erythrocytes induced by AAPH. Treatments were evaluated after 3, 4, 5 and 6 h of incubation and values are expressed as mean ± standard error. Equal letters within the same period indicate no significant differences among samples by Tukey test ($p \leq 0.05$).

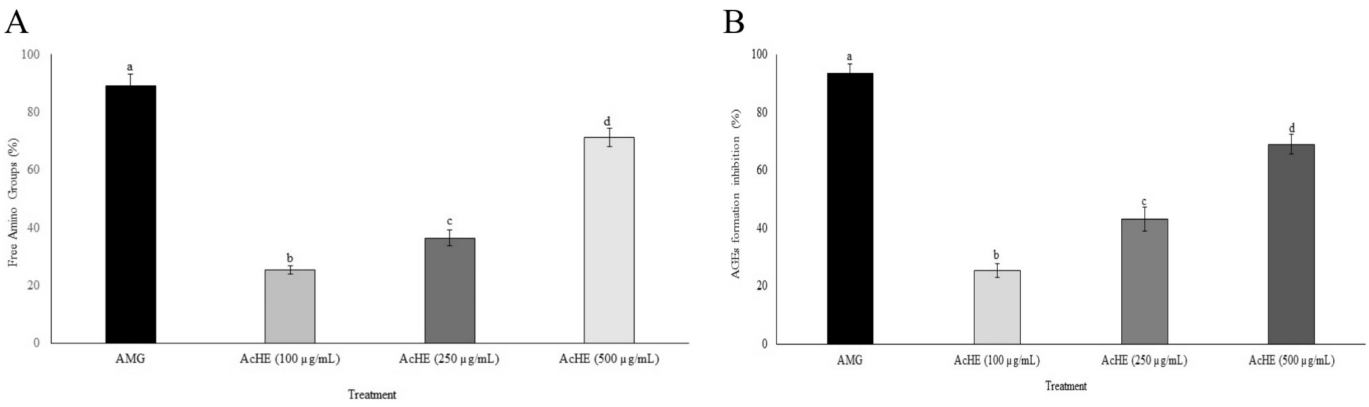

Fig. 2. Antiglycation activity of Aminoguanidine (AMG), A. crassiflora hidroetanolic extract (AcHE) at different concentrations (100, 250 and 500 µg mL^{-1}) on bovine serum albumin (BSA) exposed to ribose in the evaluation of free amino groups (A) and inhibition of advanced glycation end-products (AGEs) formation (B). Values followed by equal letters do not show significant difference by Tukey test ($p \leq 0.05$).

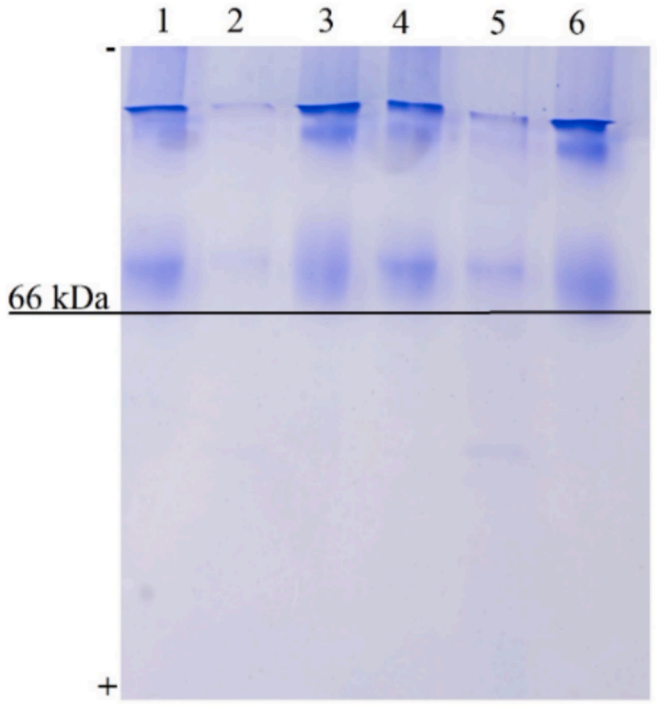

Fig. 3. Antiglycation activity evaluated by Relative electrophoretic mobility (REM), where: 1 = BSA; 2 = BSA + Ribose; 3 = BSA + Ribose + Aminoguanidine (AMG); 4 = BSA + Ribose + A. crassiflora hydroetanolic extract (AcHE) (100 µg mL^{-1}), 5 = BSA + Ribose + AcHE (250 µg mL^{-1}) and BSA + Ribose + AcHE (500 µg mL^{-1}).

degradation caused by the interaction of BSA with ribose. In contrast, bands 3, 4, and 6 reveal a reduction in this degradation, highlighting the anti-glycation properties of these samples. The strongest anti-glycation effect was observed in the sample where BSA exposed to ribose was treated with AcHE (Band 6). This band exhibited a color intensity and broadness comparable to BSA alone (Band 1), indicating that AcHE at 500 µg mL^{-1} provides a level of protection against glycation similar to the known anti-glycation compound AMG.

The REM test results highlight the antiglycation potential of AcHE compared to AMG in mitigating the effects of ribose-induced protein degradation. This finding underscores the ability of AcHE to preserve protein integrity, matching the efficacy of AMG and suggesting its potential as an alternative or complementary antiglycation agent. The results imply that AcHE may act by scavenging reactive carbonyl species or inhibiting glycation intermediates, offering promising applications in preventing glycation-related protein damage and AGE-associated diseases. These findings are supported by a study conducted by Justino et al. [58], which previously demonstrated the antiglycation activity of A. crassiflora fruit extract evaluated by REM. However, the assessment of this activity for A. crassiflora leaf extract is being presented for the first time in scientific literature.

3.4. Evaluation of anti-aging activity of AcHE

3.4.1. Evaluation of anti-aging activity of AcHE by inhibitory of aging enzyme

The inhibitory effects of AcHE on collagenase, elastase, and tyrosinase were evaluated at varying concentrations (100, 250, or 500 µg mL^{-1}), as illustrated in Fig. 4. AcHE demonstrated a dose-dependent inhibition, with the strongest activity observed at the highest concentration. However, the results differed significantly from both the positive control (PC) and the other tested concentrations. AcHE achieved inhibition rates of 75.96 % for collagenase (Fig. 4A), 65.89 % for elastase (Fig. 4B), and 81.02 % for tyrosinase (Fig. 4C).

The inhibitory effects of AcHE on enzymes associated with aging, such as collagenase, elastase, and tyrosinase, demonstrate its potential as an anti-aging agent. The results presented indicate that AcHE exhibits dose-dependent activity, with the highest concentration (500 µg mL^{-1}) achieving the most significant inhibition rates for all three enzymes. These findings align with research conducted by Chatatikun and Chiabchalard [59], Rojas-García et al. [60], and Moussa et al. [61], which demonstrated the role of bioactive compounds present in other species of the Annona genus in modulating enzymatic activity related to aging. However, this activity for the species A. crassiflora is being reported for the first time in this study.

3.4.2. Evaluation of anti-aging activity by molecular docking

Enzyme-ligand docking was performed using catechine ([(2-{3,4-Bis [(trimethylsilyl)oxy]phenyl}-3,5-bis[(trimethylsilyl)oxy]-3,4-dihydro-2H-chromen-7-yl)oxy] (trimethyl) silane) to identify their potential to bind collagenase, elastase or tyrosinase, and score hits were selected for each ligand, in which Catechine exhibited the best docking scores for Collagenase (61.1937), Elastase (70.5752) and Tyrosinase (47.4749) (Table 5).

3D and 2D visualizations of interactions between ligand with Catechine and enzymes (Collagenase, Elastase, or Tyrosinase) are shown in Fig. 5. Catechine presented bonds with residues ILE222, LEU164, PRO221, GLY162, and LEU163 from Collagenase (Fig. 5A). It exhibited bonds with residues LEU227, LYS234, SER225, ARG226, TRP179, HIS60, and VAL103 from Elastase (Fig. 5B). Catechine demonstrated bonds with residues THR391 and LYS198 from Tyrosinase (Fig. 5C).

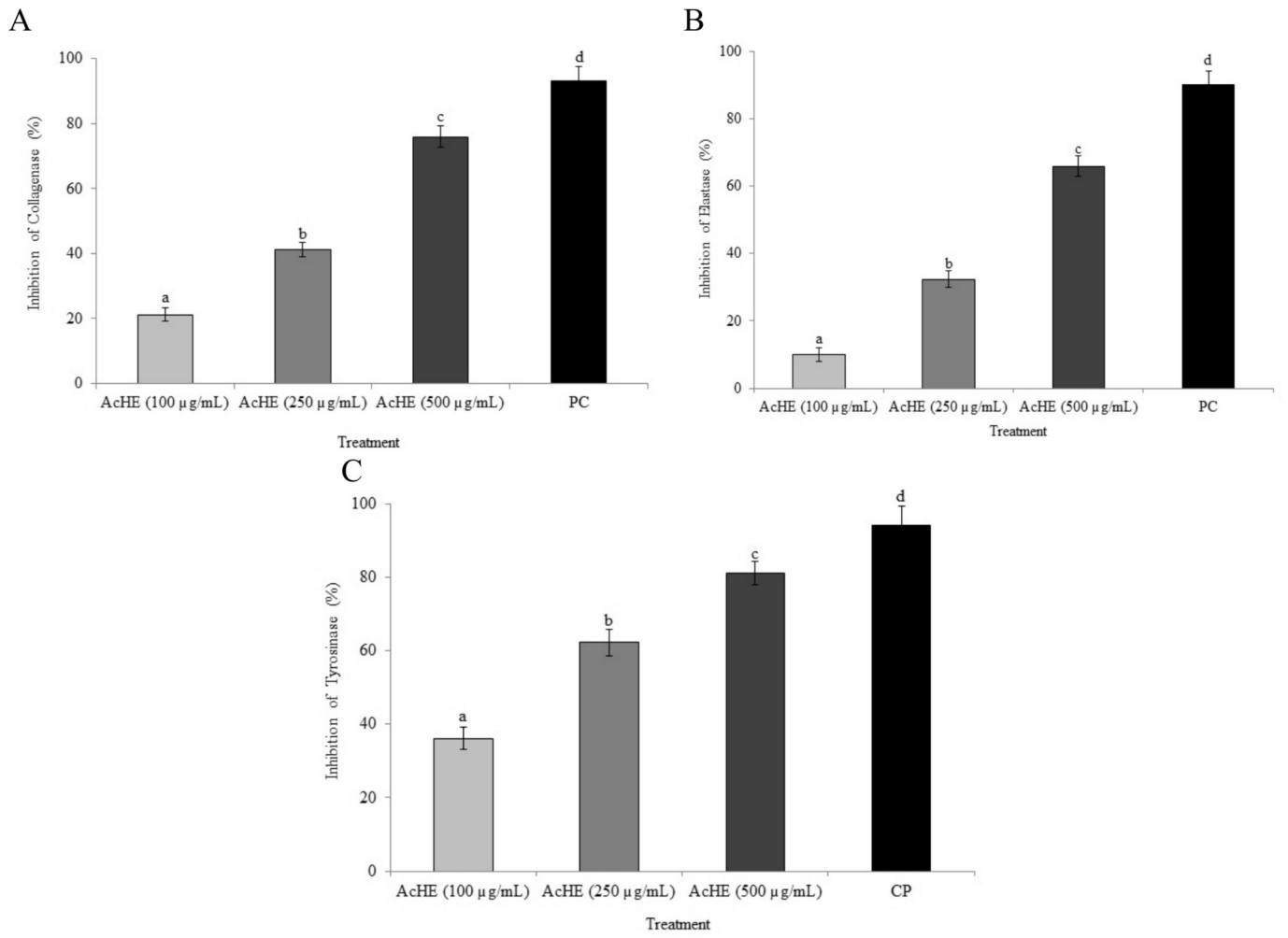

Fig. 4. The inhibitory effects of A. crassiflora hydroetanolic extract (AcHE) at concentrations of 100, 250, and 500 µg mL^{-1}, along with the positive control (PC), on collagenase (A), elastase (B), and tyrosinase (C).

Table 5
Best score hits for each ligand to enzymes (Collagenase, Elastase, Tyrosinase) involved in the aging process and oxidative stress.

Ligands	CHEMPLP		
	Collagenase	Elastase	Tyrosinase
Catechine	61.1937	70.5752	47.4749

The *in silico* assay using molecular docking provides a cost-effective method for screening compounds and visualizing interactions. This approach facilitates the design of targeted drugs and supports the development of multi-target therapies for complex conditions such as aging.[62] These findings support further experimental validation to confirm the therapeutic potential of catechin.[63] In the present study, the *in silico* assay utilizing molecular docking demonstrated strong binding affinities of catechin to key aging-associated enzymes (collagenase, elastase, and tyrosinase), highlighting its potential as a candidate for anti-aging therapies. The identification of specific amino acid interactions in collagenases provides valuable mechanistic insights for optimizing its structure, as described by Sianipar et al. [64]. These findings establish a foundation for additional experimental validation to confirm the therapeutic potential of active compounds from A. crassiflora extract.

Bioactive compounds from leaves extracts with antioxidant and antiglycation potential can be applied in several industries by acting to delay lipid oxidation and prevent AGEs formation, which would extend shelf life and maintain nutritional quality of foods. They have been used in supplements and herbal medicines to neutralize reactive oxygen species and inhibit protein glycation in order to prevent chronic diseases linked to oxidative stress. These compounds have been applied in cosmetic formulations, which have exhibited significant properties including protection of skin against free radicals and glycation of collagen and elastin and prevention of wrinkles, loss of elasticity and other signs of skin aging.

3.5. Microencapsulation of AcHE

3.5.1. Microencapsulation characterization

Microencapsulation is seen as a solution to the problem of the rapid degradation of bioactive compounds that are caused by volatilization, photodegradation and temperature.[65] Neta et al. [66] found that there were no significant differences in the amount of phenolics, flavonoids and carotenoids between microencapsulation powder and the fresh pulp of *Anonna muricata*. Spray drying of AcHE was performed using maltodextrin as coating agent. This choice of encapsulant considered the advantage of encapsulated non-toxic materials is that they can be applied more safely, with less risk of user poisoning and environmental contamination.[59] Results of runs performed according to a Box-Behnken factorial design are listed in Table 6. The effects of IT, FFR and MD, were investigated on the responses: Yield of process, WSI, WAI and WA.

The best YD values of microcapsules were obtained when used in the lowest concentration of the MD (10 %), regardless of other variables

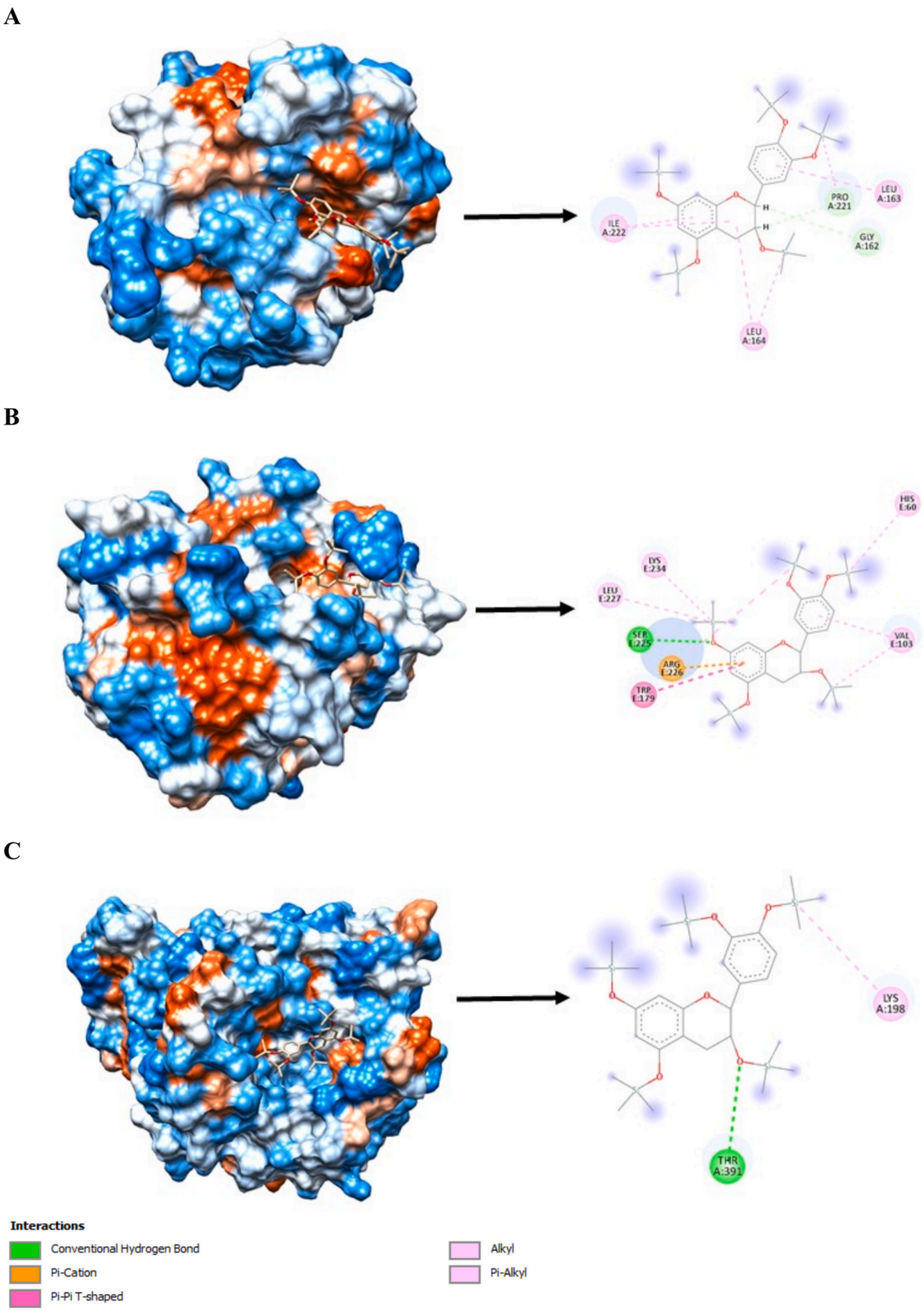

Fig. 5. 3D and 2D visualization of interactions between ligand and enzymes. A: Catechine and Collagenase; B: Catechine and Elastase; C: Catechine and Tyrosinase. 3D molecular targets are presented in hydrophobic surface visualization and 2D analyses show the main atom interactions between Catechine and enzymes.

Table 6
Microencapsulation of *A. crassiflora* leaves extract by Spray-dryer according to a *Box Behnken* factorial design.

Independent variables				Responses			
Run	IT (C°)	FFR (mL min^{-1})	MD (%)	YD (%)	WSI (%)	WAI (g g^{-1} DM)	WA (AWG)
1	120	25	10	83.2	79.96	0.80	0.345
2	180	25	10	99.4	79.42	0.79	0.274
3	120	25	50	67.7	87.44	0.87	0.354
4	180	25	50	50.3	48.41	0.48	0.266
5	120	8	30	66.2	84.41	0.84	0.235
6	180	8	30	83.3	86.99	0.87	0.273
7	120	42	30	43.5	89.87	0.90	0.287
8	180	42	30	57.7	85.49	0.85	0.325
9	150	8	10	95.0	80.94	0.81	0.310
10	150	8	50	83.8	85.79	0.86	0.196
11	150	42	10	95.4	80.95	0.81	0.336
12	150	42	50	29.3	81.27	0.81	0.214
13	150	25	30	62.1	82.32	0.82	0.319
14	150	25	30	71.0	84.93	0.85	0.217
15	150	25	30	82.2	86.65	0.87	0.298

IT: Inlet temperature, FFR: Feed flowrate, MD: Maltodextrin concentration, YD: Yield of drying, WSI: Water Solubility index, WAI: Water absorption index, WA: Water activity, DM: dry microcapsules.

(runs 2, 9 and 11). Spray drying yields ranged from 29.3 to 99.4 % (highest: yield was obtained with run 2 and the lowest with 12). WAI and WSI are shown in Table 6. The indices of water absorption are important to verify whether the powder has the capacity to remain stored without the accumulation of water, and water solubility to verify how it will be used in the future in the industry. Solubility results WSI included in Table 5 indicate high solubility to this product since, independently of the variables (excluding run 4) the microcapsule solubility was higher than 75 %. Chang et al. [67] reported WSI of *Annona muricata* powders > 93 % indicating the powders have a high dissolution ability, This can be considered because to spray-dried, the powder has a large porosity, which leads to better dissolve ability in water.

WAI shows the different runs showed values between 0.48 to 0.9 g/g DM (Table 6). The low difference between the values independent of the variables may be linked to the fact demonstrated by Kareem et al. [68] that starch obtained from fruits of *Annona* indicates capacity to agglomerate the particles and absorb water, thus, the binding and disintegrant properties have adequate to use as an excipient/additive in food, as well as non-food products.

WA talks about how the product will behave in the case, for example, of the growth of microorganisms. Silva et al. [69] obtained water activity (0.25 to 0.39) in microencapsulated propolis and evaluated the importance of wall material in decreasing water activity, emphasizing the importance of WA in maintaining the microbiological and physicochemical stability of the product.

3.5.2. Antioxidant activity and total polyphenols of microcapsules

The antioxidant activity of microcapsules was evaluated according to the ABTS method. As Table 7 shows, the reduced percentage of $ABTS^{+}$ radical were all related to the concentration of the different running conditions.

AcHE has 0.679 μM TE g^{-1} DM of AA, and the microcapsules obtained in run 2 and 11, there is no difference when compared to the extract before microencapsulated (0.547 and 0.545 μM TE g^{-1} DM, respectively). The microcapsules obtained in run 11, and in run 2 and 9, there is no difference when compared to the extract before microencapsulated. These results demonstrate that the antioxidant activity was maintained in the microcapsules under two conditions.

The total polyphenols obtained in the microcapsules produced in the different conditions varied between 33.58 and 148.00 mg GAE g^{-1} DM. Being the largest amount presented in the run 2. Efficiency of Polyphenols Encapsulation (EPE) says about the number of polyphenols that were encapsulated, and the highest percentage presented was in run 7,

Table 7
Antioxidant activity (AA) by ABTS method, Total Polyphenols (TP) and efficiency of polyphenols encapsulation (EPE) of microcapsules containing *A. crassiflora* hydroethanolic extract (AcHE).

Run	AA[1]	TP[2]	EPE[3]
1	0.996 ± 0.04a	141.13	36.2
2	0.547 ± 0.07b	148.00	87.1
3	0.217 ± 0.02c	33.58	92.5
4	0.172 ± 0.04c	36.92	91.7
5	0.309 ± 0.02 cd	57.83	91.5
6	0.263 ± 0.04c	115.67	93.2
7	0.334 ± 0.05 cd	58.17	94.9
8	0.308 ± 0.02 cd	55.08	92.7
9	0.476 ± 0.03b	109.75	80.9
10	0.242 ± 0.01c	42.25	94.2
11	0.545 ± 0.11b	121.50	84.9
12	0.276 ± 0.02c	47.58	94.1
13	0.339 ± 0.02 cd	57.17	94.3
14	0.250 ± 0.03c	57.17	94.3
15	0.306 ± 0.02 cd	58.50	94.3
AcHE (100 μg mL^{-1})	0.679 ± 0.02e	–	–

Results are expressed as mean ± standard deviation. [1]μM Trolox equivalent (TE) g^{-1} dry microcapsules (DM); [2] mg gallic acid equivalent (GAE) g^{-1} DM; [3]Percentage of encapsulation efficiency. Same letters within the same column indicate no significant differences between samples by Tukey test ($p \leq 0.05$).

with 94.9 % of the polyphenols kept inside the microcapsules. It was found that even after being encapsulated by maltodextrin the antioxidant activities and polyphenols content of *A. crassiflora* leaves remained, which has good scavenging ability for ABTS free radicals.

The encapsulation of AcHe using maltodextrin is an efficient strategy considering the encapsulating agent acted to form a protective matrix around the metabolites (mainly phenolic compounds), protecting them from environmental factors (light, oxygen, humidity, pH and temperature). This feature highlights the potential industrial use of these microcapsules as controlled release systems.

3.5.3. Response surface analysis (RSA)

Fig. 6 illustrates the variation in total polyphenol content (TPC, expressed in mg of GAE/g of product) in relation to the variables of temperature (T), feeding rate (FFR) and proportion of encapsulating agent (EA). The number of polyphenols increases progressively with increasing proportion of encapsulating agent and temperature (Fig. 1a). The combination of high temperatures and a high percentage of maltodextrin appears to improve the preservation of polyphenols, and this may be related to the protection provided by the encapsulating agent against thermal manipulation. The objective of microencapsulation is to protect the active ingredient; the greater the amount of encapsulating agent [70] and our results showed that the greater the thermal protection offered.

The interaction between temperature and feeding rates demonstrates that higher temperatures, associated with determined feeding rates (FFR), result in greater amounts of polyphenols, while very low or very high feeding rates, regardless of temperature, lead to a reduction in TPC levels, showing that feed rates influence contact time and process efficiency. Furthermore, the relationship between Encapsulating Agent Proportion (EA) and Feed Rate (FFR) shows that adequate levels of EA and FFR maximize TPC, while very low or very high EA situations, associated with extreme rates of EA FFR, resulted in a lower amount of TPC, highlighting the need for balance between the encapsulating agent and the feed rate to protect the polyphenols. In the work of Jordán Suarez et al. [71] carried out the microencapsulation of *Annona muricata* and optimized parameters were defined, such as an inlet temperature of 140 °C and a feeding rate of 7 mL min^{-1}, which maximized the desirability in terms of solubility, encapsulation efficiency (EE) and humidity. Although higher feed rates increased encapsulation efficiency and moisture content, reductions in solubility were observed. This method proved to be effective in preserving bioactive compounds with

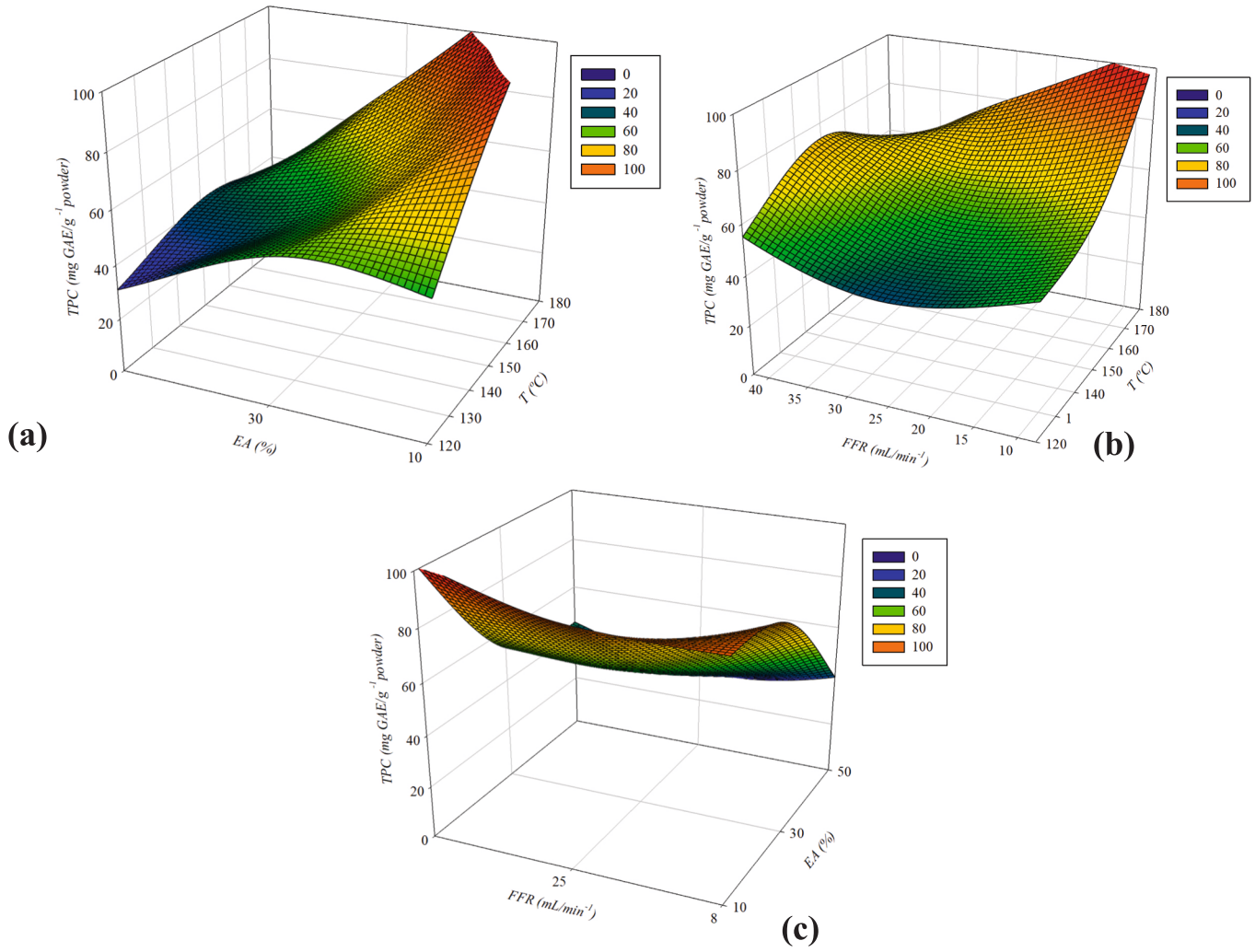

Fig. 6. Response surfaces of the total encapsulated phenolic compounds (TPC) of *A. crassiflora* leaves encapsulated by spray-drying as a function of **(a)** EA: ratio of encapsulating agents and T: temperature **(b)** FFR: feed flow rate and T: temperature **(c)** FFR: feed flow rate and EA: ratio of encapsulating agent.

therapeutic properties, such as annonacin and phenolic compounds.[65]

Fig. 7 provided present microencapsulation efficiency (%) as a function of different process variables, using three-dimensional response surfaces. The following patterns were found for each set of variables:

Fig. 7a shows that ME depends on the interaction between T and EA. At high temperatures and intermediate EA proportions, an efficiency peak close to 100 % is observed. However, very low or high proportions, regardless of temperature, and efficiency tend to be reduced, suggesting that a balance in the amount of encapsulating agent maximizes process efficiency. FFR also influences efficiency, in interaction with EA (Fig. 7b), lower FFR and appropriate ratios of EA to ME reach high values; however, very high FFR lead to a reduction in ME due to limitation in interaction time or in encapsulation capacity.

The interaction between temperature and feeding rates has an impact on microencapsulation efficiency, as at moderate to high temperatures, combined with low to intermediate FFR, efficiency reaches high levels, on the other hand, in extreme conditions (very high or low temperatures combined with rates of feed consumed), efficiency decreased substantially, as identified by Jordán Suárez et al. [71], where increasing temperature with a feed rate of 7 mL min^{-1} impairs encapsulation efficiency.

The efficiency of microencapsulation is highly dependent on the balance between process variables and optimized experimental design is indispensable for identifying optimal conditions that maximize efficiency.[72] These results provide a solid basis for precise adjustments in the microencapsulation process, allowing us to achieve better performances in industrial applications.

Similarly, polyphenol retention (TPC) is significantly influenced by process conditions. High temperatures, when combined with adequate proportions of encapsulating agent and moderate feed rates, favor the preservation of polyphenols.[73] On the other hand, extreme feed rates and inadequate proportions of encapsulating agent tend to impair retention, possibly due to thermal degradation or insufficient protection offered by the encapsulant.[74]

These observations reinforce the importance of carefully adjusting process conditions to maximize both microencapsulation efficiency and TPC, ensuring greater stability and functional quality in the final product.

The AcHE demonstrated a rich phytochemical composition, with high levels of polyphenols, flavonoids, and tannins, which exhibited a dose-dependent increase. The antioxidant activity observed across multiple assays, including DPPH, FRAP, TBARS, and NO scavenging tests, suggests a strong capacity to neutralize free radicals. GC–MS analysis identified several bioactive compounds, notably catechin, quercetin, and chlorogenic acid, which have been widely associated with antioxidants, anti-inflammatory, and antimicrobial properties. The antiglycation assays revealed that AcHE effectively preserved free amino groups and inhibited AGE formation, although its activity was lower

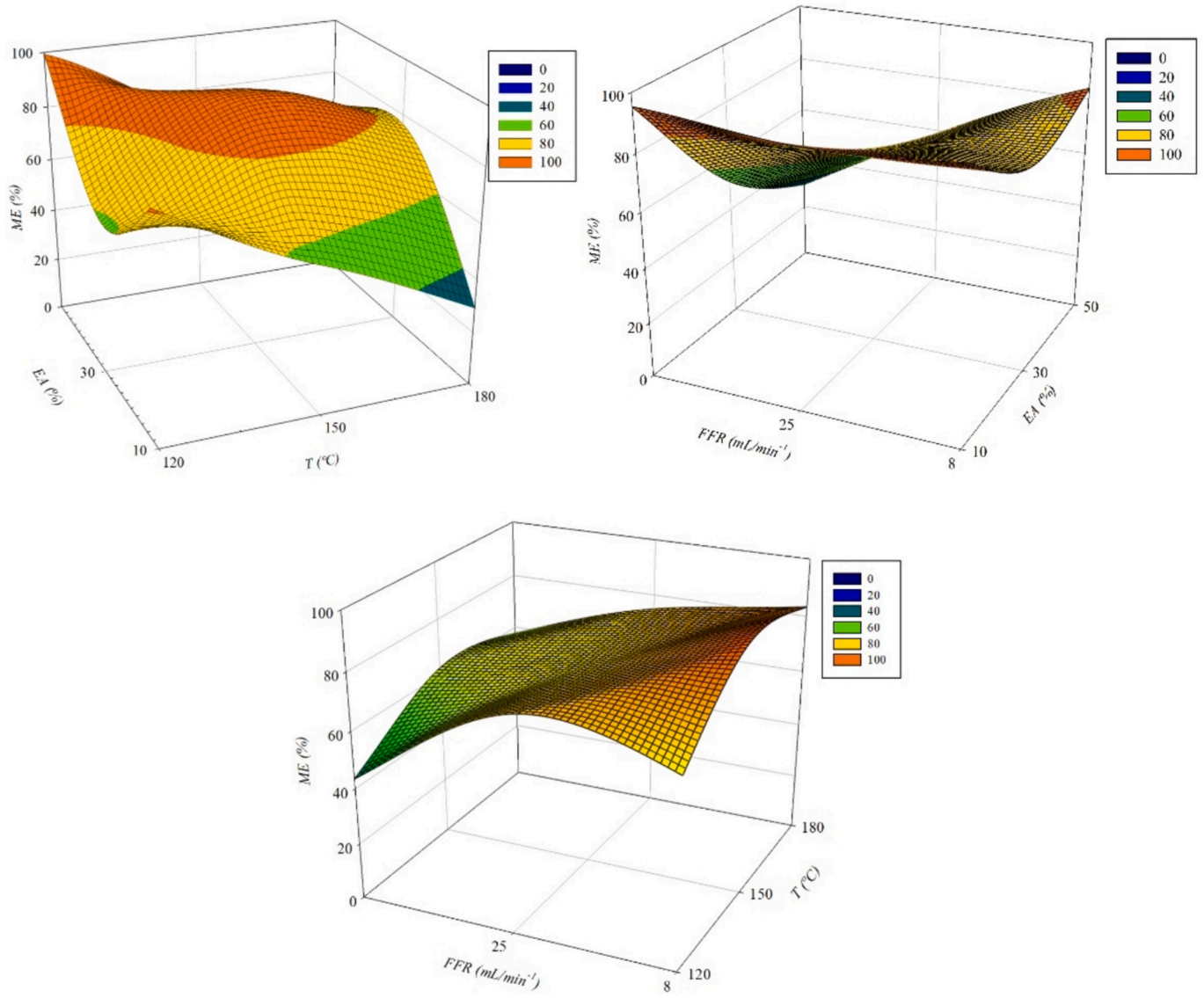

Fig. 7. Response surfaces of the efficiency of microencapsulation (ME) of *A. crassiflora* leaves by spray-drying as a function of (**a**) T: temperature and EA:ratio of encapsulating agents; (**b**) FFR: feed flow rate and EA: ratio of encapsulating agents; and (**c**) FFR: feed flow rate and T: temperature.

than that of Aminoguanidine. Additionally, the inhibitory effects on collagenase, elastase, and tyrosinase, along with molecular docking analyses, indicate potential anti-aging properties linked to the interaction of catechin with these enzymes. Microencapsulation techniques were applied to enhance the stability of AcHE, and specific conditions were found to optimize polyphenol loading and antioxidant activity, highlighting the influence of temperature, encapsulating agent proportion, and feed rate. The results support the relevance of *A. crassiflora* leaf extracts as a potential source of bioactive compounds, providing valuable insights into their chemical profile, biological activity, and possible applications.

4. Conclusion

The present study highlights the hydroethanolic extract of *Annona crassiflora* leaves (AcHE) as a novel and potent source of bioactive compounds—particularly phenolic compounds, flavonoids, and tannins—with significant antioxidant, antiglycation, and anti-aging properties. AcHE exhibited dose-dependent antioxidant potential comparable to standard controls, protected proteins from glycation, and effectively inhibited key enzymes associated with aging. Moreover, microencapsulation with maltodextrin preserved its bioactivity, enhancing stability and broadening its industrial applicability. Considering these promising findings, future studies should focus on *in vivo* validation of the biological effects of AcHE, evaluating safety and toxicity; besides, development of optimized delivery systems for pharmaceutical, cosmetic, and nutraceutical applications. Additionally, scaling up the microencapsulation process and evaluating the stability and performance of AcHE in prototype formulations will be crucial steps toward its commercial exploitation and sustainable use of an underexplored natural resource.

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Kamille Daleck Spera: Visualization, Validation, Supervision, Methodology, Conceptualization. **Pedro Henrique Gorni:** Validation, Investigation, Methodology. **João Luiz Bronzel-Junior:** Visualization, Validation, Supervision, Methodology, Formal analysis, Conceptualization. **Filipe Oliveira Granero:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Célia**

Cristina Malaguti Figueiredo: Visualization, Validation, Supervision, Methodology, Investigation, Conceptualization. **Hugo Henrique Santos:** Validation, Supervision, Software, Methodology, Formal analysis, Conceptualization. **Luciana Pereira Silva:** Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Patrizia Perego:** Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Paulo Eduardo Amaral Debiagi:** Visualization, Validation, Supervision, Software, Methodology, Formal analysis, Conceptualization. **Nilson Nicolau-Junior:** Visualization, Validation, Supervision, Software, Methodology, Data curation, Conceptualization. **Regildo Márcio Gonçalves da Silva:** Writing – review & editing, Methodology, Investigation, Conceptualization, Validation, Project administration.

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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