Food Hydrocolloids for Health 3 (2023) 100120

Contents lists available at ScienceDirect

Food Hydrocolloids for Health

journal homepage: www.elsevier.com/locate/fhfh

Formulation of double emulsions of mango seed extract (*Mangifera indica* L.) 'Ataulfo' incorporated into a mango by-product flour drink: Release kinetics, antioxidant capacity, and inhibition of cyclooxygenases

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ARTICLE INFO

Keywords:
Mango seed extract
Double emulsion
Release mechanism
Antioxidant capacity
Fickian behavior

ABSTRACT

The encapsulation and release of bioactive compounds obtained from by-products are aspects of exponential boom for several decades, as it seeks to maintain or enhance their activity. A double emulsion ($W_1/O/W_2$) was developed with mango seed extract (MS) 'Ataulfo', said extract contains gallic acid and pentagalloyl glucose as major compounds (80.16%). The double emulsion was subjected to release kinetics for 3 h in phosphate buffer (pH 6.9), presenting a release constant (k) of 35,350 ± 6,031 μg/mL/min, in addition to antioxidant capacity by the DPPH and FRAP method of 168,663 ± 4,273 and 39,718 ± 1,019 mMol/g of double emulsion respectively at 120 min of kinetics, the time of 120 min was determined as the latency time (l). The release behavior corresponds to zero-order kinetics since the release of the extract remains constant until the minimum concentration is reached to exert the antioxidant capacity mentioned above. The mechanism of release of the SM extract contained in the double emulsion is governed by diffusion (Fickian behavior), this was determined thanks to the equations of the Korsmeyer-Peppas mathematical model, obtaining a regression adjustment (R^2) of 0.9252 for said model and R^2 of 0.8126 for zero-order kinetics. The double emulsion was added to a mango peel drink formulation, to which the antitopoisomerase activity was determined in strains of S. cerevisiae (JN394 and JN362a), however, no inhibitory activity was presented towards any strain. The cyclooxygenase inhibition (COX) assay was performed on the 120-minute released fraction and the MS extract, showing that this fraction only showed 18.97% inhibition in COX-II, however, the SM extract obtained an inhibition percentage of 38.14% in COX-II.

1. Introduction

Mango seed (*Mangifera indica* L.) is by-product that has an interesting content of compounds with biological potential, among the compounds that have been identified are mangiferin and quercetin, compounds that have anticancer and chemoprotective properties (Lan et al., 2020; Zou et al., 2021), as well as ellagic acid and gallic acid (Blancas-Benitez et al., 2018; Cruz-Trinidad et al., 2019), the latter has demonstrated antioxidant (Granato et al., 2018) and anti-inflammatory (Nouri et al., 2021) activity; however, due to its low bioavailability in the human body (AL Zahrani et al., 2020) it may not provide the biological benefits already mentioned, so it is necessary to look for alternatives to favor the availability of the compound in the small intestine for absorption. In this sense, the encapsulation of compounds through the generation of double emulsions ($W_1/O/W_2$), allows the controlled release of the compounds of the system, in addition to providing protection and possibly increasing biological activity (Zambrano-Zaragoza et al., 2018), this concept is called functionalization, which, through chemical modifications or incorporation of the compound in polymer matrices, the functional properties of compounds can be improved (Makvandi et al., 2021). Double emulsion systems have already been developed where they encapsulate compounds of biological interest such as anthocyanins (Kanha et al., 2021), carotenoids (A. Rehman et al., 2020), quercetin

Abbreviations: MS, mango seed; TSP, total soluble phenols; DE, double emulsion; DE1, double emulsion 1; DE2, double emulsion 2; DE3, double emulsion 3; DE4, double emulsion 4; COX, cyclooxygenase; COX-I, cyclooxygenase-I; COX-II, cyclooxygenase-II; AOX, antioxidant activity; PVA, polyvinyl alcohol; YPD, yeast extract, peptone, and dextrose; AYPD, agar, yeast extract, peptone, and dextrose.

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https://doi.org/10.1016/j.fhfh.2023.100120
Received 16 June 2022; Received in revised form 16 November 2022; Accepted 21 January 2023

(Chouaibi et al., 2019), although plant extracts have also been encapsulated, such is the case of an olive leaf extract (Jolayemi et al., 2021) and aronia pomace extract (Eisinaitė et al., 2020), and in the case of mango by-products, a double emulsion formula ($W_1/O/W_2$) from a mango skin extract rich in polyphenols (Velderrain-Rodríguez et al., 2019). The particularity offered by encapsulation by double emulsions is the protection against adverse factors such as oxidation, pH, and hydrolysis (Singh et al., 2017), in addition, these systems can be used as ingredients for other food matrices, double emulsions of hibiscus extract have been incorporated in yogurt (de Moura et al., 2019). Although the encapsulation of compounds in emulsion systems is crucial in the stability and functional properties of these, it is also important to study the pharmacokinetic properties of the encapsulation system, to know the rate of release of the compound, the percentage of the release of the same and the latency time, that is, the time in which the concentration of the compound is sufficient to achieve an effect (Lachi-Silva et al., 2020; Rehman et al., 2022), allows to deepen the application and scope that this system can have. The use of the Korsmeyer-Peppas mathematical model for the study of the release mechanics of encapsulation systems, allows the understanding of this phenomenon in polymeric encapsulation systems, either by diffusion (Fickian behavior) or by swelling (non-Fickian behavior) (Paarakh et al., 2019), in addition to the study of the dose /effect of the compound concerning time in zero-order kinetics. In the present study a double emulsion containing mango seed extract (MS) with high content of gallates (gallic acid and pentagaloyl glucose) was developed, the pharmacokinetic properties of this system were studied using the Korsmeyer-Peppas equations, to determine the release behavior and the zero-order equations, to observe the release of the extract (dose) for the kinetic time, the release times of the extract were monitored to determine the phenolic content expressed as total soluble phenols in gallic acid equivalents, as well as the antioxidant capacity, subsequently, the double emulsion was incorporated into a beverage formulation of mango peel flour and the ability of topoisomerase inhibition and cyclooxygenase inhibition was determined, as a biological property for the potentially functional beverage.

2. Materials and methods

2.1. Mango seed extract

The process for obtaining the mango seed extract is described below: 1 g of mango seed flour (previously crushed and sieved for a particle size of 300 μm) was weighed, to which 30 mL of water/ethanol extraction solvent (80:20 v/v) was added, the mixture was subjected to ultrasound-assisted extraction with an amplitude of 100% and 100 s of pulses for 8 min, maintaining a temperature of 60 °C. The conditions mentioned above were obtained by an experimental design octagonal matrix Taguchi L18 (unpublished data).

2.2. Obtaining double emulsions of mango seed extract by ultrasound

For the formulation of the double emulsions, the methodology of Velderrain-Rodríguez et al. (2019) and Iqbal et al. (2015) was used, with some modifications. 500 μL of the mango seed extract was dispersed in a pre-emulsion containing 1250 mL of Tween 20 (polysorbate 20, BHL 16) and 10 mL of canola oil, to obtain the simple emulsion (W_1/O), subsequently dispersed 1 mL of the single emulsion in 10 mL of a second continuous phase (W_2) containing 4 mL of Polyvinyl Alcohol (PVA), 4 mL of mango pulp and 2 mL of water, to finally obtain the double emulsion, the ultrasound conditions and concentrations of PVA and pulp vary according to the statistical design (Table 1). The proportions of the different components of the single and double emulsions are presented in Table 2. To evaluate the combination of factors and the level of effect of these on the concentration of FST released, an octagonal matrix of Taguchi L8 was used and an average analysis of the level of a factor in the release of TSP, rate of release (K) and percentage of release was performed using the average per factor of the function "largest better"; that is, the higher the value of the response variable about the combination of the levels of the study factors will be taken as optimal conditions. The use of PVA is safe at the concentration used in the stucco as JECFA (Joint FAO/WHO Expert Committee on Food Additives) allocated an ADI (recommended daily intake) of 50 mg/kg body weight. The JECFA Committee established an ADI for polyvinyl alcohol of 50 mg/kg body weight, based on the NOAEL (no observed adverse effect level) of 5000 mg PVA/kg body weight/day from the 90-day and two-generation rat studies (in which it was the highest dose tested), with a safety factor of 100 (The Efsa, 2006).

2.3. Formulation of double emulsions

Based on the conditions obtained in the ETA analysis of the Taguchi L8 design, the optimal DE1 treatment was prepared, a treatment with extract, without pulp (DE2), a treatment without extract, with pulp (DE3) and a control treatment without extract and without pulp (DE4). These treatments underwent 3-hour release kinetics (the release kinetics methodology is described in Section 2.4), to obtain the kinetic data necessary for the adjustment of the Korsmeyer-Peppas mathematical model and zero-order kinetics. In preliminary tests, the release of the DE was carried out at pH 1.5 (simulating gastric conditions), however, the DE dissociated completely as soon as it came into contact with the release medium at that pH, so the release was only carried out at the aforementioned pH.

2.4. Analysis and characterization of double emulsions

2.4.1. Release kinetics of optimal double emulsions

The DEs underwent release kinetics using the methodology proposed by Calderón-Varela (2015). The DE was placed in dialysis bags (Sigma-Aldrich cellulose membrane of permeability at 13 kDa) in 200 mL of phosphate buffer (pH 6.9) at a temperature of 37 °C, aliquots of 10 mL were taken at times 0, 5, 10, 15, 20, 30, 60, 120, and 180 min compensating the volume taken of aliquot with the phosphate buffer. (se establecio este tiempo de cinetica debido a que se busca una liberacion rapida de 2 a 3 horas en pH 6.5)The aliquots were freeze-dried to later determine the Total Soluble Phenols (TSP) released using the Folin-Ciocalteu method.

2.4.2. Total soluble phenols by folin-ciocalteu

The methodology proposed by Montreau (1972) was used. The lyophilized aliquot of the release kinetics was placed in a test tube and the 1 mg/mL concentration was adjusted with phosphate buffer (pH 6.5). 250 µL of aliquot were taken, 1 mL of 7% calcium carbonate and 1250 mL of Folin-Ciocalteu reagent were added, and the test tube was homogenized at 6000 rpm in a VORTEX-GENIE equipment Model SI-0236 (G560), then incubated at 50 °C for 15 min. The tubes were cooled to room temperature and the reaction was placed on 96-well plates and read on Bio-Tek(R) spectrophotometer (Synergy HT, Winooski, VT, USA) at 750 nm. A gallic acid calibration curve of 0.2, 0.1, 0.05, 0.025, 0.0125, and 0 mg/mL was used using serial dilutions for quantification.

2.4.3. Antioxidant capacity of the released fractions

The fractions released at 120 and 180 min of release kinetics of each double emulsion were taken, Since being a system in which a rapid release is expected (2–3 h) in the small intestine (pH 6.5), these times are considered as the latency time (l), that is, the time in which the release of the compounds within the system reaches the concentration sufficient to present antioxidant activity (the sampling times before 120 and 180 min were monitored to observe the change in the referent concentration at the time of release kinetics). For the antioxidant capacity by reducing DPPH radicals, the methodology of Álvarez-parrilla et al. (2010) was followed: a Trolox calibration curve was prepared at concentrations of 600, 300, 150, 75, and 37.5 μM. 30 μL of the calibration curve and

Table 1
Experimental design using the octagonal matrix Taguchi L8.

Formulation	Emulsion (W_1/O)			Double emulsion ($W_1/O/W_2$)			
	Extract concentration (mg/mL)	Amplitude (%)	Ultrasound Time (min)	PVA (%)	Amplitude (%)	Ultrasound Time (min)	Mango pulp (%)
1	6	100	6	4	50	6	4
2	6	100	6	2	100	10	2
3	6	50	10	4	50	10	2
4	6	50	10	2	100	6	4
5	3	100	10	4	100	6	2
6	3	100	10	2	50	10	4
7	3	50	6	4	100	10	4
8	3	50	6	2	50	6	2

Table 2
Single emulsion and double emulsion content.

W_1/O		$W_1/O/W_2$	
Extract	4.26	Extract	0.39
		Tween 20	0.97
Tween	10.64	Canol oil	7.73
20		PVA	36.36
Canol	85.1	Pulpe	36.36
oil		Water	18.19
Total%	100	Total%	100

aliquots were placed on a 96-well microplate and measured at 570 nm on a spectrophotometer (Biotek, Synergy HT, Winooski VT, USA), while the team administered the DPPH radical at a concentration of 190 μM to the wells of the plate. For the antioxidant capacity by ferric reduction, the methodology proposed by Benzie and Strain (1996)) was used: The FRAP complex was prepared by adding 2.5 mL of a 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) solution 10 mM acidified (HCl, 40 mM), 2.5 mL of hexahydrate ferric chloride ($FeCl_3$) solution 20 mM and 25 mL of acetate buffer 0.3 M pH 3.6, incubated at 37 °C for 30 min. For quantification, a Trolox curve was performed at concentrations of 0.13, 0.05, 0.032, 0.016, and 0.008 mM, 24 μL of the calibration curve and aliquots were placed on a 96-well microplate and read at 545 nm on a spectrophotometer (Biotek, Synergy HT, Winooski VT, USA) while the team dosed the FRAP complex.

2.4.4. Analysis of the release profile in mathematical models

For the adjustment of the release in the mathematical model, the speed of release of the DE was calculated using Eq. (1):

$$k = \Sigma\left(\frac{\Delta C}{\Delta t}\right) \tag{1}$$

Where: k is the release constant of TSP, ΔC is the change in the released concentration of FST and Δt is the release time (Blancas-Benitez et al., 2018).

The data obtained from TSP and velocity (k) in the release kinetics were adjusted to the variants in the equation of the Korsmeyer-Peppas (Bruschi, 2015; Korsmeyer et al., 1983), by calculating the fraction of solute released at a given time (Eq. (2)), concentration of liberated compound (Eq. (3)) and compound released concerning latency time (Eq. (4)), in the same way, the exponent n was determined to determine if the kinetics model fits a Fickian model using Eq. (5) of zero-order kinetics and Eq. (6) in zero-order kinetics referring to the release of compound about the initial concentration within the DE, the equations used are described below:

$$\frac{M_i}{M_\infty} = kt^n \tag{2}$$

Where: $\frac{M_i}{M_\infty}$ is the fraction of solute released at a given time, k is the release rate constant, t is the release time, and n is the exponent describing a Fickian behavior.

$$f = \frac{M_i}{M_\infty} \tag{3}$$

For this equation; f is the concentration of the compound released, M_i is the amount of compound released at a given time, and M_∞ is the initial concentration of the compound at time 0.

$$\frac{M_{(i-l)}}{M_\infty} = k(t-l)^n \tag{4}$$

The release of the compound about the latency time is considered as $\frac{M_{(i-l)}}{M_\infty}$, where k is the constant in release rate, t is the release time, l is the latency time of the compound and n is the exponent referring to the type of release, which is determined according to the type of mechanism that governs the release of the system: if the mechanism is by diffusion, the model corresponds to a Fickian behavior where the value of $n = 0.5$. To determine whether the release mechanism corresponds to diffusion, the zero-order kinetic equation was used:

$$1 - \left(W_0 - W_i\right) = k * t \tag{5}$$

In zero-order kinetics, which is characterized by a diffusion release mechanism, W_0 represents the initial concentration, W_i is the release at a given time t and k is the release rate constant. On the other hand, Eq. (6), of order zero, is calculated by the apparent velocity k_0 referring to release time t.

$$f = k_0 t \tag{6}$$

2.4.5. Characterization by FTIR

Fourier transform infrared spectroscopy (FTIR) was performed on a JASCO infrared spectrometer equipped with a PIKE MIRacle ATR accessory in the range of 4000–450 cm^{-1}. Once the infrared spectrum data was obtained, the characteristic wavenumber regions of fingerprint and functional groups were analyzed and identified (Obregón et al., 2019).

2.5. Formulation of beverage from double emulsions of mango seed

A beverage formulation (Optimal Formulation) of mango by-product was made to which the ED was added that presented the highest antioxidant capacity by DPPH and FRAP. The formulation consists of the following: 7% mango peel flour, 0.05% sodium alginate as a stabilizer (according to CODEX STAN 192-1995 codex Alimentarius standard for food additives), 4% DE, 4% sugar, and 84.95% water. This beverage was evaluated for antitopoisomerase and anticycloxygenase activity to determine the possible functional potential of the formulation. As controls, a drink without double emulsion (White Formulation) and a formulation with double emulsion without extract (Control Formulation) were made.

2.6. Biological activity assays

2.6.1. Topoisomerase inhibition assay

The antitytopoisomerase assay was performed using genetically modified strains of *S. cerevisiae* JN362a and JN394 (John L & Karin C, 2001). The strains used were grown in YPD medium (Yeast extract,

Peptone, and Dextrose) at 30 °C for 18 h at 150 RPM, this until reaching logarithmic growth. Subsequently, the treatments were adjusted to a total volume of 10 mL with a concentration of 2×10^6 cells/mL of the strain to be used (JN394 or JN362a) and 50 μL of a sample (previously dissolved in dimethyl sulfoxide), positive control (camptothecin) or negative control (dimethyl sulfoxide) and were placed in incubation for 24 h under the same conditions of temperature and agitation. After 24-hour incubation, the treatments and controls were inoculated in YPDA medium, using dilutions 10^{-4} and 10^{-5} by serial dilution, 30 °C was incubated for 48 h and the calculation of the CFUs/mL was performed. Antitopoisomerase activity was estimated considering 100% growth from CFU/mL of negative control (that is, the yeast has no problems developing since it is not in the presence of any inhibitor). The differences in growth rates correspond to the% inhibition of the treatments evaluated. The extract concentrations used in the trial were 3, 13.34×10^{-3}, and 0.5336×10^{-3} mg/mL; which correspond to the concentration for the formulation of the DE, the final concentration of the MS extract in the DE, and the final concentration of the extract in the drink respectively, the drink containing the DE under optimal conditions, a drink with DE control (without extract) and a control formulation (without DE), and the release at 120 min of the optimal DE used in the formulation of the drink.

2.6.2. Cyclooxygenase inhibition test

The cyclooxygenase test was performed with the technique described by Créminon et al. (1995) and modified by Ramadwa et al. (2017). Treatments previously used in the antitopoisomerase trial were used for this trial. The COX-I (ovine) and COX-II (recombinant human) enzymes were inactivated by boiling for 15 min. In previously labeled test tubes, 10 μL of inactive enzymes, 10 μL of active enzymes were placed separately and in other tubes, 10 μL of treatments or inhibitors were placed. To each tube was added 10 μL Heme reagent (as a cofactor) and 160 μL of reaction buffer, and the tubes were incubated at 37 °C for 10 min. To start the enzymatic reaction, 10 μL of arachidonic acid was added to each tube and incubated at 37 °C for exactly 2 min, to end the reaction 30 μL of tin chloride was added. 10 μL were taken from each reaction tube and placed in 990 μL of ELISA buffer, then 100 μL of each dilution was added and placed on a plate of 96 wells previously identified, 50 μL of AChE tracer (Acetyl colin esterase) added after incubation by 18 to 37 °C. After the incubation time, washes of the plate with washing buffer were carried out, this action was performed 5 times. 200 μL of Ellman reagent was added to each well and incubated for 90 min at room temperature in the dark, finally, absorbance was measured at 420 nm. The results were expressed as a percentage of inhibition taking as a reference the value of the wells of the inactive COX and the wells of 100% of initial activity.

2.7. Statistical analysis

To obtain the combination of factors in the formulation of the DE, the ETA analysis (measured the effect size of different variables in ANOVA models) of the "larger better" function was used, as well as a comparison of Fisher's LSD means and analysis of variance p = 0–5. The rest of the determinations were performed in triplicate and a comparison of Fisher's LSD means and analysis of variance p = 0–5 was used. Using STATISTICA software version 12.

3. Results and discussion

3.1. Compounds of mango seed extract

The final concentration of mango seed extract in the double emulsion is 11.6 μg/mL, and the composition of the extract is 33.31% gallates (gallic acid as the majority compound) and gallotannines 46.85% (pentagaloyl glucose as the majority compound). Table 3 shows the characterization of compounds identified by HPLC-DAD/MS of mango seed extract.

3.2. Ultrasonic formulation of mango seed extract double emulsions

Table 4 shows the results obtained from the release kinetics of the 8 DE treatments, where it is observed that treatments 1, 2, 3, 4, 7, and 8 manifest a statistically similar TSP release, with treatment 2 being the lowest with an FST concentration of 75,071 ± 18,697 μg GAE/mL, on the other hand, treatments 1, 3, 4, 5, 6, 7 and 8 are similar between them (ANOVA comparison of Fisher's LSD means p = 0.05), highlighting treatment 6 with an FST concentration of 127,603 ± 13,143 μg GAE/mL. In the case of the speed of release, the treatments are classified into two groups, being the treatment 1, 2, 4, 5, 6, 7, and 8 the first group, where treatment 4 is the one that has the lowest release speed of 0.229 ± 0.144 μg GAE/mL/min, while the other group is integrated by treatments 1, 2, 3, 5, 6, 7 and 8 (ANOVA comparison of Fisher's LSD means p = 0.05) highlighting treatment 3 as the highest release rate (1.638 ± 0.634 μg GAE/mL/min). However, for the percentage of the release of TSP at 3 h of kinetics, all treatments must be statistically equal, except for treatment 1 with the lowest percentage of the release of 42.34 ± 11.25% and treatments 5, 6, and 8 (83.80 ± 21.27%, 76.07 ± 7.83% and 78.84% respectively) as those with the highest percentage of release. These data were subjected to the ETA analysis of the "bigger better" function to observe the level of effectiveness of the factors on the TSP release variable at 3 h of kinetics. According to the ANOVA obtained from the ETA analysis of the study factors for the formulation of the DE (Table 5), it is observed that the only factor that has a level of effect on the parameter of the percentage of release, is the addition of mango pulp, which will be explained in the following section. For the released TSP and release rate variables, the level of effect of the factors is not significant for those variables. It is also important to note that treatment 6 has no statistically significant difference with treatments that presented both the highest rate of release (treatment 3) and the highest percentage of release (treatments 5 and 8). Therefore, when looking for the highest concentration of TSP released in the 3 h, the combination of factors for treatment 6 was considered optimal to obtain a DE with the aforementioned characteristic.

3.3. Analysis and characterization of double emulsions

3.3.1. Release kinetics and total soluble phenols

The results of TSP release kinetics of the DE described in Section 2.3 of the methodology of this document are presented in Table 6, where the highest concentration of FST at 120 min of kinetics is observed in DE1 and DE 2 (643,919 ± 36,930 and 697,817 ± 7507 μg/mL respectively), while for DE3 and DE4 this release manifested itself at 10 min (637,647 ± 17,817 and 613,079 ± 67,356 μg/mL respectively), this type of behavior may be due to the fact that both DE1 and DE3 have fresh mango pulp in their composition, in the case of DE1 the physical phenomena (cavitation, sonoporation, erosion, shearing) present during the application of ultrasound at the time of dispersing emulsion 1 (W_1/O) in the continuous phase containing pulp, were able to release phenolic compounds present in the latter (Chemat et al., 2017), together with the reduction in pH (from 6.8 to 5.7 – 5.3) of the DE at the time of adding the mango pulp to the system, could have an effect on the acid dissociation coefficient (p*Ka*) of the PVA, causing a dissociation in the polymer, giving rise to coalescence phenomena and affecting emulsion stability (Zhang et al., 2021).

3.3.2. Antioxidant capacity of the release fraction

The antioxidant capacity was determined by the method of radical reduction (DPPH) and ferric reduction (FRAP) to the fractions of 120 and 180 min of the release of each DE because DE1 and DE2 are the ones that presented the highest concentration of TSP at these times,

Table 3
Identification and quantification of tentative compounds in mango seed extract.

No.	Tentative compound	Chemical formula	RT (min)	[M-H]-	Content
	Gallates				
1	Gallic acid	$C_7H_6O_5$	5.79	169	1449.27 ± 59.42b
2	Ethylgalate	$C_9H_{10}O_5$	16.25	191	124.66 ± 0.29a
3	Methyl gallate	$C_8H_8O_5$	12.65	183	13.27 ± 0.64b
4	Ethyl-2,4-dihydroxy-3-(3,4,5-trihydroxybenzoic)oxibenzoate	$C_{16}H_{14}O_9$	19.26	349	94.30 ± 0.28a
5	6-O-Galloil glucose	$C_{13}H_{16}O_{10}$	4.80	331	468.40 ± 68.56b
6	Galloil-di-glucose	$C_{20}H_{20}O_{14}$	5.23	493	48.07 ± 16.10b
	Total mg/100 g (%)				**2187.97 ± 145.29 (33.31%)**
7	1,2,6-Trigalloilglucose	$C_{27}H_{24}O_{18}$	13.42	635	61.78 ± 2.45b
8	Tetra-o-galloilglucose	$C_{34}H_{28}O_{22}$	16.08	787	607.60 ± 32.28a
9	Pentagalloil glucose	$C_{41}H_{32}O_{26}$	17.39	939	2407.94 ± 124.32a
	Total mg/100 g (%)				**3077.32 ± 159.05 (46.85%)**
10	Elagic acid	$C_{14}H_6O_8$	17.50	301	21.56 ± 0.76a
11	Dilactone valoneic acid	$C_{21}H_{10}O_{13}$	17.36	469	259.43 ± 28.92a
	Total mg/100 g (%)				**280.99 ± 29.68 (4.28%)**
12	Quinic acid	$C_7H_{12}O_6$	4.62	191	670.02 ± 42.29a
	Total mg/100 g (%)				**670.02 ± 42.29 (10.20%)**
	Benzophenones				
13	Maclurin3-C-glucoside	$C_{19}H_{20}O_{12}$	17.74	423	76.24 ± 3.67b
14	Maclurin galloil glucoside	$C_{33}H_{28}O_{17}$	12.01	575	2.83 ± 1.40a
	Total mg/100 g (%)				**79.07 ± 5.07 (1.20%)**
	Xanthones				
15	Mangiferin	$C_{19}H_{18}O_{11}$	14.22	421	36.88 ± 6.32a
	Total mg/100 g (%)				**36.88 ± 6.32 (0.56%)**
	Hydroxybenzoic acids				
16	Vanílic acid glucoside	$C_{14}H_{18}O_9$	11.75	329	233.41 ± 17.912a
	Total mg/100 g (%)				**233.41 ± 17.91 (3.55%)**
	Flavonoids				
17	Quercetin xiloside	$C_{20}H_{18}O_{11}$	12.90	433	2.95 ± 1.30a
	Total mg/100 g (%)				**2.95 ± 1.30 (0.44%)**
	Total phenolic compounds mg/100 g (%)				**6568.61 ± 406.91 (100%)**

Table 4
Cumulative total soluble phenol release, release rate and release percentage of double emulsions subjected to release kinetics.

Treatment	Extract concentration (μg/mL)	Amplitude (%)	Time (min)	PVA concentration (%)	Amplitude (%)	Time (min)	Pulp concentration (%)	TSP (μg GAE/mL)*	*K* (μg/mL/min)	% Liberation
1	6	50	10	2	100	6	4	80.532 ± 21.399a,b	1.281 ± 1.362a,b	42.34 ± 11.25b
2	6	50	10	4	50	10	2	75.071 ± 18.697a	1.238 ± 1.019a,b	59.35 ± 14.78a,b
3	6	100	6	2	100	10	2	82.337 ± 25.887a,b	1.638 ± 0.634b	64.71 ± 20.34a,b
4	6	100	6	4	50	6	2	103.072 ± 55.375a,b	0.229 ± 0.144a	54.40 ± 29.23a,b
5	3	50	6	2	50	6	2	87.801 ± 22.289a,b	0.589 ± 0.280a,b	83.80 ± 21.27a
6	3	50	6	4	100	10	4	127.603 ± 13.143b	0.609 ± 0.066a,b	76.07 ± 7.83a
7	3	100	10	2	50	10	4	94.256 ± 32.415a,b	1.246 ± 0.913a,b	56.07 ± 19.28a,b
8	3	100	10	4	100	6	2	102.483 ± 1.267a,b	0.587 ± 0.282a,b	78.84 ± 1.36a

The values are presented as the mean ± standard deviation of three replicates ($n = 3$) of the treatments. Per column, different letters indicate statistically different groups based on Fisher's comparison of LSD means ($p = 0.05$). equivalent micrograms of gallic acid on milliliter.

these results are presented in Table 7. DE2 stands out among the other treatments because it has the highest capacity to reduce DPPH radicals (168,663 ± 4273 mMol TE/g), exceeding by 380% the release of DE1 (44,413 ± 22,069 mMol TE/g), in terms of ferric reduction capacity (FRAP) of DE2 39,718 is observed ± 1019 mMol TE/g, being 131% greater than the ferric reduction capacity of DE1 (30,298 ± 0,048 mMol TE/g), there is a relationship between the concentration of TSP released and the antioxidant capacity obtained, since the higher the concentration of phenolic compounds, a high antioxidant capacity can be presented (Granato et al., 2018) in this particular case, it is theorized that gallic acid or some other gallate such as pentagaloyl glucose may be released (Cruz-Trinidad et al., 2019; Yang et al., 2020), compounds identified in mango seed, of which it has already been reported that the antioxidant capacity of this type of compound is dependent on the dose or concentration with which the *in vitro* assay of antioxidant capacity is carried out (Rahimifard et al., 2020). These results suggest that at 120 min of release at a pH of 6.9, the greatest release of compounds with antioxidant capacity in DE2 is presented, so the said emulsion system allows the controlled release of PC, given that the time of 120 min is in which the released compounds reach the minimum concentration to exert an effect, this was considered as the latency time (*l*) for the adjustment of the release profile in the equations of Korsmeyer et al. (1983).

3.4. Release profile in Korsmeyer-Peppas mathematical model and zero-order kinetics

To visualize the behavior of the DEs during release, the accumulated TSP results were plotted with the kinetic time, which can be seen in

Table 5
Analysis of variance of factors that affect the release kinetics.

Efect	SS	DF	MS	F	P
Total Soluble Phenols					
Extract concentration	21.3230	1	21.32305	2.584946	0.127436
Amplitude	0.0959	1	0.09592	0.011628	0.915470
Ultrasound time	4.3798	1	4.37979	0.530953	0.476737
PVA	9.9082	1	9.90819	1.201148	0.289315
Amplitude	5.9752	1	5.97523	0.724364	0.407274
Ultrasound time	0.1110	1	0.11098	0.013454	0.909102
Mango pulp concentration	6.1277	1	6.12766	0.742842	0.401485
Residual	131.9829	16	8.24893		
k					
Extract concentration	1.6273	1	1.6273	0.031322	0.861746
Amplitude	4.1062	1	4.1062	0.079034	0.782214
Ultrasound time	38.2309	1	38.2309	0.735849	0.403661
PVA	196.6654	1	196.6654	3.785317	0.069490
Amplitude	83.1897	1	83.1897	1.601194	0.223852
Ultrasound time	193.8860	1	193.8860	3.731820	0.071293
Mango pulp concentration	46.2839	1	46.2839	0.890849	0.359283
Residual	831.2768	16	51.9548		
% Liberation					
Extract concentration	63.6062	1	63.60623	7.710843	0.013469
Amplitude	0.0936	1	0.09364	0.011351	0.916477
Ultrasound time	4.2997	1	4.29967	0.521240	0.480733
PVA	10.4472	1	10.44724	1.266496	0.277024
Amplitude	5.8680	1	5.86799	0.711363	0.411424
Ultrasound time	0.1740	1	0.17402	0.021096	0.886332
Mango pulp concentration	46.8980	1	46.89803	5.685345	0.029831
Residual	131.9829	16	8.24893		

DF, degree of freedom; SS, sum of squares; MS, medium square.

Table 6
Total soluble phenols released during the release kinetics of double emulsions.

Time	Treatment			
	DE1	DE2	DE3	DE4
0	357.944 ± 62.618 g,h,i	252.372 ± 36.053c,d,e	288.067 ± 24.643d,e,f,g	200.181 ± 42.898b,c
5	108.866 ± 31.105a	162.411 ± 13.083a,b	369.422 ± 47.361 h,i.j	177.637 ± 18.931a,b
10	274.758 ± 85.656d,e,f	440.651 ± 15.264k,l	637.647 ± 17.817o,p	613.079 ± 67.356n,o
15	345.741 ± 86.734f,g,h,i	230.275 ± 33.847b,c,d	392.913 ± 44.521i,j,K	324.822 ± 28.516f,g,h,i,j
20	364.676 ± 82.430 h,i,j	474.348 ± 40.963l	580.081 ± 51.602n,o	365.110 ± 33.310 h,i
30	551.771 ± 52.677 m,n	283.275 ± 33.993d,e,f	496.241 ± 39.226l,m	386.094 ± 40.471i,j,k
60	434.965 ± 51.172j,k,l	441.966 ± 18.083k.l	302.707 ± 40.370e,f,g,h	488.722 ± 18.357l,m
120	643.919 ± 36.930o,p	697.817 ± 7.507p	197.411 ± 12.211b,c	366.735 ± 64.390 h,i,j
180	176.260 ± 25.180a,b	191.587 ± 17.559b,c	330.488 ± 29.146f,g,h.i	482.617 ± 38.463l,m
Cumulative concentration	3258.902 ± 514.506	3174.704 ± 216.356	3594.979 ± 306.901	3405.000 ± 352.695

DE1.- With extract and pulp. DE2.- With extract and without pulp. DE3.- Without extract and with pulp. DE4.- No extract and no pulp. The results are presented as the mean ± standard deviation of 3 repetitions ($n = 3$). TSP = μg GAE/g of lyophilized sample. Different letters indicate significant difference between the TSP values released during kinetics.

Table 7
Antioxidant capacity of fractions released from double emulsions.

Double emulsion	Release time (min)	Total Soluble Phenols (μg/mL)	DPPH (mmol TE/g)*	FRAP (mmol TE/g)*
DE1	120	643.919 ± 36.930a	22.564 ± 8.549a	30.298 ± 0.048d
	180	176.26 ± 25.180e	44.413 ± 22.069b	27.655 ± 0.804b
DE2	120	697.817667 ± 7.507a	168.663 ± 4.273d	39.718 ± 1.019e
	180	191.587 ± 17.559d,e	132.483 ± 6.548c	25.810 ± 0.585a,b
DE3	120	197.411 ± 12.211d	11.238 ± 0.695a	9.886 ± 0.558c
	180	330.488 ± 29.146c	18.767 ± 5.250a	11.710 ± 0.127c
DE4	120	366.735 ± 64.390c	18.219 ± 5.045a	24.104 ± 2.077a
	180	482.617667 ± 38.463b	12.137 ± 4.138a	25.667 ± 2.502a,b

DE1.- With extract and pulp. DE2.- With extract and without pulp. DE3.- Without extract and with pulp. DE4.- No extract and no pulp. The values are presented as the mean ± standard deviation of $n = 3$. Different letters indicate a statistically significant difference in the antioxidant capacity of double emulsions.

* milli moles equivalent of Trolox per gram of mango seed.

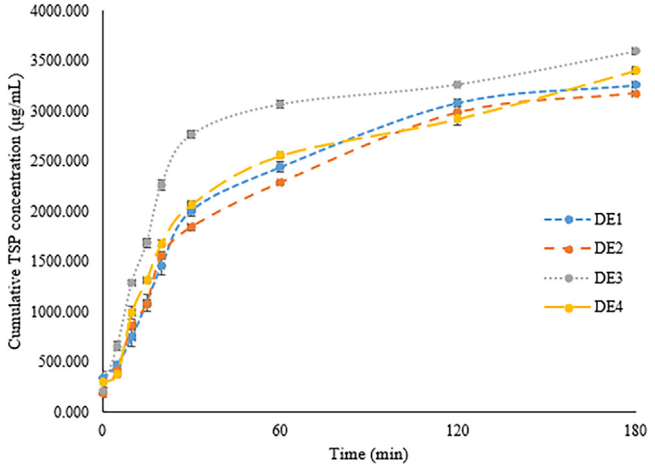

Fig. 1. Release profile of total soluble phenols accumulated in the 3 h of kinetics. DE1.- With extract and pulp. DE2.- With extract and without pulp. DE3.- Without extract and with pulp. DE4.- No extract and no pulp.

Table 8
Release rate (k) and percentage of release of double emulsions.

Double emulsion	Release rate k^*	% Liberation
DE1	41.673 ± 16.134b	19.94 ± 2.90a
DE2	35.350 ± 6.031c	22.00 ± 0.91a
DE3	75.690 ± 16.249a	5.50 ± 0.49c
DE4	76.375 ± 9.956a	10.72 ± 1.26b

DE1.- With extract and pulp. DE2.- With extract and without pulp. DE3.- Without extract and with pulp. DE4.- No extract and no pulp. The results are presented as the mean ± standard deviation of 3 repetitions ($n = 3$).

* k, release rate (μg/mL*min).

Fig. 1, where the behavior of zero order is observed in the four double emulsions (Aragón-Fernández et al., 2009; Fenner et al., 2006; Ritger & Peppas, 1987), from time 0 to the first 60 min of kinetics, a gradual release is observed in the four formulations, while in DE1 and DE2 from time 120 to 180 min the concentrations seem to be similar since, in the intervals between the penultimate and the last time, the release of PCs has no difference between them (ANOVA comparison of Fisher's LSD means $p = 0.05$), so the time of the greatest release of FST in DE1 and DE2 corresponds to 120 min. In zero-order kinetics, compounds tend to be released exponentially, when they reach a certain time, the compound reaches a certain concentration which is sufficient to exert some biological effect (Luginbuhl et al., 2017), so these last times were considered for the determination of the antioxidant capacity of the release profiles. The release rate (k) and the percentage of the release of each of the DEs were also calculated, the results are shown in Table 6. The DE2 presents a release of 35,350 ± 6031 μg/mL/min, being the lowest value of k in the formulations, however, it is the one that presents the highest percentage of the release of 22.00 ± 0.91%. Table 8

Once the release profile and characteristics of the double emulsions were established, the data obtained were processed according to the equations of the mathematical model of release kinetics; as previously mentioned, the time of 120 min is the latency time, the k values of the release profile of each emulsion were taken and according to the release behavior of the emulsions, it was defined as Fickian behavior (Ritger & Peppas, 1987), since the release process is governed by diffusion of the compound, so the value of the exponent is $n = 0.5$ (Aragón-Fernández et al., 2009; Bruschi, 2015). The linear regression adjustments of the Korsmeyer-Peppas equations (Korsmeyer et al., 1983) and zero-order equations (Bruschi, 2015; Luginbuhl et al., 2017) of the four formulations are presented in Table 9. Where it is verified that the mechanism of release is indeed by diffusion since the correlation value (R^2) corresponds to 0.9252 in all formulations and that the release of compounds remains constant as a function of time (Aragón-Fernández et al., 2009; Bruschi, 2015; Korsmeyer et al., 1983). Taking into account the TSP concentration value corresponding to the release time (M_i), the concentration value of zero time (M_∞), and latency time (l), we can suggest that DE1 and DE2 maintain a constant release until reaching the minimum concentration to exert antioxidant activity at 120 min, the regression value corresponds to 0.8151 for DE1 and 0.8126 for DE2, being the highest values of R^2 of the emulsions considering the latency time (l), so it is confirmed that this is the indicated time of the greater release of compounds that have antioxidant capacity. This release fraction, being the one with the highest antioxidant activity, was subjected to inhibition tests of topoisomerases and cyclooxygenases, also considering the different concentrations of MS extract used in the double emulsion and the by-product drink.

Table 9
Data adjustments in the Korsmeyer-Peppas equations and zero order of the four formulations of double emulsions.

Formulation	Mathematical model				
	Kosmeyer-Peppas			Order zero	
	kt^n	$f_1 = \frac{M_{(i-l)}}{M_\infty}$	$\frac{M_{(i-l)}}{M_\infty}$	kt	$1-(W_0 - W_1)$
DE1	0.9252	0.3867	0.8151	1	0.8151
DE2	0.9252	0.2427	0.8126	1	0.8126
DE3	0.9252	0.1874	0.6398	1	0.6398
DE4	0.9252	0.3034	0.7865	1	0.7865

DE1.- With extract and pulp. DE2.- With extract and without pulp. DE3.- Without extract and with pulp. DE4.- No extract and no pulp. The results are presented as linear regression (R^2) in the data adjustment of the release profiles of the double emulsions.

3.5. FTIR

The FTIR analysis of the MS extract is presented in Fig. 2A, as previously mentioned, the compound of interest in the present work is gallic acid, whose infrared (IR) spectrum has already been reported, showing characteristic signals in the wavenumbers 3407.05, 2960.40, 2931.51, 2865.75, 1639.70, 1509.58, 1420.99, 1153.78, 1097.08, 777.58, 663.54, 601.28, 462.89 cm^{-1} (Meenakshi et al., 2009; Patle et al., 2020), however, no such signals were found in the IR spectrum of the MS extract of the footprint zone 400–1500 cm^{-1} (Fig. 2B) and single bond zones 2500–4000 cm^{-1} (Fig. 2C) (Nandiyanto et al., 2019), this is because the extract does not only possess gallic acid but is a consortium of compounds in an aqueous solution, these compounds are in constant interaction, which makes it difficult to identify the intensity of a specific compound. In Fig. 3A, the IR spectra of both the MS extract and the release samples of the double emulsions at 0, 60, 120, and 180 min are appreciated, these spectra have similar behavior, however, no characteristic compounds of the extract were identified in the release fractions, however, as can be seen in Fig. 3B there are changes in the intensity of the signals of the release samples for the extract, so there is a release of compounds whose concentration varies between the release times of the optimal DE.

3.6. Biological tests

3.6.1. Topoisomerase inhibition assay

Table 10 shows the results of the antitopoisomerase activity of the extracts at different concentrations, DE (optimal and control), the formulation of the drink (optimal formulation, formulation with DE control and control formulation without emulsion), and the release fraction of the DE2 at 120 min of kinetics, since this emulsion and in that time of release was where both the greatest release of TSP (697.817667 ± 7507 μg GAE/mL) and the highest antioxidant capacity per DPPH (168,663 ± 4273 mMol TE/g). It is observed that the extract of

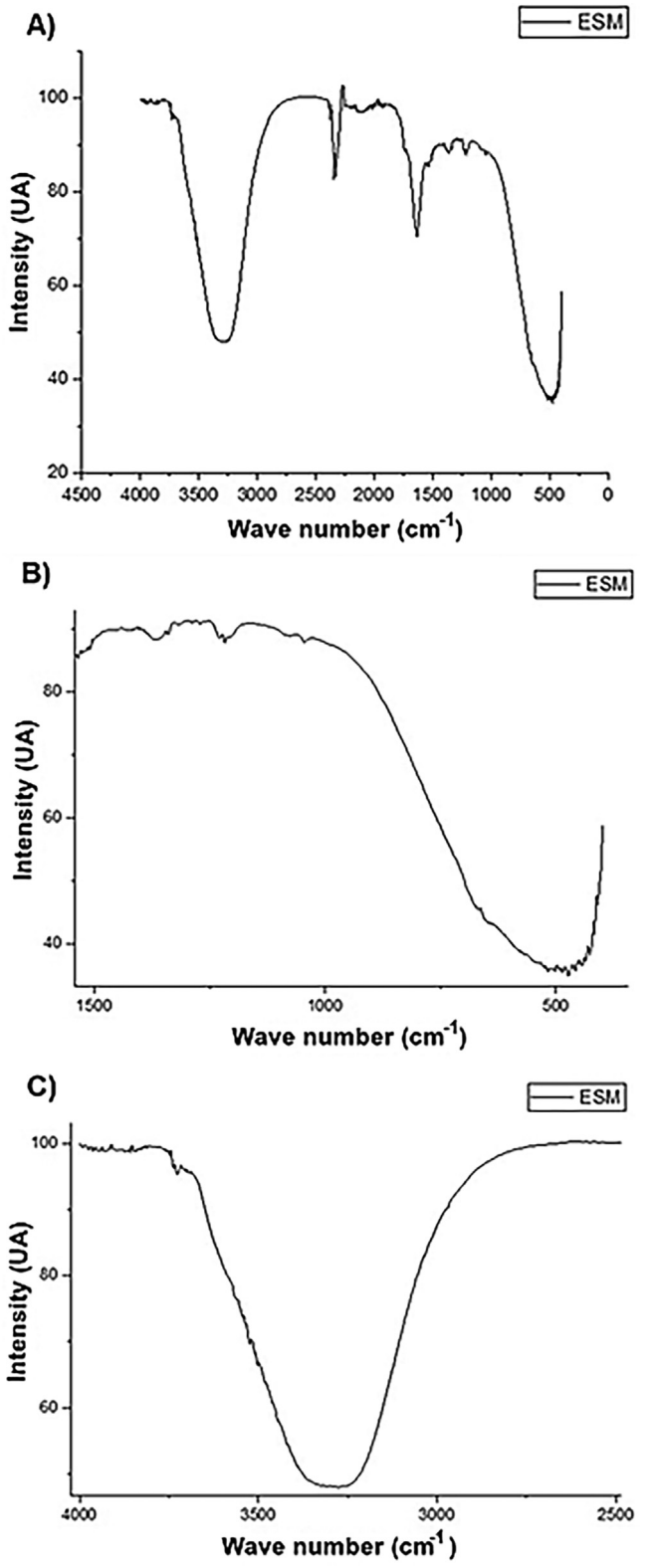

Fig. 2. Infrared spectrum, (A) Full spectrum mango seed extract; (B) Mango seed extract footprint area; C) Mango seed extract area of simple bonds.

MS at a concentration of 3 mg/mL did not affect the growth of the JN394 strain: however, the extract at a concentration of 13.34×10^{-3} mg/mL, which corresponds to the final concentration within the DE, showed a greater decrease in the growth of said strain (33% compared to the higher extract concentration of 3 mg/mL), however, the concentration of 0.5336×10^{-3} mg/mL, which is found in the formulation of the

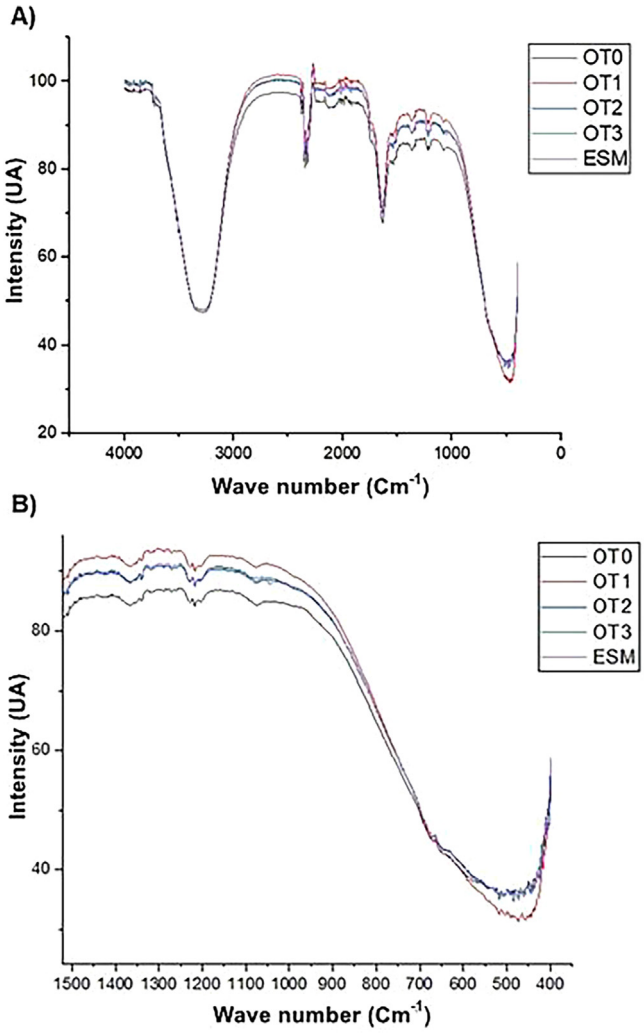

Fig. 3. Infrared spectrum of mango seed extract and double emulsion release fraction; (A) Full spectrum of mango seed extract and release fractions; (B) Footprint zone spectrum. OT0 – Optimal zero release time double emulsion. OT1 – Optimal double emulsion at 60 min of release. OT2 – Optimal double emulsion at 120 min of release. OT3 – Optimal double emulsion at 180 min of release.

Table 10
Percentage of inhibition or percentage of growth of S. cerevisiae strains JN394 and JN362a.

Treatment	Strain	
	JN394	JN362a
MS extract (3 mg/mL)	0	142↑
MS extract (13.34×10^{-3} mg/mL)	33↓	162↑
MS extract (0.5336×10^{-3} mg/mL)	6↓	120↑
Double emulsion (DE)	35↓	53↑
Double emulsion control (without extract)	7↓	15↑
Formulation with DE	31↓	24↑
Formulation with DE control (without extract)	21↓	39↑
Formulation control (without DE)	17↑	41↑
Release latency time 120 min DE2	29↑	128↑
CPT	48↓	51↑

DMSO was used as a control and all results were referred to it. ↓, decrease in growth in percentage; ↑, increase in growth in percentage.

optimal beverage, only decreased 6% of the growth compared to the 3 mg/mL extract. In this sense, the concentration of the extract in the emulsion (13.34×10^{-3}) reduced the growth of the JN394 strain by 69% compared to the camptothecin control. Optimal ED was the treatment with the greatest inhibitory effect on the growth of the JN304 strain with 35% (73% compared to camptothecin control); suggesting that during the incubation of the treatment with the strain in the YPD medium, a release of the MS extract could be presented, and considering that the extract at a concentration of 13.34×10^{-3} mg/mL is the final concentration within the DE, this concentration of the extract was the one that presented the greatest inhibition by the three concentrations of extract submitted to the test, it could be suggested in a preliminary way that the DE serves as a protection system for the extract and allows the release of the same. On the other hand, the DE control, that is, it does not have MS extract, presented only a 7% reduction in growth (15% compared to camptothecin control), so the components of the double emulsion do not have a relevant effect on the reduction of the growth of the strain. On the other hand, camptothecin control (CPT) inhibited 48% of the growth of the JN394 strain, being a Topoisomerase I poison (Razura-Carmona et al., 2019). The percentage obtained from the rest of the treatments was lower than that presented by the CPT control, so there is no antitopoisomerase effect for this assay. The JN362a strain has its repair mechanisms exacerbated, being a resistant strain, so the reduction in growth in this, would suggest an antimicrobial effect (Razura-Carmona et al., 2019; Sánchez-Burgos et al., 2013), contrary to the results obtained, in such a way they were tested against the JN394 strain, this has a deficiency in DNA repair and greater permeability due to the mutation in the Rad52 gene, this modification increases the sensitivity of the cell to the presence of some compounds (Nitiss & Wang, 1988; Ramirez-Mares et al., 2004). Being permeably sensitive, the inhibition in this strain would suggest an effect of antitopoisomerase activity, although the treatments submitted to the trial have mostly gallic acid, these do not affect said cell, since by their chemical nature they are hydrophilic compounds, although inhibition by PC towards topoisomerases has been reported, such is the case of what was reported by Sánchez-Burgos et al. (2013), where an extract of *Quercus resinosa* showed inhibition of 38.71 ± 1.92%, similarly, Cárdenas-Castro et al. (2020), reports an inhibition of 37.66 ± 5.70% in fractions subjected to fermentation for 6 h, in these fractions the presence of quercetin and capsaicin among other compounds was identified. The antitopoisomerase effect of phenolic compounds may be related to the concentration of these compounds, since in the case of extracts of *Q. resinosa* it had a concentration of 3.3 mg / mL (Sánchez-Burgos et al., 2013) and in the case of the fermentation fraction of 6 h, this had a concentration of 0.29 ± 0.02 mg GAE / mL of extract (Cárdenas-Castro et al., 2020); for the case of this study, the concentration that presented the greatest inhibition in the trial was 13.34×10^{-3} mg/mL of MS extract. In the case of the 120-minute release fraction of DE2, it only presented an inhibition of 29% (60% compared to camptothecin control); as previously mentioned, the compounds potentially released during kinetics can be mostly gallates (gallic acid and pentagaloylglucose), it has already been refueled that gallic acid can present an effect on the topo-I and II-DNA coupling thanks to the presence of pyrogallol in its structure because such activity is dependent on the redox mechanism (López-Lázaro et al., 2011). One of the proposed theories for the anticancer activity of CF, is the ability to modulate oxidative stress in cancer cells, related to the production of reactive oxygen species (ROS) (Mileo & Miccadei, 2016), this theory is supported by what was reported by Maruszewska and Tarasiuk (2019), where they tested different concentrations of gallic acid and ellagic acid in cancer cell line HL60, where they conclude that both compounds modulate the cellular level of ROS in a dose- and time-dependent manner.

3.6.2. Cyclooxygenase inhibition test

For the cyclooxygenase inhibition test, the different concentrations of the MS extract mentioned above, the release fraction at 120 min of the DE2, and the canola oil (used in the formulation of the DE) were subjected, the DE and the beverages were not analyzed, because the compounds both inside the emulsion and in the drink will be released at the intestinal level and only those compounds that can be absorbed could exert this effect on cyclooxygenases, the results are shown in Table 11. The fraction released in the latency time of 120 min showed an inhibition of 14% in COX-I and 18.97% in COX-II, which implies that the compounds released during kinetics at 120 min do not present an inhibitory effect against both enzymes. Contrary to this, the concentration of the MS extract at 13.34×10^{-3} mg/mL seems to have a selectivity towards COX-II, since it inhibited up to 33.40% of the activity of the enzyme, being markedly different from the concentration of 3 mg/mL, which does not suggest a selectivity towards any particular enzyme, since it inhibited 30.37% and 38.14% in COX-I and COX-II respectively. Although the difference may lie in the concentration of the extract, it is well known that the presence of some PCs extracted from plants can cause false positives, due to the partial denaturation of the enzyme or the interaction of these at the allosteric site, situations that may occur *in vitro* tests of crude plant extracts; in addition, some compounds can induce the inflammatory effect (Ramadwa et al., 2017). However, the fact that a compound does not present high anticycloxygenase activity in the assay does not mean that it does not have anti-inflammatory activity, since this effect can occur on the part of phenolic compounds thanks to the oxide-reduction capacity of free radicals (Lesjak et al., 2018).

Table 11
Percentage of inhibition of cyclooxygenases I and II.

Treatment	% Inhibition	
	COX-I	COX-II
Release latency time 120 min	14.00	18.97
MS Extract (3 mg/mL)	30.37	38.14
MS Extract (13.33×10^{-3} mg/mL)	2.08	33.40
MS Extract (0.5336×10^{-3} mg/mL)	20.55	27.87
Canola oil	29.45	41.30
Control	87.07	95.77

DMSO was used as a vehicle for this assay. COX-I, cyclooxygenase I; COX-II, cyclooxygenase II.

Another important point is the chemical nature, non-polar compounds have shown a greater effect on the inhibition of cyclooxygenase, as reported by Ramadwa et al. (2017), where they tested various extracts of *Funtumia africana* leaves, finding that the extracts obtained with chloroform inhibited up to 68.2% in COX-I and 59.1% in COX-II, also reports an inhibition of 45.9 and 50.4% for COX-I and COX-II respectively in extracts obtained with hexane, where they mention that the possible activity may be due to the separation of some fatty acids present in the leaves of *F. africana* and that these same could exert an effect on the isoforms of the enzyme. Related to the above, canola oil presented 41.30% inhibition in COX-II, this is because lipophilic compounds have greater ease to interact with the catalytic site of cyclooxygenases, since, near its active site, lipophilic amino acids are found, which facilitates the coupling of these compounds with the enzyme (Simmons et al., 2004).

4. Conclusions

The combination of factors used for the formulation of a double emulsion focusing on primary effects such as the release of total soluble phenols and percentage of release, allowed us to obtain a stable encapsulation system for compounds extracted from the mango seed. The release mechanism is governed by diffusion characteristic of a Fickian-type behavior and the release of the compounds remains constant as a function of time, until reaching the latency time of 120 min in which the compound reaches the concentration to exert antioxidant capacity. The addition of pulp to the continuous phase of the double emulsion system harms the release of compounds and the percentage of release thereof, causing a prompt release of phenolic compounds. Although there was no

favorable percentage of inhibition in the topoisomerase and cyclooxygenase assays in the release of latency time and extracts, the *in vitro* antioxidant capacity obtained from both the extract and the release fraction at 120 min of the double emulsion are highlighted, which can promote potential biological activity.

Ethical statement declaration

I declare that the manuscript entitled "**Formulation of double emulsions of mango seed extract (*Mangifera indica* L.) 'Ataulfo' incorporated into a mango by-product flour drink: release kinetics, antioxidant capacity and inhibition of cyclooxygenases**" submitted for Special Issue on, "Hydrocolloids for food", did not involve the participation of animals or people during its execution, so it was not required to adhere to the Helsinki protocol or any other related protocol.

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.

Data availability

Data will be made available on request.

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