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Study of the physicochemical properties of hass avocado oil encapsulated by complex coacervation

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ABSTRACT

This study aimed to encapsulate Hass avocado (*Persea americana* Mill. cv. Hass) oil through complex coacervation using a gelatin:sodium alginate (NaAlg) mixture and assess the physicochemical properties of the resulting encapsulates. Turbidity, z-potential, and efficiency analyses indicated that the optimal conditions for oil encapsulation were a pH value of 4.0 and a gelatin:NaAlg solids ratio of 3:1. Coacervates formed with lower concentrations of solids exhibited superior morphologies compared to those obtained with higher concentrations. Encapsulation yield ranged from 34.7 to 86.8%, and the encapsulation efficiency ranged between 57.4 and 92.2%. Furthermore, the samples demonstrated low water activity (ranging from 0.01 to 0.36) and moisture content (2.18–9.25%), showcasing the high microbiological stability of the powder. Overall, the encapsulates displayed low hygroscopicity (<15%), suggesting enhanced technological stability for the product over time. Additionally, they exhibited good thermal stability, with degradation initiation temperatures surpassing 190 °C. This study highlights the technological potential of complex coacervation as a method to encapsulate Hass avocado oil. This approach offers a promising and viable alternative to use this material in various industrial applications.

1. Introduction

Avocado (*Persea americana* Mill.) is a tropical and subtropical crop belonging to the Lauraceae family cultivated in more than 60 countries (FAO, 2023; Yahia & Woolf, 2011). According to the FAO, in 2021, the world production of avocado was 8.81 Mt, with Mexico (27.7%), Colombia (11.1%), Peru (8.8%), Indonesia (7.6%), and the Dominican Republic (7.2%) as the main producers with a participation of more than 50% of total global production (FAO, 2023). Among the various commercial varieties of avocado, the Hass variety stands out as the most relevant worldwide, covering 95% of the US market and 65% of the European market (Ford, Spagnuolo, Kraft, & Bauer, 2023; Naamani, 2007). Its great acceptance and importance are due to distinctive characteristics such as its thick skin, high productivity, and sensory and nutritional qualities, facilitating its transport, storage, and commercialization (Araújo, Rodriguez-Jasso, Ruiz, Pintado, & Aguilar, 2018). Hass avocado pulp represents an important source of fiber, vitamin K, folate, pantothenic acid, minerals (mainly copper and potassium), bioactive compounds (primarily phenolic compounds, carotenes, and phytosterols), and lipids (Ford et al., 2023; Viera et al., 2023). Despite the growth in Hass avocado production and its exceptional nutritional quality, certain factors, such as mechanical damage, dry matter content, and fruit color, among others, have the potential to cause the rejection of the fruit for export, contributing to the increase in postharvest losses (Ramírez-Gil, López, & Henao-Rojas, 2020). Studies such as the one of (Benítez Franco et al., 2021)Benítes et al. (2021) have reported that postharvest losses during Hass avocado export can reach up to 60%. This high loss rate highlights the need to implement strategies to mitigate these losses or repurpose the resulting biomass effectively.

Oil extraction from avocado fruits for industrial use emerges as a fundamental strategy to mitigate postharvest losses. Studies have estimated that the lipid fraction of Hass avocado fruit can amount to 31% on

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a wet basis; its composition is mainly represented by monounsaturated (mainly oleic acid) and polyunsaturated (primarily linolenic acid) fatty acids with 85% of the total lipid content and a ratio of unsaturated to saturated fatty acids of 6:1 (Alkaltham et al., 2021; Ford et al., 2023). However, the oil can deteriorate during storage and handling due to factors such as light and oxygen exposure, high temperatures, and pH changes, causing lipid oxidation. This deterioration negatively impacts oil quality and shelf life, limiting its viability for industrial purposes (Romero-Hernandez et al., 2021).

The microencapsulation of oils and lipophilic compounds has been used as an alternative to extend the useful life of ingredients or active ingredients (Tavares & Noreña, 2020). Microencapsulation techniques can be classified as chemical, physical, and physicochemical, and their use depends on the material to be encapsulated and the final properties desired to be obtained from the microcapsules (Choudhury, Meghwal, & Das, 2021; Ozkan, Franco, De Marco, Xiao, & Capanoglu, 2019). Complex coacervation is a microencapsulation method that involves electrostatic interactions between two biopolymers of opposite charges, leading to phase separation and, consequently, the formation of coacervates or complex polymers with neutral charges (Vergara et al., 2023; Yang, Gao, Hu, Li, & Sun, 2015). This method has great potential for encapsulating compounds of a lipophilic nature because it is a straightforward process with a high active ingredient loading capacity. Different authors have reported the encapsulation of fish (Yang et al., 2023), buriti palm fruit (Lemos, Mariano Marfil, & Nicoletti, 2017), pomegranate (Costa, Antunes-Gaspar, Calado, Tonon, & Guedes-Torres 2022; Costa et al., 2020), and poppy seed (Yang et al., 2015) oils using the complex coacervation method. These studies demonstrated the potential of this methodology, as evidenced by achieving high encapsulation efficiencies, favorable physicochemical properties, and good stability against degradation in the encapsulated materials. However, the success of microencapsulation using this method depends on several key factors. These include the selection of polymers or wall materials, the ratio between these polymers, the pH of the system, and the ratio between the polymers and the active ingredient. Each encapsulation system and active compound requires a unique combination of these factors for optimal results. Furthermore, compared to other encapsulation methods, complex coacervation is highly compatible with sensitive core materials, such as oils with high fatty acid content, as it does not require organic solvents or extreme reaction conditions to produce the encapsulates (Eratte, Dowling, Barrow, & Adhikari, 2018).

Regarding Hass avocado oil, to date, only the use of encapsulation by spray drying has been reported using different wall materials or the mixture of various of these, such as maltodextrin and taro starch modified with octenyl succinic acid (Romero-Hernandez et al., 2021; Sotelo-Bautista, Bello-Perez, Gonzalez-Soto, Yañez-Fernandez, & Alvarez-Ramirez, 2020), whey protein and maltodextrin (Bae & Lee, 2008), Gum Arabic/HI-CAP®100 starch/phosphorylated waxy corn starch (Espinosa-Solis, García-Tejeda, Portilla-Rivera, Chávez-Murillo, & Barrera-Figueroa, 2022), and maltodextrin/HI-CAP®100 starch (Chimsook, 2017). To our knowledge, the application of the complex coacervation method as an alternative for microencapsulation of Hass avocado oil has not been explored. Therefore, it is necessary to study the coacervation process factors, such as the type of encapsulating material, the proportions of these materials, the optimal pH values, and the relationships between the encapsulating materials and avocado oil, to establish suitable process parameters. These parameters are essential for obtaining microcapsules with technological functionality and advancing the implementation of this method as an alternative for preserving and commercializing Hass avocado oil. In this context, this study aims to establish parameters such as the ratio between polymers, the ratio between polymers and oil, and the pH to encapsulate Hass avocado oil by complex coacervation using a gelatin/sodium alginate system and evaluate how these parameters affect the physicochemical properties of the encapsulates.

2. Materials and methods

2.1. Materials

Hass avocado pulp oil (Oleo Hass, Smart Cooking, Medellín, Colombia) was purchased at a commercial store. Type B gelatin (≥270 Bloom, pH 4.2–6.5 and viscosity ≥34 mP at 6.67%; moisture content (MC) 8–12%) (CIMPA SAS, Bogotá, Colombia) and sodium alginate (NaAlg; pH: 6.0–8.0; MC <15%; melting point >200 °C) (CIACOMEQ SAS, Bogotá, Colombia) were food grade. All other reagents used in the analyzes were of analytical grade.

2.2. Oil characterization

The oil was characterized in terms of moisture content (Method 926.12), density (Method 920.212), iodine value (Method 993.20), saponification index (Method 920.160), and peroxide value (PV) (Method 965.33), according to the Association of Official Analytical Chemists (AOAC, 2000). The color was assessed using a Konica Minolta CM-5 spectrometer (Konica Minolta, Inc., Japan) on the CIELAB coordinate scale according to the methodology reported by Lemos et al. (2017). Secondary oxidation was evaluated by determining the anisidine index (ICONTEC, 2021) and carrying out a thiobarbituric acid reactive substances assay (TBARS) (Takeungwongtrakul & Benjakul, 2013).

2.3. Effect of pH, ratio, and concentration of solids on coacervation performance

The NaAlg and gelatin were dissolved in distilled water at different concentrations (0.05, 0.1, and 0.5% w/v) with magnetic stirring at room temperature for NaAlg and at 40 ± 2 °C for gelatin. The gelatin and NaAlg solutions were mixed in various proportions (1:1, 1:2, 2:1, 1:3, and 3:1 v:v) to assess the influence of the material ratio and pH on coacervate formation. Furthermore, the formation of coacervates was evaluated for each mixture obtained at different ratios, with variations in pH values (2.0, 3.0, 4.0, and 5.0). pH adjustment was performed using aqueous solutions of acetic acid (0.1 and 0.5 M) and sodium hydroxide (0.1 M). The resulting mixtures were shaken using an orbital shaker (IKA KS 4000, China) at 200 rpm for 20 min. Subsequently, aliquots (2 mL) of the samples were taken, and their absorbance was measured at 590 nm (Freitas, Albano, & Telis, 2017).

For the analysis of the total surface charge of the polymers at different pH values, solutions of each of the materials were prepared at a concentration of 0.05% (w/w), adjusting the pH to different values (3.0, 4.0, 5.0, 6.0, and 7.0). After this, the Zeta potential values were determined using a Zetasizer Nano Series ZEN 3600 (Malvern Instruments Ltd., United Kingdom). The measurements were carried out at room temperature using the methodology described by Rousi, Malhiac, Fatouros, and Paraskevopoulou (2019).

Coacervation yield for the samples that reported greater turbidity was evaluated according to Rousi et al. (2019). The samples were centrifuged at 4000 rpm at 4 °C for 40 min. The upper aqueous layer was discarded, and the lower one was dried at 60 ± 2 °C until constant weight. The yield was considered to be the ratio between the initial solids mass and the dehydrated sample mass.

2.4. Complex coacervates formation

The coacervates were prepared according to the method reported by Lemos et al. (2017) as follows. Each polymer (gelatin and NaAlg) was dissolved in water and heated to a temperature of 50 ± 2 °C (Table 1). Then, the oil was added to the gelatin solution and mixed at 15,000 rpm for 3 min using a homogenizer (Ultraturrax, IKA model T25 Digital, Germany). The resulting mixture was kept under constant stirring at 180 rpm and a temperature of 50 ± 2 °C using a stirring plate (IKA,

Table 1
Encapsulation efficiency and encapsulation yield of Hass avocado oil encapsulates by complex coacervation.

Treatment	Gelatin: NaAlg (ratio)	Total solids (%)	Avocado oil (%, w/w) [a]	Encapsulation Efficiency (*EE*, %)	Encapsulation Yield (*EY*, %)
T1	3:1	2.0	2.0 (100)	57.4 ± 1.2^{a}	62.0 ± 4.7^{b}
T2	3:1	1.6	1.2 (75)	71.3 ± 0.5^{b}	81.8 ± 5.0^{a}
T3	3:1	2.0	1.0 (50)	87.5 ± 0.1^{c}	57.5 ± 4.2^{bc}
T4	3:1	3.0	1.2 (40)	79.9 ± 0.9^{d}	48.5 ± 4.5^{cd}
T5	3:1	4.4	3.3 (75)	81.1 ± 1.7^{d}	36.6 ± 4.0^{de}
T6	3:1	4.0	2.0 (50)	86.6 ± 1.3^{c}	34.7 ± 3.0^{e}
T7	3:1	4.0	4.0 (100)	80.6 ± 1.4^{d}	58.5 ± 2.3^{b}
T8	3:1	3.0	3.3 (110)	72.3 ± 0.4^{b}	86.8 ± 1.3^{a}
T9	3:1	3.0	2.3 (75)	91.5 ± 0.3^{e}	67.9 ± 1.5^{b}
T10	3:1	3.0	2.3 (75)	92.1 ± 0.4^{e}	64.8 ± 6.2^{b}
T11	3:1	3.0	2.3 (75)	91.5 ± 1.5^{e}	63.3 ± 5.1^{b}

[a] Percentage per 100 g of solution. GB: gelatin B; NaAlg: Sodium alginate. Values are expressed as means ± standard deviation of at least three repetitions. Different letters in the same column indicate significant differences between samples ($p < 0.05$).

model C-MAG HS7, Germany). The NaAlg solution was added dropwise to the resulting mixture up to a final volume of 0.3 L. The pH of the final solution was adjusted to 4.0 ± 0.3 with glacial acetic acid. The samples were allowed to cool with constant stirring to a temperature of 10 °C using a water bath and stored at 4 ± 1 °C protected from light for 12 h. Subsequently, the supernatant was discarded, while the precipitate was transferred to aluminum trays and frozen at −80 °C for 24 h (ultra--freezer GV039P, Kaltis International Co., Ltd, Taiwan). The frozen samples were dehydrated by freeze-drying (BK-FD12PT, Biobase, China) at a temperature of −40 °C for 72 h. The samples were ground with a mortar and passed through a sieve (mesh 20 or 850 μm). All samples were stored, protected from light, and refrigerated until analysis. All treatments are shown in Table 1.

2.5. Powder characterization

2.5.1. Morphology

The morphological analyses were conducted using a scanning electron microscope (S–3400 N, HITACHI, Germany). The samples were fixed to stubs with double-sided carbon tape and coated with a layer of gold using a rotary-pump coating system (Q150R, Quorum, UK). Determination was performed with an accelerating voltage of 15 kV under a vacuum pressure of 40 Pa. Micrographs were captured at various magnification levels.

2.5.2. Yield and efficiency of encapsulation

To determine the content of surface oil (*SO*), a power sample of 0.1 g was weighed, mixed with 10 mL of hexane, and shaken for 1 min. The resulting organic phase was filtered through Whatman paper (Grade 4), and the filtrate was deposited in a pre-weighed porcelain and evaporated, first at room temperature and then in an oven at 60 °C until reaching constant weight.

The total oil (*TO*) from the capsules was determined according to Rios-Mera et al. (2019). A power sample of 0.1 g was mixed with 20 mL of 3% w/v sodium citrate. The mixture was stirred for 30 min at 250 rpm at a temperature of 60 °C. Subsequently, 10 mL of chloroform, 20 mL of methanol, and 8 mL of distilled water were added. The solution was stirred for an additional hour at room temperature. Then, 10 mL of chloroform and 10 mL of 1.5% sodium sulfate were introduced and stirred for 10 min. The mixture was left undisturbed until the phases separated. After separation, the supernatant (methanolic phase) was discarded, and the lower phase was filtered through Whatman paper (Grade 4). The resulting filtrate was transferred to a pre-weighed capsule and initially evaporated in a water bath at 30 °C, followed by further evaporation in an oven at 60 °C. Encapsulation efficiency (*EE*) and encapsulation yield (*EY*) were calculated using Eqs. (1) and (2) considering the oil initially present in the emulsion (*IO*), *SO*, and *TO* contained in the dehydrated capsules (Ferreira & Nicoletti, 2021)

$$\textit{Encapsulation efficiency}\ (EE;\%) = [(TO - SO)\ /\ TO] \times 100 \quad (1)$$

$$\textit{Encapsulation Yiel}\ (EY;\%) = (TO\ /\ IO) \times 100 \quad (2)$$

where *TO* is total oil in the microcapsules (g), *SO* is the oil on the surface of the capsules (g), and *IO* is the oil initially loaded (g).

2.5.3. Moisture content and water activity

The moisture content (MC) was determined according to Method 925.10 of the AOAC (2000). Water activity (a_w) was measured using a dew point hygrometer (Aqualab, Decagon Devices, USA).

2.5.4. Hygroscopicity and solubility

The hygroscopicity of the samples was calculated according to the methodology described by Daza et al. (2016). Thus, 2 g of each sample was weighed in a pre-weighed capsule and then stored in a plastic desiccator containing a saturated Na_2SO_4 solution (81% relative humidity) at room temperature. The samples were stored for one week and weighed. Hygroscopicity was expressed as g of water absorbed per 100 g of dry solid.

The solubility of the encapsulated samples was assessed by subjecting an encapsulated sample of 0.5 g, mixing it with 50 mL of distilled water, and homogenizing it at 100 rpm for 30 min using an orbital shaker (Shaker IKA KS 4000). Subsequently, the sample was centrifuged at 3500 rpm for 5 min (Z 326 K, HERMLE Labortechnik GmbH, Germany), and an aliquot of 25 mL of the supernatant was transferred to a pre-weighed porcelain capsule. The capsule was then placed in an oven at a temperature of 105 °C until complete water evaporation. Solubility was expressed as a percentage (%).

2.5.5. Fourier-transform infrared spectroscopy (FTIR)

FTIR spectra of the samples were acquired using a Fourier-transform infrared spectroscopy instrument (Nicolet 380, Thermo Scientific Inc., USA). The measurements were taken across the range of 4000 to 500 cm^{-1}, with a resolution of 4 cm^{-1} and 40 scans at room temperature (~20.4 °C). Before the analysis, pellets were prepared by mixing the sample with potassium bromide (KBr).

2.5.6. Thermal analysis

The thermal degradation of samples was assessed using a thermogravimetric analyzer (SDT 2960, TA instruments, Japan) according to the method described by Muhoza et al. (2019) with some modifications. Approximately 5–10 mg of the samples were placed in porcelain pans. Then, the samples were heated in a temperature range of 20–600 °C at a constant rate of 20 °C/min under a stream of nitrogen. The results were expressed as the weight loss (WL), thermal degradation temperature (T_{max}), and onset temperature (T_{onset}) of the degradation peak.

2.6. Statistical analysis

The results were reported as mean and standard deviation for at least three replicates. Analysis of variance (ANOVA) at a 95% confidence level was used to determine statistically significant differences. Multiple range tests, using the Tukey-Kramer range test, were utilized to determine which mean results differed significantly from others.

3. Results and discussion

3.1. Oil characterization

The physicochemical properties of Hass avocado oil are shown in Table 2. The oil presented adequate quality characteristics, considering

Table 2
Physicochemical characteristics of Hass avocado oil.

Parameter	Value ± SD
Moisture (%)	0.17 ± 0.01
Apparent density (ρ; g mL^{-1})	0.911 ± 0.001
Peroxide value (PV; meq. O2 kg^{-1})	4.86 ± 0.15
Saponification value (SV; mg KOH kg of oil^{-1})	200 ± 3
Iodine (I2) value (g 100 g oil^{-1})	81.2 ± 2.2
P-Anisidine Value	4.74 ± 0.42
TBARS (mg MDA kg $aceite^{-1}$)	1.73
Color parameters	
L	51.2
*a**	−1.1
*b**	27.5

*a**: CIELab coordinates (redness/greenness); *b**: CIELab coordinates (yellowness/blueness); *L**: CIELab coordinates (Luminosity). Values are expressed as means ± standard deviation of at least three repetitions.

the recommendations of Codex Alimentarius with regard to the MC (<0.2%) and the PV (15 meq O_2 kg^{-1} oil) (Brzezińska et al., 2021; Codex Alimentarius FAO-WHO, 1999; Negash, Amare, Bitew, & Dagne, 2019). The iodine index value was notably lower compared to the previously reported values for avocado oil through aqueous extraction (154 ± 4 g/100 g), hexane extraction (96.9 ± 0.63 g/100 g), and cold-pressing (139 ± 1 g/100 g), suggesting that the oil obtained in this study exhibits a lower susceptibility to oxidation. Nevertheless, the saponification value remained consistent with the reported values for these same materials (Li et al., 2019). Furthermore, the *p*-anisidine value was found to be below the recommended limit value for edible oils (<20 p-AV), aligning with findings from avocado oil obtained through conventional extraction methods, subcritical CO_2 extraction, and ultrasound-assisted aqueous extraction (Tan, Chong, Hamzah, & Ghazali, 2018).

3.2. pH effect on zeta potential, turbidity, and efficiency

The relationship between the pH and the Zeta potential of the wall materials under analysis is shown in Fig. 1A. In the pH range studied (from 3 to 7), NaAlg was negatively charged. Meanwhile, gelatin, an amphoteric protein, exhibited a positive charge region at pH values lower than its determined isoelectric point (pI = 5.4) and a negative charge above its pI. In this work, the pI value obtained for gelatin was consistent with what was previously reported by Raja and Fathima (2018). Based on this analysis, it is possible to suggest that at pH values of 3, 4, and even 5, an interaction between the polymers or wall materials may be observed due to their opposite charges. The previous behavior was confirmed by analyzing the interaction of the wall materials at different pH values (pH 2–5) and a different ratio, where the interaction of the polymers was observed through an increase in the turbidity of the system (Fig. 1B–D). In terms of the mass ratio of wall materials, the system with a higher amount of NaAlg compared to gelatin reduced the turbidity of the system. This can be attributed to the viscosity of the solution due to the high concentrations of NaAlg associated with the solubilization of the polymer and repulsion between negatively charged polymer chains, which hinder the interaction between the wall materials and the subsequent formation of coacervates (Ujwala & Mangal, 2009). Conversely, the opposite was observed when the proportion of gelatin was increased relative to NaAlg. In all cases, the gelatin/NaAlg ratio with the highest turbidity was the 3:1 ratio ($p < 0.05$). Furthermore, it was noted that the turbidity of the mixture decreased at extreme pH values (2 or 5), while at pH 4, turbidity was at its maximum since the opposite charge of the wall materials reached an electrical equivalence ($p < 0.05$) (Hernández-Nava, López-Malo, Palou, Ramírez-Corona, & Jiménez-Munguía, 2019).

The efficiency of the interaction of the wall materials was assessed by the coacervation yield in the system, confirming what was observed in the turbidity analysis. The coacervation yield values were higher in the mixtures where the mass ratio was higher for gelatin compared to its counterpart, even if a wall material with a 4:1 or 1:4 ratio is used (Fig. 1E). This result corroborates the findings stated by various authors, which have consistently demonstrated that the highest coacervation efficiency is achieved when gelatin is present in the highest proportion concerning NaAlg (Lau, Jeong, & Kim, 2018; Ujwala & Mangal, 2009).

3.3. Morphological characterization

The morphologies of the wet microcapsules obtained in this study are shown in Fig. 2. The formation of microcapsules could be observed in treatments T1 to T4 (Fig. 2T1 to 2T4), showing a spherical morphology. These results demonstrated that the best ratios between the wall material and avocado oil for encapsulation were 1:1, 1.3:1, 2:1, and 2.5:1. The formation of spherical morphologies is associated with the kinetic

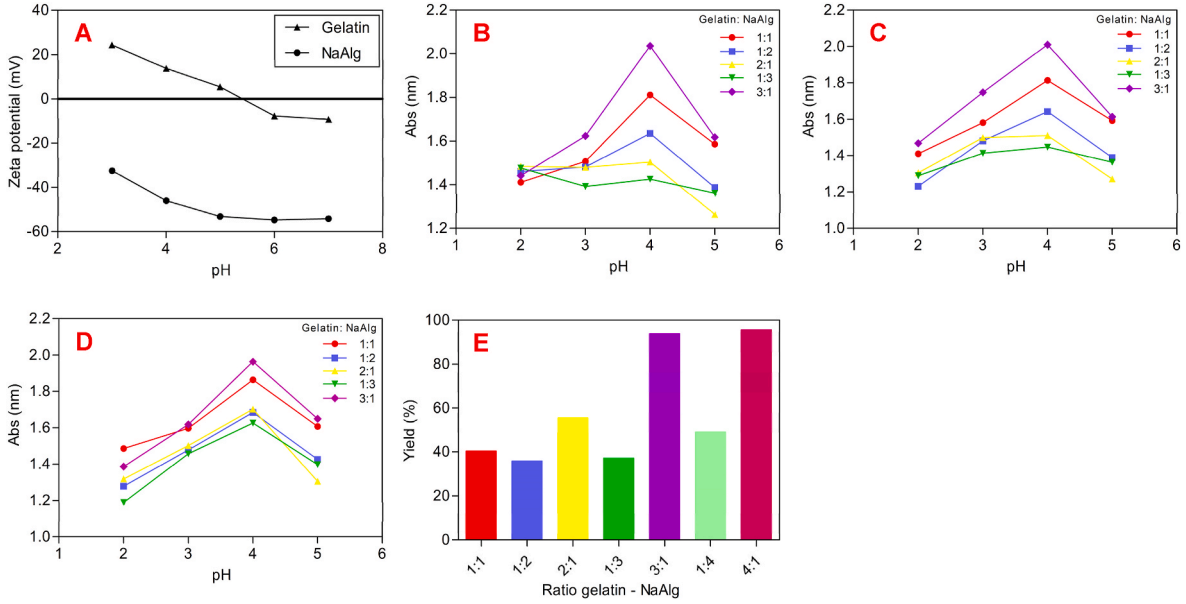

Fig. 1. Effect of pH on the Z-potential of gelatin and sodium alginate (NaAlg) (A), and on the turbidity of polymer mixtures at concentrations of 0.05% (B), 0.1% (C) and 0.5% (D) w/v, and effect of the polymer ratio on the coacervate yield (E).

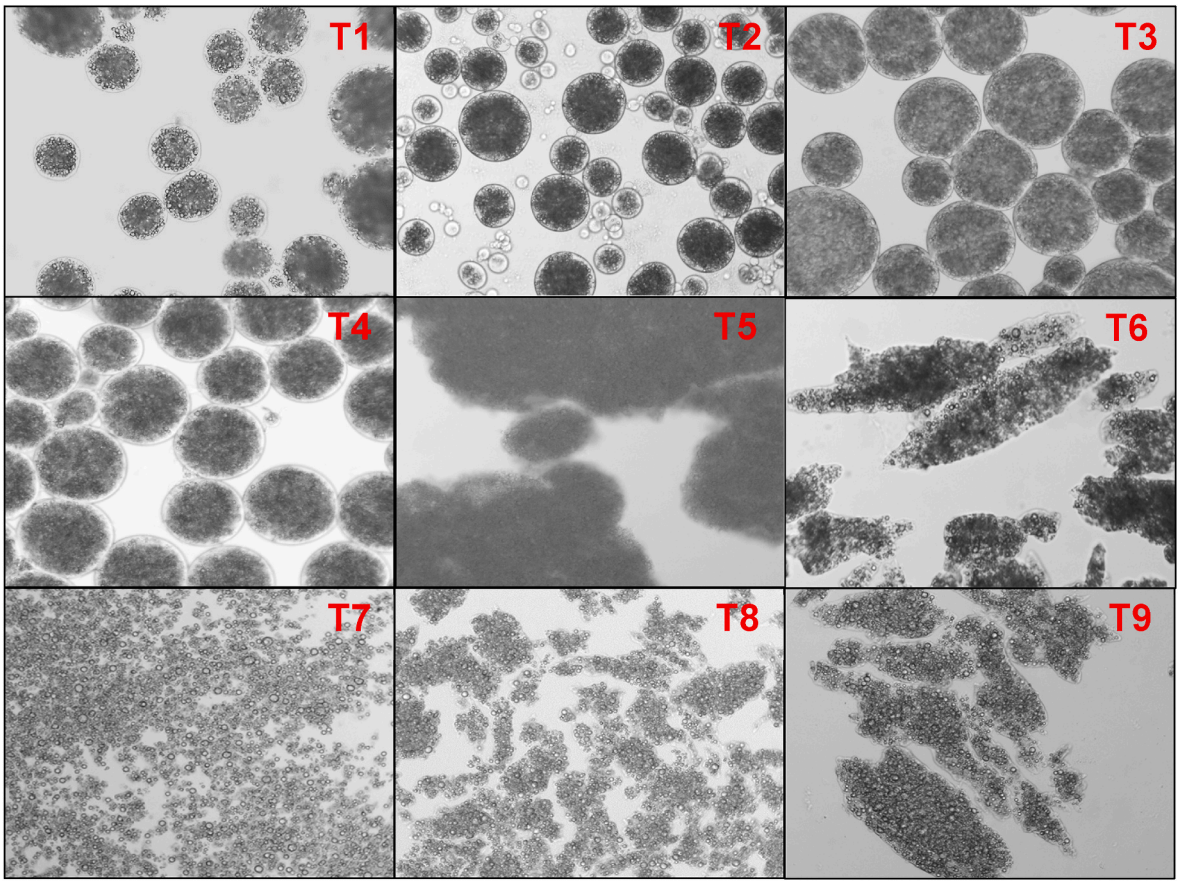

Fig. 2. Optical microscopy morphology of wet Hass avocado oil microcapsules produced by complex coacervation.

and thermodynamic stability of the system in the coacervate formation process. On the other hand, large aggregates were formed without defined geometries in treatments T5 to T11, along with deficient formation of the matrix or membrane. This poor encapsulation formation may be related to the high percentages of polymers and oil used in sample formulation. Similar observations were made for ginger oil coacervates using gum Arabic and gelatin as wall materials (Ferreira & Nicoletti, 2021). The authors observed that in the coacervates that showed deficient formation of the membrane or polymeric matrix (aggregate formation), an interaction could have occurred between gelatin segments with opposite charges due to the unfolding of the molecule during the acidification process of the previous medium prior to the mixture with gum Arabic leading to the formation of the aggregates.

The morphology of the wet encapsulates influenced the morphology of the encapsulates obtained after lyophilization (Fig. 3). Treatments T1 to T4 displayed a spherical morphology but with a rough surface and varying sizes (Fig. 3T1 to 3T4). Similar observations were reported in the coacervation of blackberry juice using pea protein concentrate with various gums as the wall material and subsequent freeze-drying (Vergara et al., 2023). As expected, treatments T5 to T11 presented particle aggregation and irregular morphologies. Previous studies have encapsulated avocado oil using the spray drying method with various wall materials and particles with a morphology similar to that observed in treatments T1 to T4 of this study (Espinosa-Solis et al., 2022; Romero-Hernandez et al., 2021; Sotelo-Bautista et al., 2020). This result suggests that the complex coacervation method has the potential for encapsulating avocado oil.

3.4. Encapsulation yield and efficiency

The coacervates assessed in this study exhibited *EY* values ranging from 34.7 to 86.8% ($p < 0.05$) (Table 1). Notably, T4, T5, and T6 displayed *EY* values below 50%, while the other treatments consistently showed *EY* values above 55%. Overall, coacervates with low or intermediate concentrations of avocado oil combined with high polymer concentrations yielded the lowest *EY*. Similar results were obtained by Wang, Yang, Cao, Zhao, and Wang. (2016) when encapsulating ginger essential oil through complex coacervation using the same wall materials as in the current study. The authors found that the optimal ratio between the core and wall materials, which provided the highest *EY*, was 1:1, with a decrease in efficiency observed as the amount of oil or wall materials increased independently. However, the mass ratio between the wall materials can also influence encapsulation efficiency. Previously, Espinosa-Solis et al. (2022) encapsulated avocado oil using the spray drying method. The emulsion was previously stabilized using high-speed homogenization and a combination of high-speed homogenization and ultrasound. The encapsulation yield obtained with the first method was 44.7%, while the samples stabilized with ultrasound ranged between 71.1 and 95.4%. Although the *EY* results observed in the current work are lower than those obtained in the encapsulation of other oils by complex coacervation where different wall materials are used (Ferreira & Nicoletti, 2021), treatments T1, T2, T8, and T9 demonstrated potential for efficient encapsulation of avocado oil through complex coacervation.

The *EE* values of the coacervates obtained in this study ranged from 57.4 to 92.2%, with statistical differences observed among the samples ($p < 0.05$) (Table 1). The oil concentration was observed to increase relative to the mass of the wall materials, and the *EE* showed a tendency to decrease. On the other hand, low or intermediate oil concentrations

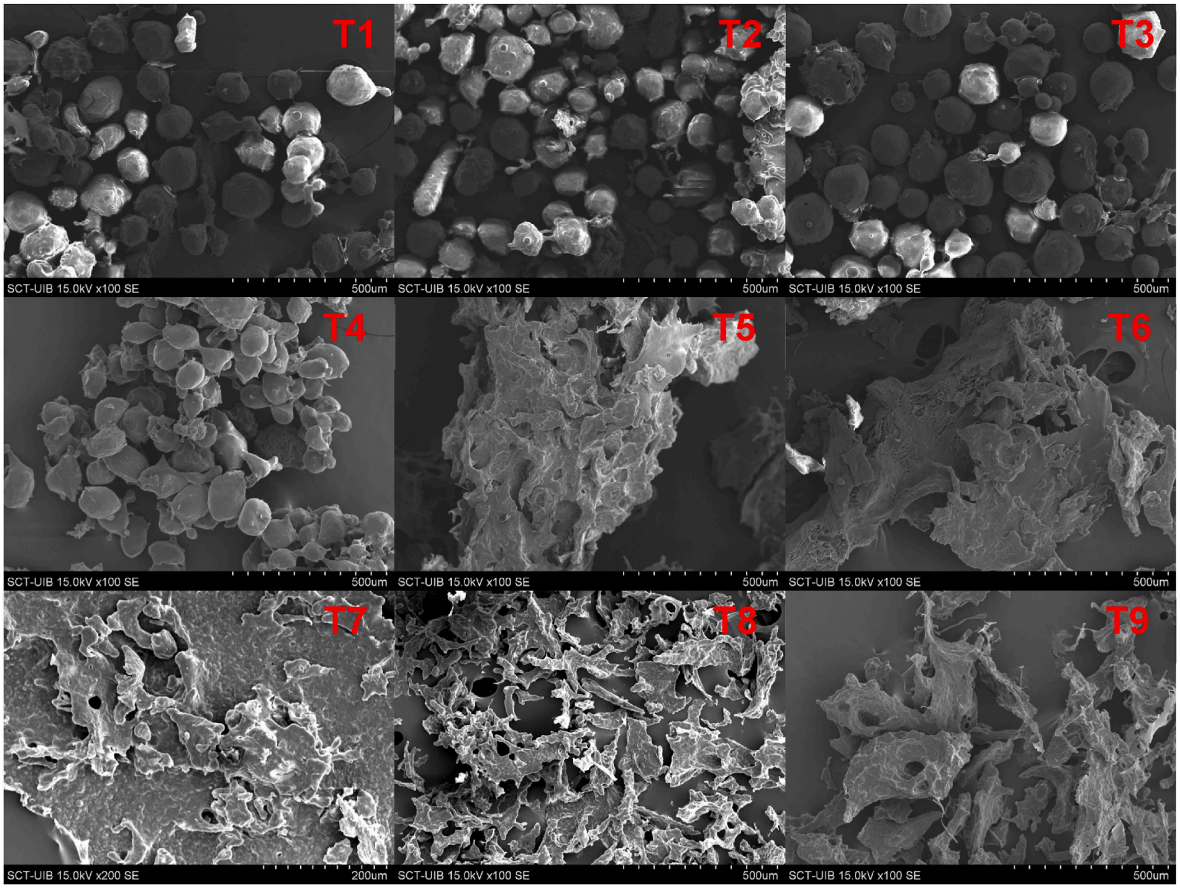

Fig. 3. Scanning electron micrographs showing morphology of the Hass avocado oil microcapsules produced by complex coacervation at magnifications of 100× (except T7) and 200 x (T7).

allowed the production of coacervates with higher efficiency in encapsulating avocado oil. A similar trend was observed in complex coacervation encapsulation accompanied by spray drying of propolis using commercial gelatin, sodium alginate, and maltodextrin as wall materials (Sukri, Putri, Mahani, & Nurhadi, 2023). The authors attribute this phenomenon to the inability of the wall materials to provide a matrix for

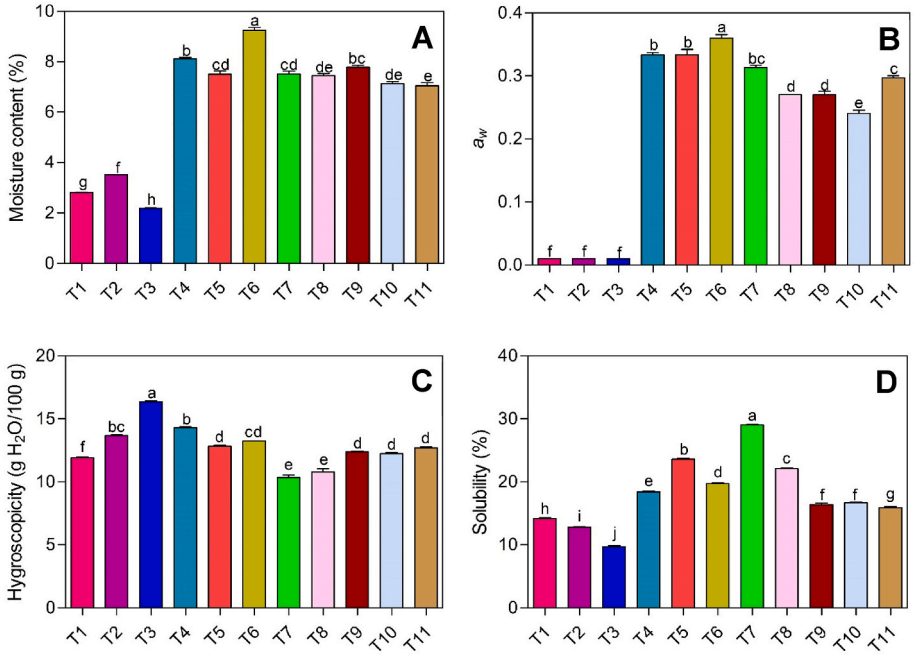

Fig. 4. Moisture content (%) (A), water activity (B), hygroscopicity (C) and solubility (%) of Hass avocado oil microcapsules produced by complex coacervation.

the core material due to inefficient interaction between them, allowing the oil to migrate to the surface of the microcapsule. The above could lead to a decrease in the protection of the active material due to interaction with the environment (oxygen and MC, among others). Additionally, a higher amount of free oil on the microcapsule surface can negatively affect properties such as dispersion, fluidity, particle size, and structural stability (Wangsuntornpakdee, Sae-tan, Iwamoto, & Peanparkdee, 2023). The *EE* obtained for the treatments evaluated in this work was higher than the results reported for avocado oil encapsulated by the spray drying method using native Taro starch (29.63%) and octenyl succinic anhydride (OSA) starch (40.01%) as wall material (Romero-Hernandez et al., 2021). The differences observed in the efficiencies obtained by these encapsulation methods are due to the high stability provided by complex coacervation compared to the instability offered by emulsion using starches and oils.

3.5. Moisture content and water activity

The MC and a_w of the coacervates obtained in this study are shown in Fig. 4A and B. Overall, all samples exhibited an MC below 10%. This low value is associated with preserving the physical quality of the encapsulates, including aspects such as flowability, dispersion, and agglomeration prevention, and providing greater storage stability to the product (Daza et al., 2016; Hazlett, Schmidmeier, & O'Mahony, 2021). There is no prior evidence of encapsulating Hass avocado oil via complex coacervation; however, several authors have explored the use of spray drying to encapsulate avocado oil, employing various types of wall materials, such as maltodextrin-Hi-Cap® 100 starch (Chimsook, 2017), maltodextrin-whey protein isolate, maltodextrin-OSA starch, maltodextrin-OSA starch-whey protein isolate (Sotelo-Bautista et al., 2020), phosphorylated starch-gum Arabic-Hi-Cap® 100 (Espinosa-Solis et al., 2022), native taro starch, and OSA starch obtained from taro (Romero-Hernandez et al., 2021). Low MC values (<5.0%) have been consistently obtained in all cases. The MC showed a certain proportion with the a_w of the samples, so treatments T1, T2, and T3 exhibited the lowest values in both parameters. The a_w of all the samples ranged from 0.01 to 0.36 (Fig. 4B). The a_w values obtained for the analyzed samples were similar to those obtained for propolis encapsulated through complex coacervation, accompanied by a spray drying process, in which a blend of gelatin/sodium alginate was used as wall material (Sukri et al., 2023). The authors established a correlation between a_w values below 0.6 and stability against microbial growth and biochemical reactions that could alter the samples.

3.6. Hygroscopicity and solubility

The hygroscopicity and solubility of encapsulates have been linked to the reconstitution potential of the capsules, influencing the resistance to particle permeability against the release of the core (Alvim & Grosso, 2010; Bakry, Huang, Zhai, & Huang, 2019). Regarding hygroscopicity, the samples displayed values ranging between 10.3 and 16.4 g of water absorbed per 100 g of dry solid, with statistically significant differences among the samples ($p < 0.05$) (Fig. 4C). According to Tavares and Noreña (2020), samples with values exceeding 15.1% can be classified as having high hygroscopicity. Based on this criterion, only T3 exhibits a high hygroscopicity. The value observed for T3 can be associated with the low oil content used in the formulation and its limited encapsulation performance, potentially resulting in a higher affinity for water in the material. In contrast, the remaining treatments, owing to their total and surface oil contents, displayed higher hydrophobicity towards the water. Similar observations were made regarding the hygroscopicity of ginger oil capsules obtained using whey protein isolate/gum Arabic and gum Arabic/chitosan (Tavares & Noreña, 2020).

The solubility values of samples are shown in Fig. 4D and range between 9.7 and 29.0% ($p < 0.05$). Sample T7 presented the highest solubility value while recording the lowest hygroscopicity value. In contrast, sample T3, despite having the highest hygroscopicity, exhibited the lowest solubility value. It is important to note that this inverse relationship was not observed in the other samples analyzed. However, a direct relationship could be observed between the solubility value of the encapsulates and the concentration of total solids (i.e., the concentration of the wall materials) in the formulations. A rise in solids concentration increased solubility, while treatments with lower solids concentrations showed the lowest solubility values (T1, T2, and T3). In general, the solubility values for avocado oil coacervates were low compared to the results reported for pomegranate oil when using gum Arabic and whey protein as wall materials (Costa, Antunes Gaspar, Calado, Valeriano Tonon, & Guedes Torres, 2022) and for encapsulated propolis through the complex coacervation of sodium alginate and gelatin (Sukri et al., 2023). However, Tavares and Noreña (2020) reported solubility values lower than 40% for ginger essential oil encapsulates using different wall materials. The authors attributed the low solubility to the complex coacervation method, which produces water-insoluble particles due to strong electrostatic interaction between the materials. Likewise, they established that one of the main advantages of this encapsulation method is the gradual and controlled release of the core in an aqueous medium.

3.7. Fourier-transform infrared spectroscopy

The FTIR spectra of samples T1, T2, T3, and T4 (selected for being the best treatments) and avocado oil are shown in Fig. 5. The FTIR spectrum of avocado oil shows an absorption band at 3008 cm^{-1} associated with *cis*–C–CH stretching. Likewise, bands are observed at 2921 and 2852 cm^{-1}, corresponding to asymmetric and symmetric –CH and $–CH_2$ stretching vibrations, respectively (Kumar, Smita, Debut, & Cumbal, 2018). Furthermore, a high-intensity peak is observed at 1745 cm^{-1} that corresponds to C=O stretching vibrations associated with the presence of ester, aldehydes, and ketones (Mahboubifar, Hemmateenejad, Javidnia, & Yousefinejad, 2017; Quiñones-Islas, Meza-Márquez, Osorio-Revilla, & Gallardo-Velazquez, 2013). A band associated with unconjugated C=C *cis* stretching was observed at 1653 cm^{-1}, while *trans* and *cis* double bonds (C=C) were observed at 968 cm^{-1} (Jiménez-Sotelo et al., 2016; Kumar et al., 2018). The bands observed at 1459, 1378, and 1239 cm^{-1} correspond to the scissor vibration of the CH_2 and CH_3 groups. Similarly, the bands observed at 1159, 1117, and 1070 cm^{-1} correspond to the C–O stretching vibrations of ester. Similar observations were previously made for avocado oil by Pérez-Monterroza, Chaux-Gutiérrez, and Nicoletti (2018). All encapsulated samples presented a slight shift in the absorption band observed in the oil at 1745 to

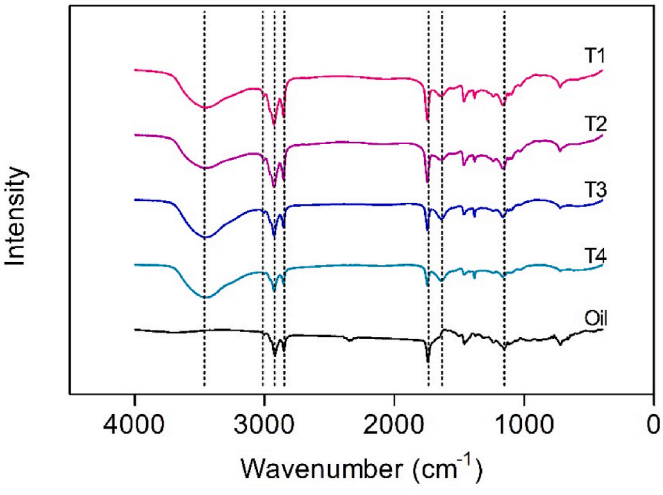

Fig. 5. FTIR spectrums of Hass avocado oil and Hass avocado oil microcapsules produced by complex coacervation (T1 – T4).

1750 cm^{-1}. Additionally, a broad band at 3440 cm^{-1} was observed in all the encapsulates, which is attributed to the stretching vibrations of hydroxyl groups (–O–H) associated with water molecules present in the encapsulates (Karaaslan et al., 2021).

3.8. Thermal stability

The weight loss (*WL*) and differential thermogravimetry (DTG) curves of samples T1, T2, T3, and T4, gelatin, NaAlg, and oil are presented in Fig. 6. Both gelatin and NaAlg showed two WL stages with a total mass loss of 76.6 ± 0.8% and 64.4 ± 1.4%, respectively. Only one degradation stage was observed for avocado oil, resulting in a total mass loss of 100%. Conversely, the coacervates showed four WL stages with total mass losses of 88.3 ± 0.8%, 87.5 ± 0.6%, 83.9 ± 0.8%, 82.1 ± 1.1%, 83.1 ± 0.6%, 81.2 ± 1.5%, 82.2 ± 0.2%, 89.7 ± 1.2%, 83.7 ± 1.0%, 88.6 ± 1.6%, and 86.6 ± 1.6% for T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, and T11, respectively ($p < 0.05$). The DTG curves reveal a first phase of degradation ranging from an approximate T_{onset} of 40.3–113 °C, which possibly corresponds to water desorption and can be associated with the water content of the encapsulates (Bordón et al., 2021). The second stage shows a T_{onset} from 198 to 238 °C with maximum temperature peaks that range between 223 and 227 °C without statistical differences between the samples ($p > 0.05$), corresponding to the decomposition of NaAlg and polyunsaturated fatty acids (Forero-Doria, García, Vergara, & Guzman, 2017). The third stage showed a T_{onset} of 274–321 °C, with maximum degradation peaks ranging between 303 and 316 °C ($p > 0.05$), which may be associated with gelatin (protein degradation) and monounsaturated fatty acids

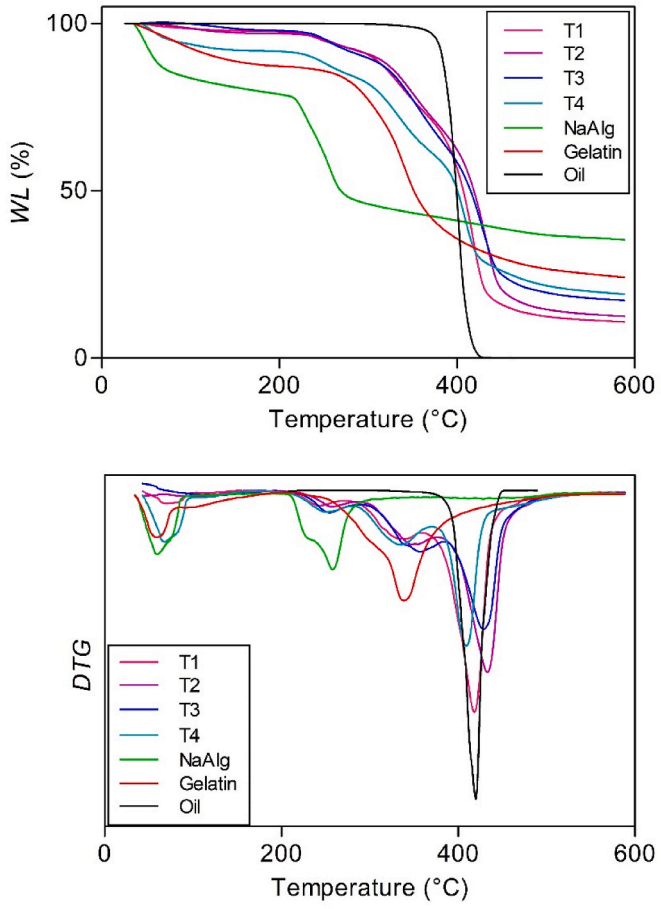

Fig. 6. Weight loss or TG (A) and DTG curves of gelatin, sodium alginate (NaAlg) and Hass avocado oil microcapsules produced by complex coacervation (T1 – T4).

degradation. The fourth stage presents the highest mass loss and occurs in a temperature range between 369 and 429 °C, with a maximum degradation peak between 393 and 407 °C and statistical differences between T4 and the other samples ($p < 0.05$). The degradation mentioned above may be linked to the saturated fatty acids present in the oil. However, considering that the highest concentration in the capsule corresponds to the wall materials, the degradation in this peak can be associated with the interaction of the gelatin/sodium alginate with the oil, generating complexes that undergo decomposition. In this study, pure avocado oil began its degradation process at a temperature above 250 °C. This indicates that the mixture with encapsulation materials increased the onset temperature of avocado oil degradation. Similar observations were made for poppy seed oil encapsulated with gum Arabic and gelatin as wall materials and by the complex coacervation method followed by spray drying (Yang et al., 2015). The authors observed that the oil degradation peak shifted towards a higher temperature in the encapsulates, which could be related to the protection of the oil present in the microcapsules compared to the crude oil.

4. Conclusions

In this study, microcapsules of Hass avocado oil were successfully obtained using a mixture of gelatin and NaAlg as wall materials and employing the complex coacervation method. A pH of 4.0 was established as the optimal coacervate formation value, with a gelatin:sodium alginate ratio of 3:1. Microscopic analyses revealed that low concentrations of solids were more efficient for coacervate formation, allowing for the electrostatic interaction of the wall materials and producing capsules with regular spherical geometries. In contrast, high concentrations limited the complete formation of coacervates, resulting in particles without defined geometries and exposing the oil to direct contact with agents that can lead to its deterioration. Overall, the samples exhibited good encapsulation efficiencies, indicating significant potential for oil protection. However, the encapsulation performances were intermediate. The low MC and a_w of the encapsulates most likely contribute to their stability during storage. Similarly, they displayed high thermal stability, thereby enhancing the protection of the encapsulated oil. This study showcased the technological potential of the complex coacervation method for encapsulating Hass avocado oil. In addition, this study is the first to evaluate the viability of complex coacervation as a method for encapsulating Hass avocado oil, establishing a baseline for implementing this methodology in the commercialization and industrialization of encapsulated avocado oil. Additionally, further analyses are necessary, such as evaluating storage stability and monitoring the physical characteristics of the oil extracted from the encapsulates. These studies will deepen the understanding of the methodology and its impact on the stabilization of Hass avocado oil.

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CRediT authorship contribution statement

Luis Daniel Daza: Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Salomé Dayana López:** Methodology, Investigation, Formal analysis, Conceptualization. **Miguel Ángel Montealegre:** Methodology, Investigation. **Valeria Soledad Eim:** Writing – original draft, Supervision, Resources, Project administration, Conceptualization. **Angélica Sandoval-Aldana:** Writing – original draft, Supervision,

Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

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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