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Encapsulation of vegetable oils in polylactic acid nanofibers to improve oil retencion in feed aquaculture

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Abstract

The feed used in fish farming contains lipids that erode and disperse in water, adversely affecting fish nutrition and contributing to water pollution. This study evaluated the encapsulation of corn and gold linseed oils in polylactic acid (PLA) nanofibers produced via Solution Blow Spinning. The nanofibers were characterized using Fourier-Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM), contact angle analysis, and encapsulation efficiency tests. Additionally, the release profiles of the oils were studied under simulated tank water and gastrointestinal conditions. The results demonstrated successful encapsulation of the oils within the PLA matrix. FTIR analysis confirmed the presence of oil in the nanofibers (wavenumbers 2925 and 2853 cm-1), with encapsulation efficiencies ranging from 81.54±10.36% to 99.14±4.55%. SEM revealed uniform nanofiber morphology with smooth surfaces and average diameters between 157±49 and 385±133 nm. All nanofibers exhibited contact angles above 90°, indicating hydrophobic behavior. Feed samples containing free oils showed significantly higher lipid release into water compared to feed incorporating encapsulated oils. The findings suggest that encapsulating vegetable oils in PLA nanofibers reduces lipid dispersion in water while maintaining nutrient availability. This polymeric system offers a sustainable alternative to enhance aquaculture feed efficiency and reduce environmental impacts, contributing to cleaner water and healthier fish.

Keywords: Farming, Formulation, Nutricion, Oil leakage rate, Pellet, Solution blow, Spinning.

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Contents

Contribution of this paper to the literature

This article proposes the use of nanotechnology as a sustainable solution for incorporating vegetable oil into fish feed, enhancing fish nutrition while reducing nutrient waste and water contamination.

1. Introduction

The fish feed should contain proteins, lipids, vitamins, and carbohydrates for efficient maintenance of adequate animal growth within the shortest time [1]. It is important to give special attention to the balance between intake, retention, and excretion, because excessive intake of certain nutrients is responsible for intoxication events and, consequently, water pollution [2, 3]. Water contamination from fish farms can occur due to therapeutic residues, fish pathogens, and feed-derived wastes, and the loss of these ingredients is also related to economic disadvantages [4]. Depending on production systems in fish farming and particularly the kind of effluent treatment, the waste load per ton of fish production can become a serious environmental challenge [3]. Monitoring and treating this feed waste in effluent directly is an inaccurate and costly process. The remainder of the undigested feed is dispersed on water (fat, vitamins, carbohydrates, and proteins) or excreted in the feces as solid waste, and the byproducts of fish metabolism are excreted as dissolved waste (ammonia, urea, phosphate). Concerns about the oils used in feed fortification also include how their waste will affect water quality since they are highly related to aquatic pollution. Oil leaching can lead to the formation of a mechanical barrier in the water surface, preventing light and oxygen from penetrates the water column and consequently reducing photosynthesis and phytoplankton formation. The decrease of phytoplankton in the aquatic medium is also responsible the reducing oxygen supply to the water column [5]. Therefore, the formulation of diets with low environmental impact is necessary to avoid damage to water quality in aquaculture production systems [6]. In this context, the use of controlled release systems containing natural products in feed formulation is an alternative to reduce water contamination from fish farming.

Fish meal and oil play an important role as lipid source in the fish diet. Because of their rich content in highly unsaturated fatty acids, particularly eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, they act as nutritional supplements [2]. Essential unsaturated fatty acids promote fish growth by regulating metabolism and improving immunity. The problem is that the overuse of fish meal and oil in farmed fish diets results in higher nitrogen and phosphorus discharges into the water systems, which causes eutrophication [7, 8]. Moreover, these products are very expensive and obtained from finite sources, therefore their use represents not only an environmental but also an economic problem. Focused on developing more sustainable aquaculture processes several authors have been studying alternative lipid sources to replace fish oil [9, 10]. Gold linseed oil (LO) and corn oil (CO) represent a good alternative to fulfill this role, since they are a sustainable source of fatty acids that are metabolized by the fish organism, leading to the formation of long-chain polyunsaturated essential fatty acids of the n-3 (DHA and EPA) and n-6 (arachidonic acid) series, respectively. Also, both corn and gold linseed oils are highly digested by all fish species. Another advantage of their use is that they do not contain dioxins and pollutants [11] and some studies have demonstrated that these oils would improve the diet conversion rate of fish as well as fish health [12]. In recent years, there has been an increasing number of authors in the literature that studying the possibility of improving the composition and quality of animal feed using nanostructured materials in feed formulations [13-17]. Oral administration of nutrients to farmed fish brings inherent challenges that must be considered when designing a nanostructured carrier. Furthermore, each compartment of the gastrointestinal tract has a unique environment that includes its complement of enzymes and specific pH levels. Nanofibers can overcome these challenges and deliver their nutrients to the intestine, where most of the absorption occurs, then traveling to the bloodstream and finally being distributed through all the organism [18].

Techniques for obtaining polymeric nanofibers have been developed in the last decade. Solution blow spinning (SBS) is a fiber manufacturing process that uses two parallel concentric nozzles. Nanofiber production by SBS depends on several factors such as concentration and viscosity of the polymeric solution, ejection rate, air pressure, and working distance. These variables have a direct influence on the fiber production rate and fiber morphology [19-21]. In comparison with other techniques, such as electrospinning, the SBS technique has a higher ejection rate, fiber production, and versatility regarding the use of solvents, polymers, and applications, in addition to its safety and low cost [22, 23]. Nanofibers obtained by the SBS technique have several applications in animal science like as controlled delivery of progesterone to control the estrus cycle in livestock animals [24] and encapsulation of diclofenac sodium to create in situ devices for pododermatitis treatment [25].

Thus, it is notable that immediate improvements in fish feed formulations are needed for a more efficient administration of nutrients, which involves reducing waste and water contamination from fish farming and increasing the biodisponibility of nutrients in the intestinal tract. With this aim, we now report the development and physicochemical characterization of hydrophobic polylactic acid (PLA) nanofibers containing different ratios of gold linseed and corn oil, produced by the Solution Blow Spinning Technique. We posit that biodegradable and hydrophobic nanofibers could protect fatty acid against release in the aquatic environment and stomach chamber, improving fatty acid release effectiveness and, thus, creating the potential for a smart fish feed with the lowest environmental impact.

2. Materials and Methods

2.1. Materials

Polylactic acid (3251 D, 66KDa, Chemical Abstracts Service number 9051-89-2) was obtained from Nature Works (USA). Corn oil (brand Lizza), gold linseed oil (brand Pazze), and the materials used in feed production were purchased locally. Chloroform (Chemical Abstracts Service number 67-66-3) was obtained from Synth (Brazil). The lipophilic dye Sudan III (Chemical Abstracts Service number 85-86-9) was purchased from Êxodo Científica (Brazil).

2.2 Solution and Fiber Production

The polylactic acid solution was prepared as follows: 12% (w/v) PLA was dissolved in chloroform under continuous stirring for 40 minutes. After the complete polymer dissolved, 50% (w/w) of vegetable oils were added to the solution [26, 27]. All the different fractions of corn oil (CO) and gold linseed oil (LO) used are shown in Table 1. The solution was kept under stirring and at room temperature for another 15 minutes. Finally, 0.125% (w/w) of the lipophilic dye Sudan III was added, and it were kept under stirring for 15 minutes.

Table 1. Different fractions of corn oil and gold linseed oil used to formulate the polylactic acid solutions that will pass through the solution blow spinning process.

Sample code	Corn oil (%v/v)	Linseed oil (%v/v)
CO:LO – 100:0	100	0
CO:LO – 75:25	75	25
CO:LO – 50:50	50	50
CO:LO – 25:75	25	75
CO:LO – 0:100	0	100
PLA nanofibers	0	0

Note: Polylactic acid (PLA), Corn oil (CO) and Gold linseed oil (LO).

The fibers were obtained using the Solution Blow Spinning technique. The spinning system was formed consisted of a source of compressed air (CHIAPERINI, model MC 12 BPV,150 liters), a polymer injection pump (NEW ERA PUMP SYSTEMS, model Syringe Pump AL1000), and a glass syringe (20 mL and 19.33 mm). A rotating horizontal cylinder covered with aluminum paper was used to collect the nanofibers. The experimental apparatus was isolated inside a glass front-opening wooden box to maintain moisture (40-50%) and temperature control (approximately 30°C, using a reflective incandescent light bulb – TOVALIGHT E.27) throughout the process.

The process took 3 hours for each sample and the spinning parameters used were adjusted based on the methodologies Nepomuceno, et al. [27] and Bonan, et al. [26]: feed rate – 6.00 mL/h; working distance – 20 cm; protrusion between the two concentric nozzles – 0.4 mm; air pressure – 172.37 kPa. The fibers were collected and stored for subsequent characterization.

2.3. Fiber Characterization

The absorption spectrum of the samples was obtained by Fourier-Transform Infrared Spectroscopy (FTIR) with the Attenuated Total Reflection (ATR) sampling technique. A total of 32 scans were considered for a wavenumber range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1} [28].

Nanofibers were coated with a gold layer by sputtering (Balzers, Sputter Coating Device 050) for the analysis of their morphology using a scanning electron microscope (SEM). The SEM system used was a LEO EVO 40 model (Carl Zeiss) equipped with Extended Variable Pressure (XVP), Bruker's Quantax EDS for X-ray microanalysis, and a cryosystem from Gatan. For each experiment, the average fiber diameter was determined with the image analysis software Image J, using approximately 100 measurements randomly taken from the samples [29].

The hydrophobicity/hydrophilicity of the nanofibers was evaluated through contact angle measurement immediately, 5 seconds, and 60 seconds after the injection of a sessile drop on the nanofiber. The experiment was performed with a Kruss Drop Shape Analyzer Goniometer – DSA25 (Hamburg, Germany).

To determine encapsulation efficiency, 30 mg of the nanofibers were dissolved in 10 mL of chloroform and the solution was quantified in a UV-Vis (Ultraviolet-Visible Spectroscopy) spectrophotometer (Shimadzu, model UV-2601) at a wavelength of 513 nm. This process was performed in 5 different regions of each sample and the encapsulation efficiency was determined through Equation 1 (Designation).

$$EE\ (\%) = {}^{C_E}/_{C_I}\ x\ 100\% \qquad (1)$$

Where: C_E = Concentration of Sudan III encapsulated in nanofibers (Average calculated using the 5 regions); C_I = Concentration of Sudan III initially added to the nanofibers.

2.4. Feed Production

A formulation simulating a conventional feed for omnivorous fish in the growth phase (the largest group of fish cultivated in the world) was produced: 45% soybean meal, 34.8% cornflour, 11.5% corn gluten meal 60, 5% corn oil/gold linseed oil, 1.5% dicalcium phosphate, 1.5% mineral and vitamin supplement, 0.5% sodium chloride, 0.19% DL-methionine, 0.125% Sudan III (a chemical marker used to track the release of vegetable oils), and water was added until the mixture obtain the proper consistency. The mixture was pelletized in an extruder (Adamo) and the product was dehydrated in a laboratory oven at 50°C for 24 hours [30]. Six feed samples were produced, containing contained the same corn oil/gold linseed oil fractions proposed in Table 1.

2.5. Assessment of Nutrient Liberation in an Aqueous Medium (In Vitro)

Approximately 30 mg of feed and feed containing nanofibers were placed into a falcon tube containing 3 mL of water, 1% (v/v) Tween 80, sodium bicarbonate (40 mg/L), and a pH equal to 7.0 [31]. The tubes were kept in an

incubator under at 25°C and 120 rpm [32]. This process represents the preparation of one aliquot that was subsequently analyzed in a UV-Vis spectrophotometer (Shimadzu, model UV-2601) around at wavelength of 534 nm. For each sample, 10 aliquots were prepared and collected after 10 minutes.

2.6. Assessment of In Vitro Digestibility

The Uv-Vis spectrophotometer (Shimadzu, model UV-2601) was used to measure absorbance around a wavelength of 534 nm. Tests of in vitro digestibility were based the methodology Wang, et al. [32]. The simulated gastric fluid of fish was prepared as follows: 20 mg of feed and feed containing nanofibers were added to an Erlenmeyer containing 7 mL of a sodium chloride (0.15 M) solution, 5% (w/v) of pepsin, and 1% (v/v) of Tween 80; the solution was posteriorly acidified with HCl until the pH of 2.0. The solution was maintained in an incubator for 1 hour in the dark (the recipients were covered with aluminum paper to prevent the passage of ambient light), at 25°C, and 100 rpm.

The simulated intestinal fluid of fish was prepared as follows: 20 mg of feed and feed containing nanofibers were added to an Erlenmeyer flask containing 7 mL of a sodium chloride (0,15 M) solution, 1.5% (w/v) of pancreatin, 0.5% (w/v) of amylase, 0.3% (w/v) of bile salts, and 1% (v/v) of Tween 80; the solution pH was posteriorly adjusted to 6.0. The solution was maintained in an incubator for 5 hours in the dark (the containers were covered with aluminum foil paper to prevent the passage of ambient light), at 25°C, and 40 rpm.

The tests described in the previous paragraphs were also performed for the 6 feed samples containing different fractions of the free vegetable oils in its formulations. The presence of lipids in the solutions was evaluated using Uv-Vis spectrophotometer.

2.7. Statistical Analysis

All data were subjected to the Shapiro-Wilk normality test and Levene's test for homoscedasticity. Parametric data were analyzed by Variance Analysis (ANOVA) (One-way or Two- way) followed by Tukey's test at 5% probability. Past software were used for data analysis.

3. Results

Fiber characterization.

3.1. Scanning Electron Microscopy (SEM)

Figure 1 shows the SEM images obtained for all the nanofibers produced in this paper, demonstrating that they exhibited uniform morphology and smooth surface. Table 2 shows average diameter values for each sample, calculated using the software Image J.

Table 2. Average fiber diameter and standard deviation of PLA nanofibers containing different fractions of vegetable oils.

Sample	CO: LO 100:0	CO: LO 75:25	CO: LO 50:50	CO: LO 25:75	CO: LO 0:100	PLA nanofibers
Average diameter (nm)	168 ± 52^{d}	209 ± 56^{c}	385 ± 133^{a}	257 ± 83^{b}	157 ± 49^{d}	167 ± 46^{d}

Note: The letters a, b, c, and d in the table indicate statistical differences between results. Results followed by different letters differ significantly between treatment (P<0.05). Ony-way ANOVA and Tukey's test were used to determine statistical significance.

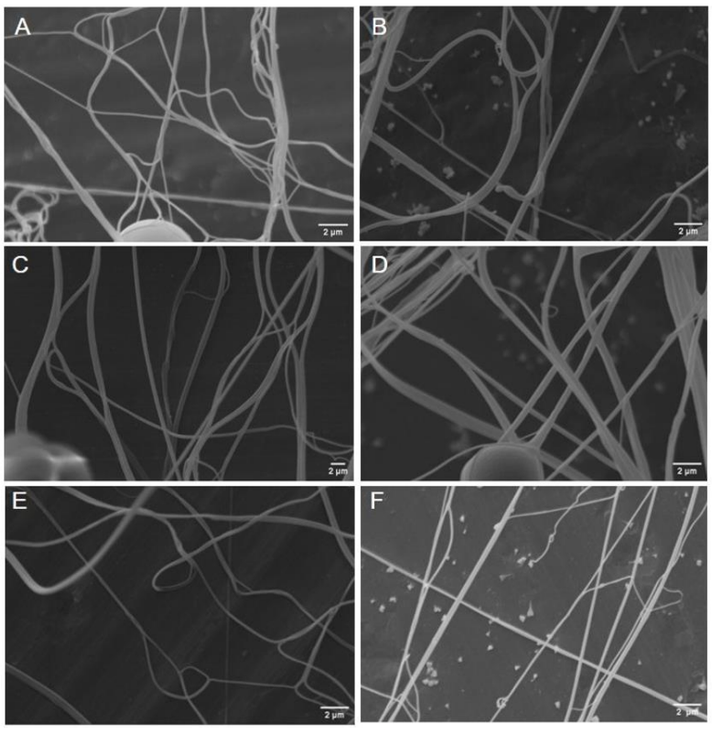

Figure 1. Scanning electron microscopy of nanofibers containing: (a) CO: LO – 100:0, (b) CO: LO - 75:25, (c) CO: LO - 50:50, (d) CO: LO - 25:75, (e) CO: LO – 0:100, (f) PLA nanofibers.

According to statistical analysis, higher values of average diameter were obtained for PLA nanofibers containing mixtures of corn oil and gold linseed oil (CL: LO – 50:50). The incorporation of pure oils did not affect

the samples average diameter and the minor values of average diameter were obtained for PLA nanofibers, CO: LO 0:100 and CO: LO – 100:0.

3.2 Fourier Transform Infrared Spectroscopy (FTIR)

Figure 2 shows the FTIR spectra obtained from the different samples. The infrared analysis reveals peaks in the spectra of the various samples produced. Notable peaks were observed in the regions around 3000, 2925, and 2853 cm-1.

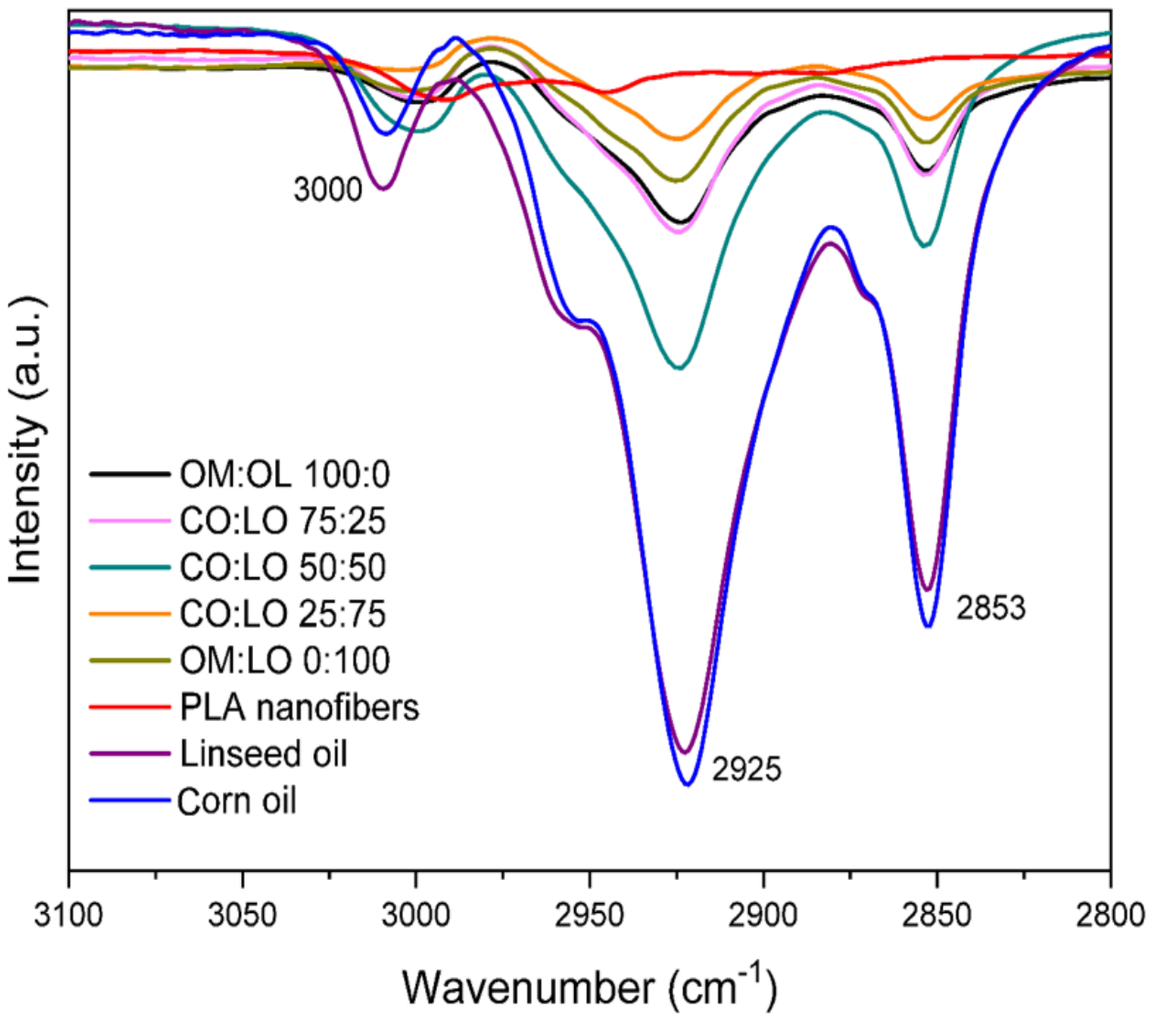

Figure 2. The infrared spectrum of PLA nanofibers containing corn oil and gold linseed oil in different proportions and of the pure vegetable oils in the wavenumber region from 4000 to 400 cm-1. This region corroborates the encapsulation of the vegetable oils.

3.3. Contact Angle

Table 3 shows the average contact angle values 60 seconds after the injection of water drop onto the surface of the nanofibers. All samples are hydrophobic since the contact angle was higher than 90° for all nanofibers. The highest contact angle were observed for the samples CO: LO – 50:150 and CO: LO – 25:75. The minors contact angle value were found for the samples PLA nanofibres and CO: LO – 0:100.

Table 3. Contact angles of PLA nanofiber mat and PLA/Vegetable oils blend nanofiber mats with their standard deviations.

Sample	Average contact angle (°)
CO: LO – 100:0	109.9±2.7 ^{ac}
CO: LO – 75:25	116.7±7.4^{ab}
CO: LO – 50:50	106.3±1.8 ^{c}
CO: LO – 25:75	107.8±3.4 ^{c}
CO: LO – 0:100	114.5±2.7^{a}
PLA nanofibers	114.6±4.8^{a}

Note: The letters a, b and c in the table indicate statistical differences between results. Results followed by different letters differ significantly between treatment (P<0.05). Ony-way ANOVA and Tukey's test were used to determine statistical significance.

3.4. Encapsulation Efficiency

Table 4 shows the encapsulation efficiency for each formulation. The results reveal that the CO: LO – 50:50 sample had the lowest encapsulation efficiency (81.54±10.36%). The highest encapsulation efficiency was obtained for samples CO: LO –100:0 (99.14±4.55 %) and CO: LO – 0:100 (97.32±11.09 %).

Table 4. Encapsulation efficiency of polylactic acid nanofibers containing different fractions of vegetable oils.

Sample	Encapsulation efficiency (%)
CO:LO – 100:0	99.14±4.55[a]
CO:LO – 75:25	94.06±9.47[abc]
CO:LO – 50:50	81.54±10.36[b]
CO:LO – 25:75	85.97±4.72 [abc]
CO:LO – 0:100	97.32±11.09 [ac]

Note: The letters a, b and c in the table indicate statistical differences between results. Results followed by different letters differ significantly between treatment (P<0.05). Ony-way ANOVA and Tukey's test were used to determine statistical significance.

3.5. Assessment of Nutrient Liberation in an Aqueous Medium (In Vitro)

In Figure 3 it is possible to observe the fraction of oil released into the water during 10 minutes of exposure of the different materials. When comparing the composition of the feed samples with and without oil encapsulated in nanofibers, it is observed that the CO: LO – 75:50 and CO: LO – 50:50 samples exhibited statistically similar oil release. The CO: LO – 100:0, CO: LO – 25:75, and CO: LO – 0:100 samples, containing oil encapsulated in nanofibers, showed lower oil release compared to the samples with free oil in the feed. When comparing oil release across different feed compositions, the CO: LO – 0:100 sample showed the highest release. For feeds with oil encapsulated in nanofibers, the CO: LO – 50:50 sample recorded the highest oil release.

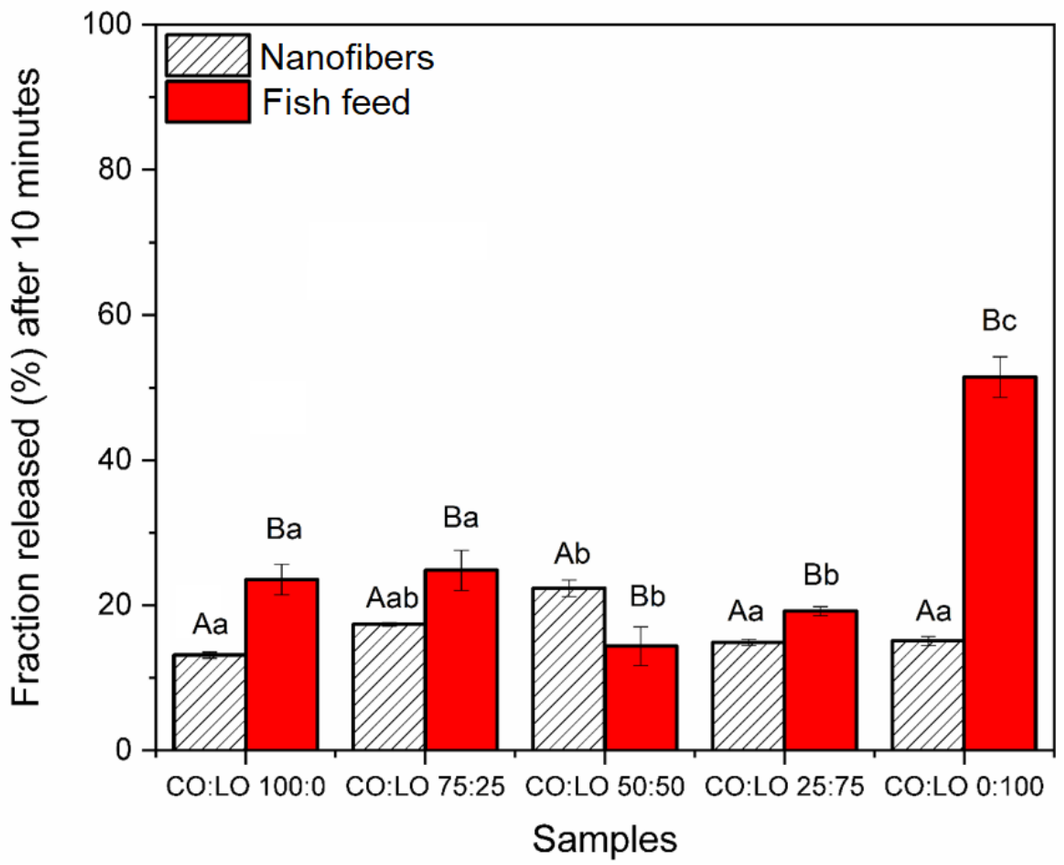

Figure 3. Comparison between the maximum quantity of vegetable oils released by the fish feed and feed containing nanofibers after 10 minutes in contact with an aqueous medium simulating fish tank condition.

Note: Results followed by different capital letters differ significantly between the different treatment (Fish feed a fndeed containing nanofibers) of the same compositions (P<0.05). Results followed by different lowercase letters differ significantly between the different compositions of the same treatment (P<0.05). Two-way ANOVA and Tukey's test were used to determine statistical significance.

3.6. Assesment of in Vitro Digestibility

Figure 4A shows the results of the digestibility test of the feeds in gastric fluid over a period of 1 hour. When comparing the same feed composition with and without oil encapsulated in nanofibers, it is observed that all feed samples without encapsulated oil exhibited higher digestibility compared to those containing encapsulated oil. When comparing the different feed compositions, the CO – 50:50 sample showed the highest digestibility. A similar pattern was observed for feeds with encapsulated oil, where the CO – 50:50 sample also presented the highest digestibility.

Figure 4B presents the results of the digestibility test of the feeds in intestinal fluid over a period of 5 hours. When comparing the same feed composition with and without oil encapsulated in nanofibers, it was found that the CO – 50:50 sample with encapsulated oil showed higher digestibility than the other feed samples. For the other compositions, all feed samples without encapsulated oil exhibited higher digestibility compared to the formulations with encapsulated oil. When comparing the different feed compositions in intestinal fluid, the highest digestibility was observed for the CO – 100:0 sample, while the lowest digestibility was recorded for the CO – 50:50 sample. Among the feeds with encapsulated oil, the highest digestibility in intestinal fluid was also observed for the CO – 50:50 sample.

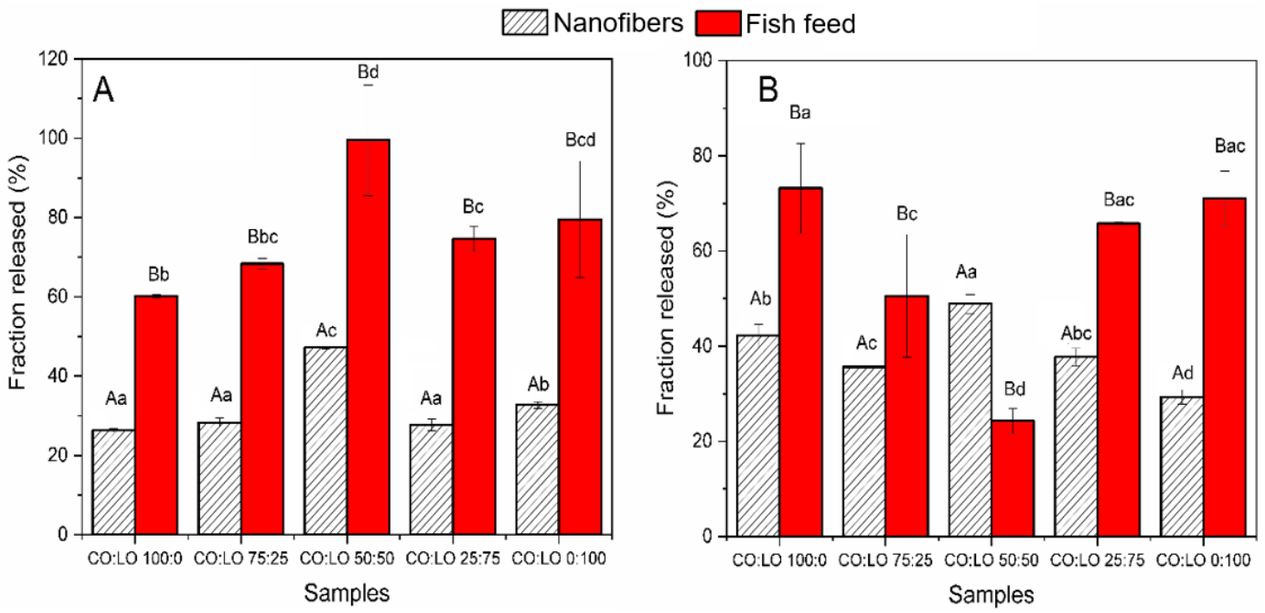

Figure 4. Comparison between fish feed and feed containing nanofibers in vitro digestibility. The test was performed maintaining the samples in simulated gastric fluid for 1 hour (A) the test was performed maintaining the samples in simulated intestinal fluid for 5 hours (B).

Note: Results followed by different capital letters differ significantly between the different treatment (fish feed a fndeed containing nanofibers) of the same compositions ($P<0.05$). Results followed by different lowercase letters differ significantly between the different compositions of the same treatment ($P<0.05$). Two-way ANOVA and Tukey's test were used to determine statistical significance.

4 Discussions

4.1. Scanning Electron Microscopy (SEM)

The study of nanofibers morphology can understand the encapsulation process. The addition of oil mixtures led to an increase in the average diameter of nanofibers (Table 2), which can be attributed to an increase in the polymeric solution viscosity. The relationship between viscosity and the average diameter of nanofibers was already noted in several papers [19, 21]. This behavior indicates that solutions containing different fractions of vegetable oils are more viscous, creating fibers with higher diameter values.

4.2. Fourier-Transform Infrared Spectroscopy (FTIR)

Infrared characterization enables the analysis of intermolecular interactions between the polymer and the active compounds through the identification of their chemical groups. The peaks observed for the polylactic acid nanofibers, corn oil, and gold linseed oil in the region from 3100 to 2800 cm-1 of the infrared spectrum agree with what is described in the literature [26, 33, 34]. The peaks show in the infrared spectrum region that confirm the encapsulation of vegetable oils in the polylactic acid matrices because it shows two signature peaks of corn and gold linseed oils (at 2925 cm-1, relative to CH3 symmetric stretch and at 2853 cm-1, relative to stretching vibration of bonds between carbon (C) and hydrogen (H) (CH stretch) in the nanofibers [33-36].

The FTIR results (Figure 2) suggest that weak interaction occurs between the gold linseed oil, corn oil, and the polylactic acid macromolecules occurs since there is no evidence of the formation of new primary bonds that can be associated with the shift or formation of new peaks in the infrared spectrum. Rahmani, et al. [33] observed alterations in the infrared spectrum of silicon nanowires (SiNWs) after the deposition of poly(3-hexylthiophene) on its surface. The formation of new peaks related to stretching vibration of bonds between carbon (C) and hydrogen (H) (C-H) and silicon (Si) and carbon (C) vibrations (Si-C) and the absence of the carbon (C) and carbon (C) (C=C) bond peak evidenced the poly(3-hexylthiophene) adsorption in the SiNWs surface through the formation of Si-C bonds after the rupture of the double bond between carbons. Another example is the paper of Yu, et al. [34], in which a shift of characteristic peaks of polyacrylonitrile nanofibers to a lower wavenumber after the encapsulation of different concentrations of acyclovir in the polymeric matrix, indicating the formation of hydrogen bonds between the carbon (C) and Nitrogen (N) (C≡N group) from polyacrylonitrile and the Oxygen (O) and hydrogen (H) (–OH group) from acyclovir.

The presence of characteristic peaks of polylactic acid and vegetable oils in the nanofibers is an indication that the Solution Blow Spinning process was efficient to encapsulate these nutrients.

4.3. Contact Angle

It was expected that nanofibers would exhibit a hydrophobic behavior, with contact angle greater than 90° (Table 3), because both polylactic acid and vegetable oils are nonpolar molecules and, therefore, are incapable of forming intermolecular interactions with water. Moreover, the interaction between a polar liquid and a nonpolar surface is weak since the interactions inside the liquid are favored, resulting in a decrease of contact with the surface [36].

Paragkumar, et al. [37] showed that PLA films had a contact angle equal to 96°. This shows that the production of materials in nanoscale can improve properties such as hydrophobicity [38].

Therefore, the results align with following what is described in the literature and it can be affirmed that all nanofibers produced in this paper have great potential to retain the diffusion of the oil fraction in aquatic environments since there will be no significant interaction between the polymeric matrix and water.

4.4. Encapsulation Efficiency

Knowing that the encapsulation process was successful, it is necessary to quantify the number of active compounds that were trapped in nanofibers. The CO: LO – 50:50 nanofiber had the one with the lowest encapsulation efficiency, which indicates that this sample will present an initial burst more evident than the other formulations, as shown by Wang, et al. [39]. In general, these results agree with what is reported in the literature about encapsulation in nanofibers. In the paper of Wang, et al. [39] the encapsulation efficiency of rosemary essential oil in polylactic acid nanofibers obtained by electrospinning was 90.18±1.77%, whereas the same encapsulation in pellets obtained by the extrusion technique led to an encapsulation efficiency of 68.77±1.41%. This behavior was also observed when comparing these two projects: Pérez-Masiá, et al. [40] obtained an encapsulation efficiency of 44.0±5.5% when encapsulating folic acid in starch microcapsules (electrospraying technique), and Fonseca, et al. [41] obtained an encapsulation efficiency of 95.6±0.0% when encapsulating folic acid in starch nanofibers (electrospinning technique). The higher values of encapsulation efficiency observed to nanofibers when compared with other polymeric matrices is due to properties like high surface-to-volume ratio, which reduce the number of interest compounds that can be present in the material surface.

Through the comparison between the values demonstrated in Table 2 and Table 4, it can be assumed that the diameters of the nanofibers do not interfere directly in the encapsulation process, since large diameters are not necessarily associated with higher encapsulation efficiency values.

4.5. Assessment of Nutrient Liberation in an Aqueous Medium (In Vitro)

In fish farming, the feed does not stay in contact with water for more than 10 minutes [42] and through analysis of Figure 3, it is notable that in this period all nanofibers released between 14-21% of the nutrients encapsulated, while all feed samples released between 14-51% of nutrients during the same time. These results show that nanofibers are more effective in protecting nutrients against premature release.

The nutrient release from nanofibers in the first 10 minutes of contact with an aqueous medium (Figure 3) has a direct connection with the high amount of vegetable oils encapsulated (50% w/w). This behavior was already explained by Karuppuswamy, et al. [43] who noticed that greater active agent concentrations led to faster burst release because there was a higher quantity of the compounds deposited in the polymeric matrix surface that could be easily dispersed in the medium [44]. The higher percentage of nutrients release observed for the feed can be explained by the fact that the feed commonly used in fish farming has approximately 40 essential nutrients that have to be present in specific quantities to ensure a balanced diet [45]. Since the formulation is extremely complex and composed of several hydrophilic and hygroscopic fractions, like soybean meal, cornflour, and dicalcium phosphate, a fast erosion of the feed granules occurs when in contact with water. This rapid erosion leads to an accelerated migration of the apolar fraction of nutrients, for example, vegetable oils. Therefore, they are dispersed in the aqueous medium before ingestion by fish. Notably, nanofibers were capable of retain the action compounds much more efficiency than the feed containing free oils. These results prove that the encapsulation of active agents was successful and confirms that the proposal to apply nanofibers in fish farming to feed fortification is promising since lower release in water is directly associated with greater availability of nutrients that can be absorbed in fish metabolism.

4.6. Assessment of in Vitro Digestibility

The fish feeding process starts when the feed comes into contact with water. At this moment, part of the nutrients added in the formulation is lost to the aquatic medium. Then, the pellets are consumed by the fish and the fraction of the active agents that were not released in water are digested in the stomach and, subsequently, in the intestine.

The analysis of Figure 3 and 4 show the absence of a release standard between the feed samples, which indicates that the formulation presents certain heterogeneity and that the chemical composition of the oil fraction added in the feed has a direct impact on the apolar nutrients release since there was a big difference between the percentage of nutrients released in the simulated mediums for each feed sample.

Therefore, considering the heterogeneity of the formulation and the loss of feed nutrients that occurs in water, each pellet has different quantities of active compounds when they reach the gastrointestinal tract. This leads to consequences in the growth, development, and nutritional value of fishes since each one will consume different concentrations of nutrients. The final weight variation of farmed fishes that are fed with the conventional feed has been already observed by several authors, such as [46-48].

Nanofibers showed a more significant release in the simulated intestinal fluid than in simulated gastric fluid, which is a promising result since nutrients stay longer in the intestine, where most of its absorption occurs [16]. Furthermore, statistical analysis shows that even with different encapsulation efficiency and average diameter values, nanofibers can have similar effectiveness.

The sample with the lowest encapsulation, efficiency (CO: LO – 50:50) was the one that released the greatest number of vegetable oils in water (Figure 3), which makes sense if we think that the lower the quantity of nutrients encapsulated the greater the fraction that is free in the polymeric matrix surface. The sample with the highest encapsulation efficiency (CO: LO – 100:0) was the one that released the greatest amount of vegetable oils in the intestinal fluid (Figure 4b), indicating that the polymeric matrix effectively protects the active agents, ensuring a pH-induced release. Since the feed components are not wrapped by a protective layer, nutrients are free and released indiscriminately both in the aqueous medium and in the simulated gastrointestinal fluids. Thus, the feed contains a small number of nutrients when reaches the intestinal fluid, which affects the growth and development of fishe. Therefore, vegetable oil encapsulation in nanofibers can be extremely advantageous, not only environmentally but also economically and nutritionally.

5. Conclusion

The results showed that the encapsulation of vegetable oils in polylactic acid nanofibers obtained by the Solution Blow Spinning technique was efficient, and the product can be applied as a controlled release system to

improve fish nutrition and reduce the environmental impact associated with aquaculture. However, future experiments are needed to verify the efficiency of the feed containing encapsulated oil on the performance and physiology of the animals.

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