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Natural Products in Favor of Food Safety: Development of Nanoparticles Based on Tea Tree Oil and Corn Starch and Their Potential for Fresh Food Protection

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ABSTRACT

Nanostructured polymeric systems based on natural resources can carry and improve the stability of natural active substances such as essential oils. Corn starch nanoparticles loaded with tree tea essential oil were prepared, characterized and reported here for the first time. The average hydrodynamic diameter and polydispersity index were determined by dynamic light scattering and the zeta potential by electrophoretic mobility. Encapsulation efficiency was determined by spectroscopy, and the in vitro antimicrobial potential was determined by agar diffusion test. Acerola cherry and grape fruits were coated by immersion into the nanoparticles, dried and kept at room temperature to analyze their behavior. The average hydrodynamic diameter was 232–559 nm, the zeta potential about −22 to −4.25 mV, polydispersity index about 0.5–1.0 and the encapsulation efficiency ranged from 46% to 15%. The highest oil concentration samples showed antifungal potential. The coating test indicated rot protection potential. The results indicated the corn starch essential oil-based system potential for application in the food industry, as well in other applications that require natural products with antimicrobial potential.

1 | Introduction

The growing world population and the increasing demand for food represent a challenge for conventional agriculture. Agriculture needs to supply the food chain and, at the same time, to overcome the problems that threaten food production, including the long-term production prospects [1]. A quarter of the world's food production is wasted post-harvest due to microbial contamination, which results in significant commercial losses [2]. The metabolic activity of microorganisms compromises the physical, chemical, and sensorial properties of food, reducing its quality and shelf life. Food contamination leads to economic losses, as well as risks to public health [3]. According to the World Health Organization [4], contaminated food causes illness in about 600 million people worldwide each year. Also, about 420 000 of these illness people die as the result of eating unsuitable food. This scenario shows the importance of preventing or inhibiting the microbial activity in the food chain. It also highlights the need for the development of novel processing or technologies within this productive sector, aiming to reduce or eliminate food-borne pathogenic and spoilage bacteria [2].

The most common method used for increasing the shelf life of fruits and vegetables is the coating with products and processes that maintain the organoleptic and visual features of the pristine products. These products should be non-toxic, free of flavor, color, or any other element that might change the properties of the natural product. They should also be edible, act as barriers to water loss and oxygen permeation, protecting against mechanical, chemical, and microbiological damage. The products conventionally used as coating for food preservation are the mineral oil, paraffin wax and petrolatum, regulated as food additives by the Food & Drug Act and Regulations [5], which sets out the quantity limits for these types of coatings. Natural resources are gaining prominence in this field as an alternative to avoid health problems caused by the use of synthetic products [6, 7].

Natural polymers such as chitosan, carboxymethylcellulose, starch, alginate, and others [8, 9], are useful as polymeric matrices for incorporating and delivering active substances [10]. The chemical nature of the natural polymers allows many of them to be solubilized, processed and mixed with additives in order to prepare these systems. Starch represents a good alternative matrix for nanostructured system development, once it is widely used as excipient in the pharmaceutical industry, because its availability, harmless, and low cost. The preparation of nanoparticles or nanostructured systems based on starch is few studied because of its intrinsic properties, mainly the gelatinization and the retrogradation, which can lead the system naturally to destabilize with time. So, to study its properties is challenging, but can lead to advances in its range of applications.

Active substances from natural sources, such as the antimicrobial essential oils have been highlighted for application in several fields because of their activity against microorganisms, including the pathogenic [11].

Although the good antimicrobial properties of these essential oils, they are volatile and unstable to oxygen, which can trigger its degradation processes, and also represents a challenge [12]. The encapsulation has been seen as an alternative to maintain the chemical properties and stability of essential oils, ensuring its efficacy and bioavailability, once the encapsulation protects them from volatilization and from oxygen. In addition, the encapsulation can improve their administration via aqueous medium [13]. Tea tree essential oil has been used in nanoparticles delivery systems for antimicrobial fruit/seed coatings [7], vegetable coatings [14], and meat preservation [15]. Tea tree oil has been encapsulated in polymers or polymers combined with other components such as alginate, chitosan/calcium chloride, and gliadin or proteins, respectively [7, 14, 15]. It represents an interesting natural product for new nanotechnological systems development because its antimicrobial property.

Systems based on natural polymers and active substances, both from natural sources, are normally harmless for humans and for the environment, and represents potential alternatives for the conventional for active substances application/delivery [16]. The nanotechnology, particularly the development of nanostructured systems for active substances delivery, has a crucial role and gain the scenario in attempt to the development of new advanced formulations.

Even though tea tree oil has been extensively studied for the development of several delivery systems, its combination with corn starch, that is widely used in the pharmaceutical industry for delivery purposes, can generate interesting results. Its demand is open to be explored, and no publication could be found in the literature in which corn starch and tea tree oil were combined for such application. With this in mind, the proposal of this work is the development of a renewable and natural based system, in which the corn starch is used as the matrix for the encapsulation of the functional tea tree essential oil, with antimicrobial potential provided by the essential oil. The formulations were prepared and the nanoparticles were characterized, as well the encapsulation efficiency. The steps for the nanoparticle's preparation represented the great challenge of this research, once the method was based on the disruption of the corn starch grains, in a top-down process, followed by the nanoparticle's preparation, in a bottom-up process from the gelatinized corn starch combined with the tea tree essential oil. The nanoparticles antifungal activity and the coating for fruit protection assays were also carried as preliminary tests at the first level of evidence for their potential of applications.

2 | Materials

Corn starch containing approximately 28% amylose and 72% amylopectin [17] (TRISANTI—Brazil), Tween–80 (Dinâmica—Brazil, CAS: 9005–65– 6), Tea tree essential oil (Condire—AGTTEC—Brazil), Agar-agar (Dinâmica—Brazil, CAS:9002-18-0), Sabouraud Dextrose Broth EP-USP (Kasvi—Spain, CAS: K25-1205), Nutrient Broth (Kasvi—Italy, REF: K25-610037), Tetracycline Hydrate 99% (Sigma-Aldrich—USA, CAS:60-54-8), *C. albicans* ATCC 10231 purchased from the American Type Culture Collection, ATCC, USA, fruits (*Malpighia emarginata* (acerola cherry) and *Vitis vinifera* L. (grape)) were purchased at a local market in the city of Araraquara, São Paulo, Brazil.

3 | Methods

3.1 | Corn Starch Swelling and Gelatinization

Starch swelling and gelatinization was standardized for the preparation of the aqueous phase for the nanoparticles preparation. Swelling was tested to follow the behavior of corns starch before heating, without heating. Gelatinization was carried out using heat treatment in the microwave. Corn starch was weighed, dispersed in distilled water and its behavior was followed by microscopic analysis. Microscopic images of each step of the corn starch swelling and gelatinization were assessed using a Leica MDi8 microscope, in the brightfield and phase contrast. Five steps were prepared (I, II, III, IV, V) with different treatments (time and heat) until the swelling and disintegration of the corn starch grains, as shown in Table 1.

3.2 | Preparation of the Coating

The nanoparticles were prepared using the microemulsion technique following the literature [18], with modifications. The influence of tea tree essential oil and the surfactant Tween-

TABLE 1 | Starch suspension composition and treatments.

Phase	Corn starch (g)	Heating time (min)	Temperature (°C)	Water volume (mL)
I	0.5	0	27	1.0
II	1.5	0	27	100.0
III	1.5	2	90	100.0
IV	1.5	5	92.5	100.0
V	1.5	10	95	100.0

TABLE 2 | Sample composition.

Sample	Corn starch (g)	Tea tree essential oil (mL)	Tween-80 (mL)	Water volume (mL)
S1	0.3	0.3	0.03	20.0
S2	0.3	0.6	0.3	20.0
S3	0.3	0.03	0.3	20.0
S4	0.3	0.9	0.9	20.0
S5	0.3	0.3	0.3	20.0
Control	0.3	—	—	100.0

80 was investigated, varying their concentrations. Two different liquid systems were used to formulate the nanoparticles, namely the aqueous phase based on the non-ionic surfactant Tween-80 solution and the oily phase. The concentrations of Tween-80 and the oil phase were varied, and the volume of corn starch solution was kept constant for all the formulations (0.3 g of corn starch/20 mL of water = 0.015 g/mL), as shown in Table 2. To prepare the aqueous phase, 3 g of corn starch was weighed and dispersed into 200 mL of distilled water. The suspension was heated and kept at 95°C for 10 min, as standardized in 3.1. At this temperature, the corn starch's polymeric matrix disintegrates, giving rise to the homogeneous suspension, corresponding to the corn starch's gelatinization. The suspension was then kept at room temperature until it reached 25°C. After cooling, the suspension was stirred at 600 rpm for 30 min, followed by the addition of Tween-80, which remained under stirring for a further 30 min. Then, the oil phase was slowly dropped into the mixture. The system was kept under magnetic stirring for an additional 1 h, leading to the formation of the nanoparticle. The samples were stored in the fridge. The corn starch concentration was kept constant in all the samples. The essential oil and Tween-80 concentration were proportionally varied, respectively, as follows: 10:1 (S1), 2:1 (S2), 0,1:1 (S3), 1:1 (S4 and S5), with increased concentrations for S4. An additional sample (S6) was planned at 1:10 proportional concentration of essential oil: Tween-80, however, this sample generated high amount of foam, which did not allow it be properly processed; thus, it was discarded.

3.3 | Atomic Force Microscopy (AFM)

For sample preparation, the nanoemulsions were deposited onto glass surfaces and dried at room temperature for 24 h. The nanoparticle profile in the polymeric system was then analyzed by topographic imaging using an atomic force microscope (AFM-SPM 9700, Shimadzu) in intermittent mode, in a field of 1 × 1 µm. The images were processed using the Gwyddion free software, version 2.45.

3.4 | Physico-Chemical Characterization

The average hydrodynamic diameter and polydispersity index (PDI) were determined by dynamic light scattering (DLS) and the zeta potential by eletrophoretic mobility using a Zetasizer (Nano-Zs Malvern Instruments). Before reading, 20 µL of each sample was diluted in 1 mL of distilled water.

3.5 | Encapsulation Efficiency

The essential oil content in the nanoparticles was determined by absorption following the method described in the literature [19, 20]. The absorption spectrum of the tea tree essential oil solution in ethanol (5 µL of oil in 950 µL of ethanol) was scanned from 190 to 700 nm using a Bel Photonics UV-M51 spectrophotometer, and the data were used to construct the calibration curve (R-value = 0.99907; y = −9.18.10-4 x + 6425.7548), using the highest absorption values, which were reached at 274 nm. To determine the encapsulation efficiency, 20 µL of the sample were diluted in 980 µL of distilled water and centrifuged at 10 000 rpm for 3 min using a KASVI K14-1215P microcentrifuge. The supernatant (composed by the free oil and water) was discarded; 500 µL of ethanol was added to the eppendorf in which the nanoparticles were deposited at the bottom, and the sample was centrifuged at 10 000 rpm for 3 min. At this stage, the microdroplets breaks up and the oil is solubilized in the ethanol for absorbance reading. The absorbance was read at 274 nm and the content of oil was determined using the calibration curve.

3.6 | Antimicrobial Potential

The antimicrobial potential of the nanoparticles samples was assessed using the diffusion method. The antimicrobial potential was investigated against the yeast *Candida albicans* ATCC 10231. The yeast was grown for 24 h in Sabouraud agar culture medium. The Petri dishes were labelled and filled with agar Sabouraud culture medium. After the agar solidification, 12 wells (6–8 mm in diameter) were made on its surface using a glass nozzle, and the yeast was inoculated by spreading it with a swab on the agar surface. The five nanoparticles' formulations were tested and the tests were carried out in duplicate. The samples (100 µL) were added to each well. The positive control was the antibiotic tetracycline (100 µL/well, 2 mg/mL), the tea tree essential oil (100 µL/well) was also tested. The plates were incubated for 24 h at 37°C. After this period of time, the samples were visually analyzed to determine the inhibition zone. *Candida albicans* was tested because it can easily contaminate fruits by hand contact in its commercial chain, from the harvest to the market.

3.7 | Evaluation of the Protective Effect of Post-Harvest Fruit

Two fruits were selected for the study of their post-harvest protective effect. The acerola cherry and grape were selected

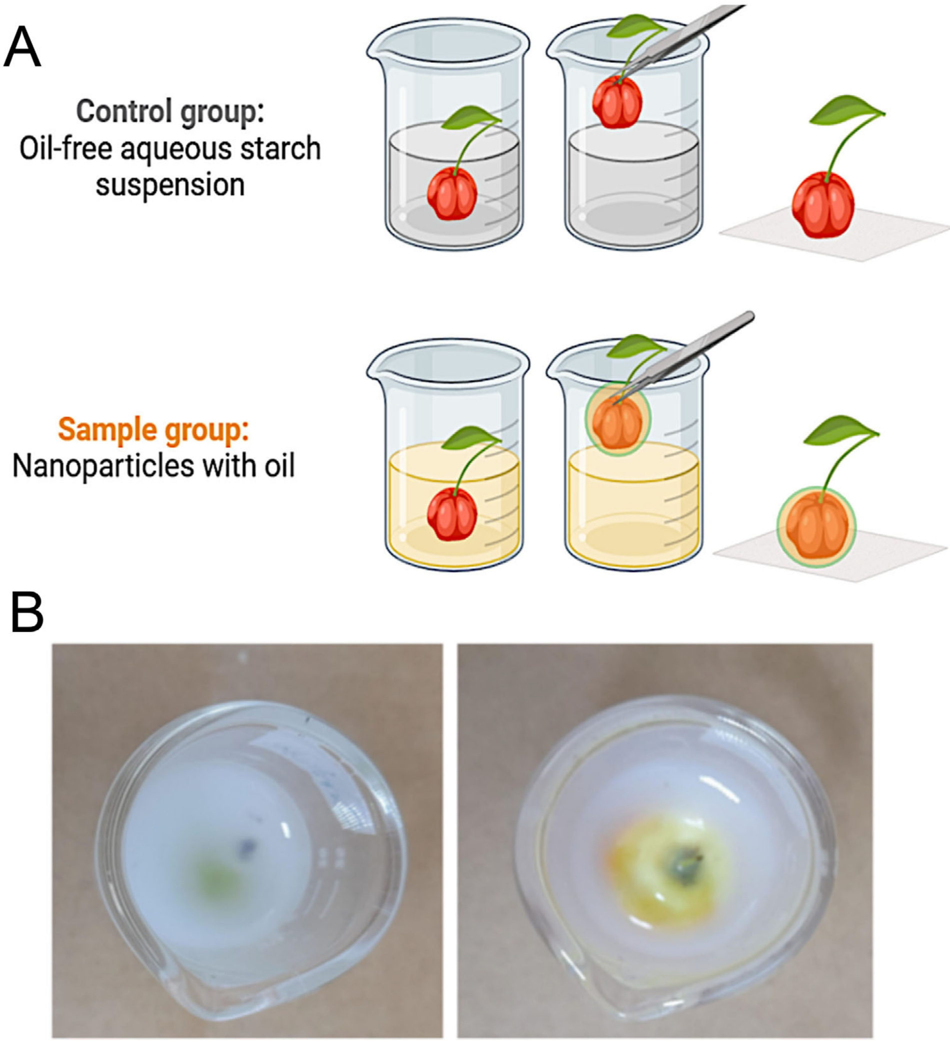

FIGURE 1 | Coating fruits by immersion into the nanoparticle (A). Picture of the fruit samples immersed into the nanoparticle (B).

because they are easy to rot at room temperature in a few days. The two fruits were purchased at a local market in Araraquara city, São Paulo, Brazil. When selecting the acerola cherry for the study, the color of its skin was taken into consideration, with the majority having only one color (the initial tone of the fruit ripening—light orange or reddish tones) or two colors, changing from the initial stage (light orange-reddish) to the ripe stage (light orange-reddish). As for the grapes, all the fruits displayed an initial post-harvest light green color. The fruits were first sanitized with distilled water and laid out on filter paper to dry. Six specimens of each species were used and divided into two groups (control and sample) to analyze the protective effect of the coating. The control group was coated with the aqueous solution of the corn starch phase (without oil). For the acerola cherry, sample S2 was used and for the grape, sample S1 was used, all with three specimens (triplicate). For the coating, the fruits were individually emerged in the nanoparticle's samples (15 mL), as shown in Figure 1, and left to rest in the emulsion for 1 min. The fruit was then removed from the beaker with tweezers and placed on the filter paper surface to dry. The samples were kept on the craft paper covered bench, at room temperature (25°C) and relative humidity of 50%, and were observed daily, photographed, for 5 days for the acerola cherry and 6 days for the grape.

4 | Results and Discussion

4.1 | Corn Starch Swelling and Gelatinization

To visualize the steps from the swelling and breaking of the granules to reach the corn starch grain gelatinization, a set of five images is displayed in Figure 2. In this Step I, the image represents the suspension immediately after its preparation, without heating, at a room temperature of 25°C. This suspension was promptly analyzed by microscopy showing the intact granules with about 10–25 µm in size, Figure 2. (I) In Step II, the granules were left to rest for 30 min in water at 25°C and then analyzed. The granules kept their structure intact, and similar to those seen in Step I, Figure 2. (II) In Step III, the corn starch was heated with water for 2 min at 90°C, resulting in the partial swelling of some granules, Figure 2. (III) In this step, the size varied between 50 and 150 µm, indicating their high swelling. In Step IV, the corn starch was heated at 92.5°C in water for 5 min, leading to partial rupture of the granules. In Step V, the corn starch was heated at 95°C for 10 min in water, resulting in a homogeneous suspension. Few very small broken grains, smaller than 50 µm, can be seen in the sample, indicated by the red arrows in images Va and Vb. These images confirm the corn starch complete disruption

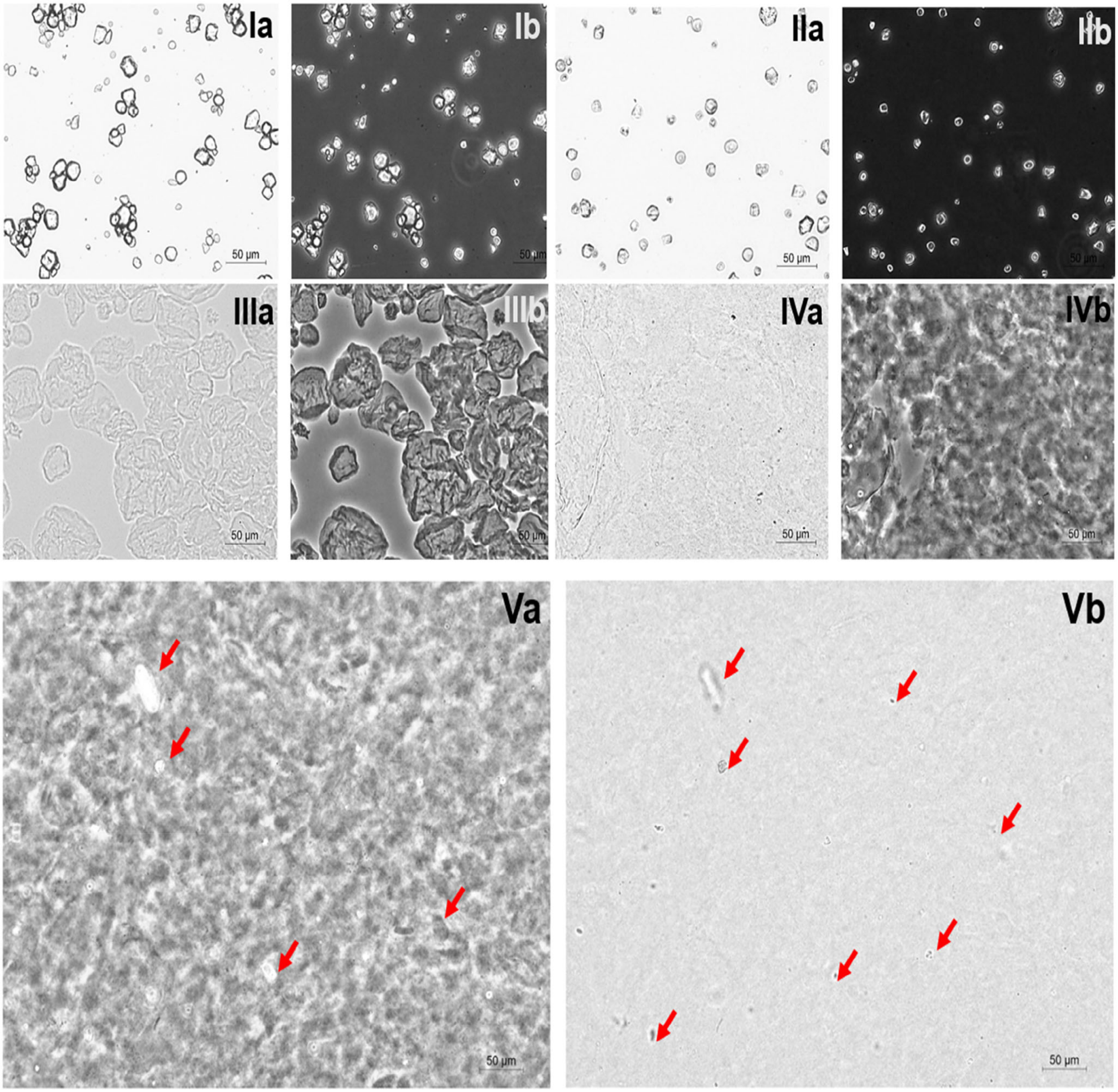

FIGURE 2 | Microscopic images of the steps of corn starch disruption process, showing the initial (Ia-Ib, IIa-IIb), partial (IIIa-IIIb, IVa-IVb), and final (Va-Vb) stages of corn starch granule breakdown process; (a) correspond to the brightfield images, and (b) correspond to the phase contrast images.

and gelatinization. Corn starch breaking and gelatinization is a well described process in the literature. Here, it disintegrated as expected, and the recorded images represent an increment of the process registration.

4.2 | Physico-Chemical Characterization

4.2.1 | Zeta Potential

The zeta potential value was determined by electrophoretic mobility and the results are shown in Figure 3. The zeta potential indicates the surface charge of the nanoparticles and is the main indicator of the potential stability of the colloidal system. The higher (negative or positive) zeta potential values indicate greater repulsion between the particles, that is, greater resistance to aggregation. Thus, when most of the particles in suspension display a large negative or positive zeta potential, the repulsive forces are dominant, leading the particles to repel each other, avoiding agglomeration. In Figure 3, the sample with the greatest stability among the formulations was S1, with a zeta potential about −22 mV, and also S2, with a zeta potential about −8 mV, which tends to be stable. The literature shows values ranging from about −1.97 mV, −3.9 mV [21] to −12.2 mV, −20.25 mV [22] for starch microparticles or nanoparticle based systems. These literature values are close to the results found here, suggesting that it can be a feature related to the starch nanoparticles. Normally, the numerical values to indicate the stability of the system are those

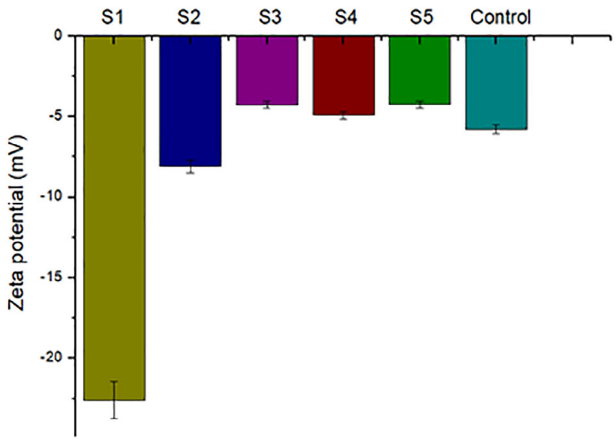

FIGURE 3 | Zeta potential values of the samples.

closest to 30 mV (+ or −); however, the zeta potential depends on the nature of the dispersant and its surface, thus indicating that sample composition must be taken into account when analyzing a specific result. Considering that the OH from corn starch is low dissociated at neutral pH, and that they represent the major component of the outer layer of the nanoparticles that gives rise to the zeta potential, it could explain the low values of the discussed samples.

The negatively charged surface layer of the nanoparticles indicated by the zeta potential was used to confirm the design of the chemical architecture of the nanoparticles, as discussed below.

4.2.2 | Nanoparticle Chemical Architecture

Zeta potential results showed that the surface of the nanoparticle displays a negatively charged surface layer, which correspond to the negative charges from the dissociated hydroxyl groups at the surface of corn starch. The corn starch solution corresponds to the higher volume of the system, thus, surrounding the core made up of tea tree essential oil. These two molecular networks are linked and stabilized by the non-ionic surfactant (Tween-80). The system stability is the result of the chemical structure of the system components, namely the Tween-80, which displays a hydrophilic moiety, with affinity for water, and a hydrophobic tail with affinity for oil, the oil, predominantly hydrophobic and the corn starch, rich in hydrophilic groups. The dominant forces that drive the hydrophilic interactions are the hydrogen bonds, and, among the hydrophobic counterparts are the dipole-dipole and Van der Waals. The hydrophilic part of the Tween-80 interacts with the corn starch hydroxyl groups and the hydrophobic part interacts with the oily core, reducing the surface tension between the phases and, leading the molecules to assemble in spheres formed by small groups of molecules. The negative charges provided by assembled corn starch molecules, exposes their hydroxyl groups at the surface of the nanoparticle. These negatively charged nanoparticles repel each other, maintaining the emulsion stability [23, 24]. The schematic representation of the nanoparticle chemical architecture can be seen in Figure 4.

4.2.3 | Average Hydrodynamic Diameter and Polydispersity Index (PDI)

The average size of the nanoparticles was determined by DLS, and is shown in Table 3. The sample with the smallest particle size (232 nm) has the highest Tween-80 content (S4), and those with intermediate Tween-80 concentration (S2, S3, and S5), showed about double size particles (Table 3), when compared to S4. These results showed that the particle size (nm) is influenced by the concentration of the Tween-80, in which the greater the amount of Tween-80, the smaller the particle size. With respect to the average size of corn starch nanoparticles, the literature shows that it can range from about 136.0–161.5 nm [21] to 343.271–855 nm [22].

The distribution of the particle size and polydispersity index are shown in Figure 5A,B, respectively. PDI values ranged from 0.4 to 1.0, including the control and the samples. Values in the range of 0.1–0.5 indicate adequate and homogeneous distributions, showing good quality in the colloidal suspension when the nanoparticles are composed of natural polymers. Values close to, or greater than 0.7 suggest large particles or aggregates in the suspension [25]. Samples S1 and S5 showed the most appropriate PDI, with respect to their physical stability in the formulations. The PDI values found in the literature for corn starch nanoparticles, is in the range of 0.3–0.5 [22].

The preparation of the corn starch nanoparticle loaded with tea tree essential oil started by heating the corn starch grains, to break it, in a typical top-down process. In this process, the whole grains were broken and then used to prepare the aqueous phase for the nanoparticle's preparation. This top-down process is inherent to the corn starch, once it is found in nature in grain shape, which need to be disrupted to expose their macromolecules that will give rise to the emulsion aqueous phase. The grain disruption can generate a suspension with small aggregates, however, well distributed and highly homogeneous. The nanoparticles preparation is based on the loading of this aqueous phase with the tea tree essential oil and the nanoparticles assembling. Thus, the properties and behavior of the nanoparticles prepared from corn starch are unique, and cannot be compared to those prepared using the polymer solubilization conventional approach. In addition, PDI and the particle size can be influenced by corn starch retrogradation, which leads to the formation of spherulitic structures filled with nanoscale substructures (primary particles) [26]. Also, amylopectin and amylose can be reassembled to form nanoscale structures, even during short period of time storage [27], influencing PDI and the particle's size.

4.2.4 | Atomic Force Microscopy (AFM)

The results of AFM are shown in Figure 6A,B. The images revealed the spherical morphology for S1, S2, S3, S5 and control samples, a good homogeneous feature for all of them, and the slightly variable size. As shown in Figure 6A, the size of individual nanoparticles in samples S1, S2, S3, and S5 ranged from about 5–30 nm, and were higher than the those in the control sample. S4 did not reveal a clear spherical pattern, thus it was not possible to determine the size. This result can be attributed to the low

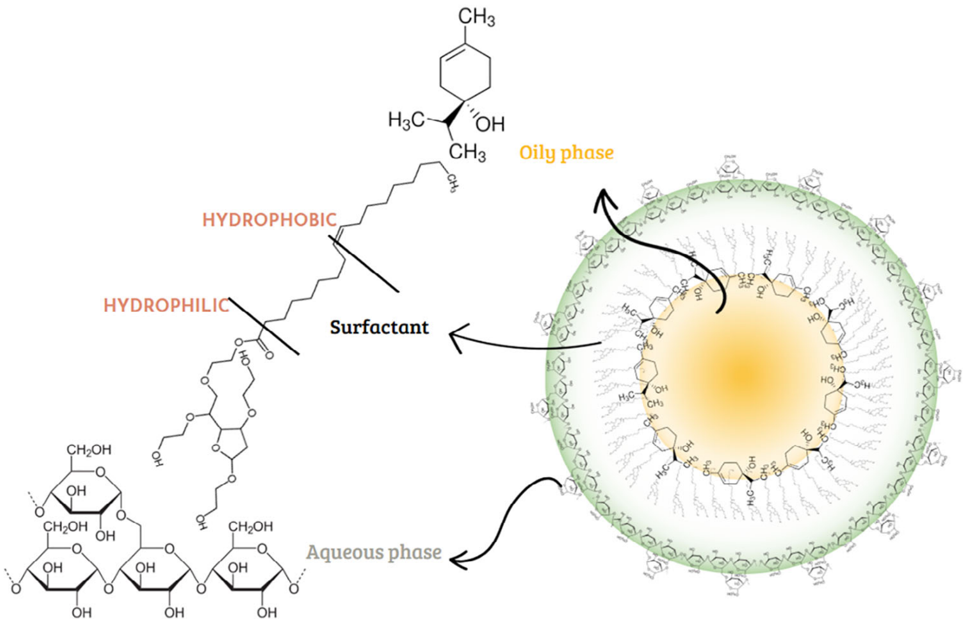

FIGURE 4 | Representation of the chemical architecture of the nanoparticle.

TABLE 3 | Average nanoparticle size of the samples.

Sample	Corn starch (g)	Tea tree essential oil (mL)	Tween-80 (mL)	Average particle size (nm)
S1	0.3	0.3	0.03	404.3
S2	0.3	0.6	0.3	497.6
S3	0.3	0.03	0.3	559.1
S4	0.3	0.9	0.9	232.3
S5	0.3	0.3	0.3	500.4
Control	0.3	—	—	230.6

encapsulation efficiency of this particular sample (item 6, below). Figure 6B shows the height, which was in the range of 155–225 nm for samples S1, S2, S3, and S5, and about 40–65 nm for the control and S4. The concentrations of Tween-80 or tea tree essential oil did not strongly influence the size of the nanoparticles. S2 showed a trend to smaller size when compared to S1, which can be explained by the smaller surfactant concentration in S1. The control, composed only by corn starch, clearly showed smaller size nanoparticles, maybe because it is not formed by assembled components, generating a more compact structure after drying. An interesting point that needs to be highlighted is the size of these nanoparticles measured by AFM compared with the size measured by DLS. When measured by DLS, the size of all the samples were much higher than their respective AFM images. This difference can be attributed to the measurement techniques, once the size of the nanoparticle measured by DLS represents its hydrodynamic radius, and by AFM it corresponds to the size of the dried nanoparticles.

The hydrodynamic radius is measured in solution; thus, the result corresponds to the size of the particle in the liquid, which is hydrated. The macromolecules dispersed in the liquid interact with the surrounding molecules, here, particularly, the macromolecules from corn starch, the tea tree essential oil

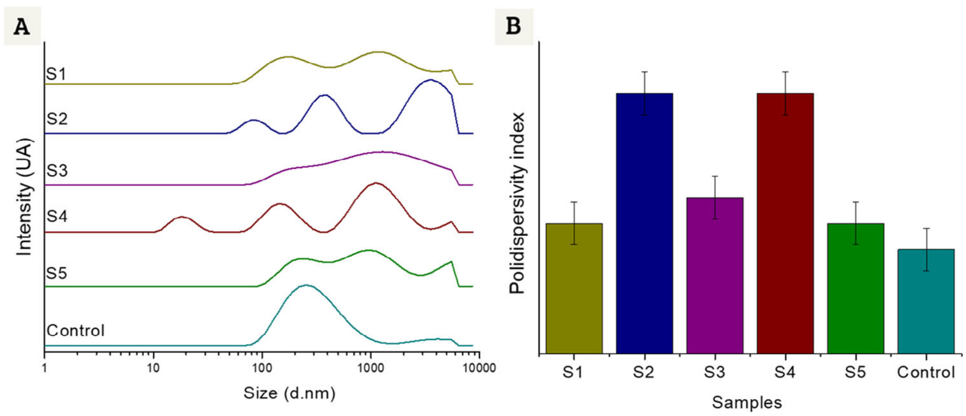

FIGURE 5 | Average particle size (A) and PDI (B).

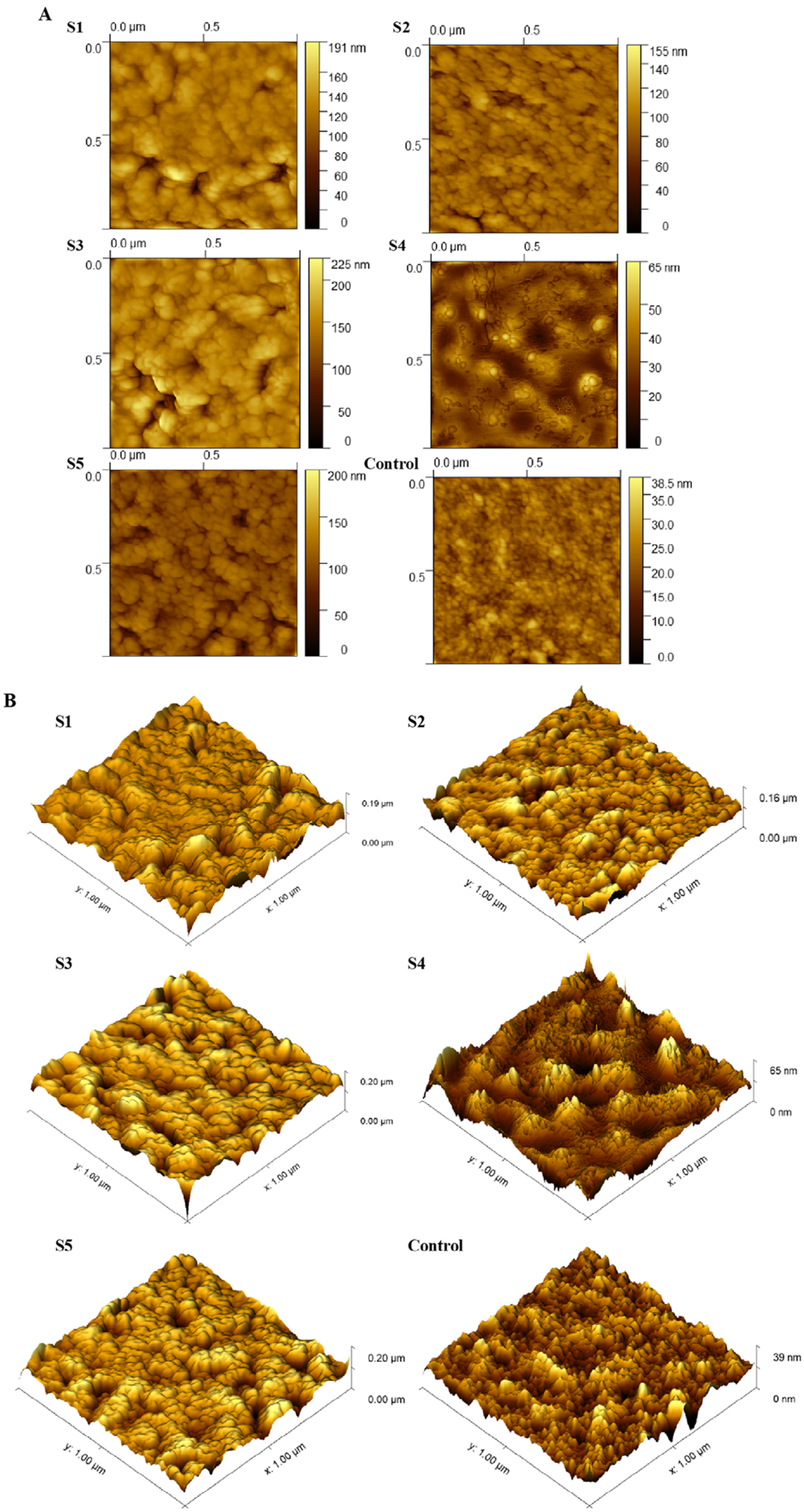

FIGURE 6 | The AFM images of the surface morphology of the samples in two-dimensional (A) and three-dimensional topography (B).

and the Tween-80, assembling the nanoparticle. Thus, when comparing the results of AFM with DLS, it is expected the smaller size particles measured by AFM, once they are dried. Also, the relationship among the particle size cannot be compared, once the DLS measured sizes represents the result of the behavior and interaction of the molecules and nanoparticles with the surrounding components. In general, the AFM provides information about the morphology of the nanoparticles and their homogeneity, and DLS results indicate the size of the hydrated particles, which should be considered for its application.

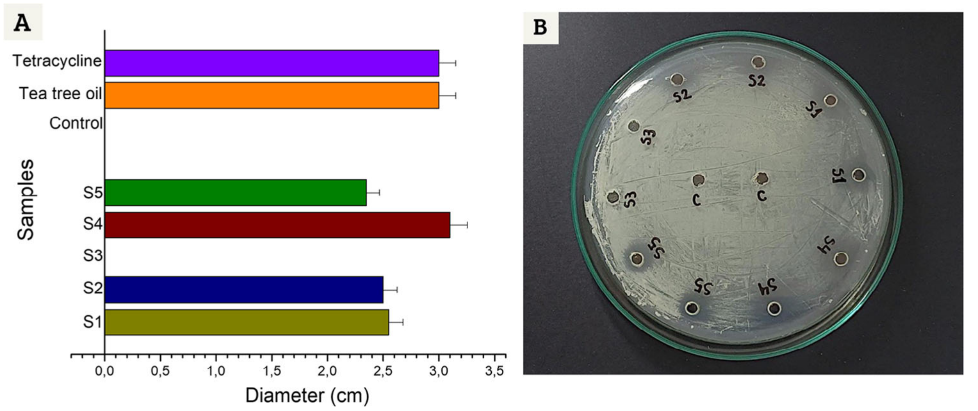

FIGURE 7 | Diameter of the inhibition zone (A) and the image of the well diffusion test showing the inhibition zone result of samples S1–S5 and the control sample (B).

5 | Encapsulation Efficiency

The encapsulation efficiency indicated the percentage of oil in the core of the nanoparticle. Samples S1, S5 (46.67%), and S2 (23.33%) showed the highest encapsulation percentage. Sample S4 displayed the lowest encapsulation efficiency (15.56%). This result is in total agreement with the AFM images, and can explain the irregular morphology of sample S4, in which few spherical structures could be seen. It was not possible to calculate the percentage of oil encapsulation for sample S3 because the low oil content in the composition.

Tea tree essential oil compounds display biological properties, however these chemical substances are susceptible to degradation by natural oxidizing agents, such as the air, destabilizing their chemical structures. In addition, many of these components are volatile. A strategy to protect the essential oil chemical components from oxidation and volatilization is the development of systems for their encapsulation. However, the processing temperature is challenging, once the components can evaporate. Here we have showed the development of a new formulation based in the natural polymer corn starch, and its processing at room temperature (25°C), to encapsulate the tea tree essential oil. The nanoparticles with high encapsulation percentages (S1, S5, and S2) can represent good systems for keeping the tea tree essential oil properties, and are expected to lead to the better performance when compared to those with low encapsulation percentages. This is the first time that corn starch was used to prepare nanoparticles loaded with tea tree essential oil using the nanoemulsion method, so, there is no data available in the literature about the encapsulation efficiency, which is reported here for the first time.

6 | Antimicrobial Potential of the Nanoparticles

The antifungal activity of the nanoparticles was assessed by diffusion assay test. Two wells were used as a control, one with the antibiotic and the other with the essential oil; the other wells were filled with the nanoparticle samples. The inhibition effect is evidenced by the growth zone of inhibition around the wells of the antibiotic, oil, samples S1, S2, S3, and S4, as shown in Figure 7A. The image in Figure 7B illustrate the inhibition zone just for the samples (S1–S5) and control, it was not used for the measurements. The sample that showed the greatest inhibition zone was sample S4 followed by sample S2. The volume of the samples was the same as the volume used in the tests. The volume of oil in S1, S2, S3, and S4 was respectively 0.3, 0.6, 0.03, and 0.9. Even with the small volume of oil in the sample's composition, all of them were capable of inhibiting microorganism growth. The antimicrobial effect with the very low amount of oil can be attributed to the increased surface area of the nanoparticles, which allowed them to get in contact in a more effective way with the microbial cell wall. In addition, the encapsulation provided an increased interaction and dispersion with the aqueous medium, which also helped to increase the contact with the microorganism, dispersed in the solid aqueous medium. An additional benefit of the encapsulation is the improvement of the stability of the active substance (oil), which makes them of great potential for use in controlling microorganisms such as yeasts.

This study shows the results related to tea tree essential oil encapsulated in corn starch nanoparticles. The methodology used to evaluate its antifungal potential was the in vitro diffusion test, in which the diffused active substance comes directly in contact with the microorganism. The results were compared with other analysis methods that also showed the antifungal potential of tea tree essential oil or its purified components, such as terpinen-4-ol. Regarding its mechanism of action, this oil or its components can penetrate the cell wall and cytoplasmic membrane of fungal strains, causing damage to these structures and leading to the loss of the cytoplasmic material. Regarding specifically the fungal strain evaluated in this study, tea tree essential oil induces changes in the permeability of *Candida albicans* cells [28, 29]. The capability of *Candida albicans* to adhere and form biofilms is one of the key features for the progression of candidiasis, and the antifungal effect of tea tree essential oil and pure terpinen-4-ol has shown efficacy in controlling biofilm proliferation under in vitro conditions [28]. Another study in the literature demonstrates that the tea tree essential oil formulation containing distilled water and Tween-80 exhibited antifungal activity against *Candida albicans*, with a minimum inhibitory concentration (MIC) of 0.1 μL/mL and the minimum fungicidal concentration (MFC) of 10 μL/mL, respectively [30].

7 | Fruit Coating and Protection Test

Edible coating is defined as a thin layer of the material that can be consumed and aims to protect the consumable by acting as a barrier to moisture, oxygen, and the movement of solutes between the food and the environment [31], as well as allowing the shelf life of the food to be extended. Coatings based on polysaccharides are versatile in terms of function and provide an ecological approach for such applications. The most common biopolymers used for coating are chitosan, carrageenan, cellulose, and others [8, 9]. Nanocarriers prepared from natural organic substances are more suitable for food applications because of their reduced environmental impact and toxicity [16].

The results of the fruit protection test can be seen in Figure 8A,B. Acerola cherry was tested for 5 days and grape for 6 days. The fruits were coated by immersing into the nanoparticle. Samples S1 and S2 were chosen for this test because they showed the highest stability, as shown by PDI results. The results indicated that the coating provided protection, preventing the fruit from rotting at room temperature, when compared to the fruits coated with a nanoparticle free of essential oil (control sample). The fruit of the control group rotted faster than that of the test groups, indicating the efficiency of the nanoparticle loaded with the tea tree essential oil in avoiding deterioration. These results mean that the coating extended the shelf life of the samples. In the field of food preservation, extending the food product shelf life by even a few days can represent considerable economic advantages for companies in the food sector [9]. The protection effect can be attributed to the tea tree essential oil antimicrobial effect, and to the formation of a protective essential oil layer on the surface of the fruits, decreasing the microorganism's proliferation rate, in agreement with the results of the antimicrobial tests in item 7.

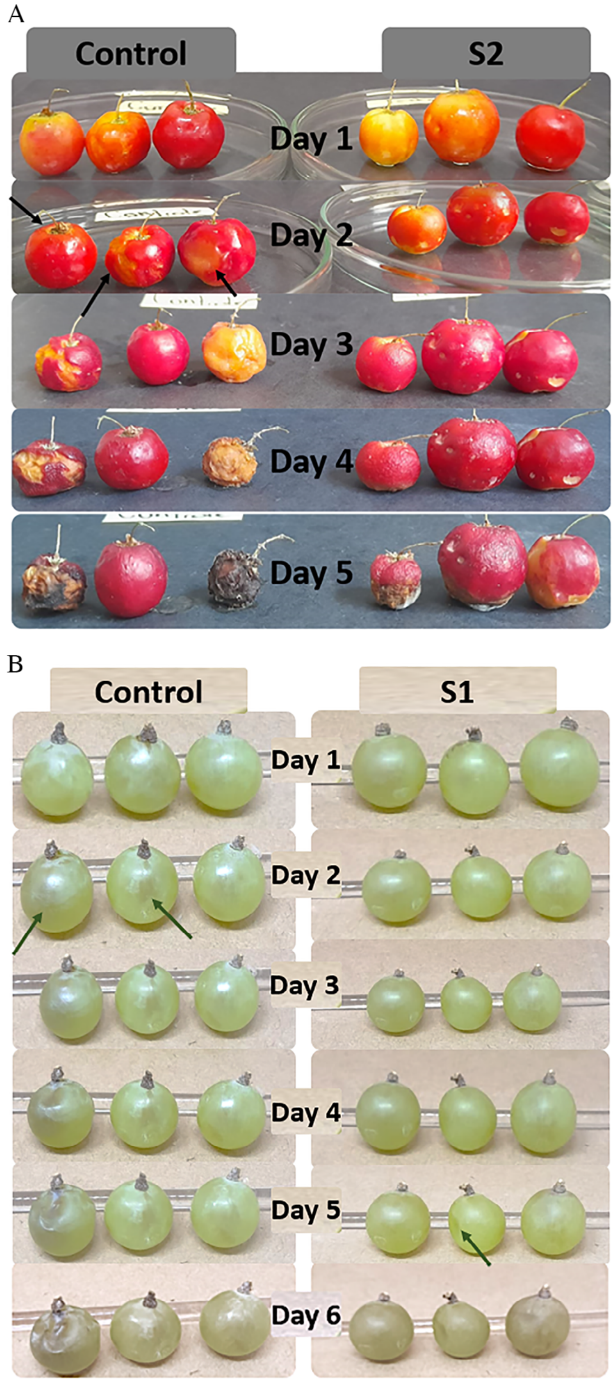

FIGURE 8 | Results of the edible coating applied on acerola cherry (A), and grape (B).

8 | Conclusion

The methodology proposed for formulating the nanoparticle was successfully carried out, once the functional and sustainable nanostructured system based on the natural polymer corn starch and the natural active substance was reached, as indicated by the results. The physico-chemical properties of the six nanoemulsion formulations, were determined by DLS, zeta potential and AFM, indicating their size in the nanoscale, ranging from 232.3 to 559.1 nm. The determination of the encapsulation efficiency showed that the nanoparticles were efficient in carrying and delivery the active components of tea tree essential oil. Samples S1 and S2 showed the highest encapsulation efficiency, ranging from about 47% to 23%, and the highest stability, as shown by Zeta potential. The samples displayed also fungal inhibition potential, as demonstrated by the antimicrobial activity test, and was effective in increasing the shelf life of fresh fruit, as shown in the preliminary tests. When used in the coating tests, demonstrated the capability to reduce the deterioration time of the fruits selected for the study. The preliminary results indicated the potential of these samples to be used as such, or improved, for future application in the field of this proposal, or in other fields. Finally, it is important to note that the development of an edible coating based on natural polymer as corn starch, and natural active substances as tea tree oil, represent an important advance in addressing the current issues related to the release of toxins and residues from synthetic materials into the environment. Preserving fruit and extending its shelf life reduces waste and ensures food safety and the food quality for consumers. In general, the nanoparticles based on corn starch and tea tree essential oil showed interesting preliminary properties that indicate their potential as a natural active system for application in fields such as food protection and others, in which the natural source of the antimicrobial formulations is valorized.

Author Contributions

Zucchini, T: methodology, data curation, writingoriginal draft, writingreview and editing, visualization, conceptualization. Nossa, TS: AFM methodology, investigation, formal analysis, data curation. Chorili M: DLS, Zeta potential methodology, formal analysis, data curation. Trovatti E: writing review and editing, data curation, resources, supervision, methodology, conceptualization, funding acquisition.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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