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Oxidative stability of sacha inchi oil microparticles covered with ovalbumin

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Abstract

The coating of microparticles obtained by ionic gelation with protein is a strategy adopted to increase the oxidative stability of different oils. In this work, the effect of different concentrations of alginate and calcium on the production of microparticles and the oxidative stability of microencapsulated sacha inchi oil coated with ovalbumin was evaluated. The sacha inchi oil demonstrated to be a source of polyunsaturated fatty acids, especially linolenic and linoleic acids. The obtained coated and uncoated microparticle showed spherical morphology with continuous walls, encapsulation efficiency about 72%, and the average size of 239 µm and 309 µm, respectively. The higher size of coated microparticle is due to high amounts of ovalbumin adsorbed, which ranged from 51.8 to 70.9%. The Oxidative stability evaluation showed that the presence of ovalbumin adsorption contributed to the stability of sacha inchi oil, when compared to the oil present in particles without protein coating.

Keywords: *microencapsulation, accelerated shelf life, egg protein, unsaturated fatty acids, oxidative stability.*

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1. Introduction

Sacha inchi oil is originally from the Amazon region and contains a high amount of Ω-3 and Ω-6 polyunsaturated fatty acids. The amount of these acids can reach 94%, with 49% of α-linolenic acids and 37% of linoleic essential fatty acids, considered essential to human health[1,2]. Thus, polyunsaturated sacha inchi oil, are subject to oxidation forming free radicals, which undergoes a reaction of oxidation responsible for producing unpleasant flavors in foods. The avoidance of its oxidation is essential, and as an alternative the microencapsulation[3] followed by coating have been proposed and systematically studied[4-7].

Among the microencapsulation techniques, the ionic gelation stands out for its simplicity and for not using toxic solvents. The production of gels as spherical microparticles can be achieved by dripping an anionic polysaccharide solution/emulsion containing the core material into a cation solution. Formation kinetics, particle volume, and stability are dependent on cation and polysaccharide concentration, which in combination with ionic strength and pH, are parameters that influence the microparticle formation[8,9].

During the gelation process, not all carboxylic groups are bound to the calcium ion, and the remaining negative charge on the particles allows another positively charged polyelectrolyte to be electrostatically adsorbed onto the microparticle surface[10]. The oppositely charged polyelectrolyte may be a protein at a pH adjusted below its isoelectric point. The interaction depends on the polysaccharide: protein ratio used, the pH and the remaining electric charge in the gelled particle and the protein used, the ionic strength, and the concentration of the polyelectrolytes[11].

Several works describe the microencapsulation of sacha inchi oil by spray drying, freeze drying and complex coacervation techniques[3,12-14], and few studies[9] report the use of ionic gelation followed by protein coating as strategy to improve the oxidative stability of sacha inchi oil and expand their use in food formulation. Hence, the

objectives of this work were to evaluate the effect of different concentrations of alginate and calcium ions on the production of microparticles containing sacha inchi oil, and then coat with ovalbumin and subsequently evaluate the properties and oxidative stability of free and encapsulated oil.

2. Materials and Methods

2.1 Material

The materials used for this study were anhydrous calcium chloride (Exodo, Brazil), pasteurized / dehydrated ovalbumin (Saltos Alimentos, Brazil), sodium alginate with high molecular weight and high guluronic acid content (Exodo, Brazil) and cold pressed sacha inchi oil with 6.50 ± 0.14 mg KOH g^{-1} of acidity and 1.73 ± 0.28 meq O_2Kg of peroxide index (Tikuna, Colombia).

2.2 Determination of fatty acid composition of Sacha inchi oil

The composition of sacha inchi oil was determined by gas chromatography (Agilent CGC 6850 Series GC System, USA), and samples were prepared as fatty acid methyl esters according to Ce 1f-96 (AOCS 1997). An Agilent Capillary DB-23 column (50% cyanopropyl and methyl polysiloxane, USA) 60 m long and 0.25 mm in diameter with 0.25 mm internal films was used to separate the methyl esters. The conditions used were: 1.0 mL/min column flow rate, 24 cm/s linear velocity, detector and injector temperature 280 °C, helium gas as the carrier gas, and injected volume was 1 µL. The column temperature was maintained at 110 °C for 5 min, then a ramp of 5 °C per min until 215 °C, remaining at this temperature for 24 min. Identification of fatty acid methyl esters was made by comparing retention times with times obtained for a standard mixture of fatty acid methyl esters (Nu Check Inc., USA).

2.3 Microparticle production by external ionic gelation

The microparticle production was performed with two concentrations of sodium alginate, 1.50% and 2.25% (w/w) and three concentrations of calcium chloride, 1.50%, 3.0% and 4.50% (w/w). Sacha Inchi oil, 0.75%, 1.50% and 2.25% (w/w), was added to the alginate solution and subjected to homogenization in a Ultraturrax at 15,000 rpm for 5 min (T-18 IKA, Germany). The emulsions were sprayed onto the aqueous calcium chloride solution, kept under constant agitation by a magnetic stirrer, using a 0.7 mm diameter double fluid atomizer (LABMAQ, Brazil), air flow of 30 L/min, and emulsion flow rate of 200 mL/h. The atomizing nozzle was kept at 23 cm above the surface of the calcium chloride solution surface. After spraying was complete, the microparticles were stirred for 30 min to ensure the migration of calcium ions into the center of the microparticles. They were then washed with deionized water (adjusted to pH 4.0) and a portion of the microparticles was lyophilized (Alpha 1-2 LDplus, Martin Christ, Germany). The wet and dry particles were packed in capped vials and kept refrigerated for further electrostatic adsorption of ovalbumin and lipid oxidation evaluation, respectively.

2.4 Electrostatic adsorption of ovalbumin on alginate microparticles

For the adsorption tests, a solution of ovalbumin (4%, w/v) and particle suspension were adjusted to pH 4.0. Fifty grams of wet microparticles were added to ovalbumin solution (200 mL) and kept under stirring for 1 h. Then the particles were washed with pH 4.0 deionized water to remove proteins that were not adsorbed by the microparticles. The amount of protein adsorbed on the microparticles was determined by the Kjeldahl method[15].

2.5 Mean diameter, size distribution, and polydispersity index

The average diameter ($D_{0.5}$) and size distribution of the microparticles with and without adsorbed proteins were determined using light scattering technique (LV950-V2, Horiba, Japan) and water as dispersing medium at pH 4.0. The polydispersity index (SPAN) was calculated by ($D_{0.9}$ - $D_{0.1}$) divided by $D_{0.5}$, where: $D_{0.9}$ - particle diameter below which 90% of the distribution was quantified; $D_{0.1}$ - particle diameter below which 10% of the distribution has been quantified and $D_{0.5}$ - considered as the average particle diameter. A minimum of six independent repetitions was performed.

2.6 Zeta potential of particles

The microparticle zeta potentials were determined using a Zetasizer Nano-Z (Malvern Instruments, UK) at pH 4.0. The microparticles were ground before dilution and reading to obtain a colloidal suspension smaller than 10 µm in size[16]. Wet microparticles were milled in a mill (IKA, Germany) at 26,000 rpm with twenty impact cycles for two sec on each impact and then sieved (62 µm) and kept in a refrigerator for sedimentation for 72 h. The colloidal supernatant was analyzed for its mean diameter in a Horiba particle diameter determiner (LV950-V2, Horiba, Japan). After size verification, the supernatant was diluted to 0.2% in deionized water adjusted to pH 4.0 and zeta potential determined with six replicates for each sample.

2.7 Encapsulation efficiency

To determine the encapsulation efficiency (EE%) of sacha inchi oil, approximately 5 g of lyophilized microparticles were subjected to a calcium citrate solution (3% w/w) for their complete solubilization and release of encapsulated sacha inchi oil. After this procedure, sacha inchi oil was extracted and quantified according to Bligh and Dyer[17], and the EE % was calculated considering the relation between extracted oil content and initial oil content[18].

2.8 Calcium adsorbed on microparticles produced by ionic gelation

Calcium quantification of the microparticles without protein-coated was performed using an atomic absorption spectrophotometer (Analytic Jena AG-NOVAA300, Germany) and a calcium solution (1000 µg/mL, SCP Science, Canada) in absorption mode with an air-acetylene flame detector. The method consisted of direct injection of a solution

prepared with 400 mg of wet particles dissolved in 25 mL of 3% (w/w) sodium citrate solution[19].

2.9 Morphology of alginate particles with and without protein coating

The morphology of the protein-coated and uncoated wet microparticles was observed with an optical microscope (BX-4, Olympus, Japan) using a 10X objective lens. Samples were illuminated by incident light provided by an optical fiber source (model LGPS, light optical fiber, Olympus 9095, Japan). Images were acquired with a digital camera (Q-Color 3, Olympus, Japan).

2.10 Oxidative stability of sacha inchi oil-free and microencapsulated

The oxidative stability of free sacha inchi oil, coated and uncoated microparticles was evaluated by peroxide index (PI) under accelerated oxidative conditions at 60 °C for 4 days[20]. Microparticles samples formed with 1.50% alginate, 1.50% calcium chloride and 1.50% sacha inchi oil were used. For uncoated microparticles, 0.5 g of sample were hydrated in 8 mL of deionized water for 18 h. Subsequently, sodium citrate (3%, w/v) was incorporated to solubilize the microparticles and release the oil. Coated microparticles (0.5 g) were suspended in 8 mL deionized water pH 7.0, and 0.5 mg/mL trypsin enzyme was added and kept for 18 h at 37 °C for protein hydrolysis, then sodium citrate solution was added to promote the release of the oil. The extraction of oil was performed according to Bligh and Dyer[17]. The PI was determined in triplicate by spectrophotometer (Libra S60, Biochrom, USA) following standard method IDF 74A (1991), and calculated using a standard $Fe^{(III)}$ curve (1 to 20 μg).

2.11 Statistical design and data analysis

2.11.1 Effect of alginate and calcium concentrations on the adsorption of ovalbumin on the particles

A 2^1x3^1 mixed factorial design with two replications was used to evaluate the effect of alginate (1.50% e 2.25%), and calcium chloride (1.5%, 3.0% e 4.5%) on the ovalbumin adsorption on the microparticles (Y_1). The experimental design and the coded and real values of the independent variables are given in Table 1. The coefficients (β) of the mathematical model (Equation 1) for each dependent variable were calculated using Statistica software (StatSoft, Tulsa, USA).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{22} X_2^2 + \beta_{123} X_1 X_2^2 \quad (1)$$

where Y is the response (protein adsorption) β_0 is grand mean, β_1 (alginate), β_2 (calcium chloride), β_{12} (alginate x calcium chloride interaction), β_{22} (calcium chloride squared) and β_{123} (alginate x calcium chloride squared interaction).

2.11.2 Effect of alginate and calcium concentrations and coating or uncoating with ovalbumin on the mean diameter, span, and the zeta potential

A 2^2x3^1 mixed factorial design with two replications was used to evaluate the effect of sodium alginate (1.50 and 2.25%), protein coating (uncoated and coated) and calcium chloride (1.5; 3.0 and 4.5%) on the mean diameter (Y_2), span (Y_3), and zeta potential (Y_4). The experimental design and the coded and real values of the independent variables are given in Table 2. The coefficients (β) of the mathematical model (Equation 2) for each dependent variable were calculated using Statistica software (StatSoft, Tulsa, USA).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{33} X_3^2 + + \beta_{133} X_1 X_3^2 + \beta_{233} X_2 X_3^2 \quad (2)$$

where Y is the response (mean diameter, span or zeta potential) β_0 is grand mean, β_1 (protein coating), β_2 (alginate), β_3 (calcium chloride) and β_{12} (coating x alginate interaction), β_{13} (coating x calcium chloride interaction), β_{23} (alginate x calcium chloride interaction), β_{33} (calcium chloride - squared), β_{133} (coating x calcium chloride - squared interaction) and β_{233} (alginate x calcium chloride - squared interaction).

3. Results and Discussions

3.1 Fatty acid composition of Sacha inchi oil

Sacha inchi oil presented 90.49% of polyunsaturated and 9.51% of saturated fatty acids, with a higher amount for linolenic fatty acid 45.51%, followed by linoleic fatty acid

Table 1. Mixed Factorial Design Matrix 2^1x3^1 - Effect of alginate and calcium chloride concentrations on the amount of protein adsorbed in the microparticles and calcium adsorbed on microparticles without adsorbed protein.

Essay	Coded Levels		Real Levels		Response	
	Alginate	$CaCl_2$	Alginate (%)	$CaCl_2$ (%)	Protein adsoption (%)	Absorbed calcium (mg Ca/g partícles)
1	-1	-1	1.50	1.50	70.97±7.63	182.94 ± 20.00
2	-1	0	1.50	3.00	62.33±3.26	117.20 ± 4.60
3	-1	1	1.50	4.50	64.30±2.10	169.05 ± 2.86
4	1	-1	2.25	1.50	69.06±1.52	149.22 ± 7.01
5	1	0	2.25	3.00	51.58±0.91	172.91 ± 7.11
6	1	1	2.25	4.50	57.84±1.32	148.34 ± 8.34

Table 2. Mixed factorial design matrix 2^2x3^1 - Effect of alginate and calcium chloride concentrations without or with protein adsorption on average diameter $D_{(0.5)}$, Span Index and Zeta potential with coded and real values used.

Essay	Coded Levels			Real values			Response		
	RP*	Alginate	$CaCl_2$	RP (%)	Alginate (%)	$CaCl_2$	$D_{0.5}$ (µm)	Span	Zp (mV)
1	-1	-1	-1	without	1.50	1.50	185.47 ± 17.99	3.18 ± 0.19	-36.00 ± 0.53
2	-1	-1	0	without	1.50	3.00	185.93 ± 20.02	2.92 ± 0.04	-16.77 ± 0.97
3	-1	-1	1	without	1.50	4.50	238.63 ± 15.09	3.05 ± 0.24	-23.17 ± 0.84
4	-1	1	-1	without	2.25	1.50	102.00 ± 9.31	3.37 ± 0.45	-16.28 ± 0.35
5	-1	1	0	without	2.25	3.00	209.49 ± 5.58	2.68 ± 0.09	-22.47 ± 1.40
6	-1	1	1	without	2.25	4.50	212.79 ± 29.17	2.63 ± 0.20	-29.33 ± 1.76
7	1	-1	-1	with	1.50	1.50	231.74 ± 11.09	2.77 ± 0.08	-27.90 ± 5.66
8	1	-1	0	with	1.50	3.00	237.56 ± 14.75	2.76 ± 0.04	-32.87 ± 0.67
9	1	-1	1	with	1.50	4.50	308.79 ± 68.41	2.32 ± 0.24	-27.93 ± 0.31
10	1	1	-1	with	2.25	1.50	213.04 ± 18.66	2.48 ± 0.08	-19.33 ± 1.10
11	1	1	0	with	2.25	3.00	204.37 ± 23.86	2.42 ± 0.10	-27.07 ± 1.16
12	1	1	1	with	2.25	4.50	221.47 ± 17.05	2.56 ± 0.05	-28.23 ± 0.45

*RP: Protein adsorption; Zp: zeta potential.

Table 3. Fatty acid composition of sacha inchi oil.

Fatty acid	(%)	Fatty acid	(%)
C12:0 – lauric	0.06 ± 0.02	C18:2 - trans-t-linoleic	0.08 ± 0.00
C14:0 – miristic	0.07 ± 0.01	C18:2 – linoleic	35.13 ± 0.02
C15:0 – pentadecanoic	0.02 ± 0.00	C18:3 - trans-t-linolenic	0.25 ± 0.00
C16:0 – palmític	5.56 ± 0.08	C18:3 – linolenic	45.51 ± 0.01
C16:1 – palmitoleic	0.12 ± 0.01	C20:0 – araquídic	0.1 ± 0.01
C17:0 – margáric	0.14 ± 0.00	C20:1 – eicosenoic	0.22 ± 0.00
C17:1 - cis-10-heptadecenoic	0.06 ± 0.00	C22:0 – behenic	0.06 ± 0.01
C18:0 – stearic	3.47 ± 0.02	C24:0 – lignoceric	0.03 ± 0.01
C18:1 – oleic	9.12 ± 0.03		

35.13% and oleic acid 9.12%, out of a total of 17 identified fatty acids (Table 3). Concentration of 59.23% linolenic acid and 33.46% linoleic acid were previously reported[12]. Minor differences in oil composition may be due to variations in lipid concentrations of sacha inchi oil due to the time of seed harvest, soil composition, climatic and geographical variations, and also different subspecies[21].

3.2 Electrostatic adsorption of ovalbumin on alginate microparticles

The amount of protein adsorbed on the ionic gelation particles ranged from 51.84 ± 0.91% to 70.94 ± 7.63% (Table 1). In a previous study, variations in protein adsorption by the particles of alginate were between 14.84 ± 0.64% to 47.37 ± 1.15%[6]. According to the factorial design model, the use of a lower concentration of calcium chloride (1.50%) and alginate (1.50%) led to higher levels of protein adsorption, and the model can be described as $Y_1 = 62.71 - 3.14X_1 - 4.46X_2 - 4.23X_2^2$. The coefficient of determination (R^2) was 0.77, and although it is a low value, it is suitable to evaluate the effect of calcium and alginate concentration on the protein adsorption. Concentrations of calcium chloride above 1.50% decreased the amount of protein adsorbed by the alginate microparticles (Figure 1a). The higher adsorption of ovalbumin at lower concentrations of calcium and alginate was due to the greater availability of non-crosslinked negative carboxylic groups allowing a higher association with the protein below its isoelectric point, and therefore positively charged. Protein adsorption on the surface of the microparticles is related to the amount of total charge available on these microparticles, where microparticles with different zeta potentials assimilated different amounts of proteins.

3.3 Average diameter size and polydispersity index

Alginate particles produced without protein coating had mean diameters ($D_{0.5}$) ranging from 102.00 ± 09.31 µm to 238.63 ± 15.09 µm (Table 2). Different sizes can be obtained due to various parameters such as atomizer nozzle tip diameter, atomizer nozzle tip distance, ion, and alginate concentration and alginate chemical composition[22]. For this response, the follow mathematical model was obtained $Y_2 = 212.61 + 23.55X_1 - 18.75X_2 + 31.18X_3 - 9.81X_1X_3 - 8.95X_1^2X_3^2 + 12.25X_2^2X_3^2$ ($R^2 = 0.72$). The coefficients indicated that the linear parameters of protein coating, alginate, and calcium chloride had a greater influence on the microparticle diameter, and there were interactions between protein coating and calcium chloride and alginate and calcium chloride.

The response surface (Figure 1b) shows that the higher the calcium concentrations, the higher the diameters of uncoated microparticles, and this parameter had the highest positive

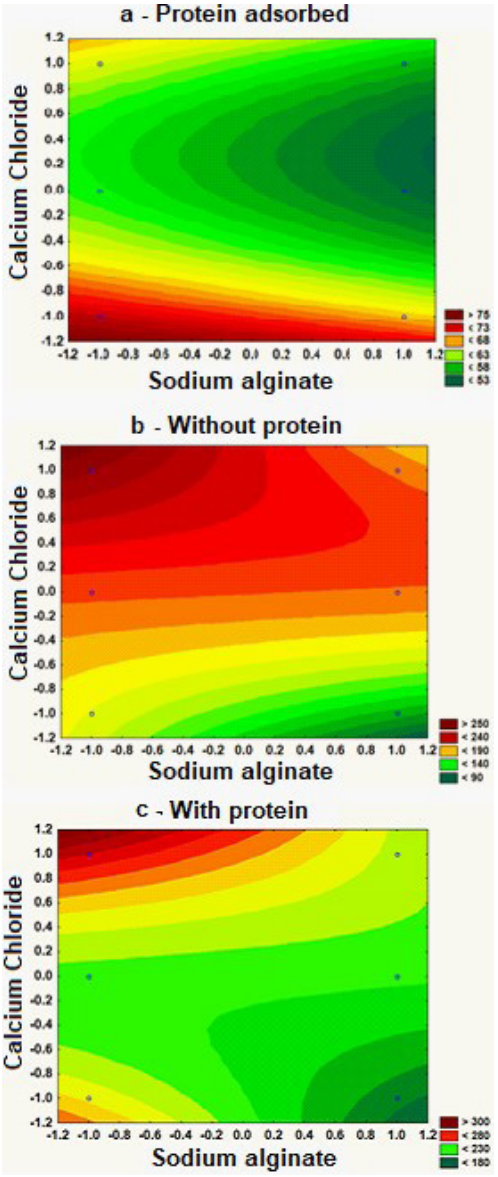

Figure 1. Effect of calcium and alginate concentration on protein adsorbed on the particles (a), mean diameter of microparticles ($D_{0,50}$) without protein adsorbed (b) and mean diameter of microparticles ($D_{0,5}$) with protein adsorbed (c).

effect on microparticle size. Smrdel[23] reported that particles produced with 0.10 and 0.27 M of calcium chloride solution were significantly smaller than particles produced with 0.50 and 1.00 M of calcium chloride solution. Zeeb et al.[24] worked with alginate and calcium chloride and obtained microparticles with smaller diameters with increasing calcium concentration. According to literature[25], higher calcium concentration enables calcium crosslinking with the alginate by increasing the number of crosslinks in the particle, producing particle-matrix compaction, which should produce a particle size decrease. In our experiment, the calcium concentrations probably were not high enough to increase the calcium-alginate crosslinking and, consequently, reduce the diameter of the particles.

The average diameter of microparticles without protein coating had decreased with higher alginate concentration (2.25%) and lower concentrations of calcium chloride (Figure 1b). The alginate concentration had a negative effect on the average microparticle diameter because of the larger number of carboxylic groups in the most concentrated solution, allowing a broader set of interactions. The gel becomes stronger as the crosslinked groups increase, reducing the spaces occupied by the alginate, decreasing particle diameters under these conditions[26]. Zeeb et al.[24] working with nanoemulsions dripped in a calcium solution, also observed that an increase in alginate concentration led to the formation of more rigid spheres, with a decrease in pore and particle size.

Adsorption of protein molecules on the surface of the microparticles by electrostatic interaction led to an increase in their mean diameter, regardless of the alginate concentration used (Table 2). With the ovalbumin coating, the microparticles produced with higher alginate concentration had a smaller average diameter (Figure 1c), but had a higher average diameter than those without protein coating. Tello et al.[6] reported similar behavior in a study with whey protein and ovalbumin, the microparticles produced with alginate and coated with protein had their diameters increased.

The coated particles diameters ranged from 231.74 ± 11.09 µm to 308.79 ± 68.41 µm (Table 2), and the coating had a significant and positive effect on the microparticle size, and negatively interacted with calcium chloride. This behavior demonstrates that the negative interaction between coating and calcium chloride leads to a limitation on the influence of the coating parameter on the average diameter of the microparticles. Particle diameter increased when 1.50% alginate and 1.50% calcium chloride particles were coated with protein (Table 2), coinciding with the point where there was higher protein adsorption by the microparticles.

The polydispersity indices (Span) were high (Table 2), ranging from 2.32 ± 0.24 to 3.37 ± 0.45, and the model can be described as $Y_3 = 2.76 - 0.21X_1 - 0.07X_2 - 0.15X_3$ (R^2 = 0.55). Ovalbumin protein coating and higher alginate and calcium chloride concentrations reduced the polydispersity index leading to the formation of particles with more uniform diameters. The microparticles produced with 1.50% alginate, without protein coating, presented the highest polydispersity indexes.

3.4 Zeta potential of particles

Particles without coating produced with the lowest alginate and calcium concentration had a significantly lower zeta potential, due to the higher binding of calcium cation with negative groups of alginate at higher calcium concentrations. More calcium available in solution provides a higher calcium ion-carboxylic group association of the guluronic acid present in the gel and thus produce less negative charge on the particle.

The zeta potentials of microparticles without protein (Table 2) were all negative as observed before[27]. Alginate, as an anionic polymer when in solution, presents charge and negative zeta potential above its pKa value as reported before, -70 mV at pH 4.0[28]. After bound to calcium ions during gelation, the zeta value decreases, meanwhile

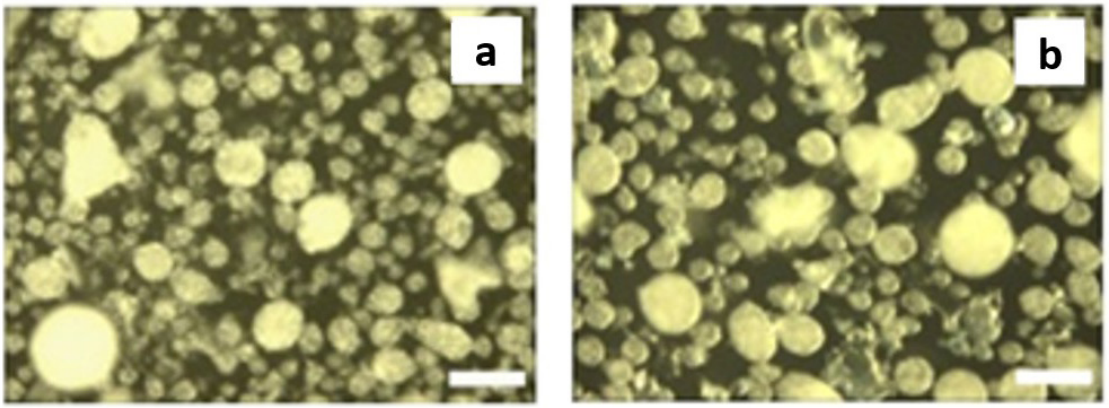

Figure 2. Morphology of microparticles produced by ionic gelation without (a) and with ovalbumin (b) adsorbed. Bars = 200 µm.

remaining negative as not all carboxylic groups bound with available ions. The remaining negative charge after gelation allows electrostatic interaction with positively charged polyelectrolytes[10].

The zeta potential values of the coated particles were negative, contradicting the theory that when a new positive polyelectrolytic layer, in adequate quantities, is adsorbed onto an oppositely charged surface produces a charge inversion of the zeta potential[16] These results suggest that the grinding augmented the surface areas containing negatively charged carboxylic groups, not associated with calcium ions, and also not associated with the positive charges present on the protein. A systematic investigation of the effect of grinding on the resulting zeta potential needs to be further investigated.

3.5 Encapsulation efficiency of sodium alginate particles

The experimental condition of lower alginate concentration (1.50%), lower calcium concentration (1.50%) and oil content of 1.50% was chosen for the determination of encapsulation efficiency (EE%). For this system, the EE% was 72.38 ± 5.53%. Chan (2011)[29] worked with 30% oil in relation to total solids and reported that encapsulation efficiency increased as the alginate concentration in solution increased, achieving a maximum encapsulation efficiency of 93%. Sacha inchi oil encapsulated by complex coacervation[30] and spray drying[31] showed encapsulation efficiency of 99.54% and 96.3%, respectively, higher than obtained in this work.

3.6 Calcium adsorbed on microparticles produced by ionic gelation

The calcium adsorbed by the particles ranged from 117.20 ± 4.60 to 182.94 ± 20.00 mg Ca/g microparticles (Table 1). Similar values (155 to 182 mg Ca /g particles) were previously reported[5] for particles produced by ionic gelation (2% of alginate solution and 0.8; 1.6 and 2.4% of calcium solution).

3.7 Morphology of alginate particles

All the other conditions studied produced spherical particles with cohesive structure (Figure 2a) due to an efficient polymeric cross-linking between the polysaccharide and calcium ions. There were enough binding sites to maintain a structure capable of trapping sacha inchi oil inside the particles, protecting the lipid reaction with the environment and providing an increase in the shelf life of the oil. The alginate particles coated with ovalbumin (Figure 2b) had the same characteristics as observed for particles without protein. Although spherical and continuous, the micrographs showed particles with a high polydispersity in their size with or without protein coating.

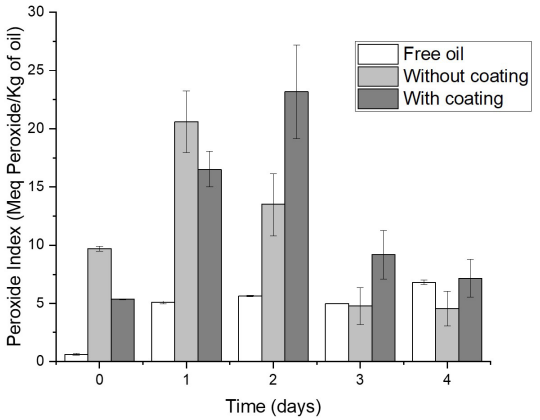

Figure 3. Peroxide Index (Meq Peroxide/Kg of oil) in accelerated oxidative stability.

3.8 Oxidative stability of free and microencapsulated sacha inchi oil

The peroxide index (PI) (Figure 3), regardless of whether the oil was free or encapsulated had oxidation peaks at different times for the different treatments and then a drop in the PI values. This behavior was previously reported[32,33], and according to the theory of lipid oxidation, in the early stage of lipid oxidation, hydroperoxides are formed until they reach a maximum oxidation value, reducing these values at the final oxidation stage due to the formation of secondary compounds not detected by the determination of the peroxide index[34].

The PI peak of the non-microencapsulated oil occurred after the fourth day, in accelerated conditions, corresponding to 120 days at room temperature[20]. For microencapsulated oil without protein coating, the PI occurred after the first day, corresponding to 30 days at room temperature and for microencapsulated with protein after the third day, corresponding to 90 days at room temperature.

The PI of the free oil occurred after the PI observed for microencapsulated oil with or without protein coating, a non-expected result, and contradicts the premise that microencapsulation would protect the oil from oxidative rancidity. According to Polavarapu et al.[32], non-encapsulated olive and fish oil were more stable to oxidation than encapsulated ones due to the smaller surface area of the non-encapsulated oils making it challenging to compare them. On the other hand, Strobel et al.[35] studied the oxidative stability of fish oil microencapsulated in alginate microparticles, and they observed the higher stability of the polyunsaturated fatty acids present in the microencapsulated oil than the non-microencapsulated ones. The PI alone is not an entirely reliable method for assessing oil quality and other tools that, as follow the secondary compounds formed during the oxidation process, could give more accurate information of the oil quality[33].

When the time of occurrence of PI peak of microparticles without protein coating is compared with microparticles with one additional layer of protein, it can be seen better protection after protein layer addition as observed before[6]. Authors used a model oil, microparticles produced by ionic gelation with and without protein coating. Both particles covered with whey protein or ovalbumin were more protective against oxidation than microparticles without protein adsorption.

At the initial time, the PI value for free oil was 0.63 ± 0.08 meq peroxide /kg of oil, and Cisneros et al.[34], reported a PI of 0.57 ± 0.01 meq of peroxide/kg of oil, close to that observed in this work. The microparticles coated with ovalbumin had a PI of 5.38 ± 0.04 meq of oil/kg, and the microparticles without coating presented a PI of 9.70 ± 0.22 meq of peroxide/kg of oil. The variations in PI at the initial time were probably due to the manipulation during the emulsification, atomization, microencapsulation, and especially during the oil removal from the particles for the subsequent determination of PI. Figure 3 also shows a variation of PI, probably because, at the later stage of the oxidation process the concentrations of primary oxidation products decrease, and the formation rates of new oxidation products are lower than their oxidation rate extinction. Smith et al.[33] observed that the primary compounds formed in the oxidation process extinguish as quickly as they are formed, which corroborates with the idea that a proper and reliable method for assessment of the oxidative products is crucial to define the oil quality.

4. Conclusions

Sacha inchi oil presented a high percentage of polyunsaturated fatty acids, and their encapsulation by ionic gelation had a high efficiency (~ 72%). Lower calcium concentration (1.50%) during ionic gelation enhanced the protein adsorption and increased the diameter of the microparticles. Particles presented a spherical format and a well-defined and stable structure, with high polydispersity. The ovalbumin adsorbed on the particles contributed to the stability of microencapsulated sacha inchi oil. However, although the peroxide index is an indicator of oils oxidation, evaluations of secondary products from oxidation are required for a more refined assessment of the actual oxidation state of oil protected by different types of microparticles.

5. Author's Contribution

- **Conceptualization** – Ermelindo de Souza Silva Neto; Carlos Raimundo Ferreira Grosso; Lyssa Setsuko Sakanaka.
- **Data curation** – NA.
- **Formal analysis** – Margarida Masami Yamaguchi; Marianne Ayumi Shirai; Izabela Dutra Alvim; Fabio Yamashita.
- **Funding acquisition** – NA.
- **Investigation** – Claudio Takeo Ueno; Izabela Dutra Alvim.
- **Methodology** – Margarida Masami Yamaguchi; Full Name.
- **Project administration** – Carlos Raimundo Ferreira Grosso.
- **Resources** – NA.
- **Software** – NA.
- **Supervision** – Carlos Raimundo Ferreira Grosso; Lyssa Setsuko Sakanaka.
- **Validation** – NA.
- **Visualization** – NA.
- **Writing – original draft** – Ermelindo de Souza Silva Neto.
- **Writing – review & editing** – Marianne Ayumi Shirai; Lyssa Setsuko Sakanaka.

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