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Polysaccharide from *Inga edulis* Pods: Emulsifying Potential and Evaluation of the Extraction Conditions Using Taguchi Approach

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Inga edulis fruits, an important cultivation commonly associated with coffee and cacao farming, were collected from Baturité, located in Ceará, Brazil. Polysaccharides from *Inga edulis* pods were extracted using citric acid solution, and the impact of the extraction conditions in the properties of the resultant polysaccharide such as the degree of methyl-esterification behavior and galacturonic content were evaluated utilizing Taguchi approach, an efficient and easy statistical tool. For the study, the extraction conditions applied were pH (2.4, 5 and 7), time (1, 2 and 3 h) and temperature (70, 80 and 90 °C). The condition which provides the higher degree of methyl esterification allied to higher galacturonic acid content was pH 2.4, time 1 h and temperature 90 °C. For this condition, the extraction yield obtained was 3.97% m/m polysaccharide/botanical material. The emulsifying capacity and emulsion stability were also evaluated, and the emulsions with lower concentrations of polysaccharide provided the best results about emulsifying action. In the best of our efforts in research, the characterization of polysaccharide from fruit pods of *Inga edulis* is reported for the first time and this research contributes to the knowledge about botanical potential of this vegetal species and reinforces its potential as industrial crop.

Keywords: *Inga edulis*, Taguchi design, polysaccharide, emulsion

Introduction

Polysaccharides are important natural polymers formed by monosaccharides units linked by glycosidic bonds and with remarkable characteristics such as low toxicity and therapeutic properties that allow their application in several areas, such as food industries, pharmaceutics, human tissue engineering and others.[1-3] Allied to biological properties, emulsifying capability is one of these interesting characteristics of polysaccharides, since their addition impacts the rheological behavior of the emulsions. Due to the toxicity of some synthetic emulsifier, natural polysaccharides and their emulsification performance are described in literature by several researchers.[4,5] In addition to this fact, the impact of the extraction methods and conditions on the structural and functional properties of polysaccharides were also evaluated in the literature. Since the extraction of polysaccharides using enzymes,[5,6] alkali,[7,8] or acid solutions[8] affect them electrostatically and modify the ability to support the interface water-oil of emulsions, they also affect the emulsifying properties.

The botanical species evaluated in the present study, *Inga edulis*, can be found in South and Central America and its culture is important for other vegetal species such as coffee and cacao trees, once *Inga* acts as a shade tree and nitrogen source.[9] Since the cultivation of *Inga* species is already associated to others industrial crops, the potential of *Inga* as resource of products with economic value is considerable, however, completely ignored. The literature also describes interesting characteristics of *Inga* species that reinforce its potential as a crop with economic value. The leaves of *Inga edulis* are rich in polyphenols and flavonoids and several studies about the extraction of those compounds and optimization of the process are reported in the literature.[10-12] Pectin from the pulp of *Inga* ssp., its

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characterization and the potential as antiviral agent was reported in literature,[13] but it was not found other studies about the pods of *Inga edulis* and its potential as source of polysaccharides. Given those considerations, the present research describes the extraction of polysaccharides from the *Inga edulis* pods, its characterization using spectroscopic and chromatographic techniques and investigation of their potential as emulsifying agent. The fruits utilized in the present study were obtained from *Inga* trees already associated with coffee farming located in an important coffee producing region from Ceará, Brazil. In addition to the study, the effects of extraction conditions in the yield, degree of methyl-esterification and galacturonic acid content of the resultant polysaccharide using experimental modeling by Taguchi approach were evaluated. The emulsifying properties were determined for the polysaccharide obtained under the determined optimal conditions of extraction. The present study aims to prove the ability of *Inga* species as commercial growing and to provide products with economical interest, since the cultivation of *Inga* trees is easy and benefic to others important industrial crops.[9]

Experimental

Inga fruits were collected in Mulungu district, located in Baturité mountain range, Ceará, Brazil. After the collection, fruit and pod were separated (Figure 1) and the pods were dried in an oven at 50 °C for 48 h. *Inga* fruit pods were grounded and extracted with ethanol (Synth, 99.5%, Rio de Janeiro, Brazil) for 24 h (500 mL of ethanol each 100 g of grounded pods) to remove the alcohol soluble residues. Then, ground pods were dried again in oven at 50 °C for 24 h. The final material was stored in plastic containers protected against exposition to solar light and identified as IDP.

Figure 1. Pods, seeds, and pulp from *Inga edulis* fruits after benefit.

Polysaccharide extraction was conducted as described: extractor solution was prepared using citric acid (Sigma-Aldrich, 99.0%, Rio de Janeiro, Brazil). It was used 200 mL of extractor solution for each 10 g of IDP. The system was kept under stirring and controlled temperature. pH was adjusted using potassium hydroxide (Vetec, P.A., Rio de Janeiro, Brazil) solution when necessary. After the extraction, insoluble material was quickly filtered and to the liquid phase was added absolute ethanol in proportion liquid phase:ethanol 1:3. Then, the resultant dispersion was kept in refrigerator overnight in order to coagulate the polysaccharide. The precipitated polysaccharide was recovered by centrifugation (8000 rpm during 20 min). The resultant precipitated was re-solubilized in distilled water, re-precipitated in absolute ethanol (Synth, 99.5%, Rio de Janeiro, Brazil) using the same proportion, liquid phase:ethanol 1:3 and recovered by centrifugation. This final process was repeated three times. The polysaccharide was dried in oven at 40 °C overnight and the yield of extraction was expressed in relation to polysaccharide mass/IDP mass. The final polysaccharide was identified as P-IDP.

Taguchi experimental design was utilized to evaluate the influence of extraction conditions in the extraction yield, the degree of methyl-esterification (DM) and galacturonic acid content of P-IDP. It was utilized three levels for the parameters. The extraction conditions applied in this study were pH (2.4, 5 and 7), time (1, 2 and 3 h) and temperature (70, 80 and 90 °C). The process variables were coded at three levels (1, 2 and 3). The experimental model contained 9 experiment runs described in Table 1 (each experiment was conducted in duplicate and expressed the average value). The data was analyzed, and the optimum conditions were obtained using statistical software Minitab 19.[14] Statistical analysis of variance (ANOVA) was utilized to determine which parameters are significant for the experiment in the statistical point of view and conducted the Fischer test (*F*-value) and *P*-value. *F*-value and *P*-value points show how the parameters affect the response and the significance of the experiment model. Analysis of the signal to noise ratio (S/N) of the parameters allows the optimized level for each parameter and the optimal conditions for the extraction can be obtained. At the end, a final experiment was conducted for validation of the Taguchi prediction of the optimal conditions of extraction.

Infrared spectroscopy was conducted using PerkinElmer® spectrometer (Massachusetts, USA) model FTIR Spectrum 1000 with a liquid film between KBr cells and the spectra were expressed by the relation between wavenumber and transmittance with 4000 to 400 cm^{-1} range. The esterification degree of the samples was determined using Fourier transform infrared (FTIR) spectra, which is

Table 1. L9 experimental matrix developed by Taguchi approach using Minitab 19.[14] The condition of each experiment and value found for degree methyl esterification (DM), galacturonic acid content and yield was also described

Run	pH	Temperature / °C	time / h	DM / %	Galacturonic acid / %	Yield / %
1	2.4(1)	70(1)	1	47.4	14.4	1.36
2	2.4(1)	80(2)	2	32.9	13.2	2.57
3	2.4(1)	90(3)	3	52.5	9.1	2.98
4	5(2)	70(1)	2	33.8	10.2	1.18
5	5(2)	80(2)	3	36.9	7.9	2.25
6	5(2)	90(3)	1	36.3	6.0	3.10
7	7(3)	70(1)	3	18.3	22.4	3.96
8	7(3)	80(2)	1	17.4	16.6	2.38
9	7(3)	90(3)	2	20.2	12.8	2.24

Levels in parenthesis.

largely discussed in literature.[15,16] For that, it was calculated the ratio between the area under the peak corresponding the methyl-esterified units at 1750 cm^{-1} and the sum of the area of methyl esterified units and non-methyl esterified units at 1650 cm^{-1}. The galacturonic acid content was determined using the method described by Blumenkrantz and Asboe-Hansen,[17] with some modifications. A small quantity of polysaccharide sample (5 mg) was solubilized using 2 mL of distilled water in a test-tube. Then, it was added 7 mL of 0.0125 mol L^{-1} sulfuric acid (Synth, Rio de Janeiro, Brazil)/sodium tetraborate (Sigma-Aldrich, 97.0%, Rio de Janeiro, Brazil) solution and the resultant solution was cooled in crushed ice. After cooling, the tube was shaken in vortex and heated in water bath at 100 °C for 5 min. The solution was cooled until room temperature and 20 µL of *m*-hydroxydiphenyl (Sigma-Aldrich, 85.0%, Rio de Janeiro, Brazil) was added to the test-tube. Then, the solution in test-tube was homogenized in vortex for 5 min and the absorbance of the sample was measured at wavelength of 520 nm in UV-Vis spectrometer Thermo Fisher Scientific (Massachusetts, USA), model Genesis 6. The blank sample was prepared by the addition of 2 mL of sodium hydroxide (Vetec, P.A., Rio de Janeiro, Brazil) 0.5% solution and without addition of pectin sample.

The monosaccharide composition of P-IDP was determined as described: a small sample of P-IDP was hydrolyzed with 4 mol L^{-1} trifluoroacetic acid (Sigma-Aldrich, 99.0%, Rio de Janeiro, Brazil) for 6 h at 100 ºC. Then, the material was submitted to rotaevaporation and washed three times with methanol (Synth, P.A., Rio de Janeiro, Brazil). The released monosaccharides were converted into alditol acetate by successive reduction with sodium borohydride (Sigma-Aldrich, 98.0%, Rio de Janeiro, Brazil) and glacial acetic acid (Synth, 100%, Rio de Janeiro, Brazil). The acetylation was conducted using anhydrous acetic (Synth, 99.0%, Rio de Janeiro, Brazil) and pyridine (Synth, 99.0%, Rio de Janeiro, Brazil) (3:1 v:v) at 100 °C for 3 h. At the end of the reaction, the acetylated derivatives were solubilized in chloroform (Synth, 99.8%, Rio de Janeiro, Brazil) and pyridine complexed was removed using solution of copper sulfate (Sigma-Aldrich, 99.9%, Rio de Janeiro, Brazil) 5%. Chloroform was removed using evaporation under reduced pressure and the final product was exhaustively washed with deionized water and dried through evaporation under reduced pressure. Chromatography analysis was performed on an Agilent (California, USA) gas chromatography coupled to mass spectrometer GC-MS model 5977 equipped with column model 5190-2293 and dimensions 30 m × 250 µm × 0.25 µm. The chromatographic conditions were: temperature program from 190 to 230 °C at 4 °C min^{-1} and helium at an inlet pressure of 15.5 psi was utilized as carrier gas and mass spectrometer utilized scan rate 1 scan s^{-1} over the range *m/z* 40-600.

Thermogravimetric analysis (TGA) was conducted on an TA Instruments equipment model Q50 V20. Synthetic air was utilized as atmosphere, platinum crucible as port sample, heat rate of 10 °C min^{-1}, and 800 °C as maximum temperature of analysis.

Oil-in-water emulsions were formulated using 15 wt.% oleic acid (Sigma-Aldrich, P.A., Rio de Janeiro, Brazil), 0.1, 0.4, 0.7 and 1.0 wt.% P-IDP, 0.1 wt.% sodium benzoate (Sigma-Aldrich, 99.5%, Rio de Janeiro, Brazil) (bactericide) and ultrapure water. Polysaccharide sample and sodium benzoate were dispersed in ultrapure water and stirred at 200 rpm for 12 h. Subsequently, oleic acid was added into the mixture, homogenized using a UltraTurrax T25 (IKA, German) at 12.000 rpm for 3 min. Then, P-IDP emulsions were sonicated (70% power, 20 s on/20 s off) for 1 min using an ultrasound sonifier W-450D (Branson, USA). The emulsions were stored at room temperature (25 °C) for 40 days. The droplet diameters (z-average), distribution

(polydispersity index, PDI) and zeta-potential (ζ) of P-IDP emulsions were measured using Malvern NanoZS90 (Malvern, UK). Emulsifying capacity (EC) and emulsifying stability (ES) after 40 days were calculated as equations 1 and 2.[18]

$$EC\,(\%) = \left(\frac{EL_i}{EH}\right) \times 100 \quad (1)$$

$$ES\,(\%) = \left(\frac{EL_f}{EL_i}\right) \times 100 \quad (2)$$

where the emulsified system volume, the initial emulsified layer volume (EL_i) and the final emulsified layer volume (EL_f) were measured during the analysis period.

Micrographs of the emulsions were taken using a Leica Microsystems microscopy (Wetzlar, Germany) model DM2500P equipped with Teledyne Photometrics Qimage digital camera (California, USA). After dilution in ultrapure water (1:5 ratio), 50 µL of each diluted emulsion were placed on a glass slide and the images captured at 100× magnification.

Results and Discussion

The infrared spectra of the samples after extraction under specific conditions were depicted in Figure 2. It was observed that the bands for methyl-esterified units were more intense for the conditions 1, 2 and 3. In common, those conditions employed the same pH, 2.4. On the other hand, in the spectra obtained in conditions that employed the highest pH utilized in the experiments, the band at 1750 cm^{-1} was gradually suppressed and the increase of the intensity of the band 1650 cm^{-1}, which corresponds to non-esterified groups, was observed. Those data suggested the pH as a factor which impacts drastically the degree of methyl-esterification. The suspect was confirmed by statistical analysis of the experiment using Minitab 19,[14] as described below. Using as desired response "larger is better" for the degree of methyl-esterification, the main effects plot for signal-to-noise ratio (Figure 3) expressed the importance of pH in the degree of methyl-esterification, once its *F*-value is expressively larger than observed to temperature and time of extraction as factors. In fact, the *P*-value observed to pH as a factor of influence in the degree of esterification was the lowest in comparison with other factors (Table 2). By the analysis of Figure 3, the larger value of DM was obtained in the lowest pH employed in the experiment, 2.4. The interactions between the factors were not plotted due to being insignificant by the statistical analysis of the data.

The main effects for signal-to-noise ratio plot (Figure 4) and analysis of variance for the content of galacturonic acid as response (Table 3) were also obtained. The response "large is better" was also choice in the analysis. Once again, the interactions between the factors were statistically insignificant. The results depicted in Figure 4 suggested the pH as the most important factor that impacts the content of galacturonic acid but the impact of the temperature was also expressive. Thus, by the analysis of variance for the content of galacturonic acid as response (Table 3), when compared pH and temperature as factor of influence, *F*-value was higher to pH, but considerable to temperature. The *P*-value observed to pH was lower than 0.05, but the value found for temperature was also closely to the interval of confidence employed in the statistical analysis.

By analyzing the impact of the factors in the yield, the *F*-value and *P*-value test showed that the factors in the level evaluated in the present study were statistical irrelevant. This way, after analysis of the Taguchi graphics obtained from degree of esterification and galacturonic acid content as response, pH 2.4 and temperature of 90 °C was determined as optimum condition; once the time was statistical irrelevant in the experiment for all the responses

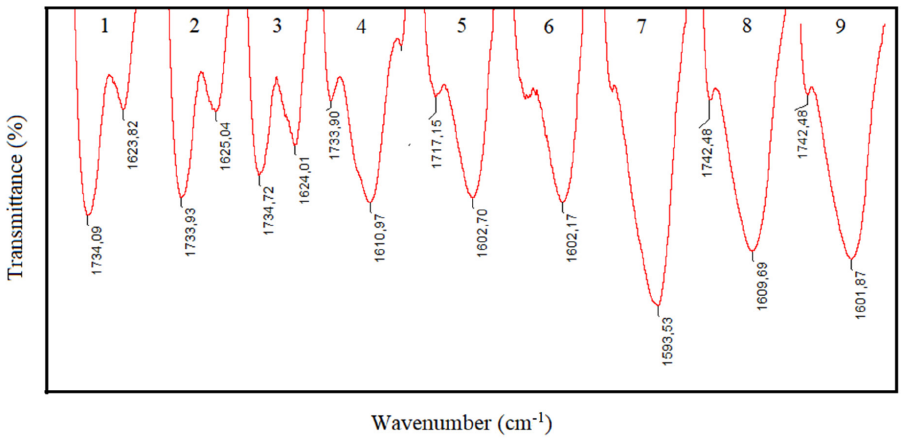

Figure 2. Infrared spectra (FTIR-KBr) of polysaccharides from *Inga edulis* under specific extraction conditions and the variation of the bands correlated to methyl esterificated group.

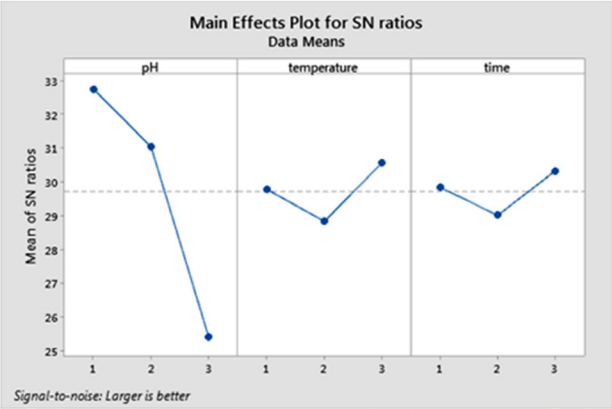

Figure 3. Main effects plot for signal-to-noise ratios when the response was degree of methyl-esterification of P-IDP using Taguchi approach as statistical tool.

Table 2. Analysis of variance (ANOVA) of the degree of methyl-esterification (DM) as response for P-IDP using Taguchi approach as statistical tool

Source	DF	Adj SS	Adj MS	*F*-value	*P*-value
pH	2	1021.16	510.58	16.65	0.057
Temperature	2	79.64	39.82	1.30	0.435
time	2	75.32	37.66	1.23	0.449
Error	2	61.34	30.67		
Total	8	1237.46			

DF: degree of freedom; Adj SS: adjusted sum of square; Adj MS: adjusted mean squares.

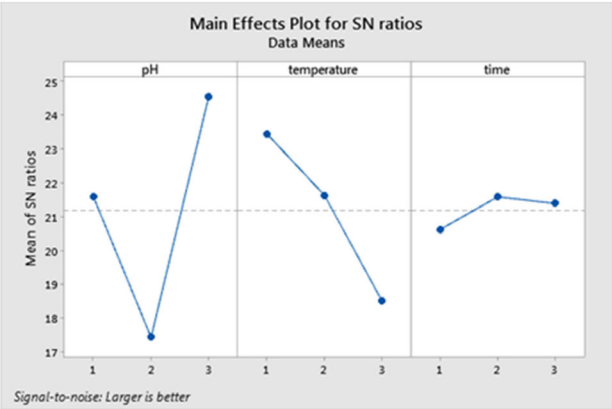

Figure 4. Main effects plot for signal-to-noise ratios when the response was the galacturonic acid content of P-IDP using Taguchi approach as statistical tool.

Table 3. Analysis of variance (ANOVA) of the galacturonic acid content as response for P-IDP using Taguchi approach as statistical tool

Source	DF	Adj. SS	Adj. MS	*F*-value	*P*-value
pH	2	138.420	69.210	23.28	0.041
Temperature	2	66.847	33.423	11.24	0.082
time	2	2.207	1.103	0.37	0.729
Error	2	5.947	2.973		
Total	8	213.420			

DF: degree of freedom; Adj SS: adjusted sum of square; Adj MS: adjusted mean squares.

analyzed, it was employed the lowest time tested, 1 h. Using those conditions, polysaccharide from IDP was extracted and submitted to structural characterization and investigation of the emulsifying properties.

Gel permeation chromatography of P-IDP was utilized to determine the values of ponderal molecular weight (M_w), numeric molecular weight (M_n) and polydispersity (M_w/M_n) and the chromatogram was depicted in Figure 5. The value found for the ponderal molecular weight was 101.4 kDa. The polydispersity was 2.12, which was slightly higher than reported from *Inga* pulp pectin.[13]

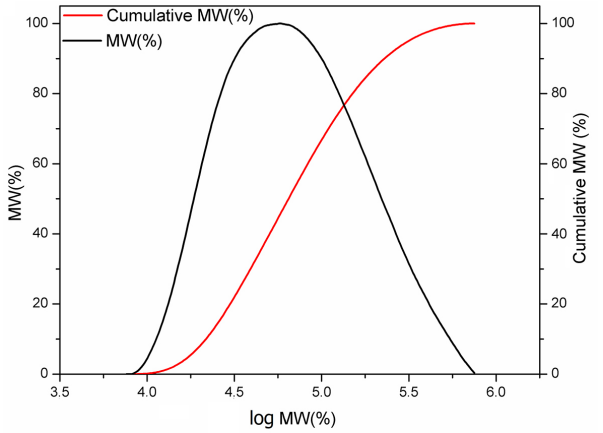

Figure 5. Chromatogram obtained by gel permeation chromatography for P-IDP.

Monosaccharide composition was determined by the analysis of the acetylated products and the composition was described in Table 4. Glucose and xylose were found as predominant, and the value of glucose content was similar to what was reported for different species of apples.[19] The quantity of galacturonic acid found was 22.3 ± 0.95% and this value was comparable to those reported for other species that were used in polysaccharide extraction.[20] When compared to pectin extracted from pulp fruit of *Inga* ssp.,[13] those values of galacturonic acid content were similar.

Table 4. Monosaccharide composition for P-IDP

Monosaccharide composition ± SD / %	
Galacturonic acid	22.3 ± 0.95
Glucose	39.6 ± 1.82
Xylose	12.9 ± 1.81
Galactose	2.79 ± 0.32

SD: standard deviation. Analysis was made in triplicate.

The complete infrared of P-IDP was depicted in Figure 6. The region 3600-3100 cm^{-1} was correlated to O–H absorptions, while the peak that occurs in 2930 cm^{-1} was due to stretching vibrations of C–H bonds of $–CH_2$ groups.[19]

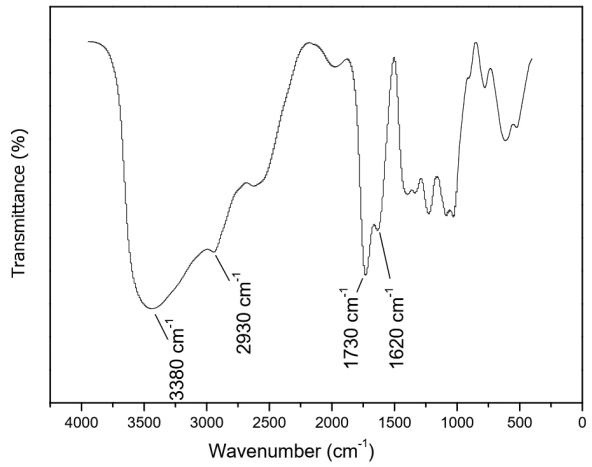

Figure 6. Infrared spectra (FTIR-KBr) from P-IDP obtained under optimum extractions conditions.

In addition, the band around 1730 cm^{-1} was attributed to the stretching vibration of carbonyl from ester.[13,19] Besides, the absorption around 1620 cm^{-1} was correlated to non-carboxylated methyl group.[13,19] This way, P-IDP under the optimum condition of extraction determined by Taguchi statistical methodology provided a well esterified polysaccharide.

The thermal behavior observed from P-IDP was similar to what was observed for other types of polysaccharides as pectins, with three distinct regions.[19,21] The first thermal event, which occurred around 70 °C, was correlated to the moisture loss. The second thermal event occurred around 190 °C and it was correlated to the evaporation of water bound to the polysaccharide chemical structure, while the third event was correlated to the quick decomposition of the polysaccharide structure. During the third thermal event, P-IDP lost about 49% of their initial weight, and that behavior similar to what was observed by Hou *et al.*[19] for several types of polysaccharides from apples. The TGA curve was depicted in Figure 7.

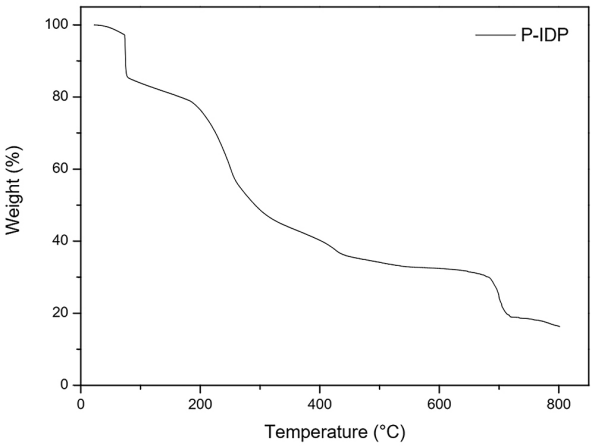

Figure 7. TGA curve of P-IDP.

After the characterization of P-IDP, oil-in water emulsions stabilized by P-IDP solution (0.1, 0.4, 0.7 and 1.0 wt.%) were prepared and stored under ambient conditions for 40 days. The stability and physical appearance of the emulsions were visually observed using photographs of the freshly prepared emulsions (1 h) and after storage for 40 days (Figure 8a). After the first hour of storage, small degrees of phase separation occurred in PE0.1% (10.7% emulsified phase), PE0.4% (28.6% emulsified phase), PE0.7% (25% emulsified phase) and PE1.0% (3.6% emulsified phase). In the first hours of preparation, the emulsion more concentrated in P-IDP showed a higher emulsifying action. More concentrated polysaccharide solutions became more viscous, thus reducing the diffusion of particulate droplets in the emulsion, according to Stokes' law.[22] Higher amounts of the polysaccharide (P-IDP) increased hydrogen interactions in the polymer network with water molecules, thus forming a more viscous hydrogel. The formulations with lower P-IDP concentration did not form a hydrogel and thus the lower viscosity reduces the stability of the formulations. However, all emulsions prepared in this work showed phase separation after storage for 40 days. The emulsions were also evaluated by microphotographs captured from the PDI-P emulsified oil droplets using optical microscopy (Figure 8c). The oil drops showed spherical shapes and different sizes in the microphotographs in the first hours of preparation of the emulsions. However, large size droplets may already be related to coalescence and Ostwald ripening processes that occurred during the storage period.

After the storage period, the PE0.1% and PE0.4% emulsions showed a higher emulsified phase compared to the others. In contrast, PE1.0% showed creaming and less emulsification throughout storage. This may be related to the presence of less soluble compounds from the extraction process that, after precipitation, could destabilize the emulsion system. These insoluble compounds may possibly be remnants of cellulosic fibrils or associated polyphenolic extracts (lignin or tannin) present in *Inga edulis* pods. Furthermore, the extracted P-IDP showed a brownish color, which may also indicate the presence of other compounds such as polyphenols (e.g., tannins, lignins and other compounds) associated to the obtained polysaccharide and which also corroborates with small insoluble fractions (sediments) observed in emulsions after the storage period. Franco *et al.*[23] reported the presence of lignin and cellulose in *Inga marginata* pods. Thus, for future work, there is a need to separate the lignin fraction which may occur in the polysaccharide samples from pods of *Inga* species. Importantly, PE0.1%

and PE0.4% maintained greater uniformity and stability throughout the experiment, and these observations intuitively indicated that P-IDP can compose emulsion stabilizing systems. Polysaccharides from *Inga marginata* pods alone do not show the ability to stabilize emulsions for prolonged time. However, they can work as co-stabilizers together with conventional surfactants or be a viscosity-enhancing agent. The reduction of synthetic additives in food products is very much desired in view of a growing market for natural products.

Figure 8d shows the results obtained on the first day of production of the emulsion and after 40 days of storage (size data over storage is shown in Table 5). Starting on the first day and for each day of analysis during storage, a bimodal distribution (or multimodal) was observed, showing one peak at about 300 nm and another between 4 and 10 μm. In general, Ostwald ripening and flocculation phenomena lead to the appearance of this second larger peak.[24] At the end of 40 days, all emulsions showed instability phenomena such as Ostwald ripening or flocculation, increasing droplet size and generating phase separation or creaming (PE0.7% and PE1.0% with emulsified layer below 70%). However, it is important to note that the overall droplet size distribution did not vary significantly after 40 days (Figure 8d). The same can be seen for the average size over storage shown in Table 5. The opposite is observed for PE0.7% and PE1.0%, which presented the main peak intensity of the particle size distribution in larger droplet diameters after the storage time (Figure 8d). Higher concentrations of P-IDP were not efficient in maintaining the emulsified phase until the end of the analysis period. However, these formulations showed more stability of the emulsified phase in the first hours (less variation in emulsification percentage). PE0.1% and PE0.4% emulsions showed better stabilization of the emulsified phase throughout the analysis. Possibly, other factors related to the polysaccharide extraction process (e.g., insoluble compounds complexed or associated with the polysaccharide chain) would be related to this change in the stability of emulsions containing a higher concentration of P-IDP.

The stability of the emulsions can also be analyzed from the zeta potential data (Table 5). The data show ζ-potential values between −33 and −43 mV for the emulsions which corresponds to a moderate stability range.[25] In pectin, the presence of galacturonic acid residues along the polysaccharide chain promotes a higher negative charge density implying negative values for the ζ-potential. This presence of charges has a significant impact on emulsifying properties due to electrostatic interactions.

In terms of EC and ES (Figure 8b), the lower concentrations (PE0.1% and PE0.4%) showed superior performance. In general, it can be concluded that P-IDP has an emulsifying action and relevant stability, which may be related to the greater presence of hydrophobic groups in the polysaccharide, which enables better interaction with the hydrophilic and hydrophobic phases. Optimized conditions (pH 2.4 and temperature of 70 °C) promoted polysaccharides with a higher degree of methoxylation (more hydrophobic regions) and showed interesting emulsifying action in O/W emulsions (being the only emulsifying agents). Moreover, polysaccharides extracted from fruit peels are rich in tannins which can perform esterification and/or intramolecular cross-linking with the hydroxyl groups of phenolic acids, forming more hydrophobic regions along the structure of the polysaccharide and improving its emulsifying action.[24,25]

Table 5. Droplet diameter and zeta-potential of emulsions (O/W) made from P-IDP and oleic acid (OA) using different concentrations

time	Droplet diameter (PDI) of emulsions / nm			
Concentration / %	0.1	0.4	0.7	1.0
1 h	357.0 ± 1.5 (0.395)	351.8 ± 13.1 (0.299)	363.3 ± 16.0 (0.179)	331.1 ± 4.0 (0.247)
24 h	301.4 ± 4.5 (0.261)	325.5 ± 9.1 (0.298)	314.7 ± 2.7 (0.306)	320.7 ± 3.2 (0.297)
7 days	298.7 ± 7.1 (0.252)	264.6 ± 1.6 (0.204)	311.7 ± 10.5 (0.312)	328.1 ± 1.3 (0.344)
15 days	344.0 ± 7.3 (0.250)	334.7 ± 2.4 (0.288)	358.0 ± 8.8 (0.265)	331.5 ± 7.6 (0.323)
40 days	291.4 ± 0.3 (0.266)	279.8 ± 4.3 (0.266)	322.5 ± 5.6 (0.352)	369.6 ± 6.0 (0.356)
time	Zeta-potential of emulsions / mV			
Concentration / %	0.1	0.4	0.7	1.0
1 h	−40.1 ± 1.1	−38.4 ± 0.5	−39.6 ± 1.2	−38.9 ± 0.3
24 h	−41.3 ± 1.4	−42.0 ± 1.4	−43.1 ± 0.4	−43.9 ± 0.7
7 days	−33.7 ± 1.2	−41.7 ± 1.3	−43.1 ± 2.2	−43.1 ± 1.3
15 days	−40.1 ± 0.7	−31.9 ± 0.9	−41.6 ± 0.3	−42.2 ± 0.9
40 days	−41.7 ± 4.8	−38.8 ± 0.6	−43.0 ± 1.1	−39.8 ± 0.4

Data is presented as mean from triplicates. PDI: polydispersity index.

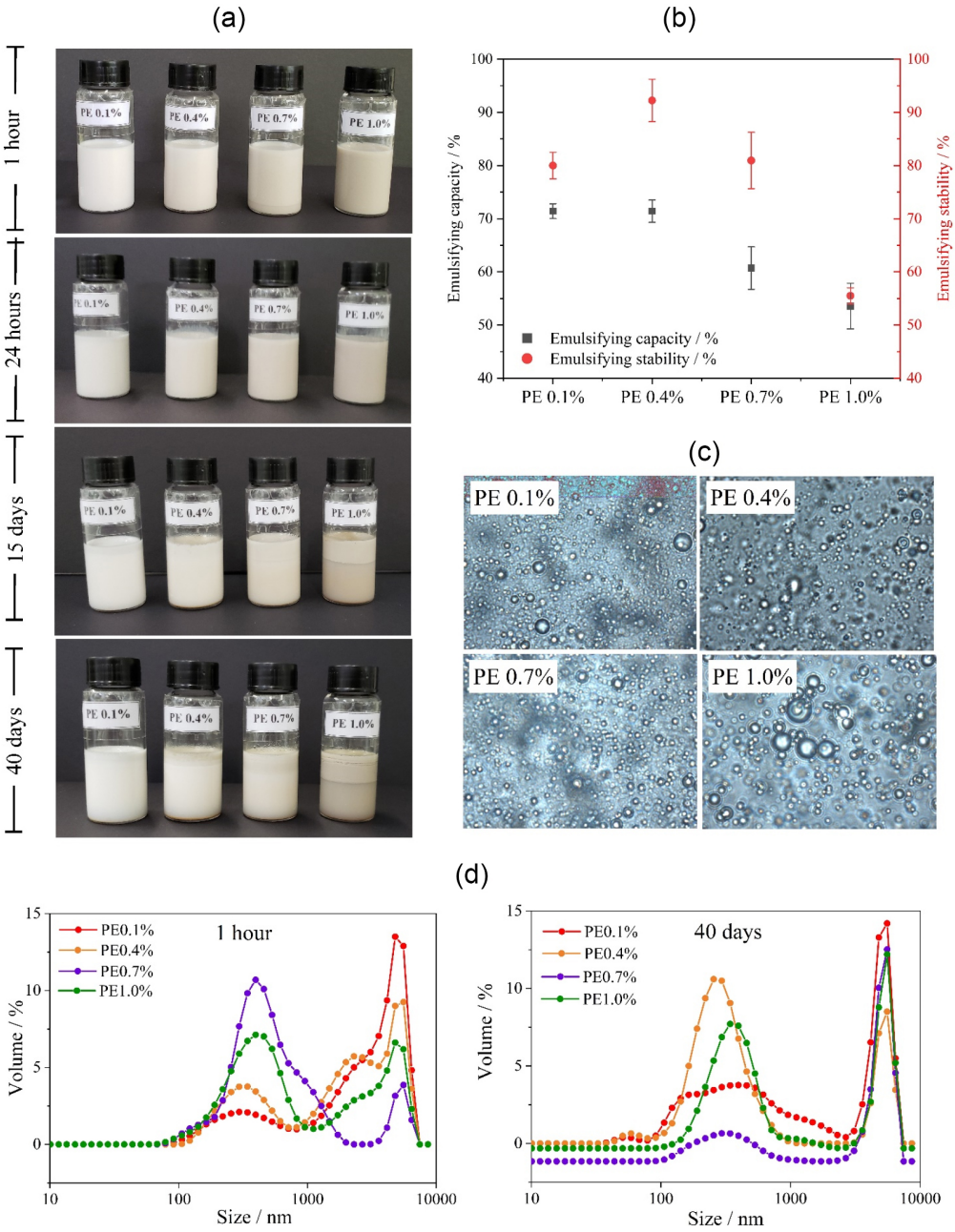

Figure 8. (a) Digital photos, (b) emulsifying properties, (c) optical micrographs and (d) droplet size and size distribution of oil-in-water emulsions prepared with different concentrations of P-IDP (0.1, 0.4, 0.7 and 1.0%). PE: polysaccharide emulsion.

Jiang *et al.*[24] analyzed the stability of persimmon pectins and observed an emulsifying and stabilizing action superior to citrus pectin. The researchers related this emulsifying action due to the presence of hydrophobic groups, such as higher acetylation and the formation of tannin-polysaccharide complexes remaining from the extraction process. Although P-IDP suspensions have properties that help in the emulsification process (viscosity and hydrophilic-hydrophobic regions), there is a need to use combined surfactant to ensure better results. Thus, the presented results suggest that the P-IDP can serve as an alternative emulsifying and co-stabilizing agent for applications in the food and pharmacological industry (reducing the amount of synthetic surfactants).

Conclusions

Using *Inga edulis* dry pods, a potential crop with cultivation fully allied to other industrial crops such as coffee and cacao, it was extracted a polysaccharide and, by Taguchi approach, the optimum level of pH, temperature and time was determined as 2.4, 70 °C and 1 h. The content

of galacturonic acid and the degree of esterification of P-IDP were influenced directly by the reaction conditions, but the yield of extraction was not statistically affected. That way, a polysaccharide with around 22% of galacturonic acid was obtained which chemical structure was analyzed about the monosaccharide composition, with glucose and xylose as majoritarian sugars. In addition, emulsifying properties of P-IDP were evaluated, and the lower concentrations used in the study showed the best emulsifying capacity and stability. The present study contributes to the knowledge of the *Inga* species and reinforces the necessity of adequate use of the *Inga* tree, once describes the polysaccharides obtained by the pods of *Inga edulis* for the first time and suggested it as a potential emulsifying agent.

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Author Contributions

All authors have given approval to the final version of the manuscript. T. B. M. G. A. was responsible for data curation, investigation, formal analysis, writing original draft; F. E. A R. for conceptualization, supervision, writing-review and editing; F. A. M. R. for investigation, writing-review and editing; P. O. B. C. for investigation; L. M. S. for investigation; N. M. P. S. R. for conceptualization, writing-review and editing, supervision, funding acquisition.

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