The influence of *Annona muricata* L. peel extract as a potent source of natural antioxidant on soybean oxidation stability

A influência do extrato de *Annona muricata* L. peel como uma potente fonte de antioxidante natural na estabilidade da oxidação da soja

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ABSTRACT
Antioxidants are an alternative to prevent or retard biodiesel degradation. Soursop (Annona muricata L.) is a native tropical fruit with important antioxidant activity, besides high concentration of phenolic compounds. Peels form about 20 % of the soursop fruit composition and are usually discarded as a waste product. In this study the antioxidant potential of different peel extracts was evaluated by oxidative stability when used as an additive into soybean biodiesel. All samples were analyzed by the Rancimat® 873, using 32 experimental design. The tests were performed with biodiesel B100 with solutions extracted in different conditions, such as pH and residue concentrations. Results demonstrated that all experiments were able to increase significantly the induction period (IP), when compared to a pure biodiesel control sample, and showed similar efficiency with synthetic antioxidants (BHT). Among all experiments, each neutral and alkaline extractions presented induction periods over 8 h, achieving and surpassing the minimum allowed by European standard. However, the highest IP was found to be 10.30 h, with 3 g L-1 in neutral extraction. Results corroborate that soursop peel has a high antioxidant capacity and efficiency to improve the oxidation stability, and could be used as an inexpensive natural antioxidant to biodiesel.

Keywords: rancimat®, Response Surface Methodology (RSM), folin- ciocalteu, Fourier Transform Infrared (FTIR), waste.

RESUMO
Os antioxidantes são uma alternativa para evitar ou retardar a degradação do biodiesel. O graviola (Annona muricata L.) é uma fruta tropical nativa com importante atividade antioxidante, além de alta concentração de compostos fenólicos. As cascas formam cerca de 20% da composição da fruta azeda e são geralmente descartadas como um produto residual. Neste estudo, o potencial antioxidante de diferentes extratos de casca foi avaliado pela estabilidade oxidativa quando usado como aditivo no biodiesel de soja. Todas as amostras foram analisadas pelo Rancimat® 873, utilizando 32 desenhos

experimentais. Os testes foram realizados com biodiesel B100 com soluções extraídas em diferentes condições, tais como pH e concentrações de resíduos. Os resultados demonstraram que todos os experimentos foram capazes de aumentar significativamente o período de indução (IP), quando comparado a uma amostra de controle de biodiesel puro, e mostraram eficiência similar com os antioxidantes sintéticos (BHT). Entre todos os experimentos, cada extração neutra e alcalina apresentou períodos de indução superiores a 8 h, alcançando e ultrapassando o mínimo permitido pela norma européia. Entretanto, o IP mais alto foi encontrado em 10,30 h, com 3 g L-1 em extração neutra. Os resultados corroboram que o soursop peel tem uma alta capacidade antioxidante e eficiência para melhorar a estabilidade de oxidação, e poderia ser usado como um antioxidante natural barato para o biodiesel.

Palavras-chave: rancimat®, Metodologia De Superfície De Resposta (RSM), folin-ciocalteu, Fourier Transform Infravermelho (FTIR), resíduos.

1 INTRODUCTION

The biodiesel has been considered as one of the most promising alternative fuels in the partial or even total replacement of diesel, presents economic competitiveness, accessible and environmentally acceptable. It appears as a nontoxic, biodegradable fuel, produced from renewable resources such as vegetable oils, animal fats and used cooking oil [1]. Offers outstanding features over conventional diesel, such as higher flash point, leading to safer handling and storage, superior lubricity, completely miscible with petroleum diesel, compatibility with the existing fuel distribution infrastructure, low or no sulfur content, no aromatics and reduces most regulated exhaust emissions. Despite its many advantages, it has inferior oxidative stability than diesel fuel [2,3].

Oxidation of biodiesel is an undesirable and prejudicial event that degrades and modifies its physicochemical properties due to long periods of storage. Therefore, is considered an essential parameter to control, in order to maintain the biodiesel quality standards [3,4]. The oxidative stability is affected by numerous factors such as the nature of the fatty acid, esters unsaturation degree, air, heat, light, antioxidants, moisture, the presence of metal ion catalysts, enzymes and other impurities [5–9].

Rancimat method (EN 14112) is the most commonly used reference test to determine the oxidative stability of biodiesel [10]. The European biodiesel standard EN 14214 minimum induction period is 8 h, whereas the Brazilian National Petroleum Agency (ANP) 12 h value is required [11–13].

In order to delay the oxidation process, natural or synthetic antioxidants are used to inhibit the initiation and propagation of free radicals, these minimize the formation of

degradation compounds improving the IP [4,14]. In general, synthetic antioxidants are the most commonly used in the biodiesel industry because of their cost, availability, and performance [15]. The main synthetic additives are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ), propyl gallate (PG), and pyrogallol (PY) [5,16,17]. However, these additives are derived from petroleum products, that are related with carcinogenic properties and environmental impacts, which reduces the renewable nature of the biofuel. Due to these reasons there is an increasing tendency to replace, partially or totally, synthetic antioxidants with natural ones, that are claimed to be safer [18].

Natural antioxidants are used as an alternative to be effective in controlling lipid oxidation, increasing the shelf-life of products [19]. The most used natural antioxidants are tocopherols, carotenoids, some organic acids such as citric acid, ascorbic acid and flavonoids. They are found especially in plant material and the extraction of natural antioxidants is being employed in the food and pharmaceutical industry to isolate these compounds, that protect and stabilize oxidizable constituents from oxidation [20].

Soursop (*Annona muricata* L.) is a fruit that is mostly distributed in tropical and subtropical regions of the world, and it is considered to be a good source of natural antioxidants [21]. Consumption of soursop has been increasing worldwide, in particular in the food industry, causing a rising amount of by-products. The fruit peel is usually a waste material and represents about 20% of the weight of the fruit [22].

Extensive studies in different parts of the *A. muricata* plant have shown high levels antioxidant compounds such as, vitamins C and B, alkaloids, flavonoids, phenolics, megastigmanes, tannins, anthocyanin, and essential oils [23–26]. However, acetogenins are the most predominant bioactive compounds only found in the Annonaceae family, as well as *A. muricata*. Previous phytochemical studies have reported more than 120 acetogenins, from the leaves, stems, bark, seeds, pulp, and fruit peel of soursop [21,25,27].

Investigations have reported that natural plant extracts have different degrees of antioxidant properties in fats and oils. Potato peel extractions were evaluated by Devi and co- workers [20] as a natural antioxidant source to enhance the oxidation stability of biodiesel produced from Nahar oil, achieved the expected oxidative stability effect [20].

Fabrice and co-workers [28] studied the antioxidant activity of methanolic soursop flowers extracts in delaying palm olein oil oxidation at frying temperature (180 °C). The results demonstrated an enhancement of the stability, delaying primary and secondary oxidation products formation of this oil than BHT.

Few studies have reported the potential capacity of the soursop peel (SP) as a natural antioxidant, and none have tested it as a possible alternative to inhibit oxidative activity in oils and biodiesel. Although, few studies have suggested to explore SP as a viable source, due to its higher antioxidant activity than the pulp, indicating that this could correspond to the presence of the phenolic compounds [23,29]. These findings strongly suggest the potential use SP as a natural source of antioxidants.

The present study evaluated the antioxidant potential of different extractions conditions of the peel of *A. muricata* in soybean biodiesel B100.

2 EXPERIMENTAL

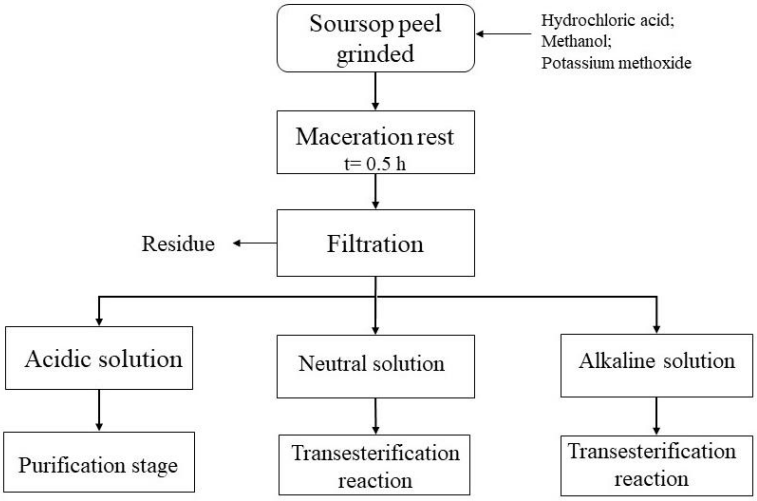
2.1 BIODIESEL

Biodiesel B100 was made through soybean oil transesterification reaction by methanolic route, using potassium hydroxide as a catalyst, in proportions of 100:30:1.5 (v/v/m). The reaction was heated and slowly agitated at 60 °C during 60 minutes. The separation of phases (biodiesel and glycerin) by decantation occurred for a period of 24 h. Purification stage was performed using solutions of 0.5% (v/v) hydrochloric acid, saturated sodium chloride and distilled water. Once free of catalyst, free fatty acids and methanol, it was heated for 15 minutes at 80 °C to remove the remaining methanol and water [12,30].

2.2 NATURAL ANTIOXIDANTS

Fresh soursop peels (SP) were washed with distilled water and dried at 45 °C in a dry matter oven for 24 h. The dried SP was milled and sieved into homogenous powdered form (20 mesh) in a mill. Extractions were perform using different pH extractions, such as hydrochloric acid (pH=1), methanol (pH=7) and potassium methoxide (pH=14), in different peel concentrations (1.0; 3.0 and 5.0 g L^{-1}). The extraction time was 30 minutes for each experiment. After filtration, soursop peel extractions (SPE) were added to the transesterification reaction (neutral and alkaline extractions) or to the purification stage (acid extraction), as shown in Figure 1. Methodology according to the one proposed by Boschen *et. al* [13,31].

Figure 1. Soursop peel natural antioxidants extractions methodology.



2.3 DETERMINATION OF TOTAL PHENOLIC COMPOUNDS

The total content of phenolic compounds was determined by the Folin-Ciocalteau spectrophotometric method. The absorbance was measured at 785 nm using a 320-G UV/Vis (Gehaka) spectrophotometer. All determinations were made in triplicate and values were estimated using a gallic acid standard curve (R^2=0.997). Results of total polyphenols content were expressed as milligrams gallic acid equivalents per gram of extract (mg GAE g^{-1}). The methodology employed was according as described by Singleton and co - workers [32].

2.4 RANCIMAT TEST

The oxidation stability was measured according to the EN 14112 standard, using the Rancimat 873 Metrohm®. This test consists in passing an air stream at the rate of 10 L h^{-1} through biodiesel sample, at 110 °C. Highly volatile products produced during the oxidation process, are transferred along with the air, into a vessel containing demineralized water, where an electrode is constantly measuring the conductivity. The determination of the induction period (IP) occurs, when the conductivity begins to increase rapidly, an oxidation curve is obtained, whose point of inflection is known as the IP.

2.5 EXPERIMENTAL DESIGN

A 3^2 experimental design was carried out to study the main effects and interactions between the SP concentration (x_1) and the solvent pH (x_2) to enhance the IP, the measured

response (Y). The variables were analyzed in three equidistant variation levels: -1; 0 and 1. For X_1 are used 1.0; 3.0 and 5.0 g L^{-1}, and for X_2 are used pH 1.0; 7.0 and 14.0. Three replicates of the central point were carried out, and all experiments were performed in triplicate. The statistical study was performed using the Statistica v. 9.0 software. Significant levels were based on the confidence level of 95 % ($p < 0.05$).

2.6 BIODIESEL PHYSICOCHEMICAL CHARACTERIZATION

Physicochemical characterization analysis were performed to the experiment that showed the highest IP and pure biodiesel control sample (CS), to corroborate whether both meet the standard quality limits. The parameters analyzed, standard test methods and equipment used for each parameter are presented in Table 1.

Table 1. Physicochemical characterization of biodiesel.

Parameter	**Standard Method**	**Equipment**
Specific weight in 20 °C	NBR 7148 (ABNT, 2001)[33]	Incoterm densimeter, scale from 0.800 to 0.900 g cm^{-3}
Electrical conductivity	ASTM D2624 (ASTM, 1995)[34]	Digimed, DM-3P-PE2
Color and aspect	Resolution n° 45 of ANP (ANP, 2018)[35]	Visual method

2.7 FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY

The FTIR spectra were determined using a Cary 600 Series Fourier transform infrared spectrometer, from Agilent Technologies. Spectral analysis range from 4000 - 600 cm^{-1}, using a calcium fluoride (CaF_2) window, with 32 scans and 2 cm^{-1} resolution. For each analysis, 50 µL of sample were injected into the sample port. The experiment that presented the highest IP, biodiesel CS, and biodiesel added synthetic antioxidant were characterized to evaluate specific molecular compounds by using FTIR spectrum data.

3 RESULTS AND DISCUSSION

3.1 EXTRACTION YIELD

The extraction yield of SP was different for each of the solvents used. The yield of SPE ranged from 7.2 – 40.0 % dry matter. The overall extraction efficiency for different solvents was observed in the following decreasing order; (40 %) hydrochloric acid > (13.8 %) methanol > (7.2 %) potassium methoxide. The methanol yield extraction value was higher than that obtained by Womeni et. al (2016) [26] that reported 8.35 % extract efficiency with methanolic extracts of soursop flowers. Also surpassed George *et. al,* [36] butanolic yield extracts of soursop leaves (10.80 %). However, it was lesser than

that of Zia-ur-Rehman (2006)[37] that reported 19.87 % yield extract using citrus peels obtained with methanol. Such differences may be attributed to the solvent, the extraction method, part and type of fruit or plant, factors that may influence the result.

Statistical analysis

An experimental design was applied to determine the optimum concentration and pH conditions for peel extractions. Encoded independent variables, variation levels and the induction period results, for the experimental design, are shown in Table 2.

Table 2. Experimental design coded variables and induction period results.

Experiments	Coded variable		Induction period (h)
	X_1	X_2	Y
1	-1	-1	7.34 ± 0.22
2	-1	0	8.25 ± 0.97
3	-1	1	8.92 ± 0.12
4	0	-1	8.30 ± 0.45
5	0	0	10.30 ± 0.36
6	0	1	10.04 ± 0.83
7	1	-1	8.89 ± 0.19
8	1	0	9.27 ± 1.02
9	1	1	9.46 ± 1.13
10	0	0	9.91 ± 0.40
11	0	0	10.75 ± 1.21
12	0	0	9.94 ± 0.23

The model predicted by ANOVA results can be expressed in terms of the coded values by the following Equation 1.

$$Y = 10.0654 + 0.5178_{X1} + 0.6494_{X2} - 0.9879_{X1}^2 + 0.6494_{X2}^2 - 0.2516\, X1\, X2 \quad (1)$$

Where Y is the induction period, X_1 is the linear term concentration of SP, X_1^2 is the quadratic term of SP concentration, X_2 is the linear term of pH solvent extraction, X_2^2 is the quadratic term of pH solvent extraction and $X_1 X_2$ is the interaction term between SP concentration and pH solvent extraction. The regression coefficient (R^2) value was 0.8875, indicating that there is significant correlation (88.75%) between experimental results and estimated values. This is because the R^2 value always ranges between 0 and 1 and for a good fit of the model, R^2 should be at least 0.80 [38]. Therefore, the closer R^2 is to 1, the stronger the model and better the prediction efficiency of the responses [39].

Analysis of variance (ANOVA), presented in Table 3, was performed to determine the important terms of the model equation.

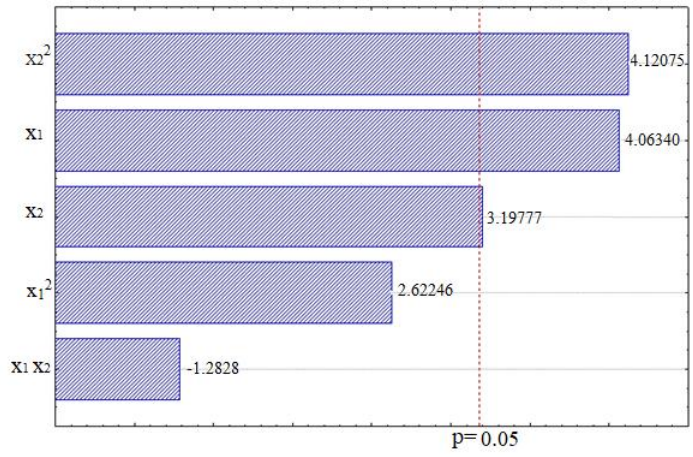
Table 3. Analysis of variance using 3^2 experimental design.

Variation Source	D.F.	Square sum	Square average	F_{calc}	F_{tab}	*p*-value
Regression	5	7.891	1.578	10.297[1]	9.01	0.039
x_1 Linear	1	1.609	1.609	10.495[1]	10.13	0.048
x_1 Quadratic	1	2.603	2.603	16.981[1]	10.13	0.026
x_2 Linear	1	2.531	2.531	16.511[1]	10.13	0.027
x_2 Quadratic	1	0.896	0.896	5.844	10.13	0.094
Interaction	1	0.253	0.253	1.653	10.13	0.289
Lack of fit	3	0.741	0.247	1.611	9.28	0.352
Error	3	0.460	0.153			
Total	11					

[1] Significant at the level of 5 %.

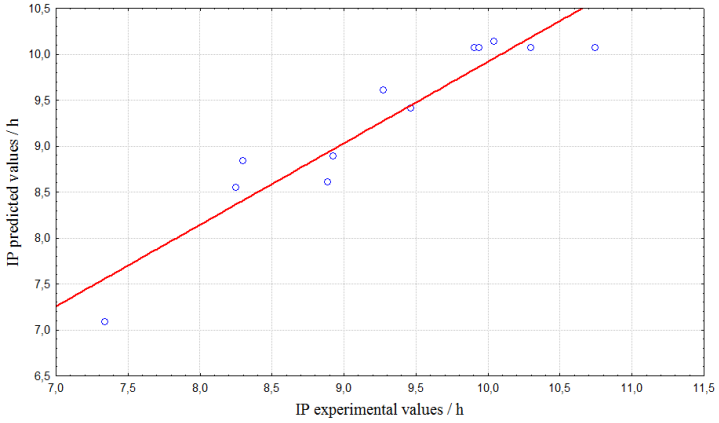
According to ANOVA, the value to regression, F_{calc}, was greater than F_{tab}, showing that the model presents a significant regression with respectives degrees of freedom. For the regression to be significant, not only statiscally, but for predictive purposes, F_{calc} must be greater than F_{tab}. Therefore, $F_{calc} > F_{tab}$ indicante that the obtained equation can be used for predictive purposes [11]. The *p*-value less than 0.05 indicates that the terms of the model are statistically significant with a 95 % confidence interval [40]. In this case, x_1, x_{12}, and x_2 are important model terms. These results show that SP concentration (x_1) and pH solvent (x_2) have significant effects on the induction period, as shown in the pareto chart (Figure 2).

Figure 1. Pareto chart for the model.



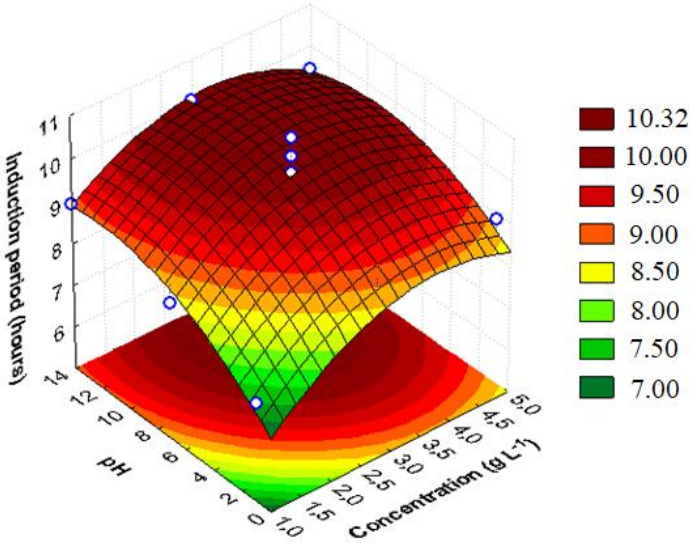
In Figure 2, the terms x_2^2, x_1 and x_2 are statiscally significant with 95 % confidence interval. Figure 3, shows the predicted values by the model *versus* the experimental values.

Figure 2. Predicted values *versus* experimental values of model.



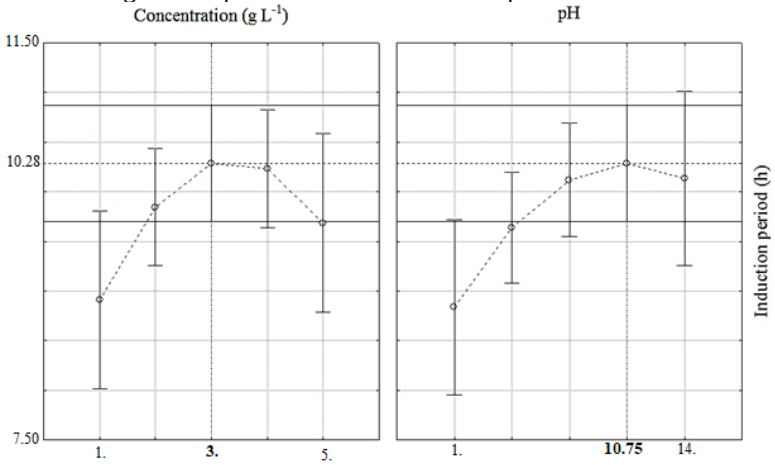
According to Figure 3, an adequate degree of similarity was observed between the predicted values from the experimental values that maintain a near distance from linearity. Due to the good regression coefficient (R^2), and since the lack of fit was not significant (p = 0.352), demonstrates that the model shows a good fit and can be used for predictive purposes [41]. Response surface of binary combination between both variables is shown in Figure 4.

Figure 3. Response surface of the experimental design for induction period (h).



In Figure 4, the curvature of the surface was the most appropriate, showing highest efficiency with SP concentration near 3 g L^{-1} and with a pH solvent extraction close to 10. The optimum conditions to reach the best possible induction period is presented in Figure 5, being that potential induction period, according to the model, 10.28 h.

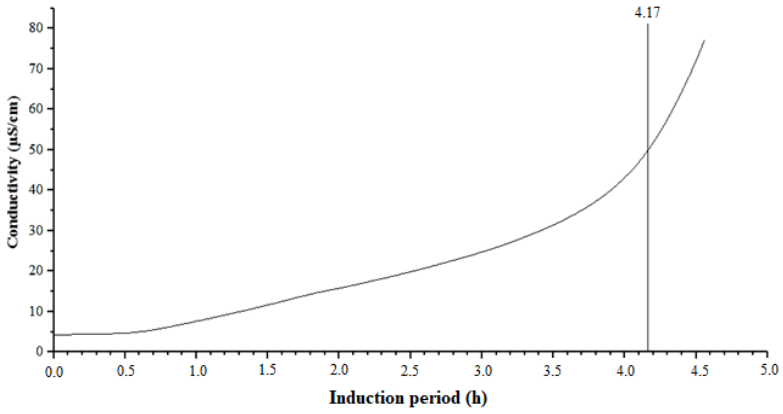
Figure 4. Optimum conditions for the process variables.



Comparison between soursop peel extracts (natural) and BHT (synthetic) as antioxidant in soybean biodiesel

Figure 6 shows the oxidation stability for pure soybean biodiesel with any antioxidant additives.

Figure 5. Induction period of biodiesel control sample.

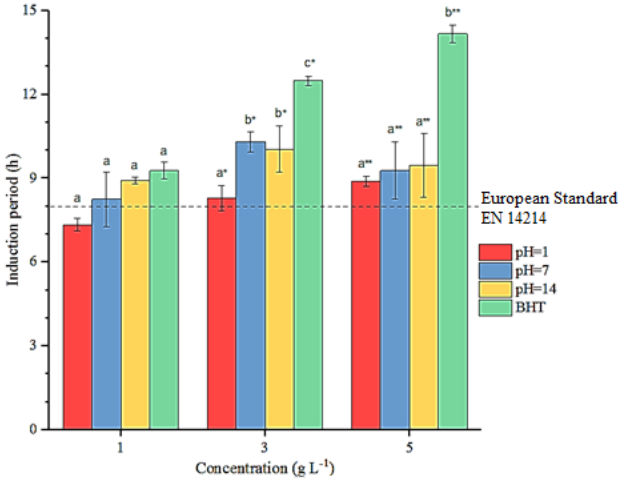


The CS induction period reached 4.17 h, below the limit established by the European Standard EN 14214 and ANP, result that was already expected due to the absence of antioxidant. Likewise, similarly results were reported for soybean biodiesel in other studies [2,6] [2,6].

Comparing all IP results of the experiments in Table 3 with the control sample (Figure 6), it may be noted that all experiments increased substantially the oxidative stability of biodiesel, and surpassed the limit of 8 h required by EN 14214, but not to the limit of ANP, with the exception of experiment 1. The highest IP (experiment 5) was achieved using a concentration of 3 g L^{-1} in neutral medium, increasing the IP over 147%.

Nowadays, synthetic additives are more commonly use in biodiesel¸ despite the efficiency shown by natural antioxidants. Figure 7, shows the comparison among induction periods of biodiesel with natural (SPE) and synthetic (BHT) antioxidants.

Figure 6. Comparison of Induction period (h) values for natural (SPE) and synthetic (BHT) antioxidants based in European limit (8 h).



These results demonstrate effective antioxidant property in soursop peel extractions. Many studies have attributed this antioxidant capacity to prevent oxidative rancidity in oils and biodiesel to the phenolic compounds present in fruit extracts [18,42]. Mainly, this capacity has been associated to the number and position of the hydroxyl groups present in the molecule, offering better antioxidant ability [43].

Some studies have found that soursop flowers methanolic extracts is a powerful and potent source of antioxidants in delaying the oxidation process in oils [26,28]. Antioxidant properties for soursop peel has been reported in other studies, exhibiting higher antioxidant activity and phytoconstituents (phenolics, flavonoids, vitamin C) than the pulp. Furthermore, revealing its ability as an electron donor that can stabilize and delay chain reactions that leads to oxidative stress, suggesting the use of the peel as a natural alternative to combat oxidation [23,29].

3.2 TOTAL PHENOLIC CONTENT

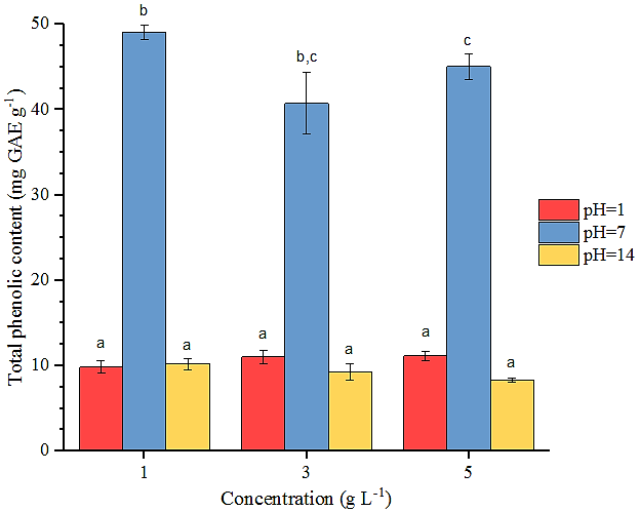
Total phenolic content (TPC) of the different peel extracts were determined by colorimetric assay (Folin-Ciocalteau) and the results are shown in Figure 8. It was observed that the TPC of the extracts obtain with different solvents and concentrations were found in the range of 8.29 – 49.08 mg GAE g^{-1}. The maximum amount of total

phenolic content (49.08 mg GAE g^{-1}) was achieve with methanol and minimum 9.84 mg GAE g^{-1} by using hydrochloric acid as solvent.

Results show that the increase of SP concentration in acid and alkaline extractions presented no significance affect in the total content of phenolic compounds. Meanwhile, neutral extractions revealed a significance influence by the alteration of SP concentration, further being significantly higher in the TPC than the other extractions methods.

The highest value of antioxidant capacity measured by total phenolic content found in the SPE were obtained with methanol mixtures. Thus, the use of methanol as solvent gave better phenolic compounds extraction results. This could be explained by the fact that phenolic compounds are often more soluble in organic solvents, in addition, methanol is considered the best solvent for polyphenols extraction [37].

Figure 7. Total phenolic content of soursop peel extracts



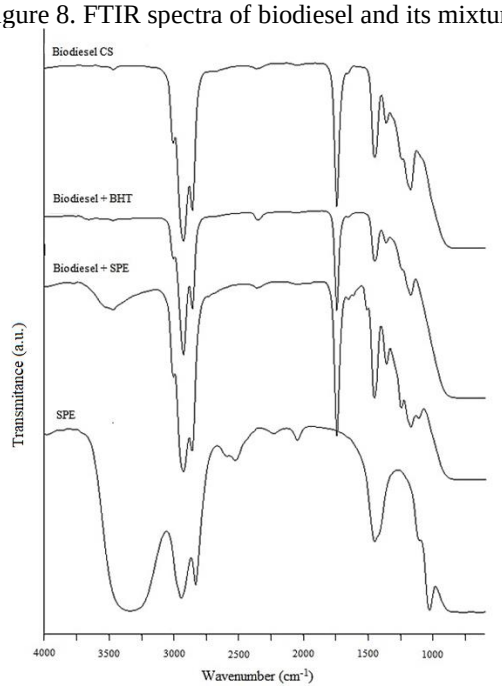
The total phenolic content varies considerably from one kind of plant extract to another, as well in different parts of the same plant source. For instance, the best result in this study indicates that soursop peel had a phenolic content of 49.08 mg GAE g^{-1}; this exceeds 28.10 mg GAE g^{-1} [44], 17.08 mg GAE g^{-1} [45] and 0.54 mg GAE g^{-1} [46] found in soursop pulp, being in accordance with Akomolafe and Ajayi (2015) [29] that determine higher antioxidant activity in peel compared to the pulp.

Nevertheless, similar content has been reported in methanolic extracts of flowers and soursop leaves of 51.33 mg GAE g^{-1} and 54.95 mg GAE g^{-1}, respectively [26,47]. This content is greater than that found in other antioxidant extracts, such as Moringa leaves 24.90 mg GAE g^{-1} [19], oregano 16.31 mg GAE g^{-1} and rosemary 17.66 mg GAE g^{-1} [41], all of them have shown adequate results increasing the biodiesel oxidation stability.

3.3 FTIR ASSESSMENT

Figure 9, compares the spectra of the infrared analysis of pure biodiesel CS, biodiesel with BHT, biodiesel with SPE, and pure SPE. The SPE selected to analyze was the one that achieve the highest IP (experiment 5). Noteworthy information was found in the infrared spectra of biodiesel and its mixtures, according to the literature, there are three spectral regions of important absorption, whose peaks have a known origin.

Figure 8. FTIR spectra of biodiesel and its mixtures.



Analising spectra of Figure 9, the highest intensity peak occurs in the region of 2945 to 2840 cm^{-1}, and can be attributed to the axial deformation vibrations of the C-H bonds of the methyl (CH_3) and methylene (CH_2) groups. Peaks with intermediate to high intensity, appear in the region of 600 to 1740 cm^{-1}, being derived from the angular deformation vibrations of the C-H bonds of the methyl and methylene groups. Low intensity peaks, but still relevant, in the region of 1000 to 1300 cm^{-1} are derived from the angular deformation vibration of the C-H bonds of the aromatic hydrocarbon ring [48].

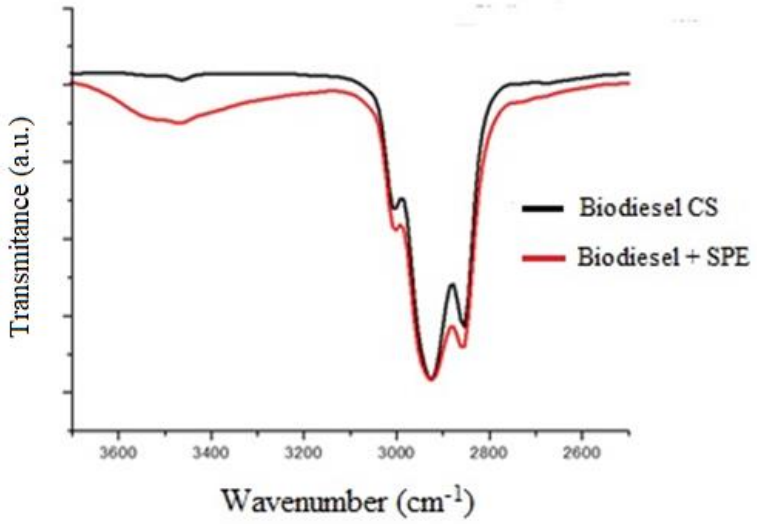
It is important to mention that the intense peak in the region of 1740 to 1750 cm^{-1} is characteristic of saturated aliphatic esters, and is due to the axial deformation vibrations of the carbonyl group bond (C=O) [49,50]. Furthermore, the peaks protruding in the region of 1165 to 1265 cm^{-1} are characteristic peaks in the biodiesel (FAME) spectra [51].

Biodiesel spectra with soursop extract, it is noted a broad and low intensity peak near 3475 cm^{-1}, which corresponds to the region of occurrence of peaks due to axial deformation vibrations of the hydroxyl group (OH). This same peak can be observed with high intensity in the spectrum of soursop extract, therefore, being characteristic of the compound. Antioxidant properties of extracts have been related to the presence of OH group found in the aromatic ring structure of the molecule, which are attributed to be responsible of free radical scavenging [52,53].

Antioxidants compounds present in soursop have been reported in this study, and several present long carbonic chain structures (phenolic compounds), that form a strong cluster of hydrogen bonds (C-H) between biodiesel and the antioxidant. These C-H molecules consume oxygen, producing carbon dioxide, delaying oxidation. Due to the low oxygen content in the fuel, free radicals become less aggressive, until the cluster is breach, providing a higher oxidative stability [54].

Figure 10 shows the enlargement of the peaks referring to the C-H bond, 2945 to 2840 cm^{-1}, showing an increase in the intensity of the peak of biodiesel with soursop extract than to the control sample. This peak increment can be considered as a reason of the important efficiency that the soursop peel extracts demonstrated in the oxidative stability of biodiesel.

Figure 9. FTIR spectra enlargement of the C-H bond region.



3.4 JBCSBIODIESEL QUALITY CONTROL

In Table 4, are shown the physicochemical parameters of biodiesel without (CS) and doped with SPE that achieved the highest induction period (experiment 5), as well as

the limits established by the ANP. The results obtained are in accordance to the ones stipulated by the Brazilian Petroleum Agency.

Table 4. Physicochemical characterization of biodiesel

Characteristics	**Unit**	**Limit**	**Biodiesel CS**	**Biodiesel experiment 5**
Specific weight @ 20 °C	kg m^{-3}	860-900	873,8	877,9
Aspect	-	Yellow	Yellow	Yellow
Color	-	CFI[1]	CFI[1]	CFI[1]
Electrical conductivity	µS m^{-1}	350 (max.)	195	168

CFI[1]= clear and free of impurities

4 CONCLUSIONS

The soursop peel showed a potential capacity to be used as a source of an antioxidant additive for soybean biodiesel. Results demonstrated that all extracts were able to increase significantly the IP, generating a protective effect against biodiesel degradation. Neutral and alkaline extractions achieved and surpassed the minimum establish by European legislation (>8 h). The best result, 10.30 ± 0.36 h, was reach with 3 g L^{-1} in neutral extraction. Results indicate that soursop peel extractions have high antioxidant capacity and efficiency to improve the oxidation stability, similar to a commonly used commercial additive (BHT), and could be used as an inexpensive natural antioxidant in biodiesel. Therefore, these findings emphasize and encourages the potential use of waste biomass resources, as promising alternatives to reduce or displace synthetic antioxidants, leading to adequate economic and environmental advantages

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REFERENCES

Jakeria, M. R.; Fazal, M. A.; Haseeb, A. S. M. A. ;*Renew. Sustain. Energy Rev.* **2014**, *30*, 154.

Saluja, R. K.; Kumar, V.; Sham, R. ;*Renew. Sustain. Energy Rev.* **2016**, *62*, 866.

Knothe, G.; Razon, L. F. ;*Prog. Energy Combust. Sci.* **2017**, *58*, 36.

Buosi, G. M.; Da Silva, E. T.; Spacino, K.; Ramazzoti, L. C. S.; Ferreira, B. A. D.; Borsato, D. ;*Fuel* **2016**, *181*, 759.

Varatharajan, K.; Pushparani, D. S. ;*Renew. Sustain. Energy Rev.* **2017**, *82*, 2017.

Maia, E. C. R.; Borsato, D.; Moreira, I.; Spacino, K. R.; Rodrigues, P. R. P.; Gallina, A. L. ;*Fuel Process. Technol.* **2011**, *92*, 1750.

Kumar, N. ;*Fuel* **2017**, *190*, 328.

Agarwal, A. K. ;*Prog. Energy Combust. Sci.* **2007**, *33*, 233.

Spacino, K. R.; Borsato, D.; Buosi, G. M.; Chendynski, L. T. ;*Fuel Process. Technol.* **2015**, *137*, 366.

Focke, W. W.; Westhuizen, I. Van Der; Oosthuysen, X. ;*Thermochim. Acta* **2016**, *633*, 116.

Dantas, M. B.; Albuquerque, A. R.; Barros, A. K.; Rodrigues Filho, M. G.; Antoniosi Filho, N. R.; Sinfrônio, F. S. M.; Rosenhaim, R.; Soledade, L. E. B.; Santos, I. M. G.; Souza, A. G. ;*Fuel* **2011**, *90*, 773.

Boschen, N. L.; Valenga, M. G. P.; Maia, G. A. R.; Gallina, A. L.; Rogério Pinto Rodrigues, P. ;*Ind. Crop. Prod.* **2019**, *140*, 111624.

Valenga, M. G. P.; Boschen, N. L.; Rodrigues, P. R. P.; Maia, G. A. R. ;*Ind. Crops Prod.* **2019**, *128*, 331.

Gregório, A. P. H.; Borsato, D.; Moreira, I.; Silva, E. T.; Romagnoli, É. S.; Spacino, K. R. ;*Biofuels* **2017**, *1*, 1.

Yaakob, Z.; Narayanan, B. N.; Padikkaparambil, S.; K, S. U. ;*Renew. Sustain. Energy Rev.* **2014**, *35*, 136.

Medeiros, M. L.; Cordeiro, A. M. M. T.; Queiroz, N.; Soledade, L. E. B.; Souza, A. L.; Souza, A. G. ;*Energy and Fuels* **2014**, *28*, 1074.

Focke, W. W.; Van Der Westhuizen, I.; Grobler, A. B. L.; Nshoane, K. T.; Reddy, J. K.; Luyt, A. S. ;*Fuel* **2012**, *94*, 227.

Chong, Y. M.; Chang, S. K.; Sia, W. C. M.; Yim, H. S. ;*Food Biosci.* **2015**, *12*, 18.

França, F. R. M.; Freitas, L. dos S.; Ramos, A. L. D.; Silva, G. F. da; Brandão, S. T. ;*Fuel* **2017**, *203*, 627.

Devi, A.; Das, V.; Deka, D. ;*Ind. Crop. Prod.* **2018**, *123*, 454.

Moghadamtousi, S. Z.; Fadaeinasab, M.; Nikzad, S.; Mohan, G.; Ali, H. M.; Kadir, H. A. ;*Int. J. Mol. Sci.* **2015**, *16*, 15625.

Schroeder, P.; Barreto, M. dos S.; Romeiro, G. A.; Figueiredo, M. K.-K. ;*Waste and Biomass Valorization* **2018**, *9*, 1459.

Lee, W. Z.; Chang, S. K.; Khoo, H. E.; Sia, C. M.; Yim, H. S. ;*Acta Sci. Pol. Technol. Aliment.* **2016**, *15*, 419.

Elagbar, Z. A.; Naik, R. R.; Shakya, A. K.; Bardaweel, S. K. ;*J. Chem.* **2016**, *2016*.

Wahab, S. M. A.; Jantan, I.; Haque, M. A.; Arshad, L. ;*Front. Pharmacol.* **2018**, *9*, 1.

Womeni, H. M.; Djikeng, F. T.; Iruku, N. S. S. P.; Karuna, M. S. L.; Prasad, R. B. N.; Linder, M. ;*Food Sci. Nutr.* **2016**, *4*, 802.

Paes, M. M.; Vega, M. R. G.; Cortes, D.; Milton, M. ;*Rev. Virtual Química* **2016**, *8*,

Fabrice, T. F.; Macaire, W. H.; Marrapu, B. K.; Karuna, M. S. L.; Prasad, R. B. N.; Michel, L. ;*Food Sci. Hum. Wellness* **2017**, *6*, 187.

Akomolafe, S. F.; Ajayi, O. B. ;*Int. Food Res. J.* **2015**, *22*, 2381.

Viomar, A. Estudo das variáveis da produção de biodiesel, Universidade Estadual do Centro Oeste, 2013.

Boschen, N. L. Estudo da aplicação de antioxidante natural para biodiesel e óleo de soja, Universidade Estadual do Centro-Oeste, 2016.

Singleton, V. L.; Orthofer, R.; Lamuela-Raventós, R. M. ;*Methods Enzymol.* **1999**, *299*, 152.

ABNT - Associação Brasileira de Normas Tecnicas Petróleo e produtos de petróleo - Determinação da massa específica, densidade relativa e °API - Método do densímetro. *NBR 7148* **2001**.

ASTM - American Society fo Testing Materials Standard Test Methods for Electrical Conductivity of Aviation and Distillate Fuels **1995**.

ANP - AGÊNCIA NACIONAL DO PETRÓLEO GÁS NATURAL E BIOCOMBUSTÍVEIS RESOLUÇÃO ANP Nº 45, DE 25.8.2014 - DOU 26.8.2014.

George, V. C.; Kumar, D. R. N.; Rajkumar, V.; Suresh, P. K.; Kumar, R. A. ;*Asian Pacific J. Cancer Prev.* **2012**, *13*, 699.

Zia-ur-Rehman ;*Food Chem.* **2006**, *99*, 450.

Bisht, D.; Yadav, S. K.; Darmwal, N. S. ;*Brazilian J. Microbiol.* **2013**, *44*, 245.

Doddapaneni, K. K.; Tatineni, R.; Potumarthi, R.; Mangamoori, L. N. ;*J. Chem. Technol. Biotechnol.* **2007**, *82*, 721.

Deveci, S.; Çetinkaya, E.; Dönmez, K. B.; Karadag, S.; Doğu, M. ;*Microchem. J.* **2018**, *143*, 272.

Spacino, K. R.; Tavares, E.; Angilelli, K. G.; Moreira, I.; Galão, O. F.; Borsato, D. ;*Ind.*

Crop. Prod. **2016**, *80*, 109.

Vijayalaxmi, S.; Jayalakshmi, S. K.; Sreeramulu, K. ;*J. Food Sci. Technol.* **2015**, *52*, 2761.

Chen, J. H.; Ho, C. T. ;*J. Agric. Food Chem.* **1997**, *45*, 2374.

Souza, V. R.; Pereira, P. A. P.; Queiroz, F.; Borges, S. V.; De Deus Souza Carneiro, J. ;*Food Chem.* **2012**, *134*, 381.

Zamudio-Cuevas, Y.; Díaz-Sobac, R.; Vázquez-Luna, A.; Landa-Solís, C.; Cruz-Ramos, M.; Santamaría-Olmedo, M.; Martínez-Flores, K.; Fuentes-Gómez, a J.; López-Reyes, A. ;*Food Funct.* **2014**, *5*, 303.

Almeida, M. M. B.; Sousa, P. H. de M.; Arriaga, Â. M. C.; Prado, G. M. do; Magalhães, C. E. de C.; Maia, G. A.; Lemos, T. L. G. de ;*Food Res. Int.* **2011**, *44*, 2155.

Vit, P.; Santiago, B.; Perez, E. M. P. ;*Interciencia* **2014**, *39*, 350.

Pavia, D. L.; Lampman, G. M.; Kriz, G. S.; Vyvyan, J. R. *Introdução à Espectroscopia*; 4th ed.; CENGAGE LEARNING, 2010.

Kudre, T. G.; Bhaskar, N.; Sakhare, P. Z. ;*Renew. Energy* **2017**, *113*, 1408.

Rabelo, S. N.; Ferraz, V. P.; Oliveira, L. S.; Franca, A. S. ;*Int. J. Environ. Sci. Dev.* **2015**, *6*, 964.

Guarieiro, L. L. .; Pinto, A. .; Aguiar, P. .; Ribeiro, N. . ;*Quim. Nova* **2008**, *31*, 421.

Devi, A.; Das, V. K.; Deka, D. ;*Fuel* **2017**, *187*, 306.

Peer, M. S.; Kasimani, R.; Rajamohan, S.; Ramakrishnan, P. ;*J. Mech. Sci. Technol.* **2017**, *31*, 455.