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Ultrasound-Assisted Extraction of Sunflower Seed Oil Enriched with Active Compounds from Jambolan Leaf

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This work aimed to incorporate the active compounds of the jambolan leaf in sunflower seed oil, in order to increase the nutritional quality of the oil. For this purpose, ultrasound-assisted extraction was conducted to establish the process conditions related to temperature, potency, solvent:sample ratio and time to obtain the enriched sunflower seed oil (ESSO). An experimental design was applied to examine the effect of variables (temperature, power, solvent:sample ratio and time) on the extraction mass yield (EMY). The application of maximum temperature, increased power, sample:solvent ratio and time resulted in higher EMY. Maximum theoretical EMY value was 35.16% (60 °C, 100%, 1:12 g mL^{-1} and 15 min). The characterization of the oils obtained from ESSO and sunflower seed oil (SSO) verified a higher content of flavonoids, phenolic compounds, a large proportion of active compounds, antioxidant activity, content of phytosterols, α-tocopherol and squalene, and induction time for ESSO.

Keywords: *Syzygium cumini*, antioxidant activity, phenolic compounds, flavonoids

Introduction

Oxidation is considered the main responsible for the loss of quality of vegetable oils, reduction of shelf life, decrease in nutritional value, generating undesirable flavors, which makes it less acceptable for consumers.[1] Control of lipid oxidation is achieved by the use of synthetic antioxidants, which have been questioned due to their toxicity and carcinogenicity.[2] Thus, natural antioxidants of plant origin have been suggested as an alternative to synthetic ones in the prevention of lipid oxidation,[3] and simultaneously increase the nutritional quality of the oil.

The leaves of *Syzigium cumini* L. (jambolan) have high biological potential with high antioxidant, anti-inflammatory and antimicrobial activity[4,5] due to the presence of phenolic compounds, such as tannic, gallic, ellagic, caffeic, ferulic and *p*-coumaric acids,[6,7] catechin, epicatechin and quercetin,[8] as well as squalene and β-sitosterol.[9]

To contribute towards increasing the nutritional quality of vegetable oils and their applicability, the recovery of active compounds present in the jambolan leaf should be considered. Recent research indicates that this procedure can be carried out simultaneously with the oil extraction from the oilseed, as performed by Massa *et al.*[10] and Jaski *et al.*[11] who obtained vegetable oil enriched with active compounds from pumpkin peels and olive leaves, respectively.

To select the extraction technique to be applied, some points must be considered, such as the requirement for high temperatures, long extraction time and high solvent consumption, factors that can compromise the active compounds.[12,13] In this way, ultrasound-assisted extraction (UAE) can be performed, so that the compounds are preserved and consequently the quality of the oil is high, since sonication implies an increase in the mass transfer rate and extraction yield.[14] The application of ultrasound generates acoustic waves between the solvent and the sample, creating regions of compression and expansion forming cavitation bubbles.[15] When these bubbles reach their critical size, they implode near the vegetative cell matrix, causing

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shock wave-induced damage, resulting in the release of extractable compounds from the sample into the solvent.[16]

UAE has already been applied to the matrices in question, as seen in the study by Sousa *et al.*[1] and Ávila *et al.*[17] However, the extraction of active compounds from jambolan leaf into sunflower seed oil using this technique has not been reported. Additionally, ethanol was applied as an extraction solvent, as it comes from a biological base, is non-toxic and considered a GRAS solvent (generally recognized as safe).[18] Ethanol is used to obtain active compounds,[19] as its polarity tends to extract polar compounds, such as phenolics,[20] and still has efficiency to extract lipids.[10]

Based on this context, obtaining sunflower seed oil enriched with active compounds from the jambolan leaf and evaluating its quality is the purpose of this study. For this, the UAE was applied under different operating conditions in order to maximize the extraction mass yield (EMY) using ethanol as extraction solvent and the nutritional characteristics of the obtained oil were determined. To evaluate the degree of increase in oil quality, the process without leaves in the extraction medium was conducted.

Experimental

Materials

Sunflower seeds (Umuarama, Brazil), and jambolan leaves (Umuarama, Brazil) (3°47'55''S and 53°18'48''W) which were identified and applied in an expository specimen in the Herbarium of the Universidade Estadual de Maringá (UEM), registration HUEM 40310, and ethanol (Riedel, Germany) were used in the extraction experiments.

In the analysis of the oil were used: methanol (Neon, Suzano, Brazil), aluminum chloride (Dynamic, Indaiatuba, Brazil), potassium acetate (Synth, Diadema, Brazil), hexane (Neon, Suzano, Brazil), Folin-Ciocalteu (Dynamic, Indaiatuba, Brazil), sodium carbonate (Anidrol, Diadema, Brazil), 2.2-diphenyl-1-picrylhydrazyl (DPPH•) (Sigma-Aldrich, Saint Louis, USA), ethanol (Riedel, Germany), trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromanwe-2-carboxylic acid (Sigma-Aldrich, Saint Louis, USA), 2,4,6-tris(2-pyridyl)-*S*-triazine (Sigma-Aldrich, Saint Louis, USA), hydrochloric acid (Anidrol, Diadema, Brazil), sodium acetate (Synth, Diadema, Brazil), glacial acetic acid (Anidrol, Diadema, Brazil), ferric chloride (Scientific Exodus, Sumaré, Brazil), *N*,*O*-bis(trimethylsilyl) trifluoroacetamide/trimethylchlorosilane (BSTFA/TMCS), 5α-cholestane and methyl heptadecanoate (Sigma-Aldrich, Saint Louis, USA), methanol suitable for high-performance liquid chromatography (HPLC) and formic acid (Merck, Rio de Janeiro, Brazil).

Sample preparation

The jambolan leaves (39.06 ± 2.74 wt.% humidity) were cleaned and the central stem of the leaf was removed, then dried in an oven with air circulation (Marconi, MA035, Piracicaba, Brazil) at 60 °C for 4 h, reaching a humidity of 4.38 ± 1.33 wt.%. Sunflower seeds (used as received with 1.83 ± 0.17 wt.% humidity) and dried jambolan leaves were ground in a multiprocessor (Walita, LiqFaz, Itapevi, Brazil) and classified granulometrically in Tyler-type sieves (Bertel, Caieiras, Brazil) to obtain particles with an average diameter of 0.841 and 0.557 mm, respectively. The sample used in the experiments was a mixture of jambolan leaves and sunflower seeds in a ratio of 1:10 (0.3 g to 3 g). This proportion was selected because it provided a homogenous sample.

Ultrasound-assisted extraction

The experiments were conducted following a Box-Behnken experimental design, generated by the Statistica 8.0 Software (StatSoft, Inc.),[21] with four independent variables, whose levels of the evaluated variables are shown in Table 1. The range of variables evaluated was selected based on a previous report[22] obtained regarding the extraction of vegetable oils using ethanol as a solvent. The equipment used in the extractions was an ultrasound bath with indirect contact (Ultronique, Q 5.9/40 A, Eco-Sonics, Indaiatuba, Brazil) with a power of 165 W.

Table 1. Levels of the variables used in the Box-Behnken experimental design to perform ultrasound-assisted extraction

Variable	Level		
	−1	0	1
Temperature (T) / °C	30	45	60
Potency[a] (P) / %	0	50	100
Solvent to sample ratio (R) / (mL g^{-1})	4	8	12
time (t) / min	15	30	45

[a]165 W.

The extraction procedure was similar to that described in previous studies[22,23] and ca. 3.3 g of sample were used in each run. After the extraction period, the solid material was removed by filtration and the solvent in the filtrate removed on a rotary evaporator (Marconi, MA120, Piracicaba, Brazil). The EMY was obtained considering the oil mass obtained and the initial mass of the sample used in the experiment.

Based on the results obtained, analysis of variance (ANOVA) was performed to assess the effects of independent variables (with a 95% confidence interval)

on the responses. The experimental data were fitted to the second-order polynomial model, according to equation 1.

$$EMY = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n} \sum_{j=i+1}^{n} \beta_{ij} X_i X_j \quad (1)$$

where EMY is the response variable; X_i and X_j are the coded independent variables (temperature, potency, solvent to sample ratio and time), β_0, β_i, β_{ii} and β_{ij} are the regression coefficients (β_0 = constant term, β_i = linear term, β_{ii} = quadratic term and β_{ij} = linear interaction term).

To determine the conditions that maximize the EMY, in the evaluated experimental range, the Derringer desirability function was applied. The predictive capacity of the equation was evaluated based on verification experiments, conducted in triplicate, under maximum extraction conditions. The prediction efficiency of the equations was verified through the Student's *t*-test. In this same experimental condition, experiments were carried out, in triplicate, to obtain oil only from sunflower seeds for comparative purposes (in terms of EMY and nutritional quality of the oil). The non-lipid fraction of the oils was determined based on the methodology of Rodriguez *et al*.[24]

Oil characterization

The analyses were performed at least in triplicate, and the results were presented as mean values ± standard deviation. The results were evaluated by analysis of variance (ANOVA) using the Statistica 8.0 software (StatSoft, Inc., Tulsa, OK, USA),[21] followed by comparison of the means using the Tukey's test (with 95% confidence interval).

Flavonoids and phenolic compounds: total content and profile

To quantify the content of total flavonoids (TF) and total phenolic compounds (TPC), it was necessary to proceed with hydromethanolic extraction of the compounds.[25] After, the content of each compound was determined as reported by Lin and Tang[26] and Singleton *et al*.,[27] with the determination of the absorbance of the solutions prepared in a UV-Vis spectrophotometer (Shimadzu UV-1900, Japan, Tokyo). The TF and TPC contents were determined using standard curves prepared with quercetin and gallic acid solutions at different concentrations, respectively.

The profile of active compounds was determined in a triple quadrupole mass spectrometer (model XEVO TQD from Waters, Massachusetts, USA) equipped with an electrospray ionization (ESI) source operating in negative ionization modes. Samples were diluted in methanol (1:9 v:v) and acidified with formic acid. The infusion in the spectrometer was at a rate of 10 μL min^{-1} and the working conditions were determined as interface temperature of 350 °C, nebulizer gas flow (nitrogen) at 600 L min^{-1} and analysis range 50 to 700 *m/z*. Data were collected and processed with MassLynx software and the PubChem database was used to identify the compounds.

Antioxidant activity

The antioxidant activity of the oils was determined by the DPPH• and ferric reducing antioxidant power (FRAP) methods, with sample preparation as indicated by Gu *et al*.[28] and Rufino *et al*.,[29] respectively.

Phytosterols, α-tocopherol and squalene content and fatty acid composition

The analyzes were conducted by gas chromatography coupled to mass spectrophotometry adopting the conditions described by Iwassa *et al*.[30] The identification of compounds was performed based on a search in the NIST Spectrum Library (2014 version). Minor compounds were quantified using 5α-cholestane as an internal standard and the fatty acid composition was determined through the relationship between the fatty acid (FA) peak area and the total peak area of the chromatogram.

Oxidative stability

The oxidative stability of the oils was determined (in triplicate) in Biodiesel Rancimat Oxidation Stability Analyzer (Metrohm, model 823, Herisau, Switzerland), with the determination of the induction time (IT) obtained automatically from the second derivative of the conductivity curve using the equipment software. In each analysis, the samples (2.5 g) were exposed to an air flow (20 L h^{-1}) at 110 ºC.[31]

Results and Discussion

Extraction mass yield

Table 2 shows the combinations of experimental variables evaluated in the experimental design to obtain sunflower seed oil enriched with active compounds from the jambolan leaf (ESSO), as well as the responses obtained regarding the EMY.

Table 3 presents the ANOVA of the data in order to evaluate the effects of each variable (linear and quadratic) and the interaction effects. From the data presented in

Table 2. Experimental conditions and results of extraction mass yield (EMY) from ultrasound-assisted extraction

Run	Variable[a]				EMY / wt.%
	(T / ºC)	(P / %)	(R / (mL g^{-1}))	(t / min)	
1	−1 (30)	−1 (0)	0 (8)	0 (30)	23.66
2	1 (60)	−1 (0)	0 (8)	0 (30)	27.93
3	−1 (30)	1 (100)	0 (8)	0 (30)	24.81
4	1 (60)	1 (100)	0 (8)	0 (30)	32.03
5	0 (45)	0 (50)	−1 (4)	−1 (15)	20.22
6	0 (45)	0 (50)	1 (12)	−1 (15)	29.80
7	0 (45)	0 (50)	−1 (4)	1 (45)	19.57
8	0 (45)	0 (50)	1 (12)	1 (45)	31.90
9	0 (45)	0 (50)	0 (8)	0 (30)	27.65
10	−1 (30)	0 (50)	0 (8)	−1 (15)	24.85
11	1 (60)	0 (50)	0 (8)	−1 (15)	27.28
12	−1 (30)	0 (50)	0 (8)	1 (45)	27.23
13	1 (60)	0 (50)	0 (8)	1 (45)	30.87
14	0 (45)	−1 (0)	−1 (4)	0 (30)	15.39
15	0 (45)	1 (100)	−1 (4)	0 (30)	19.22
16	0 (45)	−1 (0)	1 (12)	0 (30)	29.99
17	0 (45)	1 (100)	1 (12)	0 (30)	32.26
18	0 (45)	0 (50)	0 (8)	0 (30)	27.46
19	−1 (30)	0 (50)	−1 (4)	0 (30)	17.58
20	1 (60)	0 (50)	−1 (4)	0 (30)	21.90
21	−1 (30)	0 (50)	1 (12)	0 (30)	29.92
22	1 (60)	0 (50)	1 (12)	0 (30)	32.69
23	0 (45)	−1 (0)	0 (8)	−1 (15)	21.57
24	0 (45)	1 (100)	0 (8)	−1 (15)	27.21
25	0 (45)	−1 (0)	0 (8)	1 (45)	21.89
26	0 (45)	1 (100)	0 (8)	1 (45)	30.82
27	0 (45)	0 (50)	0 (8)	0 (30)	28.87
28	0 (45)	0 (50)	0 (8)	0 (30)	29.59
29	0 (45)	0 (50)	0 (8)	0 (30)	29.45

[a]As Table 1. T: temperature; P: potency; R: solvent to sample ratio; t: time.

Table 3. Analysis of variance of the regression model for extraction mass yield (EMY) from ultrasound-assisted extraction

Factor	Sum of squares	Degrees of freedom	Mean square	*F*	*p*[a]
(T) (L)	50.64	1	50.64	50.92	0.002
(T) (Q)	0.03	1	0.03	0.03	0.87
(P) (L)	55.99	1	55.99	56.31	0.002
(P) (Q)	21.31	1	21.31	21.43	0.012
(R) (L)	440.20	1	440.20	442.69	< 0.001
(R) (Q)	44.01	1	44.01	44.26	0.003
(t) (L)	10.74	1	10.74	10.80	0.030
(t) (Q)	6.59	1	6.59	6.64	0.062
T × P	2.18	1	2.18	14.87	0.061
T × R	0.60	1	0.60	0.60	0.480
T × t	0.37	1	0.37	0.37	0.577
P × R	0.61	1	0.61	0.61	0.478
P × t	2.71	1	2.71	2.72	0.174
R × t	1.89	1	1.89	1.90	0.240
Lack of fit	27.06	10	2.70	2.72	0.173
Pure error	3.98	4	0.99		
Total	656.56	28			

$R^2 = 0.99$

$R^2_{adjusted} = 0.99$

[a]Statistical significance ($p < 0.05$); L: linear effect; Q: quadratic effect. T: temperature (°C); P: potency (%); R: solvent to sample ratio (mL g^{-1}); t: time (min).

Table 3, it can be seen that the linear terms of all variables had an effect on the response variable, as well as the quadratic effect of the sample to solvent ratio and potency.

The quadratic polynomial equation adjusted to the experimental data, considering only the significant terms, is presented in equation 2.

$$EMY\ (wt.\%) = 28.02 + 2.06T + 2.16P + 6.06R + 0.95t - 1.64P^2 - 2.43R^2 \quad (2)$$

The value of $F_{calculated}$ (48.85) was > $F_{tabulated}$ (2.55) (obtained from the ANOVA), which indicates that the predictive equation is valid for predicting the extraction behavior of the studied system. Based on the generated diagnostic graphs (Supplementary Information (SI) section), it was possible to confirm the adequacy of the equation, with a high correlation between experimental and predicted data (Figure S1) and random distribution of residues close to zero (Figure S2).

Figure 1 presents the main effect of each variable expressed in a disturbance graph. In this figure, it can be seen that the temperature and power influenced from the zero factor (30 ºC and 0 W, respectively). For the solvent:sample ratio, variation occurred throughout its range, the time remained constant with a slight increase after the zero factor (15 min). The slope of the curvature of all factors indicated a positive effect on EMY, except the relatively flat time curve, which showed a smaller positive effect on EMY, suggesting that the oil was extracted quickly and efficiently in the first washing stage.

Effect of temperature

Temperature is one of the factors with the greatest influence on mass transfer in ultrasound-assisted extraction medium,[32] since the increase in this variable contributes to the collapse of the cavitation bubbles that

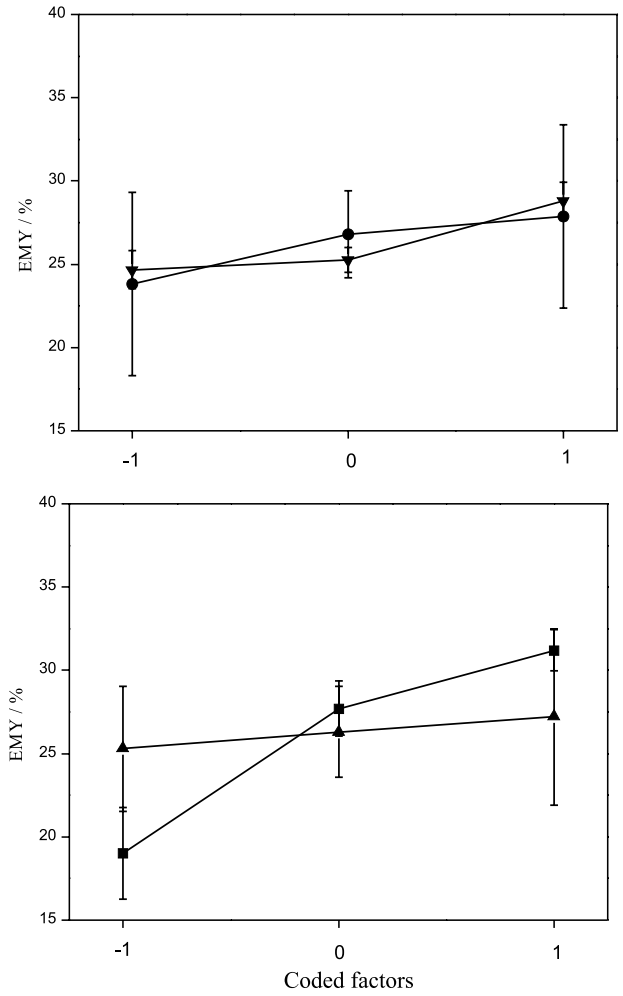

Figure 1. Perturbation graph for mass yield extraction (EMY) (results from Table 2) from ultrasound-assisted extraction: ▼: temperature, ●: potency, ■: solvent:sample ratio and ▲: time.

are generated by sonication, which damage the walls of the vegetative matrix, increasing the surface area and making the diffusion of the solvent faster inside the solid matrix.[33-35] This is attributed to the effect of temperature on oil solubilization, due to the reduction in viscosity and surface tension of the solvent.[36] Furthermore, the flow resistance is weakened and the mass transfer rate is increased due to the increased Gibbs free energy in the extraction system.[37]

Santos *et al.*[34] identified an improvement in the oil yield of favela seeds with an increase in the extraction temperature and attributed this result to the breakdown of the solute-sample interaction and an increase in the diffusion rate due to the reduction in viscosity and surface tension of the solvent. Elevated temperatures in the extractive process can soften plant tissues, increase diffusion and promote the elution of oil adhered to the interior of the solvent.[38] Sanchez *et al.*[39] and Dong and Sun[40] indicated an increase in the diffusion coefficient when the temperature changed from 25 to 60 ºC and 20 to 50 ºC, respectively, in the extraction of oil from oilseeds.

Effect of ultrasound power

Conducting the extraction assisted by ultrasound, 0 to 100% of power, favored obtaining higher EMY (for example: ca. 40%, runs 25-26). This effect is due to the increase in hydrodynamic forces in the extraction medium with the increase in power.[41,42] This occurs because the bubbles generated in the extraction medium are proportional to the power of the ultrasound, and with that, the violent implosion of the cavitation bubbles occurs, which makes the mechanical impact greater,[43] which also raises the temperature and consequently the solubilization of the oil in the solvent.[44]

Sanwal *et al.*[42] identified that ultrasound causes friction, particle rupture and cell breakage, factors caused due to the cavitation phenomenon. These effects accelerate the release of extractable compounds[45] and enhance the dissipation of energy between the cavitation bubbles and the solid material.[46] Thilakarathna *et al.*[32] carried out a scanning electron microscopy (SEM) analysis of mahua seeds submitted to extraction by ultrasound and maceration, and concluded that the application of ultrasonic power generated a porous structure in the cell wall and the process by maceration presented an image with a smooth structure, which directly implies the lack of ability to break the cell to facilitate the release of oil. Applying the same analytical technique, Pereira *et al.*[46] noticed more fragmented structures, with deformations and ruptures resulting from the increase in ultrasonic intensity, which contributes to the penetration of the solvent into the internal structures, facilitating the removal of the target compounds.

Effect of solvent to sample ratio

Increasing the volume of extraction solvent relative to the amount of solid makes diffusion more efficient,[36] and with that greater mass of oil was obtained. Among the analyzed variables, the sample to solvent ratio, in the considered experimental range, had the greatest influence on the EMY (Table 3). The greater amount of solvent in contact with soluble compounds increases the solubility between solute and solvent, which makes the process more efficient.[47]

Kostić *et al.*[48] reported an increase in the diffusion rate constant when the sample:solvent ratio was changed from 1:3 to 1:10 (g mL^{-1}), which was also evidenced by Stamenković *et al.*[49] Franco *et al.*[50] identified an increase in the diffusivity coefficient from 0.61 to 6.99×10^{-11} m^2 s^{-1}

as they increased the solvent:sample ratio from 1:15 to 1:150 (g mL^{-1}). When the proportion of liquid in relation to the solid matrix increases, the spontaneity of extraction increases as the Gibbs free energy becomes more negative with greater liquid content in the extraction solution.[49-51]

Effect of extraction time

The different extraction times evaluated in this study had little influence on the EMY, showing that oil extraction occurs mainly in the initial stage of extraction (washing). The washing step corresponds to removing the surface oil,[52] which suggests that the waves produced by the equipment have a greater effect in this first stage. Evidenced in this study by experiments in which the temperature, power and ratio were maintained and the time increased from 15 to 45 min, as well as in experiments (6 and 8) and (10 and 12) which increased by ca. 7 and 9.6%, respectively, which explains the removal of oil in the first washing stage.

Verification experiments

From the predicted equation (equation 2), the conditions that maximize the EMY were determined at 60 °C, ultrasound power at 100% (165 W), sample to solvent ratio of 1:12 g mL^{-1} and time of 15 min, resulting in the theoretical value of 35.16 wt.%. Experiments conducted under these conditions resulted in 35.59 ± 0.11 wt.%, value that does not statistically differ from the value predicted by the equation ($p > 0.05$). Oil extraction only from sunflower seeds, under these conditions, resulted in an EMY of 33.18 ± 0.68 wt.%. The literature indicates that sunflower seeds have oil contents of 23.71 to 36.37 wt.%.[53,54]

The oils obtained, ESSO and sunflower seed oil (SSO), presented 3.7 ± 0.14 and 2.4 ± 0.14 wt.% of non-lipid compounds, respectively. This is due to the characteristic of the ethanol solvent that simultaneously extracts polar lipids and other compounds such as proteins and sugars from oilseeds.[24]

Oil characterization

Table 4 presents the characterization of the oils obtained (ESSO and SSO), in which it is possible to verify that the simultaneous extraction of vegetable oil and active compounds provided a product with a higher content of flavonoids and phenolic compounds. Oil enrichment is also evidenced by higher levels of most of the identified

Table 4. Characterization of the oils obtained from ultrasound-assisted extraction: sunflower seed oil enriched with jambolan leaf compounds (ESSO) and sunflower seed oil (SSO)

Property			ESSO	SSO
Total flavonoids content / (mg QE *per* 100 g oil)			43.46 ± 0.53[a]	27.48 ± 0.64[b]
Total phenolic compounds content / (mg GAE *per* 100 g oil)			90.21 ± 1.20[a]	11.34 ± 0.19[b]
Active compound / (intensity *per* sample concentration)		*m/z*		
	quercetin	300.9	1.93×10^7	1.26×10^7
	catechin	289	1.13×10^7	9.82×10^6
	sinapic acid	223	4.58×10^8	3.09×10^8
	trans-caffeic acid	178.95	1.73×10^8	1.42×10^8
	p-coumaric acid	162.95	3.58×10^7	1.66×10^7
	quinic acid	223	4.93×10^7	2.99×10^7
	ellagic acid	300.89	1.73×10^7	1.69×10^7
	n-hentriacontane	436	7.12×10^6	7.56×10^6
	crategolic (maslinic) acid	471.3	1.52×10^7	1.26×10^7
Antioxidant activity / (μmol TEAC *per* g oil)	DPPH•		13.56 ± 0.69[a]	3.83 ± 0.65[b]
	FRAP		18.60 ± 0.05[a]	2.48 ± 0.01[b]
Compound / (mg *per* 100 g oil)	β-sitosterol		153.00 ± 5.59[a]	94.98 ± 0.28[b]
	campesterol		21.81 ± 1.82[a]	11.72 ± 0.64[b]
	stigmasterol		32.51 ± 1.04[a]	31.46 ± 1.41[a]
	α-tocopherol		104.93 ± 4.21[a]	82.13 ± 2.12[b]
	squalene		94.35 ± 2.75[a]	83.52 ± 0.60[b]
Fatty acid / %	palmitic		7.22 ± 0.06[a]	7.21 ± 0.03[a]
	stearic		3.78 ± 0.10[a]	3.99 ± 0.03[a]
	oleic		28.72 ± 0.05[a]	28.65 ± 0.19[a]
	linoleic		54.96 ± 0.09[a]	54.30 ± 0.22[a]
	others		5.32 ± 0.95[a]	5.85 ± 1.00[a]
Induction time / h			11.84 ± 0.78[a]	5.94 ± 0.56[b]

QE: quercetin equivalent. GAE: gallic acid equivalent. TEAC: Trolox equivalent antioxidant capacity; DPPH: 2.2-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power. Means followed by different letters indicate a significant difference ($p < 0.05$).

active compounds (with the exception of ellagic acid and *n*-hentriacontane) and minor compounds. Consequently, higher antioxidant activity was observed in ESSO, as well as longer induction time.

Jambolan leaves have a high content of flavonoids and phenolic compounds as indicated by Singh *et al*.[55] and Balyan *et al*.,[7] respectively. According to Correia *et al*.,[56] flavonoids from the Myrtaceae family are promising compounds in the development of new products due to their bioactive properties, as they have biological characteristics with antioxidant, anti-inflammatory, antibacterial, antiallergic and vasodilator action. These compounds, when applied to vegetable oils, act as protectors of lipid oxidation due to their ability to transfer oxygen to the oil,[57] in addition to increasing oxidative stability.[58]

Zeb and Allah[59] reported that sunflower oil can be enriched using phenolic compounds present in spinach leaves, making it resistant to lipid oxidation as well as having high antioxidant activity. Sousa *et al*.[1] supplemented sunflower oil with the active compounds found in the mixture of stems and leaves of *Crithmum maritimum* L. and identified an improvement in oxidative stability and biological value by presenting significant amounts of flavonoids and phenolic compounds.

The flavonoids identified in ESSO were quercetin and catechin, as previously reported by Balyan and Sarkar[60] and Balyan *et al*.[7] in the composition of jambolan leaves. Among the phenolic acids identified, sinapic, *trans*-caffeic, quinic, *p*-coumaric and crategolic stood out, which have antioxidant properties,[61,62] which raises the quality of the oil and increases its stability.[63]

Kiokias and Varzakas[64] observed that quercetin and catechin had an antioxidant and pro-antioxidant character in cottonseed oil. Sinapic acid has antioxidant, antimicrobial, anti-inflammatory, anticancer and anxiolytic activity.[65] *trans*-Caffeic acid was found in SSO, however, its intensity was increased when jambolan leaves were added. Some studies[66] confirm that this compound has a high free radical scavenging activity influenced by the number of hydroxyls in the aromatic ring, that is, it has a high biological action of antioxidants. Quinic acid and *p*-coumaric acid have high antioxidant power.[67,68] The *n*-hentriacontane compound is considered to be a linear alkane found in plant waxes,[69] therefore, this compound is not present in jambolan leaves, only in SSO, as well as ellagic acid and crategolic acid.

The use of compounds from jambolan leaves incorporated into sunflower seed oil provided vegetable oils with high antioxidant activity. The results indicate that compounds with antioxidant activity are fat-soluble, as an increase in this compound in supplemented oils was expected. The antioxidant activity with a lipophilic structure, which eliminates oxygen, hydroxyl radicals and lipid peroxyl radicals, consequently inhibits lipid peroxidation causing disorders of biological membrane,[70] therefore, it improves the induction time and increases the oxidative stability of the oils. As well as the presence of other compounds such as α-tocopherol, since tocopherols have antioxidant activity in lipids[71] and squalene[72] which can also increase the antioxidant capacity of vegetable oils.

The compounds β-sitosterol, campesterol, α-tocopherol and squalene were detected in greater amounts in the ESSO, due to their presence in the composition of jambolan leaves.[73,74] β-Sitosterol is the phytosterol present in greater quantity in vegetable oils,[75] as found in this study. Additionally, these compounds have the potential to protect oils from lipid oxidation and polymerization during heat treatment/light exposure.[76]

Among tocopherols, α-tocopherol is considered a natural antioxidant present in oils, which blocks the chain reaction of free radicals during oil oxidation, improving the antioxidant capacity.[77] The antioxidant properties are attributed to the hydroxyl groups of the aromatic ring, which donate hydrogen to neutralize free radicals or reactive oxygen species.[78] SSO already has α-tocopherol in its composition and with the addition of the simultaneous extraction of oil and compounds from jambolan leaves, obtain an increase of ca. 27% in this compound in the product.

Squalene is considered an intermediate hydrocarbon in the biosynthesis of phytosterols and terpenes.[79] This compound has a nutritional effect on the human body that includes a strong oxygen-carrying capacity, which can revitalize the body, promote metabolism and improve immune function,[80] in addition to increasing the shelf life of vegetable oils[81] due to its antioxidant capacity.[82]

Stigmasterol showed no difference between the SSO and ESSO samples, leading to the conclusion that the jambolan leaf does not contain a significant amount of this compound. Study reported by Martins *et al.*[83] indicates that sunflower oil can contain 26.6 to 26.74 mg of stigmasterol *per* 100 g of oil. Poulose *et al*.[84] studied the potential antidiabetic effect of stigmasterol and noticed that this compound, when ingested, had a decrease in the level of glucose in the blood, helping to reduce diabetes. Campesterol is known to have cholesterol-lowering effects due to its structural similarity to cholesterol, making it a possible antiangiogenic candidate for prevention and treatment of angiogenesis-related diseases as well as cancer.[85]

Due to the absence of lipid fraction in jambolan leaves, the oils obtained had a similar fatty acid composition, which is mostly unsaturated fatty acids with a ratio of 7.6:1 in relation to saturated fatty acids. This is relatively interesting,

as it is believed that saturated oils have a negative impact on health, while unsaturated oils improve health.[86] Linoleic acid are considered essential fatty acid, which must be ingested as the body does not synthesize them and can reduce the risk of various diseases such as heart disease and cancer.[87] Linoleic acid followed by oleic acid prevailed in the composition of polyunsaturated fatty acids, which correspond to ca. 84% of the composition of the oils.

The oils obtained have a long induction time, which is due to ethanol's ability to extract polar compounds,[24] in accordance with the polar paradox.[88] Due to active compounds present in the leaves, ESSO had an induction time ca. 2 times longer than SSO. As reported by Kasote *et al*.,[88] the presence of antioxidant acts as free radical scavengers and oxygen suppressors, consequently increasing the induction time in vegetable oils. More *et al*.[89] observed an improvement in the stability of sunflower oil with the addition of antioxidants from moringa leaves and reported that the induction time was increased from 1.99 to 5.23 h. Lafka *et al*.[90] identified an increase in the induction time of sunflower oil from 7.45 to 10.23 h using olive leaves as natural antioxidants.

Conclusions

In this work, the active compounds from jambolan leaves were incorporated into SSO to increase the nutritional quality of this oil. From the study carried out, it was found that the highest EMY (35.59 ± 0.11 wt.%) can be obtained by applying a temperature of 60 °C, power of 165 W, sample:solvent ratio 1:12 g mL^{-1} and time of 15 min. ESSO presented superior quality to SSO compared to most of the parameters analyzed, with an excess of fatty acid composition that was similar for both oils. The jambolan leaves provided phenolic acids and flavonoids in ESSO, as evidenced by the profile and total levels of these compounds.

Supplementary Information

Supplementary information (the generated diagnostic graphs, Figures S1 and S2) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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