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Research Article
Quantitative Profile of Fatty Acids and Tocopherols in Tamarind Seeds (*Tamarindus indica* L.) From Different States of Brazil

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Abstract

Background and Objective: Tamarind seeds are a by-product with great potential for industrial use. However, data on the composition of the seeds from Brazilian fruits are still scarce. In this sense, the aim of this study was to evaluate the levels of tocopherols and fatty acids of tamarind seeds from 3 states of Brazil (Minas Gerais, São Paulo and Bahia). **Methodology:** Quantitative analysis by GC-FID-MS and HPLC-FL and principal component analysis (PCA) was performed in order to identify patterns among the samples. One-way Analysis of variance (ANOVA) with F test and Tukey test ($p \leq 0.05$) were used to identify significant differences between the averages. **Results:** The seeds from Minas Gerais have the highest levels of α-tocopherol (25.5 mg $kg^{-1}_{dry\ solid}$) and γ-tocopherol (31.1 mg $kg^{-1}_{dry\ solid}$), while the lowest concentration was found in the seeds of the fruits from São Paulo (16.4 mg $kg^{-1}_{dry\ solid}$). Regarding fatty acids, linoleic and oleic acids had the highest concentrations in all samples, however, the samples from Bahia had higher concentrations of these compounds. Changes in the lipophilic profile have been observed through of the use of chemometric tools, such PCA. **Conclusion:** Tamarind seeds have been shown a source of polyunsaturated fatty acids and its use could be an alternative for the reduction of expenses with waste treatment.

Key words: Liquid chromatography, gas chromatography, mass spectrometry, seed oil, tamarind, vitamin E

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

One of the main problems encountered by agricultural industries all over the world is related to the treatment, mitigation or prevention of waste generation due to environmental effects arising from their disposal[1]. Most of the waste is generated because of the underutilization of raw material, leading to financial losses, in addition to the loss of the nutritional and industrial potential of the discarded product[2].

Brazil is one of the biggest producers of tropical fruits in the world and a major pole of processing of raw materials that generate tonnes of waste daily[3]. These wastes are products removed from the production process because they are undesirable material in the final product. However, a large part of this waste has great potential for reuse as a source of functional compounds or in the development of new products[1].

The *Tamarindus indica* belongs to the family of the Leguminosae (*Fabaceae*). It is a tree native from Africa, India and Southeast Asia that grows in tropical and subtropical regions, with an average ideal temperature of 25°C[4]. The tamarind is well adapted to the Brazilian territory being produced primarily in the Northeastern region of the country, however, it is not very explored and much of the production is destined for domestic consumption.

The pulp of the fruit has a sweet acid flavour and is widely used in the manufacturing of nectars, ice creams, pastes, sweets, liqueurs, jams and also as an ingredient in condiments and sauces[5]. The seeds represent the largest portion of the fruit, reaching up to 40% of the total weight. In its composition are present several classes of bioactive compounds, such as tocopherols and unsaturated fatty acids, to which antioxidant, anti-hepatotoxic, anti-inflammatory, anti-mutagenic, anti-diabetic and anti-atherosclerosis activities are assigned[6,7].

Despite having great potential for industrial utilization with high fatty content and bioactive compounds in the lipid fraction[8], the tamarind seed is still not very exploited, making its potential underused. In addition, taking into account that the composition of the fruits and the seeds is strongly influenced by both the climate and the soil type of the planting region and that data about the Brazilian seeds are yet to be reported, the need to characterize the seeds originated from different regions of Brazil arises in order to direct the best way of exploiting them or even their incorporation in the human diet[3].

Thus, the aim of this study was to evaluate the quantitative profile of fatty acids and tocopherols in the tamarind seed harvested in 3 different regions of Brazil, in 2 periods, to favor new alternatives of industrial application in the future.

MATERIALS AND METHODS

Reagents and standards: Methanol p.a. (Synth, Brazil), chloroform p.a. (Synth, Brazil), sodium hydroxide solution p.a. (Synth, Brazil), boron trifluoride p.a. (Merck, Germany), butylatedhydroxy toluene - BHT (Sklean, Brazil), in addition to hexane (Macron, USA), isopropanol (J.T. Baker, USA) and acetic acid (J.T. Baker, USA) were used, all chromatographic grade. For the quantifications, standards of tocopherols α, β, γ and δ(Supelco, USA), methyl esters from C4 to C24 (FAME Mix, Supelco, USA) and tricosanoic acid-23:0 (Supelco, USA) were used.

Sample collection and preparation: The tamarind fruits were collected in 2012 and 2013, in Minas Gerais (SMG), São Paulo (SSP) and Bahia (SBA), in the cities of Patos de Minas (18°34' S and 46°31' O), Campinas (22°49'3'' S e 47°4'11"W) and Tanhaçu (14°1' 11'' S e 41°14' 7'' O), respectively. The samples were sent to the Laboratory of Food Analysis of the State University of Campinas. After the collection, they were peeled and submerged in water for 24 h to hydrate them and facilitate the extraction of the seeds. Subsequently, the hydrated fruits were pulsed in an industrial blender and filtered in a common sieve to separate the pulp from the seed. The seeds were subjected to analysis of moisture and lyophilized. Next, they were crushed and subjected to the extraction of lipids through the Bligh and Dyer method[9]. The obtained oil was stored at -18°C until the moment of analysis.

Fatty acid methyl esters: The fatty acid methyl esters were obtained according to the method proposed by Joseph and Ackman[10]. To this end, 25 mg of oil extracted from the tamarind seed, 4 mL of NaOH 0.5 mol L^{-1} in methanol and a solution containing 1 mg of tricosanoic acid (internal standard) in hexane were added in test tubes with caps. The tubes were heated in a water bath at 100°C for 10 min until the obtaining of a transparent solution. Later, 3 mL of 12% BF_3 in methanol were added and heated again for 5 min. After cooling, 4 mL of a saturated solution of NaCl were added and then the mixture was homogenized. Finally, 4 mL of hexane were added followed by vigorous stirring in the vortex. The tubes were kept at rest until the separation of phases. The upper phase was collected and the residue was washed with 2 mL of hexane 3 times. The collected phases were combined, concentrated to dryness in a rotary evaporator and

re-suspended in 2 mL of hexane. The experiment was done in triplicate (n = 3).

After methylation, 1 μL of the extract was injected into the GC-FID-MS 7890A (Agilent, Germany) equipped with automatic injector, operating in the split mode (1:50), with a DB 23 column (60 m×0.25 mm×0.25 μm), following the conditions proposed by David *et al.*[11]. The equipment operated with an injector at 250°C, detector at 280°C, using H_2 as carrier gas at 1 mL min^{-1} and flow rate of the gas in the flame ionization detector (FID) of 30:30:300 mL min^{-1} (H_2: N_2: synthetic air). The oven temperature was 50°C for 1 min, followed by heating at 25°C min^{-1} up to 175°C and later increase to 4°C min^{-1} up to 230°C. The identification of the compounds was made by comparison of the standard retention times with the retention times of the compounds found in the samples, under the same conditions of separation.

The quantification of the fatty acids was performed using tricosanoic acid (0:23) as an internal standard. For the determination of the concentrations of each methyl ester, the average values of the experimental correction factor (F_{CE}) for the FID were used based on 10 injections of the fatty acid methyl ester standards[12]. The experimental correction factor was calculated according to Eq. 1.

$$F_{CE} = \frac{A_p . M_x}{M_p . A_x} \quad (1)$$

Where, Ap = Internal standard area, Mp = Mass of the internal standard, Ax = Area of the fatty acid methyl ester and Mx = Mass of the fatty acid methyl ester.

The fatty acid content (AG) was calculated in mg g^{-1} of total lipids (Eq. 2) and converted into dry solids (mg g^{-1}) of the seed.

$$AG = \frac{A_x . M_p . F_{CE}}{A_p . M_x . F_{CAE}} \quad (2)$$

Where, AG = Concentration of the fatty acids (mg g^{-1}) of the total lipids, A_X = Peak area for each compound, A_P = Peak area of the internal standard (C:23), M_p = Mass of the internal standard (mg), M_X = Mass of the oil (mg), F_{CE} = Experimental correction factor and F_{CAE} = Fatty acid methyl ester conversion factor.

The fatty acid methyl esters were identified in the mass spectrometer using electron ionization (EI) as ionization source, operating at 200°C and 70 eV. The separation of the esters was accomplished in an HP-5 column (30 m×0.32 S mm×0.50 μm). To this end, 1 μL of the samples were injected in split mode (1:45), with an injector at 250°C and programming of oven temperature starting at 50°C, increasing 1°C min^{-1} up to 110°C, followed by heating at 3°C min^{-1} up to 310°C, which was maintained for 3 min. The carrier gas flow (He) was adjusted to 0.5 mL min^{-1}.

The quadrupole was operated at 150°C in scan mode and the ions generated between 50 and 500 m/z was monitored. The identification of fatty acid methyl esters was accomplished through the NIST11 library® and through the analytical standards injected under the same conditions.

Tocopherols: For tocopherols analysis was used the methodology described by Dionisi *et al.*[13] and Pinheiro-Sant'Ana *et al.*[14]. In summary, 20 mg of tamarind seed oil was diluted with 2 mL of hexane containing 0.01% BHT. The solution was filtered on PVDF membrane of 0.22 μm (Millipore, USA) and injected into an HPLC Agilent 1100 (Agilent Technologies, Germany) coupled to a fluorescence detector and equipped with an automatic injector.

The separation was made in an isocratic system consisting of Hexane:Isopropanol:Acetic acid (98.9:0.6:0.5) with a flow rate of 1.0 mL min^{-1}, using a normal phase Hypersil column (150 mm×4.6 mm×3.0 μm). The column temperature was maintained at 30°C and the injection volume was of 100 μL. The fluorescence detector was set in $\lambda_{excitement}$ = 290 nm and $\lambda_{emission}$ = 330 nm. The identification was made through the retention time of the detected compounds in the samples compared with standards analyzed under the same conditions. All extracts were prepared in triplicate (n = 3).

Validation: The method for quantification of fatty acids was validated and described elsewhere[15]. Regarding tocopherols the method was validated according to the rules described in the Harmonized Guide[16]. The limits of detection (LD) and quantification (LQ) were estimated as being 3 and 7 times the signal/noise ratio, respectively.

Calibration curves were obtained through the random injection in triplicate of 10 concentrations of each compound studied. The linearity of the curves was evaluated and the models were validated through analysis of variance (ANOVA) and linear regression. The intraday instrumental precision was determined based on 10 injections of a solution containing four tocopherols (α, β, γ and δ-tocopherol) at 3 different concentrations, including the limit of quantification, a central point and the greatest concentration of the analytic curve. The accuracy in sample was made with 10 injections in different volumes.

The inter day instrumental precision was determined through the injection of the standards in the same concentrations of the intra day precision and in 3 consecutive days. Since there is no certified reference material for the compounds in tamarind seed, recovery tests were made through the fortification of the samples in the same concentrations used in the accuracy tests before the extraction process. The recovery was calculated for each compound, not taking into consideration contents which were naturally present in the sample. The tests were carried out in triplicate for each level established (n = 3).

Statistical and chemometrics analysis: One-way analysis of variance (ANOVA) and Tukey test ($p \leq 0.05$) was used to identify significant differences between the averages obtained for each compound determined in tamarind seeds from different batches and regions. All analyzes were performed in triplicate. Additionally, principal component analysis (PCA) was applied to identify trends or similarities between samples as well as any correlation between the variables.

RESULTS AND DISCUSSION

Method validation: The method for tocopherols quantification had LD between 0.85-3.03 ng, LQ between 1.98-7.07 ng and intraday accuracy below 5% for the four compounds studied (Table 1). All models had significant adjustment ($p>0.05$), with the exception of γ-tocopherol. The lack of fit is due to the low pure error mean square (MSPe), that consequently overestimates F and results in alack of fit of the model. However, the value of F calculated was near the threshold of F tabulated indicating that the lack of fit has no great influence on the prediction of the model. On the other hand, the ANOVA indicated a highly significant regression, with the MS_R/MS_r value higher than $F_{1,26,95\%}$ (Table 2).

In view of that, the relationship between predicted values and observed values was evaluated and did not observed behaviour outside normality or heteroscedasticity of data. Finally, since there was good agreement between the predicted and observed values, we chose to use the model to make the predictions.

Recovery ranged from 64-87% (Table 3). δ-tocopherol had the lowest rates of recovery, reaching 69% at the highest level (L3). The greatest recoveries were obtained with α-tocopherol, with 74% at L1 and 85% at L3.

The low recovery rates obtained might be attributed to the Bligh and Dyer method, which naturally increases the exposure time of the free standards of the tocopherols to oxidizing agents, such as oxygen[17].

Fatty acid composition: Ten fatty acids were identified (Fig. 1) and quantified in the tamarind seed oil (Table 4) using GC-FID. Linoleic acid (18:1n-6, cis) showed the highest concentrations in the samples ranging from 145-270 mg $g^{-1}_{d.s.}$, followed by oleic acid that ranged from 31-83 mg $g^{-1}_{dry\ solid}$ and palmitic acid, in which concentrations of 21-47 mg $g^{-1}_{dry\ solid}$ were found.

Myristic acid and α-linolenic acid had the smallest proportions in relation to other fatty acids found in tamarind seed oil, with variations from 0.35-1.07 mg $g^{-1}_{dry\ solid}$ and from 0.40-1.45 mg $g^{-1}_{dry\ solid}$, respectively. These results are in accordance with those found by Luzia and Jorge[18] for tamarinds originated from the state of São Paulo (SSP). In tamarinds from Nigeria, 11 fatty acids were found, among them, the cis-11,14,17-eicosatrienoic (20:3) and cis-11,14-eicosadienoic (20:2) acids, which were not observed in

Table 1: Validation parameters of the method to the separation of tocopherols.

Tocopherols	LD* (ng)	LQ* (ng)	Linearity (ng)	Equation	r^2	Lack of fit
α-tocopherol	0.85	1.98	47.5-902.5	y = 4.4317x-97.64	1	0.073
β-tocopherol	1.28	2.99	8-152	y = 5.3434x-20.9284	0.999	0.732
γ-tocopherol	3.03	7.07	89-1691	y = 3.7832x-61.9527	0.999	0.001
δ-tocopherol	0.90	2.10	1.25-25.65	y = 5.2638x-7.0716	0.996	0.121

*LD - Limit of Detection, LQ - Limit of Quantification

Table 2: ANOVA of the linear model used in the quantification of γ-tocopherol

				Regression		Lack of fit	
Source of Variance	QS	d.f	MS	MS_R/MS_r	$F_{1,26,95\%}$	MS_{Lad}/MS_{Pe}	$F_{8,20,95\%}$
Regression	112238070	1	112238070	123068.06	4.23	5.33	2.45
Lackof fit	6148	8	768				
Pure Error	2876	20	144				
Total QS	112247093	29					

QS: Quadratic sum, d.f: Degrees of freedom, MS_R: Regression mean square, MS_r: Residue mean square, MS_{Lad}: Lack of fit mean square, MS_{Pe}: Pure error mean square

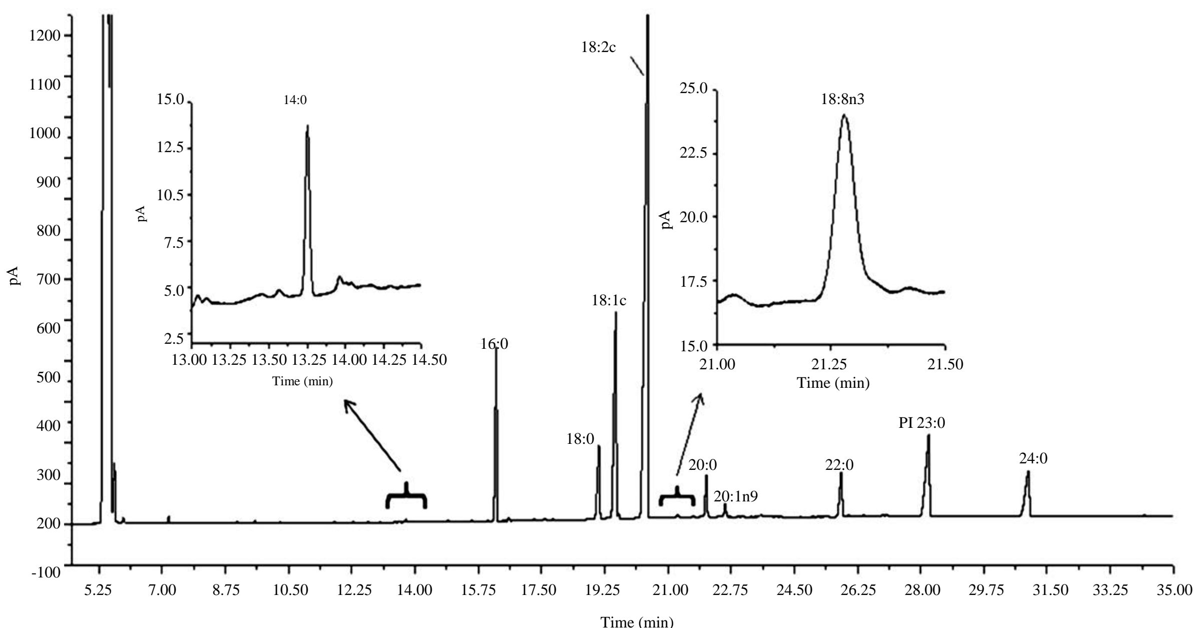

Fig. 1: Profile of fatty acids from the seed of *Tamarindus indica*

Table 3: Instrumental accuracy and recovery of the method in tamarind seeds and standard

Precision	Type	Levels	Tocopherols			
			α	β	γ	Δ
Intraday						
(%, n = 10)	Standard[a]	L1	3.00	2.38	1.84	5.66
		L2	2.77	2.82	2.84	7.6
		L3	1.98	2.70	2.69	5.88
	Sample[b]	L1	3.62	5.59	5.82	16.81
		L2	0.63	3.11	1.04	9.28
		L3	3.98	10.20	4.20	9.30
Interday						
(%, n = 5)	Standard	L1	7.08	5.36	9.61	10.60
		L2	9.46	5.25	9.02	10.89
		L3	10.58	5.57	9.25	10.72
	Sample	L1	15.41	12.05	15.90	14.64
		L2	10.60	10.82	11.29	12.31
		L3	11.00	5.60	2.70	4.29
Recovery						
(%, n = 3)[c]		L1	74.39±5.48	75.71±7.35	64.45±3.41	64.54±5.64
		L2	87.72±7.31	80.55±7.07	73.02±4.34	66.90±4.03
		L3	85.60±3.28	84.15±7.42	75.96±6.76	69.74±3.68

[a]Concentration of standards: L1: 47.5 ng of α-tocopherol, 8.0 ng of β-tocopherol, 89 ng of γ-tocopherol, 1.35 ng of δ-tocopherol. L2: 475 ng of α-tocopherol, 80 ng of β-tocopherol, 890 ng of γ-tocopherol, 13.5 ng of δ-tocopherol, L3: 950 ng of α-tocopherol, 160 ng of β-tocopherol, 1780 ng of γ-tocopherol, 27 ng of δ-tocopherol, [b]Volume of sample injection: L1: 5 μL, L2: 50 μL, L3: 100 μL, [c]L1: Same as above, L2: 332.5 ng of α-tocopherol, 56 ng of β-tocopherol, 623 ng of γ-tocopherol, 10.2 ng of δ-tocopherol. L3: 430.0 ng of α-tocopherol, 90 ng of β-tocopherol, 1000 ng of γ-tocopherol, 20 ng of δ-tocopherol

seeds originated from SMG, SSP and SBA. However, the authors did not notice the presence of myristic acid (14:0) and α-linolenic acid (18:3n-3) found in this work. Differently from the profile of fatty acids of the studied seeds from Brazil, the ones from Nigeria had similarities in the acid proportions 16:0, 18:1 and 18:2, with 27.41, 24.13 and 24.75%, respectively[19].

Regarding the origin of the seeds, the ones from SSB have been highlighted with highest concentrationsof fatty acids studied, with the exception of behenic acid (22:0) and α-linolenic acid (18:3n-3, cis), which showed higher concentrations in the SSP samples. These results may be a reflex both of the extraction method used and of the location and origin of the seeds[20,21]. On the other hand, the SMG

samples and batch 1 of the SSP samples are similar in concentration for all compounds. However, batch 1 of the SSP samples showed significantly higher values compared with batch 2 of the same state. It can also be observed that, in relation to the comparison between batches 1 and 2, there were significant variations ($p<0.05$) between concentrations for all fatty acids quantified in the SSP and SBA samples, which was not observed in the SMG samples.

Regarding the content of saturated and unsaturated fatty acids (Table 4), the seed of *T. indica* has shown about 75% of unsaturated fatty acids which linoleic acid (53%) and oleic acid (19%) are in higher concentrations, while the saturated fraction corresponds to just 25%. In the saturated fraction, about half corresponds to palmitic acid, reaching 10% of the total.

The quality of the vegetable oil is directly related to the great predominance of unsaturated fatty acids, because of its major importance in health. Conversely, the degree of saturation is related to the depreciation of the product. Recent studies suggest that, in mice, unsaturated fatty acids can directly act in the hypothalamus increasing the generation of neurones, as well as the response of the organism to leptin and lowering body weight gain. By contrast, the intake of saturated fatty acids can lead to the apoptosis of hypothalamic neurones resulting in insulin and leptin resistance, loss of control of the intake of calories and predisposing obesity[21-23].

It can also be observed in Table 4 that the origins of the samples, as well as the year of harvest, do not have significant impacts ($p<0.05$) on the total proportions of saturated and unsaturated fatty acids, however, the composition of some unsaturated fatty acids can individually vary both according to the year and the place of cultivation of the fruits, directly reflecting the quality of the oil.

Linoleic acid is one of the most important unsaturated fatty acids in human food, because of its preventive action towards diseases, reducing blood pressure and cholesterol[24,25]. Tamarind seeds might be considered a source of this compound with concentrations varying 1.45-2.69 g of linoleic acid in a portion of 10 grams[26].

Oleic acid has also been cited as being important in the human diet, with low-density lipopuotein fat-reducing action (LDL), improving symptoms of inflammatory diseases and lowering blood pressure[27]. The presence of these essential fatty acids in the tamarind seed oil makes this lipid fraction interesting from a nutritional standpoint, since these fatty acids are not produced by the organism but are responsible for the formation of cell membranes, vitamin D and various hormones[28].

Composition of tocopherols: The four isomers of tocopherol (α, β, γ and δ-tocopherol) were detected and quantified (Fig. 2) in all the samples, regardless of the origin of the fruit and harvesting time. The results (Table 5) shows that γ-tocopherol was the predominant compound found in the seed oil with concentrations ranging from 20-27 mg $kg^{-1}{}_{d.s}$. Next, α-tocopherol was quantified in the seed samples with concentrations ranging between 16 and 25 mg $kg^{-1}{}_{d.s.}$. On the other hand, β and δ-tocopherols had low concentrations.

Among the minor tocopherols, β-tocopherol had higher prevalence compared with δ-tocopherol in the samples, with

Table 4: Profile of fatty acids in seed of *Tamarindus indica*

	Fatty acid content (mg $g^{-1}{}_{d.s.}$)					
	SMG		SSP		SBA	
Fatty acids	L1	L2	L1	L2	L1	L2
14:0	$0.35\pm0.04^{eC*}$	0.32 ± 0.02^{hiC}	0.38 ± 0.05^{eC}	0.80 ± 0.07^{gB}	0.85 ± 0.09^{dB}	1.07 ± 0.04^{fA}
16:0	23.49 ± 2.63^{cdC}	21.50 ± 0.92^{cdC}	23.84 ± 1.33^{bC}	45.27 ± 2.49^{cAB}	38.70 ± 3.36^{cB}	47.28 ± 5.11^{cA}
18:0	14.12 ± 1.48^{cdeCD}	13.76 ± 0.69^{efCD}	10.13 ± 0.48^{cdD}	19.13 ± 0.88^{efBC}	22.65 ± 3.27^{cdAB}	26.03 ± 3.50^{deA}
18:1n9*c*	45.83 ± 3.76^{bCD}	40.32 ± 1.64^{bD}	31.80 ± 2.03^{bD}	60.97 ± 3.01^{bBC}	78.24 ± 13.80^{bAB}	83.06 ± 9.67^{bA}
18:2n6*c*	153.76 ± 20.13^{aB}	145.5 ± 7.14^{aB}	145.13 ± 10.20^{aB}	269.0±12.84aA	230.97 ± 44.70^{aA}	230.7 ± 21.40^{aA}
18:3n3	0.40 ± 0.03^{eC}	0.44 ± 0.05^{hiC}	0.42 ± 0.04^{eC}	1.45 ± 0.24^{gA}	0.86 ± 0.11^{dB}	1.06 ± 0.06^{fB}
20:0	5.92 ± 0.64^{eB}	5.75 ± 0.33^{ghB}	5.12 ± 0.38^{deB}	9.83 ± 0.41^{fgA}	9.30 ± 1.69^{cdA}	9.13 ± 0.46^{efA}
20:1n9	2.10 ± 0.21^{eB}	1.99 ± 0.09^{hiB}	1.57 ± 0.14^{eB}	3.12 ± 0.14^{gA}	3.30 ± 0.50^{dA}	3.29 ± 0.07^{fA}
22:0	9.75 ± 0.96^{deB}	8.96 ± 0.54^{fgB}	11.07 ± 1.03^{cdB}	21.22 ± 0.90^{eA}	11.16 ± 1.50^{cdB}	12.09 ± 1.62^{efB}
24:0	14.97 ± 1.39^{cdeB}	16.21 ± 1.05^{deB}	13.34 ± 1.08^{cB}	26.38 ± 1.18^{deA}	24.50 ± 4.46^{cdA}	24.02 ± 1.86^{deA}
Sat (%)**	26.42 ± 1.08^{bA}	27.22 ± 0.33^{bA}	27.44 ± 0.48^{bA}	27.97 ± 0.20^{bA}	26.61 ± 1.42^{bA}	27.31 ± 2.15^{bA}
Uns (%)**	73.58 ± 1.79^{aA}	72.78 ± 0.49^{aA}	72.56 ± 0.50^{aA}	72.03 ± 0.22^{aA}	73.39 ± 1.41^{aA}	73.39 ± 1.41^{aA}

*Means followed by the same lowercase letter in the comparison between fatty acids and uppercase letter for comparison between batches do not significantly differ among themselves at $p<0.05$ by Tukey test. **Saturated and Unsaturated

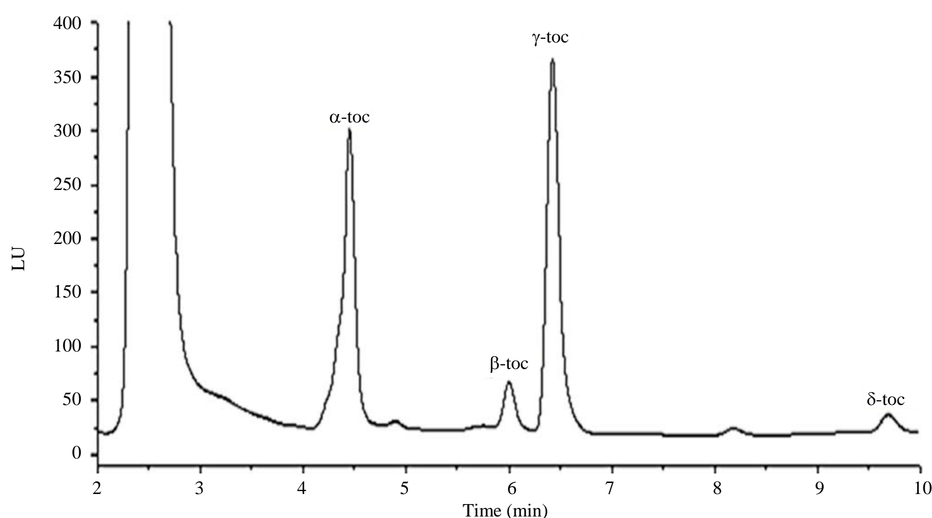

Fig. 2: Tocopherols quantified in tamarind seed through HPLC-FL

Table 5: Tocopherol content in seeds of *Tamarindus indica* from different states of Brazil

	Tocopherols content (mg $kg^{-1}_{d.s.}$)					
	SMG		SSP		SBA	
Tocopherol	L1	L2	L1	L2	L1	L2
α-toc	$21.8\pm0.98^{bAB*}$	25.5 ± 2.69^{eA}	21.3 ± 0.83^{bABC}	16.4 ± 1.90^{bC}	19.4 ± 2.50^{bBC}	17.1 ± 1.66^{aBC}
β-toc	2.3 ± 0.21^{cC}	2.8 ± 0.03^{cAB}	2.3 ± 0.05^{cC}	2.0 ± 0.16^{cC}	3.0 ± 0.18^{cA}	2.4 ± 0.33^{bBC}
γ-toc	27.0 ± 2.58^{aAB}	31.1 ± 1.35^{aA}	25.6 ± 1.22^{aABC}	23.0 ± 2.09^{aiBC}	25.9 ± 2.03^{aABC}	20.4 ± 2.60^{aC}
δ-toc	0.3 ± 0.03^{cD}	0.6 ± 0.07^{cB}	0.5 ± 0.01^{cBC}	0.4 ± 0.01^{cCD}	1.0 ± 0.11^{cA}	0.3 ± 0.02^{bD}

*Means followed by the same lowercase letter in the comparison between tocopherols and uppercase letter for comparison between batches and states do not significantly differ among themselves at $p<0.05$ by Tukey test. n = 3, Mean±SEM

the highest concentration found in batch 1 from SBA, with concentration of 2.96 ± 0.18 mg $kg^{-1}_{d.s.}$, while the maximum obtained for δ-tocopherol reached 1.03 ± 0.11 mg $kg^{-1}_{d.s.}$. Tocopherols α and γ are the homologous tocopherols most commonly found in vegetable oils[29].

Regarding the batches, variations in the concentrations of the compounds were observed, in most cases, when the fruit was harvested in different years. The SMG samples showed the highest concentrations of α and γ-tocopherol. The SSP and SBA samples did not differ between the years ($p<0.05$). For β-tocopherol, the highest concentration was found in the B2 of the SMG seeds and in the B1 of the SBA seeds, with values from 2.8-3.0 mg $kg^{-1}_{d.s.}$, respectively.

Regarding γ-tocopherol concentrations, B1 and B2 of SMG samples showed significantly higher concentrations than the others evaluated, since these results reached approximately 27 and 31 mg $kg^{-1}_{d.s.}$, respectively. The differences repeated themselves for the δ-tocopherol compound and the lowest values were found in the B1 of SMG, B2 of SSP and B2 of SBA. The tocopherols content found is proportionally compatible with data from the literature for the seed oil of *T. indica*, however, the contents of α and δ-tocopherols were higher than those reported by Luzia and Jorge[18]. Some studies suggest that the presence of compounds such as flavonoids, ascorbic acid, vitamin E, β-carotene and polysaccharides in *T. indica* give the fruit a protection power against liver diseases[30,31].

Tocopherols and tocotrienols are natural precursors of vitamin E and are chemically available in the form of isomers[32]. The effectiveness of vitamin E in the fight against liver diseases has already been reported[33,34]. In addition to pharmacological importance, tocopherols play important roles in the conservation of polyunsaturated oils, since they promote oxidative stability, inhibiting the action of free radicals.

δ-tocopherol is scarcely found in most vegetables and it is often present in low concentrations[35]. Wells *et al.*[36] mention that the presence of δ-tocopherol in the diet slows many

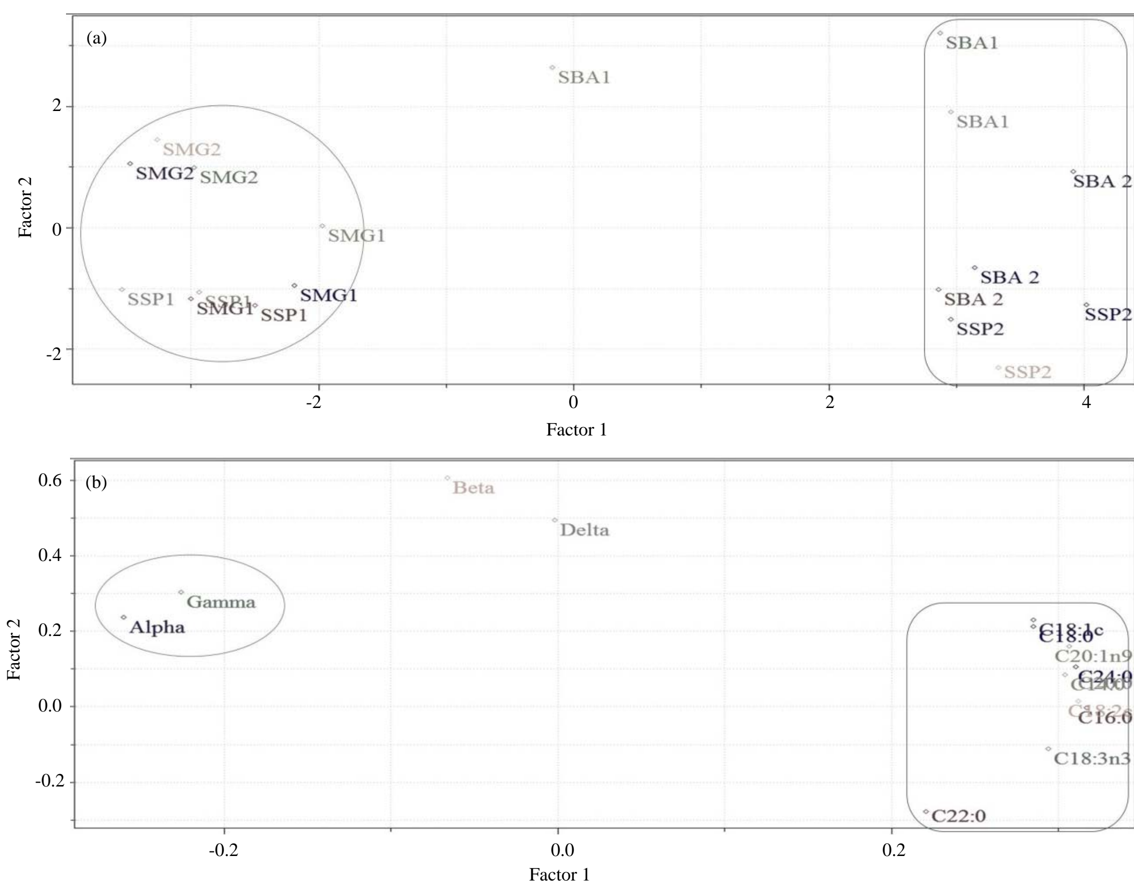

Fig. 3: Principal component analysis for seeds of *Tamarindus indica* originated from different states of Brazil

inflammatory activities. The lack of δ-tocopherol in most vitamin E formulations may be a limiting factor for the product effectiveness in health promotion[37]. Therefore, because of the predominance of the natural antioxidants α and γ-tocopherols and especially the presence of δ-tocopherol in the oil, the tamarind seed may be an important source of these compounds to be industrially exploited.

Chemometrics analysis: The principal components analysis was performed using the results from all the replicates and the concentrations of fatty acids and tocopherols as result. Four main components were required to explain 97.4% of the data, 86.49% of which were explained by components 1 and 2 (Fig. 3).

Similarities were noted between samples SMG1, SMG2 and SSP1. This similarity is the result of lower concentrations of fatty acids and higher concentrations of α and γ-tocopherol. In contrast, samples SSP2, SBA2 and SBA1 showed high fatty acid content. Because of this, there is a direct correlation that differentiates them in PC1.

In general, the SSP samples had great variation in the concentrations of fatty acids. Because of this, batch 1 is more similar to the SMG samples, while batch 2 is more similar to the SBA samples.

Plants in agricultural environments are often subjected to various types of abiotic stress, including high or low temperatures, droughts, high or low levels of light, exposure to salt and high or low levels of mineral nutrients. Several studies have been conducted to elucidate the effects of these tensions on the quantity and quality of seeds oil. Many times the environmental stresses may result in undesirable changes in the composition of the fatty acid of the seed oil[38].

Regarding tamarind seeds originated from different states of Brazil, there was greater concentration of fatty acids in the fruits from Bahia. The mechanism by which temperature influences on the oil content of the seeds has not been completely elucidated yet. This response is probably a reflex of the high temperatures, low relative humidity and high incidence of light in the place of cultivation of these fruits (Table 6). According to Singer *et al.*[38], the level of unsaturation

Table 6: Weather conditions in the years of harvest of the fruits between January and September

	Campinas-SP	Patos de Minas-MG	Tanhaçu-BA
2012			
Average temperature (°C)	22.36	21.6	25.4
Max. temperature (°C)	28.4	27.6	31.9
Relative humidity (%)	43.54	54.2	30.0
Precipitation (mm)	244	274	274
2013			
Average temperature (°C)	21.85	21.9	26.0
Max. temperature (°C)	27.0	28.0	32.0
Relative humidity (%)	53.76	55.0	33.0
Precipitation (mm)	243	273	273

Source: Agritempo[39]

of fatty acids is inversely correlated with the temperature of growth of the seeds. Furthermore, higher temperatures during the seeds development decreased levels of C18:2 and C18:3 and reductions in these fatty acids may be compensated by an increase in monounsaturated C18 content.

Regarding the content of tocopherols, differences in the concentration of these compounds according to the place of cultivation have already been reported. Depending on the desired characteristics, some regions could be more suitable than others for the production or use of seeds[40]. In this study, for example, if the demand is for a seed with a high content of α and γ-tocopherol, the seeds produced in the Southeast would have a more appropriate composition that those of the Northeast.

CONCLUSION

The oil extracted from the seed of tamarind fruits originated from 3 states of Brazil was evaluated based on validated analytical methods. The origin and the time of harvest of the fruits influenced the quantitative profile of fatty acids and tocopherols. The seeds showed high concentrations of oleic acid, α-tocopherol and γ-tocopherol, which would enable its use both as a source of essential fatty acids and tocopherols and for nutraceutical purposes. Finally, the knowledge of the quantitative profile of fatty acids and tocopherols and of the changes resulting from the place of cultivation might serve as support for the use of by-products with the seeds and, consequently, reduce the financial and environmental impacts generated because of the treatment of waste.

SIGNIFICANCE STATEMENTS

In this study Tamarinds seed, a by-product with great potential for use, was characterized regarding the fatty acid profile by GC-FID-MS and content of tocopherols by HPLC-FL. High levels of linoleic acid and tocopherols were detected in all samples, as well changing in the profile of lipophilic compounds according to the place of harvest. This information might favor new alternatives for industrial application of tamarind seed and reduce both expenses with treatment and waste generation.

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