FATTY ACID COMPOSITION OF *Capsicum* GENUS PEPPERS

Composição de ácidos graxos em pimentas do gênero *Capsicum*

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ABSTRACT

Fatty acids have a great metabolic and structural importance. Evaluation of fatty acid composition of peppers is still incomplete. Pulps and seeds from six varieties of the genus *Capsicum* were evaluated in this work with respect to their contents in fatty acids. A total of 25 different fatty acids, including some with odd number of carbons were identified in the samples. The most abundant fatty acids were palmitic (16:0), oleic (18:1n-9) and linoleic (18:2n-6) acids. The polyunsaturated:saturated fatty acid (PUFA/SFA) ratios for all peppers were high due to the elevated amounts of polyunsaturated acids, particularly linoleic acid. In the pulps, the omega-6/omega-3 ratios ranging from 1.28 to 4.33, were relatively adequate if one considers that ratios between 0.25 and 1.0 in the human diet are regarded as highly appropriate. In the seeds, the levels of omega-3 were very low whereas the levels of omega-6 were high, leading to very inadequate omega-6/omega-3 ratios ranging from 74.2 to 279.6. Principal component analysis (PCA) explained 93.49% of the total variance of the data. Considering the PUFA/SFA ratio and omega-6/omega-3 ratio, our data suggest that, among the peppers of the genus *Capsicum* evaluated in this work, the bell pepper and orange habanero pepper present the best nutritional characteristics concerning fatty acid composition.

Index terms: Polyunsaturated:saturated ratio; omega-6/omega-3 ratio; multivariate analysis.

RESUMO

Ácidos graxos tem grande importância metabólica e estrutural. Avaliação da composição de ácidos graxos em pimentas é ainda incompleta. Polpas e sementes de seis variedades de pimentas do gênero *Capsicum* foram avaliadas, neste trabalho, em relação a sua composição de ácidos graxos. Foram identificados 25 ácidos graxos diferentes nas amostras, incluindo alguns com número ímpar de átomos de carbono. Os ácidos graxos mais abundantes foram palmítico (16:0), oleico (18:1n-9) e linoleico (18:2n-6). As relações poli-insaturados:saturados foram elevadas para todas as amostras, em razão das elevadas quantidades de ácidos poli-insaturados, principalmente o ácido linoleico. Nas polpas as relações de omega-6/omega-3 variaram de 1,28 a 4,33, e foram avaliadas como relativamente adequadas, tendo em vista que, na dieta humana, relações entre 0,25 e 1,0 são consideradas altamente adequadas. Nas sementes, os níveis de n-3 foram muito mais baixos do que os níveis de n-6, que foram bem elevados, resultando em baixas proporções de omega-6/omega-3, variando entre 74,2-279,6. Análise de componentes principais (ACP) explicou 93,49% da variância total dos dados. Considerando as relações poli-insaturados: saturados e omega-6/omega-3, nossos dados sugerem que entre as pimentas do gênero *Capsicum* avaliadas, neste trabalho, o pimentão e a pimenta habanero laranja apresentaram as melhores características nutricionais em relação à composição de ácidos graxos.

Termos para indexação: Relação poli-insaturado:saturado; relação omega-6/omega-3; análises multivariadas.

INTRODUCTION

Peppers (*Capsicum* spp.), which are grown worldwide, including Brazil, are used extensively as a natural food colorant and seasoning agent due to their attractive color, flavor, and taste (Reyes-Escogido et al., 2011). *Capsicum* spp are remarkable sources of antioxidant compounds, including capsaicinoids (Ochi et al., 2003; Topuz; Ozdemir, 2007) and phenolic compounds, particularly flavonoids (Materská; Perucka, 2005). The consumption of these components has potential health benefits due to their antioxidant activity, which may help to prevent inflammatory diseases and pathologies associated with oxidative damage, such as atherosclerosis and Alzheimer's disease (Kothari et al., 2010; Topuz; Ozdemir, 2007). Peppers of the genus *Capsicum* appear to possess anti-inflammatory, antioxidant, antiplatelet, antihypertensive, hypoglycemic, and hypocholesterolemic properties and demonstrated using *in vitro* and *in vivo* models. The main pungent component, capsaicin, has been used clinically for its analgesic and anti-inflammatory properties (Rezanka; Sigler, 2009; Srinivasan, 2013).

Lipids and fatty acids represent in general a small portion of the edible portion in peppers. However, they

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have a great metabolic and structural importance. The content of lipids is an important parameter of quality, considering that several bioactives and molecules responsible for the pericarp (pulp) colour are liposoluble. Additionally, the nutritional quality of lipids is dependent on its fatty acid composition (Martínez et al., 2006; Zaki et al., 2013).

Evaluation of the fatty acid composition of peppers of the genus *Capsicum* is still incomplete and is limited to *C. annuum* (Jarret et al., 2013; Pérez-Gálvez et al., 1999). Thus, the objective of this work was to evaluate the fatty acid composition of six varieties of four species of peppers of the genus *Capsicum*.

MATERIAL AND METHODS

The standards and reagents chloroform, methanol, standard methyl esters tricosanoic acid methyl ester (23:0) were purchased from Sigma-Aldrich Co (St Louis, USA).

Four species of the genus *Capsicum,* largely produced and consumed in Brazil, were used: *C. annuum* (two varieties, cayenne pepper and bell pepper), *C. baccatum* var. *pendulum* (known in Brazil as 'dedo de moça' pepper), *C. chinense* (two varieties, red habanero pepper and orange habanero pepper) and *C. frutescens* ('malagueta' pepper). They were all produced in the Northwest Paraná region, Southern Brazil (23º21' South latitude, 52º04' West longitude and 510 m altitude), between March and May 2013. Three different samples (at least 1 Kg) of each of these six varieties were purchased. All samples were at stage and conditions appropriate for human consumption (ripened fruits). Analyses were done immediately after purchase.

Pulps (pericarp) and seeds were manually separated and grounded in a blender. Lipids were extracted using a mixture of chloroform: methanol: water (45:30:25) (Bligh; Dyer, 1959).

To determine the fatty acid composition, the lipids were converted into fatty acid methyl esters (FAME) and were methylated (Hartman; Lago, 1973). The FAME were separated using a gas chromatograph CP-3380 (Varian, USA) fitted with a flame ionization detector and a CP 7420-select Fame fused-silica capillary column (100 m x 0.25 mm x 0.25 µm cyanopropyl) (Souza et al., 2013).

The retention times were compared to those of standard methyl esters (Sigma, USA). The fatty acids were quantified using tricosanoic acid methyl ester (Sigma, USA) as an internal standard (Joseph; Ackman, 1992). The peak areas were determined with software Star 5.0 (Varian, USA) and the concentrations were expressed in mg g^{-1} of total lipid.

The results are presented as the mean±standard deviation of three replicates of each experiment. A p-value of ≤ 0.05 was used to indicate significant differences between the mean values determined by analysis of variance (ANOVA) and Tukey´s test. The methodology of principal component analysis (PCA) was used to scrutinize the relationships between variables and standards. PCA was applied to separate the samples (pulps, seeds and varieties) according to the fatty acids. Firstly a matrix was build using the samples (n=12) and the responses (n=10), totalizing 120 points. The results obtained for each parameter were used as variable columns. Pulps and seeds of peppers were used as individual samples (lines). An autoscaled pre-treatment (z-score) of data was conducted to standardize the significant importance of all variables (Zielinski et al., 2014a). Analysis was based on linear correlations and variances were computed as sums of squares/(n-1). Finally, scatter plots was built for projecting all answers and standards (Zielinski et al., 2014b). All procedures were done by using STATISTICA 7.0 software (Stat-Soft Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Considering that the fatty acids are present in both pericarp (pulp) and seed, these parts were manually separated for evaluation of the distribution of those compounds. In all peppers the seeds were richer in total lipids than the pulps (Figure 1). The highest values were found in the seeds of 'dedo de moça' (10.17%) and cayenne (6.59%). The lowest values of total lipids were found in orange habanero (1.93%). Among pulps, the highest amounts of lipids were found in the 'malagueta' pepper. Peppers contain lipids that are qualitatively similar to those found in plants in general, but their fruits are considered as poor in lipids, normally less than 2% (Asilbekova, 2003; Bosland; Votava, 2012).

The fatty acid composition of the pulps and seeds of peppers is shown in Tables 1 and 2. A total of 25 different fatty acids, including some with odd number of carbons, were identified. Such odd fatty acids occur in small quantities in vegetables and are receiving attention only now because of the technical difficulties inherent in their detection and identification (Diedrich; Henschel, 1990; Martínez et al., 2006; Rezanka; Sigler, 2009).

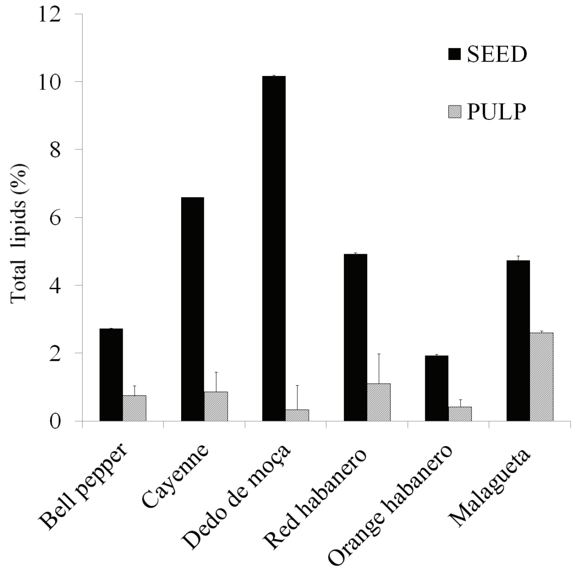

Figure 1: Total lipids of pulps and seeds of peppers of the genus *Capsicum.*

As in the majority of plants, the most abundant fatty acids in the peppers were palmitic (16:0), oleic (18:1n-9) and linoleic (18:2n-6) acids (Table 1 and 2). The last one was the most abundant in all seeds and pulps. Its contents range from 325.35 to 209.03 mg per 100 g of pulp (Table 1) and 697.29 to 669.66 mg per 100 g of seed (Table 2). In general, the major fatty acids in pulps and seeds are the unsaturated ones (apart from palmitic acid, which is the precursor of the others). As the 18:2 and 18:3 fatty acids are synthesized preferentially, they probably constitute the appropriate membrane lipids in this stage of fruit ripening (Pérez-Gálvez et al., 1999). It is interesting to note that linolenic acid (18:3n-3) is found in high amounts in pulps, but it is almost absent in seeds. This phenomenon has already been reported for other species of the genus *Capsicum* (Pérez-Gálvez et al., 1999). The reasons and implications for this particularity cannot be inferred from the hitherto available data.

The PUFA:SFA ratios for all peppers were high due to the elevated amounts of polyunsaturated acids, particularly linoleic acid (Table 3). Foods with high polyunsaturated/saturated fatty acid ratio (PUFA/SFA) are considered beneficial for health, especially because they contribute for the reduction of fat body and total cholesterol (Simopoulos, 2011). Furthermore, PUFA, especially omega-3, when incorporated into the cells can act by modulating several metabolic and signalling pathways and by exerting protective effects against inflammatory and tumoral events (Alexander, 1998; Arrigo et al., 2004; Gladine et al., 2012; Rose; Connolly, 1999).

The omega-6:omega-3 ratios in the pulps, from 1.28 to 4.33 (Table 3), are relatively adequate if one considers that ratios between 0.25 and 1.0 in the human diet are regarded as highly appropriate. In the seeds, the levels of n-3 were very low whereas the levels of n-6 were high, leading to very inadequate ratios ranging from 74.2 to 279.6. Considering that foods with a good omega-6:omega-3 ratio can act by preventing inflammatory processes and diseases, the ingestion of pulps of peppers can generate more health benefits than the ingestion of seeds (Malacrida, 2010; Simopoulos, 2002). To our knowledge, this is the first study to evaluate the omega-6/omega-3 ratio in peppers.

The application of univariate methods to a large number of variables and/or samples may not be appropriate for understanding the data structure and interrelationships. Therefore, the use of PCA can be an alternative because it allows to construct 2-D or 3-D graphs which facilitate understanding of similarities or differences (Zielinski et al., 2014a). Figure 2 illustrates the relationships of the samples with their principal fatty acid contents. PCA explained 93.49% of the total variance of the data, where PC1 explaining up 81.74% and PC2 11.75%. The samples were separated between seeds (left side) and pulps (right side). The seeds were related to the highest levels of linoleic acid, n-6, PUFA, as well as the PUFA:SFA ratio and the n-6:n-3 ratio, whereas the pulps showed higher contents of palmitic acid, oleic acid, MUFA, SFA and n-3. Among all pepper pulps, orange habanero and bell pepper pulps showed the higher levels of n-3.

Table 1: Fatty acid composition of pulps of peppers.

Fatty acid*	Bell pepper	Cayenne	'Dedo de moça'	Red habanero	Orange habanero	'Malagueta'
10:00	24.26a ± 0.31	10.06d ± 1.23	9.58d ± 1.23	14.90c ± 0.98	14.26c ± 0.87	5.43b ± 0.46
12:00	nd	nd	0.84cde ± 0.12	27.59a ± 2.28	6.50b ± 0.08	2.03cd ± 0.24
13:00	12.42a ± 0.72	6.60d ± 0.12	6.19d ± 0.86	8.91b ± 0.72	nd	3.88c ± 0.17
14:00	nd	nd	nd	24.67a ± 0.09	5.09b ± 0.61	nd
14:1n-9	47.18a ± 2.63	41.0b ± 0.86	38.52c ± 0.18	38.86bc ± 0.68	14.48d ± 0.13	13.38d ± 0.34
14:1n-5	nd	9.91b ± 0.18	9.91c ± 0.39	23.54cb ± 0.10	16.92d ± 0.28	2.71d ± 0.10
15:00	nd	2.90c ± 0.39	2.99c ± 0.03	18.93a ± 0.24	5.27b ± 0.02	nd
15:1n-9	nd	3.00c ± 0.03	3.51c ± 0.11	22.16a ± 0.09	10.19b ± 0.75	0.86f ± 0.05
15:1n-7	nd	nd	nd	81.14a ± 1.92	32.13b ± 0.08	2.46e ± 0.07
16:00	202.93a ± 2.46	174.53c ± 0.11	173.40c ± 2.70	151.52d ± 3.94	188.08b ± 1.91	200.49ab ± 0.81
16:1n-7	nd	14.12cb ± 2.70	13.67bc ± 0.54	15.39b ± 2.11	24.78a ± 0.39	11.38c ± 0.98
16:1n-5	nd	nd	nd	nd	nd	nd
17:00	nd	5.71c ± 0.54	7.44b ± 0.65	10.10a ± 0.10	8.00b ± 0.46	5.28c ± 0.28
17:1n-7	nd	nd	2.24de ± 0.05	7.79a ± 0.93	4.61b ± 0.04	2.85cd ± 0.13
18:00	67.64a ± 1.12	39.63b ± 0.65	39.25b ± 0.47	30.75d ± 0.84	35.68c ± 0.34	26.50e ± 0.54
18:1n-9	98.13d ± 0.13	158.12c ± 2.70	161.69b ± 1.61	72.06f ± 0.54	26.51h ± 0.01	232.44a ± 1.48
18:1n-7	10.18a ± 0.03	7.00b ± 0.47	5.26cd ± 0.74	7.05b ± 0.22	6.37b ± 0.52	9.72a ± 0.03
18:2n-6	270.26f ± 2.20	292.75e ± 2.30	302.80d ± 3.41	209.03g ± 0.43	272.96f ± 2.41	325.35c ± 2.00
20:00	6.67bc ± 0.14	7.28b ± 0.74	7.20bc ± 0.32	6.42bc ± 0.47	9.98a ± 0.22	3.80de ± 0.10
18:3n-3	179.74b ± 0.36	133.93c ± 3.41	137.65c ± 2.30	135.37c ± 0.51	219.17a ± 1.63	75.13d ± 0.10
20:1n-9	nd	nd	nd	nd	nd	nd
22:00	6.48b ± 1.42	6.41b ± 0.32	5.19bcd ± 0.01	6.54b ± 0.57	12.81a ± 1.14	2.40 ± 0.19
22:2n-6	nd	8.26a ± 1.61	nd	4.80b ± 2.29	6.75ab ± 0.47	nd
24:00	nd	6.35bc ± 0.01	5.71c ± 0.67	4.10de ± 0.32	9.68a ± 0.45	2.89ef ± 0.04
24:1n-9	nd	nd	nd	18.51 ± 2.38	nd	nd

nd (not detected). Results are expressed as mg fatty acid per 100 g^{-1} total lipid and represent mean ± standard deviation of three replicates. Means followed by the same letters for each fatty acid do not differ by Tukey's test ($p<0.05$). These comparisons were done simultaneously for this table and Table 2.

Table 2: Fatty acid composition of seeds of peppers.

Fatty acid*	Bell pepper	Cayenne	'Dedo de moça'	Red habanero	Orange habanero	'Malagueta'
10:00	0.92f ± 0.04	1.08f ± 0.13	0.79f ± 0.05	1.91ef ± 0.01	3.19e ± 0.03	0.65f ± 0.06
12:00	nd	nd	nd	2.71c ± 0.03	1.88cd ± 0.01	0.50d ± 0.06
13:00	nd	nd	nd	nd	nd	0.28e ± 0.03
14:00	nd	nd	nd	1.78c ± 0.14	1.08d ± 0.02	nd
14:1n-9	1.08e ± 0.07	2.05e ± 0.33	1.26e ± 0.15	2.85e ± 0.24	2.04e ± 0.02	1.91e ± 0.10
14:1n-5	0.69e ± 0.22	0.63e ± 0.03	nd	1.75e ± 0.13	3.25e ± 0.11	1.16e ± 0.31
15:00	0.35fg ± 0.02	0.35fg ± 0.07	nd	1.65d ± 0.17	1.16e ± 0.04	0.47f ± 0.03
15:1n-9	0.44fg ± 0.03	0.20fg ± 0.02	nd	1.83e ± 0.16	2.60d ± 0.05	0.65fg ± 0.05
15:1n-7	1.07e ± 0.01	nd	nd	5.40d ± 0.37	7.16c ± 0.25	1.41e ± 0.01
16:00	95.99gh ± 3.54	128.62e ± 9.12	91.23h ± 5.51	105.23fg ± 7.35	100.82fgh ± 0.05	111.40f ± 5.01
16:1n-7	2.41de ± 0.01	2.01de ± 0.16	1.72de ± 0.07	2.44de ± 0.38	4.18d ± 0.37	3.32d ± 0.02
16:1n-5	nd	0.56a ± 0.01	nd	nd	nd	nd
17:00	0.95fg ± 0.02	0.85fg ± 0.07	1.20ef ± 0.32	1.64ef ± 0.39	1.97e ± 0.03	2.98d ± 0.03
17:1n-7	1.39fg ± 0.01	0.85g ± 0.01	1.93e ± 0.06	2.64cde ± 0.04	3.44c ± 0.02	2.24de ± 0.01
18:00	27.44e ± 0.35	18.81f ± 0.43	32.10d ± 0.56	30.94d ± 0.79	36.16c ± 0.05	31.07d ± 0.49
18:1n-9	98.72d ± 0.25	74.48f ± 0.71	83.45e ± 0.81	72.32f ± 0.57	55.19g ± 0.17	81.34e ± 0.35
18:1n-7	6.18bc ± 0.08	6.54b ± 0.05	4.58d ± 0.06	5.21d ± 0.18	4.78d ± 0.25	5.37cd ± 0.11
18:2n-6	675.04b ± 3.65	677.04b ± 6.08	697.29a ± 3.29	669.66b ± 4.02	672.16b ± 0.27	669.75b ± 5.17
20:00	3.24ef ± 0.18	2.43f ± 0.25	3.99de ± 0.41	4.41d ± 0.51	6.17c ± 0.03	4.70d ± 0.25
18:3n-3	2.83f ± 0.05	3.21f ± 0.04	2.49f ± 0.06	7.21e ± 0.13	7.15e ± 0.17	9.03e ± 0.07
20:1n-9	0.83b ± 0.02	0.97a ± 0.09	nd	nd	nd	nd
22:00	2.35e ± 0.20	2.39e ± 0.39	3.22e ± 0.50	4.23cde ± 1.03	6.00 ± 0.03	3.75 ± 0.02
22:2n-6	nd	nd	nd	nd	nd	0.18c ± 0.03
24:00	2.28f ± 0.20	2.68ef ± 0.72	2.71ef ± 0.68	4.17de ± 1.08	7.35b ± 0.44	4.88cd ± 0.28

nd (not detected). Results are expressed as mg fatty acid per 100 g^{-1} total lipid and represent mean ± standard deviation of three replicates. Means followed by the same letters for each fatty acid do not differ by Tukey's test ($p<0.05$). These comparisons were done simultaneously for this table and Table 1.

Table 3: Sums and ratios of fatty acid.

Sample	MUFA	PUFA	SFA	omega-3 n-3	omega-6 n-6	PUFA:SFA	n-6:n3
Bell pepper pulp	155.48d ± 2.47	450.00d ± 2,56	320.41a ± 1.09	179.74b ± 0.36	270.26e ± 2.20	1.40c ± 0.01	1.50f ± 0.01
Bell pepper seed	112.81f ± 0.45	677.88b ± 3.71	133.53e ± 2.66	2.83f ± 0.05	675.04b ± 3.65	5.08a ± 0.13	238.39b ± 3.07
Cayenne pulp	233.31c ± 6.96	434.94e ± 7.33	259.45c ± 4.10	133.93c ± 3.41	301.01d ± 3.92	1.68c ± 0.01	2.25f ± 0.03
Cayenne seed	88.28ij ± 0.31	680.25b ± 6.11	157.23d ± 7.48	3.21f ± 0.04	677.04b ± 6.08	4.33b ± 0.25	211.08c ± 0.62
‘Dedo de moça’ pulp	243.80c ± 3.63	440.44de ± 5.72	257.79c ± 7.06	137.65c ± 2.30	302.80d ± 3.41	1.71c ± 0.02	2.20f ± 0.01
‘Dedo de moça’ seed	92.94ih ± 0.70	699.79a ± 3.35	135.23e ± 3.73	2.49f ± 0.06	697.29a ± 3.29	5.18a ± 0.17	279.63a ± 5.67
Red habanero pulp	286.50a ± 0.84	349.19g ± 1.34	304.43b ± 2.50	135.37c ± 0.51	213.82f ± 1.86	1.15d ± 0.01	1.58f ± 0.02
Red habanero seed	94.45ih ± 0.56	672.87b ± 4.16	158.69d ± 3.90	7.21e ± 0.13	669.66b ± 4.02	4.27b ± 0.13	92.91d ± 1.16
Orange habanero pulp	136.00e ± 0.79	498.87c ± 3.57	295.36b ± 2.27	219.17a ± 1.63	279.71e ± 1.94	1.69c ± 0.03	1.28f ± 0.01
Orange habanero seed	82.63j ± 0.34	679.30b ± 0.09	165.78d ± 0.57	7.15e ± 0.17	672.16b ± 0.27	4.10b ± 0.01	94.10d ± 2.33
‘Malagueta’ pulp	275.79b ± 2.17	400.48f ± 2.10	252.89c ± 0.92	75.13d ± 0.10	325.35c ± 2.00	1.58c ± 0.01	4.33f ± 0.02
‘Malagueta’ seed	97.41g ± 0.97	678.96b ± 5.27	160.69d ± 6.25	9.03e ± 0.07	669.93b ± 5.20	4.23b ± 0.13	74.16e ± 0.03

*Expressed mg fatty acid per 100 g^{-1} total lipid. Means followed by the same letters in columns do not differ by Tukey’s test ($p<0.05$). MUFA (total monounsaturated fatty acids), PUFA (total polyunsaturated fatty acids), SFA (total saturated fatty acids); n-3 (total omega-3 fatty acids) and n-6 (total omega-6 fatty acids).

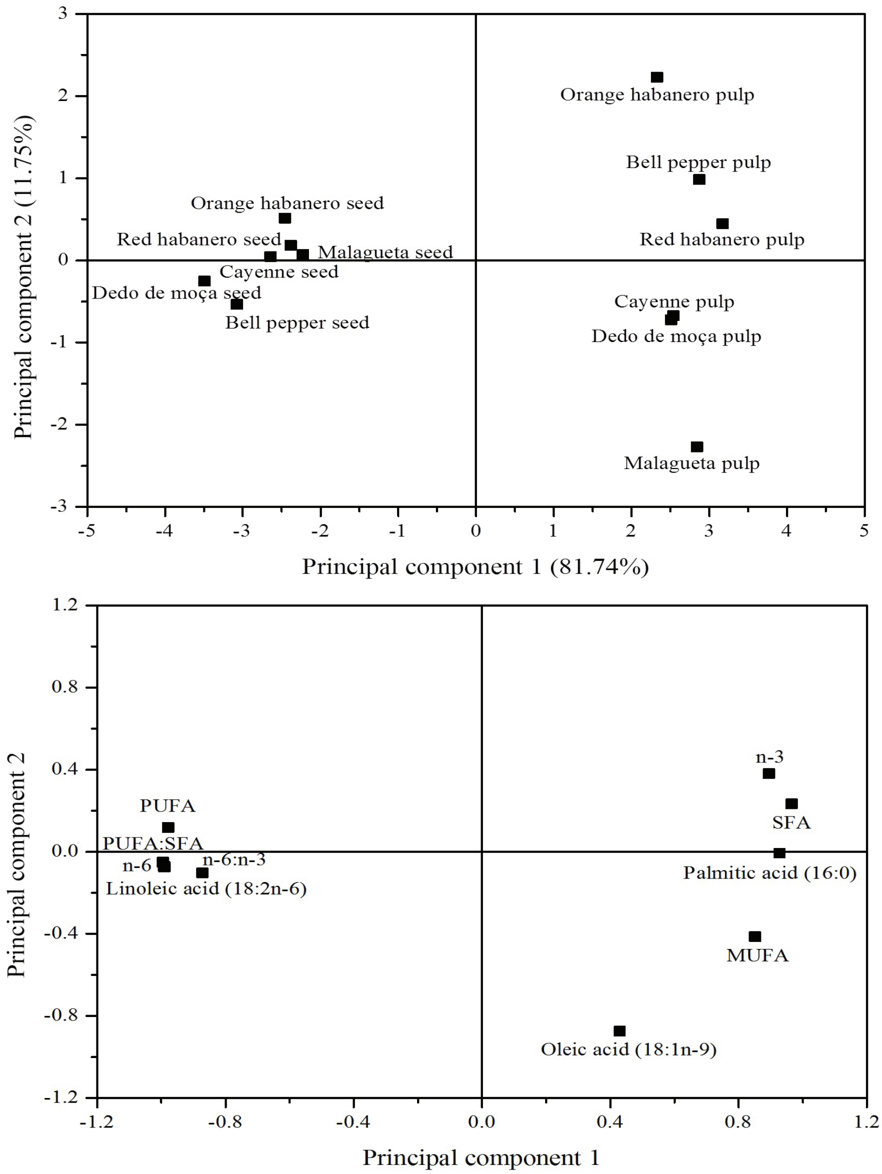

Figure 2: Scatter plots PC1 vs. PC2 on the main sources of variability among peppers samples. (A) *Scores* and (B) *Loadings* plots.

CONCLUSIONS

All peppers presented high values of PUFA, generally believed to be beneficial for health. Considering the PUFA:SFA and the n-6:n-3 ratios, our data suggest that, among the peppers of the genus *Capsicum* evaluated in this work, the bell pepper and the orange habanero pepper present the best nutritional characteristics with respect to their fatty acid composition.

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