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Identification of carotenoid isomers in crude and bleached palm oils by mass spectrometry

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ABSTRACT

In this work, a method for identification of α- and β-carotene isomers in palm oil samples was developed by UHPLC-MS/MS. The fragmentation patterns of both isomers were studied and a diagnostic ion was assigned to α-carotene (*m/z* 388). The developed method was used to identify isomers in crude palm oils from two cultivars (*E. guineensis*, here so-called native oil and *E. guineensis* × *E. oleifera*, here so-called interspecific hybrid) and bleached oil with two types of commercial bleaching earths (neutral and acid activated). It was noticed that α- and β-isomers are present in both crude oils, nevertheless β-carotene is the most abundant. The reduction of α- and β-isomers in the bleached native oil was very similar after treatment with the two different types of adsorbents. For the interspecific hybrid oil, the acid adsorbent provided a reduction of the two forms, while the neutral adsorbent reduced mostly the β-form.

1. Introduction

The oil palm is one of the most important oil crops in the world, due to its high productivity and perennial nature. The oil extracted from the fruits is currently the most consumed vegetable oil (Mozzon, Pacetti, Lucci, Balzano, & Frega, 2013). According to the most recent Statistical Yearbook from the Food and Agriculture Organization of the United Nations (Food and Agriculture Organization of United Nation, 2017), the world production of palm oil exceeded 57 million tons in 2014.

The crude palm oil (CPO), before it is ready for human consumption, undergoes a refining process that aims at removing compounds such as phospholipids, free fatty acids, oxidation products, pigments and other undesirable components. Actually, it is really important to remove carotenoids from palm oil as they are responsible for the dark orange color, that would derail its use in a wide range of food and pharmaceutical products such as ice creams, breads, margarines and cosmetics (Gibon, De Greyt, & Kellens, 2007). The α- and β-carotenes, which are precursors of vitamin A, are the main carotenoids found in CPO (Ng & Choo, 2016). CPO is submitted to a physical refining (Gibon et al., 2007) to avoid a large neutral oil loss, i.e. triacylglycerols. The physical refining comprises an integrated bleaching/degumming followed by a deacidification step. The first one aims to remove phospholipids, by precipitation, and part of carotenoids, via adsorption. The second step removes free fatty acids by volatilization at high temperature (usually above 200 °C) and low absolute pressures (below to 5 mbar). During the deacidification step, the remaining carotenoids are thermally degraded. In fact, some refineries only remove about 20% of carotenoids by adsorption mostly because after 20 min at 240 °C about 98% of carotenoids are destroyed, in a phenomenon known as heat bleaching. After the refining procedures, palm oil becomes a light-yellow oil. However, sometimes the oil color cannot be completely removed. It is well accepted that there is no correlation between carotenoids content nor color of bleached oil and the color of fully refined palm oil (Gibon et al., 2007; Silva et al., 2014).

A major problem of this procedure is the economic loss because carotenoids are valuable substrates being destroyed during the whole refining process. It is estimated a daily loss enough to meet the world population requirement of vitamin A ingestion (Mayamol, Balachandran, Samuel, Sundaresan, & Arumughan, 2007). Another drawback is the identification and quantification of carotenoid isomers in bleached oils, especially considering they will thermally undergo in varied color intensity by-products during deacidification/deodorization step, which may lead to a dark fully refined oil.

Several studies concerning the β-carotene removal from vegetable oil by adsorption are noted in the literature. For instance, the work carried out by Silva et al. (2013) investigated the total carotenoids

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removal from palm oil onto commercial bleaching earths under industrial bleaching conditions, meaning high temperatures, low pressures and without solvent. Silva et al. (2014) also studied the effect of different types of bleaching earth on the decolorization over bleached palm oil submitted to deacidification process. Pohndorf, Cadaval Jr, and Pinto (2016) described the removal of total carotenoids from rice bran oil onto commercial acid activated bleaching earth. Moreover, focused on palm oil carotenoids, Mba, Dumont, and Ngadi (2017) studied the thermostability of carotenoids in crude palm oils during deep-fat frying, concerning quality losses. Iftikhar, Tan and Zhao (2017) studied the enrichment of β-carotene by supercritical extraction. All those previous works have quantified carotenes by UV-vis spectroscopy. This analysis allows solely the total carotenes quantification. So, there is a lack in the literature about how processing may affect different carotenoid isomers.

Carotenoids isomers presents different biological activities (Rodriguez & Rodriguez-Amaya, 2007) and color (Gurak, Mercadante, González-Miret, Heredia, & Meléndez-Martínez, 2014). Any carotenoid containing an unmodified β-ionone along one polyene chain containing at least 11 carbon atoms may be metabolized to vitamin A (retinol). B-carotene contains two β-ionone ring produces two molecules of retinal, which is then reduced to retinol (Fernández-García et al., 2012). The provitamin A activity is given in relation to β-carotene, which possess 100% activity, α-carotene presents only one ring and possess 53% provitamin A activity. Carotenoids lacking this ring, such as lycopene, are not capable to form vitamin A (Saini, Nile, & Park, 2015), yet they have the ability of quenching singlet oxygen and deactivate free radicals, important to the prevention of certain types of cancer, cardiovascular diseases and macular degeneration (Müller, Caris-Veyrat, Lowe, & Böhm, 2016). The color of carotenoids is related to the number of conjugated double bounds (c.d.b.). It is necessary at least 7 c.d.b. for a carotenoid to have a perceptible color. Moreover, the carotenoids color goes from red to yellow when the values decrease (Meléndez-Martínez, Britton, Vicario, & Heredia, 2007). In this sense, β-carotene presents darker color than α-carotene. There is also a large dependence of c.d.b. on the absorption spectra: the longer the chromophore, the higher were the wavelengths of maximum absorption (Meléndez-Martínez et al., 2007).

Actually, the study performed by Silva et al. (2014) presents some evidences that the adsorbent used during palm oil bleaching catalyzes the β-carotene isomerization, interfering, therefore, in the color of fully refined palm oil. The authors carried out several fully physical refining procedures (bleaching and deacidification) using different amounts of both neutral (NBE) and acid activated (ABE) bleaching earths. The oil bleached with NBE presented lower total carotenoids content when compared with ABE. The same oil presented darker color after deacidification, showing a lower color reduction due to heat bleaching. The results were found in agreement with the established knowledge that there is no correlation between the carotenoids content after bleaching and the palm oil color after heat bleaching (Dijkstra & Segers, 2007). Only ABE proved to be efficient to remove secondary oxidation products. Carotenoids are known to be susceptible to oxidation and isomerization reactions as a consequence of their highly unsaturated chain. However, as mentioned above, the authors quantified only the total carotenes remaining in the bleached oil and suggest that the identification of the isomers is fundamental for understanding the color inversion; i.e. the remaining color of fully refined palm oil.

Advancing in the effort of clarifying the color inversion, the goal of this work was to evaluate the carotenoids isomers remaining in oil bleached with different types of adsorbents (acid activated and neutral) with identification of residual carotenoids derivatives by UHPLC-ESI-MS analysis. Furthermore, ESI-MS/MS allowed for a precise identification of main vitamin A precursors.

2. Material and methods

2.1. Oil characterization

Crude oils obtained from two different oil palm – *Elaeis guineensis* and the interspecific hybridization of *Elaeis guineensis* × *Elaeis oleifera*, here so-called native and hybrid palm oil – were kindly supplied by Denpasa (Santa Bárbara do Pará, PA, Brazil). The oil samples were characterized regarding standard quality parameters. Fatty acids profiles were determined by gas chromatography (GC-FID 7890A, HP-88 column [60 m × 0.25 mm × 0.2 μm], Agilent Technologies, USA) according to the official method Ce 1–62 of American Oil Chemists' Society (AOCS., 1998). Free fatty acid (FFA) contents were determined by titration according to the official method Ca 5–40 and results were expressed as percentage of palmitic acid (AOCS., 1998). Total carotene content, expressed as β-carotene, was determined measuring the absorbance at 446 nm of samples homogenized and diluted in hexane (Spectrophotometer SpectraMax M3, Molecular Devices, USA) (PORIM., 1990).

The deterioration of bleachability index (DOBI) was measured using a UV-vis spectrophotometer. This index corresponds to the ratio of the spectrophotometric absorbance at 446 nm (non-oxidized carotenes maximum) to the absorbance at 268 nm (oxidized carotenes maximum) of crude oil diluted in hexane. At last, the DOBI value is obtained by Eq. (1) (De Leonardis, Cuomo, Macciola, & Lopez, 2016).

$$\text{DOBI} = \frac{\text{absorbance at 446 nm}}{\text{absorbance at 268 nm}} \quad (1)$$

DOBI values between 2.5 and 4.0 indicate average to good crude oil quality. Values below 2.0 indicate a poor quality crude palm oil which is difficult to bleach (Gibon et al., 2007).

2.2. Chemical standards

Triglyceride mix (tricaprin, tricaprylin, trilaurin, trimyristin, tripalmitin) and mono, di, triglyceride mix (1-monoolein, 1,2-diolein, 1,3-diolein, triolein) were purchased from Supelco (USA). *Trans*-β-Apo-8′-carotenal, β-carotene and α-carotene were purchased from Sigma Aldrich (USA). All the chemicals (formic acid and ammonium formate) and solvents (hexane, chloroform, methanol and isopropanol) were LC-MS or HPLC grade.

Four stock solutions were prepared as followed. Stock solution of β-carotene (S1) was prepared by dissolving 2 mg of the standard in 1 mL of chloroform. Stock solution of mono-, di- and triglyceride mix (S2) was prepared by dissolving the entire ampoule content (40 mg) in 4 mL of hexane. Stock solution of triglyceride mix (S3) was prepared by dissolving the entire ampoule content (100 mg) in 4 mL of hexane:-chloroform (1:1). Stock solution of *trans*-β-Apo-8′-carotenal was prepared by dissolving 2 mg of the standard in 1 mL of chloroform (S4). Finally, a solution containing the main acylglycerols present in vegetable oils and high concentration of carotenoids was prepared by mixing 0.5 μL of S1, 0.8 μL of S2, 0.4 μL of S3 and 0.5 μL of S4, diluted with 1 mL of methanol, resulting in a final concentration of 2 μg/mL of each acylglycerol and 1 μg/mL of each carotenoid (β-carotene relative concentration to each acylglycerol similar to those reported by Mozzon et al. (2013)). This solution was used to establish the UHPLC analysis conditions to identify α- and β-carotenes in oil samples without previous derivatization neither other preparation procedure rather than solubilization.

2.3. Adsorbents

The adsorption experiments were performed using two types of commercial bleaching earths widely used in the vegetable oil industry. The acid activated adsorbent Tonsil OPT 210 FF, manufactured by acid activation of calcium bentonite, which was kindly provided by Clariant

(Germany). The neutral adsorbent Pure Flo B 80, composed by bentonite was kindly provided by Oil Dri (USA).

2.4. Bleaching experiments

Batch adsorption experiments were carried out according to methodology adapted from Silva et al. (2013), reproducing the industrial bleaching of palm oil. In each test, 50 g of crude palm oil was placed into 100 mL flasks and heated to 90 °C (Rotavapor, Gehaka, RD 180, Brazil). Then, the following steps were performed: addition of 0.09% m/m of citric acid, as a 30% m/m aqueous solution high shear mixing at 15,000 rpm (mixer, Turratec, Tecnal, Brazil); addition 3.0% m/m of the selected adsorbent (bleaching earth); maintenance of the mixture at 90 °C under atmospheric pressure during 15 min; applying vacuum (< 50 mbar of absolute pressure) during 30 min (necessary time to reach adsorptive equilibrium) at 90 °C. The adsorption process was interrupted by removing the bleaching earth by filtration over Buchner funnel and paper filter (pore size 11 μm).

2.5. Analytical techniques

2.5.1. Chromatographic separation

Chromatographic analyses were performed using a Shimadzu UHPLC System, model Nexera X2, equipped with quaternary pump, degasser, autosampler and column oven. Chromatographic separation of the compounds was achieved at 40 °C using a Shim-pack XR-ODS (C18) column (50 × 2.0 mm i.d.; 2.2 μm particle size) purchased from Shimadzu (Japan). Solvent A was methanol/formic acid 0.1%/ammonium formate 10 mM. Solvent B was isopropanol/formic acid 0.1%/ammonium formate 10 mM. Solvents were delivered at a total flow rate of 0.4 mL/min. The elution gradient used was as follows: 0–4 min linear gradient from 0% to 50% B; 4–8 min isocratic 50% A and 50% B; 8–8.01 min return to 100% A; 8.01–12 min reconditioning of the column with 100% A isocratic.

About 10 mg of crude and bleached oil samples were dissolved in 1 mL of chloroform and subsequently diluted to 1 mg/mL with methanol. The injection volume was 0.5 μL for standard mix and diluted oil samples. To preserve MS detector source from contamination, during the analysis of diluted oil samples, the chromatographic flow was directed to waste on 4.5 min by means of a diverter valve.

MS detection was carried out with maXis ESI-qTOF mass spectrometer (Bruker Daltonics, Germany) operated in electrospray positive mode. Typical instrument settings were as followed: capillary voltage, 4500 V; dry gas temperature, 200 °C; dry gas flow, 9 L/min; nebulizer gas flow, 4 bar; funnel/multipole, RF 250 Vpp. Ions were detected from *m/z* 300 to 1600 at acquisition rate of 2 Hz. The instrument was operated in auto MS/MS using stepping mode, with collision energy ranging from 15 to 60 eV. MS/MS spectra of α- and β-carotenes isomers were the average of fragmentation acquired under two collision-induced energies (CID): 15.5 and 46.6 eV. Mass calibration was performed using ESI-L Low Concentration Tuning Mix (Agilent Technologies, USA). Therefore, data were processed using the software Bruker Data Analysis 4.2 (Bruker Daltonics). Molecular formula for detected compounds was generated using SmartFormula algorithm. CompoundCrawler was employed to query correct molecular formula in open-access databases (CheBi, Kegg and Metlin).

3. Results and discussion

3.1. Crude oil characterization

Native and hybrid interspecific crude palm oils were characterized regarding fatty acids profile, FFA, DOBI value and total carotenes. Table 1 shows fatty acids profile obtained by gas chromatography. The most abundant fatty acid in the interspecific hybrid oil is oleic (corresponding to about 55% in mass), followed by palmitic acid (corresponding to about 27% in mass). For the native oil, oleic and palmitic acids were the most abundant, 37.7 and 35.4% in mass, respectively. Interspecific hybrid oil presented almost 70% of unsaturated fatty acids, whilst the native presented 50%. These differences influence on the viscosity of both oils: the native oil has solid aspect whereas the interspecific hybrid is liquid at room temperature.

Table 1
Oil Characterization: Fatty acids profile, DOBI, free fatty acid (expressed as percentage of palmitic) and total carotenes content.

Trivial Name	Number of carbon:insaturation	Mass (%)	
		Hybrid	Native
Caproic	C6:0	0.17	–
Lauric	C12:0	0.15	–
Myristic	C14:0	0.35	1.83
Palmitic	C16:0	26.81	35.43
Palmitoleic	C16:1	0.20	0.80
Stearic	C18:0	3.48	11.62
Oleic	C18:1	55.73	37.70
Elaidic	C18:1	–	0.87
cis-Vaccenic	C18:1	0.88	0.69
Linoleic	C18:2	11.16	10.10
Linolenic	C18:3	0.44	0.61
Arachidic	C20:0	0.29	0.37
cis-11-Eicosanoic	C20:1	0.16	–
Behenic	C22:0	0.06	–
Lignoceric	C24:1	0.11	–
Saturated	–	31.31	49.25
Unsaturated	–	68.69	50.75
DOBI		4.9	3.4
FFA (%)		1.7	3.1
Total Carotene (mg/kg)			
Crude Oil		1857	988
Bleached Acid		694	410
Bleached Neutral		1078	455

The DOBI values were 3.4 and 4.9 for native and hybrid, respectively (Table 1). The higher DOBI value indicates lower amount of oxidized carotene content, and consequently, it is easier to remove the color during the refining procedures (Gibon et al., 2007). Free fatty acids were 3.1 and 1.7% for native and hybrid oils, respectively. Interspecific hybrid presented two times more total carotenes then native (1857 and 988 mg/kg, respectively). Therefore, it is clear that the interspecific hybrid oil has better quality parameters, and its color will be easier to be removed during the refining steps. The removal of palm oil color is important to ensure it applicability in a wide range of industrial products, such as food and cosmetics.

3.2. Total carotene content

Table 1 shows the results of the total carotene analysis of crude oil and bleached using 3.0% (m/m) of adsorbent. According to study performed by Silva et al. (2013), after 30 min of contact between palm oil and the adsorbent Tonsil 210FF OPT, the adsorptive equilibrium for total carotenes is reached, and consequently, it is not possible any further reduction. It is possible to notice that the acid bleaching earth presented a slightly larger reduction of the total carotenes, 58 and 62% for the native and interspecific hybrid oils, respectively. Neutral bleaching earth showed a reduction of 54 and 42% for native and interspecific hybrid oils, respectively. It is interesting to notice that the total carotene content in the bleached oils with both adsorbents was very similar for the native oil. However, for interspecific hybrid oil, the difference is more outstanding, possible due to its higher initial concentration of carotenes. In fact, each adsorbent has different maximum adsorption capacities and the equilibrium of the adsorbent process will be dictated by the concentration of adsorbate on the surface of the adsorbent instead of the concentration of the adsorbent in the liquid

(Silva et al., 2013). Another possible explanation is the effect of the oil viscosity in the adsorption process. It is well established that oils containing high concentrations of unsaturated fatty acids in their composition present a lower viscosity variation at different temperatures when compared to those with lower unsaturation content (Ceriani, Gonçalves, & Coutinho, 2011; Granero et al., 2014). The viscosity of the oil is an important parameter to be considered in the adsorption process, as this will influence its mechanisms: (1) diffusion across the liquid film surrounding the adsorbent particle; (2) diffusion in the liquid contained in the pores and/or along pore walls, so called intra-particle diffusion (Gonsalvesh, Marinov, Gryglewicz, Carleer, & Yperman, 2016). Thus, it is possible that the mechanisms involved in the carotene adsorption process from the different palm oils are different due to differences in viscosity.

3.3. Chromatography

The first step was to develop a UHPLC method to separate and detect β-carotene from an oily matrix without any pretreatment. A vegetable oil with high carotenoids content was represented by a standard mixture of triacylglycerols, diacylglycerols, monoacylglycerol, *trans*-β-Apo-8′-carotenal and β-carotene. A reproducible separation of the fatty compounds was achieved with the C18 column in a total chromatographic run of 6 min. The identification of fatty compounds was carried out by comparing the retention times and the MS spectral data for oils to those obtained for the standards (for more details, see supplementary information). Peaks were sharp, symmetrical and almost all compounds were efficiently separated, except for 1,2- and 1,3-diolein, that co-eluted at 2.2 min, and tripalmitin and triolein, that partially co-eluted at 5.6 min (Fig. 1).

3.4. MS/MS analysis

Table 2 presents the data of 10 compounds separated and identified from the standard solution, including retention time, molecular formula, experimental and calculated *m/z* and the major adduct detected. Among the acylglycerols, only monoolein was identified sodiated, being all the others identified as NH_4 adduct ion. *trans*-β-Apo-8′-carotenal was identified as protonated adduct, whereas β-carotene was identified in the radical cationic form.

We also evaluated the chromatographic separation and MS identification of a mixture of α- and β-carotene (Fig. S10, available in the supplementary material), once these are the most important carotenoids in crude palm oil (Ng & Choo, 2016). The separation of the isomers α- and β-was accomplished through the developed UHPLC method, and retention times were 3.8 and 3.9 min, respectively. Both compounds could be identified by ESI-MS/MS (Fig. 2) and the α-carotene has a diagnostic ion of *m/z* 388 (Fig. 2 b). The main fragmentation of these two isomers was proposed in Scheme 1.

Both isomers (radical cations) showed a toluene (92 Da) neutral loss affording the radical cation of *m/z* 444. Nevertheless, the α-carotene spectrum has a diagnostic ion of *m/z* 388 from an isobutylene (56 Da) neutral loss affording a conjugated radical cation. If is considered that the β-carotene already has a complete conjugated system, the isobutylene loss is not a logical fragmentation, therefore the different gas phase reactivity from these isomers arise from position of the C=C bound noted in the 6-membered ring. The radical cation formation position is likely to be the same of protonated carotenes, as discussed elsewhere (Neto et al., 2016). The observed results seem to be in accordance with previous reports using FAB-MS/MS (Vanbreemen, Schmitz, & Schwartz, 1995) and other MS techniques, as reviewed (Rivera, Christou, & Canela-Garayoa, 2014). Although the similar results, the rationale for the diagnostic ion has barely been discussed.

All the other compounds of the standard mixture had their ESI-MS/MS acquired and were detected as protonated (or as Na^+ or as NH_4^+ adducts). All these spectra may be found in the supplementary material (Figs. S1–S9) and their high-resolution MS (also see Table 2) as their structural characterization were in accordance with the expected

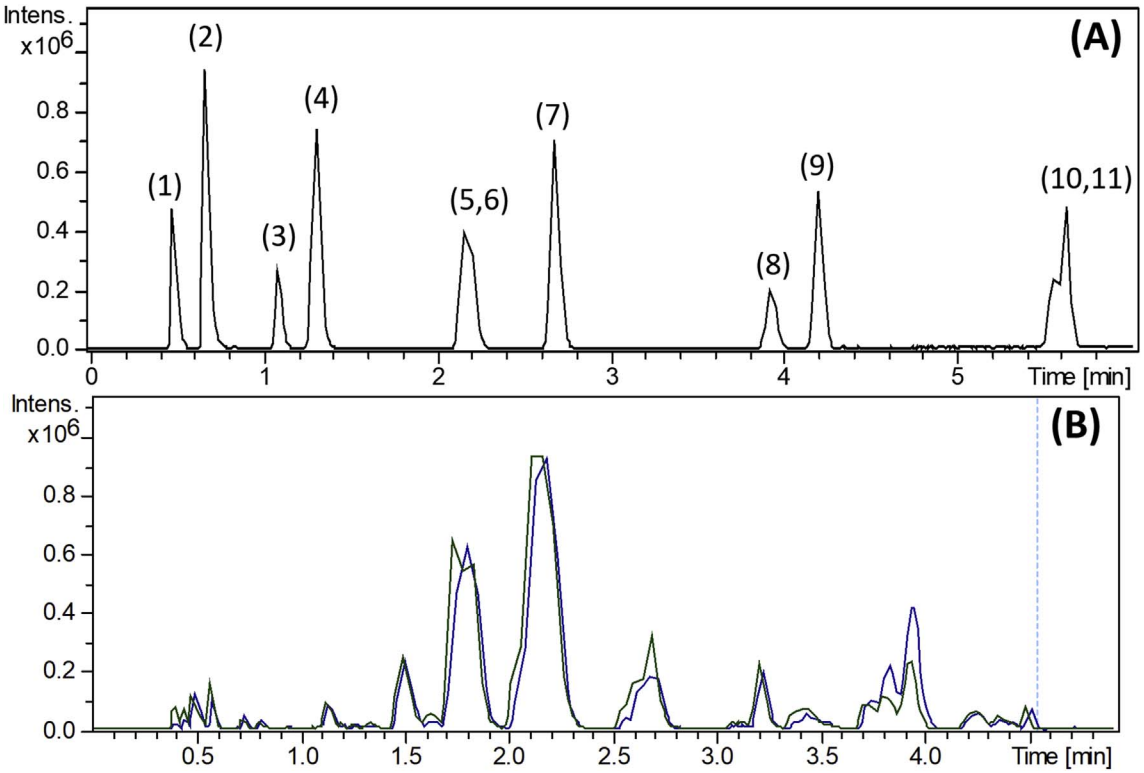


Fig. 1. Representative chromatogram of (A) model system representing a vegetable oil with high β-carotene content: (1) monoolein (RT: 0.5 min); (2) tricaprylin (RT: 0.7 min), (3) *trans*-β-Apo-8′-carotenal (RT: 1.1 min), (4) tricaprin (RT: 1.3 min), (5) 1,2- and (6) 1,3-diolein (RT: 2.2 min), (7) trilaurin (RT: 2.7 min), (8) β-carotene (RT: 3.9 min), (9) trimyristin (RT: 4.2 min), (10) tripalmitin and (11) triolein (RT: 5.6 min) and (B) crude palm oils: native (blue) and hybrid (green).

Table 2
Fatty acid compounds identified in the model system representing a vegetable oil with high β-carotene content by UHPLC-ESI-MS.

Peak #	Retention time (min)	Compound	Molecular formula	Mass to charge (*m/z*)		Ion
				Calculated	Experimental	
1	0.5	1-Monoolein	$C_{21}H_{40}O_4$	379.2819	379.2847	$[M+Na]^+$
2	0.7	Tricaprylin	$C_{27}H_{50}O_6$	488.3946	488.4003	$[M+NH_4]^+$
3	1.1	*trans*-β-Apo-8′-carotenal	$C_{30}H_{40}O$	417.3152	417.3187	$[M+H]^+$
4	1.3	Tricaprin	$C_{33}H_{62}O_6$	572.4885	572.4933	$[M+NH_4]^+$
5	2.2	1,2-Diolein[a]	$C_{39}H_{72}O_5$	638.5718	638.5748	$[M+NH_4]^+$
5	2.2	1,3-Diolein[a]	$C_{39}H_{72}O_5$	638.5718	638.5748	$[M+NH_4]^+$
6	2.7	Trilaurin	$C_{39}H_{74}O_6$	656.5824	656.5873	$[M+NH_4]^+$
7	3.9	β-Carotene	$C_{40}H_{56}$	536.4376	536.4425	$M^{+\cdot}$
8	4.2	Trimyristin	$C_{45}H_{86}O_6$	740.6763	740.6814	$[M+NH_4]^+$
9	5.6	Tripalmitin[b]	$C_{51}H_{98}O_6$	824.7702	824.7744	$[M+NH_4]^+$
9	5.6	Triolein[b]	$C_{57}H_{104}O_6$	902.8171	902.8224	$[M+NH_4]^+$

[a] Co-eluted at 2.2 min.
[b] Partially co-eluted at 5.6 min.

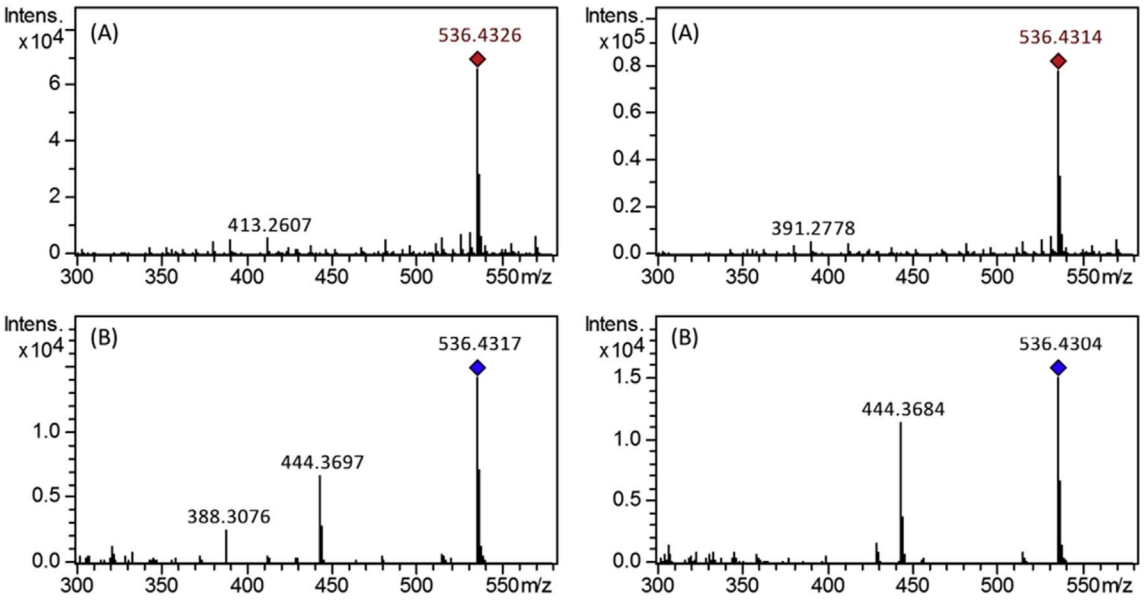


Fig. 2. (Left) (A) High resolution ESI(+)-MS spectrum of α-carotene (radical cation) (B) ESI(+)-MS/MS spectrum of α-carotene (precursor ion of *m/z* 536). (Right) (A) High resolution ESI(+)-MS spectrum of β-carotene (radical cation) (B) ESI(+)-MS/MS spectrum of β-carotene (precursor ion of *m/z* 536).

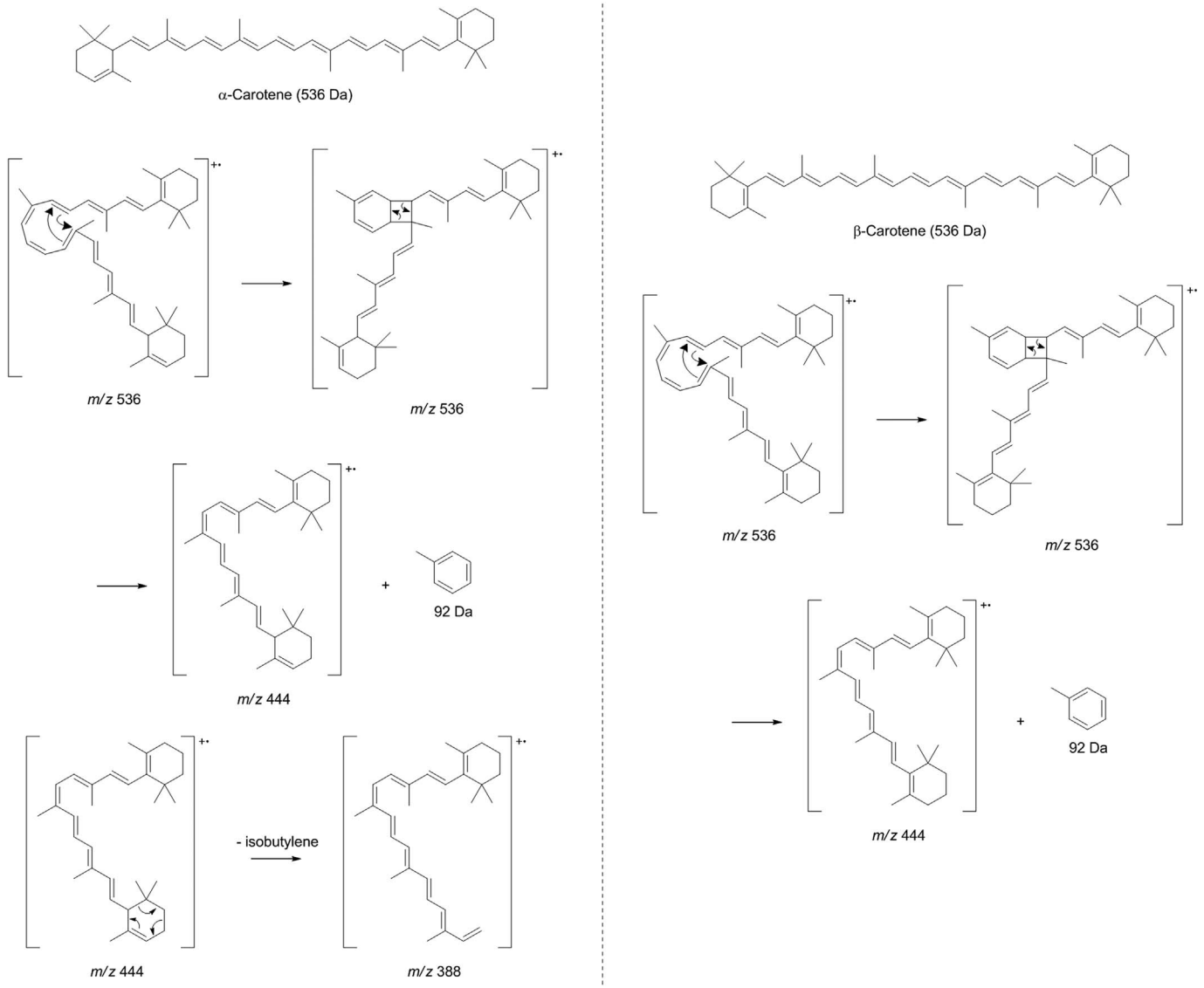
structures.

3.5. Carotenoids identification in bleached oils

The α- and β-carotene isomers identification was performed in oils obtained from native and hybrid oil palm cultivars, before and after bleaching experiments using 3.0% (m/m) of bleaching earth. Fig. 3a shows the chromatograms obtained for the native oil. It is possible to observe that the crude oil presents α- and β-carotene isomers, which correspond to the peaks in 3.8 and 3.9 min, respectively (Fig. 3a – red line). After the treatment with the adsorbents (Fig. 3a – blue and green lines), the concentration of carotenoids was reduced, and the β-isomer peak (3.9 min) was very close to the baseline. There was also a reduction, however less expressive, of the α-isomer. The peak intensity of the isomers was very similar after each treatment.

Furthermore, Fig. 3b shows the chromatogram of hybrid oil. Alike the native oil, the crude oil had both isomers. However, a different behavior was observed using different bleaching earths. Bleached oil with the neutral one (Fig. 3b – green line) had higher reduction of β isomer concentration, whereas lower difference was observed in the peak intensity of the α. Bleached oil with the acid adsorbent (Fig. 3b – blue line) presented reduction of both forms, also presenting a small reduction of the concentration of the α and substantial reduction of the concentration of β. Those results may reflect the selective adsorption between α- and β-isomers, being the last one preferably adsorbed.

From those results, it is evident that the studied bleaching earths have different capacities to remove carotene isomers, resulting in diverse carotenoid profile in bleached oils and, consequently, diverse by-products. Although, none of the previous works in literature has identified carotenoid isomers in bleached oils. This result confirms what was reported by Silva et al. (2014), which evaluated the effect of using different bleaching earth on the color of fully refined palm oil. Silva et al. (2014) have also evaluated the ability of different types of bleaching earth in the removal of total carotenes in the batch bleaching procedures, and the final color of the oils after being submitted to the deodorization process (heat bleaching). It was noted that the acid bleaching earth had a higher concentration of carotenes after bleaching, albeit, after the deodorization stage the oil had a lighter coloration. The authors suggested that this effect can be explained by the different forms of carotenes remaining in the oil after adsorption. According to Gurak et al. (2014), the color of carotenoids depend on their chemical structure, their concentration and interaction with other molecules. It is important to highlight that α- and β-carotenes are not detected in fully refined oils and the final color is mostly due to high molecular



Scheme 1. Proposed fragmentation pattern for carotene isomers main fragments. (Left) Rationale for the α-isomer fragmentation. (Right) Rationale for the β-isomer fragmentation.

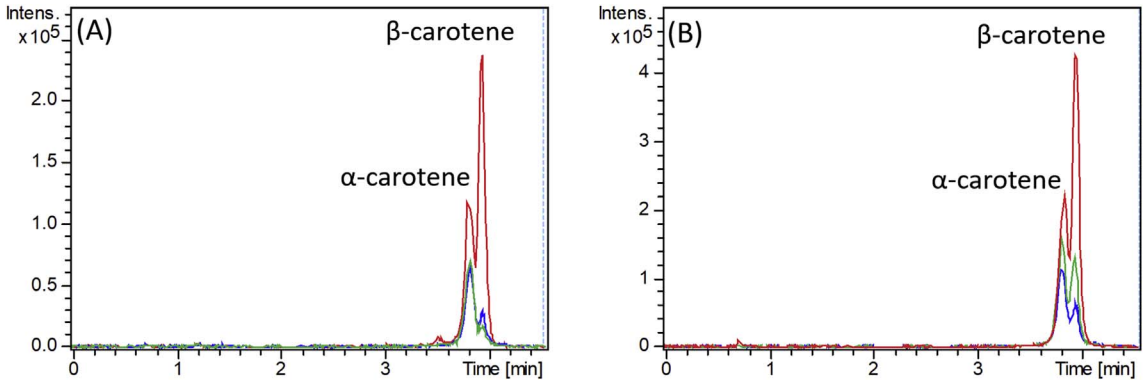


Fig. 3. Representative ion extracted chromatogram (*m/z* 536) of (A) native and (B) interspecific hybrid palm oil: crude (red); bleached with acid active adsorbent (Tonsil OPT 210 FF) (blue), bleached with neutral adsorbent (Pure Flo B-80) (green). The black line represents β-carotene standard at 2 μg/mL. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

compounds derived from oxidation reactions, especially carotenoids.

4. Concluding remarks

This work showed an important progress in understanding how the adsorption process affects the remaining carotenoids isomers in bleached palm oil, what is essential for improvements in palm oil refining towards a more cost-effective and sustainable process. A pioneer method was developed to identify α- and β-carotene in oil samples by UHPLCMS/MS, which was applied to identify them in bleached palm oils. In addition, the fragmentation pattern of carotenoids was analyzed and the rationale for the α-carotene diagnostic ion (*m/z* 388) was widely discussed. This work fulfills a gap in the literature, once none of the previous works discussed the fragmentation pattern of α- and β-carotene to this extent. The analysis was carried out on crude palm oils obtained from two different oil palm cultivars: native (*E. guineensis*) and interspecific hybrid (*E. guineensis* × *E. oleifera*). Both oils presented α- and β-two isomers, being β isomer the most important. Thereupon, the isomers were identified in bleached oils with commercial bleaching earths, that are widely used in the vegetable oil industry. It was also observed that for the native oil, the reduction of the two isomers after the adsorption process was very similar. Finally, for the hybrid oil, the remaining isomers in the bleached oil were considerably different: the concentration of the β-isomer reduced considerably after bleaching with both bleaching earths, whereas the concentration of α-isomer only showed considerable reduction after bleaching with the acid clarifying earth. Further studies are still needed to explain why there was such a difference in the behavior of different oils.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2017.11.039.

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