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Research Article

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Structural and energetic theoretical investigation of the majority triacylglycerols in olive oil

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ABSTRACT

In this work, we performed a detailed theoretical structural and energetic study of the majority triacylglycerols (TAGs) contained in olive oil, their trans isomers and the fatty acids composing them in order to better understand the chromatographic analysis. The geometries of all the studied structures, their relative equilibrium energies and their harmonic frequencies were determined. We carried out energy calculations by DFT method (B3LYP/6-31+G(d)) for fatty acids. We conducted semi empirical calculations (AM1) for larger triacylglycerol molecules. We showed that trans fatty acids are more stable than cis ones. We also showed that triacylglycerols may exist in two possible shapes. For the TAGs that contain the same number of atoms, we compared the relative equilibrium energies and we showed that when the degree of unsaturation increases, the triacylglycerol becomes less stable. The structural and energetic properties obtained in our work are a first step to understand the HPLC phenomenon because they enable us to determine all the needed theoretical parameters to correlate with the retention time of TAGs in HPLC.

Keywords: Triacylglycerols, Fatty acids, Olive oil, Theoretical investigation, Semi empirical calculation, Structural properties, Relative energy, geometry optimization.

INTRODUCTION

As all vegetable oils, olive oil is mainly composed of triacylglycerols. The content of triacylglycerols or triglycerides (TAGs) in oils is widely used in industry as marker of quality to control their purity [1]. Triacylglycerols are composed of a glycerol molecule whose the three alcohol functions are esterified by three similar or different fatty acids. The TAGs properties are related to the fatty acid nature and their positions in the TAG structure. They depend on the unsaturation degree, the configuration, the geometric isomerism and the length of the carbon chain of every fatty acid.

Among several chromatographic methods used today for analyzing TAG, the high performance liquid chromatography HPLC is mainly used [2-10]. The TAGs analysis by Non Aqueous RP-LC are usually carried out at room temperature [11-19]. There are various detection types used in this method like the refractive index detector and mainly the light scattering evaporative detection ELSD [4]. More recently, the use of mass spectrometry in coupling with HPLC becomes also widely used. This method (HPLC-MS) provides information on the structures of triacylglycerols [3, 21-25]. The identification of the peaks observed in the HPLC spectra is a complicated challenge to solve, especially for triacylglycerols having the same partition number [25-26].

So, in order to better understand and interpret the chromatographic behavior of TAG in NARP-LC, we conducted a structural and energetic theoretical study of the various more encountered triacylglycerols observed by HPLC and the fatty acids which compose them. We also studied their corresponding trans isomers. The structural data obtained in our work allow us to determinate all the theoretical properties needed to explain the retention time of each triacylglycerol.

EXPERIMENTAL SECTION

We optimized the geometries of the different fatty acids classically involved in the majority triacylglycerols. Then, we used them to determine the stable structures of the various triglycerides in order to compare them. The stability of each molecule was verified by harmonic frequency calculations. We also determined the theoretical infrared spectra of all the studied molecules, for comparing them to the previous published results when infrared detection was used for this type of analysis [20] In addition, we calculated the relative ground state energy for both fatty acids and triacylglycerols. Because of the huge size of some molecular systems, we conducted semi empirical calculations using the AM1 method as implemented in GAUSSIAN09 software.

For the fatty acids, energy calculations were conducted using the Density Functional Theory (DFT) method as implemented in the software GAUSSIAN09. On AM1 optimized structures, we performed DFT energy calculations using the B3LYP functional and the 6-31+G(d) basis set in order to compare the results found using AM1 calculations with those found by use of DFT method.

RESULTS AND DISCUSSION

The most abundant triacylglycerols contained in olive oil are respectively: OLL, PLL, OOL, POL, PPL, OOO, SOL, POO, PPO and SOO [27]. They are ordered according to their retention time [27]. These triacylglycerols are composed of four fatty acids: Oleic acid O, Linoleic acid L, Stearic acid S and Palmitic acid P. Thus we started our investigation by studying the fatty acids which form the majority TAGs contained in olive oil.

1) Fatty acids contained in the main triacylglycerols

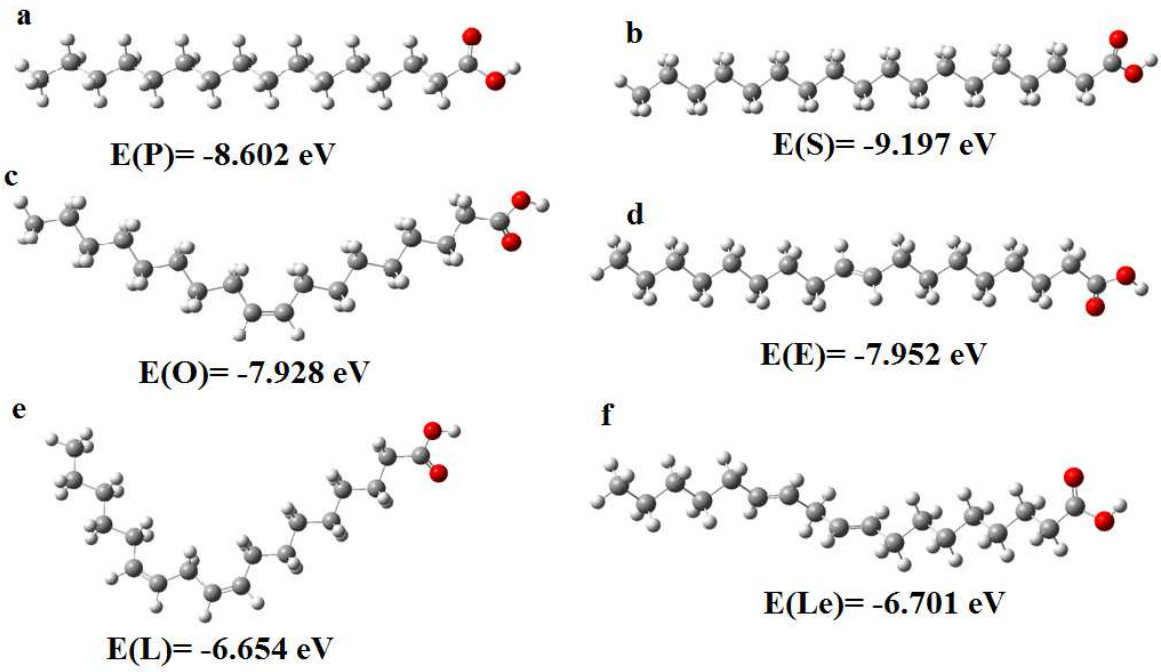
We optimized the geometries of the four fatty acids composing the majority TAGs and one of their Trans isomer using the semi empirical AM1 method. The relative equilibrium energies of the different fatty acids calculated at AM1 and B3LYP/6-31+G(d)//AM1 levels are presented in table 1.

Table 1: Fatty acid relative energies calculated by AM1 and DFT methods (eV):

Fatty acid	systematic name	AM1 energy (eV)	B3LYP/6-31+G(d)//AM1 energy (eV)
Palmitic P	hexadecanoic	-8.602	-21210.771
Stearic S	octadecanoic	-9.197	-23350.319
Oleic O	cis-9-octadecenoic	-7.928	-23316.763
Elaidic E	trans-9-octadecenoic	-7.952	-23316.869
Linoleic L	cis, cis-9,12-octadecadienoic	-6.654	-23283.187
Linoelaidic L	trans, trans-9,12-octadecadienoic	-6.701	-23283.401

The different optimized structures of the 6 fatty acids are plotted in figure 1. Observing this figure we note that the saturated acids (P and S) and the trans mono and poly unsaturated acid (E and Le), have a linear structure while the cis mono and poly-unsaturated acids (O and L) have a V shape with an angle equal to 134.2° for the Oleic acid and an angle equal to 97.5° for the Linoleic acid. We also note that the number of atoms in the studied acids is close. It ranges from 16 to 18 atoms by molecule. The Stearic acid has the highest number of atoms (18). Its relative equilibrium energy is the lowest either calculated by AM1 or DFT method (table1). The Palmitic acid has only 16 atoms, it is the smallest molecule and it has the highest DFT relative equilibrium energy.

Figure 1: Studied fatty acids and their relative energies calculated by AM1 method (eV)
a) Palmitic acid, b) Stearic acid, c) Oleic acid, d) Elaidic acid, e) Linoleic acid and f) Linoelaidic acid.



Comparing the equilibrium energies calculated by AM1 or DFT methods of the Elaidic acid E and the Oleic acid O (table 1), we note that the Oleic acid has the highest energy in both cases. Thus the trans isomer E is more stable than the cis isomer O. Also, when we compare the energies of the trans isomer Linoelaidic Le and the cis isomer Linoleic L calculated by AM1 and DFT methods (table1), we note that the trans isomer has lower energy than the cis one so Le is more stable than L. Even if trans isomers are more stable than cis ones, generally the fatty acids that occur naturally have a cis. configuration Trans fats are found in industrial foods after the thermal treatment of oils. In addition, cis fatty acids and triglycerides produced from them do not solidify as readily as trans fatty acids and their corresponding triglycerides.

2) Majority triacylglycerols

The triacylglycerols contained in olive oil are currently well separated by NARP-LC. The first one is detected at about 6.44 min with a retention factor equal to 4.1 (table2). It is the homogeneous triacylglycerol composed by three Linoleic acids LLL. The majority TAG in olive oil are later detected in the range 8.47 - 21.40 min (retention factors range: 5.6-16) (table2). In order to rationalize the retention order of TAG several properties need to be determined. So we carried out a structural and energetic investigation of these molecules as well as their corresponding trans isomers. We also studied the first detected TAG (LLL) and its trans isomer (LeLeLe) in order to compare it with the majority TAG. The structural data enable us to get all the theoretical properties needed to correlate with the retention time.

Table 2: The majority detected Triglycerides, their relative theoretical energies (eV) and their retention time by HPLC

TAGs :	AM1 energy (eV):	tr (min) [27]
LLL	-18.3111	6.44
OLL	-19.6323	8.47
PLL	-20.2555	9.11
OOL	-20.8826	11.3
POL	-21.6124	12.16
PPL	-22.2032	13.09
OOO	-22.1579	15.23
SOL	-22.1719	15.78
POO	-22.8870	16.43
PPO	-23.5572	17.80
SOO	-23.4820	21.40

In table 3, we summarize the energies of all the studied triacylglycerols (majority TAG~~s~~ : LLL, POP and PLP and their trans isomers LeLeLe, PEP and PLeP). We classified them according to their number of atoms and their

number of electrons. Observing table 3 we note that trans isomers are always less energetic than cis isomers. Thus they are the most stable.

Table 3 : The relative energies of all the studied triglycerides, their number of atoms and their number of electrons

Number of atoms:	Triglyceride:	Number of electrons:	Relative energy (eV):
169	SOO	496	-23.482
	SEE		-23.510
167	OOO	494	-22.158
	EEE		-22.283
	SOL		-22.172
	SELe		-22.263
165	OOL	492	-20.883
	EELe		-21.036
163	OLL	490	-19.632
	ELeLe		-19.757
	POO	480	-22.887
	PEE		-22.907
161	LLL	488	-18.311
	LeLeLe		-18.464
	POL	478	-21.612
	PELe		-21.687
159	PLL	476	-20.255
	PLeLe		-20.357
	POP	466	-23.481
	PEP		-23.504
	PPO		-23.557
	PPE		-23.581
157	PLP	464	-22.206
	PLeP		-22.257
	PPL		-22.203
	PPLe		-22.254

For triacylglycerols having the same number of atoms, we note that the TAGs which have the lowest number of electrons or the lowest degree of unsaturation are the most stable ones (POP is more stable than PLL, POO is more stable than OLL and POL is more stable than LLL).

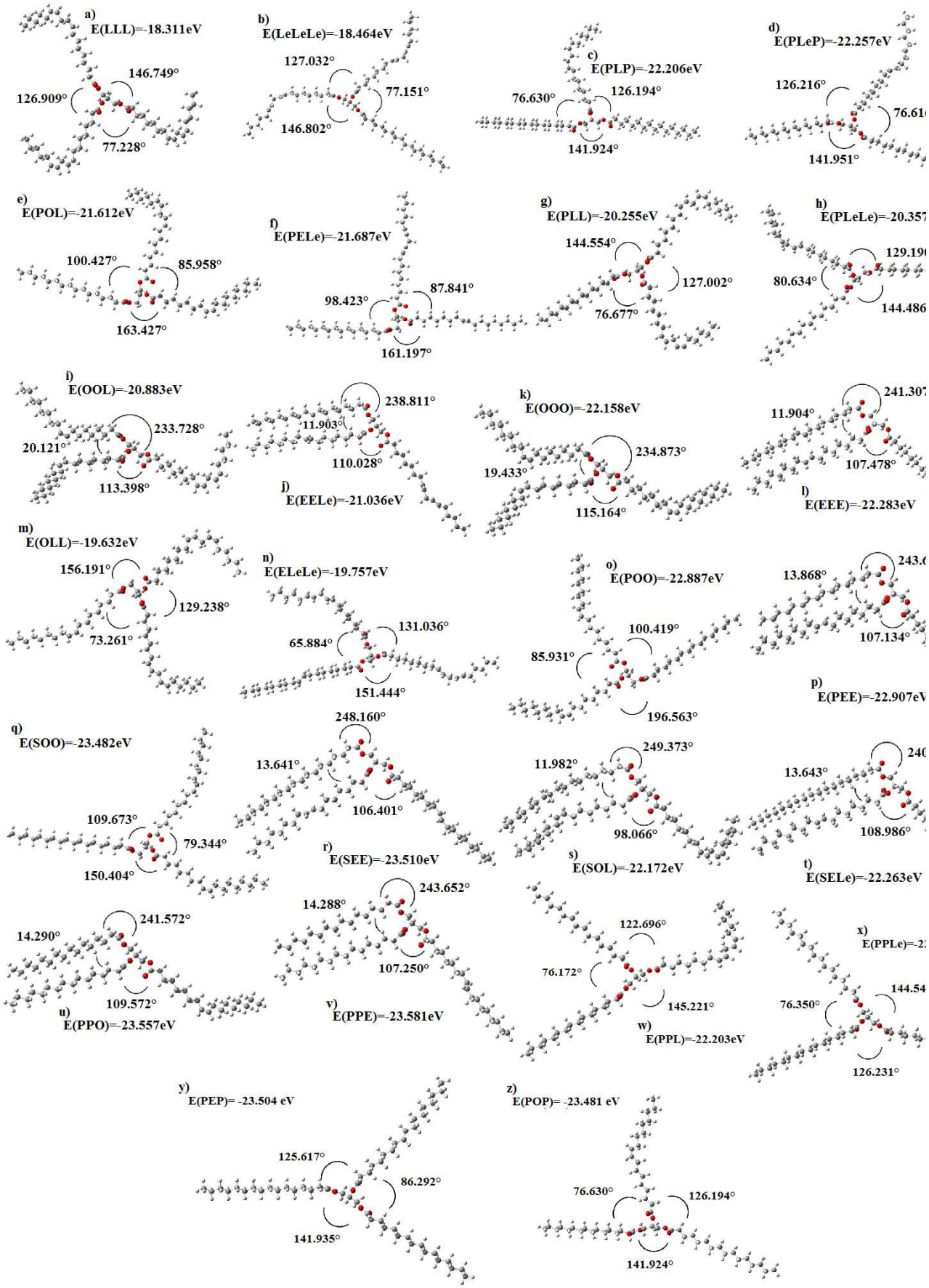
Comparing the regioisomers of TAGs which have the same electron number, PPO is more stable than POP while PLP is more stable than PPL. The energy gap is small. It is equal to 0.003 eV for PPO and POP and equal to 0.076 eV for PLP and PPL. Such pairs of regioisomers of TAG are very difficult to separate. In the experimental conditions used in the present work they are not separated. Concerning the pairs SOL/OOO SELe/EEE which have the same number of atoms and electrons, their energies are so close. Concerning the pair with cis fatty acid isomers isomer SOL is less energetic than OOO while the one with trans fatty acid isomers EEE is less energetic than SELe.

We present in figure 2 the structures of all the studied triacylglycerols. We note the existence of two possible shapes. A first shape in which the three fragments are spaced from each other and a second shape in which two fragments are very close (almost parallel) and the third one forms with them a very large angle of about 120° or more. Ten triacylglycerols have the second shape (OOL, EELe, OOO, EEE, PEE, SEE, SOL, SELe, PPO, PPE) while the other 16 ones have the first spaced shape.

The different angles between the fragments composing the triglycerides are mentioned in figure 2. Comparing the angles between the close fragments in the TAGs having the second shape, we note that OOL has the largest angle (20.121°) while EELe has the smallest one (11.903°). The gap between these angles is equal to 8.218°. OOO and EEE also have different angles between their close fragments with a gap equal to 7.529°. The structures of PPO and PPE are very close, they have close angles between their parallel fragments, the gap is equal to 0.002°.

Twenty-six triacylglycerols are studied in our work. All the studied structures are found not coplanar. Each triacylglycerol is composed by two planes, the first one is composed by two fragments and the second one contains the third fragment. The number of atoms of triacylglycerols ranges between 157 and 169 atoms and the number of electrons ranges between 464 and 496 electrons. The smallest majority triglyceride contained in olive oil is PPL. It has 157 atoms and 464 electrons. While the largest majority triglyceride is SOO with 169 atoms and 496 electrons.

Figure 2: Optimized structures of all the studied Triglycerides



Observing figure 2, we also note that the cis isomers and their corresponding trans ones have the same shape except for POO and SOO. In fact, POO and SOO have the first spaced shape, the angles between their fragments ranges between 79° and 197°. While their trans isomers: PEE and SEE, have the second shape in which two fragments are very close. The angles between their close fragments are respectively: 13.868° and 13.641°.

Concerning the triacylglycerols which have spaced structures, they have generally one angle between 65° and 87°, a second one between 98° and 131° and a third angle that ranges between 141° and 196°. POO has the largest angle (196,563°) and ELeLe has the smallest one (65.884°).

CONCLUSION

We conducted a theoretical structural and energetic investigation of the various TAGs contained in olive oil and observed by HPLC and the fatty acids which compose them [27]. We optimized the geometries of the different molecular systems and we determined their equilibrium energies. We conducted semi empirical calculations (AM1) because of the huge size of triacylglycerols.

For the fatty acids we carried out energy calculations by DFT method (B3LYP/6-31+G(d)). We showed that trans fatty acids are more stable than cis ones.

We compared the structures of the different triacylglycerols and we showed that they may exist in two possible shapes. We also compared the different angles between the three fragments of each triacylglycerol. For the TAGs that contain the same number of atoms, we compared the relative equilibrium energies and we showed that when the degree of unsaturation increases, the triacylglycerol becomes less stable.

The structural and energetic data obtained in our work are a first step to understand the HPLC phenomenon. In fact, these structural information enable us to determine all the needed theoretical parameters to correlate with the retention time in HPLC methods.

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