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Evolution of the sterolic and lipid composition of olive oils during the maturity of olives

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Abstract

Olive oil is mainly composed of triglycerides (90-99%) and natural minor constituents (1-5%) grouping compounds of various structures such as sterols, tocopherols, phenols, aromatic compounds and hydrocarbons. Several factors can influence this quality, so, in addition to climatic, geographical, pedological and genetic factors, there are other factors such as the variety cultivated, the degree of maturity of the olives, the harvesting system, the time elapsed between harvesting and crushing, the method of storing the olives, the extraction processes and the preservation of the final product. The objective of this work was to study the evolution of the sterolic composition of the oils during the maturity of the olives and then the influence of the harvest date on the different sterolic compounds. The results of this analysis revealed that the levels of β-Sitosterol and Δ-7-Avenasterol decrease with the maturity of the olives while that of Δ-5-avenasterol increase and the level of Campesterol is always higher than that of Stigmasterol. In parallel, the evolution of neutral lipids and polar lipids was monitored. The latter showed the predominance of polar lipids at the start of maturity. As the olives mature, it's the neutral lipids that become the majority compared to the polar lipids.

1. Introduction

The olive tree is the main fruit crop in Morocco [1]. The oil extracted from the fruits is highly valued for its organoleptic characteristics and nutritional value [2]. These characteristics are strongly linked to the quality which, itself, is influenced by several parameters such as variety, cultivation techniques, olive maturity, harvest period, storage conditions and extraction systems [3, 4, 5, 6]. The quality of oils has become an essential factor of competitiveness to conquer new markets at the global level and benefit from as well as the enthusiasm for this oil, known for its benefits [7]. Indeed, the Mediterranean diet is associated with a low incidence of cardiovascular disease, atherosclerosis, neurodegenerative diseases and some types of cancer [8]. The health benefits of this diet have been partially associated with the consumption of virgin olive oil by Mediterranean populations [9, 10]. The beneficial effects of virgin olive oil have been attributed to its high content of monounsaturated fatty acids, particularly oleic acid

and its minor compounds such as tocopherols, triterpene and aliphatic alcohols, sterolic compounds, phenolic compounds, chlorophylls and carotenes [11, 12].
Thus, the objective of the present work was to study first of all the evolution of the sterolic composition of the oils during the maturity of the olives, then the influence of the date of harvest on this composition and at the end the evolution of neutral lipids and polar lipids.

2. Material and methods

2.1 Plant material

This study was carried out in the region of Chaouia (central Morocco) during the agricultural campaign 2013/2014. It involved two sites: Oulad Said (site 1), located west of the city of Settat and Sidi el Aïdi (site 2), located north of the city of Settat.
The plant material studied belongs to the Moroccan Picholine population variety. The trees underwent the same treatments (pruning, phytosanitary treatments). A sample of 1 kg olives was collected from trees at both sites on a regular basis over a period of seven months (June 20 to January 20).
The samples were stored at (- 25) ° C in freezer bags for later use.

2.2 Extraction and content of olive oil

The experimental protocol is described in the article: Evolution of the Carpometric Characteristics and the Chemical Composition of Oils During the Period of Maturity of the Olive in the Chaouia Area - Morocco [13].

2.2.1 Separation of neutral and polar lipids

In a decanting bulb, a known quantity of olive oil is added to a Hexane - Methanol mixture: 1/1 (v / v). After stirring and settling at room temperature, the polar lipids are in the lower organic phase while the neutral lipids are in the upper organic phase. Each phase was recovered after evaporation of the solvents and weighed to determine the content of each class of lipids [14].

2.2.2 Thin layer chromotography

For each sample, a few µl of olive oil are diluted in hexane and deposited on a silica plate. The migration solvent consists of the mixture: petroleum ether, ethyl ether and acetic acid: 70 / 30 / 0.4 (v / v / v). After migration, the plates are dried and the different classes of lipids are revealed by iodine vapor [15].

2.3 Determination of sterolic compounds

The sterol fraction was determined according to the method described by the COI [16]. After saponification of the oils with potassium hydroxide in ethanolic solution while using α - cholestanol as an internal standard. The unsaponifiable was then extracted with ethyl ether. The sterol fraction was separated from the unsaponifiable extract by thin layer silica gel chromatography. The sterols recovered from the silica gel were transformed into trimethylsilylethers and analyzed by gas chromatography using an Agilent type chromatograph equipped with an HP 5 capillary column (5% diphenyl and 95% dimethylpolysiloxane) of 30 m long, 0.25 mm inner diameter and 0.1 µm film diameter. The temperature of the oven, the injector and the detector is 300 ºC. The gas vector is hydrogen with a flow rate of 40 ml / min. The volume injected was 1 µl. The identification of the peaks was carried out in the presence of the witnesses and the calculation of the different percentages of sterols was done using an automatic integrator.

3. Results and Discussion

3.1 Extraction and determination of the content of olive oil

The values of the oil content of olives from the two sites are described in the article: Evolution of the carpometric indices and the chemical composition of oils during the period of maturity of olives in the region of Chaouia - Morocco [13]. Indeed, the values of the oil content are close to zero during the month of June because the oil synthesis has not yet started. It is from July that lipogenesis begins and we note the presence of a small amount of oil (3.4 ± 0.3% for site 1 and 2.9 ± 0.4% for site 2). The oil synthesis continues and reaches maximum values during the month of December (22.2 ± 0.3% for site 1 and 19.2 ± % for site 2). Beyond this date, there is a slight decrease in the oil content.

3. 2 Determination of neutral lipids and polar lipids

The two neutral and polar fractions of the oil were separated by a mixture of two opposing organic polarity solvents, hexane and methanol. The results are expressed as a percentage of the weight of total lipids. Based on the results obtained, Figures 1 and 2, we note the predominance of polar lipids at the beginning of maturity for the first two samples (June 20 and July 20).

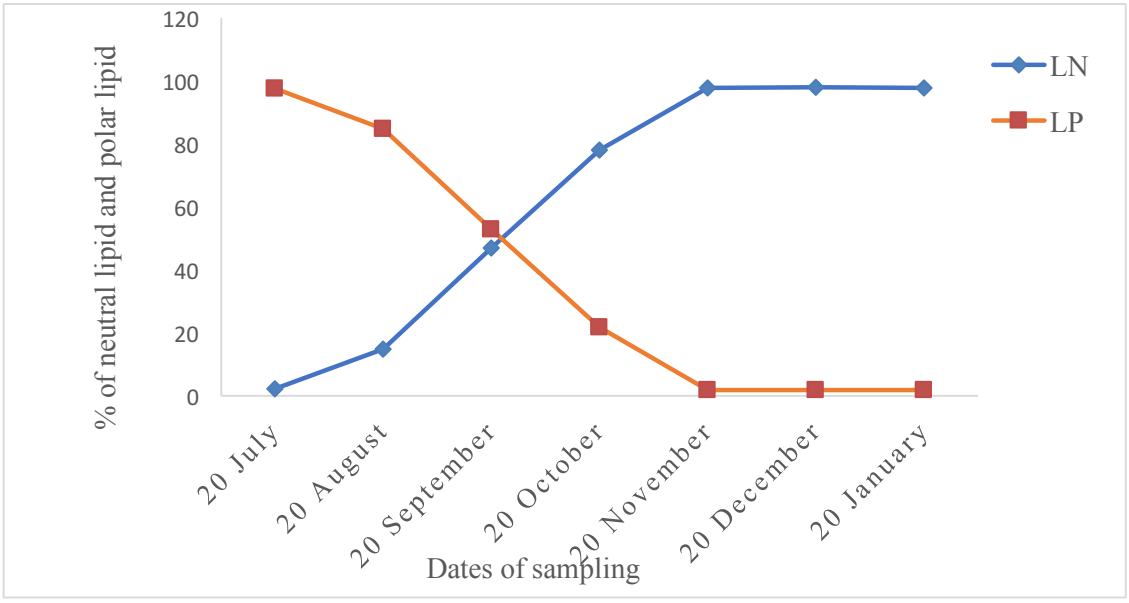

Figure 1: Evolution of the contents of neutral lipids and polar lipids according to the date of the samples for site 1

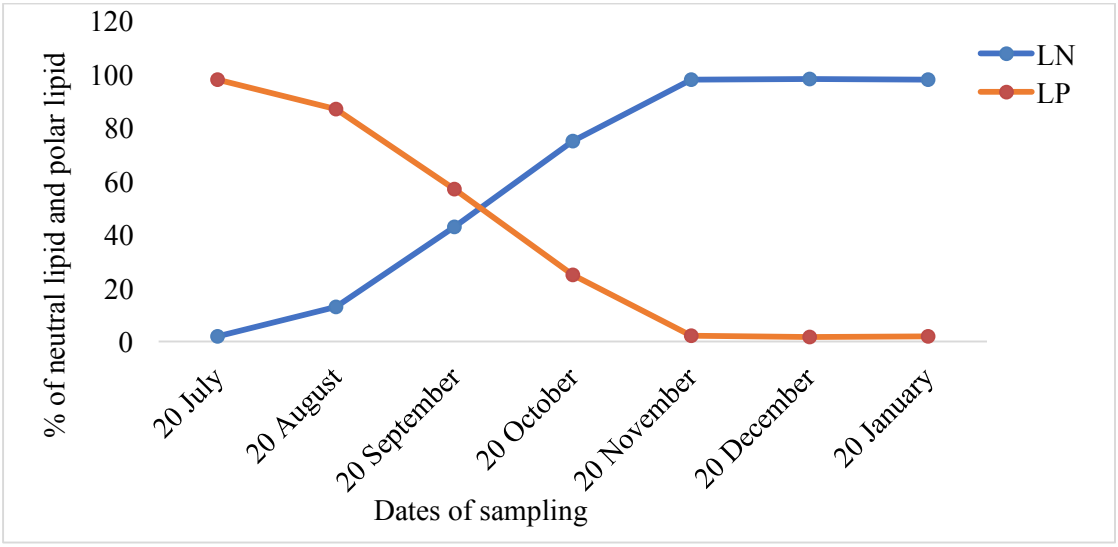

Figure 2: Evolution of the contents of neutral lipids and polar lipids according to the date of the samples for site 2

As the olives ripen, it is the neutral lipids that become the majority compared to the polar lipids (samples taken in October, November and December). In January, there is a slight decrease in neutral lipids and a slight increase in polar lipids. The results we obtained are in agreement with those reported by Ajana et al in the area of Marrakech (1991) [17] and Ait Yacine in the area of Tadla (2001) [18].

3.3 Thin layer chromatography

In order to see the evolution of the different lipid classes during the maturity of the olives, a thin layer chromatography was carried out. We note from the results obtained and shown in Figures 3 and 4, that at the beginning of July and until October, there is a dominance of phospholipids, monoglycerides, diglycerides, which is explained by the appearance of bands attributed to free fatty acids and triglycerides reflecting the ripening of olives.

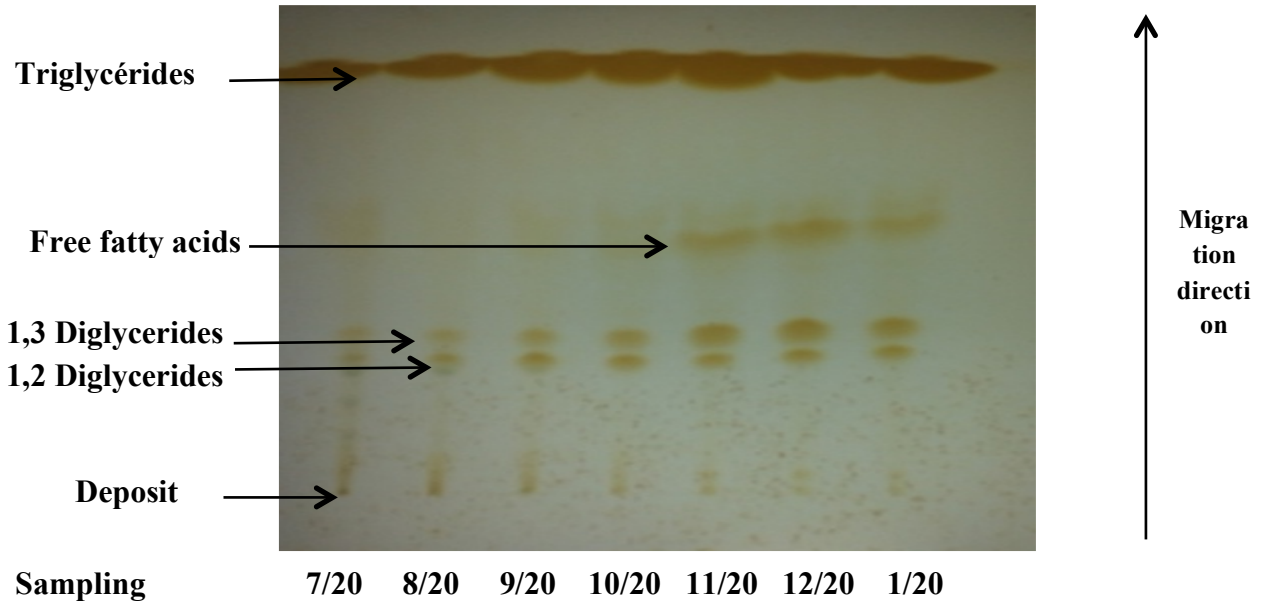

Figure 3: Thin layer Chromatography of samples of oil extracted at different stages of olive ripeness for the Oulad Said site.

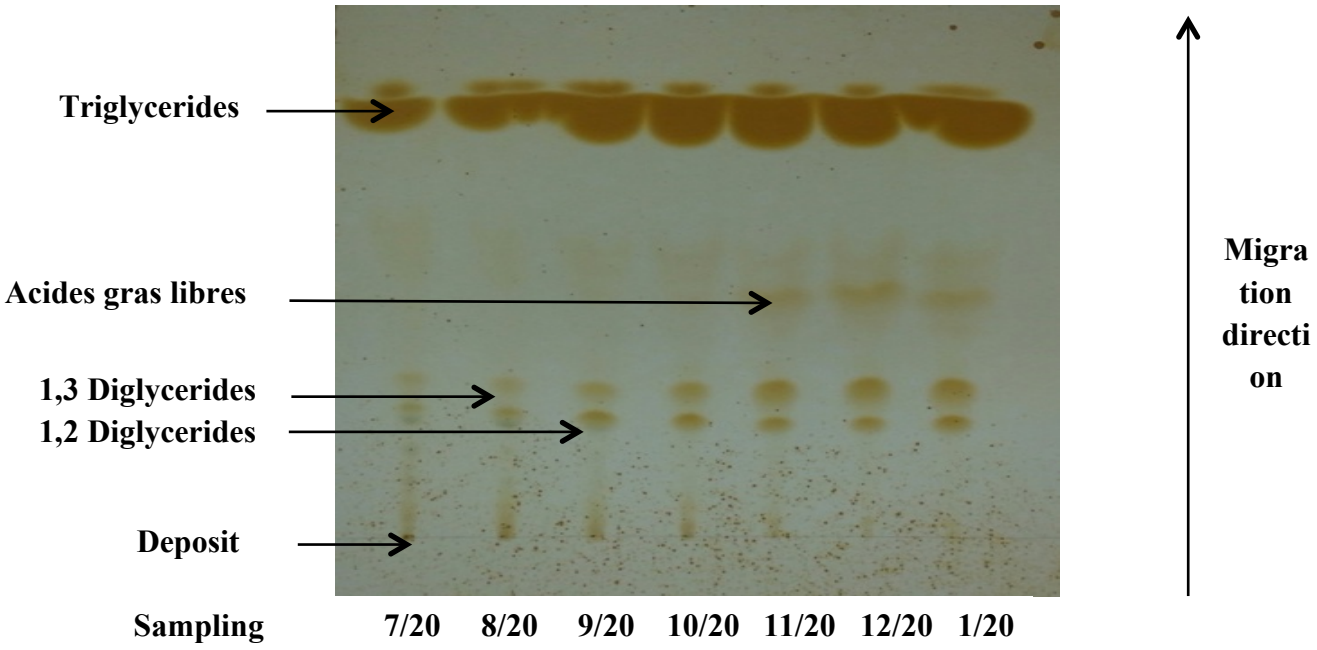

Figure 4: Thin layer Chromatography of samples of oil extracted at different stages of olive ripeness for Sidi el Aïdi site.

Moreover, by combining these results with the results of the contents of neutral lipids and polar lipids, we can subdivide lipogenesis into three phases:

- The first phase:

This phase is characterized by the biosynthesis of polar lipids involved in the formation of cell membranes (nucleus and mesocarp formation) and by the synthesis of monoglycerides and diglycerides. The synthesis of triglycerides is relatively less important [17, 18]**.**

- The second phase:

During this phase, there is a massive synthesis of triglycerides which become the majority compared to the other constituents of the oil (polar lipids). The latter accumulate at the level of the mesocarp, which consequently increases the size of the olives [17, 18]**.**

-Last phase of lipogenesis:

Lipid synthesis continues to peak in December. After this period, there is a slight decrease in the quantity of oils in the olives [17, 18]**.**

3. 4 Evolution of the sterolic composition of olive oil

Sterols are essential constituents of cell membranes. The results of the analyses show that the sterol components of the variety studied at both sites comply with those established in the IOC standard [16]**.** According to Tables 1, 2, and Figure 5, 6 and 7, we note the dominance of β-Sitosterol [19], the contents of which vary from 82.24% to 92.48% for site 1 and from 79.36% to 91.95 % for site 2. In fact, olive oil is the only oil that contains a particularly high amount of β-Sitosterol, a substance which is opposed to the intestinal absorption of cholesterol [20, 21].

Table 1: Evolution of the percentage of the different sterolic classes for site

Sterols Sampling	Campesterol	Stigmasterol	β-Sitosterol	Δ-5-Avénasterol	Δ-7-Stigmasterol	Δ-7-Avénasterol
07/20	2.75	0.60	90.81	4.11	0.09	0.40
08/20	3.07	0.57	91.99	3.38	0.09	0.24
09/20	2.52	0.71	92.48	1.88	0.27	0.08
10/20	2.91	0.90	89.67	4.26	0.06	0.17
11/20	2.51	1.18	82.24	11.41	0.07	0.18
12/20	2.67	1.40	82.35	11.68	0.03	0.25
1/20	2.81	0.94	82.66	10.97	0.06	0.12
Standards IOC	≤ 4	< Campesterol	≥ 93	-	≤ 0.5	-

From the samples taken from June to December, the β-Sitosterol and Δ-7-Avenasterol compounds show an inverse evolution with respect to Δ-5-Avenasterol. Conversely, in January, there is an increase in the levels of β-Sitosterol and Δ-7-Avenasterol and a decrease in the level of Δ-5-Avenasterol. The level of Campesterol is always higher than that of Stigmasterol. Stigmasterol is present in all the oils from the samples studied, with levels which remain within the limits established by the IOC standard [16]. For

Δ-5-Avenasterol, Δ-7-Stigmasterol and Δ-7-Avenasterol, the values found respect the limits established by the IOC.

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Table 2: Evolution of the percentage of the different sterolic classes for site 2

Sterols Sampling	Campesterol	Stigmasterol	β-Sitosterol	Δ-5-Avénasterol	Δ-7-Stigmasterol	Δ-7-Avénasterol
07/20	2.61	0.66	90.80	4.00	0.12	0.38
08/20	2.97	0.62	91.01	3.85	0.09	0.26
09/20	2.88	0.68	91.95	2.91	0.07	0.21
10/20	2.91	1.02	86.14	7.22	0.11	0.26
11/20	2.82	3.20	80.25	11.10	0.04	0.22
12/20	2.99	1.22	79. 36	11.44	0.04	0.26
1/20	3.48	3.30	88.07	1.82	0.12	0.23
Standards IOC	≤ 4	< Campesterol	≥ 93	-	≤ 0.5	-

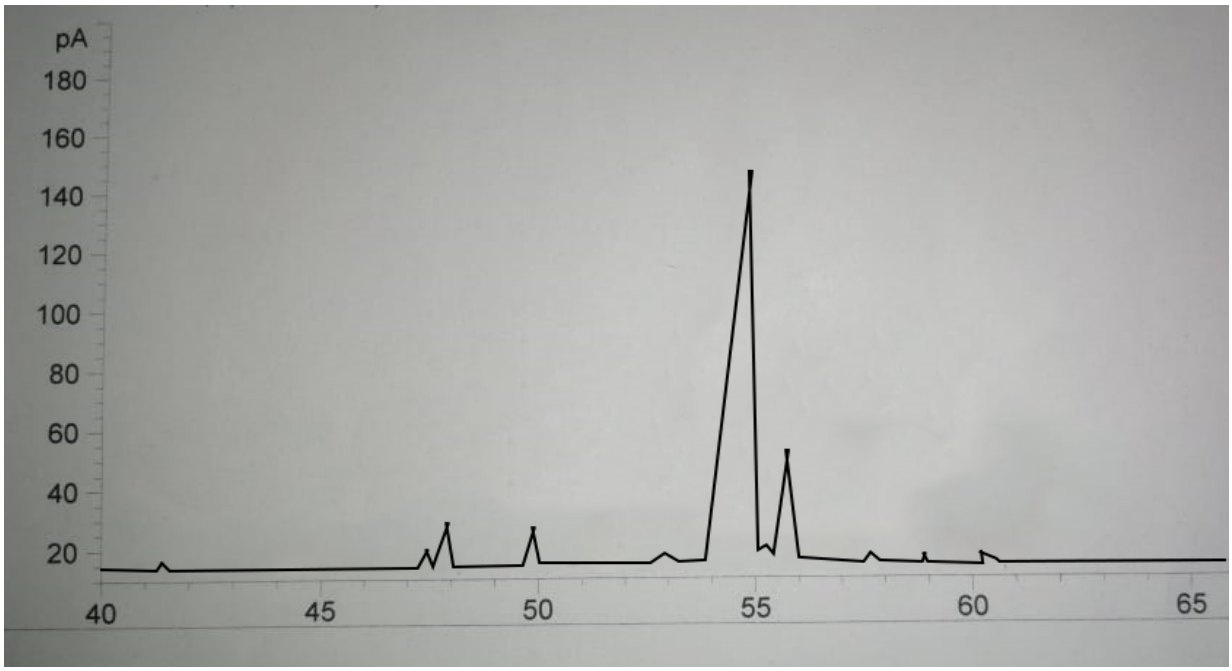

Figure 5: Chromatogram of sterolic compounds in a sample olive oil.

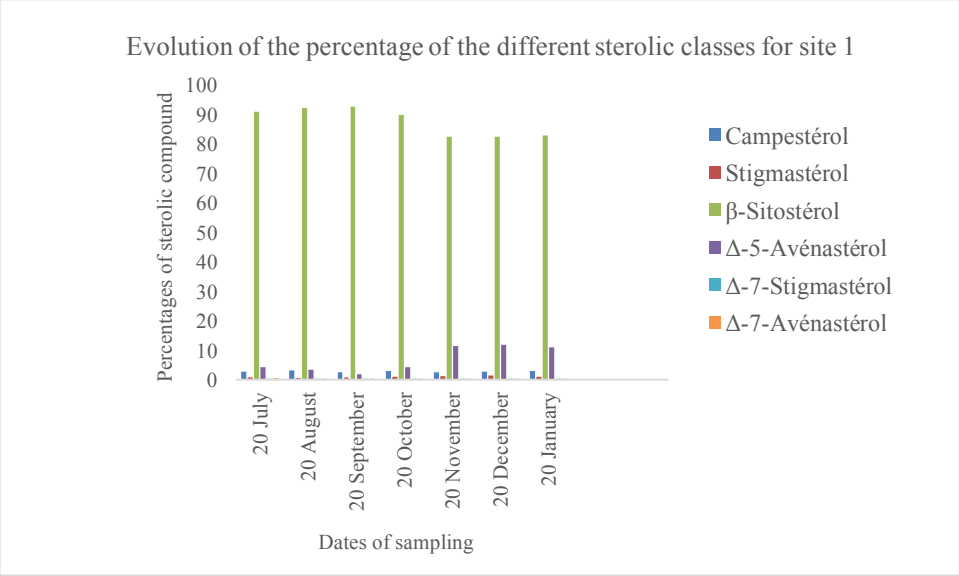

Figure 6: Evolution of the percentage of the different sterolic classes for site 1

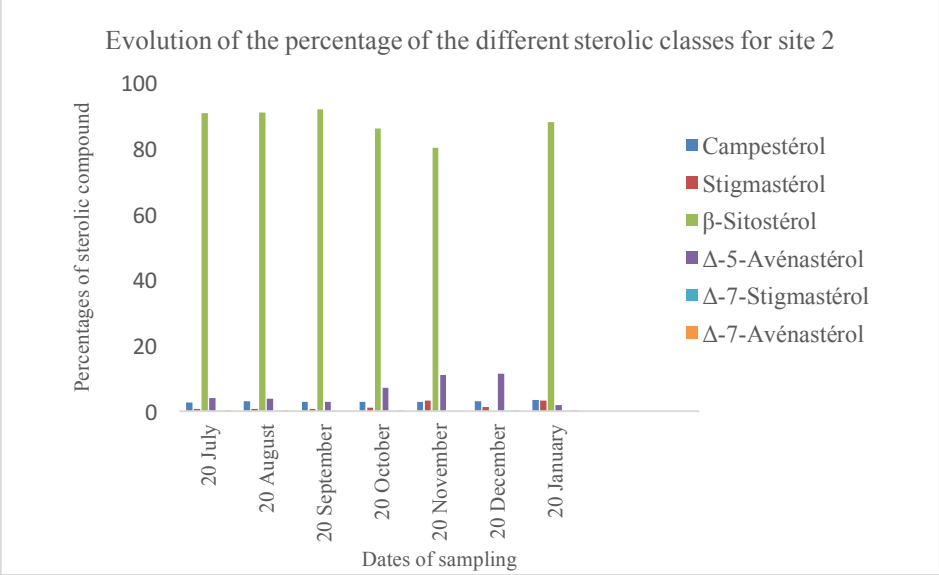

Figure 7: Evolution of the percentage of the different sterolic classes for site 2

Conclusion

Regarding sterols, we note the dominance of β-Sitosterol, the contents range from 79.36% to 92.48%, the presence of β-Sitosterol and Δ-7-Avenasterol with an inverse evolution compared to that of Δ-5-Avenasterol, β-Sitosterol and Δ-7-Avenasterol levels decrease slightly with the maturity of the olives while that of Δ-5-avenasterol is seen to increase. The rate of Campesterol is always higher than that of Stigmasterol which is present in all the oils of the samples studied, with levels that remain within the limits established by the IOC standard.

During this study, we noted that the lipid fraction of olive oils showed a predominance of polar lipids over neutral lipids at the beginning of maturity. As the olives mature, it's the neutral lipids that become the majority compared to the polar lipids.

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