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Factors Affecting D-7-Stigmastenol in Palestinian Olive Oil

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Abstract: The level of delta-7-stigmastenol (D-7-stigmastenol) contained in olive oil is a new criterion for oil quality, particularly its purity from adulteration with other seed oils. In this study, 79 olive samples were collected and analyzed from different areas of Palestine to study the factors affecting D-7-stigmastenol levels in the oil. These areas included the provinces of Jericho, Hebron, Bethlehem, Ramallah, Salfeet, Nablus, Jenin, Tulkarem and Qalqilyah. The study began in October 2007 and ended in July 2008. The following 11 factors were taken into consideration during sample collection: olive fly infection, topography, olive storage before pressing, geographical area, effect of olive seeds during oil extraction, effect of pressing temperature, presence of olive leaves during oil extraction, soil type, maturity index of the olive fruit, olive variety and oil preservation and storage in terms of storage container types. The results show that soil type, region, maturity index and olive fly infection are the main factors affecting D-7-stigmastenol. Pressing temperature, olive storage before pressing, olive variety and oil storage showed a moderate effect. Olive seeds, topography and presence of olive leaves had a negligible effect on D-7-stigmastenol levels in the oil.

Key words: Palestinian olive oil quality, D-7-stigmastenol, soil type, geographical area, maturity index, fly infection

INTRODUCTION

Olives are essential to the Palestinian economy. They are the single biggest crop in what remains a largely agricultural economy and they have deep cultural significance as a symbol of traditional society and ties to the land (The World Bank, 2006). It is estimated that the area of olive trees represents 81.1% of the area of fruit trees grown in the region (Palestinian Central Bureau of Statistics, 2009). Approximately 90-95% of the Palestinian olive harvest is used to produce olive oil, with the remainder being used for pickling and table olives (The World Bank, 2006). The amount of oil produced in Palestine in the years 2004, 2006 and 2008 was 22106, 34002 and 17584 t, respectively (Palestinian Central Bureau of Statistics, 2009).

There are many constraints on olive oil export; a significant factor is compliance with international standards (Jumana, 2004). Olive oil is often illegally adulterated with other less expensive vegetable oils (Arvanitoyannis and Vlachos, 2007). Among the various chemical and physical methods employed to detect the adulteration of olive oil by low-grade olive and seed oils are sterol, alkane, wax and aliphatic alcohol and triacylglycerol analyses (Kiritsakis and Christie, 2000).

Sterols found in vegetables and plant oils and have desirable health properties (Covas *et al*., 2006; Mailer *et al*., 2010). The International Olive Council imposes limits or ranges for each type of sterol based on the natural levels found in traditional olive oil varieties. Sterol profiles outside of these ranges could suggest that the oil is not genuine. The required sterol profile (as % of

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total sterols) is as follows: cholesterol ≤0.5%, brassicasterol ≤0.1%, campesterol ≤4.0%, stigmasterol ≤ campesterol in edible oils, D-7-stigmastenol ≤0.5%, beta-sitosterol+delta-5-avenasterol+delta-5-23-stigmastadienol+clerosterol+sitostanol+delta-5-24-stigmastadienol ≥93.0% (International Olive Oil Council, 2009).

In the years 2005 and 2006, most Palestinian olive oil contained >0.7% D-7-stigmastenol, which is higher than international standards (≤0.5% of total sterols). This problem threatens the international trading of Palestinian olive oil. In fact, some containers of oil have been rejected at European ports due to their high D-7-stigmastenol levels. This problem has also been reported in Australia (Mailer and Ayton, 2008), Syria (Codex Alimentarius Commission, 2007; Italian Ministry of Foreign Affairs, 2007) and Argentina (Carelli, 2008).

Several studies have been conducted to determine the factors affecting the quality of olive oil, particularly the sterol profile. The factors that have been investigated include harvesting, cultivation, ripeness, post-harvest storage, extraction method (Pehlivan and Yilmaz, 2010; Kiritsakis and Christie, 2000), olive varieties (Oueslati *et al*., 2009), geographical origin (Mailer, *et al*., 2010; Temime *et al*., 2008), fly attack (Tarnendjari *et al*., 2009) and packaging materials (Guil-Guerrero and Urda-Romacho, 2009). Most of the studies on D-7-stigmastenol showed great variation in D-7-stigmastenol levels in olive oil produced from different olive varieties and these values may be higher than those required by the international standards. Unfortunately, the effect of other factors on the D-7-stigmastenol level in olive oil has not been investigated in great depth because it has only been recently designated as a criterion for olive oil quality by the International Olive Oil Council. Therefore, there is a need to investigate the factors that affect D-7-stigmastenol levels in olive oil and how these factors can be optimized to solve this problem.

The aim of this study was to determine the factors affecting the D-7-stigmastenol level in Palestinian olive oil. The following 11 factors were considered: fly infection, topography, olive storage before pressing, geographical area, olive seeds, press temperature, olive leaves, soil type, harvest time, olive variety and olive oil storage. The factors were studied separately by fixing the other factors during sampling of the olive fruit and oil or the extracted oil.

MATERIALS AND METHODS

Olive and olive oil sampling: This study began in October 2007 and was completed in July 2008. Eleven factors were considered. A total of 5 kg of olives were pressed using a semi-manual press to produce approximately 1L of oil for each of the 36 samples to investigate eight of the 11 factors.

A total of 40 1-L samples of olive oil were collected from local presses on the West Bank to investigate the other three factors. The sample collection for each factor was as follows:

Effect of olive fly: Two samples of 5 kg olives were collected from the Jenin area, one was infected with olive fly and the other was not.

Topography: Two samples of 5 kg olives were collected from two different topographical areas in Jenin (level land and mountainous land).

Olive storage before pressing: Ten samples of 5 kg olives were collected from the Hebron area. Five samples were stored in plastic bags and the other five samples were stored in plastic mesh boxes for 27 days with intervals of 1 week. To investigate the effect of storage time, one sample from each storage type was pressed to extract the oil.

Geographical area: The following nine geographical areas were compared: Hebron, Bethlehem, Ramallah, Jericho, Salfeet, Nablus, Jenin, Tulkarem and Qalqilyah. A 5 kg sample of olives and 1L sample of oil were collected from each area.

Effect of olive seed presence: Two samples of 5 kg olives were collected from the Hebron area. One was pressed without removing the seeds while the other was pressed after removing the seeds.

Effect of pressing temperature: Pressing with the semi-manual press does not involve heating; hence, five 1-L samples of olive oil were collected from a press in Nablus from one batch for the same farmer and the press temperature was gradually increased to study the effect of press temperature.

Effect of olive leaves: Five samples of 5 kg olives were collected from the Ramallah area. Each sample was mixed with a different percentage of olive leaves (0.0, 1.25, 2.5, 5.0 and 10.0%).

Soil type: Two samples of 5 kg olives were collected from two different soil types (white lime soil and red clay) from the Hebron area.

Harvest time: Five 1 L olive oil samples were collected from the Salfeet area. The samples were harvested at different times starting at the beginning of October until the beginning of December.

Olive variety: Four samples of 5 kg olives were collected from the Jenin area. These samples represented different varieties of olives, namely 'Nabali', Modified 'Nabali', 'Manzanillo' and 'K18'.

Olive oil storage: A total of 30 samples of the same oil were collected from the Salfeet area; one sample was tested at zero storage time and the other 29 samples were stored in different material types and tested at 1, 2, 4 and 6.5 months of storage. The samples were stored as follows:

- Four samples stored in stainless steel containers
- Four samples stored in transparent glass bottles preserved in the light
- Four samples stored in transparent glass bottles preserved in the dark
- Four samples stored in transparent plastic bottles preserved in the light
- Four samples stored in tin plated metal drums
- Four samples stored in tin bags
- Four samples stored in ceramic pots
- One sample stored in transparent plastic bottles kept in the dark

This test ran from December 2007 to July 2008.

Methodology of oil extraction: Forty olive oil samples were collected from centrifugal olive presses on the West Bank. These presses follow the same procedures for extraction, i.e., leaf removal, washing, crushing, malaxation, solid/liquid separation and oil/water separation.

The other 36 olive samples were pressed using a small press designed by us. It consisted of a screw crusher driven by an electrical motor to crush the olive fruit. A small semi-manual mixer was used to mix the olive paste with warm water (malaxation). A manual hydraulic presser, which produced 4 t of pressure, was used to separate the liquid phase from the pomace. A small centrifuge, which provided a rotation of 6000 rpm, was used to separate the olive oil from the liquid phase.

Analytical test: D-7-stigmastenol levels (as% of total sterols) were analysed using capillary-column gas chromatography according to the IOOC method (International Olive Oil Council, 2001). The analysis was performed in the Royal Scientific Society in Jordan.

RESULTS AND DISCUSSION

Olive fly infection: Figure 1 shows the effect of olive fly infection on D-7-stigmastenol levels in olive oil. The sample from infected olive fruit contained 0.92% D-7-stigmastenol, while the sample from non-infected fruit contained much less (0.52%; almost 50% less). Several studies have reported that the degree of fly infection is negatively correlated to phenolic content in the resulting olive oil due to autoxidation (Mraicha *et al.*, 2010; Tarnendjari *et al*., 2009; Alberto *et al*., 2004). However, Gómez-Caravaca *et al*. (2008) reported that the phenolic fraction of olive oil depends on several parameters and that a clear correlation does not exist between the degree of fly infestation and phenolic content. Our apparently conflicting result can be explained by the relative oxidative stability of D-7-stigmastenol compared to other sterol components. In this study, total sterol content decreased (data not shown), while the D-7-stigmastenol fraction remained stable, which resulted in the high percentage (% of total sterols) of D-7-stigmastenol.

Topography: The effect of mountainous and level land topography on D-7-stigmastenol levels was compared. Topography had a slight effect on D-7-stigmastenol levels; these were lower in samples from level land than those from mountainous land, as shown in Fig. 2. This could be due to the lower water content of mountainous soil than level soil. This result was supported by Faci *et al.* (2002) and Stefanoudaki *et al.* (2001) who reported that oxidative stability and polyphenols are significantly higher in non-irrigated soils than in irrigated soils.

Olive storage before pressing: The results of the comparison between the two main containers (plastic bags and boxes) used for olive fruit storage are plotted in Fig. 3. Slightly higher D-7-stigmastenol levels were

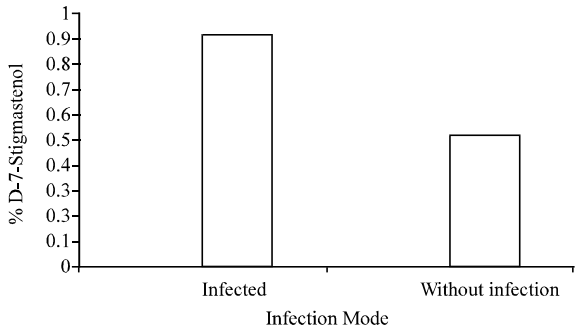

Fig. 1: Effect of olive fly infection on D-7-stigmastenol levels in olive oil

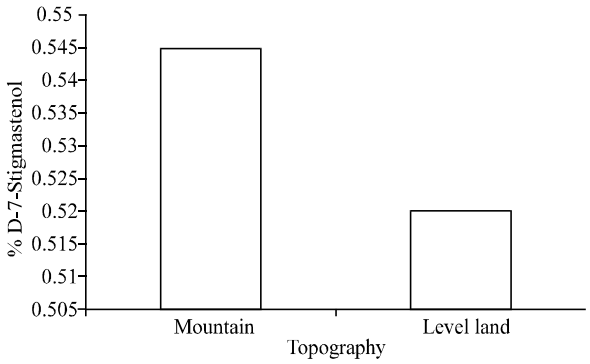

Fig. 2: Effect of topography on D-7-stigmastenol levels in olive oil

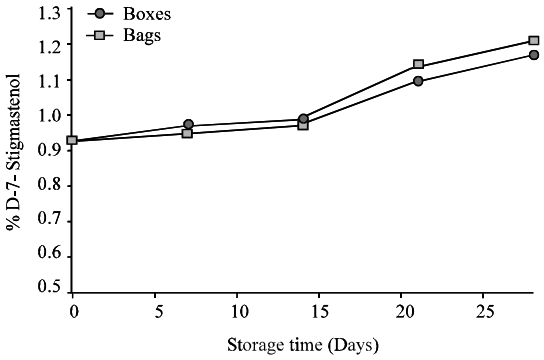

Fig. 3: Effect of olive storage material with time on D-7-stigmastenol levels in olive oil

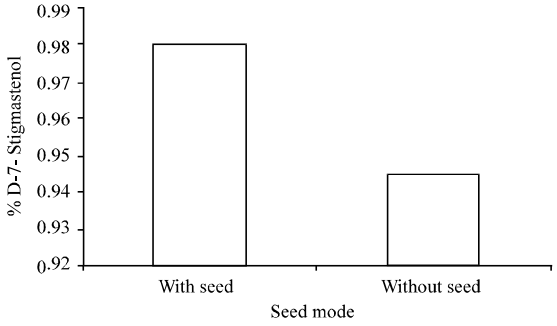

Fig. 4: Effect of olive seeds on D-7-stigmastenol levels in olive oil

obtained from samples stored in bags than from those stored in fruit storage boxes. D-7-stigmastenol levels increased with increasing storage time before pressing. In agreement with this result, Gutiérrez *et al.* (2000) reported that the total sterol content gradually increase with the olive storage time.

Geographical areas: Due to a wide spectrum of geographical areas in Palestine, from Hebron in the south to Jenin in the north, remarkable differences in D-7-stigmastenol levels were observed for olive fruit and olive oil that were subjected to the same treatments. Nine different provinces were selected. D-7-stigmastenol levels in samples from Salfeet, Ramallah and Hebron were higher than in those from other provinces. The Jericho samples had the lowest levels. The results are summarized in Table 1. A significant effect of geographical location on D-7-stigmastenol levels has also been reported by other studies (Carelli, 2008). This significant effect may be related to the different climatic conditions at each growing site, including rain fall, temperature and humidity.

Table 1: Relationship between D-7-stigmastenol levels and geographical areas

Geographic area	D-7-stigmastenol (%)
Jericho	0.48
Nablus	0.50
Jenin	0.54
Bethlehem	0.56
Tulkarem	0.61
Qalqilyan	0.64
Salfeet	0.90
Ramallah	0.96
Hebron	0.98

Olive seeds: Another factor that affects D-7-stigmastenol levels is the presence of seeds during pressing. Olive stones are comprised of a lignocellulosic material, with hemicellulose, cellulose and lignin as main components. The olive fruit can be structurally separated into the following three parts: (1) the skin or epicarp (1.0-3.0% of the drupe weight), which contains the chlorophyll, carotenoids and anthocyanins that account for the colour; (2) the pulp or flesh, called the mesocarp (70-80% of the whole fruit), the major part of the olive and (3) the stone, the woody endocarp (18-22% of the olive weight), which contains the seed (Rodriguez *et al.*, 2008).

The study involved comparison of two different processes usually performed in the pressing house, i.e., either pressing with seeds or pressing without seeds. The samples from pressing without seeds had slightly lower D-7-stigmastenol levels than the samples from pressing with seeds, as shown in Fig. 4. This may be because the D-7-stigmastenol concentration in the seeds is higher than in the fruit flesh. Conflicting results have been reported on the effect of fruit destoning on the phenolic profile of olive oil. Rodriguez *et al.* (2008) reported the results of a comparative analysis between olive oil and olive seed oil. The seed oil was found to be richer in individual sterols than olive oil, which is in agreement with the results of our study. In contrast, Luaces *et al.* (2007) reported an increase of approximately 25% in total phenolic compounds in oils obtained from destoned olive fruits. Guillaume *et al.* (2010) reported no significant differences in any of the sterols between the oils produced by crushing the entire fruit versus crushing the pitted olives.

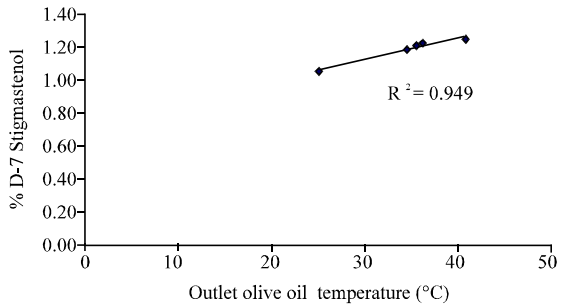

Fig. 5: Effect of pressing temperature on D-7-stigmastenol levels in olive oil

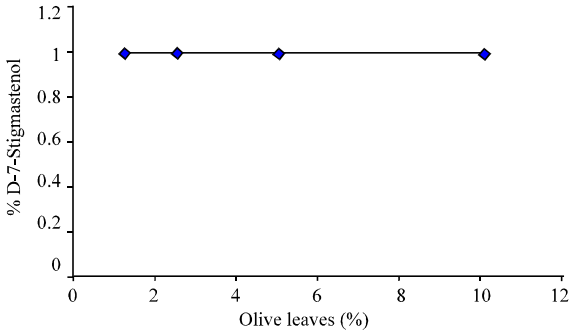

Fig. 6: Effect of olive leaves on D-7- stigmastenol levels in olive oil

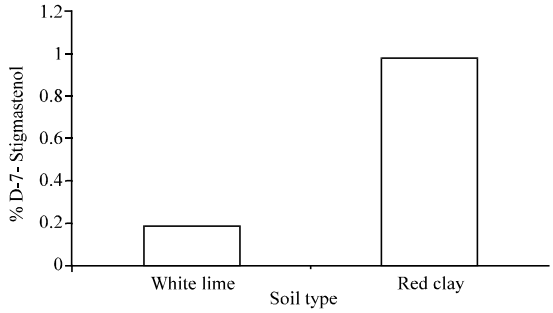

Fig. 7: Effect of soil type on D-7-stigmastenol levels in olive oil

Pressing temperature: The temperature used to extract oil affects D-7-stigmastenol levels. Different pressing temperatures were used to investigate this factor. Figure 5 shows that as pressing temperature increased, the D-7-stigmastenol levels slightly increased in a linear fashion. Ranalli *et al*. (2001**)** reported that the sterol levels in olive oil tended, in general, to increase as the kneading temperature increased. the sterol levels are significantly affected by malaxation temperature. The most affected sterols are stigmasterol and 5-avenosterol. In oils kneaded at high temperatures, stigmasterol level is high, whereas 5-avenasterol is significantly low. In contrast, Di Giovacchino *et al.* (2002) reported that variations in time and temperature of malaxation of olive paste do not significantly influence the composition of sterols in virgin olive oil.

Olive leaves: Figure 6 shows the effect of the presence of olive leaves during pressing. Three different concentrations of leaves were left in the olive samples for pressing. The results show that leaves had no significant effect on D-7-stigmastenol levels. This is in agreement with Di Giovacchino *et al.* (1996, 2002) who reported that the addition of leaves to olives does not affect the total polyphenols in the olive oil produced. This is because the concentration of the phenolic compounds (glycosides) in the leaves is similar to that in olive flesh. Leaf removal and olive washing are important operations for the mechanical safety of the olive extracting equipment, which operates at high speed and for the organoleptic quality of olive oil. Leaves crushed with olives give virgin olive oil a more green colour and the organoleptic sensation of 'green' or 'leaves' that may not be agreeable to consumers. However, the intensity of this sensation depends on the efficiency and roughness of the olive-crushing method and the comminution of leaves (Di Giovacchino *et al.*, 2002).

Soil type: Samples were collected from olive trees growing on white lime or red clay. The corresponding D-7-stigmastenol data are plotted in Fig. 7. D-7- stigmastenol level was higher in olive oil obtained from olives grown on red clay sand. This may be because white soil reflects the sun and heat and thus reduces the evaporation of the water from the soil, while the red soil does not.

Harvesting time: D-7-stigmastenol levels in olive oil samples collected on different fruit-harvesting dates were compared. Figure 8 shows that later harvesting dates resulted in lower D-7-stigmastenol levels. This is because the percentage of the non-saponifable portion of the olive oil that contains the sterols is high at the beginning of the harvesting season. With increasing maturity of olive fruits, some decomposition of these materials leads to a decrease in the percentage of non-saponifable matter, thus lowering the D-7-stigmastenol levels. This result is in agreement with the work of Tedeschini *et al.* (2003) and is supported by Lazzez *et al.* (2008), Salvador *et al.* (2001) and Andres *et al.* **(**2003), who found that the total sterols generally diminish slightly during ripening.

Olive varieties: As shown in Fig. 9. olive variety has an obvious effect on D-7-stigmastenol levels. Four olive varieties were included in this study. The oil from

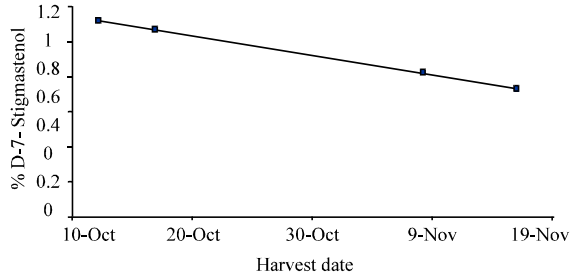

Fig. 8: Effect of harvest time on D-7-stigmastenol levels in olive oil

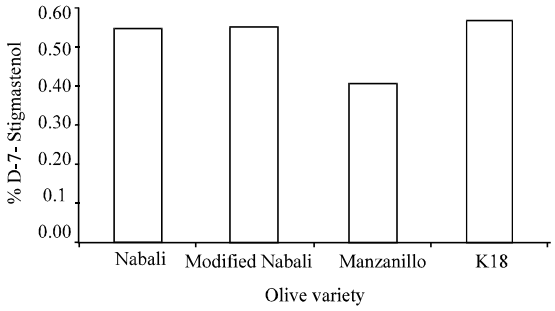

Fig. 9: Effect of olive variety on D-7-stigmastenol levels in olive oil

'Manzanillo' olive showed the lowest percentage (0.4%) of D-7-stigmastenol while other varieties had approximately 0.5% D-7-stigmastenol. This is because 'Manzanillo' fruits have a high moisture and low oil content compared to other varieties. At the same time, the percentage of non-saponifable matter in the 'Manzanillo' variety is less than that in other varieties. In comparison, the fruit of 'K18' variety is thinner with lower water content. This means that the olive seed (containing higher levels of sterols than the olive flesh) comprises a higher percentage of the fruit than in the other varieties; this may explain the slightly increased D-7-stigmastenol levels in the 'K18' variety. Several studies have reported a significant effect of variety on sterol levels in olive oil (Guillaume *et al.*, 2010; Carelli, 2008; Di Terlizzi *et al.*, 2007).

Olive oil storage: Six different storage materials (stainless steel, tin cans, tin bags, glass, plastic and ceramic) were compared. The samples were stored in good conditions in laboratory cupboard (dark). Two additional olive oil samples were placed in glass and plastic containers and stored on a shelf in the light. D-7-stigmastenol levels were measured before storage and after 1, 2, 4 and 6.5 months. The results are given in Table 2. In the second month, some samples showed an increase

Table 2: Effect of olive oil storage period and material on D-7-stigmastenol levels in olive oil

	D-7-Stigmastenol (%)				
	Olive oil storage period (months)				
Material type	0	1	2	4	6.5
Stainless Steel	1.095	1.095	1.095	1.095	1.095
Tin Plated Metal	1.095	1.095	1.095	1.100	1.135
Tin Bags	1.095	1.095	1.095	1.095	1.095
Glass in Dark	1.095	1.095	1.095	1.095	1.095
Glass in Light	1.095	1.1	1.1	1.15	1.20
Plastic in Dark	1.095	-	-	-	1.12
Plastic in Light	1.095	1.1	1.15	1.2	1.24
Ceramic	1.095	1.1	1.1	1.2	1.275

in D-7-stigmastenol levels, particularly the samples stored in glass and plastic in the light and ceramic. At the same time, D-7-stigmastenol levels were constant in the other samples. At the end of the storage period, D-7-stigmastenol levels had increased under the following storage conditions (arranged in descending order) ceramic, plastic in light, glass in light, tin-plated metal and plastic in dark. On the other hand, olive oil stored in stainless steel, tin bags and glass in the dark showed the same levels of D-7-stigmastenol throughout the experimental period (6.5 months).

These results could be due to oxidation of the sterol fraction. A number of factors affect the oxidative stability of olive oil, e.g., oxygen availability, oxygen permeability of the packaging material, storage temperature, exposure to light, degree of unsaturation of the constituent fatty acids, traces of metals, phenol content and pigment (Bendini *et al.*, 2009). These factors can explain our results because at least one of them [oxygen permeability of packaging material (for plastic and ceramics), exposure to light (for glass and plastic) and presence of metal traces (for tin cans)] affected the samples in which D-7-stigmastenol levels increased. The increase in D-7-stigmastenol levels under different storage conditions reflects the oxidation of sterols (data not shown) to different degrees (as the degree of oxidation increased, the total sterols decreased and the percentage of D-7-stigmastenol increased). These results show the relative oxidation stability of D-7-stigmastenol as discussed previously under the effect of olive fly infestation. Several studies that have reported the effect of storage period and conditions on sterol levels were in agreement with our results (Bendini *et al*., 2009; Guil-Guerrero and Urda-Romacho, 2009; Vekiari *et al*., 2007).

Based on the above results on the effects of these 11 factors on D-7-stigmastenol levels, the degree of the effect of each factor is summarized in Table 3, with the percentage effect of each factor.

Table 3: Factors affecting D-7-stigmastenol levels in olive oil according to their effect

Factor	Percentage effect
Soil type	28
Geographic area	18
Infection mode harvest time	14
Press temperature	7
Olive variety, oliv oil storage	6
Oilive storage before pressing	5
Topography, seed presence	1
Leaves	0

These results confirmed that Palestinian olive oil contains high levels of D-7-stigmastenol, exceeding the IOOC limit of 0.5%. This problem is faced by Palestine as well as many other countries. Some researchers have produced genuine extra virgin olive oils under controlled conditions (to avoid adulteration), which would be rejected on the basis of the current regulations (Codex Alimentarius Commission, 2007; Italian Ministry of Foreign Affairs, 2007; Mailer and Ayton, 2008). This study is important for oil producers and exporters of olive oil from Palestine and hopefully from other countries that are facing similar problems in selling genuine unadulterated extra virgin olive oil. It is expected that the results will be considered by the different parties responsible for setting international standards. Some producers in Australia and Syria are now blending high quality oil to meet the established standards (Italian Ministry of Foreign Affairs, 2007; Mailer and Ayton, 2008). As a result, genuine oils with exceptional organoleptic quality and oxidative stability are being blended with inferior oil to achieve compliance with inappropriate trade standards. International organisations need to continue to make changes to standards to allow the free flow of high quality olive oil products and prevent any barriers to trade.

CONCLUSION

The study confirmed that Palestinian olive oil contains high levels of D-7-stigmastenol that exceed the IOOC limit of 0.5%. In addition, D-7-stigmastenol levels are affected by different factors, in particular, soil type, geographical area, maturity index and olive fly infestation. Factors that have a moderate effect on D-7-stigmastenol are the pressing temperature, olive variety, olive oil storage conditions and olive storage before pressing. Olive seeds, topography and olive leaves do not have a significant effect on D-7-stigmastenol levels.

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