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ORIGINAL PAPER

Technological Factors Affecting Sterols in Australian Olive Oils

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Abstract Sterols are important lipids related to the quality of olive oil and broadly used for checking its genuineness. Recent analyses have identified that some Australian olive oils would not meet international standards for total content of sterols or for certain individual components. Several research works indicate that there are some significant correlations between cultural and processing practices and sterols content and composition. In this work the horticultural and processing practices that may have an impact on the sterol content and profile of the most important Australian varieties were analysed. The information generated with this study aims to solve a legislation problem as well as maximising the nutritional and health benefits of Australian olive oils. The evaluation was undertaken using three different varieties and the processing practices evaluated were: irrigation, fruit size, maturity, malaxing time, malaxing temperature and delays between harvest and process. The total content of sterols and their composition in olive oil is strongly influenced by genetic factors and year. Processing practices particularly affect triterpene dialcohols and stigmasterol while horticultural practices and fruit characteristics tend to affect more significantly other sterols such as β-sitosterol, sitostanol, Δ5-avenasterol and Δ7-avenasterol.

Keywords Fats · Oils

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Introduction

The Situation in Australia

It has been previously reported that sterol composition and total sterol content would be affected by cultivar, crop year, degree of fruit ripeness, storage time of fruits prior to oil extraction, processing and by geographical factors [1–5]. It has been found that a significant number of samples of largely cultivated varieties in Australia do not meet international standards as regards sterols. The Australian olive industry is currently targeting this problem. Research projects led by Dr Rod Mailer [6] comprehensively cover the area of variety and environment characterisation. This study complements this research by analysing the horticultural and processing practices that may have an impact on the sterol content and profile of the most important Australian varieties as well as generating biochemical and genetic information for a better understanding of the dynamics of sterols in olive oil. Recent analyses have identified that Australian olive oils have significant amount of sterols. Some Australian olive oils do not meet international standards for total content of sterols or for certain minor components [6]. The cultivar Barnea oils, in particular, contain up to 5.8% Campesterol, as confirmed by the Australian Government Analytical Laboratories (2004) [6]. Some cultivar Frantoio oil samples have shown extremely low total sterol levels, barely above or even below the minimum 1,000 ppm established as the international limit. It is extremely important to point out that Barnea oil represented 41% and Frantoio oil 26% of the olive oils produced in Australia in 2006 [7]. Exploratory research conducted by Modern Olives during the past 3 years [7] would indicate that there are some significant correlations between cultural and processing practices and sterol content and composition.

Objectives

This work complements previous research by analysing the horticultural and processing practices that may have an impact on the sterol content and profile of the most important Australian varieties, as well as generating biochemical and genetic information for a better understanding of the dynamics of sterols in olive oil. The information generated aims to solve a legislation issue, and also to maximise the nutritional and health value of the Australian olive oils.

By determining the influence of major horticultural and olive oil processing practices on total sterols and their composition in different olive varieties, growers and processors will be better prepared to plan the management and process of their fruit, minimising the amount of oil that does not meet international criteria, and maximising the nutritional value of their product.

Methodology

Horticultural and Processing Trials

The evaluation of horticultural and olive oil processing practices on total sterols and their composition was undertaken in commercial groves in Victoria. The selected groves are: Boort Estate (Boort, Victoria) and Boundary Bend Estate (Boundary Bend, Victoria).

The management and climatic conditions of each grove during the trial period was recorded. All groves had automatic weather stations, which enabled temperature, humidity, wind speed, radiation and rainfall to be recorded. Considering that most physiological aspects related to sterol formation and ripening processes in the fruit are related to one or more of those parameters, it is considered that the available information was appropriate for an adequate evaluation of the final results.

The trials were conducted following a proper statistical design. Fruit from three different varieties (Frantoio, Barnea and Picual) with clearly different sterol profiles were crushed. The significant differences between those varieties that justified their selection are well documented [6, 7].

Frantoio: average total sterol levels of 1,490 ppm and average campesterol of 3.05%.

Barnea: average total sterol levels of 1,700 ppm and average campesterol of 4.50%.

Picual: average total sterol levels of 1,500 ppm and average campesterol of 3.40%.

The fruit was processed in an experimental olive oil mill (Abencor®). The Abencor® bench top extraction system imitates the process used by the industry to extract olive oil. It consists of a hammer mill, a thermo-mixer and a centrifuge. The Abencor® system provides a fast and inexpensive means to obtain a sample of oil, operating in accordance with a well established method. The oil extraction efficiency index attained is close to the industrial efficiency to be achieved in an industrial plant for most varieties. The quantity of olives used ensures that the sample is fully representative. Oil obtained is usually enough to perform organoleptic and quality tests. The processing conditions were the standards for this extraction method apart from the variations applied while evaluating malaxing temperatures and malaxing times [8].

The horticultural and processing practices evaluated were: irrigation, fruit size, maturity, malaxing time, malaxing temperature, delays between harvest and process and storage time.

Irrigation: Kc (Crop Factor) of 0.74 during the oil accumulation period (January–April) (Normal treatment); Kc of 0.32 during the oil accumulation period (1/2 X) and Kc of 1.48 during the oil accumulation period (2 X).

Fruit size: fruit of the different varieties was classified with a table olive fruit grader into three standard sizes. For Barnea: small (<2.00 g), medium (2.00–3.00 g), large (>3.00 g). For Frantoio: small (<1.40 g), medium (1.40–2.00 g), large (>2.00 g). For Picual: small (<2.20 g), medium (2.20–3.20 g), large (>3.20 g).

Maturity: fruit from the three varieties was harvested at three different times, 2–3 weeks between harvests. Maturity was measured using the maturity index developed by the CIFA Alameda del Obispo, Spain [5]. The fruit from the early harvest typically showed a MI between 1.00 and 2.00, from the middle harvest between 2.50 and 3.50 and from the late harvest between 4.00 and 5.00.

Malaxing time: three malaxing times were utilised: 30 min (Standard), 15 min (1/2 X) and 60 min (2 X).

Malaxing temperature: three malaxing temperatures were utilised: 25 °C (Standard), 15 °C (Cold) and 35 °C (Hot).

Delays between harvest and process: three times between harvest and processing were applied: immediate processing (<12 h), medium processing (36–48 h) and delayed processing (72–84 h).

Storage time: all samples processed were analysed immediately after processing, 6 months later and 12 months later.

Three replicates of each treatment were processed. Each replicate typically consisted of two mixing units of 700 g of olive paste each.

Replicates of each treatment were processed during the 2007 and the 2008 seasons. All samples were evaluated by duplicate.

Analytical Methodology

The sterols analyses were conducted according to the official method IOC/T.20/No10/Rev. 1. [11]. The sterol

fraction was analysed by an Agilent Technology 6890N GC system, Agilent Technology 7683B series injector with a split inlet and flame ionisation detector managed by Agilent ChemStation. The analytical column was a DB-5 5% phenyl-methyl-siloxane stationary phase (30 m × 0.25 mm × 0.25 μm). The gas chromatographic conditions were as follows: inlet temperature: 280 °C; oven temperature 267 °C; detector temperature: 290 °C; split ratio: 30:1; amount injected 1:l. Hydrogen was used as the gas carrier at a flow rate of 1.2 ml/min. Sterols were quantified using 5α-cholestan-3β-ol as the internal standard.

The data subjected to a statistical analysis was assessed through an analysis of variance using the SAS version 8.02 (SAS Institute Inc., Cary, NC, USA). Separation of the means was obtained using the least square means test and significant differences were defined at $P \leq 0.05$. Every aspect was analysed separately. No interactions were evaluated in this project.

Additional Trials

In addition to the previously detailed and initially planned processing and growing parameters, Modern Olives Laboratory Services conducted further studies associated with the sterol composition of the oil extracted from different tissues of the fruit (Exocarp or skin; mesocarp or flesh and endocarp or pit/seed) and the sterol characteristics of oils produced from pitted olives in comparison with normal whole olives.

In the first case, fruit from the Barnea variety was carefully peeled with a sharp scalpel removing the skin and external (<1 mm) flesh layer and pitted. The skin, the crushed pit and the rest of the flesh components were weighed separately, dried at 100 °C until constant weight and then treated with solvent utilising the Soxhlet method in order to obtain the oil present.

In the case of the pitted olives, several large samples of Barnea fruit were pitted and processed through the Abencor® system in comparison with batches of entire fruit from the same variety. This trial was also conducted over 2 years.

Results and Discussion

Effect of Maturation Index on Sterol Composition

The evolution of sterols and triterpene dialcohols during maturation is presented in Table 1. β-sitosterol, sitostanol, Δ5-avenasterol and Δ7-avenasterol are significantly ($P < 0.001$) affected by maturity index. Among them sitostanol is the one most affected (F value of 65.2). β-Sitosterol decrease during ripening, while Δ5-avenasterol and Δ7-avenasterol significantly increase. This result agrees with other research [2]. Nonetheless, apparent

Table 1 Sterol and triterpene dialcohol concentrations (values as % total sterols) of oils processed from fruit with maturity index of <2.00, 2.00–4.00 and >4.00

	<2	2–4	>4	Std. Err.	F[a]	Significance
Cholesterol	0.18 a	0.13 b	0.12 b	0.014	2.055	0.130
24-Methylene cholesterol	0.17 b	0.22 a	0.24 a	0.012	3.375	0.038
Campesterol	3.91 b	3.92 b	4.03 a	0.071	0.300	0.740
Campestanol	0.17 b	0.17 b	0.20 a	0.007	1.930	0.150
Stigmasterol	0.75 b	0.77 b	0.83 a	0.020	1.478	0.230
Δ7-Campesterol	0.22 a	0.08 b	0.11 b	0.016	7.445	0.001
Δ7-Stigmastenol	0.37 a	0.31 c	0.34 b	0.017	1.116	0.330
Apparent β-sitosterol[b]	93.81 a	93.89 a	93.61 b	0.067	1.613	0.200
Δ5,23-Stigmastadienol	0.10 a	0.05 b	0.11 a	0.021	0.796	0.450
Clerosterol	0.91 b	0.97 a	1.00 a	0.019	2.146	0.120
β-Sitosterol	87.00 a	84.99 b	84.58 b	0.217	14.880	0.000
Sitostanol	0.95 a	0.58 b	0.56 b	0.023	65.190	0.000
Δ5-Avenasterol	4.43 b	6.83 a	6.78 a	0.221	16.560	0.000
Δ5,24-Stigmastadienol	0.42 c	0.47 b	0.57 a	0.024	3.701	0.028
Δ7-Avenasterol	0.40 b	0.55 a	0.53 a	0.016	10.700	0.000
Erythrodiol + Uvaol	1.16 a	1.02 b	0.92 c	0.028	6.424	0.002
Total Sterols (in ppm)	1,728.99 c	1,915.09 a	1,853.32 b	27.783	4.105	0.019

Mean sample size = 36. Means followed by the same Roman letter within each row do not present significant differences (Duncan's multiple range test $\alpha = 0.05$)

[a] F tests the effect of the maturity index

[b] Apparent β-sitosterol = Δ5,23-stigmastadienol + clerosterol + β-sitosterol + sitostanol + Δ5-avenasterol + Δ5,24-stigmastadienol

β-sitosterol and campesterol did not change significantly between ripening stages in disagreement with the same research [2].

Effect of Fruit Size on Sterol Composition

Campestanol, stigmasterol, β-sitosterol, sitostanol, Δ5-avenasterol, Δ7-avenasterol and erythrodiol and uvaol are significantly affected by fruit size. While β-sitosterol, sitostanol and erythrodiol + uvaol significantly decrease with fruit size, Δ5-avenasterol and Δ7-avenasterol increase (Table 2).

Effect of Irrigation on Sterol Composition

The analysis of the effect of irrigation on sterol and triterpene dialcohols concentrations is presented in Table 3. 24-Methylene cholesterol, stigmasterol, Δ7-stigmastenol, apparent β-sitosterol and Δ7-avenasterol are amongst the significantly affected compounds. It is noteworthy that while stigmasterol and Δ7-stigmastenol decrease with higher levels of irrigation, apparent β-sitosterol significantly increases.

Effect of Malaxing Time on Sterol Composition

The malaxing time at the paste preparation stage is a very important parameter of good manufacturing practice. As indicated in Table 4, erythrodiol + uvaol were the only components to be significantly affected ($P < 0.001$) by malaxing time. Stigmasterol and Δ7-stigmastenol also show to be affected but to lesser extent ($P < 0.04$). These components tend to increase with more malaxing time.

Effect of Malaxing Temperature on Sterol Composition

Similarly to malaxing time, processing temperature is another important parameter during the olive oil manufacturing process. Correspondingly, erythrodiol + uvaol were significantly affected ($P < 0.001$) by malaxing temperature and stigmasterol was one of the few sterols affected (P: 0.014) (Table 5). Once more, these components tend to increase with higher malaxing temperature. This is in agreement with other research work [2]. Additionally, the total level of sterols was significantly affected ($P < 0.001$) by this processing parameter showing increasing values at higher malaxing temperatures.

Effect of Delay Between Harvest and Process on Sterol Composition

Similar to the other processing parameters evaluated, the delay between harvest and processing significantly affected the percentage of erythrodiol + uvaol and stigmasterol ($P < 0.001$) (Table 6). Both erythrodiol + uvaol and

Table 2 Sterol and triterpene dialcohol concentrations (values as % total sterols) of oils processed from fruit of small, medium and large size within each variety

	Small	Medium	Large	Std. Err.	F[a]	Significance
Cholesterol	0.12 a	0.13 a	0.14 a	0.012	0.130	0.880
24-Methylene cholesterol	0.17 c	0.22 b	0.28 a	0.013	6.519	0.002
Campesterol	4.12 a	3.85 b	3.85 b	0.081	1.228	0.300
Campestanol	0.25 a	0.14 b	0.18 b	0.013	7.878	0.001
Stigmasterol	0.62 b	0.86 a	0.78 a	0.026	7.764	0.001
Δ7-Campesterol	0.23 a	0.26 a	0.19 a	0.031	0.343	0.710
Δ7-Stigmastenol	0.34 b	0.36 b	0.39 a	0.011	2.147	0.120
Apparent β-sitosterol[b]	93.66 a	93.83 a	93.48 a	0.087	1.326	0.270
Δ5,23-Stigmastadienol	0.03 a	0.02 a	0.03 a	0.004	1.454	0.240
Clerosterol	1.14 b	1.25 a	1.22 a	0.033	0.935	0.400
β-Sitosterol	86.88 a	85.66 b	83.52 c	0.226	28.680	0.000
Sitostanol	0.86 a	0.65 b	0.51 c	0.018	68.650	0.000
Δ5-Avenasterol	4.25 c	5.83 b	7.74 a	0.205	43.360	0.000
Δ5,24-Stigmastadienol	0.60 a	0.57 a	0.55 a	0.031	0.241	0.790
Δ7-Avenasterol	0.51 b	0.47 b	0.73 a	0.023	14.480	0.000
Erythrodiol + Uvaol	1.19 a	1.12 b	0.89 c	0.031	10.330	0.000
Total Sterols (in ppm)	1,998.16 a	2,002.29 a	1,947.13 b	23.947	0.544	0.580

Mean sample size = 36. Means followed by the same Roman letter within each row do not present significant differences (Duncan's multiple range test $\alpha = 0.05$)

[a] F tests the effect of the fruit size

[b] Apparent β-sitosterol = Δ5,23-stigmastadienol + clerosterol + β-sitosterol + sitostanol + Δ5-avenasterol + Δ5,24-stigmastadienol

Table 3 Sterol and triterpene dialcohol concentrations (values as % total sterols) of oils processed from fruit of receiving three different irrigation regimes: ½ X, X and 2 X

	½ X	X	2 X	Std. Err.	F[a]	Significance
Cholesterol	0.22 a	0.12 c	0.17 b	0.018	2.636	0.076
24-Methylene cholesterol	0.40 a	0.27 b	0.26 b	0.015	9.700	0.000
Campesterol	4.03 a	3.95 a	3.83 b	0.066	0.844	0.430
Campestanol	0.31 a	0.25 b	0.23 b	0.012	4.364	0.019
Stigmasterol	0.92 a	0.80 b	0.75 b	0.020	7.102	0.001
Δ7-Campesterol	0.15 b	0.24 a	0.16 b	0.018	2.810	0.065
Δ7-Stigmastenol	0.54 a	0.51 a	0.41 b	0.009	24.350	0.000
Apparent β-sitosterol[b]	92.85 c	93.42 b	93.73 a	0.079	13.200	0.000
Δ5,23-Stigmastadienol	0.16 a	0.08 b	0.09 b	0.018	2.084	0.130
Clerosterol	1.58 a	1.12 b	1.10 b	0.062	7.361	0.001
β-Sitosterol	83.96 c	85.29 a	84.69 b	0.203	3.737	0.027
Sitostanol	0.73 a	0.66 b	0.72 a	0.018	1.543	0.220
Δ5-Avenasterol	5.81 b	5.79 b	6.59 a	0.177	2.284	0.110
Δ5,24-Stigmastadienol	0.59 a	0.49 b	0.55 a	0.023	1.927	0.150
Δ7-Avenasterol	0.60 a	0.48 b	0.49 b	0.013	10.530	0.000
Erythrodiol + Uvaol	0.93 a	0.99 a	0.93 a	0.033	0.358	0.700
Total Sterols (in ppm)	1,933.71 a	1,851.10 b	1,992.07 a	29.511	1.954	0.150

Mean sample size = 36. Means followed by the same Roman letter within each row do not present significant differences (Duncan's multiple range test $\alpha = 0.05$)

[a] F tests the effect of the irrigation regime during oil accumulation

[b] Apparent β-sitosterol = Δ5,23-stigmastadienol + clerosterol + β-sitosterol + sitostanol + Δ5-avenasterol + Δ5,24-stigmastadienol

Table 4 Sterol and triterpene dialcohol concentrations (values as % total sterols) of oils processed at malaxing times of 15, 30 and 60 min

	15 min	30 min	60 min	Std. Err.	F[a]	Significance
Cholesterol	0.15 a	0.14 a	0.13 a	0.015	0.121	0.890
24-Methylene cholesterol	0.26 a	0.25 a	0.22 b	0.007	2.471	0.089
Campesterol	4.00 a	3.99 a	3.85 b	0.062	0.583	0.560
Campestanol	0.23 a	0.20 b	0.19 b	0.011	1.658	0.200
Stigmasterol	0.89 c	0.97 b	1.07 a	0.028	3.490	0.034
Δ7-Campesterol	0.13 a	0.13 a	0.09 b	0.012	1.082	0.340
Δ7-Stigmastenol	0.30 b	0.32 b	0.41 a	0.017	4.198	0.018
Apparent β-sitosterol[b]	93.58 a	93.55 a	93.60 a	0.057	0.008	0.920
Δ5,23-Stigmastadienol	0.17 a	0.14 b	0.13 b	0.023	0.239	0.820
Clerosterol	0.82 b	0.85 a	0.86 a	0.020	0.317	0.730
β-Sitosterol	85.23 a	84.84 b	84.67 b	0.206	0.633	0.530
Sitostanol	0.62 a	0.63 a	0.63 a	0.011	0.004	0.970
Δ5-Avenasterol	6.29 b	6.62 a	6.79 a	0.203	0.522	0.590
Δ5,24-Stigmastadienol	0.45 b	0.47 b	0.52 a	0.029	0.406	0.670
Δ7-Avenasterol	0.49 a	0.46 b	0.48 a	0.014	0.506	0.600
Erythrodiol + Uvaol	0.81 c	1.05 b	1.17 a	0.031	14.810	0.000
Total Sterols (in ppm)	1,710.33 b	1,813.10 a	1,794.17 a	24.160	1.732	0.180

Mean sample size = 36. Means followed by the same Roman letter within each row do not present significant differences (Duncan's multiple range test $\alpha = 0.05$)

[a] F tests the effect of the malaxing time

[b] Apparent β-sitosterol = Δ5,23-stigmastadienol + clerosterol + β-sitosterol + sitostanol + Δ5-avenasterol + Δ5,24-stigmastadienol

Table 5 Sterol and triterpene dialcohol concentrations (values as % total sterols) of oils processed at temperatures of 18, 28 and 38 °C

	18 °C	28 °C	38 °C	Std. Err.	F^a	Significance
Cholesterol	0.17 a	0.21 a	0.21 a	0.015	0.764	0.470
24-Methylene cholesterol	0.26 a	0.26 a	0.25 a	0.008	0.208	0.810
Campesterol	3.95 a	3.98 a	3.93 a	0.064	0.004	0.960
Campestanol	0.21 a	0.22 b	0.21 a	0.008	0.213	0.810
Stigmasterol	0.89 b	0.95 b	1.12 a	0.035	4.439	0.014
Δ7-Campesterol	0.13 b	0.09 b	0.18 a	0.013	3.798	0.026
Δ7-Stigmastenol	0.34 b	0.39 b	0.38 b	0.012	1.526	0.220
Apparent β-sitosterol[b]	93.59 a	93.35 a	93.22 a	0.066	2.706	0.071
Δ5,23-Stigmastadienol	0.23 a	0.24 a	0.28 a	0.034	0.174	0.840
Clerosterol	0.98 a	0.99 a	0.99 a	0.019	0.003	0.970
β-Sitosterol	84.21 a	84.05 a	84.33 a	0.191	0.179	0.840
Sitostanol	0.66 a	0.69 a	0.68 a	0.012	0.658	0.520
Δ5-Avenasterol	6.96 a	6.85 a	6.45 a	0.190	0.657	0.520
Δ5,24-Stigmastadienol	0.57 a	0.53 a	0.49 a	0.023	1.047	0.350
Δ7-Avenasterol	0.53 b	0.62 a	0.55 b	0.019	2.044	0.130
Erythrodiol + Uvaol	0.86 c	1.01 b	1.25 a	0.034	13.980	0.000
Total Sterols (in ppm)	1,669.97 c	1,806.86 b	1,924.26a	25.487	9.656	0.000

Mean sample size = 36. Means followed by the same Roman letter within each row do not present significant differences (Duncan's multiple range test $\alpha = 0.05$)

[a] F tests the effect of the malaxing temperature

[b] Apparent β-sitosterol = Δ5,23-stigmastadienol + clerosterol + β-sitosterol + sitostanol + Δ5-avenasterol + Δ5,24-stigmastadienol

stigmasterol levels increased with longer days between harvesting and processing. Campestanol was also significantly affected ($P < 0.001$), however it decreased with larger delays between harvesting and processing.

Effect of the Year on Sterol Composition

The variations of sterols and triterpene dialcohols between the 2 years are presented in Table 7. Most of these compounds are significantly affected by the season.

Effect of the Variety on Sterol Composition

The effect of the variety on sterol and triterpene dialcohols composition is presented in Table 8. It is important to point out that the variety has shown the most significant level of effect on the different sterols. This is in line with other authors [9, 10]. Only cholesterol and Δ7-campesterol had levels of significance higher than 0.001. Campesterol, β-sitosterol and Δ7-avenasterol were the most affected with F values of 3,125, 368 and 451 respectively. Erythrodiol + uvaol were not significantly affected by variety.

As it is indicated in Fig. 1, all processing practices had a significant impact on the concentrations of triterpene dialcohols and stigmasterol. In the particular case of the stigmasterol, these results support that the campesterol/stigmasterol ratio is an index of quality of an oil as proposed by other authors [2]. Nonetheless, this index could not be used to compare oils of different varieties as a consequence of the strong influence of genetics on campesterol content.

On the other hand, Fig. 2 shows that irrigation and fruit characteristics such as maturity and size have a significant effect on β-sitosterol, sitostanol, Δ5-avenasterol and Δ7-avenasterol. Consequently, the relationships between them could potentially be used to determine optimal harvesting times.

Finally, Fig. 3 clearly demonstrates the strong influence of the variety on sterol composition, particularly in the case of certain sterols such as campesterol, stigmasterol, β-sitosterol and total sterols. Based on this variety specificity, it is possible to include in the current legislation specific references to those varieties that do not normally comply with the authorised levels for the different sterols.

Sterol Composition in the Different Fruit Tissues

As it is indicated in Table 9, the vast majority of the oil (>75%) comes from the flesh with similar proportions of the remaining oil being contributed by the pit/seed and by the skin/outer layer of flesh. Significant differences were observed regarding certain sterols and associated substances. Stigmasterol and total sterols were significantly

Table 6 Sterol and triterpene dialcohol concentrations (values as % total sterols) of oils extracted from fruit within 12 h of harvesting, 48 h from harvesting and 120 h from harvesting

	<12 h	48 h	120 h	Std. Err.	F^a	Significance
Cholesterol	0.08 a	0.08 a	0.06 b	0.009	0.815	0.450
24-Methylene cholesterol	0.25 a	0.25 a	0.23 a	0.007	0.435	0.650
Campesterol	3.90 a	3.88 a	3.90 a	0.063	0.001	0.990
Campestanol	0.21 a	0.17 b	0.15 b	0.006	9.638	0.000
Stigmasterol	0.98 b	1.07 b	1.31 a	0.037	8.241	0.001
Δ7-Campesterol	0.07 b	0.07 b	0.14 a	0.011	5.816	0.004
Δ7-Stigmastenol	0.37 a	0.33 b	0.31 b	0.011	2.568	0.082
Apparent β-sitosterol[b]	93.56 a	93.63 a	93.42 b	0.045	1.934	0.150
Δ5,23-Stigmastadienol	0.21 a	0.25 a	0.12 b	0.025	2.517	0.086
Clerosterol	0.98 a	0.95 b	0.94 b	0.011	1.084	0.340
β-Sitosterol	84.25 a	84.66 a	84.80 a	0.220	0.552	0.580
Sitostanol	0.65 a	0.62 b	0.61 b	0.011	1.018	0.360
Δ5-Avenasterol	6.90 a	6.62 b	6.50 b	0.215	0.303	0.740
Δ5,24-Stigmastadienol	0.58 a	0.52 b	0.47 b	0.026	1.218	0.300
Δ7-Avenasterol	0.58 a	0.54 a	0.48 b	0.017	2.630	0.077
Erythrodiol + Uvaol	1.00 a	1.11 b	1.39 c	0.032	15.670	0.000
Total Sterols (in ppm)	1,817.20 a	1,802.15 a	1,776.41 a	18.0228	0.432	0.650

Mean sample size = 36. Means followed by the same Roman letter within each row do not present significant differences (Duncan's multiple range test $\alpha = 0.05$)

[a] F tests the effect of the delay between harvesting and processing

[b] Apparent β-sitosterol = Δ5,23-stigmastadienol + clerosterol + β-sitosterol + sitostanol + Δ5-avenasterol + Δ5,24-stigmastadienol

Table 7 Sterol and triterpene dialcohol concentrations (values as % total sterols) of oils processed from fruit in two different years

	2007	2008	Std. Err.	F^a	Significance
Cholesterol	0.21 a	0.07 b	0.006	126.500	0.000
24-Methylene cholesterol	0.23 b	0.25 a	0.004	2.974	0.085
Campesterol	3.98 a	3.90 a	0.027	1.617	0.200
Campestanol	0.22 a	0.16 b	0.003	57.680	0.000
Stigmasterol	1.09 a	0.84 b	0.013	72.520	0.000
Δ7-Campesterol	0.16 a	0.08 b	0.005	43.330	0.000
Δ7-Stigmastenol	0.29 b	0.41 a	0.006	91.050	0.000
Apparent β-sitosterol[b]	93.37 a	93.76 b	0.025	45.300	0.000
Δ5,23-Stigmastadienol	0.33 a	0.01 b	0.011	223.800	0.000
Clerosterol	0.93 b	0.95 a	0.007	0.961	0.330
β-Sitosterol	85.16 a	84.44 a	0.087	11.460	0.001
Sitostanol	0.64 b	0.67 a	0.006	2.838	0.093
Δ5-Avenasterol	5.94 b	7.06 a	0.085	30.970	0.000
Δ5,24-Stigmastadienol	0.38 b	0.63 a	0.010	120.100	0.000
Δ7-Avenasterol	0.49 b	0.54 a	0.007	8.120	0.005
Erythrodiol + Uvaol	0.95 a	1.18 a	0.013	62.640	0.000
Total Sterols (in ppm)	1,767.92 b	1,834.06 a	9.854	7.625	0.006

Mean sample size = 216. Means followed by the same Roman letter within each row do not present significant differences (Duncan's multiple range test $\alpha = 0.05$)

[a] F tests the effect of the year

[b] Apparent β-sitosterol = Δ5,23-stigmastadienol + clerosterol + β-sitosterol + sitostanol + Δ5-avenasterol + Δ5,24-stigmastadienol

Table 8 Sterol and triterpene dialcohol concentrations (values as % total sterols) of oils processed from fruit of three different varieties: Frantoio, Picual and Barnea

	Frantoio	Picual	Barnea	Std. Err.	F^a	Significance
Cholesterol	0.16 a	0.14 b	0.13 b	0.006	1.980	0.140
24-Methylene cholesterol	0.27 b	0.29 a	0.18 c	0.005	67.540	0.000
Campesterol	3.39 c	3.53 b	4.88 a	0.028	3125.000	0.000
Campestanol	0.23 a	0.21 b	0.17 c	0.004	16.760	0.000
Stigmasterol	0.92 b	1.07 a	0.73 c	0.013	76.050	0.000
Δ7-Campesterol	0.12 b	0.16 a	0.16 a	0.008	2.978	0.052
Δ7-Stigmastenol	0.40 a	0.39 a	0.34 b	0.006	9.235	0.000
Apparent β-sitosterol[b]	93.87 a	93.80 a	92.96 b	0.028	157.700	0.000
Δ5,23-Stigmastadienol	0.21 a	0.15 b	0.05 c	0.010	26.870	0.000
Clerosterol	1.08 a	1.11 a	0.92 b	0.014	18.050	0.000
β-Sitosterol	82.57 b	86.09 a	85.94 a	0.088	368.000	0.000
Sitostanol	0.69 a	0.62 b	0.70 a	0.007	16.120	0.000
Δ5-Avenasterol	8.61 a	5.47 b	4.93 c	0.083	451.000	0.000
Δ5,24-Stigmastadienol	0.75 a	0.39 b	0.42 b	0.011	174.600	0.000
Δ7-Avenasterol	0.66 a	0.44 c	0.48 b	0.007	127.100	0.000
Erythrodiol + Uvaol	1.08 a	1.01 b	1.05 a	0.013	2.037	0.130
Total Sterols (in ppm)	1,855.44 b	1,731.69 c	1,968.92 a	10.629	47.510	0.000

Mean sample size = 216. Means followed by the same Roman letter within each row do not present significant differences (Duncan's multiple range test $\alpha = 0.05$)

[a] F tests the effect of the variety

[b] Apparent β-sitosterol = Δ5,23-stigmastadienol + clerosterol + β-sitosterol + sitostanol + Δ5-avenasterol + Δ5,24-stigmastadienol

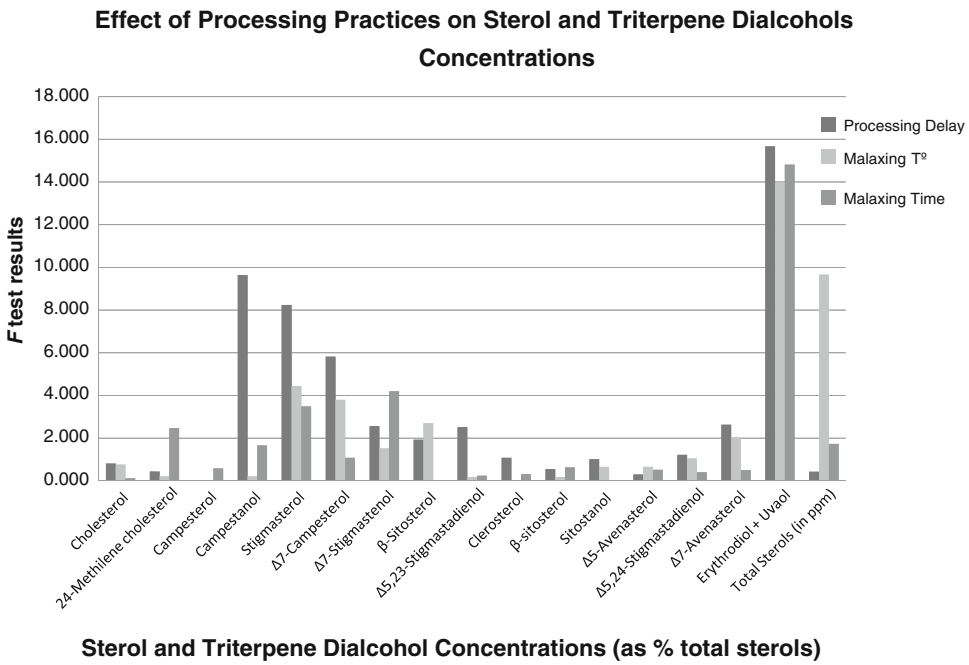

Fig. 1 Effect of processing practices on sterol and triterpene dialcohols concentrations

higher in the oil produced from the pit/seed fraction, while D7 Stigmastenol and Erythrodiol + Uvaol were particularly higher in the skin/outer flesh fraction (Table 10; Fig. 4). This difference can explain why the levels of those sterols tend to increase in the final oil produced when processing conditions deteriorate, particularly associated with higher malaxing times, temperatures or time delays between harvesting and crushing. The relatively constant proportion of Campesterol and β-Sitosterol would confirm that those two sterols could not be used as quality

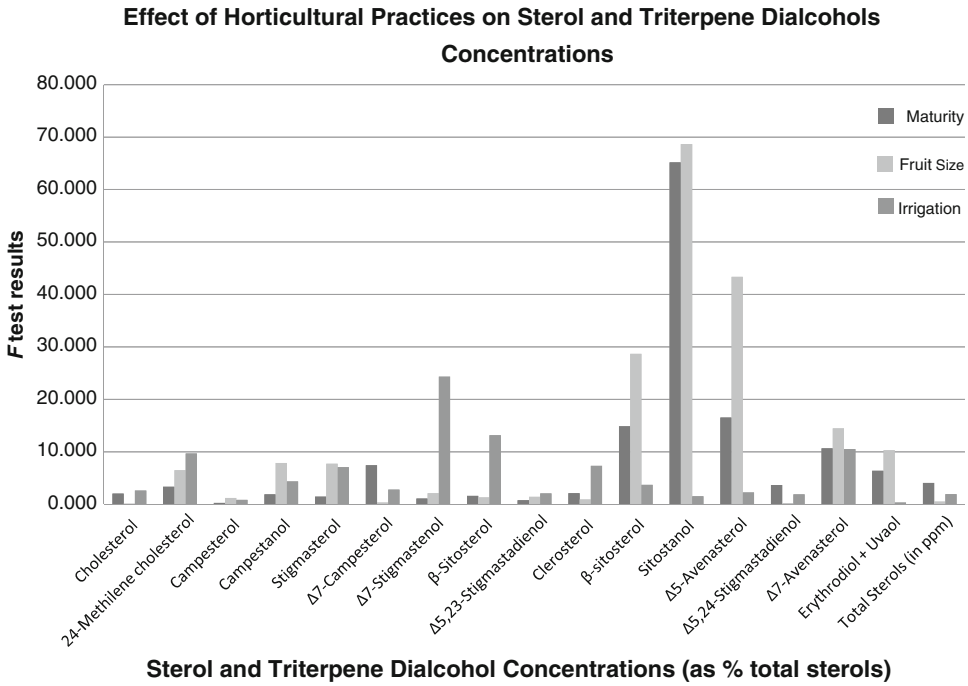

Fig. 2 Effect of horticultural practices on sterol and triterpene dialcohols concentrations

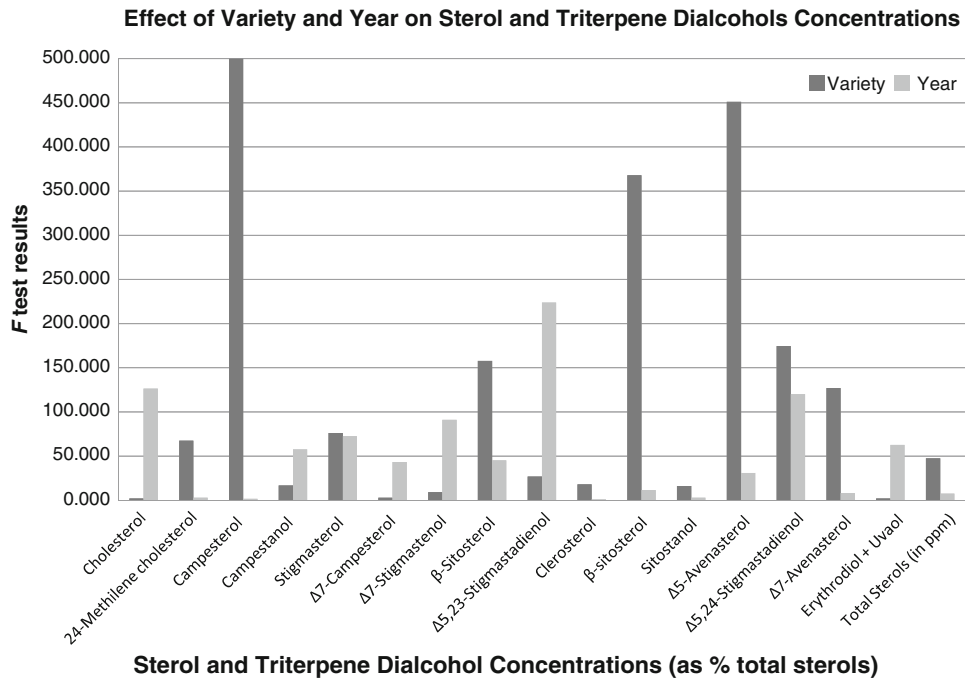

Fig. 3 Effect of variety and year on sterol and triterpene dialcohols concentrations

indicators and there is relatively little influence by processing conditions.

Sterol Composition from Pitted Versus Entire Fruit

No statistically significant differences were observed in any of the sterols between the oils produced by crushing the entire fruit (conventional method) versus crushing the pitted olives. While this commercial production technique may have an impact on other oil chemical parameters, it failed to produce significant changes in the sterol composition or in the total sterol levels of the final oils (Fig. 5).

Processing practices had a significant impact on the concentrations of triterpene dialcohols and stigmasterol. In the particular case of the stigmasterol, these results support that the campesterol/stigmasterol ratio is an index of quality of oil as proposed by other authors [2]. Nonetheless, this index could not be used to compare oils of

Table 9 Fruit composition (oil distribution)

	Weight (% of total)	Oil (% fresh content)	Oil (% of total)	Oil (% of origin)
Skin	7.7	33.5	2.6	12.3
Flesh	71.0	22.6	16.1	76.4
Pit/seed	21.3	11.2	2.4	11.4
Total	100.0		21.0	100.0

different varieties as a consequence of the strong influence of genetics on campesterol content. The sterol composition of the oils obtained from different fruit tissues support these conclusions as triterpene dialcohols and stigmasterol tend to be in significantly higher concentrations in the pit/seed and skin/outer flesh obtained oils.

On the other hand, irrigation and fruit characteristics such as maturity and size have a significant effect on β-sitosterol, sitostanol, Δ5-avenasterol and Δ7-avenasterol. Consequently, the relationships between them could potentially be used to determine optimal harvesting times.

Finally, it is clear that there is a very strong influence of the variety on sterol composition, particularly in the case of certain sterols such as campesterol, stigmasterol, β-sitosterol and total sterols.

Australian oils have shown good levels of total sterols and comparatively good campesterol/stigmasterol relationships, highlighting their healthy and high quality characteristics. Campesterol levels above most common international standards for certain Australian oils are strongly related to the combination between genetics and environment (phenotype) and they have no relationship with adulterations of any kind or with oil quality issues.

Table 10 Sterols profile of the oil obtained from different parts of the fruit

	Cholesterol (%)	Campesteroll (%)	Stigmasteroll (%)	D7 Stigmastenoll (%)	B Sitosteroll (%)	D5 Avenasteroll (%)	E + Ul (%)	Total sterols (ppm)
Skin	0.000	4.820	0.990	0.900	89.440	1.580	9.180	1,842.4
Flesh	0.000	5.140	0.740	0.160	89.770	1.750	0.630	2,596.5
Pit/seed	0.000	4.680	1.210	0.360	88.290	1.530	0.440	4,991.0
Total	0.000	5.020	0.856	0.261	89.441	1.691	1.286	3,024.450

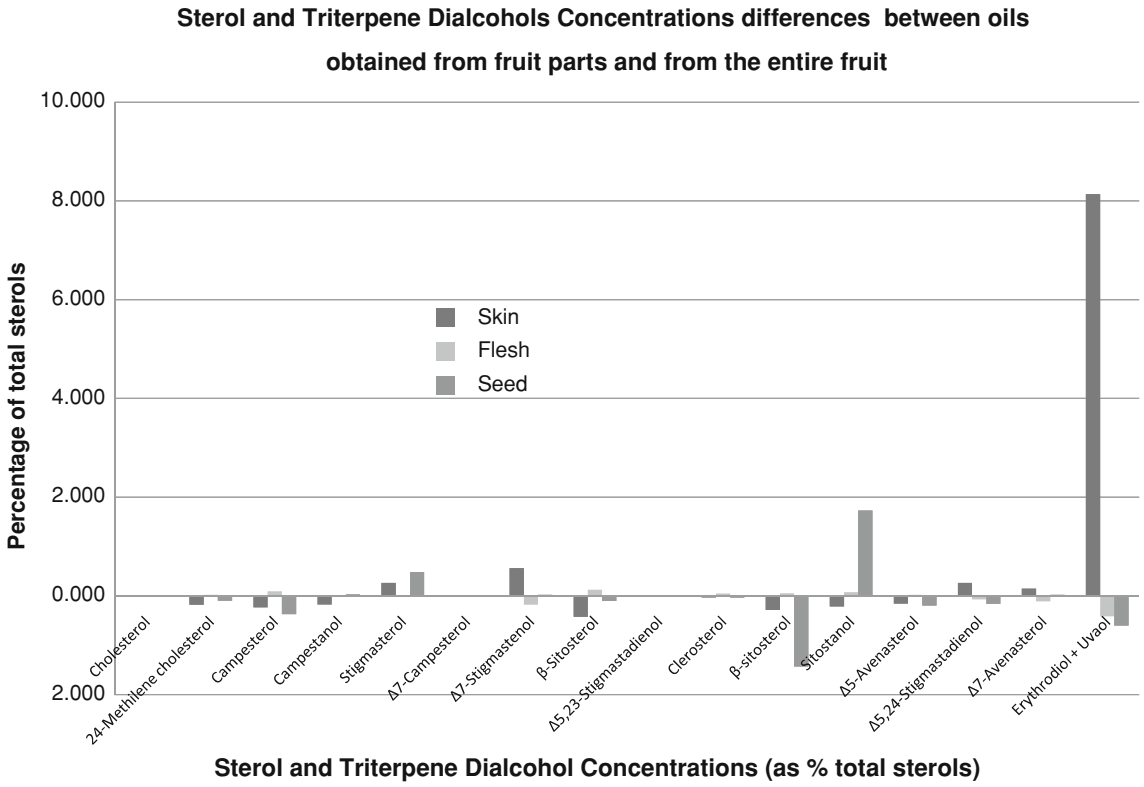

Fig. 4 Sterol and triterpene dialcohols concentrations differences between oils obtained from fruit parts and from the entire fruit

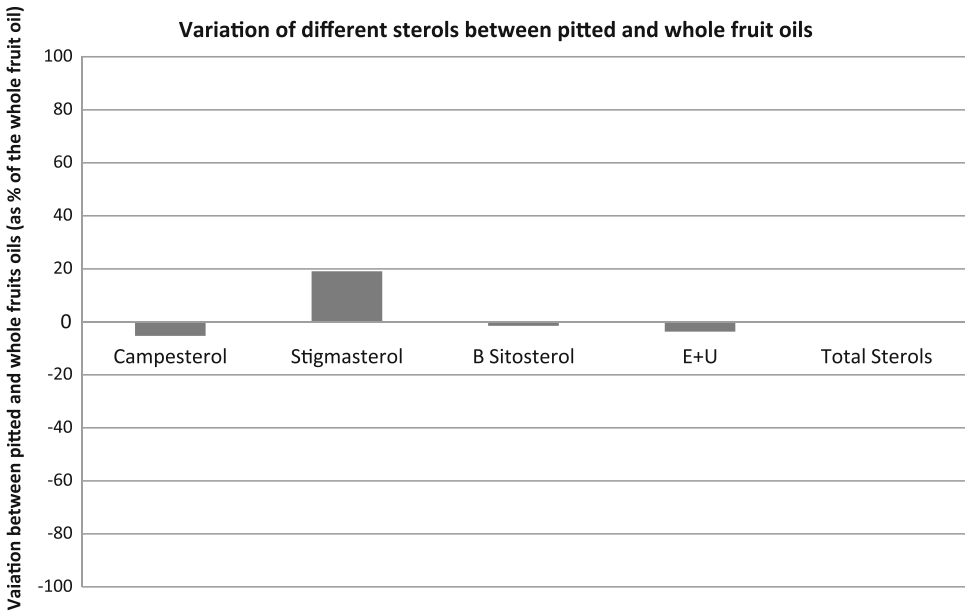

Fig. 5 Variation of different sterols between pitted and whole fruit oils

No evaluated management or processing practice seems to have contributed to reducing campesterol levels.

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