Determination of triterpenic acids in natural and alkaline-treated Greek table olives throughout the fermentation process

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Abstract

Maslinic and oleanolic acids are among the most abundant triterpenic acids found in olive fruits. Several studies have shown that both acids might be important bioactive compounds with multiple beneficial health effects for the consumers. Due to their potential importance, the aim of the present study was to assess whether processing for the production of table olives has an adverse effect on the availability of these acids in the final product. We have monitored and quantified maslinic and oleanolic acids throughout processing in Spanish-style and Greek-style preparations of the Conservolea variety that is particularly popular in Greece. Our findings clearly demonstrate that the fast de-bittering process with NaOH treatment in Spanish-style olives has a profound negative effect on the concentration of both acids. This decrease of concentration was more prominent regarding maslinic acid when compared to oleanolic acid. In contrast, the slow de-bittering during natural fermentation of Greek-style olives had no effect on the content of maslinic or oleanolic acid. To verify the broad applicability of our observation we have also looked into the Greek-style processing of the Kalamon variety. Our findings were consistent, since once again, natural fermentation did not influence the concentration of both acids.

KEYWORDS: Maslinic acid; Oleanolic acid; Table olives; Spanish-style; Greek-style

1. Introduction

For thousands of years, olive oil and table olives have been basic components of the Mediterranean diet. The two most important commercial types of table olives worldwide are the Spanish-style olives and the Greek-style olives (Gomez et al., 2006). Processing of Spanish-style green olives includes an initial step of alkaline treatment as a fast de-bittering procedure, followed by rinsing with water and fermentation in brine. Greek-style olives, on the other hand, are directly put in brine for natural fermentation, after having been first rinsed with water.

Table olives are known for their high nutritional value. They contain several nutritional components, which largely depend on the olive variety, the maturation stage of the olive fruit, the cultivating conditions and the processing method. Processed table olives are rich in vitamins, they contain reasonable amounts of minerals, while they are well-known sources of phenolic compounds, with the major ones being hydroxytyrosol and tyrosol (Kailis and Harris, 2007; Charoenprasert and Mitchell, 2012). Moreover, the consumption of table olives has been associated with a variety of health benefits which have sometimes been attributed to specific nutritional or bioactive components.

Pentacyclic triterpenes are found in abundance in a variety of plants (Jäger et al., 2009) and in the case of olive fruits represent 90-95% of their cuticle lipids (Bianchi et al., 1994). More specifically, olive fruits are remarkably rich in maslinic and oleanolic acids throughout their development (Stiti et al., 2007). These triterpenic acids are located in the epicarp of the olive fruit and they constitute the main substances of the surface waxes (Bianchi, 2003; Bianchi et al., 1994; Guinda et al., 2010). Triterpenic acids have been reported to have multiple biological effects both *in vitro* and *in vivo*. In the case of maslinic and oleanolic acids there are studies indicating their activities as anti-inflammatory, antioxidant (Ismaili et al., 2004; Liu, 1995; Tsai and Yin, 2008), antimicrobial (Horiuchi et al., 2007), antiviral (Parra et al., 2009), cardioprotective (Allouche et al., 2010), anti-hypertensive (Rodriguez-Rodriguez et al., 2006), anti-hyperlipidemic (Liu et al., 2007b), anti-diabetic (Liu et al., 2007a; Tang et al., 2008), and even anti-tumor (Hsum et al., 2011; Juan and Planas, 2010; Li et al., 2010; Reyers et al., 2006; Reyers-Zurita et al., 2009).

Based on the fact that there is a clear shift in the market for functional foods, the fate of maslinic and oleanolic acids during the preparation of table olives is of particular interest. In the present study, we report the influence of Spanish-style and Greek-style processing of table olives on the concentration of these triterpenic acids in the Conservolea variety. In order to validate our findings, we also examine the natural olives of the Kalamon variety. Our research also aims to shed further light on the differences between these two processing styles of table olives.

2. Materials and methods

2.1. Processing and sampling

For the Spanish-style green olives of the Conservolea variety, olive fruits (50 kg) with a green-yellow surface color were put into high density polyethylene (HDPE) barrels, covered with 30 L of 1.5% NaOH and maintained in the alkaline solution until the alkali penetrated 2/3 the way to the olive pit (approximately 12 h). Subsequently, olives were rinsed twice with tap water for 12 h and then covered with brine (8% NaCl and 0.3% lactic acid). Fermentation was performed under anaerobic conditions, using a tight barrel lid, for 2 to 3 months with addition, at regular time intervals, of NaCl until the concentration in the brine was slightly above 8%.

For the green olives of the Conservolea variety, olive fruits (50kg) with a green-yellow surface color were put into high density polyethylene (HDPE) barrels and covered with 30 L of acidified brine (3.5% NaCl and 0.15% lactic acid). Fermentation was performed under anaerobic conditions, using a tight barrel lid, for 6 to 8 months with addition, at regular time intervals, of NaCl until the concentration in the brine was slightly above 8%.

Finally, for the natural black olives of the Kalamon variety, olive fruits with a black surface color were processed in the same way as it is described above for the green Conservolea olives.

The samples were collected throughout the entire procedure at time points shown in Table 1. All samples were processed in the Agricultural Cooperative of Rovies and supplied to us through the Central Cooperative Union of Olives and Olive Oil Producers "ELEOURGIKI".

2.2. Extraction of triterpenic acids from the olive flesh

Extraction of triterpenic acids from the olive flesh was performed as described by Romero *et al*. (2010) with slight modifications. Ten grams of pitted olive fruits were finely chopped, dried at 105 °C until weight stabilization and pulverized. One gram of dry powder olives was mixed with 4 ml of methanol/ethanol (1:1, v/v) in a centrifuge tube, stirred for 1 min, centrifuged at 9500 rpm for 5 min at 20 °C and the solvent was separated from the solid phase. This procedure was repeated six times, and the solvent extracts were evaporated under vacuum. Subsequently, the residue was dissolved in 2 ml of methanol and centrifuged at 9500 rpm for 5 min at 20 °C. The supernatant was filtered through 0.2 μm pore size filter and an aliquot (20 μl) was used for HPLC analysis. The extraction was performed at least in triplicates for each sample.

2.3. HPLC analysis of triterpenic acids

Analysis was performed on an HPLC system consisting of a GBC LC 1150 HPLC pump and a GBC LC 1200 UV/Vis detector (GBC Scientific Equipment Pty Ltd, Dandenong, Victoria, Australia). A Spherisorb ODS-2 (5 μm, 25 × 46 mm i.d.; Waters Inc., Mildford, MA, USA) column was used. Elution was performed at 35 °C (Chemical Electronics 1270 Column Heater) with a mixture of methanol/water (92:8, v/v), acidified with phosphoric acid at pH 3.0, at a flow rate of 0.8 ml/min. The absorbance of the eluate was monitored at 210 nm. Maslinic and oleanolic acids were quantified using standard curves plotted with solutions of pure maslinic and oleanolic acid (Sigma, St. Louis, MO, USA) in a concentration range of 0-3000 mg/l.

2.4. Statistical analysis

Multiple sample comparison was performed with analysis of variance (ANOVA) for $p<0.05$ followed by the post-hoc Tukey's HSD (Honestly Significant Difference) test as those are implemented in STATGRAPHICS Centurion XV (StatPoint Technologies, Inc., Warrenton, Virginia, VA).

3. Results and discussion

The present study aims to determine the influence of Spanish-style and Greek-style preparations of table olives on two triterpenic acids, maslinic and oleanolic, for the first time throughout the entire process. The sampling schedule is indicated in Table 1. Olives were dried in order to avoid water interference during the extraction of the maslinic and oleanolic acids, which was performed with a mixture of methanol/ethanol (1:1, v/v). A good separation of peaks corresponding to the retention times of maslinic and oleanolic acids was achieved by the chromatographic system used (Figure 1).

We have initially investigated Conservolea olives prepared following the Spanish-style. The initial concentrations of maslinic and oleanolic acids in the raw fruit were 1230 ± 108 and 541 ± 44 mg/kg olive flesh, respectively (Figure 2). The concentrations in the final product (18 weeks of fermentation) were 552 ± 16 and 331 ± 33 mg/kg olive flesh, respectively, revealing a significant decrease in the concentration during processing (Tukey's test, $p<0.05$) for both triterpenic acids. Obviously, maslinic acid was affected more drastically than oleanolic acid by the alkaline procedure.

The reduction in the concentration of maslinic acid was observed in three phases (Figure 2). Initially, after the alkaline treatment for 12 h, maslinic acid was decreased by 17.8% compared to its concentration in the raw fruit. Subsequently, after the first rinse of olives with tap water for 12 h, the concentration of maslinic acid dropped further by 18.7%. Finally, at the end of the 2^{nd} week of the fermentation the total reduction on the concentration of maslinic acid was 55.1% compared to its concentration in the raw fruit. This decline can be attributed to the increase of the solubility of triterpenic acids at alkaline pH, as reported before (Romero *et al.*, 2010). The further decrease in the concentration of maslinic acid after the placement of the olives in the brine could also be related to the alkaline pH of the brine in the first days of the fermentation. Campaniello et al. (2005) has reported that the pH value increased during the first days of fermentation in Spanish style olives, both in brines and olives, as a consequence of the addition of lye. The concentration of maslinic acid remained stable after the end of the 2nd week and throughout fermentation, clearly showing that this process did not affect the concentration of the maslinic acid any further (Tukey's test, $p<0.05$).

In the case of oleanolic acid the reduction did not follow the same trend as in maslinic acid (Figure 2). The concentration of the oleanolic acid was not significantly affected during the alkaline treatment and it remained practically constant even after the 2nd rinse. However, its concentration was significantly reduced after placing the olives in the brine. At the end of the 2nd week of the fermentation the concentration of oleanolic acid had been decreased by 38.8% compared to its concentration in the raw fruit (Tukey's test, $p<0.05$). Thereafter, its concentration was constant for the remaining of the fermentation process (Tukey's test, $p<0.05$).

Regarding the natural olive samples of the Conservolea variety (Figure 3), the concentrations of maslinic and oleanolic acids in the raw fruit were the same as mentioned above, while those of the fermented final product (40 weeks of fermentation), were 1349 ± 123 and 536 ± 82 mg/kg olive flesh, respectively. During the fermentation process, the concentration of maslinic acid ranged from 1420 to 1160 mg/kg olive flesh and that of oleanolic acid from 652 to 501 mg/kg olive flesh. Statistical analysis however revealed that these variations were not significant, supporting that the concentration of the two triterpenic acids were stable and were not affected by the fermentation process (Tukey's test, $p<0.05$).

In order to verify the neutral effect of Greek-style processing on the concentration of maslinic or oleanolic acid, as observed with the Conservolea variety, we have also examined the natural fermentation of the Kalamon variety (Figure 4). The concentration of maslinic and oleanolic acids in the raw fruits were 1320 ± 256 and 794 ± 228 mg/kg olive flesh, respectively, while those in the fermented final product (32 weeks of fermentation) were 1260 ± 58 and 706 ± 48 mg/kg olive flesh, respectively. Once more, the concentration of the two triterpenic acids was not significantly affected during the entire fermentation process (Tukey's test, $p<0.05$). Our findings clearly support that the content of the final product in maslinic and oleanolic acids is not affected when the procedure used for the de-bittering of the olives does not include alkaline treatment.

Both maslinic and oleanolic acid have been identified in virgin olive oil (Allouche et al., 2009; Pérez-Camino and Cert, 1999) as well as in crude pomace olive oil (Pérez-Camino and Cert, 1999) and in olive by-products (Fernández-Bolaños et al., 2006). The concentration of maslinic and oleanolic acids in virgin olive oil is low. Allouche et al. (2009) studied virgin olive oils of forty cultivars, inter alia, for their triterpenic acid content. The values of maslinic and oleanolic acid obtained ranged from 3.93 to 49.81 mg/kg and 3.39 to 78.83 mg/kg, respectively. On the contrary, the content of table olives in these two triterpenic acids has been found to be higher (Romero et al., 2010) than that reported for olive oil. This is verified by our results as well. Therefore table olives are an appealing food vehicle for the delivery of these substances. Romero et al. (2010) have also found high quantities of maslinic and oleanolic acids in the solution used for the alkaline treatment and in the water used for the first rinse, indicating loss of these two triterpenic acids from the fruit during these washes. Our findings concerning the effect of lye used in Spanish-style preparations of olives on the concentration of maslinic acid are in accordance with these results. However, the concentration of oleanolic acid was reduced only after the lye treatment and the initiation of fermentation for two weeks. The different kinetics followed by maslinic and oleanolic acids during Spanish-style preparations deserves further investigation.

This research establishes that the natural processing of table olives does not significantly affect the triterpenic acid content of the final product. These observations, combined with the bioactivity properties attributed to the triterpenic acids, render the table olives, especially those that have not undergone alkaline treatment, an excellent natural source of maslinic and oleanolic acids and potentially, an important functional food.

4. Conclusions

The results obtained in this study indicate that two of the most economically important Greek cultivars of table olives, namely Conservolea and Kalamon (Garrido Fernández et al., 1997), are food products especially rich in maslinic and secondarily in oleanolic acids that are known to possess significant bioactive properties. However, their concentrations in table olives vary depending on the process used to produce the final product. Importantly, the fermentation process did not seem to significantly affect either of the triterpenic acids. In contrast, the alkaline treatment used to de-bitter the fruits was found to lead to a substantial decrease of both acids in the final product. This may be an essential consideration for the producers of table olives.

Acknowledgements

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References

Allouche, Y., Beltrαn, G., Gaforio, J. J., Uceda, M., & Mesa, M. D. (2010). Antioxidant and antiatherogenic activities of pentacyclic triterpenic diols and acids. *Food and Chemical Toxicology*, *48*, 2885–2890.

Allouche, Y., Jiménez, A., Uceda, M., Aguilera, M. P., Gaforio, J. J., & Beltrán, G. (2009). Triterpenic Content and Chemometric Analysis of Virgin Olive Oils from Forty Olive Cultivars. *Journal of Agricultural and Food Chemistry*, *57,* 3604-3610.

Bianchi, G. (2003). Lipids and phenols in table olives. *European Journal of Lipid Science and Technology*, *105*, 229–242.

Bianchi, G., Pozzi, N., & Vlahov, G. (1994). Pentacyclic Triterpene Acids in Olives. *Phytochemistry*, *37*(1), 205–207.

Campaniello, D., Bevilacqua, A., D'Amato, D., Corbo, M. R., Altieri, C., & Sinigaglia, M. (2005). Microbial Characterization of Table Olives Processed According to Spanish and Natural Styles. *Food Technology and Biotechnology*, *43*(3) 289-294.

Charoenprasert, S., & Mitchell, A. (2012). Factors Influencing Phenolic Compounds in Table Olives (*Olea europaea*). *Journal of Agricultural Food and Chemistry*, *60*, 7081–7095.

Fernández-Bolaños, J., Rodríguez, G., Rodríguez, R., Guillén, R., & Jiménez, A. (2006). Extraction of interesting organic compounds from olive oil waste. *Grasas Y Aceites*, *57*(1), 95-106.

Garrido Fernández, A., Fernández Díez, M. J., & Adams, M. R. (1997). *Table Olives: Production and Processing*. (1st ed.). United Kingdom: Chapman & Hall, (Chapter 3)

Gomez, A. H. S., Garcia, P. G., & Navarro, L. R. (2006). Trends in table olive production - Elaboration of table olives. *Gracas y Aceites*, *57*, 86–94.

Guinda, Á., Rada, M., Delgado, T., Gutiérrez-Adánez, P., & Castellano, J. M. (2010). Pentacyclic Triterpenoids from Olive Fruit and Leaf. *Journal of Agriculture of Food Chemistry*, *58*, 9685–9691.

Horiuchi, K., Shiota, S., Hatano, T., Yoshida, T., Kuroda, T., & Tsuchiya, T. (2007). Antimicrobial activity of oleanolic acid from *Salvia officinalis* and related compounds on vancomycin-resistant Enterococci (VRE). *Biological and Pharmaceutical Bulletin*, *30*(6), 1147–1149.

Hsum, Y. W., Yew, W. T., Hong, P. L. V., Soo, K. K., Hoon, L. S., Chieng, Y. C, & Mooi, L. Y. (2011). Cancer Chemopreventive Activity of Maslinic Acid: Suppression of COX-2 Expression and Inhibition of NF-κB and AP-1 Activation in Raji Cells. *Planta Medica*, *77*, 152–157.

Ismaili, H., Milella, L., Fkih-Tetouani, S., Ilidrissi, A., Camporese, A., Sosa, S., Altinier, G., Della Loggia, R., & Aquino, R. (2004). In vivo topical anti-inflammatory and in vitro antioxidant activities of two extracts of *Thymus satureioides* leaves. *Journal of Ethnopharmacology*, *91*, 31–36.

Jäger, S., Trojan, H., Kopp, T., Laszczyk, M. N., & Scheffler, A. (2009). Pentacyclic Triterpene Distribution in Various Plants – Rich Sources for a New Group of Multi-Potent Plant Extracts. *Molecules*, *14,* 2016–2031.

Juan, M. E., & Planas, J. M. (2010). Effects of Pentacyclic Triterpenes from olives on Colon Cancer. In R. R. Watson, & V. R. Preedy (Eds.), *Bioactive Foods and Extracts: Cancer Treatment and Prevention* (pp. 403-413). New York: CRC Press.

Kailis, S., & Harris, D. (2007). *Producing Table Olives*. Australia: Landlinks Press, (Chapter 2).

Li, C., Yang, Z., Zhai, C., Qiu, W., Li, D., Yi, Z., Wang, L., Tang, J., Qian, M., Luo, J., & Liu, M. (2010). Maslinic acid potentiates the anti-tumor activity of tumor necrosis factor a by inhibiting NF-kB signaling pathway. *Molecular Cancer*, *9*(73), 1–13.

Liu, J. (1995). Pharmacology of oleanolic acid and ursolic acid. *Journal of Ethnopharmacology*, *49*, 57–68.

Liu, J., Sun, H., Duan, W., Mu, D., & Zhang, L. (2007a). Maslinic Acid Reduces Blood Glucose in KK-Ay Mice. *Biological and Pharmaceutical Bulletin*, *30*(11), 2075–2078.

Liu, J., Sun, H., Wang, X., Mu, D., Liao, H., & Zhang, L. (2007b). Effects of oleanolic acid and maslinic acid on hyperlipidemia. *Drug Development Research*, *68*, 261–266.

Parra, A., Rivas, F., Lopez, P. E., Garcia-Granados, A., Martinez, A., Albericio, F., Marquez, N., & Muñoz, E. (2009). Solution- and solid-phase synthesis and anti-HIV activity of maslinic acid derivatives containing amino acids and peptides. *Bioorganic & Medicinal Chemistry*, *17*, 1139–1145.

Pérez-Camino, M. C., & Cert, A. (1999). Quantitative Determination of Hydroxy Pentacyclic Triterpene Acids in Vegetable Oils. *Journal of Agricultural and Food Chemistry*, *47,* 1558–1562.

Reyes, F. J., Centelles, J. J., Lupianez, J. A., & Cascante, M. (2006). (2Alpha, 3beta)-2, 3-dihydroxyolean-12-en-28-oic acid, a new natural triterpene from Olea europea, induces caspase dependent apoptosis selectively in colon adenocarcinoma cells. *FEBS Letters*, *580*, 6302–6310.

Reyes-Zurita, F. J., Rufino-Palomares, E. E., Lupianez, J. A., & Cascante, M. (2009). Maslinic acid, a natural triterpene from Olea europaea L., induces apoptosis in HT29 human coloncancer cells via the mitochondrial apoptotic pathway. *Cancer Letters*, *273*, 44–54.

Rodriguez-Rodriguez, R., Perona, J. S., Herrera, M. D., & Ruiz-Gutierrez, V. (2006). Triterpenic compounds from "orujo" olive oil elicit vasorelaxation in aorta from spontaneously hypertensive rats. *Journal of Agricultural and Food Chemistry*, *54*, 2096-2102.

Romero, C., García, A., Medina, E., Ruíz-Méndez, Mª V., de Castro, A., & Brenes, M. (2010). Triterpenic acids in table olives. *Food Chemistry*, *118*, 670-674.

Stiti, N., Triki, S., & Hartmann, M.-A. (2007). Formation of triterpenoids throughout *Olea europaea* fruit ontogeny. *Lipids*, *42*, 55–67.

Tang, X.-Z., Guan, T., Qian, Y.-S., Li, Y.-M., Sun, H.-B., Huang, J.-H., & Zhang, Y. (2008). Effects of Maslinic Acid as a Novel Glycogen Phosphorylase Inhibitor on Blood Glucose and Hepatic Glycogen in Mice. *Chinese Journal of Natural Medicines*, *6*, 0053-0056.

Tsai, S. J., & Yin, M. C. (2008). Antioxidative and anti-inflammatory protection of oleanolic acid and ursolic acid in PC12 cells. *Journal of Food Science*, *73*, 174-178.

Figure captions

Figure 1. HPLC chromatogram at 210 nm of the triterpenic acid extract of natural fermented green Conservolea olives sample (20th week of fermentation). Peaks: (1) maslinic acid and (2) oleanolic acid.

Figure 2. Concentration of maslinic and oleanolic acids of alkaline processed Conservolea olives during fermentation. All samples were analyzed at least in triplicates. Bars indicate the standard deviation. Differences in the concentrations of maslinic or oleanolic acids were assessed separately for $p<0.05$ followed by Tukey's test. Concentrations that do not differ significantly are denoted with the same letter.

Figure 3. Concentration of maslinic and oleanolic acids of natural processed Conservolea olives during fermentation. All samples were analyzed at least in triplicates. Bars indicate the standard deviation. Differences in the concentrations of maslinic or oleanolic acids were assessed separately for $p<0.05$ followed by Tukey's test. No differences were observed in the concentration of either triterpenic acid throughout the fermentation process.

Figure 4. Concentration of maslinic and oleanolic acids of natural processed Kalamon olives during fermentation. All samples were analyzed in triplicates. Bars indicate the standard deviation. Differences in the concentrations of maslinic or oleanolic acids were assessed separately for $p<0.05$ followed by Tukey's test. No differences were observed in the concentration of either triterpenic acid throughout the fermentation process.

Table 1. Sampling points during the fermentation process of table olives

Sampling points during fermentation process	Variety and processing method		
	Consevolea Green Alkaline processing	**Conservolea Green Natural processing**	**Kalamon Black Natural processing**
Raw fruit	√	√	√
After 12h in NaOH	√		
After 12h in water (1st wash)	√		
After 12h in water (2nd wash)	√		
Fermentation in brine			
2nd week	√	√	√
4th week	√	√	√
6th week	√	√	√
8th week	√	√	√
10th week	√		
12th week		√	√
14th week	√		
16th week		√	√
18th week	√		
20th week		√	√
24th week		√	√
28th week		√	
32nd week		√	√
40th week		√	

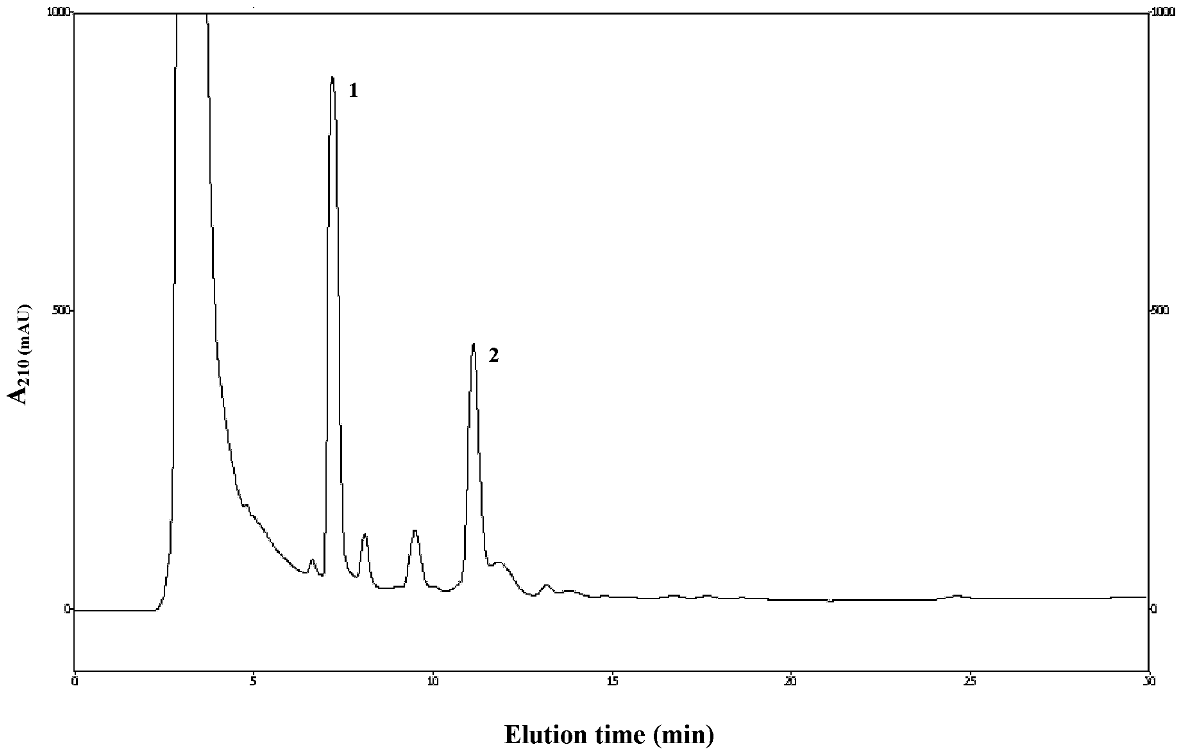
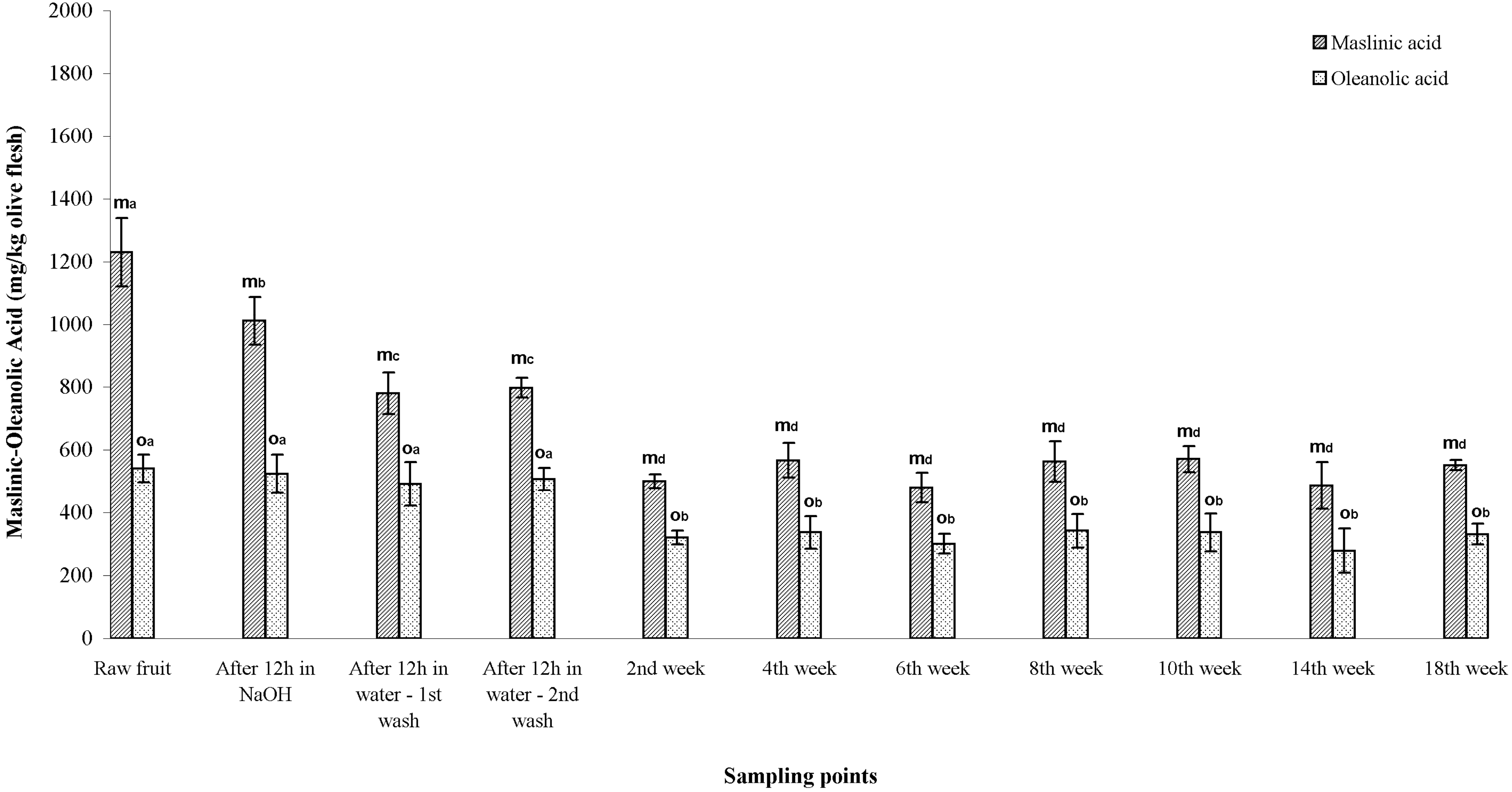
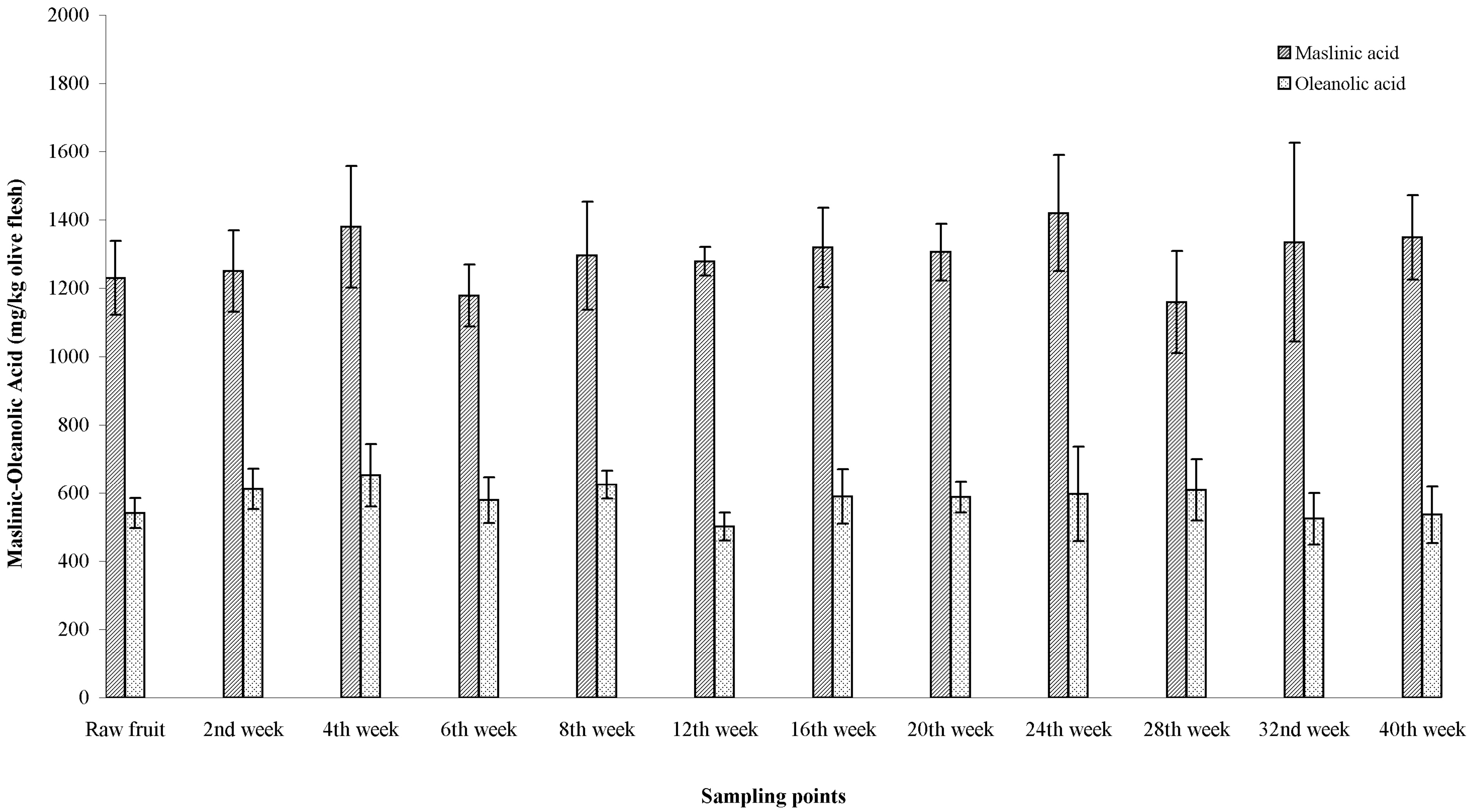


Figure 1



Maslinic acid
Oleanolic acid
Maslinic-Oleanolic Acid (mg/kg olive flesh)
0
200
400
600
800
1000
1200
1400
1600
1800
2000
ma
oa
mb
oa
mc
oa
mc
oa
md
ob
md
ob
md
ob
md
ob
md
ob
md
ob
md
ob
Raw fruit
After 12h in NaOH
After 12h in water - 1st wash
After 12h in water - 2nd wash
2nd week
4th week
6th week
8th week
10th week
14th week
18th week
Sampling points

Figure 2



Maslinic acid
Oleanolic acid
Maslinic-Oleanolic Acid (mg/kg olive flesh)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Raw fruit
2nd week
4th week
6th week
8th week
12th week
16th week
20th week
24th week
28th week
32nd week
40th week
Sampling points

Figure 3

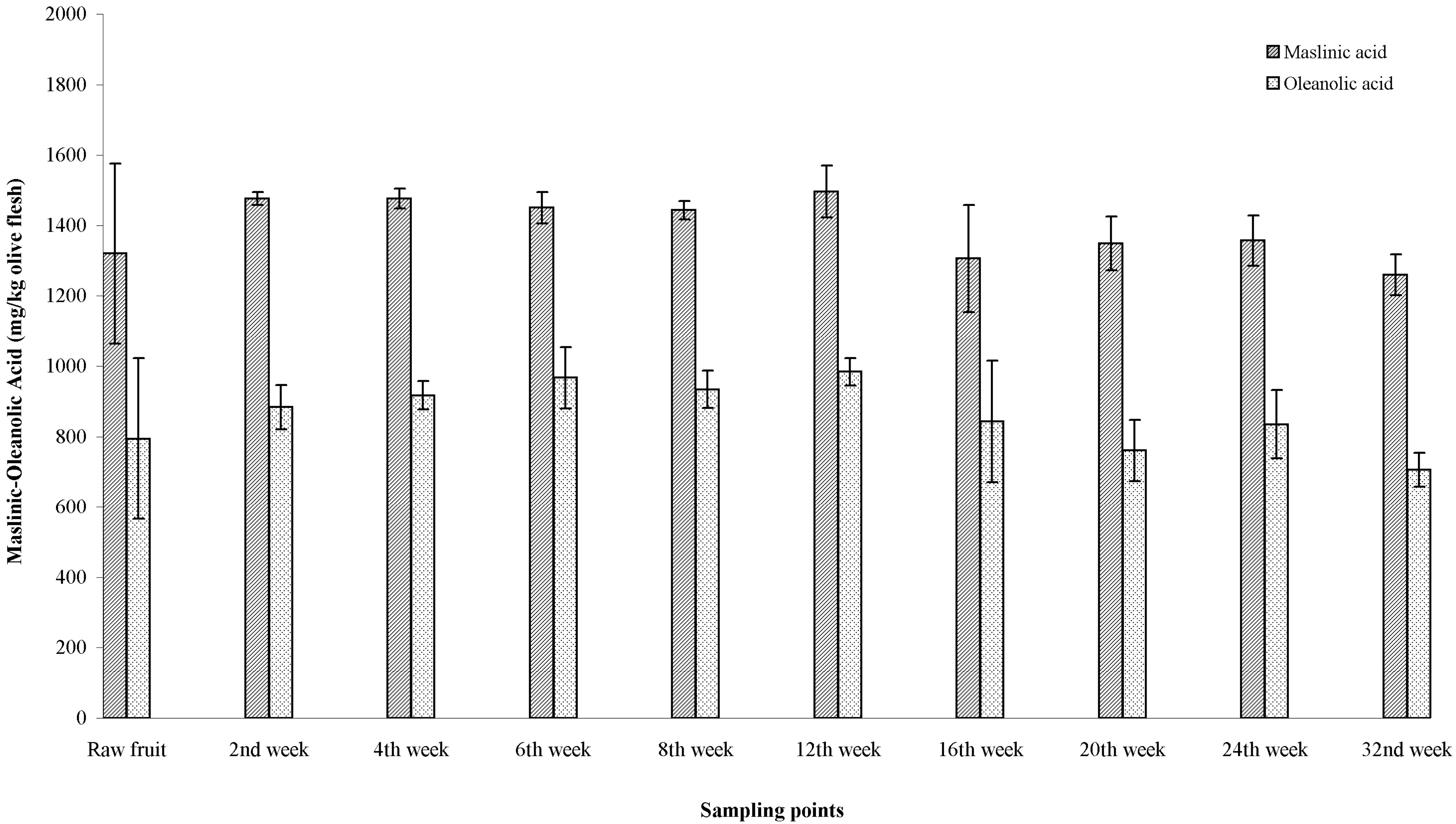

2000
1800
1600
1400
1200
1000
800
600
400
200
0
Maslinic-Oleanolic Acid (mg/kg olive flesh)
Maslinic acid
Oleanolic acid
Raw fruit
2nd week
4th week
6th week
8th week
12th week
16th week
20th week
24th week
32nd week
Sampling points

Figure 4