ELSEVIER

Food Chemistry 67 (1999) 295–299

Food Chemistry

www.elsevier.com/locate/foodchem

Effect of fruit stone removal on the production of virgin olive oil volatile compounds

Franca Angerosa*, Carla Basti, Raffaella Vito, Barbara Lanza

Istituto Sperimentale per la Elaiotecnica, Contrada Fonte Umano, 65013 Città S. Angelo (PE), Italy

Received 10 March 1999; received in revised form 15 March 1999; accepted 15 May 1999

Abstract

To verify if the quantitative composition of volatiles arising from lipoxygenase pathway was affected by the olive fruit stones, oils from whole olives and from the pulp-only tissues, respectively, were submitted to gas chromatographic determination of volatile compounds extracted by dynamic head-space. Results showed different quantitative compositions for the two oil kinds because of the greater accumulation of C_6 compounds from the lipoxygenase pathway in oils obtained from de-stoned olives, which is related to serious cellular disruption observed in the crushed pulp tissues. C_5 compounds were affected by the stone removal from fruits. Furthermore, the enzymatic oxidation of linoleic acid was promoted in oils obtained after removing fruit stones because of the more significant increase of the concentrations of corresponding C_6 metabolites.

1. Introduction

It is known that the most abundant volatile compounds present in the dynamic head-space of virgin olive oils are C_6 compounds. Among them, C_6 aldehydes are the most represented fraction. All C_6 compounds are produced through the lipoxygenase (LOX) pathway from polyunsaturated fatty acids containing a *cis-cis*-1,4-pentadiene structure (e.g. linolenic and linoleic acids) and accumulate in virgin olive oil during the physical procedures for its extraction.

The LOX pathway starts with a LOX-mediated oxidation of linolenic (LnA) or linoleic (LA) acid leading to the corresponding hydroperoxides. Their subsequent cleavage, catalyzed by hydroperoxide lyases (HPL), produces C_6 aldehydes that can either undergo isomerization of the unsaturated *cis*-3-hexenal to more stable *trans* form, or can be reduced to corresponding C_6 alcohols by alcohol dehydrogenases (ADH). The subsequent esterification of these alcohols is mediated by alcohol acetyl transferases (AAT) (Gardner, 1991; Hatanaka, 1993; Vick & Zimmerman, 1987).

Olias, Perez, Rios and Sanz (1993) found that lipoxygenases of the olive pulp give rise to both 9- and 13-hydroperoxides of linolenic and linoleic acids in a ratio ranging between 57:43 and 65:35, respectively. They also showed that hydroperoxide lyases (HPL) specifically catalyzed the cleavage of only 13-hydroperoxides, and mainly those deriving from LnA. These results are in agreement with the lack of C_9 compounds (Morales, Aparicio & Rios, 1994; Olias et al., 1993; Solinas, Marsilio & Angerosa, 1987) in virgin olive oil aroma.

Salas and Sanchez (1998a,b,c) isolated and characterized both LOX and ADH from the pulp tissue of olive fruits, pointing out that a NADP-dependent ADH is the enzyme responsible for the reduction of aldehydes involved in the LOX pathway.

Researches carried out in the last years gave evidence that the production of C_5 compounds, as in soya seeds (Salch, Grove, Takamura & Gardner, 1995), could also be explained in virgin olive oils as a result of the activity of an additional branch of the LOX pathway (Angerosa, d'Alessandro, Basti & Vito, 1998a; Angerosa, Camera, d'Alessondro & Mellerio, 1998b).

The recent isolation and partial characterization of an active 13-lipoxygenase catalyzing the production of 13-hydroperoxides of linoleic acid, probably associated with membranes of oil bodies of the mature olive endo-

* Corresponding author. Fax: +39-085-959518.
E-mail address: elaiotec@unich.it (F. Angerosa)

0308-8146/99/$ - see front matter
PII: S0308-8146(99)00138-7

sperm (Georgalaki et al., 1998), leads us to investigate whether oils extracted from the whole olives and those obtained from the only pulp tissues, after fruit stone removal, are characterized by the same accumulation of volatiles arising from LOX pathways.

2. Materials and methods

2.1. Chemicals

All solvents, for organic residual analysis, were purchased from J.T. Baker (Deventer, Holland); *trans*-2-hexenal, hexyl acetate, hexan-1-ol and actived charcoal (0.5–0.85 mm; 20–35 mesh ASTM) were from E. Merck (Schuchardt, Germany). Charcoal was cleaned by treatment in a Soxhlet apparatus with diethyl ether and tested before the analyses. Hexanal, *cis*-2-penten-1-ol, and *trans*-2-penten-1-ol were purchased from Aldrich (Steinheim, Germany); *cis*-3-hexenyl acetate from Sigma Chemical Co. (St. Louis, USA); *cis*-3-hexen-1-ol, *trans*-2-hexen-1-ol, 2-pentenal, 1-penten-3-ol and 1-penten-3-one were from Sigma Chemical Co. (Buchs, Switzerland). Pentene dimers were synthesized from *trans*-3-hexenoic acid (from Aldrich, Steinheim, Germany) according to a previous paper (Angerosa et al., 1998b). Tissue-tek OCT compound was purchased from Sakura Finetek USA Inc. (Torrance, CA, USA) and Giemsa's reagent for microscopy from Carlo Erba (Milan, Italy).

2.2. Samples

An homogeneous batch (60 kg) of olive fruits (*Olea europaea* L.) from the Italian Coratina cultivar, picked by hand at a given ripening degree (50% of green–yellow fruits and 50% of cherry olives), was divided into six lots.

A laboratory mill, equipped with a metal crusher, a mixer held at 30°C and a basket centrifuge, was used to extract oil from fruits. Three lots of de-leafed olives were submitted, without further treatment, to the extraction process, whose operative conditions only changed for the malaxation times, i.e. was 0, 15 and 30 min, respectively.

From fruits of the remaining three lots, stones were removed using a suitable tool. Particular attention was paid to limiting the total time required to perform the whole work to 4 min for each lot. Immediately after stone removal, fruits were crushed and submitted to the extraction, with the same plant, and under the same operative conditions (0, 15 and 30 min of malaxing times, respectively), adopted for the lots formed with intact olives.

Immediately after olive crushing, four samples from pastes obtained from the whole fruits and four from those without stones, respectively, were also prepared for light microscopy.

2.3. Dynamic head-space gas chromatography

Volatile compounds, stripped from the oil samples with N_2 (1.2 $dm^3\ min^{-1}$, 37°C for 2 h), trapped on 50 mg of activated charcoal and eluted with 1 ml of diethyl ether, were analyzed by GC technique using a Nordion silica capillary Carbowax 20M column (50 m length; 0.32 mm i.d.; 0.5 μm film thickness) and equipped with an on-column injection system, a CO_2 cryogenic accessory to hold the oven at 25°C and a flame ionization detector (FID). The temperature programme was in agreement with the method described in a previous paper (Angerosa, Di Giacinto & d'Alessandro, 1997a).

2.4. GC–MS analysis

The identification of volatile compounds was carried out by GC–MS in the same operative conditions adopted in gas chromatographic analysis using a HP model 5890A gas chromatograph, equipped with an on-column injection system and coupled with a mass selective detector model HP 5970B (Angerosa et al., 1997a).

2.5. Quantitation

C_6 and C_5 compounds were quantified on the basis of their gas chromatographic areas, using calibration curves previously drafted. Such curves were performed by adding known quantities of C_6 and C_5 compounds to a recently refined olive oil (Angerosa et al., 1997a; Angerosa et al., 1998b; Angerosa, d'Alessandro, Di Girolamo, Vito & Serraiocco, 1997b).

2.6. Microscopy

The cell structure of the olive tissues was observed with a Leitz Orthoplan microscope equipped with an Orthomat photoautomatic exposure control unit after the staining with Giemsa of the cryostat sections (10–15 μm thick) of material embedded in OCT tissue-tek. Representative samples were photographed for each treatment.

3. Results and discussion

Oils extracted from pastes, malaxed at 30°C for 0, 15 and 30 min, respectively, and obtained either from the whole fruits or from the pulp only tissues of a homogeneous batch of fresh healthy olives of the Coratina cultivar, were submitted to gas chromatographic analysis of the volatile components extracted by dynamic head-space. The amounts of C_6 and C_5 compounds, expressed as ppm, are shown in Table 1. The reported values were the mean values calculated from three independent experiments; the confidence limits were always below 10%

Table 1
Composition of volatile compounds from LOX pathway of oils extracted from whole fruits and de-stoned olives of Coatina cultivar at different malaxation times

Compound (ppm)	0 min		15 min		30 min	
	+S[a]	−S[b]	+S	−S	+S	−S
Hexanal	0.9	1.8	1.8	4.2	2.2	5.7
Hexan-l-ol	0.1	0.2	0.1	0.5	0.1	0.7
Hexyl acetate	Trace	Trace	Trace	Trace	Trace	Trace
ΣC_6 from LA	1.0	2.0	1.9	4.7	2.3	6.4
trans-2-Hexenal	12.8	14.9	27.2	33.4	30.7	44.8
trans-2-Hexen-1-ol	0.1	0.7	0.3	1.5	0.5	2.5
cis-3-Hexen-1-ol	0.1	0.3	0.2	0.5	0.2	0.7
cis-3-Hexenyl acetate	0.2	0.1	Trace	Trace	Trace	Trace
ΣC_6 from LnA	13.2	16.0	27.7	35.4	31.4	48.0
Σ all C_6 compounds	14.2	18.0	29.6	40.1	33.7	54.4
Pentene dimers	0.3	0.3	0.4	0.4	0.7	0.6
1-Penten-3-ol	0.1	0.1	0.2	0.1	0.2	0.2
2-Pentenols	0.2	0.2	0.2	0.2	0.4	0.4
1-Penten-3-one	0.2	0.2	0.3	0.4	0.4	0.5
2-Pentenal	0.1	0.1	0.2	0.1	0.2	0.2
ΣC_5 from LnA	0.9	0.9	1.3	1.3	1.9	2.0

[a] Whole fruits (with stone).
[b] Olive pulp (without stone).

As far as the influence of malaxation time was concerned, the results agreed with those of previous research (Angerosa et al., 1998a) that showed a greater accumulation of C_6 and C_5 metabolites, in particular carbonyl compounds and their corresponding alcohols, when the malaxation time of pastes was prolonged.

The fact that the major components of the volatile fraction of all oils are C_6 compounds showed that the heterolytic cleavage of 13-hydroperoxides of LnA and LA acids by the enzymes involved in the LOX cascade was the most important process in all experiments carried out. Besides, the greater accumulation of C_6 compounds from LnA than LA, observed in all cases, confirmed that 13-hydroperoxides of LnA were the substrate preferentially converted by the mentioned enzymes. On the other hand, experiments performed in vitro proved the greater affinities, showed by HPL, ADH and AAT of olive fruits, for unsaturated metabolites (Olias et al., 1993).

Among unsaturated components, *trans*-2-hexenal and its corresponding alcohol were the main metabolites accumulated, underlining that the isomerization of *cis*-3-enal to *trans*-2-enal was the dominant process and that only a slight activity of ADH was detected in Coratina fruits. The amount (at trace level) of hexyl acetate and *cis*-3-hexenyl acetate proved a very low content of AAT catalyzing the production of these esters. These results were in agreement with those of previous research (Angerosa, Basti & Vito, 1999).

Comparing data of volatiles extracted, by dynamic head-space analysis, from oils obtained from whole fruits and olive pulps, it was immediately clear that, regardless of the malaxation time of pastes, oils obtained from de-stoned olives generally had a greater accumulation of C_6 compounds than oils extracted by the whole fruits, whereas C_5 compounds showed no significant changes.

The higher total amount of C_6 volatile compounds in the oils extracted from the pulp only tissues could be related to a greater release of the membrane-bound enzymes involved in the LOX pathway owing to more serious cellular disruption due to a more effective grinding of the pulp tissues in de-stoned fruits.

Light microscopy (LM) observations of several sections obtained from olive pastes, from whole and de-stoned fruits, respectively, showed a very different kind of disruption of cellular tissues.

Serious mechanical damage to parenchymatous cells of mesocarp was observed when the stones were removed; in particular most tissues showed cell collapse (Fig. 1D). On the other hand, the pastes from whole fruits kept the integrity of the cell structure (cell walls and nucleus) and of annexed structures (sclereids) in most tissues (Fig. 1A). In addition, the tissue damage consisted of a cellular separation—as evidenced by the clean cuts of cell walls (Fig. 1B and C)—rather than the cell collapse observed in de-stoned pulps (Fig. 1E and F). Apart from the more serious cell damage in pastes of de-stoned olives, it was possible that some increase of C_6 compounds could be attributed to the activies of enzymes involved in the LOX pathways, released during the removal of the stone, in the 4 min before the olive crushing.

The greater release of membrane-bound enzymes in oils from de-stoned fruits explained the increase of C_6 compound concentrations and, in addition, was not in conflict with the fact that C_5 compounds remained a

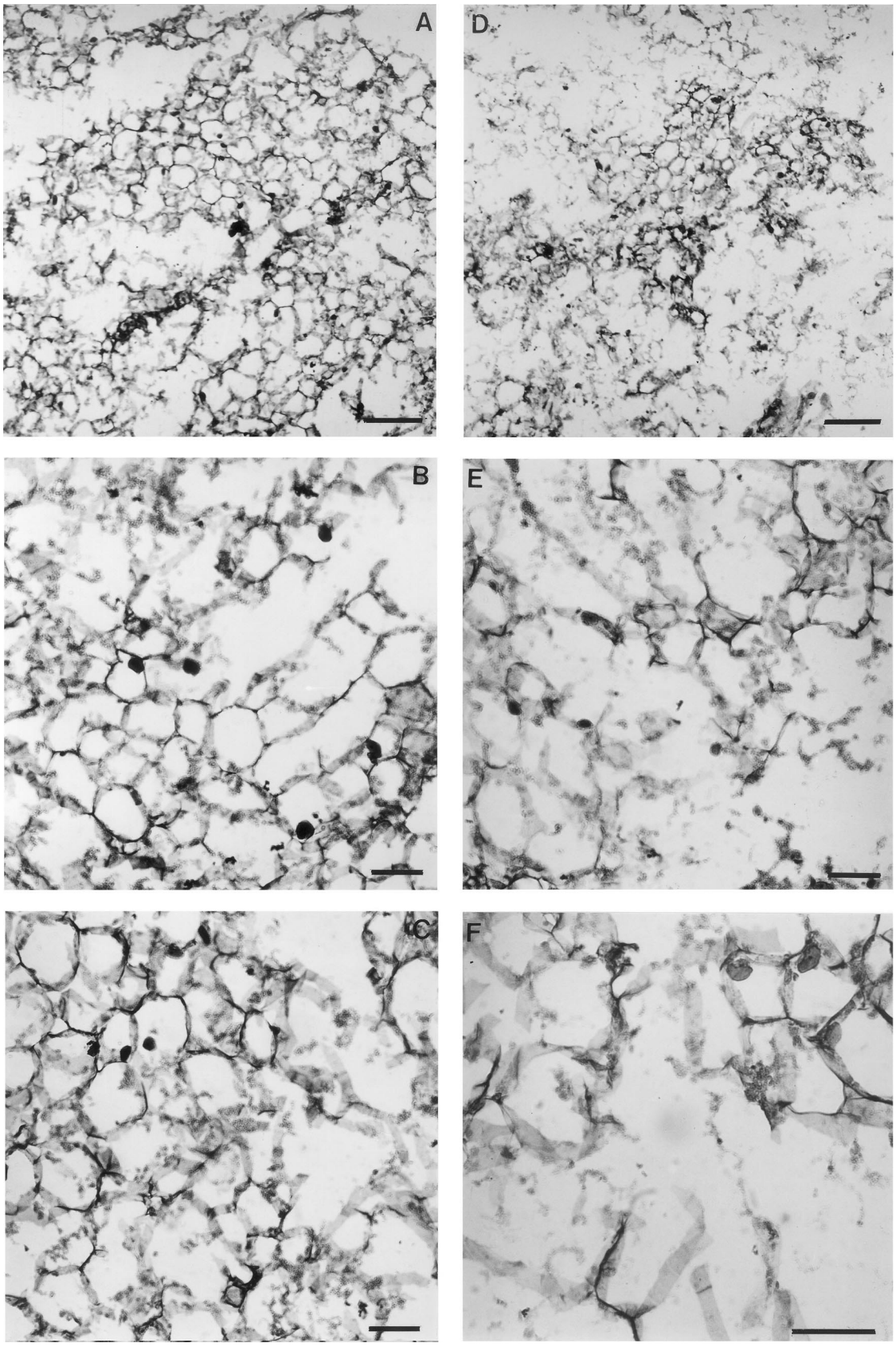

Fig. 1. LM micrographs of whole (A, B and C) and de-stoned (D, E and F) fruits. Bars in A and D = 150 μm; bars in B, C, E and F = 50 μm.

constant in all experiments carried out. In fact C_5 compounds are generated through the additional branch of the LOX pathway from LnA that involves enzyme-mediated reactions only to produce the 13-alkoxy radical. The subsequent generation of intermediates such as 1,3-pentene allylic radicals and of final products of the pathway—pentene dimers and C_5 alcohols—were only due to chemical radical reactions.

However, the increased enzymatic release, even if able to explain the greater accumulation of whole C_6 volatile compounds in oils obtained from de-stoned fruits, could not explain the quantitative distribution of each metabolite that was rather different in the two kinds of oils.

The rate of the increase of C_6 compounds coming from LnA and LA was not the same for the same malaxation time. In fact, total amount of C_6 metabolites arising from LnA increased to about 21% in oils obtained without paste malaxation, 28% at 15 min of malaxation and 53% at 30 min, whereas the content of C_6 compounds from LA showed more significant increases ranging from about 100 to 180%. Quantitatively, the most important metabolite from LA was hexanal. The results suggest that the enzymatic oxidation of linoleic acid was promoted when olive stones were not present in the pastes submitted to the oil extraction. This feature was unexpected in oils from de-stoned fruits because of the occurrence of a 13-LOX catalyzing the oxidation of LA observed by Georgalaki et al. (1998) in the endosperm of olive fruits. Our result was not in agreement with those of Georgalaki et al. (1998), and could only be explained by the removal of some inhibitor contained in the stone that would have a serious effect on the enzymatic transformation of 13-hydroperoxides of linoleic acid.

The results show an increase of the concentration of volatile compounds arising from LA, and in particular of hexanal. This compound, reminescent of green apple or green fruit odour notes (Aparicio & Morales, 1998), gives a great contribution to the olive oil flavour because of its low odour threshold mean value (Guth & Grosh, 1993). Therefore, the increase of hexanal content in oils from de-stoned fruits should have a significant positive repercussion on the flavour because hexanal is related to the fruity green aspect of the overall green aroma.

Acknowledgements

This research was supported by the European Union (Project AIR3-CT94 1967), and by the Italian "Ministero delle Politiche Agricole" (MIPA).

References

Angerosa, F., Di Giacinto, L., & d'Alessandro, N. (1997a). Quantitation of some flavour components responsible for the "green" attributes in virgin olive oils. *Journal of High Resolution Chromatography, 20*, 507–510.

Angerosa, F., d'Alessandro, N., Di Girolamo, M., Vito, R., & Serraiocco, A. (1997b). Relationship between aroma components and malaxation time. In *Proceedings of (3rd) International Symposium on Olive Growing*. Crete, 22–27 September.

Angerosa, F., d'Alessandro, N., Basti, C., & Vito, R. (1998a). Biogeneration of volatile compounds in virgin olive oil: their evolution in relation to malaxation time. *Journal of Agriculture and Food Chemistry, 46*, 2940–2944.

Angerosa, F., Camera, L., d'Alessandro, N., & Mellerio, G. (1996). Characterization of seven new hydrocarbon compounds present in the aroma of virgin olive oils. *Journal of Agriculture and Food Chemistry, 46*, 648–653.

Angerosa, F., Basti, C., & Vito, R. (1999). Virgin olive oil volatile compounds from lipoxygenase pathway and characterization of some Italian cultivars. *Journal of Agriculture and Food Chemistry, 47*, 836–839.

Aparicio, R., & Morales, M. T. (1998). Characterization of olive ripeness by green aroma compounds of virgin olive oil. *Journal of Agriculture and Food Chemistry, 46*, 1116–1122.

Gardner, H. W. (1991). Recent investigations into lipoxygenase pathway of plants. *Biochimica and Biophysica Acta, 1084*, 221–229.

Georgalaki, M. D., Bachmann, A., Sotiroudis, T. G., Xenakis, A., Porzel, A., & Feussner, I. (1998). An active linoleate-13-lipoxygenase is found in virgin olive oil. In J. Sanchez, E. Cerda-Olmedo, & E. Martinez-Force, *Advances in plant lipid research* (pp. 696–698). Spain: Secretariado de publicationes, Universidad de Sevilla.

Guth, H., & Grosh, W. (1993). Quantitation of potent odorants of virgin olive oil by stable-isotope dilution assays. *Journal of the American Oil Chemist's Society, 70*, 513–518.

Hatanaka, A. (1993). The biogeneration of green odour by green leaves. *Phytochemistry, 34*, 1201–1218.

Olias, J. M., Pérez, A. G., Rios, J. J., & Sanz, L. C. (1993). Aroma of virgin olive oil: biogenesis of the "green" odor notes. *Journal of Agriculture and Food Chemistry, 41*, 2368–2373.

Morales, M. T., Aparicio, R., & Rios, J. J. (1994). Dynamic headspace gas chromatographic method for determining volatiles in virgin olive oil. *Journal of Chromatography A, 668*, 455–462.

Salas, J. J., & Sanchez, J. (1998a). Hydroperoxide lyase from olive (*Olea europaea*) fruits. In J. Sanchez, E. Cerda-Olmedo, & E. Martinez-Force, *Advances in plant lipid research* (pp. 300–302). Spain: Secretariado de publicationes, Universidad de Sevilla.

Salas, J. J., & Sanchez, J. (1998b). Alcohol dehydrogenases from olive (*Olea europaea*) fruit. *Phytochemistry, 48*, 35–40.

Salas, J. J., & Sanchez, J. (1998c). Lipoxygenase activity from the pulp tissues of olive (*Olea europaea*) fruits. In J. Sanchez, E. Cerda-Olmedo, & E. Martinez-Force, *Advances in plant lipid research* (pp. 297–299). Spain: Secretariado de publicationes, Universidad de Sevilla.

Salch, Y. P., Grove, M. J., Takamura, H., & Gardner, H. W. (1995). Characterization of a C-5,13-cleaving enzyme of 13-(S)-hydroperoxide of linolenic acid by soybean seed. *Plant Physiology, 108*, 1211–1218.

Solinas, M., Marsilio, V., & Angerosa, F. (1987). Evolution of some components of virgin olive oil aroma in relation to ripening degree of fruits. *Rivista Italiana delle Sostanze Grasse, 64*, 475–479.

Vick, B. A., & Zimmerman, D. C. (1987). The lipoxygenase pathway. In P. K. Stumpf, J. B. Mudd, & W. D. Nes, *The metabolism structure and function of plant lipids* (pp. 383–390). NY, London: Plenum Press.