Effect of Extraction Conditions on Sensory Quality of Virgin Olive Oil

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ABSTRACT: The extraction conditions of virgin olive oil have a great influence on its sensory quality. During the centrifugation process, temperature and time of malaxing can be altered to potentially affect quality. Malaxing times (15, 30, 45, 60, and 90 min) and temperatures (25 and 35°C) were studied in an experimental oil mill. Volatile compounds, produced through the lipoxygenase pathway (hexanal, *Z*-3-hexenal, *E*-2-hexenal, hexyl acetate, *Z*-3-hexenyl acetate, hexan-1-ol, *E*-3-hexen-1-ol, *Z*-3-hexen-1-ol, and *E*-2-hexen-1-ol), were analyzed by dynamic headspace gas chromatography, gas chromatography–mass spectrometry, and gas chromatography–olfactometry. Different amounts of volatiles responsible for positive attributes of green aroma and negative attributes of astringent mouthfeel of virgin olive oil were determined. The results, after applying mathematical procedures, showed that a temperature of 25°C and a malaxing time between 30 and 45 min produced volatile compounds that contribute to the best sensory quality. High temperature ($T \geq 35$°C) with minimum values of time ($t < 30$ min) could also be useful as an alternative way to obtain pleasant green virgin olive oils.

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KEY WORDS: Extraction, gas chromatography–mass spectrometry, gas chromatography–olfactometry, headspace gas chromatography, lipoxygenase, malaxing, sensory quality , virgin olive oil, volatile compounds.

Virgin olive oil, extracted from the fruit of the olive tree, *Olea europea* L., is consumed without further refining to retain volatiles and other minor compounds that produce a fragrant and delicate flavor.

Previous works (1,2) reported different sensory notes responsible for virgin olive oil flavor including green, sweet, fruity, ripe fruit, ripe olives, undesirable, and bitter-pungent as the most prominent sensory perceptions of consumers. Most of these sensory perceptions are produced by volatile compounds (3,4). The green aroma is of great importance because the fragrant flavor of virgin olive oil is produced by the balance between green and fruity notes (5). Aliphatic C_6 compounds and the corresponding hexyl esters (5–7) are the main contributors to the unripe component of the fruit flavor; almost all volatile compounds responsible for green sensory notes are the major components of the virgin olive oil headspace (8–10). Although all of these compounds contributed to the green aroma of virgin olive oil, Aparicio *et al.* (11) found that some of them—*E*-2-hexen-1-ol, *E*-3-hexen-1-ol and hexan-1-ol—were better characterized by the global sensory perception of undesirable (e.g., astringent, rough) since they also contributed to taste. This characterization basically agreed with Bedoukian (12) who found that the *cis* forms of hexenols were characterized by a more pleasant sensory perception than their corresponding *trans* forms.

Volatile compounds are formed from C_{18}-unsaturated fatty acids in plants following the lipoxygenase (LOX) pathway (13,14). This pathway involves the actuation of different enzymes giving rise to various amounts of aldehydes, alcohols, and esters that contribute to overall flavor. In olive fruits, it has been demonstrated (15) that the LOX pathway promotes the formation of C_6 volatile compounds rather than C_9 volatile compounds and, in consequence, a great amount of those C_6 volatile compounds responsible for green sensory notes (5) can be found in fresh and high-quality virgin olive oils. On the other hand, previous studies (16,17) have shown that the kind of olive oil extraction process also affected the sensory quality of virgin olive oil. Pressure system-produced oil with flaws due to the presence of high concentration of volatiles originated from the fermentation of the olive paste residue (16). Oils obtained by a two-phase continuous system exhibited a higher content of total aromatic substances, e.g., ratios of *E*-2-hexenal/hexanal and *E*-2-hexenal/total aroma are favored by centrifugation systems (17). Thus, the goal of this research was to establish an index to the best extraction conditions (temperature and time of malaxing) based on volatile compounds that are responsible for the unique sensory attributes of virgin olive oil (11).

MATERIALS AND METHODS

Samples. Olive fruits from the Picual Spanish olive tree variety were selected because its oil represents approximately 10% of all olive oil produced in the world. Virgin olive oil was obtained from fresh, healthy fruits of good quality collected at green stage of ripeness (18). The extraction of the oil samples was carried out on an analytical scale using an experimental

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oil mill in three steps: crushing, malaxing (beating), and centrifugation. For the malaxing process, the paste of olives is stirred in a receptacle and heated by running water flowing through a water pipe. Temperature was adjusted with a valve that regulated the temperature of water flowing through the pipe. As the paste heated, larger drops appeared. As the drops appeared, yield increased. However, as malaxing temperature increased, yield also increased but olive oil quality decreased because of the deterioration in minor compounds. A similar process occurred as malaxing time was increased.

Malaxing temperatures of 25 and 35°C corresponded to cold extraction conditions and the maximum suggested by experts, respectively (19). Five malaxing times (15, 30, 45, 60, and 90 min) were selected to cover the time range used to extract oils from different types of olives. Experiments were carried out in duplicate to diminish the effect of random variables in the process.

Another experiment was carried out with intact and cut olive fruits in order to detect if the LOX pathway is induced when olive fruits are damaged (20). Twenty grams of intact fruits was analyzed following the methodology described below (dynamic headspace using Tenax TA traps). Cut olives were obtained by placing 20 g of intact fruits in the extraction vessel and cutting the pulp of each fruit five times. Cut olives were analyzed immediately after they were cut using the same methodology.

Dynamic headspace–gas chromatography. Volatile compounds of virgin olive oil were analyzed by a dynamic headspace technique previously reported (9,21). Samples of 0.5 g were heated at 40°C, swept with N_2 (200 mL/min) for 15 min, and the volatiles adsorbed on to a Tenax TA trap (Chrompack, Middleburg, The Netherlands) at room temperature. A Chrompack thermal desorption cold trap injector (TCT) was employed to carry out the thermal desorption of the trapped volatiles by heating at 220°C for 5 min. The volatiles were then condensed on to a fused-silica trap cooled at −110°C with liquid nitrogen for 5 min just before injection, which was carried out by flash heating of the cold trap at 170°C, where it was held for 5 min. The volatiles were transferred onto a fused-silica DB-WAX column (J&W Scientific, Folsom, CA) (60 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was held at 40°C for 6 min and programmed to rise at 2°C/min to a final temperature of 200°C, where it was held for 10 min. A Hewlett-Packard 5890 series II (Palo Alto, CA) with a flame-ionization detector was employed. Quantification was carried out using isobutyl acetate as internal standard.

The identification of volatile compounds was carried out by mass spectrometry using identical conditions to gas chromatography. A Fisons MD800 mass selective detector coupled to a GC 8000 series (VG Analytical, Manchester, United Kingdom) was employed. MassLab v 1.-3 (VG Analytical, Manchester, United Kingdom) was the software used. Sample components were verified by comparison of mass spectral data with those of authentic reference compounds. *Z*-3-Hexenal was synthesized (22) and its mass spectrum obtained.

Sensory properties of volatile compounds. Assessors. The sensory (odor and taste) properties of volatile compounds were evaluated by five experienced assessors and three consumers of virgin olive oil following the methodologies described below.

High resolution gas chromatography (HRGC)–olfactometry. To assess the aroma notes corresponding to olive oil volatile compounds, an HRGC–olfactometry technique was applied to virgin olive oil samples (9). The effluent of the gas chromatographic column was split 1 to 10 to the detector and the sniffing port, respectively. The descriptions of the odor-active regions of the eluate were noted on a form with a preprinted time scale from 0 to 60 min; assessors did not see the chromatogram. Assessors basically agreed on the odors of volatiles, although different semantic terms were used to describe some odors. A consensus-building discussion was held with assessors to decide the final sensory descriptors.

Threshold values and tasting of pure compounds. Odor threshold values of volatile compounds, in a matrix of deodorized sunflower oil, were determined using the method described by Guth and Grosch (7).

The assessors also carried out the smelling and tasting, in duplicate, at room temperature, of four pure volatile compounds that previously had been characterized as responsible for sensory perceptions other than green: hexan-1-ol (Merck, Darmstadt, Germany), *E*-2-hexenal, *E*-2-hexen-1-ol, and *E*-3-hexen-1-ol (Aldrich, Milwaukee, WI). The volatile compounds were previously diluted in water or paraffin oil, depending on their solubility, to the same approximate concentration found in virgin olive oil samples.

Statistical analysis. The Statistica (23) statistical package with canonical correlation and cluster analysis was used. Response surfaces were used to plot the fitted model of the selected chemical compounds in a surface along with their observed values in the experiments at different times and temperatures during the extraction process. A quadratic smoothing algorithm was applied to the standardized values of each volatile compound. Chebychev distance and the weighted pair group average was used to compute the cluster analysis. Canonical correlation was applied for assessing the relationship between sensory attributes and volatiles. This algorithm allowed investigation of redundancies between both sets of data.

RESULTS AND DISCUSSION

From the large data set of volatiles identified in virgin olive oil (9), only a few are responsible for green perceptions (11). These volatile compounds, known as green volatile compounds, are produced through the LOX pathway as soon as olive fruits are crushed (cell damage) (20). Figure 1 shows the differences in the content of volatiles produced from intact olives and cut olive fruits cv. Picual. This experiment confirmed that the volatile compounds of the LOX pathway were produced immediately after the olives had been cut. It is noteworthy that hexane was predominant in intact fruits and

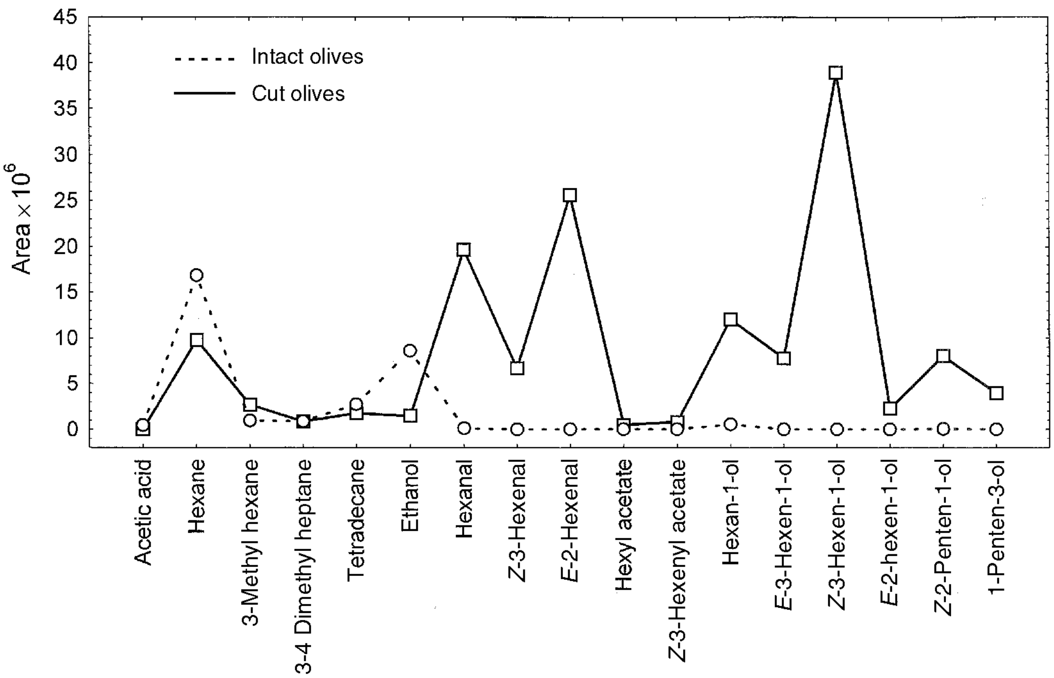

FIG. 1. Production of some volatile compounds in cut and intact olives. Quantification by dynamic headspace.

that volatile compounds responsible for the final virgin olive oil aroma were absent. The volatile profile of cut olives showed a considerable change because of the production of green volatile compounds from both linoleic and linolenic acids. In fact, only traces of hexanal and hexan-1-ol, both from linoleic acid, could be detected in intact fruits. The results also showed that the amount of *E*-2-hexenal was greater than *Z*-3-hexenal, as the latter was not stable while the former was. In fact, *E*-2-hexenal was produced from *Z*-3-hexenal by an isomerization factor.

Table 1 shows the volatile compounds analyzed in samples. The HRGC-olfactometry showed green sensory notes covering the range of sweet-fruity-green to bitter-powerful-green. The odor threshold and odor activity value (OAV) (24) are also given. A volatile compound with an OAV greater than 1.0 contributes to the green sensory perception, while volatiles having lesser OAV could contribute to virgin olive oil flavor through synergism.

TABLE 1
Sensory Descriptions, Odor Thresholds (µg/kg), and Odor Activity Values (OAV) of the Green Volatile Compounds

Volatile compounds	Odor descriptors	Odor thresholds	OAV
Hexanal	Green, apple, green fruit	60	8.91
Z-3-Hexenal	Green, cut grass	3.0	184.67
E-2-Hexenal	Green, fruity, almonds	1200	9.76
Hexyl acetate	Fruity, sweet	1040	0.04
Z-3-Hexenyl acetate	Fruity, green leaves	730	0.07
Hexan-1-ol	Fruit, banana, soft	400	0.13
E-3-Hexen-1-ol	Green	1500	0.01
Z-3-Hexen-1-ol	Grass, banana	5800	0.07
E-2-Hexen-1-ol	Green, grassy, sweet	8000	0.01

Aparicio *et al.* (11) reported a high canonical correlation (r = 0.84) between the green volatile compounds and the green sensory perception, demonstrating that the former are responsible for the latter. However, the volatiles only explained 66% of the sensory attributes (redundancies between data sets), and this could mean that the total information of green sensory attributes is not only explained by the odor of the volatiles but also by the tasting of some of these volatiles.

Previous studies using statistical sensory wheel (SSW) (1,11) demonstrated that compounds such as *Z*-3-hexenal, hexyl acetate, *Z*-3-hexenyl acetate, and *Z*-3-hexen-1-ol mainly contributed to the green sensory perception, but others, including *E*-2-hexenal, hexan-1-ol, *E*-3-hexen-1-ol, and *E*-2-hexen-1-ol, could also contribute to other sensory perceptions. The taste of these volatiles characterized them as astringent and bitter (*E*-2-hexen-1-ol and *E*-3-hexen-1-ol), rough (hexan-1-ol) and sharp, bitter, astringent (*E*-2-hexenal).

There is good agreement among consumers in relation to the sensory perceptions of olive oil. They dislike high intensities of bitter and pungent, whereas they like almost all aroma descriptors qualified with the adjective green (25,26). Therefore, the presence of volatiles responsible for pleasant sensory perceptions should be promoted. However, virgin olive oil must be extracted solely using physical means, therefore the profile of volatiles in the final product can only be varied by changing the temperature and time of the malaxing process. Depending on temperature and time of malaxing, each volatile compound showed different behavior.

Hexanal, responsible for green, apple, sweet perceptions, had an OAV of 8.91 (Table 1), thus contributing to pleasant sensory perceptions. In fact, this compound has been clearly correlated with the highest overall grading of virgin olive oil

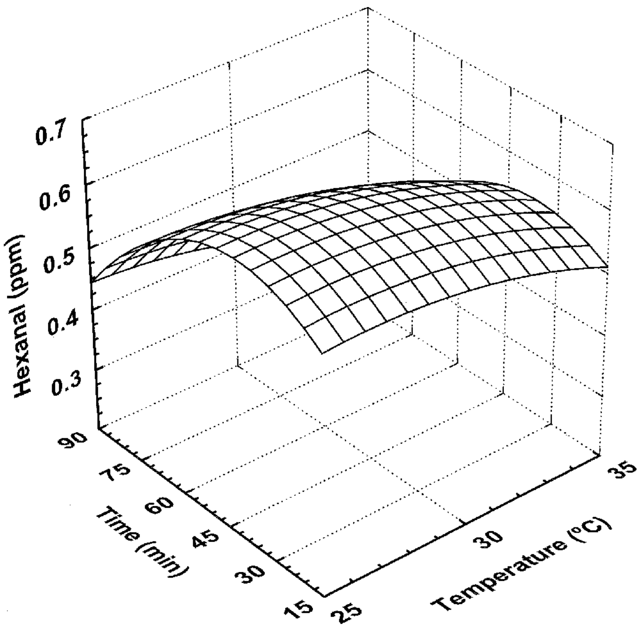

FIG. 2. Response surface of hexanal at different temperatures and times of malaxing. The surface has been fitted to the data by a second-order polynomial function.

(11), using the official panel test (27). The response surface for hexanal, time, and temperature of malaxing is shown in Figure 2. The production of hexanal was more influenced by the time of malaxing than by the temperature. Hexanal is produced during the whole process, but the higher concentrations were found after 45 min of malaxing.

A different response pattern was shown for *E*-2-hexen-1-ol (Fig. 3). This volatile is characterized by a green odor and by an astringent-bitter taste, an undesirable sensory perception for potential consumers (25). The production of E-2-hexen-1-ol was mainly affected by temperature, because the highest concentrations were measured at the highest temperature of malaxing (35°C). Hexyl acetate (Fig. 4) was also affected by temperature, but the highest concentration of this ester was measured at the lowest temperature (25°C).

The other compounds responsible for green aroma descriptors (*Z*-3-hexenal, *Z*-3-hexen-1-ol, and *Z*-3-hexenyl acetate) also showed the higher concentrations at low temperature. The influence of malaxing time was low for *Z*-3-hexenal, medium for *Z*-3-hexen-1-ol, and higher for *Z*-3-hexenyl acetate. Therefore, the production of *Z*-3-hexenal was almost immediate, whereas the production of *Z*-3-hexenyl acetate needed more time. This explanation agreed with the sequence of production of these volatiles through the LOX pathway. The first step of this biochemical pathway is the formation of the aldehyde, then it is transformed to alcohol by the action of alcohol dehydrogenase enzyme (ADH), then the ester is formed by alcohol acyl transferase enzyme (AAT).

Concerning the volatiles responsible for the undesirable sensory perceptions (*E*-2-hexenal, hexan-1-ol, *E*-3-hexen-1-ol), their concentrations were higher when the temperature of the malaxing was the highest. In this case, the time of malaxing had no influence on the formation of the alcohols, but it showed a great influence on the formation of this aldehyde. The concentration of this aldehyde was lower at high and low malaxing times and higher at medium values of time.

In general, the highest concentrations of aldehydes were measured when malaxing time was shorter, the production of alcohols was promoted at high temperature (35°C), and the concentration of esters was higher at low temperature (25°C). Malaxing at high temperatures ($T \geq 35$°C) promoted formation of green volatiles responsible for undesirable sensory

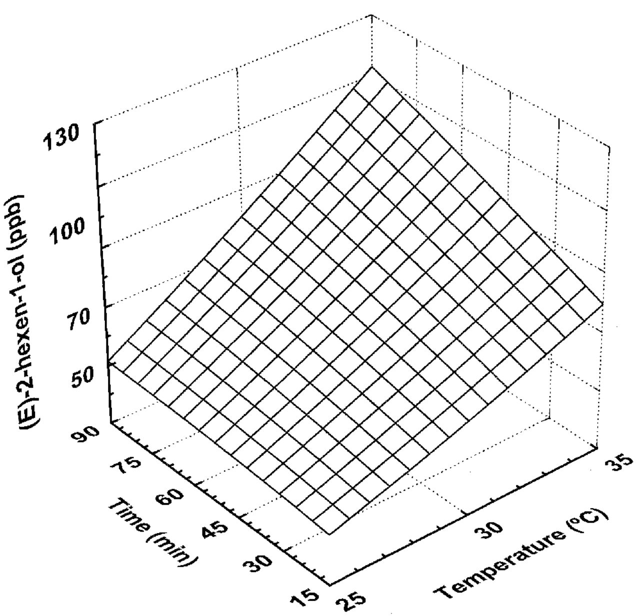

FIG. 3. Response surface of *E*-2-hexen-1-ol at different temperatures and times of malaxing. The surface has been fitted to the data by a second-order polynomial function.

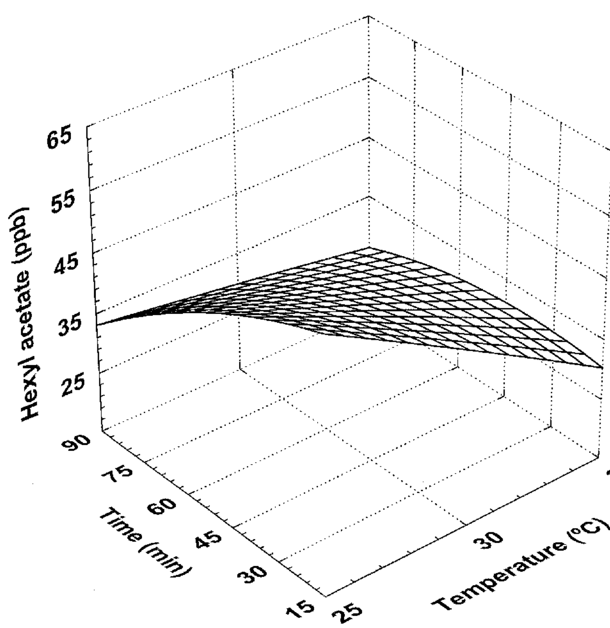

FIG. 4. Response surface of hexyl acetate at different temperatures and times of malaxing. The surface has been fitted to the data by a second-order polynomial function.

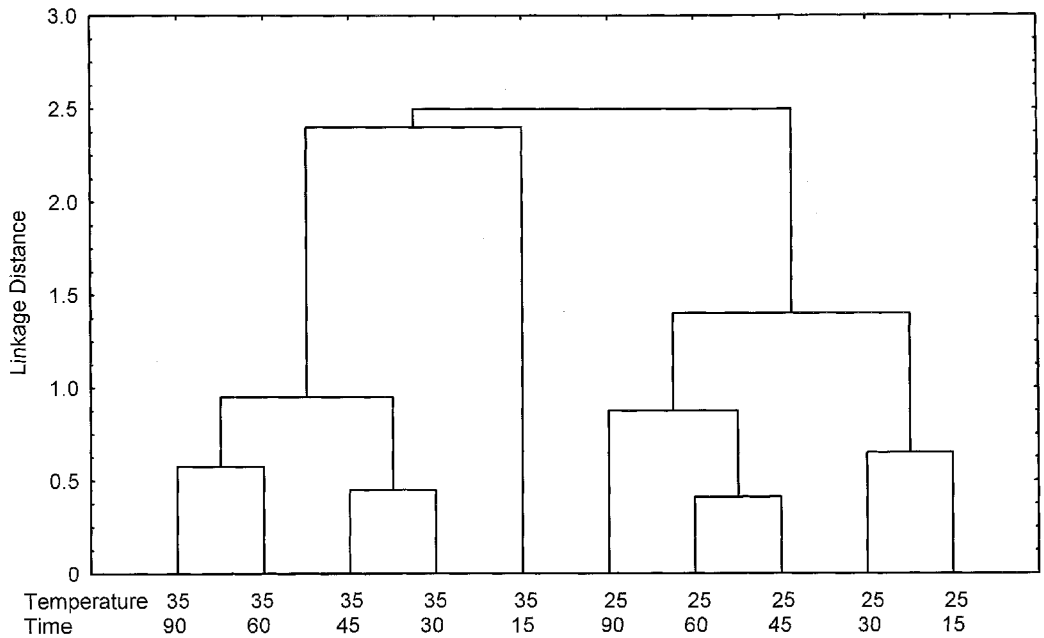

FIG. 5. Cluster analysis of the conditions of time and temperature in the malaxing process. Information from the volatile compounds produced through the lipoxygenase pathway.

perceptions, whereas low temperatures ($T \leq 25°C$) favored production of desirable green sensory perceptions. Figure 5 shows how temperature was the main factor characterizing the malaxing process. The linkage distance shows the differences between experiments in an agglomeration process, the higher the value, the greater the differences between experiments. There are two groups characterized by the temperature (25 vs. 35°C), although the experiment at 35°C and 15 min appears between the cited groups for a linkage distance lower than 2.5. These results agree with those obtained in the industrial plants using on-line fuzzy control algorithms (28).

Figure 6 shows a scheme of the LOX pathway with its three different branches for the production of volatiles. The branch coming from linoleic acid gave rise to hexanal, hexan-1-ol, and hexyl acetate; the former and the latter were responsible for desirable perceptions. This branch could be seen as the green-sweet aspect of the global green flavor. The second

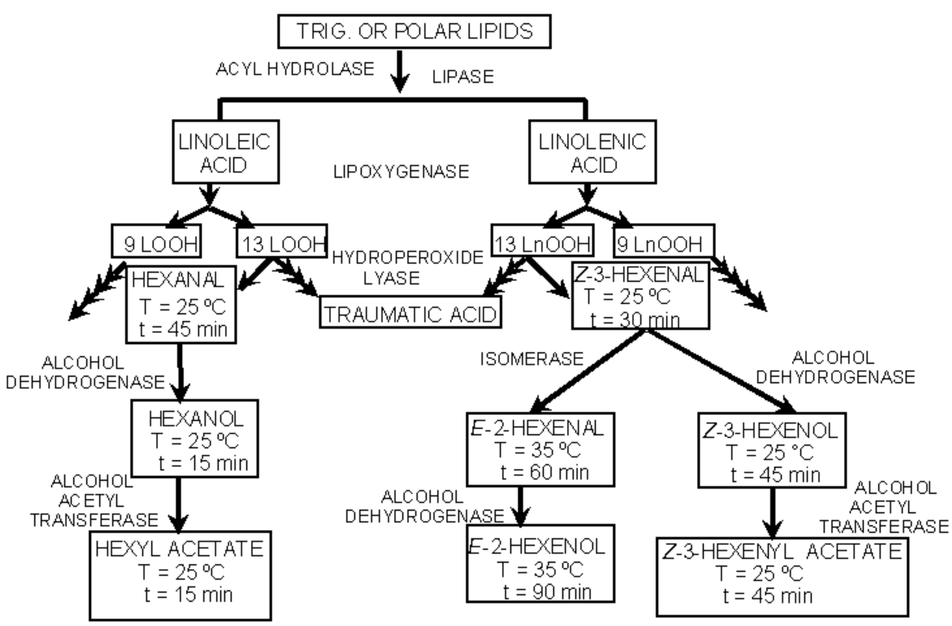

FIG. 6. Lipoxygenase pathway. Optimal malaxing conditions [temperature (T) and time (t)] for the production of volatile compounds. The sensory characterizations of volatiles are described in Table 1.

branch could be seen as responsible for the main green perception, being constituted by *Z*-3-hexenal, *Z*-3-hexenol, and *Z*-3-hexenyl acetate. The third branch, giving rise to *E*-2-hexenal and *E*-2-hexen-1-ol, could be considered as the bitter-astringent aspect of the green sensory perceptions.

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