PERFUMER & FLAVORIST

Perception of Characteristic Axillary Odors

By Ahmet E. Baydar, Martin Petrzilka and Marie-Pierre Schott
Givaudan-Roure, Grasse, France

Of all the human scents, underarm or axillary odor has been the most studied due to the billion dollar market that exists for deodorants and antiperspirants. The identification of odoriferous compounds generated by the action of micro-organisms, mainly corynebacteria, on apocrine secretion is still an area of intense activity with the ultimate aim of unravelling the secrets of the underarm odor.

There has been a lot of controversy as to exactly which compounds are responsible for the characteristic axilla odor. Reviews by Labows (1988)[1] and Gower (1989),[2] containing extensive lists of references on the subject, suggest that the characteristic odor in the underarm is due to the presence of the volatile steroids 5-α-androst-16-en-3-ol (androstenol), 5-α-androst-16-en-3-one (androstenone) and 4,16-androstadien-3-one (androstadienone) as well as isovaleric acid.

Recently, the topic of underarm odor received the attention of the world press in headlines like "Scientists find chemical clue to body odor" (*New York Times*, August 1990), "Key ingredient in armpit odor sniffed out" (*Washington Post*, August 1990), "Science sniffs out culprit in damp case" (*Herald Tribune*, European Edition, August 1990), and "Stink-tank scientist reports body-odor breakthrough" (*The Japan Times*, August 1990, referring to the work of George Preti of the Monell Centre). The new find proposed that underarm odor was mainly due to volatile C_6-C_{11} straight chain or branched unsaturated acids and that the major contributor to the characteristic odor with a high odor impact was (E)-3-methyl-2-hexenoic acid. The (Z)-isomer, found at one tenth the concentration of the (E)-isomer, also had a high odor impact, but not the underarm odor quality.[3]

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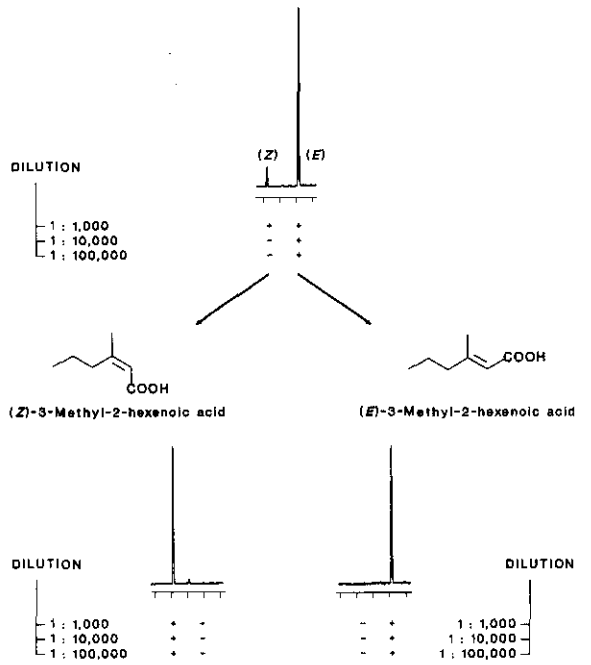


Figure 1. Separation of olfactively pure (E)- and (Z)-3-methyl-2-hexenoic acids

Preti claims that the steroids could not be important malodor contributors due to the high prevalence of specific anosmia to androstenone and to GC sniff results which showed that they did not elute at the time of the strongest axillary odors.[3]

Evidence for a high level of worldwide anosmia towards androstenone was obtained by the "*National Geographic* Smell Survey" conducted by Gilbert and Wysocki,[4,5] and has recently been confirmed by extensive olfactometric threshold measurements on a large panel population.[6]

That the individuals differ in their abilities to perceive odor is a recognized fact, with many known specific anosmias related to popular flavors and fragrances as well as human odors.[7] Preti pointed out that one of his colleagues could not perceive either of the 3-methyl-2-hexenoic acids.[3] Our assessment also indicated a great likelihood of a high level of anosmia towards these acids, which would explain inconclusive results obtained from underarm panel tests and the varied interpretation of axillary odor ranging from "flower of youth" to "goat in the armpit."[8]

In order to clarify this point we decided to carry out accurate threshold studies on pure (E)- and (Z)-hexenoic acids for a relatively large panel size to establish the level of anosmia, if any, and compare the results with the in-house threshold data on androstenone for the same panelists. A Givaudan-Roure-constructed, state-of-the-art air dilution olfactometer, which has been described by Neuner-Jehle and Etzweiler[9] and Müller[10] was used for this study.

Experimental Details

(E)- and (Z)-3-methyl-2-hexenoic acids were synthesized according to the procedure used by Wadsworth and Emmons,[11] and were separated using flash chromatography.

Olfactory purity determination indicated that the (E)-isomer had a much lower threshold than the (Z)-isomer. This explained the initial difficulties, which were later overcome, in obtaining an olfactively pure sample of the (Z)-isomer (Figure 1).

The air dilution olfactometer with a three alternative forced-choice response paradigm was used for the odor detection thresholds. It had a randomization program which loaded one of the streams with a given concentration of the odorant under investigation. The other two ports (blanks) delivered only the carrier stream. The subject sampled all three ports, with repeated sampling allowed, and selected the odorized one by pushing a corresponding button. Indicator lights next to each button lit green for correct and red for incorrect answers. There was a minimum delay of 15 seconds between trials. The process was re-started with a lower concentration and repeated down to the odor perception threshold level where the panelist started giving incorrect answers (descending staircase technique). Nine dilution steps were used as a single series of trials. The threshold

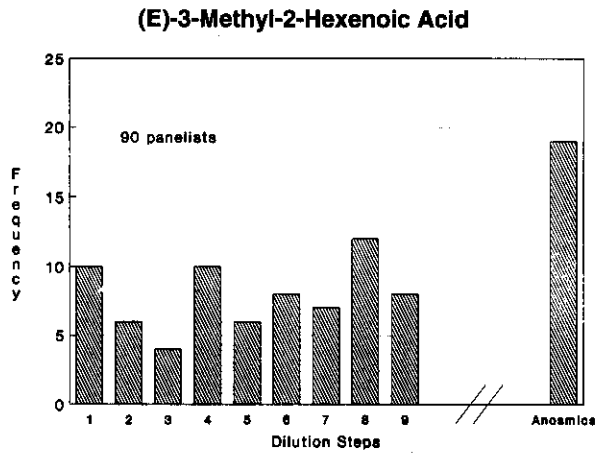


Figure 2. Threshold distribution curves for (E)- and (Z)-3-methyl-2-hexenoic acids

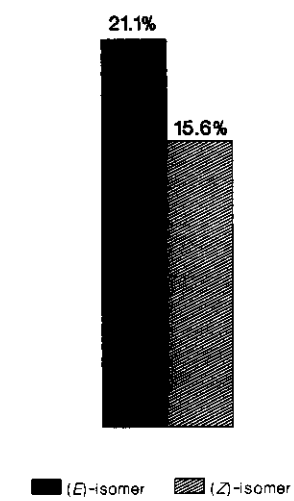
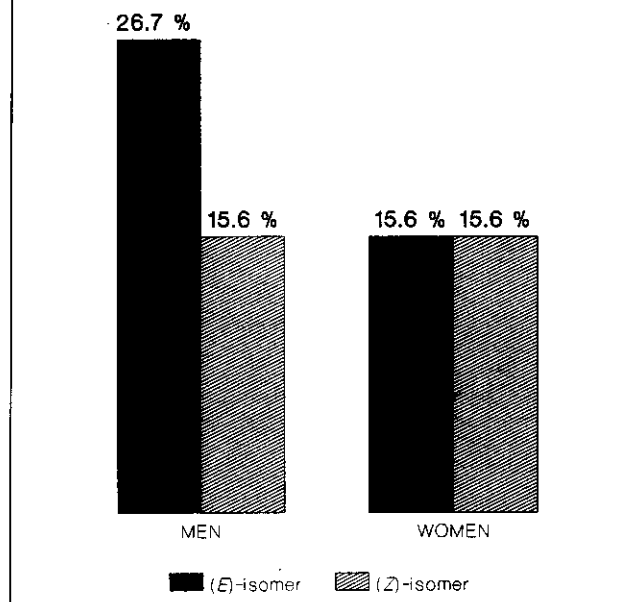


Figure 3. Percent specific anosmia for (E)- and (Z)-3-methyl-2-hexenoic acids (90 panelists)

concentration for each dilution step was calculated using a formula, which took into consideration the flow rates and actual headspace measurement of the sniffing airstream taken at the end of the sensory trials. The values throughout this article are given in ppb corresponding to 10^{-9} g/L. Experimentally measured values were treated according to ASTM (formerly American Society for Testing and Materials) standard practice E679 (ASTM, 1979). A best estimate detection threshold value for an individual subject was calculated using dilution factors (Steps 1,2,3, etc. corresponding to dilution factors 2,4,8, etc.). The threshold was calculated as the geometric mean of the dilution factor of criterion performance and the next lowest dilution factor.

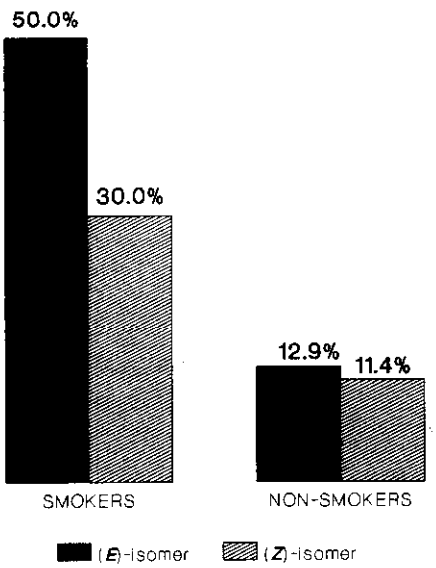
Subjects that failed to detect the (E)- or the (Z)-isomer or both at the highest concentration (step 9 = 194.3 ppb in air for (E)-isomer, and 1,597.4 ppb for (Z)-isomer) participated in a second test starting at even a higher concentration.[12] Those, who failed to detect either of the acids at level 9, could not detect them also at higher concentrations. The panel distribution histograms including smellers and anosmics for both the acids are shown in Figure 2. The subjects who were unable to achieve threshold performance at the highest deliverable vapor concentration, and could also not smell the odorant on a blotter, merited the label "specific anosmics." The relatively insensitive subjects with rather high thresholds for the acids were still considered as smellers.

A total of 90 subjects—45 men and 45 women—with a mean age of 36 years (range 5 to 56) participated in the test. All were Givaudan-Roure employees residing in France. Subjects were screened for active head colds.



	Number of panelists	Number of anosmics		
		(E)-isomer	(Z)-isomer	Common
Men	45	12	7	4
Women	45	7	7	3
Total	**90**	**19**	**14**	**7**

Figure 4. Percent specific anosmia in men and women for (E)- and (Z)-3-methyl-2-hexenoic acids



	Number of panelists	Number of anosmics		
		(E)-isomer	(Z)-isomer	Common
Smokers	20	10	6	4
Non-smokers	70	9	8	3
Total	**90**	**19**	**14**	**7**

Figure 5. Percent specific anosmia for (E)- and (Z)-3-methyl-2-hexenoic acids for smoking and non-smoking panelists

Results and Discussion

Out of the 90 subjects 19 (21.1%) were anosmic to (E)- and 14 (15.6%) to (Z)-3-methyl-2-hexenoic acid (Figure 3). Seven subjects (7.8%) were anosmic to both compounds. Thus on the basis of joint probabilities (i.e., 0.211 x 0.090 x 100 = 1.9%), the proportion of double anosmics was found to bc approximately four times that of the expected value. This gave an indication that the two isomers most likely shared a common mechanism of olfactory information processing, or even a common receptor site.

Our study showed that a high level of anosmia did exist for these two isomeric axillary acids. Previous threshold studies into androstenone and Galaxolide had confirmed that there was a good correlation between our panel data on the olfactometer and the *National Geographic* results for France.[6] Therefore, this set of data can be regarded as representative for France.

The sex-specific anosmia rates in our sample population were 26.7% for men and 15.6% for women for the (E)-isomer and 15.6% for both men and women for the (Z)-isomer (Figure 4). In smokers, a significantly high level of anosmia was found for both the isomers (Figure 5).

The mean threshold for the (E)- and (Z)- acids, calcu-

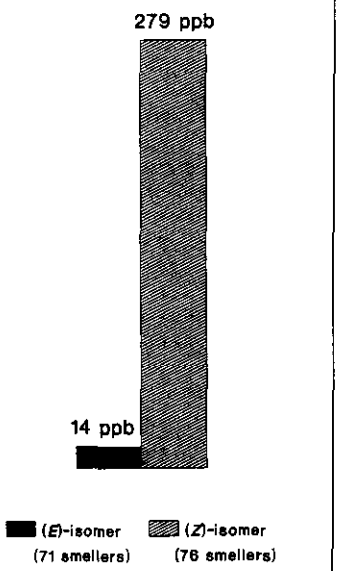
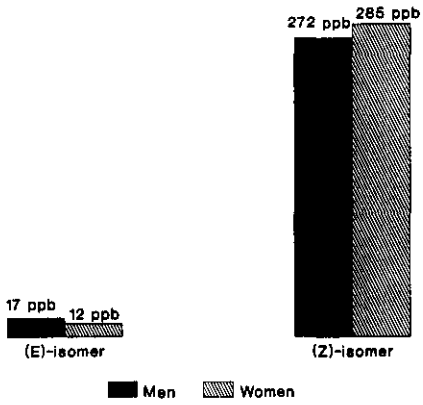


Figure 6. Threshold concentration for (E)- and (Z)-3-methyl-2-hexenoic acids (in ppb)



	Number of smellers	
	(E)-isomer	(Z)-isomer
Men	*38*	*38*
Women	33	38
Total	**71**	**76**

Figure 7. Threshold concentration sex *differences for* (E)- *and* (Z)-3-methyl-2-hexenoic acids

lated for 71 and 76 smellers respectively, was 13.8 and 278.7 ppb (Figure 6). The average threshold concentration for the (E)-isomer was, therefore, approximately 20 times lower than that for the (Z)-isomer. There were no apparent sex differences in the thresholds for either of the acids (Figure 7).

Of the two acids only the (E)-isomer was confirmed to be one of the important contributors to the underarm odor in terms of quality (our panel assessment), threshold concentration (Figure 6) and the actual amount present in the axilla according to Preti.[3] Therefore more emphasis was placed on the analysis of results obtained from the (E)- rather than the (Z)-isomer.

There is a great deal of evidence today that as humans grow older, their olfactory perception deteriorates, and perceived odor intensity decreases independent of their state of health.[12-15] Studies on age-influenced olfaction so far have mainly dealt with environmental odors and those originating from foods in the mouth with the exception of the work on androstenone.[15-17] The results of our study indicate that independent of sex, there is a gradual and rather steep age-related increase in anosmia with regard to the (E)-isomer (Figure 8)

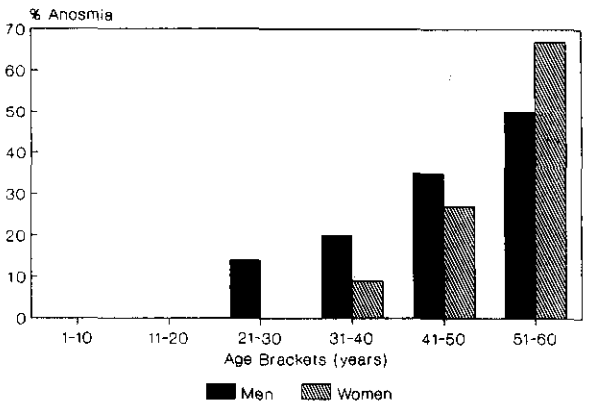
In the analyses of the results of the *National Geographic* Survey, Wysocki and Gilbert found that on a percentage change basis, the decline for androstenone was greater than that for any other compound.[15] We now find that the rate of decline for (E)-3-methyl-2-hexenoic acid is *twice* that of androstenone.

Since the present study was carried out on Givaudan-Roure employees, the panel list did not include those above the age of 60. However, within the age bracket of 50-60, 4 out of 7 (57%) were found to be anosmic, which is a very high ratio for a comparatively young population. Up to the age of 30, only 1 out of 27 (3.7%) was found to be anosmic for the (E)-isomer. Anosmia began at an earlier age in men than in women (Figure 8).

We believe that the subjective and varied results obtained in axillary odor panel tests are mainly due to two factors:

- the variety of compounds which contribute to the characteristic odor, and
- the considerably different threshold perceptions of these compounds by the participating individuals.

Labows agrees with the latter view and suggests that panel members may have different perceptions of the odor and should be screened for anosmia.[1]



Age (years)	Number of panelists		Number of anosmics	
	Men	Women	Men	Women
1-10	2	2	0	0
11-20	2	2	0	0
21-30	7	12	1	0
31-40	10	11	2	1
41-50	20	15	7	4
51-60	4	3	2	2
Total	**45**	**45**	**12**	**7**

Figure 8. Percent specific anosmia at different age brackets in men and women for (E)-3-methyl-2-hexenoic acid

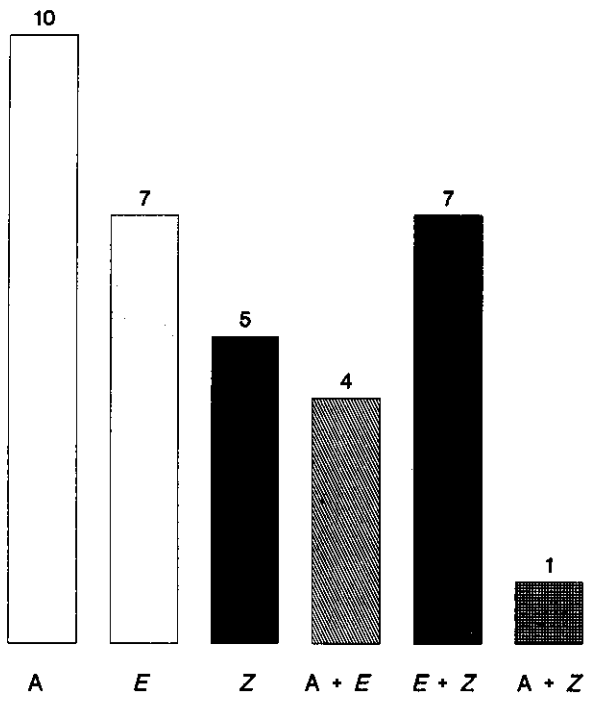


Figure 9. The distribution of total anosmics (34) for 5-α-androst-16-en-one (A), and (E)- and (Z)-3-methyl-2-hexenoic acids (out of a common panel population of 69)

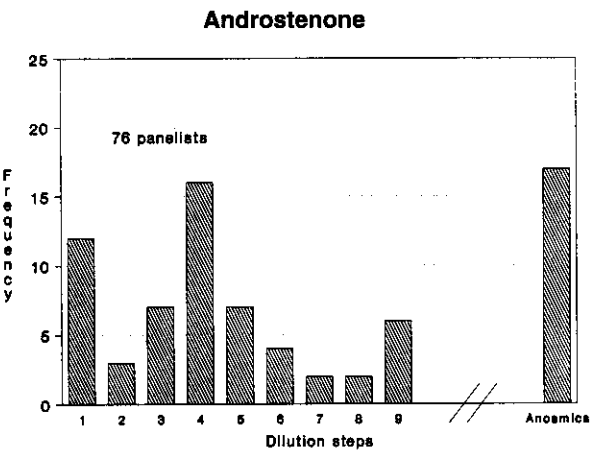


Figure 10. Androstenone threshold distribution curves

Comparison of the threshold panel list of (E)-3-methyl-2-hexenoic acid with a previous study carried out on androstenone[6] revealed that out of the total of 76 common participants, 20 (26.3%) were anosmic to (E)-3-methyl-2-hexenoic acid and 17 (22.4%) to androstenone, 4 (5.3%) being common to both. Hence there were 33 who were anosmic to one or the other compound representing 43.4% of the overall panel population.

The results of the threshold study for (Z)-3-methyl-2-hexenoic acid were also taken into consideration. In a common panel of 69 for (E)- and (Z)- acids and androstenone, 34 were found to be anosmic to either one or two of these compounds (Figure 9), representing a total panel population of 49%.

The high level of anosmia, the great variation of the odor description, and the threshold concentration of the smellers (Figures 2 and 10) clearly explain why inconclusive and often non-representative results are obtained from a high percentage of underarm panel tests. However, this does not mean that a panel should consist of those who have low threshold levels for all the important constituents of axillary odor, but rather that the panel should be representative of the population in general. What is needed is a good knowledge of the particular threshold ability of each panelist or the odor judge for the various components. This data should be obtained before commencing panel studies.

Conclusion

A significantly high level of anosmia for (E)- and (Z)-3-methyl-2-hexenoic acids, which are the key components of human axillary malodor, was found to exist among 90 subjects (45 men and 45 women). A distinct steep increase in anosmia with age was observed for the (E)-acid.

The correlation of these threshold data for (E)- and (Z)-3-methyl-2-hexenoic acids with those of androstenone for common 69 panelists revealed a very high percentage of anosmia (49%) to either one, two or three of these compounds.

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Address correspondence to Ahmet E Baydar, Givaudan-Roure, Centre de Recherche, 34 Chemin de la Madeleine, BP 72, 06332, Grasse, France.

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