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Communication

Limonene in Citrus: A String of Unchecked Literature Citings?

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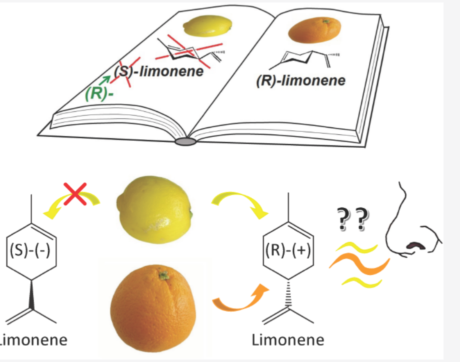
ABSTRACT: In organic chemistry textbooks (*S*)-(−)-limonene, (−)-limonene or L-limonene, is often given credit for the smell of lemons, while the *R*-analogue, (+)-limonene or D-limonene, is credited with that of oranges. Results from two odor tests revealed that few persons in the test associated (*R*)-(+)-limonene with oranges and (*S*)-(−)-limonene with lemons, when these compounds were of analytical grade. Quality and composition of standard compounds and mixtures used in these olfactory tests were corroborated by gas chromatographic analyses. The statement of (*R*)-(+)-limonene being responsible for orange odor and (*S*)-(−)-limonene for the lemon odor apparently stems from an often quoted article from 1971. This investigation was a lesson for both our students and us. Textbooks in organic chemistry that still promote (*R*)-(+)- and (*S*)-(−)-limonene as, respectively, the "orange" and "lemon" smell ingredient should be corrected.



KEYWORDS: *General Public, Organic Chemistry, Misconceptions/Discrepant Events, Textbooks/Reference Books, Chirality/Optical Activity, Natural Products, Stereochemistry, Enantiomers*

INTRODUCTION

Citrus is the largest fruit crop in the world yielding 98.3 million tons per year by February 2018/2019.[1] Juice extraction utilizes around half;[2] the rest, including the peel, segment membrane, and other byproducts, is considered as waste[2,3] and represents an economical and environmental problem if it is not used.[4,5] However, from the peel, one can isolate essential oils, which mainly contain the monoterpene, (*R*)-(+)-limonene (Figure 1). On a commercial scale, this is often achieved by a cold-extraction procedure consisting of three basic steps: crushing of the oil sacs in the peel to release the oil, using a stream of water to transport the oil, and finally centrifuging to separate the water and oil.[6] This process can be undertaken on the whole fruit in a continuous process suitable for large scale facilities, or by depulping the fruit prior to using the peel in a batch process more suitable to smaller scale productions. The latter method yields a higher quality oil.[7]

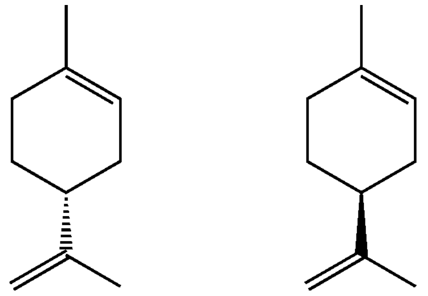


Figure 1. (*R*)-(+)-Limonene (left) and (*S*)-(−)-limonene (right).

In the laboratory citrus, essential oil can be extracted by steam-distillation, commonly in a Clevenger apparatus, or by cold-pressing. If distilled under reduced pressure, degradation of heat-labile components is diminished. More or less polar organic solvents as well as liquid carbon dioxide are used as an extraction medium of the oil.[8]

From the essential oil, (*R*)-(+)-limonene itself can be isolated through distillation under reduced pressure. Care must be taken to remove low boiling point components first. Myrcene and octanol, having boiling points similar to that of limonene, will nevertheless be distilled together with limonene. If a very high purity limonene is required, dilute NaOH or a carbonyl addition agent (e.g., hydroxylamine hydrochloride) can be used to remove aldehydes during distillation. This can yield limonene of 99.5% purity.[9] However, if not properly stored, this purified (*R*)-(+)-limonene will readily oxidize in air, leading to a lower purity of approximately 95−96%.[9]

(*S*)-(−)-Limonene (Figure 1), on the other hand, is almost nonpresent in citrus fruits. However, in the oil of citronella (*Cymbopogan nardus*) and lemongrass (*Cymbopogan citratus*), (*S*)-(−)-limonene is the major enantiomer,[10] although in modest amounts (less than 3%).[11−13] (*S*)-(−)-Limonene sold from Merck/Sigma-Aldrich is, according to Merck's Technical

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Service Scientist, synthetically made from α-pinene, acetic acid, and formamide with a distillation as a workup.[14]

The isolation of citrus oil (which often is simply referred to as limonene) from citrus peels is still a common student experiment in organic chemistry. Even in this *Journal* a range of experiments have been reported in the past decade.[4,15−21] As citrus production is so widespread and the isolation of essential oils and (*R*)-(+)-limonene therefrom so common, it is surprising that many organic chemistry textbooks, including the one used in our institution,[22] still credit (*S*)-(−)-limonene for the smell of lemons and the (*R*)-(+)-limonene for that of oranges. A consequence is that many chemists also believe this, including us before we embarked on a project on optical rotation,[23] where essential oils were tested. Then, we found that both orange and lemon oils rotate plane polarized light dextrorotatory, albeit the lemon oil rotates it to a lesser extent. This finding spurred an investigation including odor tests, chiral and achiral GC analyses, and a search for the origin of the statement that (*S*)-(−)-limonene is the trigger of lemon odor and that (*R*)-(+)-limonene, which is the dominant ingredients in orange oil (more than 98%), is the trigger of orange odor.

■ EXPERIMENTAL SECTION

Odor Tests

Two odor tests were undertaken, one before the pandemic of COVID-19 (in 2019) and the second during the pandemic (in 2020). They differed in the quality of the (*R*)-(+)-limonene and (*S*)-(−)-limonene, the matrix used to dissolve the odor compounds, and the procedure of the sniffing test. For both tests, the odor samples were presented randomly to the participants who were asked to note immediately after each odor test which citrus fruit, if any, they associated with the smell.

First Odor Test. Test solutions were made from (*R*)-(+)-limonene (technical grade) and (*S*)-(−)-limonene (*purum* grade), both from Fluka, as well as natural lemon oil and natural sweet orange oil, both from Carl Roth. Each sample (5−10 drops) was added to 15 mL of water which was then strongly shaken, before 1−2 drops of these emulsions were diluted with 10 mL water and transferred to 20 mL stopped bottles.

The participants were instructed to lift the vial about 5 cm from their nose and use a hand to fan the odor vapor toward their nose, before breathing in. The samples were shaken immediately before each odor test.

Second Odor Test. Eight odor tests were made by adding 375 μL of natural lemon oil, 375 μL of natural sweet orange oil, 375 μL of (*R*)-(+)-limonene, 375 μL of (*S*)-(−)-limonene, and 315 μL of citral to individual pumping pens (Molotow Empty pumping pens)[24] containing 3.0 mL of triacetin. A reference solution contained only 3.0 mL of triacetin. The oils were purchased from Carl Roth, and the other compounds were analytical standards and purchased from Sigma-Aldrich. The preparation of the test solutions was an adaptation from the literature.[25−27] As the orange and lemon oils are yellowish, even at this concentration, 3 drops of dilute (1:20) yellow food coloring (no smell checked by us) was added to the white felt tips of the pens, and the transparent parts of the pens were covered with white tape. The components of citral are presented in Figure 2. The concentrations were chosen from what a few people piloting the experiment found to be of a distinct intensity.

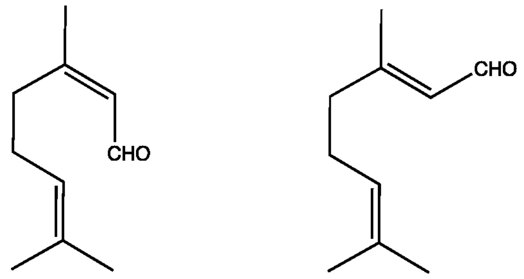

Figure 2. Citral is a mixture of neral (left) and geranial (right).

Given COVID-19 pandemic restrictions, the participants were not allowed to hold the individual pens and sniff directly from them. Instead, one drop of a test solution was transferred, by us, from the pumping pen to a brown coffee filter paper strip (approximately 2 cm × 6 cm) immediately before a test person would "sniff" the paper. In order to neutralize between the individual tests and to avoid temporary smell fatigue, the participants were asked to sniff their own body odor in the elbow joint.[28] The tests were undertaken in various rooms but all with little other smells.

A precondition for the tests was the assumption that the participants were familiar with citrus odors in general, and, in particular, with those of lemon and orange. For the first odor test (before the pandemic), 48 healthy persons participated, both students and staff, with their age ranging from 20 to 50 with an average around 25 years. In the second test, 49 participated, with an age range from 12 to 80 (average around 50 years). After the odor test had been completed, we explained the textbook statement to the participants.

GC Analyses

Chiral Analyses. The samples were dissolved at a concentration of 0.5% (v/v) in analytical grade *n*-hexane. A gas chromatography/mass spectrometry system consisting of a 7890A gas chromatograph connected to a linear quadrupole mass spectrometer (5975 Series MSD; Agilent Technologies) was equipped with a chiral Cyclosil-B column (length 30 m, i.d. 0.250 mm, film thickness 0.250 μm; Agilent Technologies). The separation was performed by temperature-programmed analysis and detection by electron ionization mass spectrometry in full-scan mode. For further details, see the Supporting Information (SI).

Nonchiral Analyses. The oils and the limonenes were dissolved at a concentration of about 10% (v/v) in analytical grade *n*-hexane, and the citral sample was diluted further by about 100-fold. GC analyses with a flame ionization detector (GC-FID) were performed on a Shimadzu 2010A gas chromatograph equipped with a TR-5 column (length 30 m, i.d. 0.25 mm, film thickness 0.25 μm; Thermo Scientific) with data collected and processed by GCSolution Software (Shimadzu). Peak areas were used for quantitation without further calibration. For further details, see the SI.

■ HAZARDS

Hexane used in the GC samples is highly flammable and harmful if ingested, inhaled, or in contact with skin. Work in a fume hood using suitable gloves and eye protection. The odor

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Table 1. Comparative Results from Odor Tests

Sample Compound	Supplier Purity Grade Designation	Odor Tests	Responses, %, Associating the Sample with Scent Categories[a]					
			Orange/ Mandarin/ Clementine	Grapefruit	Lemon or Lime	Citrus, General	Various Other Scent Perceptions/Do Not Know	No Response
Orange oil	*b*	1[c] and 2[d]	68	2	18	8	4	0
Lemon oil	*b*	1[c] and 2[d]	13	3	74	9	1	0
(*R*)-(+)-Limonene	Technical	1[c]	50	8	22	1	19	0
(*R*)-(+)-Limonene	Analytical	2[d,e]	13	5	9	13	55	4
(*S*)-(−)-Limonene	*Purum*	1[c]	16	14	18	5	45	2
(*S*)-(−)-Limonene	Analytical	2[d]	8	13	16	11	52	0
Citral[f]	Analytical	2[d]	9	0	49	28	12	2

[a]Test subjects were asked to respond to this question upon smelling a sample: "Which citrus fruit, if any, do you associate with the sample?" Answers were weighted: two suggestions for one sample gives 0.5 for each. See the Experimental Section for further details and the Supporting Information (Tables SI-1 and SI-2) for results from the individual tests. [b]Not available. [c]Odor test 1 used a water matrix; $N = 48$. [d]Odor test 2 used a triacetin matrix; $N = 49$. [e]Percentage sum is 99 rather than 100 due to rounding. [f]Citral is a mixture of neral and geranial.

Table 2. Comparison of the Quality of the Compounds Used in Tests 1 and 2

Test	Compound	Supplier Purity Grade Designation	Purity According to Supplier,[a] %	Total Limonene Content,[b] %	Enantiomeric Excess,[c] %
1	(*R*)-(+)-Limonene	Technical	~90	92.1	>99.9
	(*S*)-(−)-Limonene	*Purum*	≥95.0	95.7	80.3
2	(*R*)-(+)-Limonene	Analytical	≥99.0	99.2	>99.9
	(*S*)-(−)-Limonene	Analytical	≥99.0	99.8	82.1
1 and 2	Lemon oil	*d*	*d*	68.0	>99.9
1 and 2	Orange oil	*d*	*d*	90.5	>99.9

[a]Total limonene content as sum of enantiomers according to the supplier. [b]Determined by GC-FID; see Figure 3, and Figures SI-2 and SI-3 in the Supporting Information. [c]Determined by chiral GC−MS analyses; see Figures SI-1, SI-4, and SI-5 in the Supporting Information. [d]Not available.

samples should be prepared in a ventilated hood according to HSE specifications.

■ RESULTS AND DISCUSSION

Odor perception is a complex process, where character, threshold, and intensity of the compound are among the factors that influence a person's perceptions. The techniques used for the odor measurements and the language, memory, and emotions of the tester will also have an effect.[29] When the aim of a test is recognition and characterization, it is usually professional analysts that participate.[28] In our two tests, the participants were staff, students, pupils, and persons from all walks of life. Our results must therefore be judged accordingly. The participants would sometimes suggest more than one citrus fruit for a particular test compound or leave a blank. In the cases when they suggested more than one alternative, these were weighted. Results from the two tests are combined in Table 1, and the individual test results are found in the SI: Tables SI-1 and SI-2. These results show that there was no overall agreement between the participants. This may not be surprising given the problems associated with such tests.[29] However, the fact that 72% (35 of 49 persons) indicated no odor from triacetin (Table SI-2), a compound generally considered odorless, was reassuring, as to how the tests were undertaken. Furthermore, there were also clear trends as explained below.

Oils

A majority of the test participants associated the odor of an oil with the citrus fruit it had been isolated from in both tests: orange oil with orange/clementine/mandarin, 68% (Table 1); lemon oil with lemon/lime, 74% (Table 1). Both chiral and nonchiral GC analyses were undertaken in order to understand why the odors were perceived differently.

Chiral GC analyses (Table 2 and Figure SI-1) revealed an enantiomeric excess (ee) for (*R*)-(+)-limonene of more than 99.9% in both oils, which agrees with previous findings.[30] The difference in odor perception between the two oils can therefore hardly be explained by different enantiomers of limonene.

Nonchiral GC analyses show that both oils contain a range of minor compounds in addition to the dominant limonene (Figure 3, Figures SI-2 and SI-3). Orange oil contains about 90.5% limonene, and lemon oil contains about 68.0%; the latter also contains β-pinene (~16%) and γ-terpinene (~12%), all in accordance with previous findings.[31] The presence of many compounds in small amounts is typical for natural products. For example, in laboratory distilled sweet orange oils, more than 80 components have been identified, ranging from terpenes, alcohols, aldehydes, esters, ketones, to ethers.[32] In our analysis of orange oil, approximately 120 peaks larger than 30 ppm of the limonene peak were detected. Somewhere in this "forest" of minor components, the ones contributing to the characteristic odor are most likely hidden. We will return to this below.

(*R*)-(+)-Limonene

The results for (*R*)-(+)-limonene diverted in the two odor tests. In test 1, 50% associated (*R*)-(+)-limonene with orange/mandarin/clementine, while in test 2 this was reduced to 13% (Table 1). When all types of citrus associations are summed, the corresponding numbers were, respectively, 81% and 40%. Although different procedures were used in the two tests, this did not affect the results for the oils (see above) or for (*S*)-(−)-limonene (see below).

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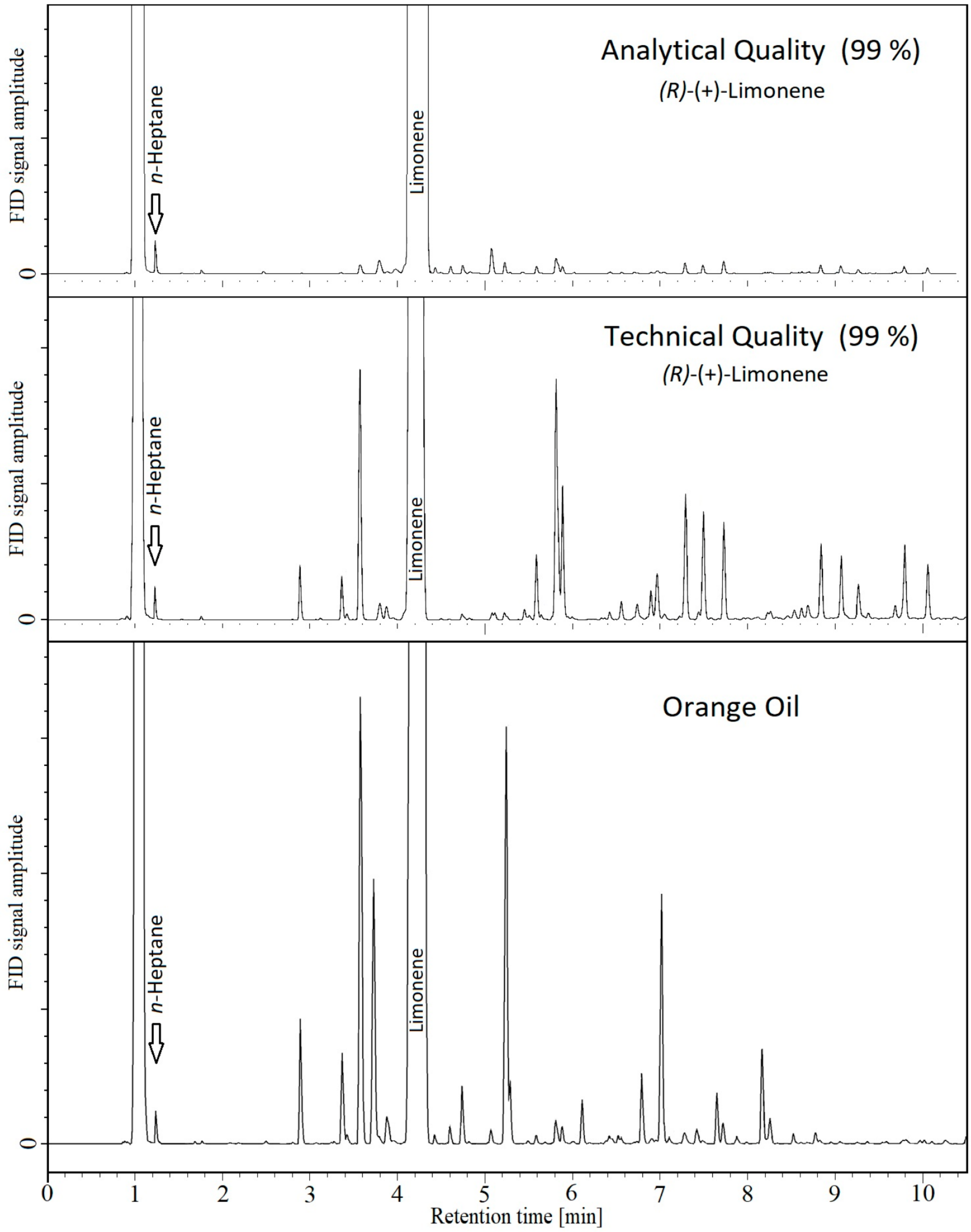

Figure 3. GC-FID chromatograms (nonchiral) of analytical (top) and technical grade (middle) (*R*)-(+)-limonene as well as orange oil (bottom) analyzed as 10% solutions in *n*-hexane. Relative intensities can be compared to the *n*-heptane peak (a typical contaminant in the hexane used as solvent) at 1.23 min acting as an internal standard here. Note that the amount of impurities in the technical grade is higher than in the analytical grade (*R*)-(+)-limonene, and that several peaks found in the technical grade (*R*)-(+)-limonene are also present in the orange oil. Among the minor compounds are the ones responsible for the orange odor.

So, how can we explain the differences in odor associations for (*R*)-(+)-limonene in the two tests? The main difference between test 1 and test 2 was the quality of the (*R*)-(+)-limonene used. The enantiomeric purity was consistent with more than 99.9% ee in both (see Figure SI-4), but the purity of the technical grade used in test 1 was 92.1% compared to 99.2% for the analytical grade used in test 2 (Table 2). Since (*R*)-(+)-limonene is produced by distillation from orange oil, impurities mainly consist of other components from the oil. Figure 3 displays GC chromatograms of analytical and technical grade (*R*)-(+)-limonene as well as orange oil at approximately the same scale. From these chromatograms, we can see that the amounts of impurities in the technical grade are around 10 times higher those in the analytical grade, actually 7.9% for the technical grade and 0.8% for the analytical grade (*R*)-(+)-limonene (see also Table 2). Furthermore, from Figure 3, we can also see that several of the peaks from the technical grade (*R*)-(+)-limonene correspond to peaks in orange oil.

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Since an increase in the purity of (*R*)-(+)-limonene and a decrease in impurities (from technical grade used in test 1 to analytical grade used in test 2) resulted in no preference for associating (*R*)-(+)-limonene with orange oil, it is unlikely that (*R*)-(+)-limonene conveys the orange odor. However, this result may explain why some associated (*R*)-(+)-limonene with orange oil: The cruder the (*R*)-(+)-limonene is, the more impurities it contains, and the more it will smell like orange oil. For the analytical grade (*R*)-(+)-limonene, the amounts of impurities were under the orange odor threshold for the testers. The textbook's statement *that (R)-(+)-limonene is the compound triggering orange odor*[22] *does therefore not hold true in this investigation.*

(*S*)-(−)-Limonene

The results for (*S*)-(−)-limonene did not divert in the two odor tests. Around half of the participants in both tests (Table 1) did not associate (*S*)-(−)-limonene (of any quality) with a citrus fruit (47% for *purum* grade in test 1 and 52% for analytical grade in test 2). Furthermore, the citrus suggestions given were arbitrarily distributed, with, respectively, 18% and 16% favoring lemon/lime, in test 1 and test 2. The textbook's statement *that (S)-(−)-limonene is the compound triggering lemon odor*[22] *does therefore not hold true in this investigation.* Nonchiral GC analyses of the (*S*)-(−)-limonenes (Figure SI-2) support these results, as none of the chromatograms of the (*S*)-(−)-limonene standards reflect those of the lemon oil, except for the main limonene peak. This should not be a surprise given that (*S*)-(−)-limonene is synthetically made from pinene.[14] The minor components of the oils convey the citrus associations, and they are not present in the (*S*)-(−)-limonene of whatever purity (Figure SI-2).

Citral

Citral (consisting of neral and geranial), which has been suggested as *the lemon odor trigger*, was found in the chromatograms of both lemon (Figure SI-2) and orange oil, but not in the standards, and in 10 times higher concentration in the lemon oil (1.4% and 2.3%, respectively, for neral and geranial) than in the orange oil. The participants (Table 1) seemed to associate citral with citrus (86%), and in particular lemon/lime (49%); thus, our result is in agreement with some previous findings.[28,33,34]

Additional Remarks

It may be worthwhile noting (for us educators) that this "forest" of tiny peaks as shown in Figure 3 and Figure SI-3 (that hide some important odor triggers) could easily be overlooked if, for example, compounds of less than around 1% of limonene were suppressed by the instrumentation or in the data analysis, a situation that could likely occur during a GC analyses after a student isolation of citrus oil from citrus peel.

A surprising observation is that the people testing, including some seasoned employees, became animated: smiling and showing eagerness to participate in this odor test. It must be admitted that some were a bit upset when being told afterward that there were no "correct" answers, thus not being able to achieve a score for their "nose". Connecting chemistry to the olfactory senses was apparently an exciting event, an experience also reported previously.[35,36] Referring to the textbook's statement, we could observe that the students felt uncomfortable. We believe that the textbook with its size, perfect print, and glossy figures is a powerful authority that students find difficult to question. The employees were clearly less surprised.

Literature: Limonene and Lemon Odor

As mentioned, there is no simple correlation between odor perception and the amount of a compound. Given the number of components present in lemon and orange oils, to single out one or a few responsible for the particular odor is definitely a challenge. Ohloff reported geranyl and neryl acetate as responsible for the fruity note; (−)-β-pinene together with (−)-terpinen-4-ol for the green, peely odor; *trans*-α-bergamotene for the basic peppery aroma; and the combination of citral (geranial and neral) and C_7−C_{13} alkanals for the odor character of lemon oil.[34] Bauer et al. also reported citral amounting to around 3%, as the compounds giving the characteristic lemon odor,[33] and so do Simat et al.[28] Our sniffing test appears to be in agreement with these reports, as citral has a high number of lemon/lime associations (49%, Table 1). None of the above references point to limonene for the lemon odor, a result also in agreement with the findings in our odor tests. In fact, Sell noticed that the purer the limonene is, the less odor it has,[37] whereas Chiralt et al. (2002) simply state that limonene is odorless at high purity.[38] On the other hand, Boelens et al., in *Perfumer & Flavorist*, refer to "very pure" (*R*)-(+)- and (*S*)-(−)-limonene both having a harsh, terpene-like odor, while the (*R*)-(+)-enantiomer has a fresh, slightly citrusy note and the (*S*)-(−)-enantiomer a turpentine-like note.[39] From the literature, it is therefore difficult to know what to believe when it comes to the odor of limonene.

Origin of Myth: From Where Does It Stem?

So, where does the myth, that (*S*)-(−)-limonene is the main component in lemon peel and that (*S*)-(−)-limonene is the molecule responsible for lemon smell, originate from? And from where does the myth stem, that (*R*)-(+)- limonene is the main component responsible for the orange odor? It is not only the textbook used in our institution that states that "(+) limonene is primarily responsible for the smell of oranges and (−) limonene of lemons".[22] In two experimental organic chemistry books, we find the following remarks: "Isomer (*R*)- has the characteristic smell of oranges, while the (*S*)- smells like lemons",[40] and "the essential oil of sweet oranges consists of about 95% (*R*)- limonene, whereas lemon peel contains (*S*)-limonene".[41] Also, Clayden et al. write that "The smells of orange and lemon differ in being the left- and right-handed versions of the same molecule, limonene. (*R*)-(+)-limonene smells rounded and orangey; (*S*)-(−)-limonene is sharp and lemony."[42] Even in a recent article in this *Journal* the (−)-isomer is being associated with a "lemony odor".[43] This paper refers to an article entitled *The Nose as a Stereochemist. Enantiomers and Odor*,[44] where the "Proof for Enantioselectivity in Odor" is traced back to 1971, when three different groups independently investigated the odor sensation for the enantiomers of carvone.[45−47] In one of the these studies, namely, that by Friedman and Miller,[45] published in *Science*, there is a table connecting (*R*)-(+)-limonene with orange odor and (*S*)-(−)-limonene with lemon odor, in addition to an explicit comment on (*S*)-(−)-limonene being associated with lemon odor, from where the origin of the misconception may stem. "Incorrect odor descriptions due to trace impurities can be perpetuated in the literature by subsequent authors citing one error" writes Sell, and continues, "For instance, it is often reported that (*R*)-(+)-limonene elicits an orange odor, whereas its enantiomer (*S*)-(−)-limonene elicits one of lemon. Where citations trails exist, they usually lead back to the paper of Friedman and Miller. Whilst this chapter describes sterling

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research on the carvone enantiomers, it also includes a comment that (*S*)-(−)-limonene elicits a lemon odor percept. Inspection of the experimental detail reveals that the sample of (*S*)-(−)-limonene was extracted from lemons. However, lemons, like all citrus fruit, produce (*R*)-(+)-limonene, and so one is forced to conclude that the lemon odor elicited by Friedman and Miller's sample was due to contamination by traces of citral."[29]

The misconception that the odor of (*S*)-(−)-limonene is associated with lemons (and (*R*)-(+)-limonene with oranges) is not written in all organic chemistry textbooks. McMurray, for example, notes that the odor of (*R*)-(+)-limonene is associated with both lemon and orange odors, and the (*S*)-(−)-enantiomer with pine trees,[48] and Vollhardt et al. (2003) associate the (*R*)-(+)-enantiomer with oranges and the (*S*)-(−)-enantiomer with cones of fir trees.[49] Other organic chemistry textbooks refer to the two enantiomers of carvone, and not limonene, to describe the impact of stereochemistry on odor perception where (*R*)-(−)-carvone and (*S*)-(+)-carvone, respectively, are associated with the smell of spearmint leaves and caraway seeds.[50−53] A video from ACS, *The Limonene Myth*, has also addressed the misconception of (*R*)-(+)- and (*S*)-(−)-limonene as the orange/lemon odor.[54]

Another finding from the literature search was that, "outside" the organic chemistry textbooks area, the notion of all citrus fruits containing predominantly (*R*)-(+)-limonene has been an accepted fact for a long time.[10,55]

■ CONCLUSION

The lessons from this investigation were the following:

1. There is hardly any (*S*)-(−)-limonene in lemons.
2. (*S*)-(−)-Limonene does not convey lemon odor.
3. (*R*)-(+)-Limonene does not convey orange odor.
4. Orange oil consists mainly of (*R*)-(+)-limonene and, in addition, more than 100 components in quantities less than 2%. Among these are those that evoke orange odor.
5. Lemon oil consists mainly of (*R*)-(+)-limonene, β-pinene, and γ-terpinene and, in addition, more than 100 minor components. Among the latter is citral (neral and geranial) that contributes to lemon odor.
6. A statement in a recognized textbook can be challenged.
7. A comment in a strong impact journal can be repeated without question for decades.
8. Knowledge from one research field appears to have problems diffusing into another.

Some of these findings are not very surprising, especially in retrospect. Still, it was a lesson for all of us. Hopefully, some textbook authors will also remove the suggestion/statement of (*S*)-(−)-limonene being the lemon odor compound and (*R*)-(+)-limonene the orange odor compound and instead caution the simple relation between structure and odor.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.1c00363.

Experimental conditions and chromatograms (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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