yhemoglobin to benzene and molecular nitrogen which also supports the above conclusion.

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Comparison of Extracted Peel Oil Composition and Juice Flavor for Rough Lemon, Persian Lime, and a Lemon–Lime Cross

Philip E. Shaw* and Charles W. Wilson, III

A hybrid (lem'n lime) from rough lemon and Persian lime fruit was evaluated. Composition of its peel oil extract was compared with that of each parent. Flavor of hybrid juice was compared with flavors of good quality lemon and lime juices. Lem'n lime peel oil extract differed from that of either parent, primarily by the presence of thymol and thymol methyl ether and the absence of neral and geranial. These differences might explain its strong, non-citrus-type aroma. A taste panel judged the juice as lemon–lime in character and mild in flavor. Thus, this lemon–lime cross will probably be an acceptable alternative to lemon–lime mixtures only when mild flavoring properties are desired.

Lemon and lime essential oils bring a premium price in flavor markets because of their desirable flavoring properties in many products such as juice drinks and carbonated beverages. A hybrid from a cross between lemon and lime with essential oil and juice having combined flavoring properties of lemon and lime would be a potentially valuable new commodity.

A lemon–lime hybrid of *Citrus jambhiri* Lush. × *Citrus latifolia* Tanaka C.V. Persian (rough lemon and Persian lime) has been developed recently and designated "lem'n lime" (Lucerne, 1973). One reason for selecting the rough lemon parent was to develop a hybrid with mild flavoring properties (Lucerne, 1975). A comprehensive analytical and flavor study of the peel oil and juice from this hybrid was needed to help assess its potential. No systematic study has been carried out comparing the composition of peel oil from a citrus hybrid with composition of oils from those two parents. For many citrus hybrids, however, composition has been systematically compared between juice sac lipids of hybrids and of parents (Nagy and Nordby, 1972, 1974; Nordby and Nagy, 1974a,b). Tatum et al. (1974) compared flavonoids and coumarins in leaves between citrus hybrids and the parent plants. In those studies, hybrids generally showed lipid and flavonoid–coumarin compositions intermediate between the two parents, although some exceptions were noted.

We have evaluated a hybrid of rough lemon and Persian lime. We compared composition of a hexane extract of its peel oil with that for each parent and flavor of its juice with flavor of good quality lemon and lime juices.

Citrus and Subtropical Products Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Southern Region, Winter Haven, Florida 33880.

EXPERIMENTAL SECTION

Samples of lem'n lime and Persian lime fruit were obtained from the Citcross Corporation, Homestead, Fla. Rough lemon fruit was obtained from Mr. Don Bridges, Florida State Department of Agriculture, Winter Haven, Fla. Commercial frozen concentrated lemonade was purchased from a local market.

Peel Oil Extraction. Whole fruit (2.3 kg each of rough lemon, lem'n lime, and Persian lime) was grated on a kitchen grater to remove the flavedo (outer portion of the peel) which was immediately placed in a beaker and covered with hexane. The flavedo–solvent mixture was filtered through a Buchner funnel and the filtrate concentrated under reduced pressure at 45 °C to remove the bulk of the solvent. Residual oils were stored at 5 °C. Oil yield was about 2.2 ml/kg of fruit.

Analytical Procedures. Oils were qualitatively analzyed with a Varian Model 1400 gas–liquid chromatograph (GLC) coupled to a DuPont Model 21-490 mass spectrometer (MS) operated at 70 eV with a source temperature of 200 °C. A 0.10 in. i.d. × 20 ft stainless steel column packed with 10% DC-200 (methyl silicone) on 60–80 mesh Gas-Chrom P was used. The He flow rate was 30 ml/min, injection port temperature was 200 °C, and a flame ionization detector was used. Oil samples of 1–5 μl were injected. For rough lemon and Persian lime oils, temperature program was: 80 °C initially, then raised at 2 °C per min to 120 °C, and then 4 °C per min to 200 °C. For lem'n lime oil, temperature programming was 70 °C initially and then raised at 4 °C per min to 200 °C.

A Hewlett-Packard Model 7620 gas chromatograph with a thermal conductivity detector at 275 °C and on-column injection was used for isolating individual components for infrared (ir) analysis and for quantitative analysis. Samples for ir analysis were isolated by use of a 0.20 in. × 20 ft stainless steel column packed with 20% UCW-98 (silicone gum) on 60–80 mesh Gas-Chrom P. Helium flow rate was 100 ml/min and 40-μl samples of oil were injected. Temperature program was 70 °C initially and then raised at 4 °C per min to 200 °C. For quantitative analyses, 1–5-μl samples of oil were injected onto the 0.10 in. × 20 ft DC-200 column. The temperature program was 80 °C initially, raised at 2 °C per min to 120 °C and then at 4 °C per min to 200 °C. Integrations were performed by a Hewlett Packard Model 3380 digital integrator.

Juice Extraction. Samples (2.3 kg each) of lem'n lime and Persian lime fruit were each extracted with a hand reamer and the juice was pasteurized at 77 °C, canned, and stored at −21 °C. Juice yield was about 1000 ml from Persian limes and 700 ml from lem'n limes.

Flavor Evaluation. Ades (sweetened, citrus based beverages), for flavor evaluation by a ten-member panel, were prepared from juice samples by adjusting each sample to 11° Brix by adding water and sucrose. Sugar:acid ratios were adjusted to the same level for all samples. Panelists were presented one lemon, one lime, and one lem'n lime sample and asked to rate each sample on a scale of 1 to 5; 1 was totally lemon-like and 5 totally lime-like. For statistical calculations, the Student's t test was used (Miller and Freund, 1965). In a preliminary screening test, potential panelists who rated lemon and lime equal or could not distinguish between them, as indicated by reversing them, were not included in the panel.

RESULTS AND DISCUSSION

Data from qualitative and semiquantitative analyses of hexane extracts of peel oils from lem'n lime and its two parents appear in Table I. Rough lemon peel oil had not been analyzed previously, but expressed Persian lime oil had been quantitatively analyzed under different conditions (Shaw et al., 1971). The compounds in Table I are listed in order of their GLC retention times on the nonpolar DC-200 column. Several differences were noted between oil from lem'n lime and oil from either parent. The most notable difference is the presence of both thymol and thymol methyl ether in the hybrid and their absence in either parent.

Thymol has been identified previously in citrus peel oil only from tangerine, where it is considered an important flavor contributor (Kugler and Kovats, 1963), and from Meyer lemon (Moshonas et al., 1972). Wenzel et al. (1958) reported, in sweetened Meyer lemon juice, an off-flavor that was intensified when Meyer lemon peel oil was added. Moshonas et al. (1972) reported 6% thymol in Meyer lemon oil and ascribed the unique flavor of Meyer lemon oil to the presence of thymol and the lack of appreciable citral (neral and geranial). Since lem'n lime peel oil extract contains considerable thymol (2.94%) and no detectable amount of citral, use of the peel oil as a flavoring agent

Table I. Composition of Peel Oil Extracts from Rough Lemon, Lem'n Lime, and Persian Lime

	Amount present, area %		
Compound	Rough lemon	Lem'n lime	Persian lime
Hexane[a]	I[b]	I[b]	I[b]
α-Thujene		0.09	0.41
α-Pinene	0.46	0.36	1.52
β-Pinene	4.85[c]		11.4
Myrcene		2.05[d]	
Limonene	92.2	81.5	47.5
γ-Terpinene	0.08	6.76	14.3
Terpinolene			1.22
Linalool	0.24	1.38	0.09
Citronellal	0.27[d]	0.21[d]	
α-Terpineol	0.05	1.13[e]	1.05[d]
Decanal	0.22[d]		
Thymol methyl ether		0.49	
Neral	0.20[d]		4.63
Geranial	0.20		6.81
Neryl acetate	0.39[d]		2.49[d]
Geranyl acetate	0.05[d]		0.55[d]
Thymol		2.94[e]	
β-Elemene		0.62[e]	
β-Caryophyllene + α-bergamotene	0.27	0.89[d]	2.46
Humulene		0.27[d]	
β-Bisabolene	0.30	0.42[e]	4.02
Total unidentified	0.22	0.89	1.62

[a] Extraction solvent. [b] I = identified, but not quantitated. [c] Combination of β-pinene and myrcene. [d] Peak confirmed by enrichment on nonpolar column. [e] Infrared spectrum obtained from 0.25 in. × 20 ft nonpolar UCW-98 column.

would probably be limited to products such as mouthwashes where the medicinal-type flavor of thymol is acceptable.

Thymol methyl ether has been previously found in citrus only as a component of tangerine peel oil (Moshonas and Shaw, 1974). Its aroma is similar to but weaker than that of thymol and would be expected to contribute the same type of off-flavor in lemon and lime products.

Other differences between lem'n lime oil and those from the parent fruit are seen in Table I. Lem'n lime oil lacks detectable quantities of β-pinene, and neryl and geranyl acetates, all present in significant quantities in oils from both parents. Two sesquiterpenes, β-elemene and humulene, were found in lem'n lime oil, but not in either parent oil. One compound, linalool, known to have strong flavor effects in citrus (Ahmed, 1973) was present in much greater quantities in lem'n lime oil than in either parent oil.

Our hexane extracts of peel oils differ from commercial citrus essential oils in method of preparation, but they should be similar in composition. For example, composition of our hexane extract from Persian lime peel flavedo was quantitatively similar to that published for commercial Persian lime peel essential oil (Shaw et al., 1971). Neither rough lemon nor lem'n lime essential oil has been prepared commercially. We concluded, however, that our samples would be representative of composition of commercial essential oils from similar sources.

Flavor studies were carried out with lem'n lime juice to evaluate the strength and degree of lemon and lime character present. Since rough lemons are not processed commercially, commercial frozen concentrated lemonade was used to prepare the lemon sample. Juices from lem'n lime and Persian lime prepared in the laboratory were adjusted to the same °Brix:acid ratio as the lemonade sample. With a limited quantity of lem'n lime juice available, adjustment to equal °Brix:acid ratios was chosen

Table II. Taste Evaluation of Commercial Lemon, Persian Lime, and Lem'n Lime Juice Ades at a °Brix:Acid Ratio of 14.4

Juice ade	Statistics of panel rankings $\bar{X}$	S	Flavor characteristics
Commercial lemon	1.0	0	Good
Lem'n lime	2.9[a]	1.2	Mild
Persian lime	5.0	0	Good

[a] Significantly different at the 99.9% confidence level from either commercial lemon or Persian lime.

as the best means of obtaining ades of approximately equal strength. The three ades were used to evaluate the lemon–lime character of lem'n lime juice (see Table II). All panelists selected the lemon and lime ades correctly, and both are judged good flavors. Seven of 10 panelists judged lem'n lime to have combined lemon and lime flavor characteristics. This judgement is significantly different from each of the lemon and lime samples at the 99.9% confidence level (Miller and Freund, 1965).

Lem'n lime juice was markedly milder in flavor than either lemon or lime juices (see Table II) and some panelists detected an off-flavor that detracted from the observed lemon and lime flavor. The juice shows promise only as a mild lemon-lime flavoring, with perhaps more lemon-like than lime-like flavor characteristics. Essential oil from lem'n lime fruit does not show promise as an acceptable alternative to lemon–lime oil mixtures in flavoring food products. The presence of thymol and thymol methyl ether and the absence of neral and geranial in the essential oil cause a strong, non-citrus-type aroma that suggests its use in products other than those for which strong lemon–lime aromas are desired such as pharmaceutical or cosmetic preparations.

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COMMUNICATIONS

Chemical Combinations of 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Polystyrene. Preparation and Application for the Control of Duckweed

By the reactions of 2,4-D with functionalized polystyrene, the herbicide was bound to the polymer via the ester and anhydride bonds. The release of 2,4-D from these preparations was estimated by the effect on the growth of duckweed (*Lemna minor*) under laboratory conditions. The anhydride released the herbicide at a faster rate than the ester. The release from both these preparations was much slower than from a mechanical mixture of 2,4-D and polystyrene.

Combinations of biologically active compounds and polymers which provide controlled release of the active compounds over an extended period have received considerable attention in recent years (*Chem. Eng. News*, 1975; Allen et al., 1971). Such preparations are especially attractive when the active agent is an insecticide or a herbicide. An ideal preparation would reduce environmental hazards by preventing the flow of large amounts of the free agent in the soil or water. As the predetermined amount is released over a long period, frequent applications are unnecessary thus saving time and labor.

Two approaches have generally been used to prepare such combinations (Allen et al., 1971). One provides the release of the active agent by diffusion through polymers in which the agent is dissolved or encapsulated. The second approach provides the release by the breakdown of a polymer (usually cellulose) containing the agent as a side chain. In this report, we wish to present our investigations where the agent is chemically bound to a nondegradable synthetic polymer by two different linkages. The purpose is to demonstrate the feasibility of obtaining control over the release by changing the nature of the covalent bond between the active agent and the inert polymer.

In our study, 2,4-dichlorophenoxyacetic acid (2,4-D) was chosen as the active agent due to its wide use as a herbicide. The carboxylic acid function provided a suitable means for its covalent bonding to the polymer matrix, which was cross-linked polystyrene. This polymer satisfied the main criterion, namely the ease of introducing different chemical functions in its backbone. It is known to readily undergo the usual nucleophilic aromatic substitution