

## Volatile Constituents of Dried Legumes

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Seven samples of lima beans (*Phaseolus lunatis*), five samples of common beans (*Phaseolus vulgaris*), two samples of lentils (*Lens culinaris*), and one sample each of mung beans (*Phaseolus aureus*), soybeans (*Glycine max*), and split peas (*Pisum sativus*) were analyzed for volatile constituents by gas chromatographic and mass spectrometric techniques. Qualitatively, the volatile constituents of these dry legumes were the same as those found in southernpeas and there were only a few significant quantitative differences. However, the concentrations of most constituents did not have normal distributions and could be divided into two or more groups with significantly different means. Low concentrations of several aromatic and chlorinated hydrocarbons, including benzene and chloroform, were found in all samples, even one grown and analyzed with little or no exposure to agricultural or laboratory chemicals.

Although dry legumes, especially dry beans, are important food products, there is very little information about their volatile constituents. Buttery et al. (1975) characterized volatiles obtained from red beans that had been soaked overnight, but their technique did not yield information on any compounds more volatile than hexane. Recently, we reported (Fisher et al., 1979) the application of a direct transfer gas chromatographic technique (Dupuy et al., 1971) and a new gas chromatographic (GC)-mass spectrometric (MS)-data system (DS) technique to the determination of volatile constituents of dry southernpeas. The results were significantly different from those obtained by Buttery et al. on red beans. In particular, carbon disulfide, chloroform, dichlorobenzene, and trichlorobenzene were not found by Buttery et al. but were detected in all samples of southernpeas. To determine whether these differences were genetic or just the result of the difference in technique, dry red beans, several other dry beans, split peas, and lentils were analyzed by the same techniques used on southernpeas.

### MATERIALS AND METHODS

**Materials.** Most of the samples were purchased locally—Fordhook and baby lima beans, pinto-1, red kidney, black, and navy beans, split peas, and lentils-1 from a grocery and pinto-2 beans, lentils-2, soybeans, and mung beans from a health food store. Portions of each lot were taken from the center of the bag and stored in closed glass jars until used. Four lots of lima beans supplied by the U.S. Vegetable Breeding Laboratory, SEA, U.S. Department of Agriculture, Charleston, S.C., included two parent lines, Hopi, which is nematode resistant, and Jackson Wonder, which is not, and two lots of seed from a nematode-resistant progeny, one (L40215-1) grown in nematode-free soil and the other (L40215-2) in infested soil. These beans were received in small manila envelopes and were stored in the original envelopes until used. The speckled lima bean seed was grown in a home garden in New Orleans without use of herbicides or insecticides. All sample storage and handling was done in rooms where use of solvents was prohibited.

**Analyses.** The apparatus and techniques are described in detail in a previous paper (Fisher et al., 1979). Briefly, semiquantitative analyses for a wide range of volatile constituents were made with a flame ionization gas chromatograph using spiking with knowns as the basis for quantitation. Peaks were identified from retention times

and mass spectra. Quantitation for 11 compounds which could not be determined by GC alone was done by a direct transfer GC-MS-DS technique using MS-DS in the multiple ion detection (MID) mode. In all cases, the seeds were ground to a fine powder and the volatiles were eluted to the GC column without addition of water.

### RESULTS AND DISCUSSION

In general, the volatiles of dry beans, split peas, and lentils (Tables I and II) were similar to those of dry southernpeas (Fisher et al., 1979), both qualitatively and quantitatively. However, there were a few significant differences between beans and southernpeas and between lima beans and common beans. The mean amounts of 1-propanol ( $P < 0.001$ ), 2-propanol ( $P < 0.001$ ), chloroform ( $P < 0.001$ ), and ethanol ( $P < 0.001$ ) were lower in beans than in southernpeas. Toluene ( $P < 0.001$ ), ethanol ( $P < 0.01$ ), benzene ( $P < 0.01$ ), and phenylacetaldehyde ( $P < 0.01$ ) were lower in lima beans than in common beans, but 1-pentanol was higher. Lima beans also contained less toluene and phenylacetaldehyde ( $P < 0.01$ ) than southernpeas did.

On the basis of GC analyses (Table I), split peas and lentils-1 were qualitatively similar to beans; but for one-third of the constituents the concentration in the split pea sample was greater than in any of the bean samples. On the other hand, lentils-1 had low concentrations for one-third of the constituents. Both split peas and lentils-1 contained above average amounts of most of the alcohols, below average amounts of most of the carbonyl compounds, more benzene and chloroform than any of the beans, and above average amounts of most of the compounds determined by MID (Table II). Lentils-2, which was analyzed only by MID, was quite different from lentils-1. This suggests that the intraspecies variabilities observed for beans and for southernpeas will be observed for other species of dry legumes. Except for a relatively high concentration of dichlorobenzene and a moderately high concentration of toluene, lentils-2 was similar to the common beans. The other samples from the health food store were also analyzed by MID. Except for pinto-2 beans, they also contained large amounts of toluene and dichlorobenzene.

As was observed for southernpeas (Fisher et al., 1979), histograms of the concentrations for several constituents had two or more reasonably well-defined maxima instead of the normal single maximum located near the mean concentration. In view of the general similarity of the various kinds of dry legumes, data from this study and the one on southernpeas were combined for closer examination. Concentrations of most of the constituents can be differentiated into two or more populations with significantly

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Table I. Compounds Identified in Dry Legumes by GC-MS and Quantitated by GC

component	concentration, ppb					
	beans				split peas	lentils
	min	max	mean	SD		
methanol	1500	7900	4200	2200	3600	4400
acetaldehyde	1200	3300	2000	700	4300	1500
ethanol	250	980	470	220	4900	2400
2-propanol <sup>a</sup>	150	710	370	160	520	280
acetone <sup>b</sup>	260	2000	880	590	530	230
1-propanol <sup>c</sup>	8	28	17	7	160	96
methylpropanal	68	140	100	27	57	28
2-butanol <sup>d</sup>	32	145	53	31	89	32
2-butanone	74	330	148	75	110	50
2-methylpropanol	22	300	72	77	140	100
1-butanol	0	7	2	3	150	120
methylbutanal <sup>e</sup>	99	250	167	54	120	75
2-pentanone <sup>f</sup>	45	245	100	64	59	29
1-pentanol	0	18	8	9	19	95
hexanal	49	220	103	48	110	59
1-hexanol	2	69	15	19	41	40
ethylbenzene	0	11	5	3	13	5
<i>o</i> -xylene	0	25	9	6	8	3
styrene <sup>g</sup>	0	17	4	5	5	5
benzaldehyde <sup>h</sup>	7	59	26	13	6	4
dodecane	8	77	30	21	38	19
phenylacetaldehyde	0	250	48	70	0	0
acetophenone	0	400	63	115	76	11

<sup>a</sup> Diethyl ether also identified in this peak. <sup>b</sup> Dimethyl sulfide and carbon disulfide also present. <sup>c</sup> Methyl acetate also present. <sup>d</sup> Chloroform also present. <sup>e</sup> Benzene also present. <sup>f</sup> Pentanal may also be present. <sup>g</sup> Cumene also present. <sup>h</sup> Dichlorobenzene also present.

Table II. Concentrations of Compounds in Dry Legumes (ppb) Determined by MID

sample	aceto-nitrile	dimethyl sulfide	carbon disulfide	chloroform	benzene	toluene	dichlorobenzene	tri-chlorobenzene	naphthalene	methyl-naphthalene
Hopi lima beans	72.2	8.7	1.8	11.5	1.2	7.3	4.3	5.7	6.4	6.0
Jackson Wonder	67.9	8.6	2.5	8.2	1.2	5.2	1.8	3.5	2.4	2.8
L40215-1 lima beans	53.0	11.1	2.4	20.3	1.4	9.7	4.8	4.9	7.2	7.9
L40215-2 lima beans	89.7	10.6	2.6	12.4	1.5	7.2	5.2	4.4	4.9	4.4
speckled lima beans	49.2	11.3	2.0	12.0	1.8	11.5	8.3	4.7	14.9	20.1
Fordhook lima beans	65.6	9.6	1.8	15.6	2.0	7.9	8.0	5.2	6.3	10.3
baby lima beans	170.9	176.7	2.3	9.6	1.3	3.9	2.5	3.8	4.4	4.8
pinto beans-1	65.2	17.3	2.3	19.7	3.4	24.7	22.1	7.8	10.2	15.6
pinto beans-2	91.0	25.3	3.1	6.1	1.1	6.4	3.9	3.7	9.5	6.8
red kidney beans	98.9	26.9	2.0	23.2	3.3	31.1	34.3	6.8	21.4	49.2
black beans	71.8	15.2	2.2	18.6	4.0	21.3	8.0	3.9	6.7	4.8
navy beans	199.0	401.7	2.3	19.9	2.8	25.0	10.0	4.5	9.2	10.0
mung beans	37.1	47.7	2.4	6.5	0.9	161.9	18.0	3.0	6.4	16.3
mean <sup>a</sup>	87	60	2.3	14	2.0	25 <sup>b</sup>	10	4.8	9	12
SD <sup>a</sup>	47	112	0.4	6	1.0	42 <sup>b</sup>	9	1.4	5	12
soybeans	94.6	48.5	2.5	12.8	4.0	239.6	32.2	3.8	7.5	5.1
split peas	170.3	8.0	2.3	74.1	10.5	32.9	36.3	8.9	17.1	17.3
lentils-1 <sup>c</sup>	174.2	10.5	2.3	57.2	0.6	50.2	9.7	4.8	8.3	16.8
lentils-2	60.4	53.6	2.4	6.6	1.5	76.9	52.9	3.1	10.2	18.6

<sup>a</sup> For the lima, common, and mung beans. <sup>b</sup> If mung bean is omitted, mean = 13 and standard deviation = 9. <sup>c</sup> Contained 85 ppb of carbon tetrachloride.

different ( $P < 0.001$ ) means. Distributions among populations were of four types: a normal distribution, e.g., ethanol and toluene, with the highest frequency in the middle; a random distribution, e.g., chloroform, with more or less equal frequencies for all classes; an asymmetric distribution, e.g., 2-butanone, with the highest frequency in the lowest concentration class; and a distribution with two high frequency classes separated by a low frequency one, e.g., benzene. In no case is a high concentration class the most frequent one. For most constituents, distribution among classes was similar for beans and southernpeas. In addition to the differences in distribution which were to be expected from the differences in the means, most of the beans contained less 2-butanol, 1-pentanol, and dimethyl

sulfide, but more 1-hexanol than most of the southernpeas. The lowest-concentration group for 1-propanol contained all the bean samples and no other samples. For many aromatic and chlorinated hydrocarbons, the Jackson Wonder lima, which is known to be susceptible to nematodes, is in the population having the lowest concentration, and the three limas which are known to be resistant are in a population having a higher concentration. However, the number of samples is too small for any significance to be attached to this observation.

In view of the similarity of all the dry legumes, the major qualitative differences between the volatiles found by Buttery et al. in red beans and those found in the present study must reflect the differences in techniques. They

were not interested in the very volatile constituents, which accounted for 90% of the volatiles in the present study, so they used a transfer technique which eliminated them. In contrast, the direct transfer technique should not have eliminated the enones and enols which were the major constituents found by Buttery et al. These constituents are more volatile and more stable than decadienals which have been recovered by this techniques (Fore et al., 1978a). Our failure to find these compounds supports the suggestion of Buttery et al. that they are oxidation products of linolenic acid, because soaking the beans overnight provided excellent opportunity for enzymatic oxidation, whereas direct transfer of the volatiles from beans to column did not. Similarly, the 2,5-dimethylpyrazine which they found may have formed during steam distillation at 50 °C for 3 h. This compound was not found in most raw peanuts by direct transfer but was found in peanut butter (Fore et al., 1978b) and in raw peanuts which has been dried at 43 °C (Brown, 1978).

Buttery et al. (1975) did not report either di- or trichlorobenzene in their beans, but we found these as well as chloroform in all dry legume samples. To establish that they were not contaminants picked up in the laboratory in spite of precautions taken to avoid this, a portion of ground beans from which the volatiles had been removed was exposed to laboratory air for about 24 h and then run in the usual way. No chlorinated compounds were detected. Further evidence that these compounds occurred naturally was provided by the speckled lima bean sample. This sample was grown without use of insecticides or herbicides in a garden plot where such products had not been used for many years, air-dried in the same laboratory used for the blank run—just described, and analyzed within a few days after harvest. The concentrations of the chlorinated compounds in this sample were not significantly lower than in the other lima bean samples.

In contrast to these differences, benzene, toluene, xylene, hexanol, benzaldehyde, phenylacetaldehyde, naphthalene, and methylnaphthalene were found in both studies. Furthermore, the approximate concentrations reported by Buttery et al. (1975) for most of these constituents were within the range we observed for beans. Their values for benzene (ca. 6 ppb) and toluene (ca. 3 ppb) were slightly outside the ranges.

As in the study of southernpeas (Fisher et al., 1979), correlations were found between concentrations of benzene and toluene ( $r = 0.89$ ), chloroform and trichlorobenzene ( $r = 0.68$ ), naphthalene and methylnaphthalene ( $r = 0.91$ ), acetone and 2-propanol ( $r = 0.83$ ), 2-butanone and 2-butanol ( $r = 0.86$ ), and methylbutanal and methylpropanal

( $r = 0.76$ ). However, the regression equations for the first three pairs were entirely different for the beans than for the southernpeas. Correlation coefficients for the last three pairs based on the pooled data from both studies were  $r = 0.80, 0.90,$  and  $0.77$ , respectively.

#### CONCLUSIONS

The volatile constituents of the dry legumes analyzed in this study were the same as those found in dry southernpeas. Except for lower concentrations of ethanol, 1-propanol, 2-propanol, and chloroform in beans than in southernpeas, there were no significant ( $P < 0.01$ ) quantitative differences. For many of the constituents, the overall concentration data from the two studies did not appear to be drawn from a single population, but could be differentiated into two or more populations with significantly ( $P < 0.001$ ) different means. Available information about the samples provided no logical explanation for these differences.

The very high concentrations of some constituents encountered in a few samples may be due to postharvest contamination, but, except for carbon tetrachloride, low levels of the volatiles reported did occur in the seeds at the time of harvest. Chlorinated constituents are produced even with minimal exposure of the bean plants to chlorinated organic compounds; it would thus appear unlikely that they can be eliminated by any practical changes in cultural practices.

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