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Analysis of the Volatile Components in Red Bean (*Vigna angularis*)

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Volatile components in red bean (*Vigna angularis*) were investigated. Extracts prepared by simultaneous steam distillation and solvent extraction were analyzed by gas chromatography/mass spectrometry. One hundred and forty-two components including alkanes/alkenes (17), aromatics (5), furans (15), miscellaneous compounds (2), other nitrogen-containing compounds (11), aldehydes (11), naphthalenes (11), alcohols (34), ketones (23), sulfur-containing compounds (5) and esters (8) were identified. Some of these components, e.g. hexanal, were known to contribute to the "beany" odor in other beans. Due to the presence of such odor, red beans may not be acceptable to some consumers.

Key words : *gas chromatography-mass spectrometry (GC-MS), red bean, simultaneous steam distillation and solvent extraction (SDE), volatile components*

The use of legumes has become popular in the 1990s [Morrow, 1991]. Cooked legumes are a good source of protein [Morrow, 1991; Nielsen, 1991]. Aside from the plant protein, vitamins, minerals, and dietary fiber were also found [Hughes, 1991]. Legumes can be processed into ingredients such as flour, protein concentrate, and protein isolate [Uebersax *et al.*, 1991]. In Asia, red beans, *Vigna angularis*, have been used as ingredients in preparing desserts and soups. The beans were reported to contain many novel components such as sterol lipids, digalactosyl ononitol [Peterbauet *et al.*, 2003], phenolic compounds [Sato *et al.*, 2005], sequiterpene glucoside [Itoh *et al.*, 2005a], and conjugated saponin [Iida *et al.*, 1997]. Hot water extract of the red beans was found to suppress the proliferation of human stomach cancer KATO III cells and tumorigenesis in the mouse forestomach [Itoh *et al.*, 2004b]. Suppression of the hyperglycemia was also observed in diabetic rats fed the hot water extract [Itoh *et al.*, 2004a]. In addition, the extract was reported to stimulate melanogenesis in the cultured mice with B16 melanoma cells and possibly contribute to anti-graying and skin protection in human [Itoh and Furuichi, 2005; Itoh *et al.*, 2005b]. The red beans have a reddish outer seed coat and are smaller than soybeans. They are prized not only by their color, but also by their delicate flavor [Hardman *et al.*, 1989]. One of the factors that determine the acceptability of beans is whether they contain undesirable flavor. Soybeans are well known to have beany, bitter, and astringent flavor, and compounds suggested to be responsible for this flavor include 1-penten-3-one, (Z)-3-hexenol, and 2-pentylfuran [Sessa and Rackis 1977; Hsieh *et al.*, 1981]. Samoto *et al.* [1998] listed 17 components identified from the off-flavor soy protein isolates. Most of the compounds were ketones, aldehydes, and alcohols. So far, many researchers have focused on the nutritional and beneficial values of the red beans [Hughes, 1991; Chau, 1997]; however, their flavor quality, which may have a larger impact on the acceptance of the red beans and is related to the presence of the volatile components, has received much less attention. Therefore, the objective of this study was to determine the profile of the volatile components in the red beans.

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Abbreviations: IS, internal standard; SDE, steam distillation-solvent extraction

Materials and Methods

Sample preparation. Red beans (*Vigna angularis*) were purchased from a local supermarket in Hong Kong SAR, China. The beans were lightly blended for 40 s using a blender (MX-T2GN, Masushita Electric Co. Ltd.,

Taiwan) and screened using a standard testing sieves (ASTM E-11 Specification no. 45 and 60, VWR Scientific, West Chester, USA) to obtain particles with sizes between 250-355 μm for extraction.

Simultaneous steam distillation-solvent extraction (SDE). The red beans were steam-distilled to mimic the food preparation method generally used by the consumers. In the current setup, blended and sieved products (35 g) were transferred to a stainless steel pocket (15 cm × 5 cm), which was then loaded on to a platform formed by placing three 20-cm spatulas in a 5-L round bottom flask [Chung *et al.*, 2001]. One milliliter of 2,4,6-trimethylpyridine (internal standard, IS) at 10 μg/mL was added to the sample. Five hundred milliliters of the double-distilled water was placed in the sample flask, and 50 mL redistilled dichloromethane was used as the extraction solvent. A Likens and Nickerson [1964] type SDE apparatus was used to extract the sample for 2 h. The extractions were carried out in triplicates. Extracts were concentrated using a stream of the prepurified nitrogen gas to reach 0.5 mL, dried by passing 2.5-g anhydrous sodium sulfate into 1.5-mL amber vials, and stored at −80°C until used.

Gas chromatograph-mass spectrometer (GC-MS). A Hewlett-Packard 6890 GC coupled with a Hewlett-Packard 5973 mass selective detector was used. A 60-m fused silica open-tubular column (Supelcowax 10, 0.25 mm i.d., 0.25 μm film thickness, Bellefonte, PA) was installed into the GC. The GC was operated under the following conditions: initial and final temperatures, 35°C for 5 min and 195°C for 110 min, respectively; ramp rate, 2°C/min; linear flow rate, 30 cm/s; split rate, 10 : 1. The MS conditions were as follows: electron ionization voltage, 70 eV; mass range, 33-450 a.m.u.; electron multiplier voltage, 1118 V; scan rate, 3.49 scans/min. Five microliters of the extract was injected into the GC-MS.

Qualification and quantification of red beans. Positive identification of each component was based on the matches of its retention times and mass spectra with those of its authentic standards. Tentative identifications were based only on the information suggested by the mass spectra library. Quantification was carried out using the internal standard method, and three-point calibration curves were prepared for the compounds with confirmed identity [Woodget and Cooper, 1987], whereas relative quantity was calculated for tentatively identified compounds.

Moisture analysis. Moisture content was determined according to the AOAC [1980].

Results and Discussion

Moisture content of the red beans was 9.7%, which was lower than that found by Chau *et al.* [1997] at 12.3%. Figure 1 shows a typical chromatograph of the volatile profile of the red beans. Table 1 shows the 142 volatile compounds identified in this study, which were divided into ten families of components, among which alcohols and ketones were the largest in numbers with 34 and 24 compounds, respectively. Alkanes/alkenes and furans had 17 and 15 compounds, respectively. Other nitrogen-containing compounds, aldehydes, and naphthalenes each contained 11 components. The rest of the groups contained less than 10 components. Each group had at least one volatile component with a very high mean concentration (Table 1).

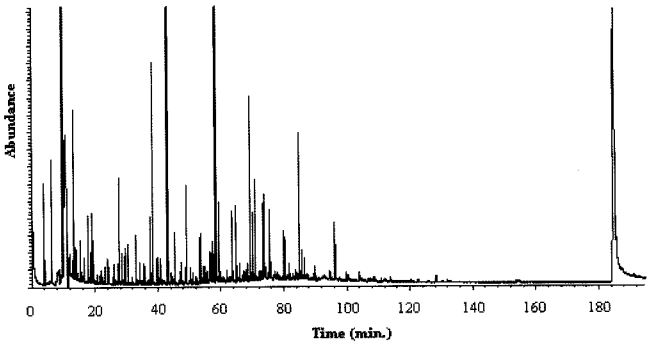

Fig. 1. A typical total ion chromatogram of red bean.

del Rosario *et al.* [1984] reported that 62 and 71 compounds were identified in the headspace of the ground raw and cooked soybeans, respectively. The raw and cooked soybeans had 47 common compounds, Twenty-one of the common compounds from both raw and cooked soybeans were also found in the red beans, which accounted for 24.4% of the total number of components identified in the red beans. In fact, 26 and 29 components in the raw and the cooked soybeans respectively (del Rosario, 1984) were also found in the red beans in this study. Samoto *et al.* [1998] listed 17 components identified from the off-flavor soy protein isolates. By removing the oil body-associated proteins, they observed a decrease in the intensities of the off-flavor. In our study, 11 of the 17 compounds were also found in the red beans, including 2-pentylfuran, *n*-hexanal, (*E*)-2-pentenal, *n*-heptanal, nonanal, benzadehyde, 1-penten-3-ol, 1-pentanol, 1-hexanol, 1-octen-3-ol and 2-heptanone.

By using gas chromatography/olfactometry (GC/O), Boatright and Lei [1999] identified 14 compounds contributing to the odor of the soy protein isolate. In the headspace analysis, *n*-hexanal, described by the group as oxidized/nutty, showed the highest intensity among the compounds of the isolate. Seven of these compounds found, including 2-pentylfuran, *n*-hexanal, benzaldehyde,

Table 1. Volatile components in *Vigna angularis*

RT[1] (min.)	RI[2]	Compound name[3]	MW[4]	*m/z*[5]	Conc.[6] (μg/kg)	RT[1] (min.)	RI[2]	Compound name[3]	MW[4]	*m/z*[5]	Conc.[6] (μg/kg)
		Alkanes/Alkenes (17)						**Alcohols (34)**			
20.19	1097	Undecane	156.19	57	1.6 ± 0.5	16.36	1035	1-propanol[a,b]	60.06	59	288.4 ± 391.3
24.16	1152	7-oxabicyclo[4.1.0]heptane	98.07	83	71.4 ± 9.1	19.68	1089	2-methyl-1-propanol[a,b]	74.07	43	137.8 ± 15.0
34.06	1298	Tridecane	184.22	85	5.3 ± 0.9	20.75	1105	3-pentanol[a]	88.09	59	20.6 ± 1.7
34.96	1311	4-formylcyclopentene[t]	96.06	67	2.0 ± 0.1	21.59	1117	2-pentanol[a]	88.09	45	15.4 ± 1.3
40.82	1398	Tetradecane	198.24	85	9.7 ± 3.7	23.23	1139	1-butanol[a,b]	74.07	56	37.4 ± 1.5
47.30	1498	Pentadecane	212.25	85	23.4 ± 10.6	24.42	1156	1-penten-3-ol[a,b]	86.07	57	2237.8 ± 456.5
53.46	1598	Hexadecane	226.27	57	23.2 ± 13.8	26.91	1189	3-hexanol	102.10	59	4.3 ± 0.2
54.73	1619	2,6,11,15-tetramethylhexadecane[t]	282.33	57	8.7 ± 4.1	27.63	1206	3-methyl-1-butanol[a,b]	88.09	70	153.4 ± 12.8
59.33	1698	heptadecane[a]	240.28	57	37.8 ± 26.5	30.62	1249	1-pentanol[a,b]	88.09	70	102.9 ± 8.5
63.49	1772	8-methyl-heptadecane[t]	254.30	57	6.3 ± 6.6	35.57	1320	methyl-2-buten-1-ol	86.07	71	38.9 ± 0.5
64.90	1798	Octadecane	254.30	57	86.1 ± 64.3	37.72	1352	1-hexanol[a,b]	102.10	56	171.9 ± 18.5
70.23	1897	nonadecane[t,a,b]	268.31	57	26.0 ± 18.5	39.88	1384	(*Z*)-3-hexenol	100.09	67	56.8 ± 14.0
75.32	1997	Eicosane	282.33	57	71.8 ± 51.6	40.49	1393	3-octanol	130.14	59	7.7 ± 3.1
80.19	2096	Heneicosane	296.34	57	14.7 ± 11.3	41.00	1401	Cyclohexanol	100.09	57	18.4 ± 1.1
84.85	2195	Docosane	310.36	57	11.5 ± 8.2	41.34	1406	(*E*)-2-hexen-1-ol	100.09	57	4.7 ± 0.5
89.87	2294	tricosane	254.30	57	6.8 ± 4.2	44.25	1451	1-octen-3-ol[a,b]	128.12	57	16.8 ± 1.8
95.35	2381	kaur-16-ene[t]	272.25	257	0.9 ± 0.3	44.49	1455	1-heptanol	116.12	83	18.5 ± 3.0
		Aromatics (5)				45.06	1463	*dl*-6-methyl-5-hepten-2-ol	128.12	95	1.6 ± 1.4
16.51	1036	toluene[a,b]	92.06	91	42.4 ± 6.0	46.75	1490	2-ethyl-1-hexanol	130.14	83	3.4 ± 0.5
22.20	1125	ethylbenzene[a,b]	106.08	106	5.4 ± 0.2	50.95	1557	1-octanol	130.14	70	3.6 ± 0.6
22.73	1133	1,4-dimethylbenzene[a,b]	106.08	106	2.9 ± 0.1	57.06	1659	1-nonanol[t]	144.15	56	6.6 ± 1.2
26.18	1180	1,2-dimethylbenzene[a,b]	106.08	106	4.7 ± 0.2	65.84	1815	.alpha-methyl-benzenemethanol	122.07	107	23.5 ± 2.4
31.40	1260	Ethenylbenzene	104.06	104	2.0 ± 1.8	68.46	1864	2-methoxyphenol	124.05	109	27.1 ± 3.3
		Furans (15)				69.23	1878	benzenemethanol	108.06	108	203.7 ± 20.2
16.17	1032	1-(2-furanyl)-ethanone	110.04	95	5.7 ± 0.2	71.03	1913	benzeneethanol	122.07	122	65.2 ± 10.3
18.13	1064	2,4-dimethylfuran[t]	96.06	96	1.7 ± 0.2	71.23	1917	2,6-bis(1,1-dimethylethyl)-4-methylphenol	220.18	205	2.2 ± 0.5
29.61	1234	2-pentylfuran[a,b,c,e]	138.10	138	37.4 ± 8.4	74.30	1977	4-hydroxy-benzeneethanol[t]	138.07	107	2.3 ± 0.4
31.83	1266	dihydro-2-methyl-3(2H)-furanone	100.05	100	16.6 ± 7.4	75.86	2008	2-methylphenol	108.06	108	9.1 ± 1.5
43.20	1435	3-furaldehyde	96.02	95	830.7 ± 173.8	75.99	2011	phenol	94.04	94	70.0 ± 8.6
45.43	1469	2-furancarboxaldehyde	96.02	96	86.5 ± 21.0	79.69	2086	4-methylphenol	108.06	108	80.2 ± 9.9
47.97	1509	1-(2-furanyl)-ethanone	110.04	95	4.3 ± 0.4	80.07	2094	3-methylphenol	108.06	108	1393.2 ± 225.7
52.21	1578	5-methyl-furfural	110.04	110	2.9 ± 0.8	84.07	2178	3-ethylphenol	122.07	108	3.6 ± 0.8

Table 1. Volatile components in *Vigna angularis*

RT[1] (min.)	RI[2]	Compound name[3]	MW[4]	*m/z*[5]	Conc.[6] (μg/kg)	RT[1] (min.)	RI[2]	Compound name[3]	MW[4]	*m/z*[5]	Conc.[6] (μg/kg)
54.39	1614	dihydro-5-methyl-2(3H)-furanone[a]	100.05	56	6.3 ± 0.8	85.01	2198	4-vinyl-2-methoxyphenol[t]	150.07	150	131.0 ± 46.0
54.66	1618	dihydro-4-methyl-2(3H)-furanone[t,a]	100.05	42	3.5 ± 3.1	90.94	2312	2,4-bis(1,1-dimethylethyl)-phenol	206.17	191	2.8 ± 0.4
55.49	1632	dihydro-2(3H)-furanone[a,b]	86.04	86	73.0 ± 12.9			**Ketones (23)**			
57.28	1663	2-furanmethanol	98.04	98	45.8 ± 7.2	18.02	1062	2,3-pentanedione[b,c]	100.05	100	248.9 ± 106.0
58.40	1682	furan-3-methanol	98.04	98	4114.0 ± 300.1	19.12	1080	2-hexanone[b]	100.09	100	7.9 ± 1.3
59.75	1705	dihydro-5-ethyl-2(3H)-furanone[t]	114.07	85	17.0 ± 1.1	20.37	1100	4-methyl-1-penten-3-one[t]	98.07	55	2.6 ± 0.7
60.67	1722	3-methyl-2(5H)-furanone[t]	98.04	98	6.3 ± 1.0	22.09	1124	(*E*)-3-penten-2-one	84.06	69	28.3 ± 6.2
		Miscellaneous compounds (2)				22.54	1130	4-methyl-3-Penten-2-one	98.07	83	2.9 ± 0.5
92.33	2334	9*H*-fluorene	166.08	166	1.1 ± 0.3	26.05	1178	2-heptanone[b,c,e]	114.10	114	36.5 ± 10.7
99.90	2440	1*H*-indole	117.06	117	21.1 ± 3.2	31.10	1256	3-octanone[b]	128.12	99	5.3 ± 0.4
		Othernitrogen-containing Compounds (11)				33.20	1286	3-hydroxy-2-butanone	88.05	88	610.3 ± 72.7
25.63	1172	Pyridine	79.04	79	25.4 ± 3.5	33.44	1289	cyclohexanone	98.07	98	10.1 ± 0.4
28.00	1211	3-methylpyridine	93.06	93	2.0 ± 1.8	34.26	1301	1-hydroxy-2-propanone	74.04	74	108.4 ± 22.0
31.68	1264	methylpyrazine[a,b]	94.05	94	11.9 ± 1.6	34.43	1304	1-octen-3-one	126.10	70	3.5 ± 0.7
35.95	1326	2,6-dimethylpyrazine[a,b]	108.07	108	41.8 ± 1.7	37.15	1344	3-hydroxy-2-pentanone[t]	102.07	59	3.6 ± 0.1
38.40	1362	2,4,6-trimethylpyridine	121.09	121	nd	37.97	1356	2-cyclopenten-1-one	82.04	82	34.8 ± 5.0
40.26	1390	2-ethyl-6-methylpyrazine[t]	122.08	121	2.3 ± 0.7	38.86	1369	2-methyl-2-cyclopenten-1-one	96.06	67	7.5 ± 0.2
44.83	1460	2,3-dimethyl-5-ethylpyrazine[t]	136.10	135	2.3 ± 0.5	44.13	1449	2,3-dimethyl-2-cyclopenten-1-one[t]	110.07	67	1.6 ± 0.2
48.76	1522	1*H*-pyrrole	67.04	67	35.2 ± 8.7	50.26	1546	4-methyl-3-penten-2-one[t]	98.07	83	14.0 ± 4.7
51.12	1560	2-methyl-1*H*-pyrrole[t]	81.06	80	8.5 ± 4.2	51.91	1573	3,5-octadiene-2-one[t,b]	124.09	95	5.2 ± 1.6
53.92	1606	1-(2-pyridinyl)-ethanone	121.05	93	7.1 ± 2.5	53.18	1593	3,5,5-trimethyl-2-cyclohexene-1-one[t]	138.10	82	4.1 ± 0.6
72.07	1933	Benzeneacetonitrile	117.06	117	2.2 ± 0.5	56.81	1655	1-phenylethanone[c,d]	120.06	105	38.0 ± 3.2
74.12	1974	1-(1*H*-pyrrol-2-yl)-ethanone	109.05	109	23.0 ± 5.2	59.17	1695	3,5,5-trimethyl-2-cyclohexene-1,4-dione[t]	152.08	68	2.7 ± 0.4
		Aldehydes (11)				65.59	1810.	2-tridecanone	198.20	58	15.2 ± 1.8
19.29	1083	*n*-hexanal[a,b,c,d,e]	100.09	72	263.8 ± 24.1	73.63	1964	maltol	126.03	126	557.4 ± 412.3
19.96	1094	2-methyl-2-butenal[t]	84.06	84	2.6 ± 0.5	76.50	2021	2-pentadecanone	226.23 156.15	58	3.5 ± 0.4
22.36	1127	(*E*)-2-pentenal[e]	84.06	69	47.5 ± 6.3			**Sulfur-containing compounds (5)**			
26.30	1181	*n*-heptanal[b,e]	114.10	70	12.7 ± 2.1	18.74	1074	dimethyldisulfide[b,c]	93.99	94	1150.7 ± 845.2
27.21	1194	3-methyl-2-butenal	84.06	84	65.5 ± 15.5	31.69	1264	1-(methylthio)-propane[t]	90.05	61	3.2 ± 0.2
28.55	1219	(*E*)-2-hexenal[t,a,b]	98.07	41	13.1 ± 2.1	56.58	1650	2-acetylthiazole	127.01	85	9.0 ± 2.8
40.68	1396	nonanal[a,b,e]	142.14	98	6.3 ± 1.3	73.22	1956	2-hexylthiophene[t]	168.10	97	6.2 ± 1.8
43.02	1432	(*E*)-2-octenal	126.10	70	1.2 ± 0.3	73.37	1959	benzothiazole	135.01	135	3.1 ± 0.5

Table 1. Volatile components in *Vigna angularis*

RT[1] (min.)	RI[2]	Compound name[3]	MW[4]	*m/z*[5]	Conc.[6] (μg/kg)	RT[1] (min.)	RI[2]	Compound name[3]	MW[4]	*m/z*[5]	Conc.[6] (μg/kg)
47.19	1496	(*E*,*E*)-2,4-heptadienal	110.07	81	6.3 ± 1.3			**Ester (8)**			
49.13	1528	benzaldehyde[b,c,e]	106.04	106	257.0 ± 4.0	50.81	1555	3-furylmethylacetate[t]	140.05	98	2.2 ± 0.3
56.44	1649	Benzeneacetaldehyde	120.06	91	18.0 ± 5.1	55.14	1626	methyl benzoate	136.05	105	2.0 ± 0.5
		Naphthalenes (11)				63.97	1781	methy 2-hydroxybenzoate[t]	152.05	120	8.6 ± 1.0
61.98	1745	naphthalene[a,b]	128.06	128	20.0 ± 0.7	85.80	2214	methyl hexadecanoate	270.26	143	57.0 ± 24.2
68.06	1857	2-methylnaphthalene	142.08	142	3.8 ± 0.8	87.61	2250	ethyl hexadecanoate	284.27	88	2.8 ± 1.4
69.93	1891	1-methylnaphthalene	142.08	142	1.8 ± 0.4	94.44	2367	diethyl 1,2-benzenedicarboxylate	222.09	149	3.0 ± 0.6
75.65	2004	1,6-dimethylnaphthalene	156.09	156	2.9 ± 2.9	104.01	2490	methyl (*Z*,*Z*)-9,12-octadecadienoate[t]	294.26	67	6.4 ± 3.4
78.40	2060	1,4,6-trimethylnaphthalene[t]	170.11	155	1.9 ± 0.8	128.40	2670	dibutyl 1,2-benzenedicarboxylate	278.15	149	9.9 ± 2.8
80.76	2108	1,6,7-trimethylnaphthalene	170.11	155	1009.4 ± 380.0						
81.07	2115	Trimethylnaphthalene	170.11	170	1822.2 ± 861.8						
82.23	2139	trimethylnaphthalene[t]	170.11	170	1.5 ± 0.6						
82.73	2150	2,3,6-trimethylnaphthalene[t]	170.11	170	3.9 ± 1.7						
83.74	2172	1,4,5-trimethylnaphthalene[t]	170.11	155	1.3 ± 0.5						
84.12	2179	1,6,7-trimethylnaphthalene	170.11	170	2354.4 ± 1019.4						

[1]Retention time (min.)

[2]Retention index

[3]Compound identified: t: tentatively identified compound; References: [a]: del Rosario *et al.*, 1984-raw; [b]: del Rosario *et al.*, 1984-cooked; [c]: Boatright and Lei, 1999-by gas chromatography/olfactometry; [d]: Boatright and Lei, 1999-by aroma extract dilution analysis; [e]: Samoto *et al.*, 1998.

[4]Molecular weight

[5]Mass/charge: specific fragment used for the determination of area ratio.

[6]Concentration and standard deviation (μg/kg) on a dry weight basis.

2,3-pentanedione, 2-heptanone, 1-phenylethanone, and dimethyldisulfide, were also identified in the red beans. When the aroma extract dilution analysis (AEDA) was carried out by Boatright and Lei [1999] on the soy protein isolate, components with the highest dilution factor were in the order of dimethyl trisulfide, (*E,E*)-2,4-decadienal, 2-pentylpyridine, (*E,E*)-2,4-nonadienal, hexanal, 1-phenylethanone, and 1-octen-3-one. However, among the compounds, only *n*-hexanal and 1-phenylethanone were found in the red beans.

Comparison of the common off-flavor components among the various typical soy-based products revealed *n*-hexanal is the only one that occurs in all samples and could be considered as a contributor to the off-flavor. *n*-Hexanal has a fatty, green, grassy, powerful, and penetrating odor [Aldrich, 2003]. Various investigators have proposed different methods to control the presence of *n*-hexanal in specific products [Maheshwari *et al.*, 1995; Peeyush *et al.*, 1997; Wang *et al.*, 1998]. In this study, the red beans contained 263.8 μg/kg of *n*-hexanal and had the highest concentration among the aldehydes. The threshold value of *n*-hexanal was low, estimated at 5.75×10^{-8} g/L [Devos, 1990]. Taking both the high concentration and the low threshold value of *n*-hexanal into account, the calculated odor activity value (OAV) would be significantly higher than the rest of the aldehydes [Guadagni, 1966]. These results indicate red beans have a detectable and distinctive odor contributed by *n*-hexanal, and thus may not be favored by consumers not familiar with the flavor.

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