Quantification and Sensory Studies of Character Impact Odorants of Different Soybean Lecithins

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Fifty-four potent odorants in standardized, hydrolyzed, and deoiled and hydrolyzed soybean lecithins were quantified by high-resolution gas chromatography/mass spectrometry (HRGC/MS). The characterization of their aroma impact was performed by calculation of nasal (n) and retronasal (r) odor activity values (OAVs). For this, the nasal and retronasal recognition thresholds of 18 odor-active compounds were determined in vegetable oil. The following compounds showed the highest nOAVs: 2,3-diethyl-5-methylpyrazine, methylpropanal, acetic acid, pentanoic acid, 2-ethyl-3,5-dimethylpyrazine, pentylpyridine, (*Z*)-1,5-octadien-3-one, 2-methylbutanal, and *β*-damascenone. In addition to the compounds above, 1-octen-3-one, 1-nonen-3-one, and 3-methyl-2,4-nonandione showed potent rOAVs. The results of quantification and OAV calculation were confirmed by a model mixture of 25 impact odorants, which yielded a highly similar sensory profile to that of the original soybean lecithin. The sensory importance of pyrazines and free acids increased through enzymatic hydrolysis and decreased by the process of deoiling. The impact of unsaturated ketones on the lecithin aroma was not changed by either process.

Keywords: *Soybean lecithin; hydrolysis; deoiling; phospholipid; aroma; flavor; odorant; OAV; recognition threshold; model mixture*

INTRODUCTION

Soy lecithins have a wide applicability because of their technological properties. They are used as emulsifier, stabilizer, or dispersing agents in a wide range of polarity, for example, in chocolate, instant products, margarine, mayonnaise, bakery products, or ice cream. Nevertheless, especially the applicability of modified, hydrolyzed lecithins (treated with phospholipase A_2), for example, in foods with a high water content (low fat products, beverages) or in bakery products (to decelerate the aging process), is partly restricted by a formation of an off-odor in the final product (Stephan and Steinhart, 1999).

The causes of this aroma (off-odor) of soybean lecithins and the differences in the aromas of various treated lecithins (hydrolyzed, deoiled) are not well-known. Except for studies of Kim et al. (1984), who examined major volatiles of deoiled soybean lecithin, there have been no systematic investigations to elucidate the causes of soybean lecithin aroma and to control the aroma generation during different treatment processes. First results on the causes of the soybean lecithin aroma were obtained in previous studies (Stephan and Steinhart, 1999) by the generation of sensory profiles of soybean lecithins, the identification of potent odorants, and the application of gas chromatography/olfactometry (GC/O) methods, such as aroma extract dilution analysis (AEDA) and modified combined hedonic and response measurement (CHARM). These investigations had revealed such odorants as 2,3-diethyl-5-methylpyrazine, (*E*)-*β*-damascenone, and 1-nonen-3-one, as having a high impact on the aroma.

Although both olfactory methods have been proven as suitable tools for recognition and identification of odor-active compounds (Acree et al., 1984; Ullrich and Grosch, 1987; Schlüter et al., 1996), the data are not sufficient to assess the degree of actual contribution of a compound to each characteristic perception. Therefore a systematic characterization of the odorants of different treated soybean lecithins on a quantitative basis is necessary. With the extensive knowledge of the concentrations and the nasal and retronasal odor thresholds of the potent odorants, it is possible to assess the causes of the lecithin aroma by the concept of odor activity values (OAV) (Rothe and Thomas, 1963; Frijters, 1978; Grosch, 1993). These data enable an evaluation of to what extent, for example, 2,3-diethyl-5-methylpyrazine, which was one of the key odorants of hydrolyzed lecithins in both olfactory methods, is actually responsible for the roasty and earthy perception. In addition, these data enable a comparison of the similarity of the original aroma of soybean lecithins with a combination of important lecithin odorants in model mixtures.

EXPERIMENTAL PROCEDURES

Lecithins. Standardized, hydrolyzed, and deoiled and hydrolyzed soybean lecithins were purchased from Lucas Meyer Company, Ltd. (Hamburg, Germany). Standardized lecithin (A) had a defined content of phosphatidylcholine and more than 60% acetone insoluble enzymatically hydrolyzed lecithin (B) (phospholipase A_2) had a grade of hydrolysis of about 40%; and oil-free lecithin (C) was the acetone deoiled version of B. The lecithins were stored air-tight, in darkness, and at room temperature (20−23 °C).

Chemicals. Diethyl ether, sodium carbonate, sodium chloride, hydrochloric acid, 2-ethylpyrazine, 5-methyl-2-hexanone,

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Table 1. Selected Target Ions, Qualifier Ions, Thin Film Capillaries, and Correction Factors for Quantification of Soybean Lecithin Odorants by GC/MS-EI

no.[a]	odorant[b]	odor description[c]	selected target ion[d] (*m*/*z*)	selected qualifier ions[e] (*m*/*z*)	thin film capillary	correction factor[f–i] (%)
1	methylpropanal	malty, biting	72	43, 41	BGB-1701	9[f]
2	2,3-butanedione	buttery	86	43, 42	BGB-1701	18[f]
3	3-methylbutanal	malty, strawy	58	71, 86	BGB-1701	63[f]
4	2-methylbutanal	strawy	57	58, 86	BGB-1701	62[f]
5	2,3-pentanedione	buttery	43	57, 100	BGB-1701	65[f]
6	dimethyl disulfide	cabbage-like	94	79, 45	BGB-1701	151[f]
7	hexanal	green	56	82, 44	BGB-1701	100[f]
8	4-methyl-3-pentene-2-one	almond-like	83	98, 55	BGB-FFAP	97[f]
9	2-heptanone	fruity, soapy	43	58, 71	BGB-1701	95[f]
10	heptanal	fatty, tallowy	70	86, 96	BGB-1701	98[f]
11	(*Z*)-4-heptenal	fishy	68	67, 84	BGB-1701	83[f]
12	diethyl disulfide	sulfury	122	94, 66	BGB-1701	102[f]
13	2-acetyl-1-pyrroline	popcorn-like	83	68, 111	BGB-1701	94[g]
14	pentylfuran	beany	81	138, 82	BGB-1701	91[f]
15	1-octen-3-one	mushroom-like	55	70, 97	BGB-1701 + FFAP	118[f]
16	1-octen-3-ol	mushroom-like	57	72, 81	BGB-FFAP	69[f]
17	(*Z*)-1,5-octadien-3-one	metallic	55	95, 109	BGB-1701 + FFAP	65[f]
18	benzaldehyde	marzipan-like	77	106, 105	BGB-1701	93[f]
19	octanal	orange-like, fatty	84	100, 110	BGB-1701	101[f]
20	acetylpyrazine	popcorn-like	122	80, 79	BGB-1701	84[g]
21	3-octen-2-one	nutty, fruity	55	111, 43	BGB-1701	114[f]
22	2-isopropyl-3-methoxypyrazine	earthy, pea-like	137	152, 124	BGB-1701 + FFAP	149[g]
23	3-ethyl-2,5-dimethylpyrazine	roasty, earthy	135	136, 56	BGB-1701	127[g]
24	2-ethyl-3,5-dimethylpyrazine	roasty, earthy	135	136, 56	BGB-1701	125[g]
25	1-nonen-3-one	mushroom-like	55	70, 111	BGB-1701 + FFAP	122[f]
26	1-nonen-3-ol	mushroom-like	57	113, 67	BGB-FFAP	110[f]
27	(*E*)-2-octenal	fatty, nutty	70	83, 97	BGB-1701	85[f]
28	nonanal[e]	tallowy, fruity	57	98, 69	BGB-1701	101[f]
29	2,3-diethyl-5-methylpyrazine	roasty, earthy	150	149, 135	BGB-1701	139[g]
30	2-isobutyl-3-methoxypyrazine	pepper-like	151	124, 94	BGB-1701 + FFAP	143[g]
31	3-nonen-2-one	nutty, fruity	55	125, 43	BGB-1701	110[f]
32	phenylethanol	honey-like	91	122, 92	BGB−FFAP	123[f]
33	(*E*)-2-nonenal	cardboard-like	83	55, 96	BGB-1701	112[f]
34	(*E*,*Z*)-2,6-nonadienal	cucumber-like	69	70, 67	BGB-FFAP	88[f]
35	(*E*,*Z*)-2,6-nonadienol	cucumber-like	79	69, 68	BGB-FFAP	153[f]
36	pentylpyridine	strawy, tallowy	93	106, 120	BGB-1701	94[g]
37	3,5,5-trimethyl-2-cyclohexen-1-one	nutty	82	138, 54	BGB-FFAP	84[f]
38	(*E*,*E*)-2,4-nonadienal	fatty	81	67, 138	BGB-1701	122[h]
39	3-methyl-2,4-nonandione	strawy	99	71, 170	BGB-1701 + FFAP	98[f]
40	*trans*−4,5-epoxy-(*E*)-2-nonenal	metallic	68	69, 125	BGB-1701 + FFAP	149[h]
41	(*E*,*E*)-2,4-decadienal	deep-fried	81	152, 95	BGB-1701	109[h]
42	γ-octalactone	coconut-like	85	55, 56	BGB-1701	94[h]
43	(*E*)-β-damascenone	baked apple-like	69	121, 190	BGB-1701	70[h]
44	*trans*-4,5-epoxy-(*E*)-2-decenal	metallic	68	69, 139	BGB-1701 + FFAP	123[h]
45	γ-nonalactone	coconut-like	85	55, 56	BGB-1701	60[h]
46	γ-decalactone	coconut-like	85	128, 55	BGB-1701	29[h]
47	acetic acid	vinegar-like	60	43, 45	BGB-FFAP	139[i]
48	propanoic acid	pungent	74	45, 73	BGB-FFAP	137[i]
49	butanoic acid	sweaty, rancid	60	73, 42	BGB-FFAP	110[i]
50	3-methylbutanoic acid	sweaty	60	74, 87	BGB-FFAP	81[i]
51	2-methylbutanoic acid	sweaty, sweet	74	57, 87	BGB-FFAP	66[i]
52	pentanoic acid	sweaty	60	73, 45	BGB-FFAP	73[i]
53	(*E*)-2-butenoic acid	biting	86	68, 69	BGB-FFAP	92[i]
54	hexanoic acid	goat-like, sweaty	60	73, 87	BGB-FFAP	104[i]

[a] Continuous numbers of odorants according to Tables 2 and 3. [b] Odorants in order of their retention index (RI) on a BGB-1701 thin film capillary (acids on a BGB-FFAP). [c] Odor description at the sniffing port. [d] Selected target ion (*m*/*z*) for quantification of the odorant. [e] Selected qualifier ions (*m*/*z*) for quantification of the odorant. [f–i] Correction factor for each compound relative to its particular internal standard: (*f*) 5-methyl-2-hexanone; (*g*) 2-ethylpyrazine; (*h*) 4-methoxybenzaldehyde; (*i*) 2-methylpropionic acid.

4-methoxybenzaldehyde, and **1**−**3**, **5**, **6**, **8**−**10**, **16**, **18**, **28**, **32**, **37**, **47**, **48**, and **53** (Table 1) were obtained from Merck (Darmstadt, Germany); 2-methylpropionic acid, **7**, **11**, **12**, **19**, **27**, **34**−**36**, **38**, **41**, **49**−**52**, and **54** were from Aldrich (Steinheim, Germany); **4**, **20**, **23**, **24**, and **29** were from ACROS (Gelnhausen, Germany); **14**, **21**, **30**, **31**, **33**, and **46** were from ABCR (Karlsruhe, Germany); **15** and **22** were from Lancaster (Mühlheim, Germany); and **26**, **42**, and **45** were from Roth (Karlsruhe, Germany). **13**, **17**, **39**, **40**, and **44** were gifts from the Deutsche Forschungsanstalt für Lebensmittelchemie (Garching, Germany), **25** was a gift from Nestec Ltd. (Lausanne, Switzerland); and **43** was a gift from Firmenich (Geneva, Switzerland).

Isolation of the Volatile Compounds. The volatiles were distilled off from 25 g of soybean lecithin (dissolved in 100 mL of diethyl ether) in a vacuum distillation apparatus (Stephan and Steinhart, 1999) under the following conditions: pressure, 1×10^{-4} mbar; temperature, 40 °C; feeding rate, 40 mL/h; falling film glass tubing, 25 cm × 35 mm; total distillation time, 3.5 h. The volatile fraction was condensed in three cooling traps, cooled by liquid nitrogen. The combined condensates were extracted with 3 × 50 mL of aqueous sodium carbonate (0.5 mol/L) followed by washing the diethyl ether fraction with 3 × 15 mL of saturated sodium chloride solution and drying through a hydrophobic filter (Schleicher & Schuell, Einbeck, Germany) (neutral/basic aroma extract).

The combined aqueous phases were adjusted to pH 2.0 with hydrochloric acid, extracted with 3 × 40 mL of diethyl ether, and dried through a hydrophobic filter (acidic aroma extract). Finally, both aroma extracts (neutral/basic and acidic) were concentrated to 0.2 mL on a Vigreux column (40 × 1 cm) and by microdistillation (10 × 1 cm column, filled with three pear-shaped glass beads).

High-Resolution Gas Chromatography (HRGC)/Mass Spectrometry (MS). HRGC/MS was performed with a Hewlett-Packard model 5890 series II gas chromatograph coupled with a HP 5971A mass spectrometer run in the electron impact (EI) mode at 70 eV. The following capillary columns were used for quantification: BGB-FFAP (polyethylene glycol, esterified with terephthalic acid) and BGB-1701 (14% cyanopropylphenylpolysiloxane) (each 60 m × 0.25 mm, 0.5 μm film thickness; BGB-Analytik, Adliswil, Switzerland). One microliter of each concentrated extract was injected into a CIS 3 cold injection system (Gerstel, Mülheim, Germany) cooled with liquid nitrogen. The injector temperature was initially set at −50 °C with a splitless time of 0.7 min, then raised at 12 °C/s to 250 °C. Subsequently, two temperature programs were used: The initial oven temperature was held at 50 °C for 3 min, then raised at 5 °C/min up to 230 °C, and held for 15 min (FFAP column); the initial oven temperature of 40 °C was held for 3 min, raised at 5 °C/min to 220 °C, then raised at 20 °C/min to 280 °C, and held for 15 min (1701 column).

Quantitative Analysis. Four compounds (5-methyl-2-hexanone, 2-ethylpyrazine, 4-methoxybenzaldehyde, and 2-methylpropionic acid) were used as internal standards. These were dissolved in diethyl ether at a concentration of 50 ppm each to make a stock solution. One-half milliliter of this solution was added before the isolation of the volatiles. The concentrations were determined by comparison of HRGC/MS-EI target ion peak areas of each sample compound with the target ion peak areas of the respective external calibration curve of the identical synthetic reference standard. The used capillaries for quantification of the odorants, their target ions, and their appropriate qualifier ions for verification of the target ion peak areas are shown in Table 1. The internal standards were used to cover a range of compounds close to their chemical−physical properties, considering retention time, functional group, and boiling point.

Correction factors were calculated for each compound relative to its particular internal standard by using known amounts of synthetic standard substances of the soybean lecithin odorants [according to Buttery et al. (1994)]. These amounts were determined in triplicates in refined, deodorized soybean oil, which was vacuum distilled and treated under the same conditions as the samples (see above). The correction factors were calculated on the basis of the determined amounts as the recovery percentages of each compound in ratio to the recovery of the related internal standards (Table 1).

Odor Thresholds. The odor thresholds were carried out as recognition thresholds for the compounds **4**, **8**, **12**, **17**, **21**, **22**, **24**, **25**, **29**, **30**, **35**, **37**, **43**, **47**, and **49**−**52** (Table 2). They were determined nasally and retronasally by sets of increasing concentrations and by triangle tests in refined vegetable oil using a panel of 18 experienced assessors. The initial concentrations of the odorants in oil were determined in preliminary experiments. A sequence of six sample dilutions (1:1, w/w) was compared by the assessors with refined vegetable oil as a blank in order of their increasing concentrations. Three questions had to be answered: the perception of a difference between the blank oil and each sample dilution (detection threshold), the attribute of perception (recognition threshold), and the intensity of the perceived difference of each concentration on a six-point scale between zero (no difference) and five (very strong difference). According to the results of the panelists with the correct quality of perception, the threshold values were calculated according to the BgVV (1997). Following this, the calculated thresholds were proved by triangle tests (blank oil and calculated detection concentration in oil) by 18 experienced assessors. The threshold was verified when a significant difference ($\alpha = 0.05$) with the correct quality of the perception was obtained.

Sensory Model Mixtures. The 25 compounds **1**, **3**, **4**, **7**, **9**, **15**−**17**, **20**, **23**−**25**, **29**, **30**, **33**, **36**, **39**, **41**, **43**, **45**, **47**, **49**, **50**, **52**, and **54** were used for model mixtures. They were dissolved in ethanol and refined vegetable oil to make a stock solution of each odorant. By this, the concentration of ethanol in the stock solutions was lower than 500 ppm. Aliquots of these stock solutions were dissolved in refined vegetable oil to get the concentrations of the odorants of the hydrolyzed soybean lecithin (B), which are shown in Table 3. After being stirred for 1 h at room temperature, 1 mL of the model mixture and of the original lecithin were put each into 10-mL amber glass flasks.

Both samples were analyzed by 20 experienced assessors. The attributes of the aroma characteristics and their intensities are presented in Figure 3. The descriptive attributes of the soybean lecithin flavor were similar to those previously reported (Stephan and Steinhart, 1999). The intensity scale stretched—similar to the recognition threshold tests—from zero (no perception of the attribute) to five (very high perception of the attribute).

RESULTS AND DISCUSSION

Quantitative Analyses. To objectify the sensory importance of the 61 odorants revealed by the olfactory methods AEDA and modified CHARM (Stephan and Steinhart, 1999), a quantification of 54 important soybean lecithin odorants was carried out by HRGC/MS-EI and selected ion monitoring (SIM). The odorants were quantified in different treated soybean lecithins (standardized, hydrolyzed, and deoiled) to get, in addition, an insight into the influence of industrial processing on the lecithin flavor and its combination. These results are presented in Table 3.

The quantification of the compounds was based on correction factors (relative to their particular internal standards) according to Buttery et al. (1994) on determination of the peak area of target ions (which were convenient for quantification in the matrix lecithin) in relation to qualifier ions (Table 1) and on external calibration curves of each compound to increase the accuracy of the measured data. The correction factors contribute to the accuracy of the quantitative data by taking into consideration the ratio between the recovery of the odorant and its related internal standard. Only the very readily volatile compounds (**1** and **2**) and the difficult volatile compounds (**46**) differ by more than a factor of 2 in their recovery from their related internal standards. These factors of more than 2 are mainly caused by losses during the concentration process by microdistillation (**1** and **2**) and by low yields during the isolation process by the used vacuum (**46**). In this connection, it has to be considered that for the evaluation of the causes of the lecithin aroma the concept of odor activity values (OAV) by Rothe and Thomas (1963) is most successful; therefore, the determination of thresholds by human nose may be the analyzed value with the highest possibility of error. Nevertheless, a precise quantification is a necessary condition for minimization of inaccuracy of the general concept.

The quantification of soybean lecithin odorants revealed that free acids (particularly **47** and **54** and in addition **48**, **49**, and **52**) are by far the class of substance with the highest amounts of all odorants, followed by the group of alcohols (**16**), lactones (**45**), and furans (**14**), and then by ketones (**9** and **21**) and aldehydes (**7**, **4**, and **1**). Nitrogen compounds appeared only to a lesser extent (Table 3). Other classes of substance were insignificant

Table 2. Odor Thresholds of Soybean Lecithin Odorants in Oil

		thresholds[c] (μg/kg)		published thresholds[d−t]	
no.[a]	odorant[b]	nasal	retronasal	nasal	retronasal
1	methylpropanal			3.4[d]	3.4[d]
2	2,3-butanedione			4.5[e]	10[e]
3	3-methylbutanal			5.4[e]	10.8[e]
4	2-methylbutanal	38	17	2.2[f]	8.2[f]
				10[d]	23[d]
				140[n]	
7	hexanal			300[g]	73[g]
8	4-methyl-3-pentene-2-one	300	450		
9	2-heptanone			1500[e]	1500[e]
10	heptanal			3200[h]	42[h]
11	(*Z*)-4-heptenal			10[i]	0.5[j]
12	diethyl disulfide	78	31		
13	2-acetyl-1-pyrroline			0.1[k]	
14	pentylfuran			1000[l]	
15	1-octen-3-one			10[g]	0.3[g]
16	1-octen-3-ol			34[m]	36[m]
17	(*Z*)-1,5-octadien-3-one	0.13	0.03	0.45[g]	0.03[g]
19	octanal			56[f]	56[f]
20	acetylpyrazine			10[k]	
21	3-octen-2-one	250	140		
22	2-isopropyl-3-methoxypyrazine	3.1	0.24		
23	3-ethyl-2,5-dimethylpyrazine			24[n]	79[d]
24	2-ethyl-3,5-dimethylpyrazine	1.8	1.2	2.2[d]	2.2[d]
25	1-nonen-3-one	0.7	0.2		
27	(*E*)-2-octenal			7000[h]	1000[h]
28	nonanal			1000[n]	320[h]
29	2,3-diethyl-5-methylpyrazine	0.5	0.5	0.5[d]	0.9[d]
30	2-isobutyl-3-methoxypyrazine	10.3	7.7	0.8[d]	0.6[d]
32	phenylethanol			211[f]	122[f]
33	(*E*)-2-nonenal			900[g]	45[o]
34	(*E*,*Z*)-2,6-nonadienal			3.8[g]	1.4[g]
35	(*E*,*Z*)-2,6-nonadienol	58	26		
36	pentylpyridine			5[k]	
37	3,5,5-trimethyl-2-cyclohexen-1-one	68	32	60[p]	
38	(*E*,*E*)-2,4-nonadienal			1000[i]	150[i]
39	3-methyl-2,4-nonandione			22.5[q]	1.5[g]
41	(*E*,*E*)-2,4-decadienal			180[g]	41[g]
42	γ-octalactone			120[d]	197[d]
43	(*E*)-β-damascenone	1.7	1.0	11.2[f]	3.7[f]
44	*trans*-4,5-epoxy-(*E*)-2-decenal			1.3[g]	3[g]
45	γ-nonalactone			148[d]	219[d]
46	γ-decalactone			320[d]	385[d]
47	acetic acid	750	1750	124[f]	378[f]
				1050[r]	7000[s]
49	butanoic acid	205	520	135[t]	660[s]
50	3-methylbutanoic acid	41	24	22[f]	26[f]
51	2-methylbutanoic acid	240	>500		
52	pentanoic acid	61	138		
54	hexanoic acid			5400[t]	2500[s]

[a] Continuous numbers of odorants according to Tables 1 and 3. [b] Odorants with known nasal and/or retronasal thresholds in vegetable oil or paraffin oil. [c] Thresholds were determined nasally and retronasally as recognition thresholds in refined vegetable oil by sequences of increasing concentrations and by triangle tests by a panel of 18 experienced sensory assessors. [d−t] The threshold values (determined in vegetable and/or paraffin oil) were obtained from the following sources: (*d*) Wagner and Grosch (1998); (*e*) Preininger and Grosch (1994); (*f*) Reiners and Grosch (1998); (*g*) Guth and Grosch (1990b); (*h*) Meijboom (1964); (*i*) Meijboom and Jongenotter (1981); (*j*) McGill et al. (1974); (*k*) Schieberle (1996); (*l*) Smouse and Chang (1967); (*m*) Kubickova and Grosch (1998); (*n*) Guadagni et al. (1972); (*o*) Widder and Grosch (1994); (*p*) Kim et al. (1984); (*q*) Guth and Grosch (1989); (*r*) Guth and Grosch (1993); (*s*) Siek et al. (1969); (*t*) Schieberle et al. (1993).

except for aldol condensation products of acetone in deoiled lecithins (**8** and **37**). The compounds **7**−**9**, **14**, **16**, **22**, and **37** were already identified by Kim et al. (1984) as volatile compounds in deoiled lecithins but not quantified. To what extent major and particularly minor classes of substance and their related single odorants contribute to the general flavor of soybean lecithins is pointed out by their OAVs in this study for the first time (Table 3; Figures 1 and 2).

Odor Recognition Thresholds. The odor thresholds (Table 2) were determined as recognition thresholds to get a better insight into the potential influence of impact odorants on the attributes of the odor profiles of the soybean lecithins (Stephan and Steinhart, 1999). They were determined nasally and retronasally to reveal both the causes of the odor and the retronasal taste of the investigated lecithins. This differentiation was necessary because the two may be very different, e.g., the thresholds of 1-octen-3-one (**15**) (Guth and Grosch, 1990b). Accordingly, retronasal thresholds may be lower than nasal thresholds (e.g., **15**, **17**, and **22**) or vice versa (particularly the acids **47**, **49**, and **52**). Furthermore, a precise differentiation between perception and recognition thresholds is useful (Ranson and Belitz, 1992) to avoid inaccuracies in OAV calculation because of insufficient definition. Apart from variations of thresholds caused possibly by the number or the quality of the panelists (e.g., sensory memory, experience, reliability,

Table 3. Concentrations and Odor Activity Values (OAVs) of Potent Odorants in Standardized (A), Hydrolyzed (B), and Oil-Free (C) Soybean Lecithin

			concentration (μg/kg)[b]			nasal OAV[c]			retronasal OAV[d]		
no.[a]	odorant		A	B	C	A	B	C	A	B	C
	aldehydes	(Σ)	1308	1124	1167	95	78	27	118	92	49
	short-chain, branched	(Σ)	640	618	111	88	73	16	94	83	15
1	methylpropanal		232	191	30	68	56	9	68	56	9
3	3-methylbutanal		58	38	30	11	7	6	5	4	3
4	2-methylbutanal		350	389	51	9	10	1	21	23	3
	saturated (aromatic)	(Σ)	558	440	895	1	1	2	5	5	10
7	hexanal		328	329	688	1	1	2	4	5	9
10	heptanal		45	19	45	<1	<1	<1	1	<1	1
18	benzaldehyde		23	20	45						
19	octanal		38	18	37	<1	<1	<1	<1	<1	<1
28	nonanal		124	54	60	<1	<1	<1	<1	<1	<1
	unsaturated	(Σ)	110	66	161	6	4	9	19	4	24
11	(*Z*)-4-heptenal		4.2	0.1	2.8	<1	<1	<1	8	<1	6
27	(*E*)-2-octenal		19	23	27	<1	<1	<1	<1	<1	<1
33	(*E*)-2-nonenal		49	15	92	<1	<1	<1	1	<1	2
34	(*E*,*Z*)-2,6-nonadienal		14	5.8	21	4	2	6	10	4	15
38	(*E*,*E*)-2,4-nonadienal		6.3	5.1	6.7	<1	<1	<1	<1	<1	<1
41	(*E*,*E*)-2,4-decadienal		14	14	8.1	<1	<1	<1	<1	<1	<1
40	(*E*)-4,5-epoxy-(*E*)-2-nonenal		0.3	0.2	<0.1						
44	(*E*)-4,5-epoxy-(*E*)-2-decenal		2.7	2.8	3.7	2	2	3	<1	<1	1
	ketones	(Σ)	1757	2145	581	25	42	23	125	147	137
	saturated	(Σ)	1607	1948	93	6	4	<1	17	15	15
2	2,3-butanedione		21	14	2.9	5	3	<1	2	1	<1
5	2,3-pentanedione		4.7	14	4.0						
9	2-heptanone		1560	1900	64	1	1	<1	1	1	<1
39	3-methyl-2,4-nonandione		21	20	22	<1	<1	<1	14	13	15
	unsaturated	(Σ)	150	197	488	19	38	23	108	132	122
15	1-octen-3-one		13	7.9	16	1	<1	2	43	26	53
21	3-octen-2-one		91	83	447	<1	<1	2	<1	<1	3
25	1-nonen-3-one		2.9	3.0	1.6	4	4	2	15	15	8
31	3-nonen-2-one		35	63	11						
17	(*Z*)-1,5-octadien-3-one		1.3	1.6	1.4	10	12	11	43	53	47
43	(*E*)-β-damascenone		6.8	38	11	4	22	6	7	38	11
	nitrogen compounds	(Σ)	181	286	16	58	150	3	49	147	1
20	acetylpyrazine		40	31	3.2	4	3	<1			
23	3-ethyl-2,5-dimethylpyrazine		29	90	0.9	1	4	<1	<1	1	<1
24	2-ethyl-3,5-dimethylpyrazine		34	54	0.5	19	30	<1	28	45	<1
29	2,3-diethyl-5-methylpyrazine		8.6	49	0.5	17	98	1	17	98	1
22	2-isopropyl-3-methoxypyrazine		0.9	0.6	0.1	<1	<1	<1	4	3	<1
30	2-isobutyl-3-methoxypyrazine		1.1	1.9	0.5	<1	<1	<1	<1	<1	<1
13	2-acetyl-1-pyrroline		0.4	0.3	<0.1	4	3	<1			
36	pentylpyridine		67	59	10	13	12	2			
	free acids	(Σ)	39039	69268	18810	81	120	37	46	67	19
47	acetic acid		26900	51200	12800	36	68	17	15	29	7
48	propanoic acid		2910	3110	1480						
49	butanoic acid		1840	1000	414	9	5	2	4	2	<1
52	pentanoic acid		1790	2160	945	29	35	15	13	16	7
54	hexanoic acid		4150	10300	1980	<1	2	<1	2	4	<1
50	3-methylbutanoic acid		292	377	118	7	9	3	12	16	5
51	2-methylbutanoic acid		230	275	149	<1	1	<1	<1	<1	<1
53	(*E*)-2-butenoic acid		927	846	924						
	alcohols, furans, lactones	(Σ)	1183	2879	710	6	32	8	6	28	7
18	1-octen-3-ol		125	834	264	4	25	8	4	23	7
26	1-nonen-3-ol		45	40	16						
32	phenylethanol		141	103	2.4	<1	<1	<1	1	<1	<1
35	(*E*,*Z*)-2,6-nonadienol		6.8	5.8	2.6	<1	<1	<1	<1	<1	<1
14	pentylfuran		305	798	25	<1	<1	<1			
42	γ-octalactone		9.7	51	14	<1	<1	<1	<1	<1	<1
45	γ-nonalactone		304	1020	75	2	7	<1	1	5	<1
46	γ-decalactone		246	27	311	<1	<1	<1	<1	<1	<1
	sulfur compounds	(Σ)	127	25	36	2	<1	<1	4	<1	1
6	dimethyl disulfide		0.8	1.5	3.5						
12	diethyl disulfide		126	23	32	2	<1	<1	4	<1	1
	miscellaneous compounds[e]	(Σ)	19	26	428	<1	<1	4	<1	<1	9
8	4-methyl-3-pentene-2-one		2.8	2.0	149	<1	<1	<1	<1	<1	<1
37	3,5,5-trimethyl-2-cyclohexen-1-one		16	24	279	<1	<1	4	<1	<1	9

[a] Continuous numbers of odorants according to Tables 1 and 2. [b] The data were mean values of at least triplicates (maximum standard deviation: ±20%). [c,d] The odor activity values were calculated by dividing the concentrations of the odorants by their nasally (*c*) and retronasally (*d*) determined detection thresholds in oil. [e] Aldol condensation products of acetone.

power of concentration, sense of responsibility, or staying power), these insufficient definitions may be one reason for considerable differences in the reported thresholds of the same odorant. For example, the nasal odor threshold of 2-methylbutanal (**4**) was determined in oil from 2.2 ppb (Reiners and Grosch, 1998), over 10

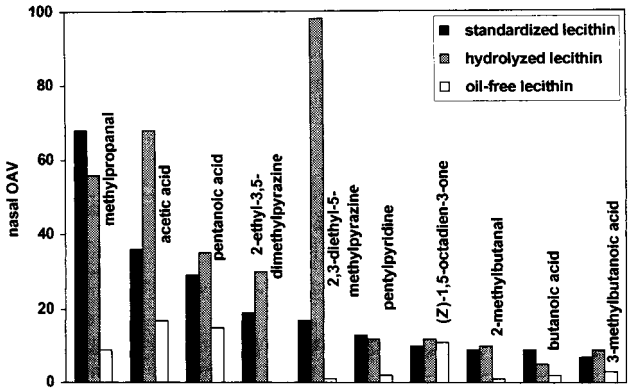

Figure 1. Odorants with the highest nasal odor activity values (nOAVs) in different treated soybean lecithins.

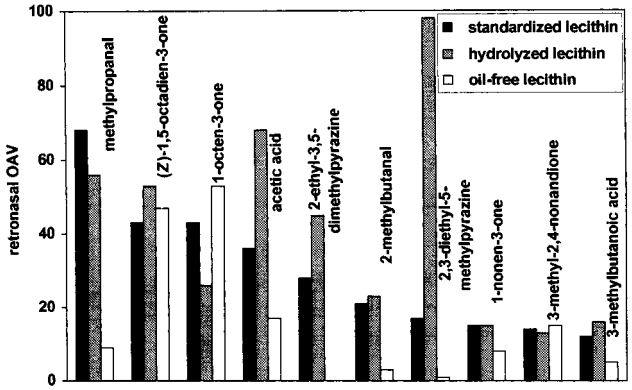

Figure 2. Odorants with the highest retronasal odor activity values (rOAVs) in different treated soybean lecithins.

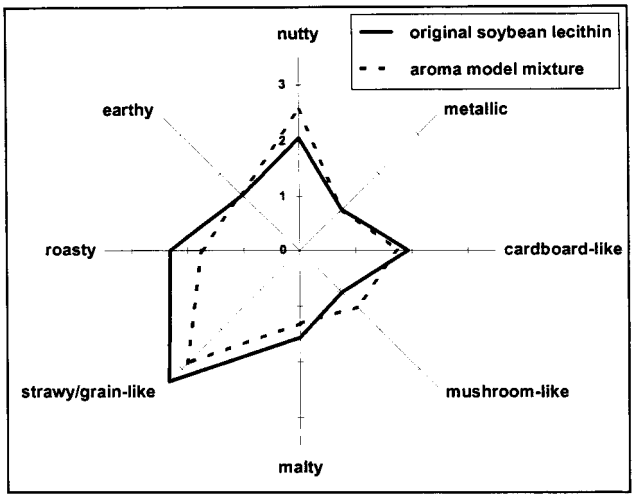

Figure 3. Retronasal odor profiles of the original enzymatic hydrolyzed soybean lecithin and the appropriate aroma model mixture in oil.

ppb (Wagner and Grosch, 1998), 38 ppb (own determination, Table 2), and up to 140 ppb (Guadagni et al., 1972); the retronasal odor threshold of acetic acid (**47**) in oil from 378 ppb (Reiners and Grosch, 1998), over 1750 ppb (own determination), and up to 7000 ppb (Siek et al., 1969).

To make the thresholds and the calculated OAVs of different odorants as comparable to each other as possible, the determination of recognition thresholds of compounds with potentially high OAVs in soybean lecithin (**4**, **17**, **24**, **29**, **30**, **43**, **47**, **49**, and **50**) was carried out as well as those of the odorants **8**, **12**, **21**, **22**, **25**, **35**, **37**, **51**, and **52** for which no nasal and/or retronasal thresholds in oil have been reported to date.

Odor Activity Values (OAVs). The OAVs (Table 3) were calculated nasally and retronasally on the basis of the threshold values listed in Table 2 and the quantitative data of Table 3. The compounds with the highest OAVs were found in the following classes of substance: aldehydes, ketones, nitrogen compounds, and free acids. The odorants of the different treated soybean lecithins with the highest nasal odor activity values (nOAVs) are presented in Figure 1, with the highest rOAVs in Figure 2.

Aldehydes. The most important aldehydes were the short-chain, branched compounds methylpropanal (**1**), 2-methylbutanal (**4**), and 3-methylbutanal (**3**) with a malty and strawy odor. Particularly odorant **1** showed one of the highest nOAVs and rOAVs in standardized and hydrolyzed lecithins. In these lecithins other aldehydes were of lesser importance. Deoiled lecithin showed considerably lower aldehyde influence on the aroma than the other investigated lecithins but a wider range of aldehydes (saturated, unsaturated, and short-chain, branched) with rOAVs of relevance (**34**, **7**, **1**, **11**, **3**, **4**, and **33**). These aldehydes develop an odor from cucumber-like, over green, malty, strawy, fishy, to cardboard-like.

Ketones. The ketones with the highest OAVs were unsaturated ketones such as (*Z*)-1,5-octadien-3-one (**17**), β-damascenone (**43**), 1-octen-3-one (**15**), and 1-nonen-3-one (**25**) containing metallic, apple-, or mushroom-like odor. Their retronasal importance was on average by a factor of 5 higher than their nasal. **17** and **15** have been determined by Guth and Grosch (1990b) as compounds with high rOAVs in stored soybean oil too. To date, **43** and **25** have not been reported as impact odorants in soybean oil or lecithin. **25**, which has been reported recently at first in yogurt (Ott et al., 1997), had the lowest retronasal threshold of all lecithin volatiles in oil (0.2 μg/kg) except for **17**. The OAVs of the odorants **15**, **17**, and **25** did not vary in the different treated lecithins by more than factor 2. These compounds were not formed by enzymatical hydrolysis and were not removeable by acetone deoiling. Their major influence on the aroma was very similar in all investigated lecithins.

Particularly the compound 3-methyl-2,4-nonandione (**39**) was identified by Guth and Grosch (1989, 1990a,b, 1991) as a light-induced, character impact compound in soybean oil that causes the strawy odor of reverted soybean oil flavor. In comparison to soybean oil (30 days daylight stored) with amounts of **39** up to 721 μg/kg (Guth and Grosch, 1990b), the investigated different treated soybean lecithins had only amounts of **39** between 20 and 22 μg/kg. Consequently, this compound had no outstanding sensory characteristics in soybean lecithins and cannot be the character impact compound that is solely responsible for the main strawy and grain-like odor of soybean lecithins. Its retronasal importance was more in the scale of the compounds **4**, **25**, or **52**.

Nitrogen Compounds. In contrast to soybean oil, nitrogen compounds had a high impact on the aroma in soybean lecithins. Roasty and earthy perceptions were caused mainly by 2,3-diethyl-5-methylpyrazine (**29**) and 2-ethyl-3,5-dimethylpyrazine (**24**). Both pyrazines are well-known as the trisubstituted alkylpyrazines with the lowest odor thresholds (Grosch, 1993). They were in a similar order of nasal and retronasal importance with thresholds in oil down to 0.5 μg/kg for

29. Enzymatic hydrolysis of lecithins increased the OAVs of both pyrazines, particularly of **29** to the highest OAVs of all, while the process of deoiling by acetone significantly removed all nitrogen compounds almost completely.

Free Acids. Free acids have lower nasal thresholds than retronasal thresholds (Table 2), but nevertheless **47**, **52**, **50**, and **49** were elucidated as both nasally and retronasally important. Particularly the nOAV of acetic acid (**47**) increased due to hydrolysis by almost a factor of 2 and decreased due to deoiling of the hydrolyzed lecithin by a factor of 4. Despite their very high amounts, free acids had due to their high thresholds a nasal importance on the general lecithin aroma only similar to that of aldehydes and nitrogen compounds and a retronasal importance lower than aldehydes and nitrogen compounds.

Miscellaneous Compounds. Other components were of little importance with the following three exceptions: 1-octen-3-ol (**18**) and γ-nonalactone (**45**) with higher nOAVs and rOAVs in hydrolyzed lecithin and isophorone (**37**) as an acetone condensation product in deoiled lecithin. Furthermore, alcohols, furans, lactones, and sulfur compounds did not yield impact OAVs.

Model Mixtures. A simulation of the original hydrolyzed soybean lecithin aroma was achieved very well in model mixture experiments by the combination of the 25 odorants with the highest rOAVs in oil (Figure 3). The profiles of the original and the model mixture aroma showed nearly identical intensities in the perception of such attributes as earthy, metallic, cardboard-like, and malty. The intensities of the perception of the other four attributes were also very similar with a deviation of less than 0.5 scale point on a six-point scale (between 0 and 5). The nutty and mushroom-like perception of the model mixture was a little more intensive; the grain-like/strawy and roasty was a little less than that of the original lecithin. The successful combination of the odorants confirmed the conclusion that by these studies the potent odorants were identified and quantified satisfactorily. The extent of the direct influence of selected compounds on single odor attributes may only be speculated on on the basis of their OAVs: In all probability the roasty, earthy attributes are caused mainly by **29** and **25**, but the major attribute strawy/grain-like is presumably caused by synergisms of a number of compounds, e.g., of **1**, **3**, **4**, **36**, **37**, and **39**.

CONCLUSION

These results pointed out that the main processes of formation of the potent odorants in soybean lecithins are caused by both the peroxidation of lipids and the thermal formation via the Maillard reaction of nitrogen containing phospholipid residues. Most of the potent aroma compounds were found in all investigated soybean lecithins but in different concentrations. The compounds with the highest nOAVs and rOAVs were found to be unsaturated ketones; short-chain, branched aldehydes; pyrazines; and free acids. While ketones and aldehydes remained almost at the same level after hydrolysis, the importance of free acids and particularly pyrazines increased significantly. Although pyrazines and most of the free acids and short-chain, branched aldehydes were successfully removed by the process of acetone deoiling, the major important unsaturated ketones were not removed by this technique. They remained in deoiled lecithin and may be significant contributors with the unsaturated aldehydes to off-flavor problems in soybean lecithin applications with higher water contents.

ABBREVIATIONS USED

AEDA, aroma extract dilution analysis; CHARM, combined hedonic and response measurement; EI, electron impact; HRGC/MS, high-resolution gas chromatography/mass spectrometry; nOAV, nasal odor activity value; OAV, odor activity value; rOAV, retronasal odor activity value; SIM, selected ion monitoring.

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