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Odor-Active Compounds in Cardboard

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The odor-active compounds of cardboard were identified by aroma extract dilution analysis and HRGC-MS analysis. In total, 36 compounds were detected with medium to high intensities during HRGC−olfactometry. The highest odor intensities were evaluated for vanillin, (*E*)-non-2-enal, (*R*/*S*)-γ-nonalactone, 2-methoxyphenol, (*R*/*S*)-δ-decalactone, *p*-anisaldehyde, 3-propylphenol, and a woody-smelling unknown compound. Most of the identified compounds were described as odor-active cardboard constituents for the first time. Sensory experiments demonstrated that extensive release of odor-active compounds occurred upon moistening of the cardboard. Accordingly, data indicated that the odorants are present in cardboard in relatively high amounts. In a further sensory study, a transfer of the released odor to food was demonstrated in a model experiment showing that cardboards with high odor potential can cause unwanted flavor changes in foods.

KEYWORDS: Cardboard; odor; aroma extract dilution analysis (AEDA); odor threshold; odorant transfer

INTRODUCTION

Paper and cardboard are very important packing materials. About half of the 384 million tons produced worldwide in 2007 was used for wrapping and packaging purposes (*1*). Alone or in combination with barrier materials, paper and cardboard are frequently used as packaging material for foods, for example, chocolate, confectionaries, sugar, liquids, and pizza boxes. Therefore, these materials need to comply with special requirements regarding food safety and sensory quality. Especially with the focus on consumer acceptance, paper and cardboard need to exhibit low or insignificant odor.

Most specifically, odor transfer from the packaging materials to foods needs to be obviated as it can negatively affect food quality. Accordingly, producers need to optimize paper production with regard to odor minimization and odor stabilization (*2*). Therefore, detailed knowledge about the sources of odor and off-flavor generation is required if producers want to control and avoid odor liberation from paper and cardboard. As an example, off-flavor formation in cardboards can sporadically occur due to microbiological and autoxidative processes during manufacturing and storage (*2*−*6*).

To characterize off-odor formation, a series of test methods has been developed. Odor measurement by a human sensory panel consisting of a number of experienced assessors is one of the standard methods (*7*) allowing the evaluation of odor intensities and the detection of off-flavors. Nevertheless, sensory tests are expensive and time-consuming, so that additional analytical methods have been applied. So-called electronic noses, consisting of, for example, metal oxides as gas sensors, were applied for volatile cardboard compound detection and were used for differentiation of several cardboard samples (*8*, *9*). However, as no molecular identification of the respective odor-inducing substances was achieved using these methods, information on the reasons for a malodor cannot be deduced.

In another approach, volatiles emitted from paper and cardboard have been analyzed by means of gas chromatography in combination with mass spectrometry (*4*, *10*, *11*). A number of compounds, for example, hydrocarbons, aldehydes, alcohols, aromatic compounds, and short-chain fatty acids, were identified using this method. Although quantitative investigations on selected odorants have been carried out and odor threshold considerations based on a water matrix have been depicted (*11*), nonetheless, no comprehensive data are at hand on the respective odor contribution of specific compounds to the characteristic cardboard odor. However, many investigations in the area of food aroma have proven that the odor activity of volatiles depends drastically on the structure (*12*, *13*). Odor thresholds can vary by a magnitude of up to 10^9 (*14*). Accordingly, as no data on air−cardboard distributions of cardboard odorants have to date been published, the contribution of previously identified cardboard volatiles could not yet be rated comprehensively.

The first investigation that accounted for different odor activities of volatiles was performed by Leitner and Pfannhauser (*15*). By application of high-resolution gas chromatography−olfactometry (HRGC-O), successful detection of odor-active compounds was achieved by sniffing the effluent during gas chromatography. The introduction of the human nose as an additional detector resulted in the identification of a number of odor-active aldehydes, for example, hexanal, heptanal, octanal, nonanal, and their (*E*)-2 counterparts. In particular, the unsaturated aldehydes were presumed to be important contributors to cardboard odor due to their low odor thresholds. In this study,

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solid phase microextraction was used to isolate the volatile compounds. This method, however, is known to be prone to discrimination effects depending on the molecular structure and the volatility of the analyzed compounds (*16*). It is therefore unclear whether all volatile compounds and odorants, respectively, were evaluated and rated appropriately according to their respective odor intensities in the samples.

Ziegleder (*2*) also used HRGC-O but performed steam distillation on the volatiles from recycled cardboard samples that were affected with an off-flavor. In the extract, some of the aforementioned aldehydes, as well as carbon acids and aromatic compounds, were identified as odorants. On the basis of a ranking of the odorants on a three-point intensity scale during sniffing, oct-1-en-3-one, benzaldehyde, and 3-methylbutanoic acid were detected with high odor activities and were therefore regarded as the main contributors to the investigated off-flavor. This study, as well as that performed by Leitner and Pfannhauser (*15*), revealed sensory information about odor-active compounds. However, it is not clear whether the data comprehensively accounted for the relative contribution of each odorant to cardboard odor.

Aroma extract dilution analysis (AEDA) is a reliable and proven screening tool to obtain insights into the relative odor potencies of selected substances in a complex odor mixture (*12*, *13*). To apply this tool, it is a fundamental prerequisite that odorant isolation from the matrix is achieved by means of a mild separation technique inducing as little heat stress as possible. Solvent extraction in combination with isolation of volatiles in high vacuum by solvent-assisted flavor evaporation (SAFE) (*17*) fulfills this requirement. Using this technique, many potent odorants were identified unequivocally in diverse food aromas, for example, grapefruit juice (*18*), cacao (*19*), beer (*20*), and coffee (*21*). The present study therefore aimed at the identification of the most odor-active compounds in cardboard using SAFE and AEDA. In addition, the characteristic cardboard smell and odor transfer to a model food system were evaluated by sensory means.

MATERIALS AND METHODS

Material. Conventional cardboard material for packaging purposes was used for the investigations. The cardboard was stored under normal storage conditions at 20 °C in the dark until use.

Reference Odorants. The compounds listed in **Table 1** were purchased from the following suppliers: compounds **1**−**3**, **5**, **6**, **8**, **12**, **13**, **15**, **16**, **18**, **20**−**22**, **24**, **26**, **27**, **29**, **31**−**35**, (*E*)-dec-2-enal, 2-propylphenol, and 4-propylphenol (Aldrich, Steinheim, Germany); **7** (ABCR, Karlsruhe, Germany); **11** (Aromalab, Freising, Germany). Silica gel (0.063−0.200 mm) and $AgNO_3$ were from Merck (Darmstadt, Germany).

Preparation of (*Z*)- and (*E*,*Z*)-Isomers by Silver Chromatography. (*Z*)-Non-2-enal (**10**), (*E*,*Z*)-deca-2,4-dienal (**19**), and (*Z*)-dec-2-enal (**25**) were prepared according to a modified procedure from Steinhaus et al. (*22*) from commercial samples of (*E*)-non-2-enal, (*E*,*E*)-deca-2,4-dienal, and (*E*)-dec-2-enal, respectively, which contained these (*Z*)-isomers as impurities.

$AgNO_3$ (10 g) was dissolved in distilled water (200 mL). Silica gel was added to the solution, homogenized by stirring at room temperature in the dark for 15 min, and finally dried at 105 °C in the dark for 18 h. A slurry of freshly prepared $AgNO_3$−silica gel (30 g) in *n*-pentane/diethyl ether (95:5, v/v) was filled in a water-cooled glass column (30 × 2 cm). The (*E*)- or (*E*,*E*)-isomers were dissolved in *n*-pentane/diethyl ether (95:5, v/v) in a concentration of 100 mg/mL, and the solutions (1 mL) were added separately onto the top of the silica gel. The gel was eluted with *n*-pentane/diethyl ether (95:5, v/v, 300 mL), and the eluate was collected in 30 fractions (10 mL each).

All solutions were analyzed by HRGC, and the highest isomeric purities were determined in fractions 11 (**10**, 100%), 12 (**25**, 95%), and 17−19 (**19**, 98%) by HRGC on the basis of the calculated area ratios of the isomers. The fractions were analyzed by HRGC-HRGC-MS-O (MS-EI and MS-CI). The linear retention indices and mass spectra (MS-EI) were in agreement with an in-house retention database and literature data of the compounds (*23*−*25*).

Isolation of Volatile Compounds. Cardboard (20 g) was cut into small pieces (5 × 5 mm), moistened with distilled water (5 mL), and extracted with dichloromethane (200 mL) at room temperature for 16 h. After filtration, the extract was distilled in high vacuum using the SAFE technique (*17*) at 50 °C. The distillate was thawed and concentrated to about 0.1 mL using a Vigreux column (50 × 1 cm) and microdistillation (*26*).

High-Resolution Gas Chromatography (HRGC). HRGC analyses were performed with a gas chromatograph type 5160 (Carlo Erba, Hofheim, Germany) equipped with a cold on-column injector and a flame ionization detector (FID). The analyses were accomplished using the capillaries DB-FFAP and DB-5 (30 m × 0.32 mm, film thickness = 0.25 μm, J&W Scientific, Folsom, CA). The helium carrier gas flow was set at 1.5 mL/min. The oven start temperature was 40 °C, held for 2 min. The oven was heated at a rate of 8 °C/min to 230 °C (DB-FFAP) or 280 °C (DB-5), respectively, and held for 5 min.

High-Resolution Gas Chromatography−Olfactometry (HRGC-O). HRGC-O analyses were performed with the GC system described above with the following modifications: the compounds eluting at the end of the capillaries were split with a Y-splitter (J&W Scientific; ratio 1:1, v/v) and transferred via two deactivated capillaries (0.5 m × 0.2 mm, J&W Scientific) to the FID and a heated sniffing port (280 °C).

Two-Dimensional High-Resolution Gas Chromatography−Mass Spectrometry−Olfactometry (HRGC-HRGC-MS-sO). HRGC-HRGC-MS-O analyses were performed with a system that consisted of two gas chromatographs type 3800 (Varian, Darmstadt, Germany). The GCs were connected with the Cryo Trap System CTS 1 (Gerstel, Mülheim/Ruhr, Germany). The first GC was equipped with the preparative capillary DB-FFAP (30 m × 0.32 mm, 0.25 μm film thickness, J&W Scientific) and the multicolumn switching system MCS 2 (Gerstel). The compounds eluting at the end of the capillary were split as described above into a FID and the sniffing port ODP (Gerstel).

The extracts were applied with the cold-on-column technique onto the capillary using the temperature program described above. Odorants were detected by sniffing the effluents at the sniffing port. In a second run, eluting odorants of a defined retention area (odorant retention time up to ±0.15 min) were transferred onto the cryo trap, which was cooled to −100 °C. After thermodesorption at 250 °C, the volatiles were flushed onto the analytical capillary DB-5 (30 m × 0.25 mm, 0.25 μm film thickness, J&W Scientific), which was installed in the second oven. The starting temperature of 40 °C was immediately raised after odorant transfer at a rate of 6 °C/min to 250 °C and then held for 5 min. The end of the capillary was split again as described above, and the eluting compounds were transferred into the mass spectrometer Saturn 2200 (Varian, Darmstadt, Germany) and the sniffing port ODP (Gerstel). Mass spectra were generated in the electron impact mode (MS-EI) and with chemical ionization (MS-CI) using methanol as the reagent gas.

AEDA. The extract containing the cardboard volatiles (cf. Isolation of Volatile Compounds) was stepwise diluted with dichloromethane (1:2, v/v), and each dilution was analyzed by HRGC-O. Flavor dilution (FD) factors of odor-active compounds were determined as previously described (*12*, *13*) and the relative retention indices (RI) of the odorants were calculated (*27*).

Identification of Odorants. The cardboard extract was analyzed by HRGC-HRGC-MS-O as described above. The odorants were detected on the analytical capillary by sniffing the effluent. In an additional GC run, the reference compounds dissolved in dichloromethane (about 2 μg/mL) were analyzed under the same conditions, and sniffing was performed again in the second dimension. The compounds were identified by comparison of the RI on capillaries DB-FFAP and DB-5, the odor quality, and the mass spectra (MS-EI) with the properties of the respective reference compound.

Sensory Analysis. All sensory analyses were performed with a trained sensory panel consisting of 12 members (2 male, 10 female, aged 27−41 years). The panelists were trained in weekly sessions in recognizing orthonasally about 120 selected odorants at different odorant concentrations according to their odor qualities. Training in these sessions was at least for half a year prior to participation in the actual sensory experiments.

Table 1. Intense Odor-Active Compounds (FD ≥ 16) in Cardboard Identified by Aroma Extract Dilution Analysis and HRGC-HRGC-MS-O Experiments

			RI[b]				
no.	compound	odor quality[a]	DB-FFAP	DB-5	FD[c]	ID procedure[d]	earlier identified by[e]
1	vanillin	vanilla-like, sweet	2559	1402	4096	A	
2	(*E*)-non-2-enal	cardboard-like, fatty, green	1525	1158	512	A	*15*
3	(*R*/*S*)-γ-nonalactone	coconut-like	2014	1360	512	A	
4	unknown	woody	2490	2012	512		
5	2-methoxyphenol	smoky, vanilla-like, sweet	1851	1086	256	A	
6	(*R*/*S*)-δ-decalactone	coconut-like, sweet	2185	1489	256	A	
7	3-propylphenol	leather-like, phenolic, ink-like	2250	1285	256	A	
8	*p*-anisaldehyde	sweet woodruff-like, sweet	2236	1259	256	A	
9	unknown	coconut-like	2217	1474	256		
10	(*Z*)-non-2-enal	cardboard-like, fatty, green	1498	1146	128	A	
11	*trans*-4,5-epoxy-(*E*)-dec-2-enal	metallic	1997	1383	128	A	
12	4-methylphenol	horse stable-like, fecal	2077	1075	128	A	
13	4-ethylphenol	horse stable-like, fecal, ink-like	2167	1165	128	A	
14	unknown	sweet, woody	2132	1481	128		
15	(*R*/*S*)-γ-dodecalactone	peach-like, fruity	2366	1690	128	A	
16	(*R*/*S*)-δ-dodecalactone	peach-like, sweet, flowery	2384	1719	64	B	
17	unknown	fatty	2400	1491	64		
18	octanal	soapy, citrus-like, green	1283	1002	64	A	*2*, *10*, *11*, *15*, *42*
19	(*E*,*Z*)-nona-2,4-dienal	fatty	1654	1195	64	B	
20	benzothiazole	rubber-like, car tire-like	1929	1231	64	A	*2*, *11*
21	nonanal	soapy, fatty, green	1385	1106	64	A	*2*, *10*, *11*, *15*, *42*
22	(*E*,*E*)-nona-2,4-dienal	fatty	1690	1214	64	A	
23	unknown	woody, sweet	2500	1814	64		
24	(*E*,*Z*)-nona-2,6-dienal	cucumber-like, green	1577	1153	32	B	
25	(*Z*)-dec-2-enal	fatty	1600	1249	32	A	
26	3-methylphenol	leather-like, phenolic, ink-like	2082	1079	32	A	
27	(*R*/*S*)-3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	spicy, savory-like	2186	1316	32	B	
28	unknown	citrus-like	2479	1574	32		
29	hexanal	grassy, green	1080	797	32	A	*2*, *3*, *10*, *11*, *15*, *42*, *43*
30	unknown	fatty	1439	1096	32		
31	(*E*,*E*)-deca-2,4-dienal	fatty	1800	1316	32	A	*2*, *11*
32	oct-1-en-3-one	mushroom-like	1291	974	16	A	*2*, *11*
33	butanoic acid	cheesy, sweaty	1621		16	A	*2*, *5*, *6*, *11*
34a/b	(*R*/*S*)-2-/3-methylbutanoic acid	sweaty, cheesy, fruity	1664		16	A	*2*, *5*, *11*
35	(*R*/*S*)-γ-octalactone	coconut-like, sweet	1884	1266	16	A	
36	unknown	citrus-like	2319		16		

[a] Odor quality perceived during HRGC−olfactometry and evaluated according to ref *14*. [b] RI, linear retention indices (*27*). [c] FD, flavor dilution factor (*12*). [d] ID, the odorants were identified using the following criteria: A, comparison of the odor qualities at the second oven during HRGC-HRGC-MS-O analysis, linear retention indices on capillaries DB-FFAP and DB-5, and the mass spectra (MS-EI) with the properties of the reference compounds; B, the MS signals were too weak for an unequivocal identification. The compounds were identified tentatively by the remaining criteria given for A. [e] The compound was previously identified in the reference given in italics.

Sensory analyses were performed in a sensory panel room at 21 ± 1 °C in three different sessions. On the basis of reference aroma solutions at defined concentrations, a flavor language was developed, defining the specific smell of a compound for a certain aroma attribute.

Determination of Odor Qualities and Odor Thresholds. The odor qualities of the odorants were evaluated according to the method given in ref *14* using aqueous odorant solutions at concentrations of a factor 100 above their respective orthonasal odor thresholds. The odor thresholds of 2-, 3-, and 4-propylphenol were determined in air according to the method in ref *28* using (*E*)-dec-2-enal as an internal odor standard. Odor thresholds in water and the purity check of the odorants were performed according to the procedures of ref *14*.

Aroma Profile Analysis. The odor characteristics of cardboard were evaluated by aroma profile analysis, which followed a detailed protocol: cardboard (dry or moistened with tap water) was cut into pieces (2 × 2 cm), placed in a glass beaker (volume = 140 mL), and presented to the sensory panel. In the first session, the panel had to describe the characteristic odor attributes they perceived while sniffing the samples. On the basis of the frequency of detection, predefined odor attributes were selected (**Figures 2** and **3**). Cardboard samples were presented again to the panel in a second session, and the selected odor attributes were evaluated on a scale from 0 (not detectable) to 1 (weak intensity), 2 (medium intensity), or 3 (strong intensity). The intensity scores of each attribute were averaged.

Odor Transfer Experiment. Odor transfer experiments were performed with the setup detailed in **Figure 1**. Cardboard squares (2 g each) were placed in a glass flask (80 mL, flask 2) and moistened with tap water (1 mL). This flask was then placed in flask 1 (500 mL) containing sunflower oil (100 mL). Flask 1 was closed with a glass lid and stored at room temperature in darkness for 2 days. An additional glass flask (500 mL) was filled with sunflower oil (100 mL), closed with a lid, and stored in parallel, under the same conditions, as a control.

RESULTS AND DISCUSSION

Sensory Evaluations. The cardboard smell was first investigated in sensory experiments. The overall odor quality was evaluated as a standard cardboard smell, and only a weak to medium odor intensity was detectable (data not shown). Aroma profile analysis (APA) provided more detailed sensory information. As illustrated in **Figure 2**, the cardboard odor was dominated by cardboard-like, woody, and musty notes, which were detectable with weak to medium intensities. Fatty, sweet, moldy, and vanilla-like qualities were found in addition, but they were only very weakly perceivable.

The aroma profile changed drastically when the cardboard was moistened. The overall odor was now described as intense immediately after moistening and was rated as an off-flavor. The intensities of the three attributes cardboard-like, woody, and musty increased to medium and intense, respectively, and the

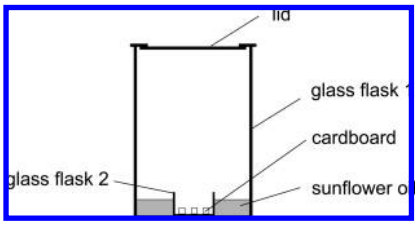

Figure 1. Setup for odor transfer experiment.

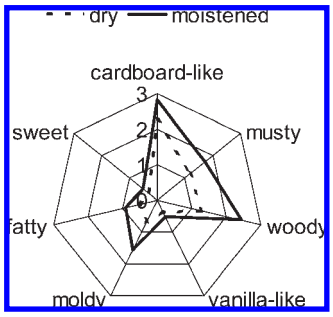

Figure 2. Aroma profile analysis of dry and moistened cardboard.

odor qualities fatty and moldy were now detectable with weak and medium intensities (**Figure 2**).

The sensory tests showed that cardboard can exhibit high odor potency. Although the specific smell of the investigated dry cardboard was low, intensities of characteristic odor attributes increased significantly upon moistening. This process is not unlikely to occur during handling and storage of packaged foods (pizza boxes, cardboard containers for ice cream) as moistening due to air humidity or other sources such as rain can readily occur.

From the presented data it becomes evident that cardboard odor consists of a large number of odor-active compounds in high amounts, but only some of them seem to be released from the dry surface. Considerable amounts of odorants might be included into the cellulose in the inner cardboard and released by water, which acts like a solvent. This assumption is supported by a study of Guth and Grosch (*29*), who quantified much higher released amounts of several odorants in oatmeal after moistening. A further indication for this hypothesis are odor thresholds, which are usually higher in cellulose than in water, for example, for oct-1-en-3-one (2.0 μg/kg of cellulose and 0.036 μg/L of water, respectively), (*E*)-non-2-enal (15 μg/kg and 0.69 μg/L), and ethyl 2-methylbutanoate (0.50 μg/kg and 0.063 μg/L) (*14*, *30*, *31*).

In an additional sensory experiment, the transfer of moistened cardboard odor to sunflower oil as a food model was demonstrated using the setup detailed in **Figure 1**. Two oil samples, one with and one without (control) cardboard exposure, were stored for 2 days and APA of both samples was performed. The test demonstrated that the control sample exhibited a predominantly oily and fatty note, which was characteristic of sunflower oil (**Figure 3**). However, the odor of the exposed sample was influenced by the moistened cardboard. Most specifically, the attributes cardboard-like, woody, and sweet were detectable with higher intensities in this sample.

Cardboard with a high odor potential can therefore be an off-odor source in the case of improper handling and storage, for example, high humidity or water contact. This is especially true for cardboards with no aroma barrier, which have more or less direct contact with the packed foods (e.g., confectionaries, sugar, pizza boxes).

Identification of Odor-Active Compounds of Moistened Cardboard. To clarify the molecular reasons for the high odor potency

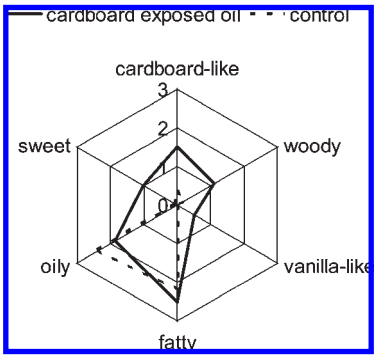

Figure 3. Aroma profile analysis of sunflower oil exposed to moistened cardboard and an untreated sunflower oil sample (control).

of the moistened cardboard sample, the volatile fraction of the moistened cardboard was extracted and carefully isolated by the SAFE technique. The extract, which elicited the typical cardboard odor, was finally concentrated and applied to AEDA. By sniffing the GC effluent after injection of the extract, a total of 62 compounds was detected. However, the number of odor-active compounds decreased drastically when the extract was diluted and AEDA was performed, so that 36 odor-active compounds were found in a FD range of 16−4096 (**Table 1**).

Identification based on HRGC-HRGC-MS-O analyses revealed vanillin (**1**) (odor quality: vanilla-like, sweet) as the odorant with the highest odor intensity (FD 4096) (**Table 1**). (*E*)-Non-2-enal (**2**) (cardboard-like, fatty, green), (*R*/*S*)-γ-nonalactone (**3**) (coconut-like), and unknown compound **4** with a woody odor impression were found as additional intense odorants with FD 512.

2-Methoxyphenol (**5**) (smoky, vanilla-like, sweet), (*R*/*S*)-δ-decalactone (**6**) (coconut-like, sweet), *p*-anisaldehyde (**8**) (sweet woodruff-like, sweet), (*Z*)-non-2-enal (**10**) (cardboard-like, fatty, green), *trans*-4,5-epoxy-(*E*)-dec-2-enal (**11**) (metallic), 4-methylphenol (**12**) (horse stable-like, fecal), 4-ethylphenol (**13**) (horse stable-like, fecal, ink-like), (*R*/*S*)-γ-dodecalactone (**15**) (peach-like, fruity), and unknown compounds **9** (coconut-like) and **14** (sweet, woody) were identified as further odorants with FD factors of 128 and 256 (**Table 1**).

MS analysis of odorant **7** (leather-like, phenolic, ink-like) showed that the mass spectrum was comparable with mass spectra of *n*-propylphenols. The concentration of the compound in cardboard seemed to be low, because the intensity of the MS signal was weak. HRGC analyses and the determination of the retention indices of the three isomers showed that 2-propylphenol was clearly separable from the other two isomers (**Table 2**). On the basis of these chromatographic data and the odor quality (**Table 2**), 2-propylphenol was excluded as a potent odorant. However, gas chromatographic separation of 3- and 4-propylphenol did not succeed on the capillaries used (**Table 2**). For this reason, the odor activities of the phenols were evaluated by determination of odor thresholds. As detailed in **Table 2**, 3-propylphenol was found with a very low threshold in air (0.098 ng/L of air), whereas the threshold of 4-propylphenol was significantly higher (11 ng/L). The results were confirmed by the respective thresholds in water. The recognition threshold of 3-propylphenol (0.62 μg/L) was lower than that of 4-propylphenol by a factor 240 (**Table 2**). In addition, only the odor quality of 3-propylphenol corresponded to that of the odorant (**Table 2**). On the basis of the sensory results and the assumed low amount of the target odorant, it was concluded that the odor-active compound **7** was 3-propylphenol. To complete the series of *n*-propylphenol, the odor thresholds of 2-propylphenol were also determined, which were comparable with those of 4-propylphenol (**Table 2**).

Table 2. Odor Qualities, Retention Indices, and Odor Thresholds of *n*-Propylphenols

				odor threshold		
		RI[b] on		in air[c] (ng/L)	in water[d] (μg/L)	
compound	odor quality[a]	DB-FFAP	DB-5		recognition	detection
7	leather-like, phenolic, ink-like	2250	1285			
2-propylphenol	smoky, smoked ham-like, sweet	2128	1244	44	64	39
3-propylphenol	leather-like, phenolic, ink-like	2250	1285	0.098	0.62	0.29
4-propylphenol	sweet, phenolic, ink-like	2250	1285	11	149	107

[a] Odor quality perceived during HRGC−olfactometry and evaluated according to ref *14*. [b] RI, linear retention index (*27*). [c] The thresholds in air were determined according to ref *28*. [d] The thresholds in water were determined according to ref *14*.

Apart from that, medium intensities according to the FD factors (range 16−64) were analyzed for (*R*/*S*)-δ-dodecalactone (**16**) (peach-like, sweet, flowery), octanal (**18**) (soapy, citrus-like, green), (*E*,*Z*)-nona-2,4-dienal (**19**) (fatty), benzothiazole (**20**) (rubber-like, car tire-like), nonanal (**21**) (soapy, fatty, green), (*E*,*E*)-nona-2,4-dienal (**22**) (fatty), (*E*,*Z*)-nona-2,6-dienal (**24**) (cucumber-like, green), (*Z*)-dec-2-enal (**25**) (fatty), 3-methylphenol (**26**) (leather-like, phenolic, ink-like), (*R*/*S*)-3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**27**) (spicy, savory-like), hexanal (**29**) (grassy, green), (*E*,*E*)-deca-2,4-dienal (**31**) (fatty), oct-1-en-3-one (**32**) (mushroom-like), butanoic acid (**33**) (cheesy, sweaty), (*R*/*S*)-2/3-methylbutanoic acid (**34a/b**) (sweaty, cheesy, fruity), (*R*/*S*)-γ-octalactone (**35**) (coconut-like, sweet), and unknown compounds **17** (fatty), **23** (woody, sweet), **28** (citrus-like), **30** (fatty), and **36** (citrus-like) (**Table 1**).

In this study, the major part of the most intense odorants was identified. Among them, phenols **1**, **5**, **7**, **12**, **13**, and **26**, aldehydes **10**, **11**, **19**, **22**, **24**, and **25**, and lactones **3**, **6**, **15**, **16**, and **35**, as well as *p*-anisaldehyde (**8**) and (*R*/*S*)-3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**27**), were identified for the first time as constituents of cardboard (**Table 1**). The identification was based on the comparison of chromatographic data, mass spectral data, and odor qualities of odorants with reference compounds. Using these criteria, identification can fail in some cases if chromatographic separation, for example, of isomers, does not succeed, as demonstrated for 3-propylphenol. The data presented here show that additional information such as odor thresholds and odor intensities as structure-specific parameters can be essential for the unequivocal structural elucidation of coeluting compounds.

Generally, the composition of intense cardboard odorants was dominated by aldehydes and phenolic compounds in our study (**Table 1**). From these substance groups, hexanal, octanal, nonanal, (*E*)-non-2-enal, and (*E*,*E*)-deca-2,4-dienal have previously been identified as odor-active constituents in cardboard by HRGC-O (*2*, *15*). In a further investigation, about 50 volatiles were identified in 8 cardboards, which exhibited good odor qualities as well as off-flavors, whereby these substances were detected in nearly every sample (*11*). Many unsaturated aldehydes were identified, and the concentrations of some aldehydes by far exceeded the corresponding odor thresholds in off-flavor samples. For that reason these compounds were assumed as off-flavor compounds. Autoxidative processes of unsaturated fatty acids were held responsible for the generation of these aldehydes. Also, various unsaturated fatty acids have already been found in spruce (*32*) and biofilms collected from paper and board machines (*33*), and model experiments using methyl linoleate and methyl linolenate demonstrated that the identified odor-active cardboard compounds were liberated by autoxidation (*28*, *34*). On the other hand, nonanal and (*E*)-non-2-enal were already found by HRGC-O in oak wood (*35*, *36*), which indicates that aldehydes may, at first hand, originate from wood used for paper processing.

Apart from that, alkyl- and methoxyphenols were identified as the second major odorant group in the present study. They had not been described as cardboard odorants so far, but a number of odor-active phenols, for example, 2-methoxyphenol and vanillin, had previously been identified as oak wood odorants (*36*). Generally, lignin might be regarded as a specific precursor in phenol generation. Lignin is one of the most abundant biopolymers with important stabilizing properties for many plants and also for wood (*37*) and is highly resistant to microbial degradation. Nevertheless, some fungi have been found to degrade the polymer (*38*). *p*-Hydroxycinnamic derivatives, which are the monomers of lignin, have been proven to be converted microbiologically by *Brettanomyces* yeast (*39*) and *Lactobacillus* strains (*40*) into odorants such as 4-ethylphenol and 4-ethyl-2-methoxyphenol. Apart from enzymatical degradation, thermal decomposition of lignin to vanillin, 2-methoxyphenol, and 4-ethylphenol has previously been confirmed in pyrolysis experiments (*41*).

In our study, comparison of AEDA (**Table 1**) and APA (**Figures 2** and **3**) provided indications on those odorants that might be responsible for the specific cardboard odor. The cardboard-like woody attributes seemed to be caused by (*E*)- and (*Z*)-non-2-enal and the unknown woody-smelling odorant **4**, respectively. However, odorants with musty and moldy notes were not detected, which might indicate that a mixture of the respective odor-active compounds described herein caused these attributes as a function of a specific quantitative blend. Interestingly, the sweet intensity was very weak in cardboard, but the quality was well detectable in sunflower oil from the transfer experiment. At first sight, odorants with sweet notes, for example, vanillin, 2-methoxyphenol, δ-decalactone, and *p*-anisaldehyde, do not seem to dominate in the orthonasal odor profile from the moistened cardboard, but obviously accumulation of such odorants in the oil was of sensory significance. Accordingly, one might assume that distinct transfer of sweet odor compounds to the oil phase took place. However, these indications have to be proven in the future by quantification and reconstitution experiments.

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