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## Determination of the overall migration from silicone baking moulds into simulants and food using $^1\text{H-NMR}$ techniques

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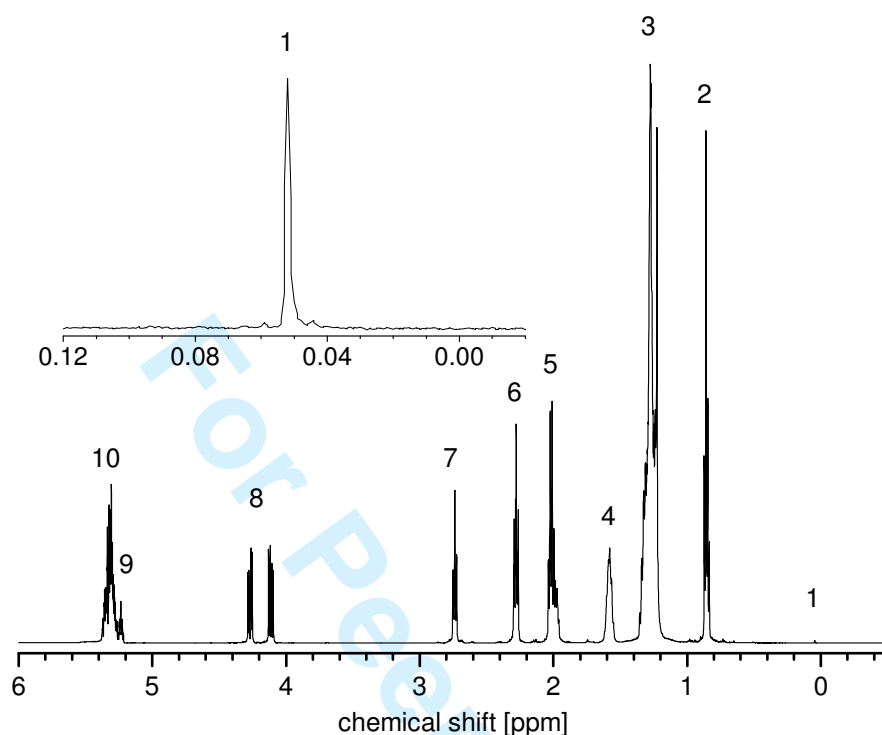
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**Determination of the overall migration from silicone baking moulds into simulants and food using <sup>1</sup>H-NMR techniques**

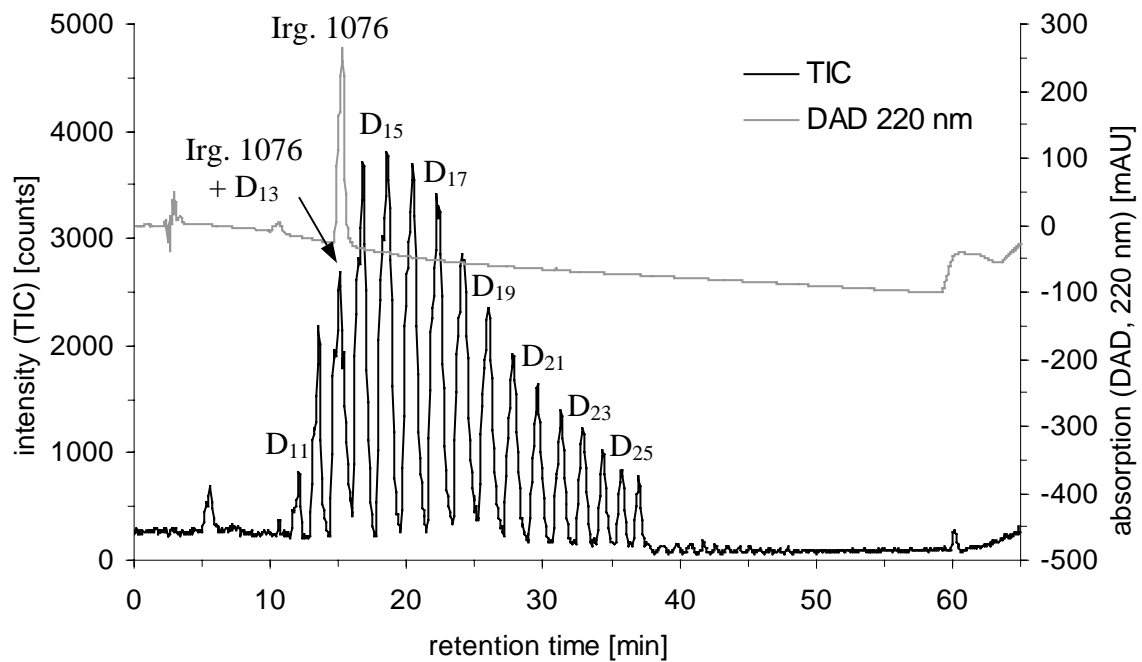
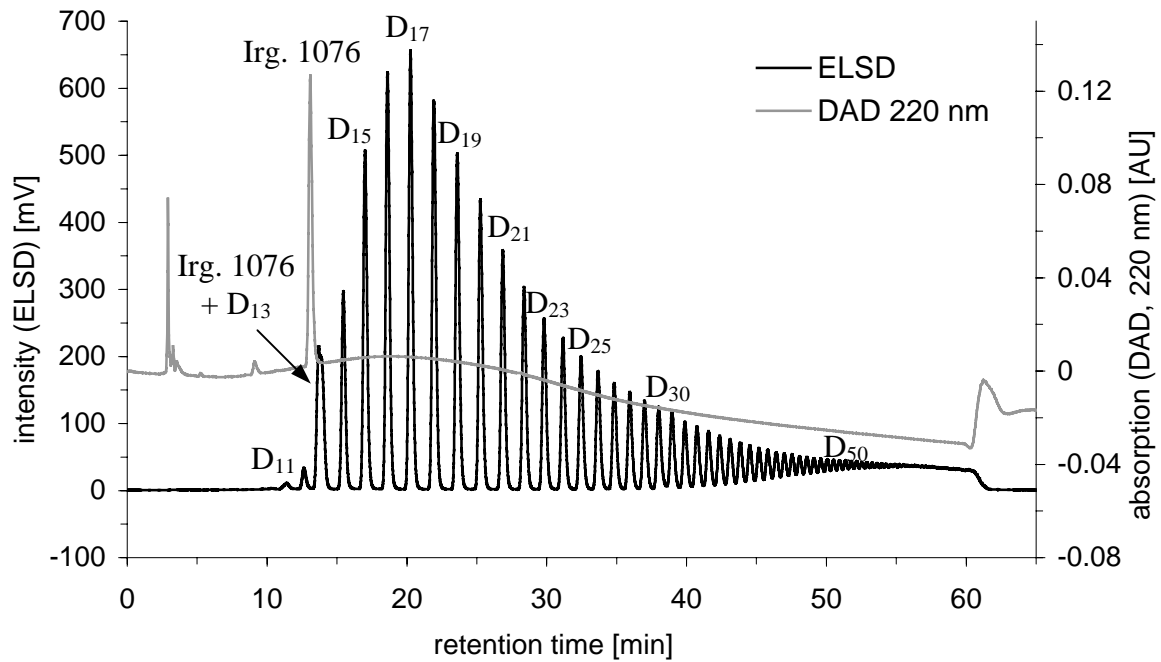
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Methods/Techniques:	Analysis - NMR, Chromatography - HPLC, Chromatography - GC
Additives/Contaminants:	Silicone, overall migration, Food contact materials, Food simulants
Food Types:	Bakery products

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**Figure 1.**  $^1\text{H-NMR}$  spectrum of a siloxane containing fat extract. The inset shows an expanded scale for the -0.01 to 0.12 ppm region.

1:  $\delta = 0.05$  ppm (s; 6 H;  $\text{Si}(\text{CH}_3)_2\text{O}$ ), 2: 0.85 ppm (m; 3 H;  $\text{CH}_3$  aliphatic), 3: 1.18-1.38 ppm (m; 2 H;  $\text{CH}_2$  aliphatic), 4: 1.54-1.62 ppm (m; 2 H;  $-\text{O}-\text{CO}-\text{CH}_2-\text{CH}_2-$ ), 5: 1.92-2.08 ppm (m; 2 H;  $-\text{CH}_2-\text{CH}=\text{}$ ), 6: 2.29 ppm (m; 2 H;  $-\text{O}-\text{CO}-\text{CH}_2-$ ), 7: 2.74 ppm (m; 2 H;  $=\text{CH}-\text{CH}_2-\text{CH}=\text{}$ ), 8: 4.05-4.35 ppm (dd; 4 H;  $-\text{CO}-\text{O}-\text{CH}(\text{CH}_2-\text{O}-\text{CO}-)_2$ ), 9: 5.25 ppm (p;  $-\text{CO}-\text{O}-\text{CH}(\text{CH}_2-\text{O}-\text{CO}-)_2$ ), 10: 5.25-5.40 ppm (m;  $-\text{CH}=\text{CH}-$ )



**Figure 2 a)** RP-HPLC-ELSD/DAD chromatogram of the migration residue;  
**b)** RP-HPLC-MS/DAD chromatogram of the same migration residue ( $m/z$  300-2000);  
Irganox 1076 was added to as an internal standard to compare both chromatograms

**Table II.** Overall migration into isooctane and ethanol (95%) determined by  $^1\text{H-NMR}$  and gravimetry. Data shown: mean  $\pm$  confidence interval ( $\alpha = 0.05$ ), threefold determination.

simulant	migrate	$^1\text{H-NMR}$	gravimetry	ratio
		$w_A$ [ $\text{mg dm}^{-2}$ ]	$w_B$ [ $\text{mg dm}^{-2}$ ]	$w_A/w_B$
isooctane	1 <sup>st</sup>	166.7 $\pm$ 10.6	208.1 $\pm$ 3.2	0.83
	2 <sup>nd</sup>	33.3 $\pm$ 1.2	38.4 $\pm$ 2.3	0.87
	3 <sup>rd</sup>	10.7 $\pm$ 2.9	12.9 $\pm$ 3.2	0.83
ethanol (95 %)	1 <sup>st</sup>	30.2 $\pm$ 1.4	35.6 $\pm$ 1.3	0.85
	2 <sup>nd</sup>	24.7 $\pm$ 3.1	27.7 $\pm$ 3.1	0.89
	3 <sup>rd</sup>	21.1 $\pm$ 3.9	24.3 $\pm$ 2.9	0.87

**Table V.** Overall migration into creamed cake (21% fat in the dough) from different silicone moulds, determined by  $^1\text{H-NMR}$ . Data shown: mean (threefold determination).

Baking dish	12-cup muffin mould		Loaf pan for cake		round cake pan	
surface/volume ratio	112 ml dm <sup>-2</sup>		171 ml dm <sup>-2</sup>		369 ml dm <sup>-2</sup>	
Baking time	25 min		40 min		45 min	
Temperature	175°C		175°C		175°C	
Migrate	[mg kg <sup>-1</sup> ]	[mg dm <sup>-2</sup> ]	[mg kg <sup>-1</sup> ]	[mg dm <sup>-2</sup> ]	[mg kg <sup>-1</sup> ]	[mg dm <sup>-2</sup> ]
1 <sup>st</sup>	10.00	1.19	9.97	1.86	8.38	2.72
3 <sup>rd</sup>	10.11	1.21	9.96	1.86	7.99	2.63

## Determination of the overall migration from silicone baking moulds into simulants and food using $^1\text{H-NMR}$ techniques

### Abstract

Different silicone baking moulds (37 samples) were characterized with respect to potential migrating substances using  $^1\text{H-NMR}$ , RP-HPLC-UV/ELSD and GC techniques. In all cases cyclic organosiloxane oligomers with the formula  $[\text{Si}(\text{CH}_3)_2\text{-O}]_n$  were identified ( $n = 6\dots 50$ ). Additionally, linear, partly hydroxyl-terminated organosiloxanes  $\text{HO-}[\text{Si}(\text{CH}_3)_2\text{-O}]_n\text{-H}$  ( $n = 7\dots 20$ ) were found in 13 samples. No other substances than siloxanes could be detected, meaning the migrants mainly consist of organopolysiloxanes. Based on this knowledge, a  $^1\text{H-NMR}$  quantification method for siloxanes was established for the analysis of both simulants and foodstuffs. Validation of the  $^1\text{H-NMR}$  method gave a suitable performance characteristics: limit of detection  $8.7 \text{ mg kg}^{-1}$  oil, coefficient of variation 7.8 % (at a level of  $1.0 \text{ mg kg}^{-1}$  food). Migration studies were carried out with simulants (olive oil, isooctane, ethanol (95 %), Tenax) as well as preparation of different cakes. Siloxane migration into cakes only slightly decreased in going from the 1<sup>st</sup> to 10<sup>th</sup> experiment with a significant dependence on fat content. Migration never exceeded a level of  $21 \text{ mg kg}^{-1}$  ( $3 \text{ mg dm}^{-2}$ ) and thus migration was therefore well below the overall migration limit of  $60 \text{ mg kg}^{-1}$  ( $10 \text{ mg dm}^{-2}$ ). However, migration behaviour into simulants differed completely from these results.

**Keywords:** Silicones, polydimethylsiloxanes,  $^1\text{H-NMR}$ , migration, foodstuffs, cake

## Introduction

Silicone elastomers are widely used as food contact materials. Because of their non-sticking properties, high temperature resistance and flexible form, their use as baking moulds and pads is very common. Their market share among baking moulds within the last few years was in a range between 23 % and 46 % (GFK Panel Services Germany).

There are only few publications describing the migration behaviour of silicone elastomers in contact with foodstuffs or food simulants. High overall migration from silicone elastomers was reported for foodstuffs containing free available fat (Forrest and Sidwell 2005, RAPEX 2007). As to be expected, no notable migration into aqueous or acidic simulants was described so far. Meuwly et al. (2005) investigated the overall migration from different silicone baking moulds into modified polyphenylene oxide (Tenax) and obtained values well above the overall migration limit of  $10 \text{ mg/dm}^2$ , for the 1<sup>st</sup> as well as for the 3<sup>rd</sup> migration experiment. Increasing temperatures caused higher migration values, the highest amount reported being  $50.5 \text{ mg dm}^{-2}$ . Characterisation of the migrate was carried out using GC-MS and MALDI-TOF and showed cyclic and linear polydimethylsiloxanes up to a molecular weight of 1500 Da as the only components.

Until 2001 silicone elastomers were covered by the Plastics Directive 90/128/EC and had to conform to the overall migration limit of  $60 \text{ mg kg}^{-1}$  ( $10 \text{ mg dm}^{-2}$ ). With Commission Directive 2001/62/EC, silicone elastomers were excluded from the plastics definition because of their physical properties, which are more similar to elastomers than to other plastics like polyolefins. Still no specific European legislation exists concerning silicone elastomers intended to come into contact with foodstuffs. This gap is filled by the Council of Europe's Resolution AP (2004) 5 on silicones used for food contact applications as well as the recommendation XV on silicone articles, set up by the Federal Institute for Risk Assessment (BfR), Germany (BfR 2007). The Resolution AP (2004) 5 limits the overall migration to  $60 \text{ mg kg}^{-1}$  food ( $10 \text{ mg dm}^{-2}$ ), whereas in the German recommendation XV limits for the loss of volatile organic compounds and extractable compounds are set up, each to  $0,5 \text{ g/100 g}$  silicone elastomer. Although the Resolution AP (2004) 5 has only a status of recommendation in most European countries, some introduced it into national legislation. So did the Swiss authorities last in December 2006 (Swiss EDI 2005).



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5 In consequence, the Rapid Alert System for Food and Feed (RASFF) of the European  
6 Commission shows notifications from EU member states who want to alert control authorities  
7 of silicone articles because of a too high overall migration in the olive oil migration test as  
8 well as the response of other countries (e.g. Germany) who declare such articles as  
9 conforming to national law (RAPEX 2006). Most notifications are on silicone materials  
10 exceeding the limit for volatile organic compounds, although it is not proven how far the  
11 amount of migration into food depends on the amount of volatile compounds.  
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19 In the field of food contact materials made of plastics, the Council Directives 85/572/EEC,  
20 82/711/EEC and Commission Directive 2002/72/EC define how to perform migration  
21 experiments and which food types and simulants should be used. Baking moulds which are  
22 used for making pastries containing free available fat should be tested with olive oil.  
23 Isooctane, ethanol (95%) and Tenax are allowed as alternative simulants if the use of olive oil  
24 is impossible for technical reasons. As required for repeat-use articles, only the result of the  
25 3<sup>rd</sup> migration experiment is of legal relevance (Commission Directive 2002/72/EC).  
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33 Silicone elastomers are not covered by these Directives, so the test procedures described here  
34 are not compulsory. On the other hand Resolution AP (2004) 5 declares, that migration tests  
35 should be conducted according to these Directives as appropriate, unless technically  
36 impracticable due to the nature of the material or the migration tests. Such a case of technical  
37 impracticability was stated for the migration test using olive oil. According to the Federal  
38 Institute for Risk Assessment (BfR), olive oil is not suitable for silicones due to the swelling  
39 of the material (BfR 2006). For that reason, most laboratories use Tenax® for migration  
40 experiments despite some others using ethanol (95%), but there is a big uncertainty  
41 concerning the interpretation and legal status of these data. Furthermore, there are differences  
42 concerning the use of a fat reduction factor as described in Council Directive 85/572/EEC. To  
43 clarify this, a comparison of all the fatty food simulants and some real foodstuffs is needed.  
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55 Until now, no validated method was published allowing the determination of the overall  
56 migration from silicone elastomers into fatty foodstuffs. Hence, there was no possibility to  
57 prove the criteria of the Resolution AP (2004) 5 in foodstuffs. Furthermore, such a method  
58 would be of great importance to assess the suitability of migration tests with olive oil or  
59 simulants for silicone elastomers by comparing these results with the amounts measured in  
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3 food. Therefore, a validated and generally accepted method for the determination of the  
4 overall migration from silicone elastomers into foodstuffs is needed.  
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9 Some analytical techniques allow the determination of siloxanes in foodstuffs. Atomic  
10 spectroscopic methods (AAS/AES, ICP-MS, ICP-OES) are most widely used (Čavić-Vlasak  
11 et al. 1996, Mccamey et al. 1986, Peters et al. 1995, Kennan et al. 1999, Dorn and Skelly  
12 1994, Biggs et al. 1987). These methods are very specific for silicon but they do not allow one  
13 to distinguish between organic and inorganic bound silicon. It is impossible to determine only  
14 the content of polydimethylsiloxanes (PDMS) as an extent of migration from silicone  
15 elastomers. Several methods based on AAS and ICP-AES techniques for PDMS  
16 determination in food after extraction with chloroform or other solvents have been described,  
17 with a recoveries from 90-95% and precision of the determination between 1 and 6% (Čavić-  
18 Vlasak et al. 1996, Mojsiewicz-Pieńkowska et al. 2003). The main drawbacks are possible  
19 interferences with inorganic bound silicon and the potential loss of siloxanes with low  
20 molecular mass before atomization. A useful, but quite expensive alternative might be the use  
21 of HPLC-ICP-AES. This technique would offer the possibility to separate organic and  
22 inorganic bound silicon first by liquid chromatography before quantification with ICP-AES.  
23 Infrared spectroscopic methods are specific for organosiloxanes but are little sensitive and  
24 need massive sample enrichment. Direct analyses in the lower  $\text{mg kg}^{-1}$  range are not feasible  
25 (Čavić-Vlasak et al. 1996). The crux is to separate the siloxanes from the big excess of fat in  
26 the food extracts which has an almost similar polarity. The main difficulty of  
27 chromatographic methods is the lack of analytical references for cyclic siloxanes in the mid  
28 and high molecular range which are essential for a reliable quantification.  
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46 For all this reasons, we decided to use  $^1\text{H-NMR}$  techniques for quantification. Mojsiewicz-  
47 Pieńkowska et al. (2003) described a method to quantify linear polydimethylsiloxanes (food  
48 additive E 900) in edible oils after saponification and extraction with tetra chloromethane.  
49 The limit of reliable detection was  $3 \text{ mg l}^{-1}$  which is well below the expected range for  
50 migrating siloxanes from silicone moulds. The advantage of  $^1\text{H-NMR}$  quantification is the  
51 specific and isolated singlet signal for the  $[-\text{Si}(\text{CH}_3)_2-\text{O}-]_n$ -group in linear and cyclic  
52 siloxanes. Furthermore, even the protons of cyclic oligomers of different size are mainly  
53 chemically equivalent, resulting in only one signal for all the oligomers and thus increasing  
54 sensitivity. No analytical reference is needed. The highly specific signal for all cyclic  
55 siloxanes should also allow a direct measurement, even without prior saponification of the fat.  
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3 This would make the resulting method significantly less time-consuming and provide the  
4 opportunity to use the triglycerides as a unique internal standard.  
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8 The objective of this work was therefore to show the applicability of  $^1\text{H-NMR}$  (without prior  
9 saponification of the fat and enrichment of the siloxanes) to determine the migration from  
10 siloxane elastomers in fatty simulants and fatty foodstuffs. Migration into simulants should be  
11 compared to migration into real foodstuffs and to the overall migration limit as given in the  
12 Resolution AP (2004) 5. Repeated use of silicone articles was examined as well as the  
13 influence of the fat content of similar foodstuffs.  
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## 19 20 21 **Materials and methods**

### 22 23 24 *Silicone moulds*

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26 The characterized silicone moulds were from various manufacturers and were all bought on  
27 the German market. More detailed investigations concerning the relation between different  
28 simulants as well as food preparation experiments were carried out with a commercial 12 cups  
29 muffin silicone mould (volume: 95.8 cm<sup>3</sup>, contact area: 0.86 dm<sup>2</sup>, thickness: 1.0 mm, Pt  
30 catalyzed cross-linked) bought on the German market, too. Single cups were cut from this  
31 sample and used for the migration tests.  
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39 All samples were first washed with warm water (45-50°C) and washing-up liquid, as  
40 recommended by the manufacturers.  
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### 43 44 *Chemicals*

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46 Olive oil was purchased from Fluka Germany, Tenax<sup>®</sup> (modified polyphenylene oxide,  
47 MPPO) from Alltech Germany. Any other chemicals were obtained from Sigma-Aldrich  
48 Germany at analytical grade or higher. All food ingredients, used for the baking studies, were  
49 of commercial quality.  
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### 53 54 *Silicone samples*

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56 In total 37 different silicone moulds were examined: 2 muffin pans with 15 cups, 4 muffin  
57 pans with 12 cups, 7 muffin pans with 6 cups, 7 bundform pans ('Gugelhupf'), 5 loaf pans, 3  
58 round cake pans, 2 mini cake moulds for 12 pieces "Madeleine" and 7 motive moulds (Disney  
59 characters, Christmas tree, teddy bear). The thickness of all moulds was in the range of 1-2  
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3 mm, maximum temperature as given by the manufacturer was from 220 °C to 260 °C. Most of  
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5 the moulds were blue coloured except three red moulds shaped like Disney characters.  
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#### 8 9 *Migration into food simulants*

10 Olive oil, isooctane, 95% ethanol and modified polyphenylene oxide (MPPO, Tenax®) were  
11 used as test media. The test conditions were based on common time-and-temperature  
12 conditions used for baking muffins and were selected according to Council Directive  
13 82/711/EEC chapter 3 N° 1 and table 4. As baking moulds are intended for repeated usage, the  
14 first, second and third overall migrate were determined in all cases (Commission Directive  
15 2002/72/EC).  
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#### 22 23 *Migration into olive oil*

24 The tests were carried out as described in part 8 of the European Standard EN 1186:2002 and  
25 Commission Directive 2002/72/EC attachment 1 N° 6 with the following modifications: The  
26 samples were filled to the brim with a pre-heated amount of olive oil (90 ml, 175°C) and then  
27 placed in a pre-heated oven at 175°C. As a blank sample, an equivalent amount of olive oil  
28 was poured into a flask and then treated identically. Time measurement was already started  
29 when the samples were put into the oven, although the examination temperature of 175°C was  
30 regained later. Contrary to repeating the migration experiment three times with one mould for  
31 25 min at 175 °C, a different approach according to Commission Directive 2002/72/EC  
32 (Annex I No. 6) was chosen. Three moulds were incubated with olive oil at 175°C for 25, 50  
33 (mimicking 1<sup>st</sup> and 2<sup>nd</sup> migration) and 75 min (mimicking 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> migration),  
34 respectively. Quantification of olive oil in the extracts of the silicone moulds was carried out  
35 by determination of the triglycerides using normal phase HPLC and evaporative light  
36 scattering (ELS) detection. Analysis were performed on a Merck HPLC-DAD 7000 coupled  
37 with an ELS-detector Sedex 75 (Sedere, Alfortville, France) with the following parameters:  
38 chromatographic column: LiChrospher 100 Diol-5 µ, 125 x 3 mm (Merck, Darmstadt,  
39 Germany), isocratic elution: 2-methoxy-2-methyl-propane/isooctane 4/96 (v/v), run time: 12  
40 min, flow: 0.5 ml min<sup>-1</sup>, injection volume: 20 µl, column temperature: 25°C, nebulization  
41 temperature: 20°C, evaporation temperature: 35°C, N<sub>2</sub> 3.5 bar, gain: 8, calibration: olive oil  
42 dissolved in isooctane. The triglyceride peak showed a retention time of about 5 min.  
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#### *Migration into isooctane and 95% ethanol*

The tests were carried out as a threefold determination as described in part 14 of the European Standard EN 1186:2002 with the following modifications: To determine the overall migration into isooctane, the samples were cut into stripes of 3 cm x 5 cm, put into 180 ml of the pre-heated solvent and then placed in a pre-heated oven at 60°C. For migration tests with ethanol/water 95/5 (v/v), the intact samples were filled to the brim with the pre-heated solvent (90 ml, 60°C). In both cases, only the inside surface area of the moulds was assumed as contact area, although during the isooctane migration the whole surface (inside and outside area) of the moulds was in contact with the simulant. Isooctane leads to an extensive, but even swelling of the complete silicone moulds, also during one-side contact. So it has to be supposed that substances may be able to migrate from the outer to the inner side of the baking moulds, resulting in nearly the same results as to be expected for a two-sided migration. A similar effect is well known e.g. for plasticizers in thin PVC-films (Kim 2003). Again, time measurement was already started when the samples were put into the oven, although the examination temperature of 60°C was regained later. An exposure time of 50 min for isooctane and 75 min for 95% ethanol was chosen, respectively. The used solvent was refilled to a defined volume (100 or 200 ml) and divided into two portions. About 2/3 of the solvent were used to determine the overall migration by the gravimetric standard method. The remaining part was concentrated by evaporation (rotary evaporator) and refilled to 5 ml with simulant solvent. 100-200 µl of this solution were mixed with 0.5-2 g ( $\pm$  0.1 mg) of sunflower oil, resulting in a mixture containing about 150-400 mg siloxane per kg sunflower oil. For <sup>1</sup>H-NMR analysis, 200 mg of this mixture were dissolved in 800 µl CDCl<sub>3</sub>, filtered and transferred into a NMR-tube. The <sup>1</sup>H-NMR signals of the simulant solvents, isooctane and ethanol, do not disturb the analysis of the siloxane and triglyceride signal.

#### *Migration into Tenax® (MPPPO)*

The tests were carried out as described in part 13 of the European Standard EN 1186:2002. For a threefold determination three flat pieces, each with an area of about 15 cm<sup>2</sup>, were cut from the bottom of the baking moulds. The samples were put into a Petri dish and covered with 0.6 g Tenax® (4 g Tenax®/1 dm<sup>2</sup>). The Petri dish was closed and then put into the preheated oven at 175°C. As a blank sample, Tenax® was filled into a Petri dish without sample material.

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3 Time measurement started when the examination temperature of 175°C was regained,  
4 exposure time was 25 min. Gravimetric determination of the migration residue followed the  
5 procedure described in EN 1186:2002 part 13.  
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10 *Qualitative analyses of the migration residue (overall migration into isooctane)*

11 For liquid chromatography, an Agilent 1100 G HPLC-DAD (Hewlett Packard, Palo Alto, CA)  
12 coupled with an ESI-TOF-MS-detector (Biospectrometry Workstation; Applied Biosystems,  
13 Foster City, CA) and a Merck HPLC-DAD 7000 (Merck, Darmstadt, Germany) coupled with  
14 an ELS-detector Sedex 75 (Sedere, Alfortville, France) were used. Irganox 1076 (octadecyl-3-  
15 (3',5'-di(tert-butyl)-4'-hydroxyphenyl)propanoate, CAS No. 2082-79-3) was added to the  
16 sample solution as a retention time standard to allow the assignment of the peaks observable  
17 in the HPLC-MS and HPLC-ELSD chromatograms. The amount of Irganox 1076 in the  
18 sample solution was about 200 mg l<sup>-1</sup>.  
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28 The measurement parameters were as follows:

29 chromatographic column: Multospher 120 RP 18 HP 5 µm 250 x 3 mm (CS-Chromatography  
30 Service), gradient elution: isopropanol(A)/methanol(B), starting with 10% isopropanol and  
31 reaching 85% within 55 min, flow: 0.5 ml min<sup>-1</sup>, injection volume: 10 µl (HPLC-DAD/ELSD)  
32 or 25 µl (HPLC-DAD/MS), column temperature: 40°C, ELSD parameters: nebulization  
33 temperature: 25°C, evaporation temperature: 30°C, N<sub>2</sub> 3 bar, gain: 8, UV: 220 nm.  
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38 Gas chromatography was done on a Hewlett-Packard 6890 GC (Hewlett Packard, Palo Alto,  
39 CA) equipped with a 5973 mass selective detector. A HP-5MS capillary column (30 m, 0.25  
40 mm) was used. The MS detector was operated in full scan mode, scan range m/z 70 – 800.  
41 Diallylphthalate (c = 5 mg/l) served as an internal standard.  
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47 Quantification experiments were performed on a Hewlett-Packard 5890 GC with flame  
48 ionization detector (FID), again using a HP-5MS capillary column (30 m, 0.25 mm). Injection  
49 was splitless using a split/splitless injection system at 280°C. High temperature GC was done  
50 at an injection temperature of 330°C, expanding the oven program to 430°C and using a high  
51 temperature GC column of adequate polarity (Phenomenex ZB-5HT Inferno, 30 m, 0.25 mm,  
52 Phenomenex, Torrance, CA). Increasing the injector temperature up to 350°C resulted in a  
53 high septum bleeding but unimproved peak shape or response.  
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### *Elemental analysis*

Elemental analysis was performed using a EuroVector EA 3000 (HEKAtech, Wegberg, Germany).

### *Migration into foodstuffs*

The baking studies were performed with two different kinds of creamed cake.

#### *Creamed cake, 21 % total fat (Das Backbuch, 1967)*

The dough contained 125 g sunflower oil, 100 g egg (homogenized), 125 g sugar, 250 g wheat flour, 8 g baking powder and 50 g water.

#### *Creamed cake, 30 % total fat (Backen und Genießen 1999)*

The dough consisted of 200 g sunflower oil, 200 g egg (homogenized), 150 g sugar, 200 g wheat flour and 8 g baking powder.

### *Baking procedure*

The samples were filled to the brim with dough and then put into the pre-heated oven at 175°C. After a suitable baking time (25 min for muffins, 40 min for creamed cake in a loaf pan, 45 min for creamed cake in a round cake pan), the samples were removed from the oven.

The cakes were removed from the moulds and each one was put into a beaker.

For the blank samples, the dough was filled into a Teflon coated metal baking mould.

### *Soxhlet extraction*

The cakes were homogenized and an equivalent (approx. 12 g of the cake) was mixed with sodium sulphate ( $\text{Na}_2\text{SO}_4$ ) (approx. 15 g) thoroughly in a mortar.

The dry powder was transferred quantitatively into a Soxhlet cartridge and extracted for 4 h with 150 ml diethyl ether in a Soxhlet extractor to isolate the fat matrix, including the siloxanes. At the end of the extraction, the diethyl ether was removed from the round-bottomed flask by distillation. Before and after extraction, the flask was dried repeatedly for 1h at 103°C until constant weight was reached. The weight of the lipid extract was determined accurate to 0.1 mg.

Sodium sulphate had to be annealed for 3 h at 550 °C in a muffle furnace prior to use to remove siloxane impurities lasting from manufacturing. Soxhlet cartridges and filter papers used to clean the mortar and pestle were extracted thoroughly in a Soxhlet extractor with diethyl ether prior to use.

### *NMR measurement*

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3 For NMR measurement 400 mg of the extracted fat were dissolved in 800  $\mu\text{l}$   $\text{CDCl}_3$ , filtered  
4 through silicone free paper and transferred into a NMR tube. The  $^1\text{H}$ -NMR spectra were  
5 recorded at 500 MHz on a DRX 500 P NMR spectrometer (Bruker). Parameter settings were  
6 as follows: temperature:  $22^\circ\text{C}$ , number of scans: 128, spectral width: 10330 Hz, acquisition  
7 time: 3.17 s, resolution: 0.158 Hz per point, frequency sample rotation: 20 Hz, pulse delay: 1  
8 s, pulse angle:  $30^\circ$ . Tetramethylsilane (TMS) was not added as a position standard for the  
9 chemical shift. Instead of this, the solvent signal ( $\delta(\text{CHCl}_3) = 7.25$  ppm in relation to TMS)  
10 was used to ascertain the position of resonance bands.  
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### 18 19 *Calculation of the siloxane content*

20 The extracted fat mainly consists of triglycerides. They serve as internal standard for  
21 quantification. They form a distinct double doublet (dd) resonance signal in a range of  $\delta =$   
22 3.95-4.45 ppm (including  $^{13}\text{C}$  satellites). For quantification, the whole double doublet signal is  
23 integrated within this range. The signal is caused by the four protons at C1 and C3 of the  
24 triglyceride backbone. The cyclic dimethylsiloxanes represent the main part of the overall  
25 migrate giving a sharp singlet at 0.05 ppm, which is formed by the six equivalent protons per  
26 [-Si(CH<sub>3</sub>)<sub>2</sub>-O-] unit.  
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35 “[Insert Figure 1 about here]”  
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38 Terminal siloxane units in linear hydroxyl terminated siloxanes give a signal at 0.16 ppm  
39 (HO-Si(CH<sub>3</sub>)<sub>2</sub>O-) and 0.11 ppm (HO-Si(CH<sub>3</sub>)<sub>2</sub>O-Si(CH<sub>3</sub>)<sub>2</sub>O-). Terminal groups in linear  
40 trimethylsilyl terminated siloxanes give a signal at 0.08 ppm (Si(CH<sub>3</sub>)<sub>3</sub>O-) and 0.03 ppm  
41 (Si(CH<sub>3</sub>)<sub>3</sub>O-Si(CH<sub>3</sub>)<sub>2</sub>O-). Their influence on the chemical shift of other methyl protons is  
42 limited to neighbouring groups, so that most methyl protons in linear siloxanes give a signal  
43 (as do cyclic siloxanes) at approximately 0.05 ppm. The amount of siloxane was calculated  
44 from the sum of all siloxane integrals in the spectrum. By approximation, the result can be  
45 expressed in [Si(CH<sub>3</sub>)<sub>2</sub>O]-groups, which corresponds to a cyclic siloxane. Using this method  
46 of calculation, the molar mass of linear siloxanes, and therefore also the siloxane amount, is  
47 slightly underestimated for hydroxyl terminated molecules (18 Da) and overestimated for  
48 trimethylsilyl terminated ones (30 Da). The resulting error can be disregarded, knowing that  
49 the migrating linear siloxanes range between 700 and well above 2000 Da.  
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3 A calibration was established using decamethylcyclopentasiloxane (D<sub>5</sub>) in sunflower oil.  
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5 Linearity was confirmed ( $r^2$  0.9975) between the amount of siloxanes per kilogram  
6  
7 triglyceride (abscissa) and the quotient of the integrals of the siloxane and triglyceride signals  
8  
9 (ordinate).

10  
11  
12 The overall migrate can be calculated as follows (without use of the calibration mentioned  
13  
14 above):

$$w_m = \frac{I_{\text{sil}} \cdot M_{\text{sil}} \cdot p_{\text{TG}} \cdot m_{\text{fat}}}{I_{\text{TG}} \cdot M_{\text{TG}} \cdot p_{\text{sil}} \cdot m_{\text{equ}}}$$

15  
16  
17  
18 where  $w_m$  is the overall migrate [ $\text{mg kg}^{-1}$  food],  $I_{\text{sil}}$  and  $I_{\text{TG}}$  are the integrals of the siloxane  
19  
20 and the triglyceride signal,  $M_{\text{sil}}$  is the molar mass of the  $[-\text{Si}(\text{CH}_3)_2\text{-O-}]$  unit [ $\text{g mol}^{-1}$ ] ( $M_{\text{sil}} =$   
21  
22  $74,2 \text{ g mol}^{-1}$ ), and  $M_{\text{TG}}$  is the average molar mass of the triglycerides [ $\text{g mol}^{-1}$ ] ( $M_{\text{TG}}$  was  
23  
24 calculated from the  $^1\text{H-NMR}$  spectrum of the lipid matrix as described below),  $p_{\text{sil}}$  and  $p_{\text{TG}}$  are  
25  
26 the number of protons responsible for the resonance signal of interest ( $p_{\text{sil}} = 6$ ,  $p_{\text{TG}} = 4$ ),  $m_{\text{equ}}$   
27  
28 is the mass of the food equivalent [ $\text{kg}$ ], which was used for Soxhlet extraction,  $m_{\text{fat}}$  is the mass  
29  
30 of fat [ $\text{mg}$ ], which was extracted from the food equivalent.  
31  
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33 Calculation of the surface related migration:

$$w_a = w_m \cdot \frac{m_{\text{food}}}{A_o}$$

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38 where  $w_a$  is the overall migrate [ $\text{mg dm}^{-2}$  contact area],  $m_{\text{food}}$  is the mass of food, which was  
39  
40 prepared in the tested silicone mould and  $A_o$  is the contact area [ $\text{dm}^2$ ] between the baking  
41  
42 mould and the foodstuff during baking.  
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46 Determination of  $M_{\text{TG}}$  from the  $^1\text{H-NMR}$  spectrum of the lipid matrix:

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48 All signals of the lipid matrix are integrated (see figure 1, signal 2-10), integrals of similar  
49  
50 subunits of the triglyceride molecule are summed up. Signals 8 and 9 are not fully separated,  
51  
52 so they are integrated as one signal for CH-groups. The carboxy group at C2 of the  
53  
54 triglyceride backbone is then regarded separately in the calculation (see table I).  
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56  
57 “[Insert table I about here]”  
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So the average molar mass of triglycerides in the used sunflower oil is:

$$M_{\text{TG}} = \sum \left( M_{\text{SU}} \cdot \frac{(I \cdot p^{-1})_{\text{SU}}}{(I \cdot p^{-1})_{\text{TB}}} \cdot 2 \right) = \underline{\underline{872,58 \frac{\text{g}}{\text{mol}}}}$$

#### *Determination of the recovery rate of cyclic siloxanes in muffins*

A baking study with a creamed cake (21 % fat) was carried out using siloxane spiked sunflower oil. As there were only low molecular, volatile cyclic siloxane compounds commercially available, spiking of the sunflower oil was done indirectly. Exactly, the sunflower oil was used to perform an overall migration experiment with a silicone baking mould for 25 min (see “migration into olive oil”). After this, the siloxane amount in the sunflower oil was determined by <sup>1</sup>H-NMR measurement (see “NMR measurement”). The sunflower oil containing the determined amount of siloxanes was then used as ingredient for a creamed cake (21 % fat). The baking study was performed as described in “migration into foodstuffs”, using a Teflon® coated metal baking mould. The siloxane content was determined again by NMR in the dried fat extract.

## **Results and discussion**

#### *Characterization of the migration residue*

Thirty-seven different baking moulds were characterized with respect to potential migrating substances. Ethanol (95%) or isooctane extracts were all analysed using RP-HPLC with UV and light scattering detection; most of the extracts were analysed by GC-MS and GC-FID, too. To compare the chromatograms of both liquid chromatography techniques Irganox 1076 (octadecyl-3-(3',5'-di(tert-butyl)-4'hydroxyphenyl)propanoate, CAS No. 2082-79-3) was used as an internal standard. Fig. 2 shows two typical corresponding chromatograms of an isooctane migration residue of a muffin mould. Cyclic oligomers below 13 siloxane units are discriminated in light scattering detection due to their relatively high volatility and are better to detect with MS techniques.

“[Insert Figure 2 about here]”

In all cases cyclic organosiloxane oligomers with the formula  $[-\text{Si}(\text{CH}_3)_2\text{-O-}]_n$  have been identified ( $n = 6 \dots 50$ ). Additionally, linear organosiloxanes ( $n = 7 \dots 20$ ) have been detected in the extracts of 13 moulds in a lower concentration (Fig. 3). This is in accordance with the

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3 migrating substances described by Meuwly et al. (2005). No other substances than siloxanes  
4 could be observed in a comparable concentration range, meaning the migrants mainly consist  
5 of organopolysiloxanes and no other substances should play an important role in the  
6 determination of the overall migration.  
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12 “[Insert Figure 3 about here]”  
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16 GC-chromatograms indicated the presence of low molecular weight siloxanes only in some  
17 moulds in minor concentrations, whereas cyclic siloxanes up from 14 monomer units were  
18 detected in all moulds investigated. Oligomers with more than 30 [-Si(CH<sub>3</sub>)<sub>2</sub>-O]-units are  
19 little volatile and hardly to be seen by GC limiting the applicability of this technique for  
20 quantification of the migration residue (Fig. 4).  
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26 “[Insert Figure 4 about here]”  
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30 To estimate the part of the overall migration which is accessible by GC a calibration was  
31 carried out with dodecamethylcyclohexasiloxane (D6) using a flame ionisation detector (FID)  
32 in the range of 0.5 to 80 mg/l. Commercial silicone oils with a mid molecular weight of 700  
33 Da (TMS-07) or 1100 Da (TMS-11) quantified under use of the D6 calibration curve gave a  
34 recovery rate of 91% and 49% respectively. The ethanol (95%) migration residue of 4  
35 different baking moulds all consisting only of cyclic siloxanes was analysed the same way  
36 resulting in recovery rates in the range of 13 – 20% compared to the gravimetric results. Thus,  
37 determination of the overall migration from silicone moulds by GC in the common  
38 temperature range up to 340°C is impossible due to the little volatility of the oligomers.  
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48 Further experiments performed with a high temperature GC column, an injection temperature  
49 of 360°C and a final oven temperature of 410°C resulted in a recovery rate for silicone oil  
50 TMS-11 of 93 % but of only up to 56 % for the ethanolic migration residue of the tested  
51 baking moulds. This can be attributed to the high molecular weight of most of the migrating  
52 cyclic siloxanes compared to TMS-11 and it limits the applicability of even high temperature  
53 GC for determination of the overall migration from silicone food contact materials.  
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60 The isooctane extract of one typical muffin mould was more detailed characterized using <sup>1</sup>H-  
NMR and elemental analysis beside HPLC and GC. The <sup>1</sup>H-NMR spectrum shows a

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3 dominating signal around 0.06 ppm chemical shift for the chemical equivalent protons of the  
4 cyclic oligomers  $[-\text{Si}(\text{CH}_3)_2-\text{O}]_n$ . Much smaller signals for the  $^{13}\text{C}$ -satellites at  $-0.07$  and  $0.18$   
5 ppm (coupling of  $^1\text{H}$ - with  $^{13}\text{C}$ -nucleuses;  $^1J(\text{C,H}) = 115$  Hz) and rotation side signals are  
6 detected. As a result of two-dimensional HMBC-NMR, it could be shown that a somewhat  
7 broad peak at  $0.13$  ppm stands for  $(\text{CH}_3)_3\text{SiO}_{1/2}$ -groups (M) beside quaternary (Q) silicium  
8 atoms (Fig. 5). These MQ-units are supposed to belong to so called MQ-resins which are  
9 widely used by industry as fillers (Vincent, 2000).  
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17 “[Insert Figure 5 about here]”  
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21 Such highly branched siloxane compounds, so called MQ-resins, are widely used as an  
22 additive in the production of silicone elastomers with high tensile strength.  
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26 Elemental analysis of the isooctane migration residue showed an C:H-ratio of 1:3 indicating  
27 the presence of  $\text{CH}_3$ -groups. The total amount of carbon and hydrogen was  $32.40\%$  and  $8.84$   
28  $\%$ , respectively, lying in the expected range for cyclic dimethylsiloxanes which theoretically  
29 contain  $32.4\%$  and  $8.2\%$ . No hints for any other compounds were found. Elemental analysis of  
30 the baking mould itself showed contents of  $23.66\%$  carbon and  $5.87\%$  hydrogen, also  
31 resulting in a C:H-ratio of 1:3 indicating  $\text{CH}_3$ -groups. The much lower total amount of carbon  
32 and hydrogen in baking moulds compared to cyclic siloxanes indicates that there are highly  
33 branched siloxanes or methylized silica used as fillers.  
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42 Quantification of the  $^1\text{H}$ -NMR signals, as described in the “materials and methods” part,  
43 resulted in a composition of the overall migration of  $89\%$  cyclic dimethylsiloxanes and  $6\%$   
44 MQ-resins related to the gravimetric result. According to Vincent (2000), a M:Q ratio of 0.9:1  
45 was assumed for the calculation of the amount of MQ resins. About  $5\%$  of the migration  
46 residue remain unexplained. In consequence of the GC-, HPLC-, NMR- and elemental  
47 analysis it seems very unlikely that this part is made of organic compounds. We suppose  
48 inorganic filling materials, e.g. pyrogenic silicic acid or chemically modified (methylated)  
49 silica gel, which are often used for silicone elastomers (BfR 2007).  
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58 Since more than  $90\%$  of the migration residue consist of polysiloxanes, the amount of these  
59 determined by NMR can be a good approach for the determination of the overall migration.  
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3 In a following migration experiment, using the same muffin baking mould as for the  
4 characterization of the residue, gravimetric results were compared with  $^1\text{H-NMR}$   
5 quantification. Because of their little amount and the broad signal the MQ-resins were not  
6 quantifiable in the sample solutions and therefore only the signal for the cyclic oligomers was  
7 integrated. Keeping in mind that the amount of cyclic oligomers is 89 % of the migration  
8 residue table 2 shows good compliance of gravimetric and NMR data. Determination of  
9 organosiloxanes can therefore be considered as a good approach for the sum of migrating  
10 substances from silicone baking moulds.  
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19 “[Insert Table II about here]”  
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### 22 *Validation of the $^1\text{H-NMR}$ quantification method*

23 No reference standards representing the migrating substances are commercially available so  
24 far. Cyclic silicones are only available up to hexamers ( $\text{D}_6$ ) which are much more volatile  
25 than the cyclic silicones up to  $\text{D}_{50}$  which we detected in the migrates investigated. Linear  
26 silicone oils (e.g. DMS-T07, DMS-T11) are available in higher molecular weight ranges but  
27 may exhibit a different mass distribution compared to the migrating silicones and result in  
28 additional  $^1\text{H-NMR}$  signals for terminal groups.  
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37  $^1\text{H-NMR}$ -signals increase linearly with the number of the excited protons. In our studies,  
38 linearity was demonstrated in the range of 20-750  $\text{mg kg}^{-1}$  fat (7 levels in duplicate,  $r^2 =$   
39 0.9975), using decamethylcyclopentasiloxane ( $\text{D}_5$ ) dissolved in sunflower oil as calibrant.  
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44 Determination of the most important validation parameters was based on nine baking  
45 experiments, each done as a threefold determination, in silicone muffin moulds using a  
46 common muffin recipe with a fat content of 21 %. The intra-day standard deviation ( $s_r$ ) of the  
47 method gave 0.92  $\text{mg dimethylsiloxanes kg}^{-1}$  food, which corresponds to a relative standard  
48 deviation of 7.3 % (average overall migration of 12.8  $\text{mg kg}^{-1}$  cake). For the uncertainty of  
49 measurement concerning the total migrate we have to take into account that the amount of  
50 dimethylsiloxanes determined by the  $^1\text{H-NMR}$  method recovers only 83 – 89 % of the total  
51 migrate determined gravimetrically (see table II). All results obtained by the  $^1\text{H-NMR}$  method  
52 reported here only refer to the amount of dimethylsiloxanes without correction of the bias  
53 towards the gravimetry. The uncertainty of measurement for the reference method using  
54 olive oil or one of its substitutes given in Commission Directive 2002/72/EC is set up to 20  
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3 mg kg<sup>-1</sup> (3 mg dm<sup>-2</sup>). This is much higher than the sum of the estimated bias and the intra-day  
4 reproducibility. The limit of detection (quantification) was calculated based on the signal to  
5 noise ratio of 3 (9) at 8.7 (26.1) mg kg<sup>-1</sup> fat or 1.9 (5.7) mg kg<sup>-1</sup> cake. Because of the  
6 enrichment of siloxanes during the fat extraction step of the food, this relatively high  
7 detection limit is suitable to determine even low levels of siloxane contamination in food and  
8 is more than sufficient to prove the overall migration limit in fatty foodstuffs according to  
9 Resolution AP (2004) 5.  
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17 The recovery was determined as described in the materials and methods part. In an  
18 experiment performed in triplicate, a recovery rate of (101.3 ± 6.7) % was reached for  
19 extraction of the oil and quantification of the siloxanes by <sup>1</sup>H-NMR. All validation parameters  
20 obtained are summarized in table 3.  
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26 “[Insert Table III about here]”  
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30 Commission Directive 2002/72/EC defines the analytical tolerance for the overall migration  
31 experiment into olive oil to 20 mg kg<sup>-1</sup>. Compared to this criteria the described <sup>1</sup>H-NMR  
32 method is suitable for analysis of the overall migration from silicone baking moulds into food.  
33 Furthermore, the method allows one to examine overall migration from silicone moulds into  
34 real foodstuffs as well as into olive oil or its substitutes. The importance is that this approach  
35 now gives the possibility of checking compliance of silicone moulds with national law or the  
36 standards described in the Resolution AP (2004) 5 as well as enabling an exposure assessment  
37 for cyclic siloxanes in prepared foodstuffs to be carried out aiding in toxicological  
38 assessment.  
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#### 48 *Siloxane migration into simulants and muffins*

49 The overall migration into olive oil and its substitutes isooctane, ethanol (95%) and Tenax®  
50 were determined gravimetrically according to the rules set out in the migration directives  
51 (Council Directives 82/711/EEC and 85/572/EEC) from the first to the third experiment. The  
52 results are shown in figure 6. Isooctane, ethanol (95%) and Tenax® are official substitutes for  
53 olive oil according to Council Directives 82/711/EEC and 85/572/EEC and therefore should  
54 give similar results. However, isooctane generated by far the highest migration giving 209 mg  
55 dm<sup>-2</sup> in the 1<sup>st</sup> experiment, compared with ethanol which gave 25 mg dm<sup>-2</sup> in the 3<sup>rd</sup>  
56 experiment. In analogy to the rules for migration from plastic materials laid down in  
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3 Commission Directive 2002/72/EC the 3<sup>rd</sup> migration is assumed of legal relevance. As to be  
4 seen both solvents overestimate the 3<sup>rd</sup> migration value. The hypothesis that the migration test  
5 using olive oil is not suitable for silicones due to the swelling (BfR 2006) of the material  
6 could not be confirmed. Although little swelling could be observed, it was not in a way  
7 making migration results unrealistic or resulting in a scenario not to be expected for the  
8 preparation of food. Similar swelling of the material was experienced in the later repeated  
9 experiments with different foodstuffs. In contrast to olive oil, isooctane caused an obvious but  
10 reversible swelling of the silicone elastomer producing the highest migration values. Tenax®,  
11 a highly porous solid, gave a significantly lower overall migration in all 3 experiments,  
12 although this tendency weakened from 1<sup>st</sup> to 3<sup>rd</sup> cycle.

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23 “[Insert figure 6 about here]”  
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26 The migration values were compared to the contamination of muffins baked in the same  
27 mould. The amounts of siloxanes detected in the muffins using <sup>1</sup>H-NMR are given in table IV.  
28 As expected, we found much lower migration (about 10 mg kg<sup>-1</sup> cake) compared to olive oil,  
29 due to the less lipophilic character and the texture of the muffin.  
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35 “[Insert Table IV about here]”  
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39 Migration was about 1/30 and 1/8 of the olive oil result for the 1<sup>st</sup> and 3<sup>rd</sup> experiment,  
40 respectively, demonstrating a completely different migration behaviour into the simulant  
41 compared with the food. Therefore, the applicability of the reduction factor of 5 according to  
42 Council Directive 85/572/EEC for bakery products is questionable. None of the simulants is  
43 suitable for imitating the migration processes in pastries. In a follow-up with 10 baking  
44 cycles, we surprisingly found the migration value to be almost constant (figure 7), indicating  
45 different migration kinetics compared to the liquid simulants as well as Tenax®. Further  
46 experiments with two larger moulds confirmed a nearly constant migration from 1<sup>st</sup> to 3<sup>rd</sup> test  
47 but were not continued to the 10<sup>th</sup> migration. More surprisingly, despite a quite different  
48 volume/surface ratio of the three compared moulds, all tested cakes contained similar siloxane  
49 amounts. More differences could be observed comparing migration values related to the  
50 surface of the moulds (table V). These almost similar migration values into creamed cake for  
51 moulds of different shape and size may result from a somewhat longer baking time needed in  
52 case of larger moulds and thus equalizing the more advantageous volume/surface ratio.  
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5 “[Insert Figure 7 about here]”  
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7 “[Insert Table V about here]”  
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10 As a first conclusion, a single instead the 3<sup>rd</sup> experiment, as claimed by law for the evaluation  
11 of plastics, could be sufficient to estimate the migration from silicone materials in muffins or  
12 cakes. Migration into cake does not differ strongly for moulds of various size making the use  
13 of small moulds as favourable as that of larger ones from the consumers point of view.  
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19 In addition, we proved the influence of the fat content in the dough using recipes with 21 %  
20 and 30 % fat. In both cases, again the results showed no significant difference between the 1<sup>st</sup>  
21 and 3<sup>rd</sup> migration for each fat content but gave a significantly higher migration for the muffins  
22 high in fat (figure 8). These results confirm the expected dependence of migration from the  
23 lipophilic character of the foodstuff and are therefore in good accordance with the literature  
24 (Forrest and Sidwell 2005). Muffins or plain cakes with much more than 30 % fat are hardly  
25 to imagine, meaning it is unlikely that the overall migration limit will be exceeded when using  
26 silicone moulds for making a cake. Even without effective compliance control in the past,  
27 most of the silicone baking moulds placed on the market should meet the criteria of  
28 Resolution AP (2004) 5 and moreover of Article 3b of Regulation (EC) 1935/2004 prohibiting  
29 an unacceptable change of the composition of foodstuffs as long as they are used for baking  
30 pastries.  
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42 “[Insert Figure 8 about here]”  
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46 It has to be outlined that there is hardly any obvious correlation between the migration data  
47 for simulants, even when data are corrected by the reduction factor which has to be applied  
48 for cake, and the amount of siloxanes found in cake, neither for the mid nor for the high fat  
49 cake. The migration kinetics as well as the amount of migrating substances differ strongly due  
50 to the less lipophilic character and the texture of the cake. In conclusion, since the  
51 requirements of article 3 of Regulation (EC) No 1935/2004 refer to food and not to simulants  
52 and there is to date no adequate testing procedure warranting comparable results between food  
53 and simulants it is mandatory to prove compliance only with food.  
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3 Intake of cyclic siloxanes from cakes was estimated to be 2 mg per portion or less, based on  
4 an assumption of a consumption of 200 g cake with 30 % of fat, made in a muffin mould with  
5 a quite adversarial volume/surface ratio of about 112 ml dm<sup>-2</sup>. This is in the same range as  
6 expected for the typical consumption of food (e.g. fruit or vegetable preserves) containing  
7 food additive E 900 (a linear polydimethyl siloxane) at its highest legal concentration of 10  
8 mg kg<sup>-1</sup> (Council Directive 89/107/EEC). Insofar, there are no toxicological concerns,  
9 although a TDI of 1.5 mg kg<sup>-1</sup> body weight was established for mid-size linear polydimethyl  
10 siloxanes as E 900 only but not for cyclic siloxanes (Synoptic Document 2005).  
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### 18 19 *Conclusion*

20 The method presented here is the first fully validated method to investigate the overall  
21 migration of silicone baking moulds into olive oil, substitutes and foodstuffs and therefore  
22 provides an essential tool to check the legal compliance of silicone moulds already placed on  
23 the market. No other suitable and validated method has so far been published.  
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29 Investigating the migration properties of silicone elastomers using gravimetric and <sup>1</sup>H-NMR  
30 analysis resulted in very different data depending on the food, simulant or substitute used.  
31 Based on our data, it has to be concluded that the migration test with olive oil, as required by  
32 Council Directive 82/711/EEC, does not give a realistic scenario of the migration into cake,  
33 even when the official reduction factor is used. This concerns the amount of migration as well  
34 as variations from 1<sup>st</sup> to 3<sup>rd</sup> migration test. There are also big differences between the  
35 migration data for olive oil and its substitutes ethanol (95%), isooctane and Tenax® according  
36 to Directive 82/711/EEC. Neither the liquid solvents nor Tenax® give similar results to olive  
37 oil as easily to expect but presupposed by the migration directives.  
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47 Consequently, the olive oil test may act only as a convention in the sense of an inertness  
48 parameter for the investigated material. It seems more appropriate to search for a simpler and  
49 better reproducible test method for silicone elastomer materials. Based on our results we  
50 suggest the much easier and more precise extraction procedure with ethanol (95%) and to  
51 focus on the 1<sup>st</sup> extract only (with a therefore new defined limit for the total extractives in  
52 ethanol). This follows the same consideration as German BfR recommendation XV (BfR  
53 2005) in which limits for both volatile and extractable substances have been defined, but not a  
54 migration limit.  
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3 To judge a silicone article with regard to the requirements of article 3 of Regulation (EC) No  
4 1935/2004 migration testing has to be done in food absolutely. This recognition well confirms  
5 with other findings proving migration into food to be sometimes completely different  
6 compared to migration into simulants as described for isopropylthioxanthone (BfR 2006),  
7 plasticizers in gaskets (Fankhauser-Noti 2006) and other (Foodmigrosure 2007).  
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13 The discussed  $^1\text{H-NMR}$ -method is suitable for examining the legal compliance of silicone  
14 articles in food contact as well as for a reliable exposure assessment of consumers. Preparing  
15 cake in a silicone mould usually will not result in exceeding the overall migration limit of 60  
16  $\text{mg kg}^{-1}$  or in a relevant contamination of the foodstuff from the toxicological point of view.  
17 Actual investigations of our research group indicate a much higher amount of migrating  
18 siloxanes for silicone moulds used for the preparation of meat loafs, even exceeding the  
19 overall migration limit. Further experiments are currently in preparation.  
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34 Chemistry) for doing the LC-MS measurements.  
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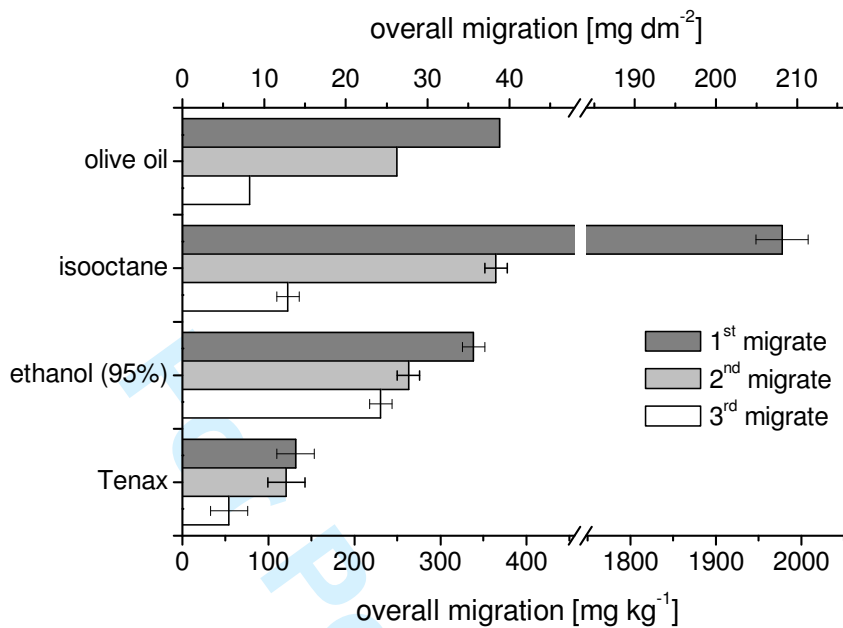
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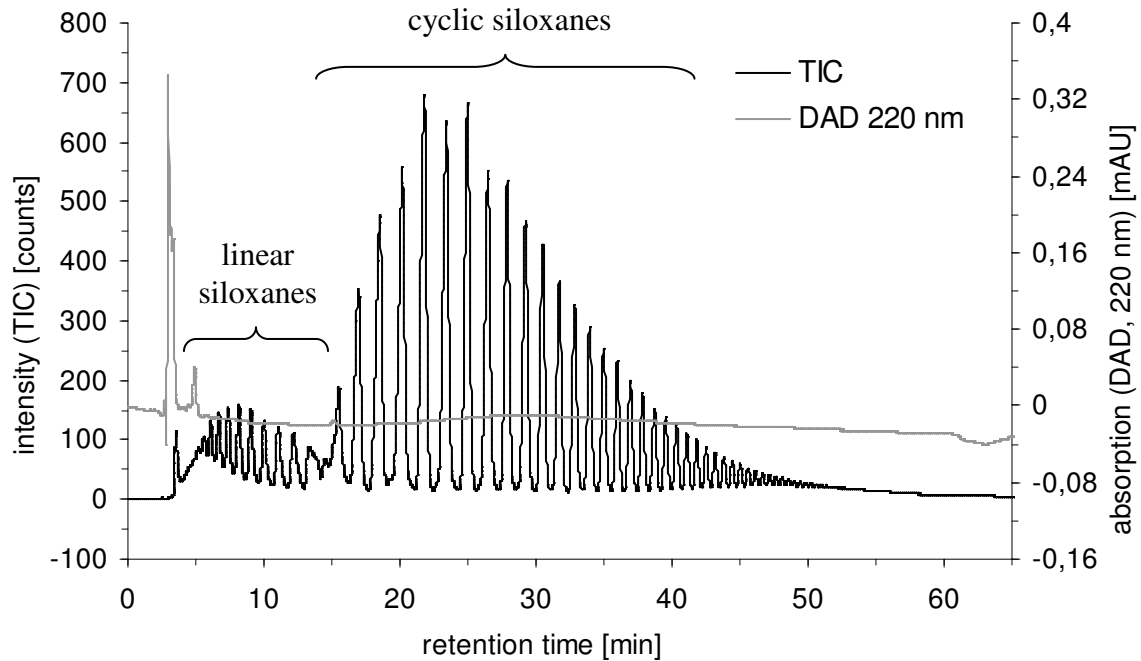
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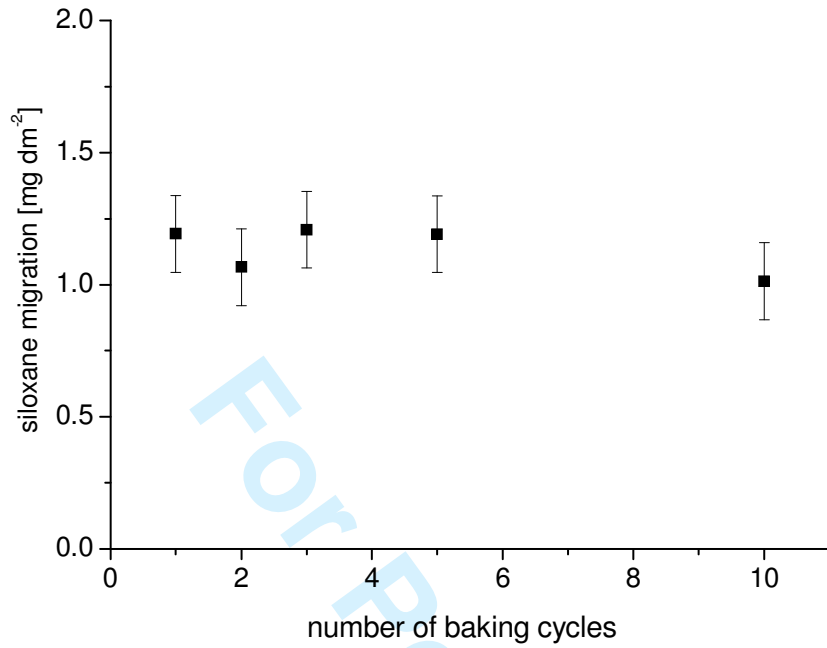


**Figure 6.** Overall migration into simulants (after gravimetric determination according to EN 1186:2002). Data shown: mean  $\pm$  confidence interval ( $\alpha = 0.05$ ), threefold determination (olive oil: single determination).

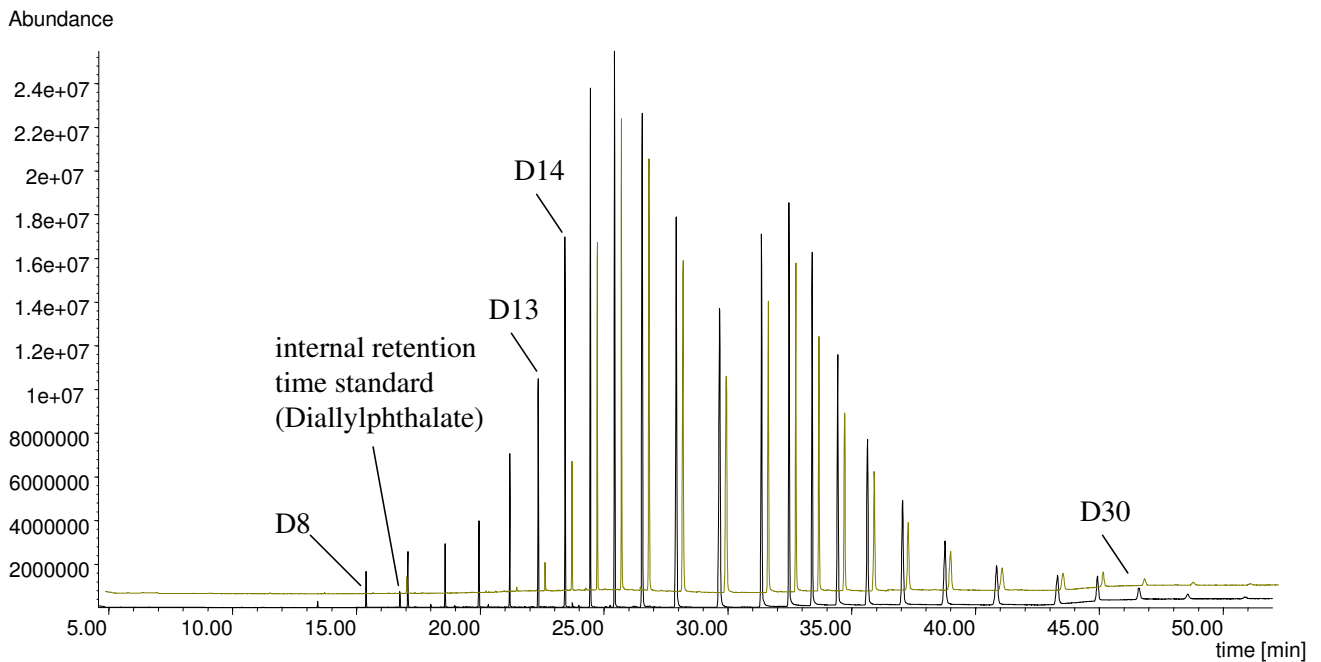


**Figure 3.** RP-HPLC-MS/DAD chromatogram of the migration residue from a silicone baking mould containing hydroxyl terminated dimethylsiloxanes ( $m/z$  300-4000)



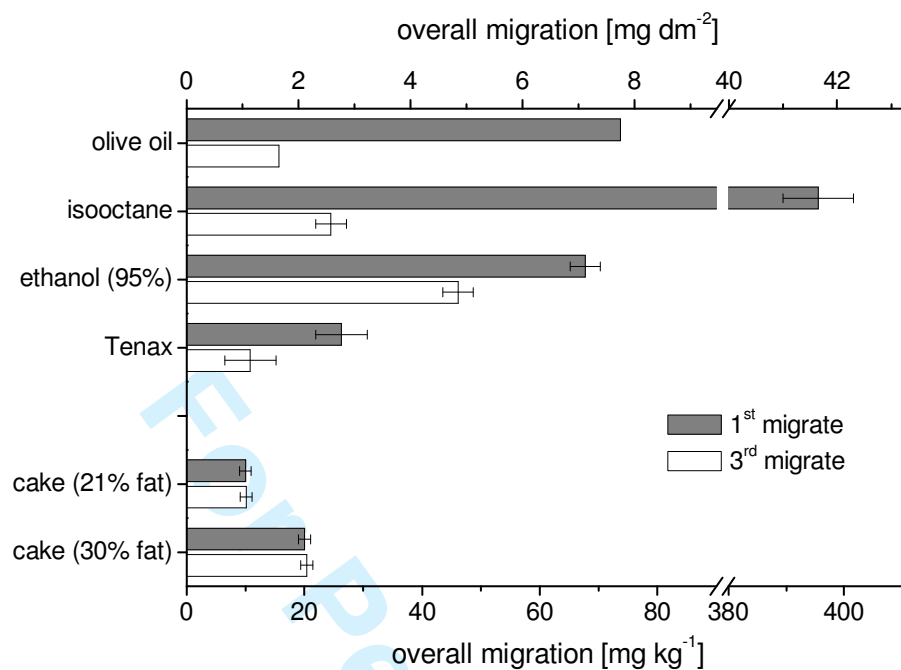


**Figure 7.** Siloxane migration into creamed cake during repeated usage. Data shown: mean  $\pm$  confidence interval ( $\alpha = 0.05$ ), threefold determination.

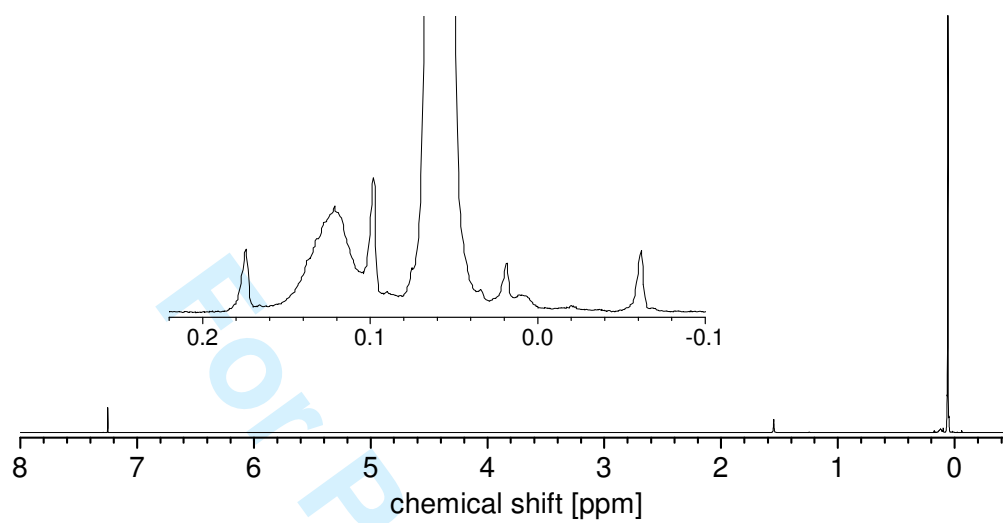


**Figure 4.** GC-MS chromatogram (m/z 65-800) of the ethanol extracts of 2 different moulds with oligomers from D8 - D32 or D 13 - D 32 respectively. Differences in the amount of low molecular weight oligomers may be caused by the process of preheating (tempering). GC-parameters: Injection splitless at 280°C, oven temperature starting at 50°C and rising up to 360°C. Column HP-5MS.

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**Figure 8.** Comparison of the overall migration into creamed cake (determination via <sup>1</sup>H-NMR) and food simulants (gravimetric determination according to EN 1186:2002). Reduction factor x/5 (Council Directive 85/572/EEC) is regarded (in contrast to Fig. 6) . Data shown: mean ± confidence interval ( $\alpha = 0.05$ ), threefold determination (olive oil: single determination).



**Figure 5.**  $^1\text{H}$ -NMR spectrum of the migration residue

**Table I.** Determination of the average molar mass of triglycerides from the  $^1\text{H-NMR}$  spectrum of the lipid matrix ( $M_{\text{SU}}$ : molar mass of the subunit [ $\text{g mol}^{-1}$ ]; I: integral of the signal(s) in the  $^1\text{H-NMR}$  spectra; p: number of protons responsible for the  $^1\text{H-NMR}$  signal; I/p: amount of substance ratio; SU: subunit of the triglyceride molecule; TB:  $\text{CH}_2\text{O-CO}$  unit at the triglyceride backbone)

signal	subunit	$M_{\text{SU}}$ [ $\text{g mol}^{-1}$ ]	I <sup>a</sup>	p	I/p	$\frac{(I \cdot p^{-1})_{\text{SU}}}{(I \cdot p^{-1})_{\text{TB}}} \cdot 2^c$
1	$\text{CH}_3$ (fatty acids)	15,03	218,1	3	72,7	2,9
2-6	$\text{CH}_2$ (fatty acids)	14,03	1912,4	2	956,2	38,2
7	$\text{CH}_2\text{O-CO}$ (triglyceride backbone)	58,04	100,0	2	50	2 <sup>b</sup>
8-9	CH	13,02	254,1	1	254,1	10,2
	COO					
	(C2 of the triglyceride backbone)	44,01	-	-	-	1 <sup>b</sup>

<sup>a</sup> The integral of signal 7 is set up to 100,0.

<sup>b</sup> Each triglyceride molecule contains two  $\text{RCH}_2\text{O-CO}$  units (at C1 and C3 of the triglyceride backbone) and one  $\text{R}_2\text{CHO-COO}$  unit (at C2 of the triglyceride backbone).

<sup>c</sup> factor 2 regarding that each triglyceride molecule contains two  $\text{CH}_2\text{O-CO}$  units

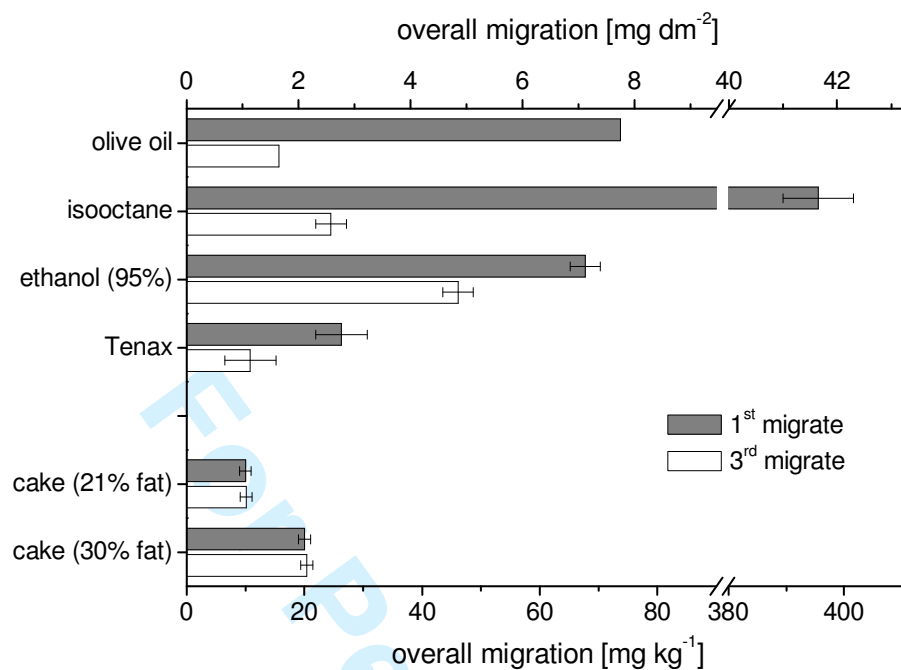
**Table III.** Analytical parameters of the  $^1\text{H-NMR}$  determination of dimethylsiloxanes in pastries (data refer to a creamed cake with 21% fat in the dough and are given mass related to avoid the uncertainty of surface calculation).

<b>calibrated range</b>	[mg kg <sup>-1</sup> triglyceride]	20-750
	[mg kg <sup>-1</sup> cake]	5-170
<b>coefficient of determination (r<sup>2</sup>)</b>		0.9975
<b>recovery rate</b>	[%]	101.3 ± 6.7*
<b>relative standard deviation (RSD)</b>	[%]	7.3
<b>standard deviation (intra day, s<sub>r</sub>)</b>	[mg kg <sup>-1</sup> triglyceride]	4.2
	[mg kg <sup>-1</sup> cake]	0.92
<b>limit of detection (LOD)</b> (S/N = 3)	[mg kg <sup>-1</sup> triglyceride]	8.7
	[mg kg <sup>-1</sup> cake]	1.9
<b>limit of quantification (LOQ)</b> (S/N = 9)	[mg kg <sup>-1</sup> triglyceride]	26.1
	[mg kg <sup>-1</sup> cake]	5.7

\*mean ± confidence interval ( $\alpha = 0.05$ , threefold determination)

**Table IV.** Overall migration into creamed cake (21% fat in the dough) (test conditions: 25 min, 175°C), determined by  $^1\text{H-NMR}$ . Data shown: mean (threefold determination). Confidence interval ( $\alpha = 0.05$ ):  $0.15 \text{ mg dm}^{-2}$  ( $1.00 \text{ mg kg}^{-1}$ ) based on standard deviation  $s$  of the procedure:  $s = 0.11 \text{ mg dm}^{-2}$  ( $0.92 \text{ mg kg}^{-1}$ )

Migrate	siloxane migration	
	$[\text{mg dm}^{-2}]$	$[\text{mg kg}^{-1}]$
1 <sup>st</sup>	1.19	10.00
2 <sup>nd</sup>	1.07	8.94
3 <sup>rd</sup>	1.21	10.11
5 <sup>th</sup>	1.19	9.98
10 <sup>th</sup>	1.01	8.46



**Figure 8.** Comparison of the overall migration into creamed cake (determination via <sup>1</sup>H-NMR) and food simulants (gravimetric determination according to EN 1186:2002). Reduction factor x/5 (Council Directive 85/572/EEC) is regarded (in contrast to Fig. 6) . Data shown: mean ± confidence interval ( $\alpha = 0.05$ ), threefold determination (olive oil: single determination).