



# Characterization of odorants from baby bottles by headspace solid phase microextraction coupled to gas chromatography-olfactometry-mass spectrometry

Wellington da Silva Oliveira<sup>a,\*</sup>, Jazmin Osorio Monsalve<sup>b</sup>, Cristina Nerin<sup>b,\*\*</sup>, Marisa Padula<sup>c</sup>, Helena Teixeira Godoy<sup>a</sup>

<sup>a</sup> Department of Food Science, School of Food Engineering, University of Campinas (UNICAMP), Campinas, 13083-862, Brazil

<sup>b</sup> Department of Analytical Chemistry, GUIA Group, Aragon Institute of Engineering Research I3A, University of Zaragoza, Zaragoza, 50018, Spain

<sup>c</sup> Packaging Technology Center, Institute of Food Technology (ITAL), Campinas, 13070-178, Brazil

## ARTICLE INFO

### Keywords:

Plackett-burman  
Silicone baby bottles  
Migration  
Volatile organic compounds  
Olfactometry

## ABSTRACT

A SPME protocol was optimised for the characterization of odorous migrants coming from baby bottles. Considering this, a Plackett–Burman design to select variables and a central composite rotatable design to define the optimal conditions were used. The method proposed by Derringer and Suich was used to simultaneously optimise the responses. After validation, the analytical method was used to characterise volatile migrants from baby bottles made of polypropylene, Tritan and silicone using 50% ethanol as a simulant. 2,4-di-*tert*-butylphenol was identified in all samples. Forty-five compounds were identified, of which thirty-eight were odorants in silicone baby bottles. Aldehydes were mainly responsible for the unpleasant odour of silicone. Moreover, silicone showed slightly higher volatile organic compounds (%) than recommended. Odours from ketones, benzophenone derivatives and alcohols were also detected by GC-O-MS, however below the LOQ obtained in the MS detector. All migration data were below the specific migration limits.

## 1. Introduction

In 2011, the European Commission (EC) and Brazil banned the sale of baby bottles containing bisphenol A (BPA). This decision was based on the precautionary principle, as there is a lack of robust data that supports the safety of BPA for babies and there are many uncertainties in the reports presented by the World Health Organisation (WHO), as well as the European Food Safety Authority [1–4].

Thereafter, many baby bottles manufactured with different plastic materials appeared on the market, among them polypropylene (PP), Tritan™ and silicone. However, these materials should be evaluated regarding the migration limits to ensure that the utensil is used safely. Since babies have a lower body weight and a less developed metabolic detoxification capability, the negative effects of migrants from food contact materials (FCM) are much greater than those induced in adult organisms. Due to this, special attention must be paid to such aspects [5].

Migration analysis from baby bottles to food can be performed in the foodstuff itself or in food simulants. The food simulants are liquid

solutions or solid compounds (Tenax) that simplify the analytical work required when working with real foods. Migration analysis from baby bottles was reported using 50% ethanol as a milk simulant followed by liquid-liquid extraction and GC-MS analysis. However, this technique requires a large volume of solvents and may present low selectivity [6–8].

Many studies have aimed the development of fast, miniaturised and eco-friendly sample preparation techniques such as solid-phase microextraction (SPME), which has great potential to reduce solvent usage. SPME has been used to identify, quantify and characterise volatile organic compounds (VOC) followed by gas chromatography-olfactometry-mass spectrometry. Many of the VOC are derived from degradation processes and may emit an odour. Odorants can modify the organoleptic properties of food and produce a negative effect on the quality of the product. For this reason, attention should be paid to VOC, as well as the formation of odorant compounds [9,10].

Although many of them have been described in the literature [11], there are no reports on the migration of these compounds from silicone baby bottles [12–14]. On the other hand, compliance assessment of

\* Corresponding author.

\*\* Corresponding author.

E-mail addresses: [wellingtonoliveira1408@gmail.com](mailto:wellingtonoliveira1408@gmail.com) (W.d.S. Oliveira), [cnerin@unizar.es](mailto:cnerin@unizar.es) (C. Nerin).

silicone food contact materials is mostly based on limits for volatile organic compounds set by the Bundesinstitut für Risikobewertung (BfR – German Federal Institute for Risk Assessment) at 0.5% [15].

Successful implementation of SPME requires careful evaluation and optimisation of factors that influence extraction efficiency (such as type of fibre coating, ionic strength, time and temperature of extraction among others) of the analytes. Multivariate statistical tools, such as Plackett-Burman (PB) and central composite rotatable design (CCRD), can determine which parameters should be evaluated, as well the simultaneous optimisation of the factors studied. Moreover, considering optimisation involving a high number of responses, it is very unlikely that the optimum region for all of them will be the same. In this case, the method proposed by Derringer and Suich [16] becomes a valuable statistical tool as it allows the simultaneous optimisation of all responses [17,18].

To the best of our knowledge, there are no studies that address multivariate optimisation of the extraction of VOC from baby bottles by SPME. Additionally, data regarding the sensory impact of the migration of VOC from baby bottles are scarce. In view of this, in this work an analytical protocol to determine VOC from baby bottles by SPME was optimised, followed by GC-MS and GC-O-MS analysis. Once optimised, the method was used to analyse 36 baby bottles made of PP, Tritan and silicone.

## 2. Materials and methods

### 2.1. Reagents

Standards of 2-nonanone (99%, CAS 821-55-6), *n*-undecane (99%, CAS 1120-21-4), camphor (95%, CAS 76-22-2), *trans*-2-nonenal (97%, CAS 18829-56-6), 1,3,5-triethylbenzene (97%, CAS 102-25-0), 1-decanol (99%, CAS 112-30-1), 2,6-ditertbutyl-1,4-benzoquinone (95%, CAS 719-22-2), diisobutyl phthalate (DIBP) (99%, CAS 84-69-5), methyl palmitate (99%, CAS 112-39-0), 4-heptanone (CAS 123-19-3), octanal (99%, CAS 124-13-0), isomentol (98%, CAS 89-78-1), isobornyl acrylate (98.5%, CAS 5888-33-5), diphenyl ether (99%, CAS 101-84-8), 2,4-di-*tert*-butylphenol (99%, CAS 96-76-4), 3,5-Di-*tert*-4-butylhydroxytoluene (BHT) (99%, CAS 128-37-0), 3,5-di-*tert*-Butyl-4-hydroxybenzaldehyde (99%, CAS 37942-07-7), benzophenone (99%, CAS 119-61-9), ethyl 4-(dimethylamino)benzoate (99%, CAS 10287-53-3) and *n*-alkanes (C8 to C20) were purchased from Sigma (Madrid, Spain). Ethanol (HPLC grade) was from Scharlau (Barcelona, Spain). Ultrapure water was obtained from a Milli-Q system (Millipore, Billerica, MA, USA).

### 2.2. Samples

Thirty-six baby bottles, from 12 different models (Table S1), intended for children between 0 and 12 months were used in this study. The baby bottles were purchased from distributors located in São Paulo, Brazil and were made of Tritan™ (*n* = 9), polypropylene (*n* = 24) and silicone (*n* = 3).

### 2.3. Determination of VOC (%)

VOC was determined only in silicone baby bottles, in triplicate, according to Hellring, Seifried, Fritzsche and Simat [19]. To do this, 3g of the silicone baby bottle were cut into 1 × 2 cm pieces and conditioned over dried CaCl<sub>2</sub> for 48 h. Thereafter, 3g were weighed into a glass bowl and heated for 4 h at 200 °C, conditioned in an exicator and weighed again. The loss of weight, calculated as the percentage, gives the amount of volatile organic compounds.

### 2.4. Migration tests

Initially, the baby bottles were boiled for 5 min as recommended by

the manufacturer. Next, the samples were submitted to migration tests at 70 °C/2 h using 50% ethanol as the milk simulant. Since baby bottles are materials for repeated use, migration tests were carried out 3 times in the same sample as recommended by Regulation 2011/10/EC and the Brazilian Health Regulatory Agency - ANVISA [20,21]. Between the tests, the baby bottles were rinsed with ultrapure water and boiled again for 5 min.

### 2.5. Extraction optimisation

#### 2.5.1. Fibre choice

Ethanol can negatively influence the adsorption of other volatile compounds as it competes directly with the analyte for adhesion sites in the SPME fibre. For this reason, the amount of organic solvent in samples should be kept to a minimum. Typically, for optimal extraction efficiencies, organic solvent should not exceed 1–5% of the sample volume [22].

In view of this, previous tests were carried out to reduce the organic solvent content and optimise the extraction of odorous compounds from 50% ethanol. To do this, 5g of the solution, obtained after the first migration test with silicone baby bottles, was used. First, the content of ethanol was removed with nitrogen stream at 40 °C followed by head space solid-phase microextraction (HS-SPME) at 60 °C/30 min using DVB/CAR/PDMS (50/30 μm). Second, 0.5g of the sample was diluted 10-fold with 4.5g of water, resulting in a sample with 5% of ethanol, followed by the extraction at 60 °C/30 min with DVB/CAR/PDMS (50/30 μm).

The first approach showed low reproducibility and a large loss of volatile analytes compared with the sample dilution. On the other hand, the dilution of the sample in water can reduce the matrix effect, favouring a greater extraction of the compounds of interest. Thus, dilution was the best alternative for SPME analysis.

After this, three types of fibres with different polarities and thicknesses were evaluated in the extraction process: PDMS (100 μm), DVB/CAR/PDMS (50/30 μm) and PDMS/DVD (65 μm). The selected fibres have different polarities, which provide different extraction capacity of the analytes.

For this purpose, 5g of a mix containing 7 mg kg<sup>-1</sup> of 2-nonanone, *n*-undecane, camphor, *trans*-2-nonenal, 1,3,5-trimethyl benzene, 1-decanol, 2,6-ditertbutyl-1,4-benzoquinone, diisobutyl phthalate and methyl palmitate in 5% ethanol was prepared in triplicate. These substances were selected based on the representativeness of the classes of compounds which had already been reported in PP, Tritan and silicone materials [7,11]. After extraction, the samples were injected in GC-MS Agilent 6890 equipped with a CTC Analytics system (Madrid, Spain).

#### 2.5.2. Experimental design

After choosing the SPME fibre, a Plackett-Burman (PB) design was used as the methodology to select variables that could affect the extraction of volatile compounds from the mix (Table S2). The significance level used to assess the effect of a variable was set to 10%, to minimise the risk of excluding any important variables of the method in the following step [23].

Next, a central composite rotatable design (CCRD), based on a 2<sup>3</sup> factorial design with six axial points and three repetitions of the central point, was used to determine the extraction conditions. Finally, the best extraction condition for each mix compound was determined using the simultaneous response optimisation technique proposed by Derringer and Suich [16]. This method is based on defining of a desirability function to each response, with values restricted to the interval [0,1], where “zero” means an unacceptable value and “one” the most desirable value.

Since the desirability function has been set for all the responses, they should be combined into an overall desirability, normally given by the geometric average of “*n*” individual desirability. The individual desirability was defined to maximise the ion abundance (by GC-MS) of

each compound. The mathematical models were evaluated by variance analysis. The proposed condition by the algorithm was experimentally validated and used to extract the odorous compounds from baby bottles.

## 2.6. Chromatographic analysis

### 2.6.1. HS-SPME-GC/MS analysis

The volatile compounds were analysed using the Agilent 6890 series GC system coupled to the 5973 series mass selective detector. The injection was performed in a HP5 MS column, in splitless mode, at 250 °C and, helium was used as carrier gas at 1 mL min<sup>-1</sup>. The ionization was performed by electronic impact at 70 eV. The chromatography started at 60 °C for 5 min, increasing 10 °C min<sup>-1</sup> to 300 °C in which it was held for 5 min.

For fibre selection and identification of volatile compounds, the SCAN mode (40–400 *m/z*) was used. Moreover, compounds were identified by matching their mass spectra vs. NIST14 and Wiley library, observing a coincidence of at least 80%. Identification was confirmed using the temperature programmed retention index (LTPRI) which was calculated using a commercial mixture of hydrocarbons (C8–C20).

On the other hand, the SIM mode was used to quantify the compounds identified. For this purpose, 2 ions were monitored: one to quantify and the other to confirm the compound identified. Therefore, the stock solution containing the following reagents: 4-heptanone, octanal, *trans*-2-nonenal, isomenthol, 1-decanol, isobornyl acrylate, 2,4-di-*tert*-butyl phenol, BHT, benzophenone, ethyl 4-dimethylamino-benzoate, 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde, DIBP, methyl palmitate, each at 1000 µg kg<sup>-1</sup>, were prepared in 5% ethanol. Six-point calibration curves were made, each in triplicate and were analysed following the same methodology described above.

The analytical procedure was validated in terms of linear range (µg.kg<sup>-1</sup>), regression coefficient (*r*), limit of detection (LOD), limit of quantification (LOQ) and reproducibility. LOD was determined as 3 times the noise signal. The LOQ was established as three times the LOD. Due to the lack of standards, some compounds were quantified using similar standards.

### 2.6.2. HS-SPME-GC-O/MS analysis

SPME-GC-O/MS analysis was performed in Agilent 7890B GC-MS coupled to a Phaser olfactory detection system and a quadrupole operating in scan mode (40–400 *m/z*). The extraction was carried out according to the method optimised in the first step. Injection was carried out using SPME manual injector where DVD/CAR/PDMS fibre was used. Odorous compounds were identified by comparing the LTPRI determined by HS-SPME-GC/MS with those obtained in the HS-SPME-GC-O/MS. Moreover, characterization of the aroma of each compound was performed and confirmed using Flavornet and Pherobase.

The aromas were described by six trained sniffers, who sniffed the migrant sample eluted from the chromatographic column and characterised them by their intensities and odour. The odour strength scale: 1–3 (1 was very weak, hardly recognisable note and 3 was very strong, intense note) was assigned to each smelled compound. Fractional values were also allowed.

After this, modified frequency was calculated according to the equation: %FM=[F(%) × I(%)]<sup>0.5</sup>; where F (%) is the percentage of the sniffers that detected the smell and I (%) is the percentage of intensity calculated by the average of the values of the intensity given by all the sniffers. The MF (%) permits to select the most important odour compounds from the samples. The compounds with MF (%) higher than 60% can be considered as the base of the aroma of the analysed samples. This methodology allowed us to check compounds which were not detected by mass spectrometry. This fact is very important in the study of compound migration to food since the organoleptic properties of food could be affected by the packaging [9,10].

## 3. Results and discussion

The main aim of this work was to develop a method by HS-SPME to determine the migration of the volatile compounds from baby bottles into food simulant. They were identified and quantified by GC-MS. Additionally, the volatiles were characterised regarding the odour by HS-SPME-GC-O/MS. The baby bottle samples under study were manufactured using PP, Tritan and silicone.

### 3.1. Determination of VOC (%)

Compliance assessment of silicone food contact materials is determined mainly by the limit of VOC(%). Originally, studies focusing on the amount of volatile organic compounds were performed as an indicator of potential high migration. A limit of 0.5% for the release of volatile organic compounds was introduced to distinguish between tempered and non-tempered materials and to avoid excessive migration. In silicone baby bottles, the release of VOC was slightly above (0.54 ± 0.02) the limit established by the Bundesinstitut für Risikobewertung [15]. Many toxic compounds might be present in the profile of volatile migrants from baby bottles [24]. Moreover, this migration might result in a change in the sensory properties of food, as well as early exposure of babies to chemical contaminants. For this reason, it is important to identify and quantify such compounds in order to mitigate the routes of exposure.

### 3.2. Fibre choice

Previous to the extraction of VOC from baby bottles, the fibre selection was performed. The choice of coating extraction phase is the first and arguably most important step in the development of any SPME method. Various coating extraction phases are available commercially. Depending on the extraction phase polarity and thickness, a given class of analytes may be favoured during extraction [25].

In this study, PDMS (100 µm), DVB/CAR/PDMS (50/30 µm) and PDMS/DVD (65 µm) (Supelco, Bellefonte, USA) were evaluated for their suitability towards the determination of the classes of compounds of interest. They present different mechanisms of extraction. In liquid SPME coatings (PMDS), the extraction occurs via partition into the extraction phase where the analyte molecules are solvated by the coating molecules. Since the diffusion coefficient of analyte is sufficiently high within the liquid coating, analyte molecules can penetrate the whole volume of the coating within a reasonable extraction time. On the other hand, in solid coatings (CAR and DVB), the analytes migrate into the pores of the adsorbent during the extraction process, and the extraction occurs only on the surface via an adsorption process through various interactions such as pi-pi bonding, hydrogen, or van der Waals interactions. Consequently, shorter extraction times are needed, since diffusion of analytes into the bulk of adsorption-type coatings does not occur [25].

Probably, for this reason, the PDMS fibre has shown the lower extractive capacity (Fig. 1). Since all fibres were exposed for the same time, due the mechanism of extraction, the liquid coating extracted less than the solid coating. Nevertheless, the better extraction of apolar compounds, such as DIBP and methyl palmitate, was obtained with this fibre. Fibre selectivity for an analyte of interest is determined on the basis of the principle 'like-dissolves-like'. Therefore, single-polymer coatings such as nonpolar PDMS is highly efficient for the extraction of nonpolar target analytes [26].

PDMS/DVB showed better extraction than PDMS fibre. However, DVB/CAR/PDMS showed better results for the extraction of all compounds except for ethyl palmitate and diisobutyl phthalate. This fibre combines the properties of solid (CAR and DVB) and liquid coatings which can extract a wide range of molecules of different sizes. Solid coatings can retain an analyte that is about half the size of its diameter. Carboxen comprises of many micropores, with opening diameters

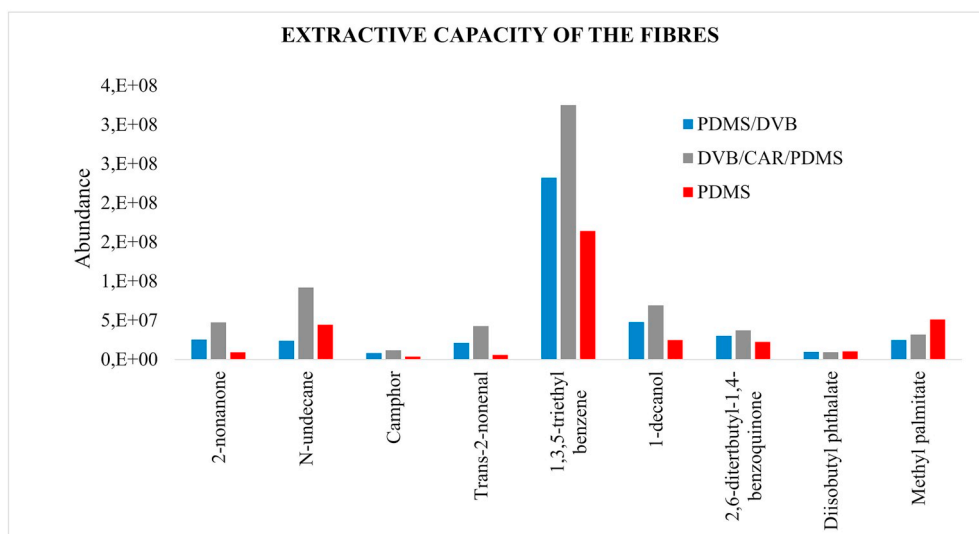


Fig. 1. Effect of coating type on extraction capacity of the mix compounds by HS SPME-GC/MS.

ranging from 2 to 20 Å. Consequently, it does not adsorb and/or desorb large molecular weight molecules efficiently. Conversely, DVB presents a high degree of mesopores (ranging from 20 to 500 Å), and therefore does not satisfactorily adsorb small molecules. For this reason, it is important to pay attention to the degree of pore distribution and pore size in the solid coating. Hence, efficient extraction of compounds with molecular weight ranging from 40 to 275 Da can be obtained [17,25,26].

### 3.3. Method optimisation

#### 3.3.1. Plackett-Burman design

PB is a saturated design that assumes there are no interactions between the different extraction variables, and each variable is tested at two experimental levels: a high level (+) and a low level (−) [27]. The NaCl concentration (%), incubation time (min), extraction time (min), extraction temperature (°C) and stirring rate (rpm) were evaluated regarding the impact on the extraction process of the mix compounds by HS-SPME (Table S2). The statistical significance of the NaCl (%), extraction time and extraction temperature were confirmed by ANOVA, with ( $p > 0.1$ ) (Fig. 2).

The NaCl concentration showed a positive effect for the compounds with low molecular weight. On the other hand, for methyl palmitate, the NaCl concentration had a negative effect. Salting out can increase or decrease the amount extracted, depending on the compound and salt concentration. In general, the addition of salt modifies the ionic strength of the medium and allows the extraction of more polar analytes through the salting out effect [26].

HS-SPME is an equilibrium extraction technique, and as such, optimisation of extraction time is a critical factor to ensure method efficiency because when the extraction time increases, the extraction efficiency also increases, until reaching a maximum (equilibrium). The extraction time showed a positive effect for the heaviest compounds of the mix. Ideally, extraction time should be chosen as the time interval that allows equilibrium to be reached for all analytes [25,28]. For this reason, this factor was also included in the next step.

Conversely, the extraction temperature had a negative effect on compounds of lower molecular mass and a positive effect on compounds of higher molecular mass. The temperature affects the extraction, decreasing the viscosity of the sample and increasing the diffusivity of the analyte, which in turn increases the quantities extracted in pre-equilibrium conditions. The increase in the temperature of the sample can also favour the release of analytes bound to the matrix in its free form in aqueous medium. On the other hand, an increase in

temperature adversely affects the distribution coefficient of the analyte ( $K_{fm}$ ) between the fibre coating and the sample. However, this will be a problem only in extractions at equilibrium conditions [26,28].

In a saturated design, such as PB, the central points may present values higher or lower than the other test conditions, indicating the possible existence of a curvature. Once there are no degrees of freedom to evaluate a second-order model, if the curvature is not evaluated, the standard error increases, as well as the p-value, masking the statistically significant factors. For this reason, all responses were evaluated taking into account the curvature. Thus, the standard error decreases, as the  $t_{cal}$  and p-value, enabling the visualisation of significant effects [23].

The curvature was significant for *trans*-2-nonenal and 1-decanol ( $p < 0.1$ ). The compounds showed  $r^2$  higher than 0.92 indicating a low error associated with the experiment. Moreover, the factors which showed a significance for both compounds are in consonance with the other compounds of the mix. For this reason, only NaCl (%), extraction time (min) and extraction temperature (°C) were evaluated in the CCRD.

#### 3.3.2. Central composite rotatable design (CCRD)

After selecting the factors that interfere in the extraction by HS-SPME, a central composite rotatable design (CCRD) was carried out (Table 1). For this purpose, the levels of the variables studied were modified taking into account that all factors showed positive or negative effects in the mix compounds. Hence, the levels were reduced in order to find the least NaCl concentration, extraction time and extraction temperature necessary to extract all compounds from the mix.

The significance of the factors studied was determined through the analysis of variance ( $p < 0.05$ ). A model was set for all compounds, except for *trans*-2-nonenal and 2,6-ditertbutyl-1,4-benzoquinone (Table S3). Both compounds have already demonstrated a negative effect regarding the extraction temperature. Probably both compounds reached equilibrium in the range of time and temperature studied. Given that the extraction process is of an exothermic nature, an increase in temperature at equilibrium could lead to a decrease in the distribution coefficient ( $K_f$ ) of the analyte between the fibre coating and headspace, generating models with a lack of fit. On the other hand, the reduction in extraction temperature could compromise the extraction of compounds with higher molecular weight. This situation is common in the development of extraction methods whose multi-residue covers a broad range of polarities. Considering this, the focus must be the overall quality of data, keeping in mind method sensitivity [26,27].

For the other compounds, optimisation showed that the linear and quadratic effects of the studied factors can affect the extraction.



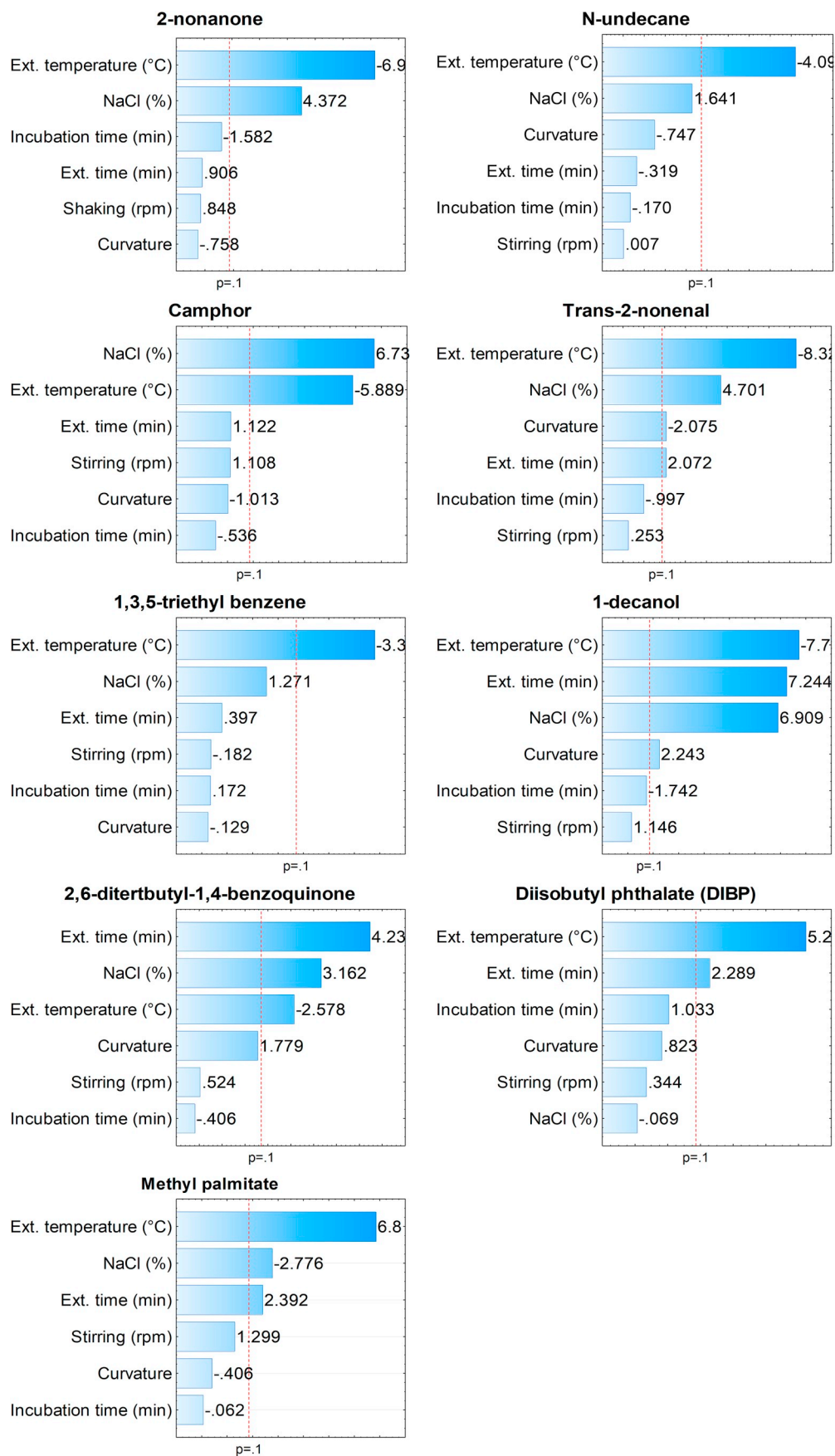


Fig. 2. Pareto diagram for each compound present in the mix.

**Table 1**

Experimental domain applied to a central composite rotatable design (CCRD).

Variables		−1.68	−1	0	1	1.68
X <sub>1</sub>	NaCl (%)	3.34	6.64	11.50	16.36	19.66
X <sub>2</sub>	Extraction time (min.)	5.00	13.10	25.00	36.90	45.00
X <sub>3</sub>	Extraction temperature (°C)	50.00	55.06	62.50	69.94	75.00

**Table 2**

Predicted values after optimisation by the Derringer and Suich tool.

Variable	Predicted values <sup>a</sup>		Observed values <sup>a</sup> (n = 3)
	Lower limits	Upper Limits	
NaCl (%)	−1	1	−
Extraction time	−1	1	−
Extraction Temperature	−1	1	−
2-nonanone	19.95	46.65	29.35 ± 3.25
N-undecane	26.24	66.75	30.97 ± 1.69
Camphor	7.13	15.08	9.86 ± 0.88
Trans-2-nonenal	31.56	73.85	33.97 ± 2.82
1,3,5-triethyl benzene	101.17	298.19	139.91 ± 13.05
1-decanol	102.00	216.15	107.93 ± 10.37
2,6-ditertbutyl-1,4-benzoquinone	55.66	108.18	52.94 ± 4.56
Diisobutyl phthalate	11.16	95.10	59.02 ± 4.98
Methyl palmitate	84.18	227.73	151.27 ± 11.95

<sup>a</sup> Values divided by 10<sup>6</sup>.

Considering several responses and different predicted extraction conditions, the Derringer and Suich [16] tool was used for simultaneous response optimisation and to find the best capable condition to extract all the compounds of the mix. A condition with desirability of 0.58 was found. The optimal extraction condition proposed was 15% NaCl, 37 min extraction time and 61 °C extraction temperature.

Three confirmatory tests were performed to experimentally evaluate the best condition given by the model. Table 2 shows a good agreement between the predicted values (for extraction and response conditions, such as peak areas) and those obtained experimentally for all the compounds.

### 3.4. Identification and quantification of odorants by HS-SPME-GC-O/MS and HS-SPME-GC/MS analysis

The optimised condition for HS-SPME was used in the extraction, identification and quantification of odorous compounds from baby bottles by GC-MS and GC-O-MS. This approach allows us to evaluate the impact that the migrants from baby bottles could have on the food, as well the compliance regarding the specific migration limits (SML) established by ANVISA [1,29].

Forty-five compounds were identified in baby bottles made of PP, Tritan and silicone (Table S4). Most of them were detected come from the silicone baby bottles (Fig. 3). The compounds were quantified after the method validation using standard or similar compounds (Table S5). The method showed LOD ranging from 7 µg kg<sup>−1</sup> up to 100 µg kg<sup>−1</sup>, LOQ between 22 µg kg<sup>−1</sup> and 295 µg kg<sup>−1</sup>, RSD (%) below 11% and r<sup>2</sup> between 0.985 and 0.999. The obtained values are of relevance for the expected use.

2,4-di-*tert*-butylphenol was detected in all samples, however, it was detected at concentrations above the limit of quantification only in samples 4 and 9. For the last one, 2,4-di-*tert*-butylphenol migrated only in the first contact (55.50 ± 5.03 µg kg<sup>−1</sup>). On the other hand, in silicone baby bottles, the compound migrated in the 3 tests with a concentration varying from 50.62 µg kg<sup>−1</sup> up to 543.16 µg kg<sup>−1</sup>. This compound might be formed through the degradation of Irgafos 168, an antioxidant widely used in polyolefins. Moreover, it has already been

reported in baby bottles, but it is not included in the positive lists of ANVISA or the European Community [6,8,21,29].

Aldehydes saturated and unsaturated such as octanal, nonanal, decanal, (E)-2-decenal, 2-undecenal and tetradecanal, were also quantified in silicone baby bottles. Octanal and decanal migrated in the first and second contact reaching concentrations of 1840.47 µg kg<sup>−1</sup> and 1037.4 µg kg<sup>−1</sup>, respectively. Notwithstanding, nonanal, (E)-2-decenal, 2-undecenal and tetradecanal migrated in the three contacts, but nonanal was the most abundant odorous compound reaching up to 5874.80 µg kg<sup>−1</sup>. Nonanal already has been reported as the most abundant odour compound in LDPE [9]. Since there is no information about the impact of migration of these volatile compounds from the silicone, only the silicone bottle has been submitted to GC-O-MS analysis.

Thirty-eight odour compounds were identified in the silicone baby bottle (Table 3). Compounds with FM (%) higher than 60% can be considered as the basis of the aroma of the sample analysed. The identification was confirmed by LTPRI and by the characterization of the aroma of each compound, using the Flavornet and Pherobase databases.

As observed in the GC-MS analysis, aldehydes were the main compound with odour detected in the silicone baby bottles. Among them octanal, (E)-2-octenal, nonanal, (E)-2-nonenal, (E,E)-2,4-decadienal, (E)-2-decenal, undecanal, (E,E)-2,4-dodecadienal, 2-undecenal, dodecanal and tetradecanal presented FM (%) varying between 62.36(%) up to 91.29 (%). Sniffers characterised these compounds as citric, fat, unpleasant, insect and plastic. These descriptions are in consonance with those reported in the Flavornet and pherobase databases. Moreover, these compounds have been widely related as odour in plastic materials [9,12–14].

The saturated or unsaturated aldehydes are not used directly in the processing of silicone. However, they can be formed after the oxidation of polyunsaturated fatty acids used in previous steps of the silicone elastomer processing. The reaction occurs after activation of the hydrosilanes, which are then added to carbon-carbon multiple bonds using a transition metal complexes as a catalyst such as Pt(0). The bond C=C found in the polyunsaturated fatty acids is the main place of activation as it is more reactive than that of the internal ones (C-C) [30].

On the other hand, the silicone elastomer might be produced from liquid silicone (PDMS). For this, the liquid silicone is subjected to the curing process using a photoinitiator. The most used is benzophenone (MF% 74.61) and derivative such as 2-hydroxy-2-methyl-1-phenylpropanone (Darocure 173) (MF% 76.45). For this, benzophenone is subjected to UV radiation and hemolytic division generating many peroxy radicals responsible for the elastomer cure. Nonetheless, peroxy radicals are a propagator of lipid oxidation, increasing the aldehyde generation [31,32].

The odour compounds from benzophenone and derivatives have also been described in the GC-O-MS analysis. Interestingly, resin, pigment and bullet paper were described by sniffers in the benzophenone characterization. Sensory descriptions are made due to many factors, among them the personal acuity of the sniffer to describe familiar odours. Since the olfactometric analysis was performed with specialists in food packaging, it is normal to use packaging materials to describe the odour from another packaging material. In this case, benzophenone was used in UV curable print inks widely applied in candy wrappers. On the other hand, the floral, spicy, sweet odour from 2-hydroxy-iso-butyrophenone might be associated to phenyl ketons, such as benzophenone, which was described with a slightly sweet geranium-like odour [33]. Although this compound has shown an apparently pleasant odour, efforts are still made towards reducing the unpleasant odour of cured formulations, such as spicy. An unpleasant odour is caused by peroxide decomposition products such as cumyl peroxides generated from benzophenone and derivatives [31].

1-hepten-3-one, 2-nonanone and 3-nonen-2-one have presented FM (%) varying between 60.18 and 76.38%. Ketones have been reported as

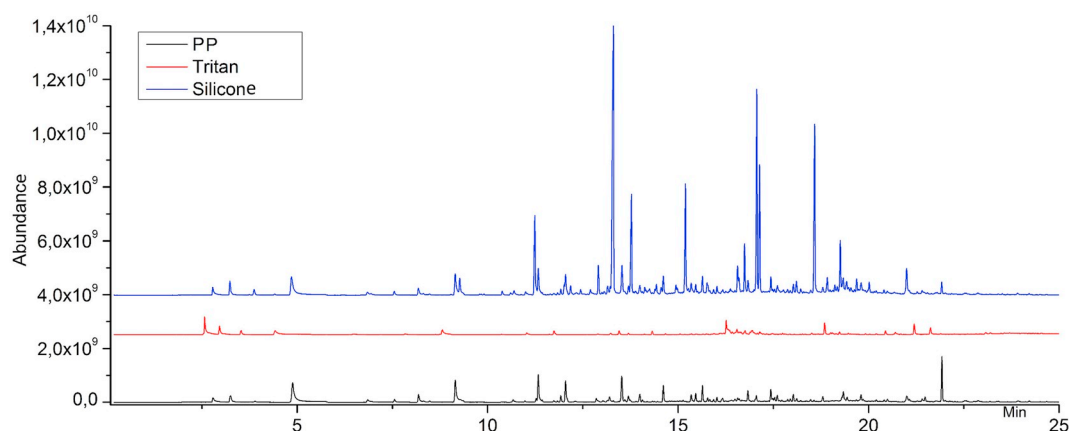


Fig. 3. Chromatographic profile of polypropylene, Tritan and silicone samples obtained by HS-SPME-CG-MS.

Table 3

Odorous compounds identified in silicone baby bottle by HS-SMPE-GC-O-MS.

Nº	TR	Compound Identified	Aroma	LTPRI Cal	LTPRI Tab	% M F
1	8.38	(E)-2-Heptenal	opium, incense, pungent	< 1100	959	40.82
2	8.87	1-Hepten-3-one	mushroom, unpleasant, fat	< 1100	1001	76.38
3	9.29	Octanal	green, chemical, lemon	< 1100	1038	79.79
4	9.93	Eucalyptol	Sweet, strawberry	< 1100	1062	23.57
5	10.47	(E)-2-Octenal	unpleasant, green, insect	< 1100	1070	74.61
6	10.83	1-Octanol	mushroom, oxidized, green	< 1100	1091	68.72
7	11.12	2-Nonanone	flower, fruity, tea	1107	1102	74.54
8	11.32	Nonanal	fruit, lemon, citric	1129	1128	52.70
9	11.7	n-Octyl formate	plastic, sweet, flower	1142	1141	33.33
10	11.99	3-Nonen-2-one	spoiled water, wet earth	1162	1162	60.18
11	12.04	D-(+)-Camphor	green, plastic	1172	1172	57.74
12	12.21	(E)-2-Nonenal	synthetic, herbal, dry, almonds	1178	1178	89.81
13	12.43	1-Nonanol	shoe polish, resin	1193	1190	52.70
14	12.45	p-Menthyl-1-ol	oxidized	1203	1186	40.82
15	12.73	2-Decanone	sweet, chemical	1211	1209	55.26
16	12.88	Estragole	burned	1210	1208	33.33
17	12.97	Decanal	fruit, lemon, bin	1251	1255	44.16
18	13.11	(E,E)-2,4-Dodecadienal	spoiled, insect, fat	1263	1263	91.29
19	13.58	Carvone	green lemon	1285	1278	83.27
20	13.76	(E)-2-Decenal	citric, chicken soap, condiment	1295	1295	91.29
21	14.08	2-Hydroxy-iso-butyrophenone	flower, spicy, sweet	1309	1309	76.45
22	14.26	2,4-Decadienol	wet earth, anise	1318	1318	57.74
23	14.42	Undecanal	plastic, soap	1341	1339	62.36
24	14.58	(E,E)-2,4-Decadienal	fat, insect, unpleasant, green	1341	1346	78.17
25	14.89	4-tert-Butylcyclohexyl acetate	green, cream, flower	1364	1361	72.65
26	15.05	1-Methoxy-4-methylbicyclo[2.2.2]octane	sweet, vanilla, honey	1382	1381	62.36
27	15.25	2-Undecenal	insect, unpleasant, flower, coriander	1428	1420	76.38
28	15.41	3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	apple, fresh, cucumber	1479	1478	78.17
29	15.99	Dodecanal	oxidized, flower, fat, wet earth	1516	1515	81.65
30	16.63	1-Dodecanol	french fries, wet earth	1540	1520	72.72
31	17.09	2,4-Di-tert-butylphenol	oxidized, lavender, unpleasant	1579	1549	62.36
32	17.44	2-ethoxy naphthalene	yellow fruit, cashew, banana	1616	1614	40.82
33	17.82	6-Tetradecanone	pigment, oxidized, lavender	1649	1655	40.82
34	18.51	Tetradecanal	wet earth, green, fresh, plastic	1678	1676	81.65
35	18.68	Benzophenone	resin, pigment, bullet paper	1710	1687	74.61
36	19.12	1-Tetradecanol	oxidized, spicy, lavender	1787	1774	37.27
37	19.38	4-(1,1,3,3-tetramethylbutyl)-phenol	parfum, flowers, yellow flower	1879	1871	40.82
38	20.10	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	clean product	1976	1970	33.33

catalyst inhibitors for silicone curing. These compounds are also formed as oxidation products in polyolefins, originating from the formation and decomposition of hydroperoxide [13,31]. The descriptions of ketones are in consonance with those reported by other researchers. The mushroom odour from 1-hepten-3-one was reported in wine cork stoppers [34], while 2-nonanone was reported as fruity/floral notes in wine [35]. Moreover, 3-nonen-2-one, one of the main odours in strawberry vinegar, was described as river water/vapour [36].

1-dodecanol, 3-hydroxy-2,4,4-trimethylpentyl 2-methyl propanoate and, 4-tert-butylcyclohexyl acetate, were detected with MF (%) higher than 70%, and similar descriptions (fatty, fresh and floral, respectively)

have already been reported [37–39].

Alcohols have also been used as inhibitors of the curing process. On the other hand, acrylates and derivatives, such as 3-hydroxy-2,4,4-trimethylpentyl 2-methyl propanoate and 4-tert-butylcyclohexyl acetate, are widely used as cross-linkers for silicone [31].

Carvone and 1-methoxy-4-methylbicyclo[2.2.2]octane were detected with MF (%) of 83.7 and 62.3. They are components of the oils extracted from *Mentha spicata* and *Cynara scolymus* L. Surprisingly, these compounds might be used for elastomersynthesis after ring-opening transesterification polymerisation followed by post polymerisation functionalisation, which might clarify its detection [40,41].

The result of the GC-O-MS analysis in silicone baby bottles is worrying matter of great concern. The German Federal Institute for Risk Assessment recommends avoiding the use of FCM with an intensive smell. In general, smells related to material emissions have hitherto been rarely investigated on a molecular basis and little is known about the exposure of consumers to such substances. Moreover, there are no data on odorous contaminants in baby bottles [14].

#### 4. Conclusion

In this work an extraction method by HS-SPME was developed to identify and quantify volatile migrants from baby bottles. To do this, multivariate statistical techniques were successfully used. The method showed to be reliable to identify and quantify a great number of odorous compounds from baby bottles. Forty-five compounds were identified, of which 84% were from silicone baby bottles. Aldehydes were the main compounds responsible for the unpleasant silicone baby bottle odour. Odours from ketones, benzophenone derivatives and alcohols have also been identified by GC-O-MS analysis, however they were detected in concentrations below the LOQ of the method developed. Moreover, VOC (%) was investigated in silicone baby bottles and values higher than those recommended were found. This emphasises the importance of olfactometry analysis, because even at a low concentration, odorous compounds from baby bottles could affect food properties and lead to rejection of the product.

#### Conflicts of interest

The authors declare no conflict of interest.

#### Acknowledgements

We would like to thank the support from the São Paulo Research Foundation-FAPESP (process n°. 2016/01099-9), National Council for Scientific and Technological Development-CNPq (process n°. 444909/2014-1) and the Fondo Social Europeo for the financial help given to the GUIA group T53-17R.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.talanta.2019.120301>.

#### References

- [1] N.H.S.A. BRAZIL, Resolution RDC N 56, from 16 November 2012. States on Positive List of Monomers, Other Starting Substances and Polymers Authorized for the Preparation Plastics Materials Intended to Produce Packages and Equipments for Contact with Foodstuffs vol. 1, Diário Oficial [da] República Federativa do Brasil, Seção, Brasília, DF, 2012.
- [2] EFSA, Scientific Opinion on Bisphenol A: evaluation of a study investigating its neurodevelopmental toxicity, review of recent scientific literature on its toxicity and advice on the Danish risk assessment of Bisphenol A, EFSA J. 8 (9) (2010) 1829.
- [3] European-Union, Regulation N. 10/2011 on Plastic Materials and Articles Intended to Come into Contact with Food, Official Journal of the European Union, 2011.
- [4] WHO, Toxicological and Health Aspects of Bisphenol A, (2010) Ottawa.
- [5] G. Russo, F. Barbato, E. Cardone, M. Fattore, S. Albrizio, L. Grumetto, Bisphenol A and Bisphenol S release in milk under household conditions from baby bottles marketed in Italy, J. Environ. Sci. Health Part B 53 (2) (2018) 116–120.
- [6] W.S. Oliveira, T.C.L. de Souza, M. Padula, H.T. Godoy, Development of an extraction method using mixture design for the evaluation of migration of non-target compounds and dibutyl phthalate from baby bottles, Food Anal. Method. 10 (7) (2017) 2619–2628.
- [7] M. Onghena, N. Negreira, E. Van Hoeck, L. Quirynen, J. Van Loco, A. Covaci, Quantitative determination of migrating compounds from plastic baby bottles by validated GC-QqQ-MS and LC-QqQ-MS methods, Food Anal. Method. 9 (9) (2016) 2600–2612.
- [8] C. Simoneau, L. van den Eede, S. Valzacchi, Identification and quantification of the migration of chemicals from plastic baby bottles used as substitutes for polycarbonate, Food Addit. Contam. A 29 (3) (2012) 469–480.
- [9] M. Wrona, P. Vera, D. Pezo, C. Nerin, Identification and quantification of odours from oxobiodegradable polyethylene oxidised under a free radical flow by headspace solid-phase microextraction followed by gas chromatography-olfactometry-mass spectrometry, Talanta 172 (2017) 37–44.
- [10] P. Vera, E. Canellas, C. Nerin, Migration of odorous compounds from adhesives used in market samples of food packaging materials by chromatography olfactometry and mass spectrometry (GC-O-MS), Food Chem. 145 (2014) 237–244.
- [11] C. Carrero-Carralero, J. Escobar-Armanz, M. Ros, S. Jiménez-Falcao, M.L. Sanz, L. Ramos, An untargeted evaluation of the volatile and semi-volatile compounds migrating into food simulants from polypropylene food containers by comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry, Talanta 195 (2019) 800–806.
- [12] P. Denk, C. Velasco-Schön, A. Buettner, Resolving the chemical structures of off-odorants and potentially harmful substances in toys—example of children's swords, Anal. Bioanal. Chem. 409 (22) (2017) 5249–5258.
- [13] H. Hopfer, N. Haar, W. Stockreiter, C. Sauer, E. Leitner, Combining different analytical approaches to identify odor formation mechanisms in polyethylene and polypropylene, Anal. Bioanal. Chem. 402 (2) (2012) 903–919.
- [14] C. Wiedmer, C. Velasco-Schön, A. Buettner, Characterization of off-odours and potentially harmful substances in a fancy dress accessory handbag for children, Sci. Rep. 7 (1) (2017) 1807.
- [15] BfR, Recommendation XV on Silicone Articles, Bundesinstitut für Risikobewertung, 2007, [https://bfr.ble.de/kse/faces/DBEmpfehlung\\_en.jsp](https://bfr.ble.de/kse/faces/DBEmpfehlung_en.jsp).
- [16] G.C. Derringer, R. Suich, Simultaneous optimization of several response variables, J. Qual. Technol. 12 (1980) 214–219.
- [17] S.G. Arcari, V. Calari, M. Sganzerla, H.T. Godoy, Volatile composition of Merlot red wine and its contribution to the aroma: optimization and validation of analytical method, Talanta 174 (2017) 752–766.
- [18] T.F.F. da Silva, A.D. Meinhardt, T.C.L. de Souza, J. Teixeira Filho, H.T. Godoy, Phenolic compounds from yerba mate based beverages – a multivariate optimisation, Food Chem. 190 (2016) 1159–1167.
- [19] R. Helling, P. Seifried, D. Fritzsche, T.J. Simat, Characterisation and migration properties of silicone materials during typical long-term commercial and household use applications: a combined case study, Food Addit. Contam. A 29 (9) (2012) 1489–1500.
- [20] ANVISA, Resolution RDC N 51 from 26 November 2010. States on Migration from Plastics Materials, Packages and Equipments Intended to Come into Contact with Foodstuffs vol. 1, Diário Oficial [da] República Federativa do Brasil, Seção, Brasília, DF, 2010, pp. 75–79.
- [21] European-Commission, Commission Regulation (EU) No 10/2011 of 14 January 2011 on Plastic Materials and Articles Intended to Come into Contact with Food, Official Journal of the European Union, Brussels, 2011, pp. 1–89.
- [22] L. Kudlejova, S. Risticovic, D. Vuckovic, 7 - Solid-phase Microextraction Method Development A2 - Pawliszyn, Janusz, Handbook of Solid Phase Microextraction, Elsevier, Oxford, 2012, pp. 201–249.
- [23] M.I. Rodrigues, A.F. Jemima, Experimental Design and Process Optimization, Taylor & Francis, 2014.
- [24] B. Mertens, C. Simon, M. Van Bossuyt, M. Onghena, T. Vandermarken, K. Van Langenhove, H. Demaegdt, E. Van Hoeck, J. Van Loco, K. Vandermeiren, A. Covaci, M.-L. Scippo, M. Elskens, L. Verschaeve, Investigation of the genotoxicity of substances migrating from polycarbonate replacement baby bottles to identify chemicals of high concern, Food Chem. Toxicol. 89 (2016) 126–137.
- [25] É.A. Souza Silva, G. Saboia, N.C. Jorge, C. Hoffmann, R.M. dos Santos Isaias, G.L.G. Soares, C.A. Zini, Development of a HS-SPME-GC/MS protocol assisted by chemometric tools to study herbivore-induced volatiles in Myrcia splendens, Talanta 175 (2017) 9–20.
- [26] J. Pawliszyn, 2 - Theory of Solid-phase Microextraction, Handbook of Solid Phase Microextraction, Elsevier, Oxford, 2012, pp. 13–59.
- [27] E. Gionfriddo, É.A. Souza-Silva, J. Pawliszyn, Headspace versus direct immersion solid phase microextraction in complex matrices: investigation of analyte behavior in multicomponent mixtures, Anal. Chem. 87 (16) (2015) 8448–8456.
- [28] É.A. Souza-Silva, J. Pawliszyn, Direct immersion solid-phase microextraction with matrix-compatible fiber coating for multiresidue pesticide analysis of grapes by gas chromatography–time-of-flight mass spectrometry (DI-SPME-GC-ToFMS), J. Agric. Food Chem. 63 (18) (2015) 4464–4477.
- [29] N.H.S.A. BRAZIL, Resolution RDC N 17, from 17 March 2008. States on Technical Regulation on Positive List of Additives for Plastics Materials Intended to Produce Packages and Equipments for Contact with Foodstuffs vol. 1, Diário Oficial [da] República Federativa do Brasil, Seção, Brasília, DF, 2008.
- [30] M. Galia, L.M. de Espinosa, J.C. Ronda, G. Lligadas, V. Cádiz, Vegetable oil-based thermosetting polymers, Eur. J. Lipid Sci. Technol. 112 (1) (2010) 87–96.
- [31] D. Wang, J. Klein, E. Mejía, Catalytic systems for the cross-linking of organosilicon polymers, Chem. Asian J. 12 (11) (2017) 1180–1197.
- [32] F. Mayer, K. Breuer, Material odor-odorant compounds identified in different materials—the surprising similarities with certain foods, possible sources and hypotheses on their formation, Indoor Air 16 (5) (2006) 373–382.
- [33] H. Surburg, J. Panten, Common Fragrance and Flavor Materials: Preparation, Properties and Uses, Wiley, 2016.
- [34] L. Culleré, J. Cacho, V. Ferreira, Comparative study of the aromatic profile of different kinds of wine cork stoppers, Food Chem. 112 (2) (2009) 381–387.
- [35] X.-j. Wang, Y.-s. Tao, Y. Wu, R.-y. An, Z.-y. Yue, Aroma compounds and characteristics of noble-rot wines of Chardonnay grapes artificially botrytized in the vineyard, Food Chem. 226 (2017) 41–50.
- [36] C. Ubeda, R.M. Callejón, A.M. Troncoso, J.M. Moreno-Rojas, F. Peña, M.L. Morales, A comparative study on aromatic profiles of strawberry vinegars obtained using different conditions in the production process, Food Chem. 192 (2016) 1051–1059.
- [37] M. Gallagher, P. Dalton, L. Sitvarin, G. Preti, Sensory and analytical evaluations of paints with and without texanol, Environ. Sci. Technol. 42 (1) (2008) 243–248.



- [38] The good scents company, (4-tert-butylcyclohexyl) Acetate, TGSC Information System, 2018, <http://www.thegoodscentscompany.com/data/rw1001372.html>.
- [39] G. Savary, A. Morel, C. Picard, M. Grisel, Effect of temperature on the release of volatile and odorous compounds in flax fibers, *J. Appl. Polym. Sci.* 133 (24) (2016) (n/a-n/a).
- [40] J. Yang, S. Lee, W.J. Choi, H. Seo, P. Kim, G.-J. Kim, Y.-W. Kim, J. Shin, Thermoset elastomers derived from carvomenthine, *Biomacromolecules* 16 (1) (2015) 246–256.
- [41] C. Saucier, A.d.S. Polidoro, A.L. dos Santos, J.K. Schneider, E.B. Caramão, R.A. Jacques, Comprehensive two-dimensional gas chromatography with mass spectrometry applied to the analysis of volatiles in artichoke (*Cynara scolymus* L.) leaves, *Ind. Crops Prod.* 62 (2014) 507–514.