UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA DE ALIMENTOS

WELLINGTON DA SILVA OLIVEIRA

ADDITIVES, AROMATIC AMINES, METALS AND NITROSAMINES IN BABY BOTTLES: ANALYTICAL METHODOLOGIES AND MIGRATION STUDIES

ADITIVOS, AMINAS AROMÁTICAS, METAIS E NITROSAMINAS EM MAMADEIRAS: METODOLOGIAS ANALÍTICAS E ESTUDO DE MIGRAÇÃO

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Thesis presented to the School of food Engineering of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Food Science.

Tese apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutor em Ciência de Alimentos.

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A ata da defesa com as respectivas assinaturas dos membros encontra-se no processo de vida acadêmica do aluno.

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RESUMO

O objetivo desse estudo foi avaliar a migração de compostos das mamadeiras, por meio da cromatografia líquida, gasosa e da espectroscopia, acopladas a espectrometria de massas de alta e baixa resolução. Foram avaliadas 196 amostras de mamadeiras fabricadas com polipropileno (n = 127), Tritan® (n = 49) e silicone (n = 20). O trabalho está dividido em quatro capítulos. O primeiro trata da caracterização das mamadeiras, da avaliação da migração específica de ftalatos por GC-MS utilizando fórmula infantil e simulante de leite (etanol 50%), bem como da avaliação da migração de metais por ICP-OES em simulante de alimentos ácidos (ácido acético 3%). A migração de metais não foi detectada. Por outro lado, em todas as amostras foi detectada a migração de dibutil ftalato (DBP). Na amostra de silicone a migração de DBP foi superior aos valores preconizados pela ANVISA. Di-isobutil ftalato, um plastificante não regulamentado para uso em mamadeiras, também foi detectado em altas concentrações nas amostras de silicone. A avaliação de risco para ftalatos demonstrou que há um potencial de efeito adverso associado ao uso das mamadeiras de silicone. O segundo capítulo trata da migração de aminas aromáticas das mamadeiras por LC-MS/MS, bem como da migração de metais a partir dos bicos, avaliada por ICP-MS e N-nitrosaminas, determinada por GC-MS. Não foi detectada a migração de aminas aromáticas e N-nitrosaminas. Por outro lado, zinco e bário, foram quantificados em 91% das amostras analisadas. Chumbo foi detectado em 16% dos bicos avaliados. Os metais migraram abaixo dos limites de migração específica preconizados pela ANVISA. No terceiro capítulo foi realizada a avaliação de compostos não voláteis, não intencionalmente adicionadas, utilizando UPLC-Q-TOF-MS. Em mamadeiras de polipropileno e Tritan® foram identificadas somente substâncias adicionadas intencionalmente (aditivos e auxiliares de processamento). Em todas as mamadeiras de polipropileno foi detectada a migração de N,N'-bis(2-hidroxietil)alquil(C8-C18)amina. Além disso, foram identificados derivados de glicerol, erucamida e NX 8000, um clarificante a base de nonitol. Todos os compostos migraram abaixo dos limites de migração específica preconizados pela ANVISA. Por outro lado, nas mamadeiras de silicone, foram identificados 17 compostos não intencionalmente adicionados. A maioria dos compostos eram derivados de acrilatos e propileno glicol. N-acetil valina também foi identificado nessas amostras. Os compostos foram quantificados e uma vez que não são contemplados nas listas positivas da ANVISA, a abordagem do limiar de preocupação toxicológica (*Threshold of Toxicological Concern - TTC*) foi

utilizada para avaliar o risco de exposição associada a migração destas substâncias das mamadeiras de silicone. Mais uma vez, foi constatado que há um potencial de efeito adverso associado ao uso das mamadeiras de silicone. Por fim, no último foram avaliadas substâncias voláteis utilizando micro extração em fase sólida (*solid phase micro-extration - SPME*) e cromatografia gasosa acoplada a espectrometria de massas e à olfatometria (SPME-GC-O-MS). Quarenta e cinco compostos foram identificados, dos quais 84% eram provenientes das mamadeiras de silicone. Aldeídos foram os principais compostos responsáveis pelo odor desagradável da mamadeira de silicone. Odores de cetonas, derivados de benzofenona e álcoois também foram identificados por GC-O-MS, no entanto foram detectados em concentrações abaixo do limite de quantificação do método desenvolvido.

Palavras-chave: migração, ftalatos, GC-MS, LC-MS/MS, mamadeiras, olfatometria

ABSTRACT

This study evaluated the migration of compounds from the baby bottles, through liquid and gas chromatography, as well spectroscopy, both coupled with high and low-resolution mass spectrometry. It were evaluated 196 baby bottles samples made with polypropylene (n = 127), Trian® (n = 49) and silicone (n = 20). The work is divided into four chapters. The first one deals with the characterization of the baby bottles, evaluation of the specific migration of phthalates by GC-MS using infant formula and milk simulant (ethanol 50%), as well as the evaluation of metal migration by ICP-OES in acid simulant (acetic acid 3%). It was not detected the metal migration. On the other hand, dibutyl phthalate(DBP) migration was detected in all samples. In the silicone sample the DBP migration was higher than the values recommended by ANVISA. Di-isobutyl phthalate, a non-regulated plasticizer for use in baby bottles, was also detected at high concentrations in the silicone samples. The risk assessment for phthalates has shown that there is a potential adverse effect associated with the use of silicone baby bottles. The second chapter deals with the migration of aromatic amines by LC-MS / MS, as well as the metals migration from teats evaluated by ICP-MS and N-nitrosamines, determined by GC-MS. It was not detected the migration of aromatic amines and N-nitrosamines. Nonetheless, zinc and barium were quantified in 91% of the samples. Lead was detected in 16% of the teats evaluated. The metals migrated below the specific migration limits recommended by ANVISA. In the third chapter, the evaluation of non-intentionally added substances (NIAS) was carried out by UPLC-Q-TOF-MS. Only intentionally added substances (additives and processing aids) were identified in polypropylene and Tritan® samples. Migration of N, N'-bis (2-hydroxyethyl) (C8-C18) alkyl amine was detected in all polypropylene baby bottles. Moreover, glycerol derivatives, erucamide and NX 8000, a clarifier based on nonitol, have been identified. All compounds migrated below the specific migration limits recommended by ANVISA. On the other hand, 17 NIAS were identified in silicone baby bottles. Most of them were derived from acrylates and propylene glycol. N-acetyl valine was also identified in these samples. Quantification was perfomed. Since these compounds are not included in the ANVISA positive lists, the Threshold of Toxicological Concern (TTC) approach was used to evaluate the risk of exposure associated with the migration from silicone bottles. The risks associate to use of silicon baby bottle was again confirmed. In the last chapter of this work volatile substances were evaluated using solid phase micro-extraction (SPME) coupled to mass

spectrometry and olfactometry (SPME-GC-O-MS). Forty-five compounds were identified, of which 84% came from silicon baby bottles. Aldehydes were the main compounds responsible for the unpleasant odor in silicone baby bottles. Odors of ketones, benzophenone derivatives and alcohols were also identified by GC-O-MS, however, they were below the limit of detection established in the method developed in the GC-MS.

Keywords: migration, phthalates, GC-MS, LC-MS/MS, baby bottles, teats

Índice de Figuras

Capítulo 1. Phthalates migration: a new problem related with the BPA-free baby bottles

Capítulo 2. Avaliação de conformidade de mamadeiras quanto a migração de aminas aromáticas, metais e N-nitrosaminas

Capítulo 3. Identification and quantification of non-intentionally added substances (NIAS) from baby bottles by UPLC-ESI-Q-TOF-MS^{E}

Capítulo 4. Characterization of odorous migrants from baby bottles by HS-SPME-GC-O-MS method

Conclusão geral

Índice de Tabelas

Capítulo 1. Phthalates migration: a new problem related with the BPA-free baby bottles

Capítulo 2. Avaliação de conformidade de mamadeiras quanto a migração de aminas aromáticas, metais e N-nitrosaminas

Capítulo 3. Identification and quantification of non-intentionally added substances (NIAS) from baby bottles by UPLC-ESI-Q-TOF-MS^E

Lista de abreviaturas e siglas

AA – Amina aromática

ADH – Alcohol dehydrogenase

AGD – Anogenital Distance

ALDH – Aldehyde dehydrogenase

ANOVA – Analysis of Variance

ANVISA – Angência Nacional de Vigilância Sanitária

AO – Antioxidante

As – Arsenic

ASTM – American Society for Testing and Materials

Ba – Barium

BBP – Benzyl butyl phthalate

BfR – Bundesinstitut für Risikobewertung

BMH – Brazilian Ministry of Health

BPA – Bisphenol A

bw – Body weight

Car – Carboxen

CCRD – Central Composite Rotatable Design

Cd – Cadmium

CHAP – Chronic Hazard Advisory Panel

CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico

CNS – Central Nervous System

CPSIA – Consumer Product Safety Improvement Act

Cr – Chromium

Cu – Copper

DBP – Dibutyl phthalate

DEHP – Di(2-ethylhexyl) phthalate

DIBP – Diisobutyl phthalate

DINP – Diisononyl phthalate

DOA – Dioctyl adipate

DOP – Dioctyl phthalate

DVB – Divinylbenzene

DI, EDI – Estimated Daily Intake

EFSA – European Food Safety Authority

EPA – Environmental Protection Agency

ESI – Electrospray ionization

ETMA – Ethoxytriethylene glycol methacrylate

F^- – Fluoride

FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo

FCM – Food Contact Materials

FDA – Food and Drug Administration

FIAS – Flow Injection Analysis System

FT-IR – Fourier Transform InfraRed

GC-MS – Gas chromatography-mass spectrometry

GC-O-MS – Gas chromatography-olfactometry-mass spectrometry

HDPE – High density polyethylene

HET – Human Exposure Threshold

Hg – Mercury

HPLC-DAD – High-performance liquid chromatography with a diode-array detector

HQ – Hazard Quotients

HI – Hazard Index

HS-SPME – Headspace-Solid Phase Microextraction

IARC – International Agency for Research on Cancer

IAS – Intentionally Added Substances

ICP-MS – Inductively Coupled Plasma Mass Spectrometry

ICP-OES – Inductively Coupled Plasma Optical Emission Spectrometry

IS – Internal Standard

LC-MS/MS – Liquid chromatography tandem mass spectrometry

LDPE – Low density polyethylene

LLDPE – Linear low-density polyethylene

LLE – Liquid-Liquid Extraction

LMT, OML – Limite de migração total

LOD – Limit of Detection

LOQ – Limit of Quantification

LTPRI – Linear Temperature Programmed Retention Index

M – Metais

MF – Modified Frequency

MRM – Multiple Reaction Monitoring

NDMA – N-Nitrosodimetilamina

NDPA – N-nitrosopropilamina

NHANES – National Health and Nutrition Examination Survey

NIAS – Non-intentionally added substances

NIST – National Institute of Standards and Technology

NO – Nitrosaminas

NPIR – N-nitroso-pirrolidina

Pb – Lead

PB – Plackett-Burman

PC – Policarbonato

PCT – Poly(1,4-cyclohexane dimethylene terephthalate

PDMS – Polydimethylsiloxane

PE – Polyethylene

PP – Polypropylene

Pt – Platinum

RDC – Resolução da Diretoria Colegiada

REACH – Registration, Evaluation, Authorisation and Restriction of Chemicals

REC – Recovery

RfD_{AA} – Reference dose for anti-androgenecity

RPM – Rotações Por minuto

RSD – Relative Standard Deviation

RT – Retention Time

RTV – Room-temperature Vulcanizing

S – Silicone

SALLE – Salting-out Liquid-Liquid Extraction

Sb – Antimony

SIM – Select Ion Monitoring

SML, LME – Limite de migração específica

Sn – Tin

T – Tritan

TDI – Tolerable Daily Intake

TPG – Tripropylene glycol

TTC – Threshold of Toxicological Concern

UPLC-QTOF-MS – Ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry

USA – United States of America

UV – Ultravioleta

UV-Vis – Espectrometria de Ultravioleta e Visível

VOC – Volatile Organic Compounds

OMS, WHO – World Health Organization

Zn – Zinc

Lista de símbolos

® - Marca resgitrada

°C – Graus Celsius

µg – Micrograma

cm – Centimetros

dm – Decímetros

g – grama

h – hora

min – Minutos

mL – Mililitros

mm – Milimetros

RF – Radio frequenia

TM – Trademark

v/v – Relação volume/volume

W – Watt

SUMÁRIO

INTRODUÇÃO GERAL

Com a proibição do uso de mamadeiras de policarbonato (PC) outros materiais plásticos têm sido utilizados para fabricação desses utensílios, entre eles o polipropileno, o silicone e um copoliéster comercializado com o nome de Tritan®.

Muitos aditivos são adicionados às resinas durante o processo de fabricação das embalagens plásticas, tais como antioxidantes, plastificantes, deslizantes, estabilizantes, lubrificantes, absorvedores de UV, antiestáticos, dentre outros, com a finalidade de melhorar as características do polímero durante sua produção, processamento e uso. Consequentemente, os plásticos são muito diversificados na sua estrutura química e apresentam propriedades variáveis em função do processamento, dos aditivos incorporados e da combinação com outros polímeros (Muncke, 2011; Coltro e Machado, 2011).

Devido ao tamanho reduzido das moléculas dos aditivos, pode ocorrer um processo indesejável de migração para o alimento acondicionado na embalagem plástica, podendo resultar em alterações de cor, sabor, odor, textura, ou a contaminação do produto por subtâncias tóxicas. Por isso, a fim de proteger os consumidores, a Agência Nacional de Vigilância Sanitária (ANVISA) regulamentou o limite de migração total (LMT) e o limite de migração específica (LME) de diversos aditivos para embalagens plásticas para contato com alimentos. O LMT é a quantidade máxima admissível de componentes do material em contato com alimentos transferida aos simulantes sob as condições de ensaio. Já o LME é a quantidade máxima admissível de um componente específico do material em contato com alimentos transferida aos simulantes, nas condições de ensaio (ANVISA, 2010).

Sob o ponto de vista da migração específica, os aditivos mais estudados têm sido os antioxidantes e os plastificantes. Os antioxidantes (AO) são ingredientes-chave na composição do polímero, devido à estabilidade limitada das poliolefinas a altas temperaturas e à radiação ultravioleta (UV). Os plastificantes reduzem a temperatura de processamento do polímero abaixo das condições de decomposição, modificam propriedades de processamento e tornam o produto final mais flexível (Wei et al., 2011; Cirillo et al., 2013).

Em mamadeiras de plástico, além dos AO e plastificantes, substâncias como as aminas aromáticas (AA), metais (M) e as nitrosaminas (NO) podem ser detectadas. As AA e os M são provenientes dos pigmentos utilizados para colorir o utensílio. As NO podem ser detectadas nos bicos de borracha e silicone que acompanham as mamadeiras.

Por fim, substâncias não intencionalmente adicionadas (NIAS), tais como oligômeros, produtos de reação e impurezas, também podem ser detectadas em materiais plásticos. Embora

no Brasil não tenha legislação que regulamente a concentração dessas substâncias em mamadeiras, elas devem ser avaliadas no sentido de garantir a segurança no uso do utensílio.

As pesquisas no campo da migração a partir de materiais plásticos em contato com alimentos são muito recentes. O baixo nível de concentração das substâncias que migram e a complexidade das matrizes (plásticos, alimentos ou simulantes) impõem o uso de método analíticos sensíveis e específicos, muitas vezes não disponíveis, requerendo assim o desenvolvimento de novas procedimentos.

Vários estudos relatam a migração desses compostos em materiais plásticos, entretanto, ainda há uma escassez de dados no tocante a migração dessas substâncias em mamadeiras de PP, Tritan® e silicone tornando-se necessário avaliar se esses materiais são de fato seguros (Aschberger et al. 2010).

Diante disso, a importância de estudos de migração se baseiam na necessidade de garantir a ausência de substâncias que possam causar problemas à saúde de bebês, uma vez que mais de 50% da população brasileira utiliza mamadeiras na alimentação de crianças entre 0 e 12 meses, sendo São Paulo a capital com maior índice de utilização (64,8%) (Ministério da Saúde, 2010).

OBJETIVOS

2.1. Objetivo geral

Avaliar a segurança dos materiais utilizados para fabricação de mamadeiras, por meio da análise qualitativa e quantitativa de migrantes, simulando a forma comum de utilização do utensílio pela população.

2.2. Objetivos específicos

- Identificar o material das seis marcas de mamadeiras mais comercializadas no Brasil por espectroscopia de infravermelho com transformada de Fourier (FT-IR).
- Caracterizar as seis marcas de mamadeiras quanto à altura, peso, capacidade volumétrica, espessura e diâmetro.
- Validar um método para avaliar, quantitativamente, por GC-MS, a migração de ftalatos a partir de mamadeiras, utilizando etanol 50% como simulante e fórmula infantil.
- Avaliar a migração de aminas aromáticas por cromatografia líquida de alta eficiência acoplada a espectrometria de massas sequencial (LC-MS/MS), utilizando ácido acético 3% como simulante.
- Avaliar por meio do ICP-OES e ICP-MS a migração de metais em mamadeiras e bicos utilizando ácido acético 3% como simulante.
- Validar uma metodologia, por GC-MS, para análise de nitrosaminas em simulante de saliva.
- Avaliar o potencial de migração de NIAS, utilizando cromatografia líquida acoplada à espectrometria de massas de alta (UPLC-Q-TOF-MS) e cromatografia gasosa acoplada a espectrometria de massas de baixa resolução (GC-MS).
- Avaliar o impacto sensorial da migração através da olfatometria

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CAPÍTULO I

Migração de ftalatos: um novo problema relacionado com mamadeiras sem bisfenol A (BPA)

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Phthalates migration: a new problem related with the BPA-free baby bottles

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ABSTRACT

The migration of plasticizers (DBP, DIBP, BBP, DOA, DEHP and DOP) in 50% ethanol and infant formula by GC-MS, and metals (As, Ba, Cr, Pt, Zn, Sn, Cu, Hg, Cd, Pb, Sb) in 3% acetic acid by ICP-OES, was investigated in baby bottles. Moreover, overall migration, dimensional characterization and, antioxidant profile by HPLC-DAD, were determined. Cumulative risk assessment was performed with the worst case of migration. Dimensional characterization has shown that there is still a lack of homogeneity in the samples of the same batch. Overall (OML) and specific migration limits (SML) for metals and antioxidant profile showed in compliance with ANVISA limits. On the other hand, the phthalates migration was detected in all samples, being above the limit established by ANVISA for DBP in the silicone baby bottles. The cumulative risk assessment was evaluated through hazard index and indicated that there is a potential for adverse effect associated with the use of the utensil.

Keywords: baby bottles, OML, phthalates, migration, risk assessment, hazard index

1. Introduction

In Brazil, plastic materials intended for food contact require overall and specific migration testing to demonstrate compliance with Brazilian Health Regulatory Agency (*Agência Nacional de Vigilância Sanitária - ANVISA*) (BRAZIL, 2010a). The overall migration limit (OML) specifies the total mass of substances permitted to migrate into food simulant (Bradley, Castle, Jickells, Mountfort, & Read, 2009). The OML to plastics is 50 $mg.kg^{-1}$ of food or food simulant or 8 $mg.dm^{-2}$ expressed on a contact area basis (BRAZIL, 1999). However, for the specific migration limit (SML), there is not a single limit for all substances. Since plastic is the most complex material that comes in contact with food, the SML has been established through toxicology studies, migration potential and risk assessment of consumer exposure. Thus, for a plastic manufactured using several different monomers and additives, testing for compliance requires an overall migration test plus individual tests of the simulant exposed to all substances which have an SML (Bradley, Castle, Jickells, Mountfort, & Read, 2009).

Baby bottles are widely used to feed newborns and infants. In 2011, Brazil banned the use of bisphenol A (BPA) in polycarbonate (PC) baby bottles (BRAZIL, 2012). Consequently, other materials, such as polypropylene (PP), Tritan (T) and silicone (S), have been used to manufacture baby bottles, diversifying the offer with the commercialization of pigmented utensils or with printing inks.

Di-esters of ortho-phthalic acids, generally recognized as phthalates, are synthetic organic chemical compounds extensively used in the manufacturing of polymers and many commonly used commercial products. Phthalates are used mainly as plasticizers worldwide but are not chemically bound to plastic polymers and over time, easily migrate out of these products and into the environment being considered a ubiquitous contaminant (Zaki & Shoeib, 2018).

Furthermore, phthalates were detected in a wide range of consumer products including personal care products, children's toys, food packaging, pharmaceuticals, nutritional supplements, cleaning materials, lubricants, insecticides, solvents, adhesives, paints, and lacquers (Qureshi, Yusoff, Wirzal, Sirajuddin, Barek, Afridi, et al., 2016).

Most of the phthalates, in the pure form, are colorless liquids that are poorly soluble in water but soluble in organic solvents. Dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), di-isobutyl phthalate (DIBP), benzyl butyl phthalate (BzBP), di-(2-ethylhexyl) phthalate (DEHP), and di-n-octyl phthalate (DOP) are the common phthalates most available in commerce (Guo & Kannan, 2012; Qureshi, et al., 2016).

Children are exposed to phthalates mainly via food, however, the air, water and other sources as baby bottle, teethers and toys might be source of contamination. Exposure to phthalates can cause serious adverse health effects on the kidney, liver, respiratory and endocrine systems (Braun, Sathyanarayana, & Hauser, 2013; Lioy, Hauser, Gennings, Koch, Mirkes, Schwetz, et al., 2015). Phthalates also exhibit serious hepatotoxic effects and the International Agency for Research on Cancer classified DEHP as class 2B probable human carcinogen (IARC, 2013).

Since infants do not have the same biotransformation and elimination mechanisms as adults, the time to eliminate phthalates is longer. Moreover, due the lower bodyweight compared with adults, their exposure to the food in contact with the plastic material is higher and it is therefore particularly important to verify the absence of possible causes for health concerns (Simoneau, van den Eede, & Valzacchi, 2012).

Inorganic compounds are used in the catalytic system or in the pigments applied in the polymeric materials. In Brazil, the SML for many metals in pigmented material has been stablished by ANVISA (BRAZIL, 2010b). Recently, the European Community has published amendments to the plastics Regulation 10/2011 and established SML for many metals used in plastic materials, including the nonpigmented (European-Commision, 2016). These amendments will only enter into force in September 2018. Although Brazil has not adopted any position on this yet, there is a trend to evaluate the migration of metal compounds from the nonpigmented plastic used as food contact material (FCM).

In view of the foregoing, the question is whether the baby bottles materials are safe to use since they are composed of a complex mixture of different monomers and a broad range of additives, such as plasticizers and pigments which might also migrate from the plastic into the foodstuffs.

Therefore, the aim of this study was to evaluate the Brazilian baby bottles regarding overall migration and specific migration of metals and plasticizers using food simulants and infant formula. For this purpose, a method was in-house validated to evaluate the specific migration of DBP, DIBP, BBP, DEHP, DOP and dioctyl adipate (DOA) by GC-MS, and As, Ba, Cr, Pt, Zn, Sn, Cu, Hg, Cd, Pb, Sb by ICP-OES, and F^- by spectrophotometric method (UV-Vis). Moreover, dimensional characterization, overall migration into food simulant, phthalates specific migration using infant formula, and antioxidant profile of baby bottles were performed.

2. Materials and methods

2.1. Sampling

Baby bottles of the same batch composed by polypropylene (PP, n = 70), Tritan (T, n = 28) and silicone (S, n = 17), of six Brazil's top-selling brands, were purchased from distributors in São Paulo, Brazil. It was used nine baby bottles of each model (Table S1) for determination of phthalates (n = 3) and metals (n = 3) migration, plus overall migration (n = 3), all using food simulants. Three silicone baby bottles were used for determination of phthalates migration using infant formula. Moreover, 5 baby bottles of each model were used in the characterization and determination of antioxidant profile, totalizing 115 samples.

2.2. Chemicals

Hexane, ethyl acetate, ethanol, dichloromethane, cyclohexane, toluene, acetic acid, were purchased in HPLC grade (Merck, DEU). Nitric and acetic acid, florisil and sodium chloride were obtained in analytical grade (Merck, DEU). Ultrapure water was obtained from a Direct-Q Millipore water purifier. EPA 506 phthalate mix (Supelco), di-isobutyl phthalate (Sigma), dibutyl phthalate-d4 (Sigma), bis(2-ethylhexyl) phthalate-d4 (Sigma), Irganox 1010 (Sigma), Irgafos 168 (Sigma), antimony (Sb), barium (Ba), boron (B), cadmium (Cd), lead (Pb), silver (Ag), zinc (Zn), copper (Cu), chromium (Cr), tin (Sn), mercury (Hg), arsenic (As) and fluoride (F^-) TraceCERT® (Fluka, Switzerland) were used for quantification.

2.3. Characterization

The baby bottles materials were confirmed using FT-IR (Perkin Elmer) in the attenuated total reflectance mode. For this, the samples were cut into small pieces in whom the spectra were measured directly with a resolution of 4 cm^{-1} in the range of 4000-650 cm^{-1}, generated from sixteen scans. The identification was made comparing the baby bottles spectrum and the spectrum of reference polymer.

For the characterization, the height, volumetric capacity and mass of the cup, thickness and diameter of the samples (n = 5) were determined previously conditioned at 23 °C

± 2 °C. The height was determined using a Mitutoyo marker. The diameter was measured in three points (base, center and top) using a pachymeter Mitutoyo. Both with resolution of 0.01 mm according with the norm ASTM D 2911 – 94 (2001).

The mass was measured in semi analytical balance. The average and minimum thickness was determined in five points (upper, middle, bottom, hell and base) (Figure 1) with a Magna-Mike sensor (Panametrics) with 0.001mm of resolution according with the methodology established by norm ASTM 4166-99 (2004). Volumetric capacity was determined according to the norm ASTM 2911-94 (2009).

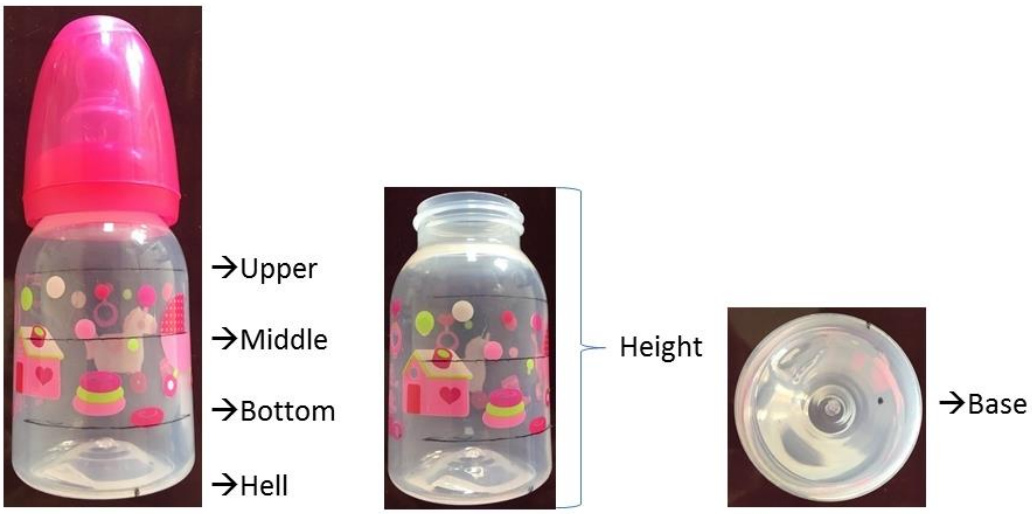

Figure 1. Points of thickness determination

2.4. Antioxidant profile

Antioxidant profile was determined according to statard procedure of ASTM D6953-03 (ASTM, 2009). For this, one baby bottle of each model was crushed in a mill and 5 g of the material was kept under reflux and agitation for 2 h with 50 mL of cyclohexane:dichloromethane (25:75, v/v). Twenty-five milliliters of extract were concentrated to 5 mL and 10 µL were injected into HPLC-DAD (Agilent 1100) operating at 200 nm. Separation was performed with RP-18 column (250 mm× 4 mm× 5 µm) using acetonitrile:water (95:5, v/v) as mobile phase in isocratic elution (1.5 mL min^{-1}).

2.5. Overall and specific migration

The overall and specific migration was carried out according with the Regulation 2011/10/EC and Resolution RDC 51/2010 (BRAZIL, 2010a; European-Commision, 2011). Briefly, migration tests were performed at 70 °C/2 h with milk simulant and acid simulant (i.e. 50% ethanol and 3% acetic acid). For the overall migration, the milk simulant (50% ethanol) was evaporated to dryness and the mass of the non-volatile residue was determined and expressed as mean of three replicates.

Phthalates specific migration has been done at 70 °C/2 h from two approaches using isotopically-labelled phthalate internal standards. This internal standard enables more accurate quantification of phthalates in food samples. The isotopically labeled and native phthalate esters can be discriminated from each other by the use of a MS detector, which eliminates potential interferences from the analysis (Guo & Kannan, 2012).

In view of this, first the specific migration was done with 50% ethanol and the concentration of phthalates was determined according to the methodology described by Oliveira, de Souza, Padula, and Godoy (2017) with minor changes. Briefly, after the migration test, ten milliliters of the simulant was spiked with 150 µL of dibutyl phthalate-d4 (DBP-d4) and di(2-ethylhexyl) phthalate-d4 (DEHP-d4) as internal standard at 1 $mg.L^{-1}$ (final concentration 150 $\mu g.L^{-1}$). Next, liquid-liquid extraction with 2 mL of ethyl acetate:dichloromethane:hexane (27.5:22.5:50) was performed. Finally, 1 µL of extract was injected in gas chromatography-mass spectrometry.

Second, specific migration into milk (infant formula) was performed using salting-out liquid-liquid extraction (SALLE) and dilution standard addition calibration as quantification method (Martins, Rizzetti, Kemmerich, Saibt, Prestes, Adaime, et al., 2016). For this, the infant formula was reconstituted as recommended by manufacturer (approximately 0.15 $g.mL^{-1}$) and kept in contact with silicone baby bottles. Next, after the migration test, 7 mL of the infant formula was spiked with DBP-d4 (final concentration 20 $\mu g.kg^{-1}$), homogenized and, subject the liquid-liquid extraction by 30 s in vortex using acetonitrile (2 g). After, salting-out was performed with 2.5 g of sodium chloride and 150 µL of acetic acid and vortexed by 1 min. The acetic acid was added due to its effect in the whey protein precipitation. The sample was centrifuged for 4 min at 4000 rpm. Two hundred microliters of supernatant were collected, diluted with 600 µL of acetonitrile (final volume 800 µL) and injected in the GC-MS.

Acetonitrile was added to reduce the matrix effect and improve the detection and shape of phthalates peaks.

A gas chromatography Agilent 7890A coupled to Triple-Axis Detector (Agilent 5975c inert) and electron impact ionization (GC-MS) was used. The samples were injected at 320 °C in splitless mode for both extracts. The plasticizers were separated on a ZB-5HT column (20 m × 0.18 mm × 0.18 µm film thickness). The chromatographic condition for samples evaluated with 50% ethanol were: temperature of GC oven at 70 °C during 0.5 min, increasing to 180 °C at 60 °C.min^{-1}, following by new increase to 320 °C at 60 °C.min^{-1} held to 3 min and, finally, increase to 350 °C at 60 °C.min^{-1} held during 2 min, totaling 10.1 min of running. The temperature programming for test with infant formula was set at 60 °C by 2 min, increasing 10 °C.min^{-1} until 300°C, totalizing 30 minutes of analysis. The migration test with infant formula was performed using only the silicone baby bottle, once this material has shown the worst case of migration. Differences in the chromatographic separation was established to avoid the coelution of the analytes with interferents from infant formula.

For metal migration, after the baby bottles-contact with 3% acetic acid, the concentration of Sb, Ba, B, Cd, Pb, Ag, Zn, Cu, Cr, Sn, Hg and As was determined by ICP-OES OPTIMA 2000DV model, from Perkin Elmer Corporation (Shelton, CT, USA). For this, a Mira Mist parallel path pneumatic nebulizer was used to quantify Sb, Ba, Cd, Pb, Ag, Zn, Cu, Cr and Sn. For boron (B) it was used the quartz cyclonic spray chamber. The cold recombination area was removed with a shear gas interface. Hg and As were quantified through hydride generation using flow injection automatic system (FIAS). The parameters employed for ICP operation are shown in Table 1. Fluoride (F^-) was determined by UV-vis spectrophotometry at 580 nm, using a double beam according to the methodology described by Rice, Baird, Eaton, and Clesceri (2012).

Table 1. ICP-OES parameters used for metals quantification

RF power (W): 1500 Observation height (mm): 15 Torch configuration: axial Auxiliary argon flow rate (L min^{-1}): 0.2		
ICP-OES with Mira Mist:		ICP-OES with FIAS:
Argon flow rate (L min^{-1}): 15 Nebulizing flow rate (L min^{-1}): 0.55 Sample flow (mL min^{-1}): 1.5		Argon flow rate (L min^{-1}): 17 Nebulizing flow rate (L min^{-1}): 0.65 Sample introduction pump (RPM): 60 Sample discharge pump (RPM): 80
Wavelength (nm):		
Sb = 217.58 Cd = 214.44 Pb = 220.35 Ag = 328.07 Sn = 189.93	Ba = 233.53 B = 208.89 Cu = 324.75 Cr = 205.56	Hg = 194.17 As = 193.70

In order to reduce the background contamination with phthalates, any contact with plastic was avoided. Moreover, all the glassware was thoroughly washed, rinsed twice with ethanol and n-hexane, heated at 400 °C for 3 hours and finally stored in glass desiccators before the use. Volumetric glasses were washed, rinsed with n-hexane and iso-octane before the use. Florisil activated (400 °C/2 h) were added to solvents used to wash the syringe auto sampler and were frequently replaced to reduce the contamination during the analysis. Furthermore, the analysis was carried out using Merlin micro seal septa to avoid the contamination from PTFE septa.

A blank injection was done after six injections to reduce the carry-over between the samples. Regarding metal determination, all glassware used was previously washed with Extran detergent (Merck, Darmstadt, Germany), decontaminated by immersion in a 20% (v/v) nitric acid solution for 12 h, and then rinsed with ultrapure water (Kiyataka, Dantas, & Pallone, 2015).

2.6. Method validation

The method for phthalates determination was in-house validated for quantification of DIBP, DBP, BBP, DOA, DEHP and DOP. Validation parameters such as the limit of detection (LOD), limit of quantification (LOQ), linearity, recovery and precision were evaluated.

All solvents were analyzed to verify the presence of contamination before set the method LOD and LOQ. The LOD was determined based on the average of ten injections (true

replicates) of blank samples, plus 3-fold the standard deviation. The LOQ was obtained as 3-fold the LOD. This procedure was done for both simulants, 50% ethanol and infant formula.

Linearity was evaluated by calibration curve in 50% ethanol or infant formula, with seven points randomly injected in triplicate. These ones were done in selective monitoring mode (SIM). For 50% ethanol (Table 2), DBP-d4 was used as internal standard to quantify DIBP, DBP, BBP, DOA and, DEHP-d4 as internal standard in the quantification of DEHP and DOP. Regarding infant formula, only DBP-d4 was used as internal standard. Calibration curves were constructed spiking the 50% ethanol with phthalates mix in the LOQ, 125, 250, 375, 500, 625 and 750 $\mu g.kg^{-1}$. For infant formula it was spiked in the LOQ, 15, 30, 45, 60, 75 and 100 $\mu g.kg^{-1}$. Then, the samples were subjected to the extraction process developed for each simulant. The lack of fit and linearity of the analytical curves were evaluated by analysis of variance (ANOVA).

Table 2. Ions monitored in plasticizers quantification

Compound	Target (m/z)	Qualifier (m/z)	IS	Target (m/z)	Qualifier (m/z)
DIBP and DBP	149	223			
BBP	149	206	DBP-d4	153	227
DOA	129	112			
DEHP	149	167	DEHP-d4	153	171
DOP	149	279			

IS – Internal standard

Recovery (n = 3) and precision intra-day (n = 10) and inter-day (3 days, n = 27) were assessed in three levels of the curve: LOQ, middle and upper of the curve. The validation parameters for the method for metals and antioxidants determination have been described elsewhere (Kiyataka, Dantas, & Pallone, 2015; Oliveira, de Souza, Padula, & Godoy, 2017).

2.7. Cumulative risk assessment – hazard quotient and hazard index

The estimation of the daily intake (DI) for phthalate diesters was carried out for each individual compound using the silicone baby bottle, the phthalates diesters concentration determined with 50% ethanol, and the average weight of babies corresponding to the 95th percentile in order to represent the worst-case of exposure (high consumers). For this preliminary exposure assessment, three age groups were considered: 0.5, 3 months (fed exclusively with infant formula) and, 6 months (fed with infant formula and other types of

food). The Dietary Guidelines for Infants and Toddlers established by the Brazilian Ministry of Health-BMH (2005) was used to determine the amount of food intake by babies, such as 590 $g.day^{-1}$, 776 $g.day^{-1}$ and 560 $g.day^{-1}$ at 0.5, 3 and 6 months, respectively.

We considered a scenario that three new baby bottles were used to feed the babies in a single day. Moreover, the amount of phthalates intake during the day was determined considering the mean of phthalates concentration determined in the 1st contact with the simulant and the total mass of simulant in 3 silicone baby bottles (450 g), plus the concentration determined in the complementary mass necessary for supply the amount of food recommended by BMH, such as 140 g, 326 g and 110 g. The mean of phthalates concentration determined in the 2nd contact was used to determine the phthalate amount in the complementary mass.

Based on DI estimate, hazard quotients (HQ) were calculated for each phthalate as follows:

$$\mathrm{HQ} = \frac{\mathrm{DI[\mu g/kg\ bw/\ day]}}{\mathrm{Reference\ limit\ value\ [\mu g/kg\ bw/day]}}$$

The reference limit values used were the tolerable daily intake (TDI) determined to DnBP, BBzP and DEHP by the EFSA and the reference dose for anti-androgenecity (RfD AA) proposed for DnBP, DiBP, DEHP and BBzP by Kortenkamp and Faust (2010). For DiBP, the TDI for DnBP, i.e., 10 μg/kg bw-day was used because DiBP has shown similar level of effects to that of DnBP (Søeborg, Frederiksen, & Andersson, 2012). HQ > 1 is deemed to be of potential risk (Kim, Lee, Park, Kim, Cho, Kim, et al., 2017).

Cumulative risk assessment was carried out by calculating hazard index (HI) for each age as the sum of the different HQ based on both acceptable tolerance values, the EFSA's TDI and the RfD AA (EFSA, 2005a, 2005b, 2005c; Kortenkamp & Faust, 2010), as follows:

$$\mathrm{HI_{EFSA\ TDI}} = \mathrm{HQ_{DiBP\ TDI}} + \mathrm{HQ_{DBP\ TDI}} + \mathrm{HQ_{BBzP\ TDI}} + \mathrm{HQ_{DEHP\ TDI}}$$

$$\mathrm{HI_{RfD\ AA}} = \mathrm{HQ_{DiBP\ RfD\ AA}} + \mathrm{HQ_{DBP\ RfD\ AA}} + \mathrm{HQ_{BBzP\ RfD\ AA}} + \mathrm{HQ_{DEHP\ RfD\ AA}}$$

3. Results and discussion

3.1. Characterization

This study has evaluated either overall migration as well as the specific migration of plasticizers and metals from baby bottles. Moreover, the polymer identification,

characterization, antioxidant profile, and phthalates specific migration, from 8 models of the baby bottle were carried out.

All baby bottles have presented the polymer indicated in the label (Figure S1). On the other hand, regarding characterization (Table S4), many variations have been detected in the batches of baby bottles. Only the samples 3 and 8 have shown measurements with RSD below 10%. The thickness determination permits to evaluate the homogeneity of the lot, which might be due to lack of fit in the transformation process. Moreover, the thickness distribution can be related to mechanical performance once the area with lower thickness might result in cracking during the handling or eventual dropped.

Regarding the volume, all samples have shown volumetric capacity higher than the nominal volume (declared on the label). Contradictory, the sample 8 (silicon) has shown the largest difference between the nominal volume and the total volume which was 61% higher than the volume declared (Table S4).

3.2. Antioxidant profile

In the presence of oxygen, polymer degradation occurs even at room temperatures and the oxidation rate is considerably enhanced with increasing temperatures. For this reason, antioxidants have been used in polymer resins to delay the overall oxidative degradation process. This process is caused by highly reactive free radicals generated by heat, radiation, and mechanical shear (often exacerbated by the presence of metallic impurities). In baby bottles, the potential for oxidation increases due to the exposure to high temperatures during the sterilization process or during the contact with hot foods (Grabmayer, Beißmann, Wallner, Nitsche, Schnetzinger, Buchberger, et al., 2015; Hahladakis, Velis, Weber, Iacovidou, & Purnell, 2018).

In all PP baby bottles (samples 1, 2, 3, 4 and 6), as well in the silicone baby bottle (sample 8) were found Irgafos 168 and Irganox 1010 (Table S2), in concentrations varying between 0.06% up to 0.14%. There are no restrictions of use for both compounds (BRAZIL, 2008; European-Commision, 2011). However, there are technical recommendations for use of antioxidants in plastics. Normally, Irgafos 168 is added from 0.05 % up to 0.2% and Irganox 1010 between 0.05 to 0.4% (BASF, 2015, 2016). These concentrations have been found in the samples. Tritan® baby bottles (samples 5 and 7) could not be analyzed due to the formation of

a fluid with a high viscosity during the extraction process. Probably, this is due to the similarity of extraction solvent (cyclohexane) and cyclohexanedimethanol, a Tritan's monomer.

Primary and secondary antioxidants have been used in polymers due to the synergistic effect of this combination. Primary antioxidants break the degradation chain by donating H-atoms to free radicals, preventing those radicals from propagating the reaction. The most ones used are hindered phenols, such as Irganox 1010. Hindered phenolic antioxidants have good thermal stability and have good nondiscoloring properties (Li, Wang, Ning, & Zhang, 2012).

On the other hand, organophosphites such as Irgafos 168, have been used to reduce hydroperoxides formed by polymers auto-oxidation and extending the performance of primary antioxidants. For this reason, blends with hindered phenols are effective to provide storage stability and long-term protection against thermo-oxidative degradation (BASF, 2016; Hahladakis, Velis, Weber, Iacovidou, & Purnell, 2018).

3.3. Overall and metals migration

All samples evaluated have shown overall migration in compliance with the recommended by ANVISA (Table S3) (BRAZIL, 1999). The same scenario was observed regarding metals migration and fluoride. OML and SML were established to ensure the inertness of plastics and prevent unacceptable adulteration of the food. For this, it's important to evaluate the compliance of food contact materials to limit the human exposure and, consequently, to ensure consumer protection (Bradley, Castle, Jickells, Mountfort, & Read, 2009).

3.4. Phthalates migration

Phthalates migration was performed using 50% ethanol and infant formula. The methods have been validated in ethanol 50% (Table 3) and infant formula. The method with 50% ethanol has shown LOQ between 1.7 $\mu g.kg^{-1}$ and 12 $\mu g.kg^{-1}$, recovery from 66 up to 109% and precision below 20% for all phthalates studied. The linearity of the analytical curves has been analyzed by ANOVA ($p < 0.05$) and was not detected lack of fit. On the other hand, only DBP and DIBP was evaluated in infant formula. For this, the validation in infant formula has shown LOQ of 15 $\mu g.kg^{-1}$, linearity between 15 $\mu g.kg^{-1}$ to 100 $\mu g.kg^{-1}$, recovery of 105% and

117% for DIBP and DBP, and precision (RSD %) below 8% for both compounds. Finally, the methods were used to measure the migration of phthalates from baby bottles in 50% ethanol and infant simulant.

DOA and DOP were not detected in the baby bottles. On the other hand, DIBP was detected in all sample analyzed (Table 4). In the first contact, DIBP concentration ranged from 29.55 $\mu g.kg^{-1}$ up to 779.05 $\mu g.kg^{-1}$. In the second and third test, DIBP migration was detected in all samples with concentrations lower than 50 $\mu g.kg^{-1}$ except for silicone baby bottle, which in the third test was still detected migration higher than 250 $\mu g.kg^{-1}$.

In migration test with infant formula, the concentration of DIBP detected was 425.57 $\mu g.kg^{-1}$, 293.02 and 243.05 $\mu g.kg^{-1}$ in the 1^{st}, 2^{nd} and 3^{rd} contact, respectively. Silicon baby bottle (sample 8) has shown the largest migration of DIBP with 50% ethanol and infant formula. Nevertheless, according to ANVISA and European Commission there is no regulation for this substance for use in FCM (BRAZIL, 2008; European-Commision, 2011).

DBP and BBP were detected in the samples 2 and 8, but highest concentration was found in sample 8 (silicon). In this last one DBP migrated above the SML (300 $\mu g.kg^{-1}$) established by ANVISA and European Commission (Table 4). BBP was detected varying between 20.38 $\mu g.kg^{-1}$ and 37.74 $\mu g.kg^{-1}$. We also detected DEHP in sample 8, however the level detected was lower than the SML (1500 $\mu g.kg^{-1}$). According to ANVISA, the SML for BBP is 30 $mg.kg^{-1}$, which is much higher than the concentrations detected. Regarding the test with infant formula, the migration of DBP have varied between 101.58 $\mu g.kg^{-1}$ to 135 $\mu g.kg^{-1}$ which is below the SML (BRAZIL, 2008; European-Commision, 2011).

Table 3. Figures of merit for the method validation in milk simulant (ethanol 50%)

	Regression equation	R^2	LOD ($\mu g.kg^{-1}$)	LOQ ($\mu g.kg^{-1}$)	Concentration ($\mu g.kg^{-1}$)	REC (%)	Intra-day precision[a]	Inter-day precision[a]
DIBP	y=0.008x-0.092	0.996	3.53	10.58	10.58	100	3.53	12.8
					375	96	1.46	7.0
					750	101	1.50	2.01
DBP	y=0.010x-0.140	0.996	1	3	3	83	5.03	2.4
					375	91	1.96	3.2
					750	100	1.20	1.70
BBP	y=0.005x-0.080	0.997	1.7	5.1	5.1	66	3.54	11.23
					375	81	4.20	20
					750	95	7.54	15.40
DOA	y=0.005x-0.094	0.997	0.56	1.7	1.7	100	4.85	6.64
					375	89	5.87	5.62
					750	87	4.69	2.23
DEHP	y=0.009x-0.172	0.997	4	12	12	109	1.35	7.32
					375	92	1.78	5.35
					750	93	0.81	2.01
DOP	y=0.012x-0.179	0.997	0.56	1.7	1.7	86	7.38	3.05
					375	91	1.80	6.94
					750	99	1.15	2.24

DIBP: diisobutyl phthalate; DBP: dibutyl phthalates; BBP: butyl benzyl phthalate; DOA: di-n-octil adipate; DEHP: diethyl hexyl phthalate; DOP: di-n-octil phthalate

LOQ: Limit of quantification, LOD: Limit of detection, REC: Recovery

[a] Precision are expressed in terms of relative standard deviation (%)

Table 4. Quantification of phthalates migration from baby bottles (µg.kg^{-1}) using 50% ethanol as simulant

	DIBP							
Samples	1	2	3	4	5	6	7	8
1st contact.	45.69 ± 5.57	32.25 ± 1.17	54.59 ± 0.77	53.97 ± 1.02	29.55 ± 1.25	66.76 ± 10.61	29.59 ± 0.56	779.05 ± 27.04
2nd contact.	39.49 ± 4.10	28.93 ± 3.35	36.54 ± 0.30	35.48 ± 0.14	45.67 ± 1.69	40.26 ± 0.33	45.67 ± 0.83	451.03 ± 50.83
3rd contact.	37.57 ± 4.97	>LOQ	28.83 ± 0.11	30.50 ± 2.32	46.67 ± 5.82	31.20 ± 1.53	46.62 ± 1.33	250.98 ± 7.30
	DBP							
Samples	1	2	3	4	5	6	7	8
1st contact.	< LOQ	20.85 ± 1.36	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	485.99 ± 41.41
2nd contact.	< LOQ	19.03 ± 0.57	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	232.12 ± 18.49
3rd contact.	< LOQ	>LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	116.54 ± 7.41
	BBP							
Samples	1	2	3	4	5	6	7	8
1st contact.	< LOQ	28.82 ± 2.15	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	37.74 ± 1.00
2nd contact.	< LOQ	22.23 ± 0.30	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	34.03 ± 1.12
3rd contact.	< LOQ	20.38 ± 0.46	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	27.44 ± 0.53
	DEHP							
Samples	1	2	3	4	5	6	7	8
1st contact.	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	125.34 ± 4.45
2nd contact.	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	78.51 ± 3.85
3rd contact.	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	78.98 ± 14.01

Phthalates are not naturally occurring materials but are intentionally created and used in specific applications. One application of phthalates in children's toys and child care articles is as a plasticizer, or softener for plastic component parts (CPSIA, 2017). In PP baby bottles the phthalates might arise from Zeigler-Natta catalyst, which is commonly used for polyolefin production. With such catalyst, PP with high isotacticity and controllable molecular weight can be produced at a very high catalytic efficiency. However, small amounts of catalyst, which can contain the prohibited phthalates, such as DBP and DIBP, may remain in a plastic resin after manufacture (CPSIA, 2017; Shamiri, Chakrabarti, Jahan, Hussain, Kaminsky, Aravind, et al., 2014).

On the other hand, in silicon baby bottles, phthalates might be used as plasticizer. The FDA authorize until 30% (w/w) of phthalates in the silicon used for food contact material. In Europe, there is no Harmonized Guide about this. However, the Germany Federal Institute for Risk Assessment (*Bundesinstitut für Risikobewertung - BfR*) recommend that silicon might contain maximum amount of 10% in food contact materials (BfR, 2007; BRAZIL, 2001; FDA, 2017).

The widespread use of phthalates resulting in potential human exposure has captured enormous attention to their monitoring by the U.S. National Health and Nutrition Examination Survey (NHANES), which have monitored human exposure through monoester phthalate determination in urine (Messerlian, Mustieles, Wylie, Ford, Keller, Ye, et al., 2017; Qureshi, et al., 2016; Silva, Wong, Samandar, Preau, Calafat, & Ye, 2017).

Recently, the U. S. Consumer Product Safety Improvement Act (CPSIA) published a final rule which determines that children's toys and childcare articles cannot contain DEHP, DBP, BBP, DOP, diisononyl phthalate (DINP) and diisodecyl phthalate (DIDP) in concentration above 0.1%. Moreover, based on Chronic Hazard Advisory Panel (CHAP) was proposed adding four new phthalates, DIBP, DPENP, DHEXP, and DCHP, to this list (CPSIA, 2017). In Europe, the prohibition was published by Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) only for DEHP, DBP, BBP and DIBP (European-Commision, 2006).

The prohibition takes into account many studies involving the toxicological aspects of phthalates. The developmental toxicity and the rat phthalate syndrome is pointed out as the point of greatest concern. Evidence suggests that the exposure to certain phthalates during the gestation causes androgen deficiency. Consequently, it might lead to altered seminiferous cords, multinucleated gonocytes, epididymal agenesis, retained

nipples, shortened anogenital distance (AGD), cryptorchidism, and hypospadias. These effects are restricted to phthalates with 3 to 8 carbon atoms in the backbone of the alkyl side chain, such as DBP, DIBP, BBP and DEHP (Lioy, et al., 2015).

3.5. Cumulative risk assessment

The highest DI estimated for children corresponded to DIBP intake, followed by DBP, DEHP and BBzP (Table 5). Only DIBP and DBP have shown DI higher than TDI established by EFSA. Infants aged under 15 days have shown highest exposure to phthalate due the low body weight. Cumulative risk assessment was evaluated calculating hazard index (HI) through of the sum of the different HQ, since the risks associated with phthalates should be evaluated taking into account combined exposures (NRC, 2008).

Only infants older than 6 months are safe to use silicon baby bottles considering the RfD AA. These results are alarming once infants and young children are generally considered to be more sensitive to endocrine disruption effects. The synergic effects of phthalates mixture on the reproductive system already were reported in rats which shown penile malformations as consequence of exposure (Kortenkamp & Faust, 2010).

Table 5. Daily intake (DI), hazard quotients (HQ) and hazard indexes (HI) based on TDI and RfD AA for infants

	DI ($\mu g.kg\ bw^{-1}\ day^{-1}$)			$HQ_{EFSA\ TDI}$			$HQ_{RfD\ AA}$			TDI ($\mu g.kg\ bw^{-1}\ day^{-1}$)	RfD AA ($\mu g.kg\ bw^{-1}\ day^{-1}$)
	0.5	3	6	0.5	3	6	0.5	3	6		
DIBP	100.9	67.24	42.48	10.09	6.72	4.25	0.50	0.34	0.22	10[a]	200[d]
DBP	61.27	39.78	26.54	6.13	3.98	2.65	0.61	0.40	0.27	10[b]	100[d]
BBzP	5.3	3.79	2.17	0.01	0.01	0.00	0.02	0.01	0.01	500[c]	330[d]
DEHP	16.44	11.08	7.07	0.33	0.22	0.14	0.55	0.37	0.24	50[a]	30[e]
		HI		16.56	10.93	7.04	1.68	1.11	0.73		

[a] Based on germ cell development (EFSA, 2005a, 2005c).
[b] Similar to DnBP (Søeborg, Frederiksen, & Andersson, 2012).
[c] Based on spermatozoa levels (EFSA, 2005b).
[d] Based on the inhibition of the fetal testosterone synthesis(Kortenkamp & Faust, 2010).
[e] Based on nipples retention (Kortenkamp & Faust, 2010)

A HI below 1 indicates a little probability to observe adverse effects from the exposure to several chemicals. On the other hand, values between 1 and 100, show that there is a potential for adverse effect. For this reason, efforts should be done toward identify possible exposure sources and mitigate the exposure among infants and toddlers (Dewalque, Charlier, & Pirard, 2014; Kim, et al., 2017).

4. Conclusion

This work has evaluated the compliance of BPA free baby bottles regarding metals and phthalates migration. Moreover, a dimensional characterization was performed. Dimensional characterization has shown lack of homogeneity in the samples. OML, SML for metals and antioxidant profile were evaluated and have shown in compliance with ANVISA limits. However, phthalate migration was detected in all samples. In silicon baby bottles the migration of high concentrations of DIBP and DBP was observed, however, only DBP is approved for use in food contact materials. This one has shown migration above the SML established by ANVISA. Cumulative risk assessment in the worst case indicated HI > 1 which might imply possible endocrine disrupting consequences. The estimated risks were mostly due to the exposure to DIBP and DBP. These findings warranted the necessity to improve the baby bottle materials to specifically reduce the intake and exposure to phthalates.

5. Acknowledgments

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<Supplementary Data 1>

Phthalates migration: a new problem related with the BPA-free baby bottles

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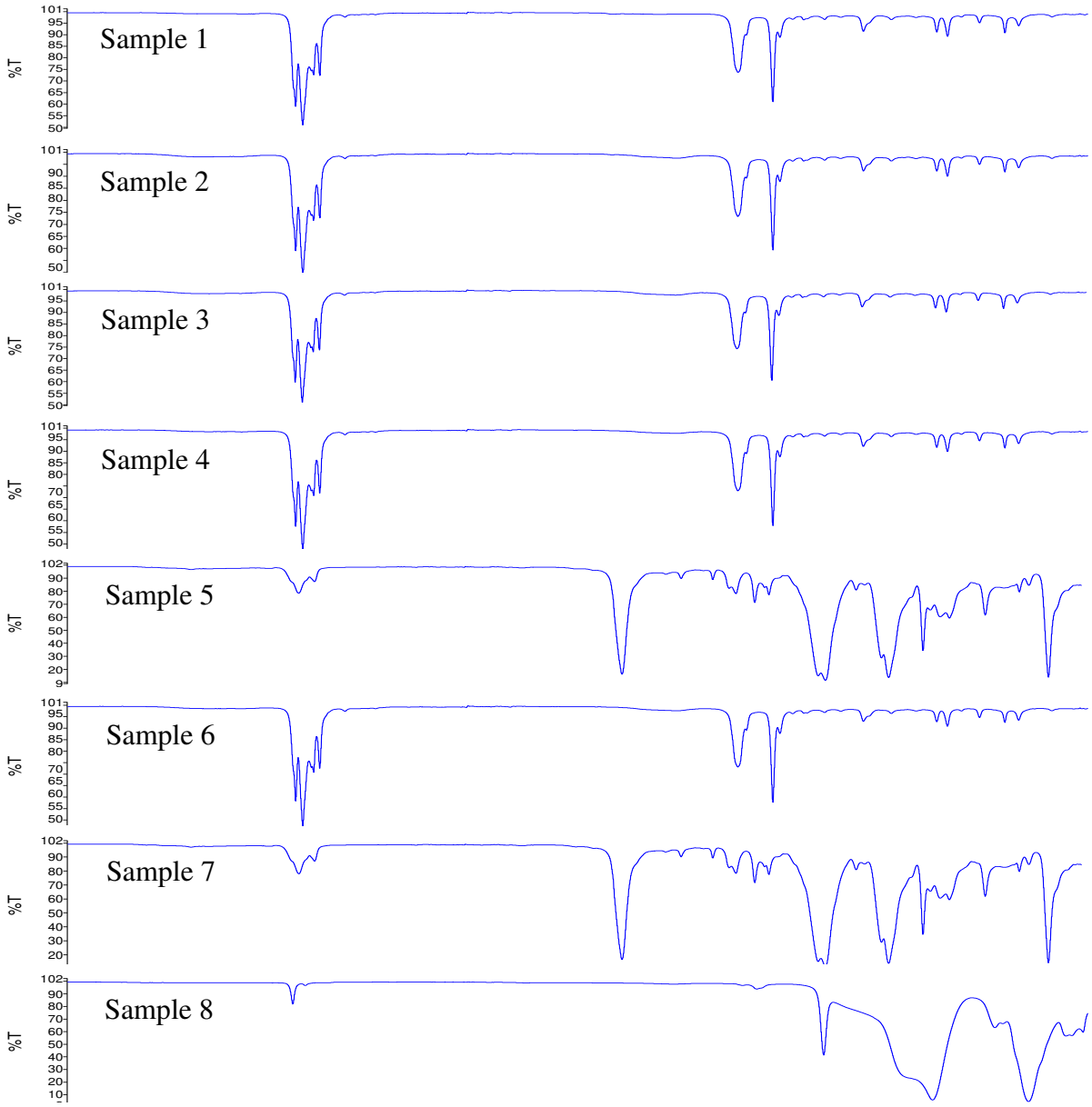

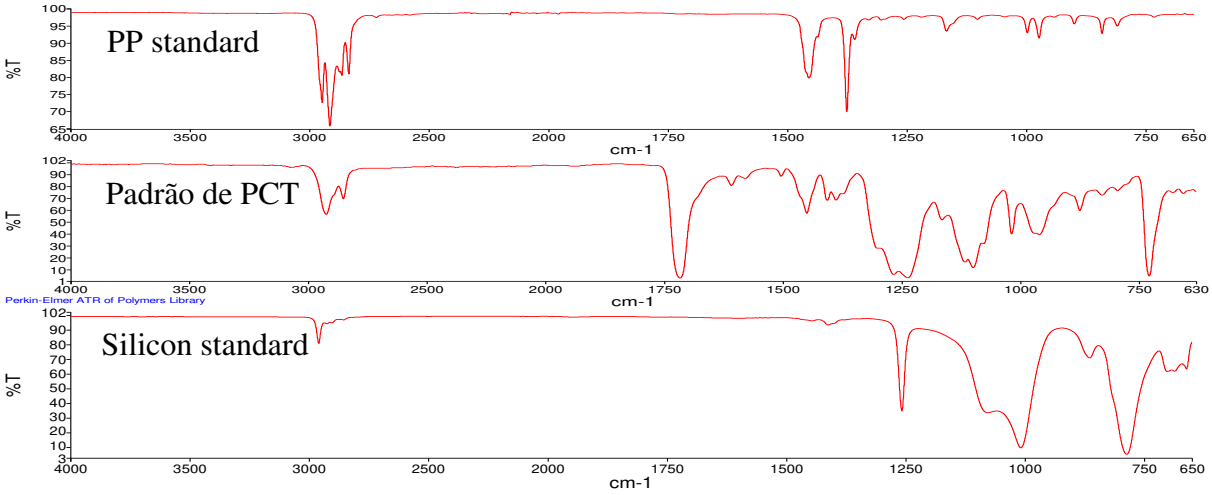

Figure S1. FT-IR spectrum of samples and PP, poly(1,4-cyclohexane dimethylene terephthalate (PCT) and silicon standards

Table S1. Baby bottle samples used in the experiment

Samples	Materials	Description	Volume (mL)
1	PP	Translucent, printed with green designs	120
2	PP	Translucent, printed with pink designs	120
3	PP	Translucent, printed with blue and green designs	70
4	PP	Translucent, pigmented with pink	70
5	T	Transparent, printed with green and purple designs	80
6	PP	Translucent, printed with blue designs	70
7	T	Transparent, printed with blue designs	80
8	S	Translucent without designs	150

PP: Polypropylene

T: Tritan

S: Silicone

Table S2. Antioxidant percentual determined in baby bottles.

Samples	Antioxidant	Mean (%)*
1	Irganox 1010	0.08 ± 0.01
	Irgafos 168	0.13 ± 0.01
2	Irganox 1010	0.08 ± 0.01
	Irgafos 168	0.13 ± 0.02
3	Irganox 1010	0.08 ± 0.01
	Irgafos 168	0.11 ± 0.01
4	Irganox 1010	0.08 ± 0.02
	Irgafos 168	0.14 ± 0.04
6	Irganox 1010	0.07 ± 0.01
	Irgafos 168	0.12 ± 0.03
8	Irganox 1010	0.06 ± 0.01
	Irgafos 168	0.12 ± 0.02

* n = 3

Table S3. Overall migration determined in baby bottles

Sample 1		
Contact	Mean (mg.kg^{-1})	SD (%)
1st.	≤ 6.67$^{(1)}$	^{2}
2nd.	≤ 7.11	0.77
3rd.	≤ 6.67$^{(1)}$	^{2}
Sample 2		
Contact	Mean (mg.kg^{-1})	SD
1st.	≤ 7.67	1
2nd.	≤ 6.67$^{(1)}$	^{2}
3rd.	≤ 6.72	0.1
Sample 3		
Contact	Mean (mg.kg^{-1})	SD
1st.	≤ 11.11$^{(1)}$	^{2}
2nd.	≤ 11.11$^{(1)}$	^{2}
3rd.	≤ 11.11$^{(1)}$	^{2}
Sample 4		
Contact	Mean (mg.kg^{-1})	SD
1st.	≤ 10.00$^{(1)}$	^{2}
2nd.	≤ 10.00$^{(1)}$	^{2}
3rd.	≤ 10.00$^{(1)}$	^{2}
Sample 5		
Contact	Mean (mg.kg^{-1})	SD
1st.	≤ 10.00$^{(1)}$	^{2}
2nd.	≤ 10.00$^{(1)}$	^{2}
3rd.	≤ 10.17	0.29
Sample 6		
Contact	Mean (mg.kg^{-1})	SD
1st.	≤ 11.11$^{(1)}$	^{2}
2nd.	≤ 11.11$^{(1)}$	^{2}
3rd.	≤ 11.11$^{(1)}$	^{2}
Sample 7		
Contact	Mean (mg.kg^{-1})	SD
1st.	≤ 10.00$^{(1)}$	^{2}
2nd.	≤ 10.00$^{(1)}$	^{2}
3rd.	≤ 10.00$^{(1)}$	^{2}
Sample 8		
Contact	Mean (mg.kg^{-1})	SD
1st.	≤ 5.00$^{(1)}$	^{2}
2nd.	≤ 5.00$^{(1)}$	^{2}
3rd.	≤ 5.00$^{(1)}$	^{2}

SD: standard deviation; Limit: 50.0 mg.kg^{-1}

(1) LOQ of the method during the analysis

(2) Not applicable

Table S4. Baby bottles dimensional characterization

	Bottom diameter (mm)							Middle diameter (mm)								Upper diameter (mm)							
Samples	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
	55.00	55.00	47.90	48.05	45.22	47.70	45.23	63.45	54.86	54.92	43.29	43.41	45.24	43.25	45.26	51.55	55.02	55.03	45.17	45.33	45.29	45.24	45.40	72.03
	55.06	55.03	47.87	49.55	45.19	47.81	45.23	62.52	54.89	55.16	43.28	44.44	45.20	43.00	45.33	51.18	54.94	55.15	45.25	46.49	45.31	44.96	45.30	71.92
	55.08	54.80	47.98	49.48	45.29	47.72	45.23	63.34	54.81	54.74	43.39	43.30	45.27	43.18	45.36	51.60	55.09	54.82	45.16	46.37	45.35	45.22	45.39	71.90
	55.02	55.01	47.88	49.53	45.24	47.67	45.21	62.65	55.00	54.72	43.28	44.46	45.28	43.33	45.24	51.31	55.02	54.84	45.22	46.47	45.34	45.11	45.33	71.66
	54.88	55.00	47.82	48.69	45.29	47.72	45.25	62.82	54.86	54.83	43.24	43.30	45.25	43.31	45.26	51.56	54.87	55.02	45.22	45.32	45.33	45.19	45.42	71.63
Mean	55.01	54.97	47.89	49.06	45.25	47.72	45.23	62.96	54.88	54.87	43.30	43.78	45.25	43.21	45.29	51.44	54.99	54.97	45.20	46.00	45.32	45.14	45.37	71.83
Deviation	0.08	0.09	0.06	0.67	0.04	0.05	0.01	0.42	0.07	0.18	0.06	0.61	0.03	0.13	0.05	0.18	0.08	0.14	0.04	0.61	0.02	0.11	0.05	0.17
RSD %	0.14	0.17	0.12	1.37	0.10	0.11	0.03	0.66	0.13	0.33	0.13	1.40	0.07	0.31	0.11	0.36	0.15	0.25	0.08	1.34	0.05	0.25	0.11	0.24
	Bottom Average Thickness (mm)							**Middle Average Thickness (mm)**								**Upper Average Thickness (mm)**							
Samples	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
	1.00	1.25	0.69	0.69	0.64	0.93	0.73	1.76	1.24	0.77	1.29	0.92	1.04	1.00	0.95	1.73	0.85	0.84	0.58	0.59	0.92	0.73	0.84	1.82
	1.19	1.24	0.65	0.76	0.57	0.56	0.55	1.75	1.39	1.19	1.07	1.05	1.05	1.16	1.04	1.77	0.82	0.85	0.68	0.63	0.96	0.87	0.89	1.81
	1.26	1.34	0.79	0.85	0.75	0.91	0.53	1.76	1.18	1.03	1.25	0.99	0.95	0.99	1.05	1.79	0.88	0.75	0.56	0.51	0.97	0.69	0.92	1.78
	1.20	1.03	0.71	0.77	0.55	0.93	0.66	1.75	1.20	0.93	1.13	0.98	1.03	0.99	0.95	1.74	0.90	0.84	0.68	0.55	0.99	0.63	0.87	1.81
	1.11	1.23	0.64	0.63	0.72	0.89	0.57	1.80	1.25	1.24	1.14	0.94	1.00	1.01	1.05	1.75	0.77	0.79	0.69	0.61	0.97	0.68	0.90	1.83
Mean	1.15	1.22	0.69	0.74	0.64	0.84	0.61	1.76	1.25	1.03	1.18	0.98	1.01	1.03	1.01	1.75	0.84	0.81	0.64	0.58	0.96	0.72	0.88	1.81
Deviation	0.10	0.11	0.06	0.08	0.09	0.16	0.08	0.02	0.08	0.19	0.09	0.05	0.04	0.07	0.06	0.03	0.05	0.04	0.06	0.05	0.03	0.09	0.03	0.02
RSD %	8.84	9.19	8.47	11.37	13.58	18.81	13.79	1.14	6.41	18.54	7.69	4.89	4.01	7.10	5.53	1.53	6.13	5.46	9.40	8.67	2.68	12.61	3.60	0.93
	Bottom Minimum Thickness (mm)							**Middle Minimun Thickness (mm)**								**Upper Minimun Thickness (mm)**							
Samples	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
	0.76	0.83	0.59	0.54	0.56	0.58	0.58	1.60	0.83	0.73	0.92	0.77	0.96	0.89	0.88	1.63	0.71	0.78	0.56	0.55	0.90	0.65	0.77	1.63
	0.58	0.94	0.57	0.46	0.48	0.45	0.49	1.57	0.77	0.96	0.87	0.71	0.96	0.97	1.02	1.61	0.61	0.75	0.64	0.50	0.91	0.83	0.85	1.70
	0.94	1.13	0.66	0.59	0.59	0.61	0.45	1.57	0.99	0.68	1.01	0.83	0.83	0.78	0.94	1.68	0.77	0.54	0.53	0.41	0.95	0.65	0.89	1.63
	0.99	0.65	0.64	0.62	0.39	0.64	0.55	1.60	0.98	0.78	0.96	0.79	0.95	0.85	0.90	1.63	0.69	0.73	0.65	0.49	0.95	0.60	0.80	1.65
	0.73	0.87	0.54	0.49	0.58	0.60	0.53	1.56	0.85	0.83	0.94	0.80	0.93	0.79	1.03	1.60	0.66	0.66	0.59	0.56	0.95	0.61	0.90	1.73
Mean	0.80	0.88	0.60	0.54	0.52	0.57	0.52	1.58	0.88	0.79	0.94	0.78	0.93	0.86	0.95	1.63	0.69	0.69	0.59	0.50	0.93	0.67	0.84	1.67
Deviation	0.17	0.18	0.05	0.07	0.08	0.08	0.05	0.02	0.10	0.11	0.05	0.05	0.05	0.08	0.07	0.03	0.06	0.10	0.05	0.06	0.03	0.10	0.05	0.04
RSD %	21.04	19.99	8.57	12.11	16.35	13.37	9.72	1.17	11.17	13.38	5.56	5.94	5.72	8.89	7.47	1.69	8.27	13.93	8.55	11.37	2.81	14.26	6.34	2.64

	Base Average Thickness (mm)								Hell Average Thickness (mm)							
Samples	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
	1.94	2.40	1.67	1.71	1.26	1.63	1.25	1.78	0.87	1.17	0.96	0.82	0.52	1.28	0.64	1.87
	1.81	2.34	1.55	1.67	1.18	1.52	1.24	1.79	1.06	1.18	0.77	0.68	0.58	0.80	0.61	1.86
	1.83	2.15	1.64	1.74	1.59	1.55	1.31	1.77	1.13	1.27	0.98	0.74	0.48	1.55	0.57	1.91
	1.79	2.45	1.60	1.76	1.18	1.51	1.32	1.77	1.25	1.03	0.86	0.67	0.52	1.21	0.64	1.95
	1.76	2.31	1.73	1.69	1.27	1.60	1.35	1.76	1.04	1.38	0.96	0.75	0.49	1.29	0.58	1.86
Mean	1.82	2.33	1.64	1.72	1.30	1.56	1.29	1.78	1.07	1.20	0.90	0.73	0.52	1.23	0.61	1.89
Deviation	0.07	0.11	0.07	0.04	0.17	0.05	0.05	0.01	0.14	0.13	0.09	0.06	0.04	0.27	0.03	0.04
RSD %	3.87	4.85	4.11	2.11	13.22	3.38	3.53	0.65	13.10	10.77	9.62	8.10	7.33	22.33	5.38	2.11

	Base Minimun Thickness (mm)								Hell Minimun Thickness (mm)							
Samples	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
	1.45	1.65	1.03	1.23	1.16	1.26	1.20	1.73	0.70	0.85	0.65	0.54	0.43	0.84	0.58	1.78
	1.50	1.77	0.93	1.39	1.14	0.99	0.83	1.75	0.66	0.99	0.64	0.53	0.50	0.51	0.51	1.77
	1.40	1.73	0.97	1.22	1.47	0.87	1.14	1.71	0.77	0.99	0.73	0.60	0.41	1.13	0.51	1.82
	1.41	1.61	1.09	1.20	1.14	0.70	1.09	1.73	0.93	0.82	0.78	0.54	0.45	0.81	0.57	1.88
	1.39	1.69	1.02	1.25	1.25	0.81	1.20	1.69	0.86	0.99	0.69	0.56	0.42	0.87	0.50	1.78
Mean	1.43	1.69	1.01	1.26	1.23	0.93	1.09	1.72	0.78	0.93	0.70	0.55	0.44	0.83	0.53	1.81
Deviation	0.05	0.06	0.06	0.08	0.14	0.21	0.15	0.02	0.11	0.08	0.06	0.03	0.04	0.22	0.04	0.04
RSD %	3.17	3.82	5.85	6.21	11.24	23.17	13.98	1.41	14.33	8.94	8.31	5.11	8.59	26.82	7.31	2.48

	Cup Volume (ml)								Cup mass (g)								Height(mm)							
Samples	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
	163.57	162.70	98.50	99.49	103.81	96.31	104.23	242.01	21.96	22.22	10.94	10.89	15.15	11.08	14.89	49.74	92.97	92.51	79.18	78.61	80.45	78.87	80.48	98.38
	163.08	163.32	98.52	103.91	103.96	95.85	103.72	241.96	22.21	22.09	10.86	10.86	15.14	11.13	14.91	49.71	92.89	92.54	79.06	78.63	80.48	78.75	80.46	98.17
	163.69	162.91	98.36	103.89	103.80	96.10	104.14	243.58	21.95	22.08	10.93	10.82	15.14	11.15	14.95	49.84	92.83	92.74	79.16	78.58	80.48	78.58	80.41	98.33
	164.07	163.70	98.55	104.01	103.94	96.09	104.45	242.04	21.94	22.10	10.94	10.84	15.11	11.05	14.85	49.65	92.83	92.58	79.31	78.59	80.52	78.61	80.47	98.15
	163.44	163.39	98.01	100.14	104.33	96.09	104.09	242.48	22.18	22.10	10.92	10.90	15.11	11.16	14.91	49.73	92.75	92.63	78.83	78.93	80.50	78.66	80.45	98.31
Mean	163.57	163.20	98.39	102.29	103.97	96.09	104.13	242.41	22.05	22.12	10.92	10.86	15.13	11.11	14.90	49.73	92.85	92.60	79.11	78.67	80.49	78.69	80.45	98.27
Deviation	0.36	0.40	0.22	2.27	0.22	0.16	0.27	0.68	0.13	0.06	0.03	0.03	0.02	0.05	0.04	0.07	0.08	0.09	0.18	0.15	0.03	0.12	0.03	0.10
RSD %	0.22	0.24	0.23	2.22	0.21	0.17	0.26	0.28	0.61	0.26	0.31	0.31	0.12	0.42	0.24	0.14	0.09	0.10	0.23	0.19	0.03	0.15	0.03	0.10

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CAPÍTULO II

Avaliação de conformidade de mamadeiras quanto a migração de aminas aromáticas, metais e N-nitrosaminas

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Avaliação de conformidade de mamadeiras quanto a migração de aminas aromáticas, metais e N-nitrosaminas.

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RESUMO

A migração de aminas aromáticas, metais e N-nitrosaminas foi avaliada em mamadeiras. Para tanto, ensaios de migração foram executados utilizando simulante de alimento ácido (70 °C/2 h para mamadeiras e 40 °C/24 h para bicos), bem como simulante de saliva (40 °C/24 h para bicos). Cromatografia líquida e gasosa acoplada à espectrometria de massas (LC-MS/MS e GC-MS) foram utilizadas para detecção de 18 aminas aromáticas primárias e 6 N-nitrosaminas. Zinco, cádmio, chumbo e bário foram avaliados por meio do ICP-MS. Os métodos foram validados e limites de detecção inferior a 5 $\mu g.kg^{-1}$ foram obtidos para todos os analitos. Não foi detectada a migração de aminas aromáticas e N-nitrosaminas. Por outro lado, zinco e bário, foram detectados em 11 dos 12 modelos avaliados, com concentrações variando de 1.25 a 10.42 $\mu g.kg^{-1}$. Chumbo foi detectado em 2 amostras em concentração abaixo de 4 $\mu g.kg^{-1}$. As amostras estão em concordância com o recomendado pela ANVISA em relação à migração de aminas aromáticas, metais e N-nitrosaminas. Entretanto, é extremamente importante o monitoramento dessas substâncias com o intuito de resguardar a saúde dos bebês.

Palavras-chave: migração, simulante de saliva, bicos, LC-MS/MC, ICP-MS

1. Introdução

Mamadeiras são largamente utilizadas na alimentação de crianças recém-nascidas. Devido à grande utilização do utensílio, os recém-nascidos entram em contato com diferentes tipos de materiais de plástico e substâncias químicas componentes destes materiais, tais como monômeros ou aditivos, os quais podem migrar para os alimentos. Como os bebês têm um peso menor em relação aos adultos, a exposição ao alimento em contato com o plástico é maior e preocupações no sentido de garantir a saúde da criança são da mais alta importância (Simoneau, Valzacchi, Morkunas, & van den Eede, 2011).

Estudos recentes ainda apontam a migração de bisfenol A (BPA) e bisfenol S em mamadeiras italianas (Russo, Barbato, Cardone, Fattore, Albrizio, & Grumetto, 2018). Em 2011 a migração de BPA de mamadeiras foi responsável pela proibição do uso de policarbonato na fabricação do utensílio (BRASIL, 2012; European-Commission, 2011). A decisão baseou-se no princípio da precaução, uma vez que faltam dados robustos que ratifiquem a segurança do BPA para bebês e há muitas incertezas nos relatórios apresentados pela Organização Mundial e da Saúde (OMS), bem como pela Autoridade Europeia de Segurança Alimentar (*European Food Safety Authority – EFSA*)(EFSA, 2010; WHO, 2010).

Como consequência desta proibição de policarbonato, materiais substitutos passaram a ser utilizados na fabricação de mamadeiras. No Brasil, polipropileno (PP), Tritan® e recentemente o silicone são utilizados na fabricação do utensílio.

Qualquer inovação na composição de materiais de contato com alimentos (*Food contact materials-FCM*) deve ser acompanhada por estudos de migração. Os estudos de migração visam garantir a segurança do consumidor em relação ao uso desses utensílios. Por isso, a fim de proteger os consumidores, a Agência Nacional de Vigilância Sanitária (ANVISA) regulamentou a quantidade máxima e o limite de migração específica (LME) de diversos aditivos para embalagens plásticas para contato com alimentos (Coltro & Machado, 2011).

Entretanto, ainda são escassos estudos de migração nas mamadeiras a base de PP, Tritan e silicone. Além disso, tanto os frascos dos novos utensílios como os bicos passaram a ser fabricados de diferentes cores. Materiais coloridos devem ser avaliados quanto a migração de metais e aminas aromáticas primárias.

Aminas aromáticas primárias (AA) são produtos químicos que contém uma amina primária (NH_2) ligada a um anel aromático que podem ser oriundos de

catalisadores, aditivos como os estabilizantes térmicos, pigmentos e corantes, e materiais como a borracha, resinas epóxi e adesivos de poliuretano (Aznar, Canellas, & Nerín, 2009; Mortensen, Trier, Foverskov, & Petersen, 2005). As fontes mais estudadas de formação de AA em materiais de contato com alimentos são os corantes e os isocianatos aromáticos.

Atualmente, a determinação das AA no Brasil é feita seguindo uma metodologia espectrofotométrica. Após o ensaio de migração, na qual o utensílio entra em contato com o simulante ácido, as AA são derivatizadas e o total de AA é determinando no simulante por meio da absorbância. O resultado é reportado como anilina equivalente. Nessa metodologia há possibilidade de outras substâncias reagirem com o pigmento usado na derivatização contribuindo para o incremento da absorbância e gerando um resultado falso positivo. Além disso, a quantificação individual de AA com maior toxicidade, como a benzidina, o 2-naftilamina e o 4-aminobifenil, que são substâncias carcinogênicas em humanos segundo o IARC (grupo 1), requer a utilização de metodologias mais seletivas.

Em relação a avaliação de metais, recentemente uma retificação do Regulamento 10/2011 da União Europeia recomenda a determinação de metais em todos os materiais plásticos destinados ao contato com alimentos (European-Commision, 2016). Por esse motivo, há uma tendência na avaliação de metais em materiais plásticos pigmentados e não pigmentados. Até o momento, não foram encontradas informações acerca da migração de metais dos bicos das mamadeiras. A avaliação deste componente do utensílio também é de extrema importância tendo em conta que também são pigmentados e estão em contato direto tanto com o alimento, quanto com a saliva do bebê.

Bicos e chupetas para crianças são feitos normalmente de silicone ou borracha natural. No Brasil a Resolução 123/2001 da ANVISA, que dispõe sobre embalagens e equipamentos elastoméricos em contato com alimentos, estabelece um limite de migração específica para N-nitrosaminas (1 mg/dm^2). Na Europa as restrições em relação à migração desses compostos também foram publicadas e determinam que 10 µg de N-nitrosaminas e 100 µg de substâncias N-nitrosáveis podem ser liberadas por kg de bicos ou chupetas feitas de elastômero ou borracha (BRASIL, 2001; European-Commision, 1993). Entretanto, não há dados sobre a incidência desses compostos nos bicos das mamadeiras comercializadas atualmente.

Diante disso, o objetivo deste estudo foi avaliar a migração de aminas aromáticas de mamadeiras utilizando a espectrometria de massas acoplada a

cromatografia a líquido (LC-MS), bem como a migração de metais e nitrosaminas nos bicos utilizando espectrometria de massas acoplada indutivamente a plasma (ICP-MS) e cromatografia gasosa (GC-MS), respectivamente.

2. Materiais e métodos

2.1. Amostras

Doze modelos de mamadeiras, destinadas a crianças com idade entre 0 e 12 meses, foram analisadas em triplicata (n = 36). As mamadeiras eram composta por polipropileno (n = 24), Tritan (n = 9), e silicone (n = 3), pigmentadas ou com desenhos impressos e foram adquiridas de distribuidores no estado de São Paulo.

Tabela 1. Amostras avaliadas quanto à migração de aminas aromáticas, metais e nitrosaminas

	Materiais	Cores	Volume (mL)
Amostra 1	PP	Azul	70
Amostra 2	PP	Rosa	70
Amostra 3	Tri	Transparente com desenho azul	80
Amostra 4	Sil	Transparente	250
Amostra 5	Tri	Transparente com desenho azul	80
Amostra 6	PP	Transparente com desenho roxo	120
Amostra 7	Tri	Transparente com desenho rosa	80
Amostra 8	PP	Transparente com desenho rosa	120
Amostra 9	PP	Transparente com desenho azul	70
Amostra 10	PP	Transparente com desenho verde	70
Amostra 11	PP	Transparente com desenho azul	70
Amostra 12	PP	Transparente com desenho verde	70

2.2. Solventes e padrões.

Foram utilizados etanol (Merck, DEU), metanol (Merck, DEU) e ácido acético (Scharlab) grau HPLC. Bicarbonato de sódio (P.A.), cloreto de sódio (P.A.), carbonato de potássio (P.A.) e nitrito de sódio (P.A.) foram obtidos da Synth. Padrões de 2,4-diaminotolueno, anilina, anilina-D5 (deuterada), 4,4’-oxidianilina, benzidina, orto-anisidina, 4,4'-diaminodifenilmetano, orto-toluidina, orto-dianisidine, 2-metoxi-5-metilanilina, 4,4-metileno-di(2-methilanilina), 4-cloro anilina, 4,4'-sulfanedildianilina, 2,6-dimethilanilina, 2-naftilamina, 3-cloro-4-fluoranilina, 4-cloro-2-metilanilina, 4-

aminobifenil, 4,4 '-metileno-di(2-cloroanilina), chumbo, bário, zinco e mix de nitrosaminas EPA 521 foram obtidos da Sigma. N-dietilamina-D10 (deuterada, NDEA-D10) foi obtido da Tedia Brasil. Água ultra pura foi utilizada no preparo dos simulantes e das fases móveis.

2.3. Ensaios de migração

Os ensaios para avaliação da migração de aminas aromáticas nas mamadeiras e metais nos bicos foram realizados utilizando simulante de alimento ácido (ácido acético 3%) de acordo com a recomendação da ANVISA (ANVISA, 2010a). Os testes foram conduzidos a 70 ºC/2 h para as mamadeiras e a 40 °C/24 h para os bicos.

O simulante de saliva foi utilizado nos ensaios de migração para determinação de N-nitrosaminas nos bicos. O simulante de saliva era composto de 4,2 g de bicarbonato de sódio, 0,5 g de cloreto de sódio, 0,2 g de carbonato de potássio e 30 mg de nitrito de sódio dissolvidos em 1 L de água destilada e com pH ajustado para 9 (European-Commission, 1993). A migração foi feita a 40 °C por 24 h.

Como as mamadeiras são utensílios de uso repetido, todos os ensaios de migração foram realizados 3 vezes no mesmo corpo de prova como recomenda a ANVISA (BRASIL, 2010a).

2.4. Preparo de amostra e análise instrumental

Após os ensaios de migração, 10 mL do simulante utilizado para determinação de aminas aromáticas (ácido acético) foi fortificado com 20 $\mu g.kg^{-1}$ de anilina deuterada (padrão interno) e injetado em um UPLC-MS/MS. Para tanto, foram utilizados um UPLC-MS/MS Xevo TQD Waters Acquity e uma coluna ACQUITY UPLC® BEH C18 1,7 µm para separação. Metanol e água acidificada foram ultilizados como fase móvel. A determinação foi realizada no modo MRM (*Multiple Reaction Monitoring*).

Para determinação de N-nitrosaminas, 10 mL dos extratos de simulante de saliva foram fortificados com NDEA-D10 (20 ppb) seguido da extração líquido-líquido (LLE) com 1 mL de diclorometano como recomenda a directiva 93/11 da União Europeia. Finalmente, 3 µL do extrato foi injetado em um cromatógrafo a gás Agilent 7890a acoplado a detector de massas Agilent 5975C (Triple-Axis) utilizando EI como fonte de

ionização (GC-MS). A cromatografia foi realizada em uma coluna capilar HP-5MS (30 m × 0,25 mm × 0,25 μm), no modo splitless, com injetor a 230 °C, utilizando hélio como gás de arraste a 1 $mL.min^{-1}$. A programação de temperatura iniciava a 40 ºC por 1 min, com incremento de 20 $°C.min^{-1}$ até 200 °C no qual permaneceu por 1 min. A quantificação foi feita em modo SIM.

A determinação de metais foi realizada em um ICP-MS Agilent 7500, equipado com um nebulizador Babington e uma câmara de pulverização de dupla passagem. Uma solução de ácido nítrico 1% (fluxo 1,2 $mL.min^{-1}$) foi utilizada como solução carreadora. Inicialmente, as amostras passaram por um *screening* para detectar quais elementos inorgânicos migraram dos bicos. Posteriormente, foi realizada uma análise quantitativa para os principais elementos detectados. Brancos do simulante e de ácido nítrico 1% foram injetados antes da análise (n = 6) e entre cada amostra (n = 2). Configurações instrumentais importantes, como posição da tocha, potência de radiofreqüência, fluxo de gás nebulizador e tensão da lente foram otimizadas com solução de tuning antes da análise. Os parâmetros de aquisição de dados foram: modo espectral, 3 pontos por pico, tempo de integração 0,2 s por ponto e 10 repetições. Todas as soluções de migração foram medidas em triplicata. Os métodos foram validados em relação aos limites de detecção, limite de quantificação, linearidade, reprodutibilidade e recuperação.

3. Resultados e discussão

A migração de aminas aromáticas em mamadeiras, bem como N-nitrosaminas e metais em bicos foi avaliada neste trabalho. Para tanto, foram desenvolvidos e validados métodos de quantificação utilizando a espectrometria de massas.

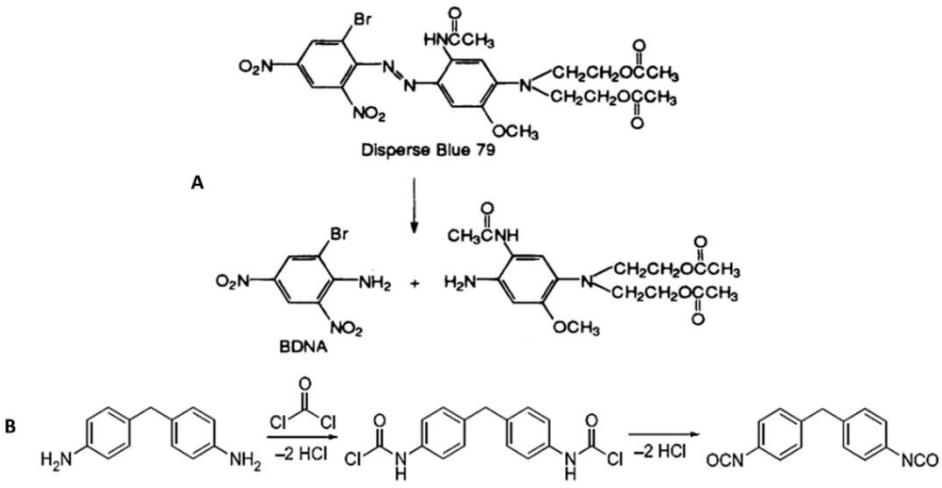
3.1. Determinação de aminas aromáticas primárias por UPLC-MS/MS

As aminas aromáticas primárias (AA) têm sido estudadas em diferentes matrizes, especialmente em materiais que entram em contato com alimento como as mamadeiras, por conta da possibilidade de migração e alta toxicidade. A contaminação de alimentos com AA pode se originar de tintas de impressão, pigmentos, adesivos à base de isocianato e monômeros presentes em plásticos, guardanapos de papel e papel impresso ou reciclado usados em embalagens de alimentos. As fontes mais estudadas de

formação de AA em materiais de contato com alimentos ainda são os pigmentos e os isocianatos aromáticos (Figura 1)(Yavuz, Valzacchi, Hoekstra, & Simoneau, 2016).

Os isocianatos são utilizados para fazer os adesivos de poliuretano e vernizes, de onde os resíduos podem migrar, por exemplo, a partir de películas plásticas flexíveis em simulantes de alimento (Figura 1b). A migração de AAP se deve, dentre outros fatores, a formação de pontes de hidrogênio entre o grupo amino, que são muito polares, com os hidrogênios presentes nos compostos dos alimentos. Além disso, a cura incompleta dos adesivos de poliuretano e a decomposição de pigmentos, que têm um grupo amino como cromóforo, também podem liberar AA (Sanchis, Yusà, & Coscollà, 2017). Considerando a toxicidade das AA, a avaliação da migração desses compostos é importante para garantir a segurança dos bebês no tocante ao uso das mamadeiras.

O regulamento 52/2010 da ANVISA determina que o conteúdo de aminas aromáticas primárias não sulfonadas solúveis em solução de ácido clorídrico 1 M, expresso como anilina, não deve exceder 500 $mg.kg^{-1}$ em massa do corante (0.05% m/m). Além disso, o conteúdo de benzidina, β-naftilamina e 4-aminobifenilo, individualmente ou combinados, não deve exceder 10 $mg.kg^{-1}$ (ANVISA, 2010b). Já o regulamento 10/2011 da União Europeia determina que materiais e artigos de plástico não devem liberar AA em quantidade superior a 0,01 mg de substância por kg de alimento ou simulante. Como informações acerca da composição dos pigmentos e tintas de impressão utilizadas nas mamadeiras não são disponibilizadas pelos fabricantes, a avaliação de conformidade desses utensilios foi avaliada por meio de estudos de migração.



Fonte: Heyn, Jacobs, and Carr (2014), Weber and Adams (1995).

Figure 1. Formação de aminas aromáticas a partir de pigmentos e poliuretanos de aminas aromáticas a partir de pigmentos e poliuretanos

Os ensaios foram executados em ácido acético 3% e as determinações foram realizadas utilizando um UPLC-MS/MS. Os compostos monitorados, as transições utilizadas no MRM (*multiple reaction monitoring*) e os limites de quantificação determinados no método estão apresentados na Tabela 2.

O método utilizado para quantificação de 18 aminas aromáticas primárias foi desenvolvido e validado por Perez, Bottoli, and Padula (2017) (Table 2). Limites de quantificação entre 1 e 5 $\mu g.kg^{-1}$ foram alcançados o que torna o método adequado para investigação das AA.

Tabela 2. Aminas aromáticas investigadas em mamadeiras

Composto	Tr (min)	Transição	LOQ (µg/kg)
2,4-diaminotoluene	1,77	123 > 105	5
Aniline	2,05	94 > 77	5
Aniline-D5	1,98	99 > 82	5
4,4'-oxidianiline	2,79	200 > 107	5
Benzidine	2,85	185 > 92	1
Orto-anisidine	3,28	124 > 109	1
4,4'-diaminodiphenylmethane	3,29	199 > 106	1
Orto-toluidine	3,50	108 > 90	1
Orto-dianisidine	4,22	245 > 230	5
2-metoxi-5-methylaniline	4,31	137 > 106	5
4,4-metilene-bis(2-methylaniline)	4,68	227 > 120	5
4-chloro aniline	5,11	127 > 93	1
4,4′-diaminodiphenyl sulfide	5,24	217 > 123	5
2,6-dimethylaniline	5,68	122 > 107	5
2-naftilamine	5,73	144 > 117	1
3-chloro-4-fluoraniline	6,02	145 > 111	5
4-chloro-2-methylaniline	6,66	141 > 107	1
4-aminobiphenyl	6,93	170 > 143	5
4,4 '-metilene-bis(2-chloroaniline)	7,56	267 > 231	5

Tr: tempo de retenção; LOQ: limite de quantificação

Não foi detectada a incidência de aminas aromáticas nas 36 mamadeiras analisadas. Entretanto, a investigação da incidência de AAP em mamadeiras ainda é extremamente importante visto que não há dados sobre a migração desses compostos em materiais de contatos com alimentos comercializados no Brasil.

3.2. Migração Específica de N-nitrosaminas por GC-MS

Nitrosaminas são compostos potencialmente carcinogênicos para o homem, as quais podem ocorrer em alimentos como produto da reação entre aminas secundárias e agentes nitrosantes, como nitrito e nitrato. A contaminação de alimentos também pode ocorrer por meio da migração de nitrosaminas formadas em materiais de contato com alimentos como a borracha.

Durante a produção das borrachas naturais e sintéticas aceleradores como os carbamatos são utilizados ocasionando a formação de N-nitrosaminas e substâncias nitrosáveis. Além disso, substâncias nitrosáveis, como a dibenzilamina, podem ser transformadas em N-nitrosaminas através da reação com o nitrito presente em alimentos ou no nitrato presente na saliva (Bouma, Nab, & Schothorst, 2003).

Na literatura já foram reportados mais de 300 tipos diferentes de nitrosaminas que podem ser agrupadas em voláteis e não voláteis. As nitrosaminas voláteis mais estudadas são a N-nitrosodimetilamina (NDMA), N-nitrosodietilamina (NDEA), N-nitrosopropilamina (NDPA) e N-nitroso-pirrolidina (NPIR). A Agência Internacional de Pesquisa sobre o Câncer classificou esses compostos no grupo 2A (NDMA e NDEA) ou 2B (NDPA e NPIR), no qual indica provável ou possível efeito carcinogênico em humanos. NDEA é a nitrosamina que possui maior potencial carcinogênico. A NDMA e as nitrosaminas heterocíclicas como a nitrosopirrolidina (NPIR), apresentam potencial carcinogênico menor (Jägerstad & Skog, 2005). Diante do exposto, fica clara a importância do monitoramento desses contaminantes em materiais elastoméricos para contato com alimentos, como os bicos de mamadeiras.

No Brasil a Resolução 123/2001 da ANVISA, que dispõe sobre embalagens e equipamentos elastoméricos em contato com alimentos, estabelece para N-nitrosaminas o limite de migração específica de 1mg/dm^2. Na Europa as restrições em relação à migração desses compostos também foram publicadas e determinam que 10µg de N-nitrosaminas e 100 µg de substâncias N-nitrosáveis podem ser liberadas por kg de bicos ou chupetas feitas de elastômero ou borracha (ANVISA, 2001).

Para tanto, os bicos foram submetidos a ensaios de migração utilizando simulante de saliva. Em seguida, um método foi validado para quantificação desses compostos em simulante. Os parâmetros de validação, bem como os íons monitorados estão apresentados na Tabela 3. A linearidade do método foi avaliada entre 5 e 100 µg.kg^{-1} para todos os analitos, com exceção da NDEA que apresentou linearidade entre 5 e 80

µg.kg^{-1}. Limites de quantificação inferiores a 10 µg.kg^{-1} foram obtidos para todos os compostos o que torna o método adequado ao objetivo proposto. Após a validação, o método foi utilizado na avaliação da incidência de nitrosaminas em 36 bicos de mamadeiras. Entretanto, não foi detectada a migração de nitrosaminas nas amostras.

Tabela 3. Parâmetros de validação do método de quantificação de nitrosaminas em bicos de mamadeiras

Composto	Ion Quant.	Ion Confi.	LOQ(µg.kg^{-1})	Curva	R^2	REC (%)*
NMEA	88	42 ; 43	5	y = 0,0204x + 0,0785	0,989	65,1
NDEA	102	56 ; 57	5	y = 0,0372x + 0,2888	0,993	86,7
NPYR	100	41 ; 42	5	y = 0,0201x + 0,0539	0,988	56,5
NDPA	70	130 ; 43	5	y = 0,0569x + 0,6793	0,993	104,0
NPIP	114	42 ; 55	5	y = 0,047 + 0,0099	0,986	81,9
NDBA	84	158 ; 116	5	y = 0,0418x + 0,270	0,991	101,2
NDEA-D10 (PI)	112	62 ; 64	-	-	-	-

REC: Recuperação
*Avaliada no LOQ

3.3. Avaliação da migração de metais em bicos por ICP-MS

A contaminação de alimentos por elementos inorgânicos tem sido amplamente relatada (Guérin, Chekri, Chafey, Testu, Hulin, & Noël, 2018; Kiyataka, Dantas, & Pallone, 2015; Sager, McCulloch, & Schoder, 2018). Dentre as várias fontes de contaminação, materiais de contato com alimento como as mamadeiras podem liberar compostos inorgânicos provenientes tanto de catalisadores quanto dos pigmentos utilizados para colorir os utensílios.

Para avaliar a incidência desses contaminantes a partir dos bicos de mamadeiras, foi realizado um *screening* com o extrato obtido a partir do primeiro ensaio de migração. Foram detectados zinco, cádmio, chumbo e bário.

Um método para quantificação desses contaminantes foi desenvolvido. O limite de detecção (LOD) foi determinado como sendo a média do branco (n = 6) mais 3 vezes o desvio padrão. O limite de quantificação foi estabelecido como 2 x LOD (Bratinova, S., Raffael, B., & Simoneau, C., 2009). A linearidade foi avaliada até 300 µg.kg^{-1} (LOQ, 50, 100, 150, 200, 250 e 300 µg.kg^{-1}) para os quatro elementos avaliados. Os limites de quantificação determinados foram de 1,40 (^{66}Zn), 1,80 (^{111}Cd,^{208}Pb) e 1,05

µg.kg^{-1} (^{137} Ba). Curvas de calibração foram construídas em triplicata e apresentaram R^2 superior a 0.999.

A migração de Cádmio (Tabela 4) foi inferior aos limites de quantificação estabelecido no método. Por outro lado, com exceção da amostra 8, foram detectados a migração de zinco e bário em todas as amostras. No Brasil os limites de migração específica para Zn e Ba são de 25000 µg.kg^{-1} e 1000 µg.kg^{-1}, respectivamente. Em 2016 uma emenda ao Regulamento 10/2011 foi publicada recomendando o limite de 5000 µg.kg^{-1} para o zinco. Esses valores são bem superiores aos encontrados nas amostras analisadas. Portanto, em relação a migração de Zn e Ba as amostras estão em conformidade ao preconizado pela ANVISA (BRASIL, 2010b; European-Commision, 2016).

Tabela 4. Migração de metais em bicos de silicone

	Minerais (µg.kg^{-1})			
Amostras	^{66} Zn	^{111} Cd	^{137} Ba	^{208} Pb
1	7,24±0,9	<LOQ	1,49±0,03	3,51±0,40
2	6,67±1,29	<LOQ	1,55±0,02	
3	10,42±1,80	<LOQ	1,53±0,05	1,95±0,17
4	4,58±0,69	<LOQ	1,25±0,01	
5	9,83±1,32	<LOQ	1,6±0,02	
6	8,11±0,07	<LOQ	1,48±0,04	
7	7,90±1,38	<LOQ	1,47±0,06	
8	-	<LOQ	-	
9	6,47±0,52	<LOQ	1,46±0,05	
10	3,11±0,69	<LOQ	1,3±0,06	
11	4,85±0,55	<LOQ	1,44±0,14	
12	5,37±0,25	<LOQ	1,32±0,12	

Por outro lado, em duas amostras (1 e 3) foi detectada a migração de chumbo. O limite de migração específica estabelecido pela ANVISA para o chumbo é de 10µg.kg^{-1}. Mesmo com migrações inferiores aos valores preconizados pela ANVISA, a migração de Pb é extremamente preocupante dada a toxicidade deste metal. O alto nível de Pb no sangue de crianças está associado a sintomas de neurotoxicidade (El-Kady & Abdel-Wahhab, 2018). Além disso, os limites de migração específica são estabelecidos com base em uma pessoa de 60 kg de peso corporal, o que não reflete o peso de uma criança. Diante disso, o monitoramento da incidência desses metais em materiais destinados a utilização por crianças é da mais alta importância alta importância.

4. Conclusão

Neste trabalho foi avaliada a migração de aminas aromáticas em mamadeiras, bem como de N-nitrosaminas e metais nos bicos. Para tanto, espectrometria de massas foi utilizada como sistema de detecção e mostrou-se uma ferramenta valiosa alcançando limites inferiores a 5 $\mu g.kg^{-1}$ para todos os analitos avaliados. Os estudos de migração avaliaram 18 aminas aromáticas em mamadeiras utilizando simulante de alimento ácido, 6 N-nitrosaminas e 4 minerais nos bicos utilizando simulante de saliva e de alimentos ácidos, respectivamente. A migração de ^{66}Zn e ^{137}Ba foi detectada em todas as amostras, exceto na amostra 8. ^{208}Pb foi detectado em 2 amostras em concentrações inferiores a 4 $\mu g.kg^{-1}$. Embora as amostras avaliadas estejam em conformidade com os limites estabelecidos pela ANVISA, a incidência desses contaminantes deve ser monitorada a fim de garantir a segurança das crianças em relação ao uso do utensílio.

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CAPÍTULO III

Identificação e quantificação de substâncias não intencionalmente adicionadas (NIAS) em mamadeiras por UPLC-QTOF-MS^{E}

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Identification and quantification of non-intentionally added substances (NIAS) from baby bottles by UPLC-QTOF-MS^{E}

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ABSTRACT

Baby bottles made of several plastic materials has been appeared on market after the ban of BPA in the utensil. However, there is a lack of information about the migration of non-volatile compound from the baby bottles. In view of this, twelve models of baby bottles made of polypropylene, Tritan® and silicone were evaluated regarding the migration of non-volatile compounds using UPLC-QTOF-MS^E. Moreover, the risk assessment was carried using the Threshold of Toxicological Concern (TTC) approach. Twenty-seven compounds were identified. In all polypropylene samples were detected the migration of 2.2'-(tridecylimino)bis-ethanol and derivatives thereof in concentrations below the SML (1.2 $mg.kg^{-1}$). Moreover, were detected clarifying agents and glycerol derivatives. Tritan baby bottle has shown migration of one slip additive. On the other hand, twenty compounds were detected in silicon baby bottles. Most of them were non-intentionally added substances, such as N-acetylvaline, acrylates and propylene glycol. The risk assessment has shown that there is a risk for babies regarding the use of silicon baby bottles since the migration of compounds with high toxicity was higher than recommended.

Keywords: silicon, migration, baby bottle, Q-TOF, mass-spectrometry, risk assessment.

1. Introduction

Food contact materials (FCM) are all materials and articles intended to come directly or indirectly into contact with food (European-Commision, 2004). FCM can be made from a range of different materials, such as plastics, paper and board, metals, glass, ceramics, cork and rubber, along with the adhesives, inks, varnishes, and coatings needed to make finished materials and articles (Driffield, Bradley, Castle, & Coulier, 2011). Among them, plastic is the most used since is cheap, can be produced easily in complex shapes with various colors or transparent (Bang, Kyung, Kim, Jung, Cho, Choi, et al., 2012).

FCM are not inert and might transfer components ("migrate") to food due to diffusion and partition mechanisms. The migration of substances from FCM should not exceed acceptable amounts because they might produce adverse effects on human health (Margarita Aznar, Alfaro, Nerín, Jones, & Riches, 2016; Hoppe, de Voogt, & Franz, 2016a). Migration from FCM has been extensively reported (Grob, 2014; Hoppe, de Voogt, & Franz, 2016b; Cristina Nerin, Canellas, Vera, Garcia-Calvo, Luque-Garcia, Cámara, et al., 2018). The increase in the investigation of migrants from FCM might be a consequence of the development of new materials and processes required by the food industry. Since any change or innovation in the composition of FCM must be evaluated, the migration studies have been performed in order to ensure the consumer safety.

Children are exposed to a wide variety of FCM. Moreover, infants normally consume higher amounts of food relative to body weight compared to adults which may increase the exposure to migrants come from FCM. Also, infants are more physiologically susceptible to endocrine disruptors than adults due to differences in their ability to metabolize, detoxify, and excrete chemicals. For this reason, the evaluation of the migration from FCM for infant is high concern.

Previous studies of children's products, such as baby bottle, have identified the migration of numerous chemicals associated with potential adverse health effects, including bisphenol A (BPA), phthalate esters, and photoinitiators (Oliveira, de Souza, Padula, & Godoy, 2017; Onghena, Negreira, Van Hoeck, Quirynen, Van Loco, & Covaci, 2016; Simoneau, van den Eede, & Valzacchi, 2012). Harmful chemicals have also been found in products not intended for children but that children often play with or on, such as lead in keys (EU 2015/628), BPA in store receipts (Hormann et al., 2014), and flame retardants in furniture and electronics (Abbasi et al., 2016; Fang et al., 2013). In view of this, there is a strict legislation in USA,

Europe, Mercosur, Australia and Euroasia as well as in many countries to ensure the food safety regarding the use of FCM (C. Nerin, Bentayeb, & Rodriguez-Lafuente, 2012).

Most of these compounds detected in baby bottles were substances intentionally added, such as antioxidants, plasticizers, stabilizers, lubricants, UV absorbers, antistatic agents, etc. However, non-intentionally added substances (NIAS) can also migrate and should be evaluated. NIAS appeared as result of the interactions between different ingredients in the packaging materials, from degradation processes and mainly from the impurities present in the raw materials used for their production (Vera, Canellas, & Nerín, 2013). In general, these ones have low molecular weight (<1000 amu) and can be detected using high sensitive technics such as chromatography coupled to mass spectrometry.

The European Regulation 10/2011 recognise that during the processing and use of plastic materials NIAS might be formed. On the other hand there is no legislation in Brazil that recommends the research or establishes the concentration of NIAS in FCM (European-Commision, 2011).

In Brazil, plastic and articles intended for food contact require overall and specific migration testing to demonstrate compliance with Brazilian Health Regulatory Agency (Agência Nacional de Vigilância Sanitária – ANVISA) (BRAZIL, 2010). The overall migration limit (OML) specifies the total mass of substances permitted to migrate into food simulant (Bradley, Castle, Jickells, Mountfort, & Read, 2009). The OML to plastics is 50 $mg.kg^{-1}$ of food or food simulant or 8 $mg.dm^{-2}$ expressed on a contact area basis. Regarding specific migration (SML), there is not a single limit for all substances. The SML has been established through toxicology studies, migration potential and consumer exposure risk assessment ingestion of the substance. Hence, plastics require testing for compliance regarding overall migration plus individual tests for all substances which have an SML (Bradley, Castle, Jickells, Mountfort, & Read, 2009). However, the identification and quantification of NIAS also should be evaluating in order to ensure the food safety.

The identification of NIAS is very difficult mainly for non-volatile compounds since there is no commercial library available. For this, it needs the use of highly sensitive analytical techniques such as high resolution mass spectrometry which permit the identification of chemical structures based on the fragmentation of mass spectra (Ubeda, Aznar, & Nerín, 2018).

More than 50% of Brazilian population use baby bottles in the breastfeeding (BRAZIL, 2009). However, there is a lack of data regarding the incidence of NIAS in this

utensil. In view of this, in this work the migration of NIAS from baby bottles of polypropylene, silicon and Tritan was studied. For this, liquid chromatography coupled with high resolution mass spectrometry was used. The formation and track of the NIAS as well the risk of exposure to the migrants quantified was investigated.

2. Materials and methods

2.1. Samples

Twelve different baby bottle models, intended for babies between 0 and 12 months were used in this work. The baby bottles were composed by polypropylene (PP), silicon (S) and Tritan (T) Table 1. These ones were purchased from distributors in São Paulo taking into account the six Brazil's top-selling brands. The determinations were done in triplicate.

Table 1. Characteristics of baby bottles analyzed

	Material	Color	Volume (mL)
Sample 1	PP	Blue	70
Sample 2	PP	Pink	70
Sample 3	T	Transparent with blue drawings	80
Sample 4	S	Transparent	250
Sample 5	T	Transparent with blue drawings	80
Sample 6	PP	Transparent with purple drawings	120
Sample 7	T	Transparent with pink drawings	80
Sample 8	PP	Transparent with pink drawings	120
Sample 9	PP	Transparent with blue drawings	70
Sample 10	PP	Transparent with green drawings	70
Sample 11	PP	Transparent with blue drawings	70
Sample 12	PP	Transparent with green drawings	70

2.2. Chemicals

Purified water was obtained with a Milli-Q 185 Plus system (Millipore, Bedford, MA, USA), and ethanol (HPLC quality) was purchased from Scharlau Chemie S.A (Sentmenat, Spain). Methanol and water for UPLC–MS analysis (ultra LC–MS quality) were purchased from Baker (Deventer, The Netherlands). Standards of 2,2'-(dodecylimino)bis-ethanol (CAS 541-67-9), 1-Stearoyl-rac-glycerol (CAS 123-94-4), erucamide (CAS 112-84-5), Triethylene

glycol (CAS 112-27-6), N-acyl-valine (CAS 96-81-1) were purchased from sigma. NX 8000 (CAS 882073-43-0) was kindly donated by Repsol SA.

2.3. Sample preparation and migration tests

First, baby bottles were boiled in distilled water for 5 minutes as recommended by the manufacturer. Next, the migration test was done at 70 ºC/2 h with milk simulant (i.e. ethanol 50%) as specified in the Regulation 2011/10/EC and the resolution 51/2010 from ANVISA. Second and third migration test were performed according to the conditions for repeated use of food contact materials (BRAZIL, 2010; European-Commision, 2011). Between the tests, the baby bottles were rinsed with ultrapure water and boiled again for five minutes. For qualitative analysis, the extracts from the migration test were concentrated twice under a gentle stream of nitrogen before the injection in UPLC-Q-TOF-MS^E.

2.4. Instrumentation

Chromatography was carried out using an Acquity system. The separation was done in a UPLC BEH C18 column (1.7-µm particle size × 2.1 mm × 100 mm) from Waters (Milford, MA, USA). The column was operated at 40 °C using flow of 0.3 mL/min. Water (A) and methanol (B) both with 0.1% of formic acid (v/v) were used as mobile phase. The gradient applied started at 95:5 (A:B) up to 5:95 (A:B) in 8 min, returning to 95:5 (A:B) in 10 minutes. Ten microliters were injected in the LC-MS/MS.

The mass spectrometer used consisted of a hexapole, a quadrupole, a collision cell, and a time-of-flight analyzer (Xevo G2) from Waters (Milford, MA, USA). Electrospray ionization in sensitivity mode with three different cone voltages (15, 30 and 70V) was used in positive (ESI+). The samples also were analyzed in negative (ESI-) mode (cone voltage 30V) in order to identify as many compounds as possible. The capillary voltage was set at 3 kV for ESI+ and 0.8 kV for ESI-. The mass range analyzed was from 50-1000 Da, with extraction cone at 4 V, source temperature at 120 °C, desolvation gas temperature at 350 °C and desolvation gas flow at 450 $L.h^{-1}$. The Acquisition was carried out in MS^E mode and the ramp from 10 to 30V was used. All analyses were carried out using a LockSpray™ which provide authenticated exact mass measurement, ensuring accuracy and reproducibility. MassLynx v.4.1 software (Waters, Milford MA, USA) was used to process the chromatographic and MS data.

Blanks of each migration test was performed to distinguish contaminants from the simulant and migrants from baby bottles. The baby bottles migrants were identified using elemental composition in the spectrum obtained from the first function chromatogram. The possible compounds for each proposed molecular formula were obtained from ChemSpider® and SciFinder® taking into account the most cited ones and literature data.

Afterwards, with the function two, the fragmentation spectra for the possible candidates were checked through MassFragment® software from Waters. This one enables to match the spectrum obtained in the high collision energy with the fragments generated from the chemical structures of the candidates proposed. Candidates with at least 2 ions (one precursor ion and one fragment ion) with a mass accuracy below 5 ppm were considered.

3. Results and discussion

Migration from 12 different baby bottles in milk simulant was evaluated in this work. Both intentionally and non-intentionally substances were identified by UPLC–Q-TOF-MS^E. Compounds detected in blank sample were not investigated. Two criteria were used to identification: the mass tolerance, which was set at 5 mDa and the i-Fit, which is the probability that the isotope pattern from the elemental composition of the list of results matches the peaks in the measured spectrum (Elena Canellas, Vera, & Nerín, 2015).

With the molecular formula determined, a research in ChemSpider and SciFinder was performed in order to find the most probable candidate. Finally, MassFragment tool from MassLynx was used to analyze the resulting ion spectra and to consider, from the fragmentation of a precursor ion, the likely structural composition involved. When it was commercially available, the standard was purchased, and the compounds were confirmed by the comparison of retention time and mass spectrum and quantified.

The migration of compounds from baby bottles was not detected in negative mode (ESI-). Moreover, was not detected migrants from sample 7. On the other hand, ten compounds were detected in the others PP and Tritan samples using positive mode (ESI+) (Figure 1). These ones were identified and quantified and the results obtained from the first contact was shown in Table 2.

2,2'-(tridecylimino)bis-ethanol (m/z 288.2916), 2,2'-(pentadecylimino)bis-ethanol (m/z 316.3221) and isomers of them (compounds 1 to 6) were detected in all PP baby bottles and in one Tritan baby bottle (sample 3). These ones have been reported as impurities or

degradation products from polyethylene materials (M. Aznar, Rodriguez-Lafuente, Alfaro, & Nerin, 2012). In fact these compounds has been used in polyolefin such as PP, PE, LDPE, HDPE and LLDPE in concentration range from 0.1 to 0.3 percent.

According to product data sheet, these compounds act as internal antistatic agent, effective mainly in films due the fast migration (AkzoNobel, 2015). Even after mixed with de polymer, the additive continuously migrates to the surface of the final product providing the antistatic performance. Furthermore, they were authorized for use in food contact materials by European Community (European-Commision, 2011) with total specific migration limit of 1.2 $mg.kg^{-1}$. In order to check the compliance of baby bottles, the quantification of these compounds was performed using a similar standard to these compounds (i.e. 2,2'-(dodecylimino)bis-ethanol).

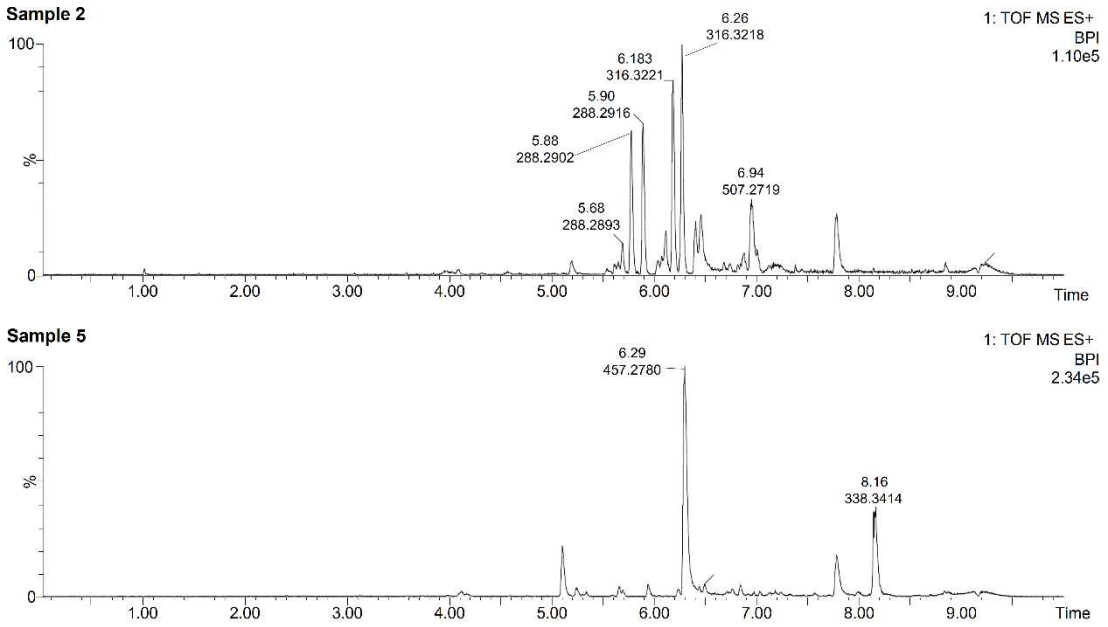

Figure 1. Profile of migrants from baby bottles obtained by UPLC-ESI-Q-TOF-MS.

In Tritan® baby bottle (sample 3) the compounds were detected below the limit of quantification stablished (0.0097 $mg.kg^{-1}$). On the other hand, total concentrations varying from 0.002 to 0.240 $mg.kg^{-1}$ were detected in the first migration for the other PP samples. Moreover, in the second migration test these compounds still were detected in the samples 1, 2 and 6 with total concentration varying from 0.035 to 0.198 $mg.kg^{-1}$.

Migration limits were established based on a person of 60 kg of body weight which do not reflect the real weight of a baby. On the other, the European Food Safety Authority suggest 6.1 kg as the reference for a baby 3-month-old child. Moreover, the safety evaluation

of substances present (intentionally or not) in the foods consumed by infants below the age of 16 weeks should be done taking into consideration existing data including a case-by-case risk assessment. However, there is no data about the incidence of this substances in baby bottles or FCM for infants (EFSA, 2010; Hardy, Benford, Halldorsson, Jeger, Knutsen, More, et al., 2017).

For this reason, even with migration below the total specific migration limit, the use of these substances in baby bottle should be monitored. Furthermore, evidences suggest that hydroxyalkylamines have an adverse effect on sexual function and fertility. In view of this there is a proposal to classify it as 1B class according to Regulation (EC) 1272/2008 (European-Commision, 2008; Harmonised Classification and Labelling, 2016).

A clarifying agent commercialized as Millad® NX 8000 (1,2,3-Trideoxy-4,6:5,7-bis-O-[(4-propylphenyl)methylene]nonitol, CAS 882073-43-0) also was detected in PP baby bottles only in the 1st migration test (Table 2). Nonitol-based clarifying agents is added to reduce the dimension of the spherulites formed during the PP crystallization and consequently reduce the opacity of the material (Sternbauer, Dieplinger, Buchberger, & Marosits, 2014). The one has been confirmed and quantified with standard and levels of migration below the specific migration limit (5 $mg.kg^{-1}$) were detected. Clarifiers' detection in polypropylene baby bottles is not surprising. This one also might be added to make new bottles similar to the older utensils which were transparent because of the polycarbonate.

Table 2. Identification and quantification of migrants from polypropylene and Tritan baby bottles by UPLC-Q-TOF-MS^{E}

Nº	RT	Mass measured and ion found	ΔmDa Δppm	Formula	Compound	LOQ (µg.kg^{-1})	SML (µg.kg^{-1})	Samples (µg.kg^{-1})									
								1	2	3	5	6	8	9	10	11	12
1	5.70	288.2910 [+H]	0.7 2.4	C17H37NO2	2,2'-(tridecylimino) bis-ethanol	10		<LOQ	<LOQ	-	-	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
2	5.78	288.2903 [+H]	1.2 4.2	C17H37NO2	2,2'-(tridecylimino) bis-ethanol isomer	10		49.36 ±1.97	27.07 ±2.12	<LOQ	-	46.39 ±3.32	39.48 ±3.39	<LOQ	<LOQ	<LOQ	4.78 ±0.29
3	5.90	288.2916 [+H]	1.3 4.5	C17H37NO2	2,2'-(tridecylimino) bis-Ethanol isomer	10	1200 (T)	33.42 ±1.32	30.37 ±1.93	<LOQ	-	51.91 ±3.10	39.04 ±1.81	4.00 ±0.16	<LOQ	<LOQ	9.22 ±0.37
4	6.11	316.3204 [+H]	1.2 3.8	C19H41NO2	2,2'- pentadecylimino) bis-ethanol	10		4.12 ±0.91	<LOQ	-	-	16.64 ±0.89	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
5	6.19	316.3221 [+H]	0.5 1.6	C19H41NO2	2,2'-(pentadecylimino) bis-ethanol isomer	10		67.57 ±3.83	36.44 ±5.56	<LOQ	-	64.31 ±2.64	54.37 ±1.24	4.03 ±0.24	9.74 ±0.27	1.35 ±0.12	18.59 ±1.65
6	6.28	316.3213 [+H]	-0.3 -0.9	C19H41NO2	2,2'-(pentadecylimino) bis-ethanol isomer	10		28.86 ±3.15	43.77 ±2.18	-	-	67.74 ±2.64	55.28 ±3.38	4.78 ±0.37	8.79 ±0.73	0.98 ±0.11	19.49 ±1.48
7	6.94	507.2738 [+Na]	1.5 3.0	C29H40O6	NX 8000	50	5000	-	300.00 ±1.40	-	-	340.10 ±32.52	368.69 ±10.20	-	-	182.10 ±6.19	195.48 ±4.14
8	7.36	353.2683 [+Na]	1.5 4.2	C19H38O4	1-palmitoylglycerol	50	60000*	132.95 ±1.85	-	-	-	-	-	-	-	-	-
9	7.75	381.297 [+Na]	-0.6 -1.6	C21H42O4	Glycerol stearate	50	60000*	578.68 ±20.25	-	-	-	-	-	-	-	-	-
10	8.15	338.3415 [+H]	-0.2 -0.6	C22H43NO	Erucic amide	35	60000*	-	-	-	384.71 ±3.19	-	-	-	-	-	-

SML: specific migration limits. Regulation 10/2011/EC and Resolution 17/2008 from Brazil.

T: Total specific migration limit. Maximum permitted sum of particular substances released in food simulant.

* Overall migration limit

Glycerol derivatives, such as 1-palmitoylglycerol and glycerol stearate were identified in the sample 1 (PP baby bottle). Glycerol stearate might be used as polymer additive acting as antifogging and antistatic agents, lubricants, plasticizers; in inks and coating additives acting as emulsifiers, stabilizers, dispersants and humectants.

Since the glycerol monostearate is synthesized by reaction of triglycerides with an excess of glycerol, the impurities are mainly diglycerides, unreacted triglycerides, glycerol, fatty acids (i.e. stearic acid. tetradecanoic acid. hexadecanoic acid) and their esters. Quantitative analysis for both compounds was performed using 1-Stearoyl-rac-glycerol as external standard. Concentrations up to 132.95 $\mu g.kg^{-1}$ to 1-palmitoylglycerol and 578.68 $\mu g.kg^{-1}$ of glycerol stearate were detected. These compounds do not have specific migration limit according to ANVISA and the European Regulation (BRAZIL, 2010; European-Commision, 2011)

Erucic amide was the only compound identified in sample 5 (Tritan). This one is used as slip additive in polyolefins, migrating to the surface of the polymer facilitating slippage. Erucic amide was confirmed and quantified using external calibration curves. However, there was no specific migration limit for this compound (BRAZIL, 2010; European-Commision, 2011).

All compounds identified in PP and Tritan baby bottles were intentionally added substances. Notwithstanding, for silicon baby bottle (sample 4) most of the compounds identified were non-intentionally added substances. Figure 2 shown the chromatographic profile of migrants from silicone baby bottles.

N-Acetylvaline was identified with the m/z 160.0973 and the migration of this compound was detected in the 3 tests performed. This one has been used in moisture curable systems for silicone compositions, commonly room-temperature vulcanizing (RTV) silicone rubbers and elastomers (Jenkins, Byrne, Patel, & Dhanabalan, 2016; Joshi, Arendt, Berry-Walker, & Butt, 2010). The extensive use of this technology is due the low cost, easy curing acceleration by increasing temperature and humidity and ease of use. On the other hand, limitations such as slow rate of curing and release of volatile organic compounds (VOCs), which have unpleasant scent, have been reported (Wang, Klein, & Mejía, 2017). Moreover, N-Acetylvaline is not regulated to use in food contact materials according to Brazilian and Europe Regulation (BRAZIL, 2001, 2008; European-Commision, 2011). External calibration curve was performed with N-acyl-valine for quantification. Concentrations varying from 1.30 up to 5.58 $mg.kg^{-1}$ were detected.

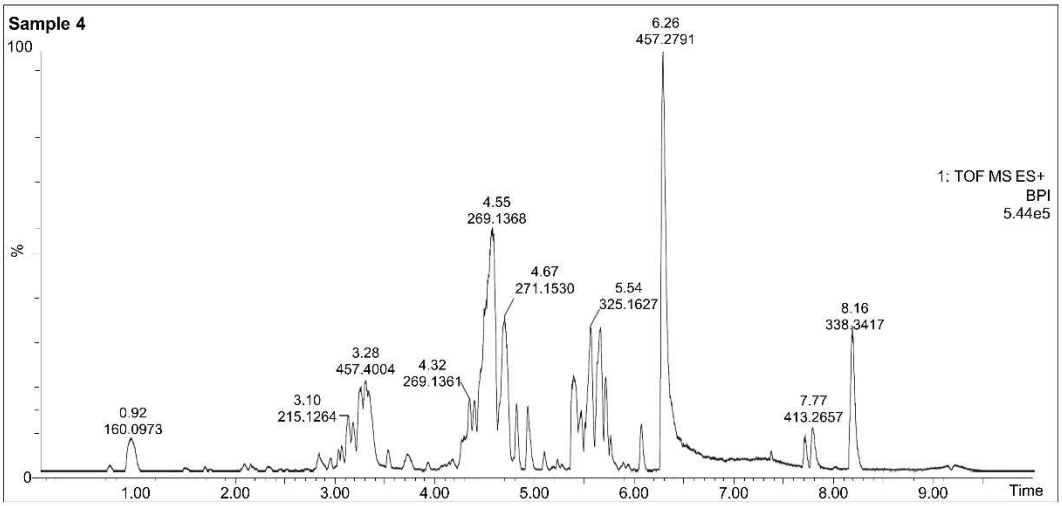

Figure 2: Chromatographic profile of silicon baby bottle migrant in ESI+ mode

The identification of others compounds from silicon baby bottles was very challenging due the formation of sodium adduct $[M+Na]^+$. The formation of sodium adducts is an important issue when it comes to the elucidation of molecular structures. This one does not fragment what makes it impossible to confirm the molecular structure of the NIAS. For this reason, some compounds such the one with m/z 457.4004 (retention time 3.28 min) was not identified. The absence of fragments from sodium adducts already has been reported and also was observed in this work. Probably, it is due the quantity of negative partial charge provided by the oxygen heteroatom which chelates the sodium atoms forming the adducts (Kruve, Kaupmees, Liigand, Oss, & Leito, 2013).

In order to overcome this drawback, the extracts from silicon baby bottle migration also were analyzed using 15V in the cone voltage. Since not all molecules form sodium adducts, the reduction of the cone energy can be an alternative to conserve the few protonated molecules formed by ESI+ ionization for later collision-induced dissociation. With this approach, acrylates in very low concentrations and derivatives thereof were identified from silicon baby bottles (Table 3).

Due to unsaturation in the structure, acrylates are arguably the most popular cross-linkers for free-radical photo-curing of silicone and adhesives formulations. Moreover, they shown high reaction rates, solvent-free conditions, ambient temperature operation, and high spatial resolution (Konuray, Fernández-Francos, Ramis, & Serra, 2018; Wang, Klein, & Mejía, 2017).

Many of acrylates derivatives detected, such as ethoxytriethylene glycol methacrylate (ETMA) (m/z 269.1371) (Figure 3), has shown a propylene glycol chain.

Propylene glycol was also detected as sodium adduct (m/z 215.1259). Acrylates with propylene glycol chain might be formed in the curing process since the last one is permitted for use as additives in silicone elastomers (BRAZIL, 2001).

On the other hand, these compounds also might be commercialized with the propylene glycol chain, such as ethoxytriethylene glycol methacrylate which is commercialized as Visiomer® ETMA. To track if the compound was added or formed in the processing is not possible since there is no information about the real composition of the different ingredients and materials used for silicon baby bottles manufacturing. Moreover, the material composition commonly is confidential, only the major components of the packaging are usually declared which makes it difficult to trace the origin of these compounds (C. Nerin, Alfaro, Aznar, & Domeño, 2013). Anyway, the identification should be performed in all compounds detected in the baby bottle to be possible to do the risk assessment resulting from the use of the material.

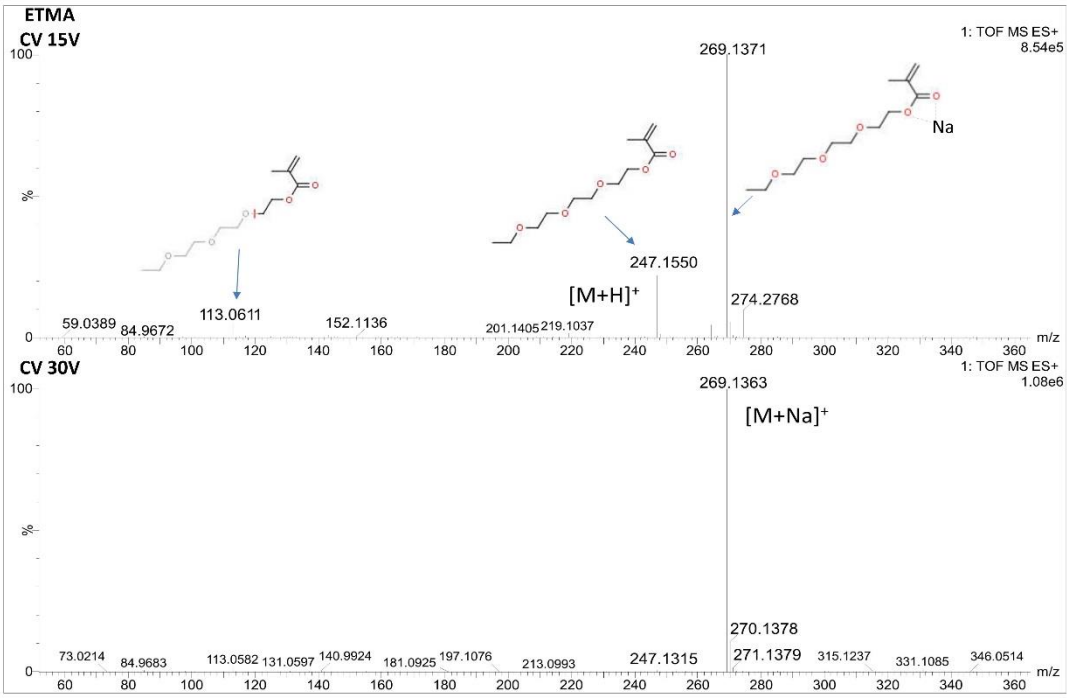

Figure 3. Identification of ethoxytriethylene glycol methacrylate using 15V and 30V cone voltage

Some works have been reported the acrylates migration from adhesive or can coatings (E. Canellas, Vera, Domeño, Alfaro, & Nerín, 2012; Paseiro-Cerrato, DeVries, & Begley, 2017). On the other hand, there is no information about the incidence of these substances in silicone for food contact material. Acrylates and propylene glycol are used in photopolymerizable resin systems for adhesives, sealants and thermoplastic polymers.

ETMA also have been recommended for use as diluents for reactive systems which also can behave as internal plasticizers and provide polymers with very low glass transition temperatures.

In view of the absence of standards to quantify the acrylates compounds, triethylene glycol was used for the semi-quantification. Tripropylene glycol (TPG), ETMA and dipropylene glycol dimethacrylate have migrated above the LOQ established (0.45 $mg.kg^{-1}$). However, only TPG is regulated for use in food contact materials and without restrictions. This one migrated in concentration varying to 2.14 up to 37.75 $mg.kg^{-1}$ (BRAZIL, 2001; European-Commision, 2011).

The high migration of propylene glycol from baby bottles requires special consideration. Propylene glycol is both eliminated by metabolism through alcohol dehydrogenase (ADH) and by renal excretion. High doses of propylene glycol can cause the nervous system (CNS) depression similar to that caused by ethanol (but about one-third as potent). Moreover, might cause acidosis through of the oxidation by ADH to form lactaldehyde, and then to lactate by aldehyde dehydrogenase (ALDH). On the other hand, infants and young children (up to the age of about four) have a lower level of ALDH/ADH activity compared with adults, so that high acute doses could lead to a propyleneglycol accumulation, cumming in adverse effects in CNS (Felter, Daston, Euling, Piersma, & Tassinari, 2015).

A comparative study reported that the mean clearance in infants born between 24-41 weeks with the weight between 630 and 3.980 g was 0.085 L/h whereas the clearance in adults ranged from 8.64–23.4 L/h. Moreover, the evidence suggests that the clearance of propylene glycol in infants is dependent on post-natal age and on body weight of the infants. For this reason, should be taken care regarding the use of propylene glycol in the manufacture of food contact materials for infants (Hardy, et al., 2017).

Regarding acrylates were detected concentrations varying from 1.08 up to 40.89 mg.kg-1. These ones have no regulation for use in FCM. Moreover, as in this case does not occur the depletion of reactive functional groups used in the polymer manufacture, genotoxicity data should be evaluated before its utilization in FCM (Bolognesi, Castoldi, Crebelli, Barthélémy, Maurici, Wölfle, et al., 2017).

9-Oxononanoic acid, 4-Vinylcyclohexene and 4-Dimethylaminobenzoic acid ethyl ester were identified in silicon baby bottles. The first one might be derived from azelaic acid which is authorized by European Regulation and can be use as catalyst (Chino & Natori, 2006; European-Commision, 2011; Shetranjiwalla, Li, Bouzidi, & Narine,

2016). 4-Vinylcyclohexene is used in the rubber manufacture and also have been reported in adhesives for FCM (Nerín, Gaspar, Vera, Canellas, Aznar, & Mercea, 2013). The last one is used as a photoinitiator for the cure of silicone resins, mainly for dental purposes (Pongprueksa, De Munck, Inokoshi, & Van Meerbeek, 2018). All compounds have migrated in concentrations below the LOQ.

Table 3. Migrants identified from silicon baby bottles

N°	Rt	Mass mensured and ion found	ΔmDa/ Δppm	Formula	Compound (Cramerclass)	CAS	LOQ ($mg.kg^{-1}$)	SML ($mg.kg^{-1}$)	Migration tests		
									1st	2nd	3rd
1	0.89	160.0971 [+H]	0.2/1.2	C7H13NO3	N-Acetylvaline (III)	96-81-1	0.43	N.A	5.58±0.57	1.50±0.09	1.30±0.02
2	1.67	157.0972 [+H]	0.7/4.5	C8H12O3	Tetrahydrofurfuryl acrylate (III)	2399-48-6	0.45	N.A	<LOQ	<LOQ	<LOQ
3	3.25	215.1259 [+Na]	0.0/0.0	C9H20O4	Tripropylene glycol	24800-44-0	0.45	60.00*	37.75±4.30	4.97±0.02	2.14±0.22
4	3.51	211.0947 [+Na]	0.1/0.5	C9H16O4	2-(2-Ethoxyethoxy)ethyl acrylate (I)	7328-17-8	0.45	N.A	<LOQ	<LOQ	<LOQ
5	3.68	287.1495 [+H]	-1.9/-6.6	C14H22O6	Triethyleneglycol dimethacrylate (I)	109-16-0	0.45	N.A	<LOQ	<LOQ	<LOQ
6	3.73	211.0946 [+Na]	0.1/0.5	C9H16O4	2-(2-Ethoxyethoxy)ethyl acrylate isomer (III)	-	0.45	N.A	<LOQ	<LOQ	<LOQ
7	4.55	269.1371 [+Na]	0.6/2.2	C12H22O5	Ethoxytriethylene glycol methacrylate (I)	39670-09-2	0.45	N.A	40.50±7.33	7.07±0.04	3.60±0.50
8	4.67	271.1526 [+Na]	-0.7/-2.5	C14H22O5	Dipropylene glycol dimethacrylate (III)	64111-89-3	0.45	N.A	10.85±1.49	0.2±0.03	<LOQ
9	4.91	173.1178 [+H]	0.0/0.0	C9H16O3	9-Oxononanoic acid (I)	2553-17-5	0.45	N.A	<LOQ	<LOQ	<LOQ
10	4.93	109.1018 [+H]	0.1/0.9	C8 H12	4-Vinylcyclohexene (I)	100-40-3	0.45	N.A	<LOQ	<LOQ	<LOQ
11	4.79	341.1610 [+H]	1.0/2.9	C17H24O7	Pentaerythritol trimethacrylate (I)	3524-66-1	0.45	N.A	<LOQ	<LOQ	<LOQ
12	4.79	267.1205 [+Na]	0.3/1.1	C12H20O5	2-(Methacryloyloxy)ethyl 6-hydroxyhexanoate (I)	85099-10-1	0.45	N.A	<LOQ	<LOQ	<LOQ
13	5.44	323.1484 [+Na]	1.3/4.0	C15H24O6	Tripropylene glycol diacrylate (III)	42978-66-5	0.45	N.A	<LOQ	<LOQ	<LOQ
14	5.69	194.1187 [+H]	0.6/3.1	C11H15NO2	4-Dimethylaminobenzoic acid ethyl ester (III)	10287-53-3	0.45	N.A	<LOQ	<LOQ	<LOQ
15	5.53	325.1635 [+Na]	0.8/2.5	C15H26O6	Glycerol tributyrate (I)	60-01-5	0.05	N.A	<LOQ	<LOQ	<LOQ
16	5.63	327.1782 [+Na]	-0.2/-0.6	C15H28O6	Glycerol tributyrate dirivative (I)	-	0.05	N.A	<LOQ	<LOQ	<LOQ
17	4.90	269.1371 [+Na]	0.6/2.2	C12H22O5	Ethoxytriethylene glycol methacrylate isomer (I)	-	0.45	N.A	40.89±6.00	1.45±0.09	1.08±0.05
18	7.36	353.2683 [+Na]	1.5/4.2	C19H38O4	1-palmitoylglycerol	542-44-9	0.05	60.00	0.11±0.01	<LOQ	<LOQ
19	7,75	381.297 [+Na]	-0.6/-1.6	C21H42O4	Glycerol stearate	621-61-4	0.05	60.00	0.14±0.01	<LOQ	<LOQ
20	8.15	338.3415 [+H]	-0.2/-0.6	C22H43NO	Erucic amide	112-84-5	0.03	60.00	0.17±0.02	0.06±0.01	<LOQ

N.A.: Not applicable. Compound without SML stablished.

*Overall migration limit

Glycerol derivatives and erucamide also were identified in silicone baby bottles. The first ones were quantified in concentrations varying from 0.11 up to 0.14 $mg.kg^{-1}$. In silicone baby bottles glycerol derivatives might be used as plasticizers (Joshi, Arendt, Berry-Walker, & Butt, 2010). However, there are no migration limits for the compounds which were quantified (BRAZIL, 2008; European-Commision, 2011). Erucamide also there is no specific migration limits and was quantified in concentrations varying from 0.06 up to 0.17 $\mu g.kg^{-1}$.

In order to evaluate the risk for babies health, the estimated daily intake (EDI) was determined with the concentration values obtained from the migration studies in silicon baby bottles. For adults, the EDI for substances from FCM is carried out taking into account that 1kg of food packaged in a cubic container of 6 dm^2 is consumed daily by a person of 60 kg body weight. Nonetheless, infants up to 16 weeks age have a higher consumption of food per kilogram body weight than adults and do not yet have a diversified nutrition. For this reason, in FCM for infants and small children, the limits should be linked to the limit of food and not to the surface area of the packaging (European-Commision, 2011). Hence, the levels of exposure have to be considered on a case-by-case basis.

In view of this were considered 2 scenarios: in the first, three new baby bottles were used to feed babies in a single day. In the second, one baby bottle was used to feed the babies in a single day. The amount of food intake was set at 200 $mL.kg^{-1}$ taking into account the hight intake of food per body weight in the first 4 months of the baby's life and 6.1 kg which is derived from the average weight of girls and boys in the 50th percentile in 3-month-old. This represent the worst case on intake for the substance quantified in the silicon baby bottle (Hardy, et al., 2017).

For determination of the mass of contaminants in the first case was considered that 3 baby bottles were prepared with milk and served for the baby until the intake of all batch. Hence, the bottles were sterilized 3 times and were taken into account the mass of the compounds found in the 1st, 2nd and 3rd contact. For the 3rd contact, only 320 mL was used since this complement the volume required by baby (1220 mL).

The second scenario do not provide the volume of milk required (1220 mL) since the baby bottle was analyzed only 3 times (total volume 450 mL). However, this approach also can provide information about the risk of the use of the utensil by mothers who use only one baby bottle for breastfeeding. For this, the average concentration

obtained in the 1st, 2nd and 3rd contact was used taking into account the volume of 150 mL for each utilization.

Since there are no experimental values reported, these values were compared with the theoretical values of Human Exposure Threshold (HET) (mg/person/day) established by the Threshold of Toxicological Concern (TTC) according to Cramer toxicity classes obtained with the Toxtree software (Ideaconsultn Ltd, 2011). The HET values are 1.8 mg/person/day for Cramer class I, 0.54 mg/person/day for Cramer class II and 0.09 mg/person/day for Cramer class III according to International Life Sciences Institute (ILSI, 2005, 2015). The Cramer values also are based on a person of 60 kg.bw and the classification for all compounds identified is shown in table 3.

The EDI for migrants without SML has shown in table 4. For this, Cramer values was corrected in order to obtain the values in kg.bw/day. The four NIAS quantified has been migrate in concentration above the established by the TTC, indicating a potential risk associated with the use of the silicone baby bottle evaluated. For N-Acetylvaline (Cramer class III), even with the use of one baby bottle per day the exposure still is more than 130-fold the threshold established.

Table 4. Exposure daily intake for NIAS from silicone baby bottle

Compounds	Cramer Limit (mg/kg bw /day) *	1st scenario (mg/kg bw /day)	2nd scenario (mg/kg bw /day)
N-Acetylvaline	0,0015	0,59	0,2
Ethoxytriethylene glycol methacrylate	0,03	3,7	1,25
Dipropylene glycol dimethacrylate	0,0015	0,81	0,271
Ethoxytriethylene glycol methacrylate isomer	0,03	3,18	1,8

* Cramer values divided by 60. I:0.03 mg/kg bw/day; II:0.009 mg/kg bw/day; III: 0,0015 mg/kg bw/day

4. Conclusion

Identification and quantification of NIAS from 12 baby bottle models made of polypropylene, Tritan® and silicone were performed. UPLC-Q-TOF-MS^E has demonstrated to be a powerful tool for this purpose. Twenty-seven compounds were identified coming from baby bottles. In polypropylene, the compounds detected were intentionally added, such as 2.2'-(tridecylimino)bis-ethanol and derivatives thereof. Moreover, clarifying agents and glycerol derivatives were also identified in PP baby bottles. In Tritan baby bottles only a slip additive was identified. All compound identified in PP and Tritan migrated below the SML established. Nonetheless, still is necessary

evaluate the safety of the use of the utensil take into account that SML was established to a person of 60 kg bw, which do not represent the infant weight. Furthermore, twenty compounds were identified in the silicon baby bottle. Most of them were non-intentionally added substances used in the silicon manufacture, such as N-Acetylvaline, acrylates and propylene glycol. Compounds not regulated were evaluated taking into account the Cramer class. Risk assessment was performed for all for compounds quantified taking into account the use of 1 or 3 new silicon baby bottles. In both cases, the migration of compounds class I and III of toxicity was higher than recommended by Cramer (0,03 and 0,0015 mg/kg bw /day, respectively). Therefore, the use of silicon baby bottle might be a risk for babies.

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CAPÍTULO IV

Caracterização de migrantes odoríferos de mamadeiras por HS-SPME-GC-O-MS

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Characterization of odorous migrants from baby bottles by HS-SPME-GC-O-MS method

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ABSTRACT

Sequential experimental designs were used to optimize an HS-SPME protocol to the odorants characterization by GC-MS and GC-O-MS analysis. For this, a Plackett–Burman design to select variables and a central composite rotatable design to define the optimal conditions were used. Derringer and Suich method was applied to simultaneously optimize the responses. The method was validated and used to characterize odorant migrants from baby bottles made of polypropylene, Tritan and silicone after the migration test with milk simulant (50% ethanol). 2,4-di-tert-butylphenol was identified in all samples. Thirty-eight odorants out of forty-five volatile compounds were identified in silicon baby bottles. Aldehydes were the main responsible for the unpleasant odour of silicone. Moreover, silicone has shown volatile organic compounds (%) slightly higher than recommended. 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.

Keywords: Plackett–Burman; Baby bottles; Silicone; Migration; Volatile organic compounds; Olfactometry

1. Introduction

In 2011, European Commission (EC) and Brazil banned the sales of BPA-containing baby bottles. This decision was based on precautionary principle, once lack robust data that supports the safety of BPA for babies and there are many uncertainties in the reports presented by World Health Organization (WHO), as well as European Food Safety Authority (BRAZIL, 2012; EFSA, 2010; European-Commission, 2011b; WHO, 2010).

Thereafter, many baby bottles manufactured with different plastic materials have appeared on the market, among them polypropylene (PP), Tritan® and silicone. However, these materials should be evaluated regarding the migration limits to ensure the safe on the use of the utensil. Since babies have lower body weight and a less developed metabolic detoxification capability, the negative effects of migrants from food contact materials (FCM) are more serious than those induced in adult organisms. For this reason, special attention must be paid to such aspects (Russo, Barbato, Cardone, Fattore, Albrizio, & Grumetto, 2018).

The analysis of migration 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 milk simulant followed by liquid-liquid extraction and GC-MS analysis. However, this technique require large volume of solvent and might present low selectivity (Matthias Onghena, Negreira, Van Hoeck, Quirynen, Van Loco, & Covaci, 2016; Simoneau, van den Eede, & Valzacchi, 2012b).

Many researches are directed towards using sample preparation fast, miniaturized and eco-friendly techniques, such as solid-phase microextraction (SPME), since this one has great potential to reduce the use of solvents. SPME has been used for identification, quantification and characterization of volatile organic compounds (VOC) followed by gas chromatography-olfactometry-mas spectrometry. Many of the VOCs are derived from degradation process and can provide 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 VOCs as well as to the formation of odorant compounds (Vera, Canellas, & Nerín, 2014; Wrona, Vera, Pezo, & Nerín, 2017).

Although many of them have been described in the literature, there are no reports on the migration of these compounds from silicone baby bottles (Denk, Velasco-Schön, & Buettner, 2017; Hopfer, Haar, Stockreiter, Sauer, & Leitner, 2012; Wiedmer, Velasco-Schön, & Buettner, 2017). On the other hand, compliance assessment of silicone food contact materials

is mostly based on the limit for volatile organic compounds set by the Bundesinstitut für Risikobewertung (BfR – German Federal Institute for Risk Assessment) at 0.5% (Bundesinstitut für Risikobewertung, 2007).

Successful implementation of SPME requires careful evaluation and optimization of factors that influence extraction efficiency (such as type of fiber 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), allow the analyst to determine which parameters should be evaluated as well as the simultaneous optimization of the factors studied. Moreover, in optimizing a high number of responses, it is very unlikely to find the same optimum region for all of them. In this case, the method proposed by Derringer and Suich (1980) becomes a valuable statistical tool, once it allows the simultaneous optimization of all responses (Arcari, Caliari, Sganzerla, & Godoy, 2017; da Silveira, Meinhart, de Souza, Teixeira Filho, & Godoy, 2016).

Up to our knowledge, there are no studies that address multivariate optimization of the extraction of VOCs and odors from baby bottles by SPME. Additionally, data regarding the sensory impact of the migration of VOCs from baby bottles are scarce. In view of this, the main objective of this work was to develop an analytical protocol for analysis of VOCs and odors from baby bottles by HS-SPME followed by GC-MS and GC-O-MS analysis. Once optimized, the method was employed in the analysis of 36 baby bottles composed by 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-triethybenzene (97%, CAS 102-25-0), 1-decanol (99%, CAS 112-30-1), 2-6-ditetrbutyl-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 purchase from Sigma. Ethanol (HPLC grade) were from Scharlau (Barcelona, Spain). Ultrapure water was obtained from a Milli-Q system (Millipore, Billerica, MA, USA).

2.2. Samples

Thirty-nine baby bottles, from 12 different models (Table S1), intended for children between 0 and 12 months were used in this work. The baby bottles were purchased from distributors located in São Paulo, Brazil and were composed of Tritan™ (n = 9), PP (n = 24) and silicon (n = 6).

2.3. Determination of VOCs (%)

VOCs were determined only in silicon baby bottles, in triplicate, according to Helling, Seifried, Fritzsche, and Simat (2012). For this, 3 g of silicon baby bottle were cut into 1 x 2 cm pieces and conditioned over dried $CaCl_2$ for 48 h. Thereafter, 3 g were weighted, added into a glass bowl and heated for 4 h at 200 ºC, conditioned in a desicator and weighted again. The loss of weight, calculated as percentage, gives the total 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 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 Brazilian Health Regulatory Agency - ANVISA (ANVISA, 2010; European-Commission, 2011a). Between the tests, the baby bottles were rinsed with ultrapure water and boiled again for 5 min.

2.5. Extraction optimization

2.5.1. Fiber choice

Ethanol can negatively influence the adsorption of other volatile compounds since compete directly with the analyte for the adhesion sites in the SPME fiber (Arcari, Caliari, Sganzerla, & Godoy, 2017). 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 (Kudlejova, Risticevic, & Vuckovic, 2012).

In view of this, previous tests were done to reduce the organic solvent content and optimize the extraction of odorous compounds from 50% ethanol. For this, 5 g of the solution obtained after the first migration test with silicon baby bottles was used. First, the content of ethanol was removed with nitrogen stream at 40 ºC followed by headspace solid-phase microextraction (HS-SPME) at 60 ºC/30 min using DVB/Car/PDMS (50/30 µm) fiber (1 cm). Second, 0.5 g of the sample was diluted 10-fold with 4.5 g of water, resulting in a sample with 5% of ethanol, followed by the extraction at 60 °C/30 min with the same fiber.

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

For this purpose, 5g of a solution in 5% ethanol containing 7 $mg.kg^{-1}$ 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 was prepared in triplicate. These substances were selected based on the representativeness of the classes of compounds which have already been reported in polypropylene, Tritan® and silicone materials (Oliveira, de Souza, Padula, & Godoy, 2017; M. Onghena, van Hoeck, Vervliet, Scippo, Simon, van Loco, et al., 2014; Simoneau, van den Eede, & Valzacchi, 2012a). After the extraction, the samples were injected in GC-MS Agilent 6890 equipped with a CTC Analytics system (Madrid, Spain).

2.5.2. Experimental design

After selecting the SPME fiber, a Plackett-Burman (PB) design was used as methodology for the selection of variables that could affect the extraction of volatile compounds from the mixture (Table S2). NaCl concentration (%), incubation time (min), extraction time

(min), extraction temperature (°C) and stirring rate (rpm) were evaluated. The significance level used to assess the effect of a variable was set to 10%, to minimize the risk of excluding any important variable of the method in the following step (Rodrigues & Iemma, 2014).

Next, a central composite rotatable design (CCRD), based on a 2^3 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 compound was determined using the technique of simultaneous response optimization proposed by Derringer and Suich (1980). This method is based on the definition 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.

Once the desirability function has been set for all the answers, they should be combined into an overall desirability, normally given by the geometric average of "n" individual desirability. The individual desirability was defined to maximize 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 applied to the extraction of odorous compounds from baby bottles.

2.6. Chromatographic analysis

2.6.1. HS-SPME-GC/MS analysis

The analysis of volatile compounds was conducted with the Agilent 6890 series GC system coupled to 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 1mL.min^{-1}. The ionization was performed by electronic impact at 70 eV. The chromatography started at 60 °C for 5 minutes, increasing 10 °C.min^{-1} to 300 °C in which it was held for 5 min.

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

On the other hand, SIM mode was used for quantification of the identified compounds. For this purpose, 2 ions were monitored: one to quantify and another to confirm

the identification. Therefore, a 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-dimethylaminobenzoate, 3,5-di-tert-butyl-4-hidroxybenzaldehyde, DIBP, methyl palmitate, each at 1000 $\mu g \cdot kg^{-1}$, was prepared in 5% ethanol. Six-point calibration curves were made, each in triplicate and analyzed following the methodology described above.

The analytical procedure was validated in terms of linear range ($\mu g.kg^{-1}$), regression coefficient (r), limit of detection (LOD), limit of quantification (LOD) 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 standards with similar chemical structure (Bratinova, S., Raffael, B., & Simoneau, C., 2009).

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 done according to the method optimized in the first step. Injection was carried out using SPME manual holder where DVB/Car/PDMS fiber was used. Odorous compounds were identified by comparison of their mass spectra simultaneously obtained by HS-SPME-GC-O/MS to those of the NIST library. Moreover, characterization of the aroma of each compound was performed and confirmed using Flavornet and Pherobase.

The aroma description was carried out by six trained sniffers, who sniffed the migrant sample eluted from the chromatographic column and characterized them by their intensities and odour. The odor strength scale:1-3 (1 was very weak, hardly recognizable 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: $\%MF = [F(\%) \times I(\%)]^{0.5}$; 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. It permits to select the most important odorous compounds from the samples. Compounds with MF (%) higher than 60% can be considered as the base of the aroma of analyzed samples. This methodology allows us to check compounds which were not detected by mass spectrometry. This fact is very important in the study of the migration of compounds

to food since the organoleptic properties of food could be affected by the packaging (Vera, Canellas, & Nerín, 2014; Wrona, Vera, Pezo, & Nerín, 2017).

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 released by baby bottles into food simulant. These ones were identified and quantified by GC-MS. Additionally, the volatiles were characterized regarding the odour by HS-SPME-GC-O/MS. The baby bottles samples under study were manufactured with polypropylene, Tritan and silicone.

3.1. Determination of VOC (%)

Compliance assessment of silicone food contact materials is determined mainly by the limit of VOCs (%). Some studies were previously carried out focused on the amount of volatile organic compounds 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 silicon baby bottles, the release of VOCs was slightly above (0,54±0,02) the limit established by Bundesinstitut für Risikobewertung (2007). For this reason, it is important to identify and quantify such compounds. Hence, it will be possible to evaluate the impact which migration could lead both the baby health and the quality of food.

3.2. Fiber choice

Previous to the selection of fiber, two approaches were carried out to reduce the effect of ethanol in the volatile organic compounds extraction. The first one (reduction with nitrogen stream) 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, favoring a greater extraction of the compounds of interest. Thus, dilution was the best alternative for SPME analysis (Arcari, Caliari, Sganzerla, & Godoy, 2017).

Next, the selection of the fiber 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 commercially available. Depending on the extraction

phase polarity and thickness, a given class of analytes may be favored during extraction (Souza Silva, Saboia, Jorge, Hoffmann, dos Santos Isaias, Soares, et al., 2017).

In this study, PDMS (100 µm), DVB/Car/PDMS (50/30 µm) and PDMS/DVB (65 µm) (Supelco, Bellefonte, USA) were evaluated for their suitability towards the determination of the classes of compounds of interest. These ones present different mechanisms of extraction. In liquid SPME coatings (PDMS) 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 SPME 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 Walls interactions. Consequently, shorter extraction times are required, since diffusion of analytes into the bulk of adsorption-type coatings does not occur (Risticevic, Vuckovic, Lord, & Pawliszyn, 2012; Souza Silva, et al., 2017).

Probably, for this reason, the PDMS fiber has shown the lower extractive capacity (Figure 1). Since all fibers were exposed for the same time, due to 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 fiber. Fiber 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 (Risticevic, Vuckovic, Lord, & Pawliszyn, 2012).

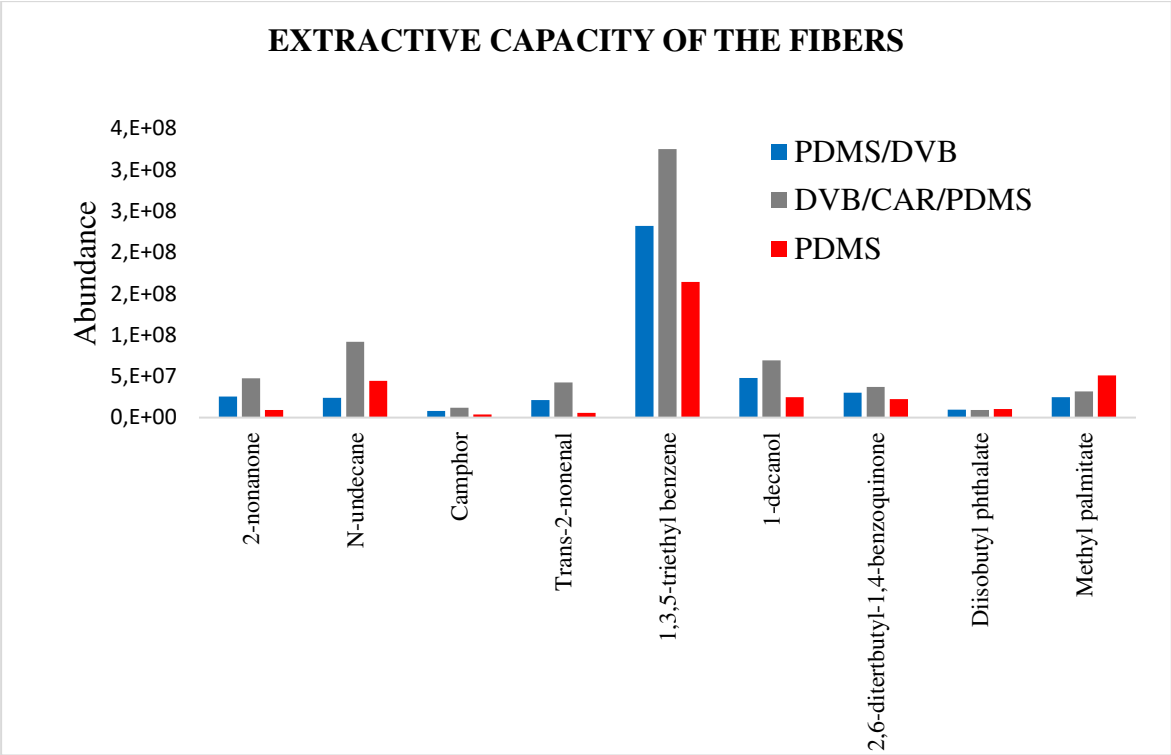

Figure 1. Effect of coating type on extraction capacity for the mix compound by HS SPME-GC/MS

PDMS/DVB showed better extraction than PDMS fiber. However, DVB/Car/PDMS has shown best results for the extraction of all compounds except for ethyl palmitate and diisobutyl phthalate. This fiber combines the properties of solid (Car and DVB) and liquid coatings which permits the extraction of a wide range of molecules of different sizes. Solid coatings can retain an analyte that is about half the size of its diameter. Carboxen is comprised of many micropores, with opening diameters 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, is possible to get the efficient extraction of compounds with molecular weight ranging from 40 to 275 Da (Arcari, Caliari, Sganzerla, & Godoy, 2017; Pawliszyn, 2012; Souza Silva, et al., 2017).

3.3. Method optimization

3.3.1. Plackett-Burman design

PB is a saturated design that assumes there are no interactions between the different extraction variables, with each variable being tested at two experimental levels: a high level (+) and a low level (−)(Gionfriddo, Souza-Silva, & Pawliszyn, 2015). NaCl concentration (%), incubation time (min), extraction time (min), extraction temperature (°C) and stirring rate (rpm) were evaluated regarding the impact in 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 ($p > 0.1$) (Figure 2).

The NaCl concentration has shown positive effect for the compounds with low molecular weight. On the other hand, for methyl palmitate the NaCl concentration had negative effect. Salting 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 (Pawliszyn, 2012; Souza-Silva & Pawliszyn, 2015).

SPME is an equilibrium extraction technique, and as such, optimization of extraction time is a critical factor to ensure method efficiency once when the extraction time increases, the extraction efficiency also increases, until reaching a maximum (equilibrium). The extraction time has shown 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 (Souza-Silva & Pawliszyn, 2015; Souza Silva, et al., 2017). 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 positive for 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 favor 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 (Kfm) between the fiber coating and the sample. However, this will be a problem only in extractions at equilibrium conditions (Pawliszyn, 2012; Souza-Silva & Pawliszyn, 2015).

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 visualization of significant effects (Rodrigues & Iemma, 2014).

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

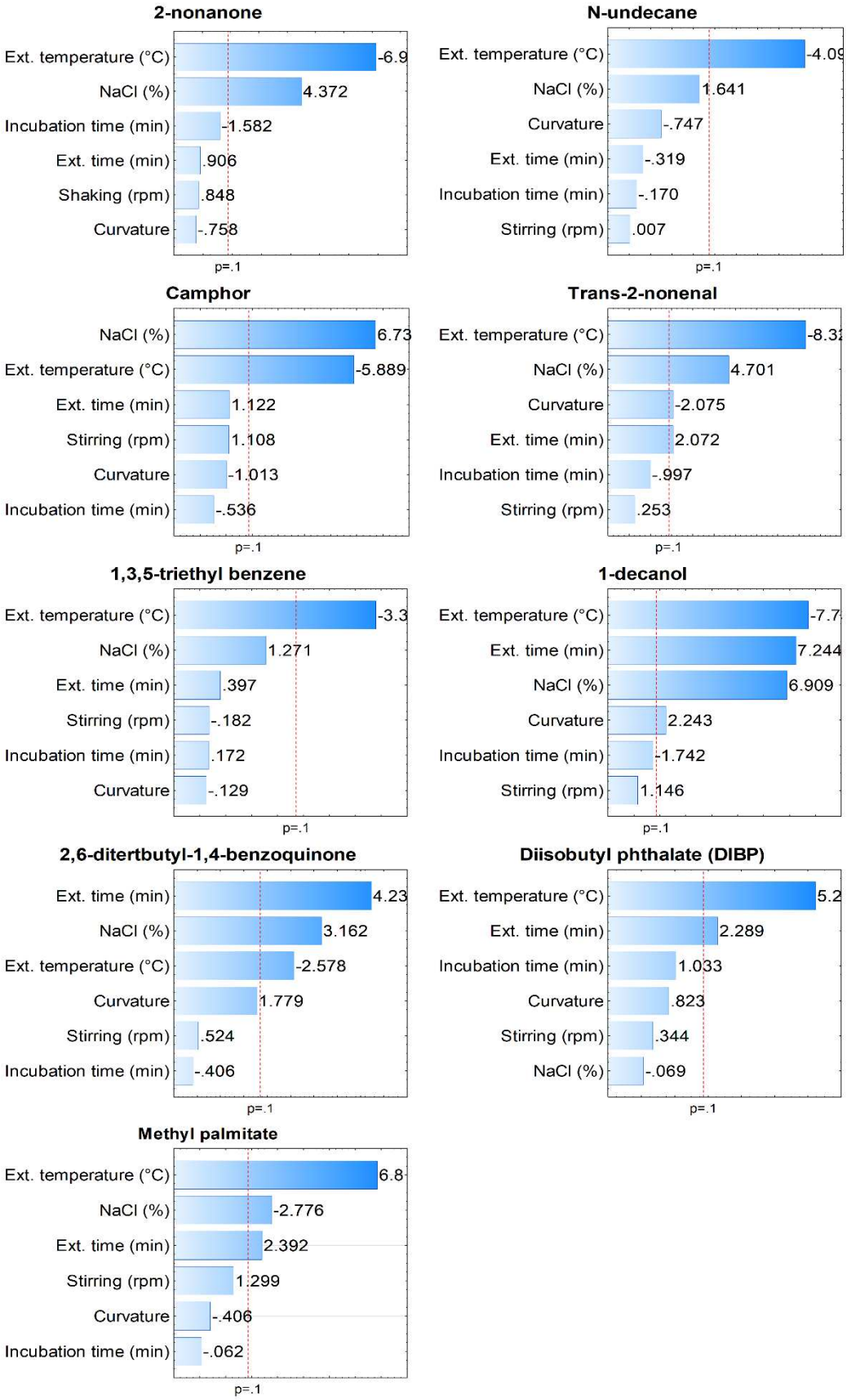

Figure 2. Pareto diagram for each compound present in the mix

3.3.2. Central composite rotatable design (CCRD)

After the selection of 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 less 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 to 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 (Kfs) of the analyte between the fiber coating and headspace, generating models with lack of fit. On the other hand, the reduction of extraction temperature could compromise the extraction of compounds with higher molecular weight. This situation is common in the development of extraction methods which multiresidue covering a broad range of polarities. In face of this, the focus must be the overall quality of data, keeping in mind method sensitivity (Gionfriddo, Souza-Silva, & Pawliszyn, 2015; Pawliszyn, 2012).

Table 1. Experimental domain aplied to a central composite rotatable design (CCRD)

	Variables	-1,68	-1	0	1	1,68
X_1	NaCl (%)	3.34	6.64	11.50	16.36	19.66
X_2	Extraction time (min.)	5.00	13.10	25.00	36.90	45.00
X_3	Extraction temperature (°C)	50.00	55.06	62.50	69.94	75.00

For the other compounds, the optimization showed that the linear and quadratic effects of the studied factors can affect the extraction. In the face of several responses and different predicted extraction conditions, the Derringer and Suich (1980) tool was used for simultaneous response optimization 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 ºC 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.

Table 2. Predicted values after optimization by the Derringer and Suich tool

Variable	Predicted values*		Observed values*
	Inferior Lim.	Superior Lim.	(n=3)
NaCl (%)	-1	1	-
Extraction time	-1	1	-
Extraction Temperature	-1	1	-
2-nonanone	19.96	46.65	29.36 ± 3.26
N-undecane	26.24	66.76	30.98 ± 1.70
Camphor	7.13	15.09	9.86 ± 0.89
Trans-2-nonenal	31.56	73.85	33.98 ± 2.82
1,3,5-triethyl benzene	101.17	298.20	139.91 ± 13.05
1-decanol	102.01	216.15	107.93 ± 10.38
2,6-ditertbutyl-1,4-benzoquinone	55.67	108.18	52.94 ± 4.56
Diisobutyl phthalate	11.16	95.11	59.02 ± 4.99
Methyl palmitate	84.19	227.74	151.27 ± 11.95

* Values multiplied by 10^6

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

The optimized conditions for HS-SPME were used in the extraction, identification and quantification of odorous compounds from baby bottles by GC-MS and GC-O-MS. This approach allowed us to evaluate the impact of the migrants from baby bottles could have on the food, as well as the compliance regarding the specific migration limits (SML) established by ANVISA.

Forty-five compounds were identified in PP, Tritan and silicone baby bottles (Table S4). Most of them were coming from the silicone baby bottles (Figure 3). The compounds were quantified after the method validation using each pure standard or a similar compound in case

the standard was not available (Table S5). The LOD ranged from 7 µg.kg^{-1} to 100 µg.kg^{-1}, and LOQ was between 22 µg.kg^{-1} and 295 µg.kg^{-1}, with RSD (%) below 11% and r^2 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 LOQ only in the samples 4 and 9. For this last one, 2,4-di-tert-butylphenol migrated only in the first contact (55.50 ± 5.03 µg.kg^{-1}). On the other hand, in silicon baby bottle this compound migrated in the 3 sequential tests, with concentration varying from 50.62 µg.kg^{-1} up to 543.16 µg.kg^{-1}. This compound might be formed through of antioxidants degradation which are widely used in polyolefins. Moreover, it has been already reported in baby bottles, but it is not included in the positive lists of ANVISA or European Community (BRAZIL, 2008; European-Commission, 2011a; Oliveira, de Souza, Padula, & Godoy, 2017; Simoneau, van den Eede, & Valzacchi, 2012b).

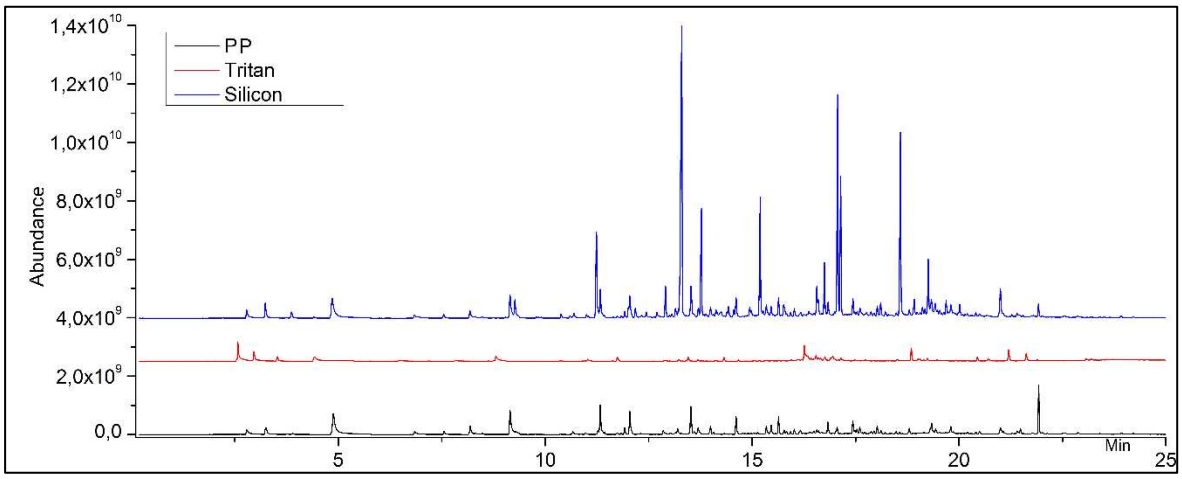

Figure 3. Chromatographic profile of polypropylene, Tritan and silicone samples obtained by HS-SPME-GC-MS

Saturated and unsaturated aldehydes such as octanal, nonanal, decanal, (E)-2-decenal, 2-undecenal and tetradecanal, were also quantified in silicon baby bottles. Octanal and decanal migrated in the first and second contact reaching concentrations of 1840.47 µg.kg^{-1} and 1037.54 µg.kg^{-1}, 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^{-1}. Nonanal already has been reported as the most abundant odorous compound in LDPE (Wrona, Vera, Pezo, & Nerín, 2017). Since there is no information about the impact of migration of these volatile compounds from silicone, only the silicone bottle was submitted to GC-O-MS analysis.

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

As observed in the GC-MS analysis, aldehydes were the main compounds with odour detected in the silicon 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 tetradecenal presented MF (%) varying between 62.36(%) up to 91.29 (%). Sniffers characterized 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 (Denk, Velasco-Schön, & Buettner, 2017; Hopfer, Haar, Stockreiter, Sauer, & Leitner, 2012; Wiedmer, Velasco-Schön, & Buettner, 2017; Wrona, Vera, Pezo, & Nerín, 2017).

The saturated or unsaturated aldehydes are not directly used in the process and manufacture of silicone. However, they can be formed after the oxidation of polyunsaturated fatty acids used in previous steps of the silicon elastomer processing. It occurs after activation of the hydrosilanes, which undergo addition across the carbon-carbon multiple bonds, using a transition metal complex, such as Pt(0), as catalyst. The bond C=C found in the polyunsaturated fatty acids is the main place of activation once it is higher reactive than that of internal ones (C-C) (Galià, de Espinosa, Ronda, Lligadas, & Cádiz, 2010).

On the other hand, the silicon elastomer might be produced from liquid silicon (PDMS). For this, the liquid silicon is subjected to the cure process using a photoinitiator. The most used ones are benzophenone (MF% 74.61) and some derivatives such as 2-hydroxy-2-methyl-1-phenyl-propanone (Darocure 173) (MF% 76.45). For this, benzophenone is subjected to UV radiation and hemolytic division generating many peroxyl radicals responsible for the elastomer cure step. Nonetheless, peroxyl radicals are propagator of lipid oxidation, increasing the aldehyde generation (Mayer & Breuer, 2006; D. Wang, Klein, & Mejía, 2017).

The odour compounds from benzophenone and derivatives have been also described in the GC-O-MS analysis. Interestingly, resin, pigment and bullet paper were described by sniffers in the benzophenone characterization. Sensory descriptions are done in function of many factors, among them the personal acuity of the sniffer to describe familiar odours. Since the olfactometric analysis was performed by specialists in food packaging, the use of packaging materials to describe the odour from another packaging material could be expected. In this case, benzophenone has been used in UV curable printing inks widely applied

in bullet paper. On the other hand, the flower, spicy, sweet odour from 2-Hydroxy-iso-butyrophenone might be associated to phenyl ketons, such as benzophenone, which was describes with slightly sweet geranium-like odor (Surburg & Panten, 2016). Although this compound has shown an apparently pleasant odor, the efforts still towards reducing the unpleasant odour of cured formulations, such as spicy. Unpleasant odour is caused by peroxide decomposition products such as cumyl peroxides generated from benzophenone and derivatives (D. Wang, Klein, & Mejía, 2017).

Table 3. Odorous compounds identified in silicone baby bottle by HS-SMPE-GC-O-MS

Nº	RT	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	sintetic, herbal, dry, almonds	1178	1178	89,81
13	12.43	1-Nonanol	shoe polish, resin	1193	1190	52,70
14	12.45	p-Menthan-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, unpleanant, 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, lavander, 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, lavander	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, lavander	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	cleaner product	1976	1970	33,33

RT: retention time

LTPRI Cal: linear temperature programmed retention índex calculated

LTPRI Tab: linear temperature programmed retention índex tabelated

M F: Modified frequency

1-hepten-3-one, 2-nonanone and 3-nonen-2-one have presented FM (%) varying between 60.18 to 76.38%. Ketones have been reported as catalyst inhibitors for silicone curing. These compounds are formed also as oxidation products in polyolefins, originating from the formation and decomposition of hydroperoxide (Hopfer, Haar, Stockreiter, Sauer, & Leitner, 2012; D. Wang, Klein, & Mejía, 2017). The descriptions of ketones are in consonance with the reported by other researchers. The mushroom odour from 1-hepten-3-one was reported in wine cork stoppers (Culleré, Cacho, & Ferreira, 2009), while 2-nonanone was reported as fruity/floral notes in wine (X.-j. Wang, Tao, Wu, An, & Yue, 2017). Moreover, 3-nonen-2-one, one of the main odours in strawberry vinegar, was described as river water/vapour (Ubeda, Callejón, Troncoso, Moreno-Rojas, Peña, & Morales, 2016).

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) already have been reported (Gallagher, Dalton, Sitvarin, & Preti, 2008; Savary, Morel, Picard, & Grisel, 2016; The good scents company, 2018).

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 (D. Wang, Klein, & Mejía, 2017).

Carvone and 1-methoxy-4-methylbicyclo[2.2.2]octane were detected with MF (%) of 83.7 and 62.3. These ones are components of the oils extracted from *Mentha spicate* and *Cynara scolymus L.* Surprisingly, these compounds might be used for elastomer synthesis after ring-opening transesterification polymerization followed by postpolymerization functionalization, which might clarify its detection (Saucier, Polidoro, dos Santos, Schneider, Caramão, & Jacques, 2014; Yang, Lee, Choi, Seo, Kim, Kim, et al., 2015).

The result of the GC-O-MS analysis in silicone baby bottles is extremely worrying. The German Federal Institute for Risk Assessment recommends avoiding the use of FCM with intensive smelling. In general, smells related to material emissions have hitherto been rarely investigated on a molecular basis and little is known about the exposure of customers to such substances. Moreover, there are no data on odorous contaminants in baby bottles (Wiedmer, Velasco-Schön, & Buettner, 2017).

4. Conclusion

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

5. Acknowledgements

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<Supplementary Data>

Development of an HS-SPME method for characterization of odorants from baby bottles

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Table S1. Composition of the baby bottles evaluated

	Material	Color	Volume (mL)
Sample 1	PP	Blue	70
Sample 2	PP	Pink	70
Sample 3	Tri	Transparent and printed with blue drawings	80
Sample 4	Sil	Transparent	150
Sample 5	Tri	Transparent and printed with blue drawings	80
Sample 6	PP	Transparent and printed with purple drawings	120
Sample 7	Tri	Transparent and printed with pink drawings	80
Sample 8	PP	Transparent and printed with pink drawings	120
Sample 9	PP	Transparent and printed with blue drawings	70
Sample 10	PP	Transparent and printed with green drawings	70
Sample 11	PP	Transparent and printed with blue drawings	70
Sample 12	PP	Transparent and printed with green drawings	70

PP: polypropylene; Tri: Tritan; Sil: silicon

Table S2. Factor levels and experimental domain applied to the Plackett-Burman experimental design.

Variables	-1	0	1
NaCl concentration (%)	5,0	15,0	25,0
Incubation time (min)	5,0	12,5	20,0
Extraction time (min)	15,0	37,5	60,0
Extraction temperature (° C)	50,0	67,5	85,0
Stirring rate (rpm)	300,0	500,0	700,0

Table S3. Statistical model coefficients ($p < 0.05$)

Compuestos	Indicated model	Regression significance	Model fit	Coefficients								
		P < 0.05	P > 0.05	$X_1(L)$	$X_1(C)$	$X_2(L)$	$X_2(C)$	$X_3(L)$	$X_3(C)$	X_1X_2	X_1X_3	X_2X_3
2-nonanone	Quadratic	0.04	0.074	2341614	-2185567	1270895	-3095252	-4366654	-2197785	2702704		2870950
N-undecane	Linear	0.001	0.278	-10981567	-	-	-	-	-	-	-	-
Camphor	Linear	0,001	0.189	1909623	-	-	-	-	-	-	-	-
Trans-2-nonenal [A]	Quadratic	0.102	0.057	2983458	-3366076	-	-4485070	-4080811	-3476044	3458651	-	-
1,3,5-triethylbenzene	Quadratic	0.036	0.098	-	-	-	-	-22416110	-20487479	-	-	-
1-decanol	Quadratic	0.006	0.128	16916782	-14388563	14221156	-18771576	-	-14268841	-	-	-
2,6-ditertbutyl-1,4-benzoquinone [B]	Quadratic	0.056	0.012	2693585	-5282707	9162739	-9931776	1878151	-7823604	3439320	-2016110	-2861550
DIBP	Linear	0.001	0.217	12704248	-	19289566	-	25238850	-	-	-	-
Ethyl palmitate	Linear	0.001	0.202	-19700486		57930261		74675993		-	-	

A: Regression model not significant ($p > 0.05$)

B: Regression model with lack of fit ($p < 0.05$)

Table S4. Volatile compounds identified and quantified in baby bottles by HS-SPME-GC-MS

Nº	R.T.	Compounds	LTPRI Cal	LTPRI Tab	Concentration (µg/kg)			Samples
					Contact			
					1st	2nd	3rd	
1	8.10	1-ethyl-3-methyl-Benzene [11]	< 1100	958*		< LOQ		7
2	8.38	(E)-2-Heptenal [4]	< 1100	959		< LOQ		4
3	8.79	1,3,5-trimethyl-Benzene [11]	< 1100	975		< LOQ		5, 7, 8
4	9.29	Octanal	< 1100	1001	1840.47±181.29	1632.05±66.99	< LOQ	4
5	9.93	Eucalyptol	< 1100	1038		< LOQ		4
6	10.47	(E)-2-Octenal [4]	< 1100	1062		< LOQ		4
7	10.83	1-Octanol [6]	< 1100	1070		< LOQ		4
8	11.12	2-Nonanona [1]	< 1100	1091		< LOQ		4
9	11.32	Nonanal [2]	1107	1102	5979.02±104.87	4838.53±212.18	782.12±54.97	4
10	11.7	n-Octyl formate [6]	1129	1128		< LOQ		4
11	11.99	3-Nonen-2-one [1]	1142	1141		< LOQ		4
12	12.21	Trans-2-nonenal	1162	1162		< LOQ		4
13	12.43	1-Nonanol [6]	1172	1172		< LOQ		4
14	12.45	p-Menthan-1-ol [5]	1178	1178		< LOQ		4
15	12.73	2-Decanone [1]	1193	1190		< LOQ		4
16	12.88	Estragole [12]	1203	1186		< LOQ		4
17	12.97	Decanal [2]	1211	1209	1037.54±28.72	893.11±39.40	< LOQ	4
18	13.11	(E,E)-2,4-Dodecadienal [4]	1210	1208		< LOQ		4
19	13.45	Hexylbenzene [16]	1242	1254		< LOQ		8
20	13.58	Carvone [5]	1251	1255		< LOQ		4
21	13.76	(E)-2-Decenal [4]	1263	1263	245.09±22.78	294.43±4.02	143.95±7.77	4
22	14.08	2-Hydroxy-iso-butyrophenone [11]	1285	1278		< LOQ		4
23	14.26	2,4-Decadienol [6]	1295	1295		< LOQ		4

24	14.42	Undecanal [2]	1309	1309		< LOQ		4
25	14.58	(E,E)-2,4-Decadienal [4]	1318	1318		< LOQ		4
26	14.89	4-tert-Butylcyclohexyl acetate [5]	1341	1339		< LOQ		4
27	15.05	1-Methoxy-4-methylbicyclo[2.2.2]octane [2]	1341	1346		< LOQ		4
28	15.10	Isobornyl acrylate	1360	1367		< LOQ		8, 9, 10, 11, 12
29	15.25	2-Undecenal [4]	1364	1361	1954,07±96.64	2632.54±256.46	1506.08±60,38	4
30	15.41	3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	1382	1381		< LOQ		4
31	15.99	Dodecanal [2]	1428	1420		< LOQ		5, 7, 8, 4
32	16.33	2,6-Di-tert-butylbenzoquinone [10]	1455	1458		< LOQ		2, 6, 7, 8
33	16.63	1-Dodecanol	1479	1478		< LOQ		5, 4
34	17.09	2,4-Di-tert-butylphenol	1516	1515	543.16±19.69	94.53±5.72	50.62±3.27	All
35	17.44	2-ethoxy naphthalene [16]	1540	1520		< LOQ		4
36	17.82	6-Tetradecanone [1]	1579	1549		< LOQ		4
37	18.51	Tetradecanal [2]	1616	1614	1051.22±68.95	678.97±11.20	639.59±34.91	4
38	18.68	Benzophenone	1649	1655*		< LOQ		4
39	19.12	1-Tetradecanol [6]	1678	1676		< LOQ		4
40	19.38	4-(1,1,3,3-tetramethylbutyl)-phenol [9]	1710	1687		< LOQ		4
41	20.10	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	1787	1774		< LOQ		4
42	21.02	Diisobutyl phthalate	1879	1871		< LOQ		1, 2, 4, 5, 6, 7
43	21.23	Palmitato de metila	1901	1913		< LOQ		1, 2, 3, 5, 6, 7, 8
44	21.96	Dibutyl phthalate [8]	1976	1970		< LOQ		1, 2, 3, 4, 5, 6, 7
45	22.16	Hexadecanoic acid, ethyl ester [3]	1996	1996		< LOQ		1

* From: http://www.pherobase.com
Quantified with:

1 - 4-heptanone
2 - Octanal
3 – Ethyl palmitate
4 - Trans-2-nonenal
5 - Isomentol
6 - 1-decanol
7 - Isobornyl acrylate
8 - Diisobutyl phthalate
9 - 2,4-Di-tert-butil phenol
10 - Hidroxitolueno butilado
11 - Benzophenone
12 - Ethyl 4-dimethylaminobenzoate
13 - 3,5-di-tert-Butil-4-hidroxybenzaldehido

Table S5. Analytical parameters of the quantification method by HS-SPME-GC-MS

Compound	Quantification ion	Qualifier íon	r^2	LOD ($\mu g.kg^{-1}$)	LOQ ($\mu g.kg^{-1}$)	%RSD
4-heptanone	71	43	0.985	30	100	4.31
Octanal	43	84	0.997	100	295	1.88
Trans-2-nonenal	41	55	0.995	60	175	8.30
Isomenthol	71	81	0.997	12	32	2.90
1-decanol	70	55	0.989	9	28	9.50
Isobornyl acrylate	55	95	0.984	7	22	10.70
2,4-di-tert-butyl phenol	191	206	0.994	7	22	10.16
BHT	205	220	0.983	9	28	3.20
Benzophnone	105	182	0.990	7	22	9.22
Ethyl 4-dimethylamino benzoate	148	193	0.994	35	100	3.01
3,5-di-tert-butyl-4-hydroxy benzaldehyde	219	234	0.992	7	22	7.14
Diisobutyl phthalate	149	223	0.991	7	22	8.48
Ethyl palmitate	74	270	0.997	35	100	5.71

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DISCUSSÃO GERAL

Neste trabalho mamadeiras de polipropileno, Tritan® e silicone, foram avaliadas no tocante à migração de substâncias para simulantes de alimento. As avaliações determinações nas quais as mamadeiras foram submetidas podem ser visualizadas na Figura I. Os ensaios de migração foram realizados seguindo as recomendações da Agência Nacional de Vigilância Sanitária (ANVISA).

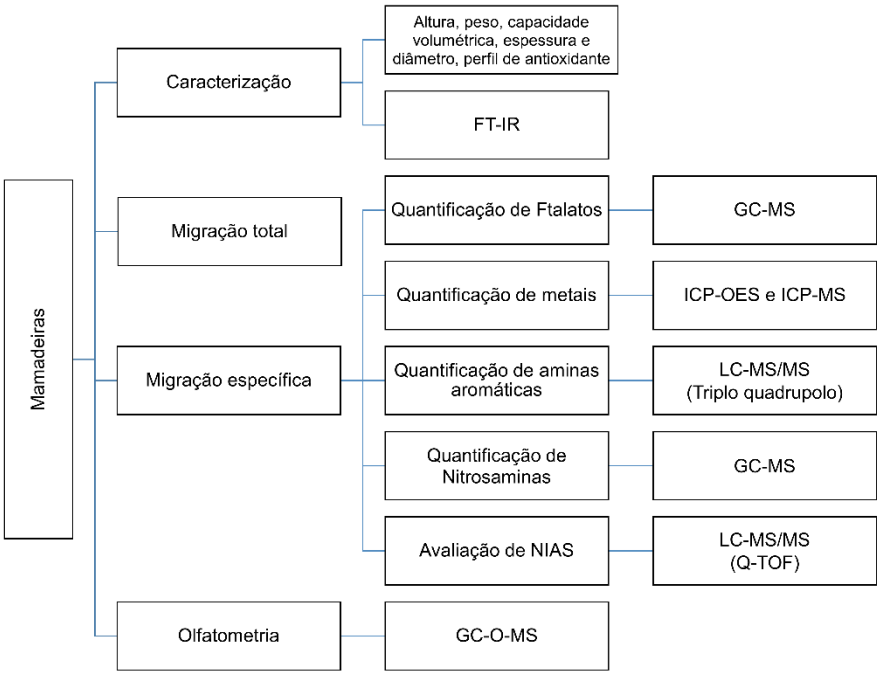

Figura I. Determinações executadas no trabalho.

Em relação à caracterização, somente duas amostras apresentaram medidas de espessura com desvios inferior a 10%. A falta de homogeneidade da espessura pode afetar o desempenho mecânico das mamadeiras, uma vez que a área com menor espessura pode resultar em rachaduras durante o manuseio ou eventual queda. Por outro lado, todos os materiais declarados na composição do produto foram confirmados no FT-IR.

O ensaio de migração total é uma das análises recomendadas para avaliação de materiais de contato com alimentos, como as mamadeiras. Deste modo, através desse ensaio é possível prevenir a contaminação excessiva ou uma mudança inaceitável na composição dos alimentos em decorrência da migração de componentes da embalagem. Em mamadeiras não foram detectadas migrações acima do preconizado pela ANVISA (LMT $\leq$ 50 $mg.kg^{-1}$).

O mesmo não ocorreu nos ensaios de migração específica. Foram detectadas não conformidades nos ensaios de migração específica de ftalatos. Em todas as amostras foi detectada a migração de dibutil ftalato(DBP). Na amostra de silicone foram detectadas migrações em altas concentrações de DBP e di-isobutil ftalato (DIBP). Para DBP a migração foi superior ao limite (300 $\mu g.kg^{-1}$) estabelecido pela ANVISA. Já o DIBP não está regulamentado para uso em materiais de contato com alimentos. Di-etil-hexil ftalato e butil benzil ftalato também foram detectados nas amostras de silicone. Uma avaliação de risco mostrou que há potencial de efeito adverso associado ao uso das mamadeiras de silicone. Por outro lado, a simples incidência desses contaminantes em mamadeiras já é um fator extremamente preocupante. Tanto a Europa quanto os Estados Unidos possuem regulamentos banindo o uso de ftalatos em materiais destinados às crianças. Os regulamentos se aplicam aos compostos detectados neste estudo (DBP, DIBP, BBP e DEHP) e outros ftalatos incidentes em materiais destinados ao uso de crianças. No Brasil ainda não temos nenhuma posição no tocante a essas proibições.

Nos bicos foram detectados a migração de zinco, bário e chumbo. Mesmo com migrações abaixo dos limites preconizados pela ANVISA, ainda é necessário o monitoramento de metais em bicos dada a neurotoxicidade associada ao chumbo, principalmente para bebês.

Em contrapartida, mesmo com a ampla utilização de tinta de impressão e pigmento em mamadeiras, não foi detectada a incidência de aminas aromáticas nos copos dos utensílios. Também não foi detectada N-nitrosamina nos bicos de borracha sintética (silicone).

No *screening* de substâncias não voláteis foram detectadas tanto substâncias adicionadas intencionalmente quanto não intencionalmente (*intentionally added substances - IAS, non-intentionally added substances - NIAS*). As IAS foram detectadas nas mamadeiras de polipropileno e Tritan. Entretanto migraram abaixo dos limites de migração específica. Por outro lado, em mamadeiras de silicone, 17 NIAS foram detectadas. Boa parte eram derivados de acrilatos e propileno glicol utilizado no processamento do silicone. Como essas substâncias não são regulamentadas para uso em mamadeiras, o Limiar de Preocupação Toxicológica (*Threshold of Toxicological Concern - TTC*) foi utilizado para avaliar a possiblidade de efeitos adversos à saúde resultante da migração. Mais uma vez os resultados indicam possibilidade de efeitos adversos associados ao uso da mamadeira de silicone. O resultado é decorrente da ingestão diária superior ao recomendado para N-acetilvalina e dipropileno glicol dimetacrilato.

Por fim o *screening* de migrantes voláteis mostrou que há uma gama complexa de compostos liberados das mamadeiras de silicone. Os resultados do percentual de compostos orgânicos voláteis (*volatile organic compounds – VOC*) foi ligeiramente superior (0,54%) ao recomendado (0,50%). A geração de altos percentuais de VOC está diretamente relacionada ao uso de N-acetilvalina no processo de cura do silicone. Por outro lado, altos percentuais de VOC também podem indicar um processo de cura incompleto.

Quarenta e cinco compostos foram identificados nas mamadeiras dos quais 84% eram provenientes dos utensílios de silicone. Os aldeídos foram os principais compostos responsáveis pelo odor desagradável da mamadeira de silicone. Odores de cetonas, derivados de benzofenona e álcoois também foram identificados. Isso enfatiza a importância da análise olfatométrica aplicada a materiais de contato com alimentos, uma vez que compostos odoríferos podem afetar as propriedades dos alimentos e levar à rejeição do produto, mesmo em baixíssimas concentrações.

CONCLUSÃO GERAL

Mamadeiras de polipropileno, Tritan® e silicone foram submetidas a análises instrumentais a fim de avaliar a conformidade dos utensílios no tocante a migração. Todos os materiais apresentaram conformidade em relação a migração de metais, nitrosaminas e aminas aromáticas. Por outro lado, a migração de ftalatos foi detectada em todas as amostras. Entretanto, somente a mamadeira de silicone liberou concentrações acima do preconizado pela ANVISA. Na avaliação de NIAS mais uma vez foi detectada não conformidade nas amostras de silicone, que apresentaram a migração de substâncias não listadas para uso em materiais de contato com alimentos. Por fim, uma complexa mistura de aromas foi detectada nas mamadeiras de silicone, os quais foram responsáveis pelo odor forte e desagradável do utensílio.

De modo geral, mesmo com limites de migração abaixo do preconizado pela ANVISA, ainda é necessário adequar os utensílios de polipropileno e Tritan para restringir a utilização de ftalatos em materiais destinados a crianças. Por outro lado, no tocante ao silicone, deve ser reavaliada a utilização do material para fabricação de materiais destinados a crianças.

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