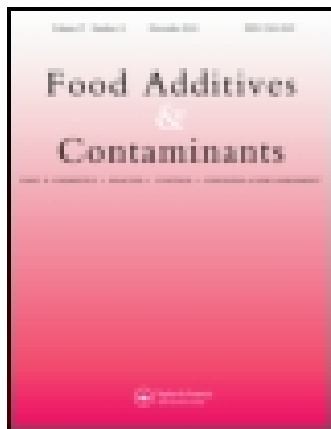


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Food Additives & Contaminants: Part A

Publication details, including instructions for authors and subscription information:
<http://www.tandfonline.com/loi/tfac20>

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Accepted author version posted online: 10 Jul 2015. Published online: 06 Aug 2015.



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To cite this article: Adriana Pavesi Ariseto, Priscila Francisca Corrêa Marcolino & Eduardo Vicente (2015): 3-Monochloropropane-1,2-diol fatty acid esters in commercial deep-fat fried foods, Food Additives & Contaminants: Part A, DOI: [10.1080/19440049.2015.1071498](https://doi.org/10.1080/19440049.2015.1071498)

To link to this article: <http://dx.doi.org/10.1080/19440049.2015.1071498>

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3-Monochloropropane-1,2-diol fatty acid esters in commercial deep-fat fried foods

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(Received 20 May 2015; accepted 3 July 2015)

Levels of 3-monochloropropane-1,2-diol (3-MCPD) fatty acid esters were evaluated in commercial deep-fat fried foods from the Brazilian market using a GC-MS method preceded by acid-catalysed methanolysis. A limit of detection of 0.04 mg kg⁻¹, a limit of quantitation of 0.08 mg kg⁻¹, mean recoveries varying from 82% to 92%, and coefficients of variation ranging from 2.5% to 5.0% for repeatability and from 3.6% to 6.5% for within-laboratory reproducibility were obtained during in-house validation. The levels of the compounds in the evaluated samples, expressed as free 3-MCPD equivalent, ranged from not detected to 0.99 mg kg⁻¹, and the highest concentrations were observed in samples of chopped onion and garlic. A preliminary estimation of 3-MCPD intake using these occurrence data suggested low risks to human health, but a potential concern may arise in particular cases of consumers of fried food.

Keywords: 3-MCPD; frying; oil refining; food safety; contaminants

Introduction

Deep-fat frying is a popular and widely used method to prepare foods. It is a cheap and fast process of simultaneous heat and mass transfer that changes the sensory and nutritional characteristics as a result of complex interactions between food and oil (Ziaifar et al. 2008). However, the amount of fat in fried foods is significantly increased, reaching in some cases a third of the total food product by weight, which could pose a risk to health since the high consumption of fat-rich foods has been linked to several adverse effects, including obesity and coronary heart diseases (Mellema 2003).

Moreover, it has been known since 2006 that high concentrations of 3-monochloropropane-1,2-diol (3-MCPD) fatty acid esters can be found in edible oils and fats (Zelinková et al. 2006; Kuhlmann 2011; MacMahon et al. 2013; Arisseto et al. 2014), including those used in deep-fat frying, which has increased the concern related to the consumption of fried foods. The possible hydrolysis of these esters by enzymes in the human gut releases free 3-MCPD (Seefelder et al. 2008; Abraham et al. 2013), a compound that has been associated with nephrotoxicity and the ability to affect male fertility (FAO/WHO 2002), and is classified as a possible human carcinogen (group 2B) by the IARC in view of its potential to induce cancer in experiments with animals (IARC 2012).

The highest concentrations of 3-MCPD esters have been frequently reported in palm oil (*Elaeis guineensis* Jacq.) and derived products, for which levels up to 10 mg kg⁻¹ have already been observed (Karšulinová et al. 2007; Kuhlmann 2011; MacMahon et al. 2013;

Yamazaki et al. 2013; Arisseto et al. 2014). The formation of these compounds can occur from triglycerides and species of organic and inorganic chlorine resulting from endogenous metabolism of the plant and environmental contamination (Nagy et al. 2011; Destailats et al. 2012). The majority of 3-MCPD esters are formed during the deodorisation step of the refining process, in which temperatures above 200°C are used (Franke et al. 2009; Hrnčirik & Van Duijn 2011).

Refined oils and fats are used in many processes and food formulations. Fats are the primary constituents of margarines, shortenings, and oils for salad and cooking. In addition to the visible fat contained in food, fats and oils are found in high quantities in many bakery goods, infant formulas, and dairy products and some sweets (FAO/WHO 1993). Palm oil and its fractions, in particular, are excellent raw materials for formulas and food applications. Among their advantages, these products do not emit undesirable odours, are highly resistant to oxidation, do not contain linolenic acid, and have a favourable nutritional composition for being free of trans-fatty acids and presenting tocopherols in its composition (Osawa & Gonçalves 2012).

As a consequence of the contamination of oils and fats with 3-MCPD esters, some authors have also found the contaminants in foods rich in these ingredients. Coffee creamers, cream aerosols and bouillon cubes presented concentrations in ranges of 130–730, 50–730 and 380–670 µg kg⁻¹, respectively (Karsulinová et al. 2007). In fried potato products, the amount of 3-MCPD esters was 27–64 µg kg⁻¹ in pre-fried French fries, 100–258 µg kg⁻¹ in

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fried French fries and 98–2201 $\mu\text{g kg}^{-1}$ in potato crisps (Ilko et al. 2011). Moreover, levels between 62 and 588 $\mu\text{g kg}^{-1}$ were reported in infant formula (Zelinková et al. 2009).

Although significant progress has been achieved in relation to 3-MCPD esters, most of the research is still conducted on oils and fats as well as on the refining process. In order to provide a better understanding and improve the database on the contamination of foods due to the use of oils and fats containing 3-MCPD esters, the objective of this work was to apply an in-house-validated GC-MS method preceded by acid-catalysed methanolysis to determine the levels of these contaminants in commercial deep-fat fried foods.

Materials and methods

Standards

The following analytical standards were purchased from Toronto Research Chemicals (North York, ON, Canada) at a purity > 98%: 3-MCPD-1,2-dipalmitoyl ester (PP-3-MCPD) and 3-MCPD-1,2-dipalmitoyl- d_5 ester (PP-3-MCPD- d_5). The stock solutions were prepared at a concentration of 0.5 mg ml^{-1} by dissolving the standards in tetrahydrofuran.

Solvents and reagents

Tetrahydrofuran (THF, analytical grade) and acid phenylboronic (PBA, 97% purity) were supplied by Sigma-Aldrich Corp. (Steinheim, Germany); methanol (HPLC grade) was acquired from Tedia Company Inc. (Fairfield, OH, USA); hexane, acetone, sulfuric acid, sodium bicarbonate, sodium sulfate and ammonium sulfate (analytical grade) were purchased from Labsynth (Diadema, SP, Brazil); diethyl ether (HPLC grade) was from Vetec (Duque de Caxias, RJ, Brazil); and ultrapure water was obtained from a Milli-Q Plus system (Millipore, Bedford, MA, USA).

Samples

A total of 85 samples of commercial deep-fat fried foods were analysed in relation to 3-MCPD esters in the present study. The samples were collected in supermarkets, restaurants, bakeries and fast-food chains from the city of Campinas, SP, between February and May 2014. The sampling included potato products, chicken nuggets, salty and sweet fried snacks, instant noodle, onion and garlic products. The samples were homogenised as purchased and kept under frozen storage until analysis.

Determination of 3-MCPD esters

3-MCPD esters were extracted from the samples according to Divinová et al. (2004), with some modifications.

The homogenised sample (5 g) was weighed into a 50 ml centrifuge tube and 100 μl of an internal standard solution of PP-3-MCPD- d_5 at 50 $\mu\text{g ml}^{-1}$ were added. Diethyl ether (20 ml) was mixed into the sample and the mixture was placed in an ultrasound bath for 5 min. The tube was then centrifuged at 3000 rpm for 5 min and an aliquot of 4 ml of the supernatant was dried over anhydrous sodium sulfate. The extract was evaporated under an N_2 flow at 25–30°C and the residue was dissolved in 1 ml of THF. Transesterification and derivatisation were performed as previously described by Arisseto et al. (2014). Since an indirect analytical approach was used, individual esters could not be identified and quantified and, therefore, the total concentration of 3-MCPD esters (including monoesters and diesters) was expressed as free 3-MCPD equivalent.

Chromatographic analysis

The analyses were conducted on an HP 7890A gas chromatograph coupled to a MSD 5975C mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). An aliquot of 1 μl of the extract was injected at 180°C in splitless mode. The separation was carried out on a capillary column VF-1 ms 30 m \times 0.25 mm (0.25 μm) (Agilent Technologies) using helium (grade 5.0, purity 99.999%) as a carrier gas at a flow rate of 1.2 ml min^{-1} . The following oven temperature programme was used: 60°C (held for 1 min), 6°C min^{-1} to 190°C, 20°C min^{-1} to 280°C (held for 30 min). The MS was operated in positive electron ionisation mode (+EI) with 70 eV of electron energy, registering the following ions: m/z 147, 91 and 196 for 3-MCPD and m/z 150, 93 and 201 for the internal standard 3-MCPD- d_5 .

Results and discussion

To date, methods to extract fats have been employed to isolate 3-MCPD esters from foods since it has been assumed that these compounds are associated with plant lipids. However, little recovery data for extraction efficiency directly from foods have been published (Hamlet et al. 2014). Therefore, in order to check the performance of the method used in the present study, an in-house validation was initially conducted in relation to linearity, LOD, LOQ, accuracy and precision (repeatability and within-laboratory reproducibility) according to the guidelines laid down by the Brazilian Institute of Metrology, Quality and Technology (INMETRO 2011), using a blank sample of French fries.

Good linearity was obtained in the evaluated range (0–1 mg kg^{-1} ; $r^2 = 0.997$). LOD and LOQ were set at 0.04 and 0.08 mg kg^{-1} , respectively, calculated as three- and six-fold the SD of six replicates of the blank sample fortified at the lowest acceptable concentration measured under repeatability conditions. Recovery and precision

Table 1. Recovery, repeatability and within-laboratory reproducibility.

Spike level (mg kg ⁻¹)	R (%)	CV _r (%)	CV _R (%)
0.08	82	3.4	3.6
0.2	91	5.0	6.5
0.8	92	2.5	3.6

Note: *R*, recovery (mean); CV_r, coefficient of variation under repeatability conditions (same day); CV_R, coefficient of variation under within-laboratory reproducibility conditions (different days).

were evaluated by spiking the sample with PP-3-MCPD at 0.08, 0.2 and 0.8 mg kg⁻¹ (six replicates for each concentration level). The results are presented in Table 1 and can be considered appropriate to ensure the reliability of the analytical procedure.

Considering that samples of processed foods containing salt would be analysed in this work, the influence of chloride in the formation of 3-MCPD during the analysis was also investigated in salted blank French fries by performing a liquid–liquid extraction with water in the organic supernatant before drying under N₂, as recommended by Ermacora and Hrnčirik (2012). No 3-MCPD was found in the samples,

indicating that the chloride content of the products may not affect the analytical result in a significant way.

Table 2 shows the levels of 3-MCPD esters, expressed as free 3-MCPD equivalent, in commercial samples of deep-fat fried foods. As can be observed, the concentrations ranged from not detected (n.d.) to 0.99 mg kg⁻¹. A total of 64 samples (75%) presented levels above the LOQ, showing a wide contamination of the products. The highest mean levels were found in chopped onion and garlic, while the lowest concentrations and percentage of positive results (> LOQ) were reported in French fries, chicken nuggets, codfish croquette, churros and banana. Some food groups presented a large range of concentration, such as potato chips and chopped garlic, for which levels of 3-MCPD esters varied from 0.11 to 0.81 mg kg⁻¹ and from < 0.08 to 0.99 mg kg⁻¹, respectively.

Limited data on the occurrence of 3-MCPD esters in fried foods are available in the literature. Some authors evaluated the presence of the compounds in potato fried products and found concentrations between 0.10 and 0.26 mg kg⁻¹ in French fries and between 0.10 and 2.20 mg kg⁻¹ in potato chips (Zelinková et al. 2009; Ilko et al. 2011), which is comparable with the results reported in the present study. In fish fried products (fish fingers and fried fish), levels below 0.60 mg kg⁻¹ were observed (Merkle et al.

Table 2. Levels of 3-MCPD esters, expressed as free 3-MCPD equivalent, in deep-fat fried foods (*N* = 85).

Sample	<i>N/N+</i>	3-MCPD esters (mg kg ⁻¹)	
		Mean	Minimum–maximum
Potato chips	9/9	0.28	0.11–0.81
Sweet potato chips	1/1	0.26	–
Cassava chips	1/1	0.26	–
Shoestring potato sticks (palha)	12/12	0.34	0.21–0.46
Shoestring cassava sticks (palha)	2/2	0.17	0.08–0.26
French fries	6/1	0.02–0.08	n.d.–0.09
Potato balls (noisette)	2/2	0.15	0.12–0.17
Chopped onion	2/2	0.64	0.57–0.70
Chopped garlic	6/4	0.49	< 0.08–0.99
Instant noodles	9/8	0.15	< 0.08–0.26
Chicken nuggets	4/1	0.02–0.08	< 0.08–0.08
Onion rings	2/2	0.14	0.14–0.14
Kibe (beef and wheat croquette)	6/4	0.12	< 0.08–0.25
Beef croquette	3/2	0.12	< 0.08–0.21
Chicken croquette	2/2	0.13	0.12–0.14
Codfish croquette	2/0	0.00–0.08	< 0.08 to < 0.08
Cassava strip	1/1	0.15	–
Rice patty	1/1	0.13	–
Pastel (cheese filled pastry)	1/1	0.15	–
Coxinha (chicken filled croquette)	4/4	0.11	0.08–0.15
Bolinha de queijo (cheese filled croquette)	4/3	0.09	< 0.08–0.13
Bolinho de chuva (sweet dough balls)	2/1	0.17	< 0.08–0.29
Churros	2/0	0.00–0.08	n.d. to < 0.08
Banana	1/0	0.00–0.08	–

Note: *N*, number of samples; *N+*, number of samples above the LOQ (0.08 mg kg⁻¹); n.d., values below the LOD (0.04 mg kg⁻¹). To calculate the mean, values below the LOD and the LOQ were treated according to the instructions for electronic submission of data on chemical contaminants in foods to GEMS/Food (WHO 2002). The range indicates different results for the lower and upper bounds for a group of data with fewer than 40% of quantifiable results.

2014). On the other hand, Svejková et al. (2004) reported concentrations ranging from 1.21 mg kg⁻¹ in doughnuts to 6.10 mg kg⁻¹ in French fries, which is higher than our results.

The contamination of fried foods with 3-MCPD esters has been mainly attributed to the use of contaminated oil during frying (Zelinková et al. 2009; Weisshaar 2011). However, considering the formation mechanism of the compounds, the presence of potential precursors (acylglycerols and chloride ions) in foods that undergo thermal treatment could also contribute to this contamination, which was not fully investigated in fried foods thus far. Therefore, the differences observed in the results of the present study, especially within certain food groups such as potato chips and chopped garlic, may be associated with several variables, including the type of frying oil and its initial content of 3-MCPD esters, fat uptake, levels of potential precursors in the raw material and process temperature, for example. Unfortunately, information regarding these variables was not available from the producers, so no direct conclusions could be drawn about those observed differences and the factors involved in the contamination of foods during frying should be further investigated.

It should be noted that the current objective of this work was to give a first general idea about the levels of 3-MCPD esters in fried foods considering as many types of foods as possible and, for some categories, the obtained results may not be considered statistically representative as only one product was sampled. Despite this limitation, a preliminary estimate of the 3-MCPD intake from fried foods was calculated by taking into account the occurrence levels reported here and national food consumption data provided by a survey on the Analysis of the Individual Food Consumption in Brazil carried out from 2008 to 2009 (IBGE 2011). The intake of 3-MCPD was estimated by considering complete hydrolysis of its esters to release the free chloropropanol.

Using a deterministic approach, mean intakes of 0.03 and 0.06 µg kg⁻¹ body weight (bw) day⁻¹ were estimated for the Brazilian population using mean and P95 (95th percentile) levels of 3-MCPD esters, respectively. Salty snacks, including beef, chicken and codfish croquette, for example, were the most important contributors, accounting for 51% of the total intake. Within subgroups, the highest exposure was observed for adolescents aged 14–18 years (0.06 and 0.10 µg kg⁻¹ bw day⁻¹ for average and high consumers, respectively). Considering the total population as well as the investigated subgroups, the estimated intakes represented 1–3% and 2–5% for average and high consumers, respectively, of the provisional maximum tolerable daily intake (PMTDI) of 2 µg kg⁻¹ bw currently established for 3-MCPD (SCF 2001; FAO/WHO 2002, 2007), which may suggest a low risk to human health.

However, a potential concern may arise in particular cases of fried food consumers. For instance, the consumption of 150 g of potato chips containing 0.81 mg kg⁻¹ could result in a 3-MCPD intake of 2.03 µg kg⁻¹ bw day⁻¹, considering an individual body weight of 60 kg. This value corresponds approximately to the PMTDI and may pose some risk for these target consumers taking into account the simultaneous exposure to other sources of this contaminant in the diet, especially margarines and similar products, which were pointed out by EFSA (2013) as being the main contributors of 3-MCPD intake. Therefore, it is highly desirable to evaluate the main factors involved in the contamination of fried foods by 3-MCPD esters as well as to apply mitigation strategies during oil refining in order to reduce consumer exposure to these contaminants.

Conclusions

The occurrence of 3-MCPD esters in deep-fat fried foods from the Brazilian market is reported in the present study. Although a limited database is available in the literature for comparison purposes, the levels of the contaminants were similar to those found in a few other recent studies. A high number of samples showed concentrations above the LOQ (75%) and a considerable variation on the levels of 3-MCPD esters of particular groups was also observed, which could be associated with several factors such as the type of frying oil, its initial content of 3-MCPD esters and fat uptake, for example. A preliminary estimation of 3-MCPD exposure from fried foods in Brazil using the reported data suggested low risks to human health, but a potential concern may arise in particular cases of fried food consumers. Therefore, additional research is needed on the main factors involved in the contamination of fried foods by 3-MCPD esters, including a full understanding on the possible formation of the compounds during frying.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the São Paulo Research Foundation (FAPESP) [grants numbers 2011/08936-0 and 2011/19043-6].

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