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Safety assessment of commercially processed foods by quantifying 3-monochloropropane 1,2-diol

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ABSTRACT

Food safety concern related to intensively processed foods in terms of unadorned processing needs consideration for process-induced compounds. Recently, food processing has accompanied safety concerns inclusive process induced contaminants like 3-monochloropropane 1, 2-diol (3-MCPD). The study was intensive to quantify 3-MCPD in selected food products. Various food samples from two different manufacturers were selected in this study including bread, cornflakes, sausages, nuggets, milk powder, canola, sunflowers, and hydrogenated oil. The gas chromatography equipped with flame ionization detector analysis was performed to quantify the 3-MCPD in extracted oil with standard compound. The analysis revealed the presence of high amount of 3-MCPD in most of the processed foods in the range of 0.98-155 µg/kg. Brands of Canola oil had the maximum 3-MCPD while the first brand for cornflakes and sunflower oil had minimum 3-MCPD. Detected concentrations were directly proportional to lipid contents for most of the products. The study revealed the presence of 3-MCPD in analyzed commercial food products. Toxicity and food safety concern of chloropropanols has been established. At present, a matter of concern is the presence of substantial amount of 3-MCPD among foods which needs future monitoring regarding exposure and risk assessment estimation.

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1. Introduction

Commercial food processing is globally adopted as a source of convenience and value addition. Recently, food processing has accompanied by safety concerns inclusive process induced contaminants like 3-monochloropropane 1, 2-diol (3-MCPD). In 1978, this was identified for the first time in hydrolyzed vegetable proteins (HVPs) (1). 3-MCPD has been found in various processed foods, i.e., cereal products, HVPs, malted products, refined vegetable oils and fats, infant formulas, carbohydrate-rich foods, human breast milk, soy sauce, and fermented products (2,3,4,5,6). In fishery products, free and esterified 2- and 3-MCPD along with esters of glycidol were also detected (7).

It is a heat-induced food contaminant believed to be produced during deodorization of edible fats and oils above 200 °C and baked items at a temperature between 100 °C and 230 °C (8,9). There are various proposed mechanisms for 3-MCPD formation among food matrices under given conditions including reactions between organochlorines and triacylglycerol. This reaction involves two steps, i.e., acyl oxonium ion formation followed by nucleophilic substitution (10). Nucleophilic substitution may be regioselective at either at sn-1, 2 or 3 positions (11). Precursors toward its formation vary depending on food type and composition, but major compounds

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include chlorine donating ions, tri-, mono and diacylglycerol (triglycerides, monoacylglycerols, and diacylglycerols), and phospholipids (12).

A lot of concern has been raised recently regarding 3-MCPD as it is declared as a carcinogen of category 2B by International Agency for Research on Cancer (13). This compound is known to induce Leydig cell, mammary glands, and kidney tumors (14). It has also genotoxic effect and induces male infertility in experimental animals (15). It can cross blood and blood-brain barrier and induces apoptosis (16,17). Due to the toxicity of 3-MCPD, European Commission has set maximum tolerable intake of 2 µg/kg body weight with a maximum of 20 µg/kg in soy sauce and acid-HVPs on 40% dry weight basis (18). This is the first study of its type as no survey has been carried out to determine "chloropropanols" in commercially processed foods in subcontinent. Keeping toxic concerns of 3-MCPD and its prevalence among foods, the present study was designed for the assessment of commercially processed foods by quantifying its level in selected foods encompassing local brands in Pakistan.

2. Materials and methods

External standard (1,2-*bis*-palmitoyl-3-chloropropanediol; minimum 97% purity) was purchased from Sigma-Aldrich (Received on order from Sci. Scientific, Lahore, Pakistan), *t*-butyl methyl ether, ethyl acetate (MERCK-Merck, KgaA, 64271 Darmstadt, Germany with minimum 96% purity), isohexane, phenylboronic acid (PBA) (LAB-SCAN, RCI Labscan Ltd, 24 Rama 1 Road, Patumwan, Bangkok 10330 Thailand with minimum 96% purity), sodium methoxide, glacial acetic acid, acetone, and toluene (BDH Laboratory Supplies, Poole, BH151TD, England). All analytical grade solvents were used.

Ethyl acetate was used to prepare a stock solution of 3-MCPD (1 mg/L) and was diluted with toluene to further prepare 1 µg/L external standard (1,2-*bis*-palmitoyl-3-chloropropanediol) (15).

The selected food products of native origin were procured comprising two brands from supermarkets located in District Faisalabad, Pakistan, between March and April 2016. The samples were dried by tray dryer (Model # R-5A) at 60° C for 3-4 hours with subsequent grinding (using lab mill). Fat was extracted by Soxhlet technique using hexane as extracting solvent (Fluka-Buchs, Switzerland) (19).

2.1. Quantification of 3-MCPD using GC-FID

Sample preparation: Using Pasteur pipette, 400 ± 5 µl of oil sample was transferred to the Pyrex test tube with a tight sealing cap. 500 ml ethyl acetate/*t*-butyl methyl ether (2:8 v/v), 1 ml sodium methoxide solution and 250 ml of 3-MCPD-d_5 solution (used as internal standard) were added with the subsequent sealing of the tube tightly. Subsequently, 3 ml of both NaCl solution (200 g/l) and isohexane were added along with 100 ml glacial acetic acid. Supernatant layer was discarded using a pipette. Using isohexanes, the aqueous layer was extracted and discarded again. Then, 250 ml derivatizing reagent (PBA; 5 g dissolved in 1 ml water and 19 ml of acetone) was added. This tube was then sealed and heated for 20 minutes at 80 °C. Phenylboronate derivatives of 3-MCPD were extracted by shaking the contents by adding 3 ml hexane after subsequent cooling to room temperature. The hexane layer was examined for 3-MCPD quantitation.

Operating conditions of GC-FID: Gas chromatography (GC), equipped with flame ionization detector (FID) was used to analyze prepared samples (Shimadzu 17A). GC-FID operated under following conditions: a capillary column with specifications; 13.0 m × 0.25 mm I.D; programming for oven temperature was: after sample injection, the column was initially held at 40 °C for 1 minute. The first heating ramp was programmed as 7.5 °C minutes/increase for 19 minutes while second heating ramp was for 20 minutes with 39.99 °C/minutes increase. At that time temperature was increased for 13 minutes at 280 °C while keeping injector and detector temperature at 200 °C and 280 °C, respectively. Nitrogen (used as carrier source); injected volume: 2 µl. Calculation based on signal-to-noise (S/N) ratio measurement using standard solution (1 µg/L) was used for a limit of detection and found to be 0.50 µg/kg when S/N ratio was 3:1. GC-FID analysis was carried out in Central Hi-Tech Laboratory, University of Agriculture, Faisalabad.

3. Results

Most of the published data regarding chloropropanols prevalence among foods are majorly about soy sauces, HVPs and refined edible oils and fats. At present, there is no published data available for chloropropanols (including 2-, 3-MCPD and 1,3 dichloropropane-2-ol [1, 3-DCP]) about Indo-Pak region (except for some surveys/studies in China).

Results of the present study are summarized in table 1. Considerable number 14 out of 16 [87.5% (inclusive of different brands)] of foodstuffs contained significant amount of 3-MCPD ranging from 0.98 to 155 µg/kg for the food products such as bread, cornflakes, sausages, nuggets, milk powder, canola, sunflower, and hydrogenated oil (all the products were of two different brands). The present study also showed the presence of 3-MCPD in cornflakes. Results showed higher 3-MCPD contents in brand 1

(S_{B1}) regarding sausages while in nuggets, 3-MCPD was not detected in brand 1. The presence of 3-MCPD among edible fats and oils was significantly higher among all tested brands of selected oils, i.e., canola, sunflowers, and hydrogenated oil in comparison to other foods.

Table 1. The concentration of 3-MCPD in processed food samples*

Food products	Brands	3-MCPD (µg/kg)
Bread	B_{B1}	23
	B_{B2}	3.5
Corn flakes	C_{B1}	0.98
	C_{B2}	9.46
Sausages	S_{B1}	6.4
	S_{B2}	2.7
Nuggets	N_{B1}	ND
	N_{B2}	1.3
Milk powder	M_{B1}	ND
	M_{B2}	ND
Canola oil	CO_{B1}	155
	CO_{B2}	62
Sunflower oil	SO_{B1}	1.17
	SO_{B2}	ND
Hydrogenated oil	HO_{B1}	1.2
	HO_{B2}	ND

B_1: Product-Brand 1; B_2: Product-Brand 2. *Standard limit was constant for all comparisons in respect to analyzed foods; MCPD: Monochloropropane 1, 2-diol

4. Discussion

The presence of 3-MCPD in bread is mostly attributed toward toasting and baking (15,20). Other bakery products including soda cracker and rolls which are heat-treated were also found to have measurable 3-MCPD levels. Other factors due to which cereal products may have 3-MCPD sauce, soup powder, and condiment (15). Per model studies regarding fermented dough products, glycerol is considered as a major precursor in baked cereal products. Moreover, lipids, sugar, and organic acids may contribute toward this contaminant in these products. There are possibilities that the processing conditions such as high moisture, temperature, pH, fermentation time, and type of yeast used during manufacturing of bread may favor 3-MCPD formation. The effect of these conditions on the concentration of 3-MCPD in product was studied (2).

Crews et al. (20) determined a range of 3-MCPD as 0.42-0.888 µg/kg in cornflakes while according to a study the levels of the 3-MCPD detected range from ND − < 10.7 µg/kg (21).

No published data are available on the formation mechanism of 3-MCPD in cornflakes and related breakfast cereals. The presence of 3-MCPD in sausages is also reported by various research groups (21,22,23) in the range of < 5-74, 6.2 and ND–15 µg/kg, respectively. One study also reported the presence of 3-MCPD in nuggets in the range of ND−1.7 µg/kg (23). One of the most interesting hypothetical mechanism of 3-MCPD formation in smoked meat products reported by Kuntzer and Weisshaar (22) is the production of 3-MCPD in smoke along with 3-hydroyacetone as a major precursor. The difference between 3-MCPD levels in sausage may attribute to the difference in salt concentration among brands. Influence of salt, moisture and temperature in relation to 3-MCPD precursors was studied and found that its formation was maximum at 4-7% NaCl, 13-17% water, and 100-230 °C (9). Moreover, detectable 3-MCPD may also be produced in meat-based processed foods due to frying, microwaving and grilling. Among meat-based foodstuffs such as salted fish, beef, and chicken, steaming, and stir-frying may also produce 3-MCPD at quantifiable levels.

Data on the formation of 3-MCPD among milk powders are very scarce, and until now, 3-MCPD formation mechanisms have not been fully elucidated and understood. Referring to formation routes of 3-MCPD among foods, there may be a possibility of its formation during heat processing due to sodium chloride and lipids which may be naturally present or as processing aids. Moreover, free 3-MCPD may also get released from its esterified form usually from stearic, oleic, and palmitic fatty acids (24). In addition, manufacturers also use unspecified or mixture of fat from vegetable sources which may contribute toward 3-MCPD formation (25).

In an earlier study, 3-MCPD was determined in canola, sunflowers, and hydrogenated oil in the range of 0.005-0.31, 0.15-0.84, and 0.009-0.92 µg/kg, respectively (26). 3-MCPD was also reported in sunflower oil in the range of 1.21-2.1 and 0.58-0.173 µg/kg (20,27). Edible fats and oils are one of the most studied subjects in relation to 3-MCPD formation due to high-temperature processing conditions. Per published literature, most of the researchers undertake deodorization step as a major felon in 3-MCPD formation. Due to very high temperature, lipid matrix may undergo structural deformities during refining (above or at 230 °C). It is also stated that processing temperature of this range increases the chances of 3-MCPD formation exponentially (28). Formation 3-MCPD is also affected by the type of oil, its origin and treatments given before deodorization (29). Major precursors, influencing 3-MCPD formation, are chloride (chlorine donating compounds) and acylglycerols (12).

5. Conclusion

The study revealed the presence of 3-MCPD in various commercial food products. Toxicity and food safety concern of "chloropropanols" have been established. At present, a matter of concern is the presence of substantial amount of 3-MCPD among foods which needs future monitoring regarding exposure and risk assessment estimation.

Conflict of Interests

Authors have no conflict of interest.

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