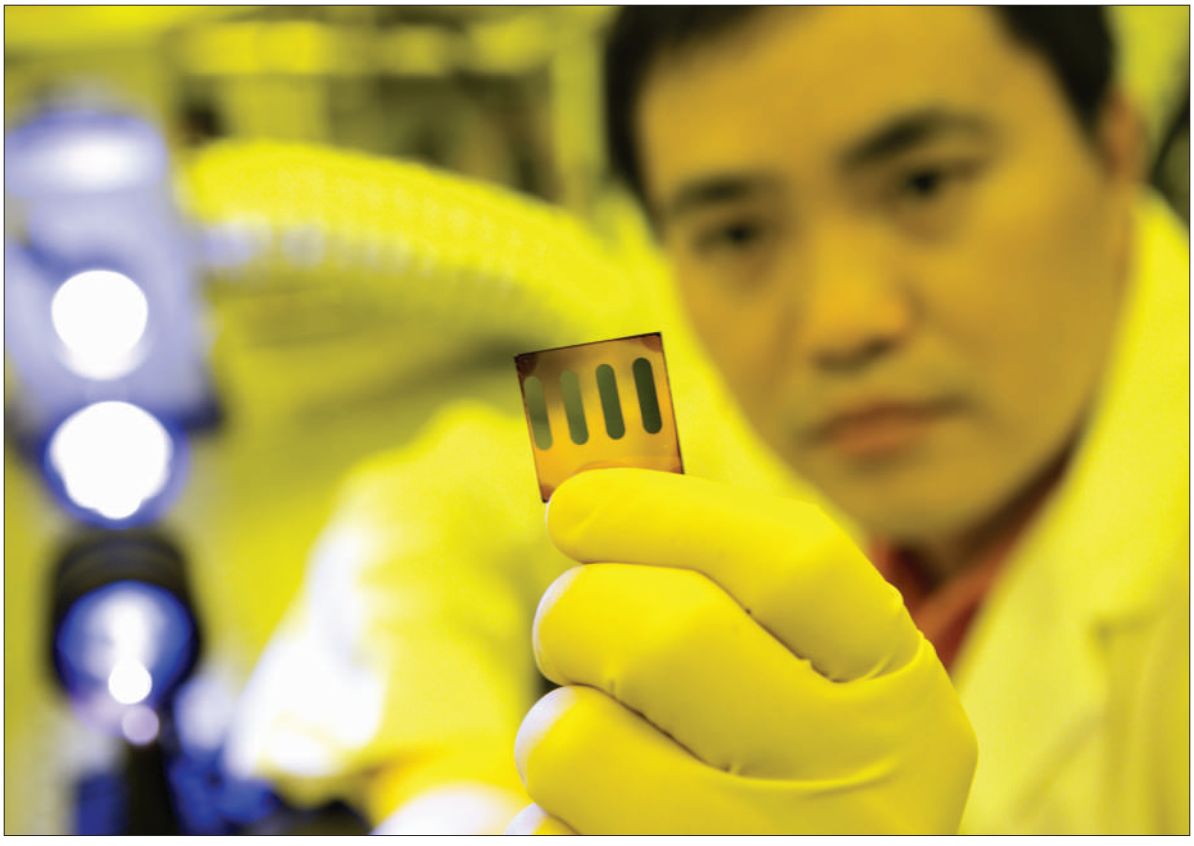

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Research Article
Levels of 3-monochloropropane-1,2-diol (3-MCPD) in Sudanese Flat Bread (Aish Baladi)

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Abstract

Background and Objective: 3-chloropropane-1,2-diol (3-MCPD) is a major food processing contaminant. The aim of this research was the detection and quantification of processing contaminants known as 3-MCPD (3-monochloropropane-1,2-diol) in flat bread. **Materials and Methods:** Twenty five samples of bread were randomly collected from different areas of Khartoum state and every sample contains 20 pieces of bread. Their moisture and oil content were determined by using (AOAC), followed by quantification of 3-MCPD by GC-MS. Dietary exposure of children and adults to 3-MCPD were estimated according to their body weight, age, amount of bread consumed per day, as well as the concentrations of the compounds in the sample. **Results:** The moisture content of flat bread samples ranged from 29.0-36.73% and their oil content ranged from 0.37- 0.92%. 3-MCPD was detected in flat bread and its concentrations were in the range of 0.15-18.27 ppm. The exposure of children to 3-MCPD ranged from 0.00063-0.5398 μg kg^{-1} b.wt., per day and that of adults ranged from 0.0044-0.6304 μg kg^{-1} b.wt., 8 per day. **Conclusion:** Therefore, it could be concluded that flat breads processing leads to the formation of 3-MCPD, however, its level in flat bread does not constitute a health risk.

Key words: Dietary exposure, 3-monochloropropane-1,2-diol (3-mcpd), Sudanese flatbread, moisture, oil content

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

3-chloropropane-1,2-diol (3-MCPD) is representative of so-called food-borne or food processing contaminants. It was identified in acid-hydrolyzed vegetable protein (acid-HVP), where it originates as a reaction product of phospholipids, acylglycerols and glycerol with hydrochloric acid. 3-MCPD occurs in a wide range of retail outlet and home-made foods as well as in various food ingredients formulated without the addition of acid-HVP[1,2]. 3-MCPD may possibly occur in products other than HVP such as thermally processed foods like bakery products, vegetable oils and fats during manufacturing or cooking and sodium chloride naturally present or added to the food[3].

Several analytical methods using ester cleavage by transesterification with sodium methoxide and derivatization with Phenyl Boronic Acid (PBA) have been presented and many samples of edible fats and oils have been analyzed[4]. The formation of 3-MCPD in food is influenced by many factors, including, temperature, pH, moisture content, sugar and lipid content, the type of processing method employed and storage conditions[3]. In general, most foods contain 3-MCPD[5] at approximately 0.0096-0.083 mg kg^{-1}.

Most methods for the analysis of 3-MCPD focus on the trace analysis at microgram per kilogram levels in various food matrices, which is relatively complicated[6]. The three main physical characteristics that contribute to the absence of a suitable chromophore, a high boiling-point and a low molecular weight[7]. Because of the absence of a chromophore, approaches based on high performance liquid chromatography with ultra-violet or fluorescence detection cannot be applied and only one such method with refractive index detection that has been used to study the kinetics of 3-MCPD formation in model systems appears to be unsuitable to determine trace quantities of the compound in food matrices[7]. The development of analytical methodology for the determination of 3-MCPD esters started shortly after Svejkovska *et al.*[5] reported the presence of 3-MCPD esters in fried food. As for free 3-MCPD, official methods are not established for the analytical determination of 3-MCPD esters. Scientific activity has taken place in recent years to develop and validate efficient and reliable methods for the analysis of 3-MCPD[6,3].

The quantification of bound 3-MCPD is based on preliminary step of fat extraction, followed by the application of one of the conventional indirect methods for the determination of 3-MCPD esters in oil and fats based on acid, alkaline or enzymatic trans esterification[8]. No study about 3-MCPD has been carried out in Sudan and awareness about the contaminant is very minimal. This research aimed to provide information about 3-MCPD levels in flat bread in Sudan.

MATERIALS AND METHODS

The study was conducted from July, 2018-October, 2019 in Food Science lab Sudan University of science and Technology.

Twenty five bread samples were randomly collected from Khartoum state (Omdurman, Khartoum and Khartoum Bahri), each of these samples contain twenty pieces of bread loaves, these samples were further divided among the area of collection as follow: Nine samples were collected from different areas of Omdurman bakeries (Alsabeel, Thawra 18, Dar-El Salam, Jikhais, Safwaa 4, Banat, Al Muhandseen, Shuhadda and Umbadda 34), followed by 8 samples collected from Khartoum Bakeries (Kalakla, Alshagara, Jabra, Mayo, Lamab, Jiraif, Remaila and Algoz 5) and 8 samples collected from Khartoum Bahri Bakeries (Samrab, Haj Yousif, Saad Guishra, Shambat, Alsafia 1, Shigla, Halfaya, Alsafia 2), then oil was extracted from these bread samples using Soxhlet apparatus and kept in plastic bottles of polyethylene, stored at temperature of (-18°C) till analysis of 3-MCPD.

Reagents and solvents: Sodium chloride (p.A), phenylboronic acid (≥98%, PBA), acetone, hexsane, methyl tert-butyl ether (MTBE), methanol and ethyl acetate, glacial acetic acid, sulfuric acid 96%, Sodium methoxide (25% w/v in methanol), 3-monochloropropane-1,2-diol (98%) and 3-methoxypropane-1,2-diol (98%). 1,2-dipalmitoyl-3-chloropropane and glycidylpalmitate were from Tokyo Chemical Industries (TCI, Tokyo, Japan). A solution of 50 µL of sulfuric acid in 5 mL of methanol was prepared subsequently for conversion of glycidyl esters (methanol/sulfuric acid). A sodium chloride solution (NaCl solution 20%) of 200 g L^{-1} was prepared in deionized water. The derivatization reagent PBA was prepared by dissolving 5 g of PBA in 19 mL of acetone and 1 mL of deionized water. Gas Chromatography-Mass Spectrometry (GC-MS), from Japan (Shimadzu Company) was used to detect the analyte.

Determination of moisture content of flat bread: Samples of flat bread were weighed in a wet basis and their weights were recorded. The samples were placed in dishes of known weight and dried in an oven at 105°C for 6 h, following AOAC.[9] method. The dried samples were then removed from the oven, cooled in a desiccator for about 20 min and weighed on sensitive weighing balance. Moisture content was calculated.

Extraction and determination of oil from flat bread: The bread loaves were dried, finely ground in a mortar, a constant weight of 500 g of ground samples was weighed and transferred into oil extraction thimble and stuffed with cotton wool to prevent particles from escaping during extraction into the flask. The Soxhlet apparatus was then properly assembled and extraction allowed to continue for 6 h, after which the solvent (hexane) was recovered. The flask was then removed from the unit and dried at ambient temperature for an hour. It was then cooled at room temperature for about 20 min and weighed. The oil percentage was calculated using the method of Zhang, *et al.*[9] and the results of oil percentage were recorded. Then the solvent was recovered by using a rotary evaporation to obtain the extracted oil, the temperature of the rotary evaporator was set at the boiling point of hexane (45-50°C), while the speed of the rotary evaporator was fixed at 20 rpm. The oil content percentage was determined.

Preparation of standard solutions: Stock solutions with concentrations of 50 mg mL^{-1} of 1,2-dipalmitoyl-3chloropropane and glycidylpalmitate were prepared in MTBE. These solutions were further diluted to 50 µg mL^{-1} (working solution for foodstuffs) as well as 5 µg mL^{-1} (working solution for fats and oils) with MTBE, expressed as 3-MCPD and glucidyl. From these solutions the calibration standards for the determination of 3-MCPD esters and glycidyl esters in fats and oils 0.01-0.3 µg mL^{-1} as well as other matrices 0.05-0.5 µg mL^{-1} were prepared by dilution with MTBE. In relation to sample preparation, standard solutions were prepared in 2 mL (liquid samples) as well as 10 mL (other samples) MTBE followed by the same treatment as samples. All solutions were stored at 4°C.

Sample preparation: 200 mg oil were dissolved in 2 mL MTBE, homogenized, 500 µL CH_3OH/H^+ (ring opening, 3 min) were added, 200 µL $NaCH_3$ (ester cleavage within 1 min, added 200 µg glacial acetic acid and 2 mL NaCl solution 20% (separate the aqueous layer) were added, 200 µL PBA was added (Derivatization at ambient temperature for 15 min) and extraction with hexane was done. All further procedures of mixing, shaking and homogenizing during sample preparation were accomplished by using a vortex mixer (2500 rpm, 30 sec).

Esters of 3-MCPD and converted glycidol were cleaved by adding 200 µL of sodium methoxide. The tube was closed tightly and the solution mixed well on a Vortex mixer for 30 sec. After one min. the reaction was stopped with 200 µL of glacial acetic acid and 2 mL of NaCl solution 20%. Extraction of the analytes was accomplished by vigorous shaking for 30 sec. After phase separation, the upper layer was discarded. The aqueous layer was derivatized as described below.

GC-MS conditions: The qualitative and quantitative analysis of the sample was carried out by using GM/MS technique model (GC/MS-QP2010-Ultra) from Japans 'Shimadzu Company, with serial number 020525101565SA and capillary column (Rtx-5-30 m × 0.25 mm × 0.25 µm).The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.20 mL min^{-1}, the temperature program was started from 100°C with rate 20°C/min to 160°C held for one minute then the rate was changed to 5°C/min-180°C, finally the rate was changed to 30°C/min reaching 300°C as final temperature degree, the injection temperature was 320°C, the ion source temperature was 230°C and the interface temperature was 300°C. The sample was analyzed by using SIM mode selecting m/z 91,147,196, the total run time was 15 min and results were recorded.

Concentration and level of 3-MCPD in flat bread: The amount of 3-MCPD is computed from the formula shown below:

Having knowing the following data:

- Area of the standard is known
- Area of the sample is known
- Concentration of the standard is known
- Concentration of the sample (amount of 3-MCPD) is unknown

Therefore:

- Area of standard = Concentration of standard
- Area of sample Concentration of sample
- By cross multiplication
- Concentration of 3-MCPD ppm = Area of sample × concentration of standard
- Area of standard

Estimation of dietary exposure for 3-MCPD in children and adults consumed flat bread: Dietary exposure for 3-MCPD concentrations per day per body weight based on the amount of breads consumed both for children and adults were determined. Knowing that the average weight of flat bread samples were 65 g, the total bread loaves consumed by individual children aged 14-15 years per day was 6 bread loaves and that of adults aged 18-20 years per day was 10 bread loaves. Their body weight for deterministic exposure

were estimated based on the EFSA[10] simulated diet, mean body weight for children was 47.67 kg and of adults was 68.03 kg. Therefore, the concentrations of 3-MCPD per body weight per day for an individual were determined according to the formula shown below[11]:

$$Ei = \frac{\sum Qi, K \times Ci, K}{Bwi}$$

Where:

Ei = Is the exposure of individual I to some chemical at some specified point in time
Qi,K = Is the amount of food K consumed by individual I
Ci,K = Is the concentration of the chemical of interesting food K, consumed by individual I and
Bwi = Is the body weight of an individual i

For deterministic (point) estimates of exposure, these parameters (concentration, food consumption and body weight) are represented by population averages or selected percentiles. For dietary modeling, food consumption and body weight will be represented by actual reported values for an individual on one particular day or on several days, depending on the structure of the dietary survey.

RESULTS

Moisture and oil content of flat bread samples: Table 1 showed the moisture and oil content of flat bread samples collected from different areas of Khartoum state. The mean moisture content in all samples studied was 33.86, while the mean oil content was 0.62. The highest moisture and oil contents were found in the samples of Haj Yousif 36.73 and 0.92%, Mayo 36.4 and 0.91%, respectively. The lowest moisture content was found in samples of Thawra18, 29.0% followed by Alsafia2 30.85%. While the lowest oil content was found in samples from Alsafia1 0.37% followed by samples from Alshagara 0.41%.

Levels and concentration of 3-MCPD in flat bread: Determination of 3-MCPD (3-monochloropropane-1,2-diol) concentrations in flat breads showed that, the highest levels of 3-MCPD were found in some bread samples (Table 2). The highest concentration of 3-MCPD in flat bread was found in samples 4 (18.27318 ppm) and sample 6 (10.68253 ppm), respectively. The lowest concentration of 3-MCPD in flat bread was found in sample 20 (0.13015 ppm) followed by sample 11 (0.15311 ppm), respectively.

Table 1: Moisture and oil content of flat bread and area of collection

Samples	Area	Moisture (%)	Oil content (g)
1	Alsabeel	35.42	0.56
2	Thawra 18	29.0	0.76
3	Dar-El Salam	35.89	0.67
4	Jikhas	34.85	0.53
5	Safwaa 4	34.05	0.44
6	Banatt	35.42	0.42
7	Al Muhandseen	32.67	0.43
8	Alsafia1	33.08	0.37
9	Umbadda 34	34.27	0.51
10	Kalakla	34.78	0.62
11	Alshagara	34.47	0.41
12	Jabra	32.27	0.92
13	Mayo	36.64	0.91
14	Lamab	35.43	0.57
15	Jiraif	31.04	0.71
16	Remaila	33.33	0.48
17	Algoz 5	33.14	0.55
18	Samrab	32.26	0.56
19	Haj Yousif	36.73	0.92
20	Saad Guishra	34.61	0.78
21	Shambat	34.47	0.51
22	Shuhadda	33.16	0.74
23	Shigla	35.75	0.74
24	Halfaya	33.11	0.60
25	Alsafia2	30.85	0.59

Mean moisture content: 33.86, Mean oil content: 0.62

Table 2: Derivatization, concentrations, retention time and areas of 3-MCPD in flat bread samples

Sample No.	Retention time	Concentration (m/z)	Area (m^2)	3-MCPD derivative concentration (ppm)
1	7.182	147.00	261209	10.35539
2	7.189	147.00	76399	3.02877
3	7.161	147.00	114525	4.54024
4	7.160	147.00	460931	18.27318
5	7.158	147.00	25530	1.01211
6	7.151	147.00	269461	10.68253
7	7.191	147.00	28305	1.12213
8	7.166	147.00	21415	0.84898
9	7.204	147.00	32976	1.30730
10	7.183	147.00	7869	0.31196
11	7.186	147.00	3862	0.15311
12	7.185	147.00	6475	0.25670
13	7.180	147.00	5995	0.23767
14	7.150	147.00	7031	0.27874
15	7.177	147.00	6503	0.25781
16	7.183	147.00	7674	0.30423
17	7.177	147.00	4386	0.17388
18	7.138	147.00	11213	0.44453
19	7.177	147.00	6463	0.25622
20	7.160	147.00	3283	0.13015
21	7.152	147.00	8100	0.32112
22	7.120	147.00	23295	0.92351
23	7.124	147.00	8913	0.35335
24	7.151	147.00	5966	0.23652
25	7.154	147.00	8049	0.31910

Estimation of dietary exposure of 3-MCPD in flat bread: Table 3 showed, the exposure of children to the 3-MCPD and there were different levels of contaminant, the reasons for this,

Table 3: Estimation of dietary exposure (flat bread) to 3-MCPD for Sudanese children with average age of 14-15 (years) and Body weight 47.67 kg

Sample No.	Age class (years)	Body weight (kg)	Exposure to 3-MCPD (μg kg^{-1})
1	14-15	47.67	0.3059
2	14-15	47.67	0.08942
3	14-15	47.67	0.13409
4	14-15	47.67	0.5398
5	14-15	47.67	0.00063
6	14-15	47.67	0.31555
7	14-15	47.67	0.03313
8	14-15	47.67	0.02503
9	14-15	47.67	0.03862
10	14-15	47.67	0.09212
11	14-15	47.67	0.00456
12	14-15	47.67	0.00753
13	14-15	47.67	0.00695
14	14-15	47.67	0.00818
15	14-15	47.67	0.00761
16	14-15	47.67	0.00892
17	14-15	47.67	0.00507
18	14-15	47.67	0.01309
19	14-15	47.67	0.00753
20	14-15	47.67	0.00376
21	14-15	47.67	0.00941
22	14-15	47.67	0.02724
23	14-15	47.67	0.01039
24	14-15	47.67	0.00695
25	14-15	47.67	0.00941

Mean exposure: 0.06843

Table 4: Estimation of dietary exposure to 3-MCPD (flat bread) for Sudanese adults with average age of 18-20 (years) and body weight of 68.03 kg

Sample No.	Age class (years)	Body weight (kg)	Exposure to 3-MCPD (μg kg^{-1})
1	18-20	68.03	0.35725
2	18-20	68.03	0.10443
3	18-20	68.03	0.1566
4	18-20	68.03	0.63041
5	18-20	68.03	0.03487
6	18-20	68.03	0.36852
7	18-20	68.03	0.0387
8	18-20	68.03	0.02924
9	18-20	68.03	0.0451
10	18-20	68.03	0.10758
11	18-20	68.03	0.00526
12	18-20	68.03	0.00879
13	18-20	68.03	0.00812
14	18-20	68.03	0.00955
15	18-20	68.03	0.00889
16	18-20	68.03	0.01041
17	18-20	68.03	0.00592
18	18-20	68.03	0.1529
19	18-20	68.03	0.00879
20	18-20	68.03	0.0044
21	18-20	68.03	0.01099
22	18-20	68.03	0.03181
23	18-20	68.03	0.1213
24	18-20	68.03	0.00812
25	18-20	68.03	0.01099

Mean exposure: 0.08128

could be the type of oil present in the flat bread samples, difference in the amount of breads consumption, dough acidity, length of, duration of bread exposure to high temperature and low pH. In the children's group, the mean exposure was 0.06843 μg kg^{-1} b.wt./day of the TDI (set at 2 μg kg^{-1} b.wt./day). Almost half of studied samples showed higher exposure of children to the 3-MCPD with samples 4 (0.5398 μg kg^{-1}), 6 (0.31555 μg kg^{-1}) and 1 (0.3059 μg kg^{-1}) as the highest. While more than half of studied samples showed lower exposure of children to the 3-MCPD with samples 5 (0.00063 μg kg^{-1}) and 20 (0.00376 μg kg^{-1}) as the lowest.

Table 4 shows adults exposure to 3-MCPD, also there were clear differences in the concentrations level of the compound and this may be due to the amount of bread consumption, baking processing conditions, such as; sodium chloride addition, duration of bread spent in the oven. It was estimated that, the mean daily adult exposure to 3-MCPD is 0.08128 μg kg^{-1} b.wt./day of the TDI.

Almost three the studied samples showed higher exposure of adult to the 3-MCPD with samples 4 (0.63041 μg kg^{-1}), 6 (0.36852 μg kg^{-1}) and 1 (0.35725 μg kg^{-1} b.wt.) as the highest. While more than three of the studied samples showed lower exposure of children to the 3-MCPD with samples 20 (0.00440 μg kg^{-1}) and 11 (0.005260 μg kg^{-1}) as the lowest.

DISCUSSION

The mean moisture content of the samples showed some variations of moisture and this may refer to the conditions of baking processing such as addition of ingredients and water (Table 1). From Table 1 there were significant differences in the mean oil content in respect to the areas due to some baking processing conditions as well as ingredient there in.

Determination of 3-MCPD (3-monochloropropane-1,2-diol) concentrations in flat breads (Table 2) showed that, the highest levels of 3-MCPD were found in some bread samples and this may refer to the baking processing conditions such as type of oil used, NaCl addition, duration of temperature exposure, time ,length, width, as well as acidity of the dough[3]. The highest and the lowest concentration of 3-MCPD in samples ranged from 0.15 to 18.27 ppm. The concentrations of 3-MCPD increased with increase in temperature in all bread samples, this indicate that the requisite precursors were present in relative excess, such as chlorine ion, phospholipids and glycerols.

In comparing these values with other studies of Dolezal *et al.*[2] Zelinkova *et al.*[12] for concentration of 3-MCPD in bread ranged from 1.56-23.6 mg kg^{-1} which is higher than the concentration of 3-MCPD in flat bread which ranged from

0.15-18.27 ppm. Other studies of Doležal *et al.*[13], was carried out for concentration of bound 3-MCPD esters in bread lipids at interval 1.56–23.60 mg kg^{-1} of fat (i.e., 5.7–84.9 µg kg^{-1} of sample). However, concentration of 3-MCPD in flat bread samples ranged from 0.15-18.27 ppm, respectively (Table 2). Tables 3 and 4 estimate the exposure of 3-MCPD in flat bread for children and adults, the findings showed that there was no much difference among both groups. However, the exposure estimate fluctuating from one group to another and this may be due to the amount of bread consumption, the ingredients therein, body weight, age, as well as the oil used in bread baking may be the fully refined or deodorized one. In the course of repeat exposure to this process contaminants, it could be possible health risk for both groups. It is obviously shown that, from the findings of the analysis, there was a gradual variation of 3-MCPD levels in these samples with the exception of sample (1) and (4) which showed a little bit high level of the contaminant of 3-MCPD and this may refer to the baking processing conditions such as duration of temperature exposure, length and width of bread, dough acidity (pH), mixture of bread flour with bran or Dura.

In comparison with international standards studies of 3-MCPD by European commission scientific committee for food, established regulations o f tolerable daily intake of (0.1-2 µg kg^{-1} b.wt./day), while concentrations of 3-MCPD in flat bread samples range from (0.15-18.27 ppm), (0.00015- 0.01827 µg kg^{-1} b.wt./day). In comparison with Karsulinova *et al.*[14], Dolezal *et al.*[2] and Zelinkova *et al.*[12] who found the concentrations of 3-MCPD in bread ranged between (1.56-23.6 mg kg^{-1} of fat which is higher than the concentration of 3-MCPD in flat bread. JECFA[10] estimated the average dietary exposure of the general population from a wide range of foods, related products which ranged from 0.06-2.3 µg kg^{-1} b.wt./day. While concentration of 3-MCPD in flat bread for children ranged between (0.00063-0.5398 µg kg^{-1} b.wt./day). While Hwang *et al.*[15], study was carried out for mean intake level of 3-MCPD in the republic of Korea was estimated in the range from 0.0009-0.0026 µg kg^{-1} b.wt./day and at the 95th percentile of consumption was 0.005 µg kg^{-1} b.wt./day and Yau *et al.*[16] secondary school student in China, Hong Kong special administrative region, have been published since that time, the average exposure was estimated to be 0.063-0.150 µg kg^{-1} b.wt./ day. While that for high consumers was 0.152-0.300 µg kg^{-1} b.wt./day. Average MCPD exposure for children from flat bread was 0.00063-0.598 µg kg^{-1} and for adults was 0.0044-0.368552 µg kg^{-1}.

CONCLUSION

It could be concluded that flat breads processing leads to the formation of 3-MCPD, however, its level in flat bread does not constitute serious health risk. Reduction of temperature in baking process of breads is desirable in order to have low content of 3-MCPD.

SIGNIFICANT STATEMENT

This study discover the flat breads processing leads to the formation of 3-MCPD, however, its level in flat bread does not constitute serious health risk that can be beneficial for flat breads processing: This study will help the researcher to uncover the critical areas of 3-MCPD formation in flat bread that many researchers were not able to explore. Thus, a new theory on reduction of temperature in baking processing of breads is desirable in order to have low content of 3-MCPD may be arrived at.

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