Sudan University of Science and Technolog

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Detection and quantification of 3-Monochloropropane-1,2-diol (3-MCPD) in bread in modern Bakeries in Khartoum state

كشف وتقدير كمية 3-MCPD في الخبز بالمخابز الحديثة في ولاية الخرطوم

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الاية

قال تعالى(وإذْ قُلْتُمْ يَا مُوسَى لَن نَّصْبِرَ عَلَىَ طَعَامٍ وَاحِدٍ فَادْعُ لَنَا رَبَّكَ يُخْرِجْ لَنَا مِمَّا تُنبِتُ الأَرْضُ مِن بَقْلِهَا وَقِثَّآئِهَا وَفُومِهَا وَعَدَسِهَا وَبَصَلِهَا قَالَ أَتَسْتَبْدِلُونَ الَّذِي هُوَ أَدْنَى بِالَّذِي هُوَ خَيْرٌ اهْبِطُواْ مِصْراً فَإِنَّ لَكُم مَّا سَأَلْتُمْ وَضُرِبَتْ عَلَيْهِمُ الذِّلَّةُ وَالْمَسْكَنَةُ وَبَآؤُوْاْ بِغَضَبٍ مِّنَ اللَّهِ ذَلِكَ بِأَنَّهُمْ كَانُواْ يَكْفُرُونَ بِآيَاتِ اللَّهِ وَيَقْتُلُونَ النَّبِيِّينَ بِغَيْرِ الْحَقِّ ذَلِكَ بِمَا عَصَواْ وَّكَانُواْ يَعْتَدُونَ).

صدق الله العظيم.

سورة البقرة الاية(61)

Dedication

I dedicate this work to my beloved family.

- To may late father Ibrahim Baher Adam.
- To my dear mother Halima Mohammed.
- To my sister and brother.
- To my great vast relatives and colleagues.
- To My friends with love and respects.

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Abstract

This study was carried out for the detection and quantification of 3-Monochloropropan-1,2-diol(MCPD) in bread in modern bahries in Khartoum state.Twenty five samples (25) of bread were collected from different modern bakries of Khartoum (Khartoum, Khartoum bahari and Omdurman), they include, round bread (13) samples and toast bread (12) samples. The moisture content and oil content of samples were determined according to the AOAC method and the quantity of 3-MCPD by Gas Chromatograpy mas Spectrometry (GC-MS).

3-MCPD exposure for individual by body weight per day for adults and children was estimated. Moisture content in round bread ranged from 31.7% to 35.7%, and in toast bread from 28.2% to 33.6%. Oil content in round bread ranged from 0.7955% to 0.9894% and oil content in toast bread from 0.7684% to 1.005%. 3-MCPD was detected in round bread and toast bread with a concentration of 0.15493-1.05873ppm to 0.11334-0.59644ppm, in round and toast bread, which were lower than that of European Commission Scientific Committee for Food Standardization. Exposure of adults and children to 3- MCPD upon consumption of bread is estimated to be (0.015446-0.031277µg\kg)-(0.002397-0.014118µg\kg)-(0.018039-0.036528µg\kg) and (0.002799-0.016488µg\kg) for adult and children, respectively. These exposure levels do not constitute a health risk.

الملخص

أجريت هذه الدراسة للكشف وتحديد كمية 3-MCPD في الخبز في مخابز الحديثة في ولاية الخرطوم، حيث جمعت خمسة وعشرون عينة (25) من مختلف مخابز ولاية الخرطوم (الخرطوم. الخرطوم بحري وامدرمان). وقسمت العينات الى الخبز المدور (13) , ثلاثة عشر عينة . وعدد عينات الخبز التوست(12) اثنى عشرة عينة. ومن ثم تم استخلاص الزيت و تقدير نسبة الرطوبة, نسبة الزيت بواسطة طريقة (AOAC)، تقدير كمية 3-MCPD بواسطة جهاز (GC:MS) وتقدير كمية تعرض الفرد من الكبار والصغار ،أظهرت النتائج عن محتوى نسبة الرطوبة في الخبز المدور في المدى بين 31.7-35.7%، و نسبة الرطوبة في الخبز التوست في المدى بين 28.2- 33.6. ونسبة الزيت في الخبز المدورة في المدى بين 0.7955-0.9894 % ونسبة الزيت في الخبز التوست في المدى 0.768-1.005%.

تركيز كمية 3-MCPD في زيت الخبز المدور 0.15493-1.05873ppm كمية تركيز3-MCPD في الخبز التوست (0.11334-0.66749ppm) وكانت التركيز اقل من التركيز الذى زكرته اللجنة العلمية الأوربية للتقييس الغذائية وكمية التعرض للفرد من تركيز كمية 3-MCPD من الخبز المدور والتوست-0.015446-0.031277µg\kg 0.018039-0.036528µg\kg, 0.002799-0.016488µg\kg, 0.002397-0.014118µg\kg- للكبار والصغار،على التوالي. هذه المستويات لا تشكل خطراً صحياً.

CHAPTER ONE

INTRODUCTION

INTRODUCTION

3-monochloropropane-1,2-diol (3-MCPD) esters are processing contaminants found in vegetable oils and foodstuffs (Crews et al. 2013). 3-monochloropropane-1,2-diol (3-MCPD)esters are also formed during refining process (deodorization) at temperature 240-270^{O}C (Biidiger et al. 2008) . The deodorization step with its high temperature of more than 200^{o}C mainly influenced formation of 3-MCPD esters in oils (Zelinkova et al. 2006).3-MCPD esters also is known carcinogen, have been detected in many refined vegetable oils such as palm oils, sunflower oil and foodstuffs, including bread, coffee, infant formula, salty crackers, dark malt, French fries, doughnuts, pickled olives, and soya sauces ….etc (Hamelet and Sadd. 2004), Pudel et al, (201)1, Svejkorska et al. 2004), Weibhaar ,(2011) , Zelinkova, et al.2009). 3-MCPD is found in human breast milk at concentration from below 300-2195ug\kg fat or 6-67ug\kg milk detection in 2008,indicating that 3-MCPD esters could be absorbed and distributed to human tissues and organs (Zelinkova et al. 2008). The European commission's scientific committee for food established maximum tolerable daily intake (TDL) for 3-MCPD of 2ug\kg of body weight per day ,and the lowest observed effect at 0.1ug\kg body weight per day . 3-MCPD is not known to occur as a natural product, and the major effect for 3-MCPD on testicular tissue, cancer in human and kidney, other effect on cell function include immunotoxicity, experimented on rats (Lee et al. 2004).

Amounts of 3-MCPD measured in a bakery fat and\or retail soft dough biscuit were 1285 and 632ug\kg and consistent with amount reported previously, application of the developed method for bound monochloropropane-1,2-diol (3-MCPD) and diesters of MCPD showed that mono-esters of MCPD accounted for 15.7% and 9.4% of the total bound MCPD in the bakery fat and biscuit, respectively ,and the concentration of free 3-MCPD in bread samples was at interval <9-54.5ug\kg. Concentration of bound 3-MCPD was at interval 1.56-23.6mg\kg of fat (i.e 5.7-84,9ug\kg of sample), (Karsuinova et al.2007,Dolezal et al ,2005, Divinova et al,2007 and Zelinkova et al. (2008) .

Hamlet et al. (2004) ,measured 3-MCPD-esters in toast bread highest levels were found in regions of the bread that attained the highest temperature, i.e the crust , and level increased from 60-160ug\kg^{-} when the bread was toasted over 40-120 seconds. The highest level of 3-MCPD-esters (6100ug\kg^{-1}) was found in a sample of French fries (Svejkovska ,et at,2004) . Detection and quantification of 3-MCPD by the several method, direct method ,indirect method(Derivatization and GC-MS analysis), DCF method C-VI 18 10, and SGS 3-in-1 method. In Sudan there is no data about the 3-MCPD, although, Sudan consumes a large amount of modern bread and vegetable oils. And consequently detection and quantification of 3-MCPD in modern bakery bread is important to evaluate the extent of potential health risk.

Objective of research

The aim is to provide information about 3- MCPD (food processing contaminant classified as a possible carcinogenic) levels in bread, in Khartoum state, and assessment of the exposure levels of the population.

Specific objectives

1- Determination of moisture and oil content of modern bakery bread samples.

2- Detection and quantification of 3-MCPD in modern bakery bread.

CHAPTER TWO

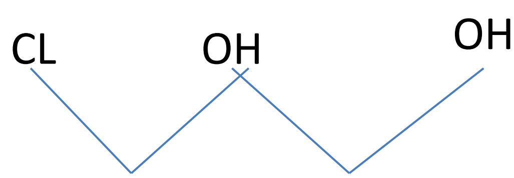
LITERATURE REVIEW

2.1 Definition:

.3-Monochloropropane-1,2-diol (known as 3-MCPD) was first identified in acid hydrolysed vegetable protein (HVP) in 1981 Daivdek et al. (1982). In view of it̓s toxicity, a regulatory limit of 0.01mg\kg was adopted for 3- MCPD in soy sauce and acid-HVP,European Commission (2001). Some studies Crews, et al. (2003) revealed that elevated levels of 3-MCPD can occur not only in soy sauces and related products ,but also occurrence in many foods and food ingredients formulated without acid –HVP (Crews et al. (2001),Hamlet et al,(2002) Breitlins-Utzmann et al. (2003). Fatty acid esters of 3-MCPD were identified in acid –HVPVelisenet et al. (1980) And Svejkovskaet et al. (2004) it has been recently found in a variety of processed foods. Thermally processed foods and refined fats and oils are the most significant sources of 3- MCPD fatty acid esters for consumer Queenswa et al. (2012).

2.2 MCPD structural and molecular formula, and relative molecular mass:

-Structure



- **Molecular formula: $C_3H_2CLO_2$**

- **Relative molecular mass: 110,54**

2.3 Chemical and physical properties of 3- MCPD esters:

From Liu et al. (2005). Merck index (2010), and Scifinder (2010), description liquid with a pleasant odour, and a tendency to turn to a straw colour, and the boiling point of 3- MCPD esters at temperature 114-120 ^{O}C and melting point decomposes at 213 ^{O}C, density 1.3218 at temperature 20 ^{O}C, refractive index 1.4831 in water ,alcohol , diethel ether and acetone. And vapour pressure for 0.195-5.445mmHg at temperature 50- 100 ^{O}C 3-MCPD esters belongs to group of chemicals called chloropropanols, at is the most widely occurring in foods , fish sauce, instant noodle, garlic powder ,spice mixture, mixes for soups, broths ,sauces and gravies ,bread, snacks etc (Queenswa et al. 2012).Processed foods reported to contain 3- MCPD esters includes edible oils and fat containing foodstuffs e.g, palm oil ,infant milk substitute, french fries, salty crackers, sausage, toasted bread etc .Fatty acid esters of 3-MCPD arise in processed foods as primary reactions of lipid and chloride, the reaction is promoted by high content of fats and salts under higher processing temperature. Same studies reported that refined oils contain higher levels of 3-MCPD fatty acid esters and suggested that the deodorization step (the last step of refining, in which unwanted aromas and off-flavorings are removed) seems to be a critical step for the formation of 3-MCPD fatty acid esters during oils processing.

2-4- Occurrence of 3- MCPD esters in vegetable oils and foodstuff:

In vegetable oils and foodstuffs 3-monochloropropanr 1,2diol (3-MCPD) has been known as a processing contaminant for decades especially in hydrolysed vegetable protein (HVP) where it was first found ,and in soya sauces, Zaizuhana and Kalanihi et al. (2008). According to the European union (EU) legislation, the tolerable daily intake (TDI) of free 3-MCPD is 0.02mg\kg^{-1}for HVP and soya sauces .it is also used by the scientific committee on food (SCF) and the joint FAO\WHO expert committee of food additives (JECFA). Recently, free 3-MCPD has also been detected in foods like bread, toast, noodle and smoked products. interestingly however, bread and noodles could be important contributors to the total daily intake especially for their strong consumption rather than for their content in 3-MCPD but the same TDL value seems to be applied .Many surveys or studies have been carried out of free 3-MCPD in foods, until recently ,where 3-MCPD esters have been found in oils and fats. Various paper have documented the presence of free 3-MCPD esters in many food products, such as cereals, roasted , coffee, malts breads, etc (Hamlet and Sadd. 2004, Dolezal et al. 2005,Divinava et al. 2002). Reported values are between 0.2-6.6mg\kg^{-1}in most analyzed foodstuffs and the levels of bound 3-MCPD are generally much higher than the free form, salami and other meat products also recorded high values of up to 6,4mg\kg^{-1}(Reece. 2005, Svejkovska et al. 2004, Zelinkva et al. 2006). Oils and fats are considered to have a higher potential of forming 3-MCPD esters upon high thermal treatments

,especially during deodorization and refining, where temperature typically reach 240C^0 and above ,some oils appear more receptive to the formation of these esters, as was discussed by Weibhaar (2011). Although palm oils have shown higher values in comparison to other refined vegetable oils, the history and source 0f oils have to be checked against the methodology used (Raznim et al. 2012). Provided details of 3-MCPD esters in refined palm oil, olein and stearin where used the BFR 008 indirect method was used, the highest recorded value was 5-7mg\kg$^{-1.}$ Currently, the palm oil industry in Malaysia is taking measures to reduce the formation of 3- MCPD esters based in research knowledge gained in recent years. The risk of exposure to 3-MCPD esters has not been fully evaluated, as potentially all vegetable oils in the presence of chlorides which are subjected to thermal treatments as in cooking, roasting, baking, and frying, will have probability of forming these components (Weibhaar (2011) , calculated to be exposed to an average of 1.5ug\kg^{-1} of body weight (bw)\day of free 3-MCPD ,assuming that the bound ester are fully hydrolysed in the gut. This works out to be the TDL value accepted by JECFA, natural occurrence 3-MCPD esters is not known or no information to occur as a natural product.

2.5 Occurrence in food

3-MCPD esters are one contaminants that can occur in various foodstuffs during the processing (Wenzl and Karasek et al. (2007). In acid hydrolysed vegetable protein (HCP), a seasoning ingredient that is widely used in a variety foods such as soups ,bouillon cubes ,sauces and soya sauce (Clta et al. 2004) 3-MCPD is the most abundant chloropropannol found in foodstuffs, (Wenzl et al. 2007). In addition, domestic processing can produce substantial increases in the 3-MCPD content of bread or cheese (Crews et al, 2004, Breilling –utzmann et al. 2003). Several studies on mechanism of 3- MCPD formation have been performed (Hamlet and Sadd, 2002, Hamlet et al. 2003, Hamlet et al,2005,Mulleret, et al. 2005).and showed that it is formed from glycerol or acylglycerols and chloride ions in heat processed foodstuffs that contain fat with low water activity as stated by Delezal et al. (2004). Although the overall levels of 3-MCPD in baking products are relatively low ,the high level of consumption of bread ,and its additional formation from toasting, indicate that this staple food alone can be a significant dietary source of 3- MCPD Breilling- Utzmann, et al. (2003). The 3-MCPD found in malt products in coloured malts and at highest levels in the most intensely coloured samples, additional heat treatments, including are significant factor in the formation of 3-MCPD in these ingredients (Hamlet et al. 2002, Muller et al. 2005). Also 3-MCPD are occurrence in beer as less than 10ug\L was reviewed recently (Iarc, 2010). Also occurrence in smoked fermented sausage concentration range above 0.02mg\kg (Kuntzer and Weibhaar, 2006, Lira. (2010). The average concentration of 3- MCPD in food or food ingredient (average < 0.3mg\kg, less than soya sauce average 0.8mg\kg (JECFA, 2007). Estimated average dietary exposures of the general population from a wide range of foods ,including soya sauce and soya sauce related products, ranged from 0.02 to 0.7 ug\kg (bw) per day ,and these for consumers at the high percentile (95^{th}), including young children ranged from 0.06 to 2.3 ug\kg (bw) per day . The exposures were calculated by linking data on individual consumption and body weight from national food consumption surveys with data on mean occurrence obtained from food contamination surveys (JECFA, 2007). The secondary school students in China , Hong Kong special administrative region ,have been published since that time ,the average exposure which was estimated to be 0,063-0,150ug\kg (bw) per day , while that for high consumers was 0.152-0.300 ug\kg (bw) per day (You et al 2008). The mean intake level of 3-MCPD in the republic of Korea was estimated to range from 0.0009 – 0.0026ug\kg bw per day and at the 95^{th} percentile of consumption was 0.005 ug\kg (bw) per day as Hwang et al. (2009).

3-MCPD esters were found in goats milk and human milk (Zelinkova et al. 2008, Rann and Yaylan. (2010). During food processing (especially during oil refining) the 3-MCPD esters may occur and various mechanism are currently under investigation that most probably in value a nucleophilic attack by chloride (Rahn and Yaylan et al. 2010). Also 3-MCPD esters found in packed cereal products Karsulinova et al. (2007). Concentration of 3-MCPD esters is highest in refined fats and oils were detected in palm oil and palm oil based fats WeiBhaar and Perz. (2010). And addition that the 3-MCPD occurs in potato products French fries and ships Hamlet. (2009), Hamlet and Sadd. 2009, Zelinkova et al. (2009).

2.6 Toxicity of 3-MCPD esters

Ingestion of free 3-MCPD is neither adsorbed by human intestinal cells , but migrate through cell monolayer barrier to increase various effects on the kidney and on the reproductive system of the mature male rate based on the increased incidences of kidney renal tubule carcinomas and leydig cell tumors Hail et al. (2017). The 3-MCPD induced morphological changes a DNA damage of leydig cells result in early apoptotic cell death (15). Lending cells are the primary source of testosteronein males and their differentiation in the testes is an important event in the re productive system development of male (Bely Hail et al. 2017). The scientific panel on contaminants in the food chain ,former European commission's (EC) scientific committee on food and the joint FAO\WHO expert committee on food additives (JECFA) set a tolerable daily intake (TDL) of 2ug\kg of body weight in 2001.The maximum allowable content of 3-MCPD in foods at 0.4mg\kg (400ug\kg)for liquid condiments (spices) sited in 2008 by (JECFA), (Bely et al 2017) the JECFA evaluated the safety of 3- MCPD in 2001 and re-evaluated in 2006 ,however , the toxicological data of 3-MCPD fatty acid esters are limited. Lin 2006, the JECFA commented that there were insufficient data to evaluated the intake or toxicological significance of 3- MCPD esters and there is no safety reference value for 3-MCPD fatty acid esters .It was reported that the primary toxicological concern of 3-MCPD is potential to release. 3-MCPD in vivo during digestion in the gastrointestinal tract. However ,the potential toxicological properties of intact 3-MCPD fatty acid esters ,actual fate and metabolism, such as the degree of hydrolysis of 3-MCPD fatty acid esters during digestion, which amount of free 3-MCPD is released are still unknown .

2.7 Kinetics and metabolism of 3-MCPD fatty acid esters

3-MCPD has been shown to be widely distributed in body fluid and cross blood –brain barrier and blood testis barrier .3-MCPD was found to be detoxified by conjugation with glutathione, and oxalic acid subsequently would be formed being a haloalcoholic, there is ample evidence that it may undergo microbial enzymatic reaction to form glycidol, which has been in vivo.

2.8 Mechanism and formation of 3-MCPD

3-MCPD esters can be formed by reaction of triglycerides (TG) with chloride (precursors at a temperature>140 ^{O}C ,hence, removing the chlorine precursors and \or avoiding acidic conditions during refining process are the most effective mitigation strategies, Maro et al. (2016).This is ,however easier said than done ,first of all ,it is very complex to determine the amount and nature of chlorine precursors in crude palm oil (CPO) and in practice the potential of a crude palm oil (CPO) for 3-MCPD ester formation is not known Maro et al. (2016) .

Applying the same refining process on different crude palm oil (CPO) (from various plantations) can give significantly different 3-MCPD levels which is quite frustrating for both the technology provider and the oil refiner Maro et al. (2016), sited in Dolezal, et al. (2009).

Thoroughly washing the fresh palm fruit bunches (FFB) defer storage and refining ,seems to be most efficient processes for the removal of chlorine precursors .As 3-MCPD esters are already formed at suite low temperature (140C^{0}) it is not possible to control or minimize their formation during deodorization, Maro et al. (2016), sited in Dolezal, et al. (2009).

Bleaching is therefore the most critical refining stage for the mitigation of 3-MCPD ester ,physical refining of freshly washed crude palm oil with use of natural bleaching earth can give 3-MCPD esters levels between 1-2ppm depending on the crude palm oil quality and the efficiency of the washing process(Maro et al. 2016) sited in Dolezal, et al. (2009).

Chemical refining has shown to be a good process for low 3-MCPD esters but good curde palm oils (CPO) quality is still required to get <1ppm 3-MCPD. Chemical interestification (CIE) is currently the only process that can be remove degrade 3-MCPD esters. interestification followed by post bleaching with non HCL activated bleaching earth (to be grade GE formed during CIE and deodorization at mild temperature (220 ^{O}C) is today the only industrial process that can give refined food oils with very low GE and 3-MCPD from standard quality (commodity) crude palm oil, Maro et al. (2016) sited in Dolezal, et al. (2009) .

The deodorization step with its high temperature of more than 200 ^{O}C mainly influenced formation of 3-MCPD esters in the oils, Frank. et al. (2009). as second refining of a pre-refined palm oil starting with a very high level of 5mg\kg had little effect on the contents ,the bleaching step leading to a decrease of the ester content and, as could be expected, the deodorization step resulted in an increase of 3-MCPD esters content. Franke, et al. (2009).

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 as stated by Baer. et al. (2010).

Heat processing of lipids with added sodium chloride occurring 3-MCPD in the present of acid hydrolyzed vegetable protein (HVP), hydrolysis of 3-MCPD esters by lipases as can occur in baked bread. (Baer, et al. 2010). 3-MCPD and other chloropropanols can be formed from the degradation of epichlorohydrin in aqueous media because of epichlorohydrins slow hydrolysis in the presence of chloride ions (Ils, 2005, sited in Fauzi, et al. (2016).

The majority of scientific research conducted on 3-MCPD esters formed during oils refining ,deodorization at temperature of 180-200 ^{O}C. Destaillats, et al. (2012 and Seefelder, et al. (2008). attempted to defer if the hydrochloride responsible for 3-MCPD esters formation was originating from the stripping steam applied during oil deodorization ,but with a similar negative result. Franke, et al,(2009) , Hrncirik and Van Duijn (2011)) demonstrated that both inorganic and organic chlorinated compounds are present at ppm (mg\kg) levels in partially refined edible oils ,further ,the authors demonstrated that the thermal decomposition of organic chloride containing compounds in crude palm oils (CPO) was pound to coincide strongly with the evolution of 3-MCPD diesters are the predominant class of MCPD fatty esters in refined oils. Nagy, et al. (2011).

The temperature may decomposed the organic or inorganic chlorinated compounds above which the released reactive chlorine and then interact with TAG and result in the formation of MCPD fatty esters at 180 ^{O}C (Nagy et al. 2011). Many of the past mechanistic studies of MCPD -FE formation are carried out in hydrophilic media, it has long been suggested that TAGs under -went hydrolysis to DAG as a first step in the MCPD-FE formation reaction, DAGs then reacted with chlorine donor compounds resulting in the formation of scylonium ion intermediates and eventual nucleophilic substitution of chloride ion on the glycerol backbone. Velisek, et al. (2002).and the TAGs can act directly as a substrate for MCPD-FE formation. Destaillats. et al. (2012). Further ,Rahn and Yaylayan (2012) proved with infrared (IR) spectroscopy that heating TAG in the presence of low acids can lead to cyclic acyloxonium ion formation ,thus ,is it theoretically conceivable to assume that the two pathways involves the reaction of TAG directly with HCL formed by thermal degradation of chlorine donors, nucleaphilic substitution of chloride ion on the glycerol backbone to form on MCPD-FE and finally acid, the second mechanism first involves the formation of an acyloxonium ion intermediate compound. These two MCPD-FE formed mechanisms were summarized by, Destaillat, et al. (2012).

2.9 MCPD-esters level in different vegetable oils and bread samples

The majority of scientific research indicated for 3-MCPD-esrers level in different vegetable oils and foodstuffs, the average content of 3-MCPD-esters in sunflower oil at 0,3mg\kg and the maximum content 1.5mg\kg and the average content of 3-MCPD-esters in cotton oil 2.8mg\kg and maximum content 7.0mg\kg, the average content of 3-MCPD-esters in palm oil 4.5mg\kg and maximum content of 3-MCPD-esters in palm oil was 13mg\kg (Fediol et al. 2009). Concentration of bound 3-MCPD esters in bread crumb was at interval 1.56-21.13mg\kg of fat (i.e, 5.7-71.9ug\kg and concentration of 3-

MCPD esters in bread crust was at interval 2.8-23.60mg\kg of fat (i.e 19.7-84.9ug\kg) as stated bay Kertisova, et al. (2009).

Malthavs, et al. (2011), reported about the 3-MCPD-esters level in vegetable oils ,the average level content of 3-MCPD-eseters in soybean oil was 0.5 mg\kg and the maximum content 0.6mg\kg ,the average level content of 3-MCPD-esters in refined sunflower 2.0mg\kg and the maximum content 4.0mg\kg ,the average content in refined corn oil 7.0mg\kg and maximum content 9.0mg\kg ,average content in refined coconut oil 7.0mg\kg and maximum content was 7.5mg\kg and the average level content of 3-MCPD-esrers in refined palm oil 6.0mg\kg and the maximum content of 3-MCPD-esters in palm oil 14.0mg\kg .

Palm Olein to palm stearin was found to be about 2.0 because Razak, et al. (2012), found 3.0mg\kg -3.8mg\kg 3-MCPD-esters in RBD (refining ,bleaching and deodorization) palm oil and only 1.6 mg\kg -1.8mg\kg in palm stearin .

The content of 3-MCPD-esters in fish oils 0.7-13mg\kg and primrose oil 0.8-5.2mg\kg ,hazelnut oil 19.0mg\kg, grape seed oil 0.8-4.2mg\kg , olive oil 0.3-3.0mg\kg and walnut oil 1.2-19mg\kg and also the content of 3-MCPD-esters in fish oil 0.1-1.2mg\kg ,walnut oil 0.7-1.4mg\kg ,grape seed oil 0.3-2.5mg\kg , palmkernal oil 0.3-2.5mg\kg and Shea butter 3.1mg\kg Kuhlmann, et al. (2011).

3-MCPD-esters and related compounds in various vegetable oils content in rapeseed oil 0.3-1.5mg\kg ,sunflower oil 1.0 -4.7mg\kg ,corn oil 2.8-7.0mg\kg and palm oil 4.5-13.0mg\kg (Fediol, 2009) .

Low level(0.5-1.5mg\kg), rapeseed, soybean and sunflower oil, medium level (1.5-4.0mg\kg), safflower ,groundnut, corn, olive ,cottonseed ,rice and bran oil and highest level (>4.0mg\kg), hydrogenated fats, palm oil and palm oil fractions and solids frying fats .

Weibhaar. (2011) also mentioned that the level of o.5-10.5mg\kg fat (median :2.3mg\kg fat) have been found in margarine ,< 0.1 -16.9mg\kg fat (median :1.5 mg\kg) in fillings and toppings of cookies

,crackers ,and bars ,2.3-10.3mg\kg fat (median :4.9mg\kg)in the sweet spreads (hazelnut ,nougats and spreads) and 0.5-8.5mg\kg fat (median :2.5mg\kg) in infant formulae (powder).

Amounts of 3-MCPD measured in a bakery fat and\or retail soft dough biscuit were 1285 and 632ug\kg and consistent with the amount reported previously, application of the developed method for bound mono-3-MCPD and diesters of MCPD showed that mono-esters of MCPD accounted for 15.7% and 9.4%of the total bound MCPD in the bakery fat and biscuit, respectively ,and the concentration of free 3-MCPD in bread samples was at interval <9-54.5ug\kg, concentration of bound 3-MCPD was at interval 1.56-23.6mg\kg of fat (i.e 5.7-84,9ug\kg of sample),(Karsuinova, et al. 2007, Dolezal et al .2005 , Divinova, et al. 2007 and Zelinkova, et al. (2008).

Hamlet, et al. (2004) ,measured 3-MCPD-esters in toast bread highest levels were found in regions of the bread that attained the highest temperature, i.e the crust , and level increased from 60-160ug\kg^{-1}when the bread was toasted over 40-120 seconds ,the highest level of 3-MCPD-esters (6100ug\kg^{-1}) was found in a sample of French fries Svejkovska, et al. (2004) .

The level of 3-MCPD-esters in coffee was relatively low and varied between 6.0ug\kg^{-1} (soluble coffee) and 390ug\kg^{-1} (detafeinated coffee) although it exceeded the free –MCPD-esters level by a factor of 8-33 times, Doleza, et al. (2005). The presence of 3-MCPD-esters in bread crumb, picked olives and herring suggests that these compounds can also from at relatively low temperature and even in acid media, Hamlet, et al. (2004).

The 3-MCPD-esters detected in human breast milk at a concentration from below 300-2195ug\kg^{-1} fat or 6-76ug\kg milk ,indicating that 3-MCPD-esters could be absorbed and distributed to human tissues and organs. Zelinkova, et al. (2008). And the content of 3-MCPD-esters in meat products is generally low, ranging from an undetectable quantity to 0.081mg\g\kg^{-1} (Crew,et al,2002,Chung, et al. 2008).

a content of 3-MCPD-esters less than 3.3mg\kg^{-1} of fat has been determined in meat products ,and a content of 3-MCPD-esres in streaks bacon was 0.15mg\kg^{-1} , Svejkovska, et al. (2004) .

2.10 Factor effecting formation of 3-monochloropropane-1,2-diol (3-MCPD) in vegetable oils and bread

The majority of scientific research indicated that, temperature can affect the 3-MCPD-esters formation the formation of 3-MCPD-esters in vegetable oils and foodstuffs is influenced by many factors, including, temperature, refining, bleaching, deodorization, (RBD) pH, moisture content, sugar and lipid

The formation of 3-MCPD-esters in oils was linked with the preliminary heat treatment of oilseeds and with the processed of oil refining , and the analysis of crude , degumming ,bleached and deodorized rapeseed oil showed that the level of 3-MCPD-esters decreased during the refining process (bioactive compounds in foods edited by John, et al, (2008) black well publishing, however, additional heating of seed oils for 30 minutes at temperature ranging from 100-280, ^{O}C and heating at 230-260 ^{O}C for up to 8 hours led to an increase of the level of 3-MCPD-esters conversely heating olive oil resulted in a decrease in the 3-MCPD-esters level.

Zelinkova, et al. (2006) reported that heat treatment of oilseed during refining could influence the formation of 3-MCPD-esters the actual mechanism of it is suggested that in addition to the thermal treatment, it could also be due to enzyme catalyzed reaction .Three mechanism of 3-MCPD-esters formation have been proposed: Acid hydrolysis of glycerol and other lipids in the presence of chloride ions ,as happens during the formation of acid-hydrolyzed vegetable protein (acid-HVP), heat processing of lipids with sodium chloride (present naturally or added), occurring in the absence of acid –HVP and hydrolysis of 3-MCPD-esters by lipases ,as can occur in baked bread. Daer, et al. (2002).some processed foods that do not contain acid –HVP, such as malt products, cold smoked meats ,sausage and fish, cooked meats (salami, bacon, hamburgers, anchovies packed in oil, melted or grilled cheese, breads and biscuits, instant coffee and roasted coffee beans reported by Hamlet, et al. (2002).

3-MCPD-esters are formed by the action of high temperatures in foodstuffs; temperature and period of heating have the greatest influence on the formation of the 3-MCPD-esters. Their total amount depends however, on the content of precursors, i.e chloride ions and the pertinent organic compounds, particularly glycerol and it is partial esters with fatty acids, their formation is also influenced by the water activity in the food and it is influence on the hydrolysis of triacylglycerols, Svejkivka, et al. (2006). 3-MCPD-esters and other chloropropanols, including 1,3 DCP (1,3dichloropripanol-2,3-DCP, and 2-monochloropropane-1,3-diol (2-MCPD) can be formed in foods during processing ,cooking and storage as a result of the reaction of chloride ions with glycerol and other lipids present in food ,WHO, (2007).

2.10.1 The major factor affecting formation of 3-MCPD-esters in vegetable oils

2.10.1.1 Effect of refining conditions

The temperature of refining, particularly that of deodorization has been suggested as the factor causing the formation of 3-MCPD-esters in oils and fats (Franke, et al. 2009). Further studies by MOPB have established that acid activated clays, especially of low PH can contribute to the formation of the esters, Ramli, et al. (2011).

The studies were conducted on a 200kg per batch pilot-scale refining plant, a comparison of the effect of acid degumming with water degumming and evaluation of acidity of bleaching earths on formation of 3-MCPD-esters were performed, and the authors reported that water degumming let to the lowest formation of the esters as compared to acid degumming. It was also observed that there was a strong correlation between acid activated clay with the formation of the esters. The authors concluded besides the high deodorization temperature, the acidity of bleaching clays and the dosage of phosphoric acid are also contributing factors to the formation of 3-MCPD-esters. Even though the application of water degumming and natural bleaching clays could reduce the formation of the esters, the colour of the refining oil exceeded the palm oil refiners association of Malaysia (PORAM) specification 3red maximum, a similar study was reported by Pudel, et al. (2011) ,using a laboratory scale experiment to simulate the ,actual chemical refining condition ,the authors compared the formation of the esters in CPO without friar pre-treatment (degumming and neutralization) with pretreated CPO, the CPO without pre-treatment contained the highest level of the esters , it was reported that water degumming (5% distilled water w\w) led to the lowest 3-MCPD-esters formation in the refined oil, the authors also varied the deodorization temperature and reported that $240^{O}C$ was an a deal temperature since the formation of the esters was negligible both groups, Ramli, et al. (2011) and Pudel, et al. (2011).

Hrncirik, (2009) monitored the formation of 3-MCPD-esters by comparing the effect of chemical refining and physical refining and reported that lower level of 3-MCPD-esters was detected in refined oil from the former method. however, Stadler, (2009) reported that the difference in the formation of 3-MCPD-esters between the two processes (chemical and physical refining) was in significant although it is difficult to explain the discrepancies in result by both groups of researchers ,refiner have in general found that chemical refining results in lower 3-MCPD esters formation , this may be due to the removal of acidity in the degummed oil, as well as to the removal of precursors during alkali-treatment and to the lower temperature of deodorization .

2.10.1.2 Effect of degumming

Degumming is the first step to removing gums from the edible oils refining, by addition of water or acid ,resulting in their precipitation from the oil , while in most vegetable oils significant amounts of gums are removed during degumming. Palm oils content of phosphorus normally is low, in the range of 10-20mg\kg, as reported by Gibon, et al. (2007), Thus for palm oils dry degumming is typically carried out by addition of phosphoric or citric acid subordinate or followed by bleaching and removal of the gums together with the spent bleaching earth, formation of 3-MCPD-esters precursors, is activated by using acid in the degumming process suggested by (Schuez, 2010). Formation of the 3-MCPD-esters decreased with lower dosage of phosphoric acid used for degumming Ranliet ,et al. (2011). although there was no a marked decrease in formation with calcium oxide prior to bleaching to neutralize the degummed oil or for water degumming ,remarkable decreases in the concentration of 3-MCPD-esters, were seen after bleaching and deodorization ,the treatments resulted in a 30-70% reduction of the 3-MCPD-esters content.

2.10.1.3 Neutralization effect

During the neutralization step of chemical refining (with sodium or potassium hydroxide or calcium oxide) ,two different effects influence the formation of 3-MCPD and glycidyl esters ,neutralization lowers the acidity of oil before deodorization , while potential precursors can be removed from the oil during the water wash to eliminate the soapstock. After crude palm oil was neutralized with potassium hydroxide, lower capability to form 3-MCPD-esters and related compounds than an untreated samples, the use of sodium hydroxide leads to a slightly lower reduction of 35%, Pudel, et al. (2011).

Lauric acid and palmitic acid ,had only a very small effect on the formation of 3-MCPD and glycidyl esters ,the authors stated that the fatty acids cannot act as direct precursors for the formation of the esters, but they could affect the PH value of oil, resulting in a higher capability of the oil to form esters during deodorization. The PH value decreased the addition of lauric acid, while the PH value did not change during addition of palmitic acid ,probably because palmitic acid was a weaker solubility. therefore, it is recommended to carry out a neutralization step prior to deodorization to avoid lower PH values resulting from higher contents of free fatty acids. From this point of view, chemical refining seems to be the better option for lowing contents of 3-MCPD-esters and related compounds in comparison to physical refining, which is the common practice for refining palm oil, Freudenstein, et al. (2012).

2.10.1.4 Bleaching effect

The primary function of bleaching is not only removing the oil color, but is the purifying the oil or fat to improve its quality and stability by removing the acids, peroxide compounds, oxidation products, colour, phosphatides, metallic compounds (Fe, Cu), FFA and tocopherols, by using HCL, H_2SO_4 ,hydrated aluminum silica and silica gel, hydrochloric acid activated bleaching earths could be sources for the chlorine-donating compounds that lead to the formation of 3-MCPD-esters, in most cases ,bleaching earths are activated either by sulphuric acid or hydrochloric acid to increase the surface area for adsorption, while acid activated clays have surfaces between 150-350m^2\g, the surfaces of natural clays have surfaces half that size or even less, nevertheless, in most cases the pore volume and the pore size of the natural clays is sufficient, Zschau, et al. (2001).

The type of bleaching earths (neutral or acidic) can be influenced the PH value of oil prior to deodorization found a good correlation between the content of 3-MCPD-esters in refining oil and the acidity of bleaching earths for acidic and water degumming, in both cases, a higher acidity of the bleaching earth resulted in higher formation of 3-MCPD-esters as stated by Ramli, et al. (2011). Therefore, the use of natural bleaching earths and acid activated bleaching earths with more neutral PH value as options to reduce the formation of 3-MCPD-esters, but they did not take to in consideration the effect of the bleaching earths on the formation of glycidyl esters. In addition good washing procedures are effective in removing residual acids , bleaching step reduces the formation of 3-MCPD and related compounds during refining , there seems to be a tendency to assume that a lower bleaching temperature would be advantageous , but the data was not evident, the authors also demonstrated that the amount of activated bleaching earths (0.7% and 1,5%,) had no significant effect on the formation of 3-MCPD and related compounds during the following deodorization step, Pudel, et al. (2011).

On the other sited Schurz (2010) described that a much higher dosage of activated clay can absorb some of the 3-MCPD –esters precursors from the oil, resulting in a lower capability to form esters. This is confirme by results the obtained by Matthaus, et al. (2011) who formed a significant decrease of 3-MCPD-esters and related compounds after deodorization at 250 ^{O}C for one hour when bleaching of water degumming palm oil was performed with 3% bleaching earth instead of 1%.

2.10.1.5 Deodorization effect

Since the formation of 3-MCPD-esters and related compounds results from heat –induced reactions ,it is clear that deodorization time and temperature are important factors when considering mitigation strategies ,up to a temperature of 240 ^{O}C, the influence of time and temperature during deodorization on the formation of 3-MCPD-esters and related compounds. The formation of 3-MCPD and glycidyl esters is relatively low and was found in the range between 2mg\kg-4mg\kg for 3-MCPD-esters and related compounds, Ppudel, et al. (2011). With longer deodorization times the values decrease, probably because some of the 3-MCPD and glycidyl esters precursors Compounds are volatilized to in the distillate or the compounds are destroyed. The concentration of the esters increases dramatically with the increasing temperature higher than 24 ^{O}C, but also with the time of deodorization , the concentration of 3-MCPD-esters and related compounds decreases, However, from a practical refining point of view the temperature has no relevance and is only a temperature ceiling or a worst case scenario for comparison. Similar results were founds by Hrncirik and Duijn (2011), who showed a stable amount of 3-MCPD-esters in neutralized bleached palm oil deodorized at temperatures between 180-230 ^{O}C and times between 1-5 hour. Same authors also found only a small effect on the content of glycidyl esters for temperature increased from 180-230 ^{O}C, while a steep increase was found for a further temperature increase above 230 ^{O}C. Destaittate, et al. (2011) reported that the critical temperature for the formation of glycidyl esters from DAG is approximately 200 ^{O}C, and above this temperature the formation accelerates rapidly with increasing temperature. However, the formation of 3-MCOD-esters seems to feature a reaction that is completed in the early of the heating period and requires only minimal heating Shimizu, et al. (2012).

The contents of 3-MCPD-esters and glycidyl esters found in the distillate of palm oils deodorization at temperature 240 ^{O}C for two hours was only very small, with amounts of about 0.01m\100g deodorized oils as stated by Matthaus, et al. (2011).

2.10.1.6 Dual deodorization

Indeed, temperature reduction is sufficient to reduce 3-MCPD and glycidyl esters, but it would also be accompanied by lowered oil quality, as an alternative, agent deodorization had been suggested by Matthaus, et al. (2011) .

Dual deodorization has already been commercially applied with the objective of inhibiting the formation of trans fatty acids and to optimize the amounts of vitamin E-active substances in oils as stated by Qibon, et al, (2007-2009). Using a short first step at a high temperature 250-270 ^{O}C combined with a second longer step at a lower temperature (200 ^{O}C), or vice versa, the total temperature load on the oil during deodorization is reduced. A comparison of different combinations of short and long term deodorization to conventional (one –step) deodorization shows significant reaction of 3-MCPD-esters and related compounds both at 250-270 ^{O}C. Furthermore, it is favorable to apply a longer deodorization at a lower temperature followed by a short high-temperature deodorization. The combination of 120 minutes at 200 ^{O}C followed by 5 minutes at 250 ^{O}C results in a reduction of one third of amounts of 3-MCPD-esters and related compounds, 3-MCPD-esters alone were two-third lower than for the conventional deodorization, at 270 ^{O}C. Dual deodorization preceded by longer deodorization at a lower temperature achieved a nearly 80% reduction of 3-MCPD-esters and related compounds, Matthaus, et al. (2011).

2.11 Methods of 3-MCPD-esters analysis

Several methods of analysis have been developed by various research organizations, these methods are based on indirect determination of bound 3-MCPD by transesterification in acid (BFR method 008)or in alkaline (BFR method 009 and 010:DGF, 2009:Weibhaar, (2008).

The principle of indirect method involves the conversion of the esters to free 3-MCPD, and then the 3-MCPD is quantified using Gas chromatography –mass selective detector (GC-MSD), and, other than that, the direct method of quantification of 3-MCPD-esters has also been developed using erating 2-monoglycerides .

Crews (2011) reported the problems which arise with a number and variety of methods as indicated indirect methods with acid or alkali method analysis and direct methods with and without clean-up .

2.11.1 Direct methods with varied LC-mstechnique

Insufficient agreement and between indirect method and direct methods These problems are caused by wide differences in analytical approaches, lack of reference compounds, lack of understanding of indirect method chemistry, large number of analytics for direct methods and problems of matrix effects in direct methods .Several methods presented (other under development) Provide a full profile, but is difficult for routine analyses. Substantial challenges (standards, instrumentation, sensitivity).

2.11.2 Analysis of 3-MCPD-esters direct method

The first direct method was presented in 2010 by Collision and Maines (ADM Research), using liquid chromatograpgy-time- flight\mass spectrometry (LC-TOF\MS) as a quantification tool, the method was developed to provide direct analysis of 3-MCPD mono-esters and di-esters without chemical modifications which can give incorrect results and also provides very specific correct identification of the analysis, dilute and shood, method Pinkston and Stoffolano, et al. (2011).

Standards are required for the mono-esters and diesters corresponding to the known fatty acid composition of the vegetable oils. However, the method require frequent instrument disassembly and cleaning, there are also other groups working on direct methods aich as Granvol, et al, (Tu Munchen):Mathieu (Nestle):and S-Macmahon, et al. (EDA) they are currently developing a direct method for determination of 3-MCPD-esters without issues related to instrument fouling .

Haines, et al. (2011) reported on the direct determination of 3-MCPD-esters in vegetable oils by LC-TOF\MS they developed this method because the DGF method gave inconsistent results when salting out conditions varied, they compared the results of the LC-TOF\MS method with the DGF method and results showed that the DGF method consistently gave results that were greater than the LC-TOF\MS method. However, there are a few challenges with the LC-TOF\MS method, the sodium in the module phase has determine effects on the MS system, and thus requires quick cleaning every day prior to use. Also certain parts in the ESI source corrode quickly and require frequent replacement, furtheremore, the need for individual standards is costly and not many labolatories can afford LC-TOF\MS equipment, thus the usage of indirect method may be limited only to a few laboratories.

2.11.3 Indirect method analysis of 3-MCPD-esters

Determination of bound 3-MCPD is based on the conversion of individual 3-MCPD-esters into a single compound, the indirect method classified to in (A) acidic tramsesterification and (B) alkaline transesterification. Generally, only two reagents are used for derivatisation , which are phenylbronic acid (PBA) and heptafiuorobutyrylimidazole (HFBL) and the salting out reagent is either sodium chloride (NaCL) or sodium sulphate (Na_2So_4). Simple to perform high, uncertainty results are dependent on sample composition, number of method available, suitable for routine analyses (total bound 3-MCPD), good sensitivity and trueness is questionable.

2.11.3.1 Acid based tranesterification

Divinova, et al. (2004) reported an improved routine, simple and sensitive method for the determination of free and bound 3-MCPD in different foods using acid- based transesterification method and quantification by GC-MS.

Hrncirik, et al. (2011), reported that the transesterification in acid medium a voids problems with selectivity and has greater robustness compared to the alkaline transesterification method, the federal german institute for sisk assessment (BFR) also developed a method based on the acid transesterification which is the BFR method 008, (BFR method-82-FC-008-01),the method involves hydrolysis step which took 16 hour incubation time, from a collaborative study which was conducted in

2009. MPOB has verified this method in food safety laboratory and the percentage recoveries of bound 3-MCPD spiked into blank sample ranged from 90% - 110%, further work by ermacora and Hrncirik (2012) resulted in an improved indirect method for the analysis of 3-MCPD-esters based on acid transesterification. The carried out a number of method which involved both indirect and direct determination of the esters, their study showed that the indirect method is of better sensitivity, although the series of chemical reactions that take place during sample preparation may affect reliability of results. Their study also showed that the interference of chloride ions can be eliminated by a single extraction step of the sample before the analysis. In addition, transesterification time can also be reduced from 16 to 4hr without any significant reduction of the accuracy and repeatability.

Method performance of acidic transesterification (MP.B) has verified the BFR method 008,though a collaborative study which was conducted in (2008), blank oil(crude palm oil) was spiked with different levels of 3-MCPD-ester and the percentage recoveries were found to range from 97%-108%, with relative standard deviation (RSD) between 0.02_0.12%, quantitative analysis was carried out by monitoring characteristic ions at m\z 91.157 and 196, respectively, for d5-3-MCPD, characteristic ions were at m\z 93.150 and 201 qualifier ions were m\z 147 towards m\z 150, calibration curve was constructed and linearity was verified (r^2 >0.999) within the concentration range .

2.11.3.4 Alkaline based transesterification

WeiBhaar in 2008 reported the alkaline –based transesterification method based on the indirect determination of total 3-MCPD in edible oils. The 3-MCPD was released from 3-MCPD-esters by transesterification with sodium methoxide in methanol. This method is adopted by the German society for fat science (DGF) and is known as method DGF C-111 18 (009), in this method, other compounds forming 3-MCPD is glycidol (oxirane -2-methanol), this means that the method is not application for oils with high glycidol compounds. The method is also used as an indirect method to measure glycidol compounds by substracting the 3-MCPD-esters from the total result.

In addition to BFR method 009, there is also BFR method 010, the principle of BFR method 009, is (a) alkaline hydrolysis with methylate solution, (b) neutralization and salting out using ammonium sulphate and sulphuric acid (c) derivatisation using PBA and (d) quantification by GC –MS, the only difference between the BFR method 009, and the BFR method 010, is that the latter uses hetafluorobutyric an hydride (HFBA) as a derivatising reagent, latest report by Hmcirik et al, (2011) showed that the method based on alkaline transesterification is particularly prone to variations in conditions, which is transesterification time and PH value of different salts.

2.12 Bread Technology

Bread-making industry is not a modern discipline, but the improvements of its techniques cause slowly-lately, the changes in human food habit. Through the increase consumption of bread is derived in part, form the convenience and whole some ness of bread and a result of the improvements in the transport infrastructure as well (Dendy, 1992). Wheat is unique among the cereals in its ability to form dough of hydrated protein and starch when its flour is mixed with water. Also, gluten-the unique structure forming protein in wheat, is responsible for the dough’s ability to leaven when fermented, and expand upon baking to form the all too familiar staple of mankind-bread (Hoseney, 1994).

2.13 Principle of Baking

The function of baking is to present cereal flours in an attractive palatable and digestible form. Bread is made by baking dough, which has for its main ingredients wheat flour, yeast and salt. Other ingredients, which may be added, include flours of other cereals, fat, malt flour, soya flour, yeast foods, emulsifiers, milk products, fruit and gluten (Kent, 1982). Zeleny (1971) reported that the quality of wheat is usually judged by its suitability for particular end use.

American hard red winter and red spring wheat and Australian prime hard wheat with 12.5% protein content at least and 62.65% water absorption is suitable for bread making (NCFM, 2003). The baker’s yeast (*Saccharomyces cervisia*) is the best micro-organism adopted for leavening of baker’s product

(Banwart, 2002). On the other hand, Roach (1989) observed increasing mixing time with the addition of sodium chloride, this mean salt also strengthen the gluten. Kim and De Ruiter (1969) reported that crumb texture gradually became finer, crumb color appeared lighter, and crust color tended to become golden brown. The taste of bread got a sweet touch. At level of 4% sugar the crumb also became softer.

CHAPTER THREE

MATERIALS AND METHODS

Material

3.1.1 Samples collection:

Twenty five samples of modern bread, were collected randomly from various bakeries of Sudan capital state (Khartoum, Khartoum Bahri and Omdurman).The samples were divided in to two groups, (12 modern toast bread and 13 round bread), 12 toast bread were collected from different areas of Khartoum, 4 samples were collected from various bakeries Khartoum (Sharwani, Burri, Manshiya and Jabelawlya), 4 samples were collected from various bakeries of Khartoum Bahri (Dar alssam, Mazad, Danagla and Margania) and four (4) sample were collected from Omdurman(Almulazmeen, Shuhada, Fetihaab and Shigla) and the second group is 13 samples modern round bread ,were collected from bakeries of Khartoum, Khartoum Bahari and Omdurman. Divided in to the 5, 4, and 4samples, respectively. Five (5) samples from bakeries of Khartoum (Duyum east, Duyum west, Hila Jadid and Alsajana) , four (4) samples from bakeries of Khartoum Bahri (Kadaru, Al-usbaa, Kobar, and Kafuri) and four sample from Omdurman bakeries(Salha, Jaddain N, Jaddain S, and Libya market). Appendices 1-3 show bread collection from different Bakeries in Omdurman, Khartoum Bahri and Khartoum. Oil was extracted from all the samples, and kept in plastic bottle (polyethylene), and stored in freezer at temperature (-18^{o}C), until analyzed.

3.1.2 Reagents and Chemicals for 3-MCOD

Sodium chloride(p.A), phenyl boronic acid (≥98%, PBA), acetone, hexane, methyl tert-butyl ether (MTBE), methanol, and ethyl acetate (all suprasolv for GC) as well as glacial acetic acid and sulfuric acid 96% (suprapur), were bought from VWR (Damstadt, Germany). Sodium methoxide (25% w/v in methanol), 3-monochloropropane-1,2-diol(98%), and 3-methoxypropane-1,2-diol(98%) were obtained from Sigma Aldrich (Weinherin, Germany). A solution of 50 μL of sulfuric acid in 5 mL of methanol was prepared subsequently for conversion of glycidyl esters (methanol/sulfuric acid). 1,2-dipalmitoyl-3-chloropropane and glycidylpalmitate were purchased from Campro Scientific (Berlin,Germany). A sodium chloride solution (NaCl solution 20%) of 200 g/L 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.

3.1.3 Equipments

Glass wool, Soxhlete, Mortar Pestle ,Sensitive Balance ,Vortex mixer (2500rpm, 30 s), Micro pipette, Beakers, Volumetric flask And Gas chromatography-mass spectrometry(GCMS). From Japan, Shimadzu Company.

3.2 Methods

3.2.1 Determination of moisture content

Sample was weighted and then registered weigh of wet samples, dried at temperature (105^{O}C) in oven for 6 hours and moisture content was determined according to AOAC method (2000).

Moisture content (%) = W1- W2 / W1 *100

W1: weight wet sample.

W2: weight of dried sample.

3.2.2 Extraction of oil

About 200g of dried bread was added inside a thimble in a soxhlet extractor, 500ml of solvent was added (hexane N) to in a distillation flask. The solvent was heated to reflux and flood into the chamber housing the thimble containing dried Bread. At this stage, oil compounds are dissolved in the warm solvent, upon reaching a certain level, the solvent is automatically emptied via the siphon side arm and flow back down to the distillation flask. This cycle was repeated for 4-6 hours at temperature 45 -50 ^{O}C.

Then, the solvent was recovered using a rotary evaporation to obtain the extracted oil. The temperature of the rotary evaporator was set at the boiling of hexane, while the speed of the rotary evaporator was fixed at 20rpm. (soxhet extraction, Malaysia).

3.2.3 Determination of oil content

Oil content was determined according to the AOAC (1990) method, after the extraction at oil and hexane evaporation, weighting oil with flask, and then takes of oil out the flask, and then weighting flask and oil. And determination oil content according to equation below.

Oil content = W1-W2\W3*100

W1: flask and oil weight

W2 : flask blank weight

W3: sample weight

3.2.4 *Determination of 3-MCPD*

3.2-4.1 Preparation of standard solutions

Stock solutions with concentration of 50 mg/mL of 1,2-dipalmitoyl-3chloropropane and glycidylpalmitate were prepared in MTBE. These solutions were further diluted to 50 μg/mL (working solution for foodstuffs) as well as 5 μg/mL (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) as well as other matrices (0.05 – 0.5 μg/mL) 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 4^0 C. (Berlin, Germany).

3.2.4.2 Sample preparation

200mg oil\fat dissolved in 2ml MTBE homogenization, added 500ul $CH_3OH\backslash H^+$ (ring opening , 3min),added 200ul $NaCH_3$(ester cleavage within 1min,added 200ug glacial acetic acid and 2ml NaCL solution 20% (separate the aqueous layer), 200ul PBA was added (derivatization at ambient temperature for 15min) and extraction with hexane. All further procedures of mixing, shaking, and homogenizing during sample preparation were accomplished using a vortex mixer (2500rpm, 30 s).

3.2.4.3 Extraction of Glycidyl Esters and 3-MCPD Esters from Oil

About 200 mg of the homogenized sample was weighed into a screw-capped centrifuge tube. Two milliliters of MTBE and 0.3 µg of the internal standard solution (60 µL of 5µg/mL working solution of 1,2-dipalmitoyl-3-chloropropane-$d_5$for fats and oils) were added and homogenized thoroughly for 30 s. No further sample preparation was required.

3.2.4.4 Conversion of the Glycidyl Moiety

2 mL of separated organic layer or solution of oil were added 500 µL of methanol/sulfuric acid, the mixture was shaken well and heated for 30 min. at 50 ^{0}C. Afterward, the esters were cleaved in the way described below without further preparation.

3.2.4.5 Ester Cleavage

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 s. After 1 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 s. After phase separation, the upper layer was discarded. The aqueous layer was derivatized as described below.

3.2.4.6 General Derivatization Procedure

Two hundred microliters of the Derivatization reagent Phenyl Bronic Acid (PBA) were added to aqueous layer, and the tube was shaken well. After a reaction time of 15 min. at ambient temperature, the cyclicphynylboronate derivatives were extracted with 2mL of hexane. The organic layer was separated and analyzed by GC-MS, according to BFR method_82_FC- 008-01, (2009).

3.2.4.7 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 Japan, Shimadzu Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30m×0.25 mm×0.25µm).The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.20 ml/min, the temperature program was started from 100 ^{O}C with rate 20 ^{O}C /min to 160 ^{O}C hold for 1 minutes then the rate was changed to 5 ^{O}C /minutes to 180^{o}C, finally the rate was changed to 30 ^{O}C /minutes reaching 300 as final temperature degree, the injection port temperature was 320 ^{O}C, the ion source temperature was 230 ^{O}C and the interface temperature was 300. ^{O}C The sample was analyzed by using SIM mode selecting m/z 91,147,196,the total run time was 15 minutes , and results were recorded.

3.2.4.8 Quantification Method

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:

$$\frac{\text{Area of standard}}{\text{Area of sample}} = \frac{\text{Concentration of standard}}{\text{Concentration of sample}}$$

3.2.4.9 Concentration of 3-MCPD exposure assessment for adults and children

For estimation exposure of individual to concentration of 3-MCPD at specified point in a day, twenty breads were give (4-6g) oil, average weight of round bread (65g) and toast bread (55g). individual consumption per day in Sudan about 10 bread, for adults 18-20+ years was estimated, and 6 bread for children 14-15 years .Body weight for deterministic exposure estimates based on the WHO, (2018) simulated diets, mean body weight for adults 68.03kg and children 47.67kg. According to information were determined concentration of 3-MCPD body weight per day for individual equation below.

$E_i = \sum Q_{i.K} \times C_{i.K} \backslash bw_i$.

Where:

E_i is exposure of individual I, to some chemical at some specified point in time, $Q_{i,k}$ is amount of food k, consumed by individual I, $C_{i,k}$ is concentration of the chemical of inters in food k, bw_i is body weight of individual I,(Vannoort and Thomson. 2005).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Moisture Content for round and toast bread from the different bakeries

4-1-1-Table 1 shows the moisture content of modern round bread (RB) and modern toast bread (TB) collected from different bakeries of Khartoum state (Khartoum, Khartoum Bahri and Omdurman), the ID number from 1-9 represents moisture content of bread collected from Omdurman, while ID number from 10-17 idealizes moisture content of bread collected from Khartoum and ID number from 18-25 represents moisture content of bread collected from Omdurman .There was no significant difference between all bread collected from different areas of Khartoum state. Lower moisture content in round bread (RB) were 31.7%, while medial moisture content in round bread were 33.56% and higher moisture content in modern round bread were (35.8%). While the lower moisture content in modern toast bread were 27.2%, medial moisture content in toast bread were 31.02%, and higher moisture content in toast bread were 33.6%. From this information moisture content in modern round bread higher than moisture content in modern toast bread.

Table 1 Moisture contents of modern round and toast bread

ID NO	**Samples**	**Moisture content %**
1	Modern TB	31.9
2	Modern TB	30.8
3	Modern TB	28.4
4	Modern TB	33.1
5	Modern RB	34.4
6	Modern RB	34.1
7	Modern RB	33.4
8	Modern RB	33.7
9	Modern RB	31.7
10	Modern RB	31.8
11	Modern RB	33.4
12	Modern TB	33.6
13	Modern TB	31.9
14	Modern TB	28.8
15	Modern TB	28.2
16	Modern RB	35.5
17	Modern RB	35.7
18	Modern RB	33.6
19	Modern RB	32.9
20	Modern RB	33.3
21	Modern RB	32.8
22	Modern TB	27.2
23	Modern TB	32.8
24	Modern TB	32.7
25	Modern TB	32.7

4.1.2 Oil content for round and toast bread from the different bakeries

Table 2 shows the oil contents of modern round bread (RB) and modern toast bread (TB)collected from different bakeries of Khartoum state (Khartoum, Khartoum Bahri and Omdurman), the ID number from 1-9 represents oil content of bread collected from Omdurman, while ID number from 10-17 idealizes oil content of bread collected from Khartoum and ID number from 18-25 represents oil content of bread collected from Omdurman .there was no significant difference between all bread collected from different areas of Khartoum state. Lower oil content in round bread (RB) was found to be 0.7955%, while medial oil content in round bread was 0.92% and higher oil content in modern round bread were 0.995%. While the lower oil content in modern toast bread as 0.894%, medial oil content in toast bread were 0.894%, and higher oil content in toast bread were 1.005%. And consequently, oil content in modern round bread was higher than oil content in modern toast bread.

Table 2 Oil contents of modern round and toast bread

ID	Samples	Oil Content
1	Modern TB	0.768
2	Modern TB	0.9465
3	Modern TB	0.998
4	Modern TB	0.9955
5	Modern RB	0.7955
6	Modern RB	0.9894
7	Modern RB	0.9305
8	Modern RB	0.9855
9	Modern RB	0.907
10	Modern RB	0.900
11	Modern RB	0.9905
12	Modern TB	0.7805
13	Modern TB	0.8305
14	Modern TB	0.9435
15	Modern TB	1.005
16	Modern RB	0.894
17	Modern RB	0.8945
18	Modern RB	0.905
19	Modern RB	0.975
20	Modern RB	0.9105
21	Modern RB	0.944
22	Modern TB	0.823
23	Modern TB	0.833
24	Modern TB	0.8305
25	Modern TB	0.8785

4.1.3 Figure 1 and 2 show full scan mass spectrum of 3-MCPD and PBA derivatives, PBA readily reacts with

3-MCPD to give a stable derivative 3-MCPD figure (2) appropriate for GC\MS analysis, PBA was derivatisation reagent of TBME as is very selective, the number on the figure (91, 147 and 196) idealizes molecular weight of treat butyl methyl esters (TBME), 3-MCPD and phenylbronic acid (PBA), respectively. The GC\MS employed was linear within the working calibration standard concentrations in a range 0.1-1ppm.

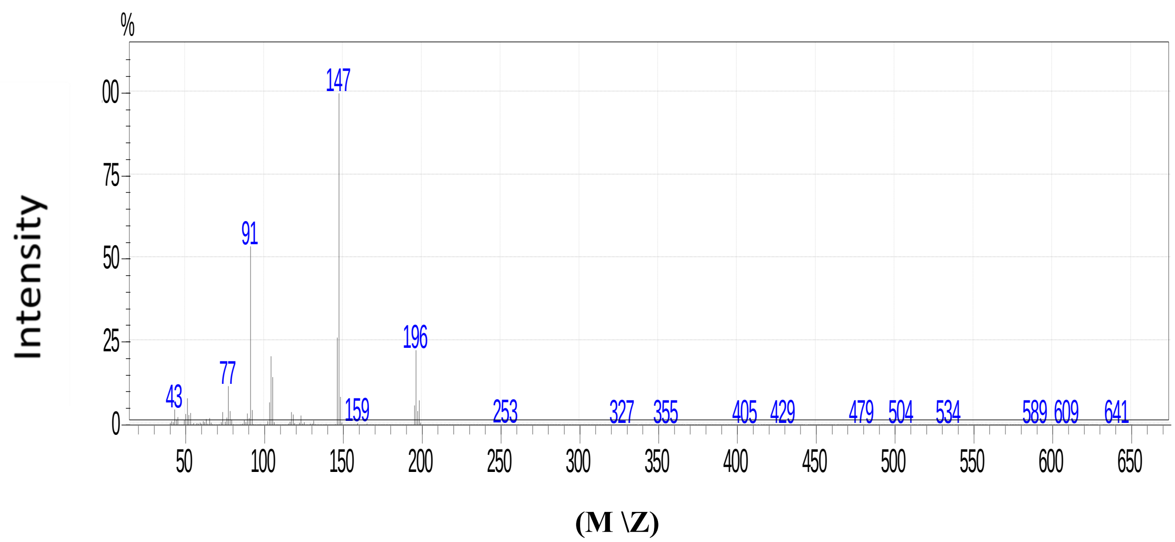


Figure 1. Full Scan mass spectrum.

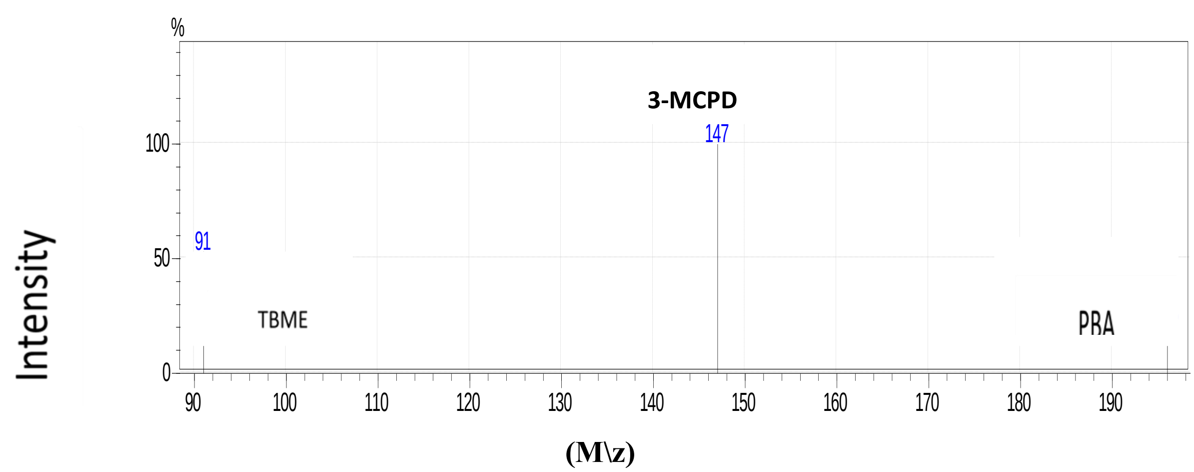


Figure 2 Selective Mass spectrum of (3-MCPD).

4.1.4 Figure 3 show Standards Chromatograms running of GS-MS by 3-MCPD, vertical column represents concentration of 3-MCPD and horizontal column idealizes retention time (min), total areas was 2408, molecular weight was 147and retention time was 7.153. By applied on equation were give concentration of 3-MCPD esters (0.1ppm).

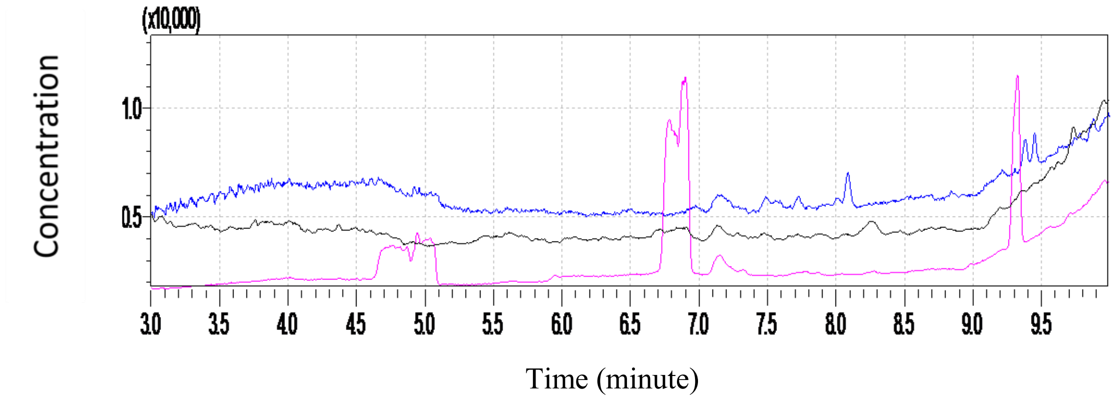


Figure 3 Standards Chromatograms, retention time (min), total areas, and concentration of 3-MCPD.

4.1.5 Figure 4 show Standards Chromatograms running of GS-MS by 3-MCPD, vertical column represents concentration of 3-MCPD and horizontal column idealizes retention time (min), total areas were 6838, molecular weight were 147.00 and retention time was 7.151. By applied on equation were give concentration of 3-MCPD esters 0.3ppm.

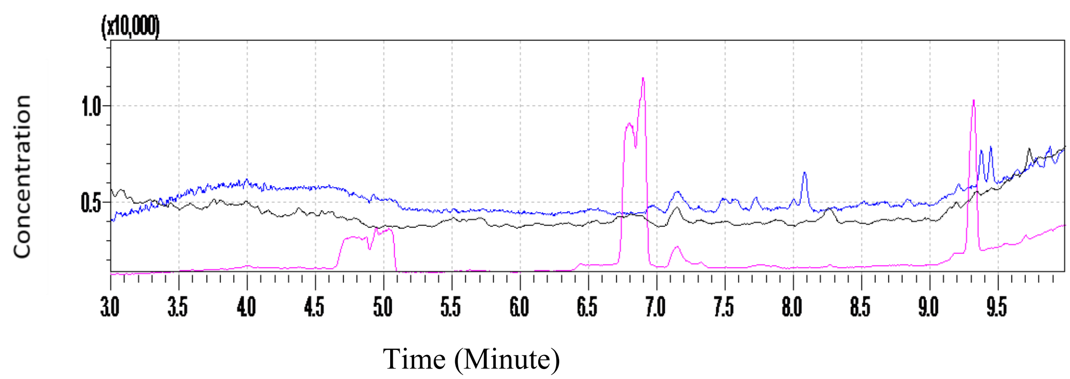


Figure 4 Standards Chromatograms, retention time, (min), total areas and concentration of 3-MCPD.

4.1.6 Figure 5 show Standards Chromatograms running of GS-MS by 3-MCPD, vertical column represents concentration of 3-MCPD and horizontal column idealizes retention time (min), total areas were 11438, molecular weight were 147.00 and retention time were 7.150. By applied on equation were give concentration of 3-MCPD esters 0.5ppm.

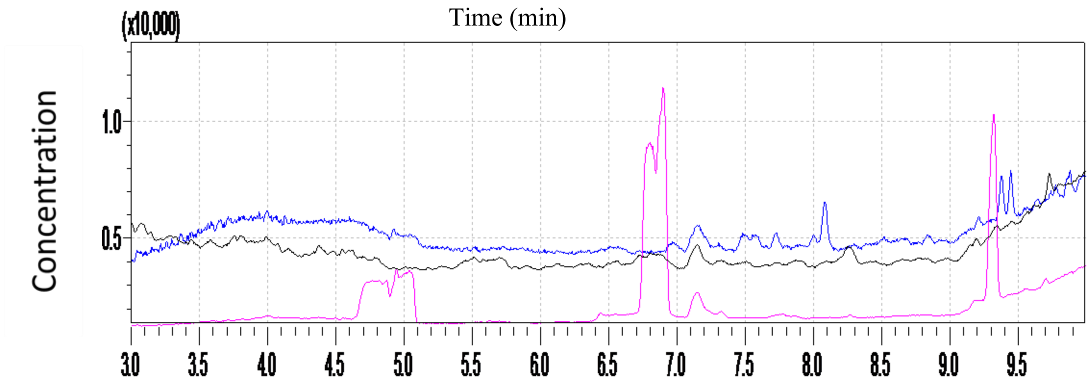


Figure 5 Standards Chromatograms, retention time (min), total areas and concentration of 3-MCPD.

4.1.7 Figure (6) shows Standards Chromatograms running of GS-MS by 3-MCPD, vertical column represents concentration of 3-MCPD and horizontal column idealizes retention time (min), total areas was 16024, molecular weight were 147.00 and retention time were 7.140. by applied on equation were give concentration of 3-MCPD esters (0.7ppm).

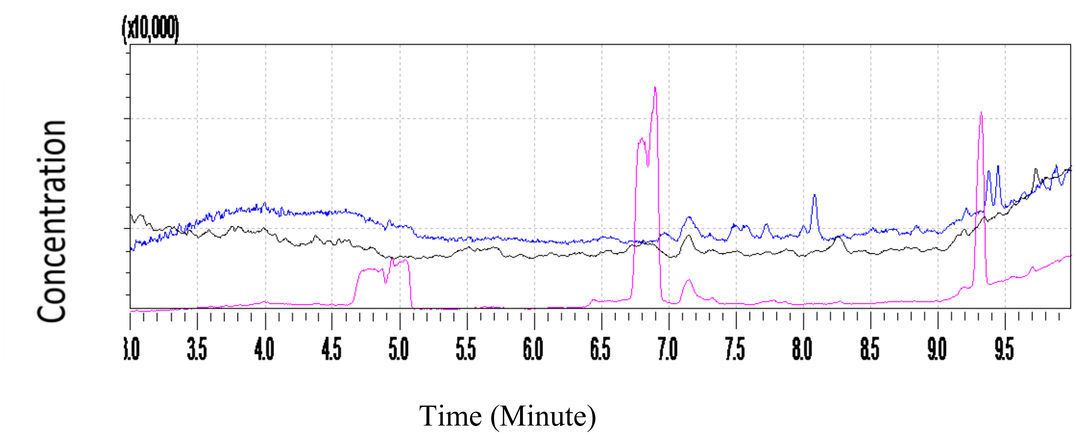


Figure 6 Standards Chromatograms, retention time, (minutes), total areas and concentration of (3-MCPD).

4.1.8 Figure 7 shows Standards Chromatograms running of GS-MS by 3-MCPD, vertical column represents concentration of 3-MCPD and horizontal column idealizes retention time (min), total areas were 23092, molecular weight were 147.00 and retention time were 7.156. By applied on equation were give concentration of 3-MCPD esters (1ppm).

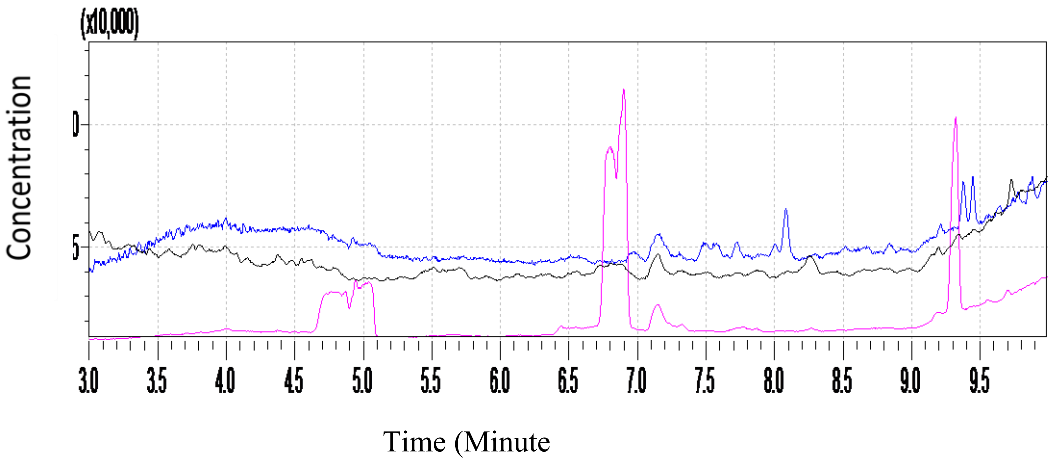


Figure 7 Standards Chromatograms, retention time (min), total areas and concentration of 3-MCPD.

4.1.9 Figure 8 shows calibration curve, the correlation coefficients were (0.9999), with samples having the level of 3-MCPD higher than (1ppm), lower of the sample were used, and relative standard deviation (RSD) were (2.3037).

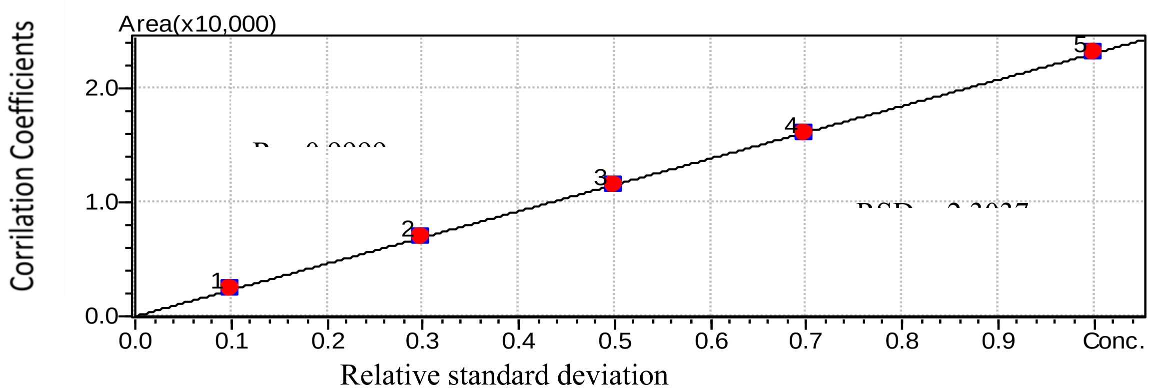


Figure 8 Calibration Curve

R = 0.9999, R = correlation coefficients. RSD = 2.3037, R = Relative standard deviation.

4.1.10 Table 3 shows 3-Monochloropropane-1,2-diol(MCPD) Derivative, running GC\MS by standard 3-MCPD repeated five times, gave concentration of 3-MCPD ranged between 0.1-1PPm, and analysis all sample by running. Concentration of 3-MCPD accounted by retention time, molecular weight, and Area of standard according to equation of quantification.

ID	Name	3-Monochloropropane-1,2-diol(MCPD) Derivative Concentration	Ret.Time	m/z	Area
1.	Std 1	0.1ppm	7.153	147.00	2408
2.	Std 2	0.3ppm	7.151	147.00	6838
3.	Std 3	0.5ppm	7.150	147.00	11438
4.	Std 4	0.7ppm	7.140	147.00	16024
5.	Std 5	1ppm	7.156	147.00	23092

4.1.11 Figure 9 Samples Chromatograms for round bread collected from Omdurman (Salha), vertical column represents concentration of 3-MCPD in sample one (toast bread) and horizontal column idealizes retention time (min), molecular weight of sample were 147.00, retention time were 7.142 and area were 13187. Concentration of 3-MCPD in sample were 0.52279ppm.

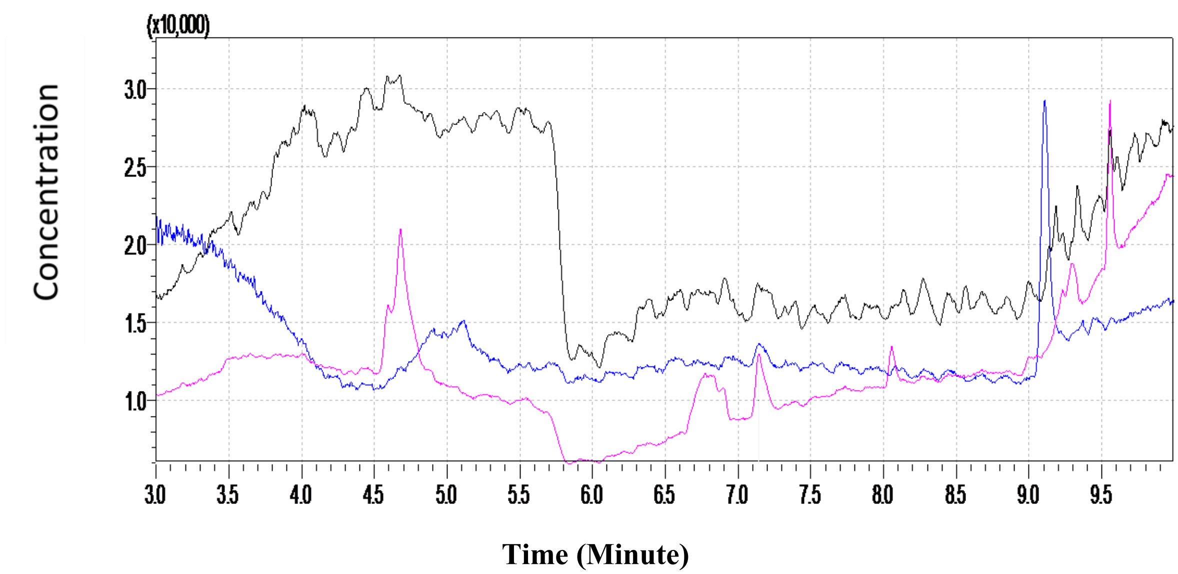


Figure 9 shows Samples Chromatograms and concentration of 3-MCPD in round bread oil.

4.1.12 Figure 10 shows Samples Chromatograms for round bread collected from Omdurman (Jaddain), vertical column represents concentration of 3-MCPD in sample two (toast bread) and horizontal column idealizes retention time (min), molecular weight of sample were 147.00, retention time were 7.155 and area were 3908. Concentration of 3-MCPD in sample were 0.15493ppm.

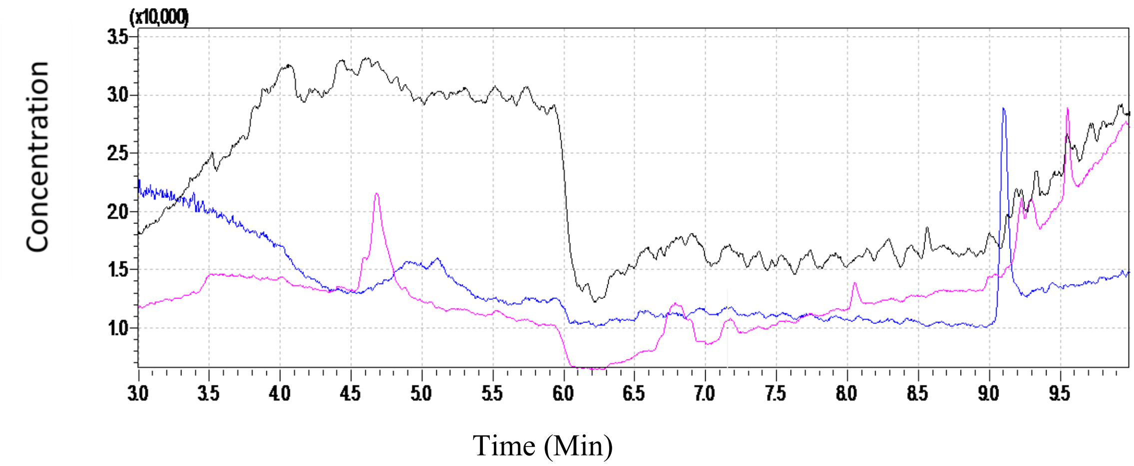


Figure 10 show Samples Chromatograms, concentration of 3-MCPD in round bread oil.

4.1.13 Figure 11 shows Samples Chromatograms for round bread collected from Omdurman (Jaddain), vertical column represents concentration of 3-MCPD in sample three (toast bread) and horizontal column idealizes retention time (min), molecular weight were 147.00, retention time were 7.189 and area were 7964. Concentration of 3-MCPD in sample were 0.31573ppm.

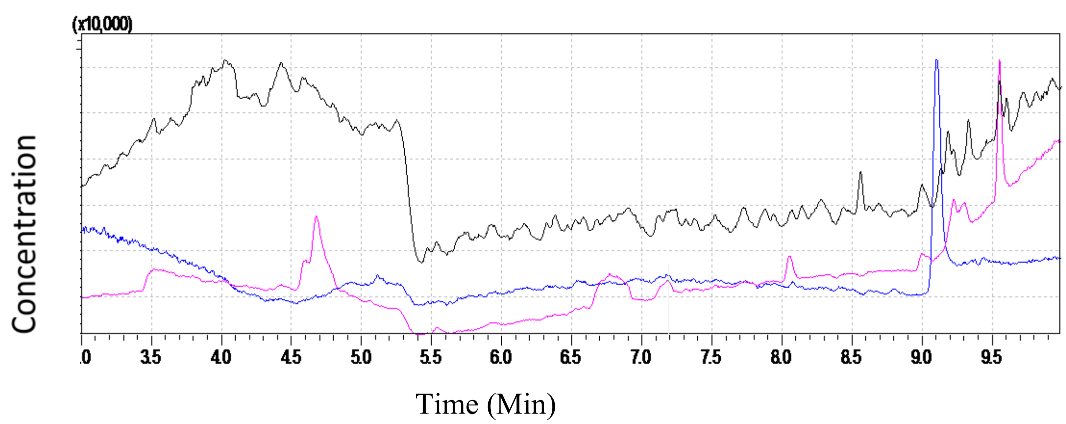


Figure 11 shows Samples Chromatograms, concentration of 3-MCPD in round bread oil.

4.1.14 Figure 12 shows Samples Chromatograms for round bread collected from Omdurman (Lybia market), vertical column represents concentration of 3-MCPD in sample four (toast bread) and horizontal column idealizes retention time (min), molecular weight of sample were 147.00, retention time were 7.189 and area were 9150. Concentration of 3-MCPD in sample were 0.36274ppm.

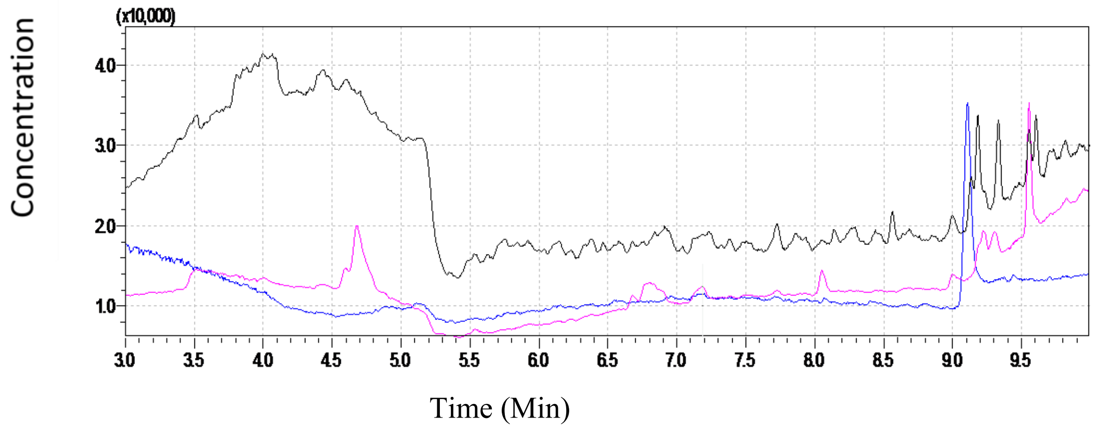


Figure 12 Samples Chromatograms, concentration of 3-MCPD in round bread oil.

4.1.15 Figure 13 shows Samples Chromatograms for round bread collected from Omdurman (Jimaihab), vertical column represents concentration of 3-MCPD in sample five (round bread) and horizontal column idealizes retention time (min), molecular weight of sample were 147.00, retention time were 7.186 and area were 8614. Concentration of 3-MCPD in sample were 0.34149ppm.

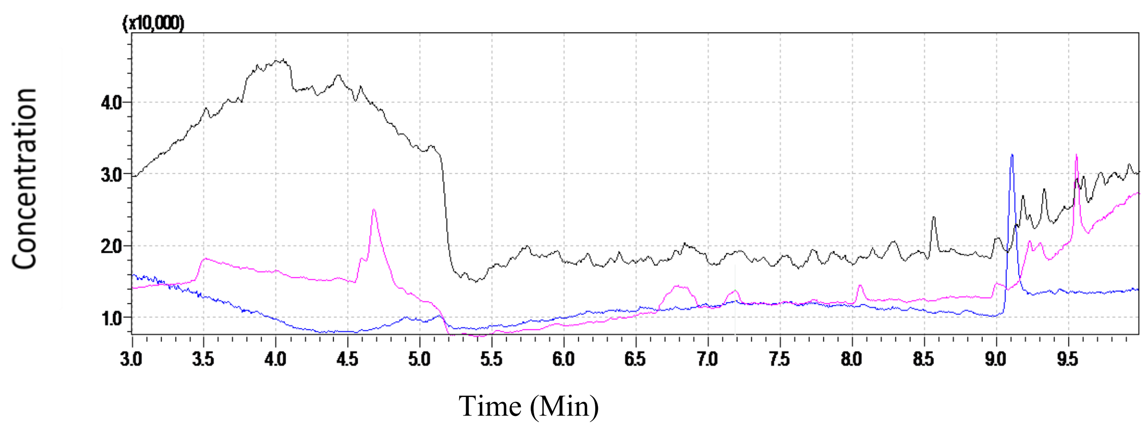


Figure 13 shows Samples Chromatograms, concentration of 3-MCPD in round bread oil.

4.1.16 Figure 14 shows Samples Chromatograms for round bread collected from Khartoum, vertical column represents concentration of 3-MCPD in sample six (round bread) and horizontal column represents retention time (min), molecular weight of sample were 147.00, retention time were 7.131 and area were 14694. Concentration of 3-MCPD in sample were 0.58253ppm.

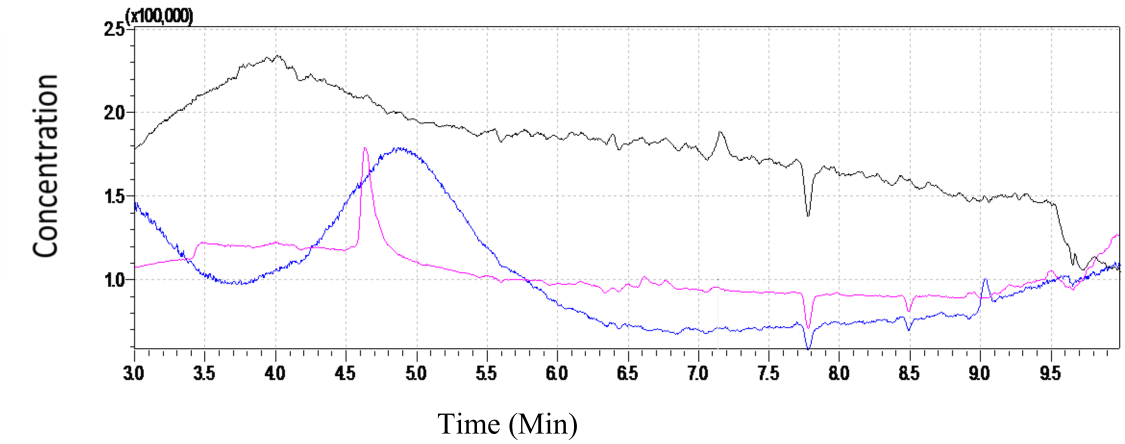


Figure 14 Samples Chromatograms, concentration of 3-MCPD in round bread oil.

4.1.17 Figure 15 shows Samples Chromatograms for round bread collected from Khartoum (Duyum east), vertical column represents concentration of 3-MCPD in sample seven (round bread) and horizontal column represents retention time (min), molecular weight of sample were 147.00, retention time were 7.178 and area were 7212. Concentration of 3-MCPD in sample were 0.28591ppm.

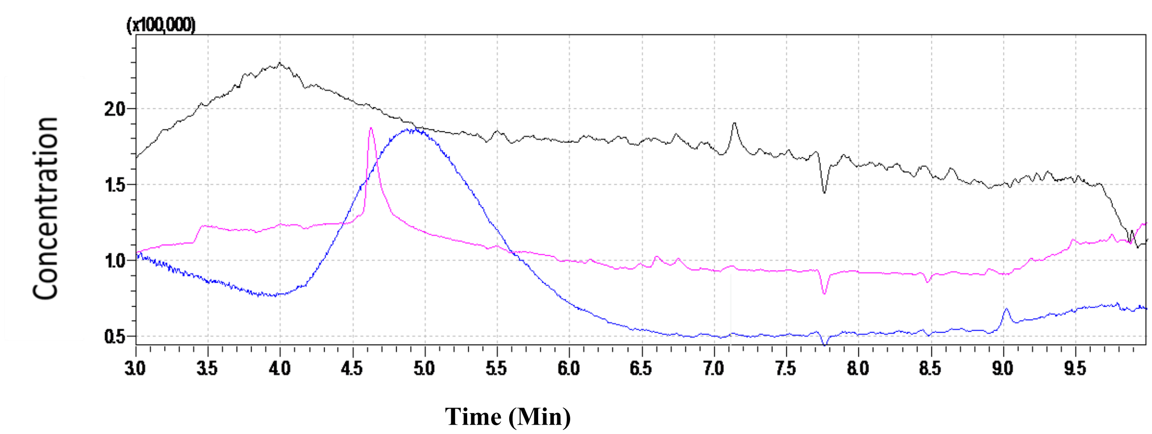


Figure 15 Samples Chromatograms, concentration of 3-MCPD in round bread oil.

4.1.18 Figure 16 shows Samples Chromatograms for round bread collected from Khartoum (Duyum west), vertical column represents concentration of 3-MCPD in sample eight (round bread) and horizontal column represents retention time (min), molecular weight of sample were 147.00, retention time were 7.148 and area were 26706. Concentration of 3-MCPD in sample were 1.05873ppm**.**

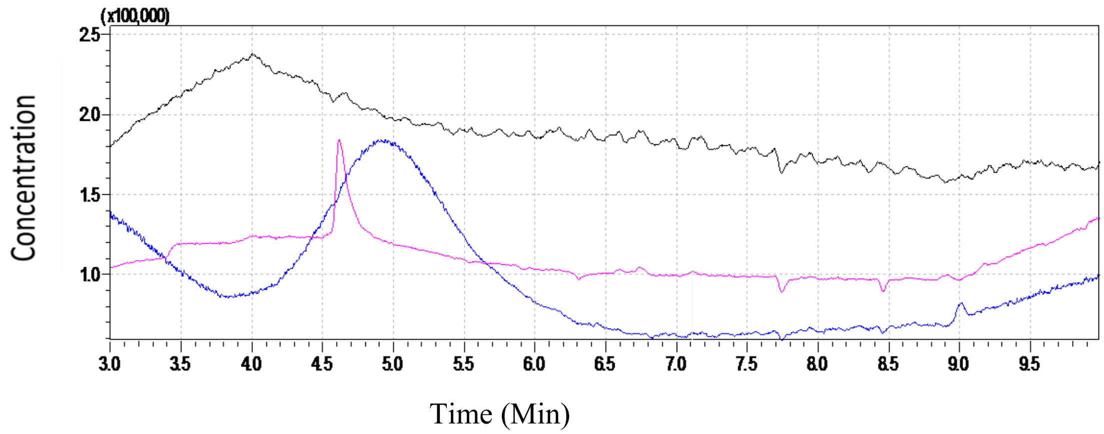


Figure 16 Samples Chromatograms, concentration of 3-MCPD in round bread oil.

4.1.19 Figure 17 shows Samples Chromatograms for round bread collected from Khartoum (Alsajana), vertical column represents concentration of 3-MCPD in sample nine (round bread) and horizontal column represents retention time (min), molecular weight of sample were 147.00, retention time were 7.138 and area were 24326. Concentration of 3-MCPD in sample were 0.96438ppm.

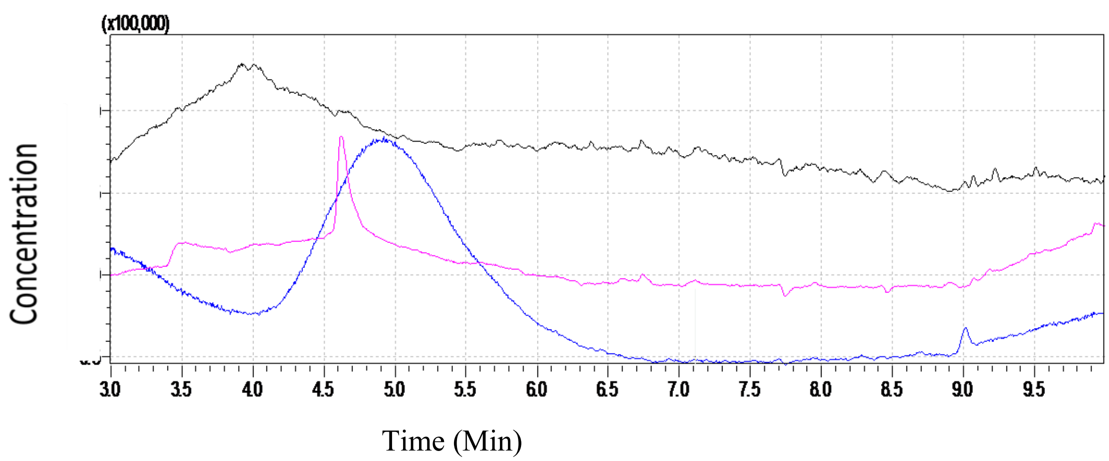


Figure 17 Samples Chromatograms, concentration of 3-MCPD in round bread oil.

4.1.20 Figure 18 shows Samples Chromatograms for round bread collected from Khartoum Bahari (Kadorow), vertical column represents concentration of 3-MCPD in sample ten (round bread) and horizontal column represents retention time (min), molecular weight of sample were 147.00, retention time were 7.135 and area were 8291. Concentration of 3-MCPD in sample were 0.32869ppm.

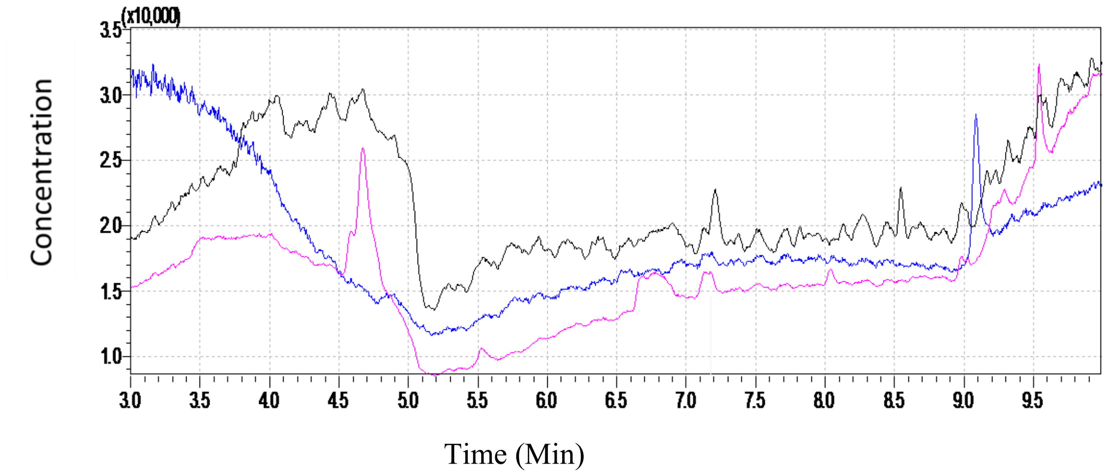


Figure 18 Samples Chromatograms, concentration of 3-MCPD in round bread oil.

4.1.21 Figure 19 shows Samples Chromatograms for round bread collected from Khartoum Bahari (Al-ubaa), vertical column represents concentration of 3-MCPD in sample six (round bread) and horizontal column represents retention time (min), molecular weight of sample were 147.00, retention time were 7.115 and area were 5906. Concentration of 3-MCPD in sample were 0.23414ppm.

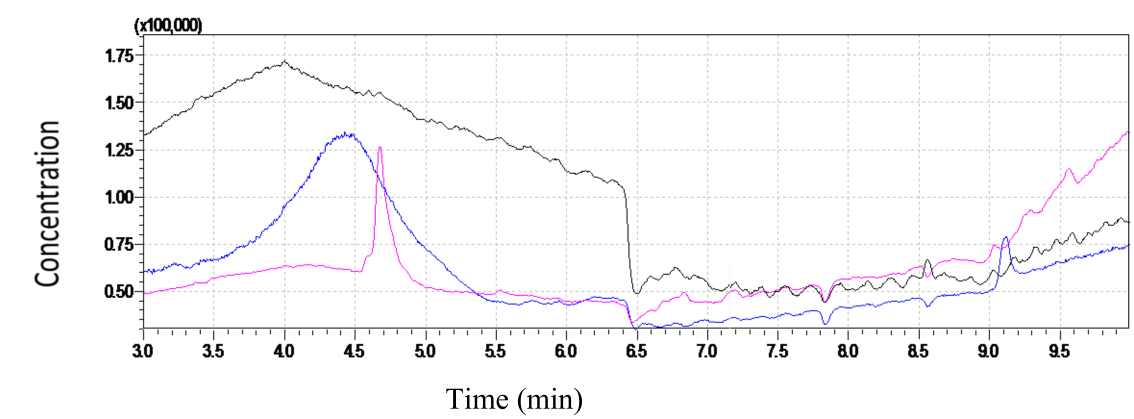


Figure 19 samples Chromatograms, concentration of 3-MCPD in round bread oil.

4.1.22 Figure 20 shows Samples Chromatograms for round bread collected from Khartoum Bahari (Kobber), vertical column represents concentration of 3-MCPD in sample six (round bread) and horizontal column represents retention time (min), molecular weight of sample were 147.00, retention time were no detected and area were no detected. Concentration of 3-MCPD in sample were no detected.

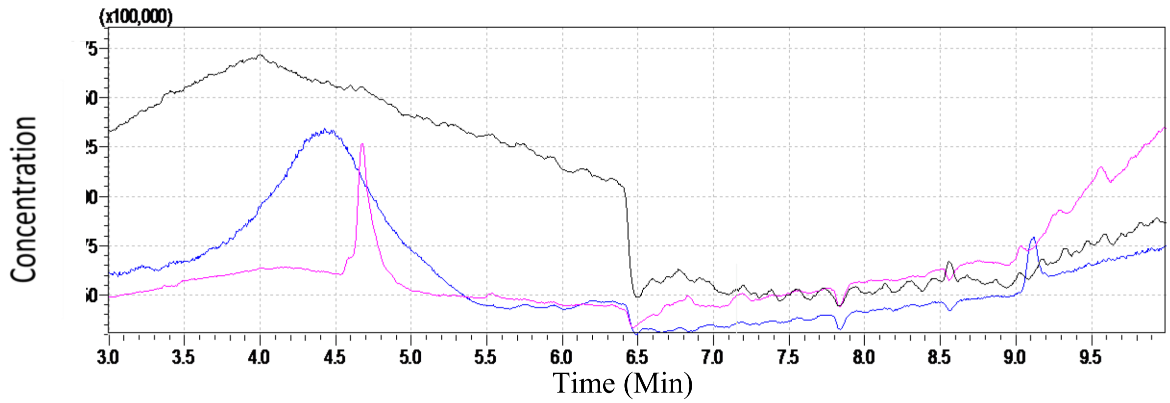


Figure 20 Samples Chromatograms, concentration of 3-MCPD in round bread oil.

4.1.23 Figure 21 shows Samples Chromatograms for round bread collected from Khartoum Bahari (Kafuri), vertical column represents concentration of 3-MCPD in sample six (round bread) and horizontal column represents retention time (min), molecular weight of sample were 147.00, retention time were no detected and area were no detected. Concentration of 3-MCPD in sample were no detected.

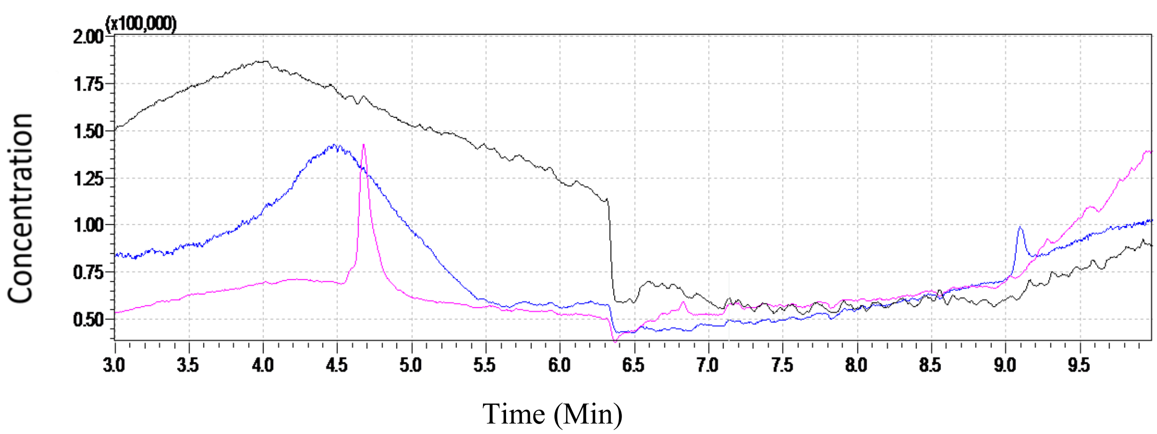


Figure 21Samples Chromatograms, concentration of 3-MCPD in round bread oil.

4.1.24 Figure 22 shows Samples Chromatograms for toast bread collected from Omdurman (Almulazmeen), vertical column represents concentration of 3-MCPD in sample one (toast bread) and horizontal column represents retention time (min), molecular weight of sample were 147.00, retention time were 7.164 and area were 14221. Concentration of 3-MCPD in sample were 0.56378ppm.

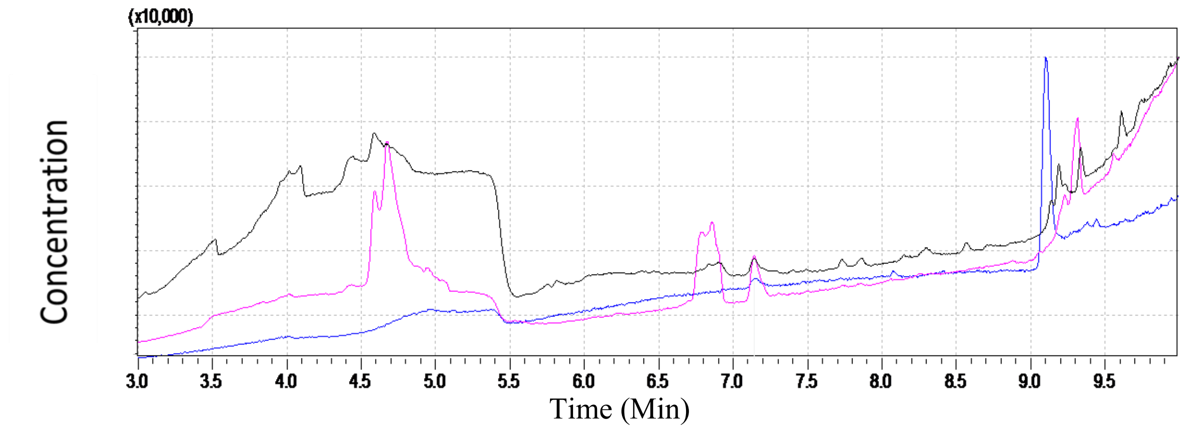


Figure 22 Samples Chromatograms, concentration of 3-MCPD in toast bread oil.

4.1.24 Figure 23 shows Samples Chromatograms for toast bread collected from Omdurman (Shuhada), vertical column represents concentration of 3-MCPD in sample one (toast bread) and horizontal column represents retention time (min), molecular weight of sample were 147.00, retention time were 7.143 and area were 2460. Concentration of 3-MCPD in sample were 0.37503ppm.

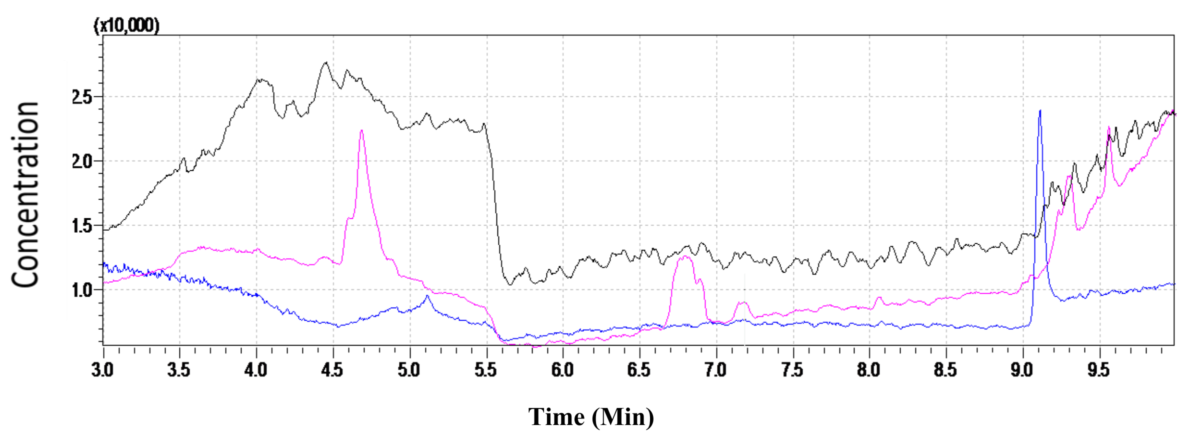


Figure 23 Samples Chromatograms, concentration of 3-MCPD in toast bread oil.

4.1.26 Figure 24 shows Samples Chromatograms for toast bread collected from Omdurman (Fitihab), vertical column represents concentration of 3-MCPD in sample three (toast bread) and horizontal column represents retention time (min), molecular weight of sample were 147.00, retention time were 7.178 and area were 5113. Concentration of 3-MCPD in sample were 0.20270ppm.

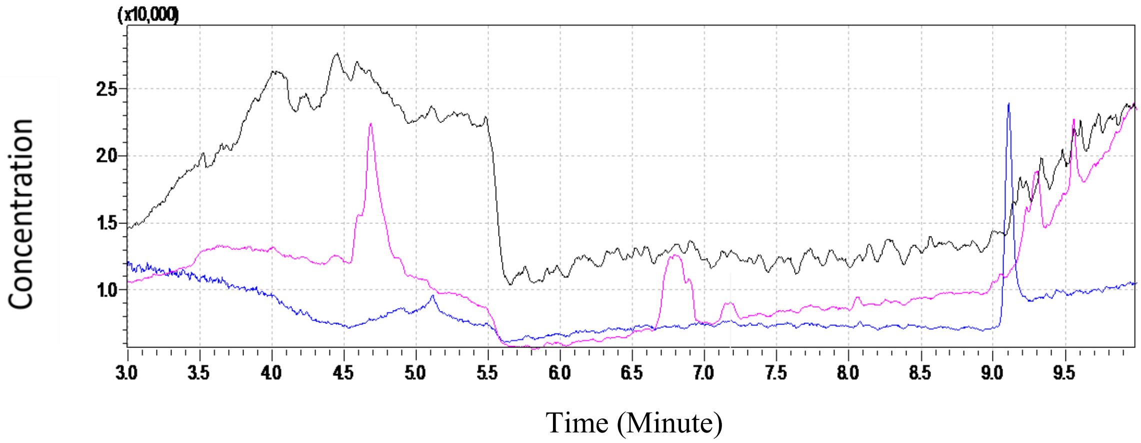


Figure 24 Samples Chromatograms, concentration of 3-MCPD in toast bread oil.

4.1.27 Figure 25 shows Samples Chromatograms for toast bread collected from Omdurman (Shiglla) , vertical column represents concentration of 3-MCPD in sample four (toast bread) and horizontal column represents retention time (min), molecular weight of sample were 147.00, retention time were 7.189 and area were 6704. Concentration of 3-MCPD in sample were 0.26577ppm.

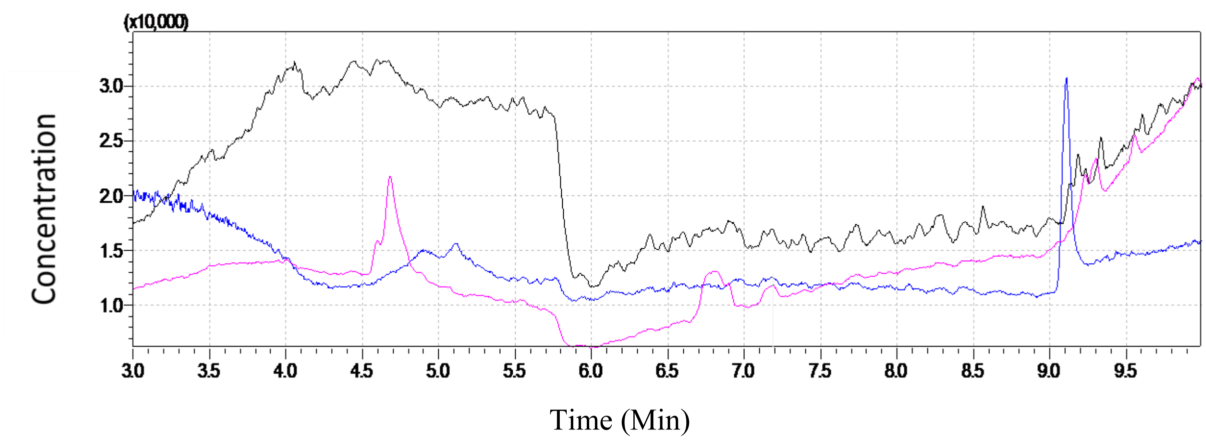


Figure 25 Samples Chromatograms, concentration of 3-MCPD in toast bread oil.

4.1.28 Figure 26 shows Samples Chromatograms for toast bread collected from Khartoum (Sharwani), vertical column represents concentration of 3-MCPD in sample five (toast bread) and horizontal column represents retention time (min), molecular weight of sample were 147.00, retention time were 7.194 and area were 16837. Concentration of 3-MCPD in sample were 0.667119ppm.

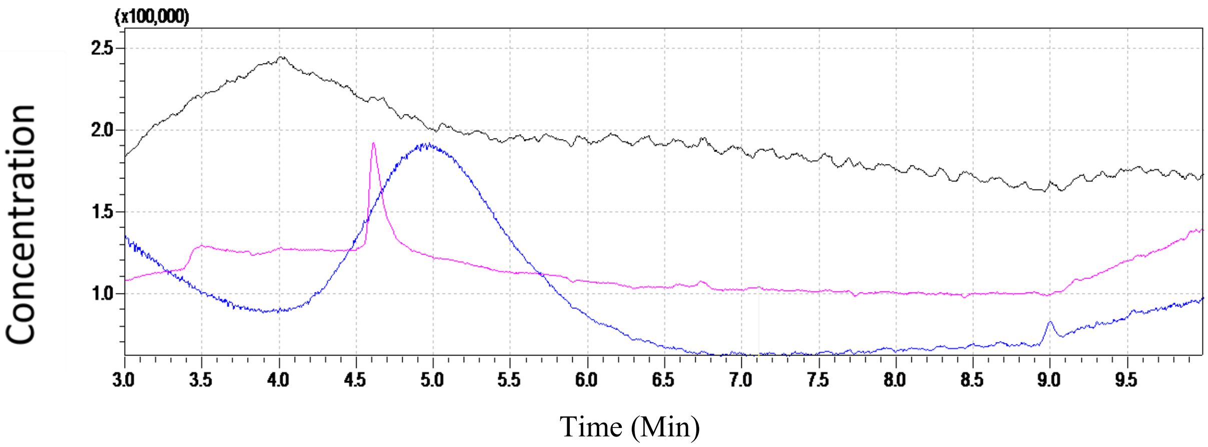


Figure 26 Samples Chromatograms, concentration of 3-MCPD in toast bread oil.

4.1.29 Figure 27 shows Samples Chromatograms for toast bread collected from Khartoum (Buree), vertical column represents concentration of 3-MCPD in sample six (toast bread) and horizontal column represents retention time (min), molecular weight of sample were 147.00, retention time were 7.164 and area were 15045. Concentration of 3-MCPD in sample were 0.59644ppm.

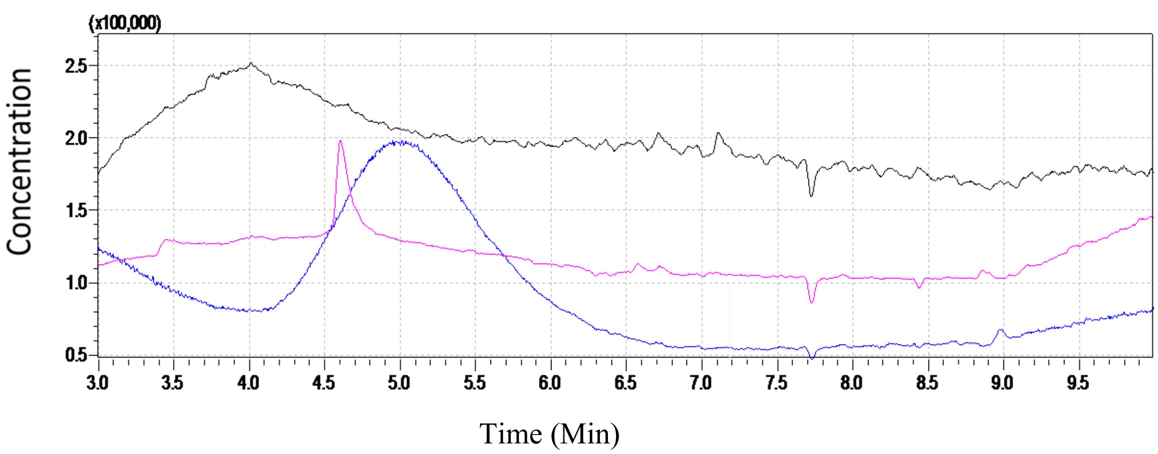


Figure 27 shows Samples Chromatograms, concentration of 3-MCPD in toast bread oil.

4.1.30 Figure (28) shows Samples Chromatograms for toast bread collected from Khartoum (Manshia), vertical column represents concentration of 3-MCPD in sample seven (toast bread) and horizontal column represents retention time (min), molecular weight of sample were 147.00, retention time were 7.155 and area were 5493. Concentration of 3-MCPD in sample were 0.21776ppm.

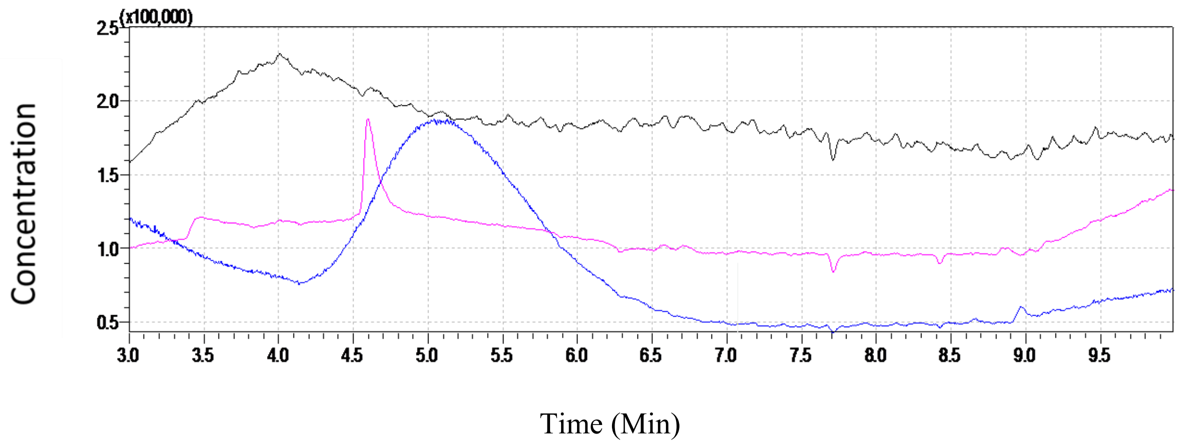


Figure 28 Samples Chromatograms, concentration of 3-MCPD in toast bread oil.

4.1.31 Figure 29 shows Samples Chromatograms for toast bread collected from Khartoum (Jabelawliya), vertical column represents concentration of 3-MCPD in sample eight (toast bread) and horizontal column represents retention time (min), molecular weight of sample were 147.00, retention time were 7.135 and area were 17440. Concentration of 3-MCPD in sample were 0.29494ppm.

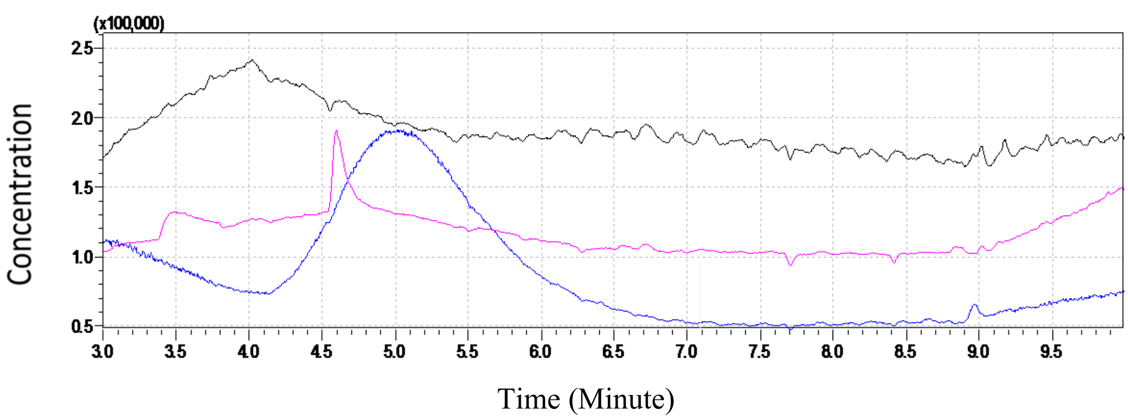


Figure 29 Samples Chromatograms, concentration of 3-MCPD in toast bread oil.

4.1.32 Figure 30 shows Samples Chromatograms for toast bread collected from Khartoum Bahari (Dar-Alsalam), vertical column represents concentration of 3-MCPD in sample nine (toast bread) and horizontal column represents retention time (min), molecular weight of sample were 147.00, retention time were 7.115 and area were 4224. Concentration of 3-MCPD in sample were 0.16746ppm.

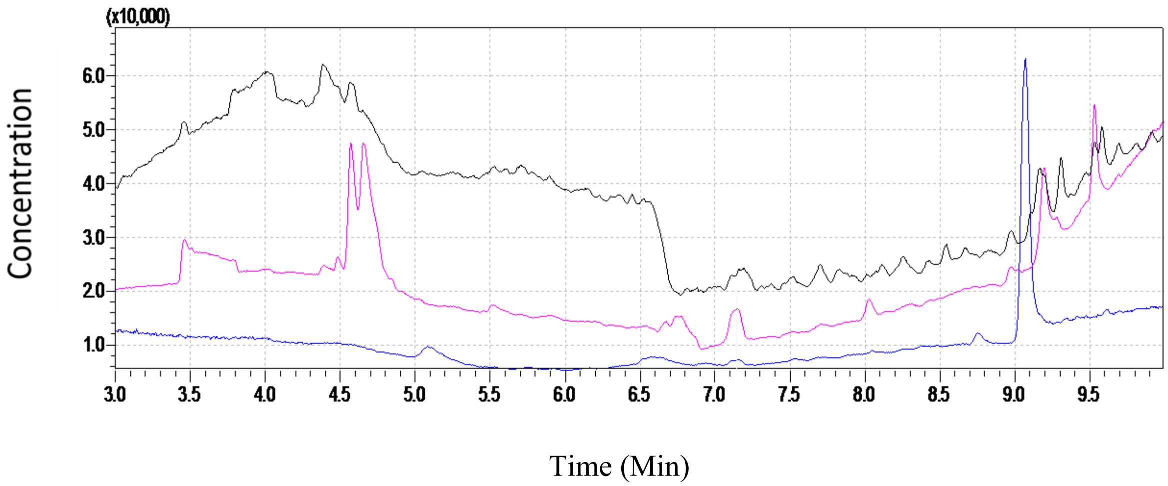


Figure 30 Samples Chromatograms, concentration of 3-MCPD in toast bread oil.

4.1.33 Figure 31 shows Samples Chromatograms for toast bread collected from Khartoum Bahari (Danagla), vertical column represents concentration of 3-MCPD in sample ten (toast bread) and horizontal column represents retention time (min), molecular weights of sample were 147.00, retention time were (ND) and area were (ND). Concentration of 3-MCPD in sample were no detected.

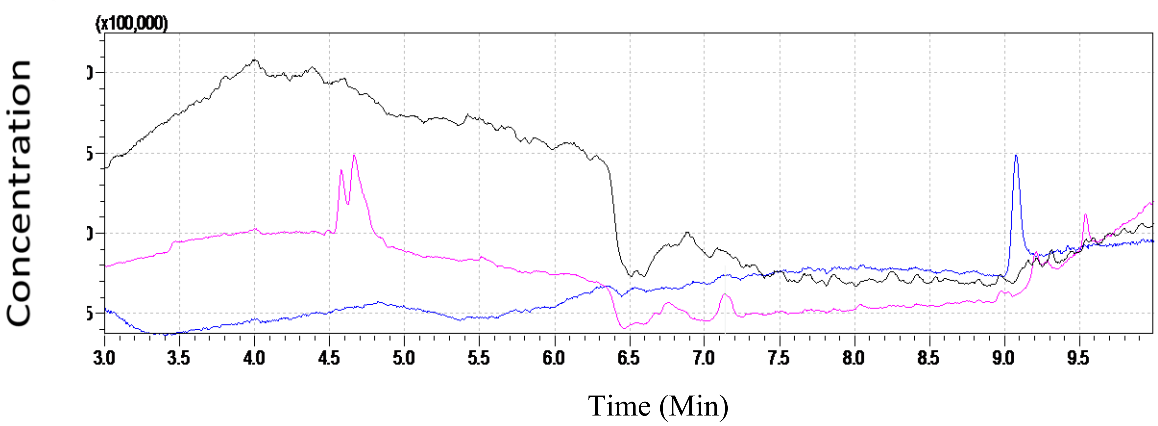


Figure 32 Samples Chromatograms, concentration of 3-MCPD in toast bread oil.

4.1.35 Figure 33 shows Samples Chromatograms for toast bread collected from Khartoum Bahari (Margania) , vertical column represents concentration of 3-MCPD in sample one (toast bread) and horizontal column represents retention time (min), molecular weight of sample were 147.00, retention time were (ND) and area were (ND). Concentration of 3-MCPD in sample were no detected.

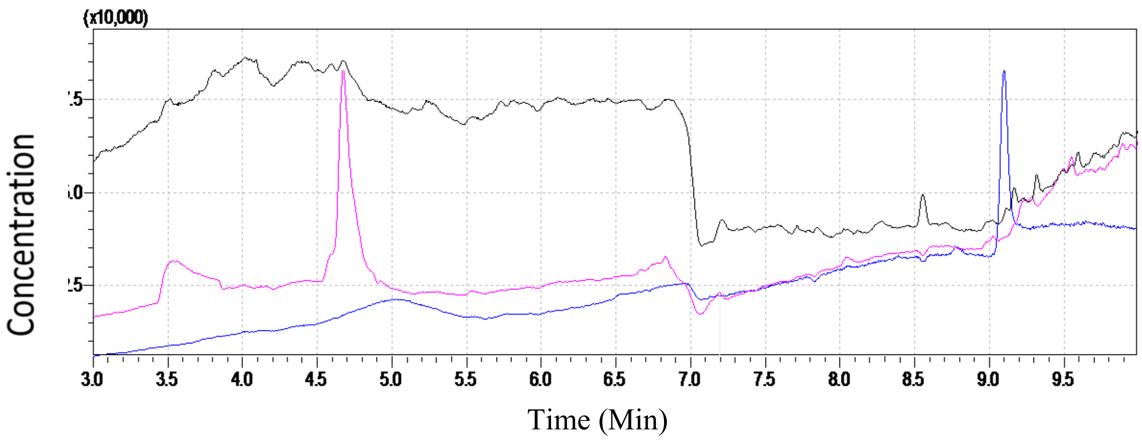


Figure 33 Samples Chromatograms, concentration of 3-MCPD in toast bread oil.

4.1.36 Figure 34 shows Samples Chromatograms for toast bread collected from Khartoum Bahari (Kobber), vertical column represents concentration of 3-MCPD in sample one (toast bread) and horizontal column represents retention time (min), molecular weight of sample were 147.00, retention time were 7.185 and area were 2859. Concentration of 3-MCPD in sample were 0.11332ppm.

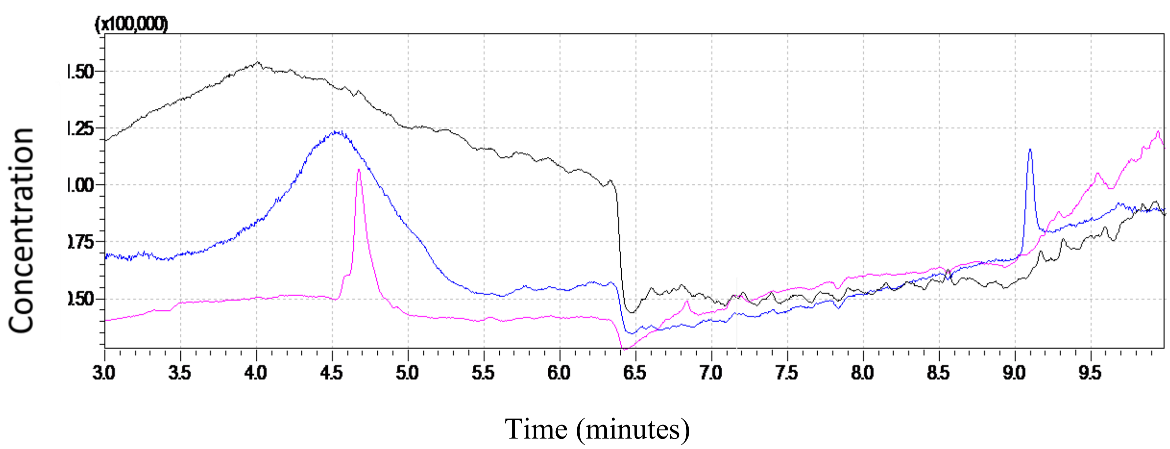


Figure 34 Samples Chromatograms, concentration of 3-MCPD in toast bread oil.

4.2 Discussion:

Table 4 shows concentration of 3-MCPD in modern round bread oil, ranged from 0.15493 - 1.05873ppm, but find two samples were not detected, the main reason due to heat treatment with short time, food processing ,moisture content. Because, the concentration of 3-MCPD increased by temperature with time increased. And traditional of oil processing and less amount of Sodium chloride were used. Dimensions (length width and thickness) with moisture content leads to increased of the concentration of 3-MCPD (if the moisture content and the length, width was high, bread needs to long time with high temperature for matured). Lower concentration levels of 3-MCPD esters in round bread oil were 0,15493ppm, average concentration levels were (0.49825ppm), and higher concentration level was 1,05873ppm.

Table 4 shows concentration of 3-MCPD (ppm) in round bread oil.

ID	sample	Concentration of 3-MCPD (ppm) of oil	Concentration of 3-MCPD mg\kg of bread
1	S5\ RB	0.52279	0.00188
2	S6\ RB	0.15493	0.00055
3	S7\ RB	0.31573	0.00114
4	S8\ RB	0.36274	0.00131
5	S9\ RB	0.34149	0.00123
6	S10\ RB	0.58253	0.00210
7	S11\ RB	0.28591	0.00103
8	S12\ RB	1.05873	0.00382
9	S13\ RB	0.96438	0.00348
10	S18\ RB	0.32869	0.00118
11	S19\ RB	0.23414	0.00084
12	S24\ RB	N.D	ND
13	S25\ RB	N.D	ND

S= Sample. R=Round. T=Toast. B= bread

Table 5 shows concentration of 3-MCPD esters in modern toast bread oil, was generally formed low ,ranging from 0.11334ppm –0.667ppm.lower concentration of 3-MCPD esters were found in modern toast bread oils, 0.11334ppm, average concentration level was 0.346472ppm, and higher concentration level was 0.66749ppm. From these concentrations result obtained it is consider no large difference between them, but find two sample were no detected, the main reason for difference quantity of 3-MCPD in toast bread oil is due to the heat treatment processing, moisture content, oil content and type of oils, (refining, partially and traditional oil) used, bread color, and its dimensions (length width and thickness).

Comparison between the quantity of 3-MCPD in modern toast bread oil and modern round bread, showed that, the quantity of 3-MCPD esters in round bread oil was higher than the quantity in toast bread oil. Because the average quantity of 3-MCPD in round bread oil were 0.49825ppm and average in toast bread oil was 0.346472ppm, while higher concentration of 3-MCPD in round bread oil was 1.05873ppm and the higher concentration of 3-MCPD in toast bread oil was 0.66749ppm and lower concentration of 3-MCPD in round bread oil was 0.15493ppm and the lowest concentration in toast bread oil was 0.11334ppm. The main differences between concentrations of 3-MCPD in modern round bread and toast bread, were mainly due to the heat treatment processing ,moisture content, oil content and type of oils, (refined, partially refined and traditional oil) used, bread color ,and dimensions (length width and thickness).the temperature ,time ,length, width, moisture content and oil content in round bread oil were (250^{o}C 7-8 minutes, 11-13cm, 4-5cm, 33.56%, and 0,924%), respectively, and brownish color and the temperature ,time ,length, width, moisture content and oil content in toast bread oil were (250^{O}C, 6-7 minutes, 20-25cm, 3-4cm, 31.02%, and 0.894%). respectively, and brownish color.

Comparison with previous studies (Karsuinova, et al. (2007), Dolezal, et al. (2005) ,Divinova, et al. (2007) and Zelinkova et al (,2008-2009), carried out for concentration of 3-MCPD in bread were formed to be ranged between (1.56-23.6mg\kg) was higher than concentrations of 3-MCPD was found in modern round bread oil and toast bread oil that ranged (0.11334-0.66749ppm) (0.000346-0.002039mg\kg) and (0.15493-1.05873ppm) (0.000559-0.003823mg\kg),

respectively.While Kertisova, et al. (2009), reported values of concentration for bound 3-MCPD esters in bread crumb ranged from 1.56 to 21.13mg\kg of fat and concentration of 3-MCPD esters in bread crust ranged from 2.82 to 3.60 mg\kg of fat. Concentrations of 3-MCPD were found in modern round bread fat and toast bread oil ranged 0.11334 to 0.66749ppm (i.e 0.000346-0.002039mg\kg) and from 0.15493 to 1.05873ppm (i.e 0.000559-0.003823mg\kg), respectively, is lowest than that reported.

Hamlet et al,(2004) ,measured 3-MCPD-esters in toast bread and, highest levels were found in regions of the bread that attained the highest temperature, i.e the crust , and level increased from 60-160ug\kg^{-1} when the bread was toasted over 40-120 seconds ,the highest level of 3-MCPD-esters (6100ug\kg^{-1}) concentration of 3-MCPD were found in modern round bread fat and toast brad oil range (0.11334-0.66749ppm) (i.e 0.000346-0.002039mg\kg) and (i.e 0.15493-1.05873ppm) (0.000559-0.003823mg\kg), respectively, were increased by temperature and time increase.

Table 5 Concentration of 3-MCPD (ppm), in toast bread oil

ID	Sample	Concentration of 3-MCPD (ppm) of oil	Concentration of 3-MCPD mg\kg of bread
1	S1\ Toast Bread	0.56378	0.00172
2	S2\ Toast Bread	0.37503	0.00114
3	S3\ Toast Bread	0.20270	0.00061
4	S4\ Toast Bread	0.26577	0.00081
14	S14\ Toast Bread	0.66749	0.00203
15	S15\ Toast Bread	0.59644	0.00182
16	S16\ Toast Bread	0.21776	0.00066
17	S17\ Toast Bread	0.29495	0.00090
20	S20\ Toast Bread	0.16746	0.00051
21	S21\ Toast Bread	N.D	ND
22	S22\ Toast Bread	N.D	ND
23	S23\ Toast Bread	0.11334	0.00034

Table (6) shows estimation of concentration of 3-MCPD (µg\kg) exposure for adults (round bread). Lower Concentration of 3-MCPD exposure for adults from round bread were (0.005345µg\kg) body weight per day, higher concentration were (0.036528µg\kg) body weight per day, and medium concentration of 3-MCPD exposure for Adults were (0.016171µg\kg).

Table 6 Estimated quantity of 3-MCPD (µg\kg) for body weight per day exposure for individual adults (Round bread oil).

ID	Name	Mean Adult Age	Body Weight	3-MCPD\µg\kg exposure
1	S5\ RB	18-20	68.03	0.01803
2	S6\ RB	18-20	68.03	0.00534
3	S7\ RB	18-20	68.03	0.01093
4	S8\ RB	18-20	68.03	0.01258
5	S9\ RB	18-20	68.03	0.01178
6	S10\ RB	18-20	68.03	0.02009
7	S11\ RB	18-20	68.03	0.00988
8	S12\ RB	18-20	68.03	0.03652
9	S13\ RB	18-20	68.03	0.03327
10	S18\ RB	18-20	68.03	0.01134
11	S19\ RB	18-20	68.03	0.00807
12	S24\ RB	18-20	68.03	ND
13	S25\ RB	18-20	68.03	ND

Mean estimated quantity of 3-MCPD exposure for Adults = 0.016171µg\kg.

Table 7 shows estimation of concentration of 3-MCPD (µg\kg) exposure for adults from modern toast bread. Lower Concentration of 3-MCPD exposure for adults from toast bread were 0.002799µg\kg body weight per day, higher concentration were 0.016488µg\kg body weight per day, and mean concentration of 3-MCPD exposure for adults were 0.0086234µg\kg.

Table 7 Estimate concentration of 3-MCPD (µg\kg) for body weight per day exposure for individual Adults (toast bread oil).

ID	Name	Body Weight	Mean Adult Age	3-MCPD\µg\kg exposure
1	S1\ TB	68.03	18-20	0.01392
2	S2\ TB	68.03	18-20	0.00926
3	S3\ TB	68.03	18-20	0.00565
4	S4\ TB	68.03	18-20	0.00656
14	S14\ TB	68.03	18-20	0.01648
15	S15\ TB	68.03	18-20	0.01473
16	S16\ TB	68.03	18-20	0.00537
17	S17\ TB	68.03	18-20	0.00728
20	S20\ TB	68.03	18-20	0.00413
21	S21\ TB	68.03	18-20	ND
22	S22\ TB	68.03	18-20	ND
23	S23\ TB	68.03	18-20	0.00279

TB : toast bread

Mean estimated quantity of 3-MCPD exposure for adults = 0.0086234µg\kg

Table 8 shows estimation of concentration of 3-MCPD (µg\kg) exposure for children from modern round bread. Lower concentration of 3-MCPD exposure for adults from round bread were 0.004577µg\kg body weight per day, higher concentration were 0.031277µg\kg body weight per day, and mean concentration of 3-MCPD exposure for adults were 0.013846µg\kg.

Table 8 shows estimated quantity of 3-MCPD (μg\kg) for body weight per day exposure for individual children, (Round bread oil).

ID	Name	Mean kids Age	Body Weight	3-MCPD\μg\kg exposure
1	S5\ RB	14-15	47.67	0.01544
2	S6\ RB	14-15	47.67	0.00457
3	S7\ RB	14-15	47.67	0.00935
4	S8\ RB	14-15	47.67	0.01077
5	S9\ RB	14-15	47.67	0.01008
6	S10\ RB	14-15	47.67	0.01720
7	S11\ RB	14-15	47.67	0.00846
8	S12\ RB	14-15	47.67	0.03127
9	S13\ RB	14-15	47.67	0.02849
10	S18\ RB	14-15	47.67	0.00971
11	S19\ RB	14-15	47.67	0.00691
12	S24\ RB	14-15	47.67	ND
13	S25\ RB	14-15	47.67	ND

RB : Round bread

Mean estimated quantity of 3-MCPD exposure for children = 0.013846μg\kg

Table 9 shows estimation concentration of 3-MCPD (μg\kg) exposure for children age (14-15) years from modern toast bread. Lower concentration of 3-MCPD exposure for children from toast bread were 0.002397μg\kg body weight per day, higher concentration were 0.014118μg\kg body weight per day, and mean concentration of 3-MCPD exposure for adults were 0.007368μg\kg. Concentration of 3-MCPD exposure for children from round bread higher than concentration of 3-MCPD exposure for children from toast bread. Consequently, concentration of (3-MCPD) boy weight per day exposure for children from round and toast bread were lower than concentration of (3-MCPD) body weight per day exposure for adults. Because, concentration of 3-MCPD body weight per day exposure increase by weight decrease and concentration of 3-MCPD (μg\kg) exposure decrease by weight increase.

Table 9 shows estimated quantity of 3-MCPD (µg\kg) for body weight per day exposure for individual children, (toast bread oil).

ID	Name	Mean Kids Age	Body Weight	3-MCPD\µg\kg exposure
1	S1\ TB	14-15	47.67	0.01192
2	S2\ TB	14-15	47.67	0.00793
3	S3\ TB	14-15	47.67	0.00468
4	S4\ TB	14-15	47.67	0.00562
14	S14\ TB	14-15	47.67	0.01411
15	S15\ TB	14-15	47.67	0.01261
16	S16\ TB	14-15	47.67	0.00460
17	S17\ TB	14-15	47.67	0.00623
20	S20\ TB	14-15	47.67	0.00354
21	S21\ TB	14-15	47.67	ND
22	S22\ TB	14-15	47.67	ND
23	S23\ TB	14-15	47.67	0.00239

TB: Toast bread

Mean estimated quantity of 3-MCPD exposure for children = 0.00736µg \kg

Comparison with the international standard of 3-MCPD**,** results of international studies of 3-MCPD by The European commission's scientific committee for food established range tolerable daily (0.1µg\kg - 2µg\kg) body weight per day. Concentration of free 3-MCPD in round bread for adults WAS formed ranged to be between (0.15493-1.05873ppm), (i.e 0.005345-0.036538µg\kg), body weight per day, while concentration of 3-MCPD in toast bread oil for adults range between (0.11334ppm – 0.667ppm), (i.e 0.002799-0.016488µg\kg) body weight per day, while concentration of 3-MCPD in round bread for children range between (0.15493-1.05873ppm), (i.e 0.004577-0.031277µg\kg), body weight per day, and concentration of 3-MCPD in toast bread oil for children ranged between(0.11334ppm – 0.667ppm), (i.e 0.002397-0.014118µg\kg) body weight per day. The concentration of 3-MCPD were found in both modern round and toast bread fat is lower than standard of European commission's scientific committee for food .

Comparison with studies of Karsuinova et al (2007), Dolezal et al ,(2005), Divinova et al,(2007) and Zelinkova et al (,2008-2009), for concentration of 3-MCPD in bread range between (1.56-23.6mg\kg) of fat (i.e 5.7-84,9ug\kg) is higher than concentration of 3-MCPD in modern round bread fat and toast brad oil were found. JECFA ,(2007) was estimated average dietary exposures of the general population from a wide range of foods, related products, ranged from 0.02 to 0.7 ug\kg (bw) per day ,and these for consumers at the high percentile (95^{th}), including young children ranged from 0.06 to 2.3 ug\kg bw per day.While concentration of 3-MCPD in round bread for children range between (0.004577-0.031277µg\kg), body weight per day, and concentration of 3-MCPD in toast brad oil for children range between (0.002397-0.014118µg\kg) body weight per day were found. While Hwang et al, (2009), reported value for mean intake level of 3-MCPD in the republic of Korea was estimated in the range from 0.0009 – 0.0026ug\kg bw per day and at the 95^{th} percentile of consumption was 0.005 ug\kg bw per day. And You et al (2008) Secondary school students in China, Hong Kong special administrative region, have been published since that time, the average exposure was estimated to be 0,063-0,150ug\kg bw per day. While that for high consumers was 0.152-0.300 ug\kg bw per day. While average MCPD exposure for children from toast and round bread were (0.007368-0.013846 µg\kg), respectively, and Mean estimated quantity of 3-MCPD exposure for Adults from toast and round bread were (0.0086234-0.016171µg\kg), respectively.

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Chapter Five

Conclusion And Recommendation

5.1 Conclusion:

3-MCPD was detected in modern round and toast bakery bread with a concentration of 0.15493-1.05873ppm to 0.11334-0.59644ppm, which are lower than that of European Commission Scientific Committee for Food Standardization.

Exposure of adults and children to 3-MCPD upon consumption of modern bakries bread does not constitute a health hazard.

5.2 Recommendation:

- Use modern toast bread instead of modern round bread because it has less 3-MCPD.
- Bakeries should reduce temperature of oven and decrease sodium chloride in bread, to reduce 3-MCPD.
- Further studies about 3-MCPD esters in different foodstuffs need to be carried in Sudan.

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Appendices

Appendix 1 shows samples collected from Omdurman.

Serial Number	Bread Type	Flour Type	Bakery Area	Heat Treatment	Time in Minute	Date of Collection	Dimensions (cm)		Color
							Length	**Width**	
1	**Modern T**	**Wheat**	**Almulazmeen**	**250**	**5-6**	**06/05/2018**	**20-25**	**3.5-4**	**Brownish**
2	**Modern T**	**Wheat**	**Shuhadaa**	**250**	**5-6**	**06/05/2018**	**20-25**	**3.5-4**	**Brownish**
3	**Modern T**	**Wheat**	**Fitaihaab**	**250**	**5-6**	**06/05/2018**	**20-25**	**3.5-4**	**Brownish**
4	**Modern T**	**Wheat**	**Shiglla**	**250**	**5-6**	**08/05/2018**	**20-25**	**3.5-4**	**Brownish**
5	**Modern R**	**Wheat**	**Salha**	**250**	**7-8**	**08/05/2018**	**11 - 13**	**5-6**	**Brownish**
6	**Modern R**	**Wheat**	**Jaddain N**	**250**	**7-8**	**08/05/2018**	**1 – 13**	**4 – 5**	**Brownish**
7	**Modern R**	**Wheat**	**Jaddain S**	**250**	**7-8**	**10/05/2018**	**11– 13**	**4 – 5**	**Brownish**
8	**Modern R**	**Wheat**	**Lybia Market**	**250**	**7-8**	**10/05/2018**	**1 – 13**	**4 – 5**	**Brownish**
9	**Modern R**	**Wheat**	**Jimaihab**	**250**	**7-8**	**10/05/2018**	**11 - 13**	**4 - 5**	**Brownish**

Appendix 2 shows samples collected from Khartoum.

Serial Number	Bread Type	Flour Type	Bakery Area	Heat Treatment (C^o)	Time in Minute	Date of Collection	Dimensions (cm)		Color
							Length	**Width**	
10	**Modern R**	**Wheat**	**Duyum East**	**250**	**7-8**	**15/05/2018**	**11-13**	**4-5**	**Brownish**
11	**Modern R**	**Wheat**	**Duyum West**	**250**	**7-8**	**17/05/2018**	**11-13**	**4 - 5**	**Brownish**
12	**Modern R**	**Wheat**	**Hila Jadid**	**250**	**7-8**	**17/05/2018**	**11-13**	**4 - 5**	**Brownish**
13	**Modern R**	**Wheat**	**Alsajana**	**250**	**7-8**	**17/05/2018**	**11-13**	**5-7**	**Brownish**
14	**ModernT**	**Wheat**	**Sharwani**	**250**	**5-6**	**17/05/2018**	**20 - 25**	**5-6**	**Brownish**
15	**ModernT**	**Wheat**	**Buree**	**250**	**5-6**	**19/05/2018**	**20 – 25**	**3.5 – 4**	**Brownish**
16	**ModernT**	**Wheat**	**Manshiya**	**250**	**5-6**	**19/05/2018**	**20 – 25**	**3.5 – 4**	**Brownish**
17	**ModernT**	**Wheat**	**Jabelawliya**	**250**	**5-6**	**19/05/2018**	**20 - 25**	**3.5 - 4**	**Brownish**

Appendix 3 shows samples collected from Khartoum Bahri.

Serial Number	Bread Type	Flour Type	Bakery Area	Heat Treatment C^o	Time in Minute	Date of Collection	Dimensions (cm)		Color
							Length	**Width**	
18	**Modern R**	**Wheat**	**Kadorow**	**250**	**7-8**	**23/05/2018**	**11 - 13**	**4 - 5**	**Brownish**
19	**Modern R**	**Wheat**	**Al-Usbaa**	**250**	**7-8**	**23/05/2018**	**11 - 13**	**4 - 5**	**Brownish**
20	**ModernT**	**Wheat**	**DarElsalam**	**250**	**5-6**	**24/05/2018**	**20 - 25**	**6**	**Brownish**
21	**ModernT**	**Wheat**	**Mazad**	**250**	**5-6**	**24/05/2018**	**20 - 25**	**5.5**	**Brownish**
22	**ModernT**	**Wheat**	**Danagla**	**250**	**5-6**	**24/05/2018**	**20 - 25**	**3.5 - 4**	**Brownish**
23	**ModernT**	**Wheat**	**Margania**	**250**	**5-6**	**24/05/2018**	**20 -25**	**3.5 - 4**	**Brownish**
24	**Modern R**	**Wheat**	**Kobber**	**250**	**7-8**	**25/05/2018**	**11 - 13**	**3.5 - 4**	**Brownish**
25	**Modern R**	**Wheat**	**Kafuri**	**250**	**7-8**	**25/05/2018**	**11 - 13**	**3.5 - 4**	**Brownish**

R= round T = toast.

Appendix 4 shows moisture content of bakery bread collected from Omdueman, Khartoum and Khartoum Bahari.

ID	Areas	Samples	W1	W2	Moisture content %
1	Omdurman	Modern T	69.6	47.7	31.9
2	Omdurman	Modern T	62.2	43.0	30.8
3	Omdurman	Modern T	50.1	46.0	28.2
4	Omdurman	Modern T	55.3	37.0	33.1
5	Omdurman	Modern R	64.3	44.1	34.4
6	Omdurman	Modern R	68.4	45.1	34.1
7	Omdurman	Modern R	75.4	50.2	33.4
8	Omdurman	Modern R	65.2	43.2	33.7
9	Omdurman	Modern R	66.3	45.3	31.7
10	Khartoum	Modern R	67.4	43.1	31.8
11	Khartoum	Modern R	69.5	46.3	33.4
12	Khartoum	Modern R	70.1	45.2	35.5
13	Khartoum	Modern R	69.2	43.2	35.7
14	Khartoum	Modern T	57.5	38.2	33.6
15	Khartoum	Modern T	56.9	38.7	31.9
16	Khartoum	Modern T	52.1	37.7	28.8
17	Khartoum	Modern T	53.2	38.2	28.4
18	Khartoum Bahri	Modern R	68.1	45.2	33.6
19	Khartoum Bahri	Modern R	66.1	44.3	32.9
20	Khartoum Bahri	Modern T	54.1	39.1	27.2
21	Khartoum Bahri	Modern T	58.5	39.3	32.8
22	Khartoum Bahri	Modern T	59.3	39.9	32.7
23	Khartoum Bahri	Modern T	57.7	38.8	32.7
24	Khartoum Bahri	Modern R	63.3	42.3	33.3
25	Khartoum Bahri	Modern R	64.2	43.1	32.8

Appendix 5 shows 0il content of bakery bread collected from Omdueman, Khartoum and Khartoum Bahari.

ID	Areas	Samples	W1	W2	W1-W2	W3	Oil content %
1	Omdurman	Modern T	128.3661	128.2125	0.1536	20	0.768
2	Omdurman	Modern T	128.4118	128.2225	0.1893	20	0.946
3	Omdurman	Modern T	128.4221	128.2225	0.1996	20	0.998
4	Omdurman	Modern T	128.5181	128.2225	0.1991	20	0.995
5	Omdurman	Modern R	128.3816	129.2225	0.1591	20	0.795
6	Omdurman	Modern R	115.4001	115.2022	0.1979	20	0.989
7	Omdurman	Modern R	128.5771	128.3110	0.1861	20	0.930
8	Omdurman	Modern R	128.5691	128.3720	0.1971	20	0.985
9	Omdurman	Modern R	128.3915	128.2101	0.1814	20	0.907
10	Khartoum	Modern R	128.4100	128.2201	0.1800	20	0.900
11	Khartoum	Modern R	128.4120	128.3139	0.1981	20	0.990
12	Khartoum	Modern R	128.4259	128.2471	0.1788	20	0.894
13	Khartoum	Modern R	128.5690	128.3901	0.1789	20	0.894
14	Khartoum	Modern T	128.4671	128.3110	0.1561	20	0.780
15	Khartoum	Modern T	128.4771	128.3110	0.1661	20	0.830
16	Khartoum	Modern T	128.4112	128.2225	0.1887	20	0.943
17	Khartoum	Modern T	128.5121	128.3110	0.2011	20	1.000
18	Khartoum Bahri	Modern R	128.5731	128.3921	0.1810	20	0.905
19	Khartoum Bahri	Modern R	128.4921	128.2977	0.1944	20	0.972
20	Khartoum Bahri	Modern T	128.3971	128.2225	0.1646	20	0.823
21	Khartoum Bahri	Modern T	128.3891	128.2225	0.1666	20	0.933
22	Khartoum Bahri	Modern T	128.4771	128.3110	0.1661	20	0.830
23	Khartoum Bahri	Modern T	128.3982	128.2225	0.1757	20	0.878
24	Khartoum Bahri	Modern R	128.3922	128.2101	0.1821	20	0.910
25	Khartoum Bahri	Modern R	128.4112	128.2224	0.1888	20	0.944

Appendix 6 shows estimated quantity of 3-MCPD (µg\kg) for body weight per bady exposure for individual Adults, (Round bread oil).

ID	Name	Concent-3-MCPD\ppm	Mean Adult Age	Boy Weight	Ei, 3-MCPD For body weight per bay\µg\kg
1	S5\ModernRB	0.52279	18-20	68.03	0.018039
2	S6\ModernRB	0.15493	18-20	68.03	0.005345
3	S7\ModernRB	0.31573	18-20	68.03	0.010930
4	S8\ModernRB	0.36274	18-20	68.03	0.012584
5	S9\ModernRB	0.34149	18-20	68.03	0.011780
6	S10\ModernRB	0.58253	18-20	68.03	0.020099
7	S11\ModernRB	0.28591	18-20	68.03	0.009889
8	S12\ModernRB	1.05873	18-20	68.03	0.036528
9	S13\ModernRB	0.96438	18-20	68.03	0.033272
10	S18\ModernRB	0.32869	18-20	68.03	0.011341
11	S19\ModernRB	0.23414	18-20	68.03	0.008078
12	S24\ModernRB	N.D	18-20	68.03	ND
13	S25\ModernRB	N.D	18-20	68.03	ND

Mean estimated quantity of 3-MCPD exposure for Adults = 0.016171µg\kg.

Appendix 7 shows Estimated quantity of 3-MCPD(µg\kg) For body weight per bay exposure for individual Adults, (toast bread oil).

ID	Name	Concent-3-MCPD\ppm	Mean Adult Age	Body Weight	Ei, 3-MCPD For body weight per bay\µg\kg
1	S1\ModernTB	0.56378	18-20	68.03	0.013929
2	S2\ModernTB	0.37503	18-20	68.03	0.009264
3	S3\ModernTB	0.20270	18-20	68.03	0.005654
4	S4\ModernTB	0.26577	18-20	68.03	0.006565
14	S14\ModernTB	0.66749	18-20	68.03	0.016488
15	S15\ModernTB	0.59644	18-20	68.03	0.014733
16	S16\ModernTB	0.21776	18-20	68.03	0.005379
17	S17\ModernTB	0.29495	18-20	68.03	0.007286
20	S20\ModernTB	0.16746	18-20	68.03	0.004137
21	S21\ModernTB	N.D	18-20	68.03	ND
22	S22\ModernTB	N.D	18-20	68.03	ND
23	S23\ModernTB	0.11334	18-20	68.03	0.002799

Mean estimated quantity of 3-MCPD exposure for Adults = 0.0086234µg\kg

Appendix 8 shows estimated quantity of 3-MCPD(µg\kg) For body weight per bay exposure for individual kids, (Round bread oil).

ID	Name	Concent-3-MCPD\ppm	Mean kids Age	Body Weight	Ei, 3-MCPD For body weight per bay\µg\kg
1	S5\ModernRB	0.52279	14-15	47.67	0.015446
2	S6\ModernRB	0.15493	14-15	47.67	0.004577
3	S7\ModernRB	0.31573	14-15	47.67	0.009359
4	S8\ModernRB	0.36274	14-15	47.67	0.010771
5	S9\ModernRB	0.34149	14-15	47.67	0.010087
6	S10\ModernRB	0.58253	14-15	47.67	0.017209
7	S11\ModernRB	0.28591	14-15	47.67	0.008467
8	S12\ModernRB	1.05873	14-15	47.67	0.031277
9	S13\ModernRB	0.96438	14-15	47.67	0.028490
10	S18\ModernRB	0.32869	14-15	47.67	0.009711
11	S19\ModernRB	0.23414	14-15	47.67	0.006917
12	S24\ModernRB	N.D	14-15	47.67	ND
13	S25\ModernRB	N.D	14-15	47.67	ND

Mean estimated quantity of 3-MCPD exposure for children = 0.013846 µg\kg

Appendix 9 shows Estimated quantity of 3-MCPD (µg\kg) for body weight per bay exposure for individual kids, (toast bread oil).

ID	Name	Concent-3-MCPD\ppm	Mean Kids Age	Body Weight	Ei, 3-MCPD For body weight per bay\µg\kg
1	S1\ModernTB	0.56378	14-15	47.67	0.011924
2	S2\ModernTB	0.37503	14-15	47.67	0.007932
3	S3\ModernTB	0.20270	14-15	47.67	0.004687
4	S4\ModernTB	0.26577	14-15	47.67	0.005622
14	S14\ModernTB	0.66749	14-15	47.67	0.014118
15	S15\ModernTB	0.59644	14-15	47.67	0.012615
16	S16\ModernTB	0.21776	14-15	47.67	0.004606
17	S17\ModernTB	0.29495	14-15	47.67	0.006238
20	S20\ModernTB	0.16746	14-15	47.67	0.003542
21	S21\ModernTB	N.D	14-15	47.67	ND
22	S22\ModernTB	N.D	14-15	47.67	ND
23	S23\ModernTB	0.11334	14-15	47.67	0.002397

Mean estimated quantity of 3-MCPD exposure for children = 0.007368µg\kg

Table 10 shows average weight by Age for children and adults belonging to the Age group (1-20) years

Age(years)	Male	Female
1	22.0 Ibs (9.97kg)	21.76Ibs (9.87kg)
2	28.4 Ibs (12.88kg)	28.4 Ibs (12.88kg)
3	33.0Ibs (14.97kg)	30.8Ibs (13.97kg)
4	35-37Ibs (15.87-15-78kg)	35.2Ibs (15.97kg)
5	41.8Ibs (18.97kg)	39.6Ibs (17.97kg)
6	46.6Ibs (20.95kg)	46.6Ibs (20.95kg)
7	50.6Ibs (22.95kg)	50.6Ibs (22.95kg)
8	57.2Ibs (25.95kg)	57.2Ibs (25.95kg)
9	61.6Ibs (27.95kg)	63.8Ibs (28.93kg)
10	70.4Ibs (31.93kg)	70.4Ibs (31.93kg)
11	77.0Ibs 34.92kg)	79.2Ibs 35.92kg)
12-13	85-100Ibs (38.55-45.35kg)	95-105Ibs (43.10-47.62kg)
14-15	105-125Ibs (47.67-56.70kg)	105-115Ibs (47.67-52.16kg)
16-17	130-150Ibs (58.97-68.03kg)	115-120Ibs (52.16-54.43kg)
18-19	150-160Ibs 68.03-72.57kg)	125-130Ibs 56.70-58.97kg)