SUDAN UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF GRADUATE STUDIES

كلية الدراسات العليا

Detection and Quantification of 3-Monochloropropane-1,2-diol (3-MCPD) in Traditional Bread in Khartoum State

كشف وتقدير كمية 3-MCPD في الخبز البلدي في ولاية الخرطوم.

A Dissertation Submitted to Sudan University of Science and Technology in Partial Fulfillment of the Requirement for the Award of M.SC. Degree in Food Science and Technology

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March, 2019

DEDICATION

I dedicate this project to my loving family who got me started.

- *To my late father Mohammed Ahmed.*
- *To my dear Mother Hasania Adam*
- *To my Grandfather and Mother.*
- *To all my beloved relatives and friends.*

ACKNOWLEDGEMENTS

My profound gratitude and exaltation goes to the almighty God for guiding me through the course of my study.

My immeasurable gratitude goes to my Supervisor, Prof. Y. M. A. IDRIS who played the role of a father and teacher as well to me. I acknowledge your great supervision, your encouragement and your selfless devotion to this work.

Much gratitude to my articulated lecturers for their immense contribution and their positive influence towards my life. This goes to Prof. Hatim Makki, Prof. Mahdi Abbas, Prof. Ahmed ELAWAD, Dr. Baraka Mohamed Kabeir, Dr. Maha Albalula, Dr. Salmma Alzain, and Dr. Ibrahim Alfaig.

With great affection and endearment, much gratitude to my loving family whose support and encouragement made everything a success to me, to my late father, my adorable love. I am highly indebted to my loving mother to whom I dedicate this work.

My sincere thanks and love to all my friends.

ABSTRACT

The aim of this research was the detection and quantification of processing contaminants known as 3-MCPD (3-monochloropropane-1, 2-diol) in traditional bread.

Twenty five samples of bread were randomly collected from different areas of Khartoum State (Omdurman, Khartoum, and Khartoum Bahri) and every sample contains twenty piece of bread. Their moisture and oil content were determined using (AOAC), followed by quantification of 3-MCPD by GC-MS. Dietary exposure of children and adults to 3-MCPD were estimated according to their body weight, age, amount of bread consumed per day, as well as the concentrations of the compounds in the sample.

The moisture content of traditional bread samples ranged from 29.0 – 36.73 % and their oil content ranged from 0.37 – 0.92 %. 3-MCPD was detected in traditional bread and its concentrations was in the range of 0.15 – 18.27 ppm.

The exposure of children to 3-MCPD ranged from 0.00063 – 0.5398µg/Kg body weight per day and that of adults ranged from 0.0044 – 0.6304µg/Kg body weight per day.

Therefore, it could be concluded that traditional breads processing leads to the formation of 3-MCPD, however, its level in traditional bread does not constitute serious health risk.

ملخص البحث

اجريت هذه الدراسة بغرض الكشف وتحديد كمية الملوث الكيميائي العضوي المعروف بالمونوكلورو بربانول الناتجة عن عملية التصنيع الغذائي بسبب التعامل الحراري في الخبز البلدي.

تم جمع خمسة وعشرون عينة من مخابز مختلفة لولاية الخرطوم كل من الخرطوم بحري وامدرمان مع العلم ان كل عينة تحتوي على عشرون قطعة خبز ومن ثم تحديد نسبة الرطوبة والزيت باستخدام النظام العالمي (A.O.A.C) وكمية المركب 3-MCPD عن طريق جهاز (GC-MS).

التعرض بالنسبة للاطفال والكبار لهذا المركب قدر استناداً لوزن الجسم ، العمر وكمية الخبز المستهلك بمعدل اليوم بالاضافة الى تركيز المركب في العينة.

نسبة الرطوبة في عينات الخبز البلدي تتراوح ما بين 29–36.73% ونسبة الزيت تتراوح ما بين 0.37–0.92% ايضاً تم تحديد تركيز 3-MCPD في الخبز البلدي فكانت ما بين 0.15–18.27% .

التعرض الاطفال بالنسبة للمركب 3-MCPD تتراوح ما بين 0.00063 – 0.5398 µg/Kg وبالنسبة للكبار يتراوح ما بين 0.0044 – 0.6304 µg/Kg .

يمكن القول ان عملية تصنيع الخبز البلدي يؤدي الي تكوين المركب 3-MCPD ومع ذلك ان الخبز البلدي لا يشكل خطورة بالنسبة لصحة المستهلك.

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CHAPTER ONE

1. Introduction

3-chloropropane-1,2-diol (3-MCPD) is representative of so-called food-borne or food processing contaminants. It was identified in acid-hydrolyzed vegetable protein (acid-HVP) in 1981 (Davideket al.1982) where it originates as a reaction product of phospholipids, acylglycerols and glycerol with hydrochloric acid.

Several recent studies have demonstrated that 3-MCPD is not only the contaminant typical for acid-HVP, soy sauce and related products, but also it occurs in a wide range of retail outlet and home-made foods as well as in various food ingredients formulated without the addition of acid-HVP (Crews *et al.* 2001, Hamlet *et al.* 2002a, 2004a, b; Breitling- Utzmann *et al.* 2003; Divinova *et al.* 2004a; Dolezal *et al.*2005).

It has been shown that edible oil, notably the refined edible oils, contain relatively high levels of bound 3-MCPD. In 2006, the first report about the occurrence of fatty acid esters of 3-monochloropropane-1,2-diol(3-MCPD esters) in fats and oils was published (Zelinkova et al.2006).

Several analytical methods using ester cleavage by transesterification with sodium methoxide and derivatization with phenylboronic acid (PBA) have been presented, and many samples of edible fats and oils have been analyzed (Kusters et al. 2010).

No study about 3-MCPD has been carried out in Sudan and awareness about the contaminant is very minimal.

Aim of The Research

The aim of this research is to provide information about 3-MCPD levels in traditional bread in Sudan.

Objectives

The objectives of this project were:

- Detection and Quantification of 3-MCPD in traditional bread
- Determination of moisture content of traditional bread
- Determination of oil content of traditional bread

CHAPTER TWO

2. Literature Review

2.1 Description of 3-MCPD

3-Monochloropropane-1,2-diol(3-MCPD) is a food processing contaminant that belongs to a group of chemicals called chloropropanols. It was first detected in (acid-) hydrolyzed vegetable protein (HVP), a seasoning ingredient, in soy sauce and similar foods, formed as a reaction product of hydrochloric acid with triacylglycerols, phosolipids, and glycerol from the residual vegetable oil (Velisek *et al.*, 1978).

Further studies showed that 3-MCPD may also occur in products other than HVP such as thermally processed foods like bakery products, vegetable oils and fats during manufacturing or cooking and sodium chloride naturally present or added to the food(Hamlet et al. , 2002; Hamlet and Sadd, 2004; Baer *et al.*, 2010).

2.2 Mechanism of 3-MCPD Formation

2.2.1 Presence of Precursors in Partially Refined Oils

According to some researchers assumption that, the precursors responsible for 3-MCPD formation such as chlorine and diacylglycerols are present in partially refined oils (Franke et al., 2009). Other went beyond the previous speculations suspected that, the refining process result in the uncontrolled addition of certain compounds to the oils (like inorganic chloride in the stripping stream), so it should be the priority to explore mitigation strategies (Pudel et al., 2011). Furthermore, analytical developments regarding MCPD-FE quantification discovered another family of compounds in refined edible oils, namely the fatty esters of glycidol (G-FE). Nevertheless, G-FE were found to be partially responsible for inflation of the results of MCPD-FE quantifications due to the generation of artifacts during sample preparation before analysis using indirect methods(BeiBhaar and Perz, 2010).

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. 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).

2.2.2 Presence of Fatty esters of Glycidol in Refined Oils

The initial detection of glycidyl esters of fatty acids in vegetable oils was the result of research to investigate the origin of 3-MCPD and 3-MCPD esters of fatty acids in these oils, in this regards, the work directed at identifying the precursors to these compounds, reported the existence of relatively high levels of glycidyl esters of fatty acids (WeiBhaarand Perz., 2010).

In addition, a survey is performed of commercial oils in Japan and reported that glycidyl esters were detected in every sample tested (Masukawa *et al.*, 2010).

2.2.3 Interference From Chloride Ions

It has been reported that acylglycerol and glycerol from oils may react with chloride ions to form 3-MCPD, which would lead to overestimations as to the quantity of 3-MCPD in oil samples (WeiBhaar, 2008). Then, the control of interference by chloride ions is removing chloride ions from the samples using the pre-cleaning process involve the addition of deionized water to spiked oil samples, this was outlined by Ermacora and Hrncirik(2012a).

2.3 Factors Affecting 3-MCPD Formation

2.3.1 Host of Lipids and Chlorides

The formation of 3-MCPD esters could only be effected in the presence of chlorides, lipids in any food matrix at high thermal processing conditions. In oils and fats, these components are clearly available, although chlorides may not have been so evident until now. As for palm oil being more susceptible to the formation of chloropropane-diol esters, although it could be due to higher levels of diacylglycerols compounds to other oils (Hamlet *et al.*, 2011).

Findings also show the mechanism of formation of 3-MCPD esters that all lipids can undergo nucleophilic substitution of the acyl group by chloride anion (Svejkovska *et al.*,2004).

Although the overall levels of 3-MCPD in bakery products are relatively low, the high level of consumption of bread, and its additional formation from toasting, indicate that this stable food alone can be a significant dietary source of 3-MCPD (Breiling-Utzmann et al., 2003). Heat treatment is considered as additional significant factor in formation of 3-MCPD in food ingredients (Hamlet *et al.*, 2002b; Muller *et al.*,2005).

2.3.2 Presence of FFA and partial Acylglycerols

Free fatty acids, diacylglycerol(DAG) and monoacylglycerols (MAG) are formed during hydrolysis of triacylglycerols and their effect contribute to the formation of 3-MCPD esters(Hrncirik, 2009). Enzymatic hydrolysis was applied to increase the FFA and DAG contents in the oil samples. Despite the increase in FFA and DAG contents, these was a slight drop in the 3-MCPD esters contents Hrncirik, 2009). A clear link between the precursor (DAG) could not be established Stadler (2009). The researchers concluded that partial acylglycerols seem to be involved in the formation of 3-MCPD esters, but they are not the critical factor determining the final levels Hrncirik and Ermacra(2010).

2.4 Occurrence of 3-MCPD in Foods

2.4.1 Vegetable Oils and Fats

In vegetable oils and fats, 3-MCPD occurs in free and esterified forms with fatty acids Seefelder *et al.*, 2008). 3-MCPD in free form is found in many of the common foods ranging from coffee, malt, bread, salami, ham, and cheese. In general, most foods contain 3-MCPD at approximately 0.0096 – 0.083 mg/kg (Svejkovska *et al.*, 2004).

During food processing most especially in oil refining, the formation of process-induced 3-MCPD esters may occur and various mechanism are currently under investigation that most probably involve a nucleophilic attack by chloride ions (Rahn,Yaylayan, 2010).

However, in refined fats and oils, the highest concentrations were detected in palm oil and palm oil-based fats (WeiBhaar&Perz, 2010). This is consistent with findings that frying oil is the major source of 3-MCPD fatty acid esters in potato products (French fries and chips) Hamlet, 2009; Hamlet & Sadd,2009; Zelinkova *et al.*, 2008).

These esters should also be treated as food contaminants because 3-MCPD may be released in vivo by a lipase-catalyzed hydrolysis reaction (Wenzl *et al.*, 2007). It was assumed that 3-MCPD esters behave similarly to triacylglycerols and undergo similar metabolism and digestion which could either lead to the release of free 3-MCPD or to its incorporation into lipoprotein particles, depending on the positioning of the 3-MCPD fatty acid esters group on the glycerol backbone Schilter *et al.*, 2010). In contrast, it was found that fat from salami contained more 3-MCPD esters of about 1.67mg/kg (Seefelder *et al.*, 2009) also showed ester levels ranging from 0.97 – 2.44mg/kg in fat mixes containing palm olein. For palm oil product, the levels of 3-MCPD esters were within the range of 1.0-5.8mg/kg (Hrncirik *et al.*, 2011 and Weibhaar). In foodstuffs, 3-MCPD occurs not only in its free form, but also as esters with higher fatty acids (so-called bound 3-MCPD). 3-MCPD esters were found in goats' milk and human milk (Zelinkova *et al.*, 2008; Rahn,Yaylayan, 2010).

2.4.2 **Bread**

In recent years studies have shown that 3-MCPD is also present in foods such as bread, other cereal-based products Crews et al. 2003). High levels of 3-MCPD esters have been reported in edible oil, as well as in other products such as crisp bread (Svejkovska *et al.*, 2004; Weisshaar, 2011). In addition, domestic processing (e.g.grilling and toasting) can produce substantial increases in the 3-MCPD content of bread or cheese(Crews et al., 2001; Breitling-Utzmann et al., 2003). In baked cereal products MCPD esters might be generated as stable intermediates or by-products of the formation reaction from mono-and diacylglycerol precursors. The esters were also found in food groups that did not contain free 3-MCPD e.g.coffee creamers, cream aerosols, boillon cubes; Karsulinova *et al* .,2007.

2.5 **Methods of 3-MCPD Analysis and Quantification**

Most methods for the analysis of 3-MCPD focus on the trace analysis at microgram per kilogram levels in various food matrices, which is relatively complicated (Wenzl et al., 2007). The three main physical characteristics that contribute to the absence of a suitable chromo-phore, a high boiling-point and a low molecular weight (Hamlet et al., 2002a). Because of the absence of a chromo-phore , approaches based on high performance liquid chromatograghy with ultra-violet or fluorescence detection cannot be applied, and only one such method with refractive index detection that has been used to study the kinetics of 3-MCPD formation in model systems appears to be unsuitable to determine trace quantities of the compound in food matrices(Hamlet &Sadd, 2002).The development of analytical methodology for the determination of 3-MCPD esters started shortly after Svejkovska et al.(2004) reported the presence of 3-MCPD esters in fried food. As for free 3-MCPD, official methods are not established for the analytical determination of 3-MCPD esters. Scientific activity has taken place in recent years to develop and validate efficient and reliable methods for the analysis of 3-MCPD in its forms reviewed in Wenzl et al., 2007; Baer et al., 2010; Crews et al., 2013).

2.5.1 Direct Method of 3-MCPD Analysis

A direct analysis method, using high performance liquid chromatograghy(HPLC)-time of flight mass spectrometry(LC-TOFMS) not required any harsh chemicals prior to analysis and had adequate detection limits for MCPDE and glycidol ester(GE) in the samples. However, both the acid and the base transesterification procedures use hazardous chemicals which may alter MCPD concentration. Acid analyzes the formation of MCPD from chloride and, even though sulfuric acid is used, chloride in the samples could result in synthesis of MCPD during the treatments. Sodium methoxide also has problems in that base catalyzed reactions can degrade MCPD, as it is not to be stable above P^H 6.0 Haines et al., 2011).

Liquid chromatograghy –tandum Mass spectrometry (LC-MS/MS) has been used to quantify 3-MCPD esters directly in edible oils from soybean, rapeseed, rice, safflower, sesame, olive, grape seed, perilla and palm. HPLC separation is performed using a reversed phase column and mass data from MS is obtained using Electro Spray Ionization (ESI) operated in positive mode (Yamazaki et al., 2013).

2.5.2 Indirect Method of 3-MCPD Analysis

Since there is no suitable chromophore in their structures, approaches based on HPLC are not applicable. Also, the low volatility of chloropropanols, especially 3-MCPD and 1,3-DCP, makes direct analysis by GC-MS difficult. Moreover, the low molecular weight of chloropropanols causes problems in a distinguishing the MS target ions from background noice. These limitations have been overcome by derivatization methods required to produce more volatile analytes and prevent the undesired interaction of chloropropanols with other components during preparation and GC analysis (Mezouaria et al., 2015).

Indirect methods of 3-MCPDesters analysis are based on a common series of steps that include the acid-or alkaline catalyzed cleavage of the esters to yield the free form, followed by the purification of the analyte from the fatty acid methyl esters formed during the methaolysis, derivatization of the free 3-MCPD using either Phenyl Boronic Acid (PBA), heptafluorobutyrylimidozol(HFBT) and acetone followed by injection of the derivatized sample in GC MS - (Hrncirik, Zelinkova

&Ermacora,(2013).

2.5.3 Methods of Quantification

Few methods have been applied to the quantification of bound 3-MCPD only (Divinova et al. 2004, Fry *et al.* 2013). They are all based on preliminary step of fat extraction, followed by the application of one of the conventional indirect methods for the determination of 3-MCPD esters in oil and fats based on acid, alkaline or enzymatic trans esterification; Hrncirik *et al* 2011).

A single method has been proposed for the quantification of glycidyl esters as well, but the accuracy of the results is questionable since the series of chemical reactions that take place during sample preparation can cause the partial conversion of glycidol to 3-MCPD. These methods rely on the assumption that native MCPD esters and glycidyl esters are extracted from the food matrix in the first stages of the sample preparation. However, this hypothesis is not always valid, since the complexity of the physical-chemical interactions of native analytes with other components of the food matrix can have a significance impact of the extraction yield and, as a consequence, on the accuracy of the result. This study aims to develop the first method for the simultaneous quantification, of 2-MCPD and 3-MCPD esters and glycidyl esters in oil-based products (Kusters *et al* 2011).

2.6 Mitigation of 3-MCPD in Vegetable Oils Refining Process

The mitigation strategies of 3-MCPD esters focused on the refining process of vegetable oils, further research showed that, more efforts have been exerted to minimize the contents of the esters in edible oils and this should begin with the careful selection of the raw material as well as with the cultivation of the seeds or fruits (Matthaus et al.,2011a).

2.6.1 Changing of Oil Extraction process During Refining

The development of the refining process began at the end of the 19^{th} century, when more vegetable oils were obtained not only by mechanical extraction with a screw press but also by solvent extraction. Such crude oil is not usable for human consumption and contains many different accompanying substances, including free fatty acid, partial acylglycerols, phosphatides, sterols glycosides, protein fragments, hydrocarbons, traces of pesticides, dioxins, and so forth, resulting from the comprehensive extraction process, thus, it is necessary to purify crude oils after solvent extraction to remove all substances that impair the safety of the oils and the acceptance by the consumer without damaging or removing valuable substance(Diks, 2010; Gibon *et al.*, 2007).

It is logical that the edible oil refining process was the first step by most scientific groups on the journey towards the mitigation of 3-MCPD esters contaminants in the food supply, given that MCPD-FE have been demonstrated to almost entirely formed during edible oil deodorization(Franke *et al.*, 2009).This unit operation has been a target for many mitigation strategies, showing that MCPD-FE are formed at temperatures as low as 200^{0}C, precluding temperature adjustment as a mitigation strategy for these contaminants in refined oils(Franke *et al.* (2009) , Destaillats, Craft, Sandoz, *et al.* (2012).

2.6.2 Removal of Chlorinated Compounds

Due to the conversion of polar chlorinated compounds into more nonpolar compounds during processing, the suggestion was that, it would be easier to remove the reactive chlorine species from the pulp during oil extraction in the oil mill rather than from crude oil, therefore, the earlier the removal of the chlorinated species takes place, the more effective it is for the mitigation of 3-MCPD (Craft *et al.*(2012a). In addition, knowledge about the compounds involved and insight into the formation path ways can help to explain the mitigation effect of processing steps like washing palm oil, it also help to improve the development of strategies to minimize the formation of 3-MCPD esters. Thus, the introduction of a washing step by Matthaus *et al.* (2011a) was an effective approach, later corrobarted by the work of Nagy *et al.* (2011) and Destaillats *et al.*(2012a).

2.6.3 Acylglycerols

Vegetable oils and fats comprised of 88-96% triacyglycerols(TAGs). For most fats and oils, the content of diacylglycerols(DAGs) is low, with amounts ranging from 1% to 2%, but for palm oil the amount of DAGs ranges from 4% to 12%, with a mean value of about 6.5%(Goh and Timms, 1985; Long et al., 2005; Siew and Ng, 1995).

2.6.4 Temperature and Time Regime

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^{0}C, the formation of 3-MCPD and glycidyl esters is relatively low and was found in a range between 2 mg/kg for 3-MCPD esters and related compounds(Pudel et al., 2011).

2.7 Estimation of Dietary Exposure To Consumed Food

In 2013, the European Food Safety Authority (EFSA) performed a preliminary dietary exposure assessment of 3-MCPD esters in Europe (EFSA, 2013). This assessment was preliminary because the analytical method to analysis 3-MCPD esters was still under development at the time of the EFSA publication. The EFSA assessment showed that Dutch children aged 1-9 have a dietary exposure to these contaminants exceeding the TDI of 3-MCPD. The exposure in adults living in the Netherlands remained below this health-based guidance value. The high exposure to 3-MCPD esters in young children was to a large extent due to the consumption of margarines (EFSA, 2013).

2.8 Toxicology Profile of 3-MCPD

The main target organ for 3-MCPD toxicity is kidney, with chronic oral exposure resulting in nephropathy and tubular hyperplasia and adenomas (as reviewed by the Joint FAO/WHO Expert Committee and Food Additives, JECFA (JECFA,2002; in particular Sunahara et al.,1993). Toxicological data on 3-MCPD esters are scarce. A sub-chronic toxicity study in rats administered equimolar doses of free 3-MCPD, or a di-ester (di-palmitate) via gavage confirmed that the kidneys and testes are the main target organs for 3-MCPD toxicity as well as for the 3-MCPD di-ester studied, although the effects were milder and proportional to the urinary excretion of metabolites, which was lower than that observed for free 3-MCPD (Barocelli et al., 2011). Benchmark doses for renal and testicular damage were higher for the di-ester compared to those calculated for 3-MCPD, probably due to a slower and/or lower bioavailability and excretion rate (Barocelli et al., 2011).

CHAPTER THREE

3. Materials and Methods

3.1 Material

Twenty five bread samples were randomly collected from Khartoum State (Omdurman, Khartoum, and Khartoum Bahri), each of these sample contain twenty piece of bread loaves, these samples were further divided among the area of collection as follow: 9 samples were collected from different areas of Omdurman bakeries (Alsabeel, Sawraa 18, Dar-Elsalam, Jikhas, Safwaa 4, Banatt, Almuhandseen, Alsafia, and Umbadda 34),followed by 8 samples which collected from Khartoum Bakeries (Kalakla, Alshagara,Jabra,Mayo,Lamab, Jiraif, Remaila, and Algoz 5), and 8 samples were collected from Khartoum Bahri Bakeries (Samrab, Haj Yousif, Saad Guishra, Shambat, Shuhadda, Shigailab, Halfay,alsafia), then oil were extracted from these bread using soxhlet apparatus and filled in plastic bottle of polyethylene, stored at temperature of (-18^0 C) and analysed for 3-MCPD.

3.2 3-MCPD Reagents

Sodium chloride(p.A), phynylboronic acid (≥98%, PBA), acetone, hexsane, methyl tert-butyl ether (MTBE), methanol, and ethyl acetate from Tokyo Chemical Industries (TCI) as well as glacial acetic acid and sulfuric acid 96% (TCI), were bought from VWR (TCI). Sodium methoxide(25% w/v in methanol), 3-monochloropropane-1,2-diol(98%), and 3-methoxypropane-1,2-diol(98%) were obtained from Tokyo Chemical Industries (TCI). 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 Tokyo Chemical Industries (TCI). 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.

3.3 Equipment

Dessicator, Petri-dishes, Oven (at 105^0C), Sensitive weighing balance, Mortar, Pestle, Soxhlet, Vortex mixer (2500 rpm, 30 s), Micro pipette, Beakers, Volumetric fasks, and Gas Chromatography-Mass Spectrometry (GC- MS), from Japan (Simadzu Company).

3.4 Methods

3.4.1 Determination of Moisture Content of Traditional Bread

Samples of traditional bread were weighed in a wet basis and recorded. The samples were placed in dishes of known weight and dried in an oven at 105^0C for six hours, using A.O.A.C method (1990). The dried samples were then removed from the oven, cooled in a dessicator for about 20 minutes and weighed on sensitive weighing balance.

Moisture content were calculated using the formula below:

%Moisture content = W1- W2 / W1 *100

W1: weight of wet sample

W2: weight of dried sample

3.4.2 Extraction of Oil from Traditional Bread

The bread loaves were dried, finely ground in amotar and pestle to a constant weight of 500 grams of ground samples was weighed, and transferred into oil extraction thimble and stuffed with cotton wool to prevent particles from escaping during extraction into the flask. The soxhlet apparatus was then properly assembled and extraction allowed to continue for 6 hours, after which the solvent (hexane) was recovered. The flask was then removed from the unit and dried at ambient temperature for an hour. It was then cooled at room temperature for about 20 minutes and weighed. The percentage oil was calculated using the method of AOAC (2000) and the results of oil percentage were recorded. 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 point of hexane (45 - 50^0 C), while the speed of the rotary evaporator was fixed at 20 rpm (Soxhlet extraction, Malysia).The oil percentage was determined according to the equation shown below.

3.4.3 Determination of Oil Content

After oil extraction, the hexane was evaporated, weighing the oil and flask then the percentage oil were determined using AOAC, (2000) method according to the equation below:

%Oil content = W1-W2\W3*100

W1: weight of flask and oil

W2: weight of blank flask

W3: weight of sample

3.5 Preparation of Standard Solutions

Stock solutions with concentrations 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/ 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.

3.5.1 Sample Preparation:

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

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 wortex 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.

3.6 GC-MS Conditions

The qualitative and quantitative analysis of the sample was carried out by using GM/MS technique model (GC/MS-QP2010-Ultra) from Japans 'Shimadzu Company, with serial number 020525101565SA and capillary column (Rtx-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 100^0 C with rate 20^0 C/min to 160^0 C held for 1 minutes then the rate was changed to 5^0 C/minutes to 180^0 C ,finally the rate was changed to 30^0 C/minutes reaching 300^0 C as final temperature degree, the injection port temperature was 320^0 C, the ion source temperature was 230^0 C and the interface temperature was 300^0 C. The sample was analyzed by using SIM mode selecting m/z *91,147,196*, the total run time was 15 minutes, results were recorded.

3.6.1 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;

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

By cross multiplication;

Concentration of 3-MCPD ppm= $\frac{\text{Area of sample x Concentration of standard}}{\text{Area of standard}}$

3.7 Estimation of Dietary Exposure (traditional bread) to 3-MCPD in Children and Adults

Dietary exposure for 3-MCPD concentrations per day per body weight based on the amount of breads consumed both for children and adults were determined. Knowing that the average weight of traditional bread samples were 65 g, the total bread loaves consumed by individual children aged 14-15 Years per day was 6 breads, and that of adults aged 18-20 Years per day was 10 breads. Their body weight for deterministic exposure were estimated based on the WHO (2018) simulated diet, mean body weight for children was 47.67 Kg and of adults was 68.03 Kg. Therefore, the concentrations of 3-MCPD per body weight per day for an individual were determined according to the formula shown below.

$$E_i = \frac{\Sigma\, Q_{i,K} \times C_{i,K}}{Bw_i}$$ (Vannort et al 2005)

Where,

E_i = is the exposure of individual I to some chemical at some specified point in time

$Q_{i,K}$ = is the amount of food K consumed by individual I

$C_{i,K}$ = is the concentration of the chemical of interesting food K, consumed by individual I, and

Bw_i = is the body weight of an individual i

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

CHAPTER FOUR

4. Results and Discussions

Table 1 below shows the moisture content of traditional bread samples collected from areas of Khartoum states of Omdurman, Khartoum, and Khatroum Bahri. The mean moisture content of three areas of Omdurman, Khartoum, and Khartoum Bahri show little bit of variations of moisture and this may refer to the conditions of baking processing such as addition of ingredients and water.

Table 1: Moisture Content of Traditional Bread and Area of Collection

Samples No	Area	W1(g)	W2(g)	% M.C
1	Alsabeel	71.7	46.3	35.42
2	Sawraa 18	81.4	57.8	29.0
3	Dar-Elsalam	73.0	46.8	35.89
4	Jikhas	68.0	44.3	34.85
5	Safwaa 4	70.2	46.3	34.05
6	Banatt	68.6	44.3	35.42
7	Almuhandseen	64.9	43.7	32.67
8	Alsafia	66.8	44.7	33.08
9	Umbadda 34	71.5	47.0	34.27
10	Kalakla	66.7	43.5	34.78
11	Alshagara	67.9	44.5	34.47
12	Jabra	75.0	50.8	32.27
13	Mayo	73.4	46.5	36.64
14	Lamab	68.3	44.1	35.43
15	Jiraif	70.6	47.1	31.04
16	Remaila	65.7	43.8	33.33
17	Algoz 5	70.0	46.8	33.14
18	Samrab	65.1	44.1	32.26
19	Haj Yousif	73.5	46.5	36.73
20	Saad Guishra	65.3	42.7	34.61
21	Shambat	70.5	46.2	34.47
22	Shuhadda	76.0	50.8	33.16
23	Shigailab	69.1	44.4	35.75
24	Halfay	77.0	51.5	33.11
25	Alsafia	60.3	41.7	30.85

Mean Moisture Content = 33.86 W1= weight of wet sample, W2 = weight of dried sample.

Table 2 shows details of extracted oil percentage of traditional bread samples from Omdurman, Khartoum, and Khartoum Bahri. There was gradual increasing in their mean oil content in respected order of the three areas, and this is due to some baking processing conditions as well as ingredient their in.

Table 2: Oil Content in Traditional Bread and Areas of Collection

Serial No	Area	W1	W2	W3	%Oil Content(g)
1	Alsabeel	128.5107	128.3989	20	0.56
2	Sawraa 18	115.3550	115.2026	20	0.76
3	Dar-Elsalam	128.3767	128.2436	20	0.67
4	Jikhas	128.2867	128.1814	20	0.53
5	Safwaa 4	115.3440	115.2561	20	0.44
6	Banatt	128.4017	128.3181	20	0.42
7	Almuhandseen	128.3315	128.2461	20	0.43
8	Alsafia	115.4012	115.3267	20	0.37
9	Umbadda 34	128.4133	128.3121	20	0.51
10	Kalakla	128.6127	128.4889	20	0.62
11	Alshagara	118.3450	118.2630	20	0.41
12	Jabra	127.4617	127.2780	20	0.92
13	Mayo	128.5933	128.4111	20	0.91
14	Lamab	118.3155	118.2012	20	0.57
15	Jiraif	127.4434	127.3005	20	0.71
16	Remaila	128.6012	128.5051	20	0.48
17	Algoz 5	118.4021	118.2918	20	0.55
18	Samrab	127.5032	127.3912	20	0.56
19	Haj Yousif	129.7812	129.5964	20	0.92
20	Saad Guishra	128.6534	128.5000	20	0.78
21	Shambat	115.3528	115.2499	20	0.51
22	Shuhadda	128.6612	128.5123	20	0.74
23	Shigailab	115.3611	115.2136	20	0.74
24	Halfay	129.6125	129.4919	20	0.60
25	Alsafia	128.6356	128.5157	20	0.59

Mean Oil Content = 0.62

W1=weight of flask and oil, w2 =weight of blank flask, W3= weight of sample

Figure 1 and 2 show the full scan mass spectrum of 3-MCPD and PBA derivatives. PBA readily react with 3-MCPD to give stable derivative of 3-MCPD. Figure 2 shows appropriate for GC-MS analysis, PBA was Derivatization reagent of (TBME), as is very selective, the numbers in figure 91, 147, and 196 show molecular weight of tert butyl methyl esters (TBME, 3-MCPD) and phenylboronic acid (PBA) respectively. The GC-MS employed was linear within the working calibration standard concentration in a range of 0.1 – 1.0 ppm.

4.1 Selective Mass spectrum

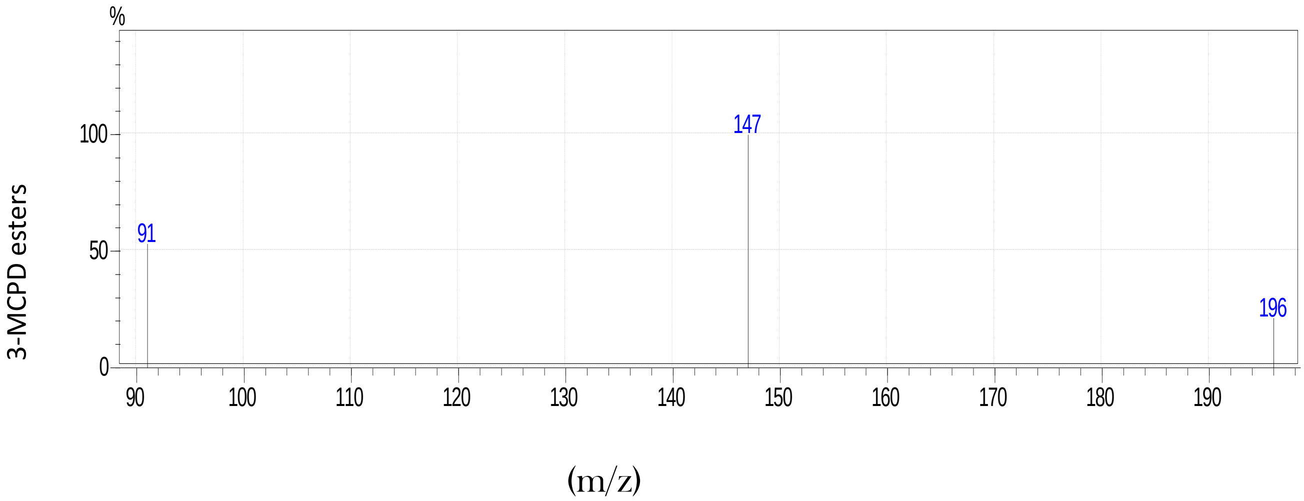
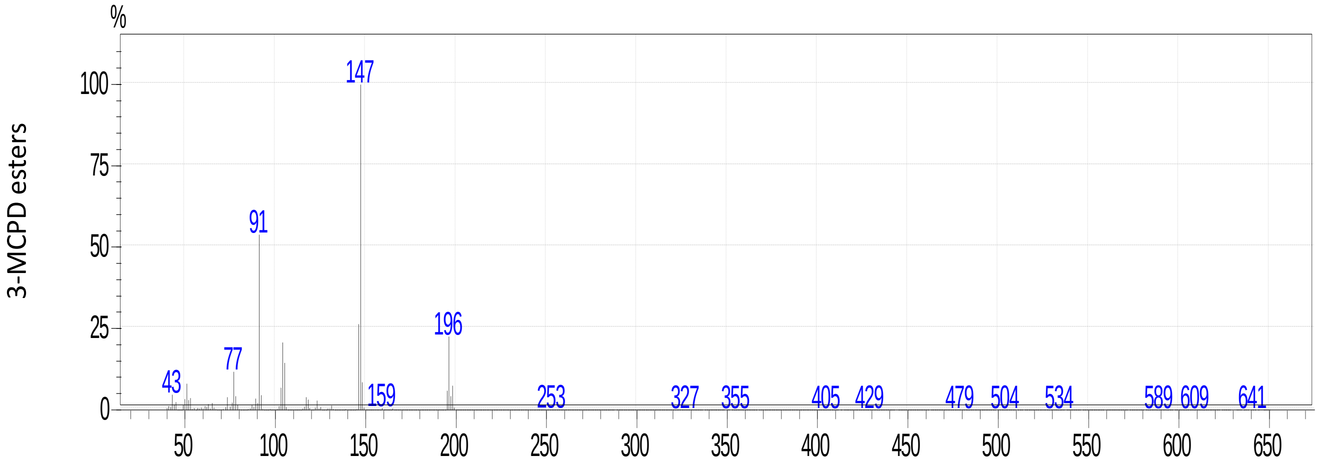


Figure 1: Selective Mass Spectrum by GC-MS of 3-MCPD esters



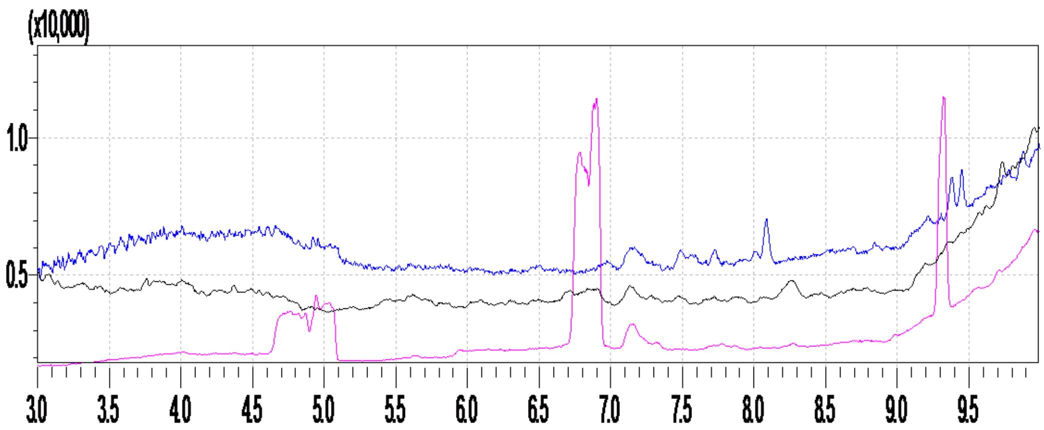
(m/z)

Figure 2: Scan Mass Spectrum by GC-MS of 3-MCPD esters

From figures 3 – 7, show the calibration curve of standards chromatogram by GC-MS of 3-MCPD esters, it was obtained by plotting the peak areas versus the concentrations of standards of 3-MCPD. A five points of 3-MCPD calibration was performed, the dynamic range of calibration is approximately 0.1 – 1.0 ppm. The 3-MCPD calibration was linear in this dynamic range, showing correlation coefficient of R = 0.9999 or better.

4.2 Standards Chromatograms

Figure 3 represents standard chromatogram run of GC-MS by 3-MCPD, Y-axis shows concentration of 0.1 ppm of 3-MCPD, and X-axis shows the retention time of 7.153 (min), total area of standard was 2408, molecular weight 147.



Retention Time (RT)

Figure 3: Standard 1 Chromatogram by GC-MS of 3-MCPD esters

Figure 4 represents standard chromatogram run of GC-MS by 3-MCPD, Y-axis shows concentration of 0.3 ppm of 3-MCPD, and X-axis shows the retention time of 7.151 (min), total area of standard was 6838, molecular weight 147.

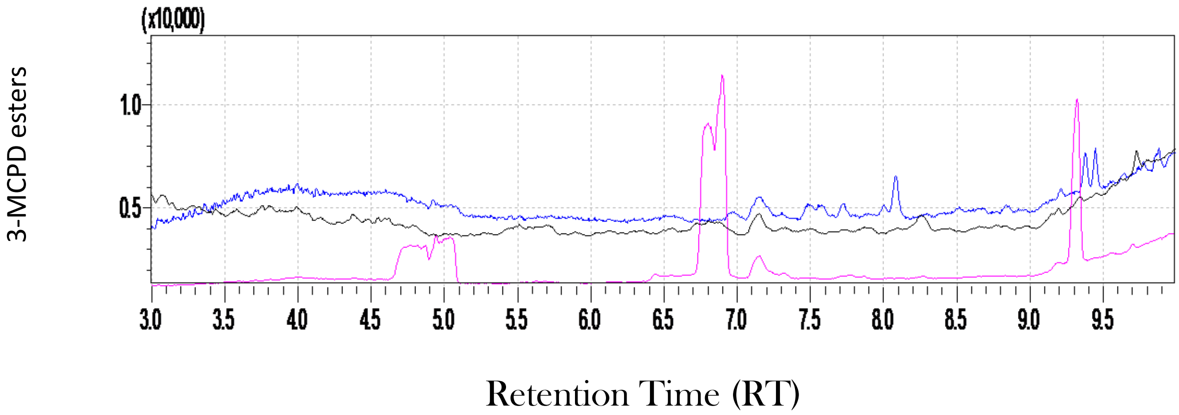


Figure 4: Standard 2 Chromatogram by GC-MS of 3-MCPD esters

Figure 5 represents standard chromatogram run of GC-MS by 3-MCPD, Y-axis shows concentration of 0.5 ppm of 3-MCPD, and X-axis shows the retention time of 7.150 (min), total area of standard was 11438, molecular weight 147.

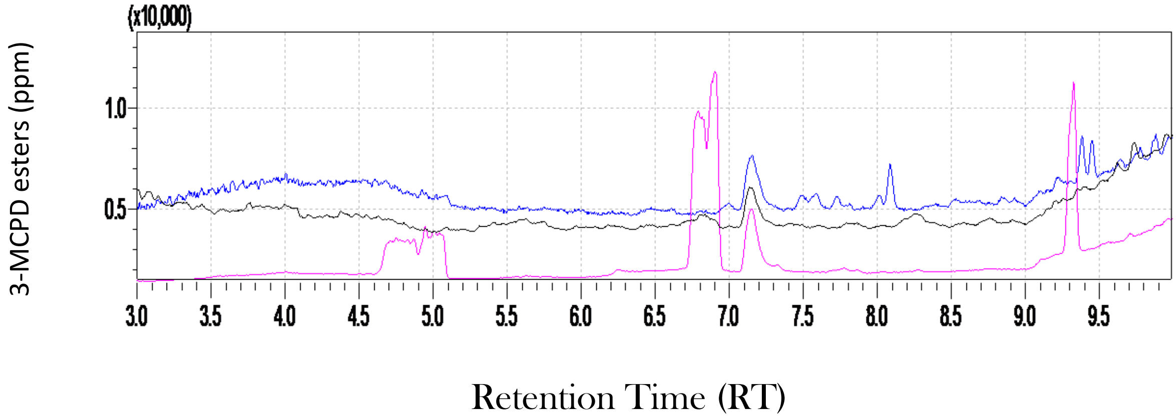


Figure 6: Standard 3 Chromatogram by GC-MS of 3-MCPD esters

Figure 6 represents standard chromatogram run of GC-MS by 3-MCPD, Y-axis shows concentration of 0.7 ppm of 3-MCPD, and X-axis shows the retention time of 7.140 (min), total area of standard was 16024, molecular weight 147.

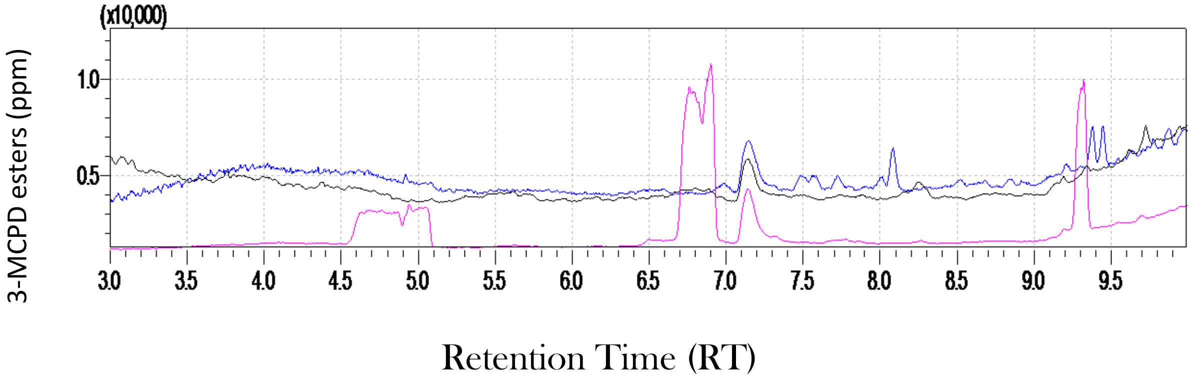


Figure 6: Standard 4 Chromatogram by GC-MS of 3-MCPD esters

Figure 7 represents standard chromatogram run of GC-MS by 3-MCPD, Y-axis shows concentration of 1ppm of 3-MCPD, and X-axis shows the retention time of 7.156 (min), total area of standard was 23092, molecular weight 147.

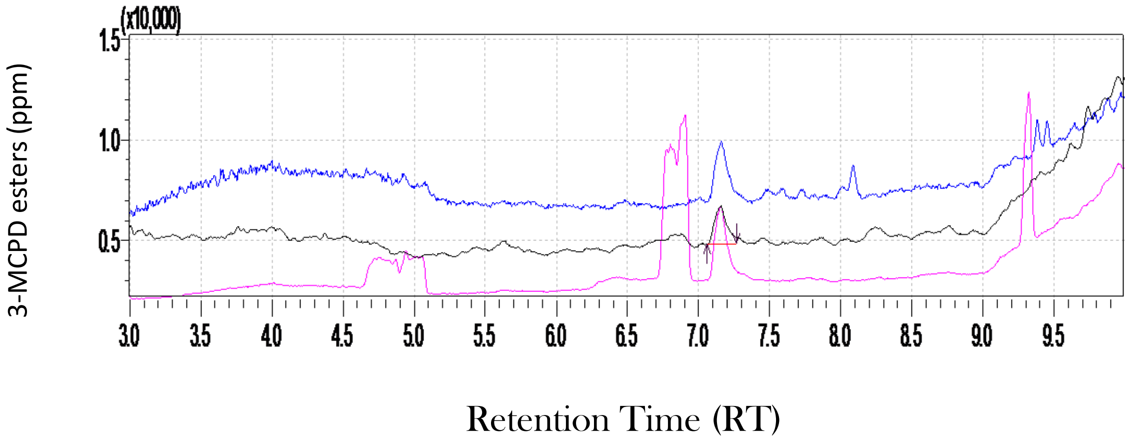


Figure 7: Standard 5 Chromatogram by GC-MS of 3-MCPD esters

Figure 8 below shows the details of the five standards, their concentrations, time retention, molecular weight due to fragmentation of the compound after derivatization and the area, samples were analysed by running concentration of 3-MCPD.

Table 3: Standards Concentration,Time Retention, Molecular Weight and Areas

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

Figure 8: Calibration Standards of 3-MCPD esters

Figure 9: shows calibration curve, correlation coefficients R= 0.9999349 of sample with higher level than 1ppm and relative standard deviation of RSD = 2.3037

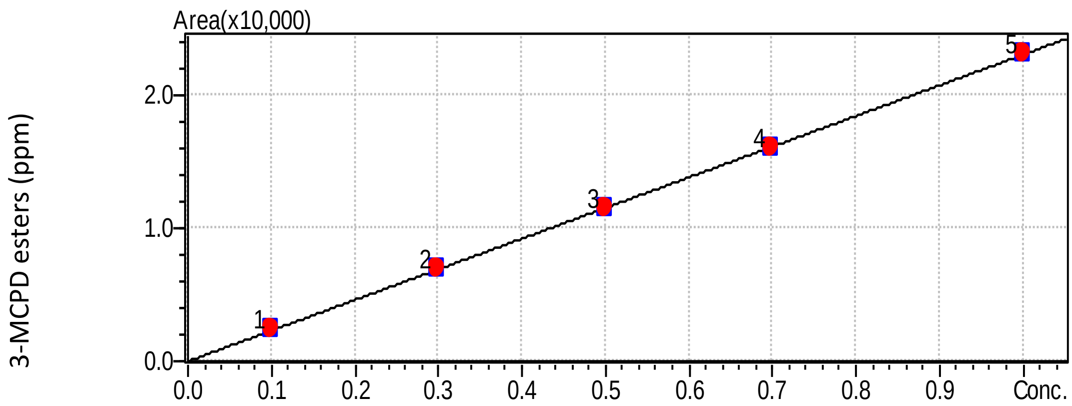


Figure 9: Calibration Curve of 3-MCPD esters Standards

R = 0.9999349 - RSD=2.3037

4.3 Samples Chromatograms

Figure 10 shows sample 1 chromatograms of traditional bread which is collected from Omdurman(Alsabeel bakery), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time 7.182 in minute, molecular weight 147, area 261209, and concentration of 3-MCPD esters in the sample 10.35539 ppm.

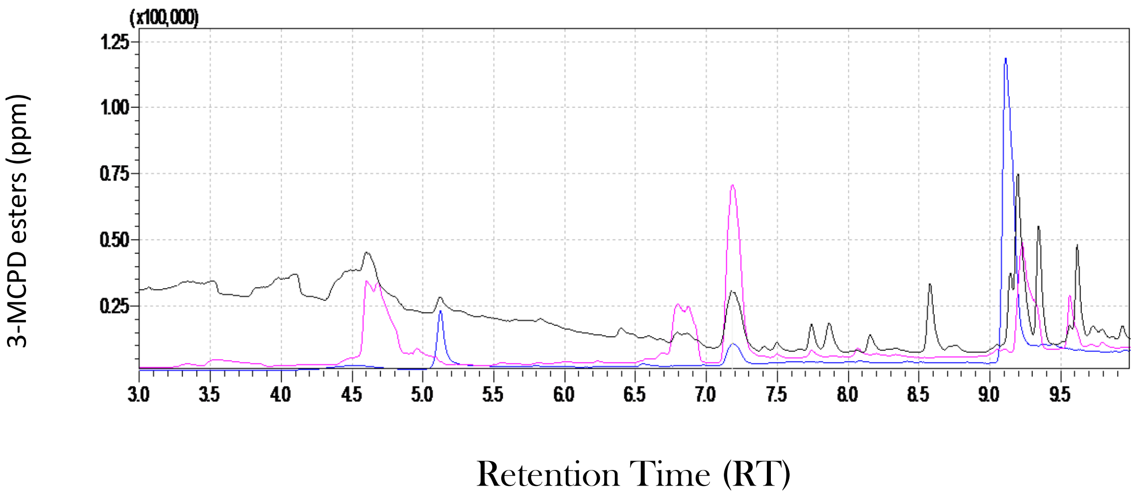


Figure 10: Sample 1 Chromatogram of 3-MCPD esters

Figure 11 shows sample 2 chromatograms of traditional bread which is collected from Omdurman(Sawraa 18), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time 7.189 in minute, molecular weight 147, area 76399, and concentration of 3-MCPD esters in the sample 3.02877 ppm.

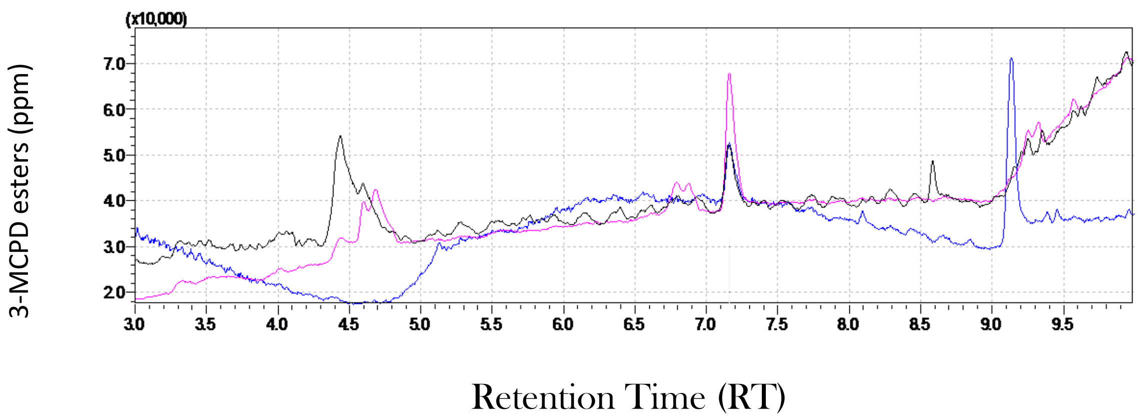


Figure 11: Sample 2 Chromatogram of 3-MCPD esters

Figure 12 shows sample 3 chromatograms of traditional bread which is collected from Omdurman(Dar-Elsalam), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time 7.161 in minute, molecular weight 147, area 114525, and concentration of 3-MCPD esters in the sample 4.54024 ppm.

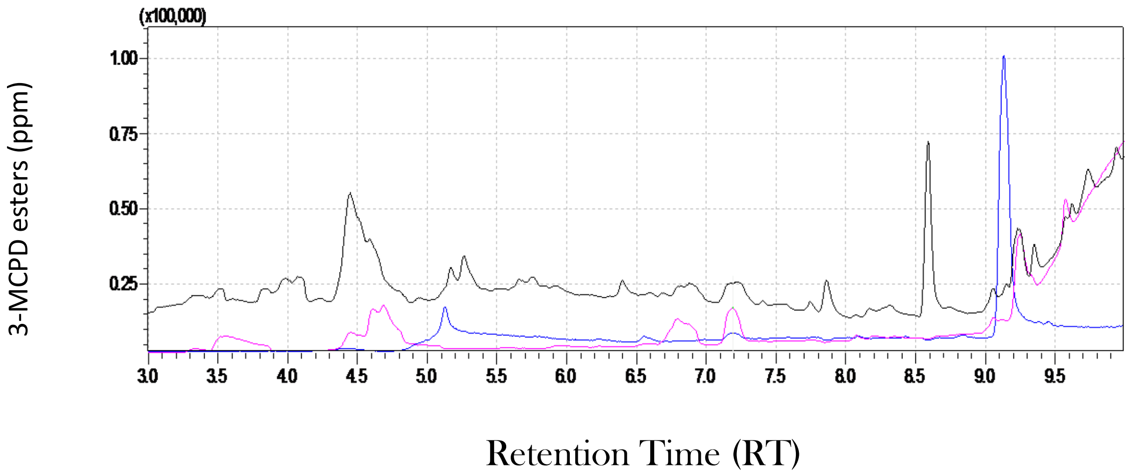


Figure 12: Sample 3 Chromatogram of 3-MCPD esters

Figure 13 shows sample 4 chromatograms of traditional bread which is collected from Omdurman(Jikhass), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time 7.160 in minute, molecular weight 147, area 460931, and concentration of 3-MCPD esters in the sample 18.27318 ppm.

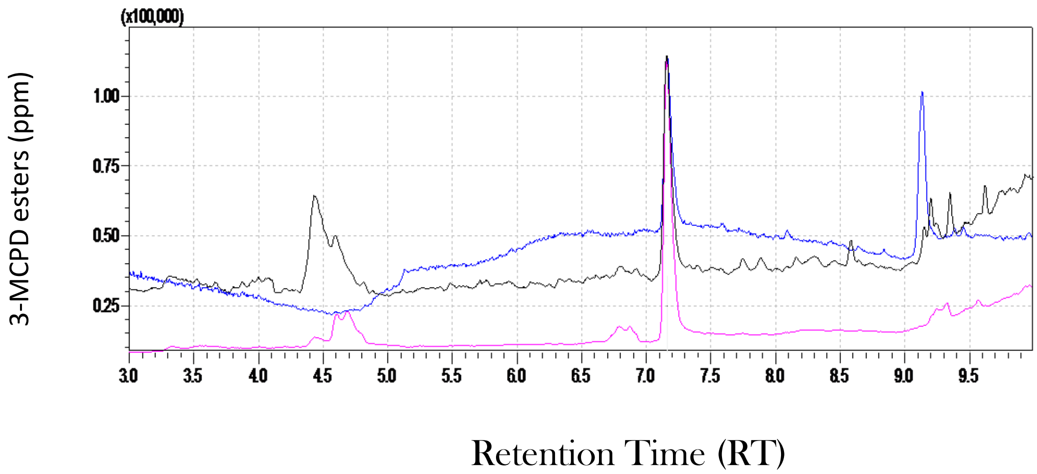
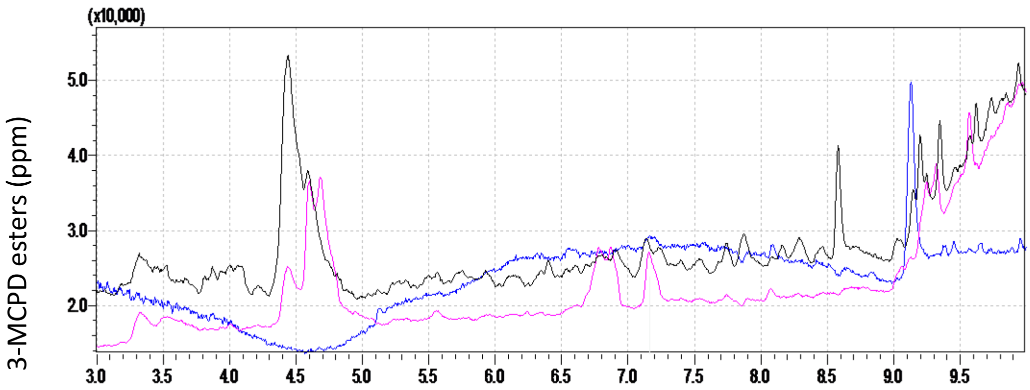


Figure 13: Sample 4 Chromatogram of 3-MCPD esters

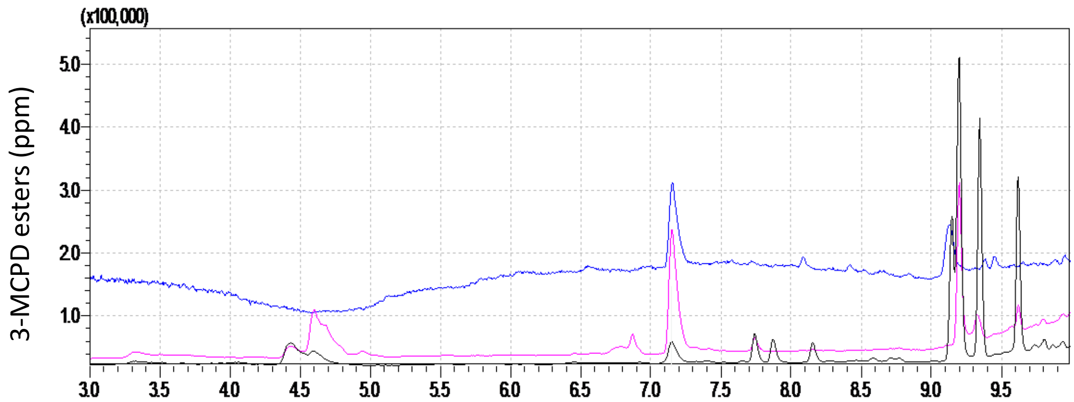
Figure 14 shows sample 5 chromatograms of traditional bread which is collected from Omdurman(Safwaa 4), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time 7.158 in minute, molecular weight 147, area 25530, and concentration of 3-MCPD esters in the sample 1.01211 ppm.



Retention Time (RT)

Figure 14: Sample 5 Chromatogram of 3-MCPD esters

Figure 15 shows sample 6 chromatograms of traditional bread which is collected from Omdurman(Banatt), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time 7.151 in minute, molecular weight 147, area 269461, and concentration of 3-MCPD esters in the sample 10.68253 ppm.



Retention Time (RT)

Figure 15: Sample 6 Chromatogram of 3-MCPD esters

Figure 16 shows sample 7 chromatograms of traditional bread which is collected from Omdurman(Almuhandssin), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time 7.191 in minute, molecular weight 147, area 28305, and concentration of 3-MCPD esters in the sample 1.12213 ppm.

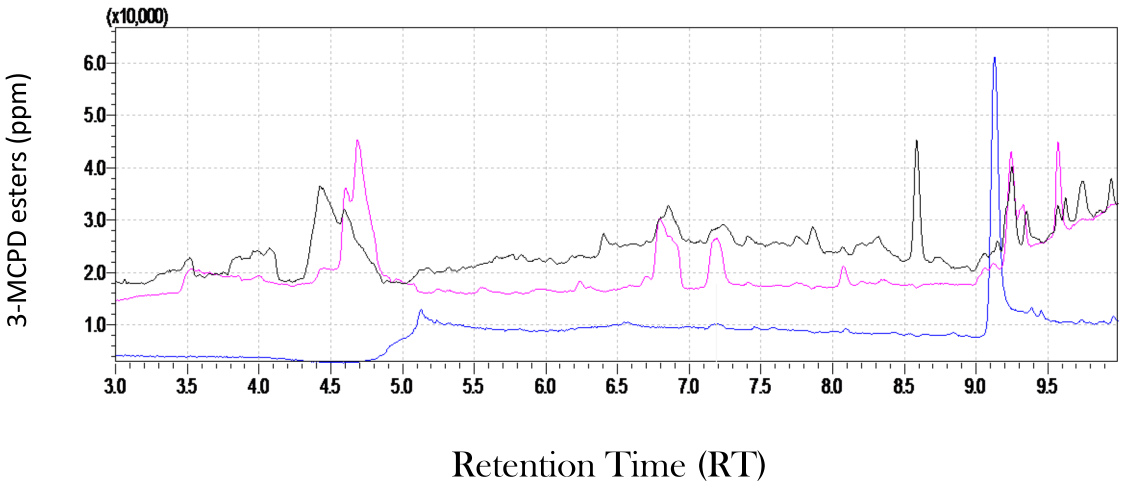


Figure 16: Sample 7 Chromatogram of 3-MCPD esters

Figure 17 shows sample(8) chromatograms of traditional bread which is collected from Omdurman(Alsafia), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time 7.166 in minute, molecular weight 147, area 21415, and concentration of 3-MCPD esters in the sample 0.84898 ppm.

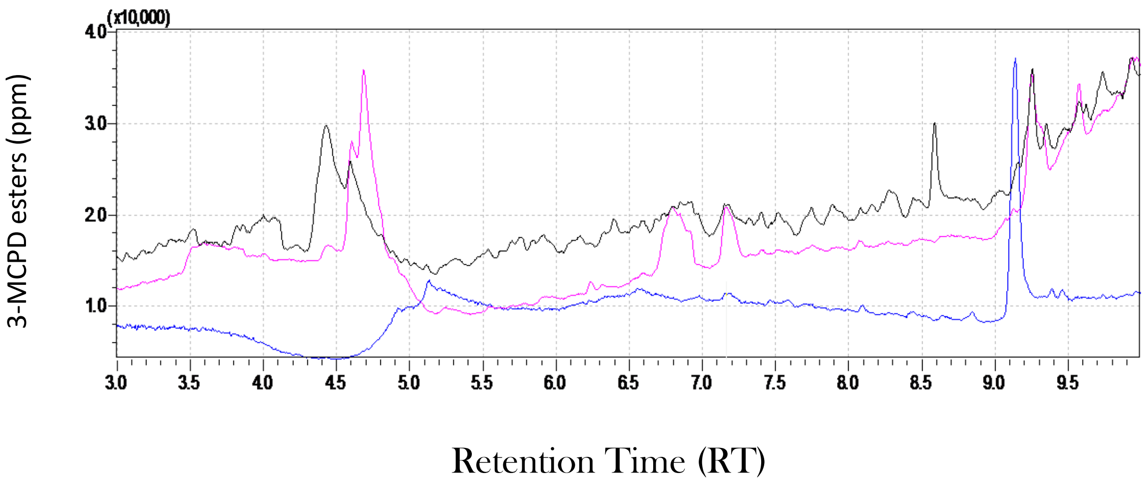


Figure 17: Sample 8 Chromatogram of 3-MCPD esters

Figure 18 shows sample 9 chromatograms of traditional bread which is collected from Omdurman (Ummbada 34), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time 7.204 in minute, molecular weight 147, area 32976, and concentration of 3-MCPD esters in the sample 1.30730 ppm.

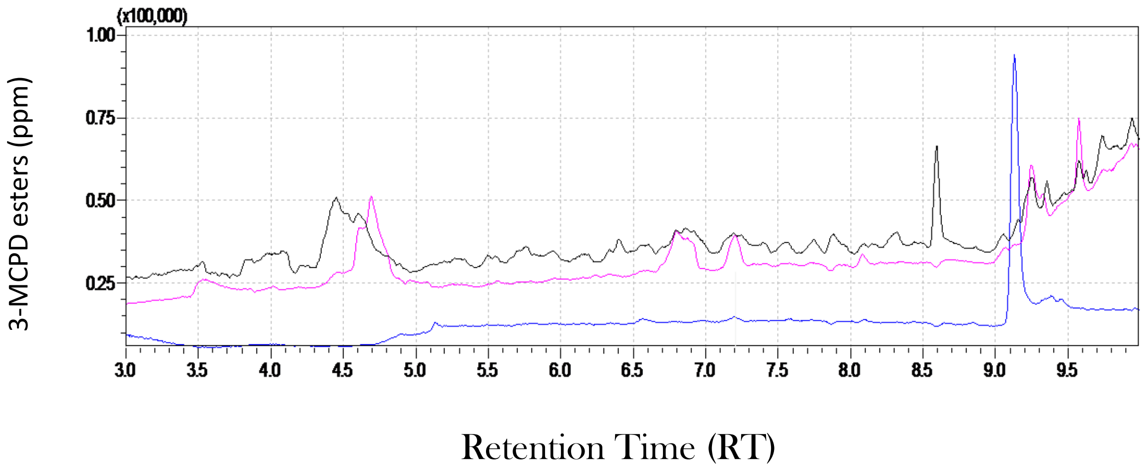


Figure 18: Sample 9 Chromatogram of 3-MCPD esters

Figure 19 shows sample 10 chromatograms of traditional bread which is collected from Khartoum(Kalakla), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time 7.183 in minute, molecular weight 147, area 7869, and concentration of 3-MCPD esters in the sample 0.31196 ppm.

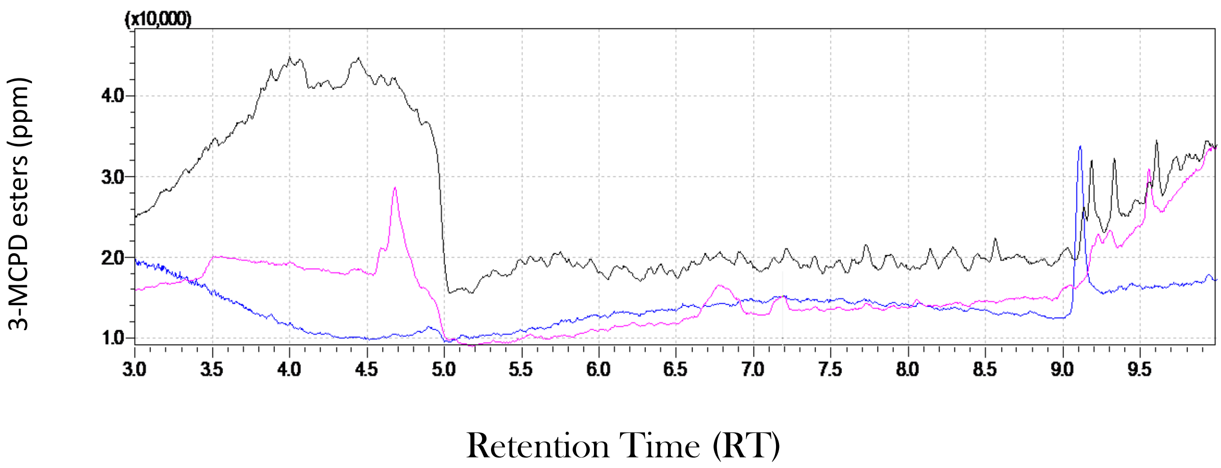
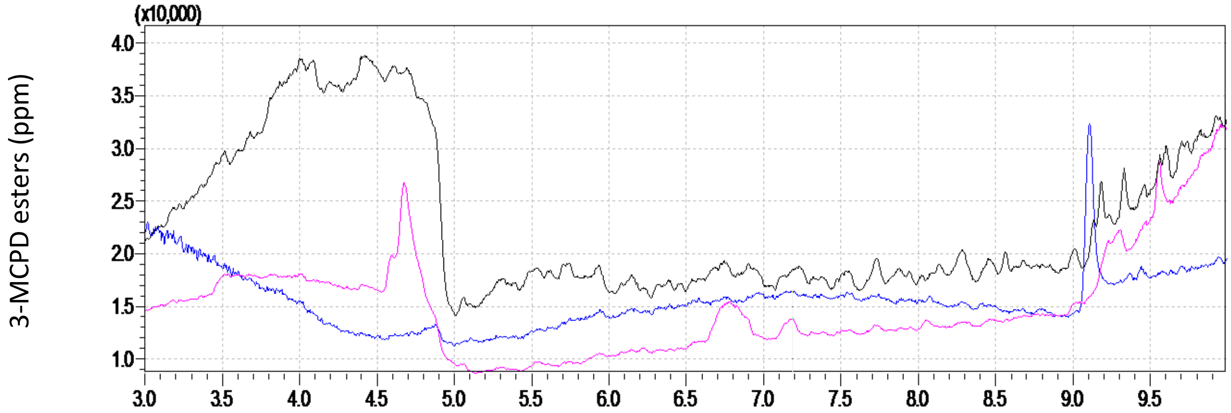


Figure 19: Sample 10 Chromatogram of 3-MCPD esters

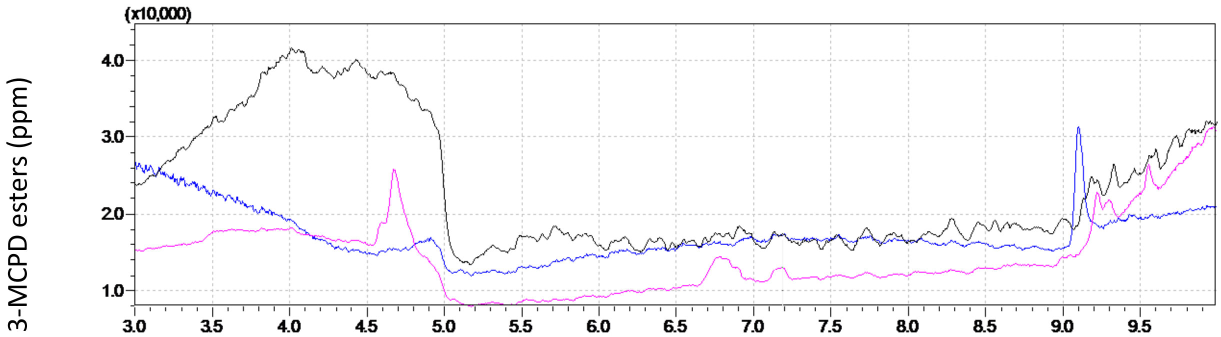
Figure 20 shows sample11 chromatograms of traditional bread which is collected from Khartoum(Alshajara), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time 7.186 in minute, molecular weight 147, area 3862, and concentration of 3-MCPD esters in the sample 0.15311 ppm.



Retention Time (RT)

Figure 20: Sample 11 Chromatogram of 3-MCPD ester

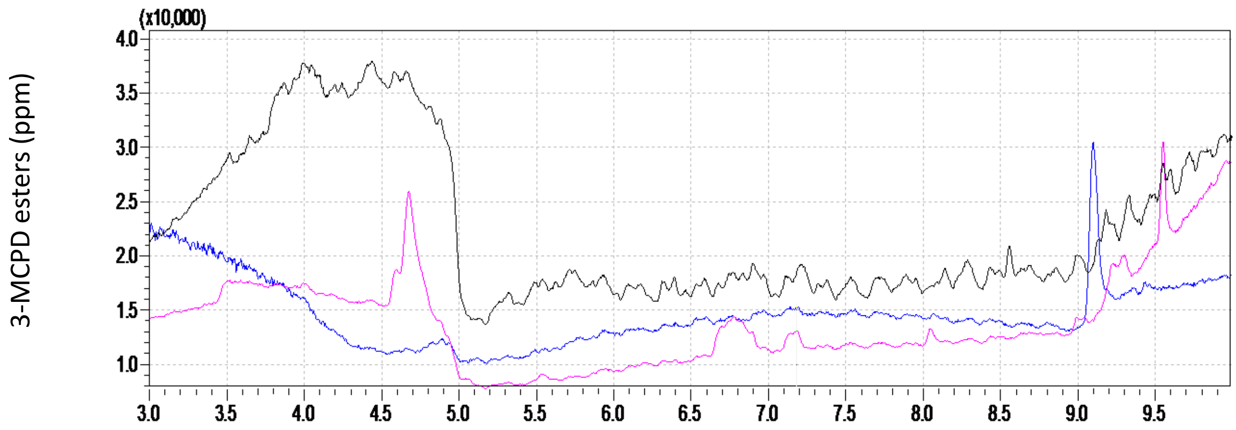
Figure 21 shows sample 12 chromatograms of traditional bread which is collected from Khartoum (Jabra), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time 7.185 in minute, molecular weight 147, area 6475, and concentration of 3-MCPD esters in the sample 0.25670 ppm.



Retention Time (RT)

Figure 21: Sample 12 Chromatogram of 3-MCPD esters

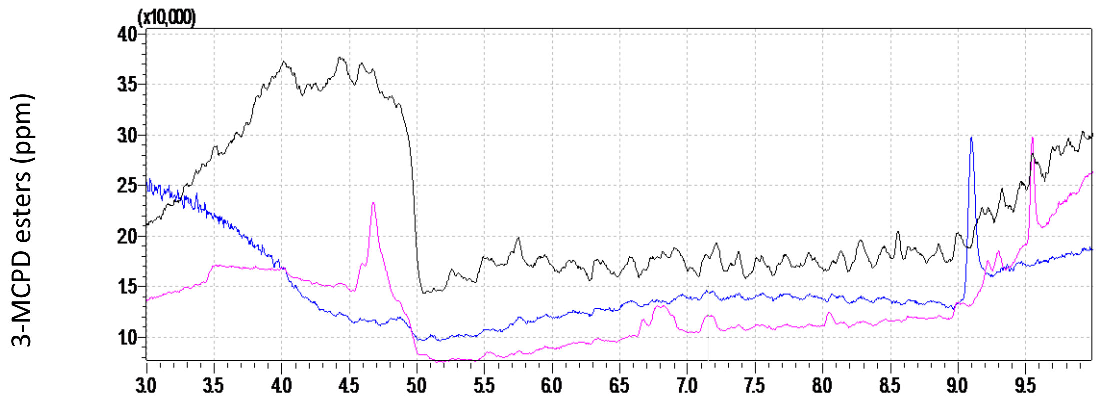
Figure 22 shows sample 13 chromatograms of traditional bread which is collected from Khartoum (Mayo), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time 7.180 in minute, molecular weight 147, area 5995, and concentration of 3-MCPD esters in the sample 0.23767ppm.



Retention Time (RT)

Figure 22: Sample 13 Chromatogram of 3-MCPD esters

Figure 23 shows sample 14 chromatograms of traditional bread which is collected from Khartoum(Lamab), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time7.150 in minute, molecular weight 147, area 7031, and concentration of 3-MCPD esters in the sample 0.27874 ppm.



Retention Time (RT)

Figure 23: Sample 14 Chromatogram of 3-MCPD esters

Figure 24 shows sample15 chromatograms of traditional bread which is collected from Khartoum (Jiraif), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time7.177 in minute, molecular weight 147, area 6503, and concentration of 3-MCPD esters in the sample 0.25781 ppm.

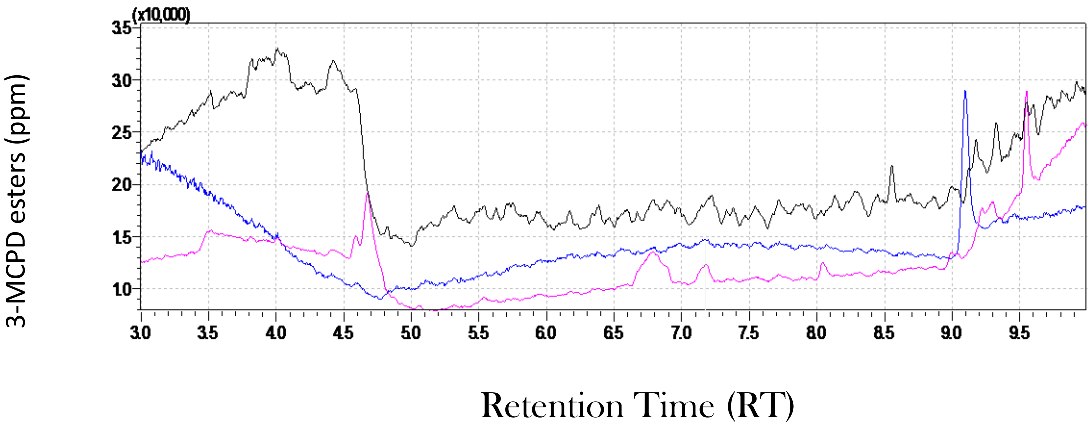


Figure 24: Sample 15 Chromatogram of 3-MCPD esters

Figure 25 shows sample(16) chromatograms of traditional bread which is collected from Khartoum(Remaila), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time7.183 in minute, molecular weight 147, area 7674, and concentration of 3-MCPD esters in the sample 0.30423 ppm.

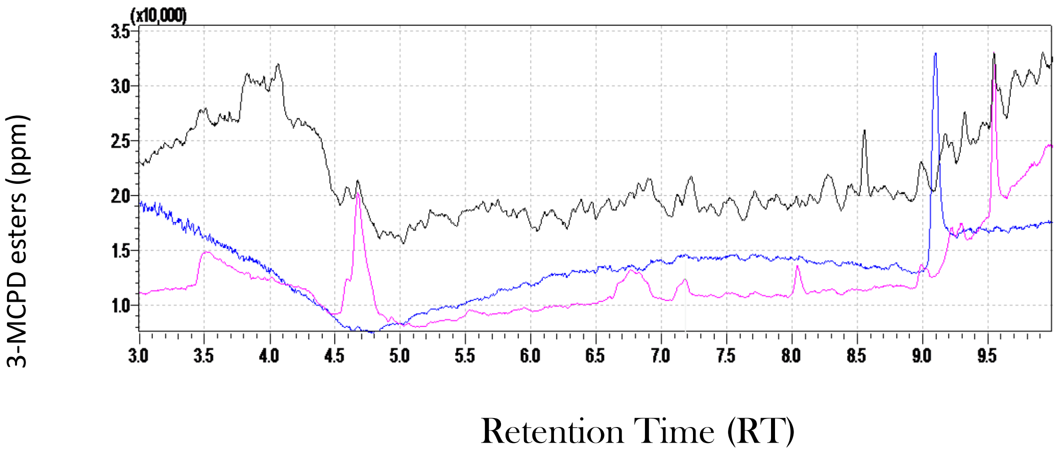


Figure 25: Sample 16 Chromatogram of 3-MCPD esters

Figure 26 shows sample17 chromatograms of traditional bread which is collected from Khartoum(Algoss 5), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time 7.177 in minute, molecular weight 147, area 4386, and concentration of 3-MCPD esters in the sample 0.17388 ppm.

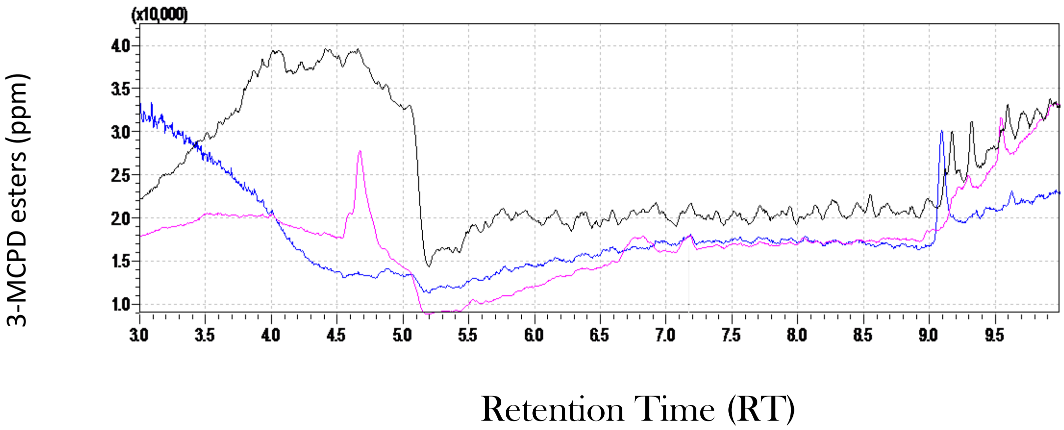


Figure 26: Sample17 Chromatogram of 3-MCPD esters

Figure 27 shows sample 18 chromatograms of traditional bread which is collected from Khartoum Bahri(Samrab), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time7.138 in minute, molecular weight 147, area 11213, and concentration of 3-MCPD esters in the sample 0.44453 ppm.

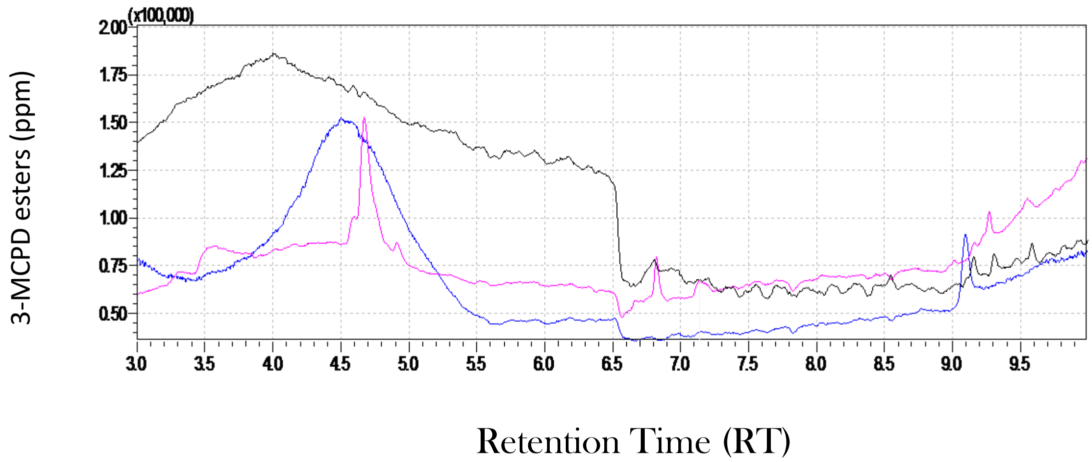


Figure 27: Sample 18 Chromatogram of 3-MCPD esters

Figure 28 shows sample 19 chromatograms of traditional bread which is collected from Khartoum Bahri(Haj-Yousif), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time 7.177 in minute, molecular weight 147, area 6463, and concentration of 3-MCPD esters in the sample 0.25622ppm.

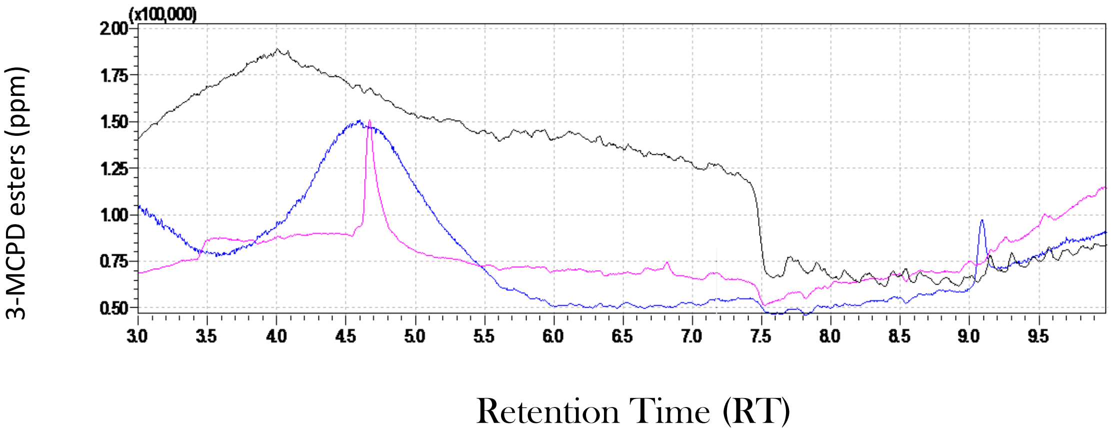


Figure 28: Sample 19 Chromatogram of 3-MCPD esters

Figure 29 shows sample 20 chromatograms of traditional bread which is collected from Khartoum Bahri(Saad-Guishra), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time7.160 in minute, molecular weight 147, area 3283, and concentration of 3-MCPD esters in the sample 0.13015 ppm.

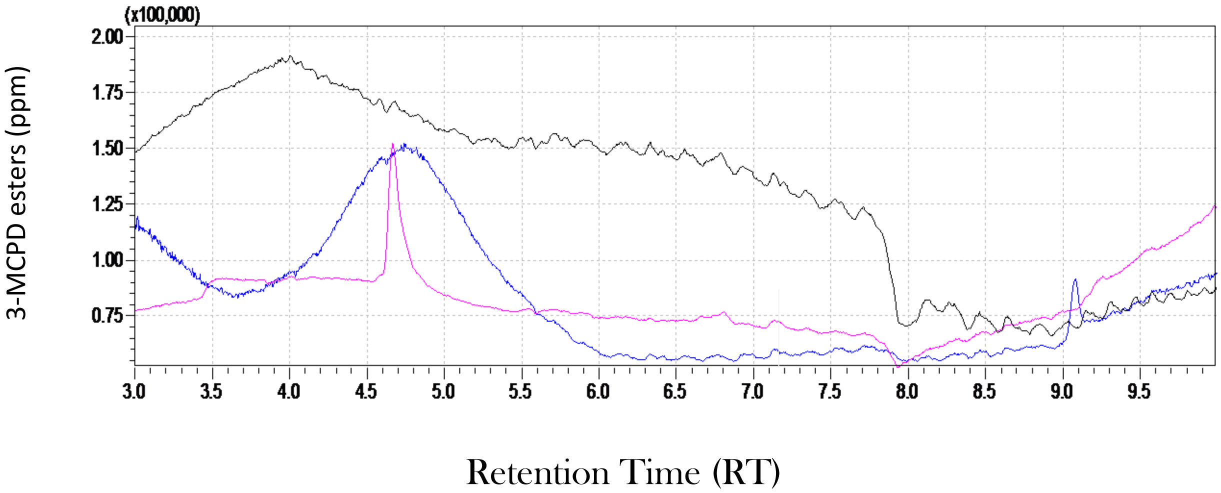


Figure29: Sample 20 Chromatogram of 3-MCPD esters

Figure 30 shows sample 21 chromatograms of traditional bread which is collected from Khartoum Bahri(Shambat), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time7.152 in minute, molecular weight 147, area 8100, and concentration of 3-MCPD esters in the sample 0.32112 ppm.

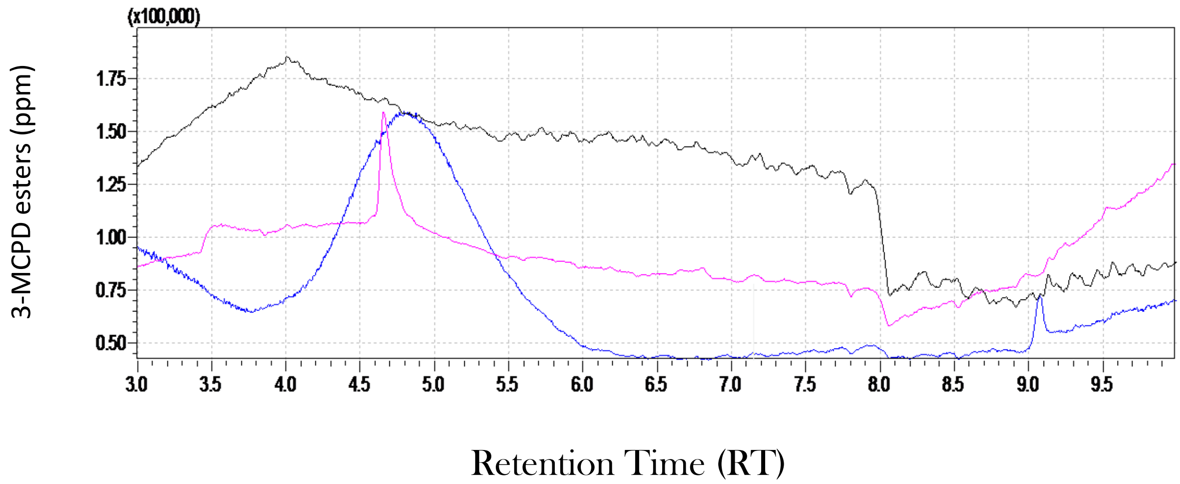


Figure 30: Sample 21 Chromatogram of 3-MCPD esters

Figure 31 shows sample 22 chromatograms of traditional bread which is collected from Khartoum Bahri(Shuhadda), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time7.120 in minute, molecular weight 147, area 23295, and concentration of 3-MCPD esters in the sample 0.92351ppm.

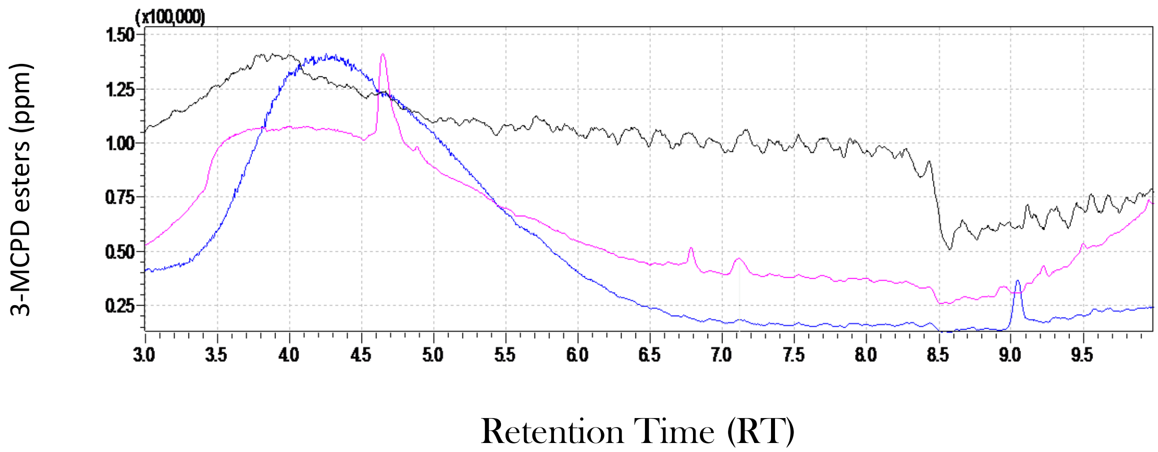


Figure 31: Sample 22 Chromatogram of 3-MCPD esters

Figure 32 shows sample 23 chromatograms of traditional bread which is collected from Khartoum Bahri(Shigailab), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time7.124 in minute, molecular weight 147, area 8913, and concentration of 3-MCPD esters in the sample 0.35335 ppm.

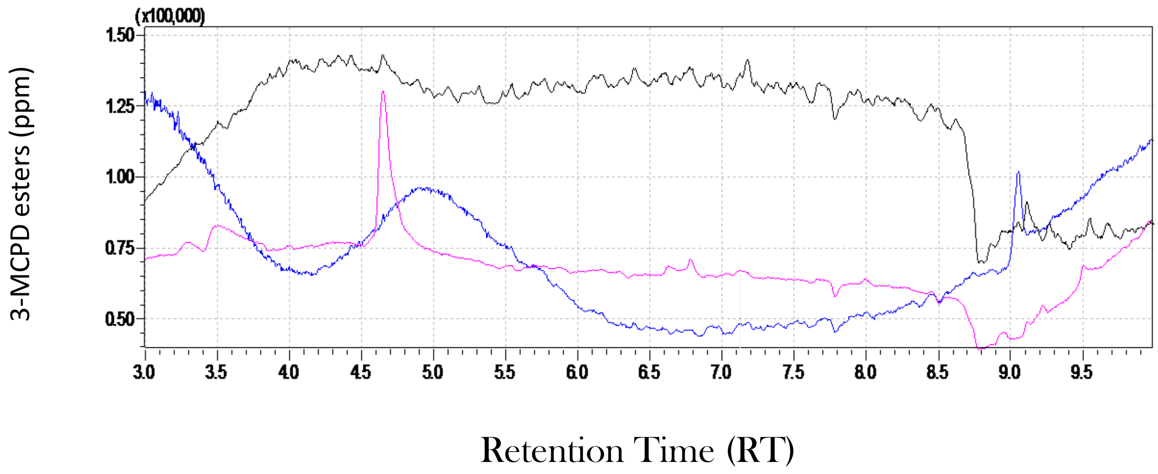


Figure 32: Sample 23 Chromatogram of 3-MCPD esters

Figure 33 shows sample 24 chromatograms of traditional bread which is collected from Khartoum Bahri(Halfayaa), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time7.151 in minute, molecular weight 147, area 5966, and concentration of 3-MCPD esters in the sample 0.23652 ppm.

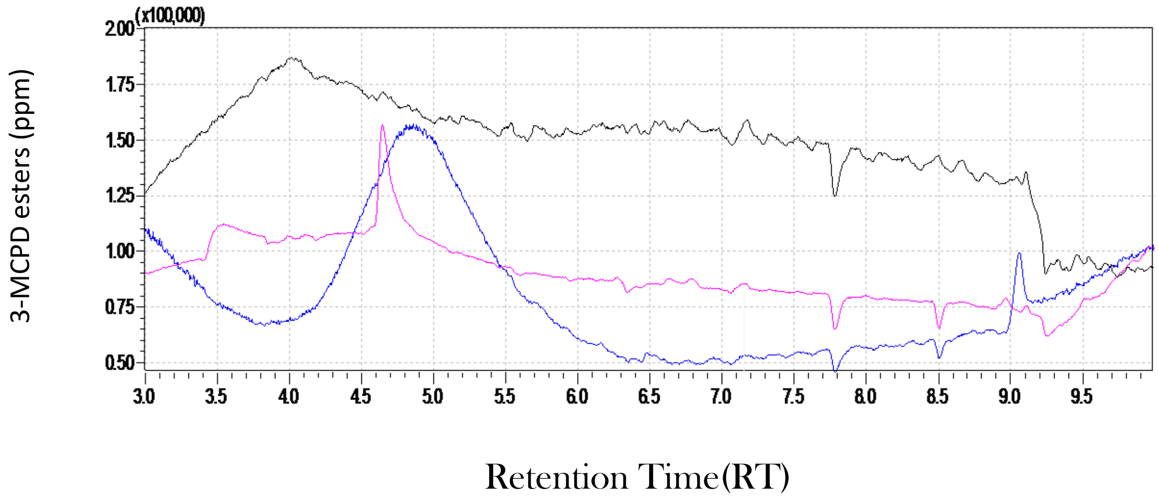


Figure 33: Sample 24 Chromatogram of 3-MCPD esters

Figure 34 shows sample25 chromatograms of traditional bread which is collected from Khartoum Bahri(Alsafiaa), y-axis represents concentration of 3-MCPD esters, while x-axis shows the retention time7.154 in minute, molecular weight 147, area 8049, and concentration of 3-MCPD esters in the sample 0.31910ppm.

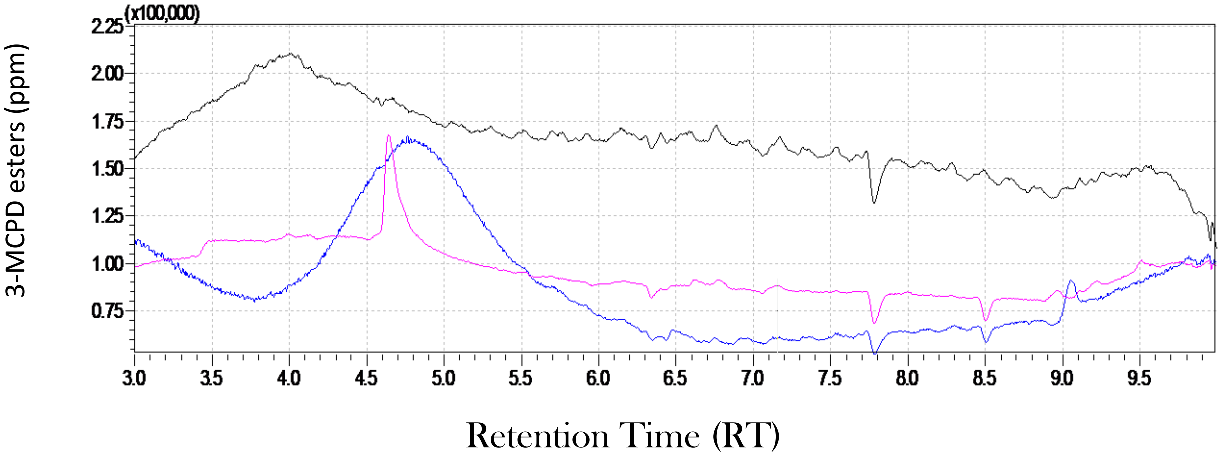


Figure 34: Sample 25 Chromatogram of 3-MCPDesters

4.4 Discussion

Determination of 3-MCPD (3-monochloropropane-1,2-diol) concentrations in traditional breads showed that, the highest levels of 3-MCPD were found in some bread samples, and this may refer to the baking processing conditions such as type of oil used, NaCl addition, duration of temperature exposure, time ,length, width, as well as acidity of the dough (Baer et al , 2010). The highest and the lower concentration of 3-MCPD in samples ranged from 0.15 – to 18.27 ppm .The concentrations of 3-MCPD increase with increase in temperature in all bread samples, this indicate that the requisite precursors were present in relative excess, such as chlorine ion, phospholipids, and glycerols.

In comparison these value with other 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 ranged from 1.56-23.6 mg/kg which is higher than the concentration of 3-MCPD in traditional bread which ranged from 0.15-18.27 ppm.Other studies of Kertisova et al. (2009), was carried out for concentration of bound 3-MCPD esters in bread crumb were 1.56- 21.13 mg of fat and concentration of 3-MCPD esters in bread crust were 2.82-3.60 mg/kg of fat. However, concentration of 3-MCPD in traditional bread samples ranged from 0.15 to 18.27 ppm respectively.

Table 4: Shows Derivatization, Concentrations, Retention Time, and areas of 3-MCPD in traditional Bread Samples

ID	Name No	Retention Time	m/z	Area	3-Monochloropropane-1,2-diol(3-MCPD) Derivative Concentrations (ppm)
1	Sample 1	7.182	147.00	261209	10.35539
2	Sample 2	7.189	147.00	76399	3.02877
3	Sample 3	7.161	147.00	114525	4.54024
4	Sample 4	7.160	147.00	460931	18.27318
5	Sample 5	7.158	147.00	25530	1.01211
6	Sample 6	7.151	147.00	269461	10.68253
7	Sample 7	7.191	147.00	28305	1.12213
8	Sample 8	7.166	147.00	21415	0.84898
9	Sample 9	7.204	147.00	32976	1.30730
10	Sample 10	7.183	147.00	7869	0.31196
11	Sample 11	7.186	147.00	3862	0.15311
12	Sample 12	7.185	147.00	6475	0.25670
13	Sample 13	7.180	147.00	5995	0.23767
14	Sample 14	7.150	147.00	7031	0.27874
15	Sample 15	7.177	147.00	6503	0.25781
16	Sample 16	7.183	147.00	7674	0.30423
17	Sample 17	7.177	147.00	4386	0.17388
18	Sample 18	7.138	147.00	11213	0.44453
19	Sample 19	7.177	147.00	6463	0.25622
20	Sample 20	7.160	147.00	3283	0.13015
21	Sample 21	7.152	147.00	8100	0.32112
22	Sample 22	7.120	147.00	23295	0.92351
23	Sample 23	7.124	147.00	8913	0.35335
24	Sample24	7.151	147.00	5966	0.23652
25	Sample 25	7.154	147.00	8049	0.31910

4.5 Estimation of Dietary Exposure of 3-MCPD in Traditional bread for Children and Adults

The findings show that there is no much difference among both groups. However, the exposure estimate fluctuating from one group to another and this may be due to the amount of bread consumption, the ingredients therein, body weight, age, as well as the oil used in bread baking may be the fully refined or deodorized one. In the course of repeat exposure to this process contaminants, it could be possible health risk for both groups. It is obviously shown that, from the findings of the analysis, there is a gradual variation of 3-MCPD levels in these samples with the exception of sample (1) and (4) which show a little bit high level of the contaminant of 3-MCPD, and this may refer to the baking processing conditions such as duration of temperature exposure, length and width of bread, dough acidity (PH), mixture of bread flour with bran or Dura. Table 4 shows, the exposure of children to the 3-MCPD and there were different levels of contaminant, the reasons for this, could be the type of oil present in the traditional bread samples, different in the amount of breads consumption, dough acidity, length of, duration of bread exposure to high temperature, and low p^H. In the children's group, the mean exposure was 0.06843 µg/Kg body weight/day of the TDI (set at 2 µg/Kg body weight/day).

Table 5: Estimation of Dietary Exposure (traditional bread) to 3-MCPD for Sudan children with average Age of 14 - 15 (years) and Body weight 47.67 Kg

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

Mean Exposure: 0.06843

Table 5 below shows adults exposure to 3-MCPD, also there were clear differences in the concentrations level of the compound, and this may be due to the amount of bread consumption, baking processing conditions, such as sodium chloride addition, duration of bread spent in the oven.
It was estimated that, the mean daily adult exposure to 3-MCPD is 0.08128 µg/Kg body weight/day of the TDI.

Table 6: Estimation of Dietary Exposure to 3-MCPD (traditional bread) for Sudan Adults with average Age of 18 - 20 (years) and Body weight of 68.03 Kg

Sample No	Age Class(Years)	Body wt(Kg)	Exposure to 3-MCPD(**µg/Kg**)
1	18 - 20	68.03	0.35725
2	18 – 20	68.03	0.10443
3	18 – 20	68.03	0.1566
4	18 – 20	68.03	0.63041
5	18 – 20	68.03	0.03487
6	18 – 20	68.03	0.36852
7	18 – 20	68.03	0.0387
8	18 – 20	68.03	0.02924
9	18 – 20	68.03	0.0451
10	18 – 20	68.03	0.10758
11	18 – 20	68.03	0.00526
12	18 – 20	68.03	0.00879
13	18 – 20	68.03	0.00812
14	18 – 20	68.03	0.00955
15	18 – 20	68.03	0.00889
16	18 – 20	68.03	0.01041
17	18 – 20	68.03	0.00592
18	18 – 20	68.03	0.1529
19	18 – 20	68.03	0.00879
20	18 – 20	68.03	0.0044
21	18 – 20	68.03	0.01099
22	18 – 20	68.03	0.03181
23	18 – 20	68.03	0.1213
24	18 – 20	68.03	0.00812
25	18 - 20	68.03	0.01099

Mean Exposure: 0.08128

In comparison with international standards studies of 3-MCPD by European commission scientific committee for food , established regulations of tolerable daily intake of (0.1 – 2 µg/kg body weight per day), while concentrations of 3-MCPD in traditional bread samples range from (0.15 – 18.27 ppm) ,(0.00015 – 0.01827 µg/kg body weight per day). In comparison with Karsuinova et al. (2007), Doleza et al. (2005), Divinnova et al.(2007), and Zelinkova et al. (2008-2009) whom carried out the concentrations of 3-MCPD in bread ranged between (1.56 – 23.6 mg/kg of fat which is higher than the concentration of 3-MCPD in traditional bread. JECFA, (2007) was estimated the average dietary exposure of the general population from a wide range of foods, related products which ranged from 0.06 – 2.3 µg/kg body weight per day. While concentration of 3-MCPD in traditional bread for children ranged between (0.00063 – 0.5398 µg/kg body weight per day). While Hwang et al.(200), was carried out for mean intake level of 3-MCPDin the republic of Korea was estimated to range from 0.0009 – 0.0026 µg/kg body weight per day and at the 95^{th} percentile of consumption was 0.005µg/kg bwt per day. And You et al. (2008) secondary school student in China, Hong Kong special administrative region, have been published since that time, the average exposure was estimated to be 0.063 – 0.150 µg/kg bwt per day. While that for high consumers was 0.152 – 0.300 µg/kg bwt per day. Average MCPD exposure for children from traditional bread were 0.00063 – 0.598 µg/kg, and for adults was 0.0044 – 0.368552 µg/kg.

CHAPTER FIVE

Conclusion

It could be concluded that traditional breads processing leads to the formation of 3-MCPD, however, its level in traditional bread does not constitute serious health risk.

Reduction of temperature in baking processing of breads is desirable in order to have low content of 3-MCPD.

Recommendations

The following measures of mitigation can significantly decrease the content of 3-MCPD in traditional breads.

- Reduction of temperature in baking processing of breads is desirable in order to have low content of 3-MCPD.
- Dough acidity and addition of sodium chloride should be minimized.
- Avoiding the use of refining oil in traditional bread baking.
- Periodic interval study is advisable to monitor and control of 3-MCPD levels.

REFERENCES

AOAC (1990): Association of Official Analitical Chemist. Official Methods for analysis (15^{th} . ed) Washington D.C, USA.

Baer, I., de la Calle, B., Taylor, P.,(2010) 3-MCPD in food other than soy sauce or hydrolysed vegetable protein (HVP). Anal. Bioanal.Chem.396,443-456.

Barocelli E, Corradi A, Mutti A and Petronini PG, 2011. Comparison between 3-MCPD and its Palmitic esters in a 90-days toxicological study. Scientific report CFP/EFSA/CONTAM/2099/01. Available online http://www.efsa.europa.eu.en/supporting/pub/187e.htm.

Breitling-Utzmann CM, Kober H, Herbolzheimer D et al (2003). 3-MCPD-Occurrence in bread crust and various food groups as well as formation in toast.} DeutlebensmRundsch, 99:280-285.

Craft, B.D.; Nagy, K.; Sandoz, L.; Destaillats,F.(2012a), Factors Impacting the formation of Monochloropropanediol(MCPD) Fatty Acid Diesters during palm(Elaeisguineensis) Oil production. *Food Addit. Contam.*,29,354-361.

Crews C, Brereton P, Davies A(2001). The effects of domestic cooking on the levels of 3-monochloropropanediol in food. Food additive Contaminants, 18: 271-280.

Crews C, Hough,P, Brereton, p, D, MacArthur, Rand Mathews,W. 2002. Survey of 3-monochloropropane-1,2-diol(3-MCPD) in selected food groups, 1999-2000. Food Additives and contaminants, 19: 22-27.

Crews C., Hasnip S., Chapman S., Hough P., Ptter N., Todd J., Brereton P., Mathews W. (2003): Survey of chloropropanols in soy sauces and related products purchased in the UK in 2000, and 2002 Food Addit. Contamin., 20: 916-922.

Davidek J, Velisek J, Kubelka V, Janicek G. 1982. New chlorine. Containing compounds in protein hydrolysates. In: Baltes W, Czedik-Eysenberg PB, Pfannhauser W, editors. Recent developments in food analysis, Proceeding of Euro food chem I. Vienna, Austria, 17-20 February 1981. Deerfield Beach, FL: Weinheim. P. 322-325.

Destaillats F, Craft B D, Dubois M, Nagy K. (2012). Glycidyl Esters in Refined Palm(Elaeis guineensis) Oil and Related Fractions. Part I: Formation Mechanis-Food Chem.131,1391-1398.

Destaillats, F.; Craft, B.; Sandoz, L.; Nagy, K. 2012a. Formation Mechanisms of Monochloropropanediol(MCPD) Fatty Acid Diesters in refined Palm(Elaeisguineensis) Oil and Related Fractions. Food Addit. Contam.,29,29-37.

Diks, R. 2010. The Effect of Oil Refining on Contaminant Removal and Secondary Product Formation. Presented at 3rd Leipzig Sumposium, processing: Quality and Safety, Leipzig, Germany, march 17-18, 2010.

Divinova V, SvejkovskaB,NovotnyO,Velisek J. 2004. Survey of 3-chloropropane-1,2-diol and its precursors in Foods in the Czech Repoblic. Czech J Foods Sci.22: 267-271.

Divinova, V.; Svejkovska, B.; Dolezal, M.; Velisek,J. 2004. Determination of Free and Bound 3-monochloropropane-1,2-diol by Gas Chromatography with Mass Spectrometric Detection Using Deuterated 3-Chloropropane-1,2-diol as Internal Standard. Czech J. Food Sci.,22,182-189.

Dolezal M, Chaloupska M, Divinova V, Svejkovska B, Velisek J. 2005. Occurrence of 3-chloropropane-1,2-diol and it's esters in coffee. Eur Food Res Tech. 221:221-225.

EFSA (2013). Analysis of occurrence of 3-monochloropropane-1,2-diol (3-MCPD) in food in Europe in the years 2009-2011 and preliminary exposure assessment. EFSA Journal 11(9):3381, 45 pp. Available online: www.efsa.europa.eu/efsajournal.

Ermacora, A., Hrncirik, K. 2012a. Evaluation of an Improved Indirect method for the Analysis of 3-MCPD Esters Based on Acid Trans esterification,J.Am. Oil Chem.Soc.89,211-217.

Ermcaora, A.; Hrncirik, K. 3-MCPD Esters Analysis: September 19,2011. An Optimized Method Based on Acid Trans esterification. Paper presented at the 7th Meeting of the AOCS Expert Panel on Process Contaminants, Rotterdam, The Netherlands,http://www.aocs.org/files/Resources PDF/notes%207th%20 expert%20 panel.pdf(accessed on December 31,2013).

Food Consumption Survey-Young children 2005/2006. Reportnr: 350070001. National Institute for Public Health and the Environment (RIVM), Bilthoven. Available online: www.rivm.nl.

Franke K Strijowski, U, (2011). Final Report: Investigation in the Formation of 3- Monochloropropane-1,2-diol Fatty Acid Esters (3-MCPDesters) in Vegetable Oils and development of Minimization Strategies. Research Association of the German Food Industry Bonn, Germany.

Franke, K.; Strijowski, U; Fleck, G.; pudel, F. 2009. Influence of Chemical Refining process and Oil Type on Bound 3-chloro-1,2-propanediol contents in Palm Oil and Rapeseed Oil. LWT-Food Sci. Technol. 42,1751-1754.

Fry H, Schodel C, These A, Preiss-Weigert A. 2013. Collaborative study for the determination of 3-MCPD and 2-MCPD-fatty esters in fat containing foods. First collaborative study-part II. Method validation and proficiency test [Internet]. Berlin: BfR-Wissenschaft 04/2013;[cited 2013 July 08]. Available from:

Gibon, V.; DeGreyt, W.; Kellens, M. 2007. Palm Oil Refining .Eur. J. Lipid Sci. Technol. 109,315-335.

Goh,E.M.; Timms,R.E. 1985. Determination of Mono-and Diglycerides in Palm Oil, Olein and Stearin,J-Amer. Oil Chem. Soc.62,730-734.

Hamlet CG (2009). Chloropropanols and their fatty acid Esters. In Bioactive compounds in foods. Gilbert J & Senyuva HZ, editors. Oxford, UK: Blackwell Publishing Ltd., pp. 323-357.

Hamlet CG and Sadd PA, 2004. Chloropropanols and their esters in cereal products. Czech Journal of Food Science, 22, 259-262.

Hamlet CG, Sadd PA (2009). Chloropropanols and chloroesters. In: process-induced food toxicants: Occurance, Formation, Mitigation and health risks. Stadler RH& Lineback DR, editors. Hoboken, NJ:Wiley, pp. 175-214.

Hamlet CG, Sadd PA, Crews C, (2002a).Occurrence of 3-chloro-propane-1 compounds in foods: 2-diol(3-MCPD) and related compounds in foods : a review. Food additcontam, 19:619-631 doi : 10.1080/02652030210132391 PMID: 12113657.

Hamlet CG, Sadd PA, Gray DA. 2004a. Generation of monochloropropanediols (MCPDs) in model dough systems. Unleavened doughs. J Agri Food Chem. 52: 2067-2072.

Hamlet CG, Sadd PA,Crews C Velisek J, Baxter DE. 2002. Occurrence of 3-chloropropane-1,2-diol (3-MCPD) and related compounds in foods: a review. Food Contam. 19:619-631.

HAMLET, G C; ASUNCION L VELISEK; DOOLEZAL, M; ZELINKOVA and CREWS, C (2011). Formation and Occurrence of esters of 3-Monochloropropane-1,2-diol(3-MCPD) in foods: What we know and what we assume. Eur. J . Lipid Sci. Technol. , 113:279-303.

Hrncirik K, Zelinkova Z, Ermacora A. 2011. Critical factors if indirect determination of 3-chloropropane-1,2-diol esters. Eur J lipid Sci Techno.113: 361-367.

HRNCIRIK, K and ERMACORA , A (2010). Formation of 3-MCPD esters in vegetable oils: Effect of partial acylglycerols. 8th Euro Fed lipid Congress. 21-24 November 2010.

HRNCIRIK, K(2009). Investigation of the mechanism of the formation of 3-MCPD esters during oil refining. 7th Euro Fed lipid Congress. 18-21 October 2009. Graz, Austria.

http://www.bfr.bund.de/cm/350/collaborative-study-for-the-determination-of-3-mcpd-and-2-mcpd-fatty-acid-esters-in-fat-containing-foods.pdf.

JECFA(2002). 3-Chloro-1,2-propanediol (addendum). Safety evaluation of certain food additives and contaminant. Prepared by sixty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives(JECFA),Geneva WHO Food Addit Ser, 58: 239-267.

K. Hrncirik, Z. Zelinkova, A. Ermacora, Euro. J. 2011.Lipid. Sci. tech. 113-361.

Karsulinova L, Folprechtova B, Dolezal M et al.(2007).Analysis of the lipidfractions of coffee, creamers, cream aerosols, and bouillon cubes for their health risk associated constituents. Czech J Food Sci, 25:257-264.

Kuhlmann, J.(2011a) Determination of Bound 2,3-Expoxy-1 propanol(glycidol) and Bound Monochloropropandiol(MCPD) in Refined Oils. Eur.J. Lipid. Technol.113,335-344.

Kusters M, BimberU,Reeser S, Gallitzendorfer R, Gerhartz M. 2011. Simultaneous determination and differentiations of glycidyl esters and 3-monchloropropane-1,2-diol(3-MCPD) esters in different foodstuffs by GC-MS. J Agric Food Chem. 59: 6263-6270.

Kusters, M.; Bumber, U.; Ossenbruggen, A.; Reeser, S.; Gallitzendorfer, R.; Gerhartz, M.2010 Rapid and simple micromethod for the simultaneous determination of 3-MCPDand 3-MCPD esters in different foodstuffs. J. Agric. Food Chem. 58, 6570-6577.

Long,K.; Jamari,M.A.; Ishak,A.; Yeok,L.J.; Lati F,R.A.; Ahmadilfitiri,A.; Lai,O.M. 2005.Physico-Chemical Properties of palm Olein Fractions as a Function of Diglyceride Content in the Starting Material.Eur.J Lipid Sci. Tech 107,754-761.

Masukawa Y, Shiro H, Nakamura S, Kondo N, Jin N, Suzuki N, Ooi N, Kudo N (2010) A new analytical method for the quantification of glycidol fatty acid esters in edible oils. J Oleo Sci 59:81–88

Matthaus, B.; Pudel, F.; Fehling, P.; Vosmann, K.; Freudenstein, A.2011a. Strategies For Reduction of 3-MCPD Esters and Related Compounds in Vegetable Oils. Eur. J. Lipid Sci. technol. 113, 380-386.

Muller RE, Boder CD, Slaiding IR et al., (2005). Modeling the formation of heat generated toxins during the processing of malt. ProcCongrEur Brew Conv, 30th : 167-1-167/12.

Nagy, K.; Sandoz, L.; Craft,B.D.; Detaillats,F. 2011. Mass-Defect Filtering of Isotope Signatures to Reveal the Source of Chlorinated Palm Oil Contaminants. *Food AdditContam*. 28,1492-1500.

Ocké MC, Hulshof KFAM, van Rassum CTM, Fransen HP, Buurma EJM, de Boer EJ, Brants HAM, Nieker KEM, van der Loan JD, Drijvers JJMM, Ghameshlou Z (2008). Dutch National

Pudel, F.; Benecke, P.; Fehling, P.; Freudenstein, A.; Matthäus, B.; Schwaf, A. 2011 On the Necessity of Edible Oil Refining and Possible Sources of 3-MCPD and Glycidyl Esters. Eur. J. Lipid Sci. Technol. 113, 368–373.

Rahn AKK &YaylayanVA(2010). What do we know about the molecular mechanism of 3-MCPD esters formation? Eur J Lipid SciTechnol, n/a.

S. Mezouaria, L. Yun,G.Paceb. 2015. Food Addit. Contam.: Part A. 32, 768.

Schilter B, Scholz G, Seefeder W (2010). Fatty acid esters of chloropropanols and related compounds in food: toxicological aspects Eur J Lipid SciTechnol, n/a

Seefelder, W., Varga, N., Studer, A., Williamson, G., Scanlan, F . P., &Stadler, R. H .(2008). Esters of 3-chloro-1,2-propanediol(3-MCPD) in Vegetable Oils: Significance in the formation of 3-MCPD. Food Additives and Contaminants, 25, 391-400.

Siew,W.L; Ng W.L.1995.Diglyceride Content and Composition as indicators of palm OilQuality.J.Sci. Food Agric. 69,73-79.

Stadler, R H (2009). Food processing contaminant- Progress and Challenges in Mitigation Strategies, Food Research in support to Science based Regulations: Challenges for Producers and Consumers. 21-22 April 2009. Prague Congress Centre, Czech Republic.

Sunahara G, perrin I and marchesini M. 1993. Carcinogenity study on 3-monochloropropane-1,2-diol(3-MCPD) administered in drinking water to Fisher 344 rats. Unpublished report No RE SR93003 submitted to WHO by Nestecltd,Research& development, Swizerland(cited in JECFA (2002).

Svejkovska, B Novoty,O ,Divinova, V, Reblova,Z, Dolezal,M and Velisek,J. 2004. Esters of 3-chloropropane-1,2-diol in foodstuffs, Czech journal of food science, 22: 190-196.

T. Haines, K. Adlaf, R. Pierceall, I. Lee, P.Venkitasubramanian, M. Collison, Am. 2011. Oil. Chem. Soc. 88, 1-14.

Van Rossum CTM, Fransen HP, Verkaik-Kloosterman J, Buuma-Rethans EJM, Ocke MC (2011). Dutch national Food Consumption Survey 2007-2010. Diet of children and adults aged 7 to 69 years. Reportnr: 350050006. National Institute for Public Health and the Environment (RIVM), Bilthoven. Available online: www.rivm.nl.

Vannort RW, Thomson BM. (2005) 2013/04 New Zealanda total diet survey. Agricultural compound,selected contaminants and nutrient. Wellington: New Zealand Food Safety Authority. MAF Technical Paper No: 2011/19.

Velisek J, Davídek J, Hajslová J, Kubelka V, Janíček G and Mánková B, 1978. Chlorohydrins in protein hydrolysates. ZeitungLebensmittelUntersForschung, 167, 241-244.

WeiBhaar R & Perz R (2010). Fatty acid esters of glycidol in refined fats and oils. Eur J Lipid Sci Technol, 112: 158-165. Doi : 10. 100/ejLt. 200900137.

WeiBhaar R. 2008. Determination of Total 3-Chloropropane-1,2-diol(3-MCPD) in Edible Oils by Cleavage of MCPD Esters with Sodium Methoxide. Eur.J.Lipid Sci. Technol. 110,183-186.

Weisshaar R,2011 fatty acid esters of 3-MCPD:Overview of occurrence and exposure estimates: Europe Journal of lipid science and technology, 113,304-308.

Wenzl T, Karasek I. (2013): Do Results for Glycidyl Esters Content Obtained by Different Analysis Methods Agree? Paper presented at the 104th AOAC Annual meeting and expo,Montereal, Canada.

Wenzl T, Lachenmeier DW, GokmenV(2007). Analysis of heat-induced contaminants(acrylamide, chloropropanols and furan) in carbohydrate-rich food. Anal BioanalChem, 389 : 119-137. Doi: 10.1007/s00216-007-1459-9 PMID:17673989

WHO, (2018). Information and data for above growth charts sourced, Gerontology Research Center (National Institutes of Health, USA), and the U.S. Center for Disease Control and Prevention (CDC).

Wohrlin, F.; Fry,H.; Preiss-Weigert, A.2011. Collaborative Study for the Determination of 3-MCPD-fatty Acid Esters in Edible Fats and Oils. Second Collaborative Study-Part I. Method Validation and Proficiency Test. Rundesinstitute Fur Risikobbewertung(BfR),Berlin(BfR-Wissenschat 04/2012). http://www.bfr.bund.de/cm/350/Collaborative-Study-fot-the-determination-of-3-mcpd-fatty-acid-esters-in-edible-fats-and-oils.pdf(accessed December 27, 2013.

Yamazaki, S. Isagawa, T. Urushiyama, T. Vkena, N. kibune. 2013. Food. Addit. Contam. :Part A. 30,52.

Zelinkova Z, Novotny O, Schurek J et al., (2008). Occurrence of 3-MCPD fatty acid esters in human breast milk. Food AdditContam Part A Chem Anal Control Expo Risk Assess, 25: 669-676. PMID : 18484295.

Zelinkova Z, Svejkovska B, Velisek J, Dolezal M. 2006. Fatty acids esters of 3-chloropropane-1,2-diol in edible oils. Food Add Contam. 23:1290-1298.

APPENDICES

Appendix 1: Determination of Moisture Content of Traditional Bread (*Omdurman, Khartoum Bahri, Khartoum)*

Samples No	Area	W1(g)	W2(g)	% M.C	Mean M.C
1	Omdurman	71.7	46.3	35.42	29.98
2		81.4	57.8	29.0	
3		73.0	46.8	35.89	
4		68.0	44.3	34.85	
5		70.2	46.3	34.05	
6		68.6	44.3	35.42	
7		64.9	43.7	32.67	
8		66.8	44.7	33.08	
9		71.5	47.0	34.27	
10	Khartoum	66.7	43.5	34.78	33.89
11		67.9	44.5	34.47	
12		75.0	50.8	32.27	
13		73.4	46.5	36.64	
14		68.3	44.1	35.43	
15		70.6	47.1	31.04	
16		65.7	43.8	33.33	
17		70.0	46.8	33.14	
18	Khartoum Bahri	65.1	44.1	32.26	33.87
19		73.5	46.5	36.73	
20		65.3	42.7	34.61	
21		70.5	46.2	34.47	
22		76.0	50.8	33.16	
23		69.1	44.4	35.75	
24		77.0	51.5	33.11	
25		60.3	41.7	30.85	

Appendix 2: Determination of Oil Content in Traditional Bread From (Omdurman, Khartoum Bahri, Khartoum)

Serial No	Area	W1	W2	W3	%Oil Content(g)	Mean Oil Content (g)
1		128.5107	128.3989	20	0.56	
2	Omdurman	115.3550	115.2026	20	0.76	
3		128.3767	128.2436	20	0.67	
4		128.2867	128.1814	20	0.53	
5		115.3440	115.2561	20	0.44	0.52
6		128.4017	128.3181	20	0.42	
7		128.3315	128.2461	20	0.43	
8		115.4012	115.3267	20	0.37	
9		128.4133	128.3121	20	0.51	
10		128.6127	128.4889	20	0.62	
11	Khartoum	118.3450	118.2630	20	0.41	
12		127.4617	127.2780	20	0.92	
13		128.5933	128.4111	20	0.91	
14		118.3155	118.2012	20	0.57	0.64
15		127.4434	127.3005	20	0.71	
16		128.6012	128.5051	20	0.48	
17		118.4021	118.2918	20	0.55	
18		127.5032	127.3912	20	0.56	
19		129.7812	129.5964	20	0.92	
20	Khartoum	128.6534	128.5000	20	0.78	
21	Bahri	115.3528	115.2499	20	0.51	
22		128.6612	128.5123	20	0.74	0.69
23		115.3611	115.2136	20	0.74	
24		129.6125	129.4919	20	0.60	
25		128.6356	128.5157	20	0.59	

Appendix 3: Estimation of Dietary Exposure (traditional bread) to 3-MCPD for Sudan children with average Age of 14 - 15 (years) and Body weight 47.67 Kg

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

Mean Exposure: 0.06843

Appendix 4: Shows Derivatization, Concentrations, Retention Time, and areas of 3-MCPD in traditional Bread Samples

ID	**Name No**	**Retention Time**	**m/z**	**Area**	**3-Monochloropropane-1,2-diol(3-MCPD) Derivative Concentrations (ppm)**
1	Sample 1	7.182	147.00	261209	10.35539
2	Sample 2	7.189	147.00	76399	3.02877
3	Sample 3	7.161	147.00	114525	4.54024
4	Sample 4	7.160	147.00	460931	18.27318
5	Sample 5	7.158	147.00	25530	1.01211
6	Sample 6	7.151	147.00	269461	10.68253
7	Sample 7	7.191	147.00	28305	1.12213
8	Sample 8	7.166	147.00	21415	0.84898
9	Sample 9	7.204	147.00	32976	1.30730
10	Sample 10	7.183	147.00	7869	0.31196
11	Sample 11	7.186	147.00	3862	0.15311
12	Sample 12	7.185	147.00	6475	0.25670
13	Sample 13	7.180	147.00	5995	0.23767
14	Sample 14	7.150	147.00	7031	0.27874
15	Sample 15	7.177	147.00	6503	0.25781
16	Sample 16	7.183	147.00	7674	0.30423
17	Sample 17	7.177	147.00	4386	0.17388
18	Sample 18	7.138	147.00	11213	0.44453
19	Sample 19	7.177	147.00	6463	0.25622
20	Sample 20	7.160	147.00	3283	0.13015
21	Sample 21	7.152	147.00	8100	0.32112
22	Sample 22	7.120	147.00	23295	0.92351
23	Sample 23	7.124	147.00	8913	0.35335
24	Sample24	7.151	147.00	5966	0.23652
25	Sample 25	7.154	147.00	8049	0.31910

Appendix 5: Estimation of Dietary Exposure to 3-MCPD (traditional bread) for Sudan Adults with average Age of 18 - 20 (years) and Body weight of 68.03 Kg

Sample No	Age Class(Years)	Body wt(Kg)	Exposure to 3-MCPD(**µg/Kg**)
1	18 - 20	68.03	0.35725
2	18 – 20	68.03	0.10443
3	18 – 20	68.03	0.1566
4	18 – 20	68.03	0.63041
5	18 – 20	68.03	0.03487
6	18 – 20	68.03	0.36852
7	18 – 20	68.03	0.0387
8	18 – 20	68.03	0.02924
9	18 – 20	68.03	0.0451
10	18 – 20	68.03	0.10758
11	18 – 20	68.03	0.00526
12	18 – 20	68.03	0.00879
13	18 – 20	68.03	0.00812
14	18 – 20	68.03	0.00955
15	18 – 20	68.03	0.00889
16	18 – 20	68.03	0.01041
17	18 – 20	68.03	0.00592
18	18 – 20	68.03	0.1529
19	18 – 20	68.03	0.00879
20	18 – 20	68.03	0.0044
21	18 – 20	68.03	0.01099
22	18 – 20	68.03	0.03181
23	18 – 20	68.03	0.1213
24	18 – 20	68.03	0.00812
25	18 - 20	68.03	0.01099

Mean Exposure: 0.08128

Appendix 6: Determination of moisture and Oil Content, 3-MCPD Concentrations, Child and Adult Exposure to 3-MCPD in traditional Bread Oil Samples Collected From ***Omdurman ,Khartoum and Khartoum Bahri***

Samples No	Moisture Content	Oil Content	3-MCPD (ppm) Concentration	Child Exposure To 3-MCPD(µg/Kg)	Adult Exposure to 3-MCPD(µg/Kg)
1	35.42	0.56	10.35539	0.3059	0.35725
2	29.0	0.76	3.02877	0.08942	0.10443
3	35.89	0.67	4.54024	0.13409	0.1566
4	34.85	0.53	18.27318	0.5398	0.63041
5	34.05	0.44	1.01211	0.00063	0.03487
6	35.42	0.42	10.68253	0.31555	0.36852
7	32.67	0.43	1.12213	0.03313	0.0387
8	33.08	0.37	0.84898	0.02503	0.02924
9	34.27	0.51	1.30730	0.03862	0.0451
10	34.78	0.62	0.31196	0.09212	0.10758
11	34.47	0.41	0.15311	0.00456	0.00526
12	32.27	0.92	0.25670	0.00753	0.00879
13	36.64	0.91	0.23767	0.00695	0.00812
14	35.43	0.57	0.27874	0.00818	0.00955
15	31.04	0.71	0.25781	0.00761	0.00889
16	33.33	0.48	0.30423	0.00892	0.01041
17	33.14	0.55	0.17388	0.00507	0.00592
18	32.26	0.56	0.44453	0.01309	0.01529
19	36.73	0.92	0.25622	0.00753	0.00879
20	34.61	0.78	0.13015	0.00376	0.0044
21	34.47	0.51	0.32112	0.00941	0.01099
22	33.16	0.74	0.92351	0.02724	0.03182
23	35.75	0.74	0.35335	0.01039	0.01213
24	33.11	0.60	0.23652	0.00695	0.00812
25	30.85	0.59	0.31910	0.00941	0.01099

Appendix 7: Traditional Bread Collection From Khartoum state

Omdurman

Serial Number	Bread Type	Flour Type	Bakery Area	Heat Treatment(C^o)	Time in Minute	Date of Collection	Dimensions (cm)		Color
							Length	Width	
1	Traditional	Wheat+Bran	Alsabeel	315	8	23/04/2018	12.8	3.1	Brown
2	Traditional	Wheat+Bran	Sawraa 18	308	7.3	23/04/2018	13.9	2.6	Brown
3	Traditional	Wheat+Bran	Dar-Elsalam	310	7.5	23/04/2018	13.1	3.6	Brown
4	Traditional	Wheat+Dura	Jikhass	320	8.5	28/04/2018	12.4	2.9	Brown
5	Traditional	Wheat+Dura	Safwaa 4	303	7.1	28/04/2018	12.7	3.1	Brown
6	Traditional	Wheat+Dura	Banatt	317	8.1	28/04/2018	13.1	2.8	Brown
7	Traditional	Wheat	Almuhandsin	309	7.5	04/05/2018	12.9	3.4	Brown
8	Traditional	Wheat	Alsafia	306	7.2	04/05/2018	12.1	3.8	Brown
9	Traditional	Wheat	Ummbada 34	310	8.1	04/05/2018	13.5	2.9	Brown

Khartoum

Serial Number	Bread Type	Flour Type	Bakery Area	Heat Treatment(C^0)	Time in Minute	Date of Collection	Dimensions (cm)		Color
							Length	Width	
10	Traditional	Wheat	Kalakla	300	7.1	13/05/2018	12.7	2.7	Brown
11	Traditional	Wheat+Dura	Alshajara	299	6.9	13/05/2018	13.0	3.4	Brown
12	Traditional	Wheat	Jabra	301	7.4	13/05/2018	13.2	3.1	Brown
13	Traditional	Wheat	Mayo	298	7.9	13/05/2018	12.5	2.9	Brown
14	Traditional	Wheat+Bran	Lamab	297	7.8	13/05/2018	12.8	3.3	Brown
15	Traditional	Wheat+Bran	Jiraif	299	7.5	15/05/2018	13.1	3.4	Brown
16	Traditional	Wheat+Bran	Remailla	289	7.9	15/05/2018	13.8	2.5	Brown
17	Traditional	Wheat+Bran	Algoss 5	300	7.4	15/05/2018	12.1	2.9	Brown

Khartoum Bahri

Serial Number	Bread Type	Flour Type	Bakery Area	Heat Treatment(C^0)	Time in Minute	Date of Collection	Dimensions (cm)		Color
							Length	Width	
18	Traditional	Wheat	Samrab	296	7.6	19/05/2018	13	3.1	Brown
19	Traditional	Wheat	Hajyussif	300	7.9	21/05/2018	12.8	3.6	Brown
20	Traditional	Wheat	SaadGuishra	298	7.4	21/05/2018	12.5	2.2	Brown
21	Traditional	Wheat+Bran	Shambat	298	7.6	21/05/2018	13.1	2.7	Brown
22	Traditional	Wheat+Bran	Shuhadda	301	8	21/05/2018	12.4	2.9	Brown
23	Traditional	Wheat+Bran	Shigaillab	298	7.3	23/05/2018	12.8	2.8	Brown
24	Traditional	Wheat+Dura	Halfayaa	291	7.7	23/05/2018	13.5	2.4	Brown
25	Traditional	Wheat+Dura	Alsafiaa	299	7.9	23/05/2018	13.1	2.9	Brown