CHAPTER I: INTRODUCTION

A Dichlorodiphenyltrichloroethane, commonly known as DDTis a colorless, tasteless, and almost odorless crystalline chemical compound, an organo-chlorine. It was originally developed as an insecticide, and then it became infamous for its environmental impacts. DDT was first synthesized in 1874 by the Austrian chemist OthmerZeidler^[11]

DDT is synthetic organic compound used as an insecticide. Like other chlorinated aromatic hydrocarbons, DDT tends to persist in the environment and become concentrated in animals at the head of the food chain. Its use is now banned in many countries. DDT was prepared by the reaction of chloral with chlorobenzene in the presence of sulfuric acid. Its insecticidal properties were discovered in 1939 by a Swiss chemist, Paul Hermann Muller.^[12]

Today DDT is manufactured in North Korea, India and China. India remains the largest consumer of the product for vector control and agricultural use. Human health effects from DDT at low environmental doses are unknown. Human symptoms can include vomiting, tremors or shakiness, and seizures are the exposure to high doses. Laboratory animal studies showed effects on the liver and reproduction. DDT is considered a possible human carcinogen. Studies show a range of human health effects linked to DDT and its breakdown product, DDE: breast and other cancers, male infertility miscarriages and low birth weight.^[13]

By October 1945, DDT was available for public sale in the United States. Although it was promoted by government and industry for use as an agricultural and household pesticide, DDT is banned for agricultural use in India, however, it continues to be used for fumigation against mosquitoes in several places in India, including Hyderabad. A partial ban on DDT was introduced in 2008 wherein it could not be used for agricultural purposes.^[11]

DDT had been shown to cause cancer and that their agricultural use was a threat to wildlife, and birds. The DDT metabolite DDE (Dichlorodiphenyldichloroethylene) was the most prevalent, occurring in 23 of the 31 foods sampled. People consume more DDT than any other persistent organic pollutant, the researchers found. Its relative abundance in food today is due to its widespread historical use. ^[14]

In general, to higher the food's fat content, the more DDT it contained. Peanut butter, ice cream, cheese, butter, oil, fish and high-fat meats were all more contaminated than low-fat milk and vegetables. Most DDT exposure is through consuming contaminated food that contains small amounts. DDT is not absorbed through the skin or lungs easily. When DDT enters the body, it tends to be stored in the fatty tissues and is excreted from the body overtime.^[15]

In human health DDT is an Endocrine disruptor. Endocrine disruptors are chemicals that can interfere with endocrine systems at certain doses. These disruptions can cause cancerous tumors, birth defects, and other developmental disorders. Anysystem in the body controlled by hormones can be derailed by hormone disruptors. Specifically, endocrine disruptors may be associated with the development of learning disabilities, severe attention deficit disorder, cognitive and brain development problems; deformations of the body; breast cancer, prostate cancer, thyroid and other cancers; sexual development problems such as feminizing of males or masculinizing effects on females, etc. ^[16]

DDT was initially used by the military in World War II to control malaria, typhus, body lice and bubonic plague. DDT was also used in buildings for pest control. The reason why DDT was so widely used was because it is effective, relatively inexpensive to manufacture, and lasts a long time in the environment. While low levels of DDT in food might be unavoidable, due to its high persistence and lipophilicity.^[14]

There have been studies indicating that levels of DDT in breast milk samples of Hong Kong mothers were high when compared with those obtained from mothers of other countries. Therefore, there are concerns on the public health implications of exposure to DDT in the population in Hong Kong^{. [15]}

The food group "sea food", particularly fish and oyster, was identified as the main dietary source of DDT. The target organ for acute effects is the nervous system. It has been reported that a single oral dose of mg/kg body weight DDT produced illness in some individuals and that mg/kg bodyweight or greater led to convulsions. DDT is not genotoxic. The International Agency for Research on Cancer (IARC) of WHO (world health organization) has evaluated DDT and its associated compounds. IARC considered that there is sufficient evidence in experimental animals but inadequate evidence in humans for the carcinogenicity of DDT and its associated compounds and classified them as group of agent that is possibly carcinogenic to humans.^[16]

FEHD (Food and Environmental Hygiene Department) had conducted a study on the dietary exposure to DDT of secondary school students. Results indicated that dietary exposures to DDT for average and high consumers of the secondary school students in Hong Kong were 0.000and 0.000mg/kg bodyweight/day respectively. Both exposure levels fell well below the PTDI (PT Dirgantara Indonesia) of 0.01mg/kg bodyweight established by the JMPR (Joint FAO/WHO Meeting on Pesticides Residues). It could be concluded that both the average and high consumers of the secondary school students were unlikely to experience major toxicological effects of DDT.^[15]

Moreover, the study finds that dietary exposure to DDT of secondary school students in Hong Kong is relatively high when compared with dietary exposure studies conducted in other countries such as Australia, Canada, Japan, the United Kingdom and the USA. This might explain other research findings that levels of DDT in breast milk of Hong Kong mothers are higher than the levels in other countries^[15]

The present study aims to do the Detection of DDT in Various Brands of Peanut Butter by Gas Chromatography. In past it has been found that there were no studies done related to detection of DDT in various brands of peanut butter.

<u>CHAPTER II: LITERATURE REVIEW</u>

Hari C. Agarwal et.al(1947)studied "distribution and metabolism of DDT in the catfish Heteropneustesfossilis in relation to the signs of poisoning". Adult male catfish, Heteropneustesfossilis (Bloch), were given a lethal dose of 800 mg DDT/kg as an im injection of 10% p, p'-DDT in peanut oil in the caudal region. The metabolites of DDT in various tissues were studied 24 hr after treatment. The results indicate that signs of poisoning in the catfish were directly related to the concentration of DDT in the brain and spinal cord.

Willis Mast Kaufman (1971) studied identification of some chlorinated pesticides by ultraviolet degradation. An increasing interest has been shown in the past few years in the degradation of certain pesticides by sunlight and laboratory ultraviolet (uv) light. Since exposure to uv light under controlled conditions results in the formation of characteristic degradation products, the use of laboratory irradiation for the identification of pesticides in environmental samples appeared promising. Seven chlorinated pesticide standards were used. These were heptachlor, aldrin, heptachlor epoxide, p, p'-DDE, dieldrin, p, p'-DDD and p.p'-DDT. An F&M research gas-liquid chromatograph (GLC) equipped with an electron capture (EC) detector provided the means for analysis. A post-column splitter constructed of stainless-steel tubing appeared to degrade DDT and its analogs but did not affect the other pesticides.

Yolanda Picoet.al (1994) studiedSolid phase techniques in the extraction (SPE) of pesticides and related compounds from foods and soils. The application of SPE technology to the isolation of pesticides and related compounds from food and soils has grown enormously in the last decade. Much of this growth has been due to the relative ease of sample handling and the wide range of solid supports currently available for a variety of applications. The aim of this review is to present the methods for solid phase extraction (SPE) of pesticide residues from soils and foods. There are three main areas according to the type of approach used to handle the sample: solid phase extraction, solid phase clean-up (SPC), and matrix solid phase dispersion (MSPD). This review covers milk products, fatty foods, fruits, vegetables, and soils. Solid phase materials are according to reach case in terms of reversed bonded-phase silica sorbents (C_{18} , C_8 , C_2 , etc.) and polar sorbents (alumina, Florisil, and silica).

B.N. Ames et.al (1979) studied identifying environmental chemicals causing mutations and cancer. Damage to DNA appears to be the major cause of most cancer and genetic birth defects and may contribute to aging and heart disease as well. The agents that cause this damage must identify. Many of these agents are natural chemicals present in the human diet as complex mixtures. The tens of thousands of man-made chemicals that have been introduced into the environment in the last few decades must also be tested for their ability to damage DNA. Existing animal tests and human epidemiology alone are inadequate for this task because of time, expense, and the difficulty of dealing with complex mixtures, newly developed short- term tests, most of them assaying for mutagen city, are discussed as keytools in identifying environmental mutagens and carcinogens.

Wu, Xiaorong et.al (2004) published the book on Purification, detection, and mutagenic activity of fusaproliferin. A method for purifying fusaproliferin from cultures of Fusarium sub glutinans E- 1583 by preparative HPLC was developed, which involved extracting culture materials with methanol, evaporating methanol extracts, transferring evaporated residues with small volume of methanol, partitioning the residues in methanol with large volumes of hexane, evaporating the hexane, and dissolving the residues in acetonitrile or methanol. The final acetonitrile solution with fusaproliferin was purified by HPLC with C 18 preparative column and mobile phase of CH3CN: water (80:20, v: v, at 2.5 mL/min). The identity of the purified fusaproliferin was verified by analytical HPLC, GC-MS, 'H NMR, and LC-MS, and its purity wereat least 99.86% when evaluated by analytical HPLC with the paired-sample method and LC-MS. The newly developed GC-FID method for fusaproliferin detection included four main steps: methanol extraction, C 18 cartridge cleanup, TMS-derivatization, and GC detection.

Tibor Cserháti et.al (2012) studied about Chromatographic determination of pesticides in food and food products. Where it explains about newest results in the chromatographic analysis of pesticides present in foods and food products are collected and the results are critically evaluated. Examples for the employment of preconcentration and prepurification technologies, gas chromatography using ECD, NPD, MS and MS/MS detection methods, liquid chromatographic methodologies such as thin-layer chromatography, high performance liquid chromatographic methods as well as electrically driven systems are presented. The advantages and disadvantages of the various chromatographic technologies are shortly discussed and the efficacies of the methodologies are compared. Pesticides included in the review are insecticides, herbicides, acaricides, organophosphorus and organochlorine compounds the application of the chromatographic methods for the determination of pesticides in a wide variety foods and food products is discussed in detail.

Stephen W.C et.al (2010) published a journal about"Validation and use of a fast sample preparation method and liquid chromatography-tandem mass spectrometry in analysis of ultra-trace levels of 98 organo-phosphorus pesticide and carbamate residues in a total diet study involving diversified foodtypes. And this paper is a reports of comprehensive sensitive multi-residue liquid chromatography-tandem mass spectrometry (LC–MS/MS) method for detection, identification and quantitation of 73 pesticides and their related products, a total of 98 analytes, belonging to organophosphorus pesticides (OPPs) and carbamates, in foods. The proposed method makes use of a modified QuEChERS (quick, easy, cheap, effective, rigged, and safe) procedure that combines isolation of the pesticides and sample clean-up in a single step. Analysis is performed by liquid chromatography-electrospray ionization-tandem mass spectrometry operated in the multiple reaction monitoring (MRM) mode, acquiring two specific precursor-product ion transitions per target compound. Two main fragment ions for each pesticide were obtained to achieve the identification according to the SANCO guidelines 10684/2009. The method was validated with various food samples, including edible oil, meat, egg, cheese, chocolate, coffee, rice, tree nuts, citric fruits, vegetables, etc. The method has been successfully applied to the analysis of 700 food samples in the course of a baseline monitoring study of OPPs and carbamates.

Sablework Mekonen et.al (2019) studied about Reduction of pesticide residues from teff (Eragrostistef) flour spiked with selected pesticides using household food processing steps. Where it explains that teff (Eragrostistef) is an ancient cereal that is indigenous from Ethiopia. Nowadays, teff grain is becoming popular to many parts of the world. Teff is gluten-free in nature, has high iron and fiber content, and many other health benefits make this crop interesting to many consumers. Since no insect pests are attacking the teff grains, farmers do not apply pesticides on it, unlike maize and other grains. Nevertheless, residues of organochlorine pesticides have been detected at an alarming level that could pose a consumer risk. Teff is often consumed as injera which is a fermented flat pancake. The main aim of the present study is, therefore, to investigate the effect of household food processing (doughing and baking) on the reduction of pesticide residues from teff.

Stephen W.C. Chung et.al (2015) studied about, Development of a Multiresidue Method for the Analysis of 33 Organochlorine Pesticide Residues in Fatty and High-Water Content Foods. Where it is a new multiresidue method has been developed and validated for the determination of 33 organochlorine pesticides (OCPs) in various fatty and high-water content food matrices. The OCP residues in foods were extracted with matrix solid-phase dispersion and cleaned up with gel permeation chromatography and Florisil solid-phase extraction. The instrumental determination was carried out by a gas chromatograph coupled to a single quadrupole mass spectrometer (MS) with runtime of 11 min. Besides, negative chemical ionization mode was also studied and evaluated for OCPs' sensitivities. The optimized MS was operated in electron ionization mode and acquiring three selected ions per target compound. The method was validated with various food samples, including edible oil, meat, seafood, eggs, coffee, tree nuts, fruits, vegetables, etc. and the developed method was successfully applied for the OCPs' determination in real samples for the first Hong Kong Total Diet Study.

Chikuni et.al (2012) studied about Organochlorine pesticide residues in Mothers' milk; evaluation of possible Drug interaction in humans. The aim of the study was to investigate exposure levels of organochlorine pesticides (OCPs) in mother's milk and in selected staple foods. The study also aimed to evaluated effects of 1,1,1-trochloro-2-bis-(4-chlorophenyl) ethane (DDT) on paracetamol half-life in highly exposed and least exposed breast-feeding mothers as a way of investigating possible drug interaction. This was an experimental study where milk and food samples from Esigodini, Harare, Kadoma Kariba Nyanga and Mudzi were collected and analysed for OCPs levels using GC-ECD. Evaluation of induction of the hepatic cytochrome P450 enzymes by DDT was carried out by pre-treatment of female rats with a single intraperitoneal dose of DDT (0.3 mg/g) body weight. Cytochrome P450 enzyme was quantified by potassium phosphate buffering the microsomal fraction followed by spectral determination of the reduction of cytochrome P450. Blood samples from selected mothers in areas showing results of higher exposure levels to DDT and selected mothers with low exposure levels to DDT (controls) were evaluated for paracetamol drug interaction.

CHAPTER III: AIM and OBJECTIVE

<u>Aim</u>

To detect the presence of DDT in various brands of peanut butter by using Gas Chromatography.

Objective:

• To observe the variations in range of DDT in various brands of peanut butter.

CHAPTER IV: MATERIALS AND METHODOLOGY

Materials Required:

Apparatus:

- 1. Micropipette
- 2. Centrifuge tube
- 3. 2ml DisQue Extraction tube (150mg MgSO4,50mg PSA)

Chemicals:

- 1. Standard stock solution of OC
- 2. 10ml of HPLC water
- 3. 1.5g sodium acetate
- 4. 6g Magnesium Sulphate (DisQuE Dispersive SPE kit)
- 5. 10ml of Acetonitrile (HPLC grade).
- 6. n-Hexane

Instrument:

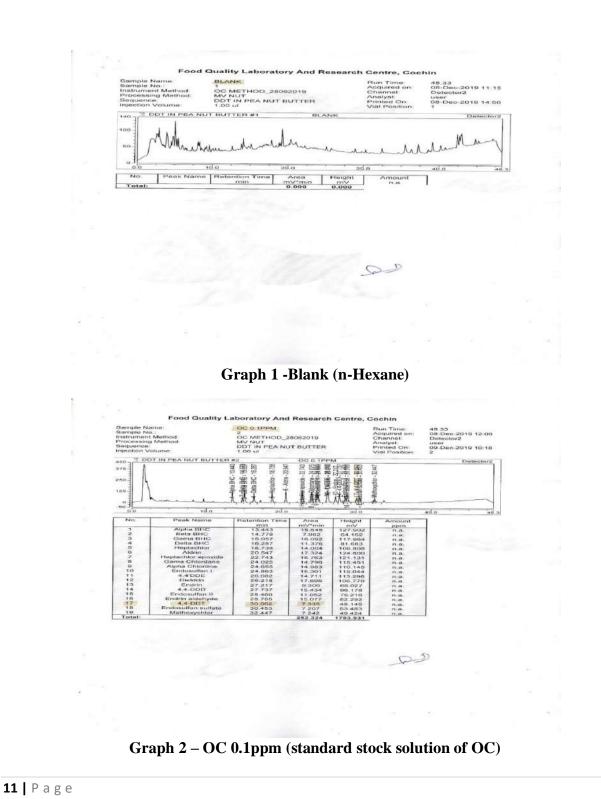
Thermo Scientific TRACE 1110 series Gas Chromatograph



Method:

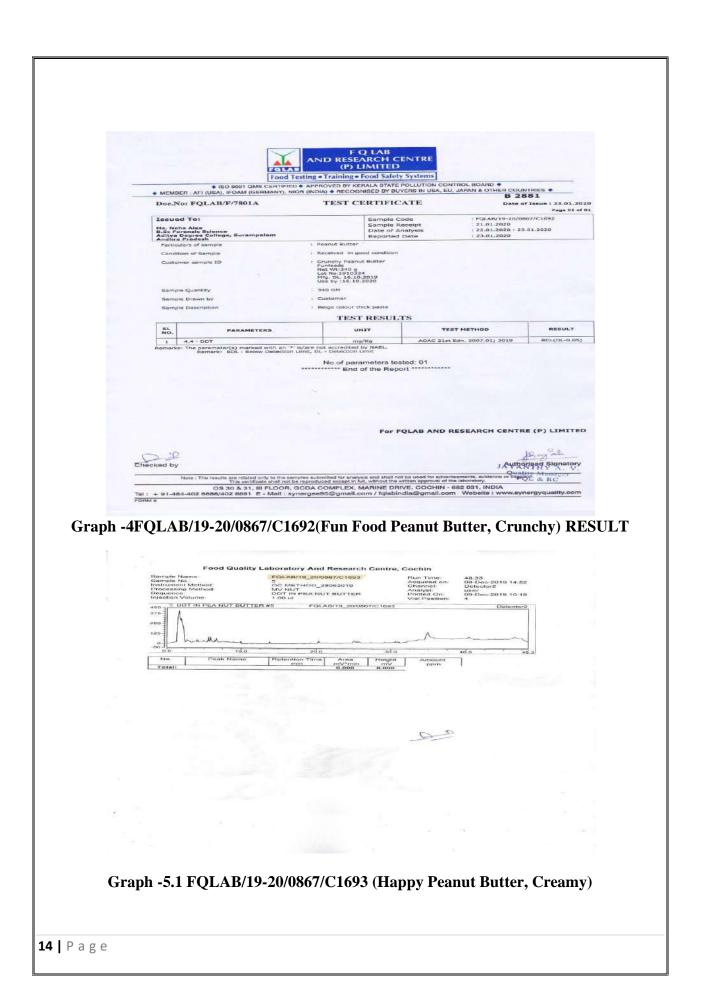
Five various brands of peanut butter were collected. Then 5g of homogenized sample transferred into 50ml centrifuge tube and treated with 10ml of HPLC water and mixed well. After that, it is treated with 1.5g sodium acetate and 6g MgSO4 (DisQuE Dispersive SPE kit), vortexed for 1min. Then added 10ml of Acetonitrile (HPLC grade) and again vortexed for 1min. Centrifuged the sample at 4500rpm for 5min. Then transferred1.0ml of centrifuged sample into a 2ml DisQue Extraction tube (150mg MgSO4, 50mg PSA) and vortexed for 1min. And then centrifuged the 2ml DisQue Extraction tube containing sample at 4500rpm for 5min and transferred to 1ml injection vial, and loaded into the autosampler tray in sealed vials. And the standard sample vials are interspersed in the tray as per analysis requirements. The operational software chromeleon7 controls the sequence of injection, sample volume, number of wash cycles and injection sequence for standards and samples.

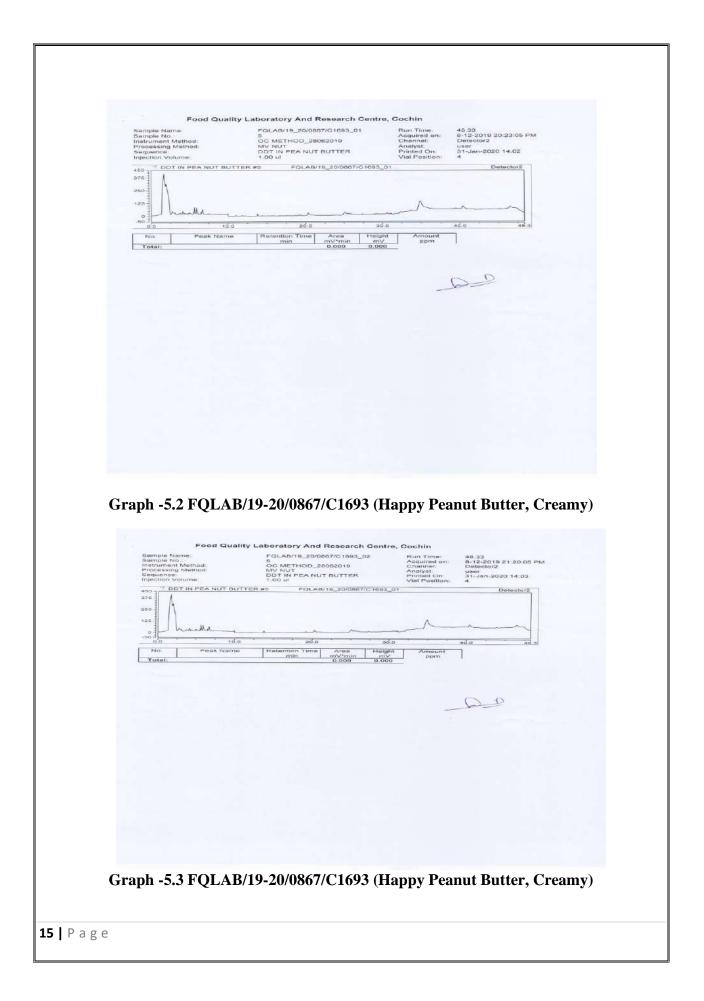
CHAPTER V: OBSERVATIONS

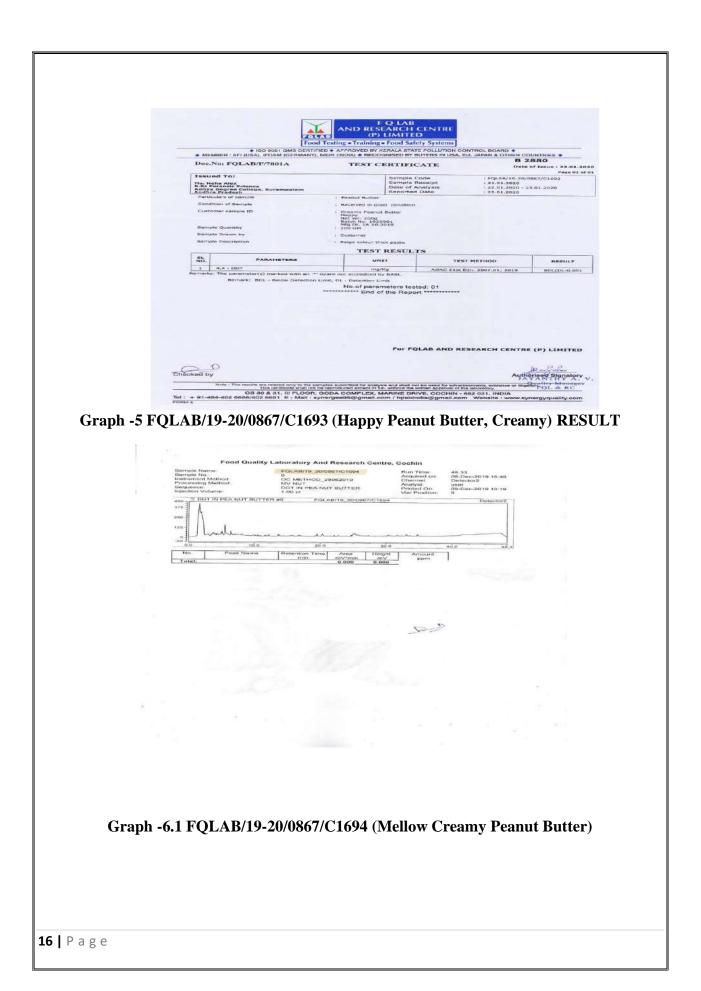


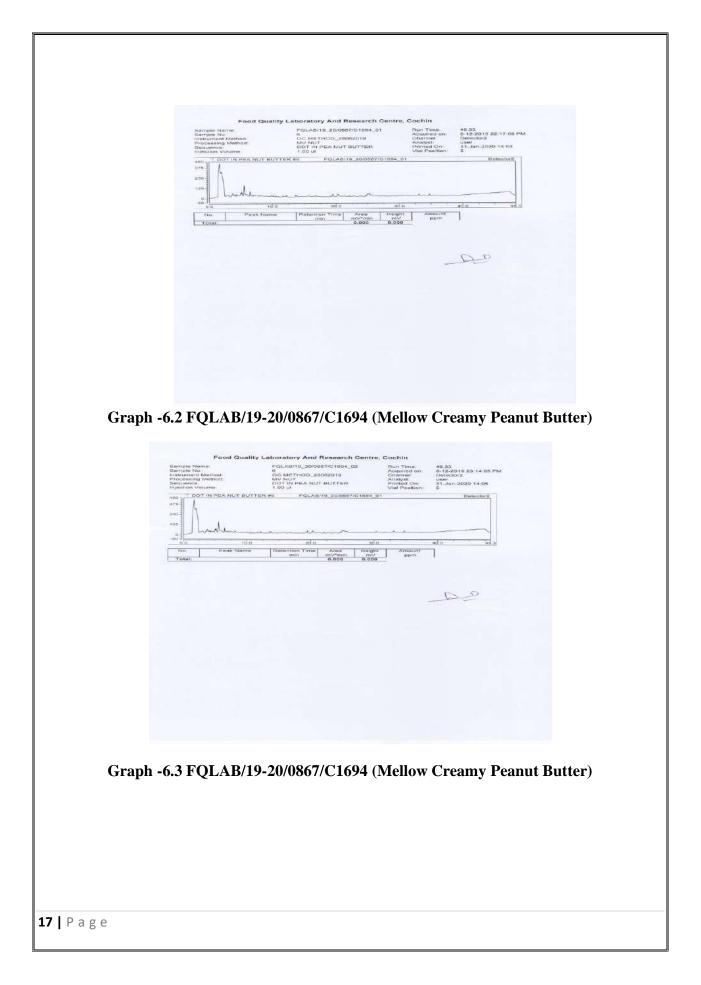
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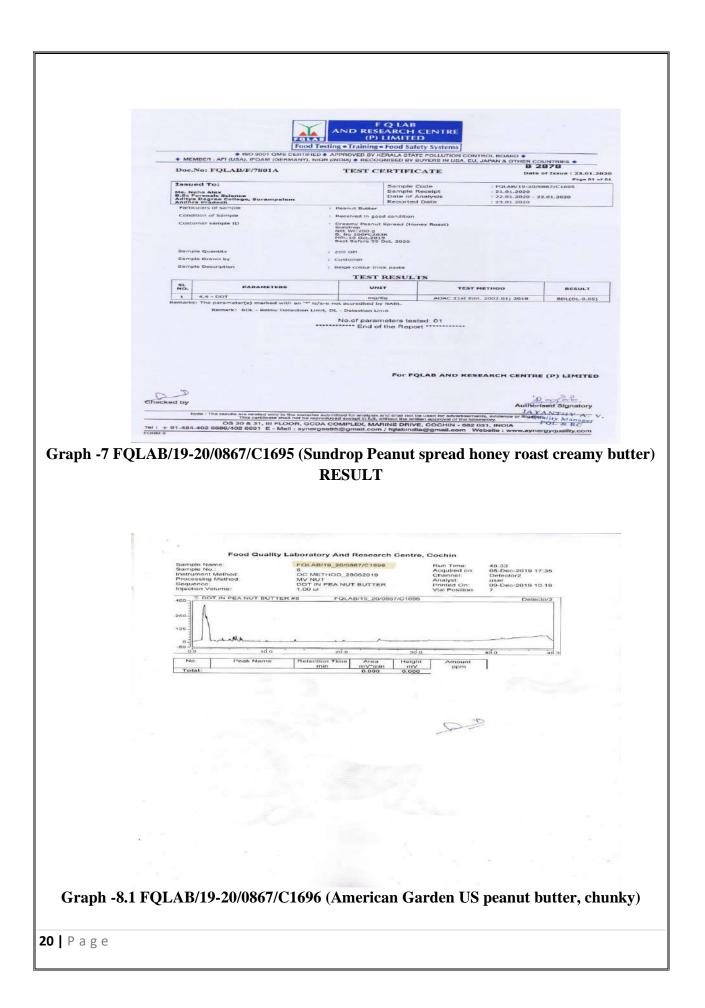








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	RESULT
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CHAPTER VI: RESULT AND CONCLUSION

Result:

The peak evaluated for the sample (4,4DDT) is 30.062. The collected peanut butter samples of various brands for the present study showed the peak in chromatogram below detection limit (DL-0.05) for DDT.

Conclusion

In the present study, the existence of DDT in the peanut butter of selected various brands is evaluated below the detection limit. As the value is below the detection limit DDT was unable to be detected in the given samples.

The study needs to analyze the DDT in various brands of peanut butter by using UV spectroscopy, IR spectroscopy to find out the amount of DDT present in that particular brand. The present study is related on five different brands as mentioned above. In future it is able to do the work on other various brands which is not included in the present study.

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