ANALYSIS OF ACRYLAMIDE MONOMER FORMATION IN REFINED, BLEACHED AND DEODORISED PALM OLEIN DURING DEEP FRYING OF FRENCH FRIES

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BY

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ABBREVIATIONS

ACR	Acrylamide
CV	Coefficient Variation
D_3	Deuterated
EI	Electron Ionisation
GC	Gas Chromatography
GC/MS	Gas Chromatography/Mass Spectrometry
GC/MS/MS	Gas Chromatography/ Mass Spectrometry/ Mass Spectrometry
HPLC	High Performance liquid Chromatography
HPLC/MS/MS	High Performance liquid Chromatography/ Mass Spectrometry/ Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
LLE	Liquid-liquid extraction
M	Molar
MS	Mass Spectrometry
mL	Milimeter
m/z	Mass/charge
µg	Microgram
RBD	Refined, Bleached and Deodorised
SPE	Solid Phase Extraction
TIC	Total Ion Chromatogram

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ANALISA PEMBENTUKAN MONOMER AKRILAMIDA DIDALAM MINYAK SAWIT OLEIN TERTAPIS, NYAHWARNA DAN NYAHBAU SEMASA PENGGORENGAN MINYAK DALAM KENTANG GORENG.

ABSTRAK

Pada April 2002, ‘Swedish National Food Authority (SNFA) telah mengumumkan penemuaan pembentukan akrilamida di dalam makanan yang berasaskan kanji, di mana ianya di bakar atau di goreng pada suhu yang tinggi terutamanya kentang goreng dan didalam pelbagai jenis makanan bergoreng. Akrilamida ialah polimer sintetik dan mempunyai jisim molekul yang rendah yang mana ianya di klasifikasikan sebagai berkemungkinan karcinogenik kepada manusia.oleh pusat Penyelidikan Antarabangsa Kanser. Penemuan ini telah menunjukan bahawa kandungan akrilamida yang terhasil adalah didalam lingkungan 10–1,800 ng/g. Persatuan Harmoni Europah telah menetapkan had yang dibenarkan pada tahap 0.5 µg/L dan Amerika Syarikat telah menetapkan pada tahap 0.1 µg/L di dalam air minuman. Semasa pengumuman ini, tiada kaedah untuk menganalisa akrilamida didalam pelbagai jenis makanan. Oleh itu, kaedah menganalisa akrilamida yang telah divalidasikan didalam pelbagai jenis makanan adalah perlu. Objektif kajian ini dijalankan adalah untuk membangun dan mengoptimunkan kaedah analisa akrilamida didalam pelbagai jenis makanan dan minyak sayuran. Sistem ‘GCMS’ yang menggunakan kaedah ‘ion trap’ dengan pengionan pelanggaran elektron digunakan untuk kajian ini (teknologi Thermo Finigan). Ion yang dikenalipasti ialah ‘2,3-dibromopropenamide’ dan D_{3}-akrilamida digunakan sebagai piawai dalaman. Had pengesanan akrilamide didalam kajian ini ialah 5 ng/g dan limit kuantitatif ialah 10 ng/g. Graf penentukuran adalah linear dan perolehan semula didalam lingkungan 82.99%-100.89%.

Penggorengan berperingkat dijalankan selama 5 hari berturut-turut, dimana potongan kentang di goreng didalam minyak sawit olein tertapis, nyahwarna dan nyahbau. Sampel minyak dan kentang yang diperolehi didalam penggorengan berperingkat ini di analisa dengan menggunakan kaedah yang telah dibangunkan didalam kajian ini. Pembentukan akrilamide didalam kentang goreng adalah didalam lingkungan 680–1449 ng/g dan didalam minyak sawit olein tertapis, nyahwarna dan nyahbau dari 21.26-159.12 ng/g. Kesimpulannya, kaedah GCMS dengan kepilihan dan kepekaan yang tinggi yang dibangunkan didalam kajian ini boleh diterimaguna.

ANALYSIS OF ACRYLAMIDE MONOMER FORMATION IN REFINED, BLEACHED AND DEODRISED PALM OLEIN DURING DEEP FRYING OF FRENCH FRIES.

ABSTRACT

In April 2002, Swedish National Food Authority (SNFA) announced the discovery of high levels of Acrylamide formation in a wide range of starch-based foods, which are fried or baked at high temperatures particularly French fries, crisps and various fried foods. Acrylamide is a synthetic monomer. This low molecule polymer was evaluated as 'probably carcinogenic to human' by the International Agency for Research on Cancer (IARC). The findings have shown that the detected amount in the above mentioned foods is in the range of 10 – 1,800 ng/g. The lowest limit of ACR in food has not been established. However, the legal limit, which is set by the Harmonized European rule is 0.5 µg/L and in United States 0.1 µg/L for drinking water. During the announcement, there was no available method of determining Acrylamide in food matrix. Therefore there has been a huge demand for the newly validated analytical method in different food matrix. The aim of this study is to develop a simple, fast and reliable method for acrylamide determination in food matrix and vegetable oil. A benchtop GC Tandem MS based on ion trap technology with electron ionization was used (Thermo Finigan). The developed method based on derivatization and the monitored ions for derivatised acrylamide for identification and quantification is known as 2,3 dibromopropenamide and Deuterated acrylamide (D_3) was used as an internal standard. The limit of detection was established at 5 ng/g and limit of quantification at 10 ng/g. The calibration curve was linear with assured recovery in the range of 82.99%-100.89%.

After the method had been validated, the batch frying was carried out for 5 consecutives days, i.e. French fries that was fried in RBD palm olein. Sampling for oil and French fries was collected and analyzed by this method. The acrylamide formation in French fries was from 680-1449 ng/g throughout the 5 consecutive days. Whereas for the acrylamide formation in RBD palm olein was from 21.26 -159.12 ng/g at the fourth and seven hours only on the second and third day respectively. This method is reliable, accurate, robust, selective and sensitive for determining acrylamide in various types of food matrix and liquid vegetables oil.

CHAPTER 1

INTRODUCTION

1.1 Acrylamide

In April 2002, Swedish scientists from the University of Stockholm and the Swedish National Food Administration (SNFA, http://www.slv.se., 2002) announced preliminary findings of significant presence of varies levels of Acrylamide (ACR) in a wide range of starch-containing foods that were fried at high temperatures or baked. The acrylamide appears to form as a byproduct of certain cooking processes such as frying, baking or grilling. However, the formation of this compound was not formed in any raw or boiled food (Swedish National Food Administration Press Release, http://www. livsmedelsverket@slv.se, 2002). This announcement received worldwide publicity and opens a completely new field of research.

ACR (2-propenamide) has been classified as “probably carcinogenic to humans” by the International Agency for Research on Cancer [Rosen and Hellenas, (2002) and Tareke et al., (2002)]. ACR is one of the many reactive compounds that react with protein, for instance the blood protein hemoglobin, and form a reaction product namely adducts.

Before this announcement was made, there has always been concerns on this monomer in occupational health as exposure to ACR is known to cause neurotoxicity and genotoxicity. For the non-occupational exposure, the only known source for low levels of ACR in food and water were through migration of food packaging material or through water treatment.

This finding was found by chance by Swedish scientists when they carried out a research study on risk assessment of workers exposed to ACR. The group at the University of Stockholm had for several years been studying the formation of hemoglobin adducts of acrylamide on humans following occupational or accidental exposure to acrylamide. According to Hagmar et al., (2002), during their study on humans occupationally exposed to acrylamide, they observed that the control human group without known exposure to acrylamide had remarkably high levels of the acrylamide adducts in their hemoglobin.

Subsequently, the scientist carried out another research on rats, which had been fed with fried and non-fried diets (Bergmark et al., 1993). Chemical analysis of rats fed with the fried diets showed that ACR was found in the hemoglobin. This research revealed that ACR had formed when feed was fried at temperatures of between 180^{O}C – 200^{O}C (Johnson et al., 1986).

Later they also demonstrated high acrylamide levels in some foodstuffs, e.g. fried, oven-baked and deep fried potatoes and cereal products that had been heated in laboratory experiments from 30-2300 ng/g (Tareke et al, 2002). These findings were quickly confirmed by the British Food Standards Agency and by other European countries, as well as by Japan, Australia and New Zealand. According to Tareke et. al., (2002), high concentrations were found in plant origin products such as potato chips, French fries, pan-fried potato products or crisp bread, whereas the ACR contents in foods that are rich in protein were low.

When this announcement was made, no one knew where the acrylamide came from, how it was formed, and if there is indeed a link between acrylamide in food and cancer. There was not much investigation and research that had been done on this monomer.

ACR is a polymer that is widely used in the treatment of drinking water as flocculants (Dhiraj and Shetty, 2003) and is also used in the manufacture of plastics. It was first evaluated as ‘probably carcinogenic’ to human in 1994 by the International Agency for Research on Cancer (IARC). But it was not known to occur at a high level in a variety of fried and baked common foods until it was announced.

These new findings raised considerable health concern worldwide due to the known toxic properties of ACR.

The lowest limit of ACR monomer in food has not been established. However the legal maximum limit that is set by The Harmonized European Committees (EU) rule for drinking water is 0.1µg/L (Ahn et al., 2002). However the World Health Organization (WHO, USA) has set that a maximum safe limit for drinking water is 0.5 µg/L (Codex Alimentarius, 2003). The limit allowed for migration of acrylamide monomer from packaging to food is 0.01 mg/kg of food (EU), (http://www.slv.se., 2002.)

At the end of December 2002, some research had shown that acrylamide is formed when starch-containing foods are fried or cooked at high temperatures. According to Mottram et al., (2002), recent model system studies have shown that acrylamide is formed during the Milliard reaction, and the major reactants leading to the release of acrylamide are sugars and asparagines.

Due to these findings, the National Food Administration and the University of Stockholm has set up a Scientific Committee and has initiated international contact, for example with the British Food Standards Agency (FSA) and The European Commission. All these agencies and the World Health Organization (WHO) have established a network for research on ACR in order to achieve a better understanding of human exposure and its possible health effects as well as the potential health risks of acrylamide in food [http://www.European Commission, (2002) and http://www.FAO/WHO, (2002)]. In other words, they urge more research on acrylamide in food as well monitoring studies and industry investigations.

The above mentioned committees have created an online research database to provide researchers with a global picture regarding this matter and have invited all the parties to share relevant data as well as to continue on going investigations. The database is comprised of studies that include levels of ACR in food, dietary exposure to ACR in food, ways to reduce level of ACR in food, mechanisms of formation, toxicology/carcinogenicity, biomarkers, method of analysis and international activities. Expert working groups have been established at the international level (e.g. FAO/WHO, JIFSAN Workshop) in order to review all the data, identify and list a number of research gaps and priorities.

During the discussion, the development and validation of sensitive and reliable analytical methods for low level quantification of acrylamide in different food matrix was considered as essential. This is because as in the past decade, methods had been developed to determine ACR in water, biological fluids and non-cooked foods such as mushrooms and field crops. Most of the developed methods are classically based on high performance liquid chromatography or gas chromatography techniques. However, these methods are not appropriate for starch based, processed and cooked foods at very low level i.e. 10 ng/g level and with an added requirement to confirm the presence of this small molecule.

The Swedish findings have attracted worldwide attention. All the concerned agencies and worldwide bodies are looking forward to receiving information on the new analytical procedure as well as on how this compound was formed. Currently, no maximum permitted concentration has been established for ACR residues in processed food (Hamide et al., 2005).

The need to develop a method by which the presence of ACR can be confirmed was deemed essential. There are primarily two approaches for the analysis of acrylamide in food, either by GCMS or HPLCMSMS. However during the past years since ACR was first found in heat treated foods in early 2002, many published papers and reviews on chromatographic techniques were reported from the year 2003 onwards.

A method, which was robust, sensitive and reliable, using GCMS, was developed in this study. This method is based on bromination of the ACR double bond and the reaction product is known as 2,3-dibromopropenamide and applicable to various types of food matrix and vegetable oils, down to the detection limit of 5 ng/g. According to Taeymans et al., (2004) and Zhang et al., (2005), the bromination method is more favoured over the non-derivatised method. This is because it will improve Gas Chromatographic characteristics and improve MS characteristic (higher mass ion and characteristic of ^{79}BR/ ^{81}BR patterns). On the other hand, GCMS without derivatisation led to the overestimation of the ACR content (Petersson et al., 2005).

1.2 Pam Oil Industry

The Palm Oil Industry in Malaysia has witnessed a prolific growth in recent years from being almost non-existent in the 1950s and now has emerged as the most profitable agricultural commodity, even overtaking the long established position of natural rubber.

The industry has undergone many significant phases with the rapid expansion from 55,000 hectares in 1960 to 3.3 million hectares in 1999. Malaysia is the world's leading producer and exporter of palm oil and accounts for more than half of the world's total output and exports. Malaysia produced about 13.98 million tones of the world's production of palm oil in the year 2004 (Selected Readings on Palm Oil and Its Uses, MPOB Publication, 2005).

Palm oil is an important revenue earner and it remains as Malaysia's second most important export commodity of which the export alone contributed to RM 10.22 billion or 75.1% of the total export revenue. Malaysian palm oil is exported to more than 100 countries all over the world.

Palm oil is one of the 17 major oils and fats produced and traded in the world. However, the main oils produced are soybean, palm oil followed by rapeseed oil and sunflower oil. In other words, palm oil is the second most consumed oil in the world after soybean oil.

Malaysia’s main products produced by the palm oil industry are crude palm oil (CPO) and crude palm kernel oil (CPKO) before further processing in downstream manufacturing activities.

Nowadays palm oil and its products have gained prominence and wide acceptance in the food industry worldwide.

Palm oil is one of the world’s leading vegetable oils in terms of both production and consumption. Almost 90% of the palm oil and its products are used for edible purposes. Palm oil has a range of distinctive properties, which enables it to meet some of the more demanding quality requirements in the edible fats products (Johari and Hishamuddin, 2003).

One of the major uses of palm oil/palm olein in food application is as a frying medium due to its stability. Palm olein or its blend with other vegetable oils are one of the standard frying oils which is commonly used for deep frying purposes, e.g. of French fries, instant noodles, potato chips, chicken, doughnuts and other snack food. About 20 million tones of oil and fats are used for cooking and frying activities around the world. In USA, Fast Food Restaurants consume several million tones of frying oils and fats. In Malaysia over 1000 Fast Food outlets use some 100,000 tons of palm oil products annually (Razali and Badri, 1993).

During deep frying, there are changes in the quality parameters such as viscosity, free fatty acids, smoke points, and the formation of the polymeric and polar compound. The formation of the polar and polymer compound is very important because of their potential toxicity to humans. In all frying systems, all the vegetable oils tend to increase in polymer content unlike palm olein because palm olein is less in polyunsaturated compound, which contributes to polar and polymer compounds (Razali and Badri, 1993).

Research studies undertaken have shown that palm oil/palm olein has good frying performance as it confers resistance to oxidation, rancidity, polymer formation and foaming. Therefore in order to compete with other oil and fat producers, we must have our own evidence on whatever arising matters or issues related to food safety.

More research is needed on different types of food to examine how ACR is formed and to understand the effects of different cooking techniques. The information available does not allow us to draw conclusions or to make recommendations for consumer or food manufacturers. In other words more research is needed to gain a complete picture.

1.3 The Objectives of The Study

The objectives of the study are

- to develop a method for determination of ACR monomer in RBD palm olein and French fries by means of Gas Chromatography Mass Spectrometry;
- to validate the method according to ISO/IEC 17025 requirements;
- to evaluate the level of ACR formation in RBD palm olein and French fries during batch frying at 178^{O}C;

- to examine the relationship between the acrylamide formation in oil and acrylamide formation in French fries during the batch frying.

1.4 The Expected Benefits

The expected benefits from this study are

- to establish a method for ACR monomer determination in RBD palm olein and French fries;
- to provide evidence of the ACR formation in French fries and RBD palm olein during batch frying at $178^{O}C$;
- to provide information on the ACR formation in French fries and RBD palm olein during batch frying at $178^{O}C$;
- to disseminate the findings to current and potential users of palm oil locally and overseas.

CHAPTER 2

LITERATURE REVIEW

2.1 Acrylamide

ACR, vinyl monomer is a chemical intermediate (monomer) produced by hydration of acrylonitrile. This monomer is odorless, occurs in a free-flowing white crystalline form and also in aqueous solution. Acrylamide monomer is used in a variety of synthetic processes to form polymers and copolymers. This low molecular weight polymer is used as a chemical intermediate in the production and synthesis of polyacrylamides.

ACR is a well-established synthetic neurotoxic chemical agent used in various industries since the early 1950s and is strictly controlled by environmental regulations. ACR had been designated as ‘probable human carcinogen’ by several scientific organizations, based on the data available at the time of these assessments [http://www.cfsan.fda.gov/dms/acrydata.html, (2002) and http://www.epa.gov/docs/oppt/chemfacts/s_acryla.txt, (2002)]. However the strength of the evidence for Acrylamide carcinogenicity is critical as there are not enough data on this evidence.

ACR is a soluble monomer and is quickly absorbed in the digestive tract. The principle end use of acrylamide is in water-soluble polymers used as a clarifier during water treatment. When it is added to water, it coagulates and traps suspended solids for easier removal. The polymer is also used to remove suspended solids from industrial wastewater before discharge, reuse or disposal. The polymers bind with particles and form heavy aggregates that rapidly settle out of the solution and leave a clear supernatant.

However, some ACR does not coagulate and remains in the water as a contaminant as there is an acceptable minimum level in water.

ACR is also used to enhance oil recovery, flocculants, papermaking aids, thickeners, soil conditioning agents, sewage and waste treatment, and ore processing and permanent press fabrics [US Food and Drug Administration (FDA), http://www.cfsan.fda.gov/dms/acrydata.html, 2002].

Polyacrylamide is also used in the washing and packaging of prepacked foods and vegetables. The US Food and Drug Administration has limited the amount of ACR monomer in polyacrylamide for use in paper or cardboard in contact with food to 2 g/kg (The International Programme on Chemical Safety, http://www.cfsan.fda.gov/dms/acrydata,html, 2002).

In the pulp and paper industry, polyacrylamide is used as binders and retention aids for fibers and to retain pigments on paper fibers. Polyacryamide is used to clarify wastewater, recover tailings and flocculants ores in mineral processing. They are incorporated in cement to slow the dehydration process to improve structural strength. Polyacrylamide when added to herbicidal gels will restrict herbicidal treatment to the bottom of lakes or reservoirs by allowing the herbicides to sink before they break up.

Ten to thirty percent of the annual production volume is used in oil recovery processes in which the polyacrylaminde increases water viscosity.

Acrylamide is also used in oil drilling processes to control fluid losses. Acrylamide is a soil stabilizer and is also used in foundry operations to facilitate free sand flow into molds. Acrylamide and polyacrylamide are also used in the manufacture of consumer products such as in textiles, contact lenses, appliances, building material, cosmetics and soap, in dental fixtures and in preshave lotions. Minor uses of acrylamide are as latex thickeners, emulsion stabilizers for printing inks, gelling agent for explosive, binders in adhesives tape, in production of diazo compound.

Acrylamide polymerization is used in the formulation of grouts for construction and repairing of sewers and tunnels. It has also been used extensively in molecular/biotechnology laboratories in preparing polyacrylamides gel (http://www.JIFSAN/NCFST, 2002).

2.1.1 Chemical and Physical Properties

The Chemical Abstracts Service (CAS) number for ACR is 79-06-1 with the molecular weight of 71.09 g/mols. The empirical molecule structure is C_3H_5NO / $CHCH_2CONH_2$. The synonym for this compound is 2-Propenamide; ethylene carboxamide, acrylic amide; vinyl amide (Merck Index, 2001). Chemical formula includes a polar amide function, which is very high water solubility, and a vinyl function allowing polymerization (Govaert et al., 2005), which is illustrated in Figure 2.1.

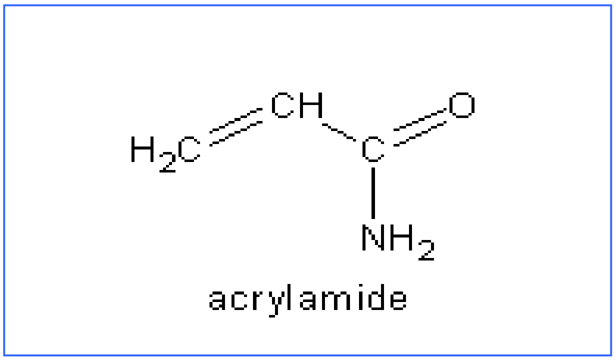

Figure 2.1: Chemical formula of the ACR

ACR melting point is at 84.5^{O}C and the boiling point is 87^{O}C. The vapor pressure is 0.009kPa at 25^{O}C. The density of this monomer is 1.22 g/cm at 30^{O}C. Acrylamide monomer is stable in the dark but it will polymerize at its melting point in solution or under ultraviolet light (Merck Index, 2001). The monomer readily polymerizes under the ultraviolet light. This monomer solubility at 30^{O}C in g/100 mL of solvent is as in Table 2.1.

Table 2.1: Solubility of ACR

AQUEOUS	**SOLUBILITY AT 30^{O} C**
Water	216 /100mL
Methanol	155 /100mL
Ethanol	86.2 /100mL
Acetone	63.1 /100mL
Ethyl Acetate	12.6 /100mL

The Merck Index, 13th Edition, 2001

2.1.2 Carcinogenicity, Neurotoxicity and Genotoxicity

ACR is classified as a substance, which may reasonably be anticipated to be carcinogenic in the Sixth Annual Report on Carcinogens, 1991 which is listed in Group 2A (IARC). Group 2A refers to the agent (mixture) which is probably carcinogenic to human and the exposure circumstances entails exposures that are sufficient probably to be carcinogenic to humans (http://www.SCF/CS/CNTM/CONT/4 FINAL.html, 2002).

ACR has been tested for carcinogenicity in rats in two long-term studies (Johnson et al., 1986). Although inadequate evidence is available from human studies, several laboratory animal studies have shown that ACR causes a variety of tumor in rats and mice. In humans, two epidemiological studies of occupational exposures to ACR are inadequate to evaluate the carcinogenic potential of the chemicals to human. The limitations included the lack of exposure data, inadequate study size and multiple chemical exposures. In several laboratories animal studies have shown that ACR causes a variety of tumors in rats and mice (Rosen and Hellenas, 2002).

The International Agency for Research on Cancer (IARC) working group reported that there are no adequate data to evaluate the carcinogenicity of ACR in humans (http://www.nsc.org/library/chemical/Acrylamide.html, 2002) but there is sufficient evidence in experimental animals for the carcinogenicity of ACR.

ACR has been shown to be a potent neurotoxin either by oral intake in animals or inhalation exposure in humans and in animal (http://www.SCF/CS/CNTM/CONT/4 FINAL.html, 2002). The presence of hemoglobin adducts of ACR was correlated with neurotoxicity in the human studies from a group of workers exposed to high levels of

ACR (Bergmark et al., 1993). It affects both the central and peripheral nervous system. Polymerized ACR is not toxic, but the monomer can cause peripheral neuropathy. The magnitude of the toxic effect depends on the duration of exposure and the total dose. The effects of neurotoxin are drowsiness, paralysis, hallucinations, distall numbness, weakness of hand and feet (http://www.nsc.org/library/chemical/Acrylamide.html, 2002).

In view of the genotoxicity, ACR can cause chromosomal aberrations, dominant lethality and DNA damage. This had been shown in studies of mice when the animal was administered with a level of 500 mg/kg of ACR everyday in their diet for 3 weeks (Johnson et al., 1986). Glycidamide is a metabolite of ACR that binds to DNA and can cause genetic damage. ACR has been shown to induce gene mutations in cultured animal cells and also treated cells of animals, (http://www nsc.org/library/chemical/Acrylamide.html, 2002). Prolonged exposure has induced tumors in rats but cancers in humans have not been convincingly shown.

According to The International Agency for Research on Cancer (IARC), no adequate data is available to evaluate the carcinogenicity of ACR in humans [http://www.cfsan.fda.gov/dms/acrydata.html, (2002) and http://www.monographs.iarc.fr/htdocs/monographs/vol60/m60-11.html, (2002)].

2.1.3 Route of Exposure

Numerous studies have been conducted to evaluate the potential dermal absorption of ACR; however, no study has attempted to directly quantify absorption of ACR following oral administration/intake or inhalation exposure. Results from distribution and excretion studies indicate that following oral administration in rats, acrylamide was readily absorbed (http://www.JIFSAN/NCFST; Worksyop on Acrylamide in Food, 2002).

ACR can be absorbed through unbroken skin, mucous membrane and lungs, and the gastrointestinal tract. In other words, ACR is readily absorbed by ingestion, inhalation and through the skin. Absorbed ACR is distributed in body water compartments and passes through the placental barrier.

The distribution of administered ACR to the blood is related to the ability of both ACR and its metabolite glycidamide to bind to hemoglobin to form adducts (http://www.JIFSAN/NCFST ;Worksyop on Acrylamide in Food, 2002). Both ACR and glycidamide are electrophilic and can form adduct with sulfhydryl groups on hemoglobin and other protein. At 24 hours, a 6-hour inhalation exposure to 3 ng/g ACR, the majority of the absorbed dose in rats was found in blood, followed by the skin, spleen and lung. In mice that were administered with the same concentration via the same protocol, a different pattern of distribution was observed, with the highest fraction of absorbed dose reported in the skin, followed by the subcutaneous fat, testes and blood (http://www.ntp-server.niehs.gov/htdocs/8_Roc/RAC/Acrylamide.html, 2002).

Human exposure to ACR is primarily occupational from dermal contact with the solid monomer and inhalation of dust and vapor (Hagmar et al., 2002). Occupational exposure to the aqueous form is primarily confined to maintenance and repair operations and connections and disconnection for transport. Workers in paper and pulp, construction, oil drilling, textiles, cosmetics, plastics and agricultural industries are potentially exposed to ACR. However data from these exposed workers are not available.

Hemoglobin adducts have been used as biomarkers of exposure and to estimate internal dose in occupationally exposed population. Exposure to ACR will irritate the nose, throat and skin. It also can cause a rash or burning feeling on contact, loss of balance, slurred speech and heavy sweating. ACR can cause chromosomal aberrations in mice when the animal was administered at a level of 500 mg/kg per day of ACR in their diet for 3 weeks (http://www.ntp-server.niehs.gov/htdocs/8_Roc/RAC/Acrylamide.html, 2002).

2.2 PALM OIL

There are two main products produced by the palm oil industry namely crude palm oil (CPO) and crude palm kernel oil (CPKO) before further processing and downstream manufacturing activities.

Palm oil is edible oil referred by FAO/WHO Codex Alimentarius as being derived from the fleshy mesocarp of the oil palm fruit (Yusof et al., 2000). In the unprocessed form, palm oil is reddish brown in color, and it has a semisolid consistency at ambient temperature.

As practiced worldwide, the CPO will undergo the refining process in order to convert the oil to finished edible food product (Yusof et al., 2000). This process is also done to meet consumer preference, flavor and stability characteristics of the oil. This oil has its own chemical composition and physical characteristics. This palm oil is used and marketed separately according to the supply and demand situation.

In this research, refined, bleached and deodorized palm olein is used because this oil is widely used in the deep-frying process as well as in domestic and industrial frying.

2.2.1 Refined Bleached and Deodorized Palm Olein

Palm Olein is a liquid fraction of the crude palm oil after the refining or fractionation process and the solid fat portion is known as palm stearin (MPOB Pocket Book, 2002). The use of the palm olein as domestic frying oil has long been established. Freshly refined palm olein is bland and has an excellent oxidative stability.

It is easy to handle and does not produce excessive smoking, spattering, foaming and forms less gum in the pan and fryer compared to polyunsaturated oil (Dimitrious Boskou and Ibrahim Elmadfa, 1999). Palm olein is comparable in terms of oxidative stability during frying with other hydrogenated vegetable oils, namely hydrogenated soyabean, hydrogenated sunflower and hydrogenated cottonseed oils.

Both palm oil and palm olein has excellent oxidative and frying stability due to its inherent composition. In other words, palm oil and palm olein have practically the same frying performance and a similar composition. The significant difference between palm oil and palm olein are degree of fluidity and iodine value. Palm oil has the melting point at about 36^{O}C and palm olein about 22^{O}C. Palm Oil has the iodine value (wijs) at the range of 50-54 (Iodine value is a measurement of the unsaturation of fatty acids). Whereas the iodine value (wijs) for palm olein varies from 56 up to 60 (MPOB Pocket Book, 2002). Palm Oil can also be blended with other vegetables oils for frying purposes and it gives the same effect.

2.2.2 Uses of Palm Olein

One of the major uses of palm oil/palm olein in food application, which has been long established, is as a frying medium. Almost all varieties of fats and oils have been or are used for frying which include vegetable oils, hydrogenated oils, animal fats (tallow and lards), animals and vegetables blends or shortenings.

Research and studies undertaken showed that palm oil/palm olein has good frying performance as it confers resistance to oxidation, rancidity, polymer formation and foaming. Deep frying using palm olein is widely used all over the world because it is quick, easily adaptable to mass production and produce tasty product or attractive appearance and possesses no health risks under controlled frying (Yusof et al., 2000).

Frying is one of the major applications and universal method for oils and fats which is the most commonly used for food preparation at home, restaurants, fast food outlets, street vending, mass catering, cottage and industrial scale operation. Frying is a popular traditional cooking process in the home and is universally used in the food industry to produce snack and convenience foods (Razali et al., 1999). Frying will give attractive flavors and textures.

Palm oils and its fats are widely used all over the world in frying foods because of its bland taste, versatility and can be easily blended with other vegetables oils (Yusof et al., 2002). Beside that, palm oil is also comparatively cheap to be used and have a long shelf live. Furthermore the palm oils, palm oil products' and its blends have excellent oxidative stability and better frying performance compared to other major vegetables oils (Razali et al., 1999).

The quality of fried foods depends heavily on the quality of the frying oil. There are two types of frying i.e. shallow frying and deep-frying**.**

2.2.2.1. Shallow Frying

Shallow frying also known as stir-frying/cooking, which usually takes place at home. Stir-frying involves a small amount of fats/oils that is placed in the pan and heated to frying temperatures of within $160^{O}C$ and $180^{O}C$ and used for a relatively short period of time or in other words used only once. During this frying almost all the fats/oils is absorbed in the food. As the oil is being used immediately, therefore its resistance and breakdown during frying is unimportant.

2.2.2.2 Deep Frying

Deep-frying is a very popular way to prepare fast food worldwide (Razali et al, 1999). Deep-frying is widely used in plant/mass catering, Fast Food Chain and industrial scale operation. This type of frying involves a large volume of oils/fats, in which the food can be totally immersed in hot oil in a fryer and will be repeatedly or continuously used at elevated temperatures for a very long period of time/cycle before being discarded. In deep-frying, effects on the oil’s quality is very important. The duration of reusable oils/fats depends on the type of outlets, foods and also the type of oils/fats and its’ blends. During deep-frying, only a small proportion of the fats are absorbed by the food, which is totally different from shallow frying.

- **Continuous Frying**

Industrial frying uses the continuous frying where a large amount of oil is used. After the frying process is over, during a particular time, the oils will be pumped up to the storage tank and it will be used for the next frying. Examples of food used in continuous frying are frying of instant noodles, potato chip/crisps and various types of snack.

- **Batch Frying**

Fast Food Restaurant uses the Batch frying method. In Batch frying, the oils will be used continuously every day where in the morning, before the frying takes place, fresh oil will be topped up to the maximum level in the fryer. Usually after the fifth day, the oils will be discarded. Examples of food used in Batch frying are French fries, fish fillet, fried chicken and nuggets.

2.2.2.3 The Process and Changes During Deep Frying

Deep-frying is a complex method of food preparation in which many reactions take place in the frying oil, where oxidative and hydrolytic degradation and polymerization occurs in the oil. These reactions produce desirable and undesirable effects in oil quality and the fried foods. It is known that oils used continuously or repeatedly at high temperatures in the presence of oxygen and water from the food being fried, is subject to oxidation, polymerization, cyclisation, pyrolysis and hydrolysis. The resultant decomposition products adversely affect flavor, taste, texture and overall quality of the oil and the fried food (Yusof et al., 2002). A good frying oil must have high oxidative stability and flavor stability as well as good taste and nutritional value (Razali et al., 2000).

During this frying process, the oils/fats are continuously or repeatedly heated to high temperatures of between 180^{O}C – 190^{O}C in the presence of water and moisture. This type of frying will produce fried foods with desirable color, appearance, flavor and texture, which can attract the consumers.

Frying process is where the heat is transferred to the product/food using oil, as oil is a good conductor of heat when the temperature is above the boiling point of water. As water moves out of the products, it will prevent burning of the outer surface of the food. Therefore, steam is generated and it moves through the hot oil to escape at the top of the fryer where there is a conduction and convection transfer of energy. Oil is absorbed by the food and forms an integral part of the deep-fried products. Fats/oils acts to remove excess water, to cook the food (gelatinize starch in the case of starchy products) and to create a characteristic taste and texture. The type and quality of the oil used for frying have an influence on the quality and shelf life of the fried products.

Under these condition, several changes occurs in food during frying, namely starch gelatinisation, protein denaturation, water vaporization and textural changes. On the other hand, the oils/fats are undergoing various stages of chemical reaction, namely primary and secondary oxidation, polymerization and interaction between fats and substrate.

The intensity of the heating process plays a role in the formation of ACR in food products. Acrylamide appears to form as a byproduct in starch-based foods when the food and heating medium are directly in contact with each other during frying. ACR formation starts as little when the temperature was above 100^{O}C, [Wicklund et al., (2005), Belcaski et al., (2002), Amrein et al., (2003) and Tareke et al., (2002)].

A number of theoretical mechanisms have been proposed for the ACR formation. According to Mottram et al., (2002), from Maillard reaction it showed that heating glucose with asparagine yielded ACR and the ACR amount will keep on increasing with the increasing temperature from 120^{O}C-170^{O}C. The reaction between amino acids (primarily asparagine) and a reactive carbonyl could be one of the possible precursor of Acrylamide (Friedman, 2003). According to Amrein et al., (2003), glucose and fructose are considered to be the main precursor and asparagine with its amide group is the backbone of the acrylamide molecule.

Belcaski et al., (2002) and Hagmar et al., (2002) suggested that ACR could be formed directly from rearrangement of nitrogen atom which is present in the food. The ACR formation in oil is non-oxidative in nature [William, (2005), Belcaski et al., (2004), Tareke et al., (2004) and Dhiraj and Shetty, (2003)].

At the stage of writing this thesis, according to Wendie et al., (2005), Yasuhara et al., (2003), Zyzak et al., (2003), Williams (2005) and Wicklund et al., (2005), there are other parameters that influence ACR formation during heating high starchy food including staple food such as time, temperature, composition of the food matrix, storage time and the processing method of the raw food. Other than asparagines as the precursors, some of the international researchers believed that reducing sugars play a vital role in ACR formation during heating starch-based foods [Francho et al.,(2005a)] or in potato crisps (Wicklund et al., 2005).

2.2.2.4 The Specification of RBD Palm Olein For Deep-Frying.

Before the frying process takes place, a few parameters need to be checked to ensure the quality of the end products. This is because the initial values of the frying oil used in deep-frying contributes to the quality of the fried food in order to produce a good end product. In other words, the frying oil is absorbed into the food during frying (Rossell, 1997). These parameters are: colour, free fatty acid and peroxide value as in Table 2.2.

Table 2.2: The specification of RBD Palm Olein for Frying

PARAMETERs	***VALUEs**
Colour (Lovibond, Red)	< 2.8R
Free Fatty Acid (mg/g)	<0.06
Peroxide (meq**/kg)	< 0.5

*Selected Readings: 26th Palm Oil Familiarization Programme. (2006). Compiled by Technical Advisory Service Unit, Malaysian Palm Oil Board.
**meq=milliequivalent

- **Colour**

The colour of the oil is measured using a Lovibond Tintometer. Colour is an important criterion in the determination of quality of finished oils and the processing efficiency. The method consists of matching the colour of the light transmitted through a specified depth of oil to the colour of the light, originating from the same source, transmitted through a standard set of coloured glass slides. The colour reading is associated with red, yellow, blue and neutral colour (MPOB Test Method, 2004).

Colour has always been associated with the oxidation process. Colour changes are not a good test for assessing the quality of frying oil, but it is important because