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ASSESSMENT AND SAFETY IMPROVEMENT OF PEANUT BASED BY-PRODUCTS FROM PEANUT OIL PROCESSING: *KULIKULI* AND *KHEBAB* POWDER

BY

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DECLARATION

I declare that this work was conducted by me under supervision in the Department of Nutrition and Food Science, University of Ghana, Legon.

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DEDICATION

I dedicate this research work to my Lord and my God for how far He has brought me, my parents: Mr. Hawkson Baah-Tuahene and Miss Comfort Appiah and my siblings especially my brother; Dr. Seth Donkor and sister; Bridget Baah-Tuahene who have been strong pillars in my life.

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ABSTRACT

Peanuts are very nutritious oil seeds grown worldwide but are however susceptible to aflatoxin contamination through the infestation of aflatoxigenic molds. These aflatoxins are known to have carcinogenic effects on humans and animals. There is therefore the need to put in measures to ensure that excessive levels of aflatoxins are reduced to the barest minimum in peanuts and peanut products to conform to the requirements of food safety regulations. The objectives of this study were to determine the aflatoxin levels in peanuts and peanut products from artisanal peanut oil processors and to identify the challenges with the implementation of a Hazard Analysis and Critical Control Points (HACCP) system as a food safety management tool to eliminate the hazards of aflatoxin contamination in peanut oil processing by-products.

A survey was done in three regions of Ghana which included the Greater Accra, Ashanti and Northern regions. Peanut processors were identified in the markets by convenience sampling and interviewed on their processing methods. A semi-structured questionnaire designed for the study was pretested in markets in the Greater Accra region that were not part of the selected markets for the study. The knowledge of the peanut processors on food safety issues was tested and the processing methods of peanut processors were observed and documented. The peanut products were characterized for their physicochemical indices. The level of microbial and aflatoxin contamination were determined on the peanut products and the challenges associated with the implementation of a Hazard Analysis and Critical Control Point system determined.

All the peanut processors were females between the ages of 20 to over 50 years. Majority (98%) of the peanut processors had no formal education. However, they were not ignorant of food safety issues associated with peanut consumption. The processing methods of the peanut processors were not different from what had been reported from earlier studies.

The peanut products had moisture content ranging between 1-4.3%, carbohydrate content of 20.1-40.1%, protein content of 26.2-40.8%, ash content of 3.8-7.1%, fat content of 20.3-33.7% and texture of 18,375.9-19,743 N/cm^2.

The microbial analysis showed that peanuts and its by-products were highly contaminated with viable cells, *E. coli*, coliforms, yeast and moulds far exceeding 100 cfu/g. Viable cell counts ranged between (1.0) 10^1-(9.7)10^6 cfu/g, *E coli* counts were between (1.0) 10^1-(7.5)10^2 cfu/g, coliform counts were between (2.0) 10^1-(5.2) 10^6 cfu/g, yeast counts were between (1.0) 10^1-(7.5) 10^2 cfu/g and mould counts were between (1.0) 10^1-(4.5) 10^2 cfu/g. *Staph aureus* and *Salmonella* were however not detected.

The aflatoxin contamination levels of the traditionally processed peanut oil and its by-products far exceeded the set limits of 20µg/kg by the United States and 4µg/kg total aflatoxin for the European Union. The aflatoxin levels for aflatoxin B1, B2, G1 and G2 were 0.05-522.1 µg/kg, 6.2-41.2 µg/kg, 0.02-25.7 µg/kg and 0.03-2.1 µg/kg respectively.

The challenges in the implementation of HACCP in the traditional peanut oil and *khebab* powder processing were identified as the level of education and adaptability of processors to the HACCP system and the ability to monitor the critical control points identified to ensure they are meeting the set standards by the HACCP team.

CHAPTER ONE

INTRODUCTION

1.1 USES OF PEANUTS

Peanuts, also popularly known as groundnuts (*Arachis hypogaea*) are among the most important oil seed crops in the world. India, China and United States of America (USA) are the largest producers of peanuts (Pazderka and Emmott, 2010). Peanuts are grown in the tropics and subtropics for their food, oil and protein rich meal (Abdulrahaman *et al.*, 2014). It is grouped among agricultural products loosely referred to as 'woman's crop' because they are the crops women actively cultivate and control the value chain (Zuberu *et al.*, 2013; Balakrishnan *et al.,* 1998). The cultivation, distribution and processing of peanuts serve as sources of income for most small scale farmers and cottage food industry owners, who are predominantly women.

Peanuts are very nutritious and serve as a good source of vegetable proteins, fats, minerals and vitamins. They serve as an inexpensive source of vegetable proteins especially in situations where people cannot afford meat. Indeed in dire situations where the diets consist largely of starchy foods, peanuts have been used to supplement animal protein to combat Protein Energy Malnutrition (PEM) (Carlberg, 2008; Debrah and Waliyer, 1996; Eshun *et al*., 2013). Peanuts are used in the production of Ready to Use Supplementary and Therapeutic Foods (RUSF and RUTF) which have been instrumental in combating malnutrition in women and children respectively (Agbemafle, 2013; Pazderka and Emmot, 2010). Furthermore weaning mothers have been advised to incorporate peanuts in the preparation of traditional foods in order to increase the protein content of the food and aid in normal growth and development of babies (Kpodo and Gyato, 1996). According to Pazderka and Emmot (2010), peanuts contain 567 (kcal) energy, 25.8g (per 100g) protein and 49.2g (per 100g) total lipid. They are also rich in vitamins such as vitamins A, E, B1,

B2, B5, B6 and minerals such as calcium, potassium, magnesium, iron and sodium (Settaluri *et al*., 2012).

Peanuts can be used in the preparation of a variety of indigenous Ghanaian foods. They are consumed in their raw state or roasted and processed into various snacks such as roasted peanuts, cookies, flakes and candies. They are also used to produce high quality cooking oil (Awuah, 2000). Peanut paste is extensively used in the preparation of soups and also serves as a bread spread. The peanut cake derived after the oil has been extracted from peanut paste is used in the manufacture of other local delicacies that are rich in proteins (Tsigbey *et. al.,* 2003; Kwanashie *et al*., 1992). Peanut cake obtained after frying the cake in oil locally called *kulikuli* is used as a delicious snack and can be used as a protein supplement in other dishes.

Peanuts are mainly produced by small holder farmers in the three administrative northern regions of Ghana (Northern, Upper East and Upper West regions) which account for 94% of peanut production in the country Ghana (MOFA, 1997). These regions have dry and transitional-Savannah weather conditions and a uni-modal rainy season which are conducive for the cultivation of peanuts (Masters *et al.*, 2013).

1.2 PROBLEMS AFFECTING PEANUT PRODUCTION IN GHANA

A major problem that affects the production of peanuts in Ghana is the limited capital available to the small scale processing groups and startup businesses (Zuberu *et al*., 2013). Furthermore the high interest rates on loans and the very short periods for repayment pushes the women processors who receive credit from micro-finance companies and banks out of business (Zuberu *et al*., 2013; Kpodo and Gyato, 1996).

Another problem in the peanut value chain is fungal infestation and mycotoxins, particularly aflatoxin contamination of the crop (Masters *et al*., 2013). This is frequently exacerbated by unseasonable weather, particularly rainfall and temperature fluctuations of the tropical climate.

1.3 RATIONALE FOR THE STUDY

Peanut is a highly nutritious legume grown in Ghana and worldwide and assures food and nutritional security especially in poor communities. Its by-products are popular in Ghana and used for *khebab* powder and *kulikuli*. Peanuts are however susceptible to aflatoxin contamination through the infestation of aflatoxigenic molds. These aflatoxins are known to have carcinogenic effects on humans and animals (Bhosa *et al*., 2013; Wild and Turner, 2002). In recent times, most of the peanuts and the by-products produced for export from West African countries including Ghana have been rejected according to Rapid Alert System for Food and Feed (RASFF) notifications (RASFF, 2012). This is as a result of exceeding levels of aflatoxins beyond the set standards used by various food safety agencies, which range from 4 µg/kg for processed peanut products in the European Union (EU), to 20µg/kg as established by the Codex Alimentarius Commission. There is therefore the need to put in measures to ensure that excessive levels of aflatoxins are reduced to the barest minimum in peanuts and peanut products to conform to the requirements of food safety regulations.

Some work has been reported on the determination of aflatoxin levels in peanut products in Ghana (Kotey, 2010; Dongdem, 2013). However, the traditional production practices of peanut products remain unchanged and the levels of aflatoxins and microbial contamination in peanut products: especially *kulikuli* and *khebab* powder remain variable and frequently high. Safety and quality management tools that can eliminate possible

hazards during processing have not yet been applied in the artisanal peanut processing industry.

1.4 MAIN OBJECTIVE

To determine the aflatoxin levels in peanuts and peanut products obtained from artisanal peanut oil processors and to identify the challenges with the implementation of a Hazard Analysis and Critical Control Points (HACCP) system as a food safety management tool to eliminate the hazards of aflatoxin contamination in peanut oil processing by-products.

1.4.1 Specific objectives

- To assess the food safety knowledge of peanut oil processors in selected communities in Ghana.
- To observe and document the traditional processing methods of peanut oil and it's by products: *khebab* powder and *kulikuli* in Ghana.
- To characterize traditionally processed peanut oil and its by-products using their physicochemical properties.
- To determine the level of microbial contamination in peanut products obtained from the traditional processing of peanut oil.
- To determine the level of aflatoxin contamination in traditionally processed peanut oil and its by-products in Ghana.
- To identify the challenges with the implementation of a HACCP system for traditional peanut oil and its by-products.

CHAPTER TWO

LITERATURE REVIEW

2.1 PEANUTS

Peanuts are major staple crops in most parts of Ghana and are used in the preparation of several indigenous foods (Tsigbey *et al.*, 2003; Pazderka and Emmot, 2010). According to Hoffman and Falvo (2004), peanuts are loosely referred to as the 'poor man's protein and is inexpensive and available at all times. In Ghana, peanut processing is mostly done by women on a small and medium scale solely based on knowledge passed down by family members (Kpodo and Gyato, 1996). Peanuts do not only produce income for the processors but are also extensively used in the production of different types of foods (Zhao *et al*., 2012; Kpodo and Gyato, 1996; Nautiyal, 2002).

There are several cultivars of peanuts in Ghana. They are classified based on their branching patterns, presence or absence of flowers on main stem and flower arrangement on leaf axils into subspecies hypogaea (Virginia market type) and fastigiata (Spanish and Valencia market types) (Asibuo *et al.*,2008). The cultivars under the sub specie *hypogaea* have alternate branching and flowering and also do not have flowers on the main stem whiles the cultivars that fall under the sub specie *fastigiata* have sequential branching and flowering and have flowers on the main stem. Work done by Asibuo *et al.* (2008) utilized about twenty cultivars of peanuts. Some of which include: *Dagomba*, F-mix, *Nkatepa*, Manipinta, *Sinkarzie, Kumawu* early, *Nkate kokoo, Baasare, Broni nkatee, Afu, Nkoranza* local, *Atebubu* local, *Aprewa*, *Kintampo* local, Shitaochi, *Broni, Kamaloo, Kofi Nsarko, Kowoka* and *Broni fufuo.* Kpodo and Gyato (1996) also found out from a survey carried out in the ten regions of Ghana that the 'China' peanut variety was most preferred in the

processing of peanut butter due to its high oil contents, accessibility, ease of dehulling and taste.

To date, no peanut cultivar that is totally resistant to the production of aflatoxins has been produced (Xue *et al*., 2004). It is therefore important to produce more aflatoxin resistant peanut cultivars, do proper sorting of raw peanuts and also develop a food safety management tool like the Hazard Analysis and Critical Control Point (HACCP) system to help reduce the production of aflatoxins as much as possible.

2.1.1 Nutritional Importance

Peanuts are known to contain a high amount of fats and proteins and can be utilized in curbing Protein Energy Malnutrition (PEM) (Eshun *et al*., 2013). According to Pelto and Armar (2011); peanuts are a rich source of protein and amino acids and help prevent malnutrition. They contain all the essential amino acids needed for normal body growth and metabolism. Ghanaian mothers have been advised in recent times to add peanuts to the foods they prepare for their babies during weaning to increase the protein content and promote growth (Kpodo and Gyato, 1996). Work done by Eshun *et al.* (2013) shows that peanuts have a high caloric content and can therefore provide a good source of energy for people in developing countries and the less privileged in the society.

Moreno *et al.* (2013) also sought to determine whether peanut consumption in Mexican-American adolescents is associated with improved weight status. They found out that Mexican-American children who consumed peanuts were less overweight and had better nutrient and lipid profile as compared to those who did not consume it. Furthermore, in the same research study Moreno *et al*. (2013) found out that peanuts had a positive effect on

cholesterol levels, nutrient intake and weight as compared to the adolescents who did not consume peanuts.

Also, work done by Eshun *et al*. (2013) to determine the nutrient content and seed characterization of seed pastes of four selected peanut varieties in Ghana indicated that the saponification values obtained for all the varieties analyzed were high enough to be used in the manufacturing of soap and other shaving creams; however Sinkarzie was more suitable for the manufacture of soaps since it had the highest saponification number.

Williams *et al.* (2004) have found that chronic exposure to aflatoxins compromises immunity and interferes with protein metabolism and micronutrients that are critical to health. Thus, this research seeks to develop a HACCP system that will serve as a food safety management tool to eliminate the health implications that come with the consumption of defective and aflatoxin infested peanuts.

2.1.2 Peanuts as a source of income for processors

Peanut processing is a lucrative venture because the processors get employment and revenue from both the major product which is the peanut oil and its by-products which include: *kulikuli* and *khebab* powder (Masters *et al*., 2013; Taru *et al*., 2010; GAIN Report, 2014). Zuberu *et al*. (2013) found that peanut processors received higher revenue from both the peanut oil they sold and the by-products which include *kulikuli* and *khebab* powder as compared to soymilk processors who only got their revenue from the soymilk they produced.

2.1.3 Challenges

There are a number of challenges that come along with peanut processing. According to Torres *et al.* (2014) and Mutegi *et al.* (2009), aflatoxin contamination in peanuts beyond set standards that are safe for both human and animal consumption is a major problem. Aflatoxins are known to be carcinogenic and may even result in death. This therefore calls for the need to put in measures to ensure that aflatoxin contamination does not occur or is reduced to the barest minimum.

Furthermore, according to Zuberu *et al.* (2013), a major problem with the production of peanuts is the lack of capital to start and expand. Also, those that are already actively involved in peanut processing are affected by the short time they have to repay the monies that have been lended to them by banks and micro credits. These challenges result in the processors not being able to sustain and expand their businesses.

In addition, tiredness and stress is faced by the processors because of the few hands they have to help them. This is because the capital they need to employ more labour to facilitate their work is limited or not available. This greatly affects the health of the processors and also reduces the level of their production.

2.2 MAJOR PEANUT CULTIVATION AREAS IN THE WORLD

African countries such as Nigeria, Gambia, Togo, Republic of Benin, Ghana, Ivory Coast, Liberia, Chad, Niger, Senegal, Mali and Upper Volta Guinea produce a lot of peanuts for domestic use and consumption (Abdulrahman *et al.*, 2014). India, China, the United States of America, and Argentina have also been ranked as the highest producers and exporters of peanuts in the world because they serve as an important part of the diets of the populace

and are used in the preparation of a lot of foods (Pazderka and Emmott, 2010). Table 2.1 shows the world ranking of the production of shelled peanuts for the 2010 crop season.

In Ghana, peanut cultivation and aggregation is mainly in the three administrative northern regions (Tsigbey *et al.*, 2003). Most of the inhabitants in these areas grow peanuts both on a small and large scale for consumption and income. Also, the environmental conditions in these places are suitable for the successful growth of peanuts.

Cotty and Jaime-Garcia (2007) have shown that aflatoxins are prevalent in warm and humid climates as well as irrigated hot deserts. Aflatoxin contamination may also occur during drought in temperate regions and is greatly affected by climate (Paterson and Lima, 2011). Also, fluctuations in climate affect aflatoxin contamination since there is a shift in aflatoxin producing fungi as the climate shifts (Cotty and Jaime-Garcia, 2007, Paterson and Lima, 2010; Magan *et al.*, 2011). The climate in Ghana is warm and humid and will highly facilitate the growth of aflatoxin producing fungi if the right control measures are not put in place. Thus, this study seeks to determine the level of aflatoxins in the different peanut products and identify the challenges that come along with the implementation of a Hazard Analysis and Critical Control Points system.

Table 2.1: FAO world ranking of shell peanut production for 2010 crop season.

Country	Total Production (MT)	World Ranking
China	15,709,036	1
India	5,640,000	2
Nigeria	2,636,230	3
United States of America	1,885,510	4
Senegal	1,286,860	5
Indonesia	779228	7
Sudan	762500	8
Argentina	611040	9
Ghana	530887	10

Source: FAOSTAT (2010).

2.3 SOME GHANAIAN TRADITIONAL FOOD PRODUCTS MADE FROM PEANUTS

There are a number of traditional food products made from peanuts in Ghana. These fall under different categories based on the processing methods and other ingredients added during the processing. Some of these peanut foods include salted and dried peanuts, fresh boiled peanuts, peanut paste, peanut soup, peanut cake etc.

2.3.1 Salted and Dry Roasted Peanuts

Salted and dry roasted peanuts can be prepared in two ways. Water with salt added to it is heated till it boils and the peanuts added to it for a few minutes' whiles the salted water is boiling before being drained off and sundried. They can also be prepared by adding salt to water and heating till it boils; the boiled salted water and then poured over the peanuts in a container and allowed to stand for a few minutes before being drained off and sun dried (Saalia *et al.*, 2008; Abdulrahaman *et al.*, 2014).

The sun dried salted peanuts are then roasted by using washed beach sand as the medium for heat transfer for pan roasting or a traditional roaster until well cooked. They are usually consumed with banana, roasted or boiled corn, and bread or eaten as a snack. Acceptability of roasted peanuts depends on the colour, crunchiness, and nutty flavor (Lustre *et al.*, 2007).

Roasting is one of the effective methods of reducing aflatoxin levels in peanut products. Aflatoxin level degradation has been found to be time and temperature dependent and roasting at 120°C for 120 minutes and 150°C for 30-120 minutes resulted in an appreciable depreciation in the level of aflatoxins (Yazdanpanah *et al.,* 2005). Aflatoxins are however stable during roasting processes but persist into finished foods such as peanut butter (Scott, 1984).

2.3.2 Fresh Boiled Peanuts

Fresh boiled peanuts are prepared by first; thoroughly washing the unshelled peanuts in clean water and then adding the peanuts to boiling water to which salt has been added to the desired taste until well cooked. The salted water is then drained from the boiled peanuts and the boiled peanut kept in a cool and dry place to prevent spoilage (Saalia *et al*., 2008; Woodroof, 1983, Abdulrahaman *et al.*, 2014). Fresh boiled peanuts are usually eaten as a snack.

Boiling reduces the level of mycotoxins but does not eliminate them totally (Bullerman and Bianchini, 2007). Njapau *et al.* (1999) found similar results where boiling of peanut meal yielded a moderate reduction in aflatoxin content as compared to roasting of whole peanut kernels which greatly reduced aflatoxin content in the raw peanuts.

2.3.3 Peanut Paste

Peanut paste can be prepared using any of the varieties of peanuts. It is produced by first sorting the peanuts and roasting them using pan roasting or the traditional roaster until it's well cooked. After roasting the peanuts, they are poured on a jute sack, dehulled and winnowed. The peanuts are then milled and kept in plastic or glass containers. It is ensured that the peanut paste does not come into contact with water so as to prolong its shelf life (Saalia *et al*., 2008; Opoku, 2013; Kpodo and Gyato, 1996).

Siwela *et al*. (2011) determined the aflatoxin carryover during large scale peanut butter production using reverse phase HPLC incorporating pre-column trifluoroacetic acid derivatization. They found that there was a total aflatoxin percentage reduction of 51% after roasting, 27% after blanching/de-skinning and 11% after grinding to make peanut butter. Thus, there was a cumulative reduction in aflatoxin levels of 89% during the processing of peanut butter.

Yentur *et al*. (2006) determined the aflatoxin levels in peanut butter using high performance liquid chromatography method and found mean levels of aflatoxins B_1, B_2, G_1 to be 15.756±3.129 ng/g, 1.232±0.244 ng/g and 9.689±1.005 ng/g respectively.

2.3.4 Peanut Soup

Peanut soup is prepared with peanut paste, tinned tomatoes, tomatoes, onions, pepper, meat, fish, salt and other spices as preferred. There are two methods that have been documented on the preparation of peanut soup. The first method is by adding water and tinned tomatoes to the peanut paste and mixing until oil separates out. An appropriate

amount of water is added to the mixture and already steamed or cooked meat and fish are added to it. This is then allowed to simmer until it's well cooked.

The second method involves steaming meat and fish with spices and adding groundnut paste and tinned tomatoes to the mixture. An adequate amount of water is then added and allowed to simmer until it's well cooked. Peanut soup can be eaten with *kenkey, gari*, rice, yam, *fufu, omotuo, banku* and many other foods as preferred (Saalia *et al*., 2008; Abdulrahaman *et al*., 2014; Opoku, 2013; Ghana recipes, 2006).

2.3.5 Saabo

Saabo is produced with corn and peanuts. The peanuts and corn are sorted. The corn is then decorticated and washed together with the peanuts. The corn and peanuts are mixed together with the peanuts put on top of the corn in a corn sheath, tied with a string and boiled in water with salt until well cooked (Saalia *et al*., 2008; Abdulrahaman *et al*., 2014; Dovlo, 1973).

2.3.6 Peanut Cake

Peanut cake locally known as *nkati* cake is prepared using the following ingredients: peanuts and sugar. The peanuts are sorted, roasted, dehulled and allowed to cool. The peanuts are then pounded in a mortar into a coarse mass or rough powdery texture. The sugar is melted, poured over the coarse mass and mixed until it becomes stiff. The mixture is then rolled into flat, kite shapes or any preferred shape for consumption or arranged in a glass framed box for sale (Saalia *et al*., 2008; Abdulrahaman *et al*., 2014; Adjou *et al*., 2012; Simmons, 1975; Kpodo and Gyato, 1996).

The incidence and consumer awareness of toxigenic *Aspergillus section flavi* and aflatoxin B1 in peanut cake from Nigeria was determined by Ezekiel *et al.* (2013). All analyzed peanut cake samples contained AFB_1 in concentrations exceeding the recommended levels reaching levels as high as 2824 µg/kg. *Aspergillus flavus* was recovered from 83% of the samples analyzed making it the most dominant across the areas of sampling. A similarly high aflatoxin level in peanut cake samples was detected by Ezekiel *et al.* (2012) which far exceeded the USDA maximum limit of 20 µg/kg in about 90% of the samples.

2.3.7 Kulikuli

Kulikuli is prepared by sorting, roasting, dehulling and winnowing peanuts. The roasted peanuts are then milled into a smooth paste and water added to it in bits whiles simultaneously kneading until the texture of the paste toughens and oil begins to separate out. After the oil is collected, the cake that is left is what is used to prepare the *kulikuli*. Powdered pepper, salt and other spices are added to the peanut cake and fried in oil until it becomes crispy. They can then be consumed or arranged in a glass box for sale (Saalia *et al.*, 2008; Kpodo and Gyato, 1996; Abdulrahaman *et al.*, 2014; Adjou *et al.*, 2012; Simmons, 1975).

Adjou *et al*. (2012), detected high concentrations of aflatoxins in *kulikuli* samples. They were between 25.54 to 455.22 µg/kg for AFB1, 33.94 to 491.20 µg/kg for AFB2, 0.41 to 100.33 µg/kg for AFG1 and 22.04 to 87.73 µg/kg for AFG2 (Adjou *et al.*, 2012).

2.3.8 Darkowa or Dzowe

This is prepared with corn, peanuts, sugar, salt, pepper and other spices. First of all, the corn and peanuts are sorted and roasted together. The peanuts are then dehulled and allowed to cool. The corn and spices are milled together (Dovlo, 1973). Subsequently, the dehulled peanuts, sugar and salt are added to the milled corn and spices and milled. The mixture is then moulded into spherical shapes and packaged in a glass frame box for sale and consumption (Saalia *et al*., 2008; Dovlo, 1973; Abdulrahaman *et al.*, 2014; Lokko *et al.*, 2004).

2.3.9 Khebab Powder or Yaji

Khebab powder is also produced with peanuts and is a by-product of peanut oil processing. It is prepared by sorting, roasting, dehulling and winnowing peanuts (Kpodo and Gyato, 1996). The roasted peanuts are then milled into a smooth paste and water added to it in bits whiles simultaneously kneading until the texture of the paste toughens and oil begins to separate out. After the oil is collected, the cake that is left is what is used to prepare the *kulikuli*; which is the fried peanut cake. The *kulikuli* is then milled into fine powder (Saalia *et al*., 2008; Kpodo and Gyato, 1996; Abdulrahaman *et al.*, 2014).

2.3.10 Peanut Oil

Peanut oil is processed by sorting, roasting, dehulling and winnowing peanuts. This is followed by milling of the peanuts into a smooth paste. Cold water is then added to the peanut paste immediately after milling and kneaded until oil separates out. Alternatively, warm water is added to the peanut paste and kneaded if the peanut paste is allowed to go cold after being milled. Water is added bit by bit to the peanut paste whiles kneading at the

same time until oil begins to separate out and the peanut paste changes texture into a tougher mass. The oil that separates out is then collected bit by bit and re-heated to remove residue water that might be left in to cause spoilage (Saalia *et al*., 2008; Kpodo and Gyato, 1996; Lustre *et al.*, 2007; Abdulrahaman *et al.*, 2014).

Peanut oil could be contaminated with aflatoxins, which are detrimental to human and animal health (Bao *et al*., 2010). Work done by Dwarakanath *et al.* (1969) showed that aflatoxins was absent in refined and hydrogenated peanut oil samples. Aflatoxins were detected in unrefined peanuts but were removed after ten minutes of frying at a temperature of 150°C. Dwarakanath *et al.* (1969) also found that to totally remove the aflatoxins from the peanut oil, they need to be heated at a temperature of 250°C.

Parker and Melnick (1966) who performed the first study of the fate of aflatoxins in vegetable oils undergoing processing found that crude peanut oil contained small amounts of aflatoxins and that majority of the aflatoxins remained in the peanut cake. They found that alkali refining, washing of the oils and bleaching reduced the aflatoxin levels to less than one (1) ppb.

2.4 PEANUT PROCESSING INDUSTRY IN GHANA

Risks that are associated with the consumption of aflatoxin infested peanuts can be reduced by using specific processing and decontamination procedures (Park, 2002). The effectiveness of these processes is dependent on the chemical stability of the mycotoxin, nature of the processes, type and interaction with the food and interaction with multiple mycotoxins if present (Park, 2002).

Peanut processing in Ghana is a batch process. It involves unit operations which include: Sorting, Roasting, Dehulling, Winnowing, Milling, Kneading, Molding and Packaging (Kpodo and Gyato, 1996).

2.4.1 Sorting

Sorting is done during peanut processing to get rid of any foreign matter that may be present in the kernels and that can also cause harm to the consumers. This step or unit operation is very essential in peanut processing since it enables the processors to get rid of the spoilt or moldy kernels from the lot thereby reducing the likelihood of aflatoxin contamination to the barest minimum (Codex, 2004; Galvez *et al.,* 2003). Hand sorting and electronic colour sorting can remove highly aflatoxin contaminated peanuts from a lot (Dorner, 2008).

Dickens and Whitaker (1975) tested the efficacy of electronic sorting and hand picking to remove aflatoxin contaminated kernels from commercial lots of shelled peanuts and found that electronic sorting was less effective compared to hand sorting which was more effective.

Kpodo and Gyato (1996) in a research found out that most of the women processors (about 91% of the respondents) sorted their peanut kernels before using them for processing. Those who did not do the sorting believed that the farmers had already done so before selling. They further revealed that there was a processor who sold her spoilt peanuts to a food joint in the vicinity to be used in the preparation of soup for sale commercially.

Galvez *et al.* (2003) have been able to develop a manual sorting procedure to eliminate aflatoxin contamination in raw peanuts which could be transferred to the peanut processing industry in Philippines. Their results demonstrated that the sorting process developed was efficient and that pre-heating the roaster to 140°C and blanching peanuts for 25 minutes at 140°C facilitated sorting of aflatoxin contaminated peanuts after de-

skinning and further reduced the aflatoxin levels of the peanuts to extremely low levels which made the peanuts safe for consumption.

Also, Whitaker *et al*. (2005) determined the effect of sorting farmer's stock peanuts by size and colour on partitioning aflatoxin into various shelled peanut grade sizes. After shelling and removal of foreign materials from the peanut lot, the shells were divided into various grades which include: Jumbo, medium, number 1, other edibles, sound splits and oil stock and colour sorted. After colour sorting of peanuts ,the aflatoxin concentration of Jumbo, medium, number 1 and other edibles grades had an aflatoxin level reduced by 37.8%,30.9%,28.8% and 32.2% respectively. This proves how important the sorting step is in reducing aflatoxin to very low levels.

Pelletier and Reizner (1992), compared fluorescence sorting and colour sorting for the removal of aflatoxin from large groups of peanut and found that fluorescence sorting was not effective as an aflatoxin control method. Hand sorting was shown to be more effective in the removal of aflatoxin in peanuts compared to machine sorting and fluorescence sorting (Pelletier and Reizner, 1992).

However sorting comes along with a lot of tedium especially where the quantity of peanuts to be processed is so much. There is therefore the need to educate the processors on the importance and health benefits of separating the bad peanuts from the good ones.

2.4.2 Roasting

Roasting in Ghana is usually done manually using aluminium pans, cast-iron pans and earthen ware pans. A piece of calabash is used in stirring during roasting. Firewood and charcoal are usually used as the main source of heat during the heating of peanuts. Just a

negligible number of processors use LPG (liquefied petroleum gas) (Kpodo and Gyato, 1996). Other processors also use the roaster which is mechanical and less tedious. Most peanuts are roasted with or without washed sea sand.

Although prevention is the most effective intervention, heat has been used to inactivate aflatoxins in contaminated foodstuffs (Yazdanpanah *et al.*, 2005). They found that roasting spiked pistachio nuts caused substantial reduction of aflatoxins at 120°C for 120 minutes and 150°C for 30-120 minutes.

During roasting amino acids and carbohydrates react to produce tetrahydrofuran derivatives which improve and develop the typical peanut flavor and taste (Woodroof, 1983). Peanut roasting dries the peanuts and causes their colour to turn brown due to the staining of the peanut cell wall with peanut oil. Roasting also reduces the microbial load to the barest minimum (Kpodo and Gyato, 1996).

Ogunsanwo *et al.* (2004) demonstrated in their research on the effect of roasting on the aflatoxin contents of Nigerian peanut seeds that roasting causes a reduction in the aflatoxin content of peanuts and thereby made the assertion that heat reduces the aflatoxin content of agricultural products. They further explained that aflatoxin G1(AFG1) was more heat labile and reactive as compared to aflatoxin B1(AFB1) (Ogunsanwo *et al.*, 2004; Bbosa *et al.*, 2013). This is as a result of differences in their chemical structure; AFG1 has two ether linkages whiles AFB1 has one ether linkage. These ether linkages are more susceptible to chemical attacks and thus the presence of two of these linkages in AFG1 makes them more thermo labile and therefore highly degraded as compared to the AFBI (Ogunsanwo *et al.*, 2004). Lustre *et al.* (2007) have also developed a roasting procedure with a roasting

temperature of 250°F and 30 minutes as the roasting time. A range of 1.1-2.2% was established as the roasted peanuts safe moisture range. In Ghana, completion of roasting of peanuts is confirmed by feeling them in the hand, the aroma and the colour.

2.4.3 Dehulling

The dehulling process during peanut processing is mainly done manually. There are three main ways of traditionally dehulling peanuts which includes: using the hands, using a wooden board to dehull and also gently pounding the roasted peanut kernels in a mortar (Kpodo and Gyato, 1996). Generally, there are two peanut peeling machine types namely: dry peeling machine; which is highly efficient and usually used to remove the red skin of peanuts and wet milling machine (Ogunwole, 2013). Ogunwole (2013) has been able to design and fabricate a manually and electrically operated roasted peanut decorticating machine to solve problems such as blowing air through roasted peanuts in order to remove chaff, reducing the stress and time involved in peeling peanuts as well as improving the cleanness of the roasted peanuts. This machine can be used in both urban and rural areas where there might be no electric power supply. However, it has been shown that using the hands to dehull greatly minimizes the amount of losses though the whole process is laborious (Kpodo and Gyato, 1996). The ease of dehulling is dependent on the variety of peanut being used, the degree of roasting, size of kernel and the amount of force applied.

Deshpande *et al.* (1982) demonstrated in their work on the effect of dehulling on certain functional properties of dry bean (*Phaseolus vulgaricus* L.) that dehulling improved the water absorption capacities as well as the oil absorption capacities of bean flours by 3-39% and 10-44% respectively.

Galvez et al. (2002) showed that de-hulled and well sorted peanuts had no aflatoxins or very low levels of aflatoxins in them. Thus, it is very important to properly dehull the peanuts to ensure that aflatoxins are greatly reduced.

2.4.4 Winnowing

After dehulling has been done, the hulls are separated from the kernels by allowing the wind to passing over it and thereby removing the unwanted hulls. If washed sea sand is used in roasting, it is sieved out before dehulling is done (Kpodo and Gyato, 1996). This processing step is very cumbersome and some of the chaff produced can enter into the eye of the processors as a result of the chaff that is produced when air is blown through the roasted and peeled peanuts. Furthermore loss of some of the peanuts may occur resulting in contamination of the products. There is therefore the need to develop cleaning and winnowing machines that can facilitate the work of the processors and also increase profit margins (Ogunwole, 2013).

Arafa *et al.* (2009) have been able to develop a local machine for winnowing and grading flax seeds (Sakha 2-variety) for better clean seeds and grades. A high seed purity of 99.33% and grading efficiency of 99.69% were reached using vibration frequency of 130 Hz, mesh hole size of 3mm, feed rate of 1400 kg/h and moisture content of 12.5%. Also, a cleaning device for threshed seeds has been developed by Muhammad *et al.*(2013) consisting of an air-blast fan and two reciprocating shakers containing replaceable sieves for different crop seeds as well as a collecting fan (Muhammad *et al*.,2013).

2.4.5 Milling

Milling is a size reduction process of the seeds into smaller particle forms. This is the only fully mechanized unit operation in the processing of peanuts. Different types of equipment have been designed for milling for household or industrial purpose. The disc attrition mill is mostly used in the milling of roasted peanuts. However peanut paste is suspected by most consumers to be highly adulterated with substances such as cassava flour and maize flour to increase the profit margin (Kpodo and Gyato, 1996).

Cheli *et al.* (2013) found in their review that milling can minimize mycotoxin concentrations in wheat fractions used for human consumption. They also emphasized on the need for mill managers to put in the needed measures to ensure that there is reduction in the risk of mycotoxin contamination (Cheli *et al.*, 2013).

2.4.6 Kneading

This is the stage at which peanut oil is extracted. This is achieved by mixing the peanut paste with small quantities of water intermittently and continually pressing the mixture in-between your fingers until oil separates out. This is a rather tedious process and therefore calls for the need for mechanization in order to make the process less laborious and also increase the profits obtained by processors (Kpodo and Gyato, 1996). Not much is known about the association between kneading and aflatoxins.

2.4.7 Packaging

The main purpose of food packaging is to maintain the safety, wholesomeness, and quality of food (Mash and Bugusu, 2007). Coles (2003) also states that the goal of food packaging is to contain food in a cost-effective way that satisfies industry requirements and consumer

desires, maintains food safety, and minimizes environmental impact. Peanut paste is usually collected in aluminium pans after milling. Some processors further dispense them into plastic buckets, plastic containers, polyethylene bags and plastic trays for retailing at the market (Kpodo and Gyato, 1996).The time taken for the peanut paste to be sold is very crucial since prolonged exposure to environmental conditions such has moisture and high temperatures supports the growth of molds which subsequently produce aflatoxins known to cause adverse health effects such as gastro- intestinal defects and liver cancer (Galvez *et al.*, 2002; Milani, 2013). Also, peanut paste is sold by being scooped with spoons and sometimes the hands which has the potential of increasing the microbial load in the paste and must be discouraged.

Mutegi *et al.* (2012) found that packaging material for peanuts significantly influenced the amount of aflatoxin in peanut products with 68% of the peanut samples stored in plastic jars having more than 10 µg/kg of aflatoxin.

2.4.8 Further Processing of Peanuts

Peanuts are further processed into peanut oil, peanut cake locally known as *tunkusa* and *khebab* powder. *Tunkusa* is partially defatted peanut paste. The preparation of this product is very similar to that of peanut paste. Peanuts are roasted, dehulled, winnowed and milled. Salt is sometimes added to the roasted and dehulled peanuts before milling. This is to increase the shelf-life of the final product. After milling, hot water is added and wooden ladles used to knead the mixture until oil separation commences (Kpodo and Gyato, 1996). Oil separation during the kneading process takes between 15 and 20 minutes. The oil is skimmed off and more hot water added and the kneading process repeated till no more oil separates out. The yield of oil ranges from 1/2 a liter to 1 liter per 2.5kg of peanuts. The residue left after oil has been skimmed off is *tunkusa*. The average lifespan of this product

is three days after which if not sold off are moulded into balls or other fancy shapes and deep fried in peanut oil into a product named *Kulikuli*. *Kulikuli* is eaten as a snack food whilst *tunkusa* is used for the preparation of soups and stews just like peanut paste. It is widely patronized by local restaurant operators because of its reduced cost when compared to peanut paste (Kpodo and Gyato, 1996).

2.5 PEANUT OIL

Peanut oil contains more oil as compared to other food crops (Abdulrahman *et al.*, 2014). Peanut oil is obtained after peanut seeds have been sorted, roasted, milled into paste and small quantities of water added to the peanut paste whiles kneading at the same time till there is a separation of phases. The oil is then collected into a saucepan and fried to remove any water that might still be present in it so as to ensure that oxidation does not occur and result in spoilage of the oil.

The seeds of most peanut cultivars contain about 50% oil (Worthington and Hammons, 1971), and therefore the quality of the oil and groundnut products depend to a large extent on the oil fraction. The oil content of peanut differs in quantity, the relative proportion of fatty acids, geographical location, seasons and growing conditions (Brown *et al*., 1975; Holaday and Pearson, 1974; Young *et al*., 1974). Groundnut seed contains 44 to 56% oil and 22 to 30% protein on a dry seed basis and is a rich source of minerals (phosphorus, calcium, magnesium, and potassium) and vitamins (E, K, and B group) (Savage and Keenan, 1994). Akuamoah-Boateng *et al.* (2007) have discovered in their research that peanut oil is energy dense. Lipid oxidation is the main cause of flavour deterioration and production of off-flavour peanuts and its by-products, due to a high content of unsaturated fatty acids (Reed *et al.*, 2002; Lee *et al.*, 2002). Oxidation of the fatty acids in peanut oil

can be caused by light, heat, air, metal contamination, micro-organisms, or enzymatic activity (Sanders *et al.,* 1993).

Work done by Zuberu *et al.* (2013) on the cost components involved in the production of groundnut oil and soya milk in Tamale has shown that the cost of raw materials needed for the production of peanut oil greatly affects the amount of profit gained by the processors. Furthermore, they found that the revenue obtained from groundnut oil far exceeded (three times more) that from soya milk because the processors were not only able to sell the peanut oil but its by-products such as the *khebab* powder (Zuberu *et al.,* 2013). The processors however sited inadequate capital, power failure, stress and tiredness due to large quantities of peanuts being produced as some of the constraints associated with peanut oil in the Tamale metropolis.

2.6 MYCOTOXINS

The term 'mycotoxin' was first used in 1961 after the outbreak of the turkey-X disease in England during which thousands of birds and domestic animals died as a result of infection with aflatoxin contaminated peanut meal fed to them (Richard, 2007). Mycotoxins are low-molecular weight compounds that are synthesized during secondary metabolism of fungi that are toxic to animals and humans when consumed in food (Paterson and Lima, 2010; Galvez *et al.*, 2002). Exposure to mycotoxins may be through inhalation, consumption of infected food and dermal contact. The mycotoxins to be expected in food differ from one country to another as a result of the different climatic conditions, crops and agronomic practices (Bryden, 2007).

Mycotoxins have attracted attention worldwide because of their economic losses that is associated with their impact on human health, animal productivity, domestic and international trade. Mycotoxins are of public health concern especially in developing countries where the environmental conditions are optimal for their growth. Currently, more than 400 mycotoxins have been found. Much research work has been focused on those that have public health concern and those that also greatly affect human food and animal feed and these include: aflatoxins, ochratoxins, fumonisins, zearalenone and patulin.

The production of mycotoxins is highly linked to temperature, moisture, water activity, pH and oxygen concentration. They have both acute and chronic effects on humans and animals and the degree of these effects is dependent on the age, sex, weight, diet, exposure to infectious agents and the presence of other mycotoxins (Zain, 2011). Aflatoxins are the best known and most widely studied mycotoxins (WHO, 2006). They are carcinogenic compounds and very toxic to humans and animals (Wogan, 1999). They usually affect crops grown in the tropics and sub tropics where the climatic conditions are optimal for their growth.

Peanuts are susceptible to mycotoxin contamination (Adjou *et al.*, 2012, Gourama and Bulleman, 1995). Mycotoxins are known to not only affect the quality of peanuts and its by-products, but also have adverse effects on the health of humans and animals. They are also serious barriers to trade from one country to another and result in great economic losses. The mycotoxins that are known to affect peanuts are aflatoxins and ochratoxin A (Pittet, 1998).

2.7 AFLATOXINS

Aflatoxins are naturally occurring mycotoxins produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Masters *et al.*, 2013, Gunstone, 2011, Gourama and Bulleman, 1995). *Aspergillus spp.* grows ubiquitously on plants and crops from tropical and subtropical areas: peanuts, figs, spices, corn, maize, wheat and grains. *Aspergillus* growth and production is dependent upon the temperature, humidity, host plant type, and the strain of fungus; high humidity usually required for growth. Consumption of 55 µg/ kg of aflatoxin daily for an unknown period of time may also result in death (FDA, 1992).

In developed countries where there is technological advancement and the necessary standards enforced in order to reduce the level of aflatoxin contamination, the level of aflatoxin in peanuts is low. However in developing countries the situation is different. Aflatoxin is usually found in the protein part of peanuts usually the peanut cake. Negligible amounts are detected in refined peanut oil. However crude peanut oil which may contain traces of peanuts may contain some aflatoxins (Gunstone, 2011).

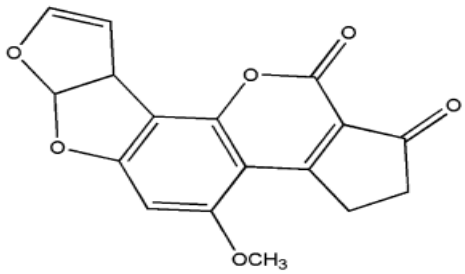
Aflatoxin contamination usually occurs after post-harvest handling of peanuts as well as during its marketing (Adu-Gyamfi, 2013).

Research has shown that about 86% of peanut processors do not dry the peanuts they purchase very well before usage; most especially those that have been dried a little before being sold (Kpodo and Gyato, 1996). Even those that are dried are mostly dried on cement floors in the homes of the processors or on polythene bags. However, the practice of drying the peanuts on the polythene bags has the potential of causing condensation in the peanut seeds and thus cause accumulation of moisture and subsequently infestation by mycotoxins; most especially aflatoxins (Kpodo and Gyato, 1996).

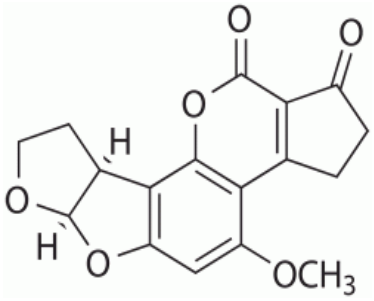
Kpodo *et al*. (2005) determined aflatoxin contamination in maize, peanuts and their products in Accra, Ghana and found that out of a total of 91 peanut samples, 37 samples contained more than 4 µg/kg (EU maximum permissible limit) for aflatoxins with 30 samples exceeding the Ghana Standard Board maximum permissible level of 15 µg/kg. The highest total aflatoxin levels recorded for raw peanuts and peanut products were 24,873 µg/kg and 1.260 µg/kg respectively.

2.7.1 Types of aflatoxins

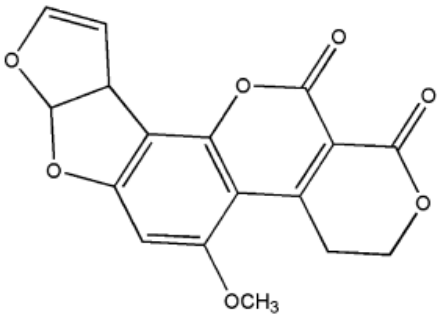
The chemical structure of aflatoxins consists of difurocoumarin derivatives that fluoresce under ultraviolet light (Figure 2.1). Depending on the colour of the fluorescence, aflatoxins are divided into aflatoxins B1 (AFB1) and B2 (AFB2) for blue; and GI (AFG1) and G2 (AFG2) for green as shown in Figure 1 (Guo *et al*., 2009). Aflatoxins M1 (AFM1) and M2 (AFM2) which are mostly known as milk-aflatoxins are the metabolites of aflatoxin B1 and aflatoxin B2 respectively (Huang *et al*., 2010). Also, aflatoxin Q1 and aflatoxicol are metabolites of aflatoxin B1. Aflatoxin B1 is the most potent and most carcinogenic amongst the types of aflatoxins (Creppy, 2002).



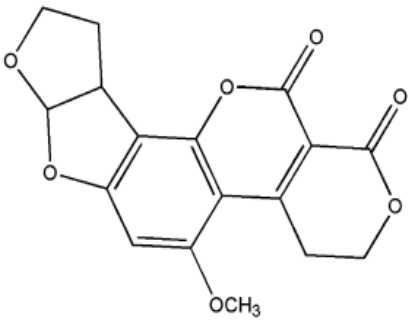
Aflatoxin B1



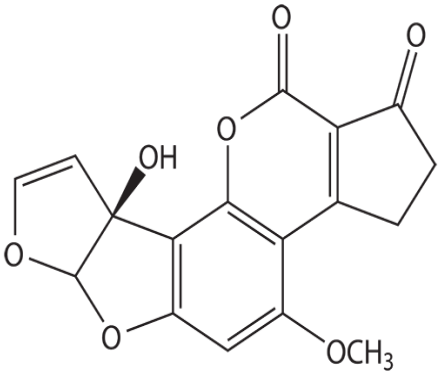
Aflatoxin B2



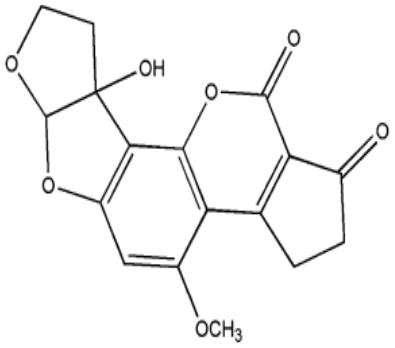
Aflatoxin G1



Aflatoxin G2



Aflatoxin M1



Aflatoxin M2

Figure 2.1: Chemical structures of six types aflatoxins

Source: Huang *et al*. (2010)

2.7.1.1 Aflatoxin B1

AFB1 is oxidized by CYT P450 in the liver into AFB1-8, 9-epoxide which is the major metabolite that exerts hepatotoxic effects (David *et al*. 1994). The 8, 9 epoxide is neutralized by conjugation in the liver with GSH (glutathione) by glutathione-S-transferase, an enzyme abundant in some animals (mice), but with rats and humans are relatively deficient (Yahl *et al.,* 1971). One of the most serious effects of the AFB1-8, 9-epoxide metabolite is it reacts with amino acids in DNA and forms an adduct. This adduct is fairly resistant to DNA repair processes and thus this gene mutation may cause carcinoma of the liver. CYP 3A4 is the major CYP 450 enzyme responsible for activation of AFB1 into the epoxide form (Yahl *et al.,* 1971). However, the liver can detoxify AFB1 by oxidizing it to other metabolites such as AFQ1 which has very little cancer-causing potential. These are usually excreted in urine with little effect on the body. The binding of the epoxide with proteins may inhibit the protein from performing its enzymatic functions and also create a reservoir of the toxin in proteins, prolonging exposure (Eaton *et al*. 1994). Both metabolized and unmetabolized aflatoxin is excreted mostly in urine. It is also excreted in milk, stool, feces, and saliva (which may be swallowed and re-enter the gastrointestinal tract). Using animal models, AFB1 has been shown to impair normal immune function either by reducing phagocytic activity or reduce T cell number and function.

Aflatoxin is shown to have a dose response relationship between exposure to aflatoxin and rate of growth among small children. In addition, it also interferes in nutrient modification such as Vitamin A or D in animal models (Yahl *et al.,* 1971).

2.7.2 Causes of aflatoxin production

Production of aflatoxins occurs when the crops are exposed to extreme conditions such as high temperatures, drought, high moisture, oxygen concentration, and infestation by insects and can occur during both the pre-harvest and post-harvest period (Masters *et al.*, 2013; Galvez, 2003; Huang *et al.*, 2010). However Wilson and Payne (1994) have shown that majority of aflatoxin contamination occurs post-harvest during transportation and storage. During storage, high levels of moisture and temperature conditions create a suitable environment for the rapid growth of molds which in turn also metabolize and produce aflatoxins. This therefore calls for the need for appropriate harvest methods and storage conditions to be used and further developed (Milani, 2013; Nautiyal, 2002).

Also, production of aflatoxin is an aerobic process hence depletion of oxygen to 0.1% in the atmosphere lowers the production of aflatoxin (Galvez, 2003). The moisture content of food samples is the most critical factor that affects the growth of aflatoxins. Maximum aflatoxin production can be attained at 25% moisture and 85% relative humidity (Woodroof, 1983). Temperature as stated earlier also influences the type of aflatoxin that is produced. They are mesophiles and grow at an optimum temperature of 36-38°C.

Furthermore, physical damage to the peanut shell during weeding, harvesting, drying, transportation and marketing can cause decay that enhances production of aflatoxin and destruction of exposed tissues. The nut also begins to go rancid and spoilage occurs. Acidic media enhance the growth of aflatoxin and matured peanut seeds are easily penetrated by aflatoxin.

2.7.3 Toxicity of aflatoxins

Aflatoxin contamination of food and feed is a serious problem worldwide. Aflatoxins can have both acute and chronic effects. Human epidemiology and experimental animal studies have provided a statistical association between aflatoxins and risk of liver cancer (Abdulkadar *et al*., 2000; Proctor *et al*., 2004; Nakai *et al*., 2008). World-wide deaths due to liver cancer amount to nearly one million per year and there is an increased incidence of liver cancer in some developing and developed countries (Abdulkadar *et al*., 2000). The International Agency for Research on Cancer has proven the carcinogenicity of aflatoxin B1 and thus placed it in group 1. Aflatoxin B1 which is the most carcinogenic type of aflatoxin is known to be an immunosuppressant and also reduces growth rate, reproductivity and causes anaemia (Wogan, 2002).

Mwanda *et al.* (2005) revealed some symptoms of acute aflatoxicosis which include; vomiting, convulsions, abdominal pain and ultimately death in humans and are serious barriers to trade.

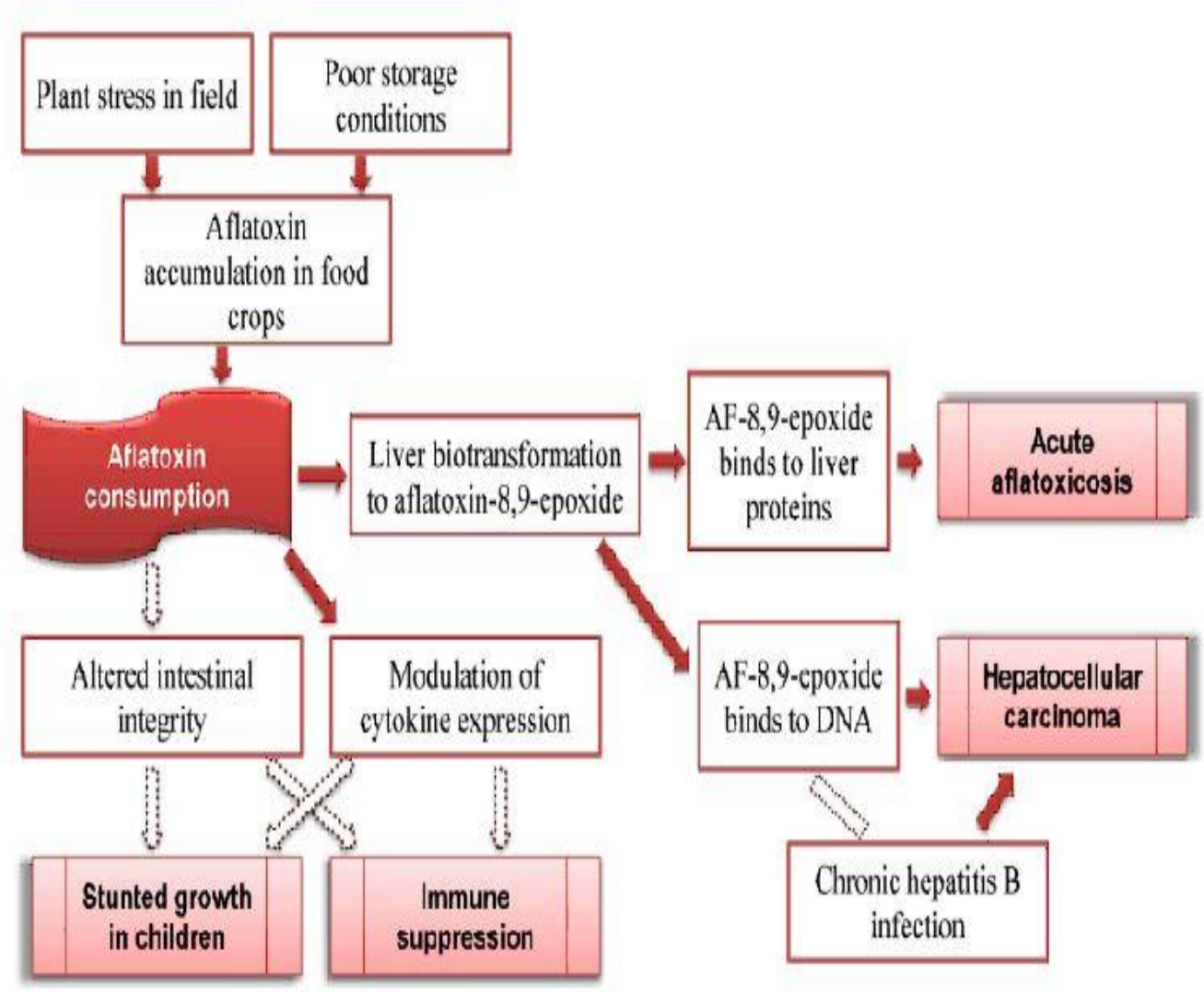


Figure 2.2: Acute and chronic effects of aflatoxins on human health

Source: Wu (2010).

2.7.4 Ways of mitigating aflatoxin contamination

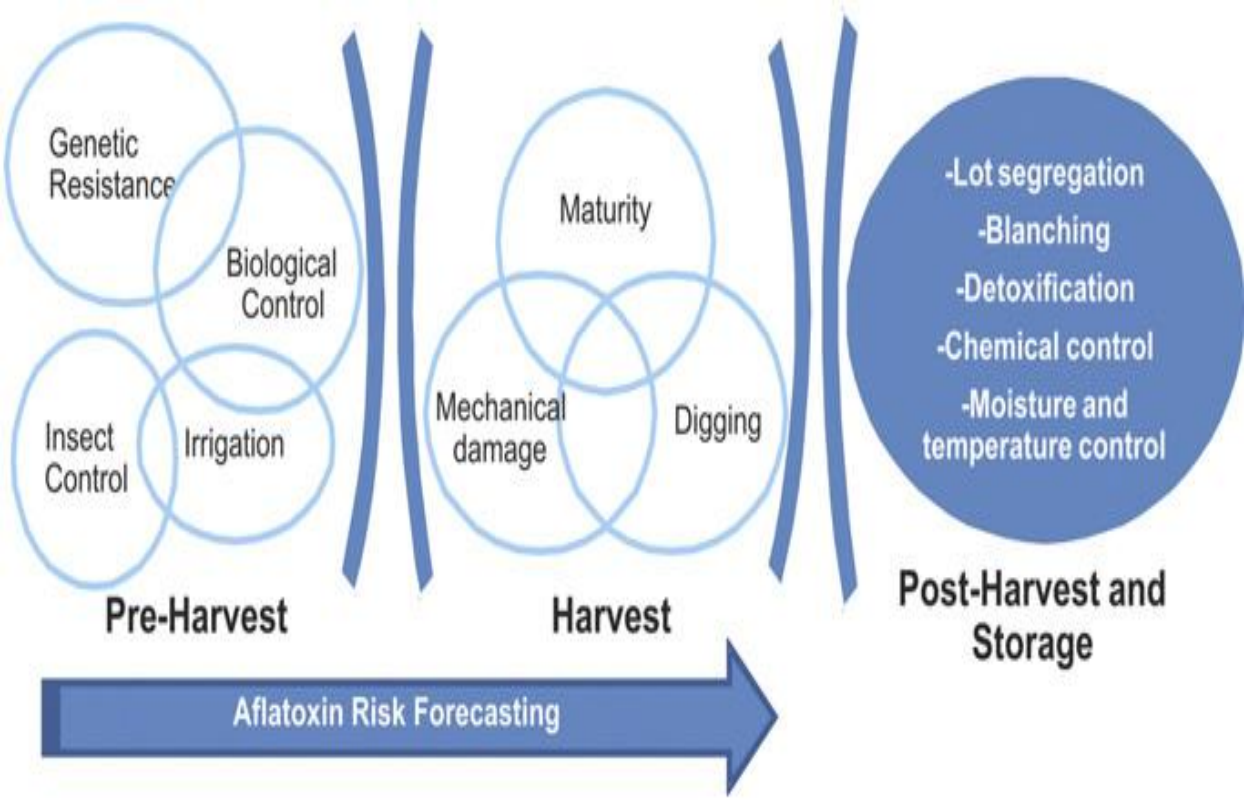


Figure 2.3: Pre- and post-harvest management of peanuts to minimize aflatoxin contamination

Source: Torres *et al.* (2014)

Aflatoxin contamination may occur right from the planting and growing stage of peanuts and throughout the transportation, storage and processing stages. There is therefore the need to put in place measures to ensure they are reduced to very low levels. Torres *et al.* (2014) has reiterated this fact in a review; for the need to reduce aflatoxin contamination before harvest, during harvest and at the post-harvest and storage stages (Figure 2.3).

2.7.4.1 Pre-harvest

The best and most economical principle to follow to alleviate aflatoxin contamination is avoidance through the use of good agricultural and storage practices. Notable in this effort is the development of cultivars of corn and peanuts resistant to infection by certain fungi or resistant to aflatoxin production (Mehan and Ramakrishna, 1986; Nigam *et al*., 2009, Dietzgen, 1999; Liang *et al*., 2006).

It is advised that proper agricultural practices such as crop rotation, tillage, management of irrigation and fertilization be done since it replenishes the soil organic matter and enhances the growth of soil microorganisms that have an inhibiting effect on *Aspergillus flavus* (Huang *et al.*, 2010; Ortiz *et al*., 2011). Dorner (2008) has reiterated *the need of* maintaining high kernel water activity on the farm until harvest time. This preserves the natural defense mechanism of peanuts against growth by aflatoxigenic fungi.

Furthermore, Dorner (2004) has shown that soil inoculation with no toxigenic strains of *Aspergillus flavus* has a carryover effect and may protect peanuts from contamination during storage. In Nigeria, Atehnkeng *et al.* (2008) have used non-aflatoxigenic *Aspergillus flavus* strains formulated into a biocontrol product named Aflasafe™. Zanon *et al*. (2013) did a two-year study to determine the efficacy of an *Aspergillus flavus* strain as a biocontrol agent to reduce aflatoxin production in peanuts under field conditions in Argentina. The results showed that *Aspergillus flavus* AFCHG2 can be applied to reduce aflatoxin contamination in peanuts.

2.7.4.2 Harvest

It is important that peanuts are harvested when matured. Delays during harvesting must be avoided as much as possible. Also, peanuts can be rapidly dried to a water activity level of 0.83 to prevent the synthesis of aflatoxin (Diener and Davis, 1970).

2.7.4.3 Post-harvest

Storage of peanuts should be in a cool and dry place with low moisture content of 8% and without insect infestation. Lots with visible molds should be separated and not used for edible purpose. Dorner (2008) has reiterated that electronic colour sorting is most effective in separating good peanuts from bad ones. It is vital that hygienic conditions are ensured during processing of peanuts and its by-products. In order to reduce the level of aflatoxin contamination in peanuts and peanut based by-products, it is important to ensure that proper sorting of the peanut grains is done. This ensures that grains that are contaminated by aflatoxins are removed, thereby resulting in reduced levels of aflatoxins and ensuring food safety (Galvez *et al.*, 2003; Codex Alimentarius, 2004).

Work done by Ogunsanwo *et al.* (2004) has shown that there is a decrease in the level of aflatoxins in peanuts and peanut based by-products as the roasting temperature is increased. Peanuts that were dry- roasted at 140°C for 40 min resulted in 58.8 and 64.5% reductions in AFB1 and AFG1. Those roasted at 150°C for 25 min resulted in 68.5 and 73.3% reductions in AFB1 and AFG1, respectively and those roasted at 150°C for 30 min led to 70.0 and 79.8% reductions in AFB1 and AFG1, respectively.

Proctor *et al.* (2004) determined the effectiveness of ozonation and mild heat in breaking down aflatoxin in peanut kernels and flour and to also quantify aflatoxin destruction compared with untreated samples. The results showed ozonation effect increased with

higher temperatures and longer times. It was further revealed that the effect of temperature lessened as exposure time increased, suggesting that ozonation at room temperature for 10-15 minutes could yield degradation levels similar to those achieved at higher temperatures with a shorter exposure time, while being more economical. Aflatoxin reduction was noticed more in aflatoxin B1 and G1 and also in the peanut kernels than in the flour (Proctor *et al.,* 2004).

Dino *et al*. (2013) also concur with the findings of Proctor *et al.* (2004). The efficiency and safety evaluation of ozonolysis of aflatoxin B1 in peanuts was determined. The results confirmed that ozone was effective in degrading aflatoxin B1 in the peanuts. Aflatoxin B1 in aflatoxin contaminated peanuts was reduced by 89.4%. Ozone was efficient in degrading aflatoxin B1 which reduced the risk of liver and kidney damages and improved blood biochemical indexes of test rats used (Dino *et al.*, 2013).

Giddey *et al.* (1977) reported an industrial process to detoxify oilseed cakes using an alkaline, nonvolatile, inorganic agent and highly alkaline, volatile organic agent. Antioxidants such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) have been used in peanut product formulations to reduce aflatoxin contamination.

2.7.5 Detection of aflatoxins

Current analysis of aflatoxins is performed by various methods and analytical techniques(Scott, 1993) including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), nanogold probe-based immunochromatographic assay and enzyme-linked immunosorbent assay (ELISA).All of these methods differ in their sensitivity. Also, new methods have been developed and applied by researchers to

facilitate the detection of aflatoxins. Huang *et al.* (2010) simultaneously detected aflatoxins B1, B2, G1, G2, M1 and M2 in peanuts and their derivative products by using ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). All analytes were successfully separated within less than 9 minutes.

Thin Layer Chromatography gives poor sensitivity, accuracy and separation of analytes and also has a high likelihood of producing false positive results (Haung *et al*., 2010). High Performance Liquid Chromatography technique fitted with fluorescence detectors is the most commonly used method for analyzing aflatoxins. However this method is time consuming because of its larger column particle sizes (5µm) in addition to a tedious pre or post column derivatization process (Haung *et al.*, 2010).

Despite its sensitivity and accuracy, HPLC method is laborious, expensive time-consuming and unsuitable for analysis of many samples. ELISA has been developed rapidly in the past years because of their simplicity, adaptability, sensitivity, and selectivity. It is reliable for the analysis of a large number of samples. Conventional ELISA for AFB1 detection, namely, the indirect competitive ELISA, requires laborious and time-consuming incubation and washing steps. Additionally, enzyme-based labels suffer from the instability due to their denaturation and degradation. Fluorescent linked immunosorbent assays (FLISAs) using fluorescence materials as labels overcome disadvantages of ELISA (Wacoo *et al.*, 2014 ; Reddy *et al*., 1988; Berthiller, 2014).

According to Adjou *et al.* (2012), the AFLACARD® kits can be used in the detection of aflatoxins in peanuts and peanut based by-products. However it has a disadvantage of being able to detect up to 4 ppb levels of aflatoxins which is not as sensitive. Therefore

only low levels of aflatoxins are detected. The LC-MS/MS method is known to be a much sensitive and effective method of determining aflatoxin levels. It gives room for the detection of higher levels of aflatoxins.

2.8 FOOD SAFETY

Food safety covers the range of food related activities from prevention and surveillance to detection and control. In 2005, it is estimated that 1.8 million people (from the United States of America) and 800,000 children (from Africa) died from diarrheal diseases and dehydration. According to the Ghana Food Safety Action Plan (2003), one in forty Ghanaians suffers serious food-borne illnesses. Food borne illnesses results in huge loss of money annually in medical costs and loss of productivity. Codex Alimentarius recommends a HACCP based approach wherever possible to enhance food safety. Codex guidelines and HACCP approach forms the core of the entire food safety program (FAO, 2005).

2.8.1 Hazard Analysis and Critical Control Points (HACCP)

HACCP is the most important and useful food safety management tool used across the globe in food industries as a result of its systematic and preventive approach. The HACCP system was first used in the 1960s by Pillsbury Company in the production of safe food for the United States Army and The United Stated National Aeronautics and Space Administration (NASA).In the 1980s and 1990s; the HACCP system was adapted by other food manufacturing companies globally (Ross-Nazzal, 2007).

2.8.2 Advantages of HACCP

HACCP has a number of advantages for food safety management which include:

- It ensures accountability (WHO, 1997).
- It ensures transparency and consistency (Sperber, 2005).
- It ensures continuous improvement and partnership building (GMA, 2010; US EPA, 2007).
- It ensures that decisions are based on reflection and best judgment (Motarjemi *et al*., 1996).
- It focuses on the critical point(s) in food processing and handling required for food safety, thus safety is designed and manufactured into the product (Talamini and Malafaia, 2010).
- It is science based and systematic allowing for the specific identification of food safety hazards thereby preventing food borne illness (Walker *et al*., 2003).
- It requires the implementation of measures to control these hazards where significant and ensures the sanitary safety of food (GMA, 2010).
- It employs the principle of risk assessment allowing prevention to be based on the control program rather than inspection and testing thereby protecting the reputation of the food industry (Talamini and Malaia, 2010).
- It reduces losses as a result of product recall (Motarjemi *et al*., 1996).
- It ensures the better use of economic resources and ensures a reduction of costs of food analysis (Yamina *et al*., 2014, Unnevehr and Jehnsen, 1999).

2.8.3 Application of HACCP

HACCP is intended to identify specific food safety hazards which can cause illness, injury or death if consumed and to put in place controls for them. It is also to build on the existing best practices employed and focus on specific hazards and steps where significant hazards need special control. Thus prior to the application of HACCP along the food chain, prerequisite programs such as good hygienic practices according to Codex General Principles of food hygiene, appropriate Codex Codes of Practice and appropriate food safety requirements need to be put in place. These should be well established, fully operational and verified in order to facilitate the success and full application and implementation of the HACCP system. Also, application of HACCP requires commitment by management and all stakeholders. Also, quality data and documentation of analysis, hazard identification, risk assessment and validation are all required (GMA, 2010).

2.8.4 Principles of HACCP

HACCP is a dynamic and preventive program composed by seven principles which have been designed to develop the level of understanding of hazards, their identification and implementation of controls (Codex Alimentarius Commission, 2004):

(1) Conduct a Hazard Analysis

(2) Critical control points (CCP) identification

(3) Target levels and Critical limits establishment

(4) Implementation of a monitoring system to control the CCP

(5) Corrective actions proposition

(6) Correction procedures establishment and

(7) Documentation and record procedures.

2.8.5 Application of HACCP principles

According to Codex (2009), the HACCP principles can further be implemented using twelve steps which include:

(1)Assemble HACCP team

(2)Describe product

(3)Identify intended use

(4)Construct flow diagram

(5)On-site verification of flow diagram

(6)List all potential hazards associated with each step, conduct a hazard analysis, and consider any measures to control identified hazards.

(7)Determine critical control points

(8)Establish critical limits for each CCP

(9)Establish a monitoring system for each CCP

(10)Establish corrective actions

(11)Establish verification procedures

(12)Establish documentation and record keeping.

Wallace *et al*. (2012) investigated on the knowledge of the HACCP team and how it affects decision making for a successful HACCP development. In their findings, they found out that the HACCP team total knowledge scores were better than or equal to the median of the individual team scores, however the team scores were poorer than the best individuals for five out of the eight teams, suggesting a leveling out of knowledge within

the group as the individual member's knowledge meets make a consensus that becomes the team decision. They further recommended that food companies recognize the importance of HACCP teams being made up of members with the correct blend of technical and HACCP principle application expertise, practical experience, team-working, administration and leadership skills, and that HACCP teams are allowed sufficient time to perform their important role in food safety management. Wallace *et al*. (2014) also holds similar views as Wallace *et al.* (2012).

Also, Perez *et al*. (2011) investigated the microbiological contamination levels in prepared or cooked foods in a HACCP environment. They found that the ingredients, time of refrigeration and degree of handling affected the microbiological quality of the samples. No significant differences were detected in the microbial levels. It was stressed that the HACCP system can be successful at minimizing the risks only when there is regular control of food production.

A study by Shih and Wang (2011) showed that differences in age, gender and job position as well as the confidence of staff in implementing HACCP are factors that may influence the application of HACCP as a food management tool in Taiwanese public health kitchens. The results showed that among managers of hospitals which had no HACCP in place, the expected level of implementation was 3.4-4.56 whiles that of managers of hospitals that implemented HACCP was 2.68-3.41.This stressed the need of hospitals to provide more funds, support, facility, HACCP training and advice from experts and consultants in order to eliminate of their staff in implementing the system (GMA, 2010).

Also, Sampers *et al*. (2012) conducted a semi-quantitative study to evaluate the performance of a HACCP-based food safety management system (FSMS) in Japanese milk processing plants and found that food safety output is not only affected by the food safety management system but also depends on the riskiness of the context characteristics. A more risky context requires a more advanced FSMS to achieve a good food safety output. The Japanese milk companies which were technologically inclined scored high on their control activities but scored less in the managerial quality assurance. Smaller companies without HACCP being implemented had lower scores for control activities and preventive measures.

Furthermore, Cerf and Donnat (2011) showed that the HACCP system is not fully applicable at the primary production level, and that food safety is obtained through the careful implementation of good hygiene practice (GHP) at the farm. Also, guides to GHP intended at primary productions cover one activity yet most farms have more than one activity and hazards from one activity can be transferred to another activity. They suggested that a horizontal complement to the Guides to GHP that would consider only the application of the Step 6 and Principle 1 of the HACCP system amended as: List all potential hazards transferred from one activity to another activity within the farm, conduct a hazard analysis, and consider any measures to control identified hazards.

Dzwolak (2014) investigated the implementation of HACCP in small Polish food businesses. A recording by exceptions method was developed in order to reduce the recording time. The need for accurate and reliable monitoring by workers in order to increase the level of food safety was emphasized.

In addition, Green and Kanek (2012) determined the effectiveness of Environmental Health Officers (EHOs), in identifying and controlling the significant risks of foodborne illness in small catering operations in the United Kingdom. The results showed a weakness in the concept of the implementation of Hazard Analysis Critical Control Points, HACCP; in that Micro Owners/Managed Catering Businesses did not have the scientific expertise to comprehensively identify the significant risks in their businesses, while EHOs had the scientific knowledge but failed to appreciate the practical differences of the systems within the business in sufficient detail to identify these potential problems. The research further highlighted the need to pool respective talents in order to effectively identify and control the significant risks of foodborne illness within the Micro Owner/Managed Catering Business sector.

Kafetzopoulos *et al*. (2013) determined the effectiveness of the HACCP Food Safety Management System and proved the three-dimensional nature of the HACCP objectives which consists of hazard identification, hazard assessment and hazard control. Further analysis of the data also revealed that HACCP can be used by a food company as a self-assessment tool and a benchmarking tool.

Osimani *et al*. (2013) evaluated the HACCP system in a university canteen and the data obtained revealed no safety risks for the consumers, since *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes* were not detected. However, there were inconsistencies in the microbial loads of mesophilic aerobes, coliforms, *Staphylococcus aureus*, *Bacillus cereus*, and sulphite-reducing clostridia due most likely to inadequate handling or processing procedures, thus suggesting the need for an enhancement of staff training activities and for a reorganization of tasks.

More so, HACCP is a more economically efficient approach to food safety regulation than command and control (CAC) interventions (Unnevehr *et al*., 1999). Another study by Marais *et al* (2007) determined hazard analysis and critical control point (HACCP) awareness among managers of food-producing small and micro enterprises (SMEs) as well as selected aspects of the knowledge, attitude and practices of respective food handlers regarding food safety. Managers had low awareness of HACCP being mandatory in South Africa. Food handlers also had inadequate knowledge of food safety issues and therefore the need to create awareness and understanding of HACCP among managers of SMEs and education regarding the control of risk factors remain crucial was emphasized.

CHAPTER THREE

METHODOLOGY

3.1 ASSESSMENT OF PEANUT OIL PROCESSING PRACTICES

This study was done in three parts. The first part involved conducting a survey on the processing methods used by peanut processors, the second part involved the laboratory analysis of the peanut products and the third part involved the identification of the challenges in the implementation of a HACCP plan in the traditional peanut processing industry. The survey was done in three regions of Ghana which includes: Greater Accra, Ashanti and Northern regions. At least three markets were visited in each of the three regions selected for the study and it was ensured that they were as wide apart from each other as possible. Peanut processors were identified in the markets by convenience sampling and interviewed on their processing methods. A semi-structured questionnaire (Appendix 1) designed for the study was pretested in markets in the Greater Accra region that were not part of the selected markets for the study, and afterwards modifications were made to the questionnaire before the survey was carried out. In all, thirty processors were interviewed.

Markets in the following areas were visited: Madina, Tankasi line, Nima, Maamobi (Greater Accra region), Kanvilli, Dungu, Kamina, Zagyuri (Northern region) and Ejura Nkwanta, Kasim, Zongo, Dagomba Line, Mempeasem (Ashanti region) as shown in Figure 3.1. The processors were interviewed at their processing sites which was usually their homes. Local interpreters were hired to help conduct interviews in the language in which the processors were most fluent.

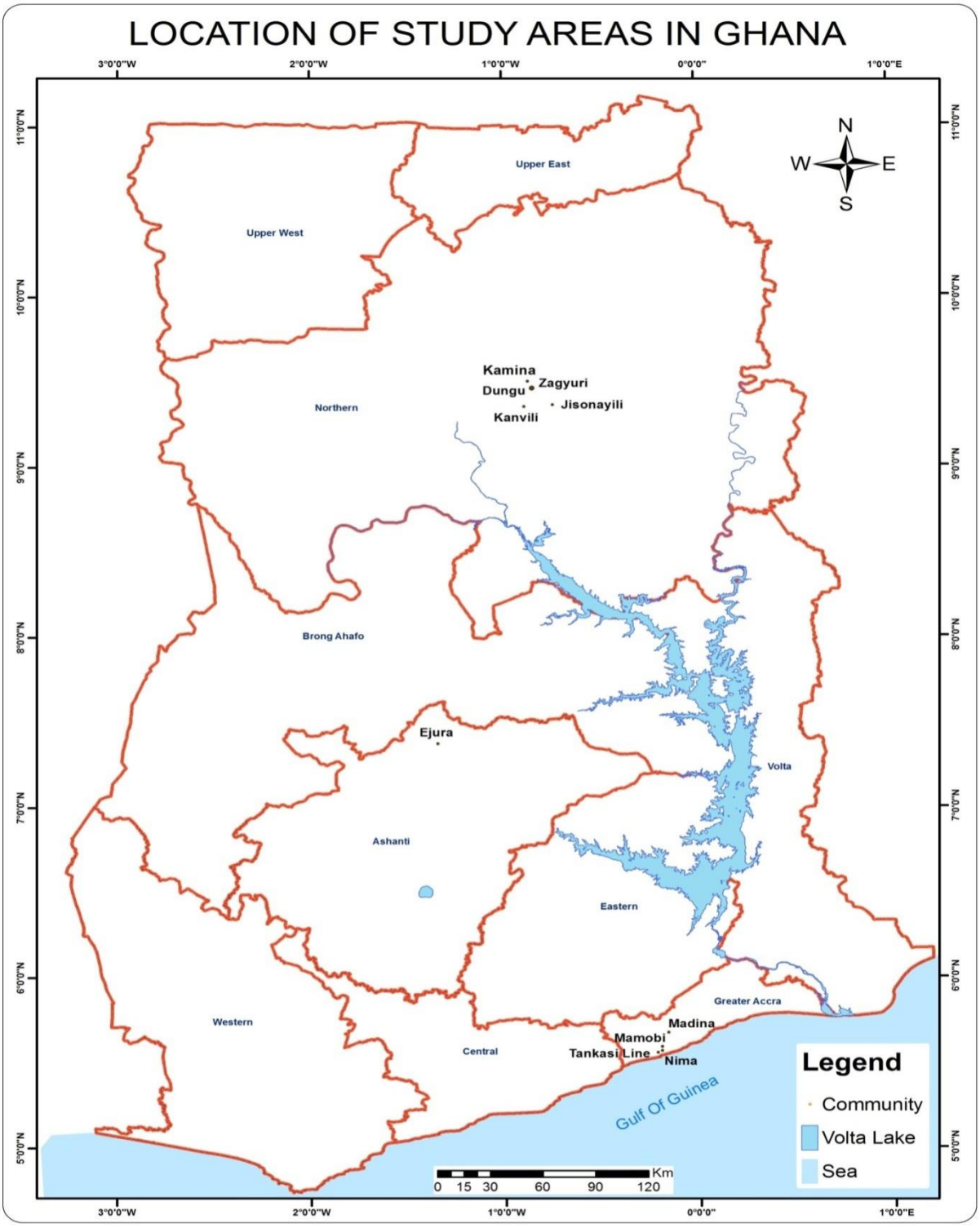


Figure 3.1: Location of study areas in Ghana

3.1.1 Sample collection

Samples were aseptically collected from ten peanut processors at the various stages of processing of peanuts into oil and by-products. The samples collected were: raw peanuts, roasted peanuts, peanut paste, peanut cake, peanut oil and by-products. Samples were placed in an ice chest with ice packs and transported to the Council for Scientific and Industrial Research (CSIR)-Food Research Institute's microbiology laboratory and the department of Nutrition and Food Science-University of Ghana in Accra for microbiological and physicochemical analyses.

3.1.2 Evaluation of the food safety knowledge of peanut oil processors

The food safety knowledge of the peanut oil processors was evaluated by conducting a cross-sectional study using a pre-tested semi-structured questionnaire (Appendix 1). For food safety knowledge questions, answers were graded by giving 1 point for the right answers and 0 point for the incorrect answers.

3.2 ANALYSIS OF SAMPLES ALONG THE PEANUT PROCESSING CHAIN

The physicochemical indices, the level of microbial and aflatoxin contamination were determined on raw peanuts, peanut paste, peanut cake, peanut oil and khebab powder which are the products found along the peanut processing chain.

3.2.1 Physical properties

3.2.1.1 Colour determination of peanut oil

The colour of the peanut oil collected from the processors was determined, using a tristimulus chroma meter (CR-310, Minolta Camera Co. Ltd., Osaka, Japan). Peanut oil samples were filled into a clean-dried glass petri dish and covered with the lid. The

chroma meter was calibrated using a white Minolta calibration plate No. 14333116 before measurements were taken. The L a*b* colour space was selected. L a*b* colour determination was done by taking measurements on three different parts of the lid covering the peanut oil samples.

3.2.1.2 Flash point of peanut oil

The flash point of the peanut oil samples was determined using the Pensky-Martens closed-cup method: EN ISO 2719 (ECS, 2008).

3.2.1.3 Refractive index of peanut oil

Refractive index of the oil was determined using the digital hand-held refractometer (Model: ATAGO® Digital Branch Refractometer RX-5000i-Plus) with a measurement range of 0-95 % Brix. One-two drops of the peanut oil were placed on the sample stage of the calibrated refractometer making sure it was free from any foreign materials and well cleaned. The cover plate was well closed. The readings obtained were recorded in % Brix @ 25°C.

3.2.1.4 Percentage (%) impurity of peanut oil

The level of impurities was measured in each peanut oil sample as described by Opoku-Boahen *et al.* (2013). Two grams (2 g) of oil was weighed into a 500 ml flask and mixed with 20 ml of a 1:1 solvent (petroleum ether and diethyl ether). The contents were vigorously shaken, covered, and allowed to stand for 24 hours. The mixture was filtered through a weighed 11 cm qualitative filter paper. The paper was then washed with 10 ml

of the 1:1 solvent and placed in an oven at 103 ºC for one hour. The dried paper was then weighed. The impurity (%) of each oil was calculated with the following formula:

$$\%\ Impurity = \frac{(W2 - W1)}{W3}$$

Where: W2= Weight of paper before filtering, W1= Weight of paper after filtering and W3= Weight of initial sample

3.2.1.5 Textural property of kulikuli

The hardness of *kulikuli* samples was determined using a TA.XT plus Texture Analyzer equipped with a knife probe of width 7mm and length 9mm. Settings for the texture analyzer were: Test Mode: Compression, Pre-test speed-1.00 mm/s, Test speed- 1.00 mm/s, Post-test speed-10.00mm/s, Distance- 3.00 mm, Trigger type- Auto (Force), Trigger force-5.0g and Force-100.0g.

3.3 PHYSICOCHEMICAL ANALYSES

3.3.1 Chemical Analysis

3.3.1.1 Proximate Analysis

Proximate analysis was conducted on the by-products of peanut oil processing. These included moisture content, protein, fat, ash and crude carbohydrates.

3.3.1.1.1 Moisture content

The moisture content was determined using AOAC method 925.40 (AOAC, 2005).

3.3.1.1.2 Crude fat determination

The crude fat content was determined using the Soxhlet method according to AOAC (2005) method 948.22.

3.3.1.1.3 Protein determination

Kjeldahl method was used to determine the nitrogen content of the by-products of peanut oil processing: *kulikuli* and *khebab* powder as outlined in AOAC (2005) method 955.04C. The protein content was then calculated as the product of nitrogen content and a factor of 5.46 for peanuts.

3.3.1.1.4 Crude ash

The ash content was obtained as the weight of the inorganic residue after the carbonization (at 600°C) of the test portion of *kulikuli* and *khebab* powder in a furnace to a constant weight of white ash.

3.3.1.1.5 Crude carbohydrate

The crude carbohydrate content was calculated as the difference between the sum of the other components and 100%, that is:

Crude Carbohydrates = 100% - (% protein + % moisture + % crude fat + % crude ash)

3.3.1.2 Free fatty acids

Percentage free fatty acid was determined using the recommended method of the American Oil Chemists' Society (AOCS, 1993). One point four grams (1.4 g) of oil was weighed into a flask containing 15 ml of hot neutralized alcohol and 0.4 ml of phenolphthalein indicator was then added. The content was titrated with 0.5 N NaOH. Percentage free fatty acid value was calculated (as oleic acid) using the formula:

$$FFA(\ as\ oleic) = \frac{V\ x\ N\ x\ 28.2}{W}$$

Where: V = volume (ml) of NaOH solution, N = normality of NaOH solution, W = weight of oil sample.

3.3.1.3 Peroxide value

The peroxide value of the peanut oil samples was determined as described by AOCS Cd 8b-90 (03).Five grams of the oil was dissolved in 30ml of glacial acetic acid: chloroform (3:2,v/v).0.5 ml of saturated KI was added and $I_2$was liberated by the reaction with the peroxide. The solution was then titrated with standardized sodium thiosulphate using starch indicator. The peroxide value was determined using the formula.

$$PV(\frac{mEq}{kg}) = \frac{(S-B)\ x\ M\ x\ 1000}{Sample\ weight\ (g)}$$

Where:

S=Sample titre value, B=Blank titre value, M=Molarity of $Na_2S_2O_3$

3.3.1.4 Iodine value

Iodine value was determined according to AOCS method (1993). An amount of 0.2 g of the oil was accurately weighed into a 500 ml flask. Fifteen millilitres (15 ml) of carbon tetrachloride was added to the sample and swirled to ensure that the sample completely dissolved in it. Twenty five millilitres (25 ml) of Wij's solution was then pipetted into the flask containing the sample. The flask was stoppered and swirled to ensure complete mixing. The sample was then placed in the dark for 30 min at room temperature. The flask was removed from storage and 20 ml of 10% KI solution added, followed by 150 ml of distilled water. The mixture was titrated with 0.1 N $Na_2S_2O_3$ solution, adding gradually and with constant and vigorous shaking until the yellow colour had almost disappeared. One and half millilitres (1.5 ml) of starch indicator solution was added and the titration

was continued until the blue colour disappeared. A blank determination was conducted simultaneously. The iodine value was calculated using the formula:

$$\text{Iodine value} = B - S \times N \times \frac{12.69}{Weight\ of\ oil}$$

Where, B = blank titre value, S = sample titre value and N = normality of $Na_2S_2O_3$.

12.69=Conversion factor from Meq. $Na_2S_2O_3$ to gram iodine, molecular weight of iodine is 126.9g.

3.3.1.5 Saponification value

Saponification value was determined according to AOCS (1993). Two grams (2.0 g) of the oil was weighed into a flask. Twenty five millilitres (25 ml) of alcoholic KOH was pipetted and allowed to drain for about 1 min into the mixture. A blank determination was prepared and determined simultaneously with the sample. A condenser was connected to the flask and the mixed sample was allowed to boil gently and steadily for 45 min for complete saponification. The flask and condenser were then cooled but not sufficient to form a gel. The condenser was disconnected and 1 ml of phenolphthalein indicator was added to the content of the flask. The solution was titrated with 0.5N HCl until the pink colour just disappeared. The saponification value was calculated using the formula:

$$Saponification\ value = B - S \times 56.1 \times \frac{N}{Weight\ of\ oil\ sample}$$

Where, B = Blank titre value, S = sample titre value and N = normality of HCl.

3.3.1.6 Acid value

The acid value of the peanut oil was determined as described by AOCS Cd 3d-63 (03).0.1 gram of the oil was dissolved in 2.5ml of 1.1 v/v ethanol: diethyl ether solvent and titrated with 0.1N sodium hydroxide while swirling using phenolphthalein as indicator.

3.3.2 Determination of the Level of Microbial Contamination along the Peanut Processing Chain

3.3.2.1 Enumeration of microorganisms

The peanut products along the peanut processing chain were sampled from the markets and from the home processors of peanuts for analysis in the selected areas of study. The samples include: raw peanuts, peanut paste, peanut cake, peanut oil, *kulikuli* and *khebab* powder.

3.3.2.1.1 Homogenization and Serial Dilution

For each food sample 10g was aseptically weighed into 90mls of sterile salt peptone solution (SPS) containing 0.1 % peptone and 0.8 % sodium chloride with pH adjusted to 7.2 and homogenized in the Stomacher (mode l 4001,Seward Medical) for 30 seconds at normal speed. Tween 80 solution was added to the SPS to ensure the samples which were oily mixed very well with the various media that were used. This provided 10^{-1} dilution. This was vortexed for about 2 min to ensure uniform mixing. Using a sterile pipette, 1 ml of the 10^{-1} dilution was pipette into 9mls of sterile salt peptone water to obtain 10^{-2} dilution. This procedure was repeated for 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions. Further, from appropriate tenfold serial dilution, 1 ml aliquot of each dilution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}

and 10^{-6}) was inoculated into sterile Petri dish plates and the appropriate media added for enumeration and isolation.

After appropriate incubation, dilutions with 30–300 colonies were selected and counted. The number of colony-forming units per gram (cfu/g) of food was calculated by multiplying the number of bacteria by the dilution. All analysis was done in duplicate for reliability of results.

3.3.2.1.2 Enumeration of Aerobic Mesophiles (Viable plate count)

Aerobic mesophiles were counted by the pour plate method (Balows *et. al*., 1991) using plate count agar medium (OXOID CM 325; Oxoid Ltd., Basingstoke, Hampshire, UK). The plates were incubated at 30 °C for 72 hours in accordance with the Nordic Committee on Foods Analysis Method (NMKL no. 86, 2006).

3.3.2.1.3 Enumeration and Isolation of Total Coliform

Coliform bacteria were counted by the pour plate method using Tryptone Soya Agar medium (OXOID CM131) and adjusted to pH 7.3 and overlaid with Violet Red Bile agar (OXOID CM 107) with pH adjusted to 7.4 and incubated at 37 °C for 24 hours. Colonies were confirmed using Brilliant Green Bile broth (OXOID CM 31) at pH of 7.4 and incubated at 37 °C for 24 hours (NMKL no.44, 2004).

3.3.2.1.4 Enumeration of Escherica coli

E. coli bacteria were counted by the pour plate method using Trypsin Soya agar medium (OXOID CM131) adjusted to the pH 7.3 and overlaid with Violet Red Bile agar (OXOID CM 107) with pH adjusted to 7.4 and incubated at 44 °C for 24 hours. Colonies were

confirmed using E.C. broth (OXOID CM 853) with pH adjusted to 6.9. Colonies that produced gas were confirmed for Indole production. This was done by sub- culturing into Tryptone water and incubated at 44 °C for 24 hours. Indole test was done by putting a drop of Kovac reagent into the culture. Red ring colouration at the surface of Tryptone indicated Indole positive (NMLK no.125, 2005).

3.3.2.1.4.1 Biochemical Confirmation of E coli

Five typical and suspected colonies of E. coli are sub cultured each in tubes of E.C. broth containing inverted Durham tubes. It is incubated at 44°C for 24 hours. Positive tubes turn cloudy and their Durham tubes traps gas as a result of the lactose fermented by the suspected *E.coli*.

0.1ml of the broth is sub cultured in tryptone broth and incubated at 44°C for 24 hours. About 2 to 3 drops of Kovac's reagent is added to each of the tubes and left to stand for about 10 seconds. Appearance of a violet or pinkish ring like structure on top of the broth indicates presence of *E.coli* in the sample.

It is then recorded as: $\frac{\text{no .of positive tubes}}{\text{total no.of tubes}}$

3.3.2.1.5 Enumeration of Yeast and Moulds

Yeast and moulds were enumerated by the pour plate method on Dichloran Rose Bengal Chloramphenicol (DRBC) medium, (Oxoid CM0727; Oxoid Ltd., Basingstoke, Hampshire, UK) to which 1% chloramphenicol in absolute ethanol was added as

supplement to suppress bacteria growth. The pH was adjusted to 5.6 and incubated at 25°C for 3-5 days in accordance with ISO 7954 (1987).

3.3.2.1.6 Enumeration of Staphylococcus aureus

Enumeration of Staph was done by the spread method using Baird- Parker agar (OXOID CM 275). 0.1 ml of the aliquot was inoculated onto the surface of Baird-Parker agar. A sterile glass rod was used to spread the medium onto the surface of the Baird Parker medium. The plates were incubated at 37°C for 48 hours. Colonies were confirmed for coagulate positive using Rabbit Plasma Serum. Coagulation of the serum indicated coagulate positive (confirmation test for staph aureus).

3.3.2.1.7 Detection of Salmonella species

Approximately 25g of the food sample were weighed aseptically into sterile Stomacher bag and 225ml buffered peptone water added to it. This was homogenized thoroughly and incubated at 37 °C for 16-21 hours. Following incubation, 0.1ml of broth (buffered peptone water) was transferred into 10ml of ml of Rappaport-Vasilliadis (RVS) broth and incubated in water bath at 42 °C ± 0.5 °C for 24 ± 3 h. Following enrichment, a loopful of the ml of Rappaport-Vasilliadis (RVS) broth culture was streaked onto XLD Agar plates, and the plates were incubated at 37°C ± 0.5°C for 24h ± 3 h. Presumptive colonies (lightly transparent zone of reddish colour and black center) were maintained on non-selective TSA agar slants for further biochemical tests.

3.3.2.1.7.1 Biochemical confirmation of *Salmonella* species

Suspected colonies were confirmed on Triple Sugar Iron medium, Urea Agar Base (UAB) medium and Lysine Decarboxylase Broth medium and incubated at 37 °C for 24 hours.

3.3.2.1.7.2 Serological confirmation

Suspected colonies were streaked onto Tryptone Soya Agar medium and incubated at 37 °C for 24 hours. A colony was picked and put onto a clean slide. One drop of anti-serum O was added onto the colony on the slide. A clean cover slip was used to mix the colony and the anti-sera. Clotting/ agglutination indicated presence of salmonella.

3.3.2.1.7.3 Triple Sugar Iron confirmatory (TSI) test

Using aseptic techniques, a colony of the test organism was picked with a sterile inoculating loop, and the slant of the media streaked. Using a sterile inoculating needle, the butt of the medial was then stabbed. Tubes were then inoculated at 37°C for 24 hours after which they were observed. A yellow butt and a red slant indicated fermentation of lactose/ or sucrose. Blackening of agar indicated the production of H_2S gas. A red colouration of the bottom and a slant indicated that none of the sugars were fermented and neither gas nor H_2S were produced. Blackening and gas formation in TSI tube indicates presence of *Salmonella.*

3.3.2.1.7.4 UAB confirmation

In the UAB confirmation, colour change from yellow to pink indicates absence of *Salmonella* species.

3.3.3 Determination of the Level of Aflatoxin Contamination along the Peanut Processing Chain

3.3.3.1 Aflatoxin Analyses

Aflatoxin analyses were carried out using the CEN (European Committee for Standardization) method with the high performance liquid chromatography (HPLC). Two processors were randomly selected out of the ten processors involved in the study in each of the three regions studied; thereby making six (6) processors in all. Samples were collected at various points of the processing chain from each processor and these samples were: raw peanuts, peanut paste, peanut cake, *kulikuli* and peanut oil for analyses. In all, thirty (30) samples were analyzed.

3.3.3.1.1 Sample preparation

The aflatoxin analyses involved three stages:

- Extraction
- Concentration and clean up using immune-affinity column specific for aflatoxins
- Quantification using the HPLC (High Performance and Liquid Chromatography)

3.3.3.1.1.1 Extraction

Twenty five grams (25g) of the unmilled samples (eg. Raw and roasted peanuts, peanut cake) and seventy five grams (75g) of the already milled samples/slurries (e.g. Peanut paste, peanut oil) was homogenized with 5g NaCl, 200ml methanol: distilled water in (80/20 v/v) and 100ml hexane (added because the samples contained fat) at low speed for 2mins and high speed for 1min in a Warring blender (Warring products division, Torrington USA). The slurry formed was filtered using whatman filter paper number 4**.**

3.3.3.1.1.2 Concentration and Clean up

10ml of filtrate was mixed with 60ml phosphate buffer saline (PBS) solution and stirred and the mixture eluted in an Easi-Extract Afla column packed with monoclonal antibodies specific to G1, B1, G2 and B2. Column was rinsed with 15ml distilled water in 5ml proportions. Aflatoxins were then eluted from the column with 0.5ml methanol followed by 0.75ml methanol HPLC grade. The eluate was collected into 5ml volumetric flask and diluted with distilled water to the mark. 2ml of the extract was filled into vial bottles and loaded onto the HPLC autosampler chamber for separation, detection and quantification. All reagents used were HPLC grade.

3.3.3.1.2 Detection and quantification of aflatoxin levels

Waters HPLC system consisting of Waters 1525 Binary HPLC pump, Waters 2707 Autosampler, and Waters 2475 Multi λ Fluorescence Detector was used. Data acquisition and analyses was done using Breeze 2 software (Waters HPLC Company). The waters HPLC was equipped with a reverse phase symmetry (C_{18}) column (sperisorb55 ODS-1) of dimensions 25cm x 4.6mm x 5µm. The fluorescence detector operated at an excitation wavelength of 360nm and an emission wavelength of 440nm at temperature of 35°C. The mobile phase was a mixture of water: methanol: acetonitrile (60:30:20) at a flow rate of 1mLmin-1 in an isocratic separation method. Linearity was estimated by injecting four Trilogy analytical laboratory aflatoxin standards: aflatoxin B1 and aflatoxin G1 at 2.0µg/ml and aflatoxin B2 (B2) and aflatoxin G2 (G2) at 0.5µg/ml. The limits of detection were determined to be: G1 and G2 = 0.13µg/kg; B1 and B2 = 0.15µg/kg. The injection volume used was 10µl. After aflatoxin readings were obtained, they were corrected by multiplying them with corresponding values for the various aflatoxin types as follows:

- B1=1.24
- B2=1.22
- G1=1.12
- G2=1.19

3.4 DEVELOPMENT OF A HAZARD ANALYSIS AND CRITICAL CONTROL POINTS (HACCP) SYSTEM FOR THE PEANUT PROCESSING CHAIN

The methodology employed in developing the HACCP plan included the procedures used by Amoa-Awua *et al.* (1998), Ackah *et al.* (2010) with modifications and the 12 tasks and 7 principles as defined in the Hazard Analysis and Critical Control Point (HACCP) system and guidelines for its application, which was adapted during the 22nd session of the Codex Alimentarius Commission in 1997.

3.5 STATISTICAL ANALYSES

SPSS software version 16 was used to analyze data collected on survey. Correlations and cross tabulations were done to determine associations between the biodata of processors and their processing methods.

Statgraphics plus 3.0 was used to analyze data obtained from the physicochemical, microbiological and aflatoxin analyses. ANOVA was done to determine if differences existed among them (the physicochemical, microbiological and aflatoxin analyses).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 PEANUT OIL PROCESSING IN SOME COMMUNITIES IN GHANA

4.1.1 Background characteristics of peanut oil processors

Results from the survey indicated that almost all the traditional peanuts processors were adult females within the age range of 36-50 years as shown in Table 4.1. All of them were Muslims, and despite where they were domiciled, most of them were originally from the Northern parts of Ghana. The number of years of processing experience of the peanut processors ranged from 1-20 years and about 50% of them indicated that they had been processing for 20 years and above. Thus the peanut processors may be set in their traditional processing methods and techniques since they have been doing it for a very long time. They may therefore find it difficult to adapt new processing techniques and this could ultimately affect their productivity (Muhammed-Lawal *et al*., 2012). Furthermore, training programs for them to appreciate and conform to quality management systems in peanut processing may have to be carefully designed since majority (93.3%) did not have any formal education and only learnt the business through apprenticeship with family members or family friends.

Table 4.1: Demographic characteristics of peanut oil processors

Biodata	Frequency	Percentage
Sex (N=30)		
Female	30	100.0
Age (N=30)		
20-25 years	1	3.3
31-35 years	1	3.3
36-40 years	6	20.0
41-45 years	8	26.7
46-50 years	6	20.0
Religion (N=30)		
Muslim	30	100.0
Home Region(N=30)		
Northern	26	86.4
Eastern	1	3.3
Upper East	2	6.6
Ashanti	1	3.3
How long have you been in the peanut oil processing business (N=30)		
1-10 years	5	16.7
11-20 years	10	33.3
More than 20 years	15	50.0
Education		
None	28	93.3
Primary	1	3.3
Middle/JHS	1	3.3

4.2 KNOWLEDGE OF FOOD SAFETY BY PEANUT OIL PROCESSORS

4.2.1 Perceptions of the causes of defects in peanuts

Table 4.2 shows that 73.4 % of the processors had perceptions of the causes of defects in raw peanuts. They identified insect attack and inadequate rains as the highest (27.3%) cause of defects in peanuts, and this was followed by improper drying (22.7%) amongst other responses shown in Table 4.2.

There were no significant associations between the age and experience of the processors and the perception of the causes of defects of peanuts respectively (χ^2=98.095, p=0.393) and (χ^2=75.714, p=0.488) as shown in Table 4.3. There were also no significant associations between the processors ethnic background (hometown) and their perception of the causes of defects (χ^2=0.0396, p=0.097). Thus most processors realized that improper drying and insect attack were the major causes of defects in peanuts (Table 4.2). Consequently post-harvest handling activities such as drying and storage of peanuts are raw material supply issues that should be addressed in the value chain using standard quality management systems.

4.2.2 Food safety issues associated with the consumption of defective peanuts

Data from the survey showed that forty percent(40%) of the respondents thought there were no food safety issues associated with the consumption of defective peanuts whiles 33.3% associated the consumption of defective peanuts to stomach pains among other health issues(Table 4.4). Chang *et al.* (2013) suggest that most peanut products do not pose food safety issues as a result of the roasting step which reduces the moisture content to 1.25% and water activity of less than 0.75. These conditions are known to inhibit the growth of moulds and bacteria.

4.2.3 Quality and stability of peanut by-products

Among the traditional peanut processors, oil yield is their chief goal. The cake obtained from the oil extraction is a by-product that is further processed as a snack (which they call *kulikuli*) or as an ingredient for other foods (*khebab* powder) (Saalia *et al.*, 2008). Most of the processors perceived the by-products (*kulikuli* and *khebab* powder) to have long

storage stability. Sixty percent (60%) of the respondents indicated that the by-products do not get spoilt whiles only 1.7% of them reported development of off-flavours and changes in texture as the main types of spoilage that could occur (Table 4.4). Even though most processors thought the by-products were stable, they also acknowledged that their stability was dependent on storage and other handling conditions. Some of the causes of spoilage were reported to be by contact with water (98%) as shown in Table 4.4.

Table 4.5 shows that the processing steps of roasting and quantity of water added during oil extraction (kneading) greatly affects the quality of peanut by-products that are obtained.

Table 4.2: Knowledge of causes of defects in peanuts

Knowledge of causes of defects in peanuts (n=30)	**Frequency**	**Percentage**
Bad farming practices	1	4.5
Improper drying	5	22.7
Inadequate rains	1	4.5
Insect attack and inadequate rain	6	27.3
Poor storage techniques	2	9.1
Insects	2	9.1
Excess moisture and lack of rain	2	9.1
Insect attack and moisture	2	9.1
Moisture	1	4.5
No idea	8	26.6
Level of knowledge (n=30)		
Adequate knowledge	22	73.4
Inadequate knowledge	8	26.6

Table 4.3: Association between perceptions of the causes of defects of peanuts with biodata of peanut processors

	χ^2,p-value			
	Age	**Hometown**	**Level of education**	**How long have you been in the peanut processing business**
What do you think may be the causes of the defects	(98.095,0.393)	(0.0396,0.097)	(60.000,0.013)	(75.714,0.488)

Table 4.4: Knowledge of food safety issues associated with consuming defective and unclean peanuts and causes of spoilage

Knowledge of food safety issues associated with consuming defective and unclean peanuts (n=30)	**Frequency**	**Percentage**
Diarrhea	3	10.0
No idea	3	10.0
Stomach pains	10	33.3
Stomach pains and diarrhea	2	6.7
There are no food safety issues and health implications associated	12	40.0
Knowledge of the types of spoilage you notice in the by-products		
Bad smell and rancidity	1	3.3
By-products do not get spoilt	18	60.0
Change in taste, rancidity	1	3.3
Kulikuli softens but does not get spoilt	2	6.7
Production of off flavours	5	16.7
Softening of the texture	1	3.3
No idea	2	6.7
Causes of spoilage sometimes experienced(n=30)		
Contact with water	30	100.0

Table 4.5: Knowledge of processing steps that influence the quality of processed peanut oil

Processing step(s) that influence the quality and safety of the processed peanut oil (n=30)	Frequency	Percentage
Addition of too much water when kneading	3	10.0
Frying of *kulikuli*	1	3.3
Kneading	6	20.0
Kneading and roasting	1	3.3
Kneading(salt must be added to the peanut cake)	1	3.3
Removal of spoilt peanuts	1	3.3
Roasting	3	10.0
Roasting and addition of water during kneading	1	3.3
Roasting and addition of water to peanut paste	1	3.3
Roasting and sorting	1	3.3
Sorting and addition of water	1	3.3
Sorting	1	3.3
Winnowing and milling	1	3.3
Roasting, sorting and addition of water during kneading	1	3.3
Addition of water during kneading	7	23.3

4.2.4 Traditional processing methods of peanut oil and its by-products: khebab *powder and* kulikuli

4.2.4.1 Scale of operations

Traditional peanut processing in Ghana is largely at very small scale, usually at the household level. This is probably due to the manual nature of the operations, and the simple equipment used in the processing operations. The processing capacity of most of the peanut processors (94%) was two bags (i.e. 100kg) or below (Figure 4.1), and as much as 60% of them had capacities well below one bag (i.e. 50kg) per batch. Consequently the quantity of peanut oil produced per day ranged from 0.5l to 3.79 liters. The raw peanuts

supply source was from the open market for most processors (96.7%), and it was largely obtained in the shelled form (90%) and not in-shell as shown in Figure 4.3.

There were no significant associations between raw material supply and ethnic background (hometown) (P-value= 0.330) of processor or years of experience (P-value= 0.905) in processing (Table 4.15) in Appendix 4. This indicates that the traditional peanut processors, no matter where they operate from had no peanut quality supply system in place and therefore did not have dedicated raw material suppliers. Consequently raw material quality is not assured as they depended on the open markets for their supplies. Peanut cake (*tunkusa*), *kulikuli* and *khebab* powder are the main by-products that were obtained by the processors (Figure 4.4).

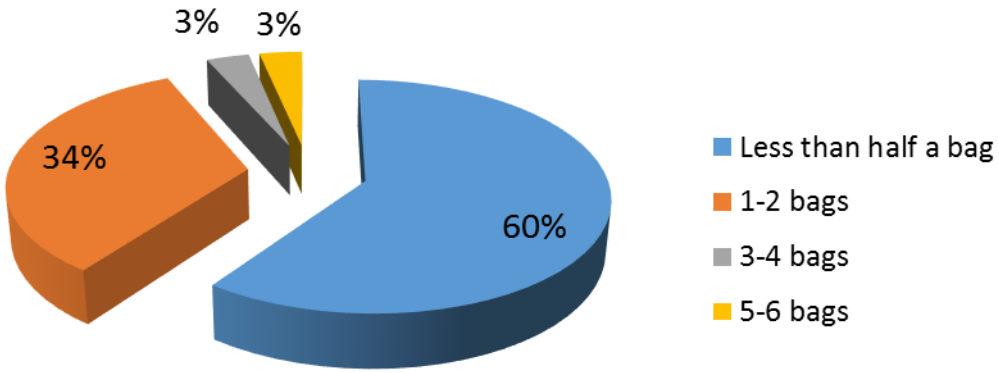


Figure 4.1: Amount of peanuts used in the processing of peanuts by peanut oil processors

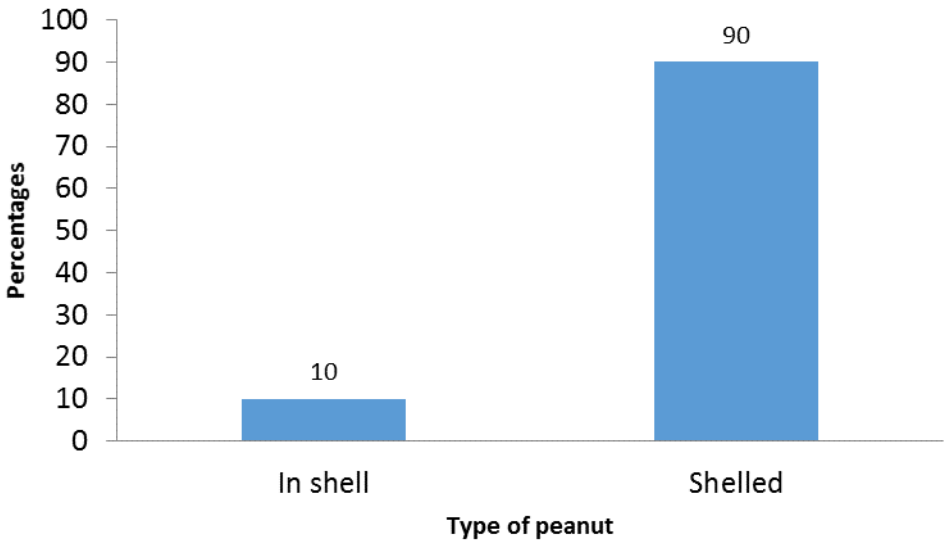


Figure 4.2: Type of peanuts used by peanut oil processors

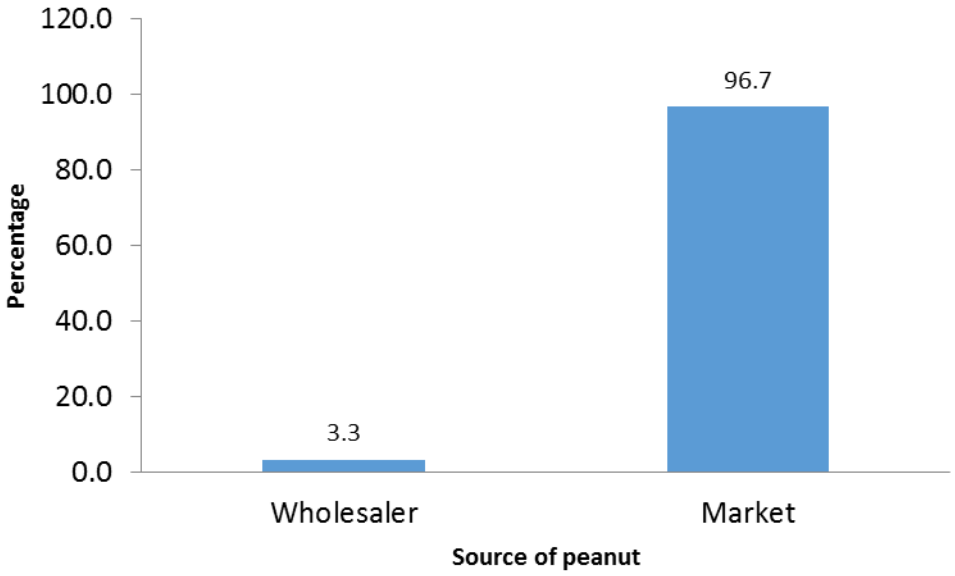


Figure 4.3: Source of peanuts used by peanut oil processors

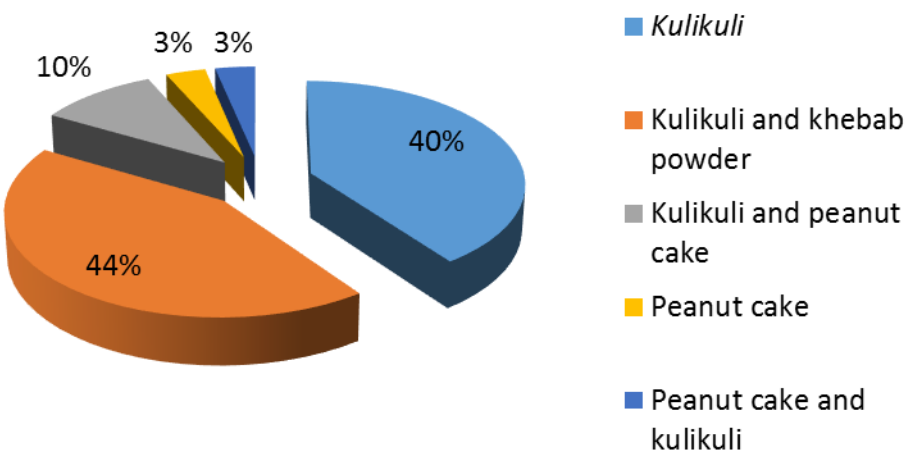


Figure 4.4: Types of by-products produced by peanut oil processors

4.2.4.2 Traditional processing methods of peanut oil and its by-products: kulikuli and khebab powder.

The traditional processing methods documented for the production of peanut oil and its by-products after the survey were not different from what has been reported by Kpodo and Gyato (1996); Mayhem (1988); Adjou *et al.* (2012) and Simmons (1975). The main raw material for peanut oil and its by-product processing was peanuts. The processing steps used to produce peanut oil and by-products: *kulikuli* and *khebab* powder are shown in Figure 4.5 and appendix 1. Processing involves sorting of the raw peanuts to remove stones, chaff and any other foreign materials that may cause hazards to consumers. The cleaned peanuts are then roasted in traditional roasters or in pans together with washed beach sand. The processors could tell when the roasting was sufficient by removing the skins of some of the peanuts and by the changes in the aroma and colour of the peanut skins. After roasting, the peanuts are dehulled and this is usually done using the bare hands or using flat wooden discs and rubbing them vigorously on the peanuts on a clean fertilizer

bag on cemented floors. After this stage, the peanuts are milled into smooth peanut paste using a commercial disc-attrition mill. For processors who further produce by-products: *kulikuli* and *khebab* powder, spices such as dried pepper as well as salt are added at the milling stage.

In the extraction of oil from the smooth peanut paste, cold water is added in little bits during kneading when the paste is still warm from milling. On the other hand in cases where the processor may not be able to process the peanut paste immediately after milling, the paste may be kept overnight and in this case warm water is added a little at a time during kneading of the paste to extract oil. This technique is usually employed in the Northern region of Ghana during the Harmattan season when the weather is dry and cold. After oil is extracted, it is boiled again to remove water that may be present, before it is cooled and bottled for sale.

After extraction of the oil, the hydrated peanut cake is shaped into cylindrical, circular or spherical shapes and deep fat fried until a crisp texture is attained. This fried peanut cake is locally called *kulikuli* and it is a ready to eat snack. They are stored in air tight containers for sale.

Khebab powder is produced by crushing the fried peanut cake (*kulikuli)* in a mortar and further milling together with spices (pepper, ginger and other mixes depending on processor) into a smooth powder which is sprinkled onto grilled or barbecued meat for flavor and zest.

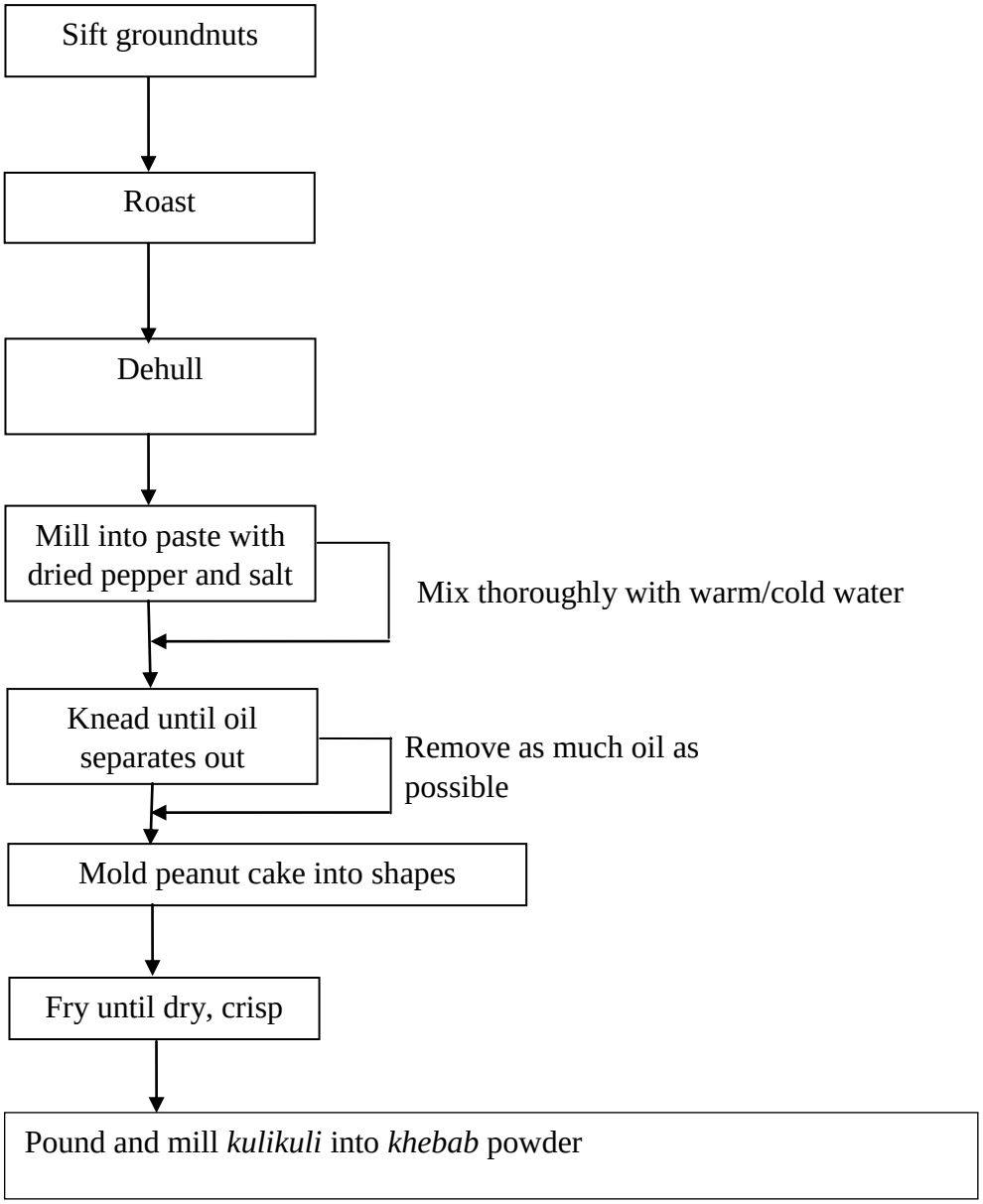


Figure 4.5: Flow diagram for the production of peanut oil, *kulikuli* and *khebab* powder

4.3 PHYSICOCHEMICAL CHARACTERIZATION OF TRADITIONALLY PROCESSED PEANUT OIL, *KULIKULI* AND *KHEBAB* POWDER

4.3.1 Physical properties of peanut oil

The physical and chemical properties of peanut oil and its by-products were determined as shown in Tables 4.6, 4.7, 4.8 and 4.9.

The physical properties of peanut oil determined were: refractive index, flash point, percentage impurity and colour as shown in Table 4.8. The physicochemical property of oils and fats greatly influences their functions in foods and may also be used to determine the purity of a substance as compared with known standards (Sikorski and Kolakowska, 2010).

4.3.1.1 Refractive index

The refractive index is usually used as a criterion for oil purity (Sikorski and Kolakowska, 2010). It measures the extent to which light is bent or refracted when it moves from air into a sample (Sikorski and Kolakowska, 2010). The more soluble substances there is in the oil, the greater the bend or refraction. There were no significant differences ($p=0.1$) in the refractive indices of the peanut oil from the different communities as they were all the same as shown in Table 4.6.

Table 4.6Physical properties of peanut oil from traditional processors in selected communities in Ghana

Region	Refractive index @25°C (n_D)	Flash Point(°C)	%Impurity	b*(yellowness index)
Northern	1.466±0.00[a]	315.3±0.5[a]	4.25±1.0[a]	+23.49±0.05[a]
Ashanti	1.466±0.05[a]	315.0±1.2[a]	3.0±0.8[b]	+23.47±0.03[a]
Greater Accra	1.466±0.06[a]	316.1±1.0[a]	5.1±0.5[c]	+23.47±0.05[a]
p-value	0.1	0.1784	0.0090	0.7428
Refined peanut oil	1.4658-1.4690	290-333	-	16-25

*p- values < 0.05 means there is significance statistically.
*Values are means of three replicates ± standard deviation
*Different letter superscripts (a, b, c) in the same column or row for each parameter indicate significant differences between means

4.3.1.2 Flash point

The flash point of oils and fats denotes the temperature at which the decomposition products formed from frying oils can be ignited (Wary, 1992). Table 4.6 shows that the flash point of the peanut oil ranged between (315-316.1) °C. Samples from the Greater Accra Region recorded the highest flash point (316.1±1.0) °C whiles those form the Ashanti region recorded the lowest (315.0±1.2) °C. There were no significant differences (p=0.1784) in the mean flash points obtained. The flash point values obtained were similar to that reported by Shahida (2005) which was between 290-333°C. Refined peanut oils have been reported to have flash point values between 314-333 °C which is not different from what was obtained from the crude peanut oil by the traditional processors.

4.3.1.3 Percentage Impurity

The percentage impurity is a measure of the amount of foreign substances such as hulls from the peanuts and sand particles in fats and oils (Dimic *et al*., 2012). The percentage impurity values ranged between (3.0-5.1) percent. Samples from the Ashanti region had the least foreign material content (3.0±0.8) % whiles those from the Greater Accra Region had comparatively the highest foreign material content (Table 4.6). There were significant differences (p = 0.009) in the percentage impurity values among communities. Samples from the Ashanti Region had relatively less impurities compared to those from selected communities in the Northern Region and Greater Accra Regions. Dimic *et al*. (2012) showed that impurities have a negative effect on sensory characteristics such as the taste and odour of the products as well as the chemical quality of the oil samples. The presence of impurities also affects the colour of the oil samples; giving them a turbid appearance (Dimic *et al*., 2012). With the increasing knowledge of consumers on food safety and

health issues and the increasing need for safe food production, it is important to ensure that edible oils are free from all forms of impurities.

4.3.1.4 Colour

Colour measurement was taken as b* indicating the level of yellowness. The yellowness value (b-value) is from –b (blueness) to +b (yellowness) (Sahin and Sumnu, 2007). The b* value for colour which indicates yellowness appeared not to be significantly different (p-value of 0.7428) for peanut oil samples from the Northern, Ashanti and Greater Accra Regions of Ghana. These values are similar to the yellowness values reported by Shahidi (2005) which was between 16 - 25.Visually, Akhtar *et al.* (2014) in their review described peanut oil as pale yellow in colour.

4.3.2 Chemical properties of peanut oil and its by-products: kulikuli and khebab powder

4.3.2.1 Moisture

Moisture content of peanut oil samples

The moisture content of the peanut oil samples are shown in Table 4.7. The moisture contents of the peanut oil samples ranged from (1.031-1.5) %. Table 4.7 indicates that there were significant differences ($p < 0.001$) in moisture contents among the peanut oil samples from the various communities. Peanut oil samples from selected communities in the Northern Region of Ghana had the least moisture content (0.030±0.007) % whiles those from the Greater Accra Region had the highest (0.5±0.5)%. The moisture content of oil samples may affect the free fatty levels with storage time, and eventually cause the oil to go rancid. It is therefore important to keep the moisture of the peanut oil samples as low as possible to increase its shelf life.

Moisture content of kulikuli

The moisture content of *kulikuli* was not significantly different (p=0.410) among communities. The values ranged between (1.21-1.214) % (Table 4.7). This indicates that irrespective of location of processors, the moisture contents were similar. The highest moisture content (1.214±0.007) % and the least were recorded for samples from the Ashanti and Greater Accra regions.

Moisture content of khebab powder

The moisture content of *khebab* powder were significantly different (<0.001) and ranged between (1.52 and 2.6) % as shown in Table 4.7. *Khebab* powder from the Northern Region recorded the lowest moisture content (1.52±0.04) % and those from the Ashanti Region recorded the highest moisture content (2.6±0.1) %. The variability in the moisture content of the *khebab* powder may be as a result of the different storage conditions employed by the processors. It is recommended that the moisture content of peanuts and its by-products be kept below 12 percent, to prevent aflatoxigenic moulds from growing (Woodroof, 1983). The moisture readings obtained for peanuts and its by-products (Table 4.7) indicate that they are far below 12% and thereby their susceptibility to support the growth of aflatoxigenic moulds is low if properly stored.

4.3.2.2 Iodine value

The iodine value is a measure of the amount of unsaturated fatty acids in the oil (Mandloi *et al.*, 2014).

Iodine value of peanut oil

Results in Table 4.7 indicate that the iodine value of peanut oil samples from selected communities in the three regions were not significantly different (p=0.267). They ranged between 91.4-91.6 mg/100g. The iodine values obtained were similar to those reported by Aluyor *et al*. (2009).

Iodine value of kulikuli

The iodine values of *kulikuli* samples from the selected areas under study were not significantly different (p=0.787) and ranged between (92.2 to 92.8) mg/100g. This shows that despite the location, the iodine values were the same.

Iodine value of khebab powder

The iodine values for *khebab* powder were not significantly different (p=0.122). They ranged between (93.9-94.0) mg/100g. Studies have indicated that the higher the iodine value, the greater the fats in products are to oxidative rancidity (Joseph, 1977). Hence it is important that the storage conditions and processing are done to ensure that the degree of unsaturation of peanut oil and its by-products are kept as low as possible.

4.3.2.3 Saponification value

The saponification value of fats and oils has no nutritional significance but is important in the determination of the efficiency of fats and oils to be used for industrial purposes (Eshun *et al.*, 2013). It provides a measure of the average size of the fatty acids in the oil.

Saponification value of peanut oil

Table 4.7 shows the saponification value of the different peanut oils from selected communities in the regions under study. They ranged between (192.9-194.2) mgKOH/g. There were no significant differences (p=0.310) in the samples. The saponification values obtained were similar to the saponification value ranges reported by Nkafamiya *et al.* (2010) which ranged between 188.1-220.20 mgKOH/g and Aluyor *et al.* (2009) who reported 1902.00.

Saponification value of kulikuli

The saponification values obtained for *kulikuli* samples ranged from (196.3-196.5) mgKOH/g with no significant difference (p=0.256). This shows that the saponification value of *kulikuli* was the same.

Saponification value of khebab powder

The saponification value for *khebab* powder samples were between 189.5-190.1 mgKOH/g with no significant differences (0.235). The saponification values obtained from the peanut oil, *kulikuli* and *khebab* flour indicate that the oils were still not oxidized and the average size of the fatty acids, irrespective of the source was still generally unchanged.

4.3.2.4. Acid value

The acid value is used to measure the extent to which glyceride in oil has been decomposed by lipase and other fatty acyl hydrolytic reactions such as light and heat (Demian, 1990).

Acid value of peanut oil samples

The acid values of the peanut oil samples from the selected communities in the various regions under study were not significantly different (Table 4.7).

Acid value of kulikuli samples

Acid values for *kulikuli* samples ranged between (4.2-4.34) mgKOH/g. There were no significant differences (p=0.440) among the *kulikuli* samples from the various regions.

Acid value of khebab powder samples

Khebab powder recorded higher acid values of 27.1-27.5mgKOH/g compared to peanut oil and *kulikuli*. There were no significant differences (0.150) among the *khebab* powder samples. While there were no significant differences in the acid values between peanut oil and *kulikuli*, that of the *khebab* powder was different. This may be as a result of the presence of higher moisture content of the *khebab* powder (Table 4.7) compared with the relatively lower moisture content of the peanut oil and *kulikuli* samples as well as the elevated temperatures during the storage of the *khebab* powder samples (Atinafu and Bedemo, 2011).

4.3.2.5 Peroxide value

The peroxide value is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation (Babalola and Apata, 2011).

Peroxide value of peanut oil

The peroxide values obtained for the peanut oil were between (1.18-1.198) meq/kg (Table 4.7). There were significant differences ($p<0.001$) between samples. The highest peroxide value (1.198±0.005)meq/kg was recorded for samples from the Greater Accra region of Ghana while samples from the Northern region recorded the lowest peroxide value (1.18±0.01) meq/kg. There were significant differences ($p<0.001$) between samples. These variations could be due to combined effect from factors like prolonged contact of the peanut oil with atmospheric oxygen and the presence of high amounts of prooxidants and moisture (Tom and Yagoub, 2007).

Peroxide value of kulikuli

The peroxide values for the *kulikuli* samples ranged between (1.0333-1.036) meq/kg with no significant differences (0.490).

Peroxide value of khebab powder

The peroxide value for the *khebab* powder samples ranged between (1.038-1.046) meq/kg as shown in Table 4.7. There were no significant differences between the samples.

The peroxide values obtained for the peanut oil and its by-products were less than 10 meq/kg and can be attributed to the fact that they were obtained fresh and had not been exposed to air to start oxidation (Babalola and Apata, 2011).

4.3.2.6 Free fatty acid

Free fatty acids are formed due to hydrolysis of triglycerides and may be promoted by reaction of oil with moisture (Frega *et al.*, 1999).

Free fatty acid of peanut oil samples

The free fatty acid values obtained for the peanut oil ranged between (2.4-2.5) % as shown in Table 4.7. There were no significant differences (p=0.255) among the peanut oil samples.

Free fatty acid of kulikuli samples

Kulikuli recorded free fatty acid values of (2.1-2.18) % with no significant differences (p=0.787) among the samples from the different regions considered.

Free fatty acid of khebab powder

The free fatty acid values obtained for the *khebab* powder samples were higher compared to the peanut oil samples and *kulikuli* and were between (13.6-13.8) %. There were significant differences in the percentage free fatty acids obtained. The highest percentage free fatty was recorded for samples from the Ashanti region (78.5±0.4) % and the lowest was recorded for samples from the Northern region (77.5±0.7) (Table 4.7). Crude oils with high free fatty acid beyond 5% are not edible for consumption and may have offensive odour and an unpleasant taste (Kirk and Sawyer, 1991).

Table 4.7: Chemical properties of peanut oil and its by-products from selected communities in Ghana

Region	Moisture (%)	Iodine value(mg/100g)	Saponification value(mg KOH/g)	Acid value(mgKOH/g	Peroxide value(meq/Kg)	Free fatty acid (%)
Peanut oil						
Northern	1.031±0.007[a]	91.4±1[a]	192.9±3.9[a]	4.8±2.8[a]	1.18±0.01[a]	2.4±1.4[a]
Ashanti	1.3±0.2[b]	91.4±2.9[a]	193.163±0.008[a]	5.0±2.1[a]	1.193±0.008[ab]	2.5±1.1[a]
Greater Accra	1.5±0.1[c]	91.6±0.5[a]	194.2±3.7[a]	5.0±1.3[a]	1.198±0.005[b]	2.5±0.6[a]
p-value	<0.001	0.267	0.310	0.525	<0.001	0.255
Kulikuli						
Northern	1.214±0.007[a]	92.3±7.8[a]	196.3±0.7[a]	4.2±1.1[a]	1.033±0.005[a]	2.1±0.6[a]
Ashanti	1.21±0.00[a]	92.2±7.9[a]	196.5±0.3[a]	4.2±0.8[a]	1.036±0.004[a]	2.1±0.4[a]
Greater Accra	1.21±0.00[a]	92.8±7.7[a]	196.4±0.6[a]	4.34±0.07[a]	1.035±0.07[a]	2.18±0.03[a]
p-value	0.410	0.787	0.256	0.440	0.490	0.787
***Khebab* powder**						
Northern	1.52±0.04[a]	93.9±0.5[a]	190.07±0.4[a]	27.1±1.7[a]	1.039±0.006[a]	13.6±0.7[a]
Ashanti	2.6±0.1[b]	93.9±0.5[a]	189.5±0.8[a]	27.5±0.8[a]	1.038±0.006[a]	13.8±0.4[ab]
Greater Accra	2.3±0.1[c]	94.0±0.2[a]	190.1±0.2[a]	27.1±0.9[a]	1.046±0.005[a]	13.6±0.5[b]
p-values	<0.001	0.122	0.235	0.150	0.129	<0.001

Values in columns with different superscripts are significantly different ($p < 0.05$)
Values are means of three replicates ± standard deviation

4.3.3 Physicochemical properties of kulikuli

The proximate analysis determined on the *kulikuli* samples included moisture, fat, protein, ash content and total crude carbohydrates. Table 4.8 shows the values for the various chemical compositions of the product. The texture of the *kulikuli* samples was determined as the physical property.

4.3.3.1 Moisture

The moisture content of the *kulikuli* samples were generally very low and ranged between (1.0-2.4) %. There were significant differences in the moisture content of the different *kulikuli* samples. The highest moisture content was recorded for samples from the Greater Accra region (2.4±1.8) % while the lowest moisture content was recorded for samples from the Ashanti region (1.0±0.6) %. The variations in moisture content of *kulikuli* from different processors may be due to the non-standardized manner in which water is added during kneading as well as the frying process.

4.3.3.2 Carbohydrates

The crude carbohydrate content of *kulikuli* samples was estimated as the percent difference after all the other chemical components were determined as shown in Table 4.8. The carbohydrate contents of the *kulikuli* samples ranged from the lowest of (20.1±4.4) % for samples from the Ashanti region of Ghana to the highest of (35.3±6.6) % for samples from the Northern region of Ghana. Significant variations ($p < 0.001$) in carbohydrate contents were observed among the samples from the various regions.

4.3.3.3 Protein

The protein values obtained were within the ranges of (31.8-40.8) % for *kulikuli*. Samples from the Northern region recorded the least protein levels (31.8±5.4) % and those from the Ashanti region had the highest protein content (40.8±3.3) %. There were significant differences (<0.001) in the protein content of the *kulikuli* samples. The protein contents of the *kulikuli* samples were within reported range of 26.4 to 39.7% as reported by Ezekiel *et al.* (2011). Peanut proteins contribute significantly to the development of the nutty flavours and colour formation through Maillard reactions during roasting of peanuts (Grosso *et al*., 2008).

4.3.3.4 Ash

The amount of ash remaining after the combustion of biological substances is often used as the measure of the mineral content of the substance (Belitz *et al*., 2009). The total ash content determined for the *kulikuli* samples from the three regions under study differed significantly (p <0.001) as observed in Table 4.8. Ash contents ranged from (3.3-4.4) %. These were the lowest and highest average amounts for samples from the Northern (3.8±1.3) % and Ashanti (4.4±1.1) % regions respectively. Belitz *et al*. (2009) suggests that variations in the mineral contents of the same raw materials could be due to genetic and climatic factors as well as the farming practices, soil composition and maturity of the produce. Minerals in foods are important for their nutritional and physiological roles.

4.3.3.5 Fat Content

Kulikuli from the Ashanti region was found to have the highest fat content (33.7±5.5) % while those from the Northern region had the lowest (27.9±4.0) %. The fat contents of the *kulikuli* samples were significantly different ($p < 0.001$) (Table 4.8). The fat levels were within the reported average range of (21.95-30.3) % reported by Ezekiel *et al*. (2011) for *kulikuli* samples in Nigerian markets. The level of fats indicates the low recoveries of fats in the cake as well as fat reabsorbed the cake during the frying process. Fats are the predominant constituents of peanuts and its products and make them an invaluable source of dietary energy for consumers. They also play an important role in achieving the texture, mouth feel and aroma of many peanut products (Mazaheri-Tehrani *et al.*, 2009).

4. 3.3.6 Hardness of kulikuli

Hardness is the force required to compress a substance between molar teeth or between the tongue and palate (Civille and Szczesniak, 1973). The hardness values for the *kulikuli* samples from the various regions were significantly different ($p=0.005$). *Kulikuli* from the Ashanti region had the least hardness of (18375.9±7191.7) N/cm^2 while *kulikuli* obtained from the Northern region had the highest hardness value of (19743.7±2824.6) N/cm^2 (Table 4.8).

Table 4.8: Physicochemical properties of *kulikuli*

Region	Moisture (%)	Carbohydrate (%)	Protein (%)	Ash (%)	Fat (%)	Texture(N/cm^2)
Northern	1.2±0.3^a	35.3±6.6^a	31.8±5.4^a	3.8±1.3^a	27.9±4.0^a	19743.7±2283.7^a
Ashanti	1.0±0.6^b	20.1±4.4^b	40.8±3.3^b	4.4±1.1^b	33.7±5.5^b	18375.9±7191.7^b
Greater Accra	2.4±1.8^c	31.2±5.0^a	34.1±8.3^{ab}	4.2±1.3^c	28.1±4.2^a	18586.5±2824.6^c
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.005

*p- values < 0.05 means there is significance statistically. *Values are means of three replicates ± standard deviation
*Different letters (a, b, c) in the same column or row for each parameter indicate significant differences between means

4.3.4 Proximate composition of khebab *powder*

The proximate analyses of *khebab* powder samples were determined. The results are shown in Table 4.9.

4.3.4.1 Moisture

The moisture content is known to affect stability, quality and texture of the peanut products (Lee and Resurrecion, 2006). There were no significant variations between the samples collected from the areas under study. The moisture content of the *khebab* powder samples was in the range of (4.0-4.3) %. Moisture contents of 9% have resulted in significant deterioration of quality of products (Pattee and Young, 1982). It is therefore important that moisture levels are kept below 9% so as to prevent the growth of aflatoxigenic moulds in peanuts and its by-products. The *khebab* powder samples were below 9% and thus their likelihood to support the growth of spoilage organisms and aflatoxigenic moulds will be lowered to a very large extent. The low moisture content also increases its shelf life and prevents the onset of rancidity (Ayoola *et al*., 2012).

4.3.4.2 Carbohydrate

The carbohydrate content of the *khebab* powder was in the range of (36.4-40.1) %. There were no significant differences in the carbohydrate values obtained. The relatively high carbohydrate content of the khebab powder samples makes it a suitable source of nutrient and energy (Ayoola *et al.*, 2012).

4.3.4.3 Protein

The protein content of the *khebab* powder samples were in the range of (26.2-30.2) %. There were significant differences between samples from the various regions. The samples from the Ashanti region of Ghana recorded the lowest protein content of (26.2±0.1) % while those from the Greater Accra region recorded the highest protein content (30.2±0.1) %. The relatively high protein content of the *khebab* powder samples shows that they will be effective in building and repairing worn out tissues and also in improving the energy density of consumers (Ayoola *et al.*, 2012).

4.3.4.4 Ash

The ash which contains the minerals can be a good source of nutrients for consumers. There were no significant differences among the *khebab* powder samples.

The ash content of the *khebab* powder samples was in the range of (7.0-7.1) %. The samples from the Ashanti and Greater Accra regions of Ghana recorded the highest levels of ash (7.1±0.2) % while those from the Northern region recorded the lowest levels (7.0±0.2) %.

4.3.4.5 Fat

The fat content of the *khebab* powder samples ranged between (20.3-25.0) percent. There were significant differences (<0.001) between samples from the various regions. The *khebab* powder samples from the Ashanti region had the lowest fat content (20.30.2) % while those from the Greater Accra region had the highest fat content (25±0.2) %. The relatively high fat content of the *khebab* powder samples makes it a suitable source of nutrient that can improve the energy density of consumers and the different cultivars of peanuts used.

Table 4.9: Proximate composition of *khebab* powder from selected communities in Ghana

Region	Moisture	Carbohydrate	Protein	Ash	Fat
Northern	4.0±0.2^{a}	38.5±0.2^{a}	28.2±0.1^{a}	7.0±0.2^{a}	22.3±0.2^{a}
Ashanti	4.3±0.4^{a}	40.1±19.0^{a}	26.2±0.1^{a}	7.1±0.2^{a}	20.3±0.2^{a}
Greater Accra	4.0±0.2^{a}	36.4±19.0^{a}	30.2±0.1^{b}	7.1±0.2^{a}	25.0±0.2^{b}
p-value	0.255	0.420	<0.001	0.887	<0.001

*p- values < 0.05 means there is significance statistically. *Values are means of three replicates ± standard deviation

*Different letters (a, b, c) in the same column or row for each parameter indicate significant differences between means

4.4 MICROBIAL CONTAMINATION OF PEANUT PRODUCTS OBTAINED FROM THE TRADITIONAL PROCESSING OF PEANUT OIL.

In microbial analysis of food, the number and type of microbes present in the food material under examination reflects the quality of the food and the extent of risk posed to the consumers (Candlish *et al.*, 2001). The microbial population obtained from peanut products taken during the traditional processing of peanut oil is shown in Table 4.10. The viable cell counts, *E. coli*, Coliform, yeast, mould, *Staph aureus* and Salmonella counts were determined and all showed significant differences.

4.4.1 Viable cell counts

The viable cell count of food products is a useful indicator of the level of microorganisms in foods (Maturin and Peeler, 1998). The viable cell counts of the peanut products ranged between 10^1 and 10^6 cfu/g (Tables 4.10). Peanut paste recorded the highest viable cell count of (6.0±0.7)10^4 cfu/g while *kulikuli* reported the least viable cell count (1.8±0.2) 10 cfu/g out of the peanut products from the Northern Region of Ghana. For the Ashanti region, peanut cake reported the highest viable cell count (3.00±1.7) 10^6 cfu/g, while peanut oil recorded the least count (1.0±0.2) 10^1 cfu/g. In the Greater Accra Region, peanut cake recorded the highest viable cell count (1.5±0.7) 10^6 cfu/g while peanut oil recorded the least count (6.5±0.9) 10^1 cfu/g.

Although plate counts do not necessarily indicate the presence of a food safety hazard, they give indications about the severity of heat treatment processes, the microbial quality of the food and the shelf-life of the food products (IFST, 1997).The high viable cell counts obtained for most of the peanut products with the exception of peanut oil are indications of unhygienic processing conditions.

4.4.2 E coli

Escherichia coli are bacteria that are commonly found in the gastrointestinal tract of humans and warm-blooded animals (Butler *et al.*, 2006). Due to its high prevalence in the gut, it is used as the preferred indicator to detect and measure faecal contamination in the assessment of food and water safety. The peanut product with the highest *E. coli* counts in the Northern region of Ghana was peanut cake (1.3±1.9) 10^3 cfu/g while the lowest was peanut oil (1.8±1.7) 10^1 cfu/g (Tables 4.10).

In the Ashanti region, the peanut product with the highest *E coli* count was peanut cake (3.0±1.7) 10^6 cfu/g while the least was peanut oil (1±0.2)10^1 cfu/g. In the Greater Accra region, the highest E coli count was found in peanut paste (8.5±0.5) 10^5 cfu/g and the lowest was found in peanut oil (2.0±0.2) 10^1 cfu/g.

The detection of *Escherichia coli* which is an enteric bacteria, confirms the poor hygienic practices among handlers of products. Also, similar results have reported by Adjou *et al.* (2012) and Ezekiel *et al.*,2011) were bacterial counts between ($5.4x10^4$-$7.2x10^6$) cfu/g was isolated from peanut cake samples and this was attributed to low level of hygiene maintained during the processing and sale of the peanut cake product. Low *Escherichia coli* counts were recorded for the peanut oil due to the high temperatures at which they are processed. The presence of *E coli* is an indication of faecal contamination of the foods and consumption of these foods can pose health issues to consumers or even result in death.

4.4.3 Coliform counts

Coliforms are bacteria that can serve as indicators of unhygienic processing environments and fecal contamination (Baylis *et al.*, 2011). The peanut product with the highest coliform count was peanut paste (1.2±0.4) cfu/g while raw peanut recorded the least coliform count (2.0±0.3) 10 cfu/g in the Northern region as shown in Tables 4.10. In the Ashanti region, peanut cake and peanut paste recorded the highest coliform counts of (8.0±0.7) 10^5 cfu/g and (5.0±2.8) 10^4 cfu/g respectively while peanut oil had the least counts (5.0±0.7) 10^1 cfu/g.

In the Greater Accra region, peanut paste and peanut cake had the highest counts with (1.4±0.5) 10^5 cfu/g and (3.7±0.7) 10^4 cfu/g respectively while peanut oil recorded the lowest counts (5.2±0.7) 10^1 cfu/g. The high coliform count in the peanut cake is similar to the findings of Adjou *et al.* (2012) in which high levels of coliform were isolated.

4.4.4 Yeast and moulds count

Yeast and moulds are a diverse group of fungi that can grow in foods within a wider range of pH from 2 - 9 especially the moulds (APHA, 2001). Table 4.10 indicates that the yeasts and moulds count were lower than the counts recorded for the bacteria. The highest yeast count was recorded for peanut cake from the Northern region of Ghana (3.0± 0.7) 10^3 cfu/g while the least count was recorded for roasted peanuts (1.0±0.8) 10^1 cfu/g from the Greater Accra region. The highest mould count was recorded for raw peanuts (3.0± 0.4) 10^3 and the least was recorded for (1.0±1.3) 10^1 cfu/g. The low yeast and mould counts could be due to the longer generation time for yeast and moulds as compared to bacteria which have a shorter generation time. The high yeast count in the peanut cake could explain why they fermented after being kept for some time. The yeasts and moulds that cause spoilage of processed foods are the aerobes (APHA, 2001). According to Prescott (2002), most vegetative yeasts and mould cells and their spores can be destroyed at temperatures between (50 – 80) °C. The survival of yeast and moulds in most processed products is usually attributed to under processing or post processing recontamination (Prescott, 2002). The high counts of (3.0± 0.7) 10^3 cfu/g for yeast and moulds (3.0±0.4)10^3 cfu/g could probably lead to spoilage of the products with extended storage.

4.4.5 Staph aureus and Salmonella

Staph aureus and *Salmonella* were not detected in all the peanut products (Tables 4.10). *Salmonella* has been implicated as one of the leading causes of foodborne diseases in the United States of America. It is an organism of public health concern and measures must be put in place to ensure they are not detected in peanuts and their by-products.

Table 4.10: Microbial population of viable cells, *E coli*, Coliforms, *Staph aureus* and *Salmonella* in different peanut products sampled from selected communities in Ghana

		Population (cfu/g)						
Region	**Peanut product**	**Viable cell count**	***E. coli***	**Coliform**	**Yeast**	**Mold**	***Staph aureus***	***Salmonella***
Northern(Tamale)	Raw Peanut	$(1.2\pm0.3)10^{3a}$	$(4.6\pm1.7)10^{2b}$	$(2.0\pm0.3)10^{1c}$	$(2.0\pm0.4)10^{2b}$	$(1.0\pm0.7)10^{2b}$	ND	ND
	Roasted peanuts	$(2.5\pm0.2)10^{2a}$	$(8.5\pm1.1)10^{1b}$	$(1.5\pm0.5)10^{2a}$	$(1.0\pm0.9)10^{2a}$	$(1.0\pm0.8)10^{1b}$	ND	ND
	Peanut paste	$(6.0\pm0.7)10^{4a}$	$(2.8\pm2.0)10^{3b}$	$(1.2\pm0.4)10^{4a}$	$(2.8\pm0.8)10^{2b}$	$(1.0\pm0.6)10^{1c}$	ND	ND
	Peanut cake	$(1.3\pm0.7)10^{4a}$	$(1.3\pm1.9)10^{3b}$	$(3.5\pm0.7)10^{3b}$	$(3.0\pm0.7)10^{3b}$	$(1.6\pm0.3)10^{1c}$	ND	ND
	Peanut oil	$(1.8\pm0.2)10^{a}$	$(1.0\pm0.2)10^{1a}$	$(2.8\pm0.7)10^{1b}$	$(2.2\pm0.8)10^{1b}$	$(1.0\pm1.3)10^{1a}$	ND	ND
	Kulikuli	$(1.0\pm0.2)10^{a}$	$(1.3\pm0.4)10^{a}$	$(8.0\pm0.4)10^{1a}$	$(1.3\pm1.2)10^{2b}$	$(6\pm0.9)10^{1a}$	ND	ND
Ashanti(Ejura)	Raw peanuts	$(3.0\pm0.2)10^{5a}$	$(2.0\pm1.7)10^{2b}$	$(4.0\pm0.3)10^{4a}$	$(5.0\pm0.7)10^{1c}$	$(3.0\pm0.4)10^{3a}$	ND	ND
	Roasted peanuts	$(7.0\pm0.6)10^{3a}$	$(3.0\pm1.1)10^{1b}$	$(3.0\pm0.5)10^{3a}$	$(2.0\pm0.9)10^{1b}$	$(2.0\pm0.5)10^{1b}$	ND	ND
	Peanut paste	$(6.0\pm0.9)10^{5a}$	$(5.0\pm2.8)10^{4a}$	$(4.0\pm0.5)10^{5a}$	$(3.0\pm0.8)10^{2b}$	$(2.0\pm0.6)10^{1c}$	ND	ND
	Peanut cake	$(3.0\pm1.7)10^{6a}$	$(3.0\pm1.9)10^{4b}$	$(8.0\pm0.7)10^{5a}$	$(6\pm0.7)10^{2c}$	$(1.0\pm0.3)10^{1c}$	ND	ND
	Peanut oil	$(1.0\pm0.2)10^{a}$	$(1.0\pm0.2)10^{a}$	$(5.0\pm0.7)10^{b}$	$(3.0\pm0.8)10^{1a}$	$(1.0\pm1.3)10^{1a}$	ND	ND
	Kulikuli	$(4.0\pm0.2)10^{2a}$	$(4.0\pm0.4)10^{2a}$	$(4.8\pm0.9)10^{2a}$	$(3.3\pm1.2)10^{1b}$	$(3\pm0.9)10^{1b}$	ND	ND

Table 4.10 Continued: Microbial population of viable cells, *E coli*, Coliforms, *Staph aureus* and *Salmonella* in different peanut products sampled from selected communities in Ghana

Region	Peanut products	Viable cell	*E coli*	Coliform	Yeast	Mould	*Staph aureus*	*Salmonella*
Greater Accra(Accra)	Raw peanuts	$(1.7\pm0.2)10^{5a}$	$(1.4\pm1.7)10^{3b}$	$(3.6\pm0.3)10^{3b}$	(3.0±0.7)10c	$(1.0\pm0.7)10^{3b}$	ND	ND
	Roasted peanuts	$(4.8\pm0.3)10^{2a}$	$(8.0\pm1.1)10^{2a}$	$(2.9\pm0.5)10^{4b}$	$(1.0\pm0.9)10^{1c}$	$(1.0\pm0.8)10^{1c}$	ND	ND
	Peanut paste	$(8.5\pm0.5)10^{5a}$	$(3.6\pm2.8)10^{4a}$	$(1.4\pm0.5)10^{5a}$	$(3.4\pm0.8)10^{2b}$	$(1.0\pm0.6)10^{1c}$	ND	ND
	Peanut cake	$(1.5\pm0.7)10^{6a}$	$(3.6\pm1.9)10^{3b}$	$(3.7\pm0.7)10^{4a}$	$(5.8\pm0.7)10^{2c}$	$(1.6\pm0.3)10^{2c}$	ND	ND
	Peanut oil	$(6.5\pm0.9)10^{1a}$	$(2.0\pm0.2)10^{1b}$	$(5.2\pm0.7)10^{1a}$	$(2.0\pm0.8)10^{1b}$	$(1.0\pm1.3)10^{1c}$	ND	ND
	Kulikuli	$(1.2\pm0.2)10^{2a}$	$(1.0\pm2.4)10^{2a}$	$(6.0\pm0.4)10^{2b}$	$(3.0\pm1.2)10^{2a}$	$(7\pm0.9)10^{1c}$	ND	ND

*p- values < 0.05 means there is significance statistically. *Values are means of three replicates ± standard deviation.

*Different letters (a, b, c) in the same column or row for each parameter indicate significant differences between means

4.4.6 Microbial population of khebab powder purchased from the market and those produced from the home

Generally, high viable cells, *E. coli*, coliforms, yeast and mould counts were recorded for market samples of *khebab* powder compared to *khebab* powder obtained directly from the processors at the point of production (ie. home), and this cut across the regions studied (Table 4.11). There were significant differences in all the samples analyzed. Viable cells recorded the highest counts compared to *E. coli*, coliforms, yeast and mould counts. For market samples of *khebab* powder, the highest viable cell count was recorded in the Greater Accra region (9.7±1.6) 10^6 while the least was recorded for samples from Tamale (6.2±1.0) 10^5 (Table 4.11). The samples obtained from processors in their homes had lower viable cell counts with the highest being (4.2±0.8) 10^4 for samples from the Ashanti region (Ejura) and the least being (1.04±1.6) 10^5 for samples from the Greater Accra region. These differences could be as a result of cross contamination of the market samples probably due to the open air exposure of the market samples of *khebab* powder compared to that obtained from the processors at home.

E coli counts for market samples of *khebab* powder was high for the Greater Accra region (Accra) (7.5±0.2) 10^2 and low in samples from the Ashanti Region (Ejura) (1.5±0.2) 10^2 (Table 4.11). The least *E coli* count for home *khebab* powder was recorded for samples from the Greater Accra region (1.0±1.2) 10^2 and the highest was recorded for samples from the Northern region (Tamale) (1.2±0.2) 10^2 (Table 4.11). There were significant variations in the *E coli* counts for the market samples and the home samples.

This may be due to the unhygienic conditions used during the processing of the samples as well as the open air exposure of the *khebab* powder.

Coliform counts for home *khebab* powder was high in samples from the Greater Accra region (7.5±0.6) 10^{2} and low in samples from the Ashanti region (3.9±0.2) 10^{4}. Market *khebab* powder samples from the Northern region recorded the highest coliform counts (7.4±0.4) 10^{2a} while the least coliform counts were recorded for samples from the Ashanti region (Ejura) (3.9±0.2) 10^{2a} (Table 4.11). There were significant differences between the samples and this could be as a result of the unhygienic practices used by the processors as well as the open air exposure of the market sold *khebab* powder samples.

Yeast counts for the market *khebab* powder were highest in samples from the Ashanti region (Ejura) (7.5±1.0) 10^{2} and lowest in samples from the Greater Accra region (Accra) (2.7±0.5) 10^{2a}. The highest yeast counts for home *khebab* powder were recorded for samples from the Ashanti region (Ejura) (6.5±1.2) 10^{2b} and the least was recorded for samples from the Northern region (Tamale) (1.5±1.2) 10^{2b} (Table 4.11). There were significant variations in the yeast counts and this could be due to the unhygienic conditions and practices employed by the processors as well as the open air exposure of the market sold *khebab* powder samples.

Mould counts for the market *khebab* powder samples were highest in samples from the Northern region (Tamale) (4.5±1.6) 10^{2b} and lowest in samples from the Greater Accra region (3.5±0.2) 10^{2b}. For home *khebab* powder samples, the highest mould counts were

recorded for samples from the Northern region (3.6±0.2) 10^{2b} and the least was recorded for samples from the Ashanti region (1.5±0.2) 10^{2b} (Table 4.11). There were significant differences in the mould counts obtained for the home *khebab* powder and the market khebab powder. This may be due to the unhygienic conditions and practices employed by the processors as well as the open air exposure of the market sold *khebab* powder samples.

Table 4.11: Level of microbial contamination in *khebab* powder purchased from the market and *khebab* powder obtained from processors at home in selected communities in Ghana

Samples	Viable Cell Count(cfu/g)	*E. coli*(cfu/g)	Coliform(cfu/g)	Yeast(cfu/g)	Mold(cfu/g)	*Staph aureus* (cfu/g)	*Salmo-nella*(cfu/g)
Accra							
Market	$(9.7\pm1.6)10^{6a}$	$(7.5\pm0.2)10^{2a}$	$(5.2\pm0.2)10^{6a}$	$(2.7\pm0.5)10^{2a}$	$(3.5\pm0.2)10^{2b}$	ND	ND
Home	$(1.04\pm1.6)10^{5b}$	$(1.0\pm1.2)10^{2a}$	$(7.5\pm0.6)10^{2b}$	$(2.8\pm0.4)10^{2b}$	$(2.5\pm0.4)10^{2a}$	ND	ND
Tamale							
Market	$(6.2\pm1.0)10^{5a}$	$(4.6\pm0.2)10^{2a}$	$(7.4\pm0.4)10^{4a}$	$(7.2\pm0.5)10^{2a}$	$(4.5\pm1.6)10^{2b}$	ND	ND
Home	$(1.8\pm0.5)10^{6b}$	$(1.2\pm0.2)10^{2b}$	$(6.1\pm0.2)10^{3b}$	$(1.5\pm1.2)10^{2b}$	$(3.6\pm0.2)10^{2a}$	ND	ND
Ejura							
Market	$(7.6\pm0.8)10^{4a}$	$(1.5\pm0.2)10^{2a}$	$(4.5\pm1.2)10^{3b}$	$(7.5\pm1.0)10^{2a}$	$(3.6\pm1.2)10^{2b}$	ND	ND
Home	$(4.2\pm0.8)10^{4b}$	$(1.1\pm0.2)10^{2b}$	$(3.9\pm0.2)10^{4a}$	$(6.5\pm1.2)10^{2b}$	$(1.5\pm0.2)10^{2b}$	ND	ND

*p- values < 0.05 means there is significance statistically. *Values are means of three replicates ± standard deviation
*Different letters (a, b, c) in the same column or row for each parameter indicate significant differences between means

4.5 AFLATOXIN CONTAMINATION IN TRADITIONALLY PROCESSED PEANUT OIL AND ITS BY-PRODUCTS FROM SELECTED SITES IN TAMALE, EJURA AND ACCRA

Tables 4.12, 4.13 and 4.14 show the aflatoxin concentrations of raw peanuts, peanut paste, peanut cake, *kulikuli* and peanut oil sampled from the various areas of study. Significant differences were obtained for all the peanut products at the different locations.

Generally, it can be seen in Table 4.12 that the highest total aflatoxin concentration was recorded for raw peanuts from Tamale in the Northern region of Ghana with a concentration of 563.33µg/kg. Similar results have been reported by Nyirahakizimana *et al.* (2013). Aflatoxins B1 and B2 recorded the highest aflatoxin concentration values of (522.1±1.2) µg/kg and 41.2±0.1 µg/kg respectively for raw peanuts. Graphs depicting the levels of aflatoxin concentrations in the various peanut products are shown in figures 4.6, 4.7 and 4.8.

Aflatoxins G1 and G2 recorded the least aflatoxin concentrations in samples from the Northern region of Ghana. Peanut cake, *kulikuli* and peanut oil also had high levels of AFB1. In Figures 4.6, 4.7 and 4.8 it can be observed that processing does not remove aflatoxins totally from food but remains throughout the various processing steps. Some food processing steps such as roasting has been reported by Siwela *et al.* (2011), Yu *et al* .(2004),Galvez *et a*l.(2003) and Okello *et al.*(2010) to reduce the levels of aflatoxins in peanuts and its products.

A similar trend can be seen in Table 4.13 for samples from Ejura in the Ashanti region as seen for the peanut products selected from the Northern Region of Ghana. A total aflatoxin

concentration of 314.43µg/kg was obtained. Aflatoxins B1 and B2 were the main aflatoxins found with 302.8±0.03 µg/kg for raw peanuts and 25.00±0.05 µg/kg for *kulikuli*. Figure 4.8 clearly shows this observation. The high aflatoxin concentration may also be as a result of the poor handling practices associated with indigenous processing. Improper or no sorting of raw peanuts as well as storage of raw peanuts in stalls where they are exposed to weather fluctuations may be contributing factors.

However, very low levels of aflatoxin were detected in peanut products from the Greater Accra region. Raw peanuts had the least concentration of total aflatoxins 0.85 µg/kg while peanut cake recorded the highest total aflatoxin concentration of 117 µg/kg (Table 4.14).

Traditionally processed peanut oil recorded aflatoxin levels of 23.69 µg/kg, 78.57µg/kg and 20.9 µg/kg in the Ashanti, Northern and Greater Accra regions which is to be expected as reported by Chang *et al*. (2013). However, aflatoxins are primarily a problem for whole peanuts but not peanut oil as observed by Parker and Melnick (1966) where peanut oil extracted from contaminated peanuts with 5500 ppb had less than 1ppb aflatoxin concentration. The levels of aflatoxins reported for peanut oil, may be related to the impurities recorded for the oil.

According to Bakole and Adebanjo (2003), *A. flavus* is the commonest of the aflatoxigenic moulds which produces aflatoxins B1 and B2 while *A. parasiticus* produces G1 and G2 in addition to B1 and B2. The results suggested that a lot of

aflatoxin production were probably the result of *A. flavus* activities as reported by Bradburn *et al*. (1993) since B1 and B2 were the major aflatoxins detected. The total aflatoxin level generally were far higher than the set maximum limit of 4µg/kg by the European Union and 20 µg/kg maximum limit by the United States of America (maximum limit) (FVO, 2007).

Table 4.12: Level of aflatoxin contamination in traditionally processed peanut oil and by-products from selected communities in Tamale

	Aflatoxin concentration(µg/kg)					
Peanut product	**B1**	**B2**	**G1**	**G2**	**p-value**	**Total aflatoxin concentration(µg/kg)**
Raw peanut	522.1±1.2^{a}	41.2±0.1^{b}	-	0.03±0.01^{a}	0.003	563.33
Peanut paste	0.05±1.0^{a}	8.2±0.02^{b}	0.02±0.01^{a}	-	0.003	8.27
Peanut cake	89.5±0.05^{a}	16.6±0.05^{b}	3.76±0.1^{c}	-	<0.001	109.86
Kulikuli	65±0.02^{a}	16.8±0.08^{b}	1.89±0.1^{c}	-	<0.001	83.69
Peanut oil	65±0.01^{a}	12.27±0.02^{b}	1.3±0.1^{c}	-	<0.001	78.57

*p- values < 0.05 means there is significance statistically. *Values are means of three replicates ± standard deviation
*Different letters (a, b, c) in the same column or row for each parameter indicate significant differences between means

Table 4.13: Level of aflatoxin contamination in traditionally processed peanut oil and it's by-products from selected communities in Ejura(Ashanti Region of Ghana)

	Aflatoxin concentration(µg/kg)					
Peanut product	**B1**	**B2**	**G1**	**G2**	**p-value**	**Total aflatoxin concentration (µg/kg)**
Raw peanuts	302.8±0.03[a]	11.6±0.1[b]	-	0.03±0.01[c]	<0.001	314.43
Peanut paste	23.23±0.05[a]	3.72±0.01[b]	0.02±0.01[c]	-	<0.001	26.97
Peanut cake	93.9±0.02[a]	13.10±0.02[b]	3.76±0.1[c]	-	<0.001	110.76
Kulikuli	133.8±0.01[a]	25.00±0.05[b]	1.89±0.0[c]	-	<0.001	160.69
Peanut oil	19.9±0.01[a]	2.49±1.1[b]	1.3±0.02[b]	-	0.002	23.69

*p- values < 0.05 means there is significance statistically. *Values are means of three replicates ± standard deviation
*Different letters (a, b, c) in the same column or row for each parameter indicate significant differences between means

Table 4.14: Level of aflatoxin contamination in traditionally processed peanut oil and it's by-products from selected communities in Accra (Greater Accra Region of Ghana)

	Aflatoxin concentration(µg/kg)					
Peanut product	**B1**	**B2**	**G1**	**G2**	**p-value**	**Total aflatoxin concentration (µg/kg)**
Raw peanuts	0.85.1±0.02[a]	-	-	-	0.159	0.85
Peanut paste	12±0.01[a]	37.0±0.05[b]	25.7±0.1[b]	2.1±0.03[a]	<0.001	76.8
Peanut cake	100.9±0.01[a]	9.9±0.1[b]	6.2±0.02[b]	-	<0.001	117
Kulikuli	45±0.02[a]	4.7±0.03[b]	7.8±1.0[b]	1.00±0.05[b]	0.002	58.5
Peanut oil	19.3±0.03[a]	1.6±0.03[b]	-	-	0.005	20.9

*p- values < 0.05 means there is significance statistically. *Values are means of three replicates ± standard deviation
*Different letters (a, b, c) in the same column or row for each parameter indicate significant differences between means

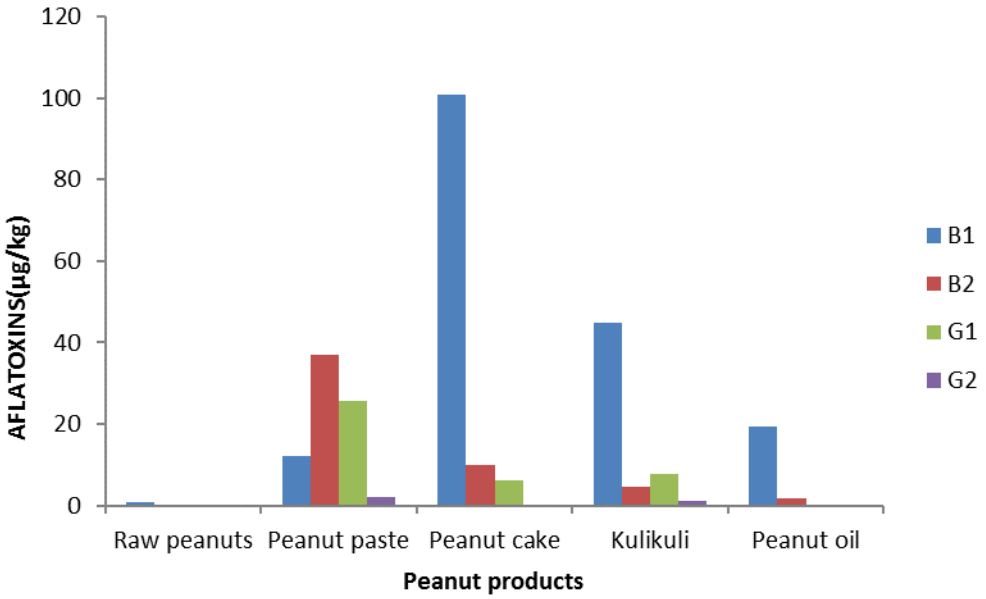


Figure 4.6: Aflatoxin levels in peanut products from selected communities in Accra

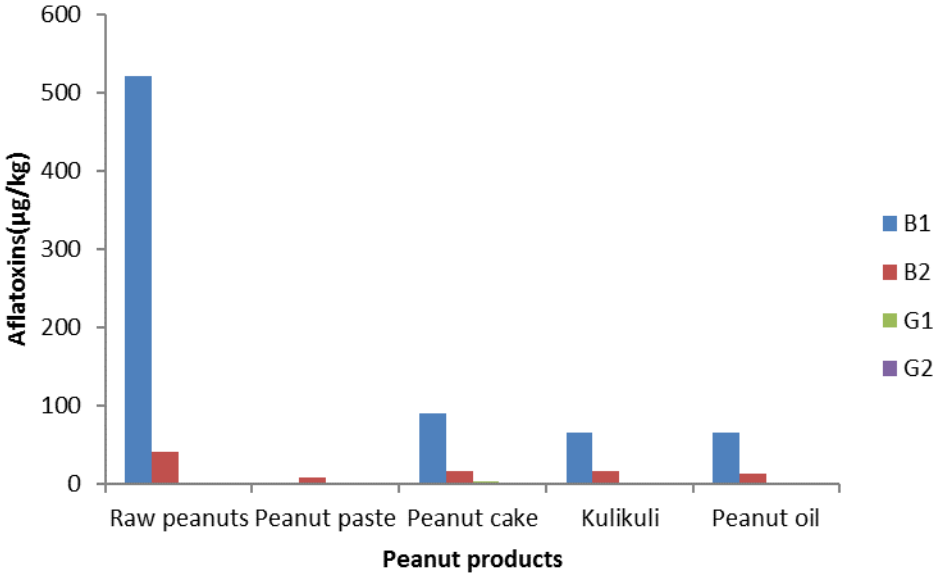


Figure 4.7: Aflatoxin levels in peanuts from selected communities in Northern Region (Tamale)

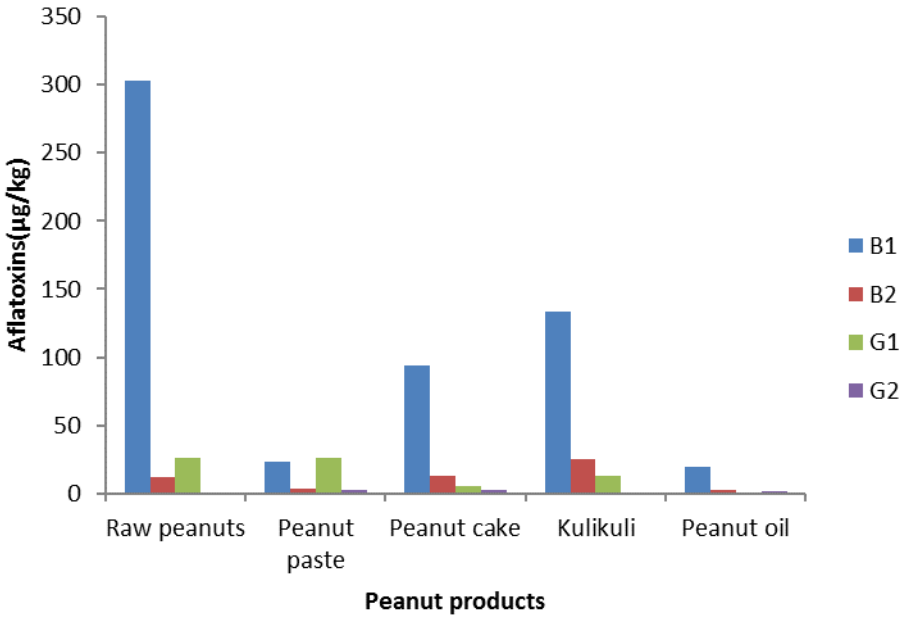


Figure 4.8: Aflatoxin levels in peanut products from selected communities in Ashanti Region (Ejura)

4.6 HACCP SYSTEM FOR THE TRADITIONAL PROCESSING OF PEANUT OIL AND ITS BY-PRODUCTS: *KULIKULI* AND *KHEBAB* POWDER

HACCP has been designed as a food safety management tool to prevent known hazards and to also reduce the risks that they are likely to occur at any point in a process through the execution of seven core steps which include:

1. Conducting a hazard analysis.
2. Determination of the critical control points.
3. Establishment of target levels and critical limits.
4. Establishment of monitoring systems for critical control points.
5. Establishment of an appropriate corrective action plan for each CCP.

6. Establishment of procedures to verify that the HACCP system is working effectively.
7. Establishment of documentation concerning all procedures and record keeping of their application.

A preliminary study on HACCP was done in order to determine the challenges associated with its implementation in the traditional peanut processing industry. The procedures followed are as discussed below.

4.6.1 Commitment of Plant Owner to HACCP implementation and maintenance

The owner of the peanut processing plant must show commitment to the implementation of HACCP in the production of peanut oil and its by-products. The owner must be willing to provide funds to implement and maintain the quality system.

4.6.2 The HACCP Team

The HACCP team must include the plant owner, general supervisor of the production process, a microbiologist, a food chemist and a quality assurance expert from any recognized institution.

4.6.3 The Products

Peanut oil and its by-products are processed by sorting, roasting, dehulling and winnowing peanuts. This is followed by milling of the peanuts together with salt (which is optional) into a smooth paste. Cold water is then added to the peanut paste immediately after milling and kneaded until oil separates out. Alternatively, warm water

is added to the peanut paste and kneaded if the peanut paste is allowed to go cold after being milled. Water is added bit by bit to the peanut paste whiles kneading at the same time until oil begins to separate out and the peanut paste changes texture into a tougher mass. The oil that separates out is then collected and re-heated to remove water that might be left over to avoid spoilage.

The peanut cake is further processed into *kulikuli* and *khebab* powder. The *kulikuli* is prepared by moulding the peanut cake into different shapes and deep frying it with peanut oil. The *khebab* powder samples are further processed by pounding the *kulikuli* and further milling in a disc attrition mill into a smooth powder. Spices such as curry powder, black pepper and white pepper are then added according to the taste and preference of the consumer.

Peanut oil and its by-products should contain less than 100 colony forming units per gram of aerobic mesophilic bacteria and should not contain any pathogenic microorganisms, such as, *Salmonella and E. coli*. Aflatoxins present in the product should not exceed 20 µg/kg.

4.6.4 Intended use of peanut oil and its by-products

Peanut oil can be used in cooking and frying of all types of foods. *Kulikuli* can be consumed as a snack and *khebab* powder used as a seasoning for barbecue meat to improve the taste.

4.6.5 Flow diagram of the products

The flow diagram must be the same as what is actually being practiced on the production plant to ensure that the diagram corresponds with the actual field work. The flow diagram is also important in the identification, evaluation and assessment of hazards which are relevant for food safety as shown in Figure 4.9 below.

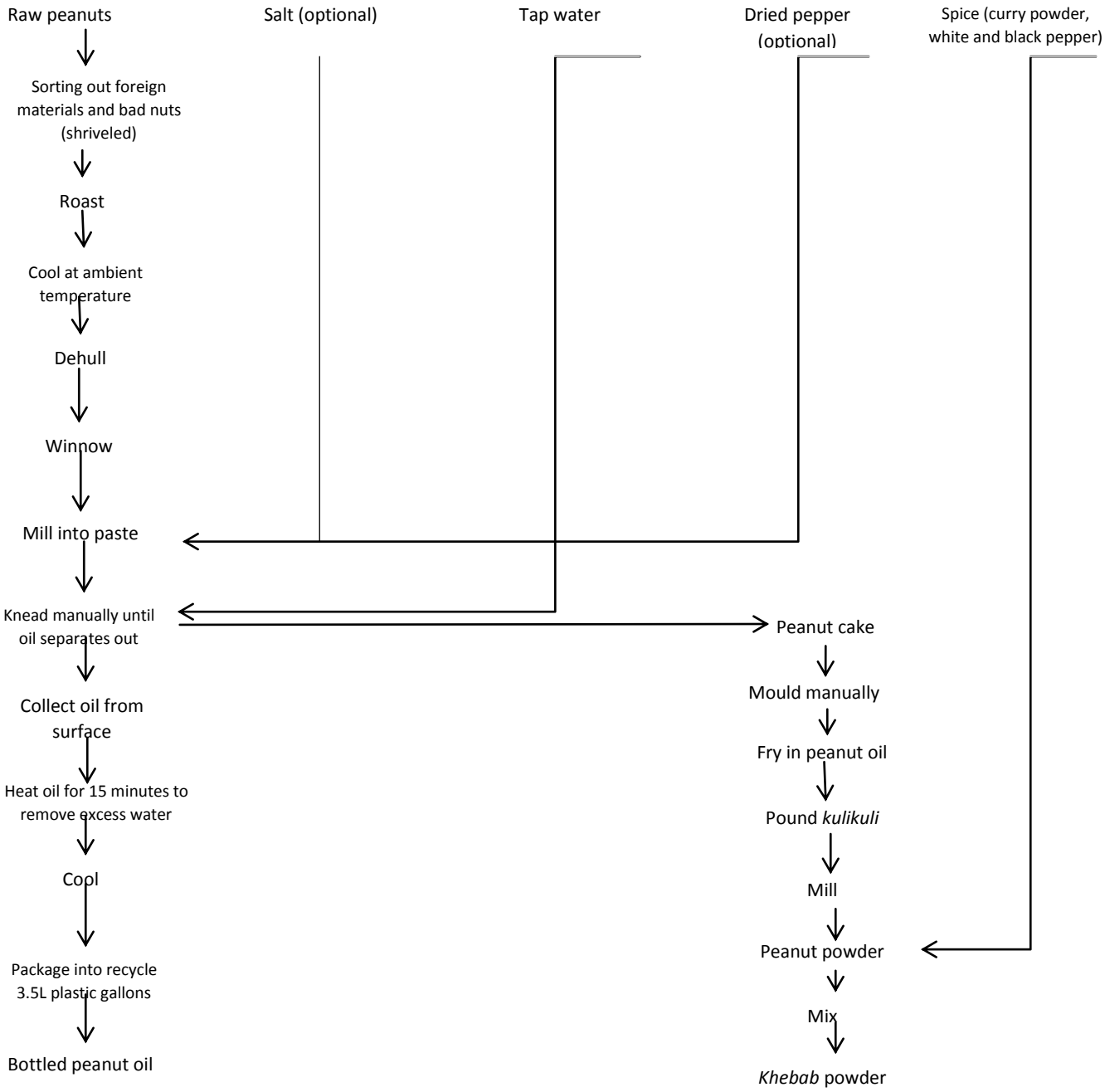


Figure 4.9: Flow Diagram illustrating the processing steps of peanut oil and its by-products: *kulikuli* and *khebab* powder

4.6.6 Identification of hazards

Hazard identification should start from the raw material and end at the finished product. During the sale and storage of peanuts, aflatoxin-producing moulds e.g. *Aspergillus* can grow and cause adverse health effects such as causing liver cancer and stunted growth when consumed over a long period. Microorganisms, in general, need a suitable environment for optimal growth such as the right levels of moisture, temperature and pH. Therefore to control microbial growth, moisture levels of the peanuts should be low by rapidly drying of the peanuts once harvested. The peanuts must be free from any visible signs of moulds. The disc attrition mill that is used for milling the roasted peanuts into paste must be thoroughly cleaned before and after use. Water used for the peanut oil production should be potable. During kneading, the growth of spoilage microorganisms can occur. The use of unwholesome water for kneading will result in the proliferation of microorganisms which may be harmful to human health. The extracted oil must be heated properly for 15 minutes to remove excess water that might be left in it. The packaging material for the peanut oil must be also thoroughly washed. Spices to be used must also be well dried and free from all visible signs of mould growth.

4.6.7 Critical Control Points, Critical Limits and Control Measures

The HACCP team will identify the CCPs and determine the appropriate control measures. The CCPs, critical limits and control measures identified for the production of peanut oil and its by-products are as follows:

• **Raw peanuts:** The raw material (raw peanut) is a CCP because the microbiological safety of the raw material which is peanuts will determine the safety of the end-product. Peanuts that are used for processing should be free from foreign materials such as stones, glass, wood and aflatoxin-producing moulds. This can be ensured by visual inspection to make sure that the peanuts are dry and not mouldy.

•**Roasting**: Galvez *et al. (*2003), Okello *et al*. (2003) and CAC (2004) have shown from studies conducted that roasting reduces the level of aflatoxin contamination in peanuts and its products. Therefore, it must be ensured that roasting is thoroughly done at the right roasting time and temperature.

• **Dehulling:** The skin or hull of the peanuts may have mould growth on them and must be removed so as to reduce the growth and transfer of aflatoxigenic moulds into the peanut oil by-products.

•**Heating of extracted oil**: The freshly extracted peanut oil must be thoroughly heated to remove any left over water that may be present to cause rancidity.

Packaging material (plastic gallon) (3.7L): The plastic gallon must be well cleaned.

Spices: Spices used in the production of *khebab* powder include curry powder, black and white pepper. These spices must be practically free from live and dead insects and moulds.

4.6.7.1 Critical limits for the production of peanut oil

The critical limits for each CCP will ensure that peanut oil produced at the processing plant will satisfy the intended toxicological, microbiological and sensory quality determined by the HACCP team. Good Hygienic Practices and Good Manufacturing Practices must be implemented by workers in the plant.

4.6.8 Establishment of monitoring systems for each CCP

All Critical Control Points identified by the HACCP Team are to be monitored with regular checks. Upon reception of the raw peanuts they should be inspected for any uncharacteristic properties, e.g. growth of moulds, presence of insects and other foreign materials like stones and glass. The water should be potable. The disc attrition mill used in grinding the roasted peanut into paste should be visually inspected for cleanliness before and after use.

4.6.9 Establishment of Corrective Measures

A corrective measure has to be taken when monitoring results show that a CCP is not conforming to the critical limits defined by the HACCP team. When the peanut that is to

be used for the processing is mouldy, it should be rejected. The storage room should be cool and dry. The water that is used for the processing of peanut oil should be clean. All the corrective actions should be recorded by the personnel in charge.

4.6.10 Establishment of verification procedures

The verification procedures should be carried out once every six months on all stages of the production of peanut oil and its by-products. It should involve microbiological and chemical analysis of the raw materials, process steps and the end product. External Audits should be carried out twice a year by a multidisciplinary team of auditors to verify that the right things are being done and the HACCP system is working effectively. Internal audits should also be done every quarter to ensure that the HACCP system is efficient. This will be done by inspecting all documents relating to the HACCP system. A new HACCP system should be created for any change in the production process. Changes may include a change in ingredients, product formulations and intended use.

4.6.11 Documentation

Every single step related to the HACCP system should be documented on appropriate forms. Forms should be appropriately coded for easy identification. Forms should be dated and the revision number stated on each of them. All the forms should have the signature of the person(s) who approve(s) them.

4.7 CHALLENGES IN THE IMPLEMENTATION OF HACCP IN THE PROCESSING OF PEANUT OIL AND ITS BY-PRODUCTS.

Peanut processing in Ghana is mainly done traditionally with no mechanized equipment apart from the disc attrition mill that is used in the milling of the roasted peanuts. Majority of the processors from the survey done had no formal education. This will most likely pose a lot of challenges in the implementation of the HACCP system and hence the processors must be well trained such that they can easily adopt it.

It is also important that all Critical Control Points identified by the HACCP team are monitored with regular checks. This may be a challenge since the processors mainly process based on knowledge passed on from one generation to the next and may not find the need to monitor the peanut products to know if they are meeting the standards set by the HACCP team.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

i. Even though most processors had no formal education, they had adequate knowledge and understanding of the causes of defects in peanuts and how to control them. They also had adequate knowledge of food safety issues related to peanuts consumption.

ii. Traditional peanuts processing in Ghana is dominated by middle aged women with very little formal education. In most cases their chief goal is to extract and sell peanut oil, and the peanut cake obtained after oil extraction is used to make *kulikuli* and *khebab* powder which also have some commercial values. Peanut is the raw material that is used in the traditional processing of peanuts and its by-products. The raw peanuts are sorted by visual inspection and roasted in pans or traditional roasters. They are then milled into a smooth paste using a disc attrition mill. Water is added to the peanut paste in bits while kneading simultaneously until oil begins to separate out. The peanut oil is collected into a plastic gallon. The peanut cake is moulded into different shapes and deep fat fried with.P.PP peanut oil into *kulikuli.* The *kulikuli* pounded in a mortar and further milled with a disc attrition mill into smooth powder. Spices such as curry powder, dried pepper and salt are added to the powder to enhance the taste.

iii. There were significant differences between the physical and chemical indices (percentage impurity, moisture, free fatty acids, carbohydrates, proteins, ash, fat and

texture) of the peanut products due to cultivar differences and differences in processing methods.

iv. The peanut by-products which are usually ready to eat snacks (*kulikuli*) and seasonings (*khebab* powder) have unacceptably high microbial loads including *E.coli* and other faecal coliforms.

v. Furthermore aflatoxin contamination levels of the products are far in excess of the regulatory limits.

vi. Consequently the traditionally processed peanuts contain hazards of both microbiological and chemical safety, and these need to be removed (or minimized) by applying quality management systems such as raw material supply quality assurance and HACCP.

vi. The challenges in the implementation of HACCP in the traditional peanut oil and by-products processing can be related to the very low level of formal education of the processors. They cannot easily adapt to the HACCP system and lack the ability to monitor the critical control points that may be identified to ensure they meet the set standards of quality. Formation of an HACCP team will require some ingenuity since the operations are usually cottage in nature and dominated by a sole processor at the home level.

5.2 RECOMMENDATIONS

i. It is recommended that the peanut oil processors are educated and given practical training on the importance of good manufacturing and hygienic practices.

ii. The manual sorting procedure developed by Galvez *et al*. (2003) and Chinnan *et al.* (2012) should be adopted by processors in order to properly sort the peanut lots so as to reduce the aflatoxin levels to acceptable levels.

iii. Strict hygienic processing and handling practices must be adhered to in the processing area to reduce the microbial counts of products to safe and acceptable levels.

iv. A HACCP system must be developed for the traditional processing of peanut oil and its by-products: *kulikuli and khebab* powder.

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APPENDICES

APPENDIX 1: SURVEY QUESTIONNAIRE

DEPARTMENT OF NUTRITION AND FOOD SCIENCE

UNIVERSITY OF GHANA, LEGON

Dear respondent, this questionnaire is aimed at collecting information on peanut oil processing methods and techniques. It is part of an MPhil Food Science Thesis on the topic above. The information you provide in this document will only be used for academic purposes and your identity will be kept confidential. Thank you.

Date: _____ Location: _____

Respondent's Code: _____

Kindly tick (√) the responses that apply to you. Where appropriate, write out your own responses in the spaces provided.

A: BACKGROUND INFORMATION RESPONSE
[For interviewer use only]

1. Sex: 1=Male 2=Female

2. Age: 1=Less than 20 years

2=20 – 25 years
3=26 – 30 years
4=31 – 35
5=36-40 years
6=41-45 years
7=46-50 years
8=51 years and above

3. Hometown, Specify ……

4. Religion

1= Christian
2= Muslim
3= Traditional African religion
4= other, specify…….

5. Highest level of education received

1= None
2= Primary
3= Middle School/JHS
4= Secondary
5= Tertiary
6= Other, specify.....

6. How long have you been in the peanut oil processing business?

1= 1-5 years
2= 6-10 years
3= 11-15 years
4= 16-20 years
5= More than 20 years

B. RAW MATERIAL ACQUISITION

7. Where do you obtain the peanuts you process from?
1 = Farm
2 = Wholesaler
= Market
= Other, specify.....

8. Do you buy in shell or shelled peanuts? .....

9. Do you inspect fresh peanuts before purchasing?
1=Yes 2=No

10. If yes to 7, what do you look out for?
1=Moulds
2=Rancidity/decay
3=Insects
4=Dirt
5=Other, specify.....

10. Do you sometimes observe peanuts with defects among your purchases?
1=Yes 2=No ☐

11. If yes to question (9), describe the look of the peanuts with defects.
……
……
……
……

12. What do you think may be the cause(s) of the defect(s)?
……
……
……
……

13. Do you remove the defective peanuts?
1=Yes 2=No ☐

14. If no to question (12), why don't you remove the defective peanuts?
.....
.....
.....
.....

15. What quantities of peanuts do you process in a batch? Tick the appropriate answer and unit.
1= Less than half a bag
2= 1 bag
3= 2-5 bags
4= 5-10 bags
5= More than 10 bags
6= Other, specify…….

16. Are you always able to process the quantities you procure in a batch?
1=Yes 2=No ☐

17. If no to question (15), indicate how you are able to store the quantity left.
.....
.....
.....
.....

C: TRANSPORTATION OF PEANUTS

18. What containers do you use to carry the peanuts to the processing site?

1= Basins
2=Baskets
3= Polythene bags
4= Other, specify.....

19. How long does it take to transport the peanuts to the processing site?

1=Less than 2 hours
2=2 hours to 6 hours
3=More than 6 hours to 12 hours
4=More than 12 hours to 24 hours
5=More than 24 hours

20. What is the mode of transportation of peanuts to the processing site?

1= By foot
2= Public transport
3= Private transport
4= Other, specify.....

D: PROCESSING OF PEANUTS

21. Please list the steps involved in the processing of peanuts

……
……
……
……
……
……

22. Please provide a detailed description of the processes

23. What kind of by-products do you produce?

……
……
……
……..

24. What do you do with them?

25. Where do you recover much of your revenue?

1=By-products 2=Peanut oil

26. How long the by-product(s) stated in question (23) stay before going bad?

1= Less than 1 day
2= 1 – 3 days
3= More than 3 days, less than 1 week
4= 1 week – 1 month
= More than a month

27. What are some of the types of spoilage you notice in the by-products?

……
……
……
……

28. Are there local standards for your type of product?

1=Yes 2=No

29. How do you control the quality of your product?

.....
.....
.....
.....

30. How did you acquire the skills involved?

1=Formal training
2=Apprenticeship
3=Self-taught
4=Traditional knowledge inherited from family

31. What are some of the food safety issues associated with consuming defective and unclean peanuts?

……
……
……
……

32. Which of the steps listed in the processing method in question (20) can influence the quality and safety of the processed peanut oil?

……
……
……
……

33. Do you sometimes have your peanut oil getting spoilt?
1=Yes 2=No

34. If yes to question (29), please estimate the quantities of peanut oil that get spoilt from the lot you process.
1= Less than 1 gallon
2= 1 – 5 gallons
3= 6 – 10 gallons
4= More than 10 gallons, specify……..

35. What causes the spoilage you sometimes experience?
……
……
……
……

36. What do you do to reduce or eliminate spoilage?
……
……
……
……..

E. HANDLING AND STORAGE OF PEANUT OIL
37. For how long do you store the peanut oil?
.....

38. Where do you store the peanut oil?
1= Regular room
2= Wooden shed
3= Other, specify.....

39. How is the processed peanut oil stored?
1=In plastic bottles
2= In glass bottles
3 = In gallons
= Other, specify.....

40. For how long after processing do you store the peanut oil before selling?
1= Less than 1 day
2= 1 – 3 days
3= More than 3 days, less than 1 week
4= 1 week – 1 month
= More than a month

F.TRANSPORTATION AND MARKETING OF PEANUT OIL
41. How do you market your products?

1= Wholesaler
2= Market
3= Other, specify..... ☐

42. Approximately how long does it take to transport peanut oil from the storage/processing site to the market?
1= Less than 30 minutes
2= 30 mins – 2 hours
3= 2 – 6 hours
4= 6 – 12 h
5= More than 12 hours ☐

43. How do you transport peanut oil to the market?
1= By foot
2= Public transport
3= Private transport ☐
4= Other, specify.....

44. Which markets do you send your peanut oil to?
.....
.....
.....

45. What do you consider as the major constraints in peanut oil processing in Ghana?
……
……
……
……..

THANK YOU

APPENDIX 2: ANOVA TABLES AND MULTIPLE RANGE TABLES

ANOVA Table for refractive index by region

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.065	2	0.0325	16.71	0.0009
Within groups	0.0175	9	0.00194444		
Total (Corr.)	0.0825	11			

Multiple Range Tests for refractive index by region

Method: 95.0 percent LSD

Region	*Count*	*Mean*	*Homogeneous Groups*
2	4	71.425	X
3	4	71.55	X
1	4	71.6	X

ANOVA Table for flash point by region

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	3.5	2	1.75	2.10	0.1784
Within groups	7.5	9	0.8333		
Total (Corr.)	11.0	11			

Multiple Range Tests for flash point by region

Method: 95.0 percent LSD

Region	*Count*	*Mean*	*Homogeneous Groups*
2	4	315.0	X
1	4	315.25	X
3	4	316.25	X

ANOVA Table for % impurity by region

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	10.1667	2	5.08333	8.32	0.0090
Within groups	5.5	9	0.611111		
Total (Corr.)	15.6667	11			

Multiple Range Tests for % impurity by region

Method: 95.0 percent LSD

Region	*Count*	*Mean*	*Homogeneous Groups*
2	4	3.0	X
1	4	4.25	XX
3	4	5.25	X

ANOVA Table for L by region

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.109017	2	0.0545083	2.66	0.1236
Within groups	0.18435	9	0.0204833		
Total (Corr.)	0.293367	11			

Multiple Range Tests for L by region

Method: 95.0 percent LSD

Region	*Count*	*Mean*	*Homogeneous Groups*
3	4	72.11	X
1	4	72.2825	X
2	4	72.3325	X

ANOVA Table for a by region

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.000216667	2	0.000108333	0.05	0.9550
Within groups	0.021075	9	0.00234167		
Total (Corr.)	0.0212917	11			

Multiple Range Tests for a by region

Method: 95.0 percent LSD

Region	*Count*	*Mean*	*Homogeneous Groups*
3	4	-6.01	X
1	4	-6.0025	X
2	4	-6.0	X

ANOVA Table for b by region

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.00111667	2	0.000558333	0.31	0.7428
Within groups	0.01635	9	0.00181667		
Total (Corr.)	0.0174667	11			

Multiple Range Tests for b by region

Method: 95.0 percent LSD

Region	*Count*	*Mean*	*Homogeneous Groups*
2	4	23.4675	X
3	4	23.4725	X
1	4	23.49	X

ANOVA Table for B. Moisture by Region peanut oil_

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.43153	2	0.215765	13.07	0.0022
Within groups	0.148631	9	0.0165145		
Total (Corr.)	0.58016	11			

Multiple Range Tests for B. Moisture by Region_ peanut oil_

Method: 95.0 percent LSD

Level	*Count*	*Mean*	*Homogeneous Groups*
1	4	0.031	X
2	4	0.28175	X
3	4	0.495	X

ANOVA Table for B.Iodine value by Region_peanut oil_

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1.55922	2	0.77961	1.24	0.3334
Within groups	5.6391	9	0.626566		
Total (Corr.)	7.19832	11			

Multiple Range Tests for B. Iodine value by Region_ peanut oil_

Method: 95.0 percent LSD

Level	*Count*	*Mean*	*Homogeneous Groups*
2	4	475.295	X
1	4	475.408	X
3	4	476.11	X

ANOVA Table for B. Saponification value by Region_ peanut oil_

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	7.01015	2	3.50508	0.28	0.7606
Within groups	111.823	9	12.4248		
Total (Corr.)	118.833	11			

Multiple Range Tests for B. Saponification value by Region_ peanut oil_

Method: 95.0 percent LSD

Level	*Count*	*Mean*	*Homogeneous Groups*
1	4	260.382	X
2	4	260.77	X
3	4	262.163	X

ANOVA Table for B. Acid value by Region_ peanut oil_

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	10.7734	2	5.38671	1.17	0.3528
Within groups	41.3575	9	4.59528		
Total (Corr.)	52.131	11			

Multiple Range Tests for B. Acid value by Region_ peanut oil_

Method: 95.0 percent LSD

Level	*Count*	*Mean*	*Homogeneous Groups*
1	4	26.6027	X
3	4	28.0093	X
2	4	28.9048	X

ANOVA Table for B. Peroxide value by Region_ peanut oil_

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.000629752	2	0.000314876	3.48	0.0761
Within groups	0.000815465	9	0.0000906072		
Total (Corr.)	0.00144522	11			

Multiple Range Tests for B. Peroxide value by Region_ peanut oil_

Method: 95.0 percent LSD

Level	*Count*	*Mean*	*Homogeneous Groups*
1	4	0.180375	X
2	4	0.192525	XX
3	4	0.19765	X

ANOVA Table for C. Moisture by Region_ *kulikuli*_

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.0000326667	2	0.0000163333	1.00	0.4053
Within groups	0.000147	9	0.0000163333		
Total (Corr.)	0.000179667	11			

Multiple Range Tests for C. Moisture by Region_ *kulikuli*_

Method: 95.0 percent LSD

Level	*Count*	*Mean*	*Homogeneous Groups*
2	4	0.21	X
3	4	0.21	X
1	4	0.2135	X

ANOVA Table for C. Iodine value by Region_ *kulikuli_*

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	22.5554	2	11.2777	0.19	0.8335
Within groups	546.296	9	60.6996		
Total (Corr.)	568.852	11			

Multiple Range Tests for C. Iodine value by Region_ *kulikuli_*

Method: 95.0 percent LSD

Level	*Count*	*Mean*	*Homogeneous Groups*
2	4	479.635	X
1	4	480.16	X
3	4	482.77	X

ANOVA Table for C. Saponification value by Region_ *kulikuli_*

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.151667	2	0.0758333	0.24	0.7903
Within groups	2.825	9	0.313889		
Total (Corr.)	2.97667	11			

Multiple Range Tests for C. Saponification value by Region_ *kulikuli_*

Method: 95.0 percent LSD

Level	*Count*	*Mean*	*Homogeneous Groups*
1	4	264.975	X
3	4	265.125	X
2	4	265.25	X

ANOVA Table for C. Acid value by Region_ *kulikuli*_

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	2.7253	2	1.36265	2.28	0.1583
Within groups	5.3833	9	0.598144		
Total (Corr.)	8.1086	11			

Multiple Range Tests for C. Acid value by Region_ *kulikuli*_

Method: 95.0 percent LSD

Level	*Count*	*Mean*	*Homogeneous Groups*
2	4	23.5566	X
1	4	24.0642	X
3	4	24.7208	X

ANOVA Table for C. Peroxide value by Region_ *kulikuli*_

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.0000116617	2	0.00000583083	0.33	0.7278
Within groups	0.000159388	9	0.0000177097		
Total (Corr.)	0.000171049	11			

Multiple Range Tests for C. Peroxide value by Region_ *kulikuli*_

Method: 95.0 percent LSD

Level	*Count*	*Mean*	*Homogeneous Groups*
1	4	0.033225	X
3	4	0.03515	X
2	4	0.03545	X

ANOVA Table for C. % Free fatty acid by Region_ *kulikuli*_

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.68819	2	0.344095	2.28	0.1583
Within groups	1.35938	9	0.151043		
Total (Corr.)	2.04757	11			

Multiple Range Tests for C. % Free fatty acid by Region_ *kulikuli*_

Method: 95.0 percent LSD

Level	*Count*	*Mean*	*Homogeneous Groups*
2	4	11.8375	X
1	4	12.0925	X
3	4	12.4225	X

ANOVA Table for D. Moisture by Region_ *khebab* powder_

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	2.30486	2	1.15243	111.89	0.0000
Within groups	0.0926981	9	0.0102998		
Total (Corr.)	2.39756	11			

Multiple Range Tests for D. Moisture by Region_ *khebab* powder_

Method: 95.0 percent LSD

Level	*Count*	*Mean*	*Homogeneous Groups*
1	4	1.52317	X
3	4	2.3	X
2	4	2.55325	X

ANOVA Table for D. Iodine value by Region_ *khebab* powder_

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.344832	2	0.172416	1.07	0.3827
Within groups	1.44927	9	0.16103		
Total (Corr.)	1.7941	11			

Multiple Range Tests for D. Iodine value by Region_ *khebab* powder_

Method: 95.0 percent LSD

Level	*Count*	*Mean*	*Homogeneous Groups*
1	4	488.33	X
2	4	488.418	X
3	4	488.725	X

ANOVA Table for D. Saponification value by Region_ *khebab* powder_

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	8837.77	2	4418.89	0.99	0.4071
Within groups	39975.2	9	4441.69		
Total (Corr.)	48813.0	11			

Multiple Range Tests for D. Saponification value by Region_ *khebab* powder_

Method: 95.0 percent LSD

Level	*Count*	*Mean*	*Homogeneous Groups*
2	4	199.05	X
1	4	256.587	X
3	4	256.65	X

ANOVA Table for D. Acid value by Region_ *khebab* powder_

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	6.65021	2	3.32511	2.29	0.1572
Within groups	13.0782	9	1.45313		
Total (Corr.)	19.7284	11			

Multiple Range Tests for D. Acid value by Region_ *khebab* powder_

Method: 95.0 percent LSD

Level	*Count*	*Mean*	*Homogeneous Groups*
1	4	154.477	X
3	4	154.603	X
2	4	156.116	X

ANOVA Table for D. Peroxide value by Region_ *khebab* powder_

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.000137807	2	0.0000689033	1.96	0.1971
Within groups	0.00031711	9	0.0000352344		
Total (Corr.)	0.000454917	11			

Multiple Range Tests for D. Peroxide value by Region_ *khebab* powder_

Method: 95.0 percent LSD

Level	*Count*	*Mean*	*Homogeneous Groups*
2	4	0.038	X
1	4	0.0393	X
3	4	0.04575	X

ANOVA Table for D. % Free fatty acid by Region_ *khebab* powder_

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1.93713	2	0.968563	3.33	0.0825
Within groups	2.61481	9	0.290535		
Total (Corr.)	4.55194	11			

Multiple Range Tests for D. % Free fatty acid by Region_ *khebab* powder_

Method: 95.0 percent LSD

Level	*Count*	*Mean*	*Homogeneous Groups*
1	4	77.5285	X
3	4	77.69	XX
2	4	78.45	X

APPENDIX 3: PEANUT OIL PROCESSING STEPS

Raw peanuts

Traditional peanut roaster being filled

Traditional roasting of peanuts

Peanut paste (milled, roasted peanuts)

Kneading of peanut paste to separate peanut oil from cake

Moulding of peanut cake into long strands and deep frying into *kulikuli*

Bottled peanut oil

Khebab powder

APPENDIX 4: Table 4.15: Association between the biodata, processing methods and processing techniques of peanut oil processor

	Hometown	Educational level	Age	Number of years in the peanut oil processing business
Where do you obtain the peanuts you process from	(21.110, 0.330)	(0.074,0.964)	(30.000,<0.001)	(1.034,0.905)
Which type of peanuts do you buy	(21.111,0.331)	(0.238,0.888)	(1.296,0.935)	(5.000,0.287)
What do you look out for when purchasing raw peanuts	(-44.667,0.212)	(4.848,0.303)	(10.708,0.381)	(7.375,0.497)
Do you sometimes observe peanuts with defects among your purchase	(25.000,0.161)	(0.536,0.765)	(7.865,0.164)	(4.583,0.333)
Describe the look of peanuts with defects	(0.04175,0.498)	(60.000,0.054)	(0.011,0.572)	(0.0101,0.168)
What do you think may be the cause(s) of the defect(s)	(0.0396,0.097)	(60.000,0.013)	(98.095,0.393)	(75.714,0.488)
What quantities of peanuts do you purchase in a batch	(32.156,0.997)	(1.429,0.964)	(41.097,0.000)	(9.089,0.695)
Are you always able to process the quantities you purchase in a batch	(26.400,0.119)	(4.286,0.117)	(2.812,0.729)	(4.875,0.300)
Indicate how you are able to store the quantity left	(0.0185,0.593)	(60.000,0.000)	(32.188,0.976)	(39.125,0.509)
What containers do you use to carry the peanuts to the processing site	(23.684,0.209)	(2.317,0.314)	(12.507,0.034)	(16.077,0.003)
What is the mode of transportation	(22.222,0.273)	(1.429,0.490)	(6.389,0.270)	(6.111,0.191)

What kind of by-products do you produce	(65.667,0.795)	(3.214,0.920)	(46.218,0.001)	(31.327,0.012)
Where do you recover much of your revenue	(19.683,0.414)	(1.639,0.441)	(2.681,0.749)	(7.330,0.119)
How long do the by-products stay before going bad	(22.222,0.273)	(3.214,0.200)	(6.389,0.270)	(5.764,0.217)
What are some of the types of spoilage you notice in the by-products	(0.0215,0.929)	(44.464,0.013)	(72.153,0.253)	(60.667,0.083)
How do you control the quality of your products	(0.0500,0.422)	(60.000,0.208)	(0.0124,0.638)	(0.0107,0.414)
How do you acquire the skills involved	(30.000,0.052)	(0.330,0.052)	(5.481,0.360)	(7.500,0.112)
What are some of the food safety issues associated with consuming defective and unclean peanuts	(0.024,0.358)	(11.321,0.987)	(71.708,0.143)	(53.133,0.283)
Which of the steps listed in processing methods can influence the quality and safety of the processed peanut oil	(0.0369,0.372)	(60.000,0.013)	(0.0123,0.030)	(0.010,0.024)
What causes the spoilage you sometimes experience	(44.464,0.218)	(30.038,0.000)	(5.625,0.846)	(7.696,0.464)
What do you do to reduce/eliminate spoilage	(0.048,0.082)	(60.000,0.81)	(0.0132,0.134)	(90.583,0.522)
How long do you store the peanut oil	(0.0318,0.089)	(60.000,0.001)	(94.896,0.060)	(76.333,0.076)
How is the processed peanut oil stored	(19.607,0.994)	(0.153,0.997)	(32.812,0.293)	(10.804,0.213)
For how long after processing do you store the peanut oil before selling	(91.267,0.112)	(14.429,0.071)	(22.917,0.293)	(19.567,0.240)

How do you market your products	(30.000,0.052)	(0.074,0.964)	(2.845,0.724)	(1.034,0.905)
Approximately how long does it take to transport peanut oil from the processing site to the market	(23.478,0.217)	(3.647,0.161)	(4.379,0.496)	(5.776,0.216)
How do you transport peanut oil to the market	(30.000,0.952)	(13.9929,0.001)	(2.596,0.702)	(2.452,0.0653)
Which markets do you send your peanut oil to	(0.0338,0.090)	(44.44,0.070)	(78.042,0.541)	(75.600,0.152)
What do you consider as the major constraints in the peanut oil processing in Ghana	(0.0540,0.396)	(30.000,0.998)	(0.0147,0.355)	(1.153E2,0.398)