FRYING OIL QUALITY IMPROVEMENT AND CONTROL OF ACRYLAMIDE FORMATION USING WHOLE PLANT EXTRACTS FROM *RENEALMIA BATTENBERGIANA*

by

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I hereby declare that this submission is my own work towards the MPHIL and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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Abstract

The study was conducted to improve upon the quality of frying oil by extending the frying life and control acrylamide formation using optimized *Renealmia battenbergiana* extract incorporated refined bleached deodorized (RBD) palm olein. A 3 × 3 full factorial design comprising of three treatment factors (extracting temperature, time and extract-oil concentration) varied at three levels respectively (25℃, 45℃, 65℃; 0.5 h, 1.0 h, 1.5 h; and 1% , 3%, 5%) was used. The optimized incorporation condition was consequently found to be 1 % extract-oil concentration at 25 ℃ under agitation contact time of 0.5 h. The frying quality of the optimized *Renealmia battenbergiana* incorporated oil compared to the native oil (N oil) and citrate treated oil (CT oil) was also evaluated. The physicochemical parameters measured were % free fatty acid (%FFA), peroxide value (PV), iodine value (IV) and acrylamide content. All frying quality parameters were found to be significant ($p<0.05$) with respect to the type of frying oil and the frying cycles. *Renealmia battenbergiana* incorporated oil effectively retarded the formation of acrylamide in fried yam slices by 14.99% compared to the native untreated oil. The native oil was found to be unfit for frying after cycle 2 whiles both *Renealmia battenbergiana* incorporated oil and citrate treated oil were fit for frying after cycle 3 of repeated frying based on the quality parameters measured. The overall sensory analysis proved that for all cycles, citrate treated (CT) oil gave the most desirable fried products, followed by the *Renealmia battenbergiana* incorporated oil.

Table of Content

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

1.0 INTRODUCTION

1.1Background

Aromatic plants are sources of diverse natural antioxidants (Exarchou *et al.,* 2002). Aromatic plants have been used since ancient times in food flavourings, pharmaceuticals, cosmetics and perfumery. The work done by Loo and Richard (1992) has shown that beyond aromatic properties of such plants, they exhibit several physiological activities including antimicrobial and antioxidant properties.

In addition, these characteristics have been applied in some plants- *thyme* and *lavender* to improve the quality parameters of frying oils and consequently extend their frying-life (Bensmira *et al.,* 2007). Furthermore, these aromatic plants have been found to significantly retard oil oxidation and deterioration comparably to 0.02 % butylated hydroxytoluene in frying experiments using *Pandanus amaryllifolius* leaf extract, rosemary and sage (Nor *et al.,* 2008; Cheman and Jaswir, 2000).

Renealmia battenbergiana is a wild aromatic plant, belonging to the Zingiberaceae family. It grows in the tropics of West Africa (Achinewhu *et al.*, 1995). *R. battenbergiana*'s flowers and leaves have been used as condiments in red palm oil, meats, soups and stews (Achinewhu *et al.,* 1995). The plant is referred to as "*Atiegya*" among the Akan speaking people of Ghana. It serves as a main source of livelihood for the women and children in the rural forest areas of Ghana who engage in the harvesting. In the crude palm oil industries, the plant is applied at the clarification stage to impact aroma to the crude palm oil before to storage.

In Ghana, deep fat frying is among one of the most popular food cooking methods because of the appetizing attributes of the deep-fried foods. Fractionation of refined bleached and deodorized (RBD) palm oil yields refined bleached deodorized palm olein (liquid) and refined bleached deodorized palm stearin (solid) fractions. RBD palm olein is largely used as cooking oil and in industrial frying. RBD palm stearin is used in biscuits, cakes and non-hydrated margarine (Cheman and Jaswir, 2000).

Despite all the these attributes of refined edible oils, deep fat frying particularly of French fries, potato chips, and certain snack foods, has been identified as one of the food heating processes leading to considerable acrylamide formation in the products (Friedman, 2003). However, it has been reported that antioxidants from bamboo leaves (plant extract) significantly reduced acrylamide formation in fried chicken wings (Zhang *et al.,* 2007).

It is presumed that the antioxidant content of *R. battenbergiana* could successfully be exploited and applied in food processing as an agent to control acrylamide and prevent the formation of undesirable oxidation products.

1.2 Problem Statement

There is growing health concern about safety of deep fried foods due to accumulation of compounds such as acrylamide, diacylglycerols, free fatty acids, monomers, polymers and other degradative products which are harmful to the human body. The presence of acrylamide which is classified as a probable human carcinogen (by International Agency for Research on Cancer, IARC, 1994; EC, 2000) in heated foods was reported in 2002 (Rosen and Hellenas, 2002; Tareke *et al.,* 2002). In addition, deep fry industries are increasingly making significant losses in oil cost by investing in fresh oil from time to time due to the development of rancidity with its attendant off flavour impact on food products (White, 1991; Lin *et al.,* 1998).

The use of exogenous synthetic preservatives such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBHQ) has not achieved much success in improving frying quality because of their safety and effectiveness particularly at elevated frying temperatures (Cuvelier *et* al., 1994). Some manufacturing industries have tried to improve upon the stability of the RBD palm olein through hydrogenation to withstand intense heat and frying for longer periods of time. However, this hydrogenation process adds unhealthy trans-fat which are harmful to the human body (Maskan, 2003).

Also, various effective ways for the reduction of acrylamide have been found during these years such as change of precursors in food materials (Fiselier and Grob, 2005), optimization of heat processing methods (Granda *et al.,* 2004; Williams, 2005), optimization of suitable cultivar and storage temperature of food materials (Biedermann-Brem *et al.,* 2003), fermentation (Fredriksson *et al.,* 2004). However, these may be difficult to achieve in an industrial setting or in commercial products.

However, in Ghana, there is insufficient scientific attention being given to indigenous aromatic plants as potent antioxidants for frying oils. Aromatic plants have found several applications in perfumery, pharmaceuticals and food flavouring but beyond their aromatic properties, they could be applied as antioxidants and antimicrobial (Bensmira *et al.,* 2007). Research work done outside Ghana has shown that indigenous aromatic plants such as *Thyme* and *Lavender* (Bensmira *et al.,* 2007*)*, Rosemary and Sage extract (Cheman and Jaswir, 2000), and *Pandanus amaryllifolius* leaf extracts (Nor *et al.,* 2008) improved the quality parameters of frying oils by retarding oxidative deterioration and acrylamide formation. Some research findings aimed at finding conditions to control or prevent acrylamide formation concluded that adding ground aromatic rosemary to olive oil resulted in 25% reduction in acrylamide formation in fried potato slices (Becalski *et al.*,

2003). Consequently, frying-life was extended and safety of fried foods improved. This provides considerable savings to the deep fat fried foods industries.

1.3 Goal/Aim

The goal of the research is to evaluate the use of *R. battenbergiana* extract incorporated in RBD palm olein as agent to control acrylamide in the cooking oil industry.

1.4 Objective

The study seeks to optimize the incorporation conditions of *R. battenbergiana* extract into RBD palm olein and compare its frying performance in terms of free fatty acid, peroxide value, iodine value, acrylamide content and sensory attributes of the fried products.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 RBD Palm olein as a Commercial Frying Oil

Refined bleached deodorized (RBD) palm olein is one of the standard frying oils which is commonly used for deep frying purposes, e.g. of French fries, instant noodles, potato chips, chicken, doughnuts and other snack foods. Many research studies have shown that refined palm olein is stable as frying medium compared to other vegetable oils. One of such research studies was conducted by Sulieman *et al.* (2006) who provided data to show that RBD palm olein was stable as frying medium compared to sunflower and cottonseed oil. This was evidenced by the lower peroxide value, colour index, viscosity, total polar compounds and UV absorbance at 232 and 270 nm.

The stable frying qualities of RBD palm olein is also supported by Boskou and Elmadfa (1999) who showed that freshly refined palm olein has excellent oxidative stability. The frying characteristics of the oil is due to the fact that it does not produce excessive smoking, spattering, foaming and forms less gum in the pan and fryer compared to polyunsaturated oil. RBD palm olein is comparable in terms of oxidative stability during frying with other hydrogenated vegetable oils namely hydrogenated soybean, hydrogenated sunflower and hydrogenated cottonseed oils (Nor *et al*., 2008). Both palm oil and palm olein have practically the same frying performance and a similar composition. The significant difference between palm oil and palm olein are degree of fluidity and iodine value. Palm oil has the melting point at about 36 ℃ and palm olein about 22 ℃. Palm oil has the iodine at the range of 50 -54 whereas the iodine value for palm olein varies from 56 up to 60 (Hussein, 2006). Friedman (2003) mentioned that deep

frying using RBD palm olein is widely used because it is quick, easily adaptable to mass production and produce tasty product or attractive appearance and possesses no health risks under controlled frying. For the large scale frying of potato chips, RBD palm olein is preferred. This is because the surface appearance of the finished product is improved.

Many studies have documented the specifications for an ideal frying medium. This is because the initial values of the frying oil used in deep-frying contribute to the quality of the fried food in order to produce a good end product (Rosell, 1997). According to Hussein (2006), RBD palm olein for frying should have free fatty acid less than 0.06 mg/g, colour less than 2.8R, and peroxide value less than 0.5 meq/kg. Pokorny (1999) also mentioned that the main requirements for a high quality frying oil are FFA of 0.05 to 0.1 %, peroxide value of 0.5 to 1 meq/kg, moisture less than 0.05 %, colour of 3 red max, smoke point greater than 220 °C, taste and flavour should be bland.

Many research works have been carried out to show the physical and chemical changes that occur in deep fat frying oils. Friedman (2003) observed that during frying, oil is transferred to the food so that the fried foods contain additional fat at a level of 10 - 40 %. Fat from the food is also transferred to the frying oil so that though oil quality is controlled at the beginning of the frying process the oil soon becomes contaminated, for example, with fish oil or with animal fat depending on the food being fried. Tarmizi and Niranjan (2010) reported a protocol involving vacuum drainage at the end of frying that significantly lowered the oil content of potato chips. Andrikopoulos *et al.* (2003) investigated the complex chemical and physical changes that take

place during deep-fat frying. These changes include oxidation, hydrolysis and polymerization which affect both the frying oil and the finished product qualities.

Research investigations conducted by Demir and Bas-han (1998) have confirmed that during frying process, complex pattern of thermolytic and oxidation reactions take place leading to the formation of new compounds such as diacylglycerols, monoacylglycerols, free fatty acids, monomers and polymers. Lin *et al.* (1998) has shown that the formation of polymers leads to an increase in viscosity. The changes in viscosity are the signs of oil deterioration. Thickening reduces the rate of heat transfer and it takes a longer time to cook and colour the food. It also increases oil absorption, and this effect carries over into the finished product.

Kochhar (2000) indicated that oxidation causes the formation of hydroperoxides and conjugated compounds, which by cleavage give aldehydes, alcohols, ketones, lactones, acids, esters and hydrocarbons. The presence of volatile oxidation products formed during the frying process was discussed by Perkins (1996) and Nawar (1998). Dimers and other oligomers have also been found in frying oil by Belitz and Grosch (1999). Numerous flavour compounds formed by caramelisation, Maillard or Strecker reactions or the oxidation of phenolic compounds and terpenes have also been found in frying foods (Pokorny, 1999). Many researchers have shown that as these reactions proceed, the quality of oil changes and eventually reach a point where it is no longer possible to prepare high quality fried products and the frying oil have to be discarded (Cheman *et al.,* 2003; Romero *et al.,* 1998; Sebedio *et al.,* 1990).

Many researchers have presented preliminary findings in relation to the presence of acrylamide in some fried foods. The data published so far indicate that a temperature above 100 °C is required for acrylamide formation (Becalski *et al.,* 2003). Levels in French fries after frying have been reported to be approximately 600 µg/kg and 900 – 1000 µg/kg in potato crisps by Yasuhara *et al.* (2003). Tareke *et al.* (2002) showed that acrylamide was formed by heating above 120 °C certain starch-based foods, such as potato chips, French fries, etc. Many research works agree that acrylamide is formed mainly through the Maillard reaction from free asparagine and a carbonyl source. (Mottram *et al.*, 2002; Stadler *et al.*, 2002).

Acrylamide can also be formed from the frying oil. During frying, lipids heated at high temperature become dehydrated leading to the formation of acrolein (Umano and Shibamoto, 1987). Acrolein can further react via oxidation to generate acrylic acid or by formation of an intermediate acrylic radical (Becalski *et al.,* 2003). Both of the intermediates could then induce acrylamide formation in the presence of a nitrogen source under favourable reaction conditions (Yasuhara *et al.,* 2003).

There are many studies in that examine the health concern of frying oils. Some of the most important aspects of the problem are thoroughly discussed in reviews by Clark and Serbia (1991), Sebedio and Chardigny (1996), Marquez-Ruiz and Dobarganes (1996), Paul and Mittal (1997) and Billek (2000). It is now established that some compounds formed during frying have antinutritional properties. These compounds might inhibit enzymes and the absorption of nutrients, destroy vitamins and potentially cause gastric distress or mutations (Friedman, 2003). The overuse of deep-frying oil causes adverse effects on flavour, stability, colour and texture of

fried product and harmful to human health. FAO/WHO (1988) reported that highly oxidized oils produce polyaromatic hydrocarbons that have carcinogenic effect in the human body. International Agency on Research Cancer (IARC) has also classified acrylamide as a probable human carcinogen and exposure to high levels has been found to cause damage to nervous system (IARC, 1994). Consequently, it has been found that human exposure to acrylamide lead to haemoglobin adducts, whereas its epoxide functionality containing metabolite, glycidamide, was found to react with DNA (Tornqvist and Landin, 1995). Detailed information about acrylamide and its toxicological properties have been recently summarized in the Scientific Committee on Food (SCF, 2002).

2.2 Approaches towards Preserving Frying Oil Quality

Oil additives have been reported as potential alternatives for inhibiting frying oil oxidation (Warner and Gehring, 2009). It has been shown that some refined palm olein manufacturers normally treat the refined oil with antioxidants to retard the undesirable changes during frying operations and eventually to prolong the shelf-life of the fried products. These antioxidants protect the frying fat from oxidation during the time that the oil is exposed to high temperatures (Augustin and Berry, 1983).

The most commonly used synthetic antioxidants are butylated hydroxylanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylated hydroquinone (TBHQ). Evaluations comparing BHA, BHT and TBHQ stabilized frying shortenings showed that TBHQ provides the maximum protection at frying temperatures as well as the best carry through effectiveness for the fried foods. An assumption for this result is that TBHQ breakdown products may also be effective

antioxidants. BHA and BHT were also found somewhat effective, but to a much lesser degree than TBHQ (Cuvelier *et al*., 1994).

However, tests performed with butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ) showed that all three phenolic antioxidants were volatilized to depletion at approximately the same rate. Furthermore, retardation of oxidation by the synthetic antioxidants occurs only at ambient temperature, thus, they are useful during storage and shipping of refined oils, but are less effective at the frying temperature due to the volatility (Nor *et al.*, 2008). It has been reported that the dietary administration of BHT to rats caused fatal haemorrhages in pleural and peritoneal cavities, as well as organs such as testes and pancreas (Deshphande *et al.*, 1996). BHA has been found to exhibit toxic and carcinogenic effects (Kahl and Kappus, 1993). These antioxidants are permitted for use within the legal limits in the food industries because of their effectiveness and low cost.

Natural antioxidative substances which are safer, healthier and less subject to hazards than synthetic antioxidants have received extensive studies owing to recent consumer interest in natural products. The technologically most important natural antioxidants are ascorbic acid and citric acid and their salts, tocopherols and plants/spices extracts (Irwandi *et al.*, 2005).

2.2.1 Naturally Occurring Antioxidants

Plant extracts, especially antioxidants obtained from plants and spices have been proposed for stabilizing frying oils. Naturally occurring antioxidants from plants have been extensively

studied for their antioxidative activity during frying (Cheman and Tan, 1999; Madsen *et al.*, 1998; Nor *et al.,* 2008;Cheman and Jaswir, 2000), in retarding thermal oxidation of frying oil (Khan and Shahidi, 2001;Shyamala *et al.,* 2005) and in improving food quality (Jaswir *et al.,* 2000;Tanabe *et al.*,2002). Plants reportedly retard lipid oxidation not only during frying but also in the fried food (Jaswir *et al.*, 2000).

Rosemary and sage extracts are two antioxidants with very good thermal resistance that have been developed. The two natural antioxidants have been reported to have strong antioxidative characteristics (Cheman and Jaswir, 2000). The two antioxidants were proven to lower the rate of oxidation of oils during frying. When four different natural antioxidants namely rosemary, α-tocopherol, ascorbyl palmitate and citric acid were studied by Hras *et al. (*2000), rosemary extract was found to exhibit the best antioxidative activity as determined by peroxide and anisidine measurements. The compounds responsible for antioxidative activity of rosemary and sage have been reported as phenolic diterpenes such as camosol, rosmanol, camosic acid (Cuvelier *et al.*, 1994).

Thyme and *Lavender* are two other natural antioxidants that have been applied to refined oil to improve thermal stability of frying oils and consequently, extend its frying life. Quality parameters were significantly improved by treatment with either *lavender* or *thyme* (Bensmira *et al.*, 2007). Lee *et al.* (2005) identified aroma compounds in the extracts of *thyme* leaves (*Thymus vulgaris*) by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). Thymol (8.55 mg/g), which constituted 70 % of quantified total volatiles, was found to be a main contributor to the antioxidant activity of volatile extract of *thyme* at 10 µg/ml, comparable to

BHT and α-tocopherol at the same concentration as found in previous study (Lee and Shibamoto, 2002).

Tomaino *et al.* (2005) studied the effect of heating on antioxidant effectiveness and the chemical composition of some spice essential oils, including thyme essential oil. They found that heating up to 180 °C of thyme essential oil does not significantly affect either its antioxidant activity or its chemical composition. Thyme essential oil was reported to be rich in phenols, and in terpenic alcohols depending on the chemotype (Stahl-Bisup, 1991). Lavender essential oil has been reported to contain linaloyl acetate, caproic acid, tannins, beta-ocimene (Bisset, 1994).

In addition, the antioxidant activity of a number of other similar plants such as curry leaves (*Murraya koenigii*), kaffir lime leaves (*Citrus hystrix*), pandan leaves (*Pandanus amaryllifolius*) and turmeric leaves (*Curcuma longa*) often used as flavourings have been investigated (Nor *et al.,* 2008). The authors reported that these plants have great potential as heat stable antioxidants and could be used as alternatives to existing synthetic antioxidants. Nor *et al.* (2008) reported that *Pandanus amaryllifolius* leave extract had a polyphenol content of 102 mg/g and significantly retarded oil oxidation and deterioration during deep frying studies.

Tsaknis and Lalas (2002) reported that Moringa extracted oil has excellent oxidative stability during frying. Compositional characterization has been conducted on Moringa oleifera oil by Anwar *et al.* (2005), Abdulkarim *et al.* (2005) and Tsaknis and Lalas (2002). The Moringa oil is reported to have different tocopherol isomers.

The mechanisms by which these antioxidants are involved in the control of autoxidation process are different. Rosemary extracts and tocopherols act as radical scavengers, while others like citric acid, act as chelating agent and oxygen scavengers (Kochhar and Rosell, 1990). The phenolic compounds in several plants have been reported to act as antioxidants due to their redox properties, allowing them to act as reducing agents, hydrogen donors, free radical quenchers and metal chelators (Cheman and Jaswir, 2000).

2.2.2 Aromatic plant- *Renealmia battenbergiana*

Aromatic plants have been used since ancient times in flavourings, pharmaceuticals, cosmetics and perfumery. Loo and Richard (1992) have shown that beyond the aromatization of these plants, they exhibit biological activities including antimicrobial and antioxidant properties.

One plant native to tropical Africa is *Renealmia battenbergiana*. It belongs to the Zingiberaceae family (Achinewhu *et al.,* 1995). It is large perennial plantaceous plant having large leaves divided into stems and leaves. It's leafy stem is about 2-4 ft long. The leaves are oblanceolate-oblong, cuspidate, 6-8 in long, 1 ½ - 2 in broad above the middle, narrowed to the base firm, glabrous with a petiole an inch long above the clasping sheath (Maas, 1997).

The aromatic plant is wildly distributed in Ghana and predominantly found in the forest zones of the *Ashanti* and *Central regions.* The plant is locally referred to among the *Akans* as "*Atiegya*". The rural folks engage in harvesting of the plant at maturity when the leaves brown. The *R. battenbergiana* leaves are often used to give a refreshing, fragnant flavour to several dishes many from cooked and mashed cocoyam as well as in soups. Some group of people use it in

cooking ordinary non-aromatic rice to imitate the more aromatic and perfumed rice. It has been reported that the pleasant aroma emitted by the long narrow blade-like leaves is due to the presence of 2-acetyl-1-pyrroline (Laksanalamai and Ilangantileke, 1993). The leaves are sometimes put into frying oils to impart flavour to fried food. In crude palm oil mills, it is added at the clarification stage to impart aroma to the finished oil.

R. battenbergiana leaves like other aromatic plants such as *Pandanus amaryllifolius* leaves, rosemary, lavender and thyme mainly contain polyphenols (Lee *et al.,* 2004) and alkaloids (Busque *et al.,* 2000). However, the antioxidative activities of the extract in frying model systems have not been determined.

2.2.3 Optimization of Extraction Conditions of Antioxidants

A study was conducted by Chan *et al.* (2009) to optimize the extraction conditions for phenolic compounds from *Citrus hystrix* peels using response surface methodology. Response surface methodology (RSM) has been introduced to overcome the weakness and limitations of classical method (Liyana-Pathirana and Shahidi, 2005). Unlike the conventional empirical method, RSM can take into account the possible interrelationship among the test variables while minimizing the number of experiments (Silva *et al.,* 2007). Thus it is a powerful tool that has provided a complete optimal condition to improve a process. In particular, Rodrigues and Pinto (2007) applied RSM in optimizing the extraction of phenolic compounds from coconut shell. Likewise, Juntachote *et al.* (2006) applied RSM to optimize the production of phenolic extracts from various plants and spices.

The experimental design applied by Chan *et al.* (2009) was divided into two major parts. Firstly, single factor experiments were performed to determine the appropriate range of conditions for *limau purut* peels phenolics extraction, namely; solvent type, solvent concentration, extraction time, and extraction temperature by varying one independent variable at a time while keeping the others constant. Secondly, the optimization of phenolic compounds extraction was carried out using RSM and a second order polynomial model was developed.

Sin *et al.* (2006) used central composite rotatable design (CCRD) comprising of a three-factor (X_1, X_2 *and* X_3) and five levels to optimize phenolic extraction from the plant *limau purut*. The independent variables studied were ethanol concentration (X_1, *%)*, extraction temperature (X_2,°C) and extraction time (X_3, *min)* while the dependent variable (response variable) measured was total phenolic compound (TPC).

2.3. Measurement of Frying Oil Quality

2.3.1 Quantitative Evaluation of the Quality of Used oil

Quality evaluation of frying oils may be carried out in many ways. Although sensory evaluation of foods is the most important quality assessment, taste evaluations are not practical for routine quality control. It is always preferred to have a quantitative method for which rejection point could be established by sensory means (Warner, 2002). Due to the complexity of the problem, there is no single procedure that will yield reliable results in all situations.

Physicochemical properties: Frying oils are monitored by measuring properties such as free fatty acid value, peroxide value, anisidine value, iodine value, conjugated diene, total polar

materials (TPM) and polymeric triglycerides (PTG). Determination of the total polar materials in frying oil provides the most reliable measure of the extent of deterioration in most cases (Abdulkarim *et al.,* 2007). Several stages of oxidation during frying are recognized and tests are available for each stage.

Oxidation products: Primary products of oxidation are allylic hydroperoxides are measured as peroxide value or as conjugated dienes formed during oxidation of polyunsaturated fatty acids. Secondary products are mainly unsaturated aldehydes and are measured by the anisidine value. Tertiary products include short chain acids measured by the Rancimat or oil stability index (OSI) or malondialdehyde measured by the TBA test (Sulieman *et al.,* 2006).

The third international symposium on deep fat frying held in 2000 at Hagen, Westphalia (Germany) worked out recommendations for frying oils analysis. It was recommended that analyses should consider use of rapid tests for monitoring of oil quality. These tests should reveal characteristics that would correlate with internationally recognized standard methods, show ease in application, provide for safe use in food processing, give quantification of oil degradation, have field rugged properties, be independent of type of food and oil, and be insensitive to water content (Sulieman *et al.,* 2006).

Several methods for the determination of the quality of deep frying fats and oils have been developed based on physical and chemical parameters. These conventional analytical methods includes titrimetric for FFA, chromatographic for total polymer content, iodometric for peroxide and iodine value determinations, spectrophotometric for conjugated diene and anisidine value.

Most researchers measure changes in peroxide value (PV), FFA, Anisidine value according to the official methods of the American Oil Chemists' Society (1998).

In peroxide value determination, the hydroperoxides reacts with acidified potassium iodide to liberate iodine that can be measured volumetrically by reaction with standardized sodium thiosulphate. The value represents milli equivalent of oxygen per kg of fat. The anisidine value is based on the measurement of the intensity of the chromophore at 350 nm arising from molecules of the type ArN-CHCH-CHR' produced by reaction of anisidine (4-$MeOC_6H_4NH_2$ represented as $ArNH_2$) with carbonyl compounds which are mainly 2-enals (R'CH-CHCHO). This value varies depending on the enals actually present and is therefore only strictly comparable across results for a single type of oil. The anisidine value test has enhanced sensitivity for unsaturated aldehydes, especially 2, 4-dienals, but does not measure the ketonic secondary products of oxidation (Augustin and Berry, 1983).

Early stages of autoxidation have been determined by measurement of ultraviolet absorption at 234 nm resulting from conjugated dienes formed during oxidation of polyunsaturated fatty acids (Warner, 1996). When linoleic acid is oxidized to form hydroperoxides, a shift in one of the double bonds occurs producing a conjugated diene that can be measured by UV absorbance at 234 nm. This method is however not suitable for frying oils that already contain conjugated dienoic acids nor oils with high content of oleic acids and consequent low levels of linoleic acid.

Oxidative stability test: In the Rancimat and Omnium oxidative stability test, measurements for tertiary stage of oxidation monitoring in frying oils, a stream of air is drawn through oil

heated at 100 -140 °C into a vessel containing de-ionized water. Short chain acids- mainly formic (HCOOH) increase the conductivity of the water and the induction period is indicated by the time that elapses before there is a rapid increase in conductivity (Dobarganes *et al.,* 2000). The oxidative stability instrument traps volatiles from the oil sample and a probe continuously measures conductivity due to the increase in organic acids as autoxidation proceeds.

These measurements may be of limited value for predicting the stability of a range of oils but for repeated samples of the same oil they give useful comparative values. The length of time before rapid acceleration of oxidation also known as induction period is a measure of the resistance to oxidation (AOCS, 1993). The development of oxidative rancidity is accelerated so that the useful life of the oil containing the material may be determined. Rancimat oxidative stability measurements have largely replaced older oxygen methods. In the older accelerated tests (Schaal Active Oxygen) the oil was held at a temperature up to 100 °C and the time taken to reach an arbitrary peroxide value was measured. This was taken as an indication of the induction period and hence shelf life under normal conditions.

Kochhar (2000) reported a new method of determining oxidative stability of fats and oils at simulated frying temperature. The known method of oxidative stability testing (Rancimat) is normally executed at lower temperatures by bubbling a stream of air. In the new method, refined vegetable fats and oils were heated at a temperature of 170 °C after adding water-conditioned silica gel for two hours. The degraded products were measured to assess the oil stability at frying temperature.

Colour determination: The colour of the oil becomes darker during frying. Many research works have monitored the change in colour using colour measuring devices such as Lovibond tintometer or Hunter colourimeter. However, Pedreschi *et al.* (2005) measured potato chip colour using the CIE lab L*, a* and b* colour scale. Colour changes were followed by the lightness parameter (L*) and redness (a*) and yellowness (b*) parameters since these colour components presented the highest and significant variations during frying due to non-enzymatic browning reactions.

Viscosity: Bensmira *et al.* (2007) carried out viscosity measurements using a HaakeRheostress RSI controlled stress rheometer equipped with temperature controller unit and a Haake thermostat. Determination was done at 25 °C using a cone-plate sensor (diameter 3.5 cm; angle 2°) and a shear rate range of 0-55 s^{-1}. Viscosity has been shown to increase as the temperature increase (Bensmira *et al.*, 2007).

Total polar compounds: Among the chemical and physical parameters, total polar compounds (TPC) content is one of the most objective and valid criteria for the evaluation of oils and fats during the deep frying process. However, the standard method for measurement of TPC by silica gel column chromatography can be accurate, but time consuming and relatively expensive. Tamizi *et al.* (2012) determined polar compounds by silica chromatography following IUPAC 2.507 (IUPAC, 1992). Nor *et al.* (2008) determined polar compounds by separation of polar and nonpolar compounds on column chromatography, followed by elution of the nonpolar compounds, and then calculation of the differences between weight of sample added to column and eluted nonpolar compounds.

Determination of polar compounds in abused oils is a well-accepted method due to its accuracy and reproducibility. It provides the most reliable measure of the extent of deterioration in frying oils in most situations (Fritsch, 1981). The level of polar compounds is a good indicator of the quality of used frying oils giving information of the total amount of newly formed compounds having higher polarity than that of triacylglycerols.

Polymer triglycerides comprise an important fraction of polar compounds for reliable monitoring of the changes in the oil during frying. This fraction is normally determined by size exclusion high performance liquid chromatography. Triglycerides in dimeric, trimeric and polymeric form are polymer compounds. Nor *et al.* (2008) determined total polymer materials (products of polymerization of triglycerides) by methanolysis with sulphuric acid in methanol. It has been recommended that polymeric triglycerides should not exceed 12 %. The oil should be discarded when the polymeric triglycerides are above specification (Berger, 2005). In most European countries, frying oil would be discarded when the total polymer compound (TPC) and polymeric triglycerides (PTG) together exceed 24-27 % (Berger, 2005).

Fatty acid composition (FAC): FAC is widely used for the establishment of oil authenticity. Additionally, FAC measurement is one factor that helps to predict the oxidative stability of oil. Fatty acid composition is quantified using a gas chromatography. Fatty acid methyl ester mixture is prepared initially for the gas chromatography analysis. This method has been described by Christie (1993). In considering fatty acid composition, attention has been given to the procedures for preparing the methyl esters, to the GC conditions and to the ways in which the results are

presented. Triacylglycerols are easily converted to methyl esters by base catalyzed transesterification. This involves reaction with excess of methanol containing sodium methoxide and is complete in a few minutes at 50 °C.

2.3.2 Current Monitoring Procedures

Several methods for determination of the degradation of deep-frying oils have been developed based on physical parameters (viscosity and dielectric changes, etc.) and on chemical parameters (free fatty acids and polymerized triacylglycerols, etc.). These conventional analytical methods are highly time-consuming and labour intensive. In addition, the possible use of large volume of solvents is considered as potential environmental problem. Other known disadvantages of the conventional methods are high purchasing cost, necessary calibration with reference substances and string influence of the water content of the oil. Other monitoring procedures have thus been developed for rapid analysis of deep frying oils.

Radical scavenging activity: Ramadan *et al.* (2003) developed the method for measuring antiradical action of vegetable oils. It was possible to dissolve polar and nonpolar lipids in the same solvent to study their radical scavenging activity. This new technique for studying the antioxidant potential of vegetable oils has been applied in studying the antioxidant potential of vegetable oils during frying by Sulieman *et al.* (2006).

Fresh oil (non-polar compounds) and decomposition products of deteriorated dried oils (polar compounds) were completely dissolved in toluene which allowed the determination of antiradical performance of fried oils very accurately. After 16 h frying, Sulieman *et al.* (2006) reported that 85 % of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicalwas quenched by palm olein

while sunflower and cottonseed oils were able to quench 77 % and 72 % respectively. The significantly stronger radical scavenging activity of palm olein compared to sunflower and cottonseed oils may be due to the differences in content and composition of bioactive compounds and antioxidants, the diversity in structural characteristics of potential antioxidants present in oils. All these factors may contribute to the radical quenching efficiency of vegetable oils (Ramadan *et al.,* 2003).

Differential scanning colourimetry (DSC): Gloria and Aguilera (1998) have proposed differential scanning colourimetry (DSC) techniques to follow changes in the thermal characteristics of commercial frying oils during heating. DSC tracings indicate that heating of the oil results in a progressive shift of the crystallization peak at 43 to 48 °C to lower temperatures and recued enthalpies of crystallization. These characteristics are well correlated with increases in viscosity, polar compounds and colour.

Spectrophotometric Absorbance: Xu (2000) developed a new quick spectrophotometric method to evaluate deep frying oil quality. The oil samples are scanned from 350 to 650 nm. Each type of oil is found to show systematic changes in; these changes are quite evident between 470 and 500 nm. According to Xu, there is a strong correlation between the absorbance and the total polar compounds and the method offers a reliable routine test for assessing frying oil quality.

Fourier transform infrared (FTIR) spectroscopy: Recently, Deyawatee *et al.* (2001) studied the deterioration of soybean oil during the frying of potato chips by. The investigators claimed

that the decrease in ester linkages of triacylglycerols and in the iodine value, and the increase in trans unsaturation and formation of unsaturated aldehydes, as measured by FTI, correlate with the chemical methods of monitoring deterioration. FTIR spectroscopy combined with a partial least squares approach was also used by Cheman and Setiowaty (1999) to predict the anisidine value. This method depends on the concentration of 2-alkenals, 2, 4-dienals; and to a lesser extent saturated aldehydes. The method is proposed to replace the laborious wet –chemical method. It is based on the influence of the carbonyl group on the C-H frequency (range, 2747 to 2619 cm^{-1}) and the carbonyl region (1715 to 1763 cm^{-1}). In another recent report, Gertz *et al.* (2000) proposed a new method to estimate the oxidative stability at elevated temperatures (OSET). The fatty material is heated at 170 °C for 2 h after the addition of water-conditioning silica gel. The polymeric triacylglycerols (mainly nonpolar dimers and oligomers) are then measured using size-exclusion high performance liquid chromatography and the oxidative stability value is calculated.

2.3.3 Acrylamide Analysis

Despite acrylamide being a relatively new contaminant for food analysts, intensive method development and refinement has been carried out since its discovery in food products in 2002. Gas chromatography with mass spectrometric detection (GC-MS) and high performance liquid chromatography (HPLC) with tandem mass spectrometric detection (LC-MS/MS) appeared to be the most widely used methods (Wenzl *et al.,* 2003).

Only a limited number of methods have been published so far on the determination of acrylamide in foods despite the large number of different matrices where acrylamide can occur.

There are only a few reports that indicate that a rigorous and systematic determination of the characteristics of the method has performed according to international guidelines such as ISO 17025, CEN and Eurachem (Schaller, 2003). Rosen and Hellenas (2002) reported results from an in-house validation study, but admitted that these would not be applicable for all matrices and that there would still be some problems with different sample types. Most significant for the comparability of results of analysis is the harmonization of the sample preparation protocols. There were large differences in the extraction procedures, e.g. variations in the composition of extractant, in swelling conditions, in the definition of extraction temperature and time, and in the mechanical treatment.

By comparing methodologies, there were huge differences in the cleanup strategies. This was found for both GC and LC based methods. Especially, in LC sample preparation; there is a large spread of different procedures used, from lavish multistage solid-phase extractions to very simple, solid phase extraction-omitting protocols. Comparability of the GC methods is difficult due to differences in the sample matrices for which they were developed.

In some articles, recoveries are reported, but often there is lack of information about how they were determined. Mostly recoveries were reported in the range 90 to about 100 %. However, 60 % has also been reported (Tareke *et al.*, 2002). Adding isotopically labeled acrylamide at the beginning of the sample pretreatment is a way to take into consideration the losses of acrylamide that can occur during the whole sample pretreatment, but invariably does not reflect correct recoveries in the case of addition before extraction. This spiking approach is based on the establishment of equilibrium in the matrix interactions between the added internal standard and

native analyte. As long as the equilibrium is not established, differences in the extraction procedure might have a great influence on recoveries (Thompson *et al.,* 1999).

Knowledge of the exact recovery achieved for an analyte when applying a certain analytical method is of paramount importance as it will strongly affect the accuracy of the results (Eurachem, 1998). Therefore, reference materials are normally accepted for comparison. In an experiment by Bent *et al.* (2012), the initial method in which the DB-1701 column was used, a Limit of detection (LOD) of 20 µg/kg was obtained. The LOD was lowered to 4 µg/kg when the column was replaced with the DB-VRX column which emphasized the importance of using a column which provides good resolution. Since there is no allowable limit yet established for acrylamide in foods, it is crucial that if acrylamide is present within a sample it should be quantified (Tareke *et al.*, 2002; Weibhaar, 2004).

2.3.4 Sensory Analysis

Many objective instrumental and chemical methods have been proposed over the years to evaluate the quality and stability of frying oils (Gan *et al.,* 2004). Gan *et al.* (2004) monitored the stability of RBD palm olein using a surface acoustic wave (SAW) sensor-based electronic nose. The results from the electronic nose data showed significant difference between fresh oil and rancid oil. A high correlation was observed between electronic nose responses and chemical test data, as well as sensory evaluation score. However, sensory evaluation still remains the ultimate measure of deterioration of frying oils as no combination of chemical or physical tests is currently capable of assessing the composite sensory profile of oils (Pedreschi *et al.,* 2005). Sensory analysis on fried products has been carried out by two main methods; quantitative

descriptive (Gan *et al.*, 2004) and preference (Nor *et al.*, 2008). Anese *et al.* (2009) used both descriptive and preference analysis to study the sensory attributes of fried potato chips.

The sensory attributes of fried products are usually evaluated based on their crispness, appearance or colour, rancid-odor, taste and overall acceptability using a scale of 1 to 9: where 1 indicate dislike extremely and 9 indicate like extremely. The most desirable texture characteristic for fried foods is crispness. It is a highly valued and universally liked textural characteristic that signifies freshness and high quality (Nor *et al.*, 2008).

The results of sensory analysis are analysed to determine preference and significant difference among sets of fried samples. Work done by Abdulkarim *et al.* (2010) on sensory and physicochemical qualities of palm olein (PO) and sesame seed oil (SSO) blends during frying of banana chips indicate that the mean scores for crispness, aroma, flavour and overall acceptability were high. The moderate to high frequencies of scores between 5 to 7 for all the sensory attributes is a measure of acceptability by the panelists of the product fried in the oils. No significant differences ($P > 0.05$) were found in the sensory attributes across the oil blends. In overall acceptability, the control (100 PO) and oil blend (90 PO: 10 SSO) had the same sensory score. Also, for aroma and flavor, scores for the (90 PO: 10 SSO) blend were higher than the other blends.

In another study by Ammawath *et al.* (2013) on the effect of oils and frying process on the quality of deep fried rice crackers, the most notable differences occurred during repeated frying was colour. Quantitative descriptive analysis showed that colour of rice crackers fried in RBD

palm olein without filtration gave the lowest among all fried samples ($P < 0.05$). While the crispness and rancidity characteristics of all fried samples were no significant difference ($P < 0.05$).

2.4 Reports on Frying Oil Quality Indices

2.4.1 Peroxide Values (PV) as Frying Quality Index

The progress of the deterioration of the oils during frying has been followed extensively by many researchers (Suleiman *et al.,* 2007; Abdulkarim *et al.,* 2007; Hras *et al.,* 2000). Cheman and Wanhussin (1998) mentioned that a good quality vegetable oil should have a peroxide value (PV) of lower than 2 meq/kg. A decrease in PV during frying has been observed by several researchers (Vieira and Regitano-D'Arce, 1999; White, 1991; Zhang and Addis, 1992).

However, others (Jaswir *et al.,* 2000; Tan and Cheman, 1999) have reported an increase in PV of oil during frying. Some researchers have observed an initial sharp increase in the peroxide value from day 0 to day 1 after which the rate slowed down throughout the frying time (Abdulkarim *et al.,* 2007). Peroxide value alone is not a suitable parameter to assess the extent of frying oil deterioration. Increase in the PV during frying period indicates increased formation of peroxides due to oxidation. However, peroxides have been reported to be unstable under deep frying conditions, and as oil deterioration continues the hydroperoxides decomposes forming carbonyl and aldehyde compounds causing the peroxide value to decrease (Shahidi and Wanasundara, 2002). Augustin and Berry (1983) reported that hydroperoxides, the products of primary oxidation, react to form secondary products of which aldehydic compounds are measured by the anisidine test.

2.4.2 Anisidine Value as Frying Quality Index

Abdulkarim *et al.* (2007) observed an increase in the anisidine value in frying oils with increasing frying time. This happened because the less stable primary oxidative products (hydroperoxide) decompose further to form aldehydic compounds. These compounds are not easily decomposed, thus the test to determine the anisidine value is more meaningful than that to determine the peroxide value. Cheman and Jaswir, (2000) reported an increase in anisidine value on the first day of frying. After day one of frying, the anisidine value for control increased from 0.96 to 32.5, while for rosemary and sage treatments, increased were from 0.95 to 25.6 and from 0.96 to 26.2 respectively. It was also shown in the studies that during frying, for all treatments, the time of frying significantly influenced the anisidine value.

Anisidine value is often used in the industry in conjunction with peroxide value to calculate total oxidation or TOTOX value given as TOTOX=2PV+AV (Shahidi and Wanasundara, 2002). TOTOX value represents the oil or fat quality, oxidation status and presence of degradation products formed from previous oxidation of oils. Typical TOTOX value reported by Ghosh *et al.* (2012) for native soybean oil was 3.02 which increased to 19.93 in deep frying, 19.37 in shallow frying and 16.70 in par frying after the fourth round of frying. Therefore, according to Ghosh *et al.* (2012) deep fried soybean oil had significantly higher TOTOX value than shallow and par fried oils. The results was in agreement with Wai *et al.* (2009) who reported that the lower the TOTOX value, the better is the quality of the oil since the given oil is more stable to oxidative rancidity.

2.4.3. Conjugated Diene as Frying Quality Index

Cheman and Jaswir (2000) reported results for conjugated diene to be closely related to the peroxide value. There was a trend of increasing diene content with progress in frying days. Augustin and Berry (1983) had explained that smaller increases in diene content occur at later stage of frying when equilibrium between the rate of formation of conjugated diene and the rate of formation of polymers is attained. Although the absorbance at 268 nm and the anisidine analyses are based on secondary oxidation of oil, the absorbance at 268 nm measures, particularly, the diethylenic ketones, whereas ketones are not monitored in the anisidine test (Augustin and Berry, 1983). Sulieman *et al.* (2006) reported increment of absorptivity at 232 nm in all frying oil systems. Ultraviolet absorbance at 232 nm was substantially increased with increasing frying time.

2.4.4. Free Fatty Acid (FFA) as Frying Quality Index

Free fatty acid (FFA) is widely used by many food processors as an indicator of oil deterioration. This is because the method is relatively fast and reliable for monitoring the acidity of oil during frying (Tarmizi and Ismail, 2008). FFA represents the percentage of fatty acids liberated from the triglyceride chain during heating due to hydrolysis and oxidation (Nor *et al.,* 2008). The formation of FFA is highly associated with the smoke point, with an increase in FFA content lowering the smoke point (Termizi and Siew, 2008).

Many researchers have reported an increase in FFA levels during frying which was due to the cleavage and oxidation of double bonds to form carbonyl compounds with the latter oxidizing to form low molecular fatty acids during frying experiments (Coppen, 1994; Irwandi and Cheman, 2000). Sulieman *et al.* (2006) observed that the FFA values showed some decreases during frying periods (after 4 and 12 h) and this may be due to the loss of low molecular weight fatty

acids through volatilization. The determination of FFA by titration does not differentiate between acids formed by oxidation and those formed by hydrolysis. For these reasons, the increase in FFA level has been reported to be a poor measure of frying oils deterioration (Nor *et al*., 2008). The development of FFA parallels to a certain extent, other degradative changes but the measurement alone does not correlate well with the quality of the frying oil.

Bensmira *et al.* (2007) evaluated the effect of Lavender and Thyme incorporation in sunflower oil on its resistance to frying temperatures. Using FFA, peroxide value and viscosity as deterioration markers, they found out for the three different frying temperatures i.e. 150 °C, 180 °C and 200 °C, Thyme and Lavender exhibited high ability in reducing FFA, peroxide value and viscosity.

Similar observation was reported by Tarmizi *et al.* (2012) when they evaluated the physico-chemical changes occurring in oil when atmospheric frying is combined with post frying vacuum application. There was steady rise in the FFA values of oil as frying progressed. It was found out that the rate of FFA formation in the case of vacuum drainage was almost 2-fold lower than that of oil processed with atmospheric drainage. They reported that applying vacuum during drainage therefore lowered the water saturation temperature and the higher degree of super heating caused water, which was initially leached out from the product during frying to evaporate from the oil, hence low FFA. According to Kun (1990), the increase in the FFA content could also be caused by further oxidation of the secondary products formed during frying.

The maximum allowed value of FFA varies depending on the type of food being fried. For the industrial production of potato chips, an FFA of 0.3 % has been reported as the threshold for discarding used frying oil, whereas a maximum value of 1 % FFA is usually allowed by the processors of pre-fried French fries (Ismail, 2005). Higher FFA concentrations are tolerated in the case of oil used for frying battered and breaded products (2 to 2.5 %). Some countries even have regulation on the FFA threshold to discard frying oil: for example, Austria (1.25 %), Belgium (2.5 %), Germany (1 %), Japan (1.25 %) and the Netherlands (2.25 %) (Rossell, 1997).

2.4.5 Colour as Frying Quality Index

Colour is a subjective indicator used by the food industry for rapid monitoring of frying oil quality. In general, colour darkening occurs when the pigments developed during oxidation and thermal decomposition of fatty acids diffuse into the oil during frying, although darkening due to traces of carotenoids may also contribute (Lalas *et al.,* 2006). Maskan (2003) also explained that oil darkening may be caused by caramelized scorched product, which accelerates the L-value reduction in oil when assessed by the Hunter colourimeter.

In the studies conducted by Tarmizi *et al.* (2012), the frying oil darkened as frying progressed with oil samples developing lower lightness (L)-value and higher redness (a) and yellowness (b) values, when assessed by the Hunter colourimeter. This indicated that the oils were darker, more reddish and yellowish compared to the colour of the fresh oil. Furthermore, they noticed that the total colour difference which combines the lightness (L), redness (a), and yellowness (b) values increased with frying time.

Aladedunye and Przybylski (2009) observed that colour development in oil undergoing frying was 67 % higher than frying performed under carbon dioxide blanketing while the oil processed by vacuum frying has the least amount of colour pigments formed. Nor *et al.* (2008) also reported darkening of oil samples treated with compared to the control. They attributed the dark colour to the presence and oxidation of phenolic antioxidants themselves while heating. BHT showed significantly lower intensity of redness when compared to the control.

In a preliminary comparative studies conducted by Sulieman *et al.*(2006) on the antiradical performance and physicochemical characteristics of vegetable oils upon frying of French fries, they observed that all the samples gradually darkened in the course of the frying period. The colour value of the vegetable oils increased with increasing frying time. This was due to brown pigments from the fried products which eventually migrate into the frying oils. Also combined results of oxidation and polymerization of unsaturated fatty acids in the frying medium is to be blamed (Irwandi *et al.,* 2000). For three different vegetable oils compared, the higher the linolenic content, the darker the final colour. It was observed that the increase in the colour of palm olein during frying was relatively faster than in other oils. However, studies have shown that although palm olein may start with a higher colour value and darkens faster, this does not affect the colour of the fried products (Razali *et al.,* 1999). The findings therefore indicate that darkening is considered useful phenomenon as it prevents the continual use of edible oils which has undergone excessive deterioration.

2.4.6. Viscosity as Frying Quality Index

Changes in viscosity appear to be a good monitor to measure differences of frying fat deterioration. Viscosity measurements are another useful index of frying oil quality marking. In

many frying studies, the viscosity values increase during the frying periods. These increases were evidence of the thermal effect, the formation of polymeric compounds and a tendency toward foaming during frying periods (Frankel *et al.*, 1984; Lawson, 1995; Tseng *et al.*, 1996). It has also been shown that the high water content of fried products increase the polymerization of frying oil within the frying process which leads to increase in viscosity (Sulieman *et al.*, 2006). In fact, in Bensmira *et al.* (2007) studies on improving the thermal stability of cooking oils by incorporation of lavender and thyme in sunflower oil, the viscosity values increased from 0.084 to 0.089 Pa s for the original sunflower oil, from 0.083 to 0.084 Pa s for sunflower oil with Lavender and from 0.083 to 0.085 Pa s for sunflower oil with thyme, when the heating temperature was varied from 25 to 200 °C respectively.

Abdulkarim *et al.* (2007) observed increase in viscosity of all oil samples studied. The more viscous the frying oil, the higher the degree of deterioration. The authors further reported that the changes in viscosity in all oil samples had a positive and high correlation with changes in total polar compounds during the frying period with correlation coefficient of 0.9966, 0.9826, 0.9796 and 0.9809 for *Moringa oleifera* oil, palm olein, canola oil and soybean oil respectively.

According to Shyu *et al.* (1998), the viscosity of palm oil, lard and soybean oil increased from 38 to 44 mPa s, from 48 to 58 mPa s and from 35 to 43 mPa s respectively after 48 h of vacuum frying. The increase in viscosity occurred because of the formation of higher molecular weight compounds by polymerization of unsaturated fatty acids (Tsaknis *et al.*, 2002). Colour and viscosity are the most common physical parameters used to evaluate the extent of frying oil

deterioration in commercial and household frying. They are the most obvious changes that can be observed even by the non-expert.

2.4.7 Total Polar Compounds as Frying Quality Index

Polar compounds in the frying oil are composed of breakdown products, non-volatile oxidized derivatives, polymeric and cyclic substances which are produced during the deep frying process (Sanibal and Mancini-Filho, 2004). An increase in total polar content was observed by Chirinos *et al.* (2011) when they studied the characterization of phenolic compounds of Inca muca (*Clinopodium bolivianum)* leaves and the feasibility of their application to improve the oxidative stability of soybean oil during frying. Total polymer compound values higher than 25-27 % imply that the oil is no longer suitable for human consumption according to Xu *et al.* (1999) and Fauziah *et al.* (2000).

In most European countries frying oil should be discarded when the total polar compounds exceed 24-27 %. In Ghana however, there is no regulation on frying oil quality threshold. Total polar compound has been reported to increase with the frying time (Abdulkarim *et al.,* 2007). In a related study, Warner and Knowlton (1997) determined the frying stability of corn oils that are genetically modified to contain 65 % oleic acid. The high-oleic corn oil was evaluated in room odour test and by total polar compound content analysis. High-oleic corn oil had significantly lower total polar compound levels after 20 h of oil heating and frying at 190 °C compared to normal and hydrogenated corn oils. In addition, Houhoula *et al.* (2003) showed that ground oregano (O. *vulgare*) added at 2 g/l to cotton oil, diminished polar compounds during frying at 185 °C for 12 h in comparison to oil without antioxidant. The results obtained by Anwar *et al.*

(2007) showed that the amounts of oxidized total polar compounds of sunflower oil and soybean oil were significantly reduced through blending with *Moringa oleifera* oil.

It has been found that the increase in polar compounds correlates well with the tendency of oil to become viscous during repeated frying. In a study conducted by Tamizi *et al.* (2012) after 80 batches of frying, polar compounds increased from 7.81 to 15.03 % in the case of vacuum drainage and from 7.81 to 17.19 % in the case of atmospheric drainage. These values are still well below the maximum level of polar compounds allowed in frying oil according to regulations in many countries i.e. 25 % for countries like Belgium, Chile, France Italy, Spain and South Africa while 27 % for countries like Austria and German (Berger, 2005). In a recent study, the increase in polar compounds was 50 and 25 % lower when frying was performed under vacuum compared to the values obtained from conventional frying and frying under carbon dioxide blanketing (Aladedunye and Przybylski, 2009).

Exposure of oil to elevated temperatures induces modification of fatty acids that contain two or three double bonds (Jorge *et al.*, 1996). Tamizi *et al.* (2012) reported that palm olein contained a balanced proportion of saturated fatty acids (46 %) and unsaturated fatty acids (54 %) which imparted stability to the oil. They further noticed an apparent increase in percentage of palmitic acid whiles the linoleic acid content significantly reduced in the case of vacuum frying experiment. Therefore, during the frying process, Tamizi *et al.* (2012) reported loss of unsaturated fatty acids. Loss of polyunsaturated fatty acids follows the increase in total polar compounds. Near the rejection point (27 % TPC), 10 to 15 % losses of polyunsaturated fatty acids has been observed (Tamizi *et al.*, 2012).

2.4.8 Iodine Value as Frying Quality Index

The iodine value is a measure of unsaturation. A decrease in the iodine value is consistent with a decrease in the number of double bonds in heated oil as it becomes oxidized. The differences in iodine values of the oil during frying are also indicative of the increased rate of oxidation during frying. According to Augustin and Berry (1983), a significant change in the iodine values can be observed when there is excessive deterioration of the oil. In a research work carried out by Cheman and Jaswir (2000), the iodine values of all treatment samples decreased significantly from day 0 to day 6. The authors reported a percentage loss of unsaturation as 27, 24 and 24 % for control, rosemary and sage-treated oils respectively.

In another studies by Abdulkarim *et al.* (2007), changes in the iodine value over the 5 days frying period from the initial values for canola oil; 109.9-103.0 was larger followed by that of soybean oil; 116.9-111.8. Lesser changes were found in palm oil; 56.8-53.7 and *Moringa oleifera* oil; 65.9-62.2. *Moringa oleifera* oil and palm oil had a longer induction period since there were no significant changes for the first two days of frying. In canola oil and soybean oil, however, the changes in the iodine values were significant after the first day of frying, indicating shorter induction periods. This was largely due to the high amounts of linolenic acid in the two oils.

2.5 Controlling Acrylamide in Deep-Fat Fried Foods

2.5.1 Physical Methods

Low frying temperatures (e.g. 120 °C) and blanching treatment in hot water before frying has been reported to decrease dramatically the acrylamide content in potato chips (Pedrechi *et al.*,

2005). Blanching treatment reduced the acrylamide content in potato chips in 68 %, 75 % and 49 % at the frying temperatures of 120, 150 and 180 °C respectively. Blanching was found to remove glucose and asparagine from the potato tissue leading to lower acrylamide formation in fried potato slices (Pedreschi *et al.*, 2004). Besides, Haase *et al.* (2003) reported that a reduction of the sugar content by blanching or soaking could reduce the acrylamide concentration by approximately 60 % according to the raw material (potato variety and field site) and the production process variable (eg. blanching conditions and frying temperatures). Granda *et al.* (2004) applied vacuum frying for producing potato chips and they could reduce acrylamide formation by 94%.

2.5.2 Chemical Methods

Jung *et al.* (2003) showed that lowering the pH with citric acid before frying was an efficient way to considerably diminish acrylamide in French fries. Shin *et al.* (2010) tested whether taurine could be applied in fried food model system using potato chips. From their studies, acrylamide formation was reported to be significantly inhibited in potato chips treated with different concentration of taurine. The fried potato slices soaked in 0.1 %, 0.5 %, 15 and 2 % taurine solution for 30 min prior to frying at 170 °C for 30 min gave 66 %, 57 %, 31% and 45 % of the control's acrylamide level respectively.

Reports on the reduction of acrylamide by the addition of flavonoids have been demonstrated. For instance, Fernandez *et al.* (2003) reported that acrylamide could be reduced by the addition of flavonoid spice mix containing green tea, apple polyphenols and onion extracts. The spice mix solution was added to potato slices before frying, and a powder spice mix was also added to the

potato slices after frying. After a 4-day incubation time the acrylamide content were reported to be reduced by up to 50 % in the spice mix treatment. In a related study, antioxidants from bamboo leaves extract (rich in flavonoids) was added to chicken wings before frying and an acrylamide inhibitory rate of 63.8 % was reported. The total flavonoids content of bamboo leaf extracts played an important role in the reduction of acrylamide.

Summa *et al.* (2006) found a direct correlation between the concentration of acrylamide and the antioxidant activity in self-prepared cookies. Addition of antioxidants would to a large degree block the oxidation of acrolein to a certain extent and further reduce the generation of acrylamide. However, both reduction and enhancement of acrylamide formation via addition of antioxidants has occurred in different published research which suggests the dual effects of antioxidants on the generation of acrylamide (Taeymans *et al.*, 2004).

Tareke (2003) found that the addition of butylated hydroxytoluene (BHT), sesamol and vitamin E to meat before frying increased the formation of acrylamide probably by protection of acrylamide against free-radical initiated reactions. On the other hand, Becalski *et al.* (2003) found about 25 % reduction in acrylamide was achieved when adding rosemary plant to the oil used for frying potato slices.

2.5.3. Biological Method

Lactic acid fermentation in the presence or absence of glycine, as well as immersion in an aqueous solution of the amino acid alone have been considered in Anese *et al.* (2009) studies as pretreatment of potato cubes before deep frying. A *Lactobacillus plantarum*20174 strain was used. The results clearly evidenced that lactic acid fermentation in the presence of glycine

resulted the most effective pretreatment in decreasing acrylamide formation during deep frying. Deep fried potatoes subjected to the glycine, fermentation and fermentation-glycine pretreatments had 60%, 70% and 82% and 35%, 50% and 70% less acrylamide content than the non-dipped and water-dipped potatoes, respectively.

2.6 Sensory Evaluation of Deep Fat Fried Foods

Anese *et al.* (2009) employed sensory analysis to ascertain the effect of chemical and biological dipping on sensory properties of deep fried potatoes. According to the results of the paired comparison preference test, no significant differences in preference were found among the samples. Also, the deep fried potato samples subjected to the dipping treatments resulted not significantly different for flavour, sourness and crispiness. The results showed that the decrease in pH as a consequence of lactic acid fermentation did not affect the perceivable acidity of the product.

Sensory attributes of fried potato crisps was also evaluated by Cheman and Jaswir, (2000). Who explained that except for crispiness attribute, the use of rosemary and sage in the frying operation significantly improved the sensory scores of fried potato crisps. In addition, the sensory score for all attributes was reported to have decreased significantly from day 1 until day 6. The decrease in all sensory attributes is closely related to the deterioration of oils during frying which caused a darkening of frying oil.

Nor *et al.* (2008) observed no significant differences in scores for oiliness, crispiness and taste between samples throughout the frying experiment. *P. amaryllifolius* extract did not show any

specific improvement of sensory score when compared to the control, however, the score for flavour, colour, oiliness, crispiness, taste and overall quality were not significantly different throughout the frying process.

Up till date, only one investigation deals with the impact of lowering acrylamide pretreatments on the sensory quality of potato crisps (Mestdagh *et al.*, 2008). Anese *et al.* (2009) reported that panelists indicated the deep fried potatoes untreated or immersed in glycine solution was the darkest ones, whiles samples subjected to fermentation or immersed in water resulted in significantly less brown colour. The fermentation in the presence of glycine resulted the most effective pre-treatment in terms of acrylamide reduction produced deep fried potatoes having an intermediate browning, which was not significantly different from those relevant to the untreated and water-dipped samples.

From the data published by Zhang *et al.* (2007) the odour and flavour of fried chicken wings processed by antioxidants from bamboo leaf extract treatment had no significant difference compared with normal food matrixes while the colour of samples achieved no obvious change. Whereas many studies (Jung *et al.*, 2003; Cook and Taylor, 2005) have found effective methods to reduce acrylamide during heat processing, their sensory evaluation was not acceptable.

For instance, the largest decrease of acrylamide content (90 %) in crisps was obtained when potato slices were soaked in acetic acid solution for 60 min at 20 °C, and a large decrease of acrylamide content (74 %) was also observed after soaking of potato slices in a 1 % NaOH solution. However, a sour and acerbic taste from both of treatments greatly influenced the

appearance as well as the taste and flavour of potato crisps, which were not sensorially acceptable (Kita *et al.*, 2004).

In the studies conducted by Zhang *et al.* (2007), although the optimal inhibitory rate (59%) of acrylamide in fried chicken samples was 34.4, 28.2 and 15.7 % lower than the optimal reduction effects reported from the contribution of Jung *et al.* (2003); Kita *et al.* (2004) and Cook and Taylor (2005), respectively, the balance between reduction and sensory acceptance was satisfactory.

2.7 Summary of Research Views on Frying Oil Quality Improvement and Grey Areas

Refined bleached deodorized (RBD) palm olein among other vegetable oils has been found to be stable frying medium worldwide (Sulieman *et al.*, 2006). When RBD palm olein is used for frying over prolonged periods, it deteriorates by darkening in colour, smoking, and foaming. Their biological value decreases because unsaturated FA and important phytonutrients, or other minor constituents, are partially or wholly lost (Nor *et al.*, 2008).

The role of oxidation retardants from natural sources has been very much appreciated by several researchers. Work done by Bensmira *et al.* (2007), Cheman and Jaswir (2000) and Nor *et al.* (2008) has pointed to the fact that extracts from plants can effectively retard the palm olein deterioration during repeated cycles of frying. The natural plants have also been proven to lower the rate of oxidation of the oil during frying. In terms of frying quality benchmarks such as peroxide value, anisidine value, free fatty acid and polymer content, research data have shown that samples prepared using oils treated with extracts from plants had lower values than the control (Cheman and Jaswir, 2000; Nor *et al.*, 2008). Organoleptically, these plants have been

proven to improve acceptability of fried potato crisps, French fires etc. According to Tarmizi *et al.* (2012), frying oil deterioration by oxidation and polymerization can also be reduced by half by the use of vacuum drainage.

Chan *et al.* (2009) found the optimum extraction conditions for antioxidants from the plant Citrus *hystrix* to be ethanol concentration of 52.9%, extraction temperature of 48.3 °C and extraction time of 126.5 min. Under this optimized condition, the experimental value for the antioxidants was 1291.8 mg gallic equivalent (GAE)/100 g, which was reasonably close to the predicted value (1268.8 mg GAE/100 g).

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Over the years, a lot of information has accumulated concerning the development and control of acrylamide in frying oils. Hussein (2006) reported the acrylamide formation in French fries from 680 -1449 ng/g throughout five (5) consecutive days of batch frying whereas for the acrylamide formation in RBD palm olein was from 21.26-159.12 ng/g at the fourth and seventh hours only on the second and third day respectively.

Tareke *et al.* (2002) measured the levels of acrylamide in a number of heated foodstuff products obtained from restaurants or from grocery stores. Acrylamide content ranged from 14 -23 , 314 - 732, 1300 -3900 and 37 -1730 µg/kg for restaurant prepared hamburger, French fries, potato crisps and crisp bread respectively. According to work done by Zhang *et al.* (2006), the addition of antioxidants extract from bamboo effectively reduced the amounts of acrylamide in fried chicken wings. The treatment could not only effectively achieve a reduction of acrylamide, but also retained reasonable sensory attributes.

Although there is much information about improving the quality of frying oil by application of natural extracts from plants, the point at which frying oil is no longer fit for use is not clearly defined. It therefore remains unclear at which stage of repeated frying should frying oil be discarded. Research papers have not been specific on the extent to which frying life could be extended by the addition of natural extracts from plants.

This project seeks to evaluate the frying performance of optimized incorporated *R. battenbergiana* extract in RBD palm olein. The emphasis of the research is to determine optimum conditions of incorporation of the extract into RBD palm olein and consequently determine to what extent it can extend the frying life.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Materials

R. battenbergiana whole plant, refined bleached deodorized palm olein and fresh yam tuber were the principal materials used for the study. Some key equipment used for the research included; High performance liquid chromatography, Lovibond colour tintometer (Model F, UK), UV-Vis spectrophotometer (Model 7305, UK) and Commercial deep fat fryer (Model DR-A1,USA).

3.2 Sources

R. battenbergiana was obtained from a local out grower at *Juaben –Ashanti*. Refined bleached deodorized (RBD) palm olein was obtained from *Juaben* Oil Mills Limited in *Juaben-Ashanti* and fresh yam tuber was obtained from a local out grower in *Juaben-Ashanti*. Acrylamide (99 %) was purchased from Sigma- Aldrich (St Louis, MO, USA) while acetonitrile (high performance liquid chromatography (HPLC) grade) was supplied by Merck (Whitehouse Station, NJ, USA). All other solvents and chemicals used for the extraction and analyses were analytical grade and purchased from Sigma-Aldrich Chemicals (St Louis, MO, USA).

3.3 Preparation of Materials

R. battenbergiana was harvested at maturity (between 3 to 4 months when the leaves were browned) by a local out grower at *Juaben-Ashanti*. A mass of 5 kg of *R. battenbergiana* were washed under 5 L of running tap water, cut into pieces and air dried for over 24 h**.** Samples of air dried *R. battenbergiana* were milled through a 1 mm mesh using a hammer mill (Model ED – 5, Germany). The milled extract (4.8 kg) was stored in a sealed plastic container at a cool, dry shelf prior to use.

About 5kg of fresh yam tuber was washed under running tap water, peeled and sliced into 0.5 cm thick and 2.5 cm wide discs using a mechanical slicer. The slices were stored in a sealed plastic container at 4 ^{o}C prior to use. Citrate treated RBD palm olein was prepared at a concentration of 100 ppm.

3.4 Methods

3.4.1 Treatment Methods

3.4.1.1 Research Design for the Extract- oil Incorporation

In order to formulate the *R. battenbergiana* incorporated refined palm olein (RB oil), 3 × 3 full factorial design was used. Three factors were varied at three levels each as presented in the table 1 and modeled Design Expert (2007) to give a total of 27 extract incorporation runs (table 4).

Table 1: Treatment factors; extract-oil concentration, extracting temperature and treatments times and their levels of variations used for the incorporation

Factors	Levels
Extract-oil concentration	1, 3 and 5 % w/v
Extracting temperature	25, 45 and 65 ° C
Treatment time	0.5, 1.0 and 1.5 h

After the extract-oil preparations, the mixtures were agitated with an orbital shaker (Gallenkamp, England) at 100 rpm according to the conditions set for each of the 27 runs. At the end of the shaking, the samples were centrifuged with a bench top centrifuge (MSE, USA) at a speed of 3000 rpm for 15 min after which the clear supernatants were collected and stored in sealed plastic containers and kept frozen for subsequent analyses.

3.4.1.2 Research Design for the Deep frying Experiment

A two category factor was used and the factors were varied at levels as shown in table 2 below. The oil levels comprise of native refined palm olein (N Oil), citrate treated refined palm olein (CT Oil) and *R. battenbergiana* incorporated refined palm olein (RB oil). The factors and levels have been completely modeled by Design Expert (2007) to give fifteen (15) runs as shown in table 6.

Table 2: Treatment factors; frying cycles and frying oil type and their levels of variations used for the frying experiment

Factors	Levels
Frying Oil type	Native oil (N oil) *R. battenbergiana* treated oil (RB oil) Citrate treated oil (CT oil)
Frying Cycles	1 – 5

The method for the frying experiment of a model yam was adapted from that developed by Tsaknis and Lalas (2002). When the oil temperature reached the temperature desired (175 ^{o}C), a 100 g batch of sliced yam was fried separately in the 1 L optimized extracted incorporated refined palm olein (RB oil), citrate treated RBD palm olein and native RBD palm olein sample using a thermostatistically controlled fryer. Five discontinuous frying was carried out in five consecutive days. The frying time was 6 min. At the end of the frying each day, a 50 g sample of sample frying oil was removed from the fryer and stored at 0 ^{o}C. The remaining frying oil was strained and a corresponding amount of yam slices added. At the end of each day's frying, the fryer was then capped with lid and the frying was continued the following day. Fresh oil was not added to the frying pan. After frying, the yam chips were removed from the fryer. The batch of

fried yam chips each day was labeled and packed in low-density polyethylene (LDPE) plastic bags for sensory evaluation. The evaluation was conducted on the same day that the frying was carried out.

3.4.2 Analytical Methods

American Oil Chemists' Society (AOCS, 2002) method was used to assess the preliminary physicochemical characteristics of the native RBD palm olein sample such as free fatty acid, peroxide value, iodine value and colour.

3.4.2.1 Colour Determination upon Extract Incorporation

The colour in terms of red, yellow and either blue or neutral slides were used to report the colour response after extract incorporation treatment. The colour was measured on the native olein and the extract incorporated refined palm olein using a tintometer (Lovibond F, England) with a 5 ¼ inch glass cuvette at room temperature of 27 °C. In its operation, the sample was placed in sample cell and the viewing tube was focused until a sharp image of the aperture of the sample oil was obtained. The tabs controlling the coloured filters were then adjusted until the proportions of red, yellow and blue combinations that matched the sample colour readings.

3.4.2.2 Antioxidant Properties Determination upon Extract Incorporation

Reducing power analysis based on the reduction of Fe^{3+} to Fe^{2+} (Jayaprakash, 2001) was used in assessing the antioxidant activity of the *RB* oils. A mass of 5 g of each RB oil sample were dissolved in 25 ml hexane and thoroughly agitated to uniform mixture and left overnight. An aliquot of 1 ml of each clear supernatant were mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1 % potassium ferricyanide solution ($K_3Fe[CH]_6(aq)$) in a test tube. The mixtures were incubated at 50 $^{\circ}$C in an incubator for 20 min after which 1.5 ml of 10 % trichloroacetic acid solution (TCA) was added and left for 10 min at room temperature (27 ^{o}C).

An aliquot of 2.5 ml of each clear supernatant were then mixed with 2.5 ml distilled water and 0.5 ml of 0.1 % ferric chloride solution ($FeCl_3$ (aq)) in a test tube. The absorbance was then measured at 700 nm using a MSE 700 series spectrophotometer. A blank determination was done using 1 ml distilled water. A standard curve was plotted using eight different concentrations of tert-butyl hydroquinone as standard antioxidant (0.01, 0.05, 0.1, 0.3, 0.5, 0.7, 0.9 and 1.2 mg/ml).

3.4.2.3 Physicochemical Analysis Deep-Fat Fried Oil

3.4.2.4 Free Fatty Acid (%FFA)

Free fatty acid content was determined in triplicate on the native olein (N Oil), *R. battenbergiana* (RB) oil and citrate treated (CT) oil after frying cycles by the titration method of AOAC (2002). Seven gram of well mixed oil was weighed into a 250 ml flask. Previously, neutralized hot ethyl alcohol (50 ml) and 1 % phenolphthalein, as indicator, were added. The mixture was titrated with 0.1 N NaOH with vigorous shaking until permanent faint pink colour appeared and persisted at least 1 min. The free fatty acid content was calculated as percentage of oleic acid according to the following equation:

$$\% \text{ FFA (as oleic acid)} = \frac{V \times N \times 28.2}{M}$$

where M is the mass of the test portion (g), N the normality of NaOH, and V the volume of NaOH consumed (ml).

3.4.2.5 Peroxide Value

Peroxide value (PV) was determined on the native oil (N oil), *R. battenbergiana* (RB) oil and citrate treated (CT) oil after frying cycles by using the AOAC method (AOAC, 2002). About 5 g of oil was weighed into a 250 ml flask. Previously prepared acetic acid–chloroform solution (30 ml), saturated potassium iodide (0.5 ml), and distilled water (30 ml) were added with occasional shaking. The mixture was titrated with 0.1 N $Na_2S_2O_3$ by shaking vigorously until yellow colour

is almost gone. Approximately, 0.5 ml of 1 % starch solution was added, and titration was continued with shaking vigorously to release all iodine from $CHCl_3$ layer, until the blue colour just disappeared. Peroxide value was calculated by using the following equation:

$$\text{PV (meq. peroxide/kg sample)} = \frac{S \times N \times 1000}{G}$$

where S is the ml $Na_2S_2O_3$ (blank corrected) , N is the normality $Na_2S_2O_3$ solution and G is the weight of oil sample.

3.4.2.6 Iodine Value

Iodine value (IV) was determined by on the native oil (N oil), *R. battenbergiana* oil (*RB* oil*)* and citrate treated olein (CT oil) after frying cycles using the AOAC method (AOAC, 2002). Approximately 0.5 g of dry oil sample was weighed into a 500 ml conical flask with glass stopper, to which 25 ml of carbon tetrachloride had been added and mixed thoroughly. About 25 ml of Wij's solution was added and the glass stopper replaced after wetting with potassium iodine solution.

The solution was swirled for proper mixing and the flasks kept in dark for one hour. After standing, 15 ml of potassium iodide solution was added, followed by 100 ml of recently boiled and cooled water, rinsing in the stopper also. The liberated iodine was titrated with 0.1N standardized sodium thiosulphate solution, using starch as indicator at the end until the blue colour formed disappeared after thorough shaking with the stopper on. Blank determinations were conducted in the same manner as test sample but without oil.

Iodine value was calculated using the following equation;

$$\text{Iodine value} = \frac{12.69\,(B-S)N}{W}$$

Where, B = volume in ml of standard sodium thiosulphate solution required for the blank.

S = volume in ml of standard sodium thiosulphate solution required for the sample.

N = normality of the standard sodium thiosulphate solution.

W = weight in g of the sample.

3.4.2.7 Determination of Acrylamide Content

The quantitative analysis of acrylamide was adopted from previous published method by Zhang *et al*. (2007). The used fried oils were taken through extraction preparations and the analysis was performed on HPLC-UV detector mode using a MSE 2695 series HPLC with an Atlantis dC18 column (150 ×2.1mm, 5 mm, USA). Initially, standard acrylamide solutions were prepared (*Appendix A*) and measured according to the HPLC-UV conditions. Using their retention times, the peak area values calculated were plotted against their respective concentration to obtain a calibration curve.

Used oil samples were then pre-treated by mixing 50 g of each used oil sample with 1 ml of methanol. About 25 ml of distilled water was added and shaken vigorously for 1 min. The vial was upturned for 20 min to allow the phases to separate. About 4 ml of the lower aqueous phase was withdrawn and filtered using an HPLC membrane filter for HPLC-UV analysis. In a much similar way, blank oil sample free of acrylamide was pre-treated following procedures for the used oil samples pre-treatment.

The aqueous extracts of the used oil samples as well as the simulant blanks were injected on to the HPLC column for analysis. The following chromatographic conditions were suitably applied. The column was dC 18 (150 ×2.1 mm), flow rate was 1.5 ml/min, detector was UV 202

nm, injection loop was 25 µl and temperature was stabilized at room temperature. The HPLC mobile phase was acetonitrile diluted with water. The acrylamide peaks were then identified based on the retention time and the respective peak area was then measured. Calculation of the acrylamide concentration in the test samples was by graphical determination in which the average of peak area values obtained from the test samples upon injection was calculated and the acrylamide concentration of the test sample was read from the calibration graph.

3.4.2.8 Sensory Analysis

Sensory evaluation was conducted on the fried yam slices using a 9-point hedonic scale (l =dislike extremely and 9=like extremely) by 9 trained panelists selected from the staff of *Juaben* Oil Mills Limited, Kumasi after each frying cycle for each frying oil type. The sensory quality of the slices was evaluated based on their odour, colour, crispiness and taste. The results of the sensory evaluation were analyzed to determine significant difference among the three oil types and the overall preference using Design Expert (2007). All panelists regularly participated in sensory evaluation and are also regular consumers of yam chips. The panelists were initially given different fried yam products to explain the sensory characteristics to evaluate.

3.4.3 Statistical Analysis

The analyses response data collected were loaded and run using Design Expert (2007) and the variations in the data collected such as coefficients of regression- (R^2), adjusted regression- (adj R^2), prediction regression-(pred R^2), and adequate precision - (adeq precision) were studied. After ANOVA studies the adequacy of the model, the level of significance ($p<0.05$) were assessed as well as any interactions that occurred among the factors that were varied. When all the model statistics and diagnostic plots were evaluated to be good, the model graphs were plotted and performance of the factors and responds made.

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1 Physicochemical Characteristics of Native RBD Palm Olein

The initial physicochemical properties of RBD palm olein used for the study are given in table 3. The results showed that the RBD palm olein was of good quality, as indicated by its initial low peroxide value of 0.8 meq/kg and % free fatty acid content of 0.1 %.

The result was within the specification for standard RBD palm olein (Hussein, 2006). This implied that the fresh RBD palm olein could suitably be applied as frying fat. The results obtained were comparable to that had been reported Gan *et al.* (2004) upon analyzing the characteristics of fresh RBD palm olein.

Table 3: The actual and expected values obtained for the physicochemical properties of the fresh RBD palm olein used for the study.

Characteristics of oil	Expected Value	Actual Value
FFA (as Oleic)	0.1 % max.	0.10 (0.03)
PV (Meq peroxide / kg oil)	1 max.	0.80 (0.1)
IV (g of I_2/ 100 g oil)	56 min.	56.1 (0.1)
Colour (5¼ Lovibond cell)	6 Red max	3.50 (0.01)

Results presented as a mean of three determinations (SD)

4.2 Extract Incorporation Treatment

4.2.1 The Red and Blue Colour

The responses for the different *R. battenbergiana* oils are shown in table 4. The minimum red colour unit achieved was 4.3R under the condition of 1 % extract-oil concentration, 25 ℃, 1.5 h. The maximum red colour unit obtained was 9.1R under the treatment condition of 5 % extract-oil concentration, 65 ℃ and 1 h. From the statistical analysis, the variations in the % extract as well temperature of the agitations were significant ($p<0.05$) and the model which was given as;

$$Y = \beta_o + \beta_1 X_1^i + \beta_2 X_1^{ii} + \beta_3 X_2^i + \beta_4 X_2^{ii}$$

was also significant ($p<0.05$), and the red unit colour as well as that of the blue, followed this regression model where Y represented the red or blue colour unit; β_o to β_4 representing the coefficient of variation and X_1 and X_2 representing % extract and temperature of agitation respectively.

For the red colour unit, statistical analysis showed that the "pred R^2" of 0.94 was in reasonable agreement with the "adj R^2" of 0.95.Since the "adeq precision" measured the signal to noise ratio of 26.87 which is above 4, it indicates an adequate signal. Similarly, the blue colour unit had "pred R^2" of 0.96 which was also in agreement with the "adj R^2" of 0.97 and the 'adeq precision" of 36.53 made the model adequate and hence the model could be used to navigate the design space and subsequently be able to make predictions.

The red colour response was found to be significant ($p < 0.05$) for both treatment temperature (℃) and extract-oil concentration (%), but insignificant ($p < 0.05$) for the treatment time. This suggest that time variation did not contribute significantly to differences in the red colour.

In comparison to the study time selected by Chan *et al.* (2009) in an optimization conditions for the extraction of phenolic compounds from *Citrus hystrix* peels, however, the extracting time was 60 – 420 min. Under optimized formulation condition of 1 % extract-oil concentration and 0.5 h, the inverse of red colour was studied in relation to the treatment temperature and extract-oil concentration.

Table 4: Extract incorporation and treatment conditions with responses for antioxidant activity, red and blue colours.

Run	A:Extract-oil conc. %	B:Temp ° C	C:Time H	Red	Blue	Antioxidant content mg/ml
1	1	65	0.5	4.5	2.3	0.61
2	5	45	1.5	7.5	7.8	0.71
3	1	25	1.0	4.5	1.1	0.55
4	5	25	0.5	7.4	6.4	0.78
5	3	65	1.5	7.3	7.6	0.54
6	1	45	1.5	4.4	2.2	0.54
7	5	65	1.0	9.1	9.2	0.57
8	3	25	1.5	7.1	5.1	0.53
9	3	25	1.0	7.1	4.6	0.60
10	5	25	1.5	7.6	7.5	0.59
11	5	65	0.5	8.6	9.8	0.58
12	3	65	0.5	8.1	7.5	0.52
13	1	25	1.5	4.3	1.1	0.67
14	5	25	1.0	7.5	6.5	0.71
15	5	45	1.0	7.6	8.5	0.59
16	1	65	1.5	4.5	2.5	0.53
17	1	45	1.0	4.4	2.0	0.56
18	3	45	1.5	6.0	5.8	0.65
19	3	45	1.0	5.8	4.5	0.63
20	1	65	1.0	4.5	2.6	0.62
21	1	25	0.5	5.1	1.0	0.71
22	1	45	0.5	4.5	3.0	0.55
23	3	45	0.5	6.2	6.5	0.55
24	3	65	1.0	6.9	7.9	0.52
25	3	25	0.5	7.2	5.1	0.62
26	5	65	1.5	8.7	9.6	0.57
27	5	45	0.5	7.6	8.1	0.70

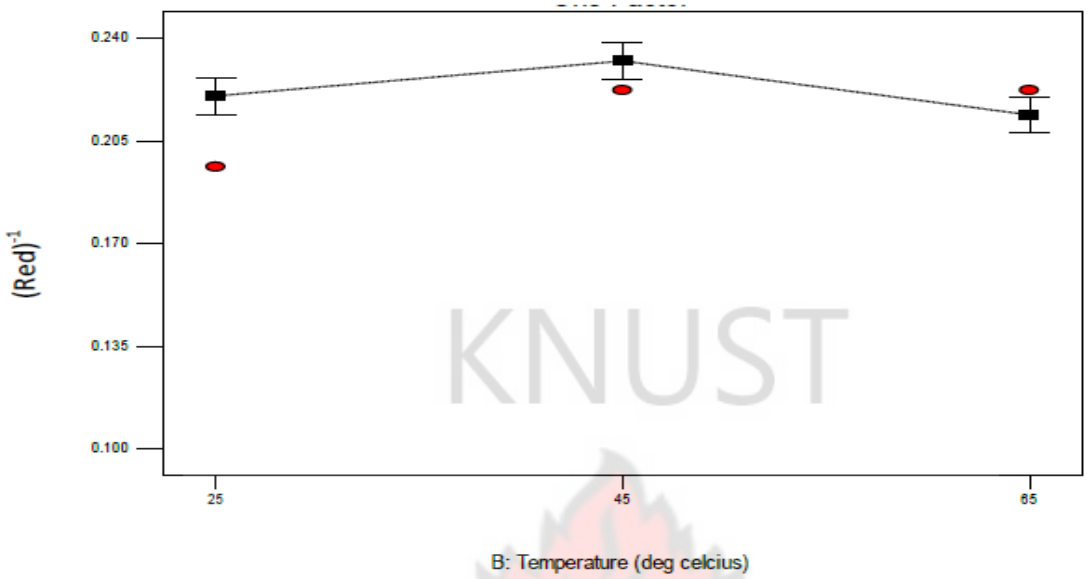

Figure 1*: Relationship between treatment temperature and inverse red colour response under optimized condition of 1 % extract-oil concentration and 0.5 h.*

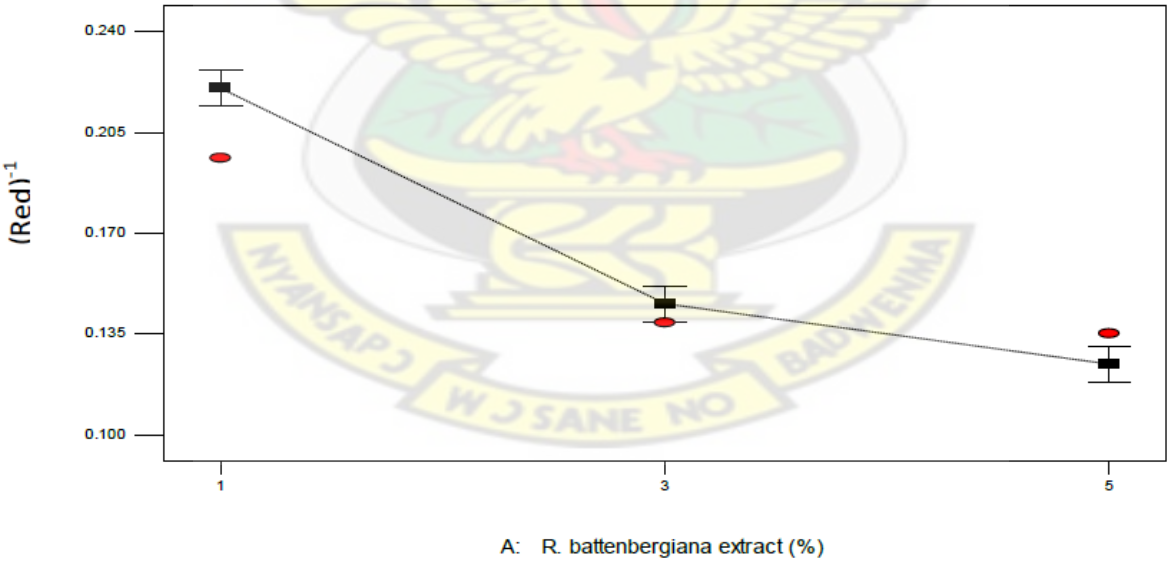

Figure 2: ***Relationship between inverse red colour response and extract-oil concentration (%) at optimized condition of 25 °C and agitated for 0.5 h.***

Refined oil processing standard select low red colour unit in the range of 2.5 – 4.5 R for consumer appeal in the oil industry (Hussein, 2006). From figure 1, it can be deduced that the red colour unit was least at 45 °C and most at 65 °C under optimized condition of 1 % extract-oil concentration and 0.5 h. At 25 °C, the red colour was expected to be lowest because temperature rapidly increases the extract incorporation treatment due to faster diffusion rate and increased mass transfer (Juntachote *et al.,* 2006).

All the red colour responses measured for the different extract incorporated refined palm olein formulations were generally above the native refined bleached and deodorised palm olein (i.e. 3.5R) and this is possibly due to the presence of chlorophyll and β- carotene pigment. In the absorption spectra, chlorophyll absorbs light in the red (long wavelength) and the blue (short wavelength) regions of the visible light spectrum (Matile, 1999). At 65 °C, the extract incorporation was highest and this could explain the greater red light absorption at that high temperature.

From figure 2, the three extract-oil concentrations (i.e. 1 %, 3 % and 5 %) were found to differ statistically from one another for the red colour response at optimized condition of 25 ^{o}C and 0.5 h. The increasing order of red colour for the extract-oil concentration was 1 % followed by 3 % and lastly 5 %. As the extract-oil concentration increased, the red colour measured also increased. This trend was usually expected because the *R. battenbergiana* extract that were incorporated could possibly contain colour pigment that absorbed in the red wavelength (Kaur and Kapoor, 2002).

However, high red colour in refined palm olein is not consumer appealing therefore at 1 % extract-oil concentration, the red colour for *R. battenbergiana* oil was most desirable while at 5 % extract-oil concentration, the red colour for *R. battenbergiana* oil was least desirable. According to Kaur and Kapoor (2002), aromatic spices contain numerous phenolic diterpenes and β-carotene and these might have absorbed energy of the magnitude of visible light leading to high red colour at higher extract concentration. Generally, the red colour unit increased for both increasing temperature and extract-oil concentrations.

Blue colour measured in refined olein samples is descriptive of the darkness or dullness attribute of the refined olein and is indicative of incomplete degumming or the presence of contaminants (Sipos and Szuhaj, 1996). The response for blue colour unit has the minimum and maximum units to be 1.0 and 9.8 respectively (table 4). These were measured under the respective formulation conditions of 1 % extract-oil concentration at 25 ℃, 0.5 h and 5 % extract-oil concentration at 65 °C for 0.5 h. This implies high extracting temperature coupled with high extract-oil concentration is very likely to cause dullness and darkness in treated refined palm olein.

The extract-oil concentration and temperature showed significant differences ($p < 0.05$) for blue colour unit. The treatment time did not vary significantly ($p < 0.05$) for blue colour unit. The extracting time (i.e. from 0.5 to 1.5 h) therefore did not produce significant differences among the different *R. battenbergiana* oil formulations. Under optimized formulation condition of 1 % extract-oil concentration, 25 $^{\circ}$C and 0.5 h, the square root of the blue colour was studied in relation to the treatment temperature and extract-oil concentration.

All the three extract-oil concentrations and temperatures were statistically different with respect to blue colour. This implies that each extract-oil concentration as well as temperature produced a blue colour reading that differ significantly from each other. From figures 3 and 4, the blue colour was increased from 1 % extract-oil concentration to 5 % extract-oil concentration and also in terms of extracting temperature from 25 $^{\circ}$C to 65 $^{\circ}$C. This trend is comparable to work done by Kaur and Kapoor (2002) who established that increase in temperature enhances pigment extraction from spices. It has also been identified by Kaur and Kapoor (2002) that aromatic plants contain secondary metabolites such as phenolics, diterpenes, β-carotene and chlorophilic compounds which are responsible for colour development.

The square root of the blue colour measured at increasing extract-oil concentration was found to be higher than that of increasing temperature. This trend shows that the extract-oil concentration has a much more significant effect on the overall colour of the treated samples. All the blue colour responses measured for all the different *R. battenbergiana* oil formulations were generally greater than that of the fresh native RBD palm olein which did not contain any blue unit. It is suggested that the blue unit measured in the *R. battenbergiana* oil formulations could be due to the components of incorporated *R. battenbergiana* extract.

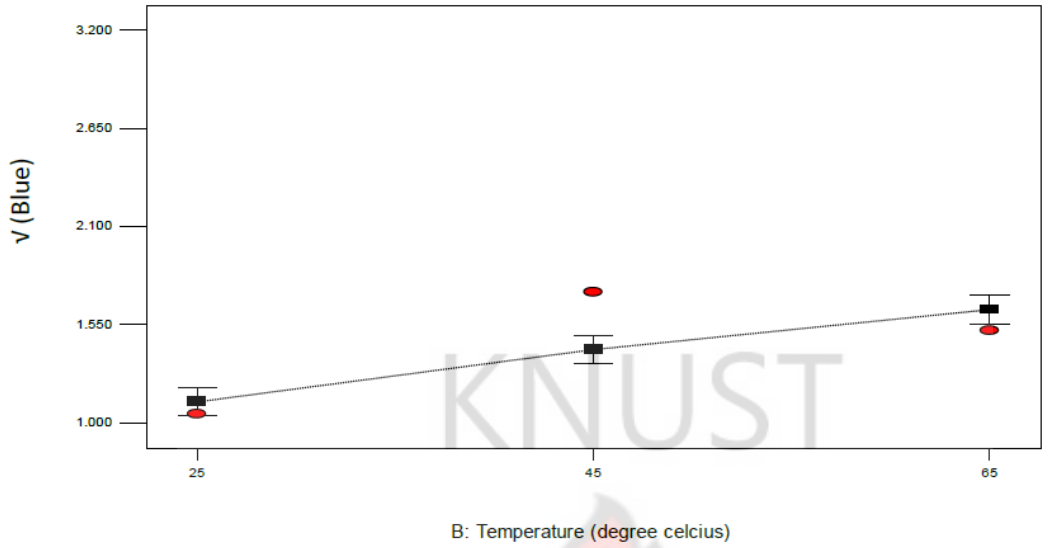

Figure 3: ***The relationship between the square root of the blue colour and the temperature (°C) at optimum condition of 1 % extract-oil concentration and agitated for 0.5 h.***

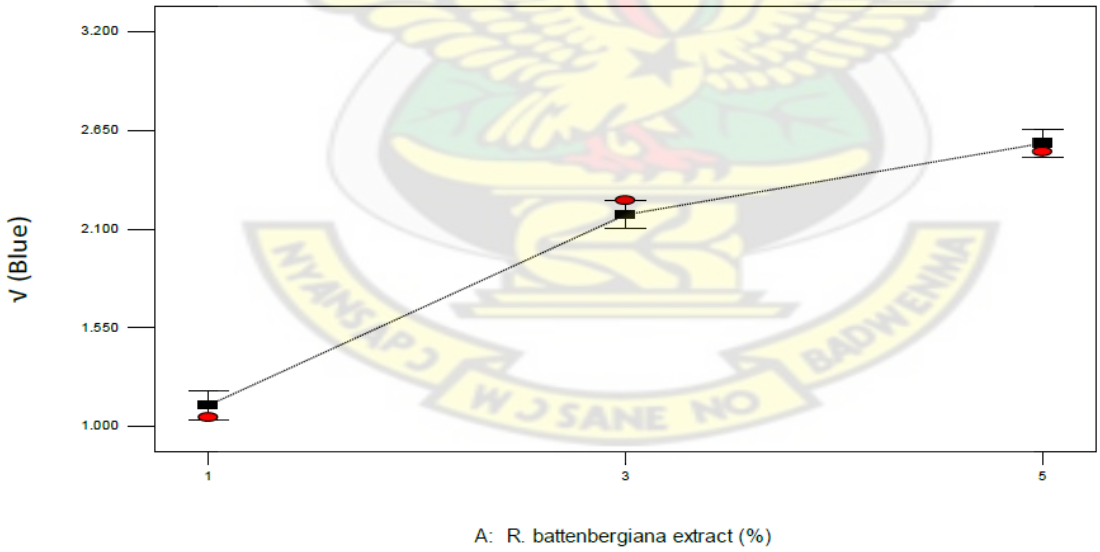

Figure 4: ***The relationship between the square root of blue colour and extract-oil concentration at optimum condition of 25 °C and agitated for 0.5 h.***

In a similar refined sunflower oil treatment with natural plants, the researchers (Bensmira *et al.*, 2007) incorporated the plants until strong and stable darkening of the oil. After this treatment, the undesirable colour observed in the mixture was removed by an additional decolourizing step to make the incorporated oil look appealing.

4.2.2 Antioxidant Property

The antioxidant activities of natural components may have a reciprocal correlation with their reducing capacity (Matthaus, 2002). In this study, the Fe^{3+} - Fe^{2+} transformation was determined as reducing capacity. From the statistical analysis, the variations in the % extract was not significant but the temperature of the agitations were significant ($p<0.05$) as well as the model which was given as;

$$Y = \beta_o + \beta_1 X_1^i + \beta_2 X_1^{ii} + \beta_3 X_2^i + \beta_4 X_2^{ii}$$

Similar to the red and blue unit colours, the antioxidant property also followed this regression model where Y represented the antioxidant property. In this case, the statistical analysis showed that the "pred R^2" of 0.2875 was in reasonable agreement with the "adj R^2" of 0.2840. Since the "adeq precision" measured the signal to noise ratio of 5.73 which is above 4, it indicated an adequate signal and hence the model could be used to navigate the design space and subsequently be able to make predictions during optimization.

The results for antioxidant properties ranged from 0.52 to 0.78 mg/ml under the treatment conditions of 3 % extract-oil concentration, 65 °C, 1 h and 5 % extract-oil concentration, 25 °C, and 0.5 h respectively. Research by Jayaprakash (2001) has shown that the antioxidant activity is a function of the resultant formulation power to reduce Fe^{3+} to Fe^{2+}. The antioxidant activity of the *R. battenbergiana* oil formulations is paramount to its ability to increase stability of the

treated oil especially during frying. From the studies, the antioxidant property response varied statistically with both treated extract-oil concentration (%) and temperature (°C) but not with time. The reducing power correlated well with increasing concentrations. Under optimized formulation condition of 1 % extract-oil concentration, 25 °C and 0.5 h, the antioxidant activity was studied in relation to the treatment temperature and extract-oil concentration as shown in figure 5 and 6.

It can be deduced from figure 5 that antioxidant response at the varying temperatures was statistically ($p < 0.05$) different from one another. This implies that the effect of each varying temperature on antioxidant property was mutually exclusive. The antioxidant property is in the decreasing order of 25 °C followed by 45 °C and lastly 65 °C. At elevated temperatures, it has been suggested by Chan *et al.* (2009) that there could be a breakdown of phenolic compounds and this might explain the trend observed.

From figure 6, the antioxidant activity was highest at 5 % extract-oil concentration and lowest at 3 % extract-oil concentration. The lowest antioxidant activity measured at 3 % extract-oil concentration was unusual since it had been reported by Morteza-Semnani *et al.* (1996) that the antioxidative effect of the species, *Phlomis bruguieri* and *Stachy slaxa*, increased as their concentrations increased in sunflower oil. However, the antioxidant activity measured at 3 % and 1 % in this research do not show statistical difference ($p<0.05$).

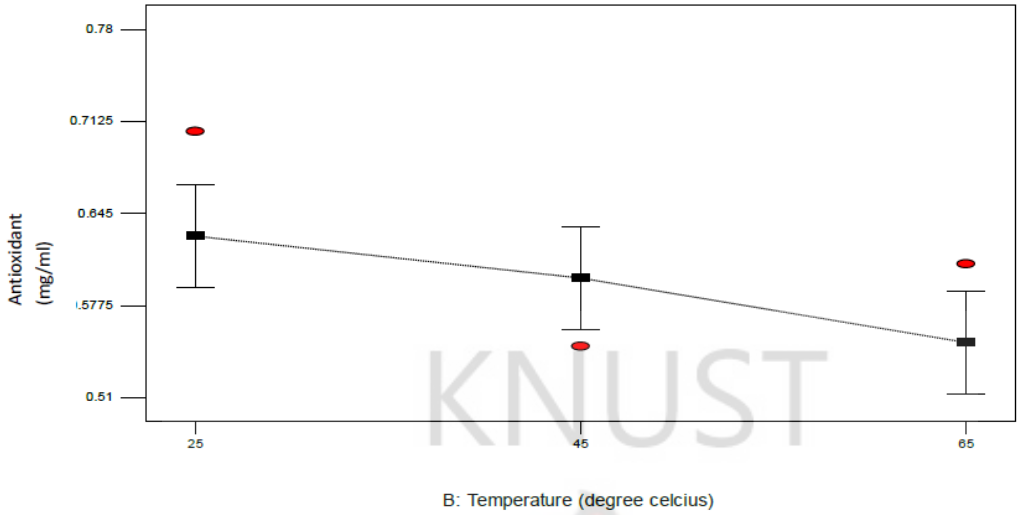

Figure 5: ***The relationship between varying temperatures (°C) and antioxidant property response (mg/ml) at optimized condition of 1 % extract-oil concentration and agitated for 0.5 h.***

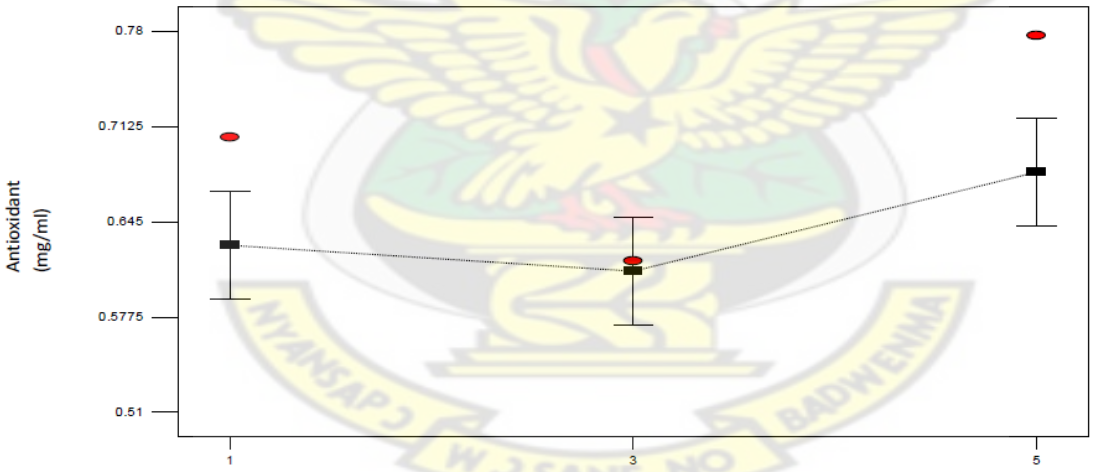

Figure 6: ***The relationship between varying extract-oil concentrations (%) and antioxidant property (mg/ml) at optimized condition of 25 °C and agitated for 0.5 h.***

The antioxidant activity of culinary plants and plantal extracts has been attributed to redox properties which function as a reducing agent, in addition to acting as a hydrogen donor, singlet oxygen quencher and metal chelators (Rice-Evans *et al.*, 1997). Numerous studies have also suggested that phenolic compounds are major components responsible for antioxidant activity in plants (Kaur and Kapoor, 2002).

The antioxidant activity measured could also be probably ascribed to the presence of aromatic compounds such as phenols, terpenic alcohols in thyme(Stahl-Biskup, 1991) and linaloyl acetate, β-ocimene and caproic acid in Lavender (Bisset, 1994). The suggestive presence of natural active substances in *R. battenbergiana* extract may help to inhibit oxidation to a certain extent in RBD-palm olein as seen in the application of aromatic plants to extend frying life in sunflower oil (Lee *et al.*, 2005). Also it has been established by Nor *et al.* (2008) that RBD palm olein samples treated with *turmeric* extract generally lowered the peroxide value significantly. In this study, tertiary butyl hydroquinone (TBHQ) was used as the standard due to its exceptional stabilizing antioxidant effect in unsaturated fats (O'Brien, 2000).

4.2.3 Optimization of Formulations

The constraints factors were based on optimum set specifications which included a minimum red and blue colour units as well as maximum antioxidant activity of 1.2 mg/ml. The optimized treatment condition was consequently found to be 1 % extract-oil concentration and 25 °C at agitation contact time of 0.5 h and the corresponding responses are as presented in table 5.

Table 5: Optimized condition of extract-oil concentration, extracting temperature and time for selected RB oils formulation.

Extract conc. (%)	Temp.(°C)	Time (h)	$(Red)^{-1}$	√(Blue)	$FeCl_3$(mg/ml)
1	25	0.5	0.22	1.12	0.63

4.3 Frying Experiment Treatment

4.3.1 Physicochemical Changes during Deep-Fat Frying Experiment

The measured changes in free fatty acid, peroxide value, iodine value and acrylamide with respect to the native, *R. battenbergiana* oil and citrate oil during five frying cycles are shown in table 6.

Table 6: Changes in %FFA, PV, IV and Acrylamide for Native, RB and Citrate treated oils during five frying cycles.

	Factor 1	**Factor 2**	**Response 1**	**Response 2**	**Response 3**	**Response 4**
Run	A:Frying cycles	B:Oil	FFA %	PV meq /kg	IV g /100g	Acrylamide µg/ml
1	cycle 1	N oil	0.11	1.62	52.00	10.23
2	cycle 1	RB oil	0.11	1.12	54.70	5.64
3	cycle 1	CT oil	0.10	1.00	55.60	4.16
4	cycle 2	N oil	0.17	2.28	48.40	14.62
5	cycle 2	RB oil	0.13	1.95	51.20	9.32
6	cycle 2	CT oil	0.12	1.56	53.70	8.59
7	cycle 3	N oil	0.22	3.00	43.40	22.05
8	cycle 3	RB oil	0.17	2.86	48.80	20.53
9	cycle 3	CT oil	0.13	1.89	50.30	15.39
10	cycle 4	N oil	0.28	4.93	41.50	25.67
11	cycle 4	RB oil	0.20	2.94	46.70	23.67
12	cycle 4	CT oil	0.15	2.24	47.70	20.15
13	cycle 5	N oil	0.33	5.63	40.00	28.89
14	cycle 5	RB oil	0.22	3.89	43.50	24.56
15	cycle 5	CT oil	0.17	2.51	44.00	22.84

4.3.1.1 %Free Fatty Acids

Free fatty acids (FFA) represent the degradation of the oil quality during heating due to oxidation and hydrolysis. Changes in percentage of free fatty acid (%FFA) of the oil samples during frying are shown in figure 7. The statistical analysis of the % FFA results indicated that the frying cycles as well as the frying oil types are both significant terms ($p<0.05$). This therefore indicated that there exists a significant difference between the control and treated palm olein samples during the frying cycles. The model which was given as;

$$Y = \beta_o + \beta_1 X_1^{i} + \beta_2 X_1^{ii} + \beta_3 X_1^{iii} + \beta_4 X_1^{iv} + \beta_1 X_2^{i} + \beta_2 X_2^{ii}$$

was also significant ($p<0.05$), and the %FFA followed this regression model where Y represented the %FFA; βo to β4 representing the coefficient of variation and X_1 and X_2 representing frying cycles and frying oil types respectively.

For the % FFA response, statistical analysis showed that the "pred $R^{2"}$ of 0.56 was not in reasonable agreement with the "adj R^{2}" of 0.78 as normally expected. This may indicate a large block effect. Since the "adeq precision" measured the signal to noise ratio of 10.45 which is above 4, it indicates an adequate signal and therefore the model could be used to navigate the design space.

In this study, the free fatty acid content of all treatments increased gradually from day 0 to day 5 of frying. In the native oil, the free fatty acid content increased from 0.11 % to 0.33 % by the end of the fifth frying cycle representing approximately 22 % increase. For *R. battenbergiana* oils, the free fatty acid increased from 0.11 % to 0.22 % after frying cycle five (5) representing 11 % increase.

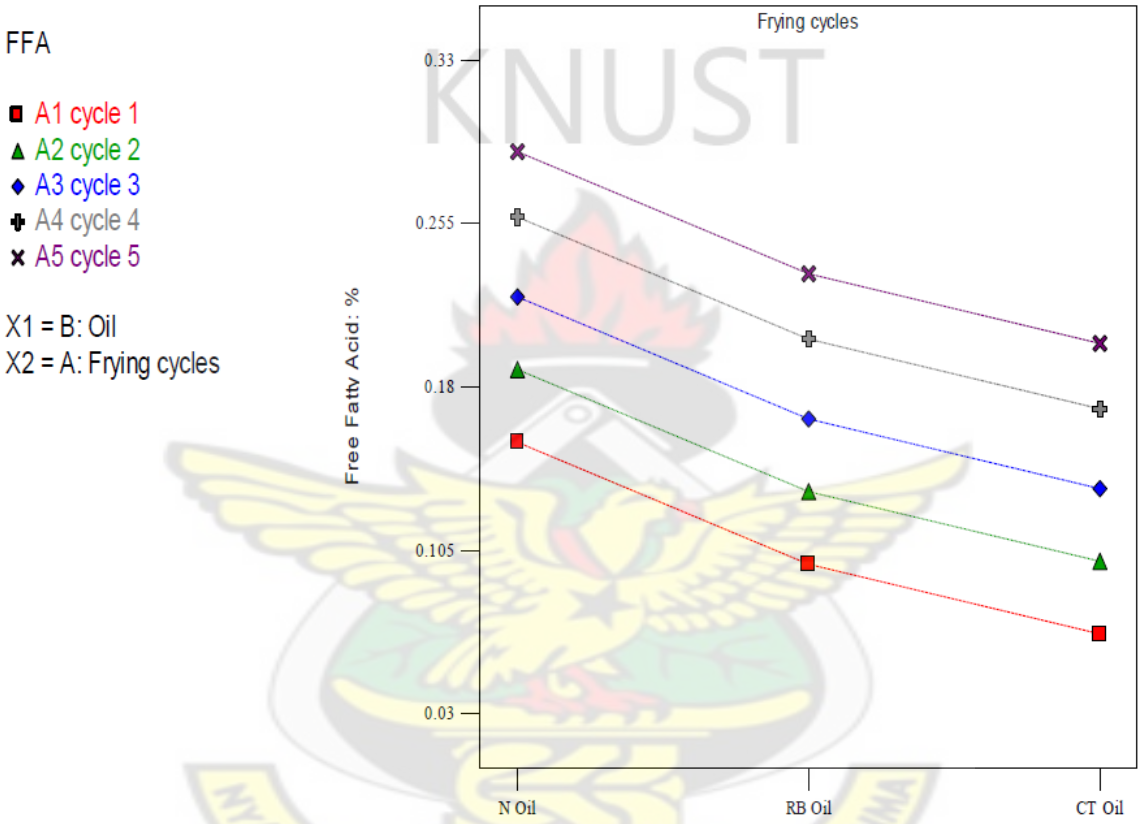

Figure 7: *Variations in % free fatty acid with respect to frying oil types and frying cycles.*

Lastly, the citrate treated oil during frying increased from 0.1 % to 0.17 % representing 7 % increase in free fatty acid. It was observed that citrate treated oil resulted in a significant lower formation of free fatty acids compared to the control

However, compared to the control, formation of free fatty acids was significantly lower in palm olein added with *R. battenbergiana* extract. This finding is in agreement with Cheman and Jaswir (2000) who reported that addition of rosemary and sage oleoresin extract at 0.4 % was able to retard the formation of free fatty acid in RBD palm olein when compared to the control. A similar finding in free fatty acid trend has been reported by Bensmira *et al.* (2007) during heating of sunflower oil with thyme and lavender extracts at elevated frying temperatures. Results also show that both *R. battenbergiana* oil and citrate treated oil samples significantly ($p<0.05$) reduce the free fatty acid contents of the oils during frying.

This might be due to their higher antioxidative activity compared to the native oil. Compounds such as various flavanoids and some other polyphenols might contribute to the antioxidative activities of the natural plant even at high temperature. Phenolics transfer the hydrogen atom to lipid peroxyl radicals and form aryloxyl which incapable of acting as a chain carrier, couples with another radical thus quenching the radical process. These compounds are particularly effective antioxidants for polyunsaturated fatty acids (Ruberto *et al.*, 2001). Generally, the increase in free fatty acid content were due to the cleavage and oxidation of double bonds to form carbonyl compounds which oxidized to low molecular fatty acids during frying treatments (Irwandi and Cheman, 2000). Furthermore it is expected that water leaching out from fried yam accelerated the hydrolysis cleavage of the oil.

The free fatty acid content, resulting from the hydrolysis of triacylglycerol as well as further decomposition of hydroperoxides, is one of the most important indicators of oil deterioration during heating. During frying, the oil is continuously and repeatedly used at elevated temperatures (160–180°C) in the presence of air and moisture (Maskan, 2003). The released fatty acids are more susceptible to thermal oxidation under frying temperatures. The oxidized products of fatty acids give the off-flavours and odours (hydrolytic rancidity) to the frying medium and fried foods (Lin *et al.*, 1998; Nawar, 1996). Therefore, controlling the level of free fatty acid within a reasonable range would prevent the breakdown of fats.

The results did not show some decrease in % FFA during the frying periods as noticed by Suleiman *et al.* (2006) after 4 and 12 h of frying treatment. This could be due to non-volatilization of the low molecular weight fatty acids. Determination of free fatty acid appears to be the method favored by many operations for quality control evaluation of used frying oil (Stevenson *et al.*, 1984). According to Kun (1990), the increase in the free fatty acid content could also be caused by further oxidation of the secondary products formed during frying.

Comparing the free fatty acid to specifications for discarding frying oils i.e. FFA below 0.3 % (Hussein, 2006) which is the value normally used by snack food producers to discard the oil, it can be deduced that the native oil was unfit for use after the fourth (4^{th}) frying cycle whereas both the *R. battenbergiana* oil and citrate treated oil were fit for re-use even after the fifth (5^{th}) frying cycle. In effect therefore, with the incorporation of the *R. battenbergiana* into the refined

bleached deodorized palm olein the frying life of the otherwise limited native oil has been extended.

4.3.1.2 Peroxide Value

The statistical analysis of the peroxide value results indicated that the frying cycles as well as the frying oil types are both significant terms ($p<0.05$). This therefore indicates that there exists a significant difference between the native and treated palm olein samples during the frying cycles. The model which was given as;

$$Y = \beta_o + \beta_1 X_1^{i} + \beta_2 X_1^{ii} + \beta_3 X_1^{iii} + \beta_4 X_1^{iv} + \beta_1 X_2^{i} + \beta_2 X_2^{ii}$$

was also significant ($p<0.05$), and the peroxide value followed this regression model where Y represented the peroxide value; β_o to β_4 representing the coefficient of variation and X_1 and X_2 representing frying cycles and frying oil types respectively.

For the peroxide value response, statistical analysis showed that the "pred R^2" of 0.55 was not in reasonable agreement with the "adj R^2" of 0.78 as normally expected. This may indicate a large block effect. Since the "adeq precision" measured the signal to noise ratio of 10.34 which is above 4, it indicates an adequate signal and therefore the model could be used to navigate the design space.

Peroxide value is a measure of the amount of peroxides formed in fats and oils through autoxidation and oxidation processes. Indirectly, it is a measure of the degree of initial oxidation of fats and oils (Cheman and Jaswir, 2000). The change in peroxide value (milliequivalent of

peroxide per kg of sample) of the oil samples during the frying cycles is shown in figure 8. The initial peroxide value for all the oils was lower than 2 meq/kg in table 3. Cheman and Wanhussin (1998) mentioned that a good quality vegetable oil should have a peroxide value of lower than 2 meq/kg.

Generally the peroxide value of all treatments increased gradually from day 0 to day 5 of frying. However, the results show that both *R. battenbergiana* and citrate treated oil samples could significantly ($p<0.05$) reduce the oil oxidation process during repeated frying cycles compared to the control. At day 1, peroxide values for RB and Citrate treated oils were 1.12 and 1.0 meq/kg, respectively, while that of control was 1.62 meq/kg. At day 5, the values were 3.89, 2.51 and 5.63 for *R. battenbergiana* treated oil, citrate treated and native, respectively.

Hindered phenols and crude tea extract were reported to be capable of lowering the peroxide value at 0.02 % concentration in oil (Naz *et al.*, 2005). This result has been similarly reported by Cheman and Jaswir (2000) where the addition of both rosemary and sage extracts to frying oils lowered the peroxide value compared to the control. Chang *et al.* (1977) has similarly reported that the addition of 0.02 % of rosemary extracts to frying oils reduced the peroxide values by 50 %. It also appeared that the *R. battenbergiana* extract was comparable to citrate treated RBD palm olein and was as effective in reducing the peroxide value.

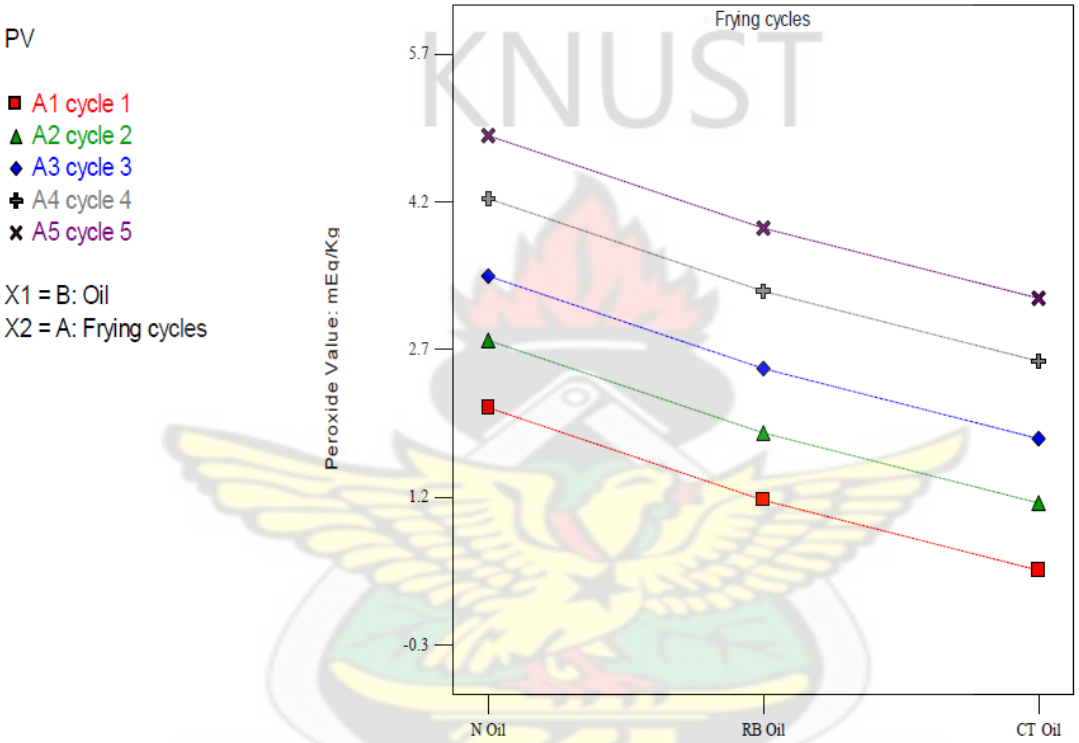

Figure 8: *Variations in peroxide value with respect to frying oil types and frying cycles.*

Inhibition of peroxide formation is through donation of the hydrogen atom from the OH group of the phenolic compounds to the lipid radical which in turn produces a stable product. The reduction in peroxide value could be due to the antioxidative activity of both materials. Their antioxidant properties can quench the initiation and propagation steps of auto-oxidation chain reactions (Marangoni, 2000).

Work done by Bensmira *et al.* (2007) reported that the addition of *thyme* and *lavender* extracts to sunflower seed oil helped to inhibit oxidation to a certain limit thus reducing the peroxides formed during its exposure to simulated frying temperatures. Compositional analysis of *thyme* and *lavender* showed that they are rich in phenols and terpenic alcohols. The phenolic compounds in plants act as antioxidants due to their redox properties, allowing them to act as reducing agents, hydrogen donors, free radical quenchers and metal chelators (Nor *et al.,* 2008). The presence of these aromatic compounds possibly present in *R. battenbergiana* extract could probably be the cause of its antioxidative property. It is indicative therefore that this *R. battenbergiana* extract can be used in the industry as it has the capability of prolonging the shelf life of cooking oil.

Results from table 6 also indicate that cycle of frying had a significant ($p<0.05$) effect on peroxide values of all samples. The peroxide values increased with the increased cycle of frying until day 5 of frying. However, the amount of peroxides formed during the frying model for the *R. battenbergiana* and citrate treated oil types were lower compared to the untreated native oil type.

A decrease in peroxide value during frying has been observed by several other researchers (Vieira and Regitano-D'Arce, 1999; White, 1991; Zhang and Addis, 1992). However, others (Jaswir *et al.,* 2000; Tan and Cheman, 1999) have reported an increase in peroxide value of oil during heating and/or frying. In fact, though the peroxide value increased with frying cycles for all samples, the evolution of the studied parameter in both treated oils was found to be slower than the one observed in the untreated oil.

Comparing the peroxide value results to standard specifications for frying oils (Hussein, 2006) it can be deduced that the native oil was unfit for use after the third (3^{rd}) frying cycle whereas both *R. battenbergiana* oil and citrate treated oil were fit for re-use even after the third (3^{rd}) frying cycle. In effect therefore, with the incorporation of the *R. battenbergiana* into the refined bleached deodorized palm olein the frying life of the otherwise limited native oil has been extended.

4.3.1.3 Iodine Value

The statistical analysis of the iodine value results indicated that the frying cycles as well as the frying oil types are both significant terms ($p<0.05$). This therefore indicates that there exists a significant difference between the native and treated palm olein samples during the frying cycles. The model which was given as;

$$Y = \beta_o + \beta_1 X_1^{i} + \beta_2 X_1^{ii} + \beta_3 X_1^{iii} + \beta_4 X_1^{iv} + \beta_1 X_2^{i} + \beta_2 X_2^{ii}$$

was also significant ($p<0.05$), and the iodine value followed this regression model where Y represented the Iodine value; βo to β4 representing the coefficient of variation and X_1 and X_2 representing frying cycles and frying oil types respectively.

For the Iodine value response, statistical analysis showed that the "pred $R^{2"}$ of 0.9401 is in reasonable agreement with the "adj $R^{2"}$ of 0.9702. The "adeq precision" which measures the signal to noise ratio and when greater than 4 is desirable was 29.207. This indicates an adequate signal and thus the model can be used to navigate the design space. As shown in figure 9, during frying the iodine values of all treatments decreased significantly ($p<0.05$) from frying cycle 1 to 5. For control, the iodine value decreased from 52 g I_2/100 g at day 1 to 40 g I_2/100 g at day 5, whiles for samples with treatments for *R. battenbergiana* and citric acid, they decreased from 54.7 to 43.5 and 55.6 to 44 g I_2/100 g respectively.

The percentage loss of unsaturation was 23.07 %, 20.47 % and 20.86 % for native, *Renealmia battenbergiana* oil and citrate treated oil respectively. The percentage loss implied that citrate treated oil was least susceptible to oxidation, however compared to the control; the *Renealmia battenbergiana* oil was less susceptible. The decrease in iodine value can be attributed to the destruction of double bonds by oxidation and polymerization.

Iodine value is a measure of the total number of unsaturated linkages in fats and is expressed in terms of percentage of iodine absorbed (Hussein, 2006). The differences in iodine values of the oil during frying are also indicative of the increased rate of oxidation during frying. A significant ($p<0.05$) change in iodine values can be observed when there is excessive deterioration of the oil (Augustin and Berry, l983). Similarly to peroxide and free fatty acid values in this study, both *R. battenbergiana* oil and citrate treated oil gave a significant effect on iodine value. *R. battenbergiana* and citrate could significantly ($p<0.05$) protect the oil from further oxidation, from first to fifth cycle of frying with better protection by citrate.

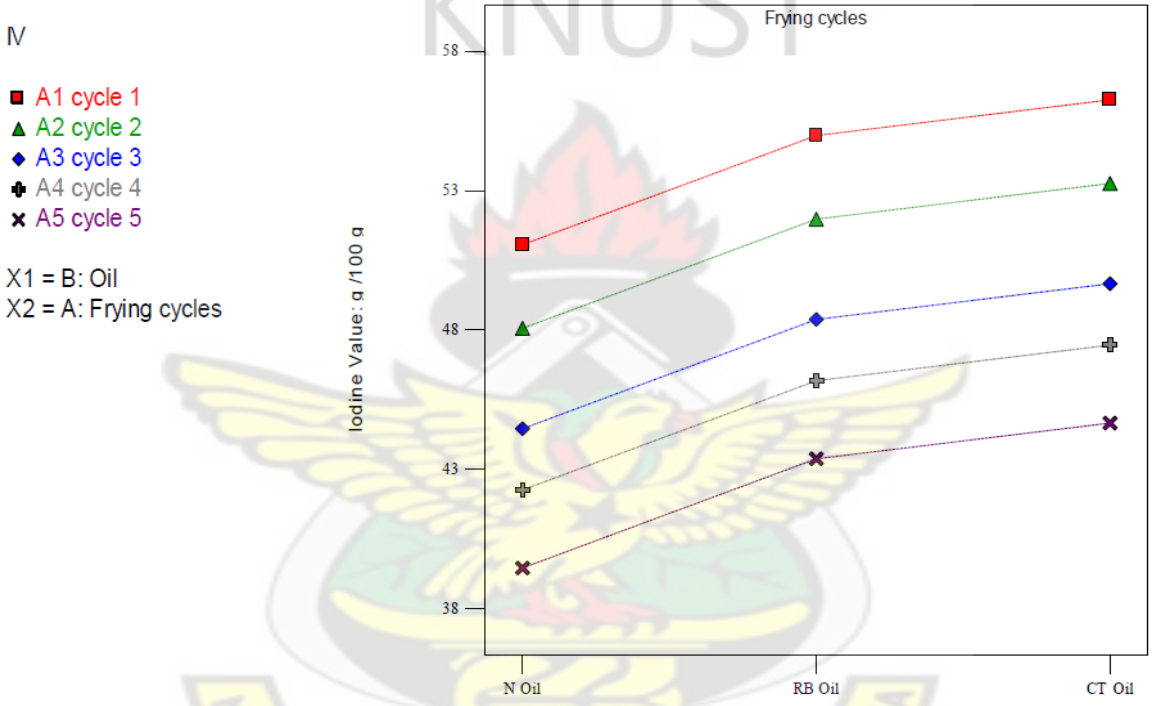

Figure 9: *Variations in iodine value with respect to frying oil types and frying cycles.*

This trend reduction in unsaturation during frying was comparable to findings reported by Cheman and Jaswir (2000) during treatment of frying oil with rosemary and sage extracts. In addition, the statistical analysis of results indicated a significant difference ($p<0.05$) between the iodine value of untreated and treated oil samples during frying.

Iodine value decreases are indicative of decreased unsaturation during frying and are attributed to reactions involving double bonds, whether through direct interaction across the bond to form 1, 2-diols or through chain reactions adjacent to the double bond to form volatile degradation products. When food is fried, water, steam and oxygen initiate the oxidation and hydrolysis degradation reactions in the frying oil. Water, a weak nucleophile, attacks the ester linkage of triacylglycerol and produces di- and monoacylglycerols, glycerol, and free fatty acids. Other plant extracts, such as rosemary and sage oleoresin, reportedly retard the release of free fatty acid in RBD palm olein at 0.4 % concentration (Cheman and Jaswir, 2000). The changes in iodine values showed that during the frying period, oil samples have been significantly degraded with the control being the most degraded.

The frying oils have rapidly undergone oxidation, polymerization and other chemical changes which resulted in an increase in viscosity of the frying oil and decrease in iodine value (Tan *et al.*, 1985). This seems to be due to the process of polymerization via Diel-Alder interactions and also to oxidation, hydrolysis and isomerization (Lin *et al.*, 1998).

During frying process, the oil is really a heat transfer medium. The reduction in iodine value indicates reduction in the rate of heat transfer and this can affect the frying time and colour of the

food. Also with reduction in the iodine values significantly, there can be an increase in oil absorption, and this effect will carry over into the finished products.

However, from the studies, it can be explained that the natural antioxidants present in *R. battenbergiana* probably reacted with the initiating and propagating radicals to produce harmless low molecular weight polymers thereby lowering the rate of oil degradation. Similarly, additives and phenolic compounds, such as catechin and composites of catechin, also caused significant improvement in the stability of canola oil, and in palm super olein frying performance (Razali *et al.*, 2003; Chen and Chan, 1996).

Other works have reported composites of several antioxidants that synergistic effects in increasing oil stability (Anwar *et al.*, 2007). Interaction between the compounds and existing tocopherols and tocotrienols in RBD palm olein may have resulted in some synergistic effect that retarded oil degradation.

Comparing the Iodine value results to standard specifications for frying oils (Hussein, 2006) it can be deduced that the native oil was unfit for use after the third (3rd) frying cycle whereas both *R. battenbergiana* oil and citrate treated oil were fit for re-use even after the third (3rd) frying cycle. In effect therefore, with the incorporation of the *R. battenbergiana* into the refined bleached deodorized palm olein the frying life of the otherwise limited native oil has been extended.

4.3.1.4 Residual Acrylamide

The residual acrylamide determination method was estimated by taking into account the precision of the retention time, the interference of co-elution and the peak purity of acrylamide. A high repeatability of the retention time was obtained with RSD <1 % for both non-labeled acrylamide standard and frying oil samples. Impurities or co-elutions were not observed in the HPLC-UV chromatogram of frying oil samples.

The calibration curve for the determination of acrylamide in oil samples (y= 0.02861x + 0.00) was linear over the range of 1–40 µg/ml with a coefficient of determination (r^2) of 0.9610 (n = 5). Some of the oil samples containing relatively very low levels of acrylamide were concentrated before injection in order to match the linear range of calibration.

The statistical analysis of the acrylamide results indicated that the frying cycles as well as the frying oil types are both significant terms ($p<0.05$). The statistical analysis of the results indicated a significant difference ($p<0.05$) between the residual acrylamide content of untreated and treated oil samples during frying.

The model which was given as;

$$Y = \beta_o + \beta_1 X_1^{i} + \beta_2 X_1^{ii} + \beta_3 X_1^{iii} + \beta_4 X_1^{iv} + \beta_1 X_2^{i} + \beta_2 X_2^{ii}$$

was also significant ($p<0.05$), and the iodine value followed this regression model where Y represented the residual acrylamide; βo to β4 representing the coefficient of variation and X_1 and X_2 representing frying cycles and frying oil types respectively.

For the residual acrylamide response, statistical analysis showed that the "pred $R^{2"}$ of 0.9670 is in reasonable agreement with the "adj $R^{2"}$ of 0.9836. The "adeq" precision which measures the signal to noise ratio and when greater than 4 is desirable was 35.8. This indicates an adequate signal and thus the model can be used to navigate the design space.

From figure 10, during the frying cycles, the acrylamide content of native oil (N oil), *R. battenbergiana* oil (RB oil) and citrate treated oil (CT oil) increased significantly ($p<0.05$) from frying cycle 1 to 5. For control, the acrylamide content increased from 10.23 at day 1 to 28.89 µg/ml at day 5, whiles for oils treated with *R. battenbergiana* and citrate, it increased from 5.64 to 24.56 µg/ml and 4.16 to 22.84 µg/ml respectively.

However, the results indicated *R. battenbergiana* oil (RB oil) and citrate treated oil (CT oil) induced acrylamide retardation by 14.99 and 20.94 % respectively against native oil (N oil). The acrylamide contents in *R. battenbergiana* oil were significantly different from that of the native ($p <0.05$). This reduction in acrylamide with *R. battenbergiana* oil could be probably attributed to the antioxidant compounds such as phenols, flavonoids and terpenic alcohols (Bensmira *et al.*, 2007).

The formation of acrylamide in the frying oil systems is via the acrolein pathway formed from the degradation of lipids, mainly oxidized fatty acids or glycerol at high temperature (Umano and Shibamoto, 1987). Acrolein further reacts via oxidation to generate acrylic acid or by formation of an intermediate acrylic radical (Becalski *et al.*, 2003). Both of the intermediates then induce

acrylamide formation in the presence of a nitrogen source under favourable reaction conditions (Yasuhara *et al*., 2003).

Therefore, addition of antioxidants would block the oxidation of acrolein to a certain extent and further reduce the generation of acrylamide. However, both reduction and enhancement of acrylamide formation via addition of antioxidants occurred in different published research, which suggested the dual effects of antioxidants on the generation of acrylamide (Taeymans *et al*., 2004).

Tareke (2003) found that the addition of butylated hydroxytoluene (BHT), sesamol and vitamin E to meat before heating increased the formation of acrylamide, probably by protection of acrylamide against free radical-initiated reactions. Similar reports on the reduction of acrylamide by the addition of flavonoids have been demonstrated. For instance, Ferna´ndez *et al*. (2003) reported that acrylamide could be reduced by the addition of a flavonoid spice mix containing green tea, apple polyphenols and onion extracts. The spice mix solution was added to potato slices before frying, and a powder spice mix was also added to the potato slices after frying. After a 4-day incubation time, the acrylamide contents were reported to be reduced by up to 50 % in the spice mix treatment.

On the other hand, Becalski *et al*. (2003) found acrylamide could be reduced when adding rosemary plant to the oil used for frying potato slices. Biedermann *et al*. (2002) showed a weak inhibition of the acrylamide formation by the addition of ascorbic acid in a potato-based model.

In the present work, sufficient evidence demonstrated that *Renealmia battenbergiana* oil has a good retardation control of acrylamide compared with the native oil sample.

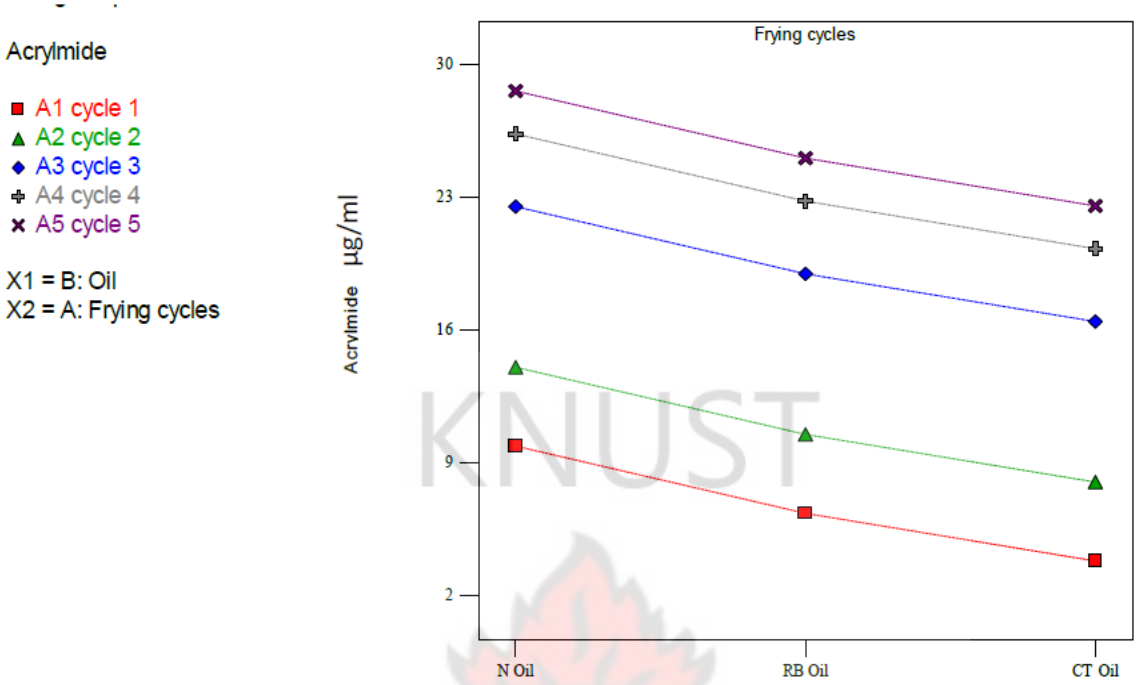

Figure 10: *Variations in the residual acrylamide of frying oil types during the frying cycles.*

4.3.2 Overall Frying Performance of Frying Oil Types

The overall frying performance of the native oil (N oil), *R. battenbergiana* oil (RB oil) and citrate oil (CT oil) was assessed by the Design Expert (2007) statistical tool. Certain constraints were selected for statistical screening of the fifteen runs.

The selection criteria based on literature specifications for discarding frying oil included a goal of maximized iodine value and minimized residual acrylamide, peroxide value and free fatty acid. Upper limit for frying cycle was five (5), for peroxide value was 3 meq/kg and free fatty acid was 0.3 % (Hussein, 2006).

Table 7: Constraints Solutions for the Overall Performance of Frying Oil Types

Number	Frying cycles	Oil	FFA (%)	PV (meq/kg)	IV(g I_2/100g)	Acrylamide (µg/ml)
1	cycle 3	CT oil	0.13	1.79	49.66	16.46
2	cycle 3	RB oil	0.16	2.50	48.38	18.98

From table 7, citrate (CT) oil and *R. battenbergiana* (RB) oil were statistically judged favorable in terms of free fatty acid, peroxide value, iodine value and acrylamide residue after frying cycle three (3). The native oil was found not to be suitable after frying cycle two (2). This implied that with the incorporation of *R. battenbergiana* extract into native refined olein, there was considerable extension of the frying cycle of the oil. However, it was found that none of the oil types were overall suitable after the fourth (4^{th}) and fifth (5^{th}) frying cycles.

4.4 Sensory Evaluation

All the attributes evaluated i.e. odour, colour, crispiness and taste were found to be statistically significant ($p<0.05$) in scores for the frying oil type and the frying cycles.

4.4.1 Odour

The change in odour with respect to the panelists and frying cycles for native (N) oil, *R. battenbergiana* (RB) oil and citrate (CT) is shown in figure 11. The odour scores differed statistically ($p<0.05$) between the type of frying oil and the frying cycles. From figure 11, as the frying cycles increased, all the oil types showed decrease in the odour acceptability of the fried yam slices. This trend was not unusual as the odouriferous compounds such as free fatty acids, aldehydes, ketones and oxidized products increase along increasing frying cycles (Brooks, 1991).

The increasing order of odour rating for each frying cycle was found to be native oil fried yam product which was scored the lowest followed by *R. battenbergiana* (RB) oil and citrate treated (CT) oil.

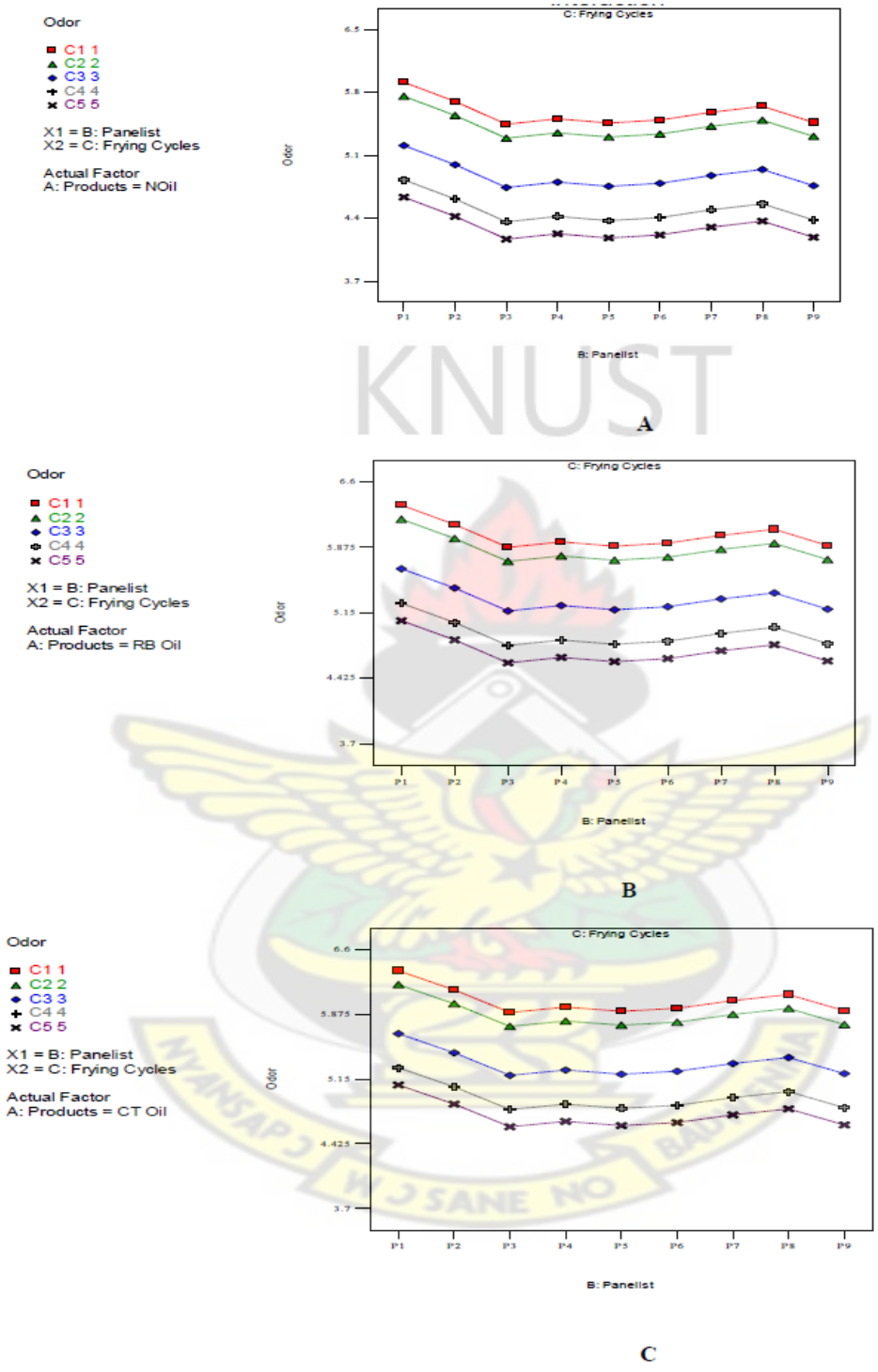

Figure 11: *Variations in odour scores with respect to panelists and frying cycles for native oil (A); Renealmia battenbergiana oil (B) and citrate treated oil (C).*

4.4.2 Colour

The change in colour scores for fried yam slices with respect to panelists and frying cycles for native (N) oil, *R. battenbergiana* (RB) oil and citrate (CT) oil are shown in figure 12. The colour scores differed statistically ($p<0.05$) between the type of frying oil and the frying cycles.

From figure 12, the colour for all the frying oil types i.e. native (N) oil, *R. battenbergiana* (RB) oil and citrate (CT) oil decreased as the frying cycle increased from 1 to 5. However, it can be seen that, the native oil fried yam product showed lowest colour acceptability from cycle one to five of the frying period whiles citrate treated oil showed the highest colour acceptability.

The decrease in the colour scores was closely related to the deterioration of the oils during frying which caused a darkening of the frying oils. This colour trend was also observed by Cheman and Jaswir (2000) when they studied the effect of rosemary and sage extracts on sensory acceptability of potato crisps during deep-fat frying.

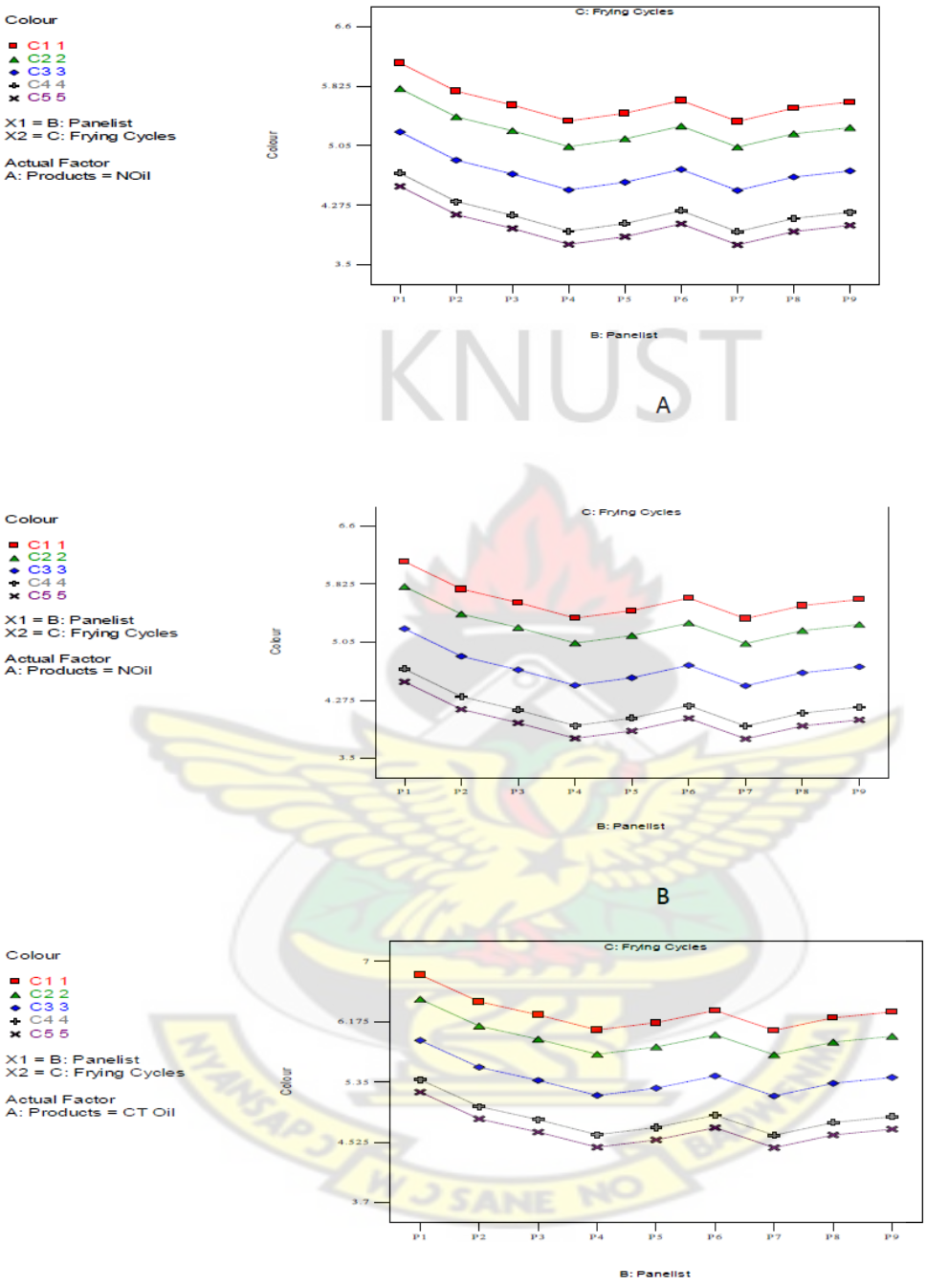

Figure 12: *Variations in colour with respect to panelists and frying cycles for native oil (A); Renealmia battenbergiana (B) and citrate treated oil (C).*

4.4.3 Crispiness

The change in crispiness score with respect to panelists and frying cycles for native, *R. battenbergiana* oil and citrate oil are shown in figure 13. The crispiness scores for the frying oil types differed statistically ($p<0.05$) between the type of frying oil and the frying cycles.

From the figure 13, the crispiness decreased as the frying cycle also increased from one to five for all the frying oil types. This occurs due to increased polymerization of the oil leading to increased viscosity and soaking of the fried product in the viscous oil (Cheman and Jaswir, 2000).

However, the decrease in crispiness was slowest in citrate oil fried yam product followed by *R. battenbergiana* and native oil fried yam product accordingly. This could be attributed to slower rate of oxidation of citrate and *R. battenbergiana* oils due to their antioxidative potentials.

A score of more than 4.5 was considered acceptable on a 9-point hedonic scale test. Therefore, no frying cycle was found to be unacceptable for the native, *R. battenbergiana* and citrate oils even after the 5^{th} frying cycle.

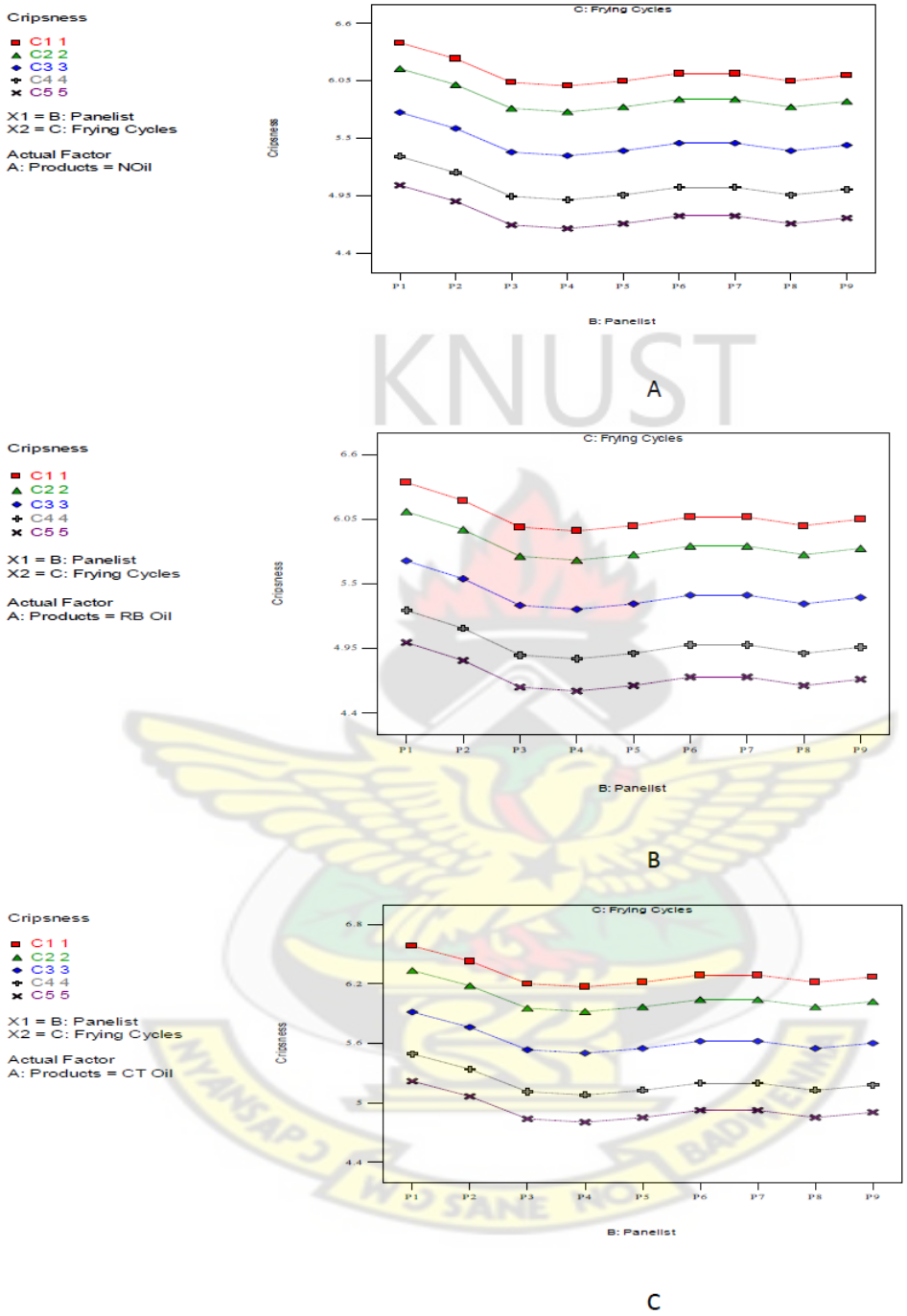

Figure 13: *Variations in the crispiness score with respect to panelists and frying cycles for native oil (A); Renealmia battenbergiana (B) and citrate treated oil (C).*

4.4.4 Taste

The change in taste scores with respect to the panelists and frying cycles for native oil, *R. battenbergiana* oil and citrate oil are shown in figure 14. The taste scores for the frying oil types differed statistically ($p<0.05$) between the frying cycles and the type of frying oil.

From figure 14, as the frying cycle increases from 1 to 5, the taste acceptability also decreases. This is because of the deterioration of the oil at repeated frying temperatures. The native oil was found to be least acceptable for the taste throughout the five frying cycles whiles the citrate treated oil was most acceptable throughout the five frying cycles.

Similar decrease in taste acceptability during deep-fat frying has been reported by Nor *et al.* (2008) and Cheman and Jaswir (2000). They also attributed this observation to the deterioration of the oil during frying.

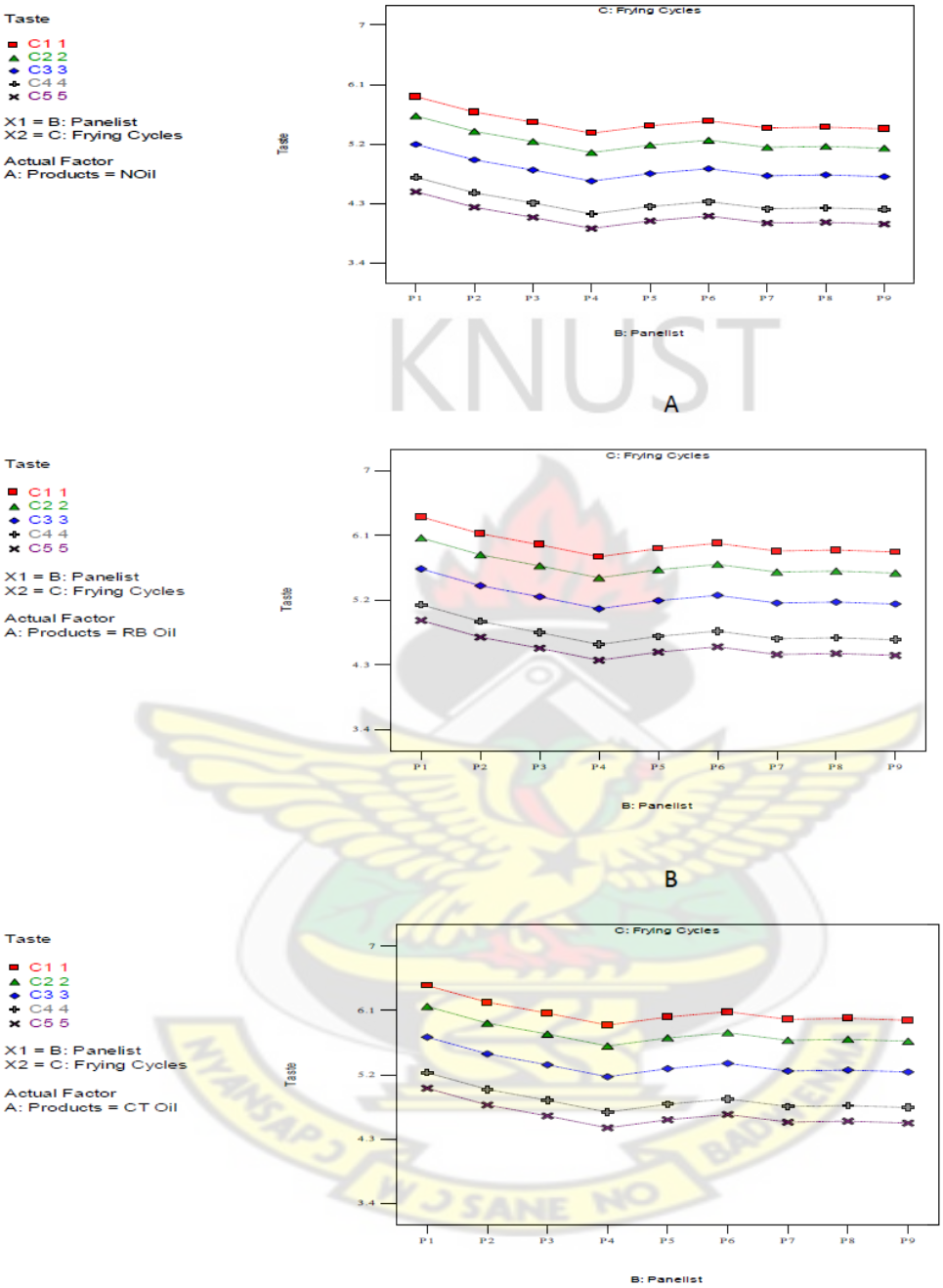

Figure 14: *Variations in taste scores with respect to panelists and frying cycles for native oil (A), Renealmia battenbergiana (B) and citrate treated oil (C).*

4.4.5 Overall Sensory Acceptability

Design Expert (2007) was used to statistically analyze the sensory data for the overall sensory rating of the frying oil types throughout the five cycles of frying. The selection criteria for the overall sensory acceptability were a goal of maximized odor, colour, crispiness and taste at each frying cycle.

The overall sensory analysis proved that for all cycles, citrate treated oil gave the most desirable fried products followed by *R. battenbergiana* oil and native oil. The overall desirability as per the selection criteria statistically was 0.95, 0.90 and 0.80 for citrate treated oil, *R. battenbergiana* oil and native oil respectively. Desirability of the native (N) oil, *R. battenbergiana* (RB) oil and citrate (CT) oil fried yam products however decreased from cycle 1 to cycle 5 of frying.

The results of the sensory evaluation showed that the treated oils i.e. citrate and *R. battenbergiana* treated oils could improve upon the sensory characteristics of fried products in terms of colour, taste, odour and crispiness compared to native oil when used repeatedly.

In the present work, *R. battenbergiana* oil was able to significantly ($p<0.05$) improve the quality of the fried yam slices whiles effectively achieving a reduction of acrylamide. Hitherto, many studies (Jung *et al*., 2003; Cook and Taylor, 2005) found effective methods to reduce acrylamide during heat processing, but their reported sensory evaluation was not acceptable.

For instance, the largest decrease of acrylamide content (90 %) in crisps was obtained when potato slices were soaked in acetic acid solution for 60 min at 20 ^{o}C, and a large decrease of acrylamide content (74 %) was also observed after soaking of potato slices in a 1 % NaOH

solution. However, a sour and acerbic taste from both treatments greatly influenced the appearance as well as the taste and flavour of crisps, which were not sensory acceptable (Kita *et al*., 2004).

CHAPTER 5

5.0 CONCLUSION AND RECOMMENDATION

The optimized treatment condition for the incorporation of *R. battenbergiana* extract into refined bleached deodorized (RBD) palm olein was consequently found to be 1 % extract-oil concentration at 25 °C under agitation contact time of 0.5 h. Under this conditions, the *R. battenbergiana* oil yielded acceptable colour and acceptable antioxidant properties.

The physicochemical parameters studied in this work showed gradual increase for all frying oil types from day 0 to day 5 of frying. Free fatty acid increased from 0.11 % to 0.33 % by the end of the fifth frying cycle representing approximately 22 % increase for native oil. For *R. battenbergiana* oils, the free fatty acid increased from 0.11 % to 0.22 % after frying cycle five (5) representing 11 % increase. Lastly, the citrate oil during frying increased from 0.1 % to 0.17 % representing 7 % increase in free fatty acid. After day 1, peroxide values for *R. battenbergiana* and citrate treated oils were 1.12 and 1.0 meq/kg, respectively, while that of native oil, was 1.62 meq/kg. After day 5, the values were 3.89, 2.51 and 5.63 for *R. battenbergiana*, citrate treated and native oil respectively.

For native oil, the iodine value decreased from 52 g I_2/100 g at day 1 to 40 g I_2/100 g at day 5, whiles for samples with treatments for *R. battenbergiana* and citrate, they decreased from 54.7 to 43.5 and 55.6 to 44 g I_2/100 g respectively. The percentage loss of unsaturation during frying was 23.07 %, 20.47 % and 20.86 % for native, *R. battenbergiana* oil and citrate treated oil respectively. For control, the acrylamide content increased from 10.23 at day 1 to 28.89 µg/ml at day 5, whiles for samples with treatments for *R. battenbergiana* and citrate, acrylamide increased

from 5.64 to 24.56 µg/ml and 4.16 to 22.84 µg/ml respectively. *R. battenbergiana* oil therefore showed improvement in the frying qualities. Furthermore, the results showed that *R. battenbergiana* oil (RB oil) and citrate treated oil (CT oil) were able to retard acrylamide formation by 14.99 and 20.94 % respectively compared to the native oil (N oil).

Native oil was therefore unfit for frying use after cycle 2 whereas *R. battenbergiana* oil and citrate oil were both fit for frying use after cycle 3. This implied that native oil can be used for repeated frying only two times after which it must be discarded. However, *R. battenbergiana* oil can be used for repeated frying three times after which it must also be discarded. In effect *R. battenbergiana oil* can extend the frying life of native frying oils by one frying cycle. The optimized *R. battenbergiana* oil retained reasonable sensory attributes. The overall sensory analysis proved that for all cycles, citrate treated oil gave the most desirable fried products followed by *R. battenbergiana* oil and native oil. The desirability as per selection criteria statistically was 0.95, 0.90 and 0.80 for citrate treated oil, *R. battenbergiana* oil and native oil respectively.

It is recommended that this pilot scale frying experiment using optimized *R. battenbergiana* oil would be scaled up with the use of commercial and industrial scale fryers.

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APPENDIX A

3.3.3 Preparation of Citrate incorporated refined palm olein (*CTO*)

Pure citric acid was used to prepare a 10% w/v solution stock. This solution stock was used to treat 1L native refined palm olein at the rate of 3kg per ton of processed RBD oil as practice in industrial vegetable oil refineries.

3.3.4 Solutions preparation for Acrylamide determination in frying oil samples

3.3.4.1 Stock solution of acrylamide in methanol (500 mg/ml)

About 0.05 g of acrylamide was weighed into a 100 ml volumetric flask and dissolved in methanol. It was filled up to the mark with methanol, closed and mixed thoroughly.

3.3.4.2. Diluted stock solution (10 mg/ml)

Using a graduated pipette, 1.0 ml of the acrylamide stock solution was transferred into a 50 ml volumetric flask and filled to the mark with methanol. This solution contains 10 mg/ml acrylamide.

3.3.4.3 Intermediate standards

Using graduated pipettes 0, 0.5, 1.0, 2.0, 3.0 and 4.0 ml of the 10 mg/ml diluted stock solution was transferred into a series of 10 ml volumetric flasks and diluted to the mark with methanol and mixed. These standards corresponded nominally to 0, 0.5, 1.0, 2.0, 3.0 and 4.0 mg/ml acrylamide.

3.3.4.4 HPLC mobile phase

Using a measuring cylinder, 70 ml of 0.05 mol/l sulphuric acid was transferred into a 1 litre volumetric flask and diluted to about 500 ml with water, followed by the addition of 70 ml of acetonitrile and diluted to the mark with water.

3.3.4.5 Calibration Sample Preparation

About 50 ± 0.5g of acrylamide-free fat simulant was weighed into a series of 120 ml vials followed by the addition of 1.0 ml of each intermediate standard using a graduated pipette and mixed well. Approximately 25 ± 0.5 ml of water was added and shaken vigorously for 1 minute. The vials were turned upside down to allow the phases to separate for about 20 minutes. About 4 ml of the lower aqueous phase was withdrawn using a syringe and filtered using an HPLC membrane filter. The standards corresponded nominally to approximately 10, 25, 30, 35, and 40 µg/ml of fat simulant.

APPENDIX B

4.0 **Frying Experiment Response**

Response 1	FFA					
ANOVA for selected factorial model						
Analysis of variance table [Classical sum of squares - Type II]						
	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	
Model	0.053867	6	0.00897778	9.335644	0.0030	significant
A-Frying cycles	0.034027	4	0.00850667	8.845754	0.0049	
B-Oil	0.01984	2	0.00992	10.31542	0.0061	
Residual	0.007693	8	0.00096167			
Cor Total	0.06156	14				

Response 2	PV					
ANOVA for selected factorial model						
Analysis of variance table [Classical sum of squares - Type II]						
	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	
Model	21.43939	6	3.573231	9.147316	0.0032	significant
A-Frying cycles	14.57331	4	3.643327	9.326757	0.0042	
B-Oil	6.86608	2	3.43304	8.788432	0.0096	
Residual	3.125053	8	0.390632			
Cor Total	24.56444	14				

Response 3	IV					
ANOVA for selected factorial model						
Analysis of variance table [Classical sum of squares - Type II]						
	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	
Model	327.088	6	54.51467	76.88952	< 0.0001	significant
A-Frying	253.68	4	63.42	89.44993	<	

cycles					0.0001	
B-Oil	73.408	2	36.704	51.76869	< 0.0001	
Residual	5.672	8	0.709			
Cor Total	332.76	14				

4.1. **Sensory Evaluation Response**

Response 1	Odour					
ANOVA for selected factorial model						
Analysis of variance table [Classical sum of squares - Type II]						
	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	
Model	42.44193	14	3.031566	25.696077	< 0.0001	significant
A-Product	5.787704	2	2.893852	24.528787	< 0.0001	
B-Panelist	2.817926	8	0.352241	2.985653	0.0044	
C- Frying Cycles	33.8363	4	8.459074	71.700571	< 0.0001	
Residual	14.15733	120	0.117978			
Cor Total	56.59926	134				

Response 2	Colour					
ANOVA for selected factorial model						
Analysis of variance table [Classical sum of squares - Type II]						
	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	
Model	68.49852	14	4.89275	35.95228	< 0.0001	significant
A-Product	10.74978	2	5.37488	39.49506	< 0.0001	
B-Panelist	6.452	8	0.8065	5.92621	< 0.0001	
C- Frying Cycles	51.29674	4	12.82419	94.23303	< 0.0001	
Residual	16.33081	120	0.13609			

Cor Total	84.82933	134				

Response 3	Cripsness					
ANOVA for selected factorial model						
Analysis of variance table [Classical sum of squares - Type II]						
	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	
Model	37.88696	14	2.70621	26.93409	< 0.0001	significant
A-Product	1.18533	2	0.59266	5.89862	0.0036	
B-Panelist	2.07466	8	0.25933	2.58106	0.0123	
C- Frying Cycles	34.62696	4	8.65674	86.15789	< 0.0001	
Residual	12.05704	120	0.10047			
Cor Total	49.944	134				

Response 4	Taste					
ANOVA for selected factorial model						
Analysis of variance table [Classical sum of squares - Type II]						
	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	
Model	50.08281	14	3.57734	25.77566	< 0.0001	significant
A-Product	7.19511	2	3.59755	25.92129	< 0.0001	
B-Panelist	3.39333	8	0.42416	3.05622	0.0036	
C- Frying Cycles	39.49437	4	9.87359	71.14172	< 0.0001	
Residual	16.65452	120	0.13878			
Cor Total	66.73733	134				