PHYSICO-CHEMICAL CHARACTERISTICS AND ANTIMICROBIAL EFFECTIVENESS OF A FOOD GRADE DETERGENT DEVELOPED FROM LOCAL RAW MATERIALS

BY

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DECLARATION

This is to certify that this thesis is the result of research undertaken by Ivy Osei- Ampong towards the award of an MPhil. Food Science degree in the Department of Nutrition and Food Science, University of Ghana, and that this work either in whole or in parts has not been presented for another degree elsewhere.

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ABSTRACT

Cleaning is central to any food process operation and it entails the removal of soils from processing equipment, food contact surfaces and production floor. It is done with the aid of a cleaning agent and key among several are the soaps and detergents. Most detergents currently used in the industry are petroleum based, which are not easily biodegradable. Moreover they are imported, and add costs to the operations of industry. The use of locally available material that are biodegradable to produce effective cleaning agents will offer more environmentally friendly (or "green") alternatives. Ash derived alkalis from agricultural wastes, contain high levels of potash which are, inexpensive alternatives to imported potassium and sodium hydroxide, which form part of the key ingredients of detergents.

The main objectives of this study were to develop and characterize a food grade detergent from locally available materials and examine its antimicrobial efficacy against common food borne pathogens. Dried cocoa pods, obtained from the Cocoa Research Institute (Tafo in the Eastern Region), were processed into potash. Two edible oils: palm kernel and coconut oil were mixed in various proportions (0, 30, 50, 70, and 100%). A total of five soap samples were replicated three (3) times, using a combination of both the cold and hot processes of saponification. A control food grade detergent (petrochemical based) was obtained from Cleaning Solutions Ltd –Tema, near Accra, Ghana. Differences between treatments were assessed using ANOVA procedures.

The apparent viscosity of the coconut based liquid soaps were light (K=5.869 Pa.s). Addition of palm kernel oil thickened the apparent viscosity of the soaps obtained. Soaps obtained from using only palm kernel oil showed a high apparent viscosity, K of 48.057 Pa.s The flow behavior of liquid soap made using coconut oil changed from non-Newtonian, shear thinning flow and

approached Newtonian, shear independent flow as the composition of palm kernel oil increased in the oil blends. The soaps made using higher proportions of coconut oil showed better foaming capacity. As the proportion of palm kernel oil increased the maximum foam capacity decreased. Increasing viscosity of the detergent decreased the foam capacity. Soaps made with high proportions of coconut oil (100-70%) exhibited characteristics of a good food grade detergent in forming good foam volumes with low foam stability. Soap made using only coconut oil showed the lowest pH (9.3) and the minimum total free alkaline (TFA). With all the different detergents formed from the various oil mixtures, the total free alkaline (TFA) levels were well below the maximum acceptable limits of 0.6% for TFA as stipulated by the Ghana Standard for alkaline soaps. Excess free alkali in the detergent will be corrosive on metallic food contact surfaces. Soap formulated using 100% coconut oil showed a much greater inhibition against *bacillus species* as compared to its inhibition against *Salmonella* and *Proteus spp*. The antimicrobial activity of the soaps depended on the oil type used in their formulation. The detergents with 100% coconut oil and 100% palm kernel oil soaps were relatively effective against the gram positive bacteria. Except for the detergent mixture with equal amount of both oils, all the other detergents with the oil mixtures also showed a relatively higher inhibition against the gram positive bacteria tested. There is a great potential in using local agricultural wastes and vegetable oils to develop food grade detergents with effective antimicrobial activity.

DEDICATION

This work is dedicated to my lovely husband Jones Appiah,to my wonderful parents Mr. and Mrs. Osei- Ampong, to my children Caleb and Estherbel, my siblings Derrick, Emma, Steve, Esther and Benjamin and friends Afia, Akua and Abere.

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1.0 INTRODUCTION

Cleaning is an integral part of any food process operation and it entails the removal of soils from processing equipment, food contact surfaces, production floor and the surrounding environment. If standard cleaning protocols are followed and the appropriate detergents are used for the right food soils or contaminants on food contact surfaces, contamination on these surfaces could be reduced to levels that the food would be virtually harmless to the consumer. "If food soils are not properly removed, bacteria may survive the sanitation phase because the microorganisms may not only be shielded by the food" (Augustin *et al*., 2004), but will serve either as culture for growth and multiplication or anchors for microfilm development under non ideal conditions for growth. Consequently, the US Food Code (2009) and NSF International (ANSI/NSF 3, 2009) require the complete removal of food soil from food contact surfaces after the completion of cleaning protocols. There are two common cleaning methods in industry; manual and clean-in-place (CIP). Manual cleaning involves selecting the right cleaners and applying mechanical cleaning methods to remove the soil from the surfaces. The CIP system is a method of cleaning pipes and equipments that use recirculation of cleaning and sanitizing solutions. This method of cleaning is used for equipment that cannot be easily broken down so is designed to be adequately cleaned in place. Failure of CIP can be costly, and potentially catastrophic to public health with the survival of unwanted pathogenic micro-organisms in plant or product (Augustin *et al*., 2004).

In Ghana, a survey on food hygiene awareness and practices by food handlers revealed that 89% of food service centers do not observe standard protocols during cleaning, hence the use of an appropriate detergent for a particular food soil would definitely not be practiced by such food centers (Ababio and Adi, 2012). An affordable detergent that would be readily available locally, and exhibit microbial inhibition to some extent would therefore be necessary to ensure reduction of microbial load on work surfaces especially for small scale food industries to harmless levels.

Cleaning is done with a cleaning agent that removes food, soil, or other substances, and that is compatible with the food contact surface because not all cleaning agents can be used on food-contact surfaces. Among several cleaning agents, key among them are the soaps and detergents. They are primarily surfactants, which lower the surface tension of water, essentially making it 'wetter' so that it is less likely to stick to itself and more likely to interact with oil and grease and loosen them. Detergents have several properties that aid in cleaning. Some reduce the hardness of the water; others tie up metal ions in the water, increase wetting ability, or emulsify fats. Most detergents used in the food industry are petroleum based, and are not easily biodegradable and will eventually be detrimental to the environment (Gebelein, 1996). Moreover they are imported, and can be very expensive to the operations of the local industry. The uses of locally available material that are biodegradable to produce effective cleaning agents will not only save cost but will also offer a more environmentally friendly alternative.

The manufacturing of a food grade detergent from ingredients that would not leave harmful residues on food contact surfaces is crucial to the safety of every food industry .According to Ababio and Adi, in 2012, it is observed that the handling of detergents used in the cleaning of most small scale food industries in Ghana require extra safety measures to be undertaken to ensure that detergent residues left on food contact surfaces do not become hazardous to final consumer. The crude non- edible part of some of the oils extracted in Ghana could be channeled into the formulation of detergent, hence providing employment and avoiding waste. It has been observed over the years that most food industries, especially those operating on small scale use ordinary household detergent that contains ‘SLES’ (Sodium laureth ethanolamide sulphate an imported petrochemical agent that is less non- biodegradable)

(Gebelein, 1996) in carrying out the cleaning protocols and most of these cleaning protocols observed by most of these local industries do not fit into the standard cleaning procedures specified by Ghana Standards Authority (Ababio and Adi, 2012). There is therefore the need to provide the local food industry with a detergent in which the raw materials could be obtained locally hence a readily available detergent, that can clean as well as inhibit microbial growth to some extent and also provide a biodegradable product (environmentally friendly).

1.1 Main objective

The main objective of this study was to develop and characterize a food grade detergent from locally available materials and examine its antimicrobial efficacy against common food borne pathogens.

1.2 Specific objectives

a) Develop a food grade detergent from local raw materials
b) Characterize the physical and chemical properties of the detergent
c) Test the efficacy of the detergent against some food borne pathogens

2.0 LITERATURE REVIEW

2.1 Background to the study

According to a 2004 report published by the U.S. Food and Drug Administration (FDA), "Out of Compliance" percentages for contaminated equipment were high for food facilities observed as part of a study to determine foodborne illness risk factors (Fraser and Pascall, 2010). Specifically, improper cleaning and sanitization of food-contact surfaces before use were commonly observed, with Out of Compliance values ranging from 25% in school kitchens to 58% in supermarket deli departments. The authors contend that the 2004 data are consistent with previous findings, indicating that proper implementation of cleaning and sanitization methods needs to be better emphasized in food service departments and processing facilities to protect the public (Barker et al., 2004). Even in the home a major concern is the transmission of foodborne pathogens by cross-contamination of foods via food contact surfaces, particularly chopping boards, which is found to be one of the top five sites most contaminated with heterotrophic bacteria in the kitchen (Josephson and Rubino, 1997). The highest occurring cause for foodborne outbreaks in Taiwan was reported to be cross-contamination, which mostly occurred on the surfaces of kitchen utensils (Lin *et al*., 2010). The survival of these microorganisms during washing up of kitchen surfaces was reported in 2003 by the International Journal of Food Microbiology. According to the report, there is the potential for the survival and cross contamination of food borne pathogens in the kitchen environment after a typical washing up of kitchen surfaces with water detergent and sponge (Mattick *et al*., 2003).The purpose of cleaning food contact surfaces is to remove soil and debris, and this involves washing with an appropriate detergent, rinsing with potable water and then sanitizing with an appropriate sanitizer. A detergent that also provides sanitizing capability will be very useful in the food industry.

2.2 Microbial contamination of food through food contact surfaces

Food contact surfaces are those surfaces with which food normally comes into contact with (Kusumanigrum *et al*., 2003). They include work benches, cutting boards, utensils, tables, etc. and may also include surfaces onto which food may splash and drip (such as microwaves and fridges), flow or pass through before it is finally packaged. The cutting board poses constant risk of infection in the domestic environment (Rusin *et al*., 1998). In the home, transmission of foodborne pathogens by cross-contamination of foods via food contact surfaces, particularly chopping boards was found to be one of the top five sites most contaminated with heterotrophic bacteria in the kitchen (Josephson and Rubino, 1997).

Several studies have reported cross-contamination via domestic hand and food contact surfaces to be a significant contribution to cross-infection and there is a constant risk of microbial transfer from these surfaces (Bloomfield and Scott, 1997).

Effective cleaning and sanitization of food contact surfaces of equipment and utensils serve two primary purposes: (a) It reduces chances for contaminating safe food during processing, preparation, storage and service by physically removing soil, bacteria and other microorganisms; and (b) it minimizes the chances of transmitting pathogens to the consumer by assuring bacteriologically safe eating utensils. For the most part, chemistry plays a very important part in the cleaning and sanitization process. A good understanding of the type of soil, as well as the food contact surface is required for determining the cleaning and sanitization procedures and agents. Kusumanigrum *et al*., in 2003 tested the influence of surface finish on the ease of cleaning of stainless steel soiled with either cultured milk inoculated with spores of *Bacillus stearothermophilus* or by growth of *Pseudomonas sp*. of biofilms. The research conclusions indicated a higher significance of surface defects/roughness on the ease of surface cleaning rather than the surface finishing type.

2.2.1 Biofilms

It is a natural tendency of microorganisms to attach to wet surfaces, to multiply and to embed themselves in a slimy matrix composed of extracellular polymeric substances (EPS) that they produce, forming a biofilm (Simoes *et al*., 2010). Bacteria in a biofilm are not effectively removed with common soap and water cleaning procedures. The use of enzyme-based detergents as bio-cleaners, also known as ‘‘green chemicals’’, sometimes serve as a viable option to overcome the biofilm problem in the food industry. However, due to the EPS heterogeneity, a mixture of enzymes is usually required for sufficient biofilm degradation. Augustin *et al.,* (2004) demonstrated the potential application of enzymatic cleaning products against biofilms formed by microorganisms commonly found in dairy products (*Lactobacillus bulgaricus, Lactobacillus lactis, and Streptococcus thermophilus*). The main strategy to prevent biofilm formation is to clean and disinfect regularly before bacteria attach firmly to surfaces (Midelet & Carpentier, 2004; Simoes *et al*., 2006). A systematic cleaning routine should be followed to remove food soils.

2.3 Food Soils

Food "soil" may be regarded as any food material that is in an undesired place. It is generally defined as unwanted matter on food-contact surfaces, and it may be visible or invisible, the latter being primarily microorganisms, such as bacteria, yeasts, and molds (Kaoru, 1998). The primary source of food soil is the food product being handled. Food soils may be classified as soluble in water (sugars, some starches, most salts), soluble in acid (limestone and most mineral deposits), soluble in alkali (protein, fat emulsions), soluble in water, alkali, or acid (Kaoru, 1998). This classification method is critical in the determination of the type of detergent, the cleaning procedure and the systems and equipment that need to be employed in the cleaning process (Parker, 2007). Films on food contact surfaces may also vary in their solubility properties depending upon such factors as heat effect, age, dryness, time, etc. Since soils and films vary widely in composition, no one detergent is capable of removing all types. For example, biofilms are problematic in particular food industry sectors such as brewing, dairy processing, fresh produce, poultry processing and red meat processing (Chen *et al.*, 2007; Frank *et al.*, 2003; Jessen & Lammert, 2003).It is therefore important to have a good understanding of the nature of the soil to be removed before selecting a detergent or cleaning regime (Parker, 2007).

2.4 Cleaning and sanitization in the food industry

The surfaces of the equipment used in the manufacture of foods inevitably become soiled and require cleaning. Cleaning is the removal of organic matter, using appropriate detergent chemicals under recommended conditions. It is accomplished using a cleaning agent that removes food, soil, rust stains, minerals, or other deposits. Furthermore, clean, sanitary contact surfaces are fundamental to the control of pathogenic micro-organisms (Parker, 2007). Cleaning is so central to food plant operations that every effective sanitation standard operations procedures outline the basic cleaning and sanitizing schedule. The right cleaning agent must be selected because not all can be used on food contact surfaces (Miller and Baker, 1940). Moreover, water and detergent alone may not be enough to kill all the microorganisms present during cleaning and rinsing, so the use of a disinfectant is also necessary (Zelma.,1941). It is important that cleaning is properly done before sanitization. Organic matter from food residues such as oils, grease and protein not only harbors bacteria but can actually prevent sanitizers from coming into physical contact with the surface to be sanitized. In addition, the presence of organic matter can inactivate or reduce the effectiveness of some types of sanitizers, making sanitization ineffective (Moyo and Baudi, 2001). The schedule typically involves several steps, critical among which are detergent application, rinsing and sanitizing. Cleaning is performed at regular and frequent intervals so that a consistently good quality of product is maintained.

How the cleaning is done depends principally on:

- the nature of the soil or contamination to be removed;
- the type of surface to be cleaned;
- the materials used for cleaning;
- the degree of water hardness; and
- the standard of cleanliness required.

The basic steps in cleaning can be summarized as:

- the removal of gross soil or dirt;
- the removal of any residual soil with detergent; and rinsing to remove detergent and soil.

2.4.1. Detergents and surfactants used in cleaning

Water, the liquid commonly used for cleaning, has a property known as surface tension. This tension causes water to bead up on surfaces (e.g. glass), which slows wetting of the surface and inhibits the cleaning process. To facilitate cleaning, the surface tension must be reduced so water can spread and wet surfaces. Chemicals that are able to reduce the surface tension effectively are called surface active agents, (or surfactants) and they act to make water "wetter" (Rogers, 1920). Surfactants perform other important functions in cleaning, such as loosening, emulsifying (dispersing in water) and holding soil in suspension until it can be rinsed away. They have a molecular structure comprising a hydrophilic and a hydrophobic portion. One end of the molecule is thus attracted by water and the other end is repelled but is attracted by fat and oil and this is the basis of the cleaning action of surfactants (Ross and Miles, 1941). Surfactants are classified by their ionic (electrical charge) properties in water: anionic (negative charge), non-ionic (no charge), cationic (positive charge) and amphoteric (either positive or negative charge). There are many hundreds of surface active agents now available which are incorporated into detergent formulations. Surfactants are usually excellent emulsifying agents, they have good wetting and penetrating powers, they are non-corrosive, non-irritating and readily rinsable. In addition, they are highly soluble in cold water, are largely unaffected by hard water and many are stable in both acid and alkaline conditions. Whilst the bactericidal activity of both the anionic and non-ionic detergents is poor, that of some cationics is excellent although the latter are not as good as detergents. For this reason the cationic surfactants are used as disinfectants and sterilizers (Fujimoto, 1985). The principal anionic surfactants used today are alkyl sulphates or alkylbenzene sulphonates. The hydrophobic portions of the molecules are represented by the alkyl (i.e. lauryl) and benzene groups whilst the hydrophilic portions are represented by sulphate and sulphonate; the cations are commonly sodium or potassium. All anionic surfactants are high foam producers and none can be combined with a cationic detergent (Eshrat, 2011).

2.4.2 Types of surfactants

Linear alkyl benzene sulfonate, alcohol ethoxysulfates, alkyl sulfates and soap are the most common anionic surfactants ((Ross and Miles, 1941). The non-ionic surfactants are not dissociated in solution and can be used in conjunction with either anionic or cationic agents. They are powerful emulsifying agents, are unaffected by hard water and vary considerably in their foaming characteristics. As with the anionic agents, the list of non-ionic surfactants is an extensive one. Two of the major categories are based on products formed by condensation reactions between ethylene oxide and synthetic long chain alcohols (e.g. lauryl alcohol ethoxylate) or between ethylene oxide and alkyl phenols (e.g. nonyl phenol ethoxylate). The former represent the major group of low foaming surfactants in many countries.

Amphoteric surfactants can exist in solution in either the cationic or anionic form depending on the pH ((Ross and Miles, 1941). They are based on amino acids and have the general formula R-NH-CH_2-

COOH where R is usually an alkyl radical. An example is dodecyl diaminoethyl glycine. The amphoteric agents are relatively good emulsifiers, are stable in both acids and alkalis and show a good tolerance to hard water (Thomssen, 1922).

Sequestering agents are added to detergents to prevent calcium and magnesium salts by binding to their ion and chelating them out of solution. The principal organic sequestering agents, also called chelating agents, are ethylene diamine tetra acetic acid (EDTA) and its sodium and potassium salts, and the sodium salts of gluconic and heptonic acid. In spite of their relative expense they are fairly widely used, because of their high solubility in liquid detergent formulations. Among the inorganic sequestering agents, sodium polyphosphates are widely used (Thomssen, 1922). They are also good emulsifiers, dissolving and dispersing agents and also generally enhance rinsability. Modern detergent formulations are carefully blended mixtures of different chemicals each contributing to the desired properties of the detergent. A general-purpose cleaner may contain alkali builders to break up grease, surfactants to improve wetting, dispersion and rinsability, and sequestrants to stabilize magnesium and calcium; the level of the sequestrants should be carefully adjusted depending on the degree of water hardness and in-use concentration (Thomssen, 1922).

2.5 The theory of traditional soap making

The making of soaps from ash-derived alkalis has been an age-old craft in Ghana, Nigeria and many West African countries (Nwoko, 1982). Ash derived alkalis offer inexpensive alternatives to imported ones such as potassium and sodium hydroxide, etc. Agricultural wastes such as plantain peels, cocoa pods, .maize cobs, cassava peels and numerous others contain high levels of potash. According to Onyegbado *et al.* (2002), when they are burnt in air, the resulting ashes contain oxides of potassium and sodium which when dissolved in water yields the corresponding hydroxides according to the following equations:

$$Na_2O + H_2O \longrightarrow 2NaOH \qquad 1$$

$$K_2O + H_2O \longrightarrow 2KOH \qquad 2$$

Locally manufactured soaps are made from lye obtained from ash of burnt agricultural wastes and other plant debris and are known to have some antimicrobial properties processes produces soap (equation 3) which can lather and exhibit detergency action (Beetseh and Anza, 2013).

$$\text{OIL + LYE} \longrightarrow \text{SOAP + GLYCEROL} \qquad 3$$

Generally, the fat is sourced from tallow, lard, palm oil, palm kernel oil, coconut oil, marine oil, etc. Some of the oils employed in soap making are also known to exhibit antimicrobial properties and in combination with lye are expected to produce a detergent that can stop or minimize the growth of food borne pathogens. Antimicrobial studies of traditionally extracted palm kernel oil (*Adwe ngo*) and two other oils; palm oil and coconut oil, on some microorganisms revealed that "*Adwe ngo*" was active against *Escherichia coli*, alpha and beta hemolytic streptococci, *Aspergillus fumigatus* and *Staphylococcus aureus (*Ekpa and Ebana, 1996).

It has also been established (*Krueger et al*., 1973) that unsaturated fatty acid soaps are more effective against gram-positive bacteria while soaps of saturated fatty acids are more active against gram-negative bacteria. The type of oil or fat that is used has a significant and direct influence on the final characteristics and qualities of the detergent produced. According to Duke and Wain (1981), palm kernel oil contains about 50% saturated fatty acids. Similar to coconut oil with high content of saturated fatty acids, mainly lauric, it is solid at room temperatures in temperate areas, and is nearly colorless varying from white to slightly yellow. Palm oil, palm kernel oil and coconut oil are among the most commonly extracted edible oils in Ghana. Their characteristics are such that they could be employed in the manufacture of detergents and soaps that could provide both cleaning and sanitizing actions.

2.5.1. Comparison of soaps to detergent properties

The classical detergent or surface active agent is soap which is usually composed of sodium or potassium salts of fatty acids such as stearic, palmitic and oleic acids. Soaps are reasonably effective in soft water but their reduced solubility in cold water constituents a disadvantage; in addition, soaps form precipitates with calcium in hard water to give insoluble deposits (Kaoru, 1998; Fujimoto, 1985). For these reasons they have been largely replaced by synthetic detergents which are anionic, cationic, non-ionic or amphoteric depending on their active electrical charge when in solution. When negative charges predominate the surfactant is classed as anionic, when positive cationic, whilst surfactants that do not dissociate in solution are termed non-ionic. Where the predominant charge varies according to pH the surfactant is then termed amphoteric.

Non soap detergents are similar to soap, but they are less likely to form films (soap scum) and are not as affected by the presence of minerals in water (hard water). Detergent surfactants were developed in response to a shortage of animal and vegetable fats and oils during World War I and World War II. In addition, a substance that was resistant to hard water was needed to make cleaning more effective (Augustin *et al*., 2004). At that time, petroleum was found to be a plentiful source for the manufacture of these surfactants. Currently, detergent surfactants are made from a variety of petrochemicals. A detergent is an effective cleaning product because it contains one or more surfactants. Because of their chemical makeup, the surfactants used in detergents can be engineered to perform well under a variety of conditions. Such surfactants are less sensitive than soap to the hardness minerals in water and most will not form a film. General detergents are composed of four main parts: surface active agents or surfactants, chelating agents, builders, and solvent (Piyali, 1999). Detergents must be capable of removing many different types of soil under a variety of conditions. According to Parker (2007), a good detergent must have the following characteristics: rapid and complete solubility in water, good swelling of soil, good wetting capability of surfaces, good dispersion and suspension properties, good rinsing properties, non-corrosive to surfaces, non-toxic and cost effective. They should, ideally, also be:

- odourless;
- biodegradable;
- economical in use;

In general, detergents are not expected to possess bactericidal properties although in practice some of them do. Some bacteriostatic organic acids such as gluconic, hydroxyacetic, citric and tartaric acid have been incorporated in detergent formulations. Such acid detergents usually incorporate corrosion inhibitors and wetting agents and as such can be employed in the removal of inorganic deposits. However, detergents do physically remove a large number of bacteria during cleaning and this makes subsequent disinfection that much easier. Foam is an important aspect of detergent products and surfactants are mainly responsible for its generation. Two important properties are the speed of foam generation and foam stability. The factors that affect these properties are soil in the wash load and the concentration of hardness ions (Piyali *et al.*, 1999)

2.6 Raw Materials for soap formulation

2.6.1 Alkalis

Many detergents incorporate an alkali as one of the principal ingredients and these detergents are generally termed as “soaps”. Sodium hydroxide (caustic soda) is the strongest of the alkalis and inexpensive. It has excellent dissolving properties, is a very strong saponifier and has added advantage of being strongly bactericidal. It is, however, highly corrosive to metals especially aluminium and extreme care must be taken when handling as it can cause severe burns to the skin (Oghome *et al.*, 2012). As with all the alkali detergents sodium hydroxide precipitates insoluble calcium and magnesium salts from hard water so that sequestering agents need to be incorporated with alkali cleaners in any detergent formulation. Sodium metasilicate, although a strong alkali, is non-caustic and therefore much less corrosive than sodium hydroxide. It suppresses the corrosive activity of sodium hydroxide and the two are often combined in detergents for this reason. Sodium carbonate (soda ash) is a relatively weak detergent, is somewhat corrosive and precipitates calcium and magnesium salts from hard water. However, it is inexpensive and has a good buffering capacity and is frequently included in detergents. Trisodium phosphate (TSP) is a good emulsifier and saponifier has strong dispersive properties and has the ability to soften water by precipitating the salts. Although somewhat corrosive, it is often incorporated in detergents (Thomsson, 1922).

Soap formed using soda and potash is soluble in water, unlike those from the other bases and they constitute the soap of commerce. These reagents are always used in sufficient quantity to combine with the whole of the fatty acids contained in an oil or fat, though doubtless, by the use of considerably smaller quantities, under pressure, complete resolution of the fatty matter into fatty acids and glycerol could be accomplished. They are, by far, the most important saponifying agents employed in the soap manufacturing processes over a long period of time (Oghome *et al.*, 2012).

2.6.2 Oils

The type of oil or fat that is used has a significant and direct influence on the finished characteristics and qualities of the detergent product. Some fats and oil are better for bubbles and others are better for cleaners. Examples of fats and oils that are suited for the traditional soap making process are: neem, coconut, tallow, palm oil, palm kernel, ground pea nut, shea butter, and cocoa butter (Ekpa and Ekpe, 1995). The palm kernel and coconut oils are good for soapy bubbles, while the neem oil is good for an antiseptic.

Besides the various physical properties of oils and fats, such as color, specific gravity, melting point, solubility, etc., they may be distinguished chemically by a number of chemical constants. These are the iodine number, the acetyl value, saponification number, Reichert-Meissl number for volatile acids, and Hehner number for insoluble acids. These constants, while they vary somewhat with any particular oil or fat, are more applicable to the edible products and form a criteria where any adulteration of fat or oil is suspected (Thomssen, 1922).The various oils used in detergent and soap making have similarities and differences that make them exhibit unique chemical and physical properties. Due to the triglyceride composition, the oils used in soap making exhibit a steep melting curve and melt below body temperature. Their low degree of unsaturation gives them a high oxidative stability. The characteristics exhibited by oils used in soap making are due to their high content of saturation (Webb, 1926).

The characteristic behavior of any oil is due to its fatty acid content and the percentage ratios of saturation levels to unsaturation levels. From table 1, coconut oil has a higher level of saturation as compared to palm kernel oil which has a higher level of unsaturation. These observations show that the oils are highly related since their fatty acid values are close. The differences however affect their saponification values, their viscosities, their acid values etc.

Table 1:Fatty acid content of various fats used for soap making

	Lauric acid	Myristic acid	Palmitic acid	Stearic acid	Oleic acid
Fats	C_{12} saturated	C_{14}saturated	C_{16}saturated	C_{18}saturated	C_{18} monounsaturated
Tallow	0	4	28	23	35
Coconut oil	48	18	9	3	7
Palm kernel oil	46	16	8	3	12
Laurel oil	54	0	0	0	15

Adapted from Wikipedia, May 2013.

2.6.2.1 Role of fatty acids in soaps

Soap is a function of acids and fatty acids are functions of fats and oil. In the simplest sense, oils that are solid at room temperature are hard whereas those that are liquid at room temperature are soft. The degree of hardness and softness differs according to their sources and other parameters. Oils that are hard contributes to hardness and/or lather in soap. Oils that are soft contribute to conditioning. Oils are made up mainly of a variety of fatty acids. The main conditioning fatty acids are oleic (1 unsaturated bonds), linoleic (2 unsaturated bonds) and linolenic (3 unsaturated bonds). The more unsaturated bonds, the better the conditioning and the more easily it is absorbed by the skin, but the softer the oil is in soap, the more prone to oxidation. Making soap therefore means choosing a combination of oils with different degrees of hard/soft, conditioning and lather, to get the particular product that fits best and provides the best result (Oghome *et al*., 2012). According to Oghome *et al.,* (2012), the chief fatty acids in soap making are lauric acid, myristic acid, palmitic acid, stearic acid and oleic acid. They are obtained from mutton tallow, beef tallow (animal fats), palm oil, and palm kernel oil.

Lauric acid is a saturated fatty acid whose single bond helps in soap hardening. It also has good cleansing agent and supports foaming. Saturated fatty acids themselves are solids at room temperature.

As they increase in size from lauric to stearic, the melting point of the oil increases. Saturated fatty acids in soap have good cleaning properties and support foam. The longer chains also tend to harden soap. Unsaturated fatty acids are liquids. They tend to have good cleaning power, but lather poorly. These fatty acids also tend to make milder soaps (www.soap-making-resource.com/fatty-acids).

The percentage of palmitoleic acid is between 0.00-2.20 percent. This acid is unsaturated. It makes soap to be mild, have good cleaning power but foams poorly (Oghome *et al*., 2012).

2.6.2.2 Coconut oil

Desiccated coconut contains about 69% coconut fats. Approximately 50% of the fatty acids in coconut fat are lauric acid. Some studies have shown some antimicrobial effects of free lauric acid. It is a medium chain fatty acid, which has the added beneficial function of being formed into monolaurin in the human or animal body (Hautfenne, 1982). Monolaurin is the antiviral, antibacterial, and antiprotozoal monoglyceride used by the human or animal to destroy lipid-coated viruses such as HIV, herpes, cytomegalovirus, influenza, various pathogenic bacteria, including *listeria monocytogenes* and *helicobacter pylori*, and protozoa such as *giardia lamblia* (Ekwenye and Ijeomah, 2005).

2.6.2.3 Palm kernel oil

Palm kernel oil is manufactured from the kernel of the oilseed palm. It is very similar in composition to coconut oil. Palm kernel and coconut oils are known as lauric fats (Pantzaris and Ahmad, 2004). Lauric fats, Capric acid and Caprylic acid, are known for their natural antifungal and antimicrobial properties. Among the 17 major oils and fats in world trade, there are only two lauric oils: coconut oil (CNO) and palm kernel oil (PKO) and they are called lauric because lauric acid is the major fatty acid in their composition at about 50%, while no other major oil contains more than about 1% (Ekwenye and Ijeomah, 2005). .

2.6.2.4 Other oils employed in soap making

There are other oils that can equally be used in soap manufacturing but they offer different characteristics to the final soap formed based on chemical constants of the oils such as its saponification value, iodine number, peroxide value etc. The various and most important oils and fats used in the manufacture of soap are, tallow, coconut oil, palm oil, olive oil, poppy oil, sesame oil, soya bean oil, cotton-seed oil, corn oil and the various greases. Besides these the fatty acids, stearic, red oil (oleic acid) are more or less extensively used. These oils, fats and fatty acids, which vary from time to time and as to their colour, odor and consistency can readily be distinguished by various physical and chemical constants (Thomssen, 1922).

Tallow is the name given to the fat extracted from the solid fat or "suet" of cattle, sheep or horses. The quality varies greatly, depending upon the seasons of the year, the food and age of the animal and the method of rendering. The better quality is white and bleaches whiter upon exposure to air and light, though it usually has a yellowish tint, a well-defined grain and a clean odor. It consists chiefly of stearin, palmitin and olein. Tallow is by far the most extensively used and important fat in the making of soap (Thomssen, 1922).

According to Mak-Mensah and Frempong (2011), neem oil has been used in the manufacture of natural cosmetics, soap, toothpaste, hair and skin care products, emulsions, liquors, ointments and medicinal cosmetics. However neem oil can be produced mechanically (hot or cold press) or chemically (solvent extraction) from dried neem seeds. The best quality neem oil with a majority of phytoconstituents intact is obtained through cold press. In cold press the oil is lighter in colour and has a milder odour. Moreover potential residual solvents in chemical extracted oil that may pose health hazards to consumers are eliminated since solvents are not used in the pressing techniques.

2.7 Soap manufacturing

In soap making the properties of the fats and oils are important; the fatty acid composition in oil determines its properties (Nwoko, 1982). The acids may be distributed at random in the triglycerides. In the soap making, it is the fatty acid content that matters the most. The chain length (C number) is usually cited and helps describe the molecule's properties in relation to others in its same series. Saturated fatty acids contain no double bonds. They are stiff molecules which tend to increase the melting point of oils.

Soap-making was an established craft in Europe by the seventh century. Vegetable and animal oils were used with ashes of plants, along with fragrance. Italy, Spain and France were early centers of soap manufacturing, due to their ready supply of raw materials such as oil from olive trees. A major step toward large-scale commercial soap-making occurred in 1791 when a French chemist, Nicholas Leblanc, patented a process for making soda ash, or sodium carbonate, from common salt. Soda ash is the alkali obtained from ashes that combine with fat to form soap. The chemistry of soap manufacturing stayed essentially the same until 1916, when the first synthetic detergent was developed in Germany in response to a World War I-related shortage of fats for making soap. Known today simply as detergents, synthetic detergents are non-soap washing and cleaning products that are "synthesized" or put together chemically from a variety of raw materials (Onyegbado *et al*., 2002).

2.7.1 Classification of soap-making methods

Oil and alkali based soap making involves the hydrolysis of the triacyl glyceride molecule of the fat into fatty acids and glycerol and the subsequent saponification of the fatty acids by the alkali to form soap according to the following equations (Fig. 1) adopted from Beetseh and Anza (2013).

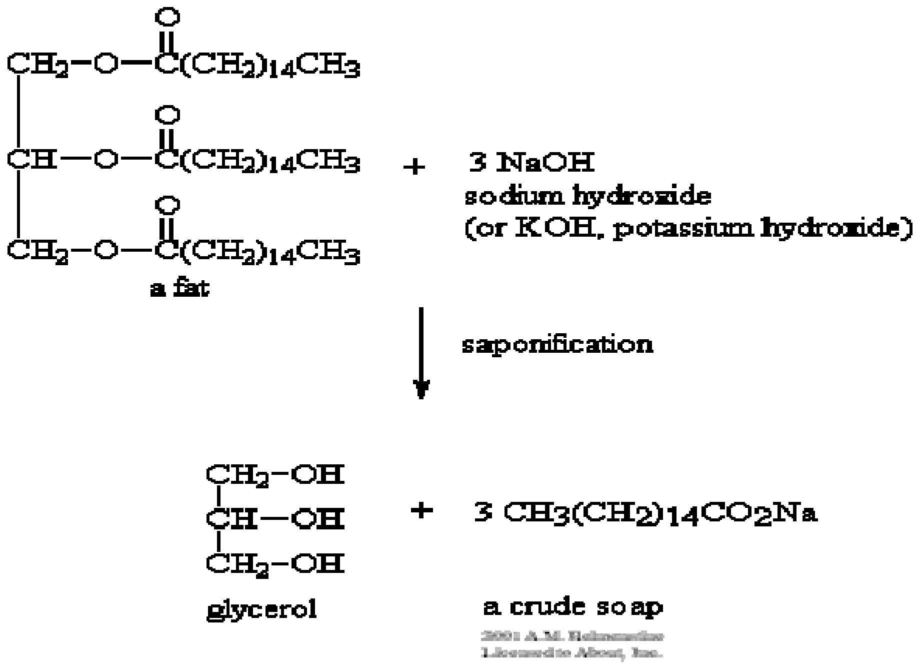

Figure 1: Soap formation reaction

The various methods adopted in soap making may be thus classified:

1. Boiling the fats and oils in open kettles by open steam with indefinite quantities of caustic alkali solutions until the finished soap is obtained; ordinarily named *full boiled soaps*. These may be sub-divided into (a) hard soaps with sodium hydrate as a base, in which the glycerine is recovered from the spent lye; (b) hard soaps with soda as a base, in which the glycerine remains in the soap, e. g., marine coconut oil soaps; (c) soft potash soaps, in which the glycerine is retained by the soap (Webb, 1926).

2. Combining the required amount of lye for complete saponification of a fat therewith, heating slightly with dry heat and then allowing the saponification to complete itself. This is known as the cold process.

3. Utilizing the fatty acid, instead of the neutral fat, and combining it directly with caustic alkali or carbonate, which is incorrectly termed *carbonate saponification*, since it is merely neutralizing the free fatty acid and thus is not a saponification in the true sense of the word.

In the methods outlined the one most generally employed is the full boiled process to form sodium soap. The stock, strength of lye, heat, amount of salt or brine added, time of settling, etc., are all influencing factors (Webb. 1926).

2.7.2 Cold process for soap making

The cold-process requires exact measurements of lye and fat amounts and computing their ratio, using saponification charts to ensure that the finished product does not contain any excess hydroxide or too much free unreacted fat. Excess unreacted lye in the soap will result in a very high pH and can burn or irritate skin; not enough lye and the soap is greasy. Most soap makers formulate their recipes with a 4–10% deficit of lye so that all of the lye is converted and that excess fat is left for skin conditioning benefits.

Once the oils are liquefied and the lye is fully dissolved in water, they are combined. This lye-fat mixture is mixed until the two phases (oils and water) are fully emulsified (Webb, 1926). Coconut oil is used very largely in the manufacture of cold-made soaps as it is well adapted for this purpose, although it is by no means true that other oils may not be employed.

2.7.3 Properties of Soaps and Detergents

Soaps or detergents are cleansing agents that are capable of reacting with water to dislodge these foreign particles from a solid surface (e.g. cloth or skin). Soaps have their origin in oils and fats present in the animal and plant kingdom and synthetic detergents find their source in mineral oils (hydrocarbon compounds of petroleum or coal). Chemically speaking, Soaps are sodium or potassium salts of higher fatty acids like stearic, palmitic and oleic acids can be either saturated or unsaturated. They contain a long hydrocarbon chain of about 10-20 carbon with one carboxylic acid group as the functional group.

Saturated fatty acids such as stearic and palmitic etc. contain only single bonds in their molecule, while unsaturated fatty acids such as oleic, linoleic etc., contain one or more double bonds. Thus, soaps are usually a mixture of the sodium salts of the following acids:

- Stearic acid as sodium stearate (C17H35COONa) - saturated fatty acid; from vegetable oils like linseed oil, soyabean oil.
- Palmitic acid as sodium palmitate (C15H31COONa) - saturated fatty acid; Palm oil, animal fat
- Oleic acid as sodium oleate (C17H33COONa) - unsaturated fatty acid; Vegetable oils like linseed oil, soyabean oil.

When soap is made from the sodium salts of the acids of cheap oils or fats, the resulting soap is hard. These soaps contain free alkalis and are mainly used as washing bars for laundry. When soap is prepared from the potassium salts of the acids of good grade oils and fat, it results in soft soap. These soaps do not contain free alkalis. They produce more lather and are used mainly as toilet soaps, shaving cream and shampoos. A soap molecule has a tadpole shaped structure, whose ends have different polarities that enhances its cleansing action. At one end is the long hydrocarbon chain that is non-polar and hydrophobic, i.e., insoluble in water but oil soluble. At the other end is the short polar carboxylate ion which is hydrophilic i.e., water soluble but insoluble in oil and grease. The subsequent mechanical action of rubbing or tumbling dislodges the dirt and grease from the fabric and surfaces. These get detached and are washed away with excess of water leaving the surface and fabric clean.

2.7.4 Antibacterial Soap

An antibacterial soap is a cleansing product designed to kill germs on the hands or body. These soaps are made in either liquid or bar form by blending detergent additives with ingredients, which have antimicrobial properties. An antibacterial soap is designed to safely kill germs and cleanse the skin. It must therefore consider the types of organisms the product should be effective against and how much time is required for the product to work. It must also consider factors related to cleansing such as foam quality, speed of foaming, rinsability, and skins feel etc. In addition, the product's aesthetic qualities (how it looks and smells) must also be evaluated (Jungerman, 1996).

2.7.4.1 Detergents inhibition of pathogens

The extent of microbial control or inhibition can be determined by the use of a paper disc method. The paper disc method is the most used commonly technique for determining microbial susceptibility. The paper disc is impregnated with a known amount of chemical known to exhibit antimicrobial properties and placed on the surface of an inoculated plate (Barnes et al., 1996).. After incubation, the plates are observed for any zone of inhibition surrounding the disks. A zone of inhibition (a clear area) around the disk is an indication that the organism has been inhibited by the antimicrobial agent. The type of oil and most importantly the types of fatty acids that constitutes the oil used in the soap formulation plays a significant role on the final properties that would be exhibited by the soap or detergent (Zelma *et al*., 1941).

3.0 MATERIALS AND METHODS

3.1 Raw Materials

Palm kernel oil and coconut oil were obtained from local oil processors at Nsawam in the Eastern region. Dried cocoa pods were obtained from the cocoa research Institute (of the Council for Scientific and Industrial Research) Tafo in the Eastern region and processed into potash at Bojuase in the Central region under supervision. Sodium hydroxide (reagent grade) was obtained from a chemical reagent distributor in Accra. The control detergent was obtained from Cleaning Solutions Ltd –Tema, near Accra, Ghana.

3.2 Sample Preparation

The oils employed were cleaned with various methods for oil clarification such as heating with activated charcoal, filtering, drying etc., and the alkalis employed were taken through various stages of processing.

3.2.1 Oil clarification

About 100g of oil was heated at different temperatures (100^{o}C, 120^{o}C and 150^{o}C), mixed with 4g of granulated activated charcoal (fine particle size) and stirred for ten minutes. The mixture was allowed to stand at room temperature (25^{o}C± 2) and filtered through whatman No. 4 fluted filter paper (Onyegbado, 2002) to obtain a much cleaner oil but there was no significant colour change in the oil mixtures as they passed through the oil clarification process.

3.2.2 Oils preparation

The cleaned palm kernel and coconut oils were mixed in various proportions according to the experimental design and used in the saponification processes. The proportions were as follows:

i. 100% coconut oil-A
ii. 70% coconut oil 30% palm kernel oil-B
iii. 50% coconut oil 50% palm kernel oil-C
iv. 30% coconut oil 70% Palm kernel oil-D
v. 100% palm kernel oil-E

3.2.3 Potash Preparation

The preparation method described here was adopted from the traditional soap preparation methods described by Onyegbado (2002). Fifty (50) kilograms of dried cocoa pod was weighed and burnt to ashes. According to Kuye and Okorie (1990), "a particle size of 1.06 x 10^{-4} of the potash gave the highest concentration of potassium hydroxide when slurry of the ashed sample containing 0.15kg of the ashes in 2.5dm^3 of distilled water was kept for eight hours at 60^oC". About 20kg of the resulting ash was washed with 20litres of water and filtered through a bamboo basket to obtain potash solution. The potash solution was kept for 60 hours and the filtrate evaporated to obtain crystals of potash. 1.1kg of the potash crystals was re- dissolved in 3.9litres of distilled water to obtain 28.2%w/v concentration and allowed to stand for 48hours at room temperature of 25^oC.

3.2.4 Sodium Hydroxide preparation

Approximately 23.5%w/v of a solution of sodium hydroxide was prepared whilst stirring for one (1) minute (Mak-Mensah and Frempong, 2011).

3.3 Detergent processing

The detergent manufacturing involved saponification reaction, using a combination of both the hot and cold processes of soap making to achieve desired results.

3.3.1 Hot process

About 500ml of palm kernel oil was heated to a temperature of 200°C and 350ml of potash solution of 28.2%w/v added slowly to prevent excessive vapour evaporation. In this process solid detergent was formed. 200ml of distilled water was added to 100g of the solid detergent and was allowed to sit for 24 hours after which a liquid detergent was obtained. The process was repeated for all the oil mixtures and the results were recorded.

3.3.2 Cold process

About 320ml of the oil mixtures were gently stirred for three (3) minutes and 160ml of the 28.2%w/v potash was gently poured into the oil in a 1 liter beaker whilst stirring. Stirring continued quickly and continuously for 15 seconds and the mixture was gently poured into a two (2) liter rubber bowl, lined with a polyethylene sheet and stood for twenty four (24) hours. The detergent formed had a gel-like consistency characteristic of most food grade detergents. There was separation in the detergent with loss of gel-like consistency over a period of 6 weeks. The process was repeated for all the oil mixtures. The trend was observed for all oil combinations and the results were recorded. The gel- like detergent formed was unstable over a period of six (6) weeks. This resulted in the modification of the method employed by the addition of a solution of sodium hydroxide with a combination of the hot and cold processes in the formulation.

3.3.3 A combination of both the hot and the cold processes

A total of 160ml of potash and caustic soda (in the ratio 3:7 respectively) was measured into 500ml beaker. About 320ml of oil was measured into a one liter beaker. The oil was gently stirred for three (3) minutes after which the base mixture (sodium and potassium hydroxide) was gently poured into the oil whilst stirring briefly and continuously for 15 seconds. The mixture was allowed to stand for twenty four (24) hour at room temperature (25^{o}C$\pm$ 2). The resulting detergent was a semi- solid product with high viscosity. About 20ml of distilled water was added to 100g of the detergent formed and heated for 15minutes until a gel-like consistency was obtained at a temperature of 70°C which was characteristic of most liquid detergents. The pH was stabilized by the addition of 0.3g/ml citric to 200ml each of detergent mixture as indicated in Fig. 2. The process flow diagram (fig. 2) indicated below shows the procedures followed to achieve the final product used for analysis.

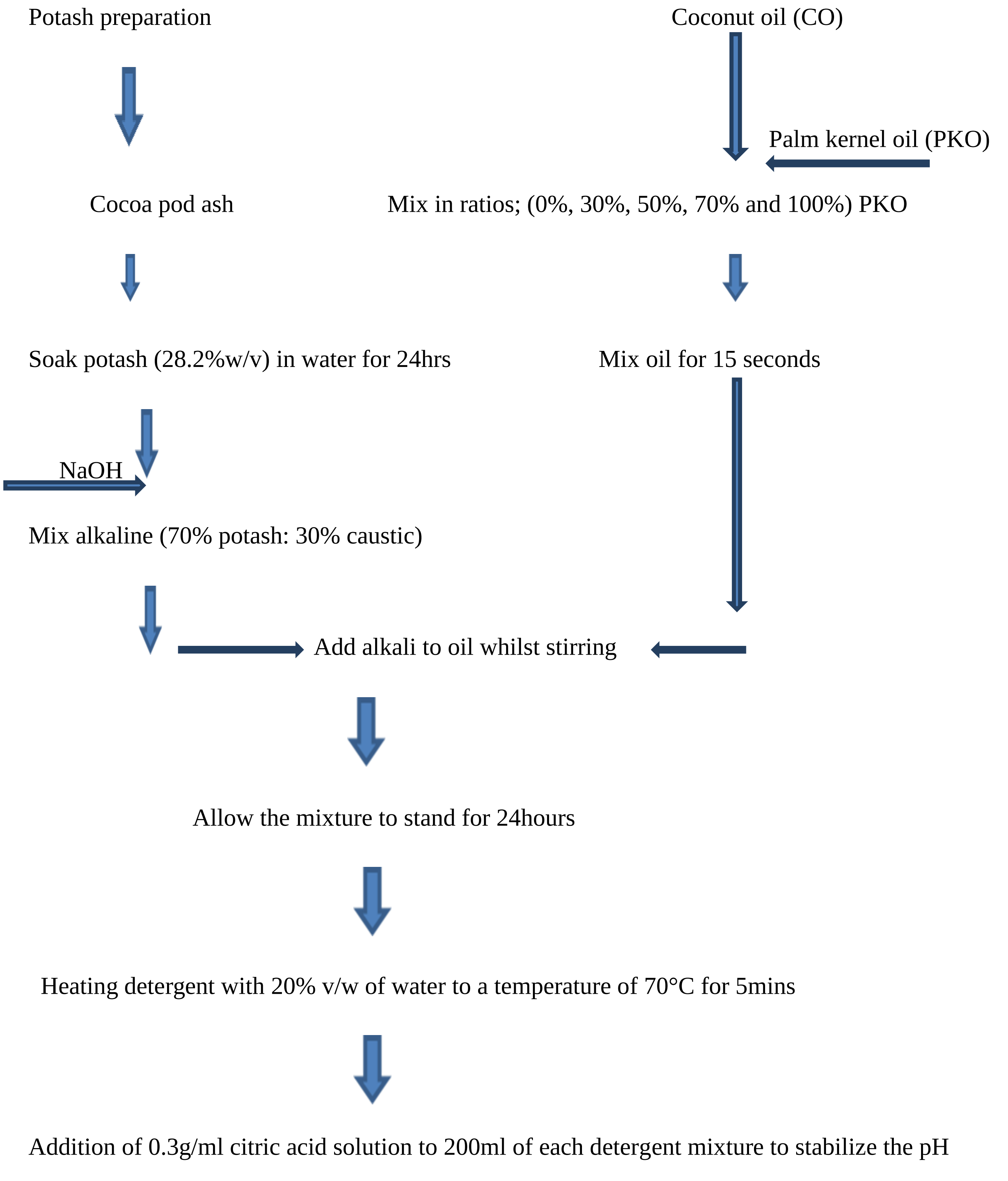

Figure 2: Process flow chart of the development of the food grade detergent

3.4 Analysis of physical properties of detergent samples

The detergents were analysed for their physical characteristics, foaming properties and stability, colour, viscosity and moisture content.

3.4.1 Measurement of viscosity of the detergents

The Hake viscometer D (thermo scientific Haake viscometer D) was employed in the measurement of apparent viscosity of the detergent samples and control. The spindles size labeled 'L3' was employed to test the viscosities of the various test samples and the control and run at speeds or 'RPM's of 10, 12 20 and 30 in detergent volumes of 400ml. The data obtained was fitted to the power law model (Steffe *et al*., 1989) and the consistency index as well as the flow behavior index of the detergents was determined.

3.4.2 Determination of foaming capacity and stability

In the determination of the foam formation of the soap samples, 10ml of the soap sample was placed in 1 liter measuring cylinder. Ninety (90) ml of distilled water was added to make up to the 100ml mark. The resulting solution was vigorously shaken 20 times (up and down, with care that it did not spill). The foam height was taken from the 100ml mark on the measuring cylinder to the foam height level and recorded as the foam capacity (Ross and Miles, 1941). The time taken for the foam to disappear as soon as the measuring cylinder was placed on the bench after shaking was also recorded as the foam stability. The difference gave a measure of rate of foam disappearance which was recorded (Ross and Miles, 1941).

3.4.3 Measurement of moisture content

Moisture content of the samples was determined according to AOAC methods (AOAC 1990).

3.4.4Determination of colour

The Minolta CR- 310 tristimulus colorimeter (Minolta camera Co. Ltd., Osaka, Japan) was used to determine the colour of the detergent samples formed and the control sample recording the L, a and b values. The calibration of the chroma meter was done against a white tile. The colour space parameters, L*, a* and b* were calculated as: L* (L_s – L_o) representing lightness (with 100 = perfect/brightness to 0 = darkness/ blackness); a* (a_s -a_o) representing the extent of green colour (negative = green to positive = redness) and b* (b_s-b_o) representing blue colour (negative = blue to positive = yellow)

3.5 Chemical Analysis

3.5.1 Determination of total free alkali

About 10g of the detergent sample was weighed. 100ml of 96% Ethanol was added and boiled for 10 seconds. 3ml of 0.5M H_2SO_4 was added and the resulting mixture was allowed to boil until the detergent sample dissolved over a period of 5mins. The hot solution was titrated with 1M NaOH till a pink colour showed at the end point using phenolphthalein as an indicator (Mak-Mensah and Frempong, 2011). The process was repeated three times. The percentage total free alkali (%TFA) was calculated as follows:

%TFA = 3.1 (V H_2SO_4 X fa-V NaOH X fb)/Sw

Where Sw is the weight of the detergent and fa= 1.007 and fb=0.998 (constant factors of the acid and base respectively)

3.5.2 Determination of free caustic alkaline

Approximately 10g of sample was weighed and dissolved in 100 ml of 96% ethanol according to the method employed by Mak-Mensah *et al*. (2011). Five (5) ml of 10% BaCl was added. A few drops of phenolphthalein indicator were added and the resulting pink solution was titrated with 0.1M HCl till the pink colour disappeared. The process was repeated three times for each detergent mixture.

The free caustic alkaline (NaOH):

NaOH = $\frac{0.31XVa}{W}$ where Va = Vol. of acid and W = Weight of detergent

3.5.3 Determination of free fatty acids

Five (5) g of each detergent sample was well mixed and weighed. Twenty-five (25) ml of 95% ethanol/ether (1:1) was added using phenolphthalein as an indicator. The resulting solution was titrated with 0.1N NaOH shaking constantly until a pink colour showed and persisted for at least 30seconds

(Mak-Mensah *et al*., 2011).The process was repeated three times for each of the detergent mixtures and the percentage free fatty acid expressed by its lauric acid content and acid values were determined by the formula below:

$$\%FFA \text{ (as lauric)} = \frac{vol.\ of\ NaOH\ (ml)\ x\ Normality\ of\ NaOH\ x\ molecular\ weight}{\text{Sample weight}}$$

Acid value = %FFA x 1.99

3.5.4 Determination of total fatty matter

Ten (10) g of the sample was dissolved in 100ml distilled water by heating. The mixture was transferred into a 500ml separating funnel and 2-4 drops of methyl orange indicator was added. Two (2) M H_2SO_4 was added in drops and the mixture was shaken until a pink colour appeared. The flask was allowed to cool and 100ml of diethyl ether was used for the extraction process. The extraction process was repeated three times and all the extracts combined and washed in distilled water. The washing was complete when the mixture was neutral to methyl orange. The extract was passed through Na_2SO_4 on filter paper into previously conditioned and weighed conical flask. (Hautfenne, 1982). The extract was evaporated on water bath and 10ml acetone was used to complete the evaporation.

The percentage total fatty matter was calculated as follows:

$\% \text{ TFM} = \frac{W1\ X\ 100}{W2}$ Where W_1=weight of fatty matter and W_2= weight of soap.

3.5.5 Determination of pH

The pH was determined according to the AOAC method (AOAC 1990) using pH meter (pH 210, microprocessor pH meter, Hanna Institute, 1999).The pH meter was calibrated with buffers at pH 4 and 9.

3.6 Antimicrobial effectiveness of the detergents

3.6.1 Microorganisms employed in the analysis (how authentic were the culture)

The following microorganisms were used to test how antimicrobial effectiveness the detergents were in of the detergents:

i. *Bacillus spp.*

ii. *Enteric bacteria*

iii. *Staphylococcus aureus*

iv. *Proteus mirabilis*

v. *Salmonella Typhi*

vi. *Escherichia. Coli*

3.6.2 Determination of inhibition zones of detergents against selected food borne pathogens

The microorganisms employed were all 24-hour-old cultures obtained from the Biochemistry Department of the University of Ghana, which were revived in nutrient broth.

3.6.2.1 Procedure for microbial analysis

1. Liquefied nutrient agar was prepared and sterilized according to the manufacturer's instructions. It cooled to 50^{o}C and inoculated with one loop of each microorganism.
2. Seeded poured into agar plates and allowed to set. Using a lightly flamed forceps, a sterile disc (autoclaved at 121^{o}C for 15mins) was picked and dipped halfway into labeled beakers containing the formulated soap at a concentration of 10%w/v of distilled water.

3. The impregnated disc was placed respectively in the center of each nutrient agar. In each of the cases the dipped disc was firmly fixed unto the media.
4. The media was incubated at 37^{o}C and observations were made after 24 to 48 hours respectively.
5. The zone of inhibition was measured as the distance between the edge of the disc and edge of the growth using a pair of calipers and a ruler (Barnett, 1992).

3.7 Design of experiments and data analyses

Two types of edible oils: palm kernel and coconut oil were mixed in various proportions (0, 30, 50, 70, and 100%). Sodium hydroxide and potassium hydroxide (as potash) were also mixed in the ratio of 30: 70 respectively (as determined from preliminary work). A total of five samples were replicated three (3) times. Differences between treatments were assessed using ANOVA procedures on the MINITAB version 14 platform.

4.0 RESULTS AND DISCUSSION

4.1 Physico-chemical characteristics of oils used in detergent development

The physical and chemical properties of the palm kernel and coconut oil used as the source of fatty acids in the soap making process are presented in Table 2. Both oils were relatively crude with free fatty acids (FFA) levels of 2.25% (as lauric acid) and 1.05% (as lauric acid) for coconut and palm kernel oils respectively. Color determinations using L* a* b* values showed the palm kernel oil to be relatively darker L*=42.42 than coconut oil L* = 67.68 (Plantazari and Ahmad, 2004). The viscosity of oil is a property of commercial significance, especially to manufacturers of non-food industrial products such as lubricants and related products. Olive oil is known to have the highest viscosity of any of the common vegetable oils.

Table 2 Indices of Coconut and Palm kernel oils after activated charcoal cleanup

Index		Coconut oil		Palm kernel oil	
		Before cleanup	After cleanup	Before cleanup	After cleanup
FFA %(as lauric acid)		2.25	2.25	1.05	1.05
Iodine value mg/100 g		17.6	17.6	33.3	33.3
Saponification value		215	215	225	225
Color	L*	67.68	69.15	42.42	51.01
	a*	-2.15	-1.42	-0.02	-0.99
	b*	+6.72	+5.39	+18.76	+7.62

The iodine values of 17.6mg/100 g for coconut oil and 33.3 mg/100 g for palm kernel oil together with the fluidity of the oils at room temperature suggest some level of unsaturation of the fatty acids in the triacylglycerides (TAGs) (Hautfenne, 1982). Indeed, while lauric acid (C12:0) and myristic acid(C14:0) are the main components of the oils of both palm kernel and coconut oil, palm kernel oil with 12% oleic acid (18:1) composition has a higher level of unsaturated fats than coconut oil which has 7% oleic acid. The relatively low iodine numbers in coconut oil compared to palm kernel oil confirms the presence of fewer unsaturated fatty acids in coconut oil and hence a relatively lower susceptibility to oxidative rancidity (Ekpa,1995). Polyunsaturated oils easily turn rancid, darken in color, and can change characteristics when acted upon by oxygen. Moreover, it has been determined that oils and fats with a high proportion of saturated fatty acids and fatty acid chains of 12 to 18 carbons make the most suitable soaps and detergents (Oghome *et al*., 2012).

The peroxide values indicate that the oils were still stable and not oxidized (Hautfenne, 1982). Indeed oxidized oils are not suitable for use in soap/detergent making because of flavor and other quality defects (Alsberg and Taylor, 1926). Even though it was not done in this study, several reports have indicated the fatty acid composition of both coconut and palm kernel oil to be predominantly lauric acid (C12:0) being 48 and 46% respectively (Piyali, 1999). Oils with high lauric acid content are highly priced in the soap and detergent industry because of their functionality as they have more desirable carbon chain lengths (C12—C14) for good detergency (Piyali, 1999). Table 2 also shows the saponification numbers of coconut and palm oil to be 215 and 225 mg KOH/g respectively. The saponification number gives information about the character of the fatty acids of the fat, and in particular concerning the solubility of their soaps in water. It is widely known that the higher the saponification number of a fat, the smaller the average size of the fatty acids in the TAG (Plantazari, 2004) and also the more soluble the soap that can be made from it (Alsberg and Taylor,1926).The high saponification number of coconut and palm kernel oil is largely due to the high proportion of lauric acid (C12:0) and myristic acid (C14:0) that they contain and would therefore yield quite soluble soaps in water. On the other hand the high saponification numbers also suggest that using coconut and palm kernel oils in the soap making process with lye will require high intake of metallic base as (sodium and or potassium) hydroxide, yielding high viscosity soaps (Oghome *et al*., 2012).

4.2 Detergent Development

4.2.1 Cleaning of oil

Considering that the oil samples were dark, with low L* values (Table 2) they were first cleaned up (using activated charcoal) before using them for the soap making process. The second part of Table 2.shows that the cleaning process marginally improved the L* values of the oils. The poor bleaching rate of the oils used in the soap making process suggest they were non-bleaching oils, probably due to their treatment/extraction history.

4.2.2 Development procedure of the Detergents

Both the hot and cold process of making soap using strong alkali and oil was explored (Kelly *et al*., 1957). Observations on the color and acceptability of the soap obtained using the hot process is presented in Table 4.3. The products obtained from the hot process when coconut oil alone was used were dark, but the detergent formed got even darker as the proportion of palm kernel oil increased in the oil mix. It may be surmised that heating oil in strong alkali would likely produce carboxylic acids (free fatty acids) and glycerol. The carboxylic acids, apart from undergoing saponification reactions, would also form a number of cyclic carbon compounds due to β-elimination or abstraction of protons from the anomeric carbons in hot, strongly alkaline environments. The cyclic carbon compounds would then polymerize in reactions similar to the Amadori reactions to form dark colored compounds. It is not very clear why increasing the palm kernel oil composition in the oil mix increased the darkening of the product. On the other hand considering that palm kernel oil has higher proportion of long chain oleic (C18:1) acid than coconut oil (Wikipedia, 2013), might explain the trend in the observations that increasing palm kernel oil composition decreased the L* values of the soap.

Table 3. Qualitative description of crude detergent obtained using the hot and cold processes using Palm kernel oil and coconut oil and Potash

	Product Description	
Samples	Hot Process	Cold Process
A (100% CO; 0%PKO)	Dark brown coloration	Light cream coloration
B (70% CO; 30% PKO)	Dark brown coloration	Cream coloration
C (50% CO; 50% PKO)	Dark brown coloration	Cream coloration
D (30% CO; 70% PKO)	Black coloration	Light brown coloration
E (0% CO; 100% PKO)	Black coloration	Light brown coloration

CO- Coconut oil and PKO- Palm kernel oil

The cold process of detergent formulation resulted in a lighter colored product that had a gel-like consistency. The color of the final product changed from light cream to light brown as the percentage of palm kernel oil increased in the detergent formulation (Table 3). Onyegbado *et al.* (2002) using a similar process in the saponification of palm kernel and palm oil blends with plantain peel ash (as source of alkali) also described the color of the products as milky white. The implementation of the hot process of soap making (method 1) and the cold process (method 2) separately yielded an unstable detergent after a period of six (6) weeks which affected some physical properties such as viscosity etc. There was therefore the need to modify the method in order to achieve a more stable gel. A combination of both processes (method 3) was finally employed to obtain a more stable and consistent gel.

The results are shown in Table 4. The gel formed was more stable and its physical properties were comparable to that of the control detergent that is popularly employed in the food industry and its colour variation is shown by plate 1.

Table 4. Qualitative description of the products obtained using both the cold and hot processes

SAMPLE	Description
A (0% PKO)	Light cream coloration and stable gel
B (30% PKO)	Cream coloration and stable gel
C (50% PKO)	Cream coloration and stable gel
D (70% PKO)	Light brown coloration and stable gel
E (100% PKO)	Light brown coloration and stable gel

PKO- Palm kernel oil

Plate 1. Samples of detergent mixtures; A (0% PKO; 100% CO), B (30% PKO; 70% CO), C (50% PKO; 50% CO), D (70% PKO; 30% CO), E (100% PKO; 0% CO) and the Control detergent.

4.3. Physical Analysis

4.3.1. Viscosity of the Liquid Detergents made by both the hot and cold processes

Viscosity is an important parameter in liquid detergent quality characteristics since it is an indication of the flow properties. It affects package selection and design as well as consumer acceptance. Therefore, controlling the viscosity of liquid soap is important in creating a product that meets consumer needs. It has been well established that different oils impart different rheological characteristics to soaps and detergents. Webb in 1926 observed that different oils produce soaps of varying hardness, odour and lathering.

The oil mixtures employed in this study produced a varying range of viscosities, which were shear dependent, except for the control detergent which showed more consistent viscosity trends at varying shear rates (Figure 3).

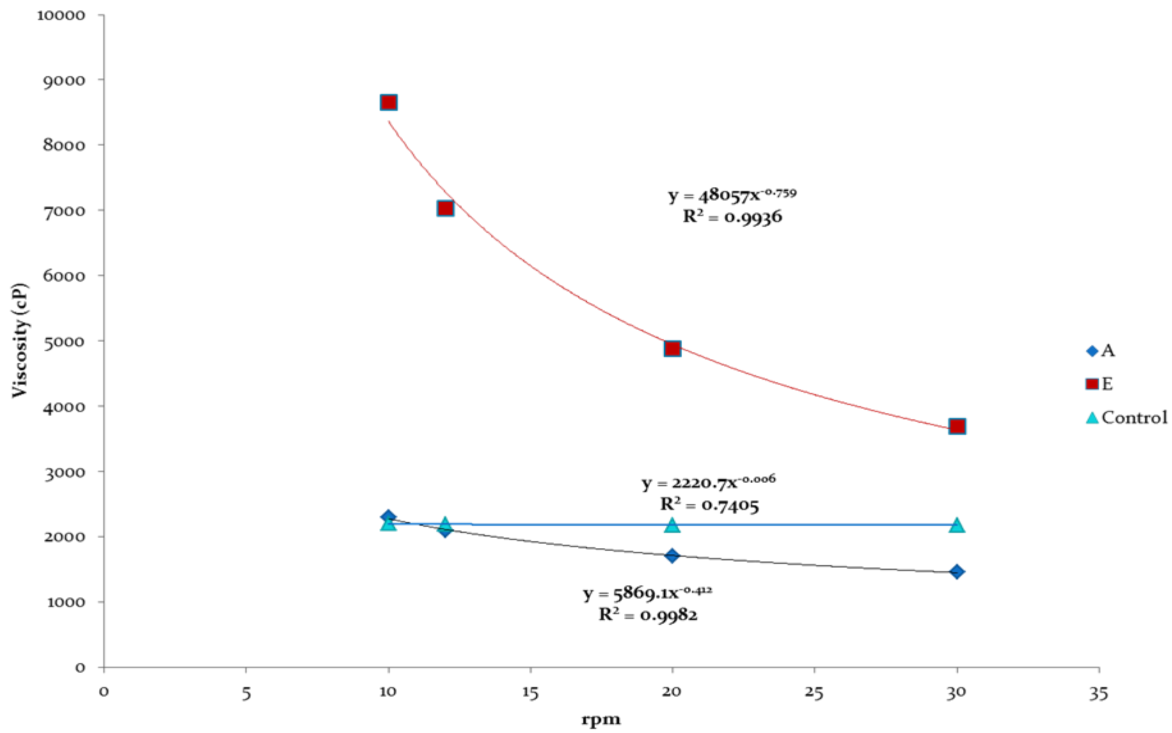

Fig. 3 Flow behavior of test samples comparing A and E with the control (A- 0% PKO and E- 100% PKO)

The viscosity data (i.e. shear rate versus shear stress) were adequately fitted to the power law model, as shown in the high R-squared values in Table 5. The table shows that while the oil based liquid detergents (i.e. samples A to E) showed non-Newtonian behavior (i.e. n<1) and thus will practically exhibit shear thinning when squirted out of a bottle, or pumped, the synthetic control detergent exhibited Newtonian flow, with flow behavior index n=1. Its flow behavior will not change with a change in shear rate (i.e. the viscosity will not change with different dispensing mechanisms). Table 5 also shows that increasing the Palm kernel oil composition in the soap mixture raises the flow behavior index towards Newtonian behavior.

The apparent viscosity data (i.e. the consistency index, (K, Pa.s) shows that while only coconut based detergents will be light (K=5.869 Pa.s); adding palm kernel oil thickened the apparent viscosity. Detergents made using only palm kernel oil showed a high apparent viscosity, K of 48.057 Pa.s, while the synthetic (petrochemical) non-oil based control detergent, with sodium laurel ethanolamide sulphate (SLES) as the main ingredient, showed the lowest apparent viscosity of 2.221 Pa.s. The apparent viscosity of the liquid detergents is probably influenced by the fatty acid chain length in two ways:

1. Increasing the average fatty acid chain length will increase the Stoke's radius, and consequently increase the apparent viscosity.

2. Reduction of the chain length in the TAG (as demonstrated by increased saponification number) increases the amount of metallic base intake in the soap with a consequent increase in apparent viscosity.

Considering that coconut oil generally has higher levels of short chain fatty acids (lauric and myristic) while palm kernel oil has higher amounts of longer chain fatty acids (oleic acid), it seems plausible that the former might explain the increase in viscosity of the soap made using palm kernel oil.

Table 5.Apparent viscosity parameters of liquid detergents at 25°C

Detergent Samples	n	K (Pa S)	R^2
A (0% PKO)	0.4	5.869[a]	0.998
B (30% PKO)	0.4	5.735[a]	0.997
C (50% PKO)	0.6	12.767[b]	0.996
D (70% PKO)	0.6	19.497[b]	0.993
E (100% PKO)	0.7	48.057[c]	0.994
Control	1.0	2.221[d]	0.741

4.3.2. Foam Forming Capacity of samples

Wet foams are disperse systems consisting of gas bubbles, separated by liquid films. It is not possible for pure water to foam unless a surface active material is present to reduce the surface tension (Saouter, *et al*., 2001). Table 6 shows the maximum foam capacities of the experimental samples. Although the control contained artificial foaming agents (like SLES) to enhance its foaming, the detergents made using higher coconut oil showed better foaming capacity. Saouter, *et al*., in 2001 suggested that foaming may be enhanced by the presence of surfactants, but the largest effect and best foaming capacity were obtained with shorter fatty acid chain length surfactants or polymer systems. Oghome *et al.* (2012) noted that saturated fatty acids in soap have support foam formation and have good cleaning properties. On the other hand while soaps made using unsaturated fatty acids have good cleaning power, they lather poorly. As the proportion of coconut decreased and palm kernel oil increased the maximum foam capacity decreased. Different oils with different properties have different outcomes on foam capacity when used for soap. The foaming ability of a solution is a property characterizing each particular surfactant solution, and it is a function of the lowering capacity of the surface tension. This is because the surfactant (or soap) decreases the surface tension and enhances the foaming capacity. Ordinarily, the cleansing capacity of soap is determined by its maximum foaming capacity (Yamada et al, 1981).

A study of the viscosity data (Table 5) and the foam capacity data (Table 6) reveals that increasing viscosity of the detergent decreased the foam capacity. This observation is in line with Yamada *et al.* (1981) who showed that the elasticity of the foam and the reciprocal of the bubbles size decreased with increasing viscosity of the soap.

Table 6. Foam capacity of the test samples and control

SAMPLE	**FOAM Volume (ml)**
A (0% PKO)	500±7.07
B (30% PKO)	500±7.07
C (50% PKO)	340±3.54
D (70% PKO)	300±3.54
E (100% PKO)	270±7.07
CONTROL	400±7.07

4.3.2.1. Foam Stability of samples

Foam stability usually refers to the ability of foam to maintain its parameters constant with time (i.e. bubble size, liquid content and total foam volume). In this work, foam life time (i.e. volume) was monitored as the stability index. This may be compared by shaking equal volumes of solutions of different samples having the same concentration with same force for the same amount of time. The solutions are then allowed to stand when the foam produced during shaking disappears gradually (Sardashi et al, 1998). The longer the time taken for the disappearance of the foam for the given sample of soap, the greater is its foaming capacity or cleansing action. If the foam takes a shorter time to disintegrate, it is preferred to be used as a food grade detergent since the food industry would prefer detergents that are efficient with little lather so they do not have to spend time and energy to rinse foam after cleaning. The foam volumes of all the five detergent formulations did not last longer than 10 minutes although the rate of foam volume disintegration was different among them (figure 4). It appeared that the higher the composition of palm kernel oil, the lower the foam volume and also the less stable it was. Thus similar to the foam capacity, the foam stability generally decreased with decreasing percentage of coconut oil employed in the detergent formulation (Figure 4).

Sample A which is 100% coconut oil and B which is 70% coconut: 30% palm kernel oil exhibited characteristics of a good food grade detergent in forming good foam volume that did not last very long. It would take less time, water and energy to rinse since the foam disappeared in a relatively short time. The foam volumes in Table 6 show that the control gave a relatively lower foam volume than the sample A (100% coconut oil) but also showed the highest foam stability (Fig. 4). Thus using the control as a food grade detergent in a processing factory will require longer rinsing time, more water and energy utilization.

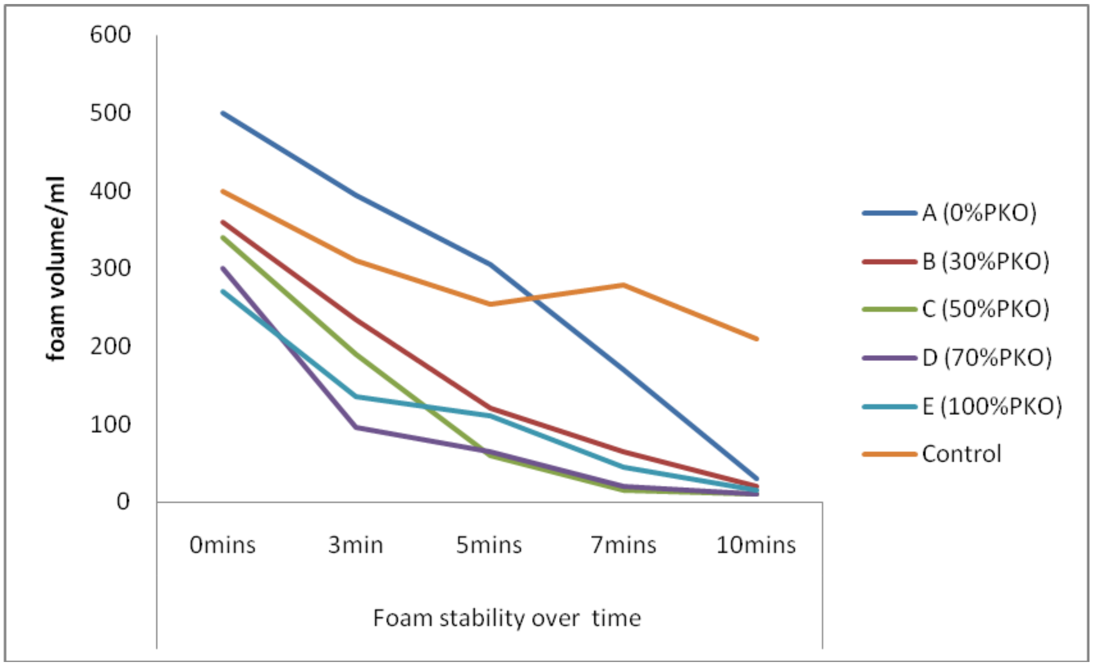

Fig. 4. Foam stability of test samples (A-E) and Control

4.3.3. Determination of Moisture Content

The moisture content influences the physical properties of a substance such as weight, density, viscosity, refractive index, electrical conductivity and many more. Chemical, thermo gravimetric or losses on drying techniques are used to determine this content. Its value is very important in detergent analysis since it is related to its storability, microbial stability, flow properties, viscosity, dry substance content, concentration, commercial grading and general acceptability of the detergent by the user.

Tale 7: Comparison of results of physical analysis of detergents

Sample	Colour			% Moisture	Foam Volume (ml)
	L	a	b		
A (100% CO; 0% PKO)	61.93	-1.82	+9.74	47.88±0.19	500±7.07
B (70% CO; 30% PKO)	50.35	-1.21	+8.39	47.25±0.16	500±7.07
C (50% CO; 50% PKO)	46.63	-1.47	+13.19	44.82±0.67	340±3.54
D (30% CO; 70% PKO)	46.56	-0.32	+10.55	42.30±0.34	300±3.54
E (0% CO; 100% PKO)	46.28	-0.17	+12.49	41.74±0.05	270±7.07
Control	63.94	+0.62	-2.15	88.94±0.03	400±7.07

Test samples from table 7; show that the test samples generally had lower moisture content as compared to the control. Comparing the test samples the moisture content generally decreased as the percentage of palm kernel oil increased in the detergent formulation. This could be due to the higher molecular weight of the palm kernel oil as compared to the coconut oil. The longer chain fatty acid of palm kernel oil that contributed to its higher viscosity values as compared to coconut oil could have been the reason for a lower moisture content of detergent sample E (100% PKO) as compared to the other detergent samples.

4.3.4. Determination of Colour

The hunter Lab colour system employed was used to determine how close the degree of difference in measured values match the degree of perceived colour difference. The colour of a product is very significant to the acceptability of the product since the preference of most consumers is based on it. Although this experiment did not cater for the sensory evaluation of the detergent samples developed, its significance on any product development process cannot be overemphasized.

The detergent made from 100% coconut oil (Sample A), had the highest 'L' value showing lightness in colour and it was comparable to the control which gave a value of 63.94 although the control was colourless. It was generally observed that the 'L' value decreased as the percentage coconut oil employed in the detergent preparation decreased. Hence the presence of 100% palm kernel oil (detergent sample 'E') showed the least value. Detergent sample 'C' (50% PKO) 'D' (70% PKO) and 'E' (100% PKO) gave higher b* value of +13.19, +10.55, +12.49 respectively but was comparatively lower for detergent samples 'A' (0% PKO) and 'B' (30% PKO). These observations could imply that the detergent samples were more yellow in colour than blue as compared to the control that was colourless. This colour variation was due to the fact that the oil clarification processes employed in the earlier stages of the experiment did not yield a high 'L' value as observed in Table 7. This trend of palm kernel oil showing a lower 'L' value compared to coconut oil, was observed in the final detergent samples as palm kernel oil increased in the detergent formulation.

4.4 Chemical analysis of the detergent samples

4.4.1 Total Free Alkaline (TFA)

The total free alkali (TFA) content is the quantity of free caustic alkali and carbonated alkali expressed as a percentage (m/m) as either sodium hydroxide for sodium soaps or potassium hydroxide for potassium soaps (Hautfenne, 1982). It was determined by neutralization of the total free alkali by boiling under reflux with a known amount of fatty acids, added in excess, and then determination of the excess of fatty acids by titration with ethanolic potassium hydroxide solution. The method employed in the detergent development process in this study combined potassium hydroxide and sodium hydroxide in a ratio of 70%:30% respectively, with the aim of keeping the final detergent stable as a gel over a longer period. Table 8 shows the total free alkali content of the samples. While there was no particular trend in the TFA, it appears that products made using only coconut oil had minimum total free alkaline (TFA), while those made with Palm kernel oil showed some level of TFA, even though in all cases the levels were well below the maximum acceptable limits of 0.6% as stipulated by the Ghana Standard for alkaline soaps (GSA 132, 2008). The lower %TFAs recorded by the samples could be because more of the alkaline combined with the fatty acids leaving less of the alkaline free in the final detergent formed. The control did not show any value for the total free alkaline because it was formulated using artificial surface active agents or chemical based surfactants. It was not made by the saponification of fat with alkali.

Table 8: Total free alkali content of samples and control

SAMPLE	AVERAGE %TFA
A (0% PKO)	0.050
B (30% PKO)	0.080
C (50% PKO)	0.054
D (70% PKO)	0.083
E (100% PKO)	0.075
Control	Nil

4.4.2Free caustic alkaline

The free caustic alkali content of sodium soaps of ordinary quality is the quantity of free caustic alkali, expressed as a percentage of sodium hydroxide (Hautfenne, 1982). In other words it is the amount of alkali free to prevent soap from becoming oily. The Ghana Standards for soap require them to have maximum limit of free alkali of 0.07. (GSA 132, 2008). Table 9 shows that there was no free or unbound caustic alkali for any of the samples indicating that all the alkali used in the detergent formulation reacted to form soap leaving no trace to be detected. The significance of this is that in its application on food contact surfaces or in the determination of its bactericidal efficacy, the effects observed will not be due to excess alkali but due to the detergency and sanitization effects of the soap. Excess free alkali in the detergent will be caustic on metallic food contact surfaces. Free caustic alkali was not detected in the control detergent because it was formulated from petrochemicals and not made from saponification reaction of oils and strong alkali. It was used as a control by virtue of its function and popular usage as a food grade detergent in many food industries.

Table 9: Free caustic alkali of the samples

SAMPLE	AVERAGE %FCA
A (0% PKO)	0.00
B (30% PKO)	0.00
C (50% PKO)	0.00
D (70% PKO)	0.00
E (100% PKO)	0.00
Control	ND

ND- Not Detected

4.4.3 Free Fatty Acids (FFA)

The free fatty acid content is the amount of alkali consumed by components in a filtrate (solvent) of a certain amount of sample and is calculated as percentage fatty acid. Sometimes the use of either free fatty acid or of filler (e.g., organic particles, polymers, wax, sugar, etc.), individually, in a fatty acid soap bar is a common practice ("superfatting") used to make soaps with enhanced user properties like enhanced lather and/or feel. However, unlike toilet soaps that require some amount of excess free fatty acids as emollient for moisturizing effects, detergents must have negligible amounts of free fatty acids. Table 7 shows that indeed the amount of free fatty acids in the soap was negligible. From Table 7, the free fatty acid was higher for the detergent sample 'A' which has 100% coconut oil as compared to detergent sample 'E' (100% PKO). With lauric acid being the dominant saturated fatty acid in both oils but higher in coconut oil, it could be deduced that the higher FFA observed in detergent sample A was due to the higher levels of saturated fatty acids in coconut oil as compared to palm kernel oil. The detergent sample C showed the lowest FFA level.

Table 10: Results of free fatty acid determination and acid value

DETERGENT SAMPLES	FFA/ Lauric acid	Acid value
A (0% PKO: 100% CO)	0.011	0.020
B (30% PKO: 70% CO)	0.010	0.019
C (50% PKO: 50% CO)	0.006	0.012
D (70% PKO: 30% CO)	0.008	0.015
E (100% PKO: 0% CO)	0.009	0.018
CONTROL	ND	ND

ND- Not Detected

4.4.4. Total Fatty Matter (TFM)

The total fatty matter determination is crucial in the soap development process since it defines the quality of the soap and helps in soap grading. It is the water-insoluble fatty material obtained by decomposing the soap with a mineral acid under the specified conditions or the total amount of fatty matter, mostly fatty acids, that can be separated from a sample after splitting with mineral acid, usually hydrochloric acid.TFM can be as low as 50%. Low TFM is usually associated with hardness and lower quality. Soap with TFM 75% minimum was referred to as Grade 1 and 65% minimum as Grade 2 (Wikipedia, 2013). Table 8 shows the TFM of the samples. Soap made with only coconut oil had a lower TFM than that made using palm kernel oil.

4.4.5 pH determination

The samples made using coconut oil recorded the lowest pH reading (9.30) and as the proportion of palm kernel oil increased in the mix, the pH also increased (Table 11). Soaps made using only palm kernel oil showed the highest pH of 9.7. The Ghana standard for soap (GSA 132, 2008) pH ranges from 6 to 9. The control which was formulated using various chemical based surfactant molecules gave a pH value of 9.07 which was closer to the GSA standard of 6 to 9 for liquid detergent. The presence of potash and caustic mixture as the alkaline base for the sample product preparation could have resulted in the high pH readings although from Table 11, all the free caustic alkaline reacted to form soap.

Table 11: Comparison of chemical characteristics of the detergent samples and control

Samples	Total fatty matter (%)	Total free alkaline (%)	FFA/ lauric acid (%)	pH
A (0% PKO: 100% CO)	39.6±0.3	0.05±0.005	0.011±0.0014	9.30±0.07
B (30% PKO: 70% CO)	52.7±0.1	0.80±0.003	0.010±0.00	9.6±20.02
C (50% PKO: 50% CO)	46.0±0.12	0.54±0.006	0.006±0.0014	9.64±0.04
D (70% PKO: 30% CO)	44.5±0.2	0.83±0.004	0.008±0.00	9.67±0.02
E (100% PKO: 0% CO)	58.1±0.2	0.75±0.007	0.005±0.00	9.760±.02
CONTROL	ND	ND	ND	9.07±0.01

ND- Not Detected

4.5 Antimicrobial effectiveness studies

4.5.1 Inhibition of detergent against microbial cultures

There was a general trend of the detergent types showing a higher inhibition against gram positive bacteria as compared to the gram negative bacteria employed in the experimental analysis as observed in Table 12. The detergent formulated using 100% coconut oil (sample A), showed different ranges of inhibition against various microbial isolates. It showed a much greater inhibition against *Bacillus species* as compared to its inhibition against *Salmonella* and *Proteus spp*. The *Bacillus spp*. and *Staphylococcus aureus* were the only gram positive microorganisms among the test organisms and they showed a higher susceptibility to the detergent sample A (100% coconut oil) than the gram negative microorganism. Among the gram negative bacteria, enteric bacteria showed the highest susceptibility to detergent sample A (Table 12). The overall inhibition of the detergent sample 'A' made using 100% coconut oil

(100% CO) on all microorganisms was comparable to that of detergent sample E formulated with 100% palm kernel oil (100% PKO). The trends show that detergents made using pure oils (i.e. either palm kernel or coconut oil alone) showed greater microbial inhibition than detergents obtained from the oil blends.

The detergent formulation B (30% PKO) showed a very strong inhibition against *Staphylococcus aureus* and *Bacillus species* though both are gram positive bacteria. The detergent type C (50% Palm kernel oil; 50% coconut oil) was found to exhibit the least microbial inhibition and was comparable to that of the control. The detergent sample D (70%PKO) was very effective against bacillus spp. And its effectiveness was comparable to that of detergent sample E (100% PKO) as observed from Table 9. The inhibition of detergent sample D (70%PKO) against *Staphylococcus aureus*, a gram positive microorganism was the lowest amongst the detergent samples although its inhibition against the gram negative microorganisms followed the general trend of lower inhibition zones as compared to the gram positive microorganisms. The detergent sample E (100% PKO) was generally most effective against all the food borne pathogens employed. It showed a stronger inhibition against the gram positive bacteria following the general trend of a higher inhibition of the detergent against such bacteria. Its effectiveness against gram negative bacteria however was generally higher than the other detergents but comparable to detergent sample A (0% PKO). Detergent sample 'E' also showed a good inhibition against E. coli confirming what has been established by Ekpa *et al*. in 1996 that "palm kernel oil could the growth of against E. coli". The control detergent showed the poorest effectiveness against the microorganisms employed but its inhibition zone was comparable to that of detergent sample C (50% PKO; 50% CO).

The detergent' formulated with 100% palm kernel oil (Detergent E) was generally effective against the food borne pathogens employed. This pattern was also observed in the detergents formulated with 100%

of each oil (coconut and palm kernel oil) as compared to the detergents that employed a combination of the oils. This could be due to an interaction between the oil mixtures which affected some chemical properties of the oil mixtures which subsequently affected the antimicrobial properties of the final detergent produced. Some of the chemical properties of the oil which could have been affected by the oil combination may include level of saturation of the fatty acids, the saponification value, the acid value etc.

Table 12: Comparing the efficacy of the detergents against the microorganisms employed

	Zone of inhibition (cm)					
Organism	Sample A 0% PKO	Sample B 30% PKO	Sample C 50% PKO	Sample D 70% PKO	Sample E 100% PKO	Control
Bacillus spp.	1.5±0.1	1.1±0.1	0.6±0.17	1.3±0.1	1.3±0.1	0.6±0.1
Enteric bacteria	1.0±0.1	0.5±0.17	0.3±0.1	0.3±0.17	0.7±0.1	0.3±0.1
Stapylococcus aureus	1.0±0.00	1.3±0.1	0.7±0.17	0.5±0.00	1.1±0.17	0.4±0.1
Proteus mirabilis	0.4±0.1	0.4±0.00	0.4±0.12	0.3±0.1	0.5±0.1	0.2±0.1
Salmonella spp.	0.4±0.2	0.6±0.2	0.3±0.00	0.1±0.00	0.5±0.00	0.3±0.0
E. coli	0.5±0.00	0.5±0.2	0.5±0.17	0.4±0.1	1±0.17	0.2±0.1

Antimicrobial studies of the traditionally extracted palm kernel oil (*Adwe ngo*) and two other oils; palm oil and coconut oil, on some microorganisms revealed that "Adwe ngo" was active against *Escherichia coli,* alpha and beta hemolytic *streptococci, Aspergillus fumigatus* and *Staphylococcus aureus* (Ekpa *et al.*, 1996). The results of the soap type E (100% PKO) showed a higher inhibition zone against *E. coli* as compared to all the gram negative bacteria employed in the research though it showed a generally higher inhibition against the gram positive bacteria employed as observed from table 12. It can be observed that the inhibition trend was the same for both *Salmonella typhi* and enteric bacteria which gave an inhibition zone of 0.3cm followed by *Proteus mirabilis* and *E. coli* which gave the same inhibition zone of 0.2cm. These gram negative bacteria gave an inhibition zone that was comparatively lower than the gram positive bacteria (*Bacillus species and Staphylococcus aureus*) that gave a zone of inhibition of 0.6cm and 0.5cm respectively

It has been established that unsaturated fatty acid soaps are more effective against gram-positive bacteria while soaps of saturated fatty acids are more active against gram-negative bacteria (Krueger *et al.*, 1973). The level of unsaturated fatty acids in coconut oil is 9% which is relatively lower than that of palm kernel oil at 14% unsaturated fatty acids. This suggests that soap made using palm kernel oil with a higher level of unsaturated fatty acids are more likely to be effective against gram positive bacteria which were *Staphylococcus aureus* and *Bacillus spp.* From Table 12, it is also observed that the detergent with 100% palm kernel oil inhibited *Staphylococcus aureus* better than *Bacillus spp.*, which is also gram positive as observed on plates 2 to 5 in appendix 3.

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

i. Soaps obtained from blends of coconut and palm kernel oil with cocoa pod ash and potash showed varied characteristics depending on the proportions of the oil type. While soap from only coconut oil was creamy in appearance, with relatively low viscosity and had a good foam volume and stability, increasing the palm kernel proportion darkened the color, increased the viscosity and reduced the foam capacity.

ii. The soaps were of good quality and met the Ghana Standards specifications for soaps of similar category. They generally had low total alkaline values, with pH between 9.3 -9.7 and will not be corrosive to food contact surfaces. The total fatty matter however needs to be increased.

iii. The antimicrobial activity of the soaps depended on the oil type used in their formulation. Soaps made from 100% coconut oil and 100% palm kernel oil soaps were more effective against gram positive bacteria compared to gram negative bacteria. Soaps made using blends of coconut and palm kernel oils gave a comparatively lower inhibition against the growth of both bacteria types employed in the study, irrespective of the proportion of palm kernel oil.

iv. Soaps made using locally available oils and alkali from cocoa pod ash showed effective antimicrobial activity against some pathogenic microorganisms likely to be present on food contact surfaces at food service centers.

v. There is a great potential in using local agricultural wastes and vegetable oils to develop food grade detergents with effective antimicrobial activity.

5.2 Recommendations

i. Further studies should be conducted in assessing the product stability over a longer period.

ii. Decolorization and deodorization studies on oils to produce odorless and colorless soaps/detergents should be undertaken.

iii. The lower inhibition zones of the oil mixtures used in the detergent formulations should be further investigated to ascertain the cause.

iv. There should be further studies to practically confirm the effectiveness of the detergent on food contact surfaces of food service centers.

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APPENDIX

Appendix 1: Viscosity results of the various detergent formulations at different RPMs

RPM	Average Viscosities/Cp Of Test Samples					
	A(0% PKO)	B(30% PKO)	C(50% PKO)	D(70% PKO)	E(100%PKO)	Control
10	2295.1	2280.9	2193.8	2907.6	4818.0	8656.0
12	2088.0	2063.6	2469.2	4073.6	7035.4	2182.4
20	1700.3	1698.5	1788.8	2998.9	4876.4	2178.0
30	1451.9	1447.5	1403.4	2407.1	3685.6	2176.5

Appendix 2: Analysis of qualities of coconut oil most suitable for soap making (extracted from; the project Gutenberg on the handbook of soap manufacture, 1908)

	Saponification Equivalent.	Acidity (as Oleic Acid) Per Cent.	Titre, °C.	Refractive Index at 25° C.
Cochin oil	215.5	1.5	23.5	1.4540
Cochin oil	214.3	2.6	22.1	1.4541
Ceylon oil	214.6	5.47	23	1.4535
Ceylon oil	216	3.95	22.75	1.4535
Belgian coprah	214.2	1.65	23	1.4541
Belgian coprah	215	2.60	22.1	1.4540
French coprah	214.2	6.55	23	1.4535
French coprah	214.8	7.42	22	1.4540
Pressed coprah	215.8	7.45	22.2	1.4542
Pressed coprah	216	9.41	22	1.4555

Appendix 3: Observation of inhibition zones of detergent samples against some microorganisms

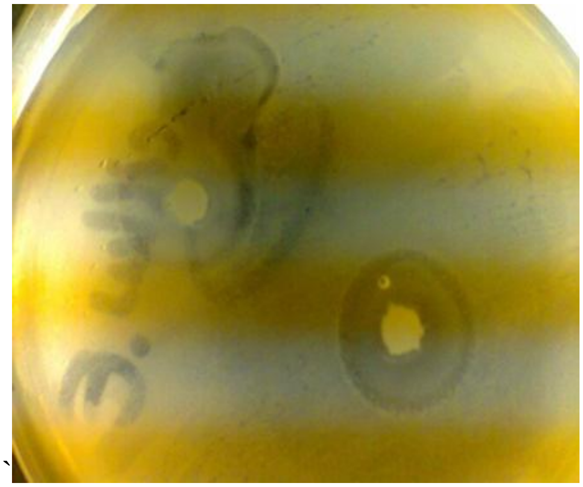

Plate 2 Sample E (100% PKO) on enterobacteriacea

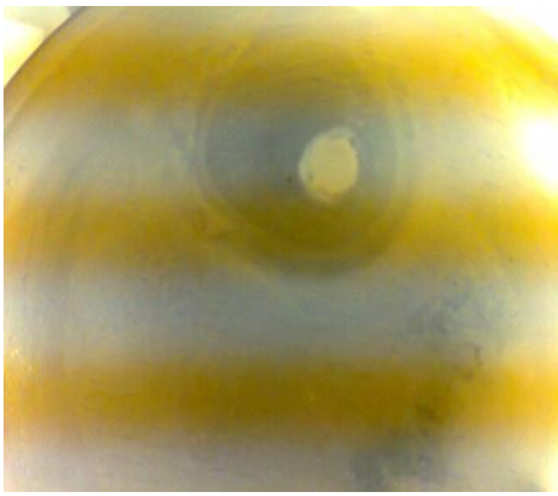

Plate 3 Sample E (100% PKO) on Bacillus spp.

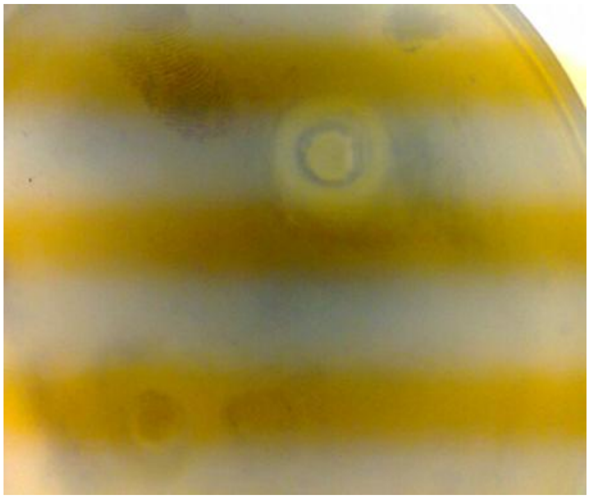

Plate 4: Sample D (70% PKO; 30% CO) on Salmonella spp.

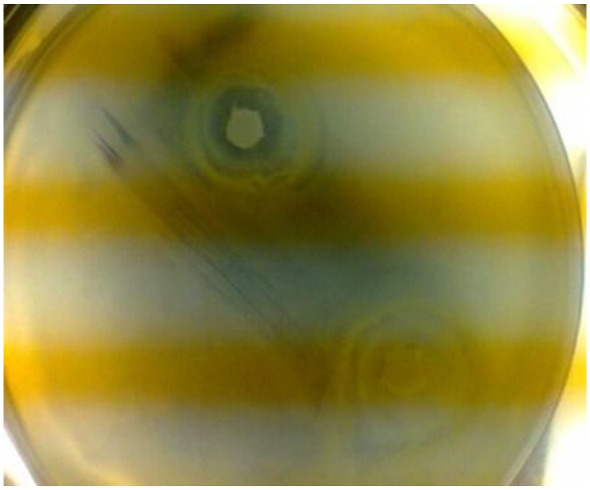

Plate 5: Sample B (30% PKO; 70% CO) on Enterobacteriacea