THE PREPARATION AND ANTIBACTERIAL ACTIVITIES OF ANIONIC SURFACTANT BASED LIQUID HAND SOAPS

by

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THE PREPARATION AND ANTIBACTERIAL ACTIVITIES OF ANIONIC SURFACTANT BASED LIQUID HAND SOAPS

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ABSTRACT

THE PREPARATION AND ANTIBACTERIAL ACTIVITIES OF ANIONIC SURFACTANT BASED LIQUID HAND SOAPS

Soap is the essential cleansing material which is necessary for prohibiting diseases and the propagation of harmful microorganisms. For 30 years, the usage of antibacterial soaps has increased as these products are claimed to be highly effective against bacteria and inhibit their reproduction. Several synthetic chemicals such as triclosan (TCS) and triclocarban (TCC) have been introduced within formulation of these antibacterial soaps as an antibacterial and antifungal agent. However, FDA banned several chemicals including TCS and TCC in 2016 due to lack of sufficient data on their safety and effectiveness. Additionally, it is well known that antibacterial liquid hand soaps have relatively lower foaming performances due to the presence of cationic surfactants. The purpose of this study is to produce an anionic liquid hand soap which bears adequate antibacterial activity and contend with commercial alternatives along with sufficient cleansing and foaming performances in the absence of banned antibacterial agents. For this purpose, synthetic surfactant (LS), fatty acids (FAS) and coconut oil (NLS) based liquid hand soaps were prepared in the presence and absence of essential oils (lavender and cinnamon) which have previously shown to exhibit antibacterial activities. The viscosity measurements indicated that LS in the presence and absence of cinnamon and lavender oil showed convenient thickness as the amounts of salt and thickener optimized while these samples offer high foaming performances. Moreover, the addition of lavender and cinnamon oils enhanced the antibacterial activity of LS on Escherichia Coli and Staphylococcus Aureus where even plain LS presented inhibition effect comparable to the commercial market product. Finally, the panel test revealed that the synthetic anionic surfactant based soaps prepared in the presence of natural and biocompatible essential oils exhibit almost identical consumer satisfaction as compared to commercial alternatives. All these performed analysis and tests pointed out the possibility of producing a competent natural antibacterial liquid hand soap in the absence of detrimental and banned antibacterial agents simply by using natural alternatives.

ÖZET

ANYONİK YÜZEY AKTİF MADDE BAZLI SIVI EL SABUNLARININ HAZIRLANMASI VE ANTİBAKTERİYEL ÖZELLİKLERİ

Sabun en temel temizleme malzemesi olmak ile beraber, zararlı mikroorganizmaların üremelerinin ve hastalık oluşumunun engellenmesi adına da kullanılmaktadır. Yaklaşık 30 yıldır antibakteriyel özellikli sabunların kullanımı, bakterilere ve üremelerine karşı oldukça etkili olduklarının iddia edilmeleri sebebiyle artış göstermiştir. Geçmiş yıllarda, bu sabunların formülasyonlarına, antibakteriyel ajan ve mantar önleyici olarak çoğunlukla triklosan (TCC) ve triklokarban (TCC) gibi sentetik kimyasallar eklenmekteydi. Ancak, 2016 yılında FDA, TCS ve TCC ile beraber bir çok antibakteriyel ajanı, sağlığa zararları ve etkinlikleri ile ilgili yeterli veri olmaması sebebiyle yasaklamıştır. Antibakteriyel sıvı el sabunlarının içerdikleri katyonik yapıdaki yüzey aktif maddelerden ötürü daha az köpürmeleri tüketici açısından da olumlu karşılanmamaktadır. Bu projenin amacı, yüksek temizleme ve köpürme kapasitesine sahip, antibakteriyel etki anlamında piyasada sununlan alternatifleri ile karşılaştırılabilir anyonik yapıda sıvı el sabunlarının üretililmeleri ve analizlerinin gerçekleştirilmesidir. Bu amaç için, sentetik yüzey aktif madde (LS), yağ asitleri (FAS) ve hindistancevizi yağı (NLS) bazlı sıvı el sabunları hem yalın hem de antibakteriyel etkileri önceden gösterilmiş lavanta ve tarçın yağları ile beraber hazırlanmıştır.Viskozite ölçümleri, tüm sentetik yüzey aktif madde ile hazırlanmış örneklerin yeterli koyuluğa sahip olduklarını göstermiştir. Ayrıca bu örnekler köpürme testleri sonucunda da oldukça yüksek değerler ortaya koymuşlardır. Saf LS örneklerinin yaklaşık olarak ticari market ürünlerinden daha düşük olsa da, uygun antibakteriyel özellik gösterdikleri belirlenmiştir. Hazırlanmış olan sabun örneklerine lavanta ve tarçın yağının eklenmesi ile antibakteriyel özelliğin daha da arttırıldığı *koli basili* ve *stafilokok aureus* üzerinde gösterilmiştir. Gönüllüler üzerinde panel testi sonucunda doğal ve esans yağları içeren anyonik yüzey aktif madde bazlı sıvı el sabunlarının, ticari ürünler ile aynı tüketici memnuniyetine yol açtığı saptanmıştır. Tüm bu analizler ve testler, ticari antibakteriyel sıvı el sabunları ile yarışabilecek düzeyde ve zararlı ve yasaklı maddeleri içermeyen doğal antibakteriyel ajanlar ile formüle edilmiş sıvı el sabunlarının üretilebilmesi potansiyelini ortaya koymuştur.

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LIST OF SYMBOLS/ABBREVIATIONS

Cfu	Colony forming units
ºC	Celcius
Cm	Centimeter
cP	Centipoise
Min	Minutes
mL	Milliliter
µL	Microliter
CAPB	Cocamidopropyl betaine
C_n	Carbon number
EDTA	Ethylenediaminetetraacetic acid
FA	Free fatty acid based soap
FDA	Food and Drug Administration
LS	Synthetıc surfactant soap
NL	Natural liquid soap
NS	Natural solid soap
PEG	Polyethylene glycol
pH	Power of hydrogen
RPM	Revolution per minute
SAP	Saponification value
SLES	Sodıum lauryl ether sulphate
TCC	Triclocarban
TCS	Triclosan
wt.	Weight
wt. %	Weight percent

1. INTRODUCTION

Soap has been the basic cleansing material since BC 4000 and the first samples were seen in Mesopotamia. Historical sources shows that Sumerians made it with natural components such as ashes and versatile oil sources for cleaning equipments, clothes and personal hygiene.

Today, various types of natural and synthetic soaps are produced in solid bar and liquid forms. Natural soaps are mainly produced by the saponification reaction of strong base sand oil sources where the type of alkali determines the final form of the soap. On the other hand, the synthetic soaps are prepared by the direct usage of anionic or cationic surfactants such as SLES an SLS. These soaps also contain several additives for thickening, preservation etc.

For the last 30 years, antibacterial products gained a lot of attention among consumers as these products are claimed by the producers to be highly effective against harmful bacteria and fungi. The manufacturers which hold 1.8 billion US$ market share used several chemicals that have antibacterial activities on gram positive and negative bacteria within the formulation of these products. However, in 2013 FDA demanded sufficient clinical data on the safety and effectiveness of these chemicals including triclosan and triclocarban which are frequently used in antibacterial hand soaps and consequently due to lack of justifications all these chemical were banned in 2016. Today all manufacturers replaced their antibacterial agents with alternatives which may potentially unknown to be detrimental.

The objective of this project is to produce and analyze natural and biocompatible antibacterial agents containing liquid hand soaps that exhibit high foaming and cleansing performances. For the analysis of the samples, the viscosities, foaming performances, pH, antibacterial activities were measured and accelerated stability tests were performed in order to comment on the shelf life of the products.

In the theoretical background necessary information about surfactants, soaps, bacterial strains, antibacterial products and their impacts, potential of essential oils and FDA regulations are given.

Chemicals and Methods section includes the used chemicals and detailed information of equipments used during the analyses. Synthesis of soaps and characterization methods include formulations and methods used for the characterization of the samples. All results related with the products and corresponding discussions are given in the discussion part.

2. THEORETICAL BACKGROUND

2.1. SURFACTANTS

Surfactant is derived from the phrase “surface active agent” and represent substances, which have the ability to alter the interfacial properties of the liquid. There are different types of surfactants all of which can be classified according to their properties. Considering the water affinity of the molecules (hydrophilic part), surfactants may be classified as anionic, cationic, non-ionic and amphoteric[1].

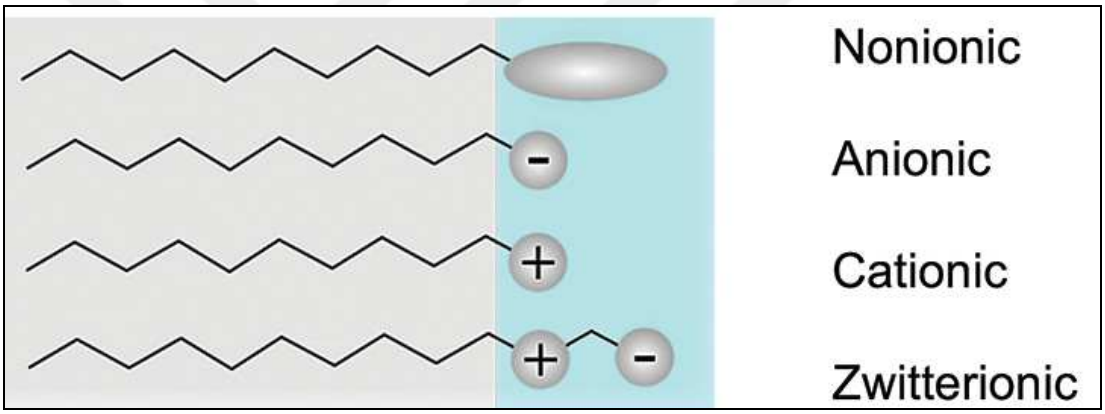


Figure 2.1. Surfactant types[2]

2.1.1. Anionic Surfactants

Anionic surfactants are composed of hydrophilic head groups and hydrophobic tail groups. The name anionic represents the negatively charged hydrophilic head group of the molecule (Figure 2.1). The most commonly used anionic surfactants in the industry contain hydrophilic head groups such as carboxylic acids and sulfuric acid derivates.

2.1.1.1. Carboxylic Acids

As mentioned before, the hydrophilic negatively charged part of an anionic surfactant molecule can be carboxylic acid. Carboxylic acids, which are organic acids contain one or more carboxylic acid groups as shown in Figure 2.2.

The carbon atom, which is located at the center is bonded to an alkyl group (R), a hydroxide group and also makes double bond with another oxygen atom. Well known examples of carboxylic acid are acetic acid, fumaric acid and acrylic acid. Additionally, fatty acids are in the carboxylic acid group. They are long chained carboxylic acids with varying 8 to 22 carbons.

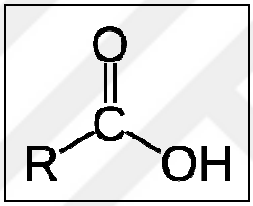


Figure 2.2. The structure of basic carboxylic acid[3]

Free fatty acids are not preferred to be used as surfactant due to low solubility. But, water soluble carboxylic salts, which are produced by alkine hydrolysis such as saponification reaction are used as surfactants. The carbon number also have big role for the solubility of carboxylic salt in water. C_8 is extremely soluble in water, but when the carbon number reached 18, the solubility begin to decrease.

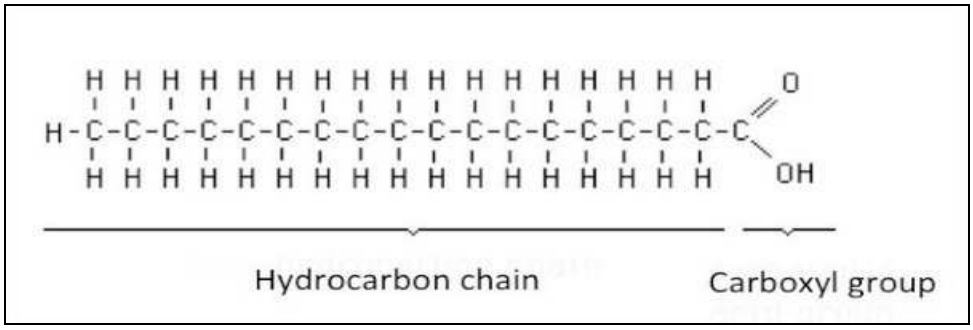


Figure 2.3. The structure of fatty acid chain[4]

2.1.1.2. Sulfuric Acid Derivatives

The esters of sulfuric acid are called as sulfuric acid derivates or alkyl sulfates. The structure is composed of a sulfur atom that is connected to carbon atom of the hydrocarbon chain with oxygen atom.

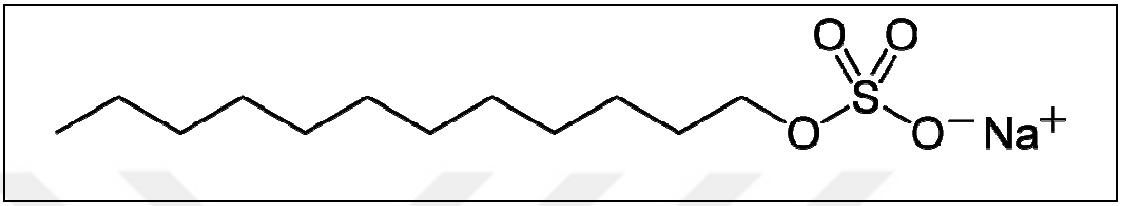


Figure 2.4 The structure of SLS molecule[5]

The reaction of the sulfation of the fatty alcohols creates alkyl sulfates. Most known family member of that category is SLS (Sodium Lauryl Sulfate). These surfactants are used in cosmetics and personal care market due to their relative advantages such as price, foaming capacity, versatility and ability of using in different formulations.

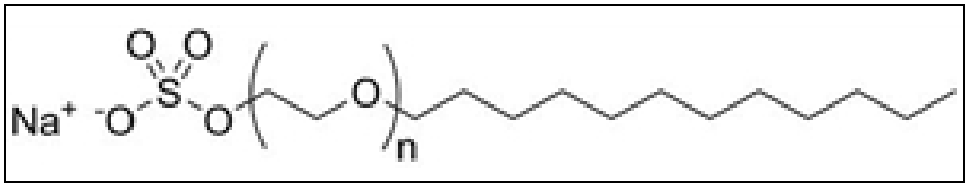


Figure 2.5 The structure of SLES molecule[6]

Another anionic surfactant group is alkyl ether sulfates. Most known family member of that category is SLES (Sodium Ether Lauryl Sulfate). The foaming capacity of SLES is very high and viscosity can also be enhanced easily by using ionic salts such as NaCl(Sodium Chloride) as a result of the sensivity of electrolytes to ether sulfates[1].

2.1.2. Cationic Surfactants

Cationic surfactants contain positively charged head groups and a hydrophobic tail groups. Generally, surface active agents that contains nitrogen compounds are in cationic surfactant category. Fatty amine salts, quaternary ammoniums and alkyl amine chains can be represented within this group.

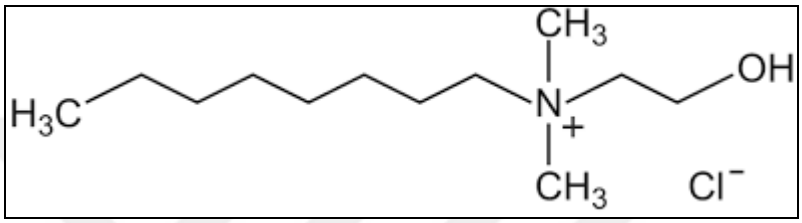


Figure 2.6. The structure of quaternary alkyl cationic surfactant[7]

Cationic surface active agents have great role on bactericidal applications for instance, they show significant performance such as germicides and fungicides in cosmetic and antiseptic preparations. Not only personal care purposes , cationic surfactants are also used on many fields such as fabric softeners, asphalt and crude oil additives[8]. However these surfactants cannot be used in combination with anionic surfactants as these oppositely charged entities interact within solution and produce goodness, which drops out of solution afterwards.

2.1.3. Amphoteric Surfactants

The surfactant type that has both anionic and cationic groups at two different edge of the molecule is called amphoteric surfactant. If there is no presence of pH domination, the net charge of the molecules is 0 due to cationic and anionic group confliction. Hence, pH of the surroundings determine the surfactant's charge. For instance, if the pH is alkali, the molecule is negatively charged and acts as anionic surfactant. If it is acidic, surfactant would behave as cationic one because of its positive charge.

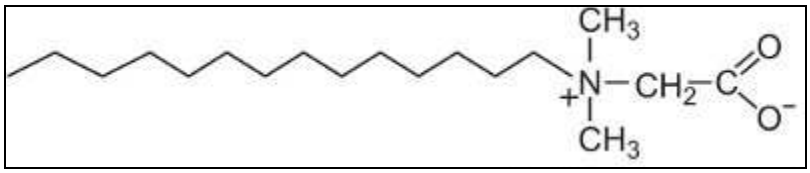


Figure 2.7. The structure of alkyl betaine[6]

The most known types are betaines and sulfobetaines. Amphoteric surfactants are found in softeners for textiles, hair rinse formulas, and corrosion inhibition additives. In the detergent industry, cocoamidebetaine is the most used chemical because of its viscosity enhancement role and foaming capacity[9].

2.1.4. Non-Ionic Surfactants

In today's industry, the most common surfactant type, which is used in various products is non-ionic surfactants that do not produce any ions in their aqueous solutions. Considering the surfactant market, non-ionic agents have 40% share in surfactant production[10]. The essential members of non-ionic surfactants are alcohol ethoxylates and alkyl phenol ethoxylates, alcoxylated alcohols, alkyl polyglucosides and alkanolamides etc.

Alkoxylated alcohols are one of the main types of non-ionic surfactants and are produced by a reaction involving a fatty alcohol and ethylene oxide [1]. This sub group`s most known member is polyethylene glycol(PEG).The alcoxylated alcohols are favorable as compared to other non-ionic surfactant types due to their properties such as water solubility as a result of their head groups and high temperature resistivity, which is crucial in food industry where spray drying is commonly employed. These types are also used as thickening agents in some formulations.

2.2. THE HISTORY OF SOAP

For the whole history of human nature, it has been a necessity to be neat and clean. However, due to lack of knowledge and technology, it was a tough task to overcome this problem. Later on Sumerians found a solution for cleansing. Considering the geographic nature of the Mesopotamia, ashes were abundant in the area. They realized that ashes could clean stuffs and human bodies. The ash is composed of two parts, which are hydrophilic and hydrophobic. Thus, the alkali parts of the ash, which is hydrophilic dissolve in the hydrophilic part of the dirt and the greasy part of the dirt dissolves byte hydrophobic part of the ash.

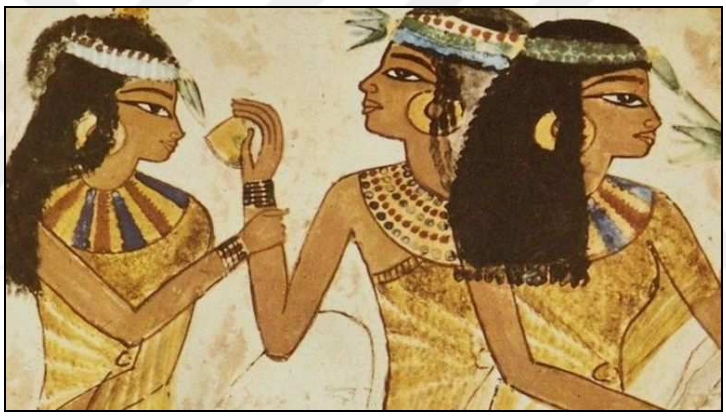


Figure 2.8. The ancient scrap about soap using[11]

Although it was a simple solution, which worked in general, the performance of the ancient cleaning agent needed to be developed. They realized that it is possible to produce soap by using solid animal fats from sheeps and cattles and vegetable oils such as palm oil or coconut oil. In the beginning soap was not meant to be used for personal hygiene but rather for cleaning equipments or for clothes [12]. The word of soap comes from famous Roman baths. The word ‘soap’ is adapted from Mount Sapo (Sapo means soap in Latin), where animals were sacrificed, and from where rainwater washed a mixture of melted animal fats and wood ashes into the River Tiber below.

Spread of soap usage to other civilizations took a long time. French people began to produce soap in 15th century. The recipes of soaps varied for each civilization. In France, they mixed the soap with essential oils but, the vegetable oils and herbs were used to produce soap in China. The reason of limited usage of soap was due to production cost. In the 17th century, the optimization of production with decreased cost led to broad availability. After the industrial revolution, the better quality soaps were produced with affordable prices. Especially, world wars and increasing epidemic diseases changed the importance of using soap from luxury to necessity. Later on, more versatile products such as laundry soaps, hand soaps, bath soaps, cleanser and detergents etc. were invented after surfactant production started. Today's most common product ,which is liquid hand soap was invented in 20th century and thereafter used widely in 1970s[13].

2.3. SOAP TYPES

Soap is the product of a saponification reaction, which is the alkaline hydrolysis of esters. For the soap making, ester hydrolysis of fats take place in the presence of an alkali. The saponification reaction may be performed with two different ways. In the first one, free fatty acids and base solution could be reacted to obtain soap. The alkali atom of the base replaces the hydrogen atom of the free fatty acid to produce soap and water as shown in Figure 2.8.

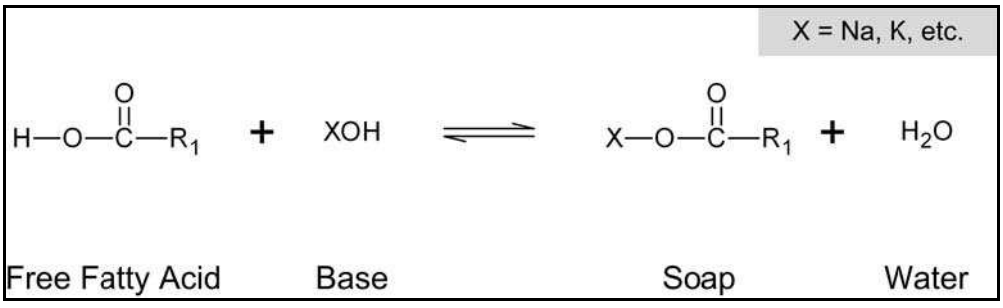


Figure 2.9. Saponification reaction with free fatty acid[14]

In the second alternative, triglyceride, which is an ester reproduced from three fatty acids and glycerol, reacts with a base solution (Figure 2.9). Two different base, which are potassium hydroxide (KOH) and sodium hydroxide (NaOH) could be used for saponification reaction. The type of lye determines the physical shape of soap. In other words, the introduced lye type characterize the soap as solid (Figure 2.10) or liquid (Figure 2.11). After the reaction, soap is obtained along with glycerol as a byproduct.

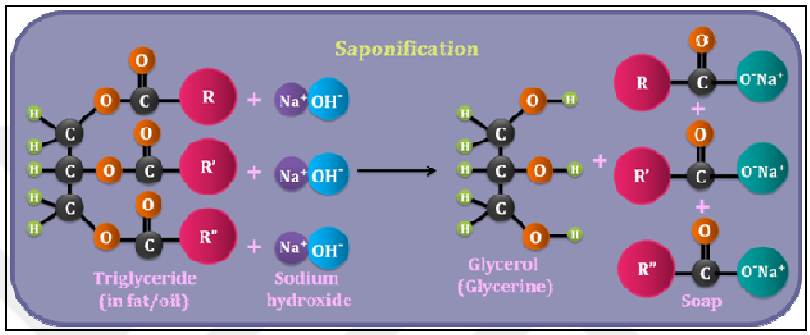


Figure 2.10. Saponification reaction with triglyceride[15]

There are essentially two different soaps according to the preparation method. The first one is natural soaps, which are produced by traditional saponification reaction of free fatty acids or triglycerides with base. However, it is also possible to obtain synthetic soaps by mixing artificial cleaning agents (such as surfactants as SLS) with viscosity enhancers, preservatives etc. Additionally it is possible to obtain both liquid and solid soaps by using these two alternatives.

2.3.1. Natural Soaps

Natural soaps are the basic and the ancient product for hygiene of human being. As stated before, ancient people made soap by mixing animal fats with lye. The developing technology lead to the production of natural soaps with new techniques. Nowadays soaps are commonly made from fats, fatty acids or fatty alcohols. Considering cost of the materials and maintenance during the process, companies prefer fatty alcohols and fatty acids in the production of natural soaps. However, synthetic soap production is more popular in terms of cost and facility.

The natural soap can be produced in two forms as solid and liquid. Basically, the properties of fatty acid based soaps depend on the type of lye, which is used during the saponification process.

Sodium hydroxide makes the soap hard while potassium hydroxide base makes the soap soft. Both of them can be used as bar but soft one can degrade easily. It is diluted with water and variant additives to gain liquid soap. So, potassium hydroxide is used for producing liquid soap. The reason behind the effect of different base over the properties of soap is related with the polarity. By going down the periodic table, the reactivity and size of atoms in 1A group increases. Hence, for potassium, the polarity is higher and the interaction with water molecules is smoother than sodium ion so, the crystal strength of potassium salt is looser than salt of sodium [16]. Additionally, the solubility of potassium salts of fatty acids are higher than sodium salts, thus remain in solution better.

Figure 2.11. Bar soap[17]

2.3.2. Synthetic Soaps

In the detergent industry, both bar soap and liquid soap can be produced by anionic, cationic, non-ionic and amphoteric chemicals such as SLES, CAPB, and Sterats etc. The first liquid hand soap was produced in 1940 for using as a hygienic agent in hospitals. In 1970s, the companies produced liquid soap for public as it is more convenient to be used due to hygiene concerns on bar soaps.

Today, the liquid soap industry holds influential share in the detergent market. Mainly, the ingredients of a regular liquid hand soap contain skin cleaning agents(SLES,SLS or quertanized ammonium), skin conditioning agents(glycerin), rheology agents(PEGs),color, fragrance, preservatives and others like antibacterial agents[18].

All these ingredients can be mixed by pre-determined amounts to form synthetic soaps. As a consequence of simplicity, cost and time efficiency and production capacity, many companies favor the production of synthetic soaps rather than natural ones.

Figure 2.12. Synthetic liquid soap[19]

2.4. BACTERIAL STRAINS

There are many organisms in the nature such as viruses, bacteria, algae etc. Some of them are beneficial for humans but some of them lead to severe diseases. The harmful organisms are called pathogens. According to the studies, humans meet some specific pathogens during daily life. Bacteria are just one of the pathogens and removal and prevention is relatively easier than others. Bacteria are one of the smallest and basic living organisms. They have just one cell, which is 1-2 μm of diameter so, volume of a thousand bacteria could fill 10^{-12} mL. As stated in the literature, 1-2 x 10^{8} cfu/ml of bacteria are found on human skin. Bacteria could be classified as beneficial and harmful [20]. For instance, nitrogen fixing bacteria helps to grow the plants while the intestine bacteria in human system aid digestion [21]. On the other hand, harmful bacteria may cause many animal, plant and human diseases, which could lead to fatal disorders.

2.4.1. *Escherichia Coli*

Escherichia Coli is a common bacterial strain in the world. Not all bacteria types are hazardous but *Escherichia Coli* is a pathogenic one that creates risk on environment and human life. This strain causes diarrheal diseases that lead death generally in non-developed or 3rd degree countries. It is estimated that roughly 1.8 million people died as a result of diseases caused by this bacteria. *Escherichia Coli* essentially spreads by common water sources and reach human habitats [22]. This strain accumulate due to fecal contamination where water system is insufficient.

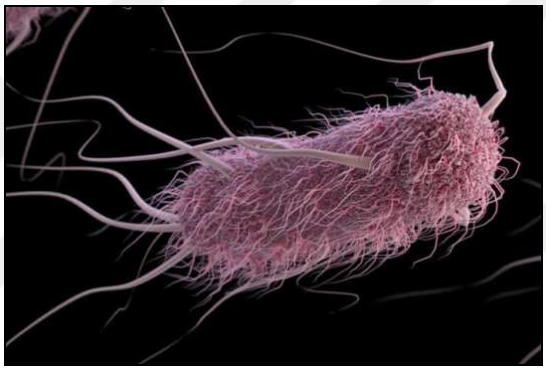


Figure 2.13. *Escherichia Coli*[23]

The strain has a rod shape (Figure 2.12) and it is a gram-negative bacterium being a member of Enterobactericeae family. *E.coli* can live in poor and extreme conditions with limited sources such as at high and low temperatures, low humidity, limited source in soil and in the presence of solar radiation [24]. The growing temperature interval is 7.5-49.5 ^{o}C, but the optimum temperature is 37.5 C. Also, many studies stated that the strain has prolonged life time [25].

2.4.2. *Staphylococcus Aureus*

Another dangerous pathogen is *staphylococcus aureus,* which is a spherical gram-positive bacteria and has 20 different mutants that can be found in the nature (Figure 2.13). It can also survive in extreme conditions by having the ability to grow by utilizing oxygen and even without it. In other words, it can replicate itself with aerobic and anaerobic growth[26, 27]. Although the strain can grow best at 37.5 ºC, it can also survive within a temperature range of 6-46 ºC. This strain prefers roughly neutral pH however it has the ability of survival at 4.0-9.3 pH range.

Staphylococcus Aureus can lead to skin, soft tissue, bone and respiratory diseases. The bacteria spreads usually by human skin such as hands, wounds, noses etc. Roughly, 20 percent of humans carry this strain[28]. Van Hal et.al. stated that between 10-30 percent of patients who is infected by a disease caused by *staphylococcus aureus* die in a year[29].

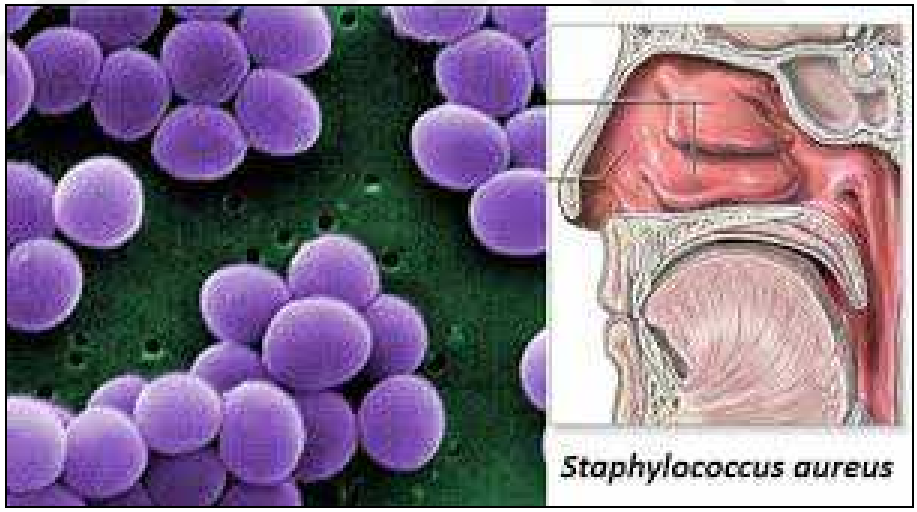


Figure 2.14. *Staphylococcus Aureus*[30]

2.4.3. *Pseudomonas Aeruginosa*

Another common malignant bacteria is *Pseudomonas Aeruginosa,* which is a rod-shaped, gram-negative bacteria (Figure 2.14). It belong to Pseudomonadaceae bacteria family and the average size is roughly 1.5µm long and 0.5-1µm wide. It can resist to the environment and prefers to live in the temperature range of 25 - 42 ºC where the optimum temperature is 37.5 ºC. The survival pH range is 4.5 to 9.5[31].

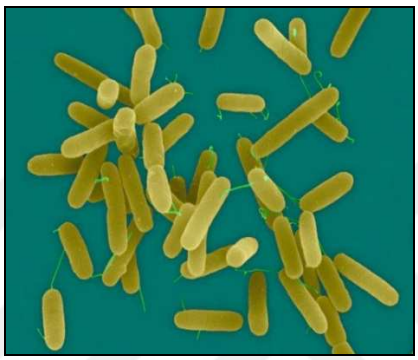


Figure 2.15. The view of *Pseudomonas Aeruginosa* organism[32]

Epidemiologically, *Pseudomonas Aeruginosa* is found in soil with living organisms, water, plants and animals. The pathogen mainly infects upper respiratory tract, skin (Figure 2.15), external ear and large intestine [33]. It is an opportunistic infection maker thus occur frequently and cause more acute infections on people with weakened immune system. As a consequence, the possibility of getting the pathogen is higher in the hospitals [34].

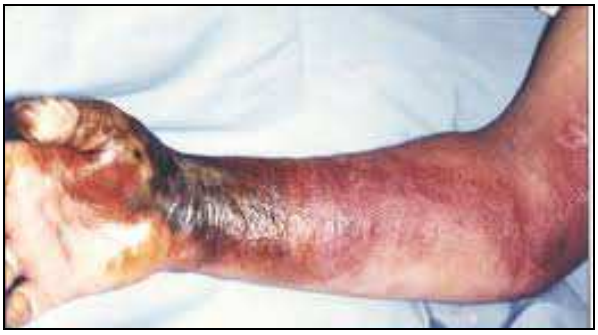


Figure 2.16. Skin infection caused by *Pseudomonas Aeruginosa* organism[35]

2.5. SHARE OF THE ANTIBACTERIAL PRODUCTS IN MARKET

The inclination of antibacterial household products such as fabrics, toothpastes, liquid soap and plastics have begun since 45 years ago. In the common household products, triclosan (2,4,4"-trichloro-2"-hydroxydiphenyl ether) have been used as an antibacterial agent (Figure 2.16 and Table 2.1)[36]. Triclocarban ,which is a derivate of TCS(triclosan) have also been used to remove gram-negative bacteria in deodorants and soaps [37]. Not only soap and deodorants, but also many personal care products contain antibacterial agents such triclosan and triclocarban separately or in combination. Other triclosan including products are listed as[38] ;

- Soaps
- Deodorants and antiperspirants
- Socks and undershirts
- Hand-washes
- Cosmetics and shaving creams
- Hot tubs, plastic lawn furniture
- Dish-washing products
- Acne treatment products
- Impregnated sponges
- Laundry detergents and softeners
- Hair conditioners
- Surgical scrubs
- Plastics
- Bedding
- Implantable medical devices
- Toothpaste and mouth washes
- Trash bags
- Pesticides

Figure 2.17. Triclosan containing products[39]

The usage of sanitizers is common for sterilization in hospitals, epidemic areas etc. by the application of antibacterial products. Thereafter, demand on these antibacterial products increased by the community as a result of obsession on cleaning. Therefore, the market of antibacterial products developed rapidly and nowadays became ample. The revenue of antibacterial soaps have great share in this market as compared to other listed products in Table 2.1. According to Perencevich et. Al., liquid soaps, which contain antibacterial agents among all others were 75.9 percent in 2001 in USA (Table 2.2)[40]. Additionally, the sales revenue of antibacterial products was 1.8 billion US$ in 2014 [41].

Table 2.1. The share of antibacterial soaps in the market [40]

Store Type	**Liquid Soaps (%)**	**Bar Soaps (%)**	**All Soaps (%)**
National	75.7	26.4	43.5
Regional	76.4	32.5	47.7
Internet	75	24.2	44.1
Cumulative	75.9	29.1	45.48

2.6. MECHANISMS OF TRICLOSAN AND TRICLOCARBAN

Triclosan (TCS) (Figure 2.17) and triclocarban (Figure 2.18) are antiseptic chemicals that are used frequently in the antibacterial products usually at 0.3 percent (wt.). In the literature, many studies show that antifungal and antibacterial activities were possed by both agents. Considering the usage of these antibacterial agents, according to McAvoy et.al, an average American gets5 mg/day of these agents. Moreover, 1500 kg of TCS are used in a day[42].The influence of triclosan bearing products have been shown as 5 to 10 times more effective than other non-antiseptic alternatives by Irish et.al and Zafar et.al.[43, 44].

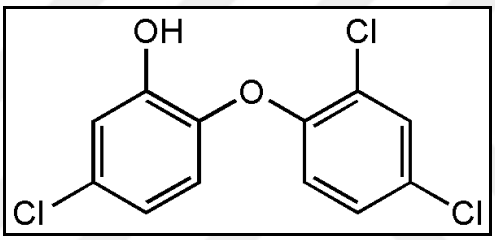


Figure 2.18. The structure of Triclosan[45]

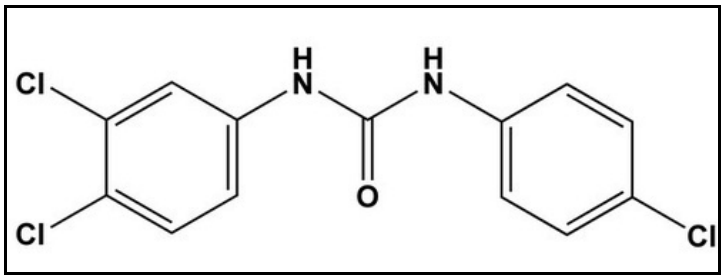


Figure 2.19. The structure of Triclocarban[46]

The basic mission of antibacterial agents are stopping the growth of bacteria. The bacteria increases its population by enzyme excretion and the enzyme leads to the production of fatty acid (Figure 2.20). Fatty acids are highly important for building cell membranes and the cloning of bacteria. Actually, the purpose of introducing antibacterial agents is to prevent further fatty acid production disrupt the chain[47].

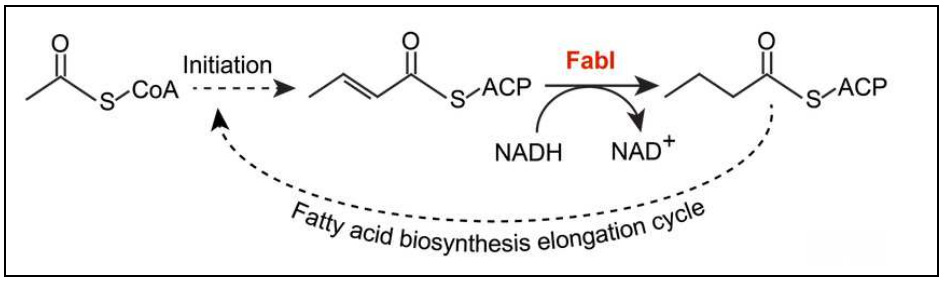


Figure 2.20. Fatty acid production in bacteria[48]

In the fatty acid production, there are two significant stages, which are enzyme condensing cycle and enoyl-ACP reductase cycle. Many antibacterial chemicals target these cycles (Figure 2.21). The active sites ,which are enoyl-acyl carrier protein reductase enzyme are blocked by the TCS and consequently the fatty acid synthesis is stopped thus the new cell formation and growth are prevented leading to the disruption of bacterial activity [49]. As an alternative of triclosan, triclocarban also shows the same mechanism on bacteria and fungies. The bacteria types, which are affected by triclosan and triclocarban are gives as;

- Staphylococci
- Streptococci
- Methicillin-resistant Staphylococcus aureus (MRSA)
- Enterococci: e.g. Escherichia coli
- Proteus spp
- Acinetobacter spp
- Proteus mirabilis
- Mycobacteria

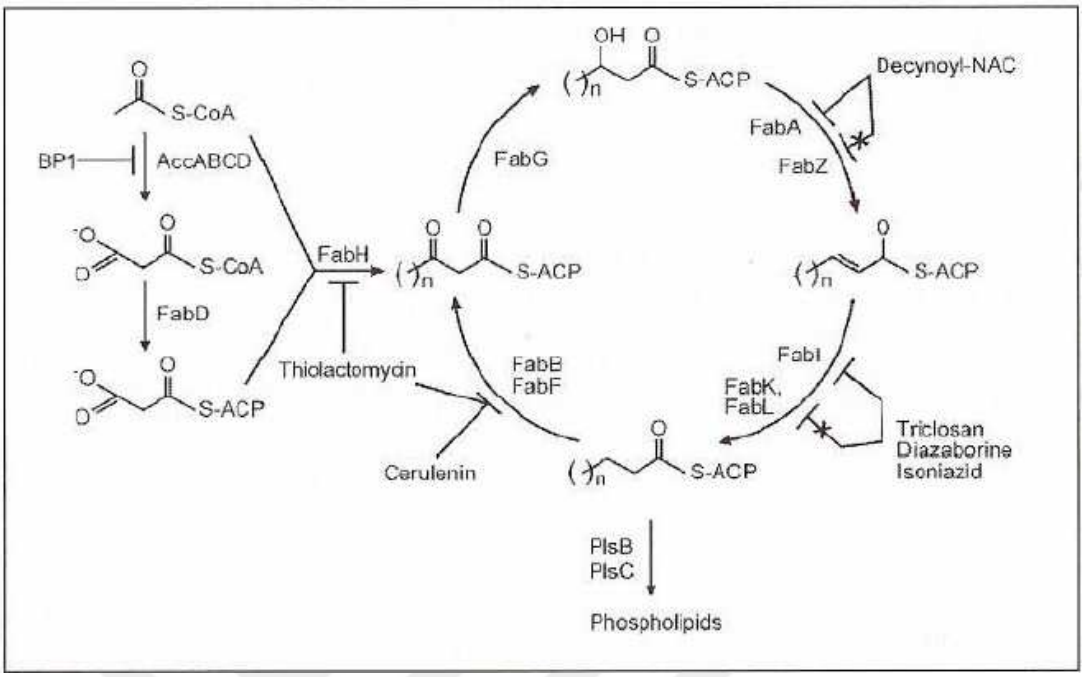


Figure 2.21. Fatty acid breakdown in bacteria by the TCS and TCC[49]

2.7. IMPACTS OF TRICLOSAN AND TRICLOCARBAN ON ENVIRONMENT

The common use of triclosan and triclocarban eventually lead to the emission to the environment and these agents were detected in many rivers, in USA. Moreover, it was proved that the pollution was not particular for USA but the fishes contain methyl-TCS (Figure 2.19), which is the byproduct of the triclosan, also in Tokyo [50].

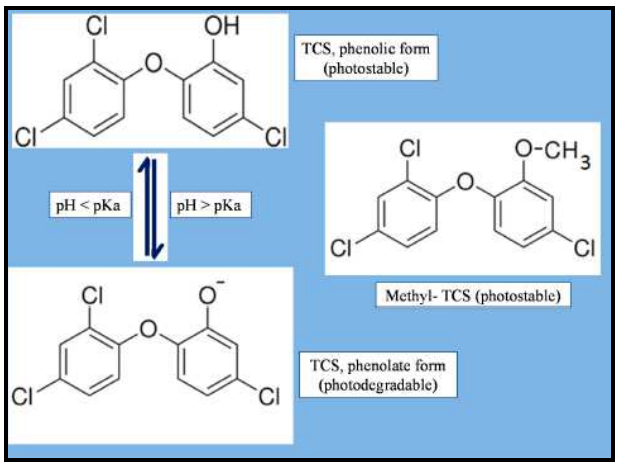


Figure 2.22. Degradation of TCS [51]

The evidences showed that the aquatic biota were under a serious risk as triclosan is not biodegradable. According to Kolpin et.al., TCS is one of the top 10 hazardous contaminants in rivers in 2002 and present roughly in 60 percent of streams in USA [52]. Additionally, TCC pollution were valid for the groundwater, drinking water and waste water in Baltimore and Maryland[53]. The by-products of TCS have also significant contributions to the environmental pollution. Types of by-products are methyltriclosan, and derivatives of dioxins, chloroform and chlorophenols. Although the chemicals are not toxic in low levels, increasing trend of consuming TCS and TCC products are risky for the environment [54]. The strongest evidence of bioaccumulation are shown on Table 2.2.

Table 2.2. Concentrations of Triclosan (TCS) in aquatic organisms

Organisms	**Sample**	**Site Description**	**TCS(µg kg^{-1})**	**Reference**
Algae and intervertebrates Filamentous algae (*Cladophora* spp.)	Whole organism	Receiving stream for the city of Denton WWTP(TX,USA)	100 - 150	[55]
Freshwater snails vertebrates(*Helisoma trivolvis*)	Whole organism	Receiving stream for the city of Denton WWTP	50 - 300	[56]
Rainbow trout(*Oncorhynchus mykiss*)	Muscle	Upstream from WWTP, Sweden(caged): Downstream 2 km from WWTP(caged)	710 17 000	[57]
Killers whale (*Orcinus Orca*)	Plasma	Marine Centre	9.0	[58]

2.8. THE EFFECT OF TRICLOSAN AND TRICLOCARBAN ON HUMAN

According to some studies, TCS was found in human breast milk, which supports the bioaccumulation of TCS in human body. Adolfonsson-Erici *et al*. conducted a study, which compares women who used TCS containing household products and who did not. The results suggests concentration of TCS in human breast milk was comparably low for women who did not used TCS containing products [57].

Many other studies questioned the effects of TCS on endocrine hormones both in-vivo and in-vitro. TCS has shown to possess antiestrogenic and antiandrogenic impact while interacting with estrogen and androgen receptors[59]. Also in pregnant rat model experiment showed that T4 and sex hormones decreased dramatically [60]. Same situation is also valid for TCC as studies showed that TCC has great impact on human health and lead to adverse health effects [61].

Another problem of TCS and TCC is the origination of antimicrobial resistance. Beier *et al.* stated that vancomycin-resistant Enterococcus faecium strain increased the endurance of TCS and 14 other antibiotics which indicates that TCS and its derivate of TCS have a role for antibacterial resistance and affect human health indirectly[62]. TCS also disorder the microbial flora in the environment. Drury et. al stated that the antibacterial variety disturbed by TCS [63].

2.9. THE ANTIBACTERIAL POTENTIAL OF ESSENTIAL OILS

Essential oils have been extracted and used for many purposes such as pharmaceutical agents and aromatic additives in beverages etc. People extracted essential oils from barks, leaves, herbs and roots by using various methods for almost 2000 years. The extraction and the usage of essential oils for pharmaceutical purposes goes back to 13th century in Europe [64].

Essential oils are complex natural mixtures and may contain 20–60 species at various concentrations. Mainly, essential oils are characterized by top primary components appear at relatively higher concentrations (20–70 percent) as compared to other trace constituents.

Today, essential oils are also used for aromatherapy, cosmetic products and food preservative agents[65]. Some studies were performed to reveal the performance of essential oils potential as a natural antibacterial agent as other chemical antibacterial agents and their by-products adversely affect the environment and human health. Researches show that essential oils have bactericidal and anti-fungal activity, but every essential oil are not comparably effective for all types of bacteria.

Rohraff et al. studied the antibacterial activities of pine (Pinus Sylvestris), cinnamon bark (Cinnamomum cassia Blume), spearmint (Mentha spicata), peppermint (Mentha piperita), juniper berry (Juniperus communis), lavender (Lavandula officinalis) and ginger (Zingiber officianale) oils.

Cinnamon oil was shown to possess great inhibition zone in E.coli and S.aeurus strains. The study also showed that the lavender oils could be an effective agent as generating a significant inhibition zone in S.aeurus and E.coli respectively[65].

Ooi et al. stated that trans-cinnamaldehyde, which is the major component in cinnamon bark oil bear antibacterial activity [66]. Also, the study ,which were conducted by Raeisi et al. approves antibacterial activity of the cinnamon extract and also showed it could be used in mixtures at different compositions in different media[67].Raeisi et al. and Prabuseenivasan et al. reported the antibacterial activity of lavender oil.

Unlike E.coli, the growth of S.aeurus, P.aeruginosa and other strains diminish in the presence of lavender oil[68]. Most of the studies pointed cinnamon oil as the most effective essential oil among others.

Although, the effect of essential oils on bacteria have been presented, the storage is crucial for the stability. Shah et al. stated that the volatility of major component of essential oils could be a problem for the biological activity and besides Desai et al. indicated the presence of oxygen and light might disturb the stability of the compounds[69, 70].

The presence of antibacterial activity of essential oils lead to the study of antibacterial products that contain these additives as agents. Lertsatitthanakorn et al. studied antibacterial soaps involving essential oils and demonstrated the activity by these natural agents. In this study, E.coli, S.aeurus, P.aeruginosa and S.epidermis were selected as bacteria culture. They formulated a synthetic soap, which was not prepared by

saponification reaction and added cinnamon and lavender oils. The results showed that synthetic soap containing essential oils inhibited the growth of four aforementioned bacteria.

The product stability and antibacterial activity tests were conducted in a 90-day period. Although there was a decreasing antibacterial activity of essential oils on S.aeurus and S.epidermis, the activity on E.coli and P.aeruginosa was stable during that period[71].

2.10. FDA REGULATION ON ANTIBACTERIAL PRODUCTS

Triclosan and triclocarban are the major substances that are used in antibacterial products approximately for 40 years. They were also listed as eligible agents in antiseptic wash by FDA (Food and Drug Administration) in 1994 as the scientific analysis methods were not sophisticated and their effect on the environment and human health was not known.

In 2013, FDA requested sufficient clinical data for the safety and effectivity from suppliers on 19 different chemicals including triclosan and triclocarban that are used in consumer products due to uncertainty and suspicion. After the call of FDA, the companies did not give sufficient data about the positive effects of 19 chemicals. On September 2, 2016, FDA banned 19 chemicals due to lack of clinical data. FDA finalized the rule that finds that all these active ingredients are not Generally Recognized as Safe and Effective (GRASE) for usage in health care and hygiene products. In addition to this regulation, many studies additionally validated the impacts of triclosan and triclocarban on the ecosystem and the role on enhancement of antibacterial resistance. This ban excepts health care area, but valid on household and personal care products.

This rule was postponed for one year for three specific agents ,which are benzalkonium chloride, benzethonium chloride and chloroxylenol (PCMX) for requesting additional safety and effectivity data[72]. The banned chemicals are listed as;

- Cloflucarban
- Fluorosalan
- Hexachlorophene
- Hexylresorcinol

- Iodine complex (ammonium ether sulfate and polyoxyethylene sorbitan monolaurate)
- Iodine complex (phosphate ester of alkylaryloxy polyethylene glycol)
- Nonylphenoxypoly (ethyleneoxy) ethanoliodine
- Poloxamer-iodine complex
- Povidone-iodine 5 to 10 percent
- Undecoylium chloride iodine complex
- Methylbenzethonium chloride
- Phenol (greater than 1.5 percent)
- Secondary amyltricresols
- Sodium oxychlorosene
- Tribromsalan
- Triclocarban
- Triclosan
- Triple dye

3. MATERIALS AND METHODS

3.1. CHEMICALS

Table 3.1. Structures and suppliers of chemicals used in this study

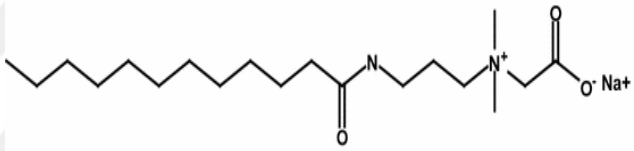
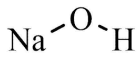
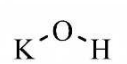
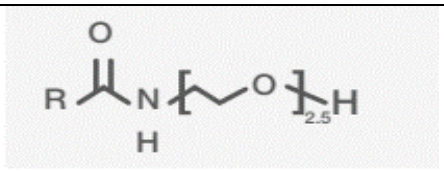
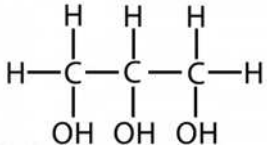
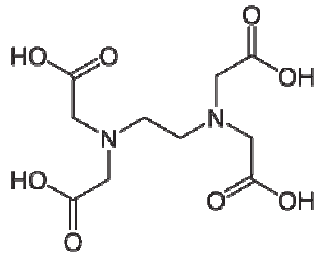
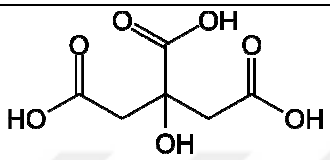
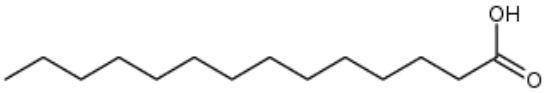
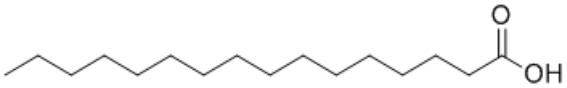
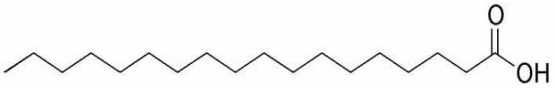
Chemicals	Structure	Supplier
Sodium Laureth Ether Sulfate (SLES)		EVYAP
CAPB (Cocamidopropyl Betaine)		EVYAP
Sodium Hydroxide (NaOH)		J.T.Baker
Potassium Hydroxide (KOH)		Sigma-Aldrich
AMIDET (PEG-4RAPESEEDAMIDE)		Kao Chemicals
Sodium Chloride (NaCl)	Na-Cl	Sigma-Aldrich
Glycerol (C_3H_8OH)		EVYAP

Table 3.1. Structures and suppliers of chemicals used in this study (Contd.)

Ethylenediaminetetraacetic acid (EDTA)		EVYAP
Citric Acid ($C_6H_8O_7$)		Sigma-Aldrich
Cinnamon Oil	-	Arifoğlu
Lavender Oil	-	Arifoğlu
Myristic Acid		EVYAP
Palmitic Acid		EVYAP
Stearic Acid		EVYAP
Lauric Acid		EVYAP

3.2. METHODS

3.2.1. Rheometer

Viscosity is a measure of a fluid's resistance to flow. The viscosities of liquid soap samples were measured with Brookfield DV-III Ultra Programmable Rheometer (Figure 3.1), which measures fluid parameters of shear stress and viscosity at given shear Rates and RPMs.

Figure 3.1. Brookfield Rheometer DC- III Ultra

The principle of operation of the DV-III Ultra is to drive a spindle, which is immersed in the test fluid through a calibrated spring. The viscous drag of the fluid against the spindle is measured by the spring deflection. Spring deflection is measured with a rotary transducer.

In this project, the samples had different viscosities and the range of the values were very high. The Rheometer can detect from 0.4 to 50000 cP but at specific RPM levels. When the RPM is decreased to low levels, very viscous substances could be measured. Hence, 0.05 to 0.3 RPMs were used in viscous samples and 1 to 2 RPMs were used in less viscous samples. For the calibration, 0.4 µL of water was added into the cap of the rheometer and then viscosity was measured at 70 RPM and at room temperature. According to the measured viscosity value, a correction is performed.

Finally for the measurement of the samples, 0.4 µL of sample was inserted into the cap of the rheometer and the viscosity measurement was performed at a specific RPM as described previously.

3.2.2. pH Meter

pH is a scale, which indicates the acidity or alkalinity of a solution. A pH meter measures the voltage difference between a reference solution in the probe and the solution it is immersed in. The voltage is translated to a pH reading and it is read from electronic control unit screen. pH is an essential property in many applications especially for skin care products. The pH of skin is 5.5 so the pH of the product must be skin friendly otherwise, it could be corrosive and harmful. For this study, the pH of the samples were measured by using a benchtop Hanna Instruments pH Meter (Figure 3.2).

Figure 3.2. Hanna instruments pH meter

3.2.3. Densitometer

Densitometer is a device to measure turbidity ,which is belonged to cell suspension of 0.3–5.0 McFarland units (Figure 3.3).Although the device has the ability for detecting concentration up to 15 McFarland, higher concentrations are detected with big standard deviations.

Densitometer is commonly used in the analysis of cell concentrations of bacteria during fermentation process, tolerance of antibiotics and microorganism characterization. The working principle relies on the measurement of the optical density of the samples, which has specific turbidity levels with respect to concentration at 565 nm. The optical density are converted to McFarland unit form and displayed on the device. 0.5 McF is equal to $1x10^8$ cfu/ml. At least 2 mL of sample is prepared in a tube and put into the cap of the device. The McF unit appears on the screen of the device as measurement concludes.

Figure 3.3. Densiometer

3.2.4. Soap Formulation Software

In this project, the natural and fatty acid soaps were synthesized and the formulations were generated such that the properties such as bubbling, cleansing, lathering etc. sort together with public needs. For this purpose, a dedicated software (soapcalc.net) was used to formulate the final product. The software works in a trial and error method. The fatty acid or oil types and mass percentages are entered (Figure 3.4) and it calculates the final properties of the corresponding soap (hardness, conditioning, bubbling, cleansing, lathering etc.) (Figure 3.5).Additionally, it calculates the required alkali amount for the soap making process by considering the SAP (saponification) values of oils, fats, and waxes etc., which are tabulated by Randall Engel. The figure 3.4 shows the software, which was used during this study.

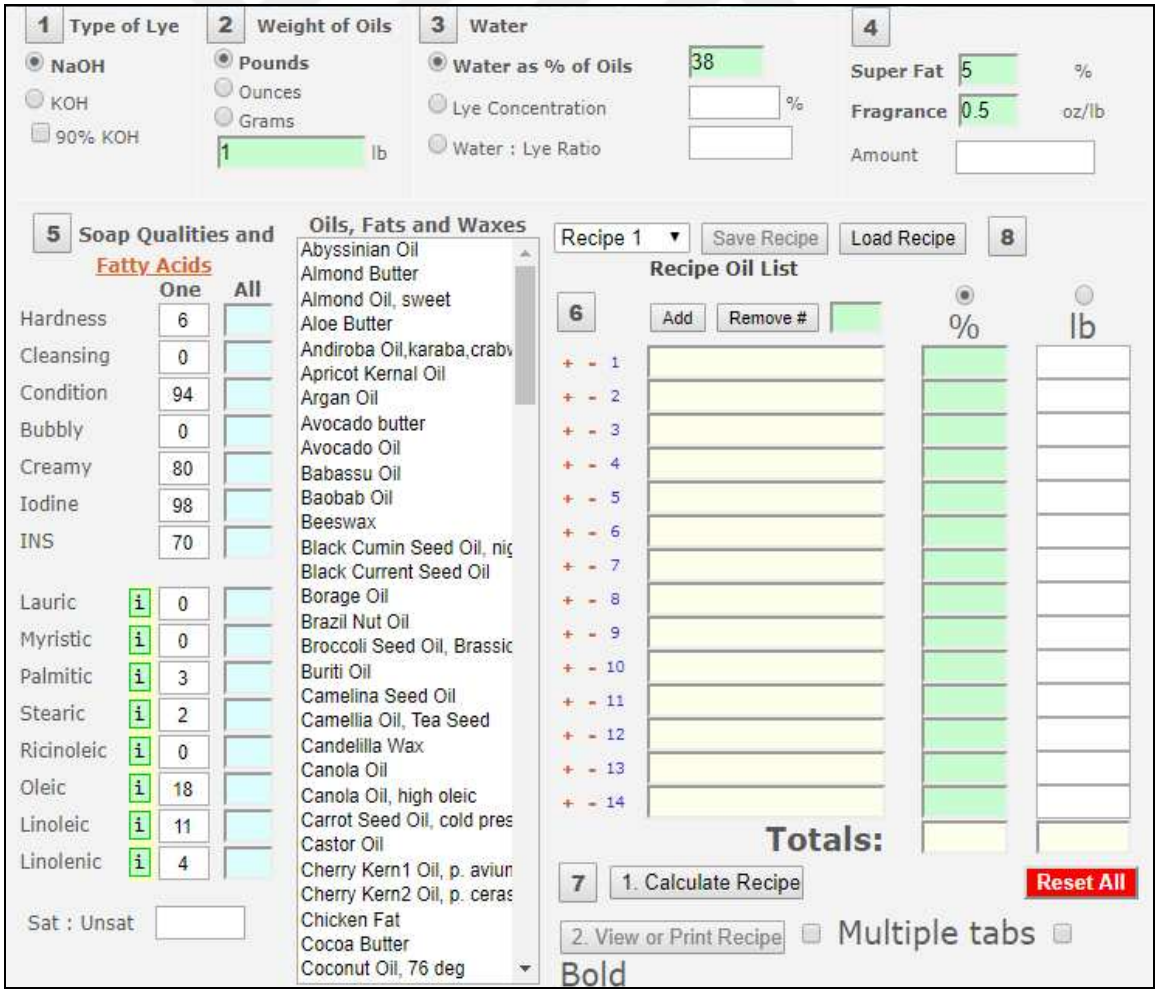
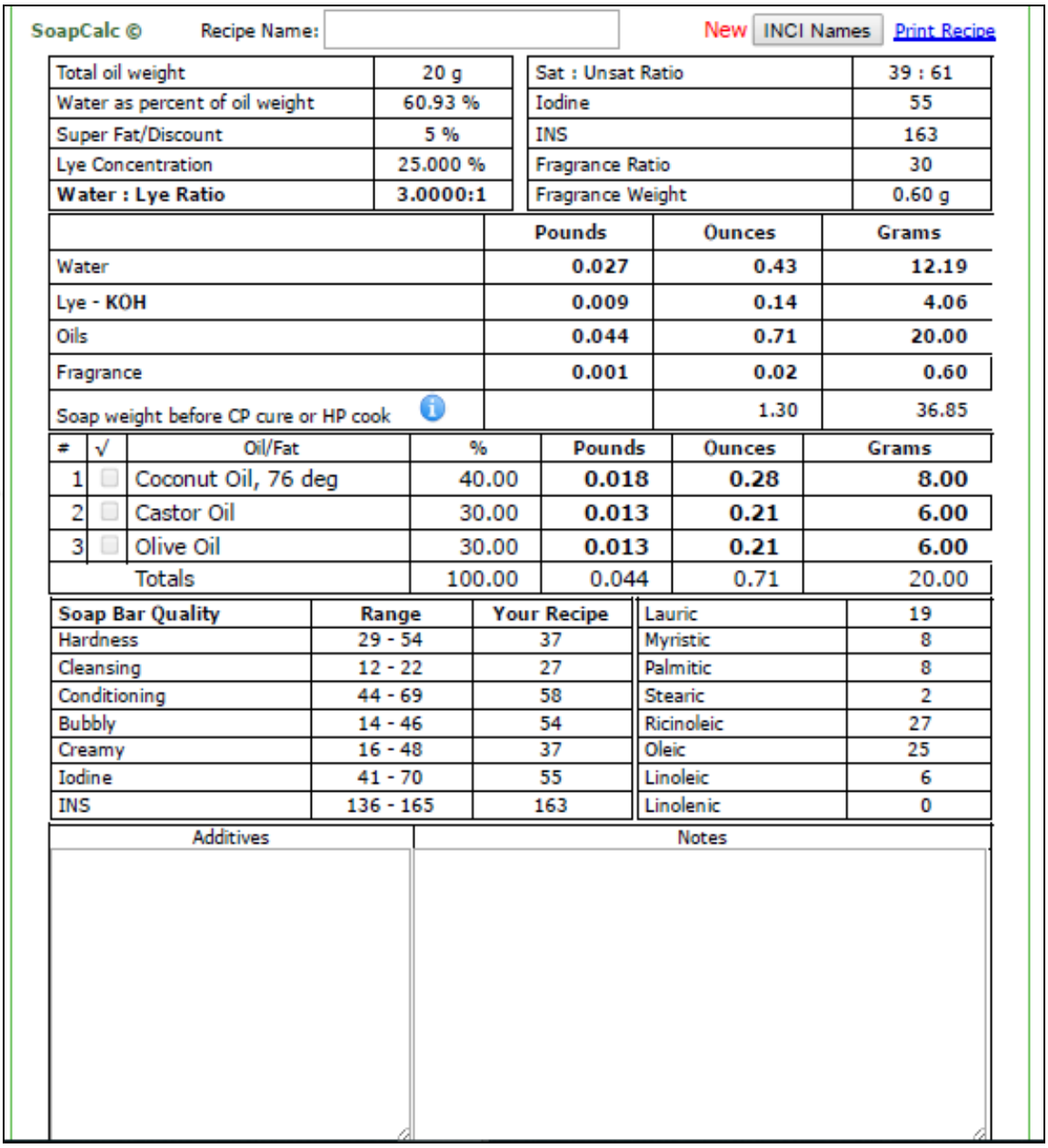


Figure 3.4. SoapCalc software



SoapCalc © Recipe Name: New INCI Names Print Recipe

Total oil weight	20 g	Sat : Unsat Ratio	39 : 61
Water as percent of oil weight	60.93 %	Iodine	55
Super Fat/Discount	5 %	INS	163
Lye Concentration	25.000 %	Fragrance Ratio	30
Water : Lye Ratio	**3.0000:1**	Fragrance Weight	0.60 g

	Pounds	Ounces	Grams
Water	**0.027**	**0.43**	**12.19**
Lye - **KOH**	**0.009**	**0.14**	**4.06**
Oils	**0.044**	**0.71**	**20.00**
Fragrance	**0.001**	**0.02**	**0.60**
Soap weight before CP cure or HP cook		1.30	36.85

#	√	Oil/Fat	%	Pounds	Ounces	Grams
1	☐	Coconut Oil, 76 deg	40.00	**0.018**	**0.28**	**8.00**
2	☐	Castor Oil	30.00	**0.013**	**0.21**	**6.00**
3	☐	Olive Oil	30.00	**0.013**	**0.21**	**6.00**
		Totals	100.00	0.044	0.71	20.00

Soap Bar Quality	Range	Your Recipe		
Hardness	29 - 54	37	Lauric	19
Cleansing	12 - 22	27	Myristic	8
Conditioning	44 - 69	58	Palmitic	8
Bubbly	14 - 46	54	Stearic	2
Creamy	16 - 48	37	Ricinoleic	27
Iodine	41 - 70	55	Oleic	25
INS	136 - 165	163	Linoleic	6
			Linolenic	0

Additives	Notes

Figure 3.5. SoapCalc property screen

3.2.5. Foaming Test

Foaming is an important for property for surfactant involved customer products. Even foaming seems advantageous, this property is not preferred for some products. If the soap is considered, high foaming capacity and stability are necessities for customer pleasure. However, the dishwasher detergents or motor oils are produced with less foaming capacity in order to get high efficiency.

The foaming test measures the foaming level and the stability of the soap or detergent in question with respect to time. 2 grams of soap sample was weighed and transferred to a 600 mL beaker. Then, 250 mL of tap water was added into the beaker and the solution was poured from 55 cm high and the foam level and the liquid level were measured by using a ruler at 3, 5 and 8 minutes later. The foam stability was calculated by the ratio of liquid level to foam level.

Figure 3.6. Foaming test

3.2.6. Antibacterial Assay

Antibacterial assay was divided into several sub phases such as agar preparation, bacteria cultivation, well plate-based method and inhibition zone measurement.

3.2.6.1. Agar Preparation

Agar plates were prepared by Tryptonic Agar. 50g of Tryptonic Agar were weighed and transferred to 1 L of distilled water then, exposed to 70 ºC in an incubator for 15 minutes. After that, the agar solution was poured to the empty plates and stored at 4 ºC.

Figure 3.7. Agar plate

3.2.6.2. Preparation of Bacteria

The bacteria, which were cultivated in lag phase were collected from the source. The solid bacteria was dissolved in PBS, which is used as a carrier fluid. The final concentration was arranged via densitometer either by adding extra PBS solution or adding solid bacteria depending on the case. For all antibacterial assay in this study, the final concentration was arranged as $1x10^8$ cfu/mL.

3.2.6.3. Agar Well Plate Diffusion Method

The bacteria suspension for, which the concentration was adjusted previously spread on the agar plate by cotton swap stick (Figure 3.8). After the inoculation, the agar plates were scraped by sterilized glass Pasteur pipets. Then, 70 µL of samples were transferred to the wells. After transferring, the plates were kept to 36 ºC for 24 hours and the inhibition zones were measured (Figure 3.9).

Figure 3.8. Addition of bacteria on agar plate

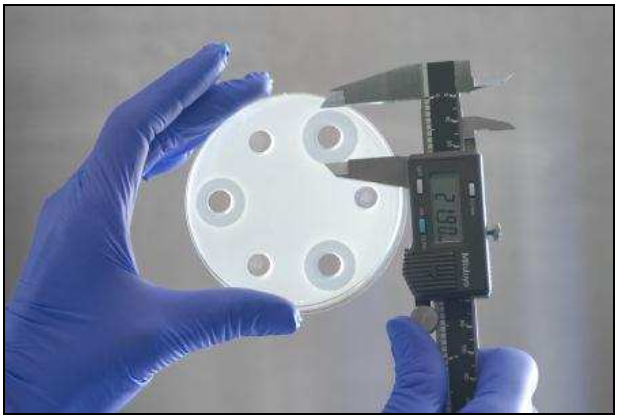


Figure 3.9. Inhibition zone recording

3.2.7. Sensory Test

Sensory test is an analysis, which measures the customer satisfaction and contentedness of consumer with a product (Figure 3.10). The statistical data, which are gained from volunteers feedbacks are generally used for the improvement and reformation of the product. Sensory test is used in many area such as food products, personal care products etc. and each specific product requires corresponding exclusive questions to be asked. For this study, liquid hand soap related properties were analyzed such as foaming capacity, viscosity, appearance, odor and cleansing by using panel test. Additionally, demographic questions such as gender, age etc. were also added in order to classify the results.

Figure 3.10. Sensory test[73]

3.2.8. Accelerated Stability Test

Stability test is a study to observe products shelf life at specific conditions. However, accelerated stability test gives approximate idea about shelf life of the product within shortened period of time. An average stability test lasts roughly 3 months to 6 months while accelerated stability test lasts just 10 days and yield the necessary information about the product. The test consist of 4 cycles and for each cycle, initially the product is kept at 45 ^{o}C for 24 h. Later on, the ambient temperature is altered to 4 ^{o}C and again the product is kept at that temperature for 24 h. By doing this, the product come up against possible extreme temperatures consecutively at a short period of time. At the end of the cycles, the viscosity and pH of the product were measured along with the observation of odor and color.

4. SYNTHESIS OF SOAPS AND CHARACTERIZATION METHODS

For this study, synthetic surfactant bearing (LS), fatty acids derived (FA) and conventional liquid soaps (Natural) were prepared and in this chapter, the details of preparation, optimization and characterization steps for the samples are given.

4.1. SYNTHETIC SURFACTANT SOAP (LS)

Liquid hand soap formula was designed shown below.

Table 4.1. The formulation of LS

Chemical	**Activity/Effect**
Distilled Water	Base Fluid
Sodium Lauryl Ether Sulfate (SLES)	Surfactant/Cleansing
CAPB	Surfactant/Cleansing
Tetra Sodium Edta	Chelating Agent
Glycerin	Conditioner
Citric Acid	pH Regulator
NaCl	Thickener
PEG-4 RAPESEEDAMIDE	Thickener

Figure 4.1. Preparation of base LS

Figure 4.2. Liquid hand soap sample (LS)

4.1.1. Adjusting the Viscosity of LS

The recommended formulation of LS, which is given in Table 4.1 was incapable for the formation of a viscous product. As a consequence, some variations in the formulation was performed in order to optimize the viscosity of base LS. PEG-4RAPESEEDAMIDE is an imperative thickening agent for the viscosity of cosmetic formulations. In the literature, synergic effect of PEG-4 RAPESEEDAMIDE derivatives and salt solutions was shown to enhance the viscosity of soap products [74].Thus, the effect of PEG-4 RAPESEEDAMIDE over the viscosity of water was analyzed by changing the corresponding mass percentage within the formulation at various salt concentrations and intrinsic viscosity of samples were measured. The results indicate, an increase in the concentration of PEG-4 RAPESEEDAMIDE from 0.5 wt. percent to 5 wt. percent leads to a linear enhancement in the viscosity of water as salt concentration simultaneously increases from 1.0 wt. percent to 2.0 wt. percent (Table 4.2 and Figure 4.3),which clearly indicates the synergic effect of salt and PEG-4.

Table 4.2. Intrinsic viscosity values for PEG-4 RAPESEEDAMIDE and NaCl

	NaCl Concentration (wt. %)		
PEG-4 RAPESEEDAMIDE (wt. %)	**1.0**	**1.5**	**2.0**
0.5	1.17 s	1.17 s	1.24 s
1.0	1.34 s	1.34 s	1.42 s
2.0	1.65 s	1.92 s	2.1 s
5.0	3.39 s	3.39 s	3.55 s

Considering the intrinsic viscosity results and observations, although the simultaneous enhancement in the concentrations of salt and PEG-4 RAPESEEDAMIDE increases the viscosity of the base fluid, the resulting viscosity and thickness of the base fluid was inadequate when compared with commercial liquid soap products.

Therefore, the concentration of salt further increased to 2.5 wt. percent while keeping the PEG-4 RAPESEEDAMIDE concentration constant (1 wt. percent as given in the recommended formulation) due to economic feasibility. For the measurement of the viscosity of base LS, samples were prepared as stated in the previous section (Table 4.1) and the salt concentration was further increased to 2.5 wt. percent (1.25 g NaCl in total) by the addition of solid salt crystals to the soap samples under stirring. As shown in Table 4.3, the average viscosity of base LS was found as 2845 cP, which is comparably similar to the viscosity of commercial liquid hand soap. As a consequence, in order to enhance the thickness and viscosity of base LS samples it was decided to introduce 2.5 wt. percent of salt simultaneously with 1 wt. percent of PEG-4 within formulations.

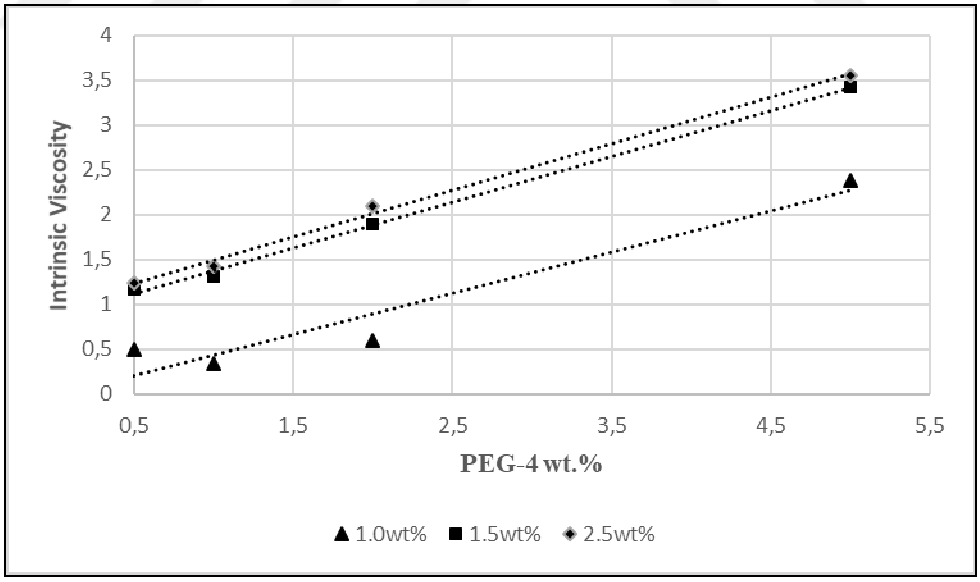


Figure 4.3. Intrinsic viscosity of LS at various PEG-4 RAPESEEDAMIDE and salt concentrations wt. percent

Table 4.3. Viscosity measurements of base LS samples

Sample	Viscosity (cP)	RPM	Temperature (°C)	Torque (%)	Viscosity Average (cP)
1.25 g 1st Trial	2804	0.01	24.1	16.2	2845.67
1.25 g 2nd Trial	2978	0.01	24.1	17.1	
1.25 g 3rd Trial	2755	0.01	24.1	15.9	
Commercial LS 1st Trial	2324	0.02	25.7	28.2	2474.67
Commercial LS 2nd Trial	2645	0.02	25.7	30.5	
Commercial LS 3rd Trial	2455	0.02	25.7	28.7	

4.1.2. Selection of Antibacterial Agents

The essential aim of this study is to replace hazardous chemicals such as triclosan, triclocarban etc., which are for the most part used within commercial household products by natural antibacterial agents. For this purpose, some suitable and alternative natural agents, which possess antibacterial properties were selected. According to the studies given in the literature, the most efficient natural antibacterial agents are lavender oil, cinnamon oil, tea tree oil, thyme oil and linseed [62, 71, 75, 76].

Initially, the physical interaction between the liquid hand soap (LS) and the essential oils was observed by incorporating 2 wt. percent of oil within soap samples. The final samples were analyzed according to the formation of different phases and turbidity. The results indicated homogenous liquid soap samples in the presence of lavender oil and cinnamon oil while linseed oil and tea tree oil led to the formation of a two phase suspension with high turbidity (Figure 4.4). Additionally, the presence of thyme oil also disrupted the stability of LS and caused turbidity. As a consequence, in order to replace triclosan and triclocarban, lavender oil and cinnamon oil were selected as suitable candidates for antibacterial activity.

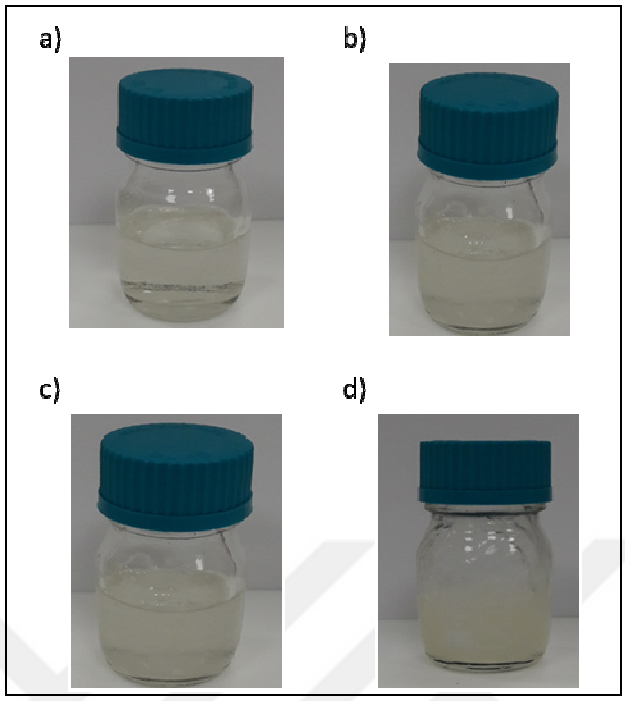


Figure 4.4. The addition of various essential oils within LS samples
a) Lavender Oil b) Cinnamon Oil c) Thyme Oil d) Tea Tree Oil

4.2. FATTY ACID BASED LIQUID SOAP (FA)

Liquid hand soap was also prepared by using fatty acids and all corresponding properties were compared with synthetic surfactant soap (LS). The formulation of the soap was again performed by using SoapCalc software according to the contents and individual properties of the fatty acids. For instance, lauric acid and myristic acid aids in the cleansing and foaming of the final product while palmitic acid and stearic acid enhances hardness and creaminess. After successive trials, final formulation of the fatty acid based liquid soap is given in Table 4.4

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Table 4.4. The synthesis of fatty acid soap

Chemicals	**Amount (g)**
Distilled Water	4.76
Myristic Acid	1.9
Palmitic Acid	0.7
Stearic Acid	2.3
Lauric Acid	5.3
KOH	3.57

For the preparation of the fatty acid based liquid hand soap, 10.2 g of a solid fatty acid mixture was placed in a jacketed reactor where the outside temperature was maintained at 70 ^{o}C by using a water circulator bath. At this temperature, the solid fatty acid mixture melts as all the ingredients have a melting point below this temperature. The obtained liquid mixture of fatty acids then mixed by using a mechanical stirrer set at 250 RPM and potassium hydroxide base solution was added in order to initiate the saponification reaction. After 30 minutes, the reaction between fatty acids and potassium hydroxide was completed and the soap paste, which will be used for the preparation of liquid hand soap was taken out from the reactor and cooled down to room temperature.

After the preparation of the soap paste, it is necessary to regulate the dilution medium and conditions of the paste. The dilution medium for the fatty acid soap paste was principally distilled water. However, it was important to analyze the mixing rate and the temperature of the medium. Experimental conditions are tabulated in Table 4.5.

Table 4.5. Various mixing conditions for the preparation of fatty acids based liquid soap

	Alternative 1	**Alternative 2**
Mixing Temperature	Room Temperature	70 ºC
Mixing Rate	Without mixing	400 RPM

For the mixing process, 4 different procedures were tried and the results indicated mixing at a rate of 400 RPM via mechanical stirrer yields uniform soap sample as compared to static dissolution. On the other hand, the effect of temperature could not be observed for the samples prepared at room temperature and at 70 ºC. Thus for the final mixing procedure, it was decided to perform all the steps at room temperature in order to be cost efficient.

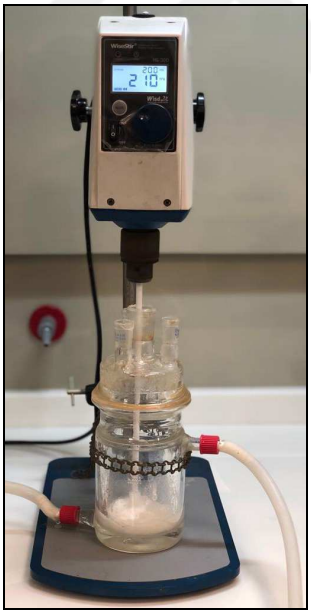


Figure 4.5. The experimental setup used for the preparation of fatty acids based liquid hand soap

Table 4.6. Formulation of fatty acid based soap

Chemical	**Percent Weight**	**Activity/Effect**
Distilled Water	73.5	Base Fluid
Soap Paste	15.00	Surfactant/Cleansing
Tetra Sodium Edta	0.05	Chelating Agent
Glycerin	0.5	Conditioner
Suttocide A	0.1	Preservative
Lauric Acid	0.05	pH Regulator
NaCl	2.5	Thickener
PEG-4 Rapeseedamıde	8.33	Thickener

For the preparation of final liquid hand soap sample, various additives were mixed with the obtained soap paste according to the percentages given in Table 4.6. Briefly, 10 g of soap paste was dissolved in distilled water and PEG-4 Rapeseedamide and sodium chloride was added to adjust the thickness (viscosity) of the sample. Later on, EDTA and glycerin was added to the soap sample and finally the pH of the soap was adjusted to 9.5 by using lauric acid, which also draws away the excess base from the soap sample. For the preparation of fatty acids based antibacterial liquid hand soap, essential oils such as cinnamon and lavender oils were mixed with the formed base liquid soap and the final concentration of the essential oils was adjusted to 2 wt. percent (Figure 4.6).

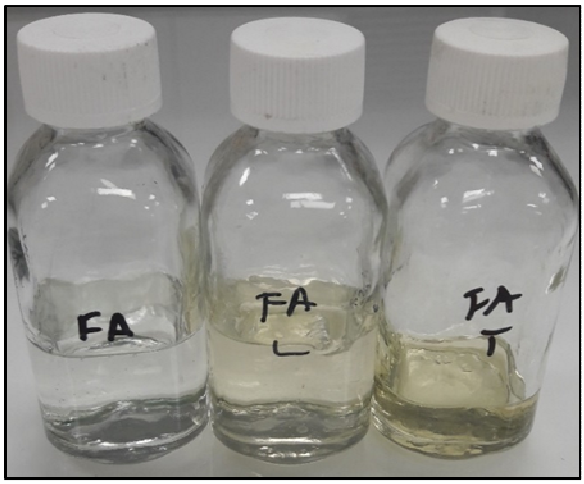


Figure 4.6. Fatty acids based liquid soap (FA) – (pure, lavender, and cinnamon)

4.2.1. The Adjustment of Formulation for Fatty acids Based Liquid Soap

The Table 4.4. shows the final formulation of the fatty acid based liquid hand soap but initially some trials were performed in order to optimize the formulation in order to achieve the best performance considering the viscosity, foaming and stability of the soap. The theoretical amount of base necessary for the complete reaction was calculated by using the SoapCalc. However, the liquid fatty acid soap obtained by using this amount was not transparent and tend to precipitate due to lack of stability. The reason of this turbidity can be explained due to the presence of exact stoichiometric amounts within the reaction. When an excess amount (1.5 times) of KOH was used, the turbidity problem was overcame and homogeneity was achieved.

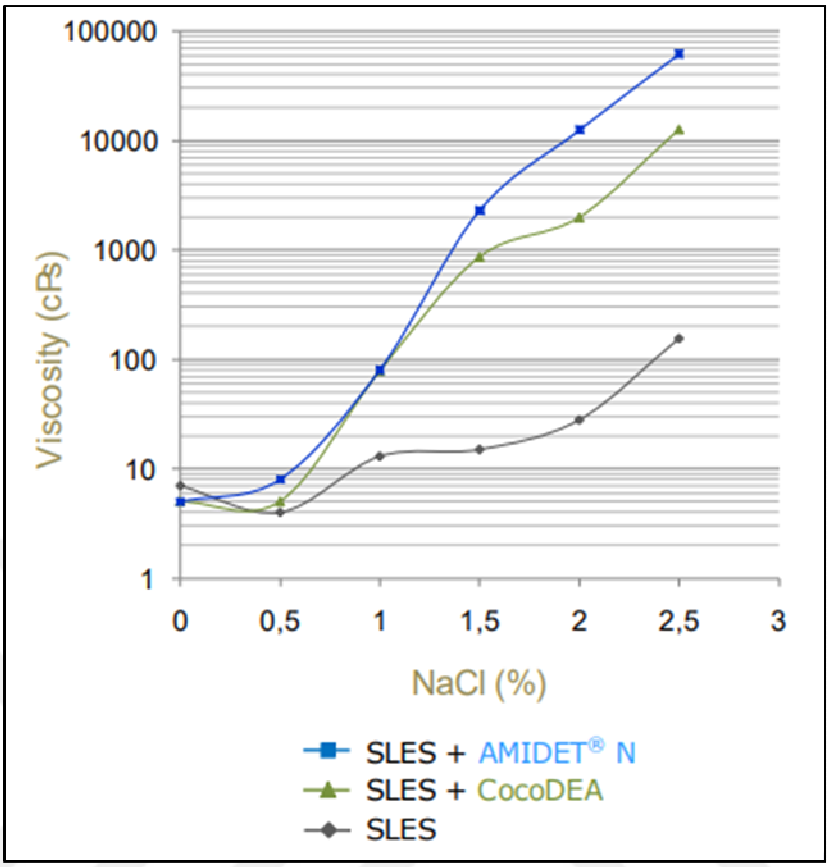


Figure 4.7. The effect of salt addition to thickeners on viscosity enhancement

The viscosity of fatty acids based liquid hand soap was regulated by PEG-4 Rapeseedamide and sodium chloride as shown in Table 4.7. The manufacturer of the thickener states that PEG-4 Rapeseedamide is capable of enhancing the viscosity of products more than SLES and CAPB. The Figure 4.7 shows the synergetic effect of thickeners (SLES, SLES plus CocoDEA (CAPB) and SLES plus AMIDET N (PEG-4 Rapeseedamide) and salt on the viscosity of samples. It is clearly seen from the figure that the addition of sodium chloride along with various thickeners enhances the viscosity remarkably.

According to Figure 4.7, the maximum viscosity is achieved when 2.5 wt. percent sodium chloride is applied along with SLES/Thickener ratio of 3:1. For these measurements the active matter (a.m.), which is the soap paste is fixed at 15 wt. percent. As a consequence, for the formulation of fatty acids based liquid hand soap, the paste concentration was chosen as 15 wt. percent. For comparison, 25 wt. percent soap paste formulation was also prepared and the corresponding viscosity measurements were performed.

The essential purpose of this step is to optimize the viscosity of the product by using the synergetic effect of the salt and thickener to ensure product quality while using less amount of soap paste to minimize the cost.

Table 4.7 shows the viscosity measurements for the soap samples, which was prepared with 25 wt. percent soap paste and various amounts of salt and/or PEG-4 Rapeseedamide. The results indicate that the presence of PEG-4 Rapeseedamide and salt individually enhances the viscosity of the samples. Additionally, an increase in the amount of both the salt and the thickener increases the viscosity of the soap. When salt and PEG-4 Rapeseedamide is applied in combination (8.33 wt. percent and 2.5 wt. percent respectively), the viscosity of the product increases dramatically up to 182.7 cP. This thickness in the sample could not be achieved solely with salt and PEG-4 Rapeseedamide at any concentration, which illustrates the synergetic effect as shown in Figure 4.7.

Table 4.7. Viscosity measurements of samples containing 25 wt. percent soap paste and various compositions of PEG-4 Rapeseedamide and salt

PEG-4 Rapeseedamide (wt. %)	**Salt (wt. %)**	**Viscosity (cP)**
8.33	-	33.1
5	-	6.5
2.5	-	3.2
-	2.5	3.6
-	1.5	2.6
-	0.5	2.0
8.33	2.5	182.7
8.33	1.5	37.5
8.33	0.5	4.9
5	2.5	10.5
5	1.5	3.8
5	0.5	2.8

Table 4.8 shows the viscosity measurements for the soap samples ,which was prepared with 15 wt. percent soap paste and various amounts of salt and/or PEG-4 Rapeseedamide. Again the addition of salt and PEG-4 Rapeseedamide individually enhances the viscosity of the samples and the synergetic effect is valid. Inherently, the viscosity values obtained at this concentration of the soap paste is lower than higher concentration. However, when the samples were prepared by using 15 wt. percent of soap paste along with 8.33 wt. percent PEG-4 Rapeseedamide and 2.5 wt. percent of salt, the viscosity of the sample reaches 550.4 cP, which is the highest value of viscosity obtained for fatty acids based liquid hand soaps. It should also be noted that when the liquid hand soap is prepared by using 15 wt. percent soap paste, the cost of the product also decreases, which makes the product more customer friendly. Although the viscosity (thickness) of the samples was adequate, it was comparably lower than the values obtained for LS.

Table 4.8. Viscosity measurements of samples containing 15 wt. percent soap paste and various compositions of PEG-4 Rapeseedamide and Salt

PEG-4 Rapeseedamide (wt. %)	**Salt (wt. %)**	**Viscosity (cP)**
5	-	18.2
3	-	3.8
-	2.5	3.5
-	1.5	2.4
-	0.5	2.3
8.33	2.5	550.4
5	2.5	36.9
5	1.5	5.3
5	0.5	2.7
3	2.5	10.3
3	1.5	2.5
3	0.5	2.2

As the viscosity and the dilution factor of the fatty acid based liquid soap were regulated, the other ingredients such as chelating agent and pH regulator was added to the soap samples. For the regulation of pH, lauric acid was used as it is also capable of reacting with excess base present within the samples. As an alternative citric acid was also used however those samples showed turbidity. In addition, glycerin amount was also changed and it was seen that when the samples contain more than 0.5 wt. percent of glycerin, turbidity was inevitable.

4.3. NATURAL LIQUID SOAP

For comparison, another liquid hand soap (NLS) samples was prepared by using coconut oil, which is a natural essential oil. The preparation of the soap is slightly different than LS and FAS according to the reaction pathway and its by-products. LS contains only distinct chemicals and surfactant, which yields only the soap sample and no by-products. Although the free fatty acid soap was produced as a result of saponification reaction, there was no by-products due to free fatty acid reactants. On the other hand, during the preparation of natural oil based liquid soap, both glycerol and tripalmitin were produced as by products after the saponification reaction.

Table 4.9. The synthesis of natural liquid soap

Chemicals	**Amount (g)**
Distilled Water	4.88
Coconut Oil	10
KOH(Potassium Hydroxide)	2.44

For the production, SoapCalc software was used in order to determine the required base amount as well as final soap properties as indicated previously. Briefly, 10 g of coconut oil was placed inside a jacketed reactor maintained at 60 ^{o}C and waited till complete melting. This part is performed under constant mechanical stirring of 300 RPM.

After complete melting of the natural oil, the calculated base solution is added gradually to the reaction vessel (Table 4.9)

Finally, as shown in Table 4.10, 10 g of the soap paste was dissolved in water andPEG-4 Rapeseedamide was added along with NaCl to the soap sample in order to adjust viscosity (thickness) of the sample. After that, EDTA and glycerin was added to the mixture and the pH was adjusted to 9.5 by using lauric acid as it also contributes to the foaming of the liquid soap. For the preparation of fatty acids based antibacterial liquid hand soap, essential oils such as cinnamon and lavender oils were mixed with the formed base liquid soap and the final concentration of the essential oils was adjusted to 2 wt. percent (Figure 4.6).

Table 4.10. Formulation of natural liquid soap (NLS)

Chemical	**Percent Weight**	**Activity/Effect**
Distilled Water	71.8	Base Fluid
Soap Paste	15.00	Surfactant/Cleansing
Tetra Sodium Edta	0.05	Chelating Agent
Glycerin	0.5	Conditioner
Suttocide A	0.1	Preservative
Lauric Acid	0.05	pH Regulator
NaCl	2.5	Thickener
PEG-4 Rapeseedamide	10	Thickener

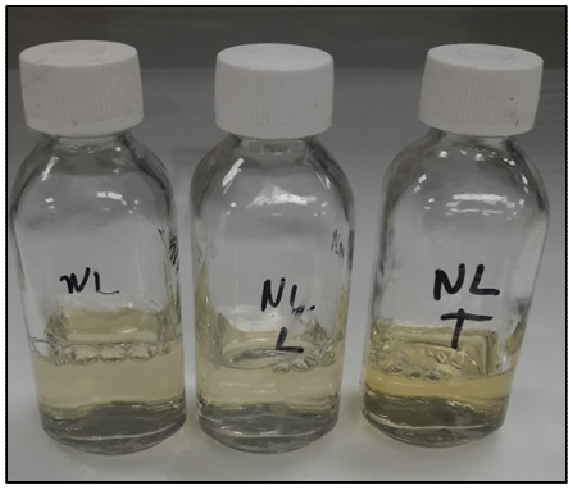


Figure 4.8. Natural liquid soap (NLS) – (pure, lavender, and cinnamon)

4.3.1. The Adjustment of Formulation for Natural Liquid Soap

The viscosity of the natural liquid soap samples were adjusted by using different concentrations of PEG-4 Rapeseedamide and/or salt.

Table 4.11. The effect of PEG-4 Rapeseedamide and salt concentrations on NLS viscosities

Salt / **PEG-4 Rapeseedamide**	**0 wt.%**	**1 wt.%**	**2 wt.%**	**3 wt.%**
0 wt.%	-		-	1.59 cP
3 wt.%	-	3.03 cP	-	3.72 cP
5 wt.%	-	3.21 cP	-	6.17 cP
10 wt.%	21.6 cP	7.48 cP	396.53 cP	495.2 cP

As shown in Table 4.11, the addition of salt and PEG-4 Rapeseedamide individually increases the viscosity of the soap samples. Additionally, when salt and PEG-4 Rapeseedamide is used together, the synergic effect of these to additives enhances the viscosity of the samples remarkably.

The highest viscosity is achieved when using 10 wt. percent of PEG-4 Rapeseedamide along with 3 wt. percent of salt. However the soap sample obtained by using these concentrations were not transparent. As a consequence for the final formulation 10 wt. percent of PEG-4 Rapeseedamide and a range of 2-3 wt. percent of salt was chosen.

4.4. NATURAL SOLID SOAP (NSS)

In order to compare liquid hand soaps, which were prepared by using 3 different routes, a natural solid antibacterial soap was also produced. The essential difference during the preparation was using NaOH rather KOH as it is capable of forming a solid state soap after saponification reaction.

Table 4.12. The synthesis of natural liquid soap

Chemicals	**Amount (g)**
Distilled Water	3.8
Coconut Oil	10
NaOH(Sodium Hydroxide)	1.74
Glycerin	2.5
Ethanol	5
Sucrose	2.5

As shown in Table 4.12, 10 g of Coconut oil was melted in a jacketed reactor at 60 ^{o}C and mixed at 300 RPM by using a mechanical stirrer. After complete melting of coconut oil, the calculated NaOH solution (by using SoapCalc) was added into reactor. After 20 minutes, ethanol, sucrose and glycerin were mixed and added into reactor. Finally, 2 percent essential oils (cinnamon and lavender oils) for a final concentration of 2 wt. percent were introduced into the soap and the product was cooled down at soap molds.

5. RESULTS AND DISCUSSION

5.1. VISCOSITY OF SOAP SAMPLES

5.1.1. Viscosity of LS

The viscosity of soap products is essential and viscous products are generally necessity for customer satisfaction. Additionally, effective cleaning requires sufficient contact time and viscous products stays on the surface of the skin much more than non-viscous alternatives.

The viscosity measurements of the samples were performed during 90 days in order to validate the stability of the products and also the viscosity of samples ,which were kept at lightless condition were measured as this technique is also performed by manufacturers for controlling the storage conditions and their possible corresponding effects on the products[77].

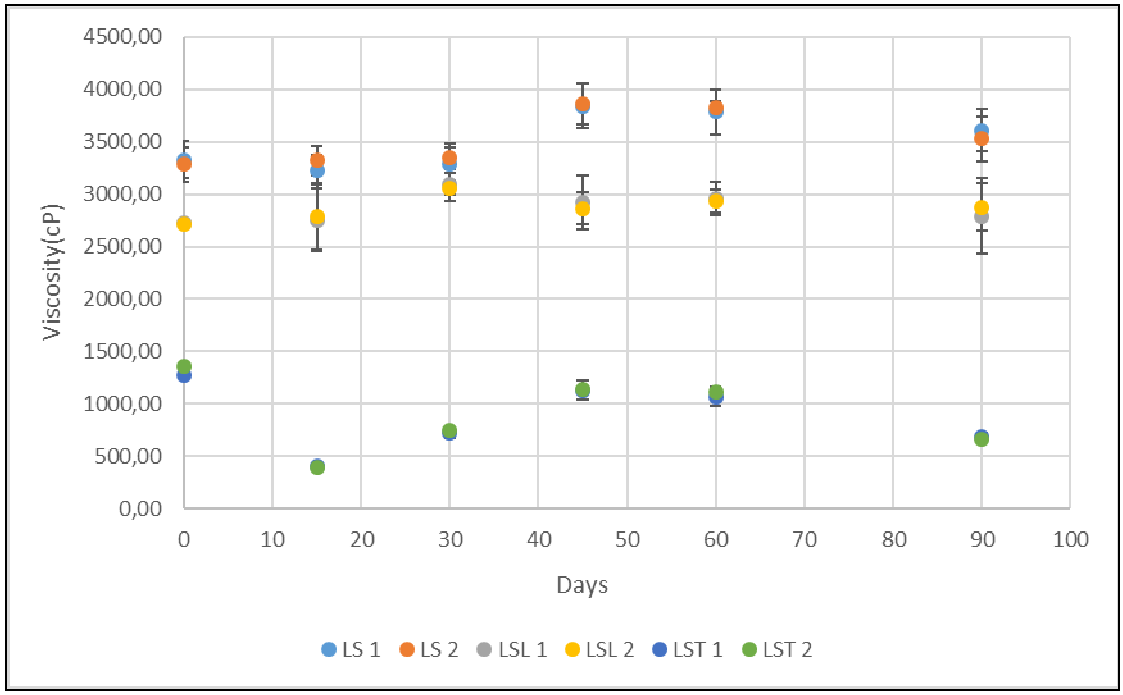


Figure 5.1. Viscosity of LSs (2wt. percent essential oils)

Figure 5.1 shows the viscosity measurements of LS samples, which contains 2 wt. percent of different essential oils in 90 days period. The results indicate that antibacterial agent (essential oils) free LS samples have viscosity of 3308 cP in average.

The addition of lavender oil decreased the viscosity of LS to roughly 2720 cP and the addition of cinnamon oil further decreased the viscosity to 1313 cP. It is obvious that used essential oils deteriorated the viscosity of the products. The analysis on the 15thday indicates the viscosity of bare LS and LSL stayed almost stable with 2.11 percent and 1.88 percent variance respectively. However, LST decreased considerably as compared to initial measurements and fluctuates for the rest of the analysis till 90th day but stays fairly stable. On the other hand, LS and LSL samples show almost constant viscosity values, which indicates stability over 3 month's shelf life. For the samples, which were not exposed to natural light and kept at dark yield average viscosity values of 3400, 2800 and 1100 cP for LS, LSL and LST samples respectively, which also indicates the nonexistent light exposure effect over the products stability.

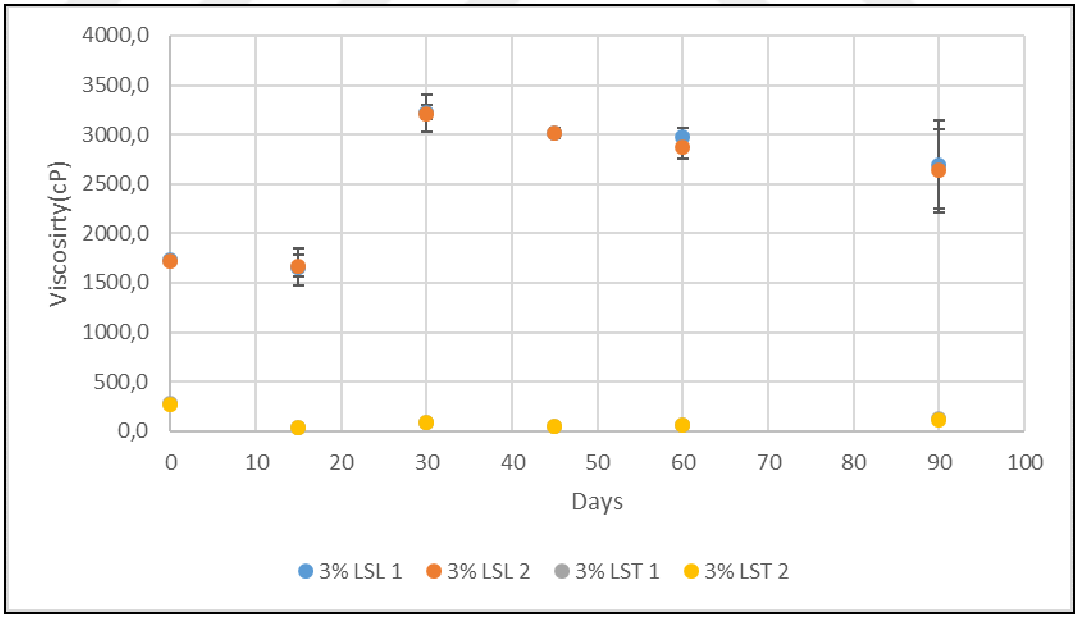


Figure 5.2. Viscosity of LSs (3 wt. percent essential oils)

When the essential oil amounts were increased to 3 wt. percent in order to bear more antibacterial activity (larger antibacterial inhibition zone), the viscosities of LSL stayed almost the same at an average value of 2535 cP while for LST samples the average viscosity was found to decrease to roughly 110 cP.

The viscosity of samples, which were held without light exposure showed viscosity values of 2575 and 145 cP for LSL and LST samples respectively indicating a stability over direct light exposure for products.

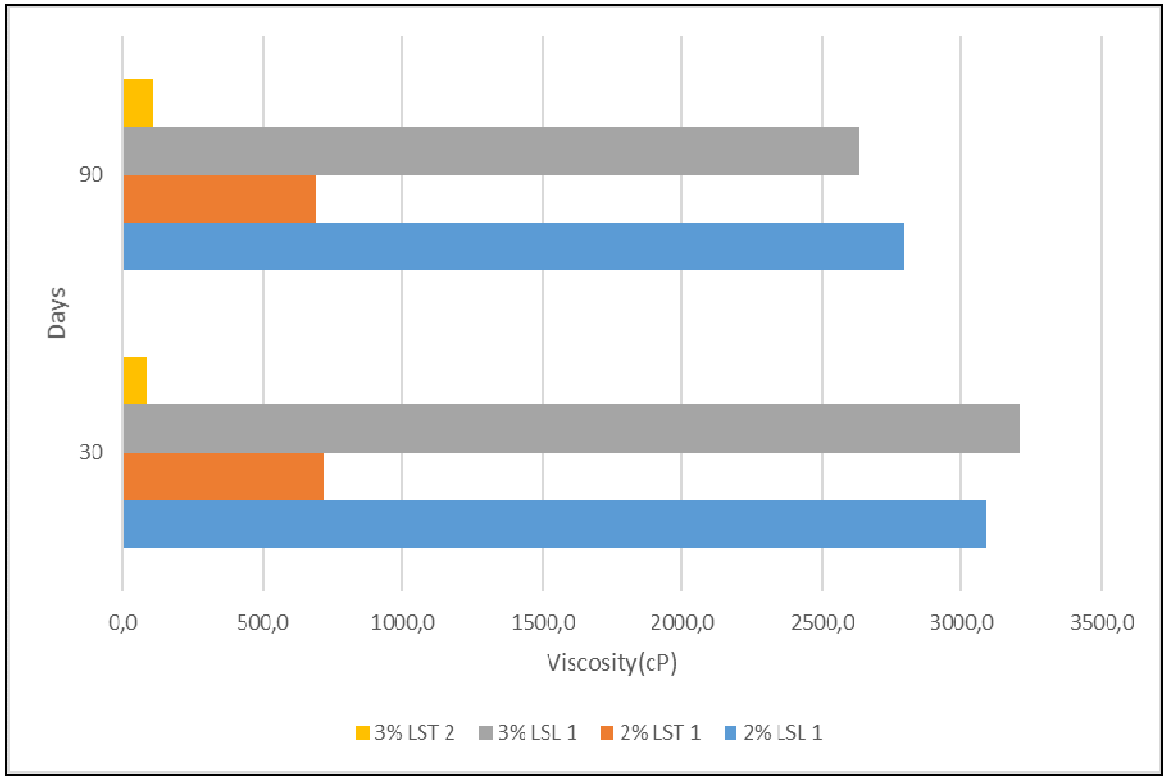


Figure 5.3. The viscosity of LS samples containing different concentrations of essential oils

Figure 5.3 shows the viscosity measurements for LS samples containing 2 wt. percent and 3 wt. percent of lavender and cinnamon oils as antibacterial agents. The results indicate an increase in the concentration of lavender oil did not influence the viscosity of the product whereas an increase in the cinnamon oil concentration decreases the viscosity of the samples remarkably.

As a consequence, it is also obvious that an addition of any essential oil decreases the viscosity of liquid soap samples, which was prepared by direct addition of surfactants.

There are not sufficient knowledge in the literature about the effect of essential oils over the properties of soaps. According Mei et. *al*, essential oils reduced the densities and viscosities of organic solvents up to 50 percent[78].

The reason of this deterioration in viscosity is due to the molecular interaction of essential oils with long chain organic compounds. The lavender oil and cinnamon oil have predominantly terpenes and the interaction of the terpenes are very weak with corresponding surfactant molecules present in LS, which leads to the decrease in viscosity of soap samples. It should also be noted that some fluctuations was observed during viscosity measurements especially at longer terms. This fluctuations were attributed to the volatility of essential oils, which lead to the enhancement of viscosity.

This argument is also in line with previous claim over the viscosity deterioration due to the addition of essential oils to soap samples. When essential oil concentration decreases due to volatility the viscosity of samples increases. Likewise when the concentration of essential oils increases from 2 wt. percent to 3 wt. percent the viscosity of samples nearly decreases.

5.1.2. Viscosity of Fatty Acids Based Soaps (FA) and Natural Liquid Soaps (NLS)

The viscosities of bare and essential oil containing fatty acids based liquid soaps (FA) were also measured at ambient and lightless conditions. Soap samples, which do not contain any additives showed viscosities around 730 cP. On the other hand, the addition of essential oils such as cinnamon and lavender oils decreased the viscosities of soap samples remarkably to 250 cP where the viscosities of FAL and FAT samples were comparable. This result is in agreement with viscosity measurements performed for LS samples. However, the decrease in viscosities of FA samples, which contain lavender oil is more pronounced than LSL. Similarly, the maximum viscosity deterioration was found for samples containing cinnamon oil following the stabilization of the viscosities after 60 days.

The results indicated that the thickness of bare FA samples remain almost constant after 60 days at around 580 cP while modified FA soap samples (FAL and FAT) showed viscosity enhancement during the initial 60 days possibly due to the volatility of essential oils from the soap samples after ,which viscosity remains constant around 500 – 650 cP.

This result in combination with previous findings indicated that liquid soap samples ,which contain essential oils as antibacterial agents may experience viscosity changes for a limited time after ,which thickness remains constant. Similar fluctuations of viscosities for cleansing formulations was also indicated by other studies[77]. The viscosities of fatty acids based soap samples, which were not exposed to ambient light conditions were also measured at 90th day. The viscosities were measured as 597 cP, 485 cP and 409 cP for FA, FAL and FAT samples respectively, which are comparable to the samples exposed to natural light.

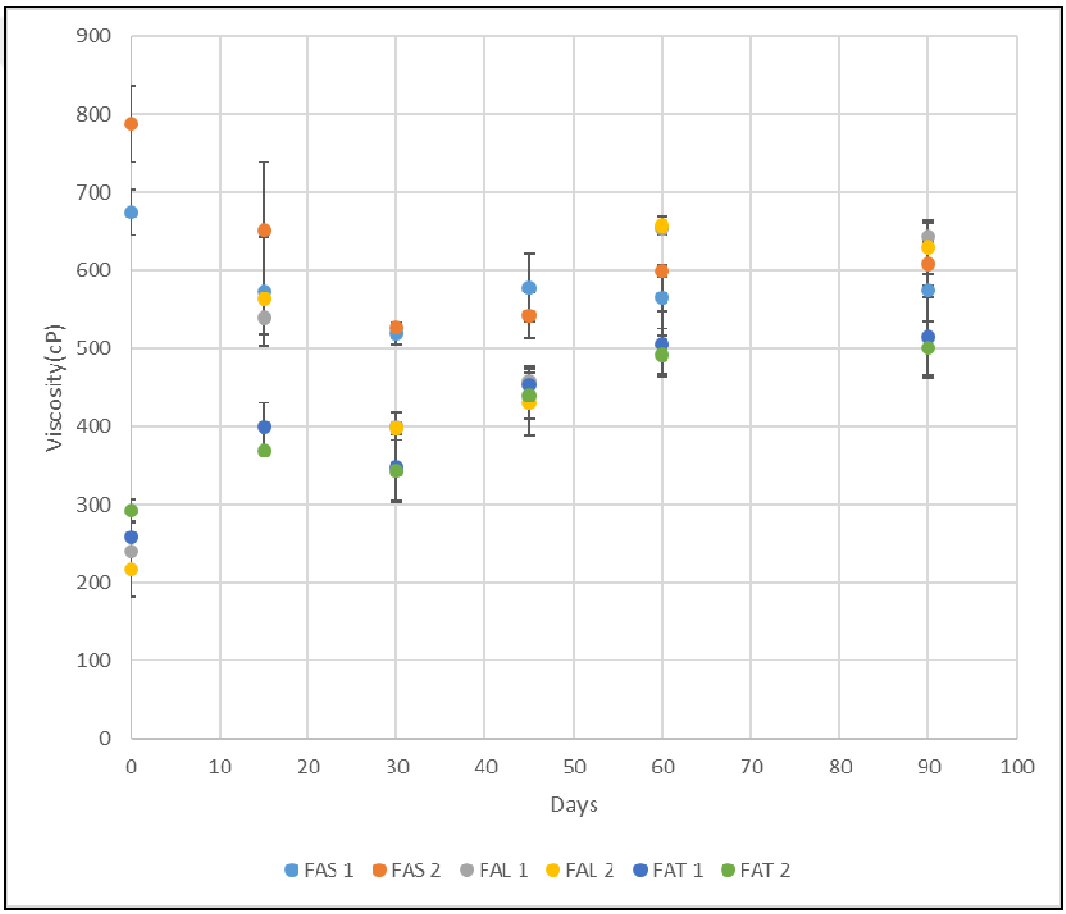


Figure 5.4. The Viscosity of fatty acids based soaps (FA) (2wt. percent essential oils)

The viscosities of bare and essential oil containing natural liquid soaps (NLS), which were exposed to ambient and lightless conditions were also measured for 90 days. The Figure 5.5 shows the viscosity behavior of the samples during 90 days period while exposed to natural light. Unlike other liquid soap samples, the viscosities of both bare and lavender oil

introduced NLS are initially close to each other as viscosities were measured as 172 cP and 128 cP respectively. On the other hand, the viscosity of NLT, which contains cinnamon oil was found as 27 cP, which is the lowest viscosity bearing sample among all formulations.

The viscosity deterioration upon addition of cinnamon oil to soap samples was validated for both LS and FAS samples previously and this result is also in line with those findings. Similar to FAS, the thickness of natural liquid soap samples again remain constant after 30 days and were comparable to viscosities of FAS samples.

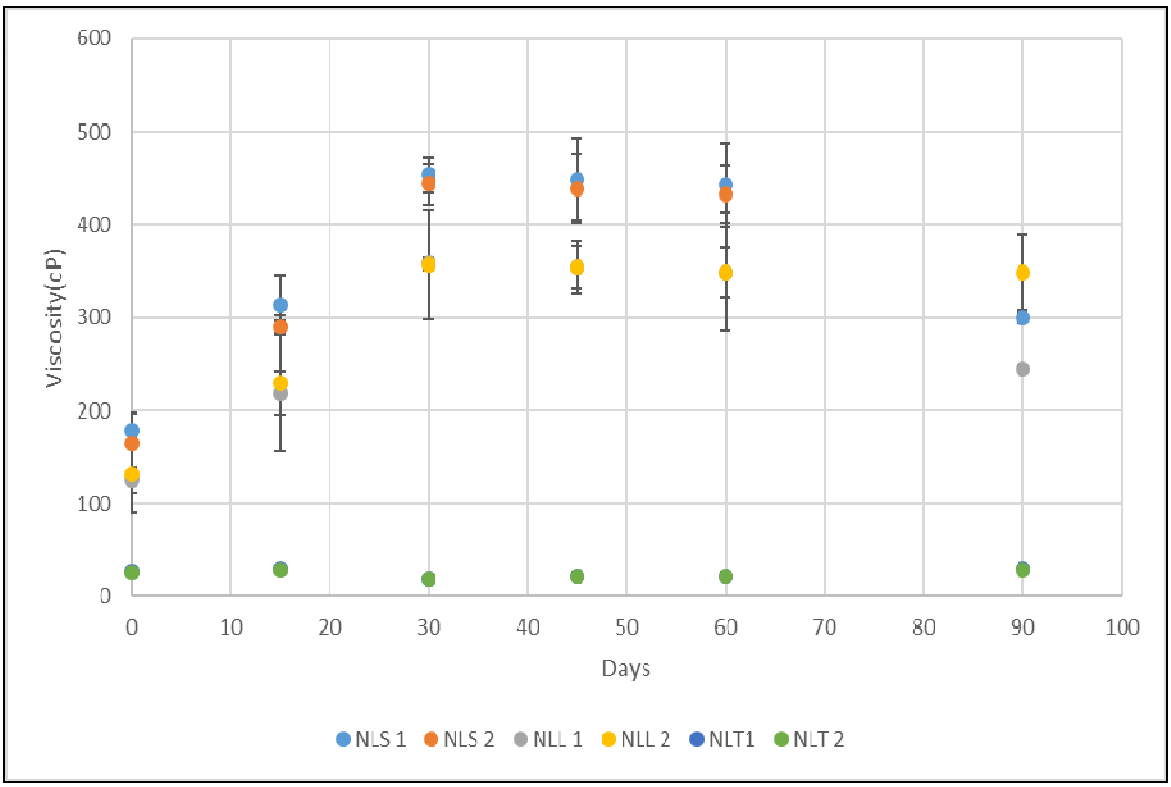


Figure 5.5. The viscosity of NLS samples

5.2. pH OF THE PRODUCTS

Health care and hygienic products such as shampoos, soaps, creams etc. must be manufactured under strict regulations. Along with the toxicity of chemicals within the formulations, the pH of the final product is an important factor. It is known that the pH of human skin is around 5.5 so the pH of the products should be regulated to 5.5 accordingly. Generally, the surfactant based liquid synthetic soaps are produced at pH 5.5 but the pH of natural liquid soaps are around 8.5-9.5 interval. In this study, the pH of LS, FAS and NLS samples were measured during 90 days by exposing to and without natural light.

5.2.1. pH of the LSs

The pH of LS samples were measured for both bare and essential oil containing samples. For comparison, the concentration of essential oils were selected as 2 wt. percent and 3 wt. percent. The Figure 5.6 shows the pH of LS samples during 90 days and it is clearly seen that the pH of bare LS samples are around 5.3 and remains almost constant for 90 days. The addition of lavender and cinnamon oils into LS samples decreased the pH slightly to the range of 5.05 – 5.20 (3 – 5 percent) and also stays constant during measurement interval.

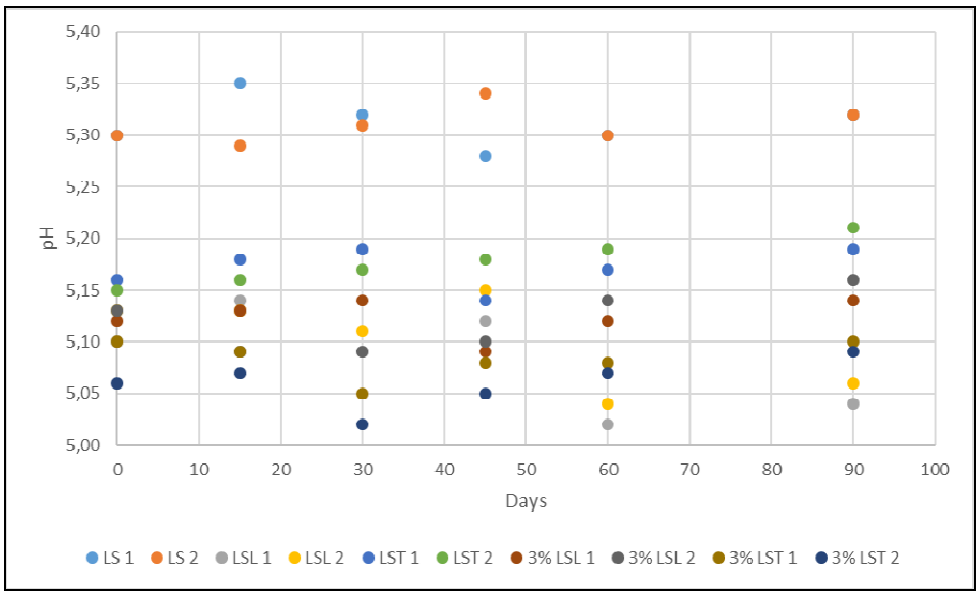


Figure 5.6. The pH of LS samples

The reduction for the samples, which contain lavender oil as antibacterial agents is higher than the ones containing cinnamon oil however this downtrend is not significant as the pH of bare LS is similar.

5.2.2. pH of the FAS and the NLS Samples

The pH of fatty acids based liquid soaps, which contain essential oils as antibacterial agents were also measured during 90 days by exposing to ambient conditions. As mentioned before, LS and FAS samples are quite different in terms of ingredients and synthesis methods.

LS is prepared by using synthetic surfactants such as SLES while FAS is prepared via saponification reaction of fatty acids with a strong base (KOH).

For the cleansing procedure where potassium soaps (liquid form) are used, the polar carboxylate end of the potassium salt is attracted towards water and should be suspended in water and repel all the remaining micelles in suspension. In order to have a stable suspension, the polar carboxylate end of the surfactant molecule should be charged and this can only be achieved at basic pH for anionic surfactants. When the synthesis pathway for FAS is considered, the saponification reaction is performed at high pH thus the soap has a basic pH at the end unlike LS samples. Figure 5.7 indicates that the pH of pure FAS samples are around 9.3 in average and during 90 days the pH remains almost constant as the change from the initial day is roughly 2.75 percent. When essential oils are introduced to the samples, the pH's are similar to the bare FAS and changes occurring during measurement are 2.13 percent and 0.75 percent for FAL and FAT samples respectively. These results indicate that the alkalinity of FAS samples are quite stable for several months and the use of essential oils as antibacterial agents did not influence the pH significantly.

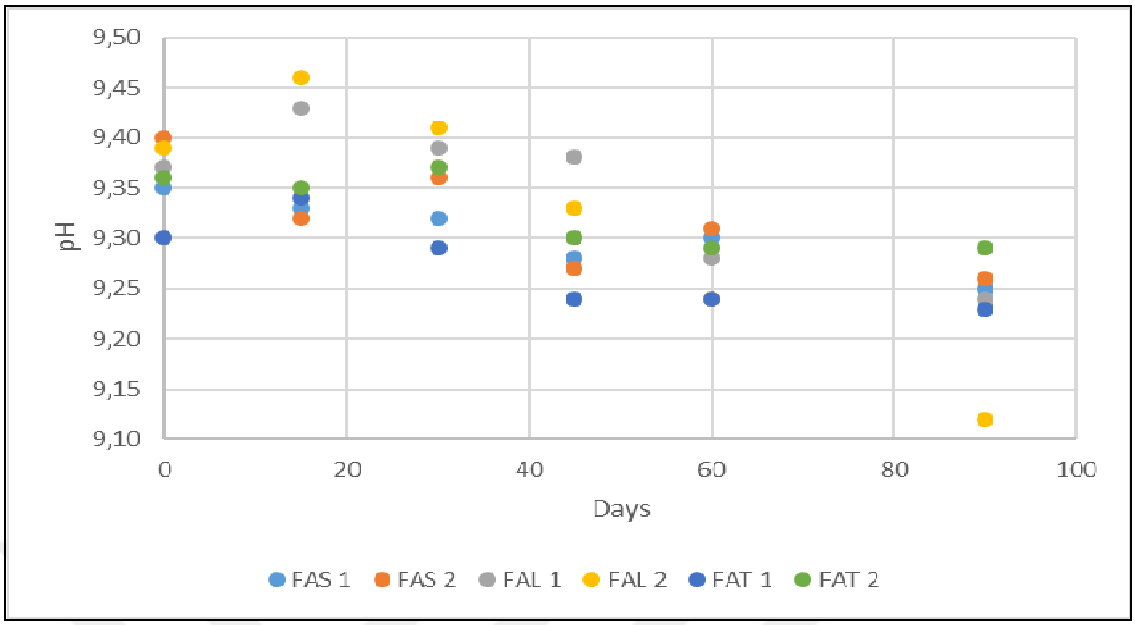


Figure 5.7. The pH of FAS sample

The pH of NLS samples are also higher than LS samples and quite similar to FAS as the formation pathway is comparable. The average pH of bare NLS samples are roughly 9.3 and the change occurring during the measurement for 90 days is 2.18 percent, which indicates a stable product when alkalinity is concerned.

The addition of essential oils also did not influence the pH of the products and the alterations for NLSL and NLST are 0.87 percent and 0.22 percent respectively indicating a steady and consistent product.

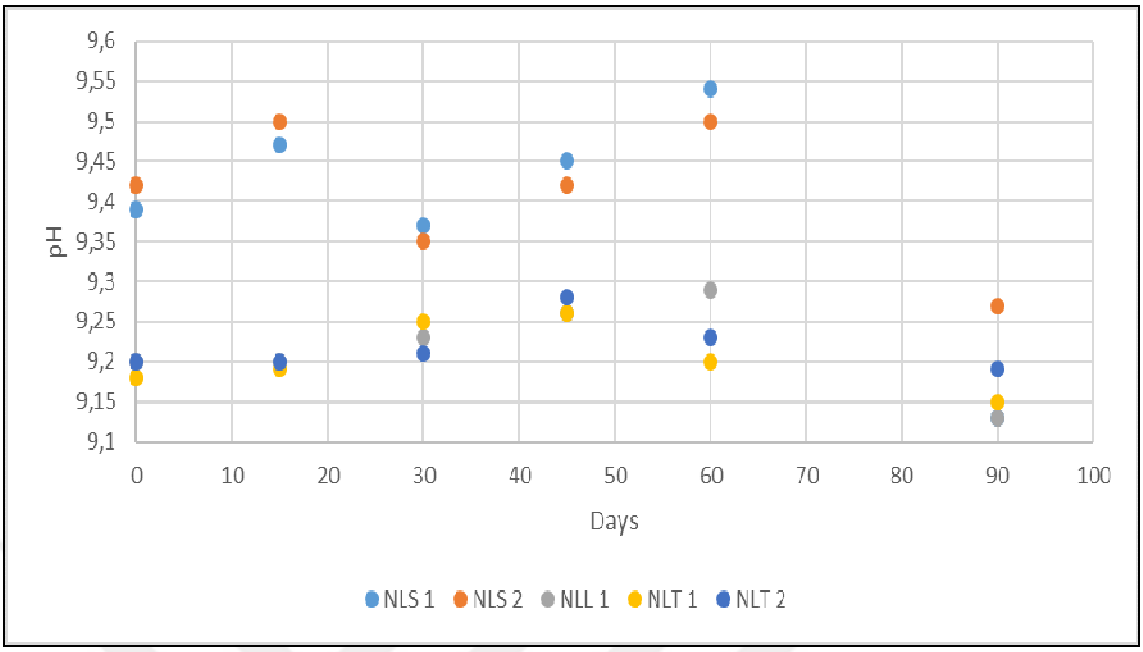


Figure 5.8. The pH of NLS samples

5.3. FOAMING PERFORMANCE OF THE PRODUCTS

Foaming is an essential property of many common hygiene and cleaning products. In different cases, foam may be a desired option whereas it can also be needless. For instance, laundering and dishwashing detergents are prepared by using foam suppressers in order to get rid of foam as it can damage the dishwasher or cause overflow. On the other hand, personal care products such as shampoos are prepared by formulations where bubbling is enhanced as it is requested by consumers due to the common belief that if a product such as a shampoo or a soap is more bubbly, it would have act more efficiently.

The cleaning performance of soaps depends on surfactant characteristic and its amount in the mixture. Especially in antibacterial soaps, the main surfactant is cationic such as quaternary ammonium and its derivatives. The surveys show antibacterial soaps foam less as compared to plain soaps.

This less foaming capacity is essentially due to the cationic antibacterial agents such as triclosan. As the antibacterial agents are cationic in nature, for the stability of soap products cationic surfactants are preferred. It is well known in the literature that cationic surfactants lead to deteriorated foaming capacity as compared to anionic surfactants.

As a consequence, it is a necessity to obtain a soap product ,which has high foaming capacity by using an anionic surfactant and a compatible antibacterial agent (not cationic) as some studies indicate cationic – anionic interaction may decrease foaming capacity [79]. The foaming capacity of LS, FAS and NLS samples were measured for 90 days interval where all of, which was exposed to ambient and lightless conditions. The corresponding foaming capacity of the samples are calculated according to Eq. 5.1.

$$\%\text{foaming capacity} = \frac{\text{height of foam(mm)}}{\text{height of residual liquid under the foam(mm)}} x100 \quad (5.1)$$

5.3.1. Foaming of LSS

The main components in LSs are SLES and CAPB, which are known for their foaming capacities and it is expected for LS samples to have high foaming properties. Figure 5.9 shows foaming capacities of bare LS samples. Results indicate that initially LS has a foaming capacity of 24 percent and then slightly decreases to 20.6 percent after 10 minutes, which was expected as foaming decreases as time progressed. This nominal decrease in foaming capacity of LS indicates the high bubbling performance of the soap.

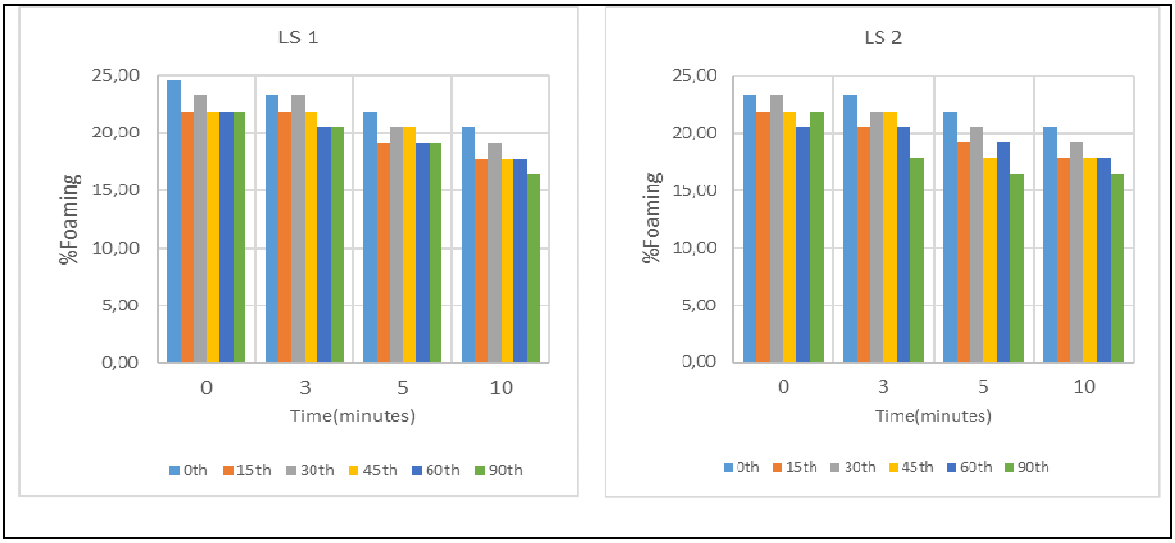


Figure 5.9. Foaming performance of LS

Figure 5.9 also shows that the foaming capacity of bare LS samples decrease slightly as the product is kept for 3 months. This deterioration in the foaming can be attributed to the thickness enhancement occurring during the stated time interval as mentioned previously. Similar alteration in the foaming capacity was also observed for LS samples prepared by the addition of lavender and cinnamon oils as antibacterial agents (Figure 5.10 and Figure 5.11). At the starting day, the foaming capacity of LSL samples was around 21.9 percent and LST samples was around 23.3 percent, which are slightly lower than bare LS. As expected, the foaming decreased to 17.8 percent and 20.6 percent after 10 minutes for LSL and LST respectively. At 90th day, the foaming for both samples were found to be deteriorated like LS but not influential to cause limitations in the usage of the product. However, the foaming performance of LST was a bit more than that of LS, which cannot be readily noticeable in daily use.

All these results for LSL and LST are comparable to bare LS samples indicating although the addition of essential oils causes a slight weakening in foaming capacities, performance is still adequate for a liquid hand soap.

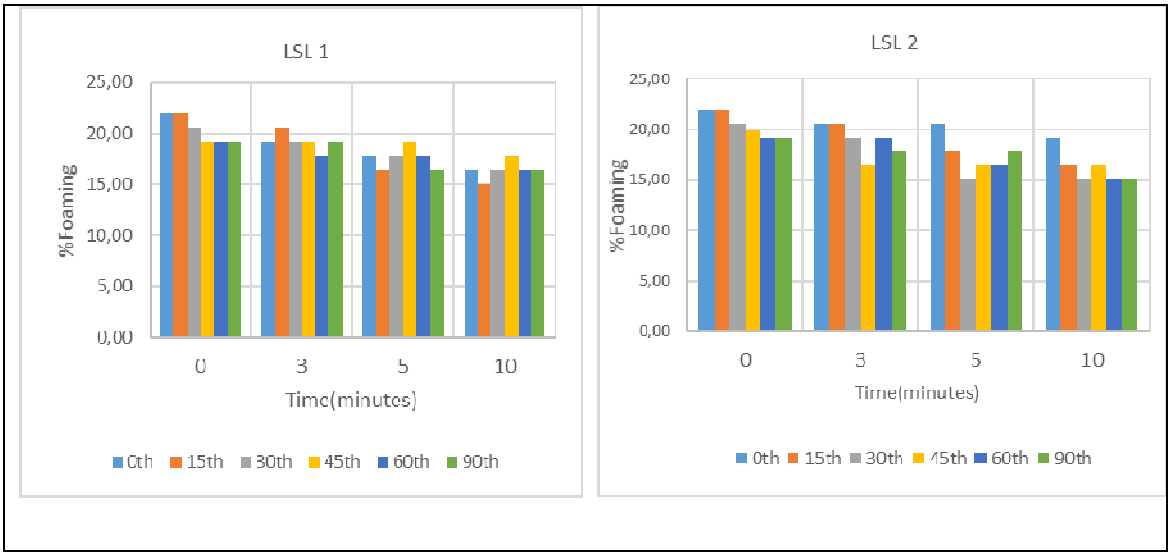


Figure 5.10. Foaming performance of LSL

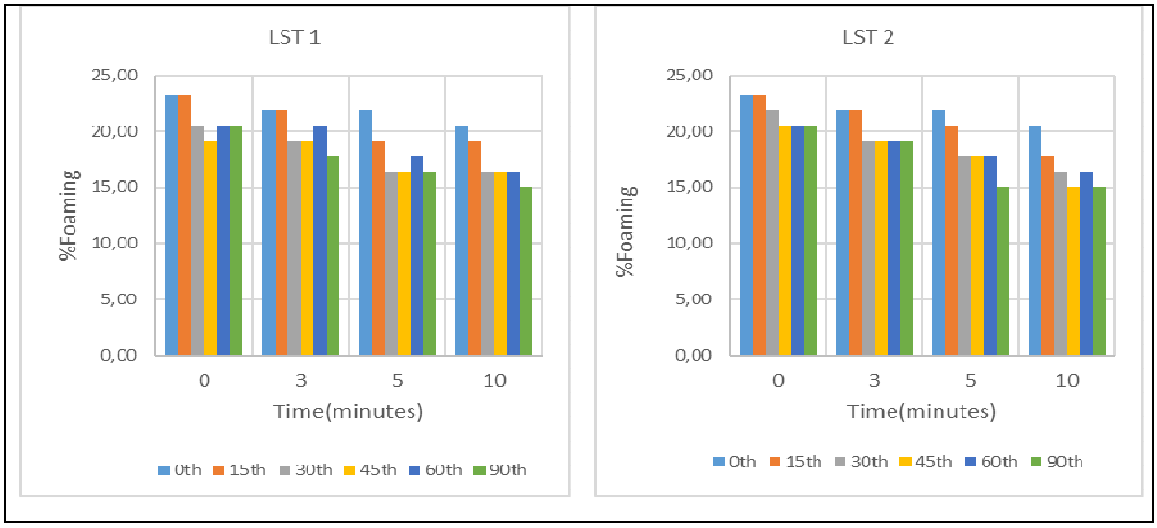


Figure 5.11. Foaming performance of LST

5.3.2. Foaming of FAS and NLs

The foaming capacities of fatty acids based and natural liquid soaps were also analyzed for 90 days and compared with their derivatives made by the addition of essential oils as antibacterial agents. As compared to LS, which is a product of synthetic surfactants, these products are made by saponification reactions of fatty acids and coconut oil with strong bases. As a consequence it was possible to alter the formulations of FAS and NLS such that the output possess high foaming capacities.

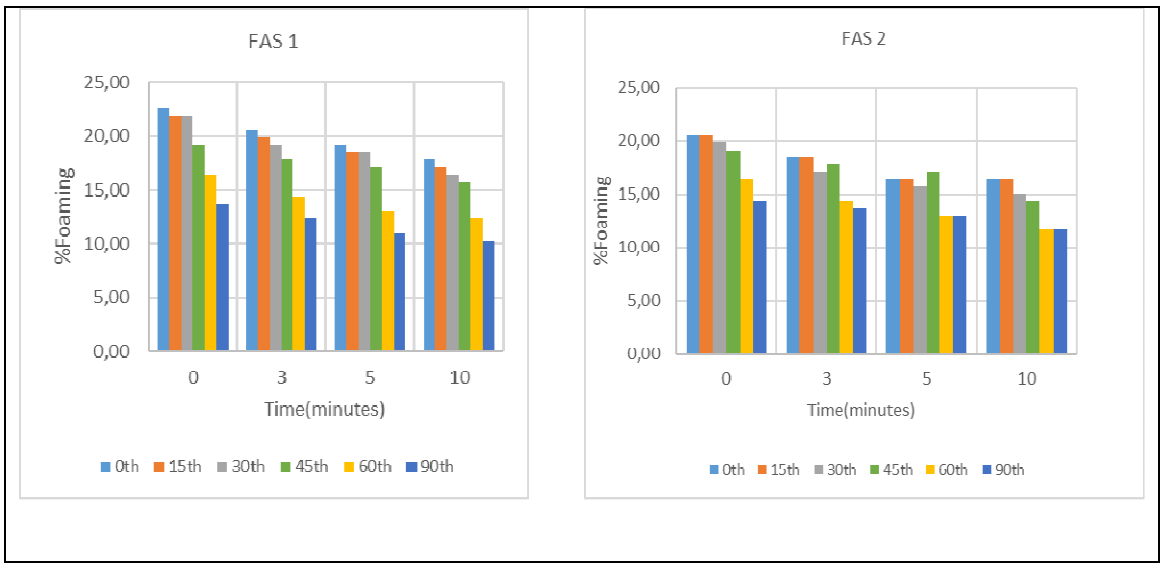


Figure 5.12. Foaming performance of FAS

The foaming capacities of fatty acids based liquid soaps were found as 21.6 percent initially while it decreases to 17.1 percent after 10 minutes indicating an adequate performance and comparable properties with LS (Figure 5.12). This result also indicates the sufficiency of the formulation as it was regulated to yield similar foaming capacity of LS. The time effect over foaming of FAS samples also indicated a relative decrease with respect to the outset as expected. Approximate results were also obtained with LS samples as mentioned previously. FAL samples prepared with lavender oil produced also efficient and comparable foaming capacities with 19.2 percent initially, which was decreased to 17.1 percent after 10 minutes. The foaming capacity was also degraded up to 15.1 percent during 3 months of storage, which was valid for both bare LS and its essential oil derivatives.

The foaming of FAT was even better than FAL as the capacity was 22.6 percent initially, which was decreased to 17.4 percent after 10 minutes of measurement (Figure 5.14).

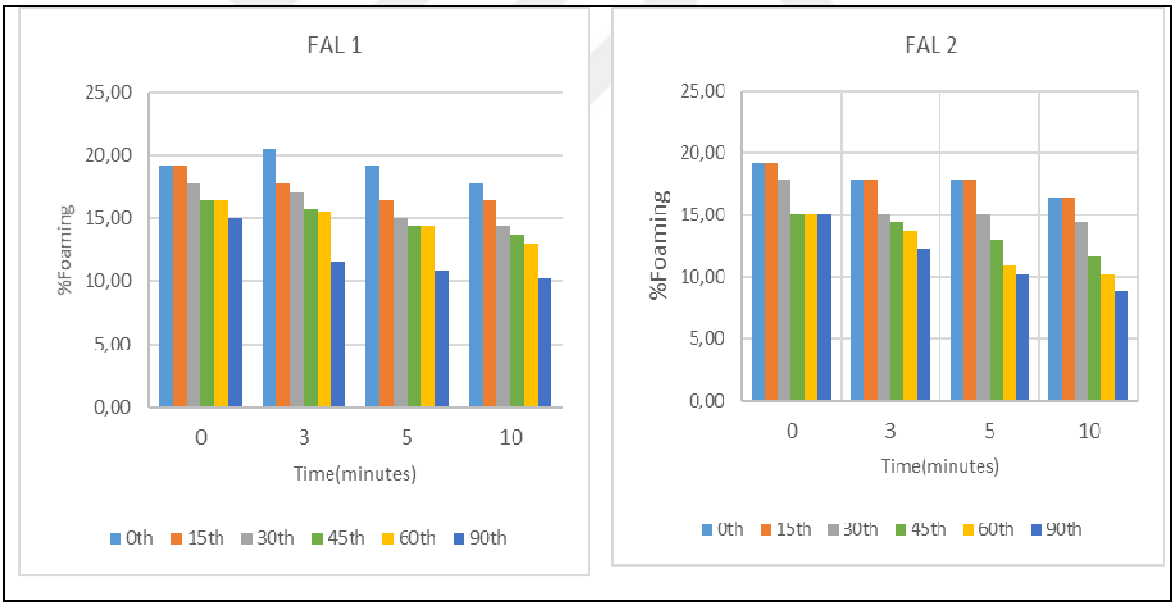


Figure 5.13. Foaming performance of FAL

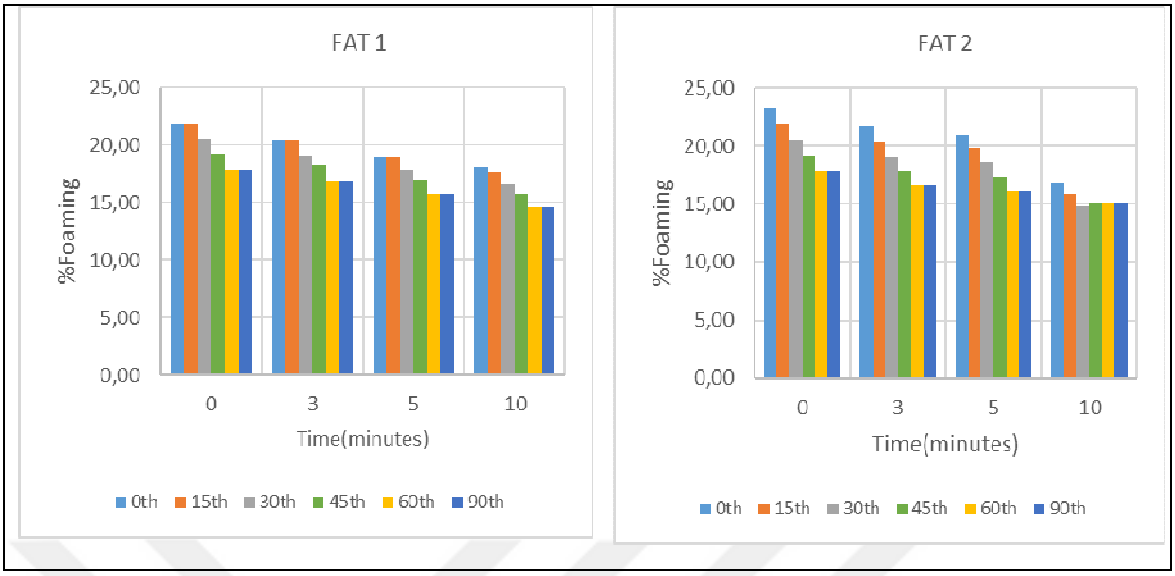


Figure 5.14. Foaming performance of FAT

The foaming capacities of NLS were also investigated throughout 90 days. It was clearly seen that the foaming performance was the lowest as compared to LS and FAS. Pure NLS gave 17.8 percent of foaming capacity in the beginning of measurement while it was decreased to 14 percent after 10 minutes. After 90 days the performance dramatically deteriorated to 7.5 percent.

However, NLL and NLT ,which contain lavender and cinnamon oils as additives were capable of producing adequate foaming capacities with respect to their bare counter parts as results of measurements were 17.8 percent and 19.2 percent respectively initially (Figure 5.16 and Figure 5.17). Additionally, after 3 months of storage NLL and NLT foaming performances (15.9 percent and 15.8 percent respectively) were better than NLS indicating that the addition of essential oils lead to the improvement of the corresponding properties.

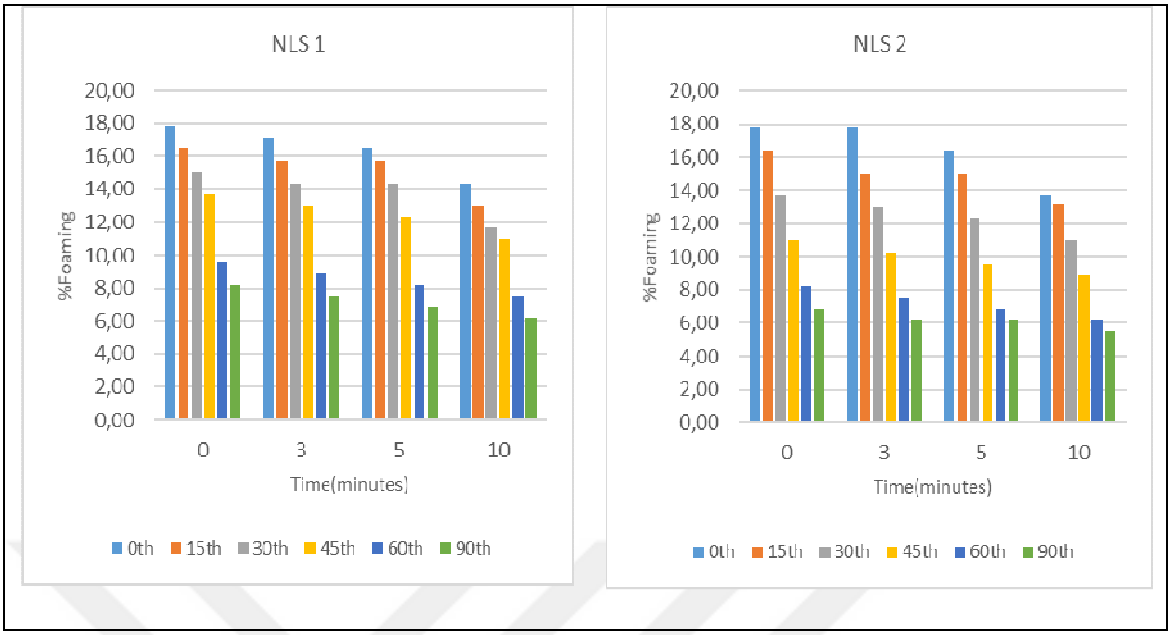


Figure 5.15. Foaming performance of NLS

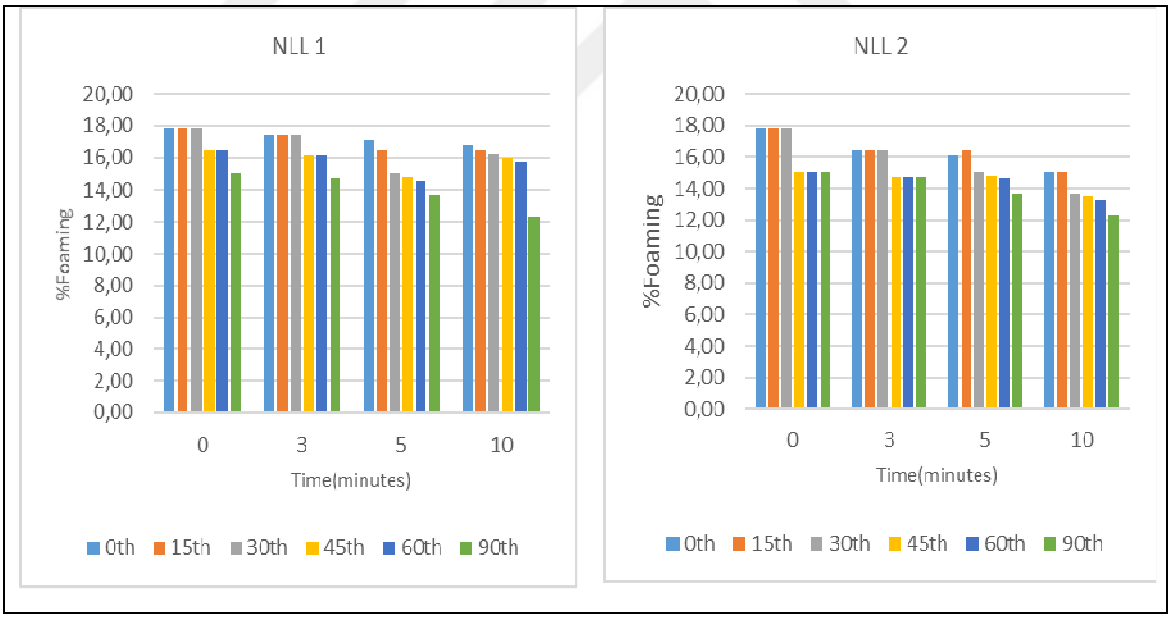


Figure 5.16. Foaming performance of NLL

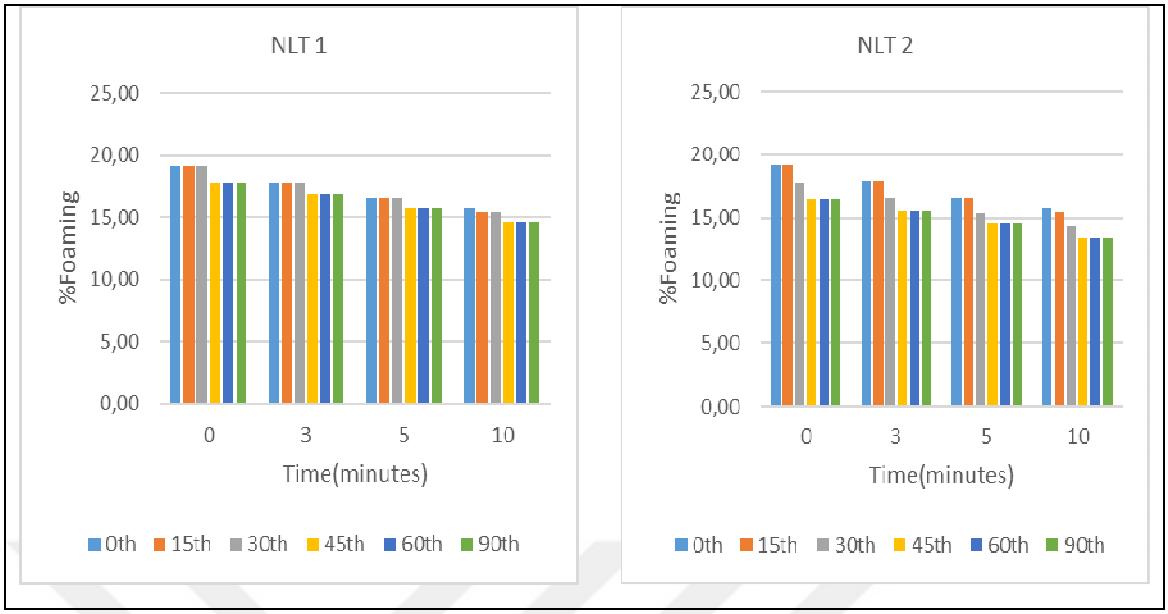


Figure 5.17. Foaming performance of NLT

In this section foaming capacities of different products prepared via different routes were investigated for 90 days. It was actually necessary to obtain a product with highest possible foaming performances along with sufficient antibacterial activities as it is a highly stated demand of consumer for the products supplied in the market.

Table 5.1. Initial foaming capacities of LS, FAS and NLS samples

Sample	**Initial Foaming Capacity (%)**
LS (Bare)	24.0
LSL (Lavender Oil)	21.9
LST (Cinnamon Oil)	23.3
FAS (Bare)	21.6
FAL (Lavender Oil)	19.2
FAT (Cinnamon Oil)	22.6
NLS (Bare)	17.8

Table 5.1. Initial foaming capacities of LS, FAS and NLS samples (Contd.)

NLL (Lavender Oil)	17.8
NLT (Cinnamon Oil)	19.2

The overview of foaming performances of both bare and essential oil derived liquid soap samples prepared via different formulations are given in Table 5.1. According to the results, the highest foaming was achieved by LS and although the additives caused a slight reduction, the overall capacity was still comparable. Results also indicated that fatty acids based liquid soaps (FAS) presented better foaming performances than coconut oil based liquid soaps (NLS) due to variation of fatty acid contents as different types provide distinctive foaming performances. For both cases, the essential oils reduced the corresponding properties where cinnamon oil gave better results for all samples. As a whole, all samples regardless of essential oil contents, showed adequate foaming capacities around 20 percent, which is analogous to commercial liquid soap (LS).

It is essential to consider the economical aspect of a product while designing a new formulation. In general, maximum efficiency is expected with minimum material and cost. When formulations of LS, FAS and NLS are considered, LS contains roughly twice as much of foaming agent than other two alternatives.
However, results showed that it is still possible to obtain similar foaming performances by lesser amounts of ingredients via configuring the quantities of fatty acids, which in overall might lead to cost reduction.

5.4. ANTIBACTERIAL ACTIVITIES OF THE PRODUCTS

The essential aim of this study is to formulate a liquid hand soap alternative, which contain biocompatible antibacterial agent rather than banned chemicals such as triclosan and triclocarban due to their cancerogenic effects and hormone disruption. The antibacterial liquid hand soaps offered at the consumer market mainly composed of these or similar antibacterial agents so the usage of especially natural agents, which are capable of the same impact is important.

At the same time, the commercial antibacterial consumer products have poor foaming properties. The main reason behind this fact is the existence of cationic surfactants, which are compatible with cationic antibacterial agents. It is well known that cationic surfactants have comparably lower foaming performances than anionic ones. As a consequence the foaming along with antibacterial effect is significant for consumer satisfaction. In the previous parts, the details about the formulation of liquid hand soaps and their corresponding properties including thickness (viscosity) and foaming were presented and all of which were fairly adequate. In this section, the related antibacterial effects of 3 different formulations (LS, FAS and NLS) made by the addition of lavender and cinnamon oil as antibacterial agents are illustrated.

Many different essential oils such as thyme oil, lavender oil, cinnamon oil etc. were studied in the literature for their bactericidal activities. In this study, lavender and cinnamon oils were selected as a consequence of their high antibacterial effects and costs. *Escherichia Coli*, *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* were selected as bacterial strains during these tests. For the analysis duplicate samples were prepared and tested by agar well plate method against these bacterial strains for 90 days both in the presence and absence of ambient light during storage.

5.4.1. Antibacterial Activity of LSs

The antibacterial effect of LS samples both in the presence and absence of essential oils at different concentrations (2 wt. percent and 3 wt. percent) were tested on three different bacterial strains *Escherichia Coli*, *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* at $1x10^8$ cfu/mL. The concentration of bacteria was arranged such that it matches the concentration on human body ($1\text{-}2x10^8$ cfu/mL).

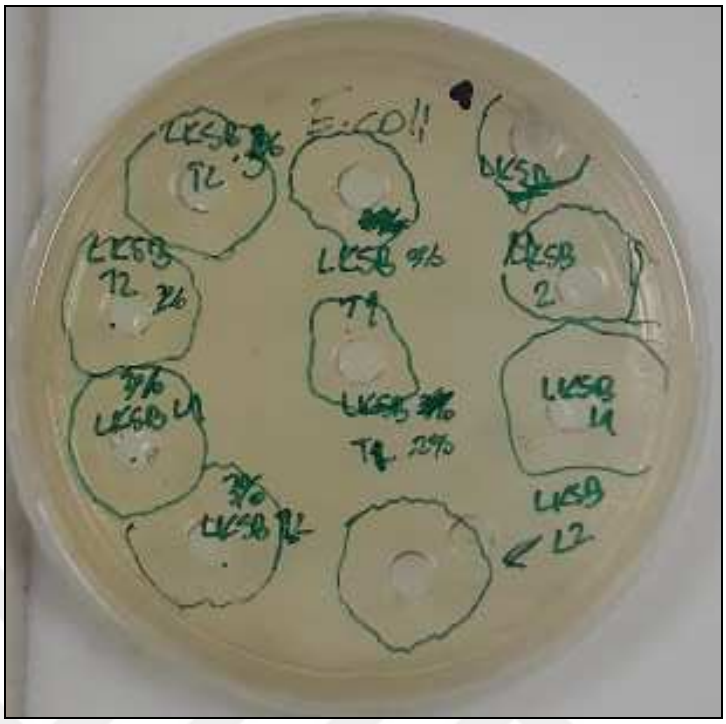


Figure 5.18. Antibacterial activity of LS containing 2 wt. percent and 3 wt. percent essential oils on *Escherichia Coli*

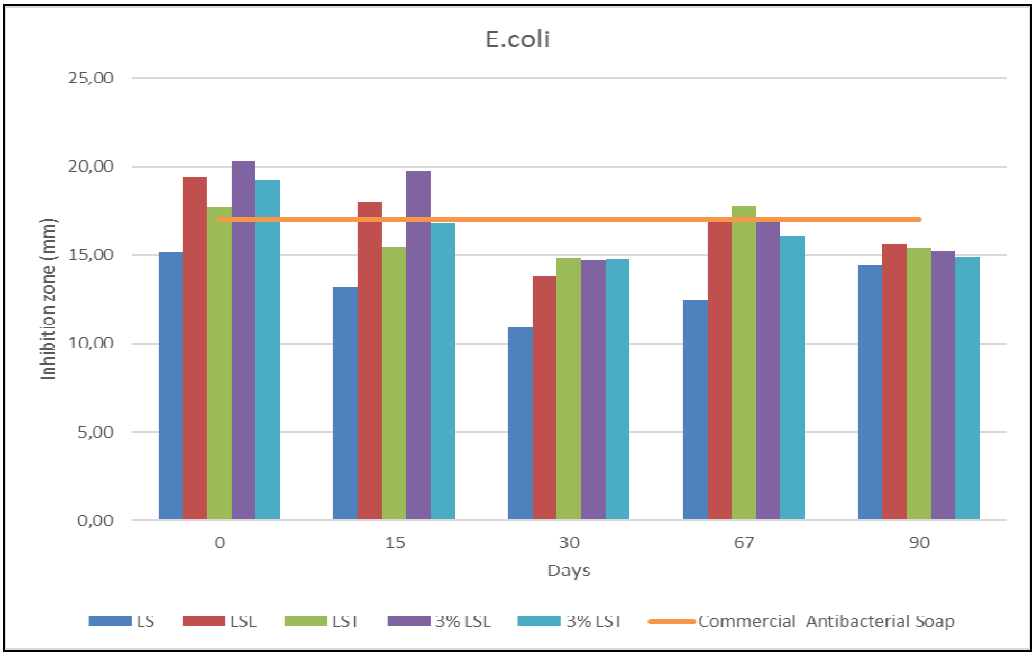


Figure 5.19. Inhibition zones of LS on *Escherichia Coli*

Figure 5.19 shows Antibacterial activity of LS containing 2 wt. percent and 3 wt. percent essential oils on *Escherichia Coli* for 90 days. The activity of samples were evaluated according to the corresponding average dimensions of inhibition zones occurring for duplicates on the plate. The results showed that all samples even bare LS (15.2 mm) possessed antibacterial activity on *Escherichia Coli* while inhibition zones for 2 wt. percent LSL (19.4 mm) and LST (17.7 mm) were comparably higher indicating an enhancement in antibacterial activity of the product. Additionally, this enhancement further increases with increasing concentration of essential oils to 3 wt. percent. where inhibition zones for LSL and LST were 20.3 mm and 19.2 mm respectively. After 3 months of storage it was found that the activity of products decreased roughly 20 – 25 percent, which was also valid for bare LS. However, the activities of essential oil derivatives were still higher than that of bare LS. For comparison, antibacterial activity of a commercial product containing TCS and benzalkonium chloride was also tested and added to the plot as a green line. The comparison showed the possibility of obtaining almost the same antibacterial effect by using a simple and natural essential oil (especially lavender oil at 3 wt. percent) in a plain liquid hand soap. Although the activities of some LSL and LST samples were slightly lower as compared to the commercial alternative, comparable results indicate the incoherent use of harsh and detrimental products for personal hygiene purposes. Moreover, the antibacterial activity of bare LS was even close to the commercial antibacterial liquid hand soap, which also supports this claim.

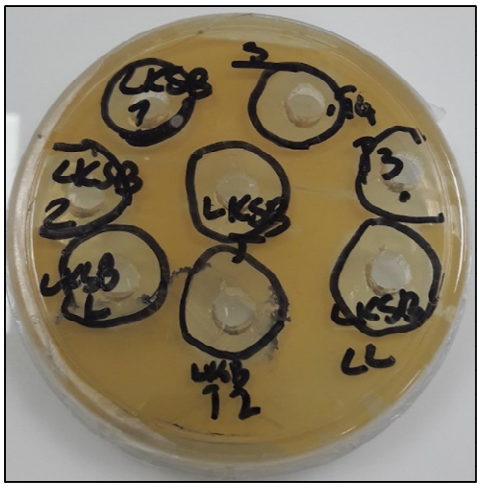


Figure 5.20. Antibacterial activity of LS containing 2 wt. percent and 3 wt. percent essential oils on *Staphylococcus Aureus*

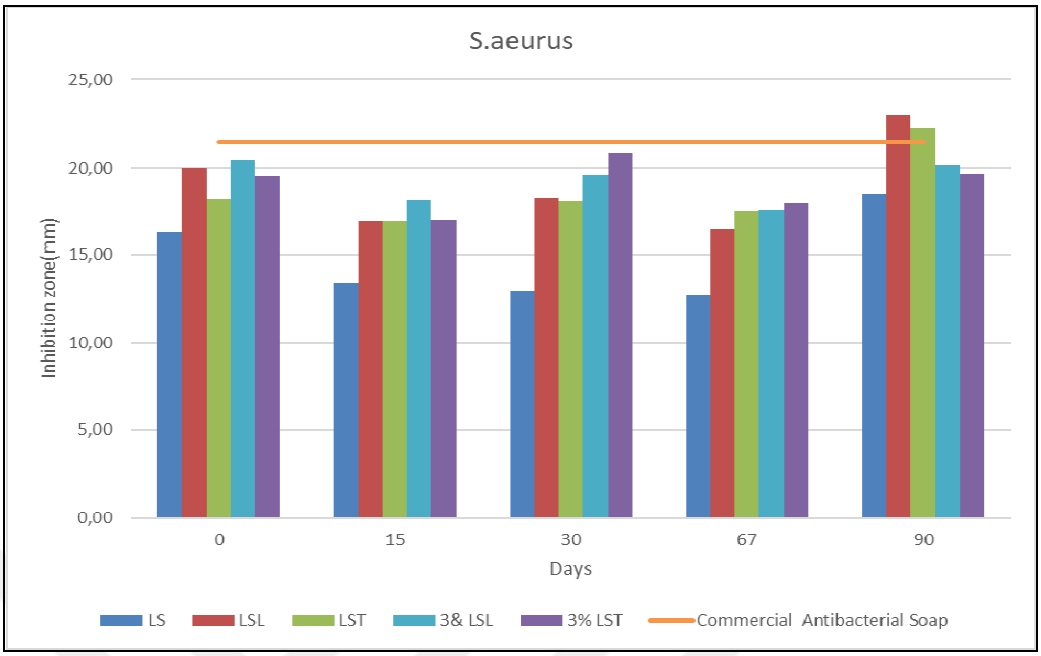


Figure 5.21. Inhibition zones of LS on *Staphylococcus Aureus*

The antibacterial activity of LS on *Staphylococcus Aureus* was also tested for 90 days and the corresponding inhibition zones are given in Figures 5.20 and 5.21. Results indicated an enhancement in the antibacterial activity of samples containing essential oils as compared to bare LS regardless of essential oil concentration. A similar trend was also obtained for *Escherichia Coli*, which reveals the effective impact of essential oils on bacterial strains. The assays show a slight increase in the inhibition zones when the concentration of essential oils increases from 2 wt. percent to 3 wt. percent as lavender oil and cinnamon oil yield 18.96 cm and 18.61 cm inhibition zones at 2 wt. percent and 19.18 cm and 19 cm at 2 wt. percent where bare LS reaches only 14.78 cm in average for 90 days. These results indicate a comparable and preferable effect of these natural essential oils on *Staphylococcus Aureus.* Unlike *Escherichia Coli*, the commercial market product, which is indicated by the green line in Figure 5.21, showed a better inhibition effect as compared to LS but LSL and LST were again as effective as commercial market product on this bacterial strain.

The inhibition performance of LS, LSL and LST were finally tested against another dangerous pathogen Pseudomonas Aeruginosa (Figure 5.22 and Figure 5.23). Lavender oil and cinnamon oil containing samples showed approximately 10percent higher antibacterial effect as compared to LS while all these samples were 10 percent ineffective with respect to the commercial market product.

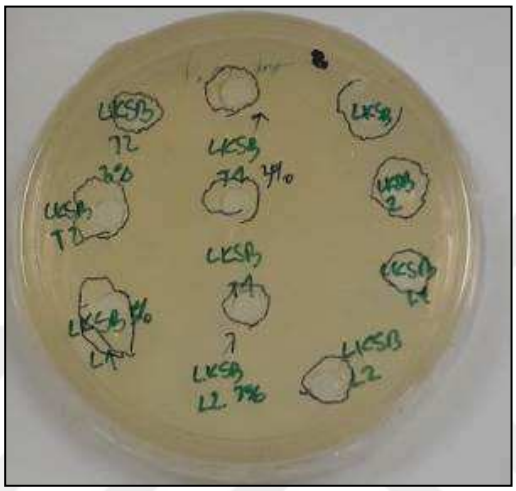


Figure 5.22. Antibacterial activity of LS containing 2 wt. percent and 3 wt. percent essential oils on *Pseudomonas Aeruginosa*

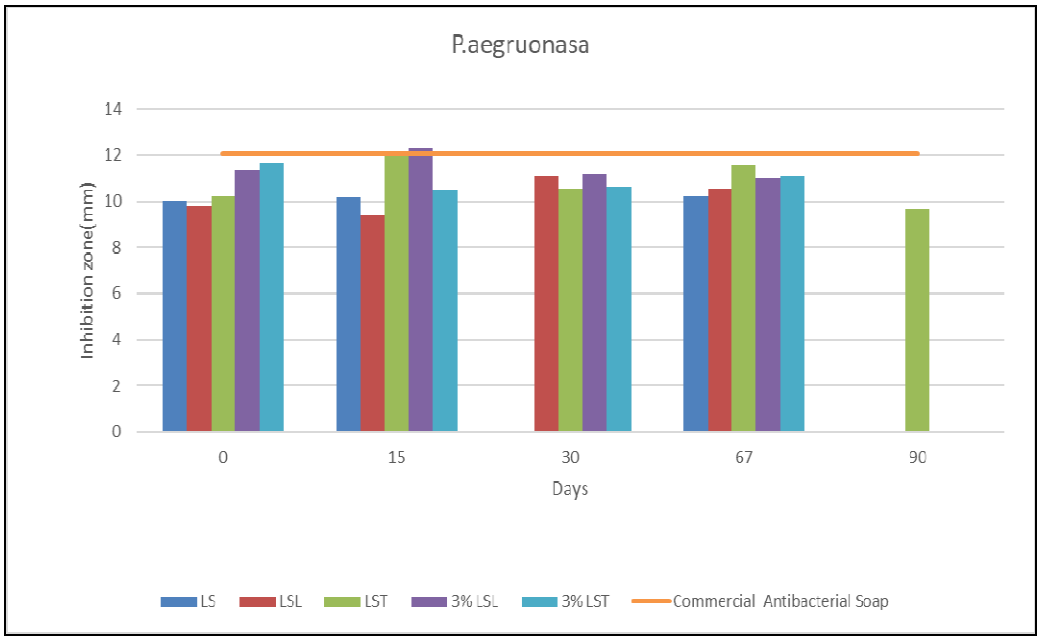
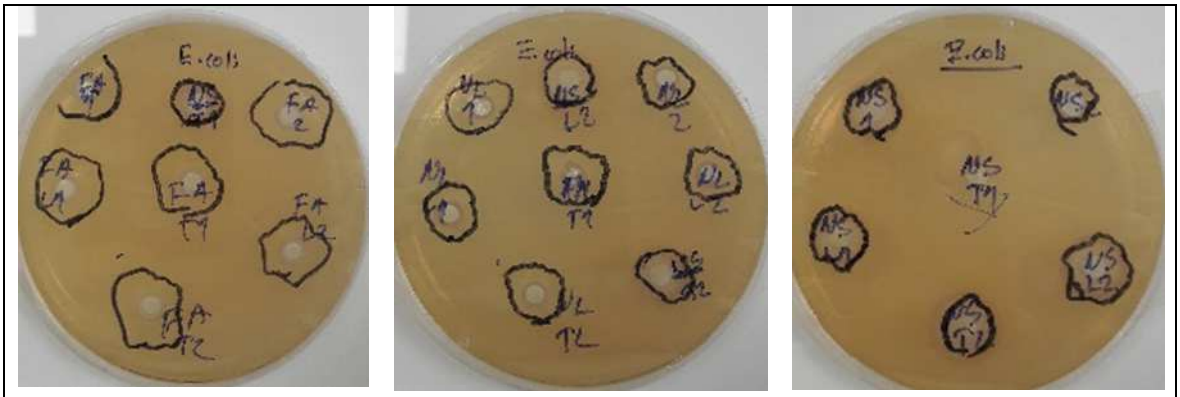


Figure 5.23. Inhibition zones of LS on *Pseudomonas Aeruginosa*

In this section, the antibacterial performances of surfactant based soaps containing different essential oils as antibacterial agents were tested against various bacterial strains. The results clearly indicated an improved effect as cinnamon and lavender oils were simply introduced into the formulation of LS, which is a plain liquid hand soap presented in the market. Although the activities of these liquid hand soaps enhance with an increase in the concentration of essential oils, the incremental effect is not significant as the concentration increases from 2 wt. percent to 3 wt. percent. Thus the variation in the antibacterial effect should be considered economically while designing a new formulation as it might not be feasible to utilize higher concentrations of antibacterial agents.

5.4.2. Antibacterial Activity of FAS, NLS and NSS

Essentially many soap products both in solid and liquid form in the market are made of synthetic surfactants due to production and raw material costs. On the other hand, it is also common to produce these personal hygiene products at home by using traditional methods. In order to represent the similarities and variations, the antibacterial effect of fatty acids based (FAS), natural coconut oil based (NLS) and solid soap samples containing cinnamon and lavender oil as antibacterial agents were also tested.



(a) (b) (c)

Figure 5.24. Antibacterial activity of samples containing 2 wt. percent and 3 wt. percent essential oils on *Escherichia Coli*
a) FAS b) NLS c) NSS

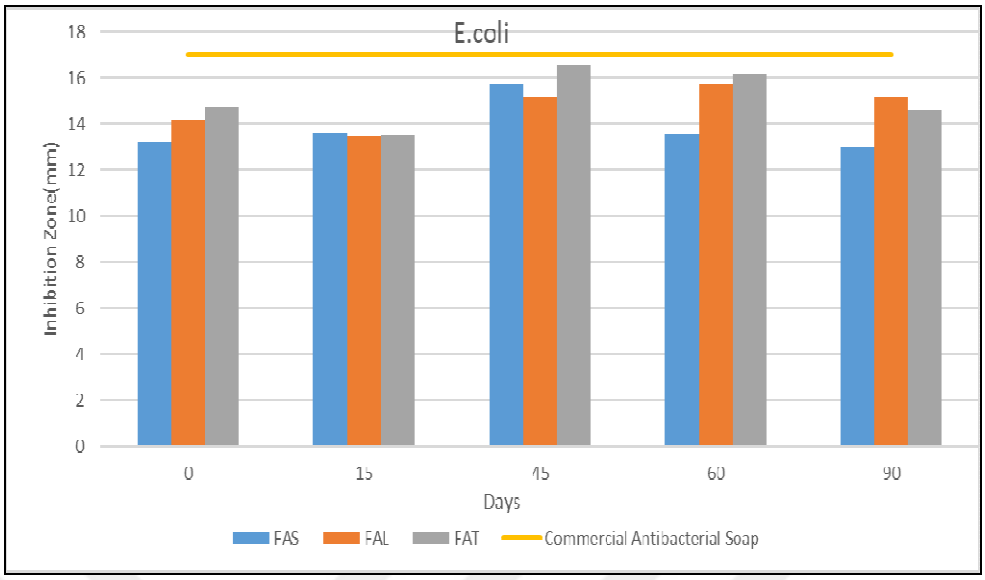
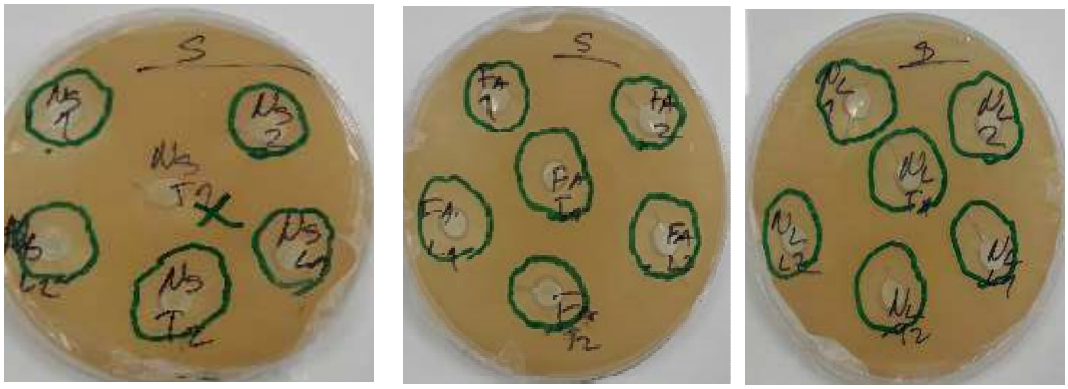


Figure 5.25. Inhibition zones of FAS on *Escherichia Coli*

Figures 5.24 and 5.25 present the performance of fatty acids based soaps including essential oils for 90 days on *Escherichia Coli*. As the saponification reaction is performed by using various fatty acids and an excess amount of strong base (KOH), the final pH of the products are higher than the alternatives made by using synthetic surfactants (LS). It should also be noted that high pH can individually inhibit the growth of microorganisms. As a consequence, the pH of the FAS were regulated at the end of production and decreased to values that is safe for human skin. According to the results, both bare FAS and essential oil derivates had similar effect on *Escherichia Coli* but roughly 15 percent less effective as compared to the commercial product. On the whole, the addition of essential oils to the surfactant based soap (LS) caused a significantly better impact for the inhibition of *Escherichia Coli* than fatty acids based soap (FAS).



(a) (b) (c)

Figure 5.26. Antibacterial activity of samples containing 2 wt. percent and 3 wt. percent essential oils on *Staphylococcus Aureus*
a) FAS b) NLS c) NSS

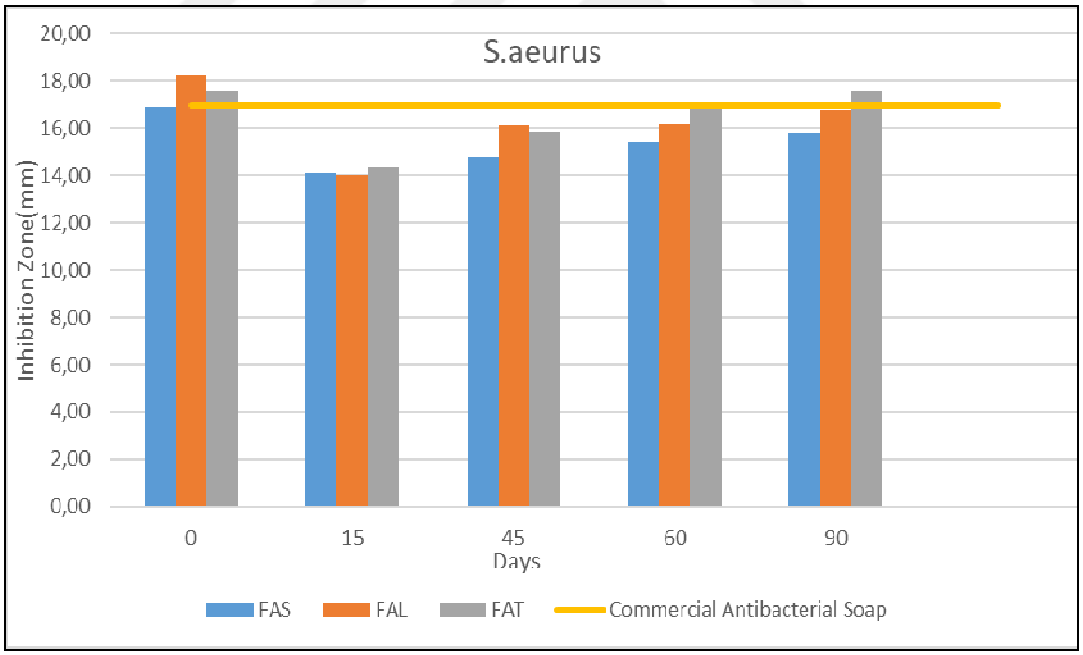


Figure 5.27. Inhibition zones of FAS on *Staphylococcus Aureus*

The inhibition effect of FAS on *Staphylococcus Aureus* was also analyzed and the results are given in Figure 5.27. As compared to *E.coli*, lavender and cinnamon oil containing fatty acids based liquid soaps presented enhanced antibacterial activity than bare FAS on *Staphylococcus Aureus*.

Additionally, the commercial market product exhibited a zone of 16.98 cm where FAL and FAT samples gave 16.26 cm and 16.45 cm, which are almost identical to the commercial alternative. These results indicate only a 4 percent and 3 percent difference in between FAL and FAT samples respectively as compared to market product when antibacterial effect is considered, which can be evaluated as insignificant.

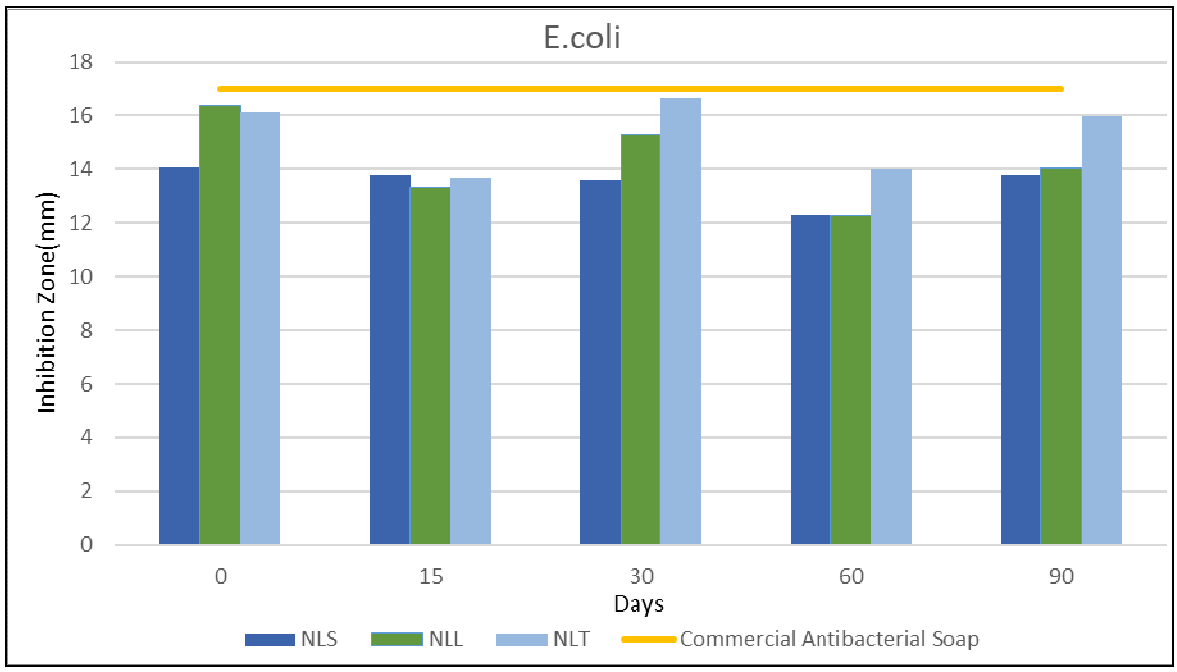


Figure 5.28. Inhibition zones of NLS on *Escherichia Coli*

The liquid soap samples prepared by using only a single type of natural oil (NLS) were tested against different bacterial strains both as pure and essential oil derived forms. Although, bare NLS sample exhibited sufficient antibacterial activity against *Escherichia Coli*, the sample containing cinnamon oil as additive (NLT) showed enhanced inhibition effect while lavender oil (NLL) could not improve the performance of the plain soap (Figure 5.28). When these samples are compared with the commercial market product, the antibacterial activities were slightly lower but comparable even for plain soap prepared without any additives. It should be noted that the addition of cinnamon oil further increases the performance by roughly 15 percent.

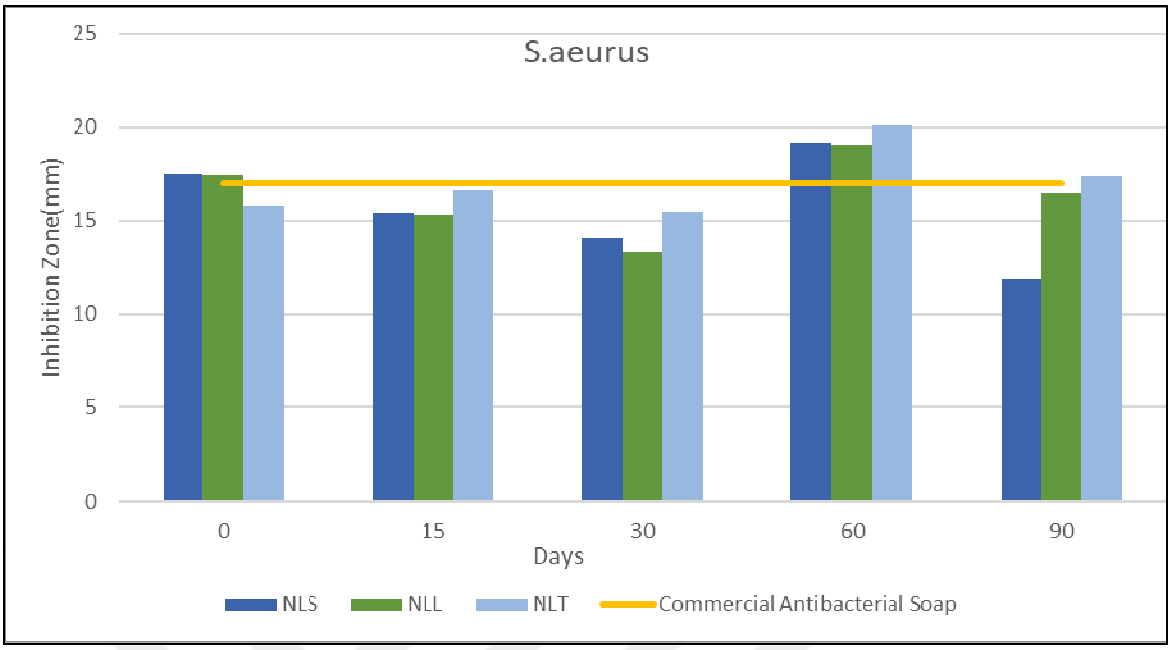


Figure 5.29. Inhibition zones of NLS on *Staphylococcus Aureus*

Natural liquid soaps were also tested against *Staphylococcus Aureus*, both in the presence and absence of essential oils as antibacterial agents. As shown in Figure 5.29, all NLS samples showed better inhibition performances on this bacterial strain as competed to *Escherichia Coli*. Comparably, the cinnamon oil derived sample (NLT) exhibited enhanced performance as compared to bare liquid soap sample (NLS). According to the inhibition zone calculations, NLS, NLL and NLT gave 14.92 cm, 16.1 cm and 17.12 cm respectively, which indicates almost 14.7 percent enhancement in the antibacterial activity of NLS by the addition of cinnamon oil as additive. This sample also exhibited completely similar inhibition effect on this strain when compared with the commercial market product (16.98 cm). Consequently, all these results once again implies the overrated performance of commercial products containing harmful antibacterial agents as a simple natural oil based liquid soap may perform analogous by the addition of an ordinary essential oil.

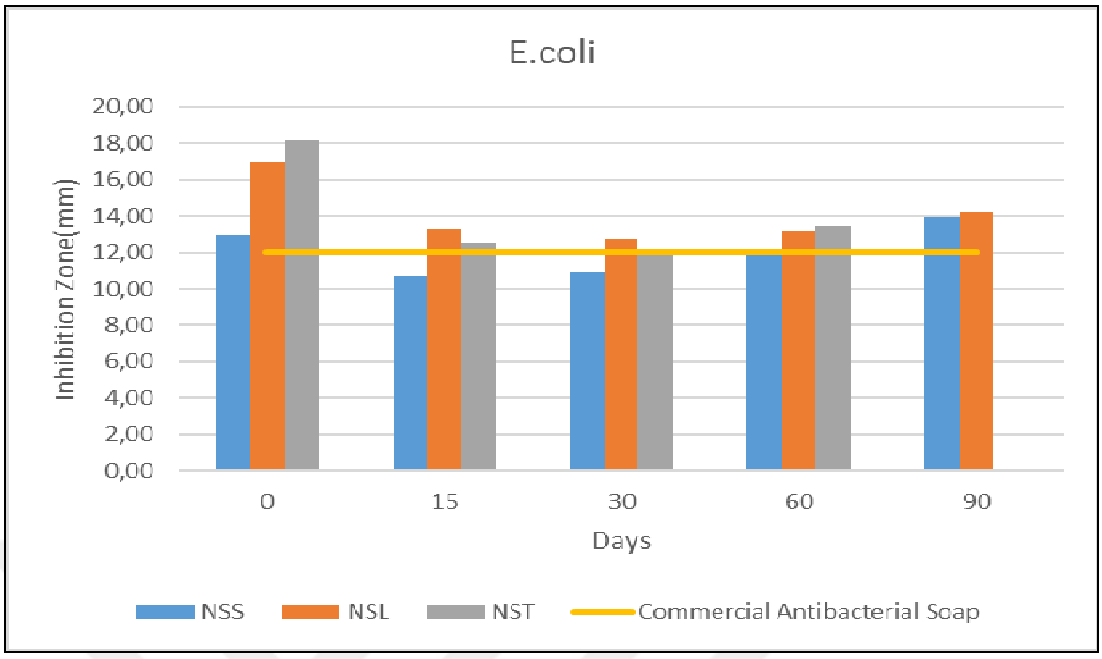


Figure 5.30. Inhibition zones of NSS on *Escherichia Coli*

According to customer preferences, liquid hand soaps are the most widely used type as compared to bar soaps due to hygiene related concerns. However, solid bar soaps are still an option within the market. Therefore, solid bar soaps were also prepared in the presence of natural essential oils in order to evaluate the performance with respect to the commercial alternatives presented for antibacterial effect. In order to prepare the bar soap, the same fatty acids and essential oils as antibacterial agents were used but for the reaction sodium hydroxide replaces potassium hydroxide in order to achieve solid form. Figure 5.30 shows the antibacterial activity of solid bar soaps both in the absence (NSS) and presence of lavender oil (NSL) and cinnamon oil (NST). The results indicated a significant effect of all samples on *E.coli* where both bare and essential oil derived solid hand soaps exhibited similar or better antibacterial effects. Lavender and cinnamon oils further enhanced the activity of plain soap by 17.1 percent and 17.2 percent respectively but pure form was still adequate in order to demonstrate the same performance as compared to the commercial product as those samples revealed almost the same inhibition zones in average (12.13 cm and 12.02 cm respectively).

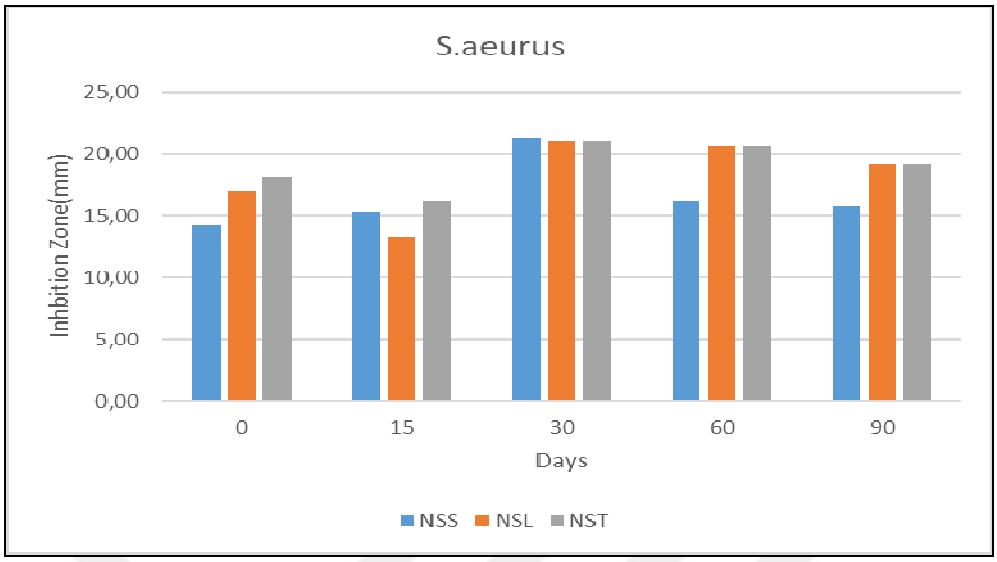


Figure 5.31. Inhibition zones of NSS on *Staphylococcus Aureus*

The antibacterial performance of natural solid soaps were also tested on *Staphylococcus Aureus and* corresponding inhibition zones are given in Figure 5.31. The results indicate that the solid soap prepared in the presence of lavender oil (NSL) exhibited enhanced antibacterial activity than plain soap (NSS) where cinnamon oil derived soap (NST) also presented satisfactory performance. It is also concluded that the activities of antibacterial solid soaps remain effectual at least for 3 months.

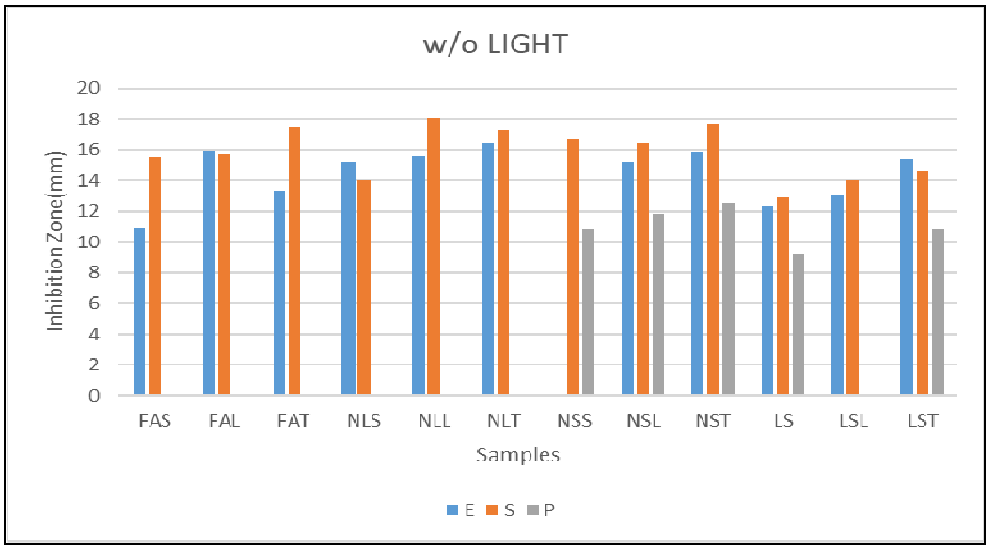


Figure 5.32. Inhibition zones of all soap types (LS, FAS, NLS and NSS) on *Escherichia Coli* (E), *Staphylococcus Aureus* (S) and *Pseudomonas Aeruginosa* (P) at dark conditions

Figure 5.32 and Table 5.2 show the antibacterial performance of all samples, which were kept at dark conditions for 90 days on *Escherichia Coli* (E), *Staphylococcus Aureus* (S) and *Pseudomonas Aeruginosa* (P). Results are generally diverse for different soap samples as exposure to ambient light had both enhancive and deteriorative effect on the antibacterial effects. For surfactant based (LS) and fatty acids based (FAS) liquid hand soaps, exposure to ambient light had improving inhibition activity for both bacterial strains whereas the activity of natural essential oil based liquid soap (NLS) and its derivatives containing cinnamon (NLT) and lavender (NLL) oils increased at dark conditions. According to the results it can also be concluded that the addition of natural essential oils as antibacterial agents to all samples, which are kept at dark conditions increased the antibacterial performance of their corresponding base where cinnamon oil acted more effective. This analysis also pointed out the inequality of storage conditions for different products.

Table 5.2. Comparison of inhibition zones of samples kept at dark and ambient conditions

Sample	**E**	**S**	**P**	**E**	**S**	**P**
	Inhibition Zones of Samples at Lightless Condition (mm)			**Inhibition Zones of Samples at Ambient Condition (mm)**		
FAS	10.9	15.5	-	12.9	15.8	-
FAL	15.9	15.7	-	15.2	16.8	-
FAT	13.3	17.5	-	14.6	17.6	-
NLS	15.2	14.0	-	13.8	11.9	-
NLL	15.6	18.1	-	14.1	16.4	-
NLT	16.5	17.3	-	15.9	17.3	-
NSS	-	16.7	10.9	13.9	15.3	11.9
NSL	15.2	16.5	11.9	14.2	18.3	11.8
NST	15.9	17.7	12.5	-	19.9	12.3
LS	12.3	12.9	9.2	14.4	18.5	-
LSL	13.0	13.9	-	15.6	23.0	-
LST	15.4	14.6	10.9	15.4	22.3	-

5.5. ACCELERATED STABILITY TESTS

For all soap samples prepared via different routes and ingredients stabilities, foaming capacities, pH, viscosities and most importantly the antibacterial activities were analyzed for 90 days. Additionally, for a commercial product it is necessary to perform an accelerated stability tests in order to foresee its shelf life. This test is performed by 4 cycles and the details of the procedure is given in Section 3.2.6

5.5.1. Viscosity

The viscosities of soap samples after 4 cycles of accelerated stability test are given in Table 5.3. The viscosity of plain surfactant based liquid hand soap (LS) was initially 3425 cP and increased roughly 12.9 percent after the test ,which is comparable to the previous findings for 90 days suggesting the perfect stability of the product over an extended period of time. On the other hand, the viscosities of LSL and LST were found to decrease 62 percent and 30.1 percent respectively and these values are substantially lower than viscosities obtained after 90 days, which demonstrates the instability of samples after considerable time and indicates lower shelf life.

Table 5.3. Viscosities of liquid soap samples after 4 cycles of accelerated test

Sample	Initial Viscosity (cP)	Final Viscosity (cP)	% Change
LS	3425	3867	12,9
LSL	2855	1085,3	-62,0
LST	995	695,4	-30,1
FAS	760	1330	75,0
FAL	214	1025	378,8
FAT	196	2432	1141,0
NLS	155	57	-63,4
NLL	117	54	-53,6
NLT	87	78	-10,6

The accelerated test of fatty acids based soaps, especially lavender oil (FAL) and cinnamon oil (FAT) derived samples showed a significant viscosity enhancement. Similar increase in the thickness of the samples also observed for 90 days period and attributed to the high concentration of stearic acid in the formulation, which causes a waxy phase and prevent extended shelf life of the product. Finally, the viscosities of both bare and essential oil modified coconut oil based liquid soaps (NLS) were found to decrease after 4 cycles, which is contradictory to the results obtained after 90 days. However, it should be noted that the accelerated stability test does not rely solely on time span but also on temperature changes. Thus, it is possible to obtain divergent results on properties of the products. The accelerated stability test in general reveled LS as the most stable product as viscosity is considered.

5.5.2. Foaming

The foaming capacities of different liquid soap samples containing essential oils as antibacterial agents were tested after accelerated stability test and the results are given in Table 5.4. It is clear that the foaming performances of all liquid soap samples decrease as 4 cycles of a stability test is applied. Especially, the deterioration is more pronounced for fatty acids (FAS) and natural essential oil (NLS) based liquid soaps than LS, which is actually a replica of a commercial market product. Inherently, it is expected for such a sample to exhibit higher performance and extended shelf life than its ordinary alternatives. Nevertheless, the reduction in the foaming performance obtained for FAS, FAL and FAT samples after accelerated stability test are around 10 percent and this also indicates a relatively adequate shelf life.

Table 5.4. Foaming capacities of liquid soap samples after 4 cycles of accelerated test

Samples	Initial Foaming Capacity (%)				Final Foaming Capacity (%)			
	0 min	3 mins	5 mins	10 mins	0 min	3 mins	5 mins	10 mins
LS	26.39	23.61	22.22	20.83	21.53	18.75	18.75	16.67
LSL	25.00	23.61	22.22	20.83	20.83	20.14	16.67	15.97
LST	22.22	19.44	18.06	16.67	20.14	18.06	16.67	15.28
FAS	15.28	12.50	11.81	9.72	13.89	12.50	11.11	0.00
FAL	13.89	11.81	9.72	7.64	13.89	11.11	9.72	0.00
FAT	16.67	15.28	15.28	14.58	15.28	13.89	12.50	9.72
NLS	15.28	9.72	9.03	9.03	9.72	8.33	6.94	0.00
NLL	14.58	13.19	12.50	11.81	8.33	6.94	5.56	0.00
NLT	16.67	15.97	15.28	15.28	13.89	12.50	9.72	6.94

5.5.3. pH

The variation of pH after accelerated stability test for samples were also analyzed and the results are given in Figure 5.5.Actually, the pH of liquid soap products were roughly constant as LS, FAS and FAL revealed only 6.8 percent, 2.8 percent and 3.9 percent changes respectively. These results point out the fact that it is possible to use LS or its derivatives for an extended shelf life at skin friendly pH's without any modifications while the pH of FAS and NLS products should be regulated before storage. As the changes occurring for FAS and NLS samples were quite nominal after accelerated stability test, it is also expe1cted for the pH of these sample to stay constant after regulation.

Table 5.5. pH of liquid soap samples after 4 cycles of accelerated test

Sample	Initial pH	Final pH	% Change
LS	5.7	5.94	4.21
LSL	5.2	5.52	6.15
LST	5.15	5.66	9.90
FAS	9.64	9.84	2.07
FAL	9.45	9.74	3.07
FAT	9.24	9.54	3.25
NLS	9.8	10.07	2.76
NLL	9.38	10.02	6.82
NLT	9.25	9.04	-2.27

5.5.4. Antibacterial Assay

Finally, the antibacterial activities of fatty acids (FAS), natural essential oil (NLS) and synthetic surfactant based liquid hand soaps prepared in the presence and absence of cinnamon and lavender oils as antibacterial agents were tested on Escherichia Coli and Staphylococcus Aureus. The results indicated that the antibacterial effect of plain fatty acids based liquid soap disappears while especially cinnamon oil derivative (FAT) still exhibits adequate activity on both bacterial strains. On the other hand, natural coconut oil based liquid soap (NLS) sustain its activity even without essential oils as antibacterial agents. Moreover, synthetic surfactant based liquid hand soap maintain its inhibition effect where cinnamon (LSL) and lavender (LST) oil derivatives present more effective performances.

Table 5.6. Inhibition zones of liquid soap samples after 4 cycles of accelerated test

Samples	*Escherichia Coli* (mm)	*Staphylococcus Aureus* (mm)
FAS	-	16
FAL	10.92	16.64
FAT	16.53	18.13
NLS	17.15	18.94
NLL	14.51	17.71
NLT	-	14.31

Table 5.6. Inhibition zones of samples after 4 cycles of accelerated test (Contd.)

LS	15.22	19.625
LSL	16.23	20.525
LST	16.17	22.395

5.6. PANEL TEST

Panel test is a type of analysis that quantify pleasure of consumers by using specified criterias. In this test, it is aimed to evaluate liquid hand soaps prepared via different routes according to the appearance, odor, cleaning performance, viscosity and foaming capacity and reveal the most consumer friendly sample after feedbacks. The sample size for the test is in between 25 – 30 volunteers and the tested samples were LS, LSL, LST, commercial antibacterial soap and plain commercial liquid hand soap analogous to LS.

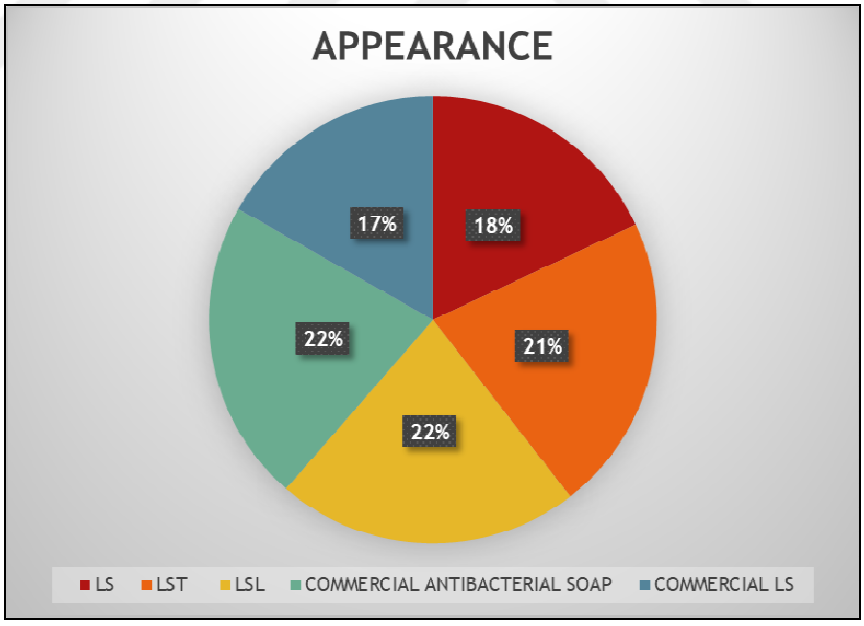


Figure 5.33. Consumer feedback on the appearances of the samples after the panel test

Figure 5.33 shows the pie chart constructed from the feedbacks of volunteers on the appearance of the samples. According to the results, there are no distinction in between prepared samples and the commercial alternatives.

Moreover, lavender (LSL) and cinnamon (LST) oil derivatives of LS are quite competitive with the commercial antibacterial soap presented in the market. As illustrated in Figure 5.34, odor of a personal care product has a key role on the pleasure of customers. Plain LS and commercial plain soap (without antibacterial agent) were not preferable due to lack of odorizer. Additionally, LST was not the first option as cinnamon has a dominant scent, which might not be favorable for a group of volunteers.

On the other hand, commercial antibacterial soap and LSL were amongst the highest rated alternatives due to their odor where LSL remains as a strong alternative to the commercial product.

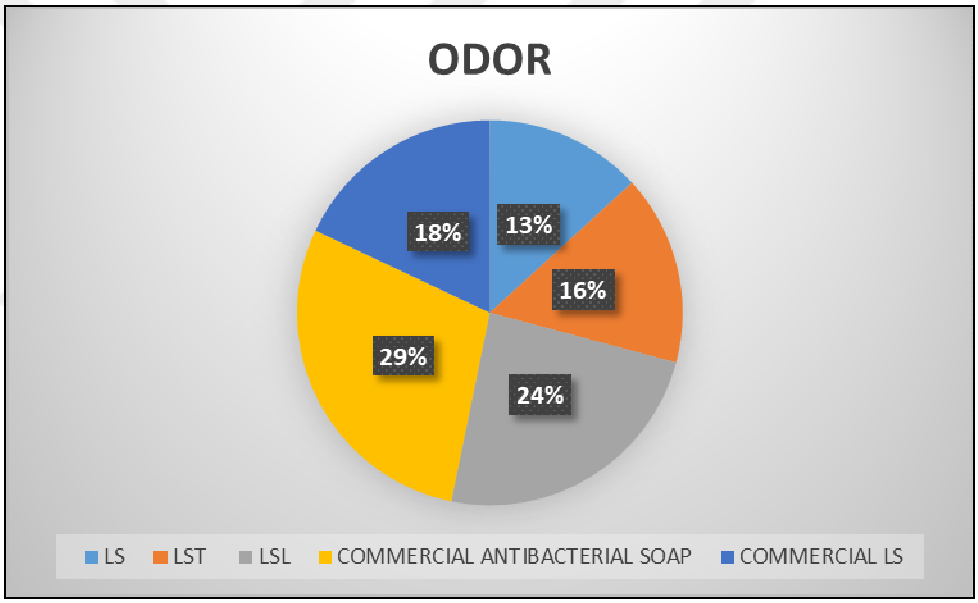


Figure 5.34. Consumer feedback on the odor of the samples after the panel test

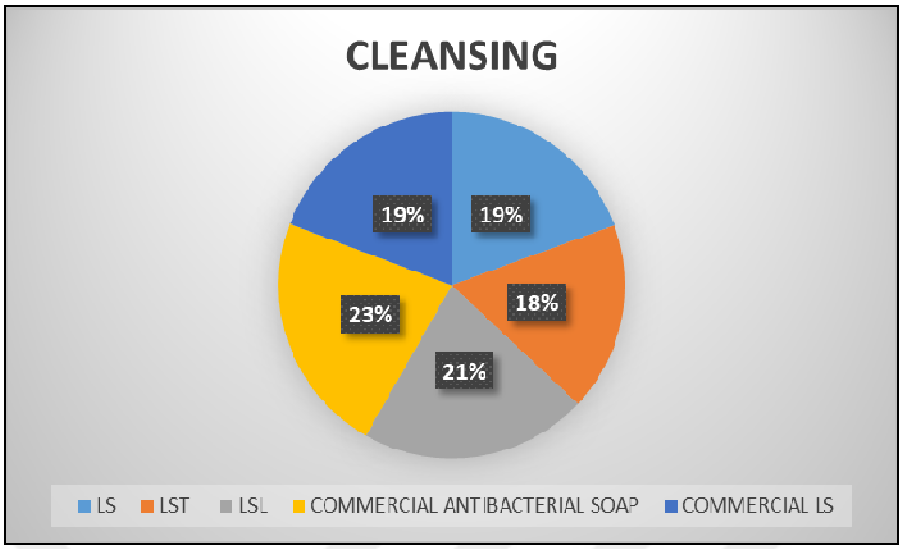


Figure 5.35. Consumer feedback on the cleaning performance of the samples after the panel test

The feedbacks on the cleaning performances of the samples are shown in Figure 5.35 and all samples exhibit approximately the same cleaning effect on volunteers where commercial antibacterial soap and LSL possess the highest percentages again.

However, when the foaming is concerned, all the prepared synthetic surfactant based liquid soaps (LS derivatives) presented preferable consumer satisfaction than their commercial alternatives (Figure 5.36).

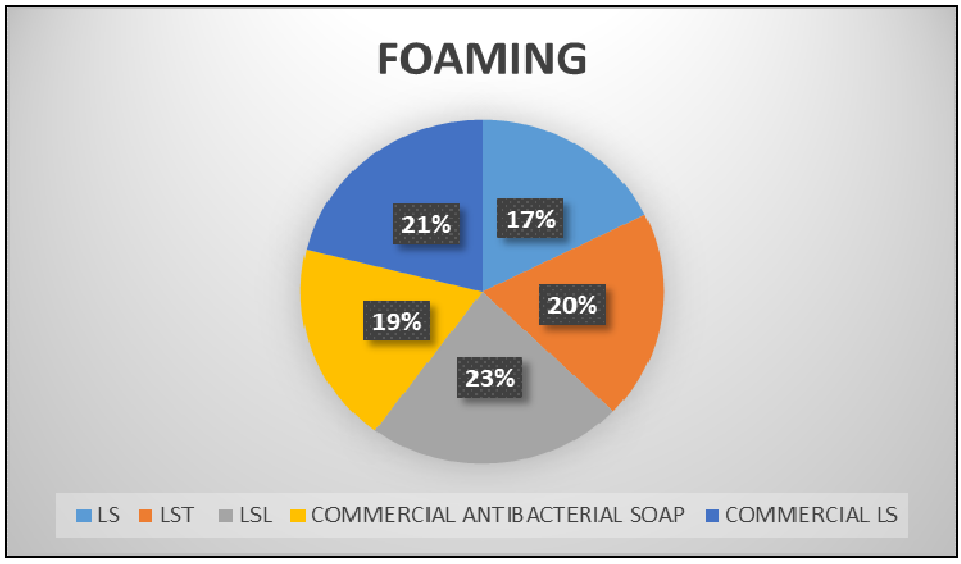


Figure 5.36. Consumer feedback on the foaming of the samples after the panel test

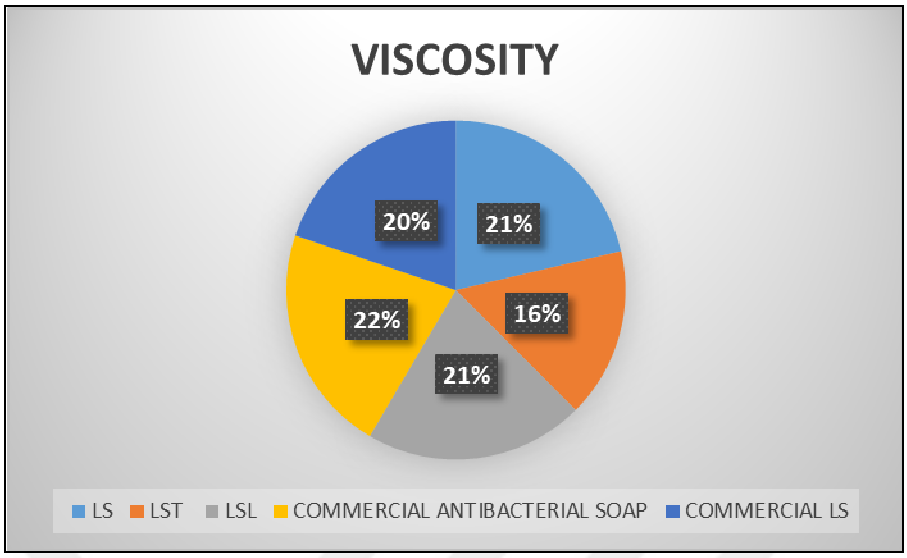


Figure 5.37. Consumer feedback on the viscosity of the samples after the panel test

The viscosity (thickness) of the samples were also evaluated by the volunteers and the results showed insignificant differences in between the prepared samples and their commercial alternatives (Figure 5.37). Solely the LST was slightly unpreferable but this can be attributed its corresponding viscosity, which was considerably lower than all other samples.

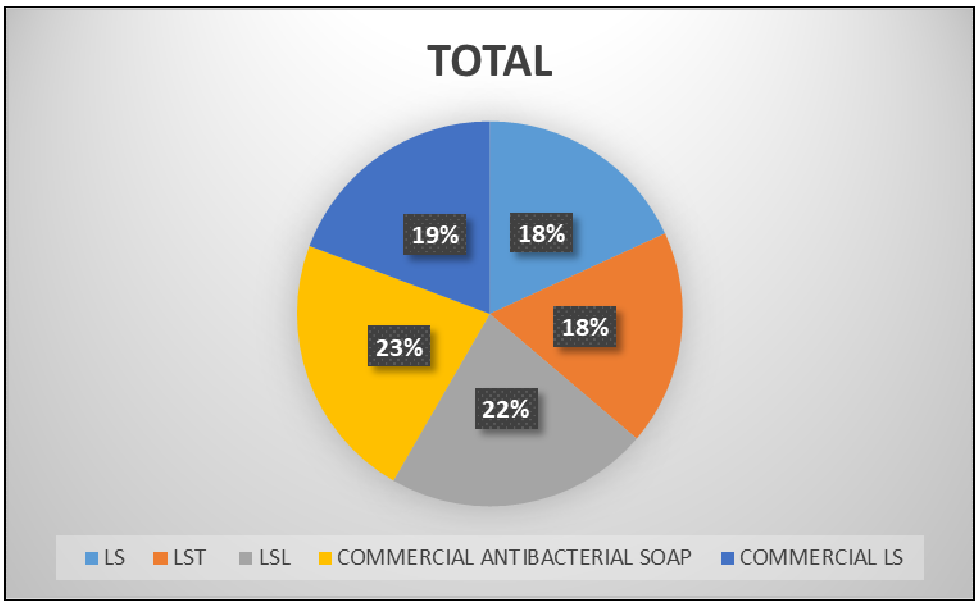


Figure 5.38. Overall consumer satisfaction after the panel test

Finally, consumers were asked to put forward a single sample amongst the group by considering all corresponding properties and the results are given in Figure 5.38. Roughly, half of the testers preferred LSL and commercial antibacterial as their percentages are 23 and 22 respectively. These similar figures along with all other panel test outcomes suggest that the addition of a simple natural essential oil into the formulation of a synthetic surfactant based liquid soap exhibit almost identical consumer satisfaction as compared with a commercial antibacterial liquid soap. By considering the antibacterial activities, all these results consequently put forward the possibility of producing a natural antibacterial liquid soap in the absence of detrimental and banned antibacterial agents simply by using biocompatible and natural alternatives.

6. CONCLUSION AND FUTURE WORK

Soap has been one of the essential materials as cleaning is crucial for humans and necessary in order to prevent diseases and the reproduction of many harmful microorganisms. Today, manufacturers produce various types of soaps mostly by using synthetic surfactants as these chemicals are relatively cheaper and the production costs are comparably lower than saponification processes, which requires high temperatures and extended reaction time.

The usage of antibacterial hygienic products especially soaps is common for roughly 30 years and attracted attention since then. The essential purpose of these products is to kill bacteria and inhibit their reproduction while providing sufficient cleaning performances and there is no doubt that antibacterial products are extremely needed in hospitals and highly contaminated areas. Several chemicals, which are known for their effective antibacterial potential have been used in hygiene products for many years. However, FDA banned 16 such chemicals in 2016 including triclosan (TCS) and triclocarban (TCC), which are commonly used in daily antibacterial products presented in the market due to insufficient clinical and scientific data on the safety of these agents. It was also shown that these banned chemicals affect the environment along with their byproducts, which cannot degrade totally and affect aquatic life. These antibacterial agents were also shown to increase the bacterial resistance in the environment.

Consumer satisfaction is also another aspect when designing antibacterial products especially liquid hand soaps. There is a clear demand on enhanced foaming performance of liquid antibacterial hand soaps as these products remain incapable due to their contents. Triclosan and its alternatives are cationic and require also cationic surfactants within formulations as anionic surfactants cause ionic interactions and lead to instability of the product. However, cationic surfactants exhibit lower foaming performances as compared with anionic alternatives.

The aim of this study is to prepare liquid hand soaps, which possesses antibacterial activities, adequate cleaning and high foaming performances in order to satisfy consumer demands and compete with commercial alternatives present within the market. It is also

aimed to reveal the possibility of exhibiting similar antibacterial activities with commercial products on bacterial strains by using natural alternatives and avoiding banned agents.

For these purposes, synthetic surfactant (LS), fatty acids (FAS) and coconut oil (NLS) based liquid hand soaps along with solid bar soap were prepared in the presence and absence of essential oils (lavender and cinnamon) ,which have previously shown to exhibit antibacterial activities. The prepared soaps were analyzed according to their viscosity, pH, foaming performances and antibacterial activities. In order to reveal the long term stability of these products, accelerated stability tests were also performed. Finally, panel test were conducted for determining the overall performance of these samples when compared with their commercial market alternatives.

The thickness (viscosity) of liquid soaps are important and mainly requested by consumers. It is also necessary to supply adequate contact time between the product and the surface, which can only by obtained by viscous liquid soaps. The viscosity measurements showed that LS and all its derivates prepared by the addition of cinnamon and lavender oil exhibited suitable viscosities as the amounts of salt and thickener (PEG-4 RAPESEEDAMIDE) optimized. Additionally, LS and LSL had almost constant viscosity for 3 months indicating sufficient shelf life. On the other hand, fatty acids (myristic, stearic, palmitic and lauric acids) and coconut oil based soaps had viscosities much lower than LS and the addition of essential oils further decreases the thickness of the samples.

For health care products such as shampoos, soaps, etc. the pH of the product is also important. As a consequence of production methods, synthetic surfactant based liquid soaps have pH similar to the human skin whereas saponification products have basic pH due to the presence of excess base. It is also necessary to maintain constant pH for the products during storage. The measurements for LS showed that the pH is close to 5.5, which is highly skin friendly. On the other hand, FAS and NLS exhibited higher pH values as expected. Most importantly, all these values remain almost constant for 3 months indicating an adequate and stable shelf life.

Foaming is a fundamental property of many common cleaning products. For personal care products such as soaps and shampoos formulations are mainly optimized in order to enhance bubbling after consumer feedbacks.

It is well known that antibacterial liquid hand soaps have lower foaming capacities as compared to plain soaps due to their cationic surfactant ingredients. Thus it was one of the purposes to produce liquid hand soaps, which contain antibacterial agents and exhibit sufficient foaming capacities.

The foaming capacity tests showed that LS and the essential oil derivatives offer high performances where the presence of additives cause a slight decrease. Additionally, fatty acids based liquid soaps also revealed comparable foaming capacities. However, coconut oil based liquid soap exhibited the lowest performance as compared to LS and FAS. This fact can be attributed to the variation of fatty acid contents in FAS, which can provide distinct foaming performances.

The main purpose of this project is to prepare liquid hand soaps by using biocompatible and natural antibacterial agents, which can be alternative to commercial liquid hand soaps containing detrimental chemicals such as triclosan and triclocarban. Various essential oils such as thyme oil, lavender oil, cinnamon oil etc. were shown in the literature to exhibit bactericidal activities. In this study, lavender and cinnamon oils were introduced into the formulations of different liquid hand soaps and the samples were tested especially against *Escherichia Coli* and *Staphylococcus Aureus*. For comparison commercial antibacterial liquid hand soap offered in the market containing benzalkonium chloride was also tested. The results indicated that even plain LS presented antibacterial activity on these strains. Furthermore, the addition of lavender and cinnamon oils further enhanced the inhibition performance of the product. The overall performance of synthetic surfactant based soaps were approximately close to the fatty acids based soaps. The comparison in between LS derivatives and the commercial antibacterial soap indicated the possibility of obtaining similar antibacterial effect by introducing natural essential oils in a plain liquid hand soap.

In addition to the performed series of analyses, accelerated stability test were performed in order to comment on the shelf life of the samples. It is clear that foaming performances, viscosities and antibacterial activities decreases as storage time extends.

For antibacterial activities, fatty acids based liquid soaps presented variable performances while coconut oil (NLS) and synthetic surfactant (LS) based soaps maintain their inhibition effect on bacterial strains where cinnamon (LSL) and lavender (LST) oil derivatives

present more effective performances. In general, LS were pointed out to be the most stable product amongst all other alternatives.

Finally, sensory assessment test were performed on prepared samples along with commercial alternatives for analyzing the consumer satisfaction by quantifying predetermined criteria. According to the results, all plain soaps were not preferred due to lack of odor while lavender oil derivatives were the most consumer friendly samples. For the cleansing effect, all samples presented almost similar performances but commercial antibacterial soap and LSL obtained the highest percentages while for foaming, all synthetic surfactant based liquid soaps (LS derivatives) had better feedbacks than their commercial alternatives. The overall evaluation of volunteers showed that the presence of natural essential oils in the formulation of a synthetic surfactant based liquid soap exhibit almost identical consumer satisfaction as compared to commercial antibacterial liquid soaps.

In the near future, the determination of certain and novel regulations will surely impact the products on the market and all manufacturers will adopt their productions accordingly. As a consequence all these performed tests and evaluations revealed the possibility of producing a less effective natural antibacterial liquid soap in the absence of detrimental and banned antibacterial agents simply by using biocompatible and natural alternatives but in exchange for a shorter shelf life.

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