

***Curcuma Longa L.* vegetable oil characterization as cutting
fluid base and *Curcuma Longa L.* essential oil
antimicrobial properties analysis for machining
application**

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application**

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RESUMO

A demanda global por lubrificantes gira em torno de 40 milhões de toneladas e cerca de 40% dessa produção é perdida em processos ou acidentes. Essa alta demanda e desperdício torna inevitável considerar outra possibilidade de substituir os lubrificantes à base de óleo mineral por uma fonte renovável. Muitas técnicas estão sendo desenvolvidas para alcançar alternativas mais sustentáveis, portanto a produção de biolubrificantes a partir de óleos vegetais é um mercado promissor devido à sua biodegradabilidade e grande número de fontes. Este trabalho tem por objetivo investigar o óleo vegetal de *Curcuma Longa L.* como potencial base lubrificante e o óleo essencial de *Curcuma Longa L.* como potencial aditivo biocida, ambos para aplicação em usinagem. No método propõe-se avaliar as propriedades físico-químicas do óleo vegetal de *Curcuma Longa L.* relacionadas com as características do lubrificante (viscosidade, estabilidade térmica e molhabilidade) e avaliar a atividade biocida de três óleos essenciais comerciais de *Curcuma Longa L.* por meio da concentração mínima inibitória (MIC), concentração mínima bactericida (MBC) e disco difusão. Pelos resultados, o óleo vegetal *Curcuma Longa L.* apresentou alto valor de viscosidade, alta molhabilidade para aço 1045 e alumínio e boa estabilidade térmica, características promissoras para ser utilizado como base lubrificante e apenas um óleo essencial apresentou boa atividade biocida contra *Klebsiella pneumoniae* e *Staphylococcus aureus*, o que indica que diferentes fontes podem levar a diferentes atividades bactericidas. Logo se conclui que a os óleos extraídos da planta de *Curcuma Longa L.* tem potencial para ser utilizado como biolubrificante.

Palavras chave: Óleo vegetal, Fluido de corte, Usinagem, Óleos essenciais, Antimicrobiano, Curcuma.

ABSTRACT

The global demand for lubricants is around 40 million tons and about 40% of that production is lost in processes or accidents. This high demand and waste makes it inevitable to consider another possibility of replacing mineral oil-based lubricants with a renewable source. Many techniques are being developed to achieve more sustainable alternatives, so the production of biolubricants from vegetable oils is a promising market due to its biodegradability and large number of sources. This work aims to investigate the vegetable oil of *Curcuma Longa L.* as a potential lubricant base and the essential oil of *Curcuma Longa L.* as a potential biocidal additive, both for machining applications. The method proposes to evaluate the physicochemical properties of vegetable oil of *Curcuma Longa L.* related to the characteristics of the lubricant (viscosity, thermal stability and wettability) and to evaluate the biocidal activity of three commercial essential oils of *Curcuma Longa L.* using minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and disk diffusion. From the results, the vegetable oil *Curcuma Longa L.* showed high viscosity value, high wettability for 1045 steel and aluminum and good thermal stability, promising characteristics to be used as a lubricant base and only one essential oil showed good biocidal activity against *Klebsiella pneumoniae* and *Staphylococcus aureus*, which indicates that different sources can lead to different bactericidal activities. It is concluded that the oils extracted from the *Curcuma Longa L.* plant have the potential to be used as a biolubricant.

Keywords: Vegetable oils, Cutting fluids, Machining, Essential oils, Antimicrobial, Curcuma.

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SIGNS

DMHB	Difco Mueller Hinton Broth
DMSO	dimethylsulfoxide
DSC	Differential Scanning Calorimetry
ITA	Aeronautics Institute of Technology
MBC	Minimal Bactericidal Concentration
MFU	Mcfarland unit
MIC	Minimal Inhibitory concentration
MLQ	Minimum Quantity Lubrication
MWF	Metalworking Fluid
pH	Hydrogen ion concentration
<i>sp.</i>	Specie
TGA	Thermogravimetry
Vc	Cutting velocity [mm/min]
θ	Contact angle (°)
Q'	[mW/g]
Q	[J/g]
C	Culture density
P	Percentage of bactericidal activity [%]

1. INTRODUCTION

1.1 CONTEXTUALIZATION

Metal working fluids (MWFs) have been largely used in a significant number of industrial processes and products. Reports indicate that the global demand for lubricants amounted over 36 million metric tons [1]. They influence heat generation by reducing friction between tool and workpiece by dissipating and conducting the generated heat. Lubricating and cooling properties, MWFs contribute to avoid thermal damage in the working piece and reduce tool wear [2].

More than 50% of metalworking lubricants are petroleum-based [3], which can cause negative impacts such as water and groundwater contamination, air pollution, soil contamination, besides environmental problems, it is reported that 80% of all occupation diseases of operators were caused due skin contact with cutting fluids [4]. For those reasons there is an increasing demand for environmentally acceptable and biodegradable that can replace petroleum-based fluids. Vegetable oils based fluids have shown potential to be used as cutting fluids since they can offer 95% biodegradability, high viscosity index, high lubricity, low volatility, low toxicity and it is a renewable resource [3]. Also those oils are suitable as base stock due the facility to blending with different kinds of environmentally additives, besides additives prepared from vegetable oils demonstrated excellent tribological properties and good performance at extreme pressures [5]. There are other environmentally friendly technics being developed such as dry machining and minimum quantity lubrication (MQL), however, in some operations as grinding, cutting fluid is necessary due to high amount of heat generated [6].

Vegetable oils can be classified in edible and nonedible, edible have high commercial demand such as coconut, soybean, canola, corn oils and nonedible are less expensive such as castor, linseed, jatropha, nowadays the production of vegetable oils in the world is around 198 million of tons, giving a wide and consolidate market with a miscellaneous sources of vegetable oils [7]. Many researches are in progress to develop bio based cutting fluids, which in terms of performance have good tribological properties, corrosion resistance and increases tool life [5].

In this work is proposed to investigate a new source of vegetable oil, *Curcuma Longa L.* plant, as lubricant base and verify the potential and limitations of this fluid,

the results can give new possibilities to develop and improve physico-chemical properties through chemical modification or investigate other applications [8]. Also from the same source it is possible to obtain essential oil with different methods of extraction, those types of oils have shown a remarkable biocidal activity [9]. This study has the purpose of analyzing antimicrobial potential from Curcuma plant against pathological bacteria from contaminated cutting fluids and also substitute harmful biocidal additives, the results can also indicate methods to improve antimicrobial activities such as chemical modification, methods of extraction and others.

1.2 JUSTIFICATION

The global demand for lubricants is around 40 million of tons, being 56% automotive lubricants and 44% industrial applications and around 40 % is lost in use or accidents. With this high demand and waste for lubricants is essential to consider the use of renewable resources to compete or even replace mineral based lubricants. Also, the pollution associated with use and discharge of these petroleum-based lubricants can generate direct and indirect negative effects on nature due to the poorly biodegradable and high toxic characteristics. Some base fluids and additives have found to present potential hazards related to inhalation of mists and skin contact, causing long term diseases on skin and lungs. Biolubricants can be a revolutionary market to a recyclable, nontoxic, renewable product in addition with high demand it can improve and amplify natural resources agriculture or even incentive family farming, which represents 70% of aliment consumed in Brazil [8-12].

Biolubricants have some good lubricant properties, however they suffer from poor low temperature fluidity and poor thermal and oxidative stability which as consequence leads to rapid degradation [13]. But those issues are being outlined through chemical modification of vegetable oils and genetic modification of oil seeds with the intention to improve those properties giving possibilities to new researches and formulations for renewable sources [4]. Epoxidized soybean oil demonstrated an improvement for thermal and oxidative instabilities for high temperatures by reducing significantly unsaturation in fatty acid chains [12]

Another issue that can be solved through the use of natural products is biodeterioration from lubricants. Part of occupational diseases is caused by harmful additives but also it is been reported that exposure to contaminated lubricants is related

to respiratory diseases [14]. Essential oils have demonstrated biocidal potential against pathogenic bacteria and are not a nocive product for environment and operator [15].

Through this context it is noted that are still demand for multidisciplinary researches for vegetable oils and essential oils to develop a biolubricants with physico-chemical properties, technical characteristics, ecological and economic viability to substitute mineral based lubricants in industry.

1.3 OBJECTIVES

The objectives of this thesis can be separated in two parts, first is to study, analyze and evaluate physico-chemical properties from *Curcuma Longa L.* vegetable oil comparing to commercial synthetic vegetable oil and literature to verify if this fluid have potential as lubricant base for machining purposes.

The second objective is to analyze and evaluate *Curcuma Longa L.* essential oils potential as antimicrobial and how effective is this activity, with the purpose apply as a biocidal additive for cutting fluids.

With good results there will be a possibility to produce a bio-lubricant using consequently as base for lubricant and additive the *Curcuma Longa L.* plant.

1.4 THESIS STRUCTURE

The thesis is structured in 6 chapters. Chapter 1 gives an introduction to the theme approached, with a motivation background and the main objectives of this research, considering the benefits of using vegetables oils as a form to replace non-renewable sources as petroleum-based oils for lubricant applications.

Chapter 2 is the bibliographic review that first explains machining process and heating problems associated to this process and how metalworking fluids are applied to reduce those issues and the potential of vegetable oils as cutting, considering lubricant properties and advantages and disadvantages of those fluids. Then an introduction to essential oils and the potential as biocidal additive to metalworking fluids.

Chapter 3 describes the materials and methods used to develop this research. First with characterization of *Curcuma Longa L.* vegetable evaluating physical-chemical properties through fatty acid profile, wettability, thermal stability and viscosity. And antimicrobial activity of essential oils by analyzing three different commercial *Curcuma*

Longa L. essential oils through minimal inhibition concentration (MIC), minimal bactericidal concentration (MBC) and disk diffusion test, associating this activity with gas chromatography test by comparing composition.

Chapter 4 contemplates results and discussions about the physical-chemical properties compared to literature and a commercial synthetic vegetable oil to evaluate performance and potential for industrial application. The biocidal activity from each vegetable oils comparing bacterial inhibition and associating performance with composition.

In chapter 5, are presented the main conclusions of this research, and are also presented some suggestions for future work.

2. BIBLIOGRAPHIC REVIEW

2.1 MACHINING ISSUES

Machining is a fabrication process that removes chip, this operation has the objective to give the desirable form, dimension and finishing to the workpiece. Chip is a portion of material removed with a cutting tool which is characterized through its irregular form [16].

The chip formation occurs initially when the cutting edge is pressed against the workpiece, through this contact the material suffers a plastic deformation which progressively raises until it ruptures, then the crack spreads defining a shear region. Continuing the tool penetration movement, it will occur a partial or complete rupture, the chip size is determined by material properties, cut velocity and advance conditions. Due to the relative movement between the tool and the workpiece, the deformed and broken portion of material slips on the tool's outlet surface. It's possible to observe in Figure 1 the chip formation and shear region [16].

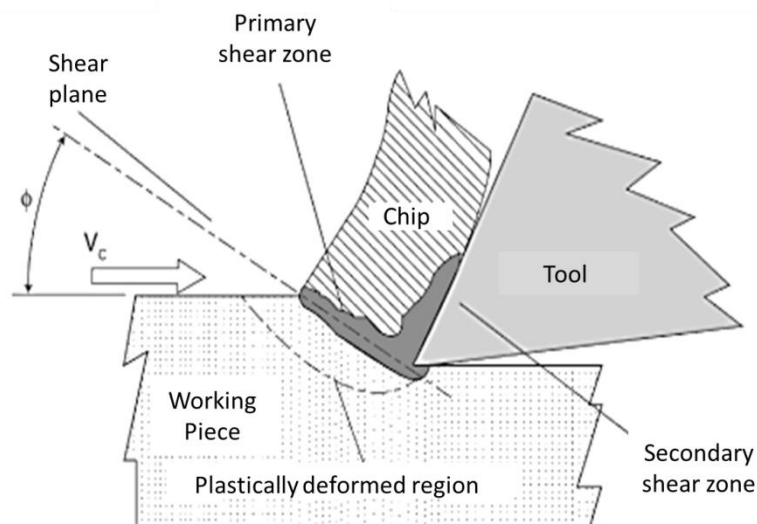
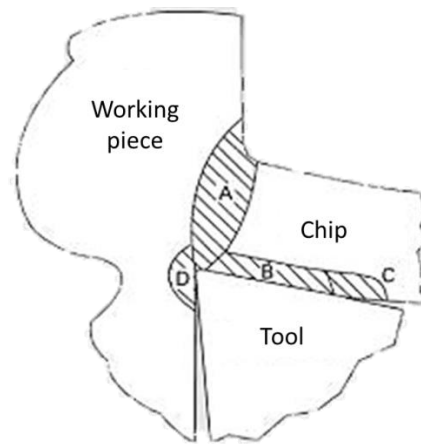


Figure 1. Chip formation mechanism [16].

To perform machining movements a great amount of energy is demanded, however most of consumed power is converted into heat near the cutting edge. Tool and chip interface can reach temperatures around 1100°C depending on the workpiece

material and cutting parameters used. Machining forces causes deformations on chip, workpiece, cutting tool and even in the machine, the work from deformation and friction forces are converted into heat, due to the complex geometries and variables this phenomenon isn't complete predictable. Figure 2 illustrate different heating regions during chip formation [16].



perFigure 2. Shear regions during chip formation [16].

The energy spent in the chip formation process is released as heat in three different regions in the chip formation area: in the area of the shear plane or primary shear zone (region A); at the interface between the chip and the tool's exit surface or secondary shear zone (B and C regions); and at the contact interface of the part with the tool clearance surface or tertiary shear zone (region D) [16].

The heat, in the primary zone, from shearing comes from the internal shearing work of the material, while in the other two zones the heat comes from friction and internal shearing caused by the adhesion between the surfaces. The heat in the shear plane can even be beneficial for machining, as it increases the temperature in the primary and secondary zones and decreases the mechanical resistance of the part material to continue the plastic deformation (softening effect). The heat generated in the secondary shear zone has a greater influence on the performance of the tool and is practically a limiting factor for the material removal rate, especially by increasing cutting speed. The contact between the clearance tool surface and the workpiece (tertiary zone) can occur even in the case of tools without flank wear, since minimal deformation is required for chip formation and it can also contribute to increase temperature of workpiece and tool. The cutting fluid function starts on tertiary zone

assisting the tool slide on surface contact against workpiece and cooling the region, on secondary zone it helps to expel chip during its formation [16].

High temperatures can cause issues such as poor surface finish, residual stresses, cracks, affect workpiece mechanical properties and, in some cases, diffuse tool components with workpiece material. Normally, after machining some post processes have to be done with the purpose to achieve demanded dimensional tolerances and surface harshness. To achieve the best machining performance by reducing temperature during machining some studies have been developing materials for tool and workpiece that can reduce friction coefficient and improving cooling technics including new cutting fluids [17].

2.2 METALWORKING FLUIDS

The necessity of using fluids in machining processes originated, when the American F. W. Taylor used water in the contact between tool and workpiece, he observed that he could raise 33% of cutting velocity without harming the tool. Besides dissipating heat, another benefit is that the water cleans undesirable remaining chips, which can damage tool and workpiece on cutting region. However, for lubrication purposes only water is quite deficient making the cutting region susceptible to have corrosion problems [18].

Presently, metalworking fluids (MWFs) are part of a large family of lubricants, due the existence of a great number of different metals cutting processes. With the advance of technology, industries are requiring developed fluids with specific functions, for different materials. Moderns MWFs have a complex mix of different components, each one fulfils specific functions. The development and application of MWFs it's an interdisciplinary field which includes technology of metals processing and tribology therefore is possible to develop different types of MWFs [19].

The selection and application of cutting fluids in industry when properly applied, can increase productivity and reduce costs by making possible the use of higher cutting speeds, higher feed rates and greater depths of cut. Effective application of cutting fluids can also increase tool life, decrease surface roughness, increase dimensional accuracy and decrease the amount of power consumed [20].

2.2.1 Function

The main functions of MWFs can be separated in three segments, physical, chemical and mechanical interaction [18]. The fluid acts physically through lubricating and cooling machining processes by reducing tool and workpiece friction and dissipating generated heat, respectively [2]. The Chemical action occurs when MWFs contains components that can react chemically with nascent chip surface in contact with the tool. This reaction can reduce friction coefficient in chip-tool interface by producing a low hardness compound in the chip, reducing chip deformation and, consequently, the cutting force, that's additives have their importance. The mechanical action occurs when MWFs expels chip from cutting region, if the chip builds up it can impair the machining operation. In figure 3 is possible to observe the adhesion and sliding regions where cutting fluids act during chip formation [18].

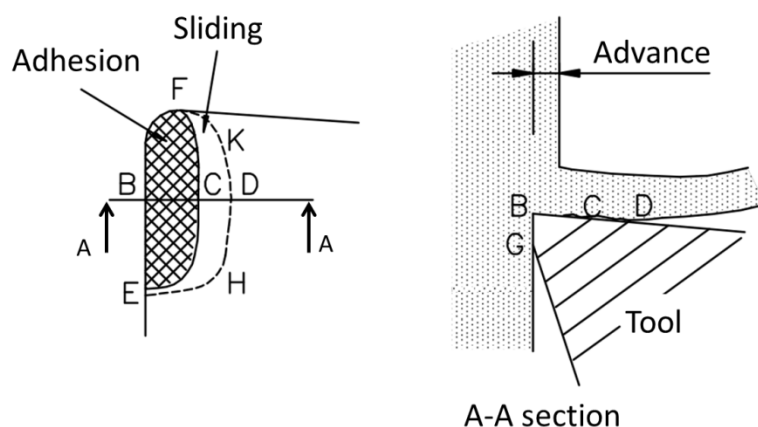


Figure 3. Adhesion and sliding zones [21].

2.2.2 Classification

Lubricants can be separated into two major groups, automotive lubricants and industrial lubricants. Industrial lubricants can be divided into industrial oils and industrial specialties, which are principally greases, metalworking fluids and solid lubricants [22]. Between Metalworking fluids there are three main groups [16]:

- Straight or neat oil: Predominantly mineral based oil, they may be wholly mineral or contain carrying additive due to your low thermal conductivity and their low specific heat make them ineffective in conducting heat outside the cutting region;

- Emulsion: Biphasic compounds of mineral oils added to water at ratio of 1:10 to 1:100, with emulsifying agents or surfactants that guarantee the uniformity of the mixture, forming smaller oil droplets resulting in translucent emulsions;
- Solution: Monophasic oils compounds that dissolve completely in water, also called as synthetic oils, without mineral oils, they consist of organic and inorganic salts, lubricity additives, biocides, corrosion inhibitors, among others, when added to water [16].

For each machining process demands is necessary to select the most suitable fluid in order to have the best performance, which includes considering the chemical additives used to formulate MWFs to serve various functions. These include emulsification, corrosion inhibition, lubrication, microbial control, pH buffering, coupling, defoaming, dispersing, and wetting [23].

The functional additives used in metalworking fluids each contribute to the total composition. The effect of the addition of an additive is tested by the chemist to ensure that optimal properties of a fluid are maintained. In general, a fluid should be stable, low foaming, and waste treatable. Many of the properties of additives are mutually exclusive, if a fluid has excellent biological and hard-water stability, it may be difficult to waste treat. If it provides excellent lubricity, it may be difficult to clean [23].

2.2.3 Microbiological degradation

Inside manufacturing environment MWFs becomes conducive to microorganism proliferation, experiencing oscillation of temperature, humidity and exposure to factory environment [24]. Microbes are primarily agents of biodeterioration, capable of degrade commercial value of coolants, tools, finished parts and fluid systems. Those uncontrolled microbial effects can cause an annual expense in the tens of millions of dollars, associated to coolant and waste disposal, lost productivity, decrease of tool life, failure rate and others [23].

Biodegradation occurs in two stages, first microbes consume completely or partially some MWF components, second is when microbial metabolites react with MWF components, most microbes excrete a variety of weak organic acids as metabolic waste. These acids may react with neutralizing amines directly or may react with inorganic ions, such as chloride, sulfate, and nitrate that are present in the MWF [23].

Biological debris for example endotoxins from degraded cell walls are ubiquitous in MWF systems and are potent toxins causing compromise of lung function and sensitization [24]. Besides several components of MWFs already may be irritants even some biocides may cause injuries for the operator when evaporated [25].

There are some common biodeterioration indicatives that MWFs can exhibit which are: production of malodourous, volatile organic chemicals, pH decreases and emulsion instability. Also, by consequence of those changes, some visible issues start to appear such as corrosion of working piece and decrease of machine tool life [26].

2.2.4 Bacteria present in MWFs

There are two types of microorganism that contaminate MWFs that are bacteria and Fungi. Bacteria are single-celled microorganism, for this reason they have distinct cell wall structures depending on the environment that are submitted, making difficult to fully identify a given bacterium [27], it is believed that less than 1% of the population present in the environment is isolated and identified by means of culture-based techniques [28]. The bacteria commonly found in contaminated MWFs are: *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumonia*, *Desulfovibrio sp.* [27]. In a study with 150 samples of contaminated emulsions indicated that *Pseudomonas aeruginosa* and *Escherichia coli* have the most frequent presence on Figure 4 several samples presented more than one bacteria in the analysis [29]. They are categorized as gram positive or gram negative gram positive have a thick and rigid cell wall and presence of teichoic acid, and contrary to what it seems they have permeable cell wall for biocides, gram negative have opposite characteristics thin and limp cell wall and absence of teichoic acid which offers a complex barrier for biocides [28, 29].

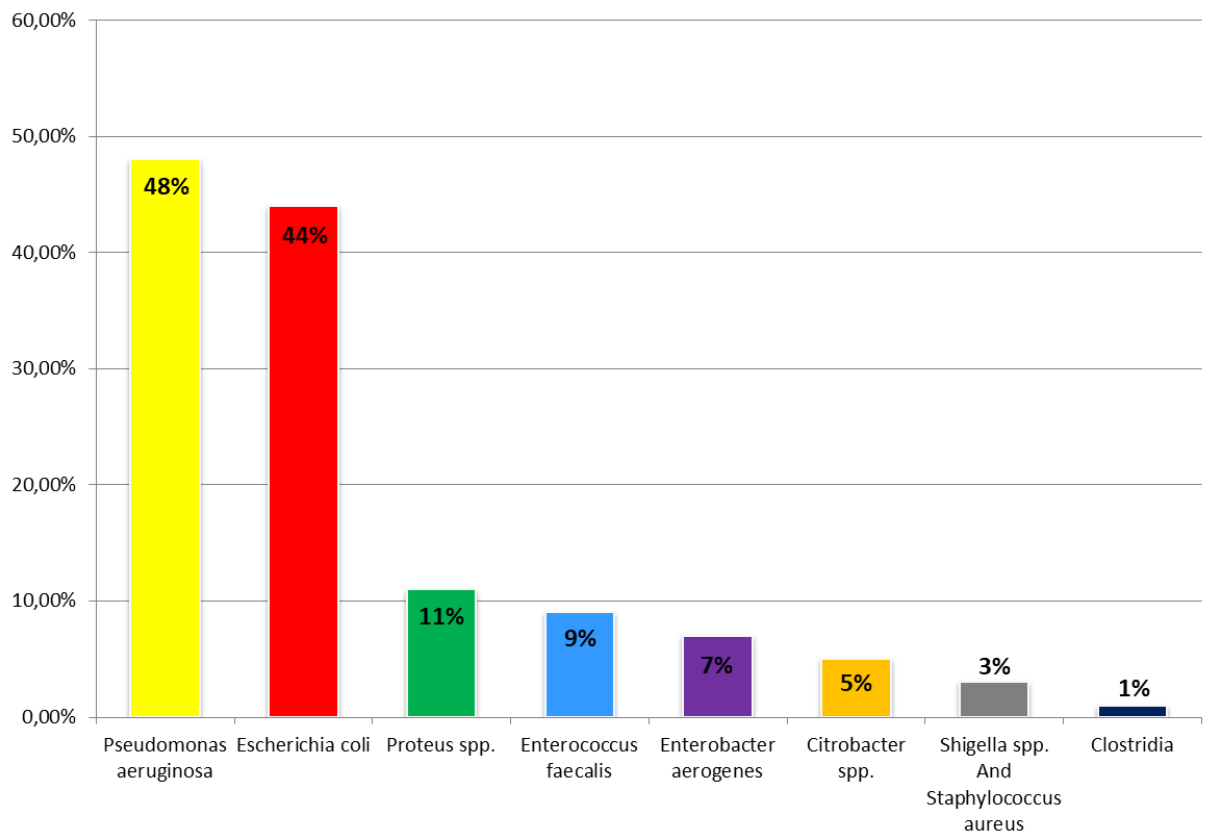


Figure 4. Proportion of bacteria found in 150 samples of MWFs emulsions [29].

The dominant group of bacteria in contaminated MWFs is *Pseudomonas sp.* capable of growing in pH 9.0 to 11.0, considered as primary colonizers its growth reduces pH, provide by-products and neutralize biocides favoring other bacteria to grow and establish [30]. As gram negative type, it seems that this specie is capable of surviving harsh environments, even with high concentrations of biocides. Otherwise *Pseudomonas sp.* in general are not pathogenic for humans, but some infections were reported from few specific species [31, 32]. *Pseudomonas aeruginosa* causes the degradation of the mineral oil and have been shown to possess natural resistance to many commonly used antimicrobial agents and biocide [31], for machine operator may cause festering wounds on damaged skin and intestinal infections of dysenteric nature [29].

Proteus Mirabilis and *Escherichia Coli* are also gram negative bacteria, but unlike most *Pseudomonas sp.* they are pathogenic for humans being associated to infections of the urinary tract associated with catheter use being able to cause severe sequelae [32]. *Escherichia Coli* produce enterotoxins whose properties and role in diarrheal disease have been investigated [33].

progression of pits and asperities on the metal surface [41]. The presence of a polar group with a long hydrocarbon chain makes vegetable oil amphiphilic surfactant by nature, allowing it to be used as a boundary lubricant, providing high strength lubricant films reducing friction and wear [35,36].

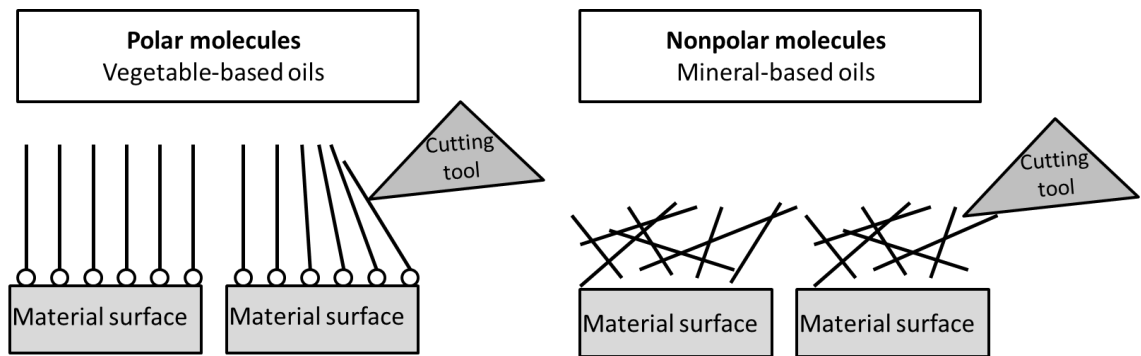


Figure 7. Polar molecules alignment compared to nonpolar molecules. Adapted from Kuroda (2006) [40].

Physicochemical properties from vegetable oils also influence lubricant performance. Viscosity is the most significant property of lubricant oil, it indicates the internal friction within a liquid due to molecular interaction, influencing lubricant film formation during machining. The high degree of long chained fatty acids implies in high viscosity index, higher density and lower volatility. Vegetable-based oils have relatively high viscosity index compared to mineral-based oils, this enables to operate in wider temperature range, which is a desirable characteristic [37,42].

For high temperatures volatility and flash point are important properties to evaluate thermal stability [43]. Volatility is the tendency of the lubricant to vaporize and flash point is the temperature point at the formed vapor is ignitable in the presence of air. Good high temperature properties have volatility and flash point above operating temperature. For low temperatures cloud point and pour point are important properties. Cloud point is the temperature at which starts to become hazy due to the crystallization process. Pour point is the temperature below cloud point which lubricant stops to flow. With low pour point it is possible to work with the fluid without clogging of filters [36].

The vegetable oils properties are directly influenced by fatty acids composition and saturation degree (Table 1), by consequence their efficacy and performance allowing them to be used as lubricant. Fatty acids composition varies due to a wide number of sources that vegetable oils can be extracted and biological factors such as

temperature, climate, light, water, soil, for this reason is important to analyses and understand the fundamentals of saturated and unsaturated fatty acids [44].

Table 1. Influence of fatty acids in vegetable oil properties.

Properties	Condition	Influence	References
Viscosity	High unsaturation degree	Lower viscosity	[44] [45]
	High monounsaturated FA	Superior lubricity at room temperature	
	Long hydrocarbon chain length	Increases viscosity	
Oxidative stability	Low unsaturation	Increases oxidative stability	[46]
Pour point	High unsaturation degree	Lower pour point	[47]
Biodegradability	linear chained compounds	Better biodegradation	[48]
Volatile	short hydrocarbon chains	Higher volatile	[43]
Thermal stability	High unsaturation degree	More susceptible to thermal degradation	[49]

Compared to mineral-based lubricants, vegetable oils in general possess high flash point, high viscosity index, higher lubricity, low evaporative losses, and good metal adherence [50]. In Table 2 there are the principal advantages and disadvantages of using vegetable oils instead of mineral based oils for machining applications. To overcome those disadvantages modification of the chemical structure is already been used as an alternative. For example, unsaturated alkene groups of fatty acid chains in triglyceride molecules can be altered to improve low temperature and oxidative stability properties [51].

In literature can be found some potential vegetable oils that demonstrated good machining performance besides being viable technologic and economically. An example is the non-edible vegetable oil from pine nut with high lubrication capacity and high resistance to oxidation, compared to others vegetable oils, also it can be cultivated in desert climate and encourage familiar agriculture from necessitous regions [21].

Table 2. Advantages and disadvantages of vegetable-based oils as lubricants [36, 42].

Advantages	disadvantages
High biodegradability	Low thermal stability
Compatibility with additives	Poor oxidative stability
Wide production possibilities	Poor low temperature properties
Low toxicity	Poor corrosion protection
High flash point	
Low volatility	
High viscosity indices	

It's been reported in various literature that metalworking fluids can be environmentally friendly with similar or better performance obtained using mineral-based oils, in Table 3 there are some applications and studies found in literature.

Table 3. Application of vegetable oils in literature.

Vegetable oil	Application	Reference
Palm	Engine oil, Hydraulic fluids, MWF	[52], [53], [54]
Soybean	Hydraulic fluids, Compressor oil, MWF	[55], [56], [57]
Castor	Engine oil, Grease	[58], [59]
Corn	Insulating film	[60]
Coconut	Automobile lubricant, Nano fluid	[61], [62]
Peanut	Asphalt release agents	[63]
Sunflower	Asphalt release agents	[64]
Rapeseed	Grease, compressor oil, Hydraulic fluid	[65], [66], [67]
Canola	MQL	[68]
Jatropha	Engine oil	[69]

2.4 ESSENTIAL OIL

Essential oils are one of the most important resources for industries such as food, cosmetics and pharmaceuticals, the word “essential” is postulated by Paracelsus in the theory of quinta essential. They are predominantly constituted of monoterpenes, sesquiterpenes, phenylpropanoid and esters. The strong essence and volatile nature from those oils is due its process of extraction usually through steam drag with organic matter

that could be leaves, roots, fruits, parts of plants in general. There are many applications and studies about antimicrobial, antifungal, antioxidant, anti-inflammatory, antiviral, anesthetic and others activities [70,71].

2.4.1 Biocidal potential

Some bacteria, due to massive use of traditional antibiotics have created resistance to them, essential oils besides being a natural product also demonstrated antimicrobial activity. Plants and other extracts can provide a complex and structurally diverse compounds which can provide new resources of antimicrobial compounds. *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* are amongst some of the main bacteria with multidrug resistance. A characteristic of essential oils is hydrophobicity of their components, which enables them to partition with lipids in cell membrane of bacteria, giving them more permeability to disturb cell structures [15].

Gram positive bacteria are more susceptible to essential oils than gram negative, this is due the outer membrane which is rigid, rich in lipopolysaccharide and more complex, limiting the diffusion of hydrophobic compounds through it, while this characteristic is absent in gram-positive bacteria which instead are surrounded by a thick peptidoglycan wall not dense enough to resist small antimicrobial molecules, facilitating the access to the cell membrane. Due to the lipophilic ends of lipoteichoic acid present in cell membrane, gram-positive bacteria are ease to infiltrate with hydrophobic compounds of essential oils [15].

Essential oils compounds can contain about 20 to 60 components with different concentrations, they are characterized with two or three major components at fairly high concentrations (20% to 70%) compared to the others. The components mainly are terpenes and aromatic, terpenes are made from combinations of isoprene and with oxygen present it is a terpenoid, aromatic compounds are derived from phenylpropane, usually occur less frequently the terpenes [72].

Most terpenes do not present high antimicrobial activity, otherwise tepenoids due to their functional groups and the hydroxyl groups of the phenolic terpenoids with the presence of delocalized electrons are important elements for their antimicrobial activity. Phenylpropenes antimicrobial activity of these molecules is due to their free hydroxyl groups and also depends on the type and number of substitutions on the aromatic ring. Because of the wide variety of molecules present in the natural extracts,

the antimicrobial activity of the essential oils cannot be attributed to a single mechanism, which is the reason to study each type of essential to discover which is the best application [70].

2.4.2 *Curcuma Longa L.* essential oil

Curcuma longa L. belongs to the Zingiberaceae family and is a perennial herb, normally found in tropical and subtropical regions, is widely cultivated in Asiatic countries as a spicy seasoning (figure 8) [71]. *Curcuma Longa L.* essential oil can be extracted with two main technics hydrodistillation and extraction with ethanol which consequently presents differences in composition. Through hydrodistillation aromatic turmerone is the predominant component, with ethanol it is α -turmerone. When extraction is from rhizomes or leafs also can influence to have different composition. Leafs produce oils with terpinolene and 1,8-cineole as predominant components and rhizomes produce with high percentage of α -turmerone, those differences can influence in biocidal activity from those oils [73].

The principal activities found in literature from curcuma essential oils are antioxidant, anticancer and antibacterial [73]. In table 4 there are some biocidal applications found in literature with curcuma essential oils with different extractions and microorganism.



Figure 8. Rhizome and powder from *Curcuma Longa L.* plant species [74].

Table 4. Application of essential oils in literature with bactericidal activities.

Bacteria	type of extract or component	reference
<i>Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli</i> and <i>Pseudomonas aeruginosa</i>	Methanol	[75]
<i>Escherichia coli, Salmonella enteritidis, Clostridium perfringens, Staphylococcus aureus, Campylobacter jejuni</i> and <i>Bacillus cereus</i>	Methanol	[76]
<i>Staphylococcus aureus, Micrococcus luteus</i> and <i>Bacillus cereus</i>	Methanol	[77]
<i>Staphylococcus aureus</i>	Ethanol	[78]
<i>Helicobacter pylori</i> and <i>Campylobacter jejuni</i>	hydroethanol	[79]
<i>Staphylococcus aureus</i>	ethyl acetate, methanol and water	[80]
<i>Escherichia coli, Staphylococcus aureus</i> and <i>Klebsiella pneumoniae</i>	Water	[81]
<i>Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae</i> and <i>Staphylococcus epidermidis</i>	Water	[82]
<i>Staphylococcus aureus</i> and <i>Bacillus cereus</i>	curry paste extract	[83]
<i>Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa</i> and <i>Salmonella typhimurium</i>	rhizome	[84]
<i>Bacillus cereus</i>	alcoholic and aqueous	[85]
<i>Listeria monocytogenes</i> and <i>Salmonella Typhimurium</i>	isopropanol, hexane or a mixture of isopropanol-hexane	[86]
<i>Helicobacter pylori</i>	water	[87]
<i>Staphylococcus spp.</i> and <i>Pseudomonas aeruginosa, Acinetobacter spp.</i>	Methanol	[88]
<i>Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Bacillus cereus, B. subtilis</i> and <i>Staphylococcus aureus</i>	Acetone	[89]
<i>Bacillus cereus, Bacillus coagulans, Bacillus subtilis, Staphylococcus aureus, Escherichia coli</i> and <i>Pseudomonas aeruginosa</i>	Hexane	[90]
<i>Escherichia coli, Staphylococcus aureus, Bacillus cereus</i> and <i>Listeria monocytogenes</i>	Hydrodistillation, petroleum ether and ethanol	[91]
<i>Bacillus subtilis, Staphylococcus aureus, Escherichia coli</i> and <i>Pseudomonas aeruginosa</i>	hexane, dichloromethane (DCM), ethyl acetate, ethanol, methanol and water	[92]

3. MATERIALS AND METHODOLOGY

In this chapter is detailed the experiments and procedures used to characterize *Curcuma Longa L.* vegetable oil physico-chemical properties and evaluate biocidal potential from three commercial *Curcuma Longa L.* essential oils, on experiments description will have materials, equipment, technics used to perform them.

3.1 CURCUMA LONGA L. VEGETABLE OIL LUBRICANT PROPERTIES EVALUATION

3.1.1 Wettability

The lubricant affinity with working piece material surface can be evaluated with wettability analysis. Wettability is the ability of a fluid to spread out, penetrate and cover the tool and workpiece, quantitatively is defined by the contact angle between a liquid droplet and a solid surface in thermal equilibrium with each other [93]. The complex effects of roughness and chemical composition of the surface develop intermolecular forces (Van der Waals' forces, hydrogen polar bonding, electrostatic attraction, etc.) with the lubricant determine an equilibrium condition in which how much the surface will be wetted [94]. In Figure 9 is possible to see how the droplet behaves from low to high surface affinity.

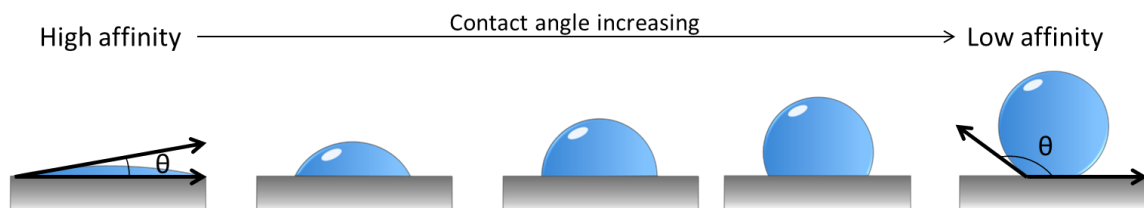


Figure 9. Correlation between contact angle and affinity with material surface.

For the analysis five different materials (Figure 9) commonly used in machining processes were prepared with Galdino's metallurgic equipment to have similar surface roughness. *Curcuma Longa L.* vegetable oil was compared to deionized water and commercial synthetic vegetal-based cutting fluid on different surface materials (Figure 10). Wettability tests were done in Ramé-Hard Model 500 advanced goniometer,

equipment from Laboratory of plasmas of the Physics Department at Aeronautics Institute of Technology (ITA) (Figure 11).



Figure 10.Materials surfaces used for wettability analysis.

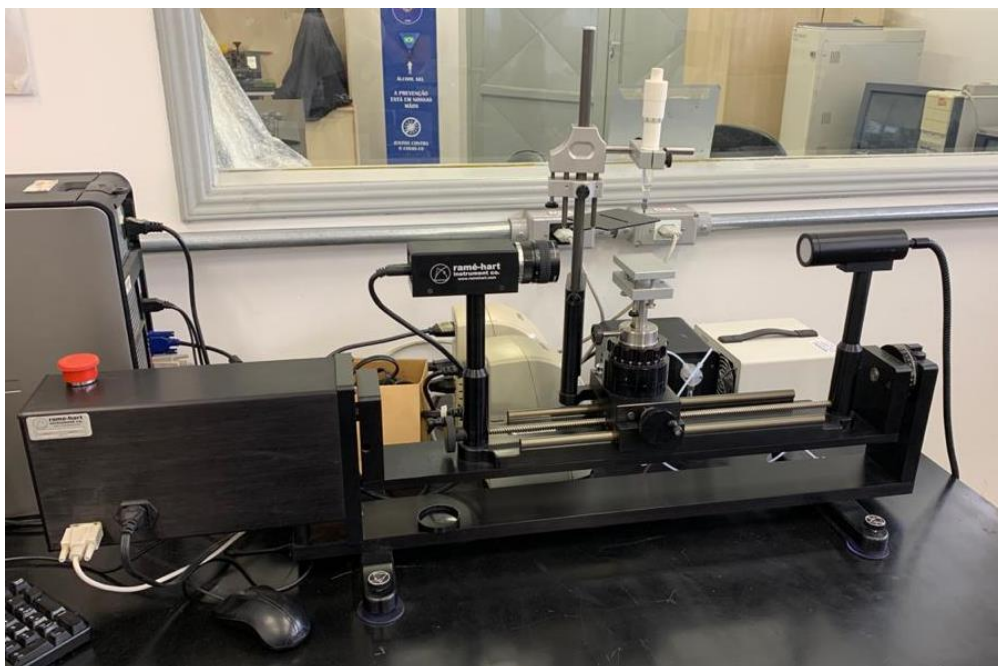


Figure 11. Ramé-Hart Model 500 advanced goniometer.

3.1.2 Thermal stability

Thermogravimetry (TGA) and Differential Scanning Calorimetry (DSC) evaluate the loss of mass as a function of heating to verify the thermal decomposition from vegetable oils [95]. *Curcuma Longa L.* vegetable oil is going to be compared with commercial cutting fluid (Quimatic 1) which is composed with synthetic vegetable oil, with the purpose of comparing thermal stability. The TGA and DSC were performed in TGA-51 Shimadzu and DSC-60 Shimadzu, respectively (Figure 12) from characterization laboratory localized in material engineering department, UTFPR-Londrina. The tests for both equipment were executed in an atmosphere with nitrogen, with flow rate of 30mL/min, at a heating rate of 10°C/min, from 25°C to 300°C for curcuma vegetable oil and 25°C to 200°C for synthetic vegetable oil. An aluminum crucible perforated lid was used as a recipient to perform DSC tests with 30 µL sample volume. For a TGA an open melting pot was used to perform tests with 12 mg of sample mass.

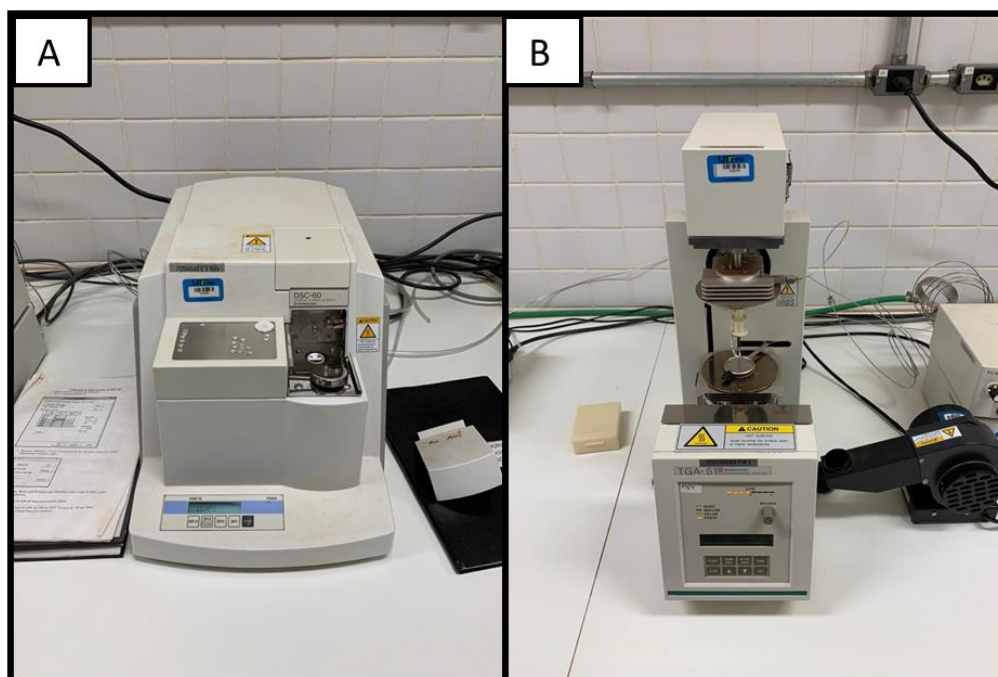


Figure 12. a) DSC-60 Shimadzu b) TGA-51 shimadzu.

3.1.3 Viscosity

Viscosity is a measure of fluid resistance to flow or shear and is often represented as a coefficient of friction. As seen before viscosity is a property associated with fatty acid profile from vegetable oils, in addition it is well established that temperature has a strong influence on viscosity. There are two types of viscosity, dynamic and kinematic, for the experiment proposed will be measured dynamic viscosity that doesn't depend on fluid's density [96] [97]. For this experiment, *Curcuma Longa L.* vegetable oil is compared with commercial synthetic vegetable oil. To evaluate the influence of temperature and velocity it is proposed for the experiment to measure viscosity at three different temperatures (25°C, 50°C and 80°C) varying for each temperature the rotational velocity from 0 to 500 rpm. The equipment used is a Brookfield rheometer R/S plus, the spindle used for curcuma vegetable oil is CC25 filled 16 mL sample fluid volume and for synthetic vegetable oil, an OS DIN 54453 filled with 17 mL sample fluid volume, a micropipette is used to achieve the exact volumes (Figure 13).

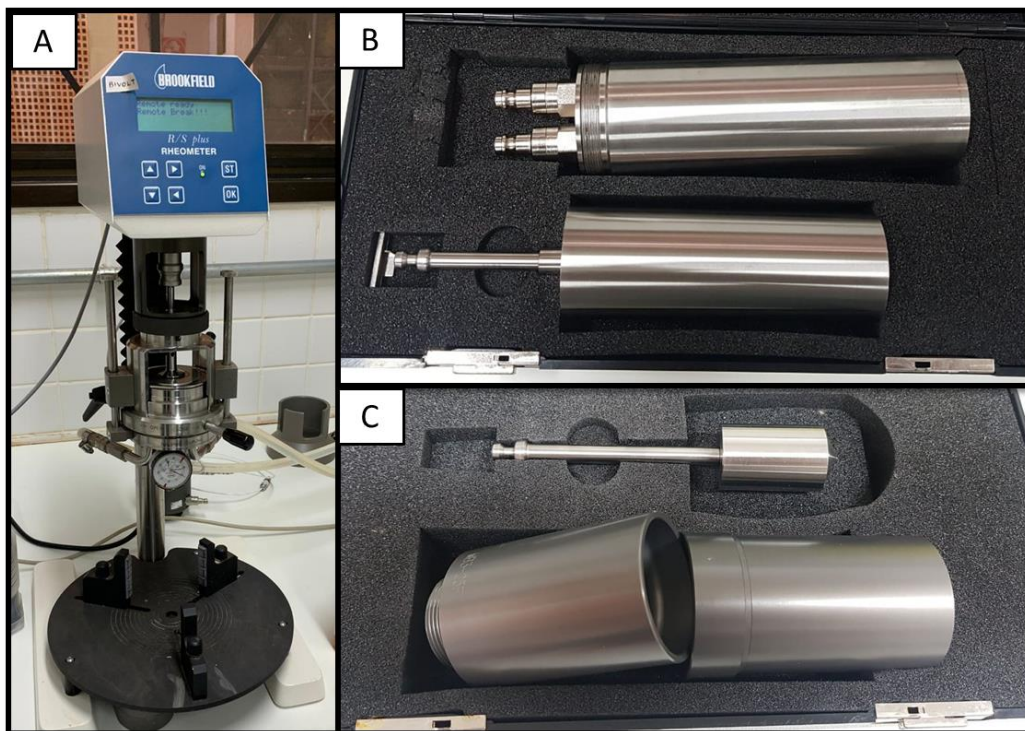


Figure 13. a) Brookfield rheometer b) OS DIN 54453 spindle c) CC25 spindle.

3.2 CURCUMA LONGA L. ESSENTIAL OIL ANTIMICROBIAL PROPERTIES EVALUATION

3.2.1 Microbiological analysis

According to bibliographic review *Pseudomonas aeruginosa* and *Escherichia Coli* are predominantly found in contaminated MWFs, for the study both of them were chosen to evaluate the potential from *Curcuma Longa L.* essential oils, also they are gram negative bacteria, for a better perspective of biocide potential a gram positive *Staphylococcus aureus* and a gram negative *Klebsiella pneumoniae* due to its encapsulated characteristic, are also going to be tested. The bacteria and analysis methods are from basic and applied bacteriology laboratory data situated inside State University of Londrina (UEL). For the evaluation, three different commercial essential oils are going to be tested, all of them were extracted with steam drag, the difference is in the components used (roots, leafs, etc.) to obtain those oils, in Figure 14 is possible to observe even different colors from the essential oils. The methods chosen for microbiological analysis are: minimal inhibitory concentration (MIC), minimal bactericide concentration (MBC) and disk diffusion. Bacteria description are *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia Coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 10031).

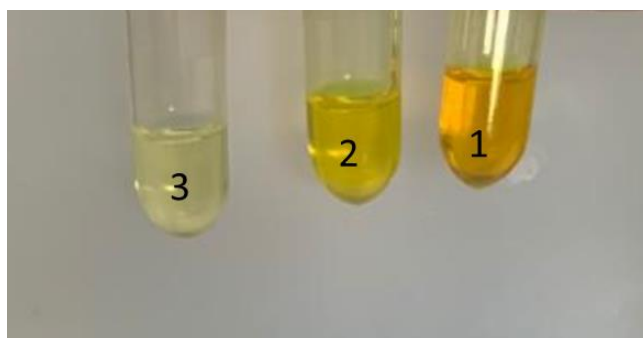


Figure 14. Samples from three different commercial essential oils used in experiment.

Minimal inhibitory concentration

The method consists in determine the minimal concentration of essential oil capable of inhibit bacteria growth. For each essential oil eight concentrations are going to be tested (10%, 5%, 2.5%, 1.25%, 0.6%, 0.3%, 0.16%, and 0.08%) in triplicate. The first step is to prepare essential oils solutions separately to turn into an aqueous solution in order to make it soluble in bacteria culture, this is made by diluting 500 μL of each essential oil separately with 500 μL of dimethylsulfoxide (DMSO), producing a 50% concentration solution. Then use this solution to prepare the initial concentration of 20% by mixing 400 μL of 50% solution of each essential oil with 600 μL of Difco Mueller Hinton Broth (DMHB) (figure 15) a bench vortex is used to homogenize the solutions.

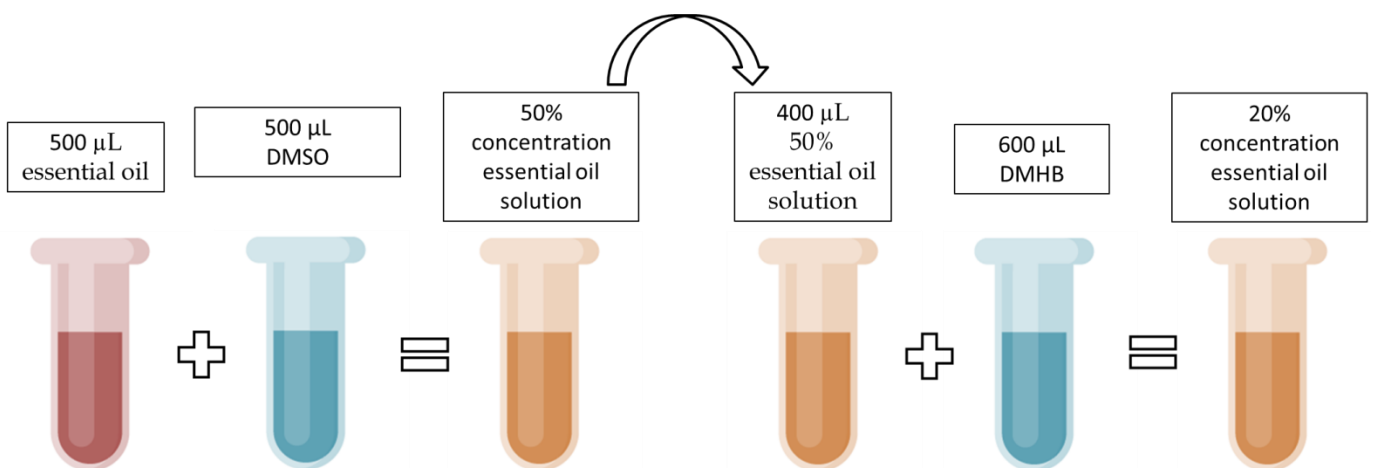


Figure 15. Preparing 20% concentration solution of each essential oil.

The second step is to perform a **serial dilution** of each 20% essential oil solution. To obtain the eight concentrations proposed in triplicate a microplate with 96 wells and a multichannel pipette to auxiliary in this process (Figure 16).

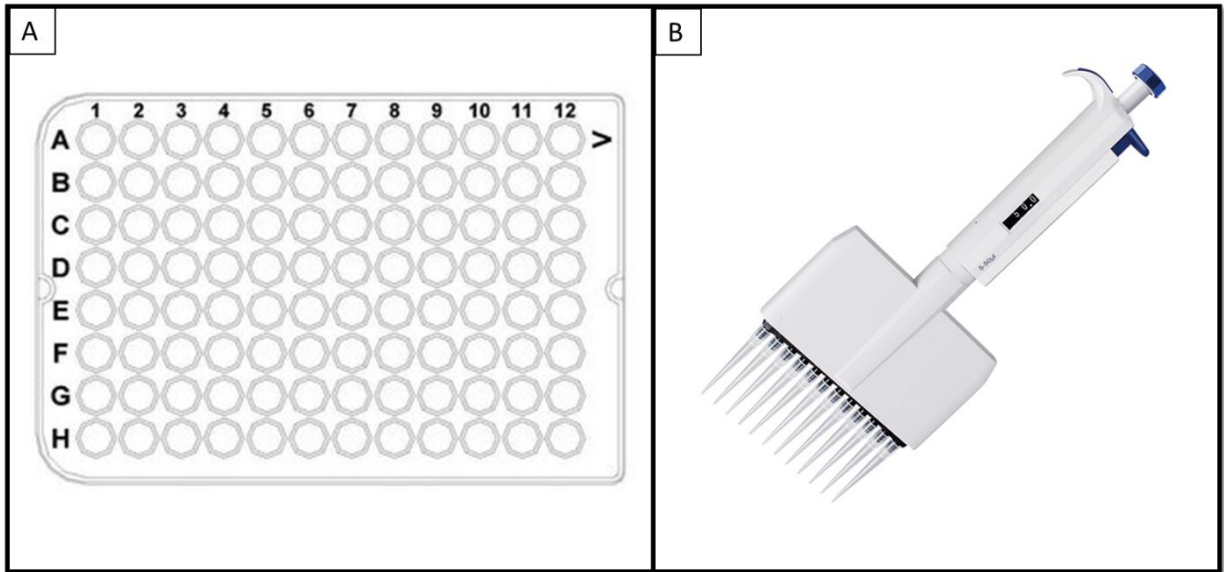


Figure 16. a) microplate with 96 wells; b) multichannel pipette.

The serial dilution demands several steps, first is to fulfill lines B to H and columns 1 to 10, from microplate with 50 μL of DMHB. Then the first line A and columns 1 to 9 will be added 100 μL of 20% essential oil solution previously prepared, from this line using multichannel pipette, 50 μL will be taken and diluted in line B from columns 1 to 9 producing a 10% concentration solution, this process will be repeated from line B to line C and C to line D and so forth (Figure 17). At the end of this process from line A to H will have the respective concentrations 20%, 10%, 5%, 2.5%, 1.25%, 0.6%, 0.3%, 0.16%, 0.6%, 0.3%, 0.16%.

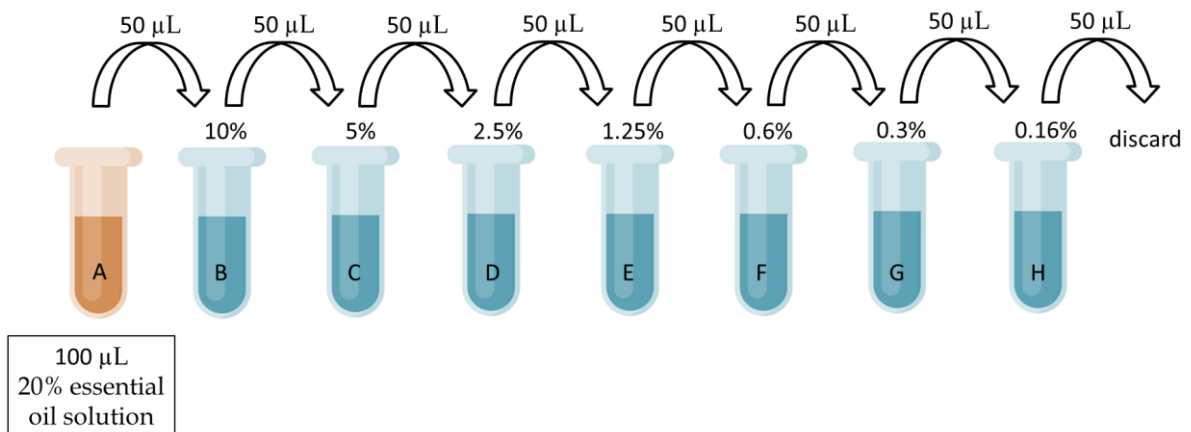


Figure 17. Serial dilution of essential oils inside microplate.

To add bacteria inside microplate wells is necessary to prepare it as an aqueous solution to guarantee that each well will receive around the same amount of bacteria. A little amount of colony bacteria is taken from the plate and then is dissolved in DMHB, after homogenization a slight turbidity will appear and then compared with McFarland scale, this procedure will be repeated until turbidity achieve 0.5 Mcfarland unit (MFU) which correspond a culture density of 1.5×10^8 cells/mL (Figure 18).



Figure 18. McFarland scale turbidity level for different values of MFU.

After achieving 0.5 MFU 10 μ L of this solution is diluted in 990 μ L of DMHB reducing culture density to 1.5×10^6 cells/mL, from this new solution only 50 μ L will be added in each microplate well from line A to H and columns 1 to 9, that were previously filled with essential oils solutions. By adding 50 μ L of bacteria solution each concentration of essential oils inside the wells will reduce to half, achieving the concentrations proposed for MIC evaluation (10%, 5%, 2.5%,..., 0.16%, 0.08%), and also bacteria solution is being diluted, for this reason culture density inside the wells is 1.5×10^5 cells/mL. The column 10 from line A to H is used to as a control to verify if DMSO and DMHB are not interfering on results with contamination or even the opposite by inhibiting bacteria growth, lines A, B and C are filled with DMSO, DMHB and bacteria, lines D, E and F are filled with DMHB and bacteria, lines G and H are

filled with only DMHB (Figure 19). After finishing filling and sealing with plastic film the microplate is reserved inside a kiln with constant temperature of 37°C for 24h.

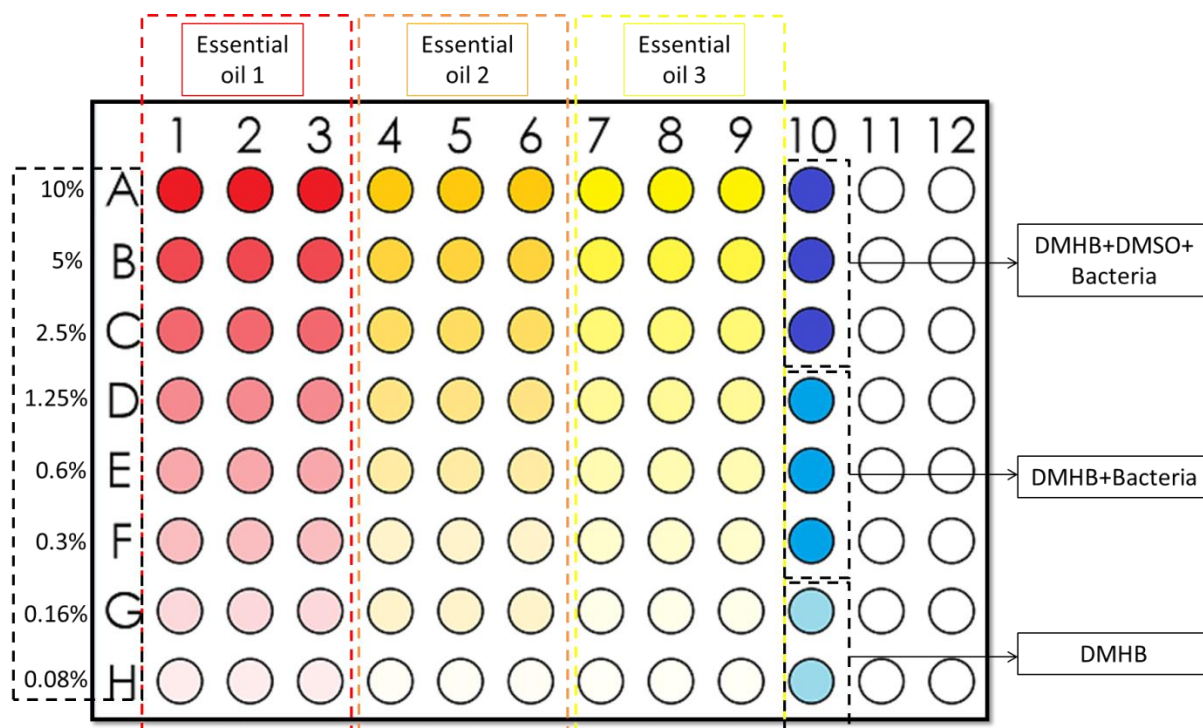


Figure 19. Final configuration of microplate before reserve it inside the kiln.

Minimal bactericidal concentration

The minimal bactericidal concentration (MBC) is to determinate the lowest concentration used for MIC that is capable of killing above 90% of bacteria growth. This analysis is made by subculturing 10 μ L of MIC concentration results of each essential oil into agar plates and after 24h inside a kiln with constant temperature of 37°C the interest is to observe if still resulted in microorganism proliferation, this test is made in triplicate (Figure 20).

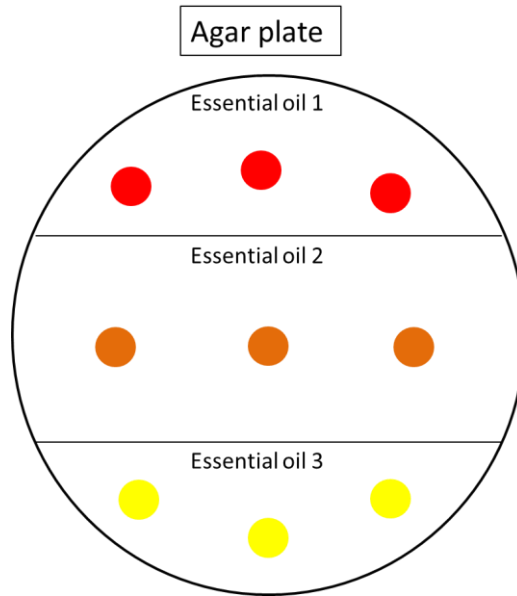


Figure 20. MBC test in agar plate with aliquots of MIC essential oils.

After this period colonies of bacteria might appear and to measure MBC is necessary to count separately from each aliquot the number of colonies that appeared, then for each essential oil calculate the mean between numbers of colonies. This value is equivalent to number of bacteria in equation (1) to calculate final density culture inside contaminated essential oil. Remembering that the initial value of culture density is 1.5×10^5 cells/mL.

$$C = (N^\circ \text{ Bacteria}) \cdot (\text{Dilution}) \cdot (\text{Aliquot}) \left[\frac{\text{cells}}{\text{mL}} \right] \quad (1)$$

For this experiment no dilution is made then this value can be replaced by 1, and for aliquot correction is to transform 10 μL in 1mL, so is necessary to multiply by 100 resulting in cells/mL density culture. Then using equation (2) to calculate how much the biocide agent caused bacteria death in percentage, if this value is superior to 90% MBC is equal to MIC subcultured in agar plate.

$$P = 100 \cdot \left(1 - \frac{C}{1.5 \cdot 10^5} \right) [\%] \rightarrow \text{MBC} = \text{MCI if } P \geq 90\% \quad (2)$$

Disk diffusion

Disk diffusion test is to evaluate bacteria sensibility to antimicrobials which in this case is the essential oils. For this experiment is necessary to use four Agar plate one for each bacteria. Four solutions with *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia Coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Klebsiella pneumoniae* (ATCC 10031) patronized with 0.5 McFarland scale are used to contaminate agar plates using a sterilized swab. An aliquot of 10 μ L with pure essential oil is dropped on a filter paper disc (16 discs total) this process is made for each essential oil. Those discs are disposed on contaminated agar plates, one disc in each agar plate is used as control, without essential oil, to certificate that the filter paper is not interfering on results. After finishing this procedure, the agar plates are reserved inside a kiln with constant temperature of 37°C for 24h. It is expected that an inhibition halo grows around the filter disk demonstrating antimicrobial activity, the results are measured calculating the difference between final halo diameter and initial paper filter disc diameter (Figure 21).

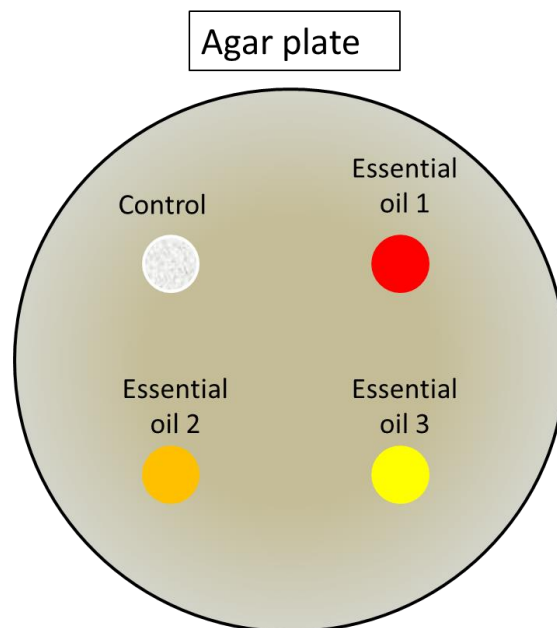


Figure 21.Contaminated Agar plate with control and essential oils paper filter discs.

4. RESULTS AND DISCUSSIONS

4.1 CURCUMA LONGA L. VEGETABLE OIL LUBRICANT PROPERTIES EVALUATION

4.1.1 Wettability analysis

For a lubricant is important to have low values of wettability (preferable contact angle under 45°), besides contributing with cooling and lubricating the workpiece it helps to build up a tribofilm that is able to carry the load and separate surfaces. A surface is considered highly hydrophilic when angle contact is between 0° and 45° [98]. The wettability of deionized water, *curcuma Longa* vegetable oil and Synthetic vegetable oil were evaluated in five different surfaces. For all the surfaces with both vegetable oils it was observed that the contact angle reduced with time as a consequence of surface and droplets interaction spreading the droplet, for this reason it was analyzed contact angle during one second since the first liquid and surface contact.

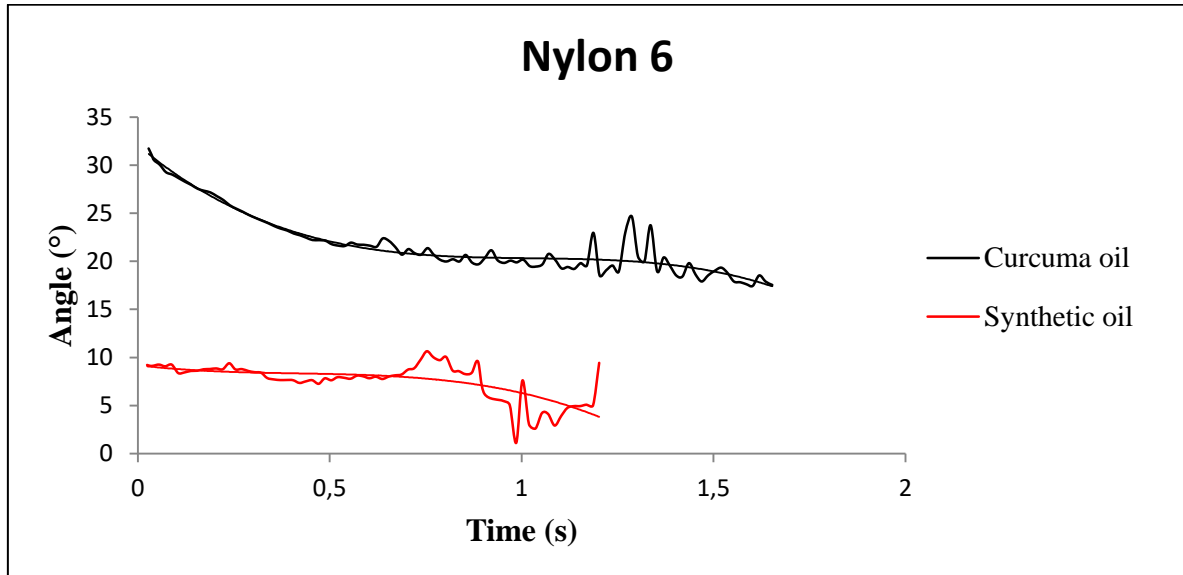


Figure 22. Contact angle of vegetable oils with nylon 6 surface.

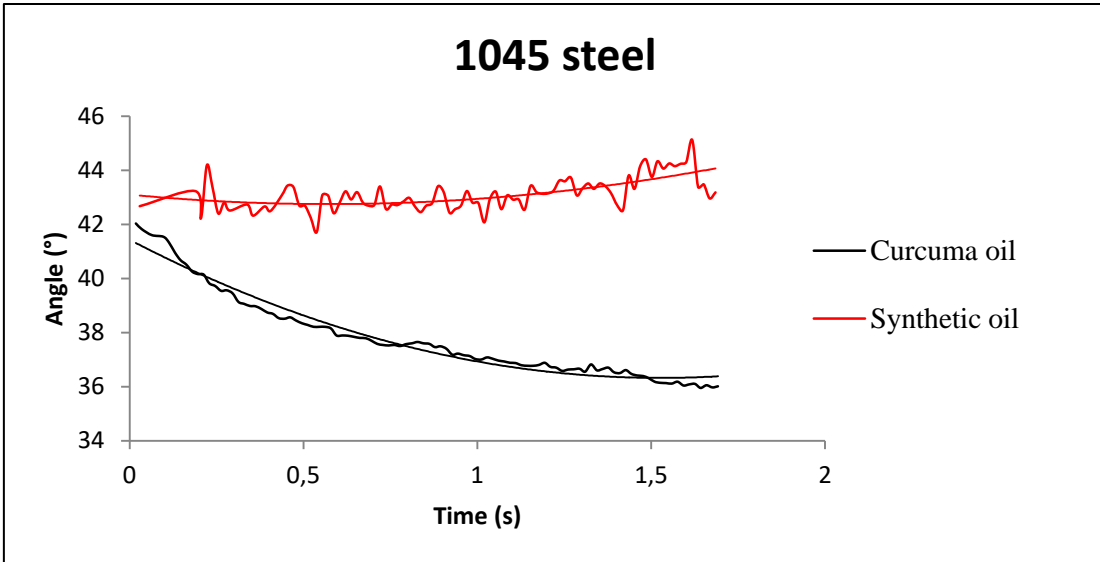


Figure 23. Contact angle of vegetable oils with 1045 steel surface.

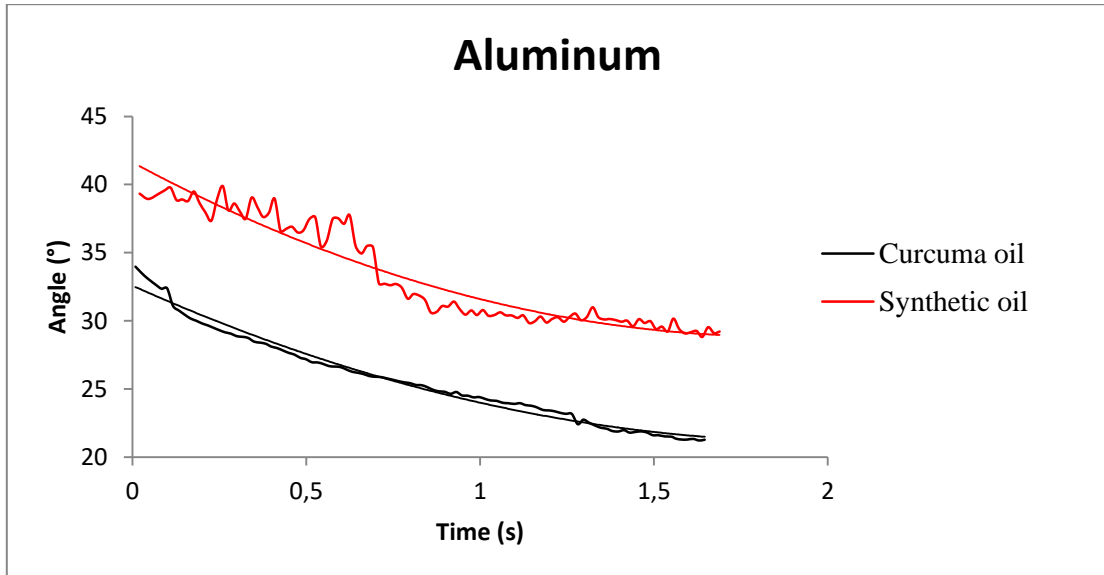


Figure 24. Contact angle of vegetable oils with aluminum surface.

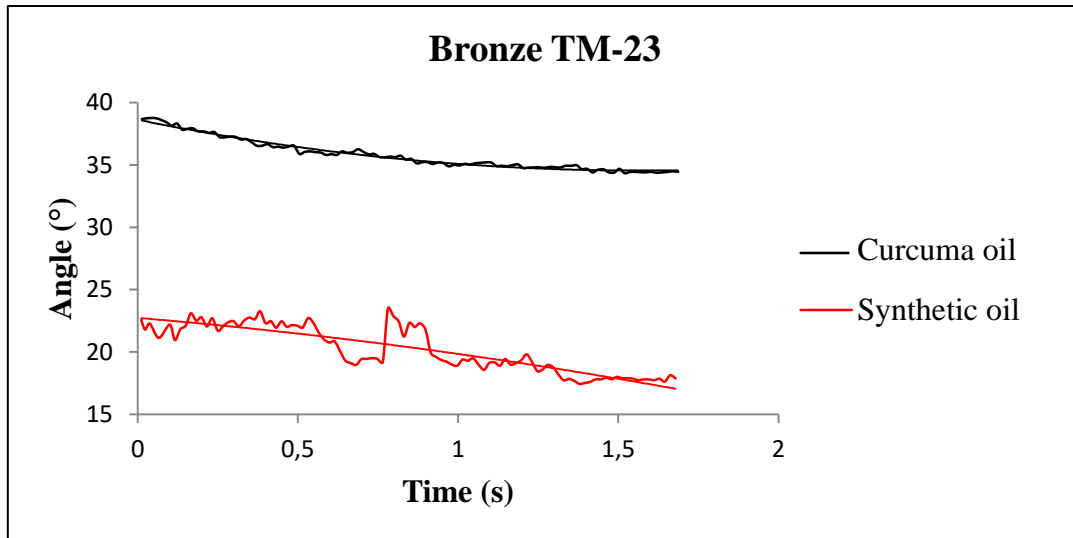


Figure 25. Contact angle of vegetable oils bronze TM-23 surface.

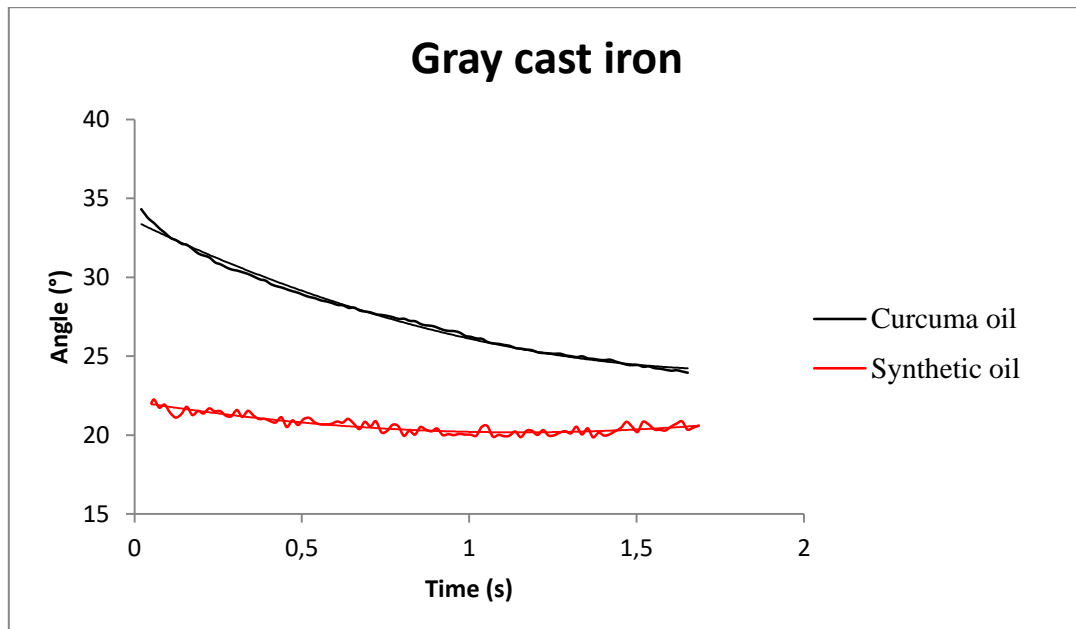


Figure 26. Contact angle of vegetable oils with gray cast iron surface.

To analyze contact angle between vegetable oils it was calculated pattern deviation and coefficient of variation from each data to ensure if mean values could be used to compare contact angles (Figure 27). From the Figure 23 and 24, *Curcuma Longa L.* vegetable oil presented higher wettability for aluminum and 1045 steel, with gray cast iron surface it presented a close contact angle with synthetic vegetable oil. For gray cast iron, bronze TM-23 and nylon 6 synthetic oil presented better wettability (Figure 22, 25, 26).

	Deionized water			Synthetic Vegetable oil			Curcuma vegetable oil		
	Contact angle (°)	Pattern Deviation	Coefficient of variation (%)	Contact angle (°)	Pattern Deviation	Coefficient of variation (%)	Contact angle (°)	Pattern Deviation	Coefficient of variation (%)
Aluminum	85.87	0.71	0.83	33.36	3.79	11.35	25.75	3.33	12.94
1045 steel	78.82	0.29	0.37	43.11	0.60	1.38	37.78	1.50	3.96
Nylon 6	69.99	1.77	2.53	7.61	1.98	25.96	21.88	3.38	15.5
Bronze TM-23	77.25	0.58	0.76	20.19	1.89	9.36	35.75	1.20	3.36
Gray cast iron	79.93	0.75	0.93	20.64	0.56	2.72	27.62	2.76	10.00

Goniometer:

Ramé-hart advanced goniometer model 500

Drops Volume: 3 μ L

Temperature: 25°C

Software: DROPimage Advanced

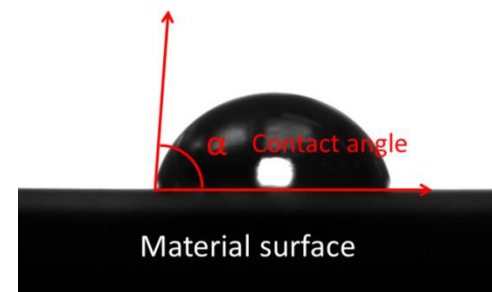


Figure 27.Contact angles of deionized water, synthetic vegetable oil and *curcuma Longa* vegetable oil for different surfaces.

4.1.2 Thermal Stability

In machining process is common to achieve high temperatures in tool and workpiece surface contact, so it is important to evaluate thermal stability from vegetable oils. For this purpose, Curcuma Longa L. vegetable is compared to commercial synthetic vegetable oil when the temperature increases. According to TGA and DSC results the vegetable oils demonstrated a significant difference in thermal stability (figure 28).

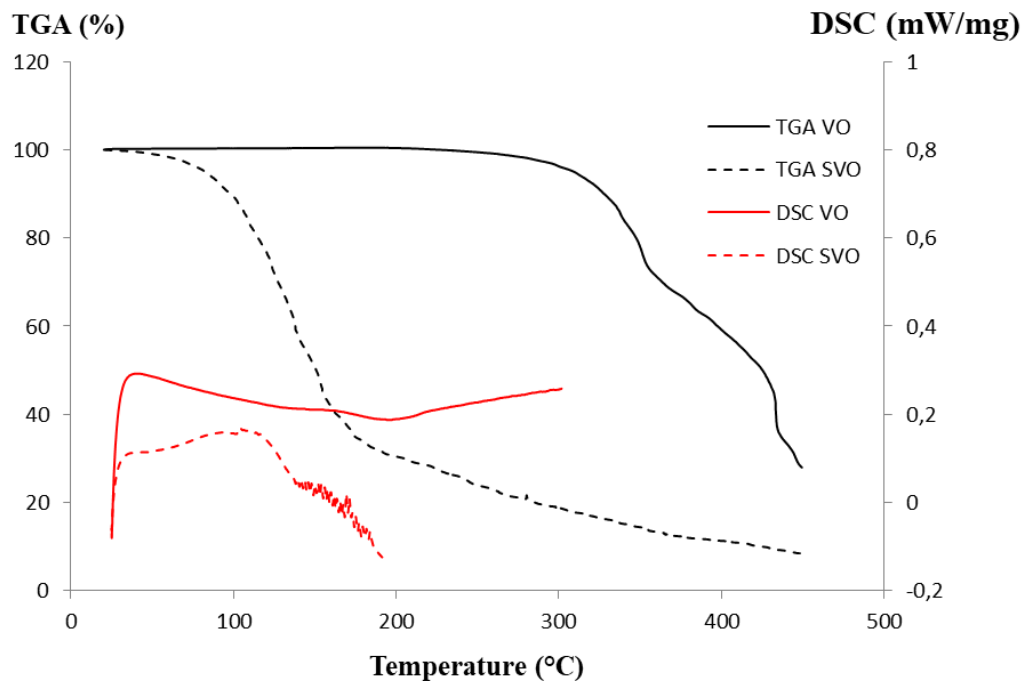


Figure 28. TGA and DSC analysis from *Curcuma Longa L.* vegetable oil (VO) and synthetic vegetable oil (SVO).

First comparing thermogravimetry (TGA) curcuma vegetable oil starts to lose mass with a considerably percentage around 330°C, before it has presented a stable behavior, for the synthetic vegetable oil mass decrease starts around 100°C. For DSC curves, the same variation of temperature curcuma vegetable oil is capable of absorbing more energy per mass (endothermic reaction) for 100°C vegetable oil presented $Q'=0.25$ mW/mg and for synthetic vegetable oil $Q'=0.15$ mW/mg, if considered a time interval of 1 min with constant temperature of 100°C curcuma vegetable is capable of absorbing $Q=15$ J/g and synthetic vegetable oil $Q=9$ J/g. Therefore, as it was expected,

considering TGA and DSC curcuma vegetable oil presented better thermal stability capable of work in a higher range of temperature before mass degradation and absorb higher amount of energy when submitted to the same variation of temperature.

4.1.3 Viscosity

Viscosity is a relevant property that influences the ability to form lubricant film in surfaces, which contributes to friction reduction. For lubricants high values of viscosity allows the fluid to work in a higher range of temperature by reducing friction between tool and working piece [99]. This experiment has the objective of evaluate viscosity behavior in function of temperature and shear stress. The synthetic vegetable oil presented low viscosity values, this could mean a considerable presence of water classifying it as an emulsion. The graphic presented same behavior for the three temperatures, while velocity is progressively raising shear stress values also increased and viscosity demonstrated a tendency to stabilize in a constant value, concluding that velocity doesn't influence considerably on viscosity values (Figure 29, 30, 31).

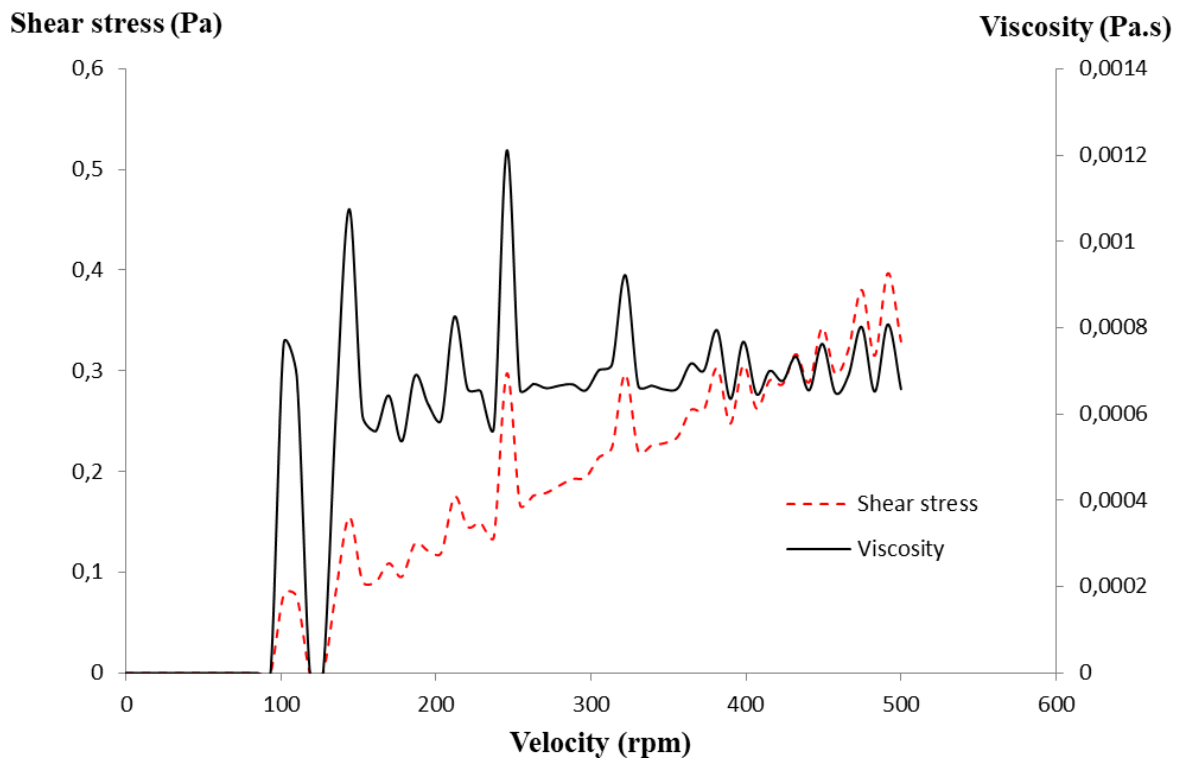


Figure 29. Viscosity and shear stress in function of the velocity of synthetic vegetable oil at 25°C.

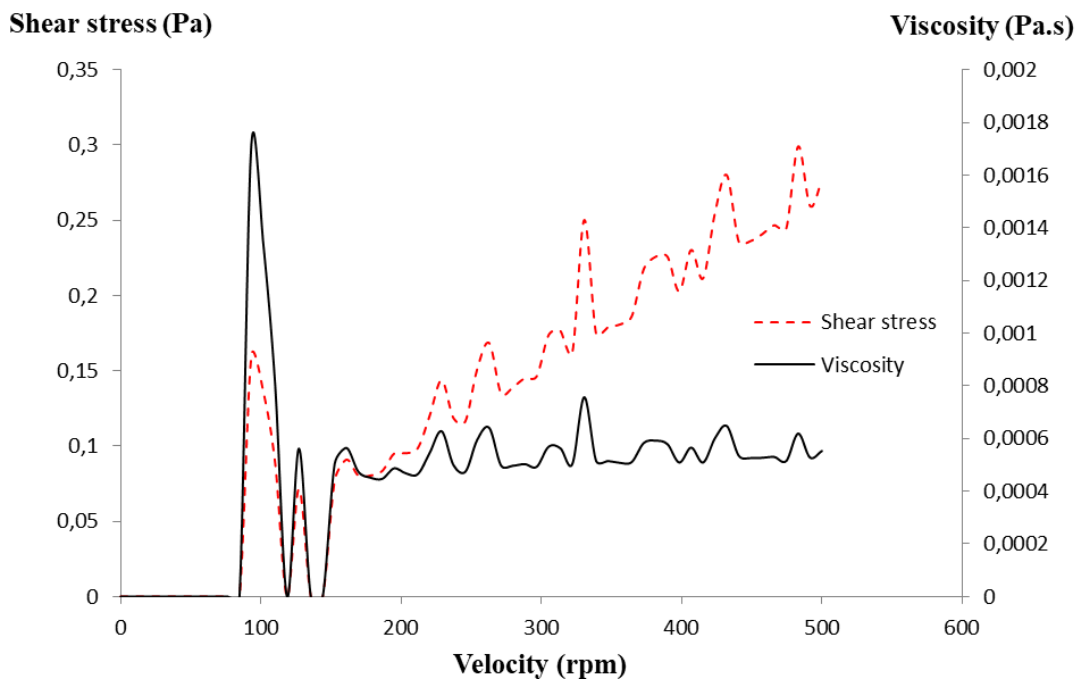


Figure 30. Viscosity and shear stress in function of the velocity of synthetic vegetable oil at 50°C.

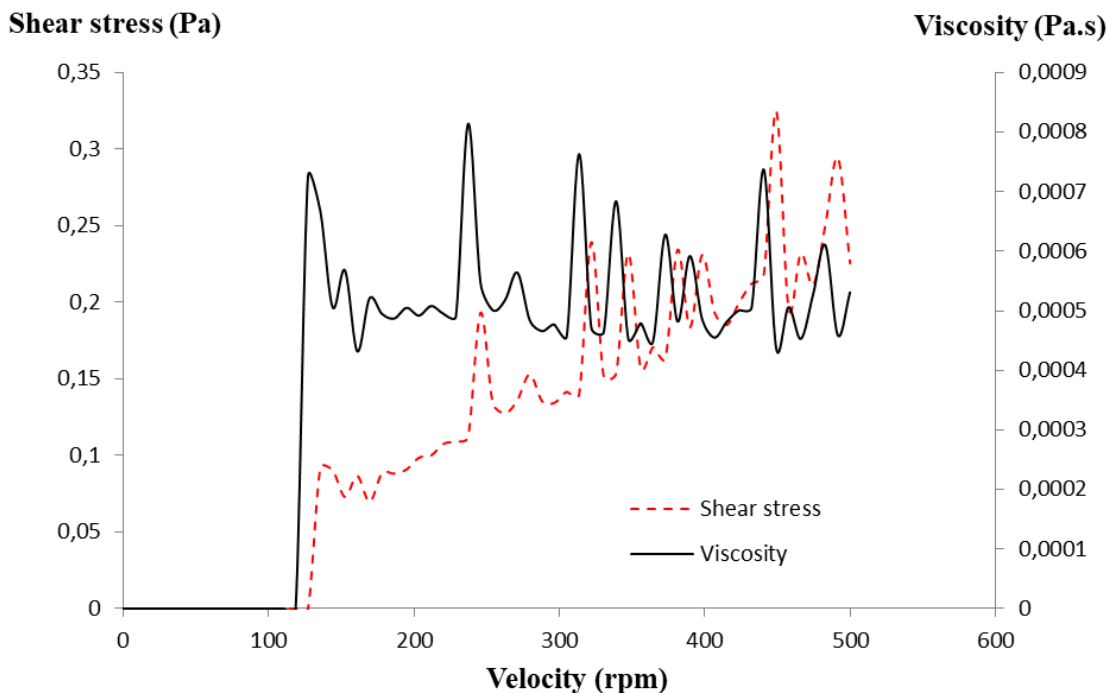


Figure 31. Viscosity and shear stress in function of the velocity of synthetic vegetable oil at 80°C.

For *Curcuma Longa L.* vegetable oil presented high values of viscosity as expected. For the three temperatures graphics presented a behavior similar to synthetic vegetable oil, with velocity raise values of shear stress also increased and viscosity

stabilized in a constant value, indicating that velocity do not influence considerably viscosity values.

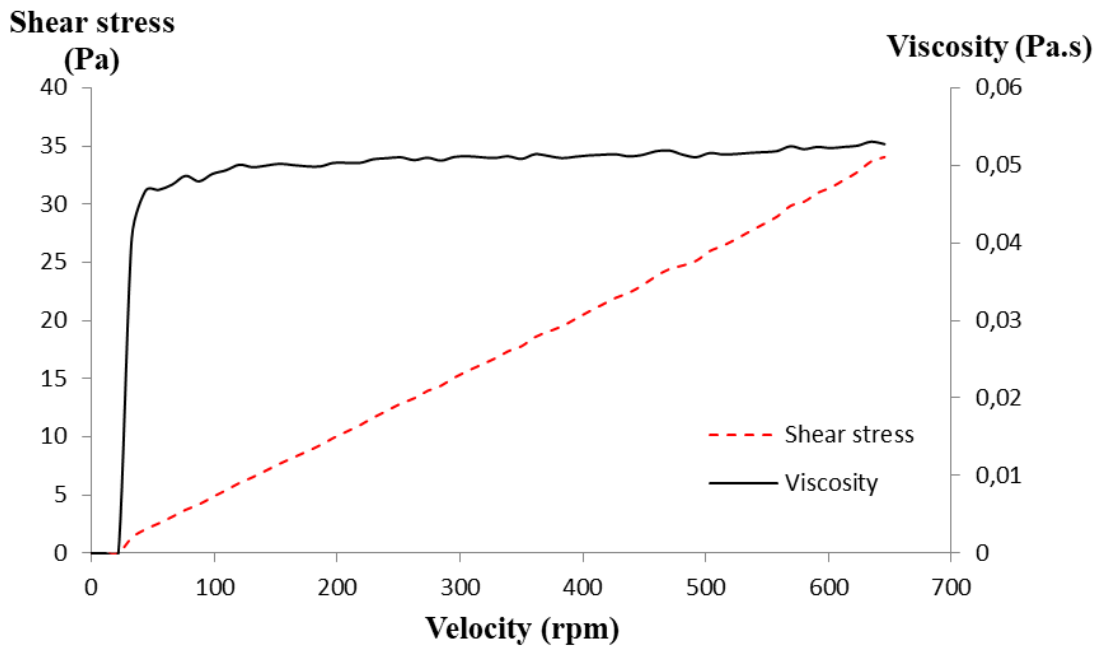


Figure 32. Viscosity and shear stress in function of the velocity of *Curcuma Longa L.* vegetable oil at 25°C.

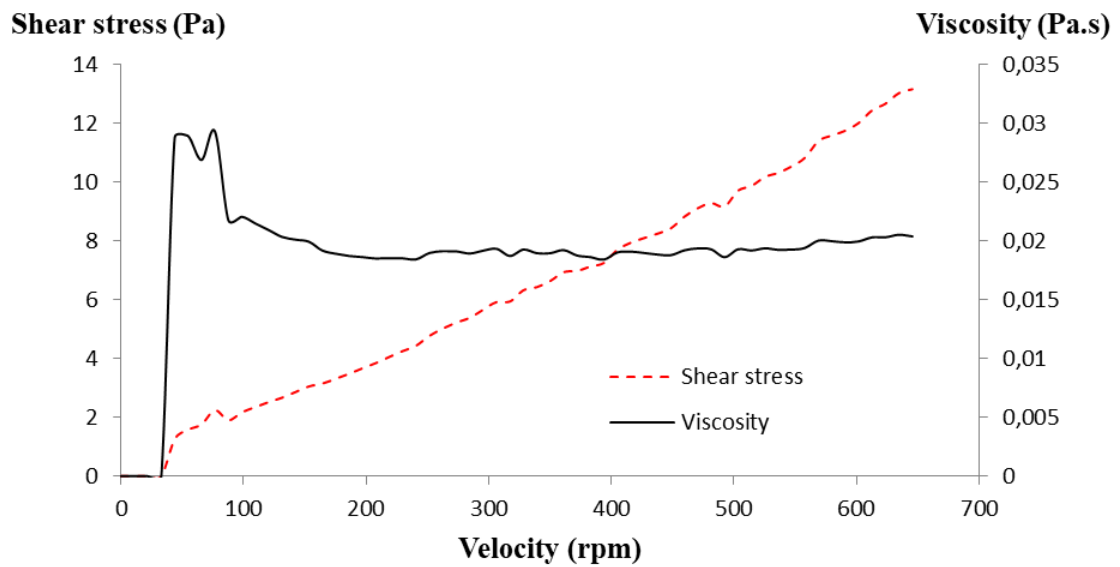


Figure 33. Viscosity and shear stress in function of the velocity of *Curcuma Longa L.* vegetable oil at 50°C.

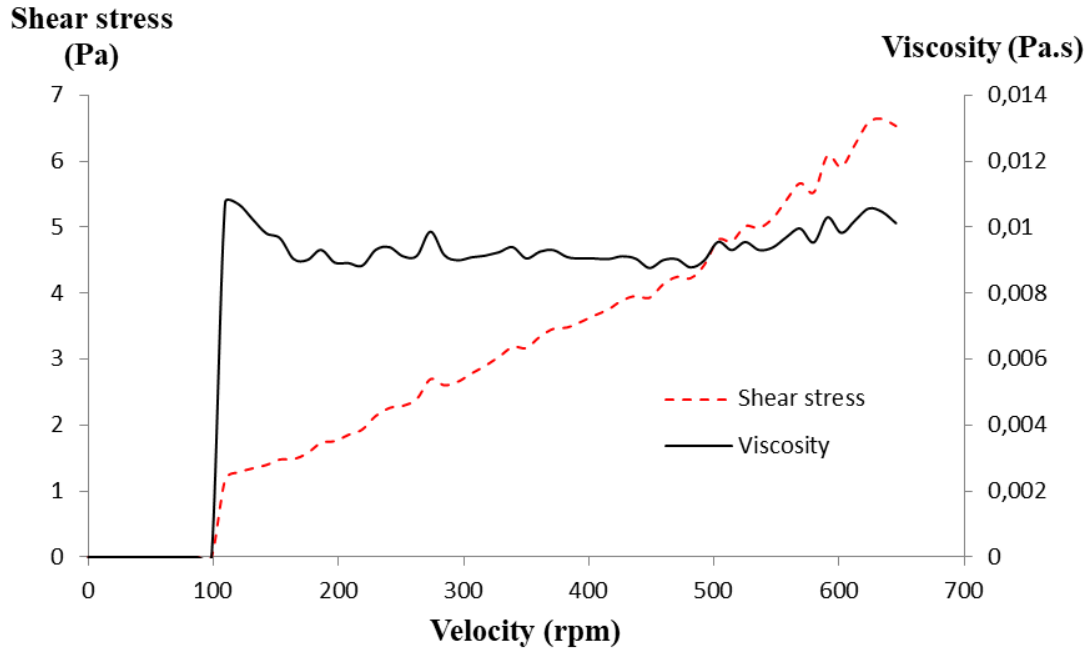


Figure 34. Viscosity and shear stress in function of the velocity of *Curcuma Longa L.* vegetable oil at 80°C.

In table 5 it is possible to compare the values of viscosity for each temperature, first curcuma vegetable oil compared to synthetic vegetable oil exhibit high values of viscosity, comparing with literature at 50°C curcuma vegetable oil has similar values to walnut (21.2 mPa.s) and safflower (22.32 mPa.s) [100]. Also, as expected for both oils viscosity values decrease with temperature raise, which indicates temperature dependence with inversely proportional relation.

Table 5. Values of vegetable oils samples viscosity in different temperatures.

sample	Viscosity (mPa.s)		
	25°C	50°C	80°C
Curcuma Vegetable oil	50.62656	20.07741	9.43056
Synthetic vegetable oil	0.70213	0.538558	0.530489

4.2 CURCUMA LONGA L. ESSENTIAL OIL ANTIMICROBIAL PROPERTIES EVALUATION

4.2.1 Microbiological analysis

Four species of bacteria were evaluated: *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia Coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC10031) and *Staphylococcus aureus* (ATCC25923), three gram negative and one gram positive respectively. Those bacteria were chosen due to recurrence in contaminated fluids and also to verify *Curcuma Longa L.* effects in different species. Gram negatives due its complex cell wall barrier that implies in more resistance to conventional biocides are more likely to resist microbiological action from three different commercial essential oils, so it is expected to have more biocide activity for gram positive *Staphylococcus aureus* due its more permeable cell wall [101].

Minimum inhibition concentration

This experiment has the objective to determinate for which concentration of essential oil (10%, 5%, 2.5%, 1.25%, 0.6%, 0.3%, 0.16%, 0.08%) is capable of inhibit bacteria growth. After 24h inside microplate is possible to observe through turbidity and colony formation, for which wells grew bacteria, to consider a concentration as MIC the three wells from each essential oil must have a limpid appearance. Microplate from *Staphylococcus aureus* (Figure 35) it was observed that only oil 1 presented inhibition activity MIC concentration of 1,25%. For *Escherichia Coli* (Figure 36) all oils didn't present inhibitory activity giving a 10% MIC for all three oils. *Klebsiella pneumoniae* (Figure 37) had one low concentration from oil 1 MIC=0,625% and oil 2 presented some inhibition activity with MIC=5%. *Pseudomonas aeruginosa* (Figure 38) for all three oils didn't present inhibition activity with MIC=10%.

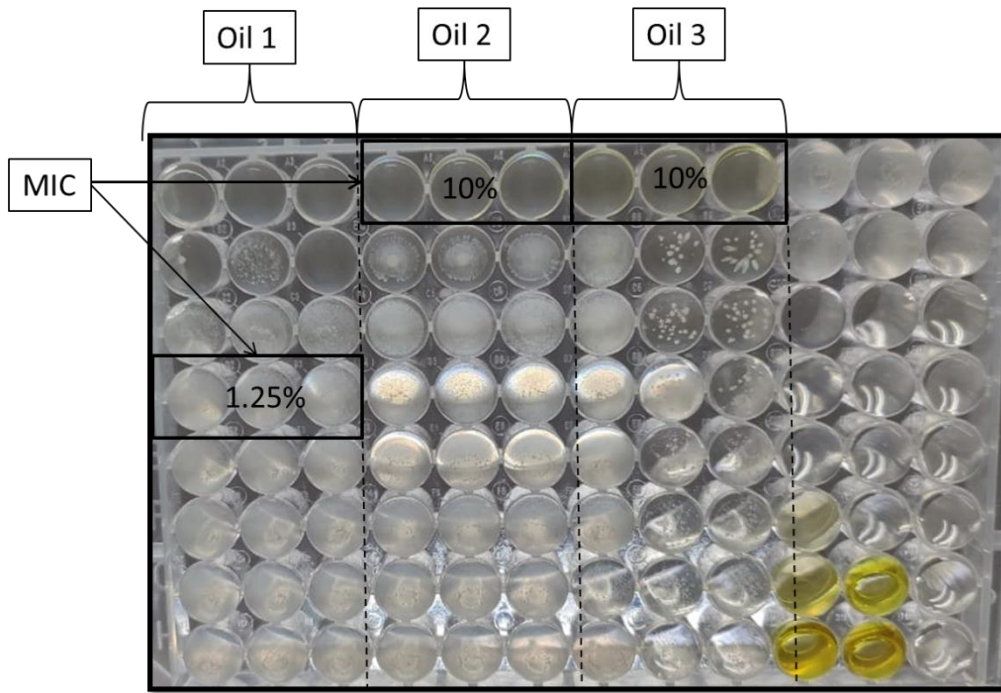


Figure 35. MIC with *Staphylococcus aureus* (ATCC25923).

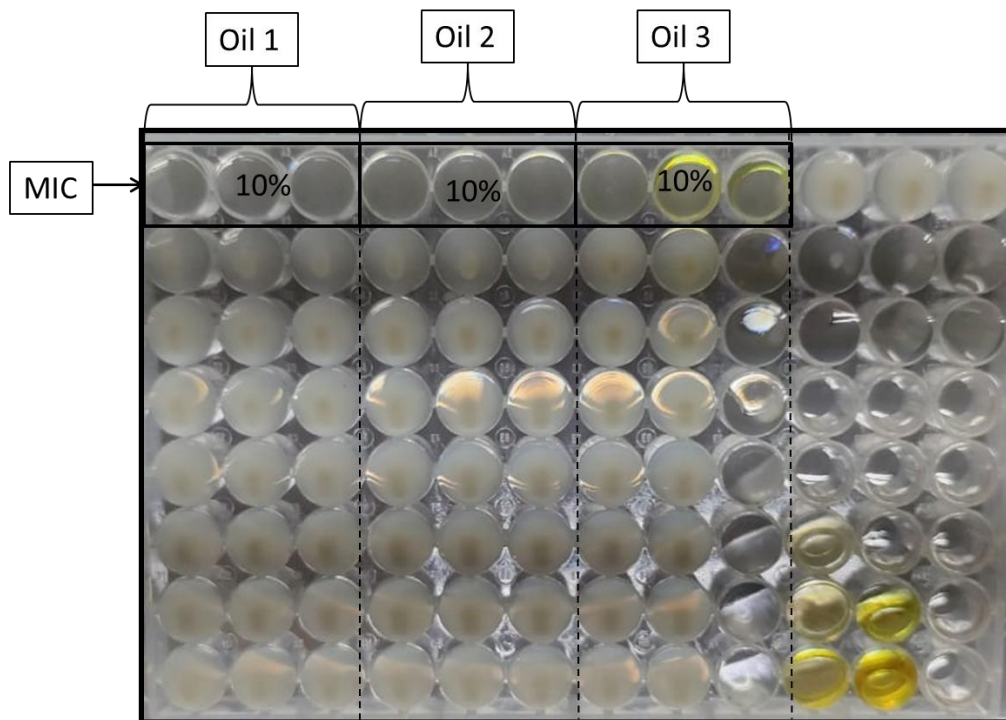


Figure 36. MIC with *Escherichia Coli* (ATCC 25922).

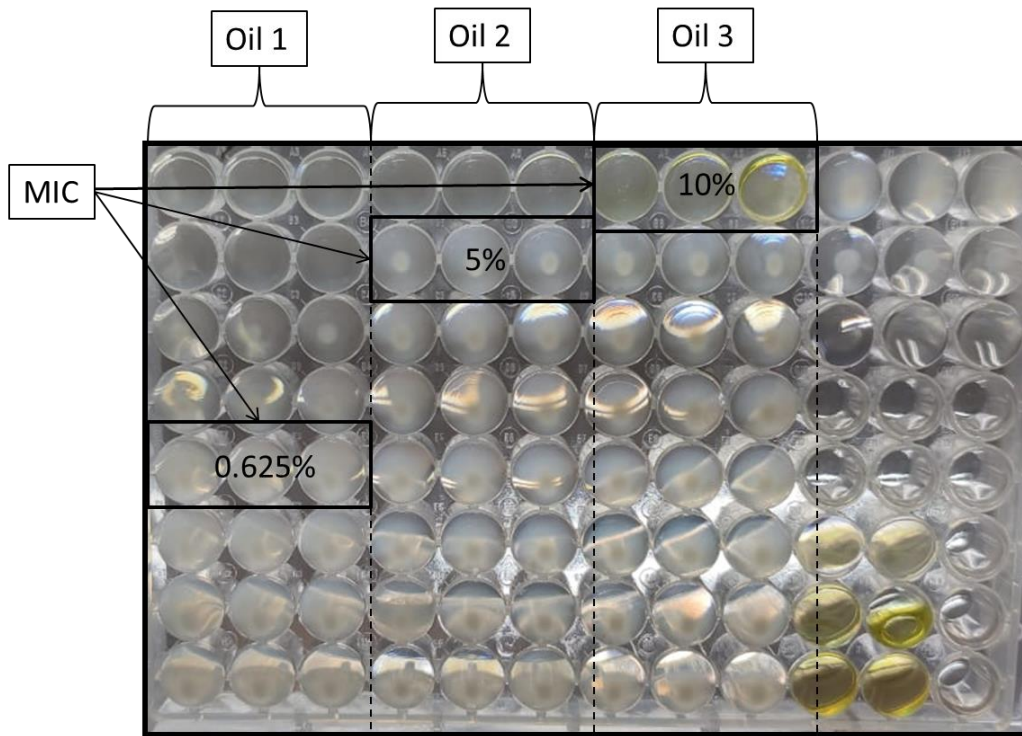


Figure 37. MIC with *Klebsiella pneumoniae* (ATCC10031).

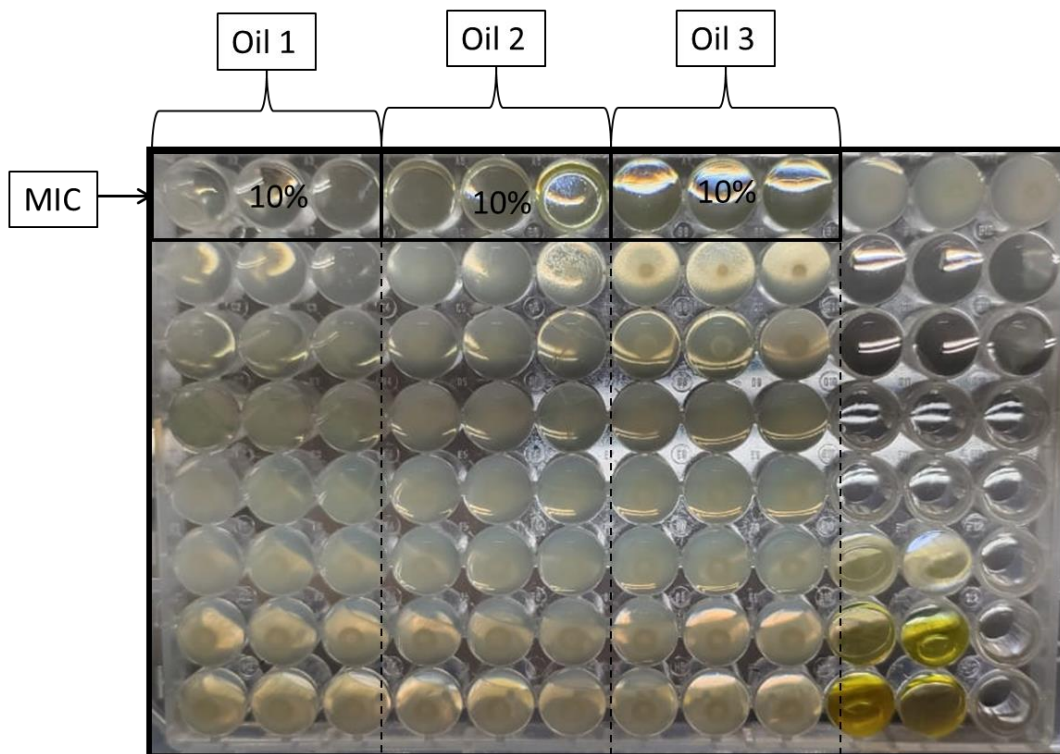


Figure 38. MIC with *Pseudomonas aeruginosa* (ATCC 27853).

Table 6 have a resume of results to compare MIC between the essential oils, it is possible to observe that essential oil 1 presented the higher inhibition activity among them.

Table 6. Results from MIC tests.

Bacteria	MIC		
	Essential oil 1	Essential oil 2	Essential oil 3
<i>Staphylococcus aureus</i>	1.25%	10%	10%
<i>Escherichia Coli</i>	10%	10%	10%
<i>Klebsiella pneumoniae</i>	0.625%	5%	10%
<i>Pseudomonas aeruginosa</i>	10%	10%	10%

Minimal Bactericidal concentration

The minimal bactericidal concentration (MBC) has the objective to determine for which essential oil concentration it was capable of killing above 90% of bacteria comparing to initial value of culture density is 1.5×10^5 cells/mL. The concentrations used to MBC are determined by MIC results; normally is tested MIC concentration and two concentrations above and under in triplicate. After 24h is observed where 10 μ L aliquot was dropped if bacteria colonies have grown.

First for *Staphylococcus aureus* (ATCC25923) (Figure 39) oil 1 that presented MIC=1.25% it was tested concentrations 0.625%, 1.25%, 2.5%, 5% and 10%, observing agar plate A in line with 1.25% concentration it has fully grown bacteria colony in the middle aliquot, so MBC is above this concentration, then analyzing line with 2.5% concentration it was counted and averaged 11 colonies giving a culture density of 1100 using Equation 1. For oil 2 and 3 MIC resulted in 10%, so MBC tested is only with 10% concentration, is possible to observe in agar plate B that didn't grow bacteria, so in this case for oil 2 and 3 MBC=MIC=10%.

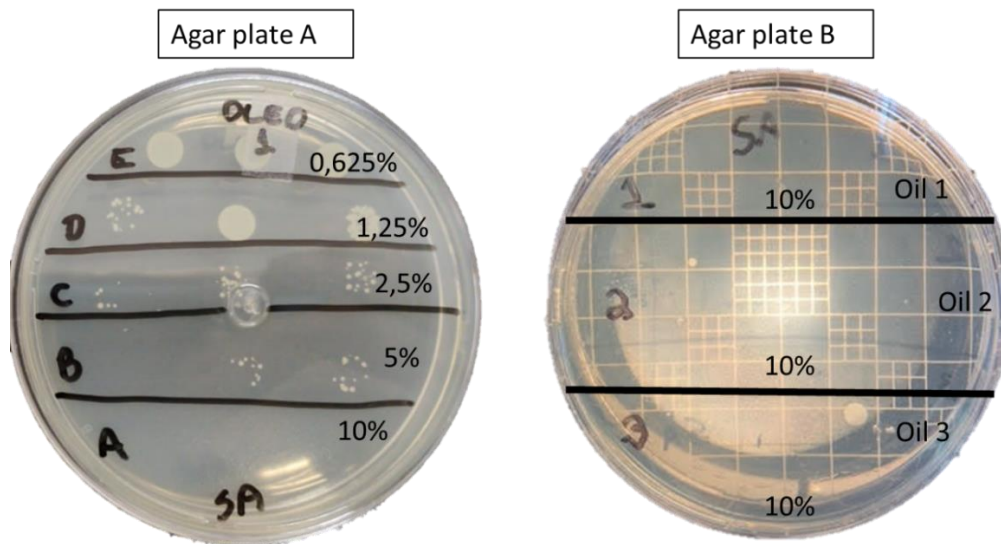


Figure 39. MBC with *Staphylococcus aureus* (ATCC25923), Agar plate A with oil 1 and Agar plate B with Oil 1, 2 and 3.

With *Escherichia Coli* (ATCC 25922) (Figure 40) it can be noticed that for oil 2 there wasn't bacteria colony formation, directly concluding that $MIC=MBC=10\%$, the same that has happened with *Staphylococcus aureus*. With oil 1 it is possible to observe some colonies with whitish aspect have grown after 24 h, for oil 1 it was counted and averaged 6 colonies giving a culture density of 600 using Equation 1. In oil 3 doing the one of the aliquots had fully colony grow, for this reason $MBC>10\%$.

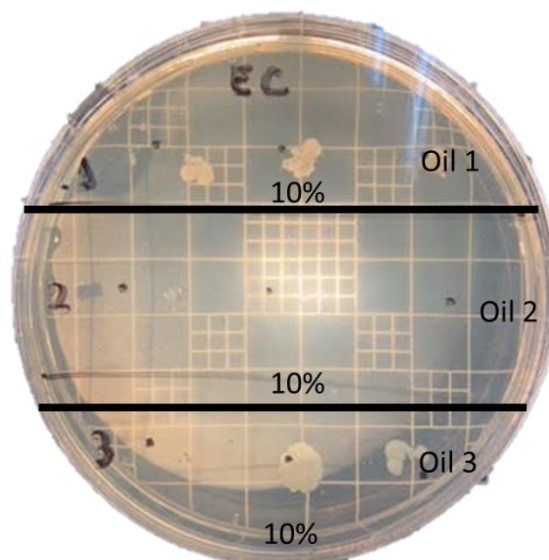


Figure 40. MBC with *Escherichia Coli* (ATCC 25922) Agar plate with Oil 1, 2 and 3.

For *Klebsiella pneumoniae* (ATCC10031) (Figure 41) observing agar plate A with oil 1 the concentration 2.5% presented a fully-grown bacteria colony in the first aliquot which indicates that MBC is higher, for 5% concentration no bacteria has grown which means that MBC=5%. In agar plate B the oil 2 presented no bacteria grow for 10% and 5% concentration so in this case MBC=5% and for oil 3 with 10% concentration also there was no bacteria grow concluding that MBC=10%.

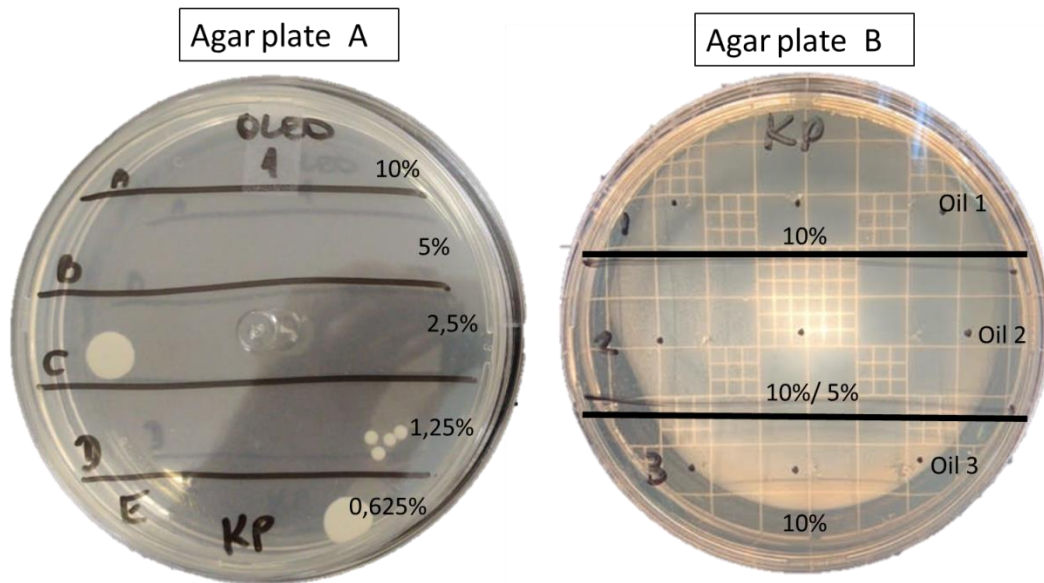


Figure 41. MBC with *Klebsiella pneumoniae* (ATCC10031).

Pseudomonas aeruginosa (ATCC 27853) (Figure 42) where the aliquot was dropped for all three essential oils solution have grown a fully colony being unable to count which means that for 10% concentration the oils weren't able to have a bactericidal activity above 90%, so MBC is above MIC concentration, giving the answer of MBC > 10%.

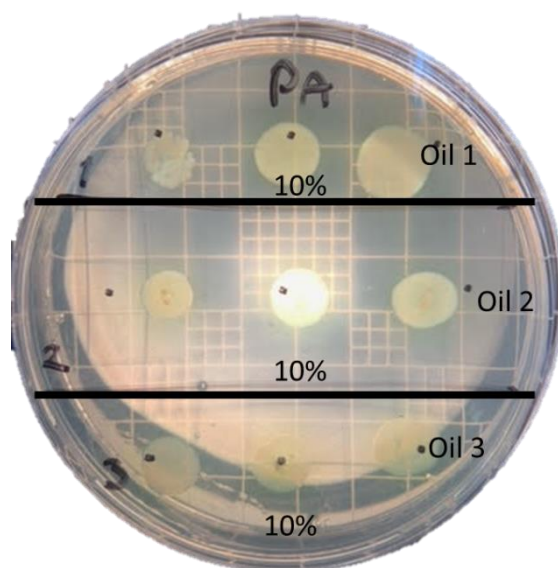


Figure 42. MBC with *Pseudomonas aeruginosa* (ATCC 27853).

Using Equation (1) and (2) to calculate percentage of bacteria that was killed with each oil and final MBC results of essential oil 1, 2 and 3 respectively in tables 7, 8 and 9.

Table 7. Results of MBC test for essential oil 1.

	Essential oil 1		
	C	P	MBC
<i>Staphylococcus aureus</i>	1100	99.3%	2.5%
<i>Escherichia Coli</i>	600	99.60%	10%
<i>Klebsiella pneumoniae</i>	0	100%	5%
<i>Pseudomonas aeruginosa</i>	1.50E+05	0%	>10%

Table 8. Results of MBC test for essential oil 2.

	Essential oil 2		
	C	P	MBC
<i>Staphylococcus aureus</i>	0	100%	10%
<i>Escherichia Coli</i>	0	100%	10%
<i>Klebsiella pneumoniae</i>	0	100%	5%
<i>Pseudomonas aeruginosa</i>	1.50E+05	0	>10%

Table 9. Results of MBC test for essential oil 3.

	Essential oil 3		
	C	P	MBC
<i>Staphylococcus aureus</i>	0	100%	10%
<i>Escherichia Coli</i>	1.50E+05	0%	>10%
<i>Klebsiella pneumoniae</i>	0	100%	10%
<i>Pseudomonas aeruginosa</i>	1.50E+05	0%	>10%

Disk diffusion

Disk diffusion is intended to evaluate which oil had more potential of inhibition through measuring halo that have grown radially around paper filter, which have a diameter of 6 mm, this halo represents where bacteria wasn't able to grow after 24h. From Figure 43 is possible to observe that for all three essential oils there was almost no formation of halo, maybe this is a consequence of the volatile characteristic of those oils. With the aid of a caliper halos were measured, in table 10 there are the results from halo size for each bacteria. Only for *Staphylococcus aureus* and *Klebsiella pneumoniae* there was halo formation, through inhibition diameter is possible to observe that they presented similar activity, comparing to initial diameter of 6 mm from paper filter it is a low activity.



Figure 43. Disk diffusion test with pure essential oils.

Table 10. Inhibition halo result from essential oils in disk diffusion test.

Oil	Inhibition halo diameter (mm)			
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>
1	6.85	6	6.4	6
2	7	6	6.3	6
3	6.6	6	6.4	6

5. CONCLUSION AND FUTURE WORKS

In this work it has been achieved all objectives to evaluate *Curcuma Longa L.* vegetable oil as potential lubricant base and essential oil as potential biocidal additive for cutting fluids applications.

Curcuma Longa L. vegetable oil for wettability comparing to the synthetic vegetable oil demonstrated better wettability for aluminum and 1045 steel which means that for those materials a better affinity for cooling and forming lubricant film than the synthetic oil. Also, for TGA and DSC tests curcuma vegetable oil have shown better thermal stability capable of work in a higher range of temperature before mass degradation and absorb higher amount of energy when submitted to the same variation of temperature when compared to synthetic oil. For the viscosity as, expected curcuma vegetable oil had a considerable high value compared to synthetic vegetable oil and when the temperature raises both values of viscosity decreased, high values of viscosity enables the fluid to work in a higher range of temperature. In a general view from the physico-chemical properties evaluated *Curcuma Longa L.* vegetable oil have great potential as base for lubricants to substitute mineral based lubricants.

Curcuma Longa L. essential oil for MIC tests essential oil 1 presented the best performance for gram positive *Staphylococcus aureus* and gram negative *Klebsiella pneumoniae* with 1.25% and 0.625% MIC values, it was expected that for gram positive inhibition activity were higher from gram negative, which means that also for gram negative this specific commercial oil have biocidal effects on *Klebsiella pneumoniae*. Essential oil 2 also presented biocidal effect for *Klebsiella pneumoniae* which means that is possible they have common antimicrobial components but in different proportion.

MBC analysis also indicates that essential oil 1 had better performance for gram positive *Staphylococcus aureus*, indicating that with 2.5% concentration this specific oil was capable of killing 99.3% of bacteria. Disk diffusion otherwise for all three oils almost didn't had inhibition, there was only a low halo formation for *Staphylococcus aureus* and *Klebsiella pneumoniae* for all three essential oils, from this test is concluded that the volatile components from *Curcuma Longa L.* essential oil are mostly responsible for the biocidal activity.

On Figure 44 is structured a resume of all results obtained and general conclusions considering *Curcuma Longa L.* plant as a multiple source to be applied inside MWFs context and potential to develop more researches from this initial step.

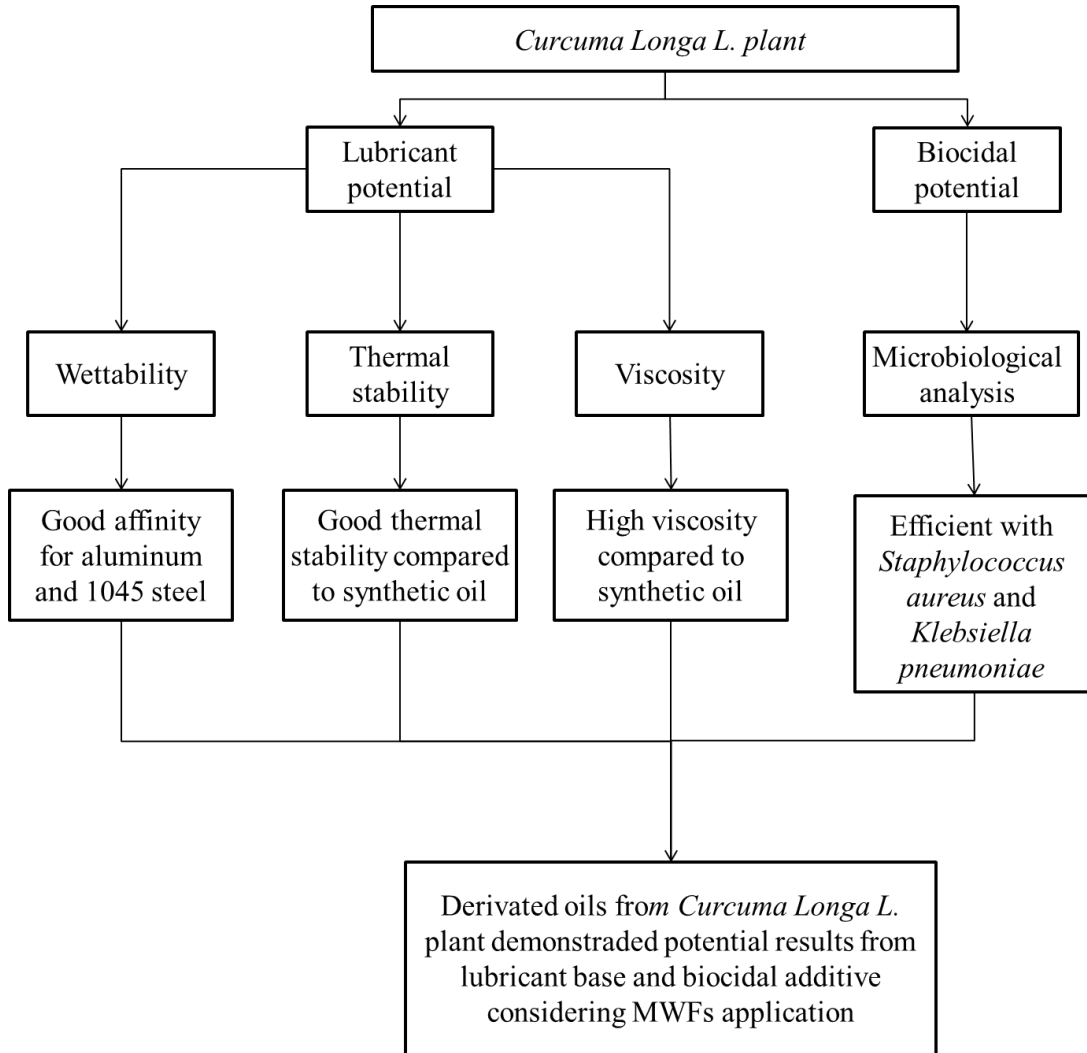


Figure 44. Resume from results obtained in this research.

Suggestions for future works

- Evaluate tribological characteristics from *Curcuma Longa L.* vegetable oil for machining applications;
- Evaluate fatty acid profile as a possibility to apply chemical modifications to achieve better lubricant performance;

- Analyze composition through gas chromatography from *Curcuma Longa* *L.* essential oils to understand the main substances with biocidal potential;
- Evaluate antimicrobial potential with different microorganism;
- Propose different types of extractions and compare biocidal activities between them.

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