Development of Environmentally Compatible Detergents Using Biosurfactants

Lénia Maria Amado Durão

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Supervisors: Prof. Dr. Nuno Ricardo Torres Faria

Dr. Ana Rita Ramos Diniz de Quadros e Costa

Examination Committee

Chairperson: Prof. Dr. Ana Cristina Anjinho Madeira Viegas

Supervisor: Prof. Dr. Nuno Ricardo Torres Faria

Members of the Committee: Dr. João Pedro da Fonseca Anes

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Preface

The work presented in this thesis was performed at the Institute for Bioengineering and Biosciences of Instituto Superior Técnico (Lisbon, Portugal) and at SGS Portugal, during the period March – October 2022, under the supervision of Prof. Nuno Faria and Doctor Ana Rita Costa.

Declaration

I declare that this document is an original work of my own authorship and that it fulfils all the requirements of the Code of Conduct and Good Practices of the Universidade de Lisboa.

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Resumo

Lípidos de manosileritritol (MELs) são um bio tensioativo com excelentes características, nomeadamente biodegradabilidade, baixa toxicidade e biocompatibilidade. Podem ser usados em diversas aplicações como, por exemplo, em detergentes.

Durante o desenvolvimento das formulações vários testes (pH, viscosidade, espuma e determinação de CMC) e vários ingredientes foram avaliados para perceber quais formulações eram as mais indicadas, assim chegamos às formulações finais do detergente de loiça manual (SLES+CB+MELs (5 g/L)) e limpa vidros (SDBS+MELs (2 g/L)).

A formulação final do detergente de loiça tem um pH de 5.99, viscosidade de 688.4 cP e poder espumante de 5.25 cm que se manteve durante os 5 minutos. A formulação final do limpa-vidros tem um pH de 8.11. Para ambos os detergentes, o índice de emulsificação a 24h foi de 47.69% e, relativamente, ao ensaio de sobrevivência de *Galleria mellonella* os resultados não foram conclusivos.

As formulações foram submetidas a ensaios de estabilidade acelerada durante três meses e ambas demostraram estabilidade. Em relação aos testes de performance realizados, o detergente de loiça manual necessita de melhorias, pois a sua capacidade de limpeza é inferior à marca líder de mercado e de uma formulação com o certificado ecolabel. O limpa-vidros tem uma boa capacidade de limpeza semelhante à líder de mercado e também à formulação com certificação ecolabel.

Por último, foi realizado um questionário para obter opiniões externas à cerca da aparência e da performance dos detergentes.

Palavras-chave: tensioativos naturais; lípidos de manosileritritol; detergentes; formulações; performance

Abstract

Mannosylerythritol lipids (MELs) are biosurfactants with excellent characteristics, namely biodegradability, low toxicity, and biocompatibility. It can be used in several applications, such as in detergents.

During the development of the formulations, several tests (pH, viscosity, foam and CMC determination) and several ingredients were evaluated to understand which formulations were the most suitable, thus we arrived at the final formulations of the manual dishwashing detergent (SLES+CB+MELs (5 g/L)) and glass cleaner (SDBS+MELs (2 g/L)).

The final dishwashing detergent formulation has a pH of 5.99, viscosity of 688.4 cP, and foam test of 5.25 cm which was held for 5 minutes. The final glass cleaner formulation has a pH of 8.11. For both detergents, the emulsification index at 24h was 47.69% and for the *Galleria mellonella* survival test, the results were not conclusive.

The formulations were subjected to accelerated stability tests for three months and both proved to be stable. About the performance tests performed, the hand dishwashing detergent needs improvement, as its cleaning ability is lower than the benchmark and an ecolabel certified formulation. The glass cleaner has a good cleaning ability similar to the market leader and also to the ecolabel certified formulation.

Finally, a questionnaire was conducted to obtain external opinions about the appearance and performance of the detergents.

Keywords: Biosurfactants; Mannosylerythritol lipids; Detergents; Formulations; Performance

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List of Abbreviations

AISE- International Association for Soaps, Detergents and Maintenance Products
BPR- Biocidal Products Regulation
CAGR- Compound Annual Growth Rate
CB- Cocamidopropyl betaine
CMC- Critical micellar concentration
CPCH- Cosmetic, Personal Care and Household products
CLP- Classification, Labelling and Packaging Regulation
DG- Decyl glucosidase
DGAE- General Directorate of Economic Activities
ECAT- EU Ecolabel Online Catalogue
EI- Emulsification Index
EU- European Union
GC- Gas chromatography
GMP- Good Manufacturing Practice
IKW- Industrieverband Koerperpflege- und Waschmittel
ISO- International Organization for Standardization
LAS- Linear alkylbenzene sulfonate
MELs- Mannosylerythritol lipids
MWTPs- Municipal wastewater treatment plants
REACH- Registration, Evaluation, Authorization and Restriction of Chemical Substances
RH- Relative Humidity
rpm- Rotation per minute
SCS- Sodium coco sulfate
SDBS- Sodium dodecyl benzene sulfonate
SGS- General Society of Superintendence
SLES- Sodium lauryl ether sulfate
TLC- Thin layer chromatography
USD- United States dollar

1. Introduction

1.1 Overview

Industrial processes to produce goods are a significant source of pollution, that has been intensified due to rapid economic growth as a consequence of excessive consumption, leading to a significant increase in environmental degradation.

Recently, the detergent industry has undergone some alterations to respond to consumer preferences, with recent trends aiming at becoming more sustainable and safer for the environment while maintaining product quality and efficiency. According to the forecasts, and although detergents can be found in different markets, the market for household cleaning products, the most representative detergents market, will increase with a CARG (compound annual growth rate) of 4.5% from USD 235.76 billion in 2021 towards USD 320.82 billion in 2028 [1]. Household cleaning products are used in domestic and industrial cleaning and can be classified, according to their applications, as laundry detergents, dishwashing detergents and hard surface cleaners. Cleaning formulations are expected to remove all dirt quickly, effectively, and safely. The capacity of a detergent to fulfil its purpose leans on its composition, the usage circumstances, the nature of the surfaces to be treated, and the type of substance to be cleansed [2]. Owing to the complexity of the detergent formulation process, the formulation was influenced by the final user's specific needs, economic and environmental factors, and the availability of ingredients that could offer the needed functionality [3]. The main component of detergents are surfactants, which are, typically, derived from petroleum. They have a diverse and significant role in several industries like household soaps and detergents, personal care, food processing, agricultural chemicals, and fuel additives. Nevertheless, some concerns about its use began to arise due to environmental problems and the sustainability of its production, namely ecological and toxicological problems.

Technological advances and learning from nature enable the arising of new solutions, such as the surfactants. The biosurfactants are, usually, subproducts of the metabolism of microorganisms and present several advantages such as non-toxicity, biodegradability, biocompatibility, and ecological acceptability [4]. However, its production is still limited, mainly due to high production costs and lack of a process optimization. It is necessary to make this industry more economically viable to aim to large scale utilization of such ingredients [5]. Nevertheless, in the past years, a few companies started biosurfactant production. The market for biosurfactants was estimated to be around USD 16.5 million in 2022; by 2032, it was predicted to be worth USD 24.3 million, growing at a 3.9% CAGR [6].

This growth is based on the increasing consumers awareness and their preference to products that are sustainable as well as safe for the environment. In this regard, the ecological labels in the final products are of paramount importance as a tool to deliver certified claims and improve consumers trust, while avoiding dishonest marketing strategies of greenwashing.

1.2 Objectives

Nowadays, most detergents have surfactants derived from petroleum in their composition, thus, their use is not the most compatible with the environment. Surfactant alternatives are starting to emerge, namely biosurfactants. This class of amphiphilic molecules can represent different molecular families, such as lipoproteins, phospholipids, and glycolipids. Within the glycolipids, sophorolipids, rhamnolipids and mannosylerythritol lipids (MELs) are the best known, and those that have the highest microbial biosurfactants market share.

In the case of MELs, it is an extracellular product of non-conventional yeasts, such as *Moesziomyces* spp. and possess interesting properties, such as versatile biochemical functions, and biocompatibility with the environment and could be used in many different areas. The main objective of this work was the development of new detergent formulations using MELs as an active ingredient and the characterization of these formulations. The other goal was to study and implement ecological certifications and labels, namely EU Ecolabel. To achieve these goals, several surfactants were tested, in order to optimize their properties to obtain a formulation with excellent performance and environmentally compatible, as demanded by the environmental labels.

1.3 Research questions and research strategies

This work addresses the following questions:

1. How to formulate an environmentally friendly detergent?
2. What MELs concentration should be used in the formulation?
3. How are detergents performing?
4. What is the economic viability of the formulation under study?

To address these questions, the following experiments were performed:

- Several environmentally friendly surfactants were tested in various formulations in order to understand which are the most suitable for the formulations in question, various parameters were analysed, such as pH, viscosity, and foam test.
- After obtaining a base formulation, several concentrations of MELs (10 g/L, 5 g/L, and 1 g/L) were tested to find out which would be the most suitable, several parameters were evaluated such as pH, viscosity, and superficial tension.
- To address the performance of the detergents, tests were carried out based on the IKW protocols (Industrieverband Koerperpflege- und Waschmittel), the German Association for Cosmetics, Toiletries, Fragrances and Detergents, was also compared with a reference detergent.
- The production cost of formulations was simulated to understand the economic viability of the production on a large scale and the economic analysis to obtain the EU Ecolabel certification.

2. Company

2.1 SGS

The General Society of Superintendence (SGS) was established in 1878 in the city of Rouen, France, starting its activity in the grain trade sector and providing agricultural inspection services. In the mid-twentieth century, there is a diversification of services in the areas of inspection, testing, and verification in various sectors, such as energy, environment, health and nutrition. Nowadays, SGS is the world's leading inspection, verification, testing, training, and certification organization. The company's main objectives are to continually improve its skills, to be the most competitive and productive service organization in the world and to offer a service consistently throughout the world [7].

SGS Portugal was founded in 1922 and developed its activity on the same principles as the group itself. Over time, it has gradually extended its activity to other sectors, following the changes and demands of the market. So today it extends to the most diverse fields of economic activity. It offers a comprehensive portfolio of services in the areas of inspection, verification, tests and laboratory tests, consultancy and certification in the most diverse references, there is also a SGS training academy (SGS Academy) that bets on training with a strong practical component, making the courses prove to be tools to support efficiency, effectiveness, and innovation. There are also accredited laboratories in the food, agricultural and mineral, environmental, CPCH (Cosmetic, Personal Care and Household products) and medical devices, chemical and petroleum fields [7].

2.1.1 Cosmetics and hygiene group

During the internship at SGS the student integrated the cosmetics and hygiene group, this group is supporting the settling on the market of cosmetics, household products, biocides, and medical devices. SGS helps companies to verify the safety and efficacy of products, performance, and support claims and, helps to ensure the chosen packaging that offers the protection needed for the product. Also, assist with the verifications of product agreement with the requirements of applicable regulations as cosmetic regulations, detergents regulations and other relevant legislation, including REACH (Regulation, Registration, Evaluation, Authorization and Restriction of Chemical Substances) and GMP (Good manufacturing practice).

In summary, SGS accompanies all stages of the product, through tests, safety assessments, documentation review (EU market), audits and inspections. Safety and quality are vital for the products and the end consumers are increasingly demanding proof of quality, compliance, and efficacy from raw materials to finished products [8].

2.2 Accomplished activities

During the internship, several activities were developed, such as:

- Development of detergents formulations
- Characterization tests (pH, viscosity, microbiologic tests)

- Performance tests
- Stability tests
- Study and implementation of ecolabel certification

For its realization, it was necessary to obtain knowledge about cosmetics regulation, detergents regulation, and ecolabel certification. I had the opportunity to be a speaker in the Webinar organized by SGS "Detergents and Cosmetics: learn more about the Ecolabel - the EU Ecolabel", with 93 participants. Which I first addressed the topic of the EU Ecolabel and how certification can be obtained, then criteria for obtaining certification for detergents, and the performance tests required for each category of detergent.

3. Literature review

3.1 Detergents

According to the Detergents Regulation (EC) No 648/2004, detergent is any substance or mixture containing soaps and/or surfactants intended for washing and cleaning processes. They can be found in different forms (liquid, powder, paste, bar, moulded piece) and marketed for or used in household, institutional or industrial purposes [9]. It is expected that the detergents remove all dirt quickly, effectively, and safely.

Detergency is a process that consists of removing dirt with the application of a mechanical force, in the presence of a chemical substance. This detergency mechanism involves three phases. The first stage consists of wetting the surface to be cleaned, allowing the adsorption of the detergent at the dirt/surface interface. The second step is the removal of the dirt, through mechanical action, which causes the surfactant to drag the dirt particles with it. Finally, the dirt particle is kept stably inside a micelle (Figure 1) [10].

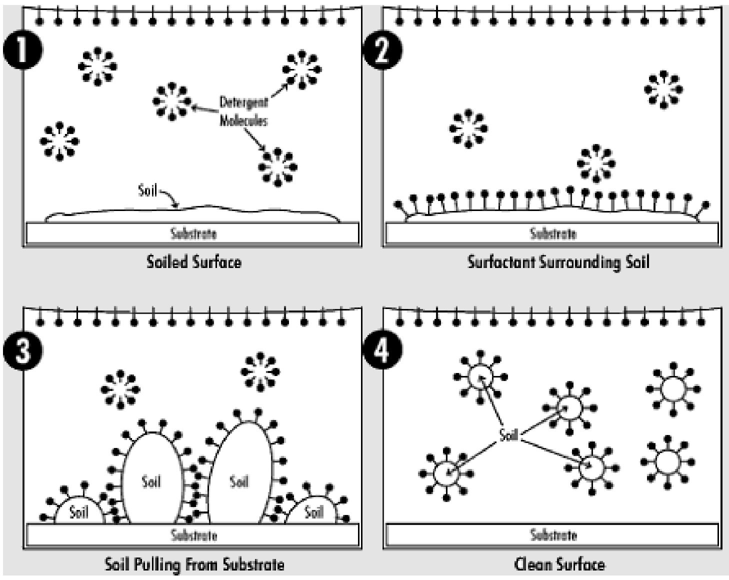

Figure 1 - Detergency mechanism: 1- Addition of detergent; 2- Interaction of the detergent with the surface; 3- Removal of surface dirt; 4- Stable integration of dirt particles.

The material to be cleaned, the type of dirt to be removed, and the apparatus to be used (manual or machinery) are the main factors that determine a detergent's composition [11].

The main constituent of detergents are surfactants or surface-active agents, they are amphiphilic molecules with polar and non-polar domains that preferably partition at the interface between liquid phases with different degrees of polarity, such as oil/water or air/water interfaces [12]. This feature reduces the surface tension of liquids through specific and preferential interactions at surfaces and interfaces due to hydrophilic and hydrophobic moieties in the same molecule. The nonpolar part of a surfactant is often a hydrocarbon chain, while the polar part (hydrophilic head group) can be ionic (cationic or anionic), non-ionic or amphoteric [13].

A surfactant's efficiency is determined by reducing surface tension, which is the mechanical energy required to create one unit of new liquid surface area. Surfactants increase the aqueous solubility of hydrophobic molecules. The surface tension decreases with increasing surfactant concentration in the aqueous medium until the formation of micelles, which are aggregated structures with the hydrophilic portion positioned outside the molecule and the hydrophobic portion placed inside. The critical micellar concentration (CMC) is the concentration that corresponds to the point at which the surfactant reaches the lowest stable surface tension, this is the minimum concentration of surfactant necessary for the maximum reduction of surface tension. Micelles are usually formed when the critical micellar concentration is reached (Figure 2) [14].

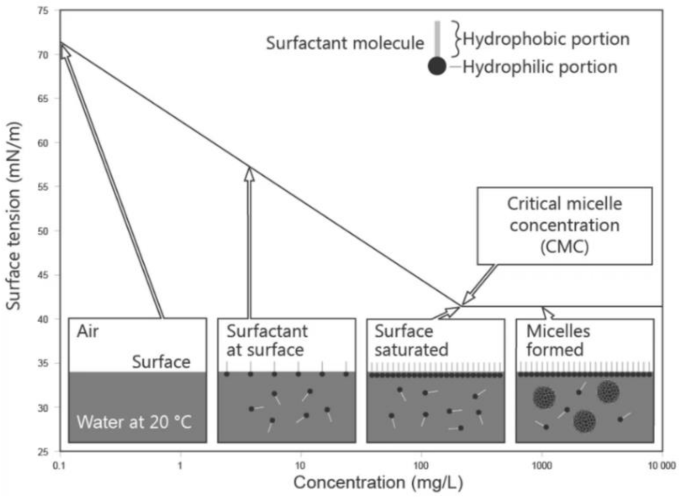

Figure 2 - The surface tension of a surfactant solution with increasing concentration and formation of micelles when the CMC was reached.

According to the charge of their hydrophilic portion, surfactants can be grouped into four categories: anionic, non-ionic, cationic, and amphoteric. The anionic surfactants have a negative charge on their hydrophilic end; this helps the surfactant molecules lift and suspend soils in micelles. Because they can attack a broad range of soils, they are used frequently in soaps and detergents. Anionic surfactants typically produce more foam than other categories of surfactants [15]. They contain anionic functional groups at their head, such as sulfonate, phosphate, sulphate, and carboxylates. Alkyl sulphates, alkyl ethoxylate sulphates are the most used in detergents [16]. In non-ionic surfactants the hydrophilic part is uncharged, they are lower foaming and are less affected by water hardness ions. They offer properties such as wetting, dispersion, emulsification, and detergency. The non-ionic surfactants include ethoxylates, alkoxylates, and cocamide [16]. The anionic and non-ionic surfactants cover the majority of industrial surfactant needs. The selection of surfactants for cleaning applications is based on specific needs and, often, mixtures of surfactants are used to attain the required properties and performance [17].

The cationic surfactants on their hydrophilic part are positively charged. They are applied as bactericides in disinfectants and fabric conditioners due to their ability to adhere to surfaces. These surfactants do not react with water hardness ions and have tiny or no detergency properties. It’s not possible combine cationic and anionic surfactants. The quaternary ammonium derivatives, in which the N atom is linked to four alkyl groups, are the most typical cationic surfactants [16], [17]. The amphoteric surfactants have a negative or positive charge; the pH of any given solution will determine how the surfactants react. They acquire a positive charge in an acidic solution and behave as cationic surfactants, and in an alkaline solution, they become negatively charged like anionic surfactants [16]. They offer mildness, improved wetting properties, low foaming characteristics, stability, and good hydrotrope or coupling ability. Alkylaminopropionates and alkylbetaines are examples of amphoteric surfactants. Cationic and amphoteric surfactants cover the smallest market segment [15].

Other ingredients that usually are present are builders, the most important function is the sequestrating agent of Ca^{2+} and Mg^{2+} ions that could interfere with the surfactant, also enhance the surfactant performance, and provide an alkaline environment. Sodium tripolyphosphate-STP, tetrasodium pyrophosphate-TSPP, zeolites, and sodium carbonates are examples of builders [11].

Thickening agents regulate the viscosity of liquid formulations, sodium chloride is highly common.

Enzymes are used to promote soil removal by the catalytic breakdown of specific soil components. Proteases are the most common of all the detergent enzymes, but amylases, lipases, and cellulases are also used [18]. pH adjusters help to balance the pH of the formulation, making it either more basic or acidic considering the formulation type. Citric acid and sodium/potassium hydroxide usually are used. Preservatives are needed to keep an adequate shelf life, otherwise, bacteria and fungi destroy the product. For industrial detergents, the most common preservative is formalin [2].

To improve the appearance and the experience of the consumer, the fragrances give a nice smell to the product and could be used to mask odours caused by any of the other ingredients. Dyes are utilized to give a detergent an appealing colour. It also gives identity to a product, making it easier to distinguish it from other products.

3.1.1 Environmental impact of detergents

Recently there has been an increased concern about the pollution of water resources, due to the high toxicity and bioavailability of chemical compounds. The environmental impact of detergents has focused on the discharge of industrial and domestic wastewater into receiving waters [19]. Detergents can alter some water parameters such as pH, salinity, temperature, and turbidity, which leads to a decrease in water quality. In general, one of the most common problems is caused by the formation of a foam layer on the water surface that decreases the oxygen rate in the water, so there will be poor oxygen adsorption by aquatic organisms.

For fish, the accumulation of detergents in the water in a short period can disturb their vision and can also cause damage to their gills. Regarding fungi, they are fundamental in food chains and play an important role in bioremediation of pollutants [19]. Studies have been conducted to understand the ability of different fungal species to degrade pollutants, namely detergents, the decomposition of detergents depends on the metabolic activities of fungi. In another study it was possible to observe that the amount of fungal biomass decreases by increasing detergent concentration [20].

Detergents are also harmful to plants, especially aquatic plants. The eutrophication process results from detergent discharge into water bodies due to a high concentration of phosphates [21], however currently the use of phosphates in detergents is already banned in developed countries. In addition to the direct effect of detergents on aquatic crops, decreasing populations of marine plants indirectly affect species that depend on them for food, protection, or a spawning site.

Water resources contain different types of microorganisms, such as bacteria, the diversity and density of bacteria can be affected by detergent discharge, which can have inappropriate biological effects [22]. Several detergents have bactericidal properties, so they can limit the metabolic activities and growth rate of aquatic bacteria [23]. Detergents could destroy bacterial cell cytoplasmic membrane proteins, inhibit cellular metabolism. When water contaminated with detergents meets soil it will cause a negative effect on flora, particularly on plant germination [24] and can also lead to a gradual destruction of soil structure due to the increase in pH.

The linear alkylbenzene sulfonate (LAS), alkyl ethoxy sulphate (AES), alkyl sulphate (AS), alkylphenol ethoxylate (APE), alkyl ethoxylates (AE), and quaternary ammonium-based structures (QAC) are the most purchased and used surfactants globally [25]. Due to the many applications of surfactants, their high concentration in mainly urban, industrial, or domestic wastewater can end up in municipal wastewater treatment plants (MWTPs) or discharge directly into the environment [26]. There are possibilities that surfactants can penetrate drinking water through MWTPs, thus representing a risk to human, animal, and aquatic health [27]. Some of the commercially available surfactants cause serious environmental and public threats to humans and ecosystems. For example, anionic surfactants, predominantly linear alkylbenzene sulfonates (LAS), cause biochemical, pathological, physiological, and other impacts in aquatic and terrestrial ecosystems [28]. In addition, LAS causes skin irritation and respiratory problems [29] and reduces the resistance of aquatic biota against environmental stress, reproduction, and growth processes [30]. Surfactants generally increase the solubility of contaminants and thus facilitate eutrophication [31]. The vast impacts consequently raise public and environmental health concerns with the high concentration of surfactants. Several techniques are used to decontaminate surfactants from wastewater. Some of these remediation technologies include physicochemical processes, like membrane filtration and flocculation-adsorption, ultrafiltration, electrocoagulation, chemistry, and electro-oxidation [32]–[34]. However, when surfactant concentrations are high, the techniques are less effective [35].

In resume, industrial and domestic wastewaters release varying amounts of detergents into the soil and receiving waters, which have a variety of consequences on the fauna and flora in natural ecosystems, in addition to affecting wastewater treatment processes. However, detergents are essential for human life, and its manufacture and consumption cannot be eliminated, its impacts on human health, the environment, and biological treatment processes must be minimized. Biotechnology-based cleaning products, such as bio detergents, are more environmentally friendly, and biodegradable, and have better cleaning characteristics than synthetic cleaning agents [36].

3.1.2 European measures

To control the adverse effects of detergents on the environment, the Detergents Regulation (EC) No 648/2004 was created and valid from October 2005 onwards. This regulation establishes common rules to enable detergents and surfactants to be sold and used across the EU while protecting the environment and human health. For instance, it stipulates that the surfactants used in detergents must be fully aerobic biodegradable. In addition, it regulates how products should do the additional label, including fragrance allergens and the information that manufacturers must hold at the disposal of the member states competent authorities and medical personnel [9].

Over the years this regulation has been updated, in 2006, Regulation (EC) No 907/2006 corrected annexes III and VII related to the biodegradability tests of surfactants and the labelling/ingredient data sheet, respectively [37]. Another actualization was Regulation (EC) No 1336/2008 which adapts the detergents regulations by the Classification, Labelling and Packaging (CLP) [38]. Furthermore, Regulation (EC) No 551/2009 indicates the derogation and banned or restricted detergent surfactants [39]. Another actualization was Regulation (EU) No 259/2012 limited the phosphate and phosphorus concentration in domestic laundry and dishwasher detergents [40].

In addition, to provide clearer information to consumers, some tools have been created such as the CLP; Regulation, Registration, Evaluation, Authorization and Restriction of Chemical Substances (REACH) and the Biocidal Products Regulation (BPR). The purpose of the regulation (EC) 1272/2008 CLP is to harmonize the criteria for the classification of substances and mixtures and the rules on labelling and packaging for hazardous substances and mixtures. It also aims at establishing a classification and labelling inventory of substances [41]. The REACH is a European regulation that covers the safe use of chemicals over their entire life cycle, helping to ensure the safe use of chemicals for consumers and the environment [42]. The BPR regulates biocidal products in a harmonised way across Europe to ensure the protection of human health and the environment [43].

Currently, the Detergents Regulation (EC) 648/2004 is being updated, and the main goals are the improvement of the consistency and simplification of the regulatory framework for detergents as well as adapt the legislation to technical progress.

There also exist the criteria related to the attribution of the EU ecolabel certification, which are stricter criteria aimed at the existence of more environmentally friendly and sustainable products. These criteria set out general requirements for some substances, exclude the use of some specified substances and restricted the concentration of other substances (Appendix A).

3.2. New biobased products

3.2.1 New trends

One of the most active industries on constantly seeking to boost its output levels is the detergent industry [44]. Due to technological advances, industries have been adapting and the household cleaning products industry was no exception. In recent years there has been a larger concern with the environment and new trends emerged, such as:

- The “green” movement and consumer preference is driving through an increased interest in products that are more sustainable to manufacture and safer for the environment, such as biosurfactants, when released in wastewater.
- Appliance technologies, the detergent formulations will continue to be driven by changes in the machines that the consumers are using. The move to high-efficiency washing machines, which use relatively little water, has already resulted in the formulation of new high-efficiency detergents.

Nowadays, a greater concern for packaging to be made of recycled materials or at least be recyclable is a reality. Refill systems and packaging have also been used in this sense, for example ECOX® has a refill system implemented in some supermarkets. Capsule detergents are also more environmentally friendly, as the packaging does not require as much plastic and they are usually more concentrated. Capsule detergents are already common in laundry detergents, however recently this concept has also emerged for other types of detergents such as multi-purpose detergents. Clean Essence® is one of the brands with these products.
Natulim® is another brand concerned about environmental issues and the impact of their products, this brand has detergents in strips both for clothes and for the floor, this type of detergent has no plastic in the packaging and is compact which greatly reduced the pollution emitted during transport. Novozymes is a company with an innovative concept that produces enzymatic solutions that are used as detergents. Through these examples it is noticeable that there is a greater environmental concern and that the detergent market is changing.

3.2.2 Biosurfactants

Biosurfactants are compounds of microbial origin with diverse structures and surface properties [12]. Five classes classify them, namely lipopeptides; glycolipids; fatty acids including neutral lipids and phospholipids; polymeric; and particulates (Table 1). The majority of the currently utilised and existing biosurfactants are glycolipids, which have a low molecular mass [45].

Table 1 - Main five types of biosurfactants, their characteristics and examples.

Class of biosurfactant	Characteristics	Examples
Glycolipids	Composed of carbohydrate and lipid, the linkage is by ether or an ester group	MELs, Rhamnolipids, Sophorolipids, Trehalolipids
Lipopeptides and lipoproteins	Contain a lipid connected to a polypeptide chain	Gramicidins, Polymyxins Surfactin, Viscosin,
Fatty acids, phospholipids, and neutral lipids	Unsaturated fats and phospholipid surfactants amid the development of n-alkanes	Fatty acids, Phospholipids
Polymeric	Formed between saccharide unit and fatty acid residues	Alasan, Emulsan, Liposan
Particulate	Extracellular film vesicles segment hydrocarbons from a microemulsion	Vesicles and fimbriae

They have aroused interest due to their unique properties such as high biodegradability, low toxicity, great structural diversity, and effectiveness at extreme temperatures [54]. Additionally, they can possess lower CMC values than synthetic surfactants, improving their efficiency in various applications [48]. Biosurfactants play important roles in industrial [49], pharmaceutical [50], and environmental applications [51], such as detergency, foaming, wetting [52], emulsification, bioremediation [53], stabilization [54], lubrication, dispersion, and solubilization of hydrophobic compounds, thanks to their structural diversity and functional properties. They are nontoxic to the environment and represent possible alternatives for synthetic surfactants in the production of laundry detergent [55]. The use of biosurfactants in the medical field is increasing due to their antibacterial, antifungal, antiviral, anticancer and antiadhesive effects [50]. Remediation techniques using biosurfactants help remove hydrocarbons and metals from contaminated areas [56].

While there are many emerging applications for biosurfactants, due to their unique properties and low toxicity compared to synthetic surfactants, and emerging consumer preferences for "natural" ingredients, the commercial availability of microbial biosurfactants is quite limited. Large-scale production of microbial biosurfactants can be difficult to achieve at competitive costs [48], however, there are already some companies producing biosurfactants (Table 2). There are markets interested in exploring the biosurfactant industry, as there is a growing receptivity among consumers for environmentally friendly compounds [57]. Biosurfactants are the multifunctional biomolecules/materials of the 21st century [58].

Table 2 - Some of the biggest microbial biosurfactant-producing companies.

Company	Location	Product(s)	Application(s)
AGAE Technologies LLC	USA	Rhamnolipids (R95, an HPLC/MS grade rhamnolipid)	Pharmaceutical, cosmeceutical, cosmetics, personal care, bioremediation (*in situ* & *ex situ*), Enhanced oil recovery
BASF	Germany	Sophorolipids	Household products, Personal Care
Ecover	Belgian	Sophorolipids	Household products
Evonik	Germany	Rhamnolipids Sophorolipids	Household products
Groupe Soliance	France	Sophorolipids	Cosmetics
Henkel	Germany	Sophorolipids, Rhamnolipids, Mannoslyerthritol lipids	Glass cleaning products, laundry, beauty products
Holiferm	UK	Sophorolipids	Personal care, home care, industrial cleaning, agriculture
Jeneil Biosurfactant Co. LLC	USA	Rhamnolipids (ZONIX, a bio-fungicide and RECO, a rhamnolipid)	Cleaning products, Enhanced oil recovery
TeeGene Biotech	UK	Rhamnolipids and Lipopeptides	Pharmaceuticals, cosmetics, and anti-cancer ingredients

3.2.2.1 Mannosylerythritol lipids

Mannosylerythritol lipids (MELs) are one of the most promising biosurfactants, glycolipids produced by *Moesziomyces* spp. (former *Pseudozyma* spp.), *Ustilago* spp., and related yeasts and filamentous fungi, contain 4-O-β-D-mannopyranosyl-meso-erythritol or 1-O-β-D-mannopyranosylerythritol as hydrophilic group and fatty acid and/or an acetyl group as the hydrophobic moiety (Figure 3) [46]. MELs generally have one or two acetyl groups at C-4 and/ or C-6 of the mannose moiety. Based on the degree of acetylation at the C-4 and C-6 positions, and their order of appearance on the thin layer chromatography, MELs are classified as MEL-A diacetylated, the most common, while MEL-B and MEL-

C are monoacetylated at C-4 and C-6, respectively. MEL-D has a completely deacetylated structure, generally, it can only be derived by enzymatic synthesis of MEL-B [59]. The production of MELs aren't associated with growth, so MELs is only produced when the yeast is in a stationary phase [60].

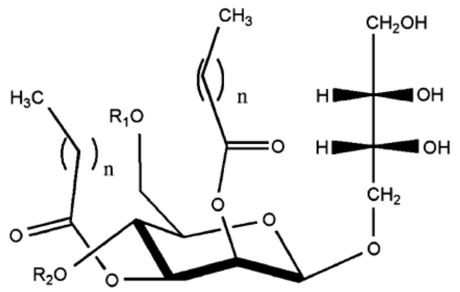

Figure 3 - Structure of MELs (MEL-A: R1 = R2 = Ac; MEL-B: R1 = Ac, R2 = H; MEL-C: R1 = H, R2 = Ac: n = 6–10)

MELs has been applied in many fields, due to their exceptional interfacial properties, biocompatibility, self-assembling properties, antimicrobial activities, and biochemical functions. MELs has several pharmaceutical applications such as antimicrobial activity against gram-positive bacteria [61], differentiation-inducing activities against human leukemia cells [62], mouse melanoma cells [63], and high-affinity binding for different immunoglobulins and lectins [64]. MELs can also be used in cosmetics as they have an amphiphilic structure similar to ceramide-3 [65]. In resume, MELs could be used in pharmaceutical industries, cosmetics, food, household products, environmental protection and energy saving technologies.

3.3.3 Market assessment

In 2021, the market for household cleaning products was worth USD 235.76 billion. The market is projected to grow from USD 247.94 billion in 2022 to USD 334.16 billion in 2029 at a CAGR of 4.4% in the forecast period. The impact of COVID-19 on the world has been unprecedented, the pandemic has resulted in a rise in demand for household cleaning products all over the world. In 2021, the global market showed a 6.5% increase. The quick increase in CAGR is due to the demand and expansion of this market, which will return to pre-pandemic levels once the pandemic is over.

This increase in the market is also due to the growing demand for more natural and eco-friendly products. It's predicted that the market would continue to be led by the sector of laundry detergents [1].

Regarding, the surfactants market size was estimated at USD 44.99 billion in 2021 and it is expected to surpass around USD 81.7 billion by 2030, growing at a CAGR of 4.9% during the forecast period 2022 to 2030. One of the most widely produced and used chemicals worldwide is surfactant. They are compounds with unique characteristics and are used in a wide range of areas, like home care and personal care, food processing, oil field chemicals, and agricultural chemicals. The consciousness regarding personal hygiene and cleaning has increased in the current situation, which has stimulated the demand for personal and household cleaning products, thus enhancing the market growth of surfactants. The growing personal care industry in Asia-Pacific and the growing demand for oleochemicals are driving the growth of the surfactant market (Figure 4) [66].

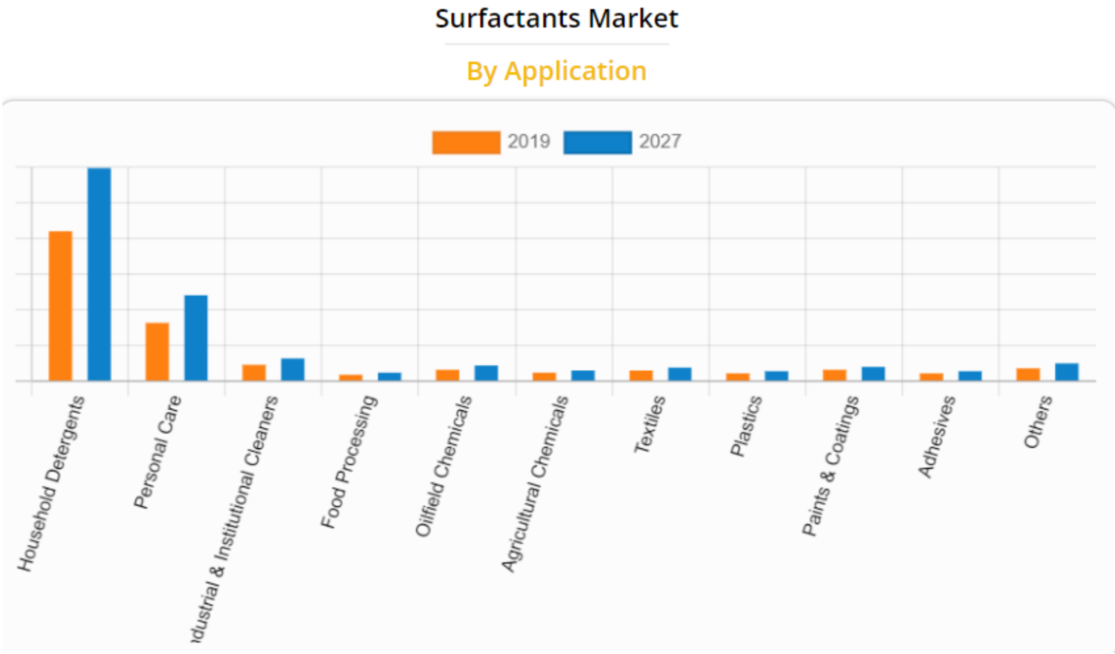

Figure 4 - Surfactants market prediction (2019 - 2027) by application.

Considering, that the biosurfactants market size was USD 24.40 billion in 2021 it is expected to register a revenue CAGR of 15.7% until 2030. This high growth is due to the increasing consumer awareness about environment-friendly and sustainable product alternatives, emerging trends and novel strategies related to biosurfactant manufacturing or industrial production processes, and rising demand from end-use industries are crucial factors driving the market growth. In addition, increasing commercialization of biosurfactants in personal care and food industries and oil field applications is another factor contributing to growth (Figure 5) [67]. The glycolipids are the most studied biosurfactants and the most used. In 2021, the Europe market accounted for the largest revenue share in the field of green surfactants, due to an increase in research and development activities and government-funded research projects in this field [67]. The industries of detergents and cosmetics is expected have an important impact on biosurfactants demand over the years. However, the expansion of the worldwide biosurfactants market is anticipated to be constrained by the limited features of biobased surfactants.

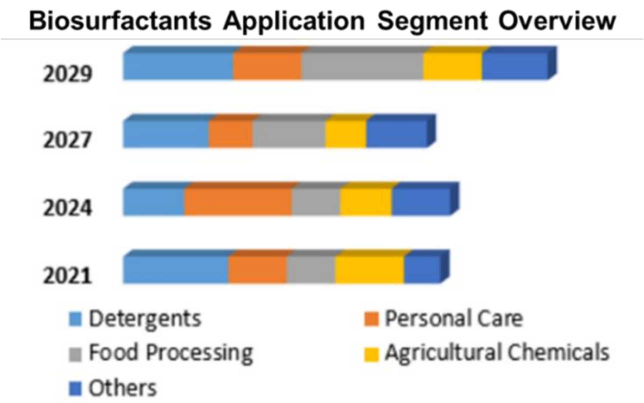

Figure 5 - Biosurfactants application prediction from 2021 to 2029.

3.3 Ecological labels

3.3.1 Ecolabels

Concern about environmental degradation has increased over the years, thus ecological labels began to appear, as they indicate products that are compatible with the environment. An ecological label is defined as a logo, symbol or proof of authorization given to the product that informs consumers about the better ecological quality and communicates information about the environmental attributes of the goods [68]. The main objective of ecological labels is the protection and preservation of the environment, which leads to more efficient use of resources, reduction of waste, materials reuse, and recycling. As well as inform manufacturers and traders of products and processes compatible with the environment. Finally, the other goal is to make the information easier to understand, accessible, trustworthy, and credible to consumers, several ecolabels have been created over the years to inform consumers aware of environmentally friendly choices [69]. There are three different types defined by the International Organization for Standardization (ISO): Type I, II and III. Type I (ISO14024) is based on multiple criteria, where a third party awards the use of the label to indicate overall environmental preferability within a particular product category based on life cycle assessment, which is the most monitored [69]. Type II (ISO 14021) is an environmental self-declaration with no verification. Type III (ISO 14025) provides environmental product data in different categories based on a life cycle meeting predefined parameter and must be verified by a qualified party. This type of label gives all the environmental information and leaves it up to consumers. Among the three classes, the foremost reliable and trustworthy are type I ecolabels. These category objectives are based on the product's life cycle, differentiate from products that are harmful to the environment, licenses are valid, transparency in all stages of the process of obtaining the label, compliance with standard indicators, and it can be ordered by any company [69]. Nowadays, with increasing environmental concerns and the search for more sustainable products, several ecolabels have emerged over the years. In Europe, exist two large programs: the EU ecolabel and the Nordic ecolabel. The EU ecolabel is one of the most popular ecolabelling programs in Europe and the Nordic Swan is a scheme created by the Nordic Council of Ministers for the Nordic countries:

Denmark, Finland, Iceland, Norway, and Sweden. Creates sustainable solutions based on a life cycle assessment and an overall goal to reduce the environmental impact of producing and consuming goods [69].

There are also ecolabels at national level, like Blue Angel (Gernamy), NF environment (France), AENOR Medio ambiante (Spain), Umweltzeichen Baume (Austria) and Milieurkeur (Netherlands). The goal of each national authority is to maximize the welfare of its own country [68]. There are also companies, such as Ecocert, which issue certifications that guarantee and highlight the best environmentally friendly and socially conscious practices. Related exclusively to detergents and soaps there is one label that is The Charter for Sustainable Cleaning which was created by the Association for Soaps, Detergents and Maintenance Products (AISE). The objective is to encourage consumers to adopt more environmentally friendly washing and cleaning, as well as to push the entire industry to pursue continuous sustainability improvement. Globally, there is a wide variety of environmental labels, the most critical aspects are cooperation between eco-labelling programs, the development of mutual criteria, and mutual recognition [69].

3.3.1.1 European Ecolabel

The EU Ecolabel, also known as "The Flower", was established in 1992 and is recognized across Europe and worldwide. This label is given to products and services that meet high environmental standards throughout their life cycle, from raw material extraction to production, distribution, and disposal. It covers 24 different categories of products and services, for example, detergents, cosmetics, clothes, textile products, and paints. These products and services must meet specific criteria, which differ by product group. The EU Ecolabel certification promotes the circular economy by encouraging producers to generate less waste and carbon dioxide during the manufacturing process. The EU Ecolabel requirements also motivate businesses to create products that are durable, simple to repair, and recyclable. This label addresses five main critical points which are: the environmental impact of toxic substances, deforestation and degradation, poor waste disposal and lack of recycling, inefficient use of resources and products and finally, unnecessary carbon emissions [70].

The main advantages of obtaining this label are the guarantee to consumers that the product has a lower environmental impact, a high level of transparency, reliability, and scientific credibility, which meets customers green demands. No technical knowledge is required to read and understand the label. By choosing eco-labelled products, it is easy for consumers to make an environmentally friendly choice [70].

3.3.1.2 How to apply

There are a few steps to obtain EU Ecolabel certification, the first step is to contact the competent body, which is responsible for assessing, awarding, and managing ecolabel applications and licences at a national level, in Portugal it is represented by Dr. Carla Pinto from the General Directorate of Economic Activities (DGAE). It provides guidance on the necessary documents to be submitted. The second step is to register on the EU Ecolabel online catalogue (ECAT). Is a database of listing goods and services which have this certification and corresponding information. This catalogue can also be used as a marketing tool to promote your products to consumers. The next step is the creation of a dossier with all the necessary documents from relevant declarations, data sheets and test results. This dossier is necessary to assess compliance with all the criteria. After, submit the application is submitted and the payment of the fees must be made [78].

The next step is the assessment, in this step all documentation will be analysed, the competent body will give feedback within two months maximum. After this time further documentation may be requested. After all the documentation has been assessed. Then, approval of the application and allocation of the license. After approval of the application the contract and licence are issued. At this point the products will become visible in ECAT. The time this process varies from case to case, but usually takes between two to eight months depending on whether additional information is required and how quickly that information is delivered [78].

3.3.1.3 Criteria

To be approved for the EU ecolabel certification, the products need to respect some criteria, the criteria presented are specific to detergents.

Criteria 1- Toxicity to aquatic organisms: estimates the impact of a product on aquatic freshwater ecosystems by calculating the volume of water required to dilute a quantity of the product to a concentration with any predictable harmful effect on aquatic species.

Criterion 2- Biodegradability: All surfactants must be biodegradable, and the organic substances that are not biodegradable should not exceed the allowed values.

Criteria 3- Sustainable sourcing of palm oil, palm kernel oil, and their derivatives: substances which use palm oil or palm kernel oil must only contain ingredients that come from plantations that have been certified to produce sustainably.

Criteria 4- Excluded and restricted substances: Some substances indicated shall not be included in the product formulation regardless of concentration, and other substances will not be included above a specific concentration.

Criteria 5- Packaging: Firstly, packaging must be easily recyclable, which means avoiding contaminants and incompatible materials. Preferably, they should be made from recycled materials. Packaging should be refillable and reusable when it’s possible, and in some cases, namely for hard surface cleaners and industrial detergents, a packaging take-back system could be applied. The weight/utility ratio for primary packaging should also be considered.

Criteria 6- Fitness for use: The product should have a satisfactory wash performance at the lowest temperature and dosage recommended by the manufacturer. The fitness for use tests changes accordingly to the product category, however, all are compared to reference detergents. The performance test for laundry detergents shall show that laundry detergents achieve good washing performance according to soil and stain removal, basic degree of whiteness, colour maintenance, dye transfer inhibition criteria and not damage the fabric. The detergents for dishwashers are assessed by cleaning efficiency with several different and specific soils. Hand dishwashing detergents are evaluated in a dishwashing solution, soiled plates are washed by hand until the foam layer collapses. For hard surface cleaning products exist several categories, the evaluation depends on the type of the product, for bathroom cleaners the assessment is realized through lime soap and limescale removal, for kitchen cleaner's and all-purpose cleaners fat removal is assessed, finally, for glass cleaners is evaluated for remotion of light fat and the strip-less drying.

Criteria 7- User information: The label should include instructions for proper use to maximize product performance, minimize waste, reduce water pollution, and use resources. These instructions shall include dosing instructions, and packaging disposal information.

Criteria 8- Information appearing on the EU Ecolabel: The logo and EU Ecolabel registration/license number must be visible and legible.

4. Materials and methods

4.1 Materials

Reagents: Yeast Extract (Oxoid), Malt Extract (Sigma Aldrich), D-Glucose anhydrous (Fisher Chemicals), Agar (LabChem), $NaNO_3$ (PanReact AppliChem), $MgSO_4$ (LabChem), KH_2PO_4 (Chem-Lab), Cocamidopropyl betaine (Plena Natura), Citric acid (Sigma-Aldrich), Decyl glucoside (Plena Natura), Ethanol (Fábrica do Álcool), Eugon (Sigma-Aldrich), Kerosene (Sigma-Aldrich), SAB (Sigma-Aldrich), Sodium benzoate (Sigma-Aldrich), Sodium chloride (PanReac AppliChem, ITW Reagents), Sodium coco sulfate (Plena Natura), Sodium dodecyl benzene sulfonate (Sigma-Aldrich), Sodium hydroxide (Fischer Chemical), Sodium lauryl ether sulphate (Gran Velada), Xanthan gum (Sigma-Aldrich), TSA (Sigma-Aldrich).

Organic Solvents: Acetyl Chloride (Sigma Aldrich), Chloroform (Fisher Chemicals), Ethanol (Fábrica do Álcool), Ethyl Acetate (Fisher Chemicals), Hexane (Fisher Chemicals), Kerosene (Sigma-Aldrich), Methanol (VWR Chemicals), Heptanoic Acid (Sigma Aldrich), Naphthol (Sigma Aldrich).

Equipment: Centrifuge (Eppendorf), Climatic chamber (FITOCLIMA 1200 PH PHARMA, Aralab), Fridge (Bosh), GC system (Hewlett-Packard, HP 5890), Heating plate (MR Hei-Tec, Heidolph), Orbital shaker (Optic invymen sydtem), Oven (Binder), pH Meter (744, Metrohm), Tensiometer (KRÜSS GmbH), Viscosimeter (Brookfield DV-II+ Pro).

Microorganism: *Moesziomyces* yeast strain was provided by Portuguese Yeast Culture Collection (PYCC), CREM, FCT/UNL, Caparica, Portugal: *M. antarcticus* PYCC 5048^{T} (CBS 5955).

4.2 Methods

4.2.1 MELs production and characterization

Microorganisms and maintenance

The yeasts strains were provided by the Portuguese Yeast Culture Collection (PYCC), CREM, FCT/UNL, Caparica, Portugal: *Moesziomyces antarcticus* PYCC 5048^T. The strain was plated in YMAgar (yeast extract 3 g/L, malt extract 3 g/L, peptone 5 g/L, D-glucose10 g/L and agar 20 g/L) and incubated for 3 days at 25°C. Cultures were kept at 4°C and renewed every 2 weeks and stored at − 80°C in 20% glycerol to be recovered when is required.

Medium and cultivation conditions

The inoculum was prepared as described in Faria et al., 2014 (3 g/L $NaNO_3$, 0.3 g/L $MgSO_4$, 0.3 g/L KH_2PO_4, 1 g/L yeast extract, 40 g/L glucose) and incubated at 27°C and 250 rpm for 48 hours. The inoculum was prepared in Erlenmeyer flasks with 1/5 working volume of the medium described above. To start the fermentation, 1/10 of the inoculum was transferred in fresh media and incubated for 10 days at 27°C and 250 rpm. The condition uses an initial concentration of 40 g/l of glucose and at day 4, 40 g/L of glucose were added to fermentation broth. At the end of fermentation, the fermentation broth was centrifuged, the supernatant was collected and used in the indicated formulations. All media were sterilized in an autoclave (AJC, Uniclave 88) at 121ºC and 1 bar for 20 minutes.

MELs quantification by GC analysis

Firstly, pure methanol was cooled to 0°C under a nitrogen atmosphere, then 20/1 (v/v) of acetyl chloride was added while stirring over a period of 10 minutes, this created a water free acetyl chloride/methanol solution. To the MELs sample was added 2 mL of acetyl chloride/methanol solution and 100 μL of internal standard (composed by 4% (v/v) of heptanoic acid on 96% (v/v) of n-hexane). Afterwards, for conversion to methyl esters, the samples were incubated at 80°C for 1 hour. It was added 1 mL of hexane and 1 mL of water to the resulting product that was extracted, then the organic phase was collected and 1 μL was injected in a GC system (HP5890, Hewlett-Packard) equipped with an FID detector and a HP-Ultra 2 column. The oven was programmed for 140ºC, and temperature raised to 170ºC at 15ºC/min, to 210ºC at 40ºC/min and 310ºC at 50ºC/min. Nitrogen gas was used at a flow rate of 50 mL/h. MEL was quantified by the amount of C8, C10 and C12 present.

Thin layer chromatography

Thin layer chromatography (TLC) was performed to characterize the MELs present in the formulations and thus observe the different types of biosurfactant present. Aluminium sheet (TLC Silica Gel 60 F_{254}, Sigma-Aldrich) was cut with the dimensions of 8x6 cm with mobile phase consisting of 6.5 mL of chloroform, 1.5 mL of methanol and 0.2 mL of water. By heating the plate after spraying with a solution composed of 1.5 g of naphthol, 6.5 mL of sulphuric acid, 51 mL of ethanol and 4 mL of water, the eluted compounds were revealed.

4.2.2 Development of formulations

The development of formulations was performed under classic methods [2,3]. In order to obtain a good and environmentally friendly formulation several ingredients were tested, such as anionic, non-ionic and amphoteric surfactants and viscosity modifiers (Table 3), using different mixing profiles and temperatures. The formulations were built using a base formulation, they were characterised through various tests, such as pH, viscosity, foam, and surface tension to define the critical micellar concentration. A reference formulation was also created, for both formulations, that uses decyl glucosidase instead of MELs. For both final formulations were used a mixing procedure was started at 40ºC gradually increasing to 60°C and 500 rpm.

Table 3 - Ingredients used in the development of formulations for both detergents and respective functions.

Ingredient	Abbreviature	Function
Sodium coco sulfate	SCS	Anionic surfactant
Sodium dodecyl benzene sulfonate	SDBS	Anionic surfactant
Sodium lauryl ether sulfate	SLES	Anionic surfactant
Mannosylerythritol lipids	MELs	Non-ionic surfactant
Decyl glucoside	DG	Non-ionic surfactant
Cocamidopropyl Betaine	CB	Amphoteric surfactant
Sodium Chloride	-	Viscosity modifier
Xanthan gum	-	Viscosity modifier
Ethanol	-	Solvent
Citric acid	-	pH adjuster
Sodium hydroxide	-	pH adjuster

4.2.3 Characterization of developed formulations

pH

The pH was measured with help of a pH Meter (744, Metrohm). Firstly, the device was calibrated using solutions with a specific pH, namely 4, 7 and 10. Subsequently, the sensor was placed on the sample to measure the pH after it stabilized, and the sensor is cleaned after each measurement.

Viscosity

The viscosity is measured with a viscometer (Brookfield DV-II+ Pro). The measurements were performed at room temperature, using the CPE-52 spindle and 20 rpm. The spindle is placed in the viscometer and subsequently, a 1 mL sample is also placed. The measurement conditions are set, and the measurement is started by starting the engine, the viscosity value is measured, and the viscometer is cleaned after each measurement.

Foaming test

The Ross-Miles method was adapted from the Kruss Benchmark test following the well-established ASTM D 1173-07 Ross-Miles standard for foam analysis [73]. In a measuring cylinder containing 25 mL of the solution in the test, 125 mL of the same solution was dropped from a height of 40 cm (Figure 6). The foam was measured at the instant when all the solution was added and after 5 minutes. Water was used as a negative control and commercial detergent was used as a positive control.

The height of foam produced was calculated by:

$$Net\ foam\ height = Total\ height - Liquid\ height \quad (1)$$

Figure 6 - Setup used in foam test.

Surface Tension

The surface tension was measured in a tensiometer (KRÜSS GmbH). In this method, the platinum ring was immersed in the sample solution until the meniscus is observed. After this, the ring was continuously and slowly pulled upwards until the meniscus broke. At this point, the surface tension value was observed.

The surface tension values decrease with increasing surfactant concentration until a stable value of this parameter is reached, which corresponds to the CMC. The critical micelle concentration (CMC) is defined as the minimum concentration of the surfactant at which micelle formation occurs. The CMC results from the intersection between the regression straight line of the linearly dependent region and the straight line passing through the plateau. The aqueous solutions of the formulations were analysed in the concentrations: 1 mg/L; 0.1 mg/L; 0.01 mg/L; 0.001 mg/L and 0.0001 mg/L. The goal is to obtain a formulation that has a reduced surface tension. As controls were used Mili-Q water and commercial detergents.

Emulsification potential

The emulsifying potential of the final detergent formulations was determined with the use of a kerosene. It's a hydrophobic compound derived from petroleum. The emulsification was measured by mixing equal volumes (4 mL) of kerosene and the final formulations. As negative control was used Mili-Q water, was and as positive control was used commercial detergents. The mixture was vortexed for 2 minutes at 2400 rpm and left to stand for 24, 96 and 168 hours at room temperature (25°C). Emulsifying Index (EI) was calculated using the equation (2).

$$Emulsification\ Index\ (EI) = \frac{Height\ of\ emulsion\ layer}{Height\ of\ liquid\ column} \times 1000 \quad (2)$$

***Galleria mellonella* survival assay**

Galleria mellonella larvae were raised in insectaries at a temperature of 25°C in the darkness, along with a pollen grains diet. For the dishwashing detergent, a solution with the dissolved detergent was placed in the petri dishes at a concentration of 3 mL/L. For the glass cleaner, they were set directly on the plates. In both cases, the plates were left to dry.

This experiment was performed in triplicate and on each plate 10 larvae (90 ± 10 mg) were placed in the dark at 25°C. For one month the larval health score was evaluated. The health score consists in several parameters such as larval activity, cocoon formation, melanisation, and survival (Table 4). The controls utilized were water and commercial detergents.

Table 4 - Health index scoring system of *G. mellonella*.

Category	Description	Score
Activity	No movement	0
	Minimal movement on stimulation	1
	Move when stimulated	2
	Move without stimulation	3
Cocoon formation	No cocoon	0
	Partial cocoon	0.5
	Full cocoon	1
Melanisation	Black larvae	0
	Black spots on brown larvae	1
	≥ 3 spots on beige larvae	2
	< 3 spots on beige larvae	3
	No melanisation	4
Survival	Dead	0
	Alive	2

4.2.4 Performance test

The hand dishwashing detergent performance test was based on SOFW-Journal, 128 Jahrgang, 5-2002 [74]. The soil composition was margarine (17%), olive oil (17%), flour (24%), and water (42%), the fat was melted in the microwave, other ingredients were added after and everything was homogenised. It's frozen for at least 12 hours and defrosted overnight. In each dish were placed 5 g of soil.

The 5 L reservoir was placed 90 centimetres from the washbasin (Figure 7) where the detergent is (reference detergent dosage it is 4 mL) between the centre and the wall of the washbasin. In dishwashing, solution-soiled plates are washed by hand, with the help of a brush, until the foam layer collapses. When washing, 20 movements are performed on the front of the dish and six on the back, the dish and hands must be completely immersed in the dishwashing soak. When the foam layer permanently breaks down on the surface of the wash bath, the wash bath is considered exhausted, and the endpoint has been reached. The number of dishes washed was accounted for. The test was performed with hot water (37ºC) and cold water (21ºC). Between each test, the washbasin and brush were cleaned.

Figure 7 - Set up for hand dishwashing performance test

Regarding, the glass cleaner performance test was based on SOFW-Journal, 148, 4-22 [75]. In this test, glass tiles (15x10x0.1cm) were used with three different soils: soap, cream gel, and hair tonic (40%), flour (20%) and water (40%). Tiles with soil were dried at room temperature for two hours.

To carry out the cleaning, microfibre cloths were used, on which 5 mL of detergent was placed, on each tile 15 strokes are performed (1 stroke = movement back and forth). The cleanliness of the tiles was assessed visually. Between tests, the tiles are cleaned with ethanol. The performance tests were carried out three times for each detergent and condition.

4.2.5 Accelerated stability

The accelerated stability study ensures that the product maintains its physical appearance, chemical, aesthetic properties, functionality, and microbiological characteristics when stored under normal conditions. It also aims to provide data to predict the stability of the product over its shelf life and its compatibility with packaging. This method assesses the detergent parameters that are most susceptible to change during the shelf life of the product, and that may influence characteristics related to quality, safety, and performance. This study includes organoleptic characteristics (appearance, color, odor) and physicochemical characteristics (pH, viscosity).

In an accelerated stability study, the detergents samples were stored in a refrigerator and in climatic chambers with 24h control of temperature and humidity. The test conditions were 4ºC; 25ºC / 60% relative humidity (RH) and 40ºC / 75% RH, this study lasts for three months. At the beginning of the study, a measurement is carried out and at the end of each month. For the manual dishwashing detergent, the parameters analysed were organoleptic (colour, aspect, odour), pH, viscosity and, lastly, microbiologic control for diverse bacteria's, yeasts, and moulds, only in the beginning and in the third month. The glass cleaner parameters were the organoleptic (colour, aspect, odour) and pH. The acceptance limit was stipulated to within ± 20% variation from the initial test value [76].

5. Results and Discussion

5.1 MELs production and characterization

The MELs used in the formulations was obtained by fermentations realized in reactors with D-glucose (40 g/L) and feeds of 20 g/L of waste frying oil at days 0, 4 and 7, where occurred the formation of beads which uses an in-situ harvesting process of MEL-rich beads from fermentation broth, in the context of the thesis "Reengineering production of Mannosylerythritol lipids (MELs): a holistic approach" realized by Petar Keković in September 2022 (Confidential work).

To characterize the MELs present in the formulations two analyses were performed: a gas chromatography (GC) and thin layer chromatography (TLC). Through GC it is possible to realize that the sample used contains 65% MELs and 35% fatty acids; C_8, C_{10}, C_{12} corresponds a 5%, 33%, 7%, respectively. It is possible to obtain purer MELs, but as detergents are low value applications, it is not necessary in this case. TLC was performed to understand what types of MELs are used in the formulations, it is mostly made up of MEL-A and also MEL-B (Figure 8).

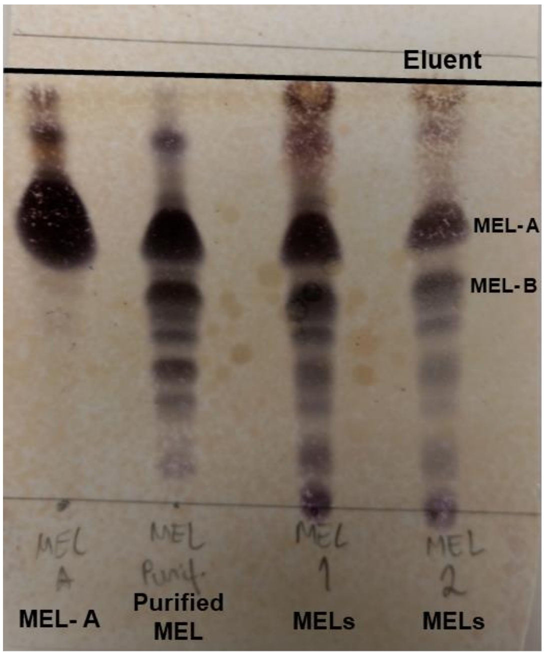

Figure 8 - TLC picture that permits the comparison between MELs used in formulations, MEL-A, and purified MELs.

In order to optimise the detergent production process and to lower the detergent costs were tested the hypothesis of producing detergents directly with the supernatant where the MELs is, having only removed the cells by centrifugation. The MELs used in the supernatant, originating from fermentations realized with *M. antarcticus*, with an initial concentration of 40 g/L of glucose and at day 4, 40 g/L of glucose were added to fermentation broth. The supernatant was 3.24 g/L of MELs, which is a low concentration, but it was expected that this value would be lower compared to the previous MELs used since this culture medium does not contain oils and these are favourable for MELs production.

5.2 Development of formulations

Currently there is a great concern with the environment, so there has been a superior demand for greener and more sustainable solutions. Biosurfactants are one of the possible solutions to be used in several products to reduce environmental impacts of such products. MELs are an example of these molecules and possess several interesting characteristics such low CMC, emulsification, and biodegradability to be used in several applications, namely household cleaning products [46].

One of the main objectives of this work was to develop and test new ecological formulations that include MELs, namely hand dishwashing and glass cleaner formulations. To achieve this objective, several surfactants were tested towards the development of a formulation that can accomplish both environmental and technical performance. Both anionic (SCS, SDBS, SLES) and non-ionic (DG and MELs) surfactants were here considered. A formulation can benefit from overall advantages and benefits, such mildness, wetting, foam volume, and foam stability, when anionic and non-ionic surfactant mixtures are used [77]. These surfactants were chosen due to their low environmental impact. SLES offers excellent cleaning and environmental qualities, is made from plant oils like coconut oil, biodegrade swiftly. SCS helps to generate foam and has a good cleaning action, it comes from a mixture of all the fatty acids contained in coconut oil. SDBS is a cleaning agent used in bath products, cleansing products, shampoos, and hair conditioners. It is used to remove dirt and deposits. DG is a vegetable-based surfactant derived from coconut and biodegradable, and it was used in formulations as a non-ionic surfactant.

Several formulations were developed to figure out which one was most suitable in terms of homogeneity, viscosity, pH, foaming ability. Figure 9 shows the formulations that were tested for the hand dishwashing detergent formulation.

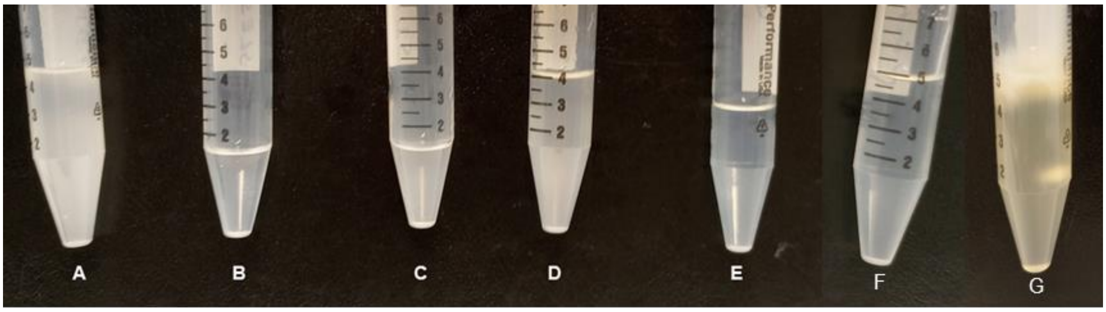

Figure 9 - Test formulations for hand dishwashing detergents. A) SDBS+DG; B) SLES+DG; C) SDBS+MELs; D) SLES+MELs; E) SCS+MELs; F) SLES+MELs+CB; G) SCS+Supernatant.

In the following tables 5 to 11 it is possible to observe the composition of each formulation tested which are represented in figure 9.

Table 5 - Composition of SDBS+DG formulation.

This formulation became very turbid, and the foam is not stable. The pH is very alkaline due to the presence of sodium hydroxide.

SDBS+DG	
Composition (% of active matter)	**Concentration (% w/w)**
SDBS	8
DG (50%)	2
Xanthan Gum	3
Sodium Hydroxide (50%)	1
Water	to 100

Table 6 - Composition of SLES+DG formulation.

This formulation showed good results, so it is used as a control to see the differences between presence and absence of MELs.

SLES + DG	
Composition (% of active matter)	**Concentration (% w/w)**
SLES (30%)	28
DG (50%)	2
Sodium Chloride	3
Water	to 100

Table 7 - Composition of SDBS + MELs formulation.

This formulation turned a bit turbid.

SDBS + MELs	
Composition (% of active matter)	**Concentration (% w/w)**
SDBS	8
MELs	1
Xanthan Gum	3
Water	to 100

Table 8 - Composition of SLES + MELs formulation.

It has a good appearance and with stable foam.

SLES + MELs	
Composition (% of active matter)	**Concentration (% w/w)**
SLES (30%)	28
MELs	1
Sodium Chloride	3
Water	to 100

Table 9 - Composition of SCS+MELs formulation.

It has a good appearance, but the foam stability could be improved.

SCS + MELs	
Composition (% of active matter)	**Concentration (% w/w)**
SCS	8
MELs (65%)	1
Sodium Chloride	3
Water	to 100

Table 10 - Composition of SLES+MELs+CB.

This formulation has a slightly high viscosity and stable foam.

SLES + MELs + CB	
Composition (% of active matter)	**Concentration (% w/w)**
SLES (30%)	8
MELs (65%)	1
CB	1
Sodium Chloride	3
Water	to 100

Table 11 - Composition of SCS + Supernatant formulation.

This formula is more yellowish and does not have stable foam.

SCS + Supernatant	
Composition	**Concentration (% w/w)**
SCS	8
CB	1
Sodium Chloride	3
Supernatant	88

In addition to the different surfactants, the presence or absence of sodium hydroxide was also studied. This question arose because it is a component of the base formulation and helps in fat removal. However, it was removed because in all formulations that contain MELs, formed a tiny foam layer, and the foam was not stable.

The viscosity modifier was also evaluated, sodium chloride is the most used in common detergents and xanthan gum is more used in greener formulations. The xanthan gum made the formulations turbid, so sodium chloride was used, it gave the formulations the desired viscosity without compromising their appearance. The appearance is one of the most important characteristics that is a relevant factor, because it determines whether the detergent is bought or not. Besides, it was also tested the presence or absence of CB, this amphoteric surfactant can decrease skin irritation and positively influence foam stability.

In order to optimise the detergent production process the hypothesis of using directly the supernatant where the MELs are, was tested. In figure 9 it’s possible to observe the formulations, the formulation G is more yellowish, however the dishwashing detergent formulation has a white mass, probably protein precipitation occurred, that should not be present.

For this formulation work, a supernatant with a higher concentration should be used and this is a hypothesis that needs further testing to verify that it is reliable and does not affect the performance of the detergent.

The parameters evaluated to determine the final formulation were pH, viscosity, foam, and CMC, and the results are presented in the table 12. Regarding pH very diverse values were observed, from acid to alkaline formulations. More alkaline detergents are considered good candidates that allow the removing of fats, grease, and oils, but detergents with a pH above 11 are very irritating to the skin, being the most common pH for this type of detergent between 7-9. The optimal pH to avoid irritating the skin is between 4.7 and 5.75 [77].

Viscosity is a rheological property and is one of the main characteristics of hand dishwashing detergents. Viscosity levels allow the consumer to regulate the products dosage, as well as the feeling on how much is the product concentrate. However, it should be mentioned that extremely high viscosity may result in a slow rate of dissolution in water and, as a result, poor utilization of the dose of the product during dishwashing. An fairly acceptable viscosity is usually between 400-600 cP [78]. The differences in viscosity were observed in the test formulations were due to small changes in the addition of sodium chloride.

Detergent foam is a mass of gas separated by thin films of liquid generated on the surface of the liquid [77]. The foam is not directly related with cleaning performance; however, the consumer believes if the foam is poor, the cleaning performance will be low [77]. The adaptation of Miles-Ross method was used to characterize the capacity of test formulation produce foam and its stability. Through the results obtained it is possible to observed that the formulations developed have a lower foaming power and lower stability than benchmark and even ecolabel detergent. The formulations that were developed that demonstrated greater foaming capacity and foaming stability were SLES+DG, SLES+MELs and SLES+MELs+CB, as shown in table 12. In this regard it is confirmed that SLES is an anionic surfactant with excellent foaming capacity and MELs do not enhance both foam formation and its stability in a formulation.

Table 12 - Characterization of test formulations for hand dishwashing detergent.

Hand dishwashing detergent					
Formulations	**pH**	**Viscosity (cP)**	**Foam (cm)**	**Minimum surface tension measured (mN/m)**	**CMC (mg/L)**
Benchmark	8.5	473.9	t0= 6.3 t5= 6.3	23.3	0.120
Ecolabel	4.2	403.4	t0=5.9 t5=5.9	28.5	0.190
SDBS + DG	12.7	333.7	t0= 4.0 t5= 3.0	33.2	0.190
SLES + DG	8.5	508.4	t0= 5.6 t5= 5.6	41.3	0.370
SDBS + MELs	6.8	522.8	t0= 4.2 t5= 3.9	46.3	0.300
SLES + MELs	5.5	237.6	t0= 5.1 t5= 4.7	37.6	0.180
SCS + MELs	7.6	436.8	t0= 5.5 t5= 3.3	38.3	0.380
SLES + MELs + CB	5.0	785.3	t0=5.1 t5=5.0	34.9	0.380
SCS + Supernatant	7.35	521.7	t0= 5.4 t5= 5.1	39.3	0.210

The surfactant concentration in an aqueous solution at which it is started to form micelles structures is referred as the CMC. Surface tension reduces with increasing surfactant concentration as the amount of surfactants increases at the interface below the CMC. Contrarily, the solution's surface tension remains constant above the CMC because the interfacial surfactant concentration no longer changes [79]. The goal is to obtain a detergent that has a reduced surface tension, in appendix B are presented the graphs used to obtain the CMC value. The detergent with the lowest surface tension and CMC is the benchmark, so it is the detergent with the highest amount of surfactants. The surface tension of the detergents is a little high compared to the benchmark and the ecolabel detergent, so there should have a larger amount of surfactants to reduce the surface tension.

MELs are glycolipid molecules reported as having a low CMC. In this regard, different MELs concentration (1 g/L, 5 g/L, and 10 g/L) were tested with the surfactants SLES (Figure 10) and SCS (Figure 11).

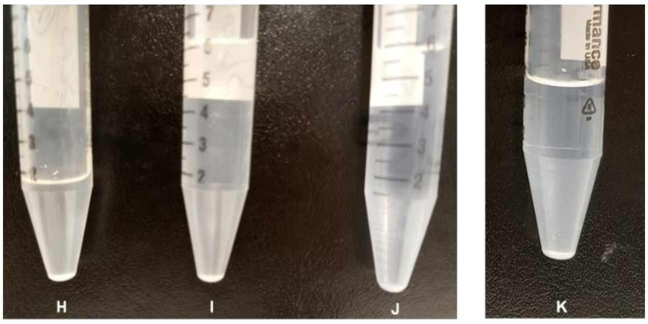

Figure 10 - Test formulation for SLES and different MELs concentrations.

H) SLES+MELs (10 g/L); I) SLES+MELs (5 g/L); J) SLES+MELs (1 g/L); K) SLES+CB+MELs (5 g/L)

The SCS+MELs formulations (5g/L and 1 g/L) are not stable as they formed two phases a clear liquid and a more viscous off-white phase (green circle), so they were not tested.

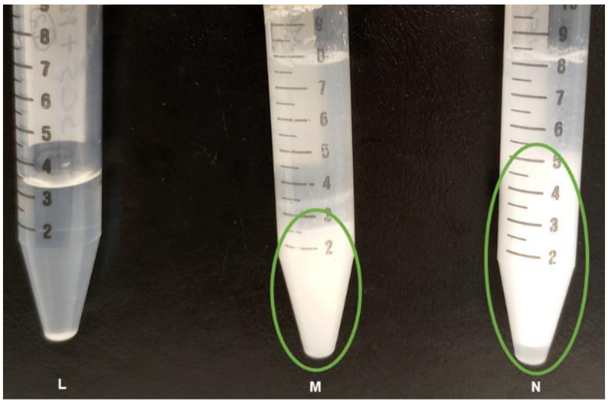

Figure 11 - Test formulation for SCS and different MELs concentrations

L) SCS+MELs (10 g/L); M) SCS+MELs (5 g/L); N) SCS+MELs (1 g/L)

In table 13 it is possible to observe the different characterizations regarding foam, surface tension and CMC, for the formulations with different MELs concentrations. Considering these results, the formulation that best corresponded to the intended purpose was SLES+MELs+CB, with a concentration of 5 g/L of MELs, which present a good stable foaming ability and low surface tension.

Table 13 - Characterization of test formulations for different MELs concentrations for hand dishwashing.

Formulations	Foam (cm)	Minimum surface tension measured (mN/m)	CMC (mg/L)
SLES + MELs (10 g/L)	t0= 5.1 t5= 4.7	37.6	0.180
SLES + MELs (5 g/L)	t0= 5.1 t5= 5.1	36.4	0.270
SLES + MELs (1 g/L)	t0= 5.5 t5= 5.5	42.1	0.380
SLES+CB+MELs (5 g/L)	t0=5.2 t5=5.2	35.3	0.220
SCS + MELs (10 g/L)	t0= 5.5 t5= 3.3	38.3	0.380

Also, several glass cleaner formulations were developed, are presented in figure 12. The glass cleaner formulations were also evaluated with various parameters, namely pH, foam, and CMC.

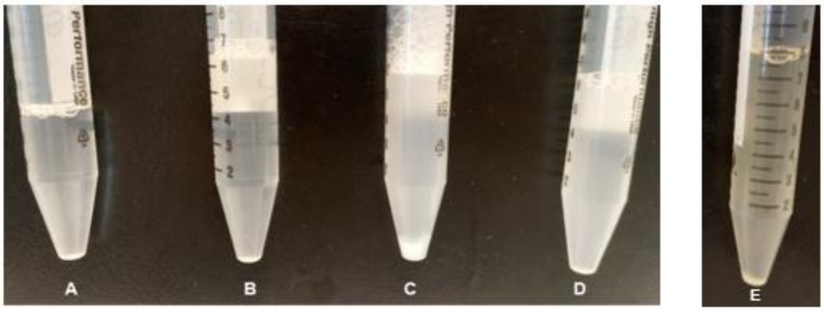

Figure 12 - Test formulations for glass cleaner.

A) SCS+DG; B) SCS+MELs; C) SDBS+MELs; D) SLES+MELs; E) SDBS+Supernatant

Tables 14 to 18 present the composition of each glass cleaner test formulation.

Table 14 - Composition of SCS+DG glass cleaner formulation.

Composition	Concentration (% w/w)
SCS	2
DG	4
Ethanol 96%	8
Water	to 100

Table 15 - Composition of SCS+MELs glass cleaner formulation.

Composition	Concentration (% w/w)
SCS	2
MELs	1
Ethanol 96%	8
Water	to 100

Table 16 - Composition of SDBS+MELs glass cleaner formulation.

Composition	Concentration (% w/w)
SDBS	2
MELs	1
Ethanol 96%	8
Water	to 100

Table 17 - Composition of SLES+MELs glass cleaner formulation.

Composition	Concentration (% w/w)
SLES	2
MELs	1
Ethanol 96%	8
Water	to 100

Table 18 - Composition of SDBS+Supernatant glass cleaner formulation.

Composition	Concentration (% w/w)
SDBS	2
Supernatant	90
Ethanol 96%	8

Table 19 shows the results for the characterization of the glass cleaner formulations, with regard to pH there is a wide range of values, the most common values are between 6 and 8 so that there is less likelihood of leaving residue, however there are also formulations with pH between 3-5, but these are less frequent [80].

Table 19 - Characterization of test formulations for glass cleaner.

Glass cleaner				
Formulations	**pH**	**Foam (cm)**	**Minimum surface tension measured (mN/m)**	**CMC (mg/L)**
Benchmark	3.68	t0= 1.0 t5=0.4	59.8	41.00
Ecolabel	9.73	Foam falls apart	61.3	43.00
SCS + DG	10.88	t0= 3.6 t5= 2.9	35.2	2.20
SCS + MELs	7.30	t0= 2.9 t5= 1.1	34.6	0.23
SDBS + MELs	7.02	t0= 2.8 t5= 2.1	37.4	0.30
SLES + MELs	4.95	t0= 2.5 t5= 2.7	33.8	0.28
SDBS+ Supernatant	6.28	t0= 3.6 t5= 2.5	37.7	2.50

Both the benchmark and the detergent with ecolabel certification have high surface tension and CMC values, the values for the formulations tested are much lower. This difference should not affect the performance as the soiling expected for a glass cleaner is relatively easy to clean. Different concentrations of MELs were also tested to understand which one was most suitable, the concentrations tested were 10 g/L, 8 g/L and 2 g/L (Figure 13).

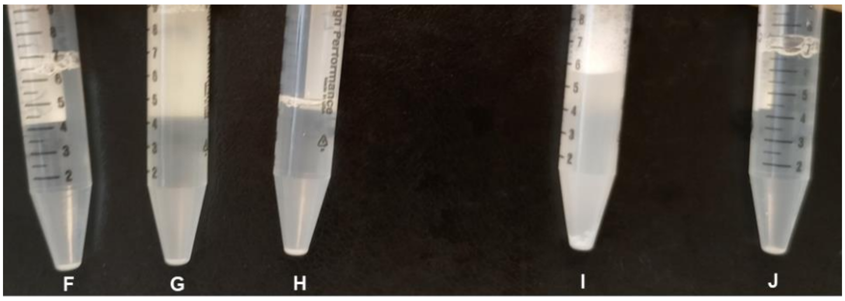

Figure 13 - Test formulation for SCS e SDBS and different MELs concentrations

F) SCS+MELs (10 g/L); G) SCS+MELs (8 g/L); H) SCS+MELs (2 g/L); I) SDBS+MELs (10 g/L); J) SDBS+MELs (2 g/L)

In table 20 it is possible to observe the different characterizations regarding surface tension and CMC, for the formulations with different MELs concentrations. The surface tension of all formulations is quite similar. For the formulation of the glass cleaner, the surface tension is not a decisive factor since dirt on these surfaces is easy to clean. Therefore, surface tension values are usually higher. The formulation with the highest CMC 4.10 mg/L, was the selected formulation corresponding to SDBS+MELs (2 g/L).

Table 20 - Characterization of test formulations for different MELs concentrations for glass cleaner.

Formulations	**Minimum surface tension measured (mN/m)**	**CMC (mg/L)**
SCS+MELs (10 g/L)	34.6	0.23
SCS+MELs (8 g/L)	30.6	1.80
SCS+MELs (2 g/L)	32.9	2.10
SDBS+MELs (10 g/L)	37.4	0.30
SDBS+MELs (2 g/L)	34.0	4.10

5.3 Characterization of final formulations

Through the studies presented above it was possible to develop an environmentally compatible formulations, having in its composition a biosurfactant, MELs. In Figure 14 it is possible to observe a final formulation of the manual dishwashing detergent produced and table 21 shows its composition.

Figure 14 - Final formulation developed of hand dishwashing detergent.

The formulation was homogeneous, viscous, light yellow and has a characteristic odour, this formulation has a pH of 5.99, viscosity of 688.4 cP. This pH is not irritating to the skin; however, it can slightly decrease the cleaning ability of the detergent. The viscosity of this detergent is higher than the viscosity of existing detergents on the market, but still has an acceptable value for easy dosing.

Table 21 - Composition of final formulation of hand dishwashing detergent.

Composition (% of active matter)	Concentration (% w/w)
SLES (30%)	28
MELs	0.5
CB	1
Sodium Chloride	3
Water	to 100
Sodium Benzoate	0.01

The other detergent developed was glass cleaner, the final formulation is a homogeneous, light-yellow mixture with a characteristic smell (Figure 15), in table 22 have the composition.

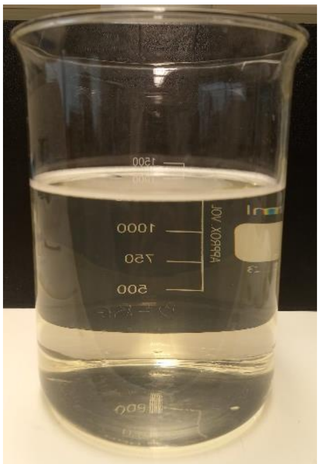

Figure 15 - Final formulation developed of glass cleaner.

The pH is 8.11, which is suitable because this value reduces the possibility of residue formation on the glass.

Table 22 - Final formulation of glass cleaner.

Composition	Concentration (% w/w)
SDBS	2
MELs	0.2
Ethanol 96%	8
Water	to 100
Sodium Benzoate	0.01

The foam test determines the amount of foam formed and whether the foam is stable. This test is most suitable for dishwashing detergents because it is one of the important characteristics for the client. In addition, it is also interesting to understand the difference between this detergent and the glass cleaner. Regarding, the foam test of hand dishwashing detergent (Figure 16) the foam height has been maintained after 5 minutes, so the foam of the detergents is stable. But less foam was formed than in the benchmark (1.05 cm) and ecolabel certified detergent (0.8 cm). This result may be indicative of cleaning efficiency.

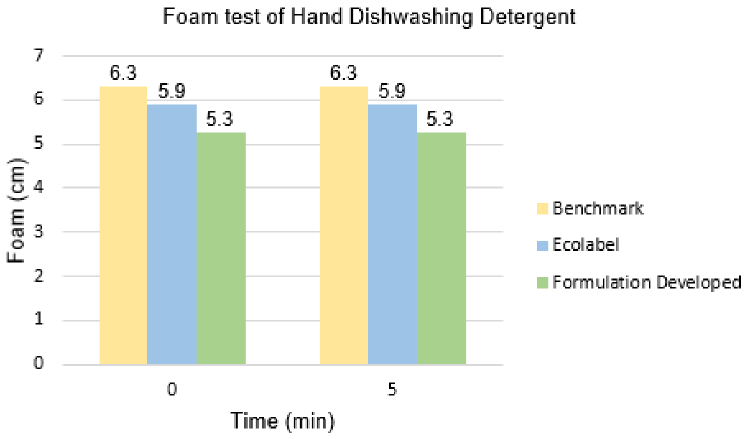

Figure 16 - Foam test of hand dishwashing detergent final formulation comparing with benchmark and ecolabel detergent.

The foam test for glass cleaner formulation (Figure 17) demonstrates the developed glass cleaner formed much more foam (3.1 cm) and more stable compared to the benchmark (1cm), to decrease this discrepancy the SDBS concentration used should be lower (only 1%).

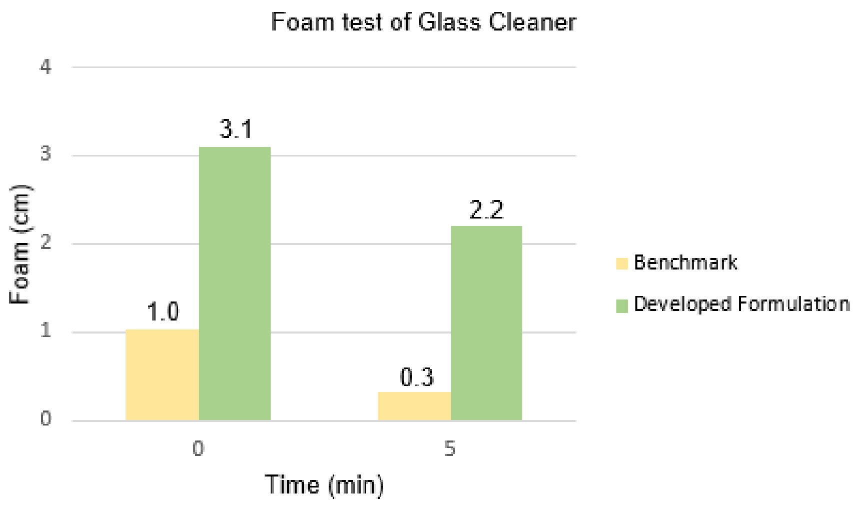

Figure 17 - Foam test of glass cleaner final formulation comparing with benchmark and ecolabel detergent. The foam formed by the ecolabel detergent dissolves quickly.

Considering the emulsification index, the most frequently analysed parameter is EI24, representing the emulsion in liquid that remains after 24h. Longer experimental times, 96 and 168 h, were evaluated to understand the performance of the formulations over a longer time. This test is useful to understand the interaction between dirt and detergent. Water was used as a negative control, and no emulsion formed. For hand dishwashing detergent (Figure 18), the benchmark showed the highest emulsification index after 24h (64.1%). However, the developed formulation (47.9%) showed an emulsification index very similar to the ecolabel detergent (48.4%). Over time the benchmark detergent showed a reduction in the emulsification index, however, the developed detergent continues to show an emulsification index similar to the ecolabel detergent, which proves the stability of our formulation.

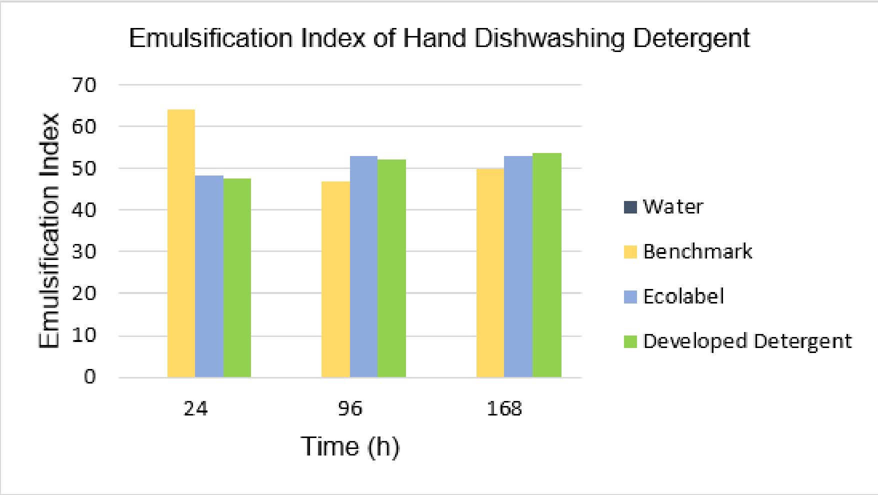

Figure 18 - Emulsification index of hand dishwashing detergent: benchmark, ecolabel and developed formulation for 24h, 96h e 168h. Water as used was control.

The emulsification index for the three glass cleaners is similar (Figure 19). For the ecolabel certified detergent, it is higher in EI24 (53%), however for EI 96 and EI168 the emulsification value is higher for the developed detergent. The benchmark presents the lowest emulsification index. All emulsions can be considered stable.

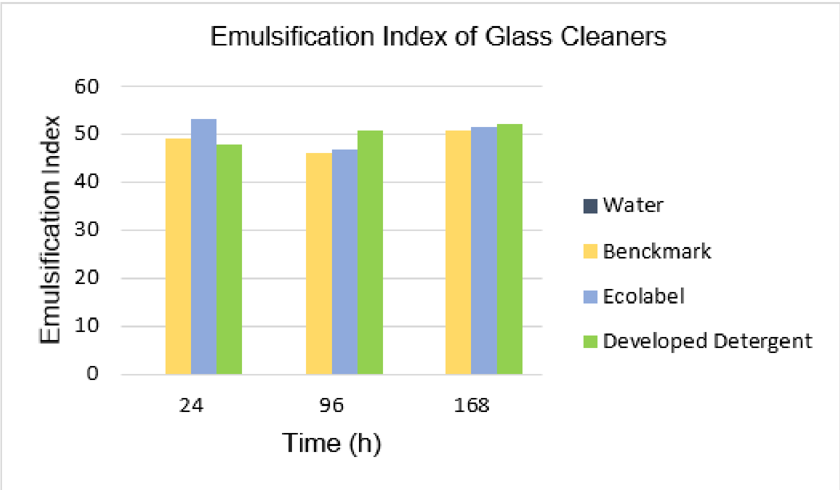

Figure 19 - Emulsification index of glass cleaner: benchmark, ecolabel and developed formulation for 24h, 96h e 168h. Water as used was control.

To understand what the impact of the developed detergents in ecotoxicological terms, a *in vivo* survival assay using *G. mellonella* was made, this preliminary study was important to comprehend their impact on the environment during use and disposal phases. The assays started with *G. mellonela* in larval stage and conducted until the end of the life cycle, for approximately one month.

The results obtained in this assay were not as expected, because the larvae started cocoon formation early, on day 8 they already had their full cocoon, which was expected happen only after day 20.

Because of the early cocooning, the butterflies that hatched were small, some didn't hatch. It's not possible to confirm that the death of the larvae was caused by the detergents, since even in water exist deaths (negative control). In Figure 20 are demonstrate the results obtain for the hand dishwashing detergents. The detergent that showed the lowest survival rate was the detergent developed in this work (90%), the detergent SLES+DG showed the highest survival rate (97%).

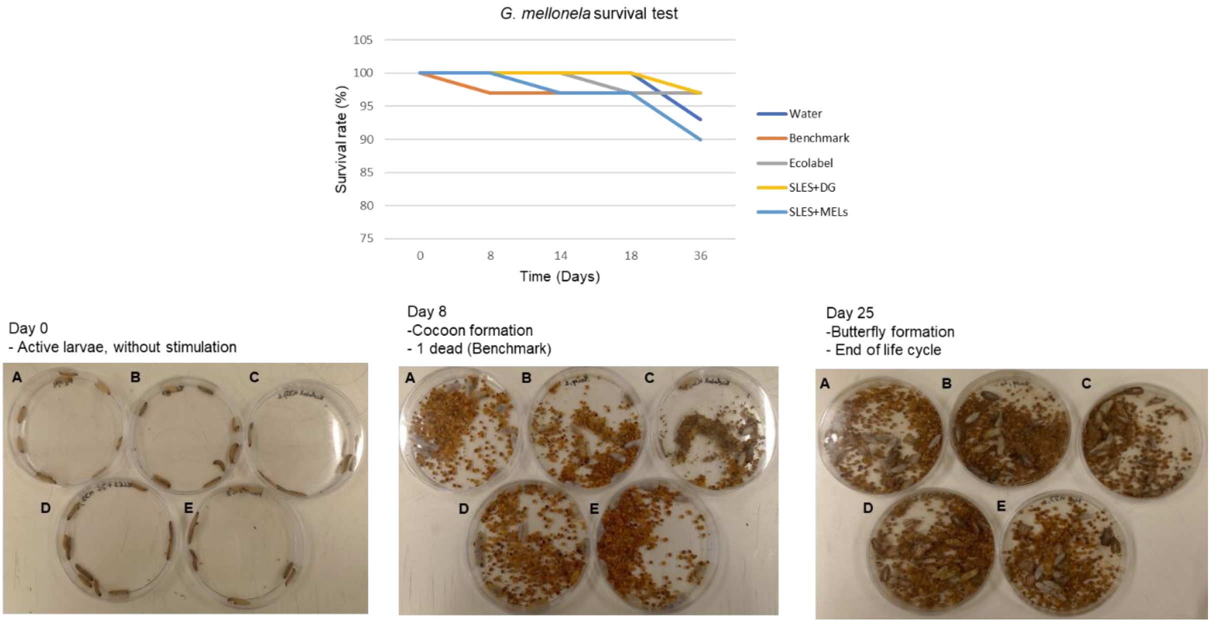

Figure 20 - Assessment of hand dishwashing detergents on *G. mellonela* survival test.

However, in all other conditions there were deaths and due to the abnormal duration of life cycle stages, the assay is considered inconclusive.

For the glass cleaner the result of the survival assay was presented in Figure 21. The precocious cocoon formation and the hatch of small butterflies also occurred as described for the hand dishwashing detergent assay. In general, survival rates were lower in this assay, which is expected due to the presence of solvents in some formulations. The two detergents with the lowest survival rate were SDBS+MELs (80%) and SDBS+DG (83%), with survival rates of 80% and 83%, respectively. This result may be due to the presence of SDBS, this detergent can be slightly toxic, however it is easily biodegradable. The ecolabel certified detergent has the highest survival rate (97%), the negative control, water had a survival rate of 93%. With these results it is not possible to conclude the toxicological impact of the detergents tested, this test should be repeated. In this case, also exist deaths in all conditions and abnormal duration of life cycle stages, this assay was also considered inconclusive. These inconclusive results may have occurred due to the stress the larvae were subjected to on day 0 with the preparation of the assays due to changes in location and being exposed to light for a long time.

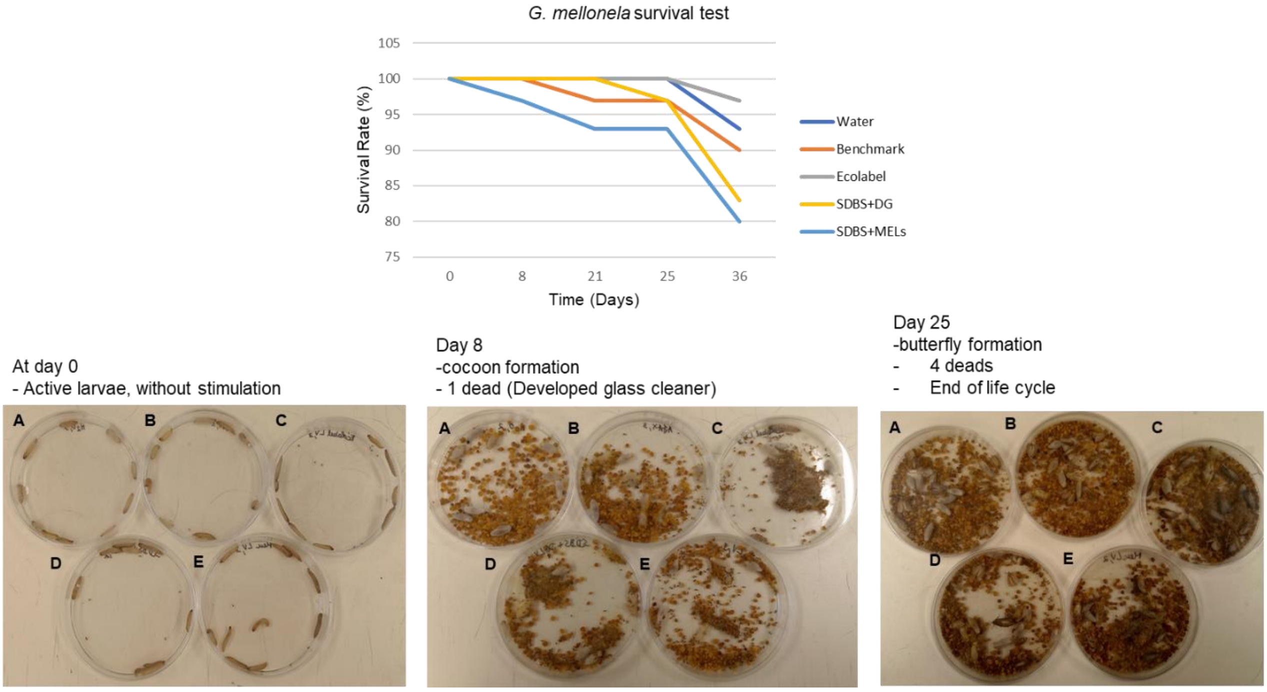

Figure 21 - Assessment of glass cleaners on *G. mellonela* survival test.

5.4 Performance

Cleaning products are indispensable in our daily life. Their capacity to clean dishes, clothes, and other items is critical to enhancing cleanliness and health. The developed detergents efficiency was evaluated, by assessing their performance during the cleaning process, which is one of the most crucial qualities. The effectiveness of the product under test was evaluated based on its capacity to remove soil and keep a clean surface, compared to a reference product (in this case a benchmark product and a detergent with Ecolabel certification) [74].

Regarding, the performance test of hand dishwashing detergent, the first step was determining the dosage of developed detergent that should be used, several quantities were used in 5 litres of hot water, first 5 mL of detergent, then 7 mL and finally 10 mL, the results obtained were presented in table 23.

Considering the results, the determined dosage was 10 mL of detergent per 5 L of water, this dosage was used in the following experiments. Through the dosage of the product, it is already possible to observe that the formulation needs some improvements because the established dosage is more than double the dosage established for the reference detergents. This problem did not affect the following tests as the defined dosage for each detergent was used.

Table 23 - Developed detergent dosage determination, at 37°C.

Developed Detergent	
Water Temperature= 37°C	
Dosage in 5 L (mL)	**Clean plates**
5 mL	6
7 mL	7
10 mL	10

After that, was proceeded to evaluate the performance of the developed detergent by comparing the performance with the benchmark and with an ecolabel certified detergent, at 37°C, the result obtained was presented in table 24.

Table 24 - Performance tests of hand dishwashing detergent, at 37°C.

	Benchmark	Ecolabel Certification	Developed Detergent
Water Temperature	37°C		
Dosage in 5 L (mL)	4	4	10
Clean Plates	10	10	10

The results showed no variation in the number of clean dishes, which is a positive result, however, as already mentioned, the detergent developed requires a higher dosage and is therefore less effective.

The ability to clean in cold water, namely at 22°C, was also evaluated. The life cycle of hand dishwashing detergents has the biggest environmental impact in the use phase, which contributes 86-98% for most categories due to the energy required to heat the water [81]. The results were shown in table 25, the performance of the developed detergent and the ecolabel certified detergent decreases slightly, it is normal because grease removal is less effective at lower temperatures, however the benchmark was able to maintain its performance.

The final appearance of the dishes in all tests was extremely similar for all detergents. Through this test, was possible realize that the detergent developed needs some improvements to be able to simultaneously increase performance and reduce the dosage used, in this case the concentration of anionic surfactants should be higher, as this type of surfactant is the main responsible for foam formation. The two main options would be to increase the concentration of SLES, as this surfactant is somewhat irritating, it might not be the best solution. The other option would be to add another anionic surfactant such as SCS or Sodium Cocoyl Isethionate, this surfactant is obtained from the fatty acids presents in coconut oil and has good foaming power. With the addition of another surfactant would create a synergistic effect with the SLES in order to improve the performance of the detergent.

Table 25 - Performance tests of hand dishwashing detergent, at 22°C.

	Benchmark	Ecolabel Certification	Developed Detergent
Water Temperature	22°C		
Dosage in 5 L (mL)	4	4	10
Clean Plates	11	8	9

Glass cleaners were created specifically to remove mild soiling from smooth, washable surfaces like glass [75]. The cleaning performance of the developed glass cleaner was tested using three different types of soils, soap, cream gel, and, lastly, hair tonic (40%), flour (20%) and water (40%). The performance was compared with the benchmark and ecolabel certified glass cleaner. The results for all detergents were very satisfactory, with no significant differences between the various detergents in the different soils tested. Figure 22 shows the glass tiles before the start and at the end of the soil soap test. The hair tonic (40%), flour (20%) and water (40%) soil required more effort during cleaning due to the presence of the tonic, since it was oily, however all three detergents performed well. Appendix D presents the results for the remaining soils.

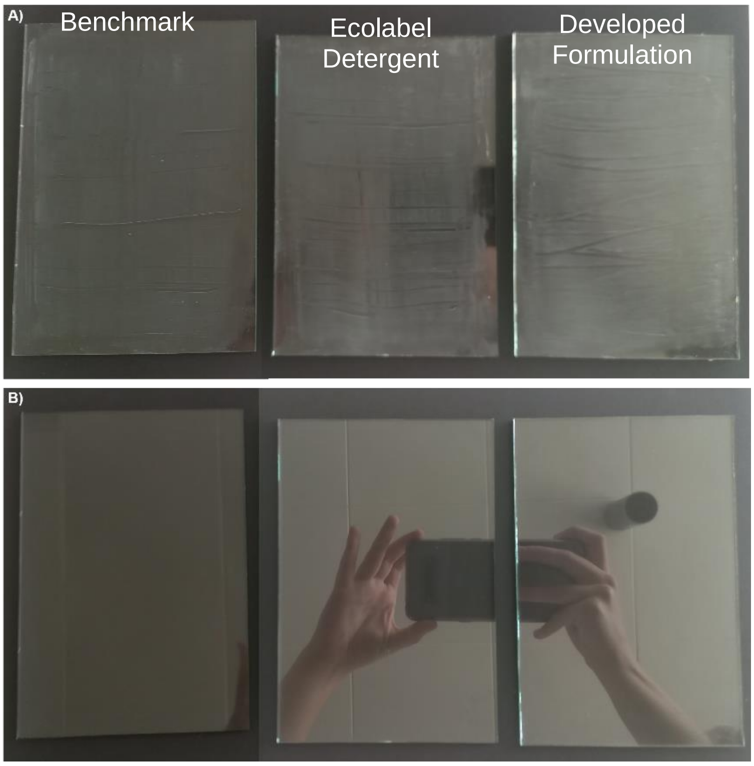

Figure 22 - Performance test of glass cleaner, using soap as soil. Each glass tile corresponds to the benchmark, ecolabel certified detergent and the developed detergent, respectively. A) Appearance of the tiles at the beginning of the test, B) Appearance of the tiles at the end of the test.

Therefore, the developed detergent proved to have adequate performance and consequently a suitable formulation for its function, however, to reduce a slight foam that forms during cleaning, the SDBS concentration would decrease to 1%. Nevertheless, this foam does not stain the glass and disappears during the cleaning process.

5.5 Accelerated stability study

The accelerated stability study was used to observe and predict the changes that may occur in products over time and predict the shelf life. This study increases the rate of chemical degradation and physical change of the detergents by using exaggerated storage conditions.

Regarding the organoleptic parameters, both detergents had a homogeneous liquid appearance, with the dishwashing detergent obviously more viscous, a light-yellow colour and, finally, a characteristic smell. All these parameters remained constant over the three months of the study. In relation to the other parameters analysed, it was possible to observe some variations over time. Figures 23 and 24 show the results of the measurements taken.

In relation to the dishwashing detergent, the pH variations were minimal in all conditions throughout the study, however the sample with the highest variation was 40ºC/ 75% RH, which was expected as it is the sample that is under the most stress. Regarding viscosity, there was a high variation in the first month, this variation happened due to the short waiting time at room temperature before the measurements (Figure 23). In the following months this time increased, so in the following measurements the variations were not significant. Regarding the microbiological analysis, there was no growth of any microorganism both at the beginning and at the end of the study, namely various bacteria, moulds and yeasts, which revealed the effectiveness of the preservative, sodium benzoate, and the application of a correct dosage.

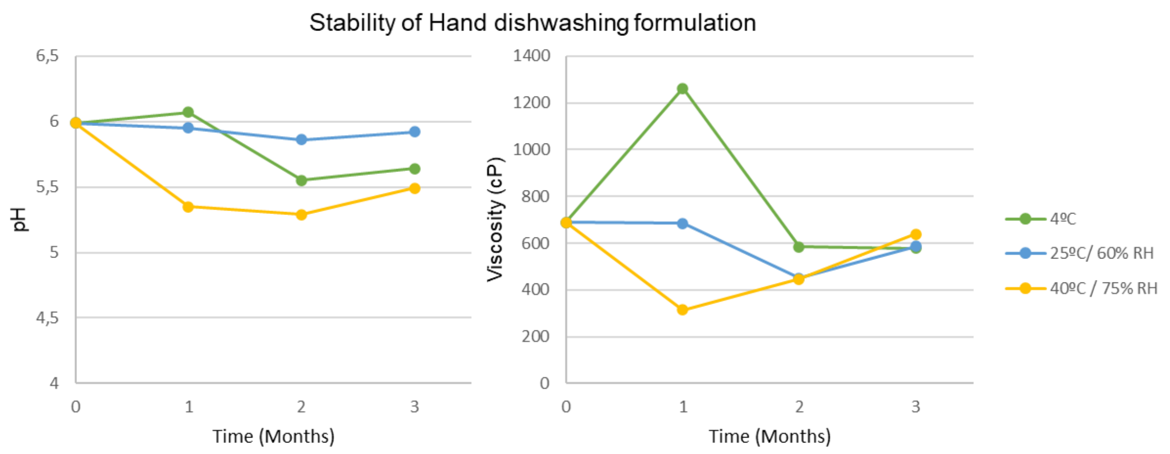

Figure 23 - Stability of hand dishwashing detergent over 3 months for pH and viscosity.

Regarding the glass cleaner, the pH variations were also not significant, the samples at 40ºC/75%RH also having the greatest variation, this condition represents the accelerated storage condition (Figure 24).

Figure 24 - Stability of glass cleaner for pH.

The variations between the first and the last measurement should not exceed 20%, the variations obtained are described in table 26 and 27.

Table 26 - Variation of stability of hand dishwashing detergent (pH and viscosity).

	4ºC		25ºC/60%RH		40ºC/75%RH	
	pH	Viscosity	pH	Viscosity	pH	Viscosity
Variation of initial value (%)	5.84	16.12	1.17	14.82	8.35	7.19
Acceptance criteria <20%	OK	OK	OK	OK	OK	OK

Table 27 - Variation of stability of glass cleaner (pH).

	4ºC	25ºC/60%RH	40ºC/75%RH
	pH	pH	pH
Variation of initial value (%)	0.61	2.84	8.51
Acceptance criteria <20%	OK	OK	OK

At the end of the 3 months, all organoleptic and physic-chemical parameters were in accordance with the specifications and in accordance with the acceptance criteria, for the samples tested under the three different conditions. There was some variation in the pH and viscosity results at the various checkpoints, however, the results are within the acceptance criteria.

Concluding, the hand dishwashing detergent and glass cleaner maintained its characteristics throughout the study and demonstrated satisfactory stability over a period of 3 months under accelerated storage conditions.

5.6 Production cost

Liquid detergents contain mostly water, the other part is a combination of surfactants, builders, hydro-tropes, colorants, perfume, and preservatives, among others. The selection of ingredients must be careful to obtain a stable product. The production of liquid detergents can use batch or continuous equipment. Both the liquid and the solid raw materials are accurately weighed and added to a mixer, where they are properly blended. Stabilizers can be introduced at any point in the process to guarantee the consistency and stability of the final product. The main machinery used are static or in-line mixers. High energy mixing techniques are used to create liquid detergents with high concentrations [11].

For the formulations developed to be produced, raw materials, energy, labour, and mixing equipment are needed. This is followed by packaging and labelling. To have an idea of the manufacturing price of the products developed, a simulation was performed to produce one litre of each detergent, using the prices per ton of raw materials. The prices used are described in table 28.

Table 28 - Price per ton in euros for each ingredient.

Ingredient	Price / Ton (€)
Water	100
SLES	997
SDBS	1210
MELs	70 000*
CB	500
Ethanol 96%	1000
Sodium chloride	382
Sodium Benzoate	1050

Note: All prices except MELs price were taken from this website [82].

*Value obtained from the thesis "Mannosylerythritol Lipids production from *Moesziomyces* spp. Bioreactor studies, economic analysis, and their application in the remediation of crude oil contaminated sand" (Confidential work).

The price is based on one litre of detergent; however, the price does not consider the energy used in the manufacturing process, nor the labour. Selected packaging is recyclable, this is an important issue due to the impact on the environment, because the packaging, it has the biggest impact on the environment during the life cycle of glass cleaners [81].

The price of hand dishwashing detergent is €1.21 (Table 29), and glass detergent is €1.24 (Table 30) for one litre, this is a very reasonable base price to be able to compete with market prices. From this analysis it can be concluded that it is not considerably more expensive to produce a detergent that pays attention to environmental issues. Therefore, if the quantity of the most environmentally friendly surfactant used is not high, as is the case with the formulations indicated above, the price variation is not significant. However, if the concentration of this surfactant is high, the production cost of the detergent will increase, and hence the price to the consumer.

Table 29 - Determination of hand dishwashing price for 1litre.

Hand Dishwashing Detergent		
Ingredients	**Quantity for 1L of formulation**	**Price (€)**
Water (mL)	670	0.07
SLES (mL)	280	0.28
MEL (g)	5	0.35
CB (mL)	10	0.01
Sodium Chloride (g)	30	0.01
Sodium Benzoate (g)	0.1	0.0001
Packaging	1	0.37
Label	2	0.12
Total		1.21

Table 30 - Determination of glass cleaner price for 1litre.

Glass Cleaner		
Ingredients	**Quantity for 1L of formulation**	**Price (€)**
Water (mL)	898	0.09
Ethanol 96% (mL)	80	0.08
SDBS (g)	20	0.02
MELs (g)	2	0.14
Sodium Benzoate (g)	0.1	0.0001
Packaging	1	0.79
Labels	2	0.12
Total		1.24

This may be a problem, but consumers are currently willing to pay a bit more for an environmentally friendly product. Furthermore, obtaining a certification, such as the EU Ecolabel, is an important way to communicate and inform consumers properly. In table 31 are the expenses needed to obtain this certification.

Table 31 - Cost for the EU Ecolabel certification.

Awarding of EU Ecolabel certification [83]	Application Fee	300 €
	Extension/Modification Fee	300 €
	Annual Fee	750€
Fitness for use [84]	Performance test for hand dishwashing detergent	1160 €
	Performance test for glass cleaner	400 €

The most expensive is the performance test, which is used to understand if the detergent being certified is up to standard in terms of effectiveness, but this test only needs to be done once for each product. For sure, certification is an important step forward today and a great way to market and inform consumers.

5.7 Questionnaire

It was relevant to have an external opinion about the products, in both terms of appearance and performance, so a questionnaire was prepared comparing the formulated detergent with the benchmark and other with the ecolabel certification. The questionnaire was carried out by five people, which independently used the three detergents twice. The opinions obtained were in line with the results obtained in the performance tests carried out.

Regarding the dishwashing detergent questionnaire, the respondents prefer the traditional aspect of the detergent. The evaluation of the detergent during the washing shows the benchmark was the favourite, followed by the performance of the ecolabel certified detergent and lastly, the formulated detergent, the respondents said this detergent foamed less. However, the appearance of the crockery at the end of the wash was equally for all detergents. The preferred detergent, for all the participants, was the benchmark.

Considering the glass cleaner questionnaire, the appearance and colour is concerned it's a personal choice, the result was quite identical. All detergents are very effective in cleaning and consequently the appearance of the cleaned glass is also very good. The preferred detergent was the benchmark, but not unanimously, followed by the developed detergent as second choice.

In conclusion, in view of the opinion of the respondents, the manual dishwashing detergent needs some improvements, as previously stated, however the glass cleaner showed quite satisfactory results. Another conclusion that can be retained is the fact that exist a growing environmental concern regarding the use of cleaning products.

Appendix E (Figure E.1 and E.2) and F (Figure F.1 and F.2) demonstrate the questionnaire, and the answers given by our participants.

6. Conclusions and Future perspectives

Most of the detergents contain surfactants made of petroleum and using them is not the most environmentally friendly practice. Alternatives to the traditional surfactants are beginning to appear such as MELs that is a biosurfactant. One of the objectives was the development of a new detergent formulations using MELs as an active ingredient.

After several tests with different ingredients, the formulations that initially best met the criteria were SLES+CB+MELs (5g/L) for the dishwashing detergent and SDBS+MELs (2g/L) for the glass cleaner.

The dishwashing detergent formulation could have a slightly higher pH, around 7-8 and a higher foaming power. The performance can also be considerably improved, the SLES concentration should be higher or SLES should have been used in synergy with another anionic surfactant.

The glass cleaner formulation has an adequate pH value; however, it forms too much foam for this type of formulation, but it does not affect the good performance and doesn't leave residues.

In conclusion, both formulations need improvements. Nevertheless, this work was an important start for new and better formulations to be developed, there exist numerous options that can be explored.

Regarding future perspectives, the formulations developed can be improved and subsequent characterisation and performance. For the hand dishwashing detergent, an increase in the concentration of the anionic surfactant and for the glass cleaner, a decrease in the concentration of the anionic surfactant or even its replacement by one with less foaming power. Another test to be carried out would be to assess the biodegradability of detergents.

Following the detergents context, it would be interesting to use the MELs in other formulations, for example, laundry detergents or multipurpose detergents. Another option could be in the developed formulations to use other biosurfactants, like sophorolipids or rhamnolipids and compare them, in order to understand which would be the most viable to be commercialized later.

Due to the incredible properties of MELs, it has several applications. Another hypothesis to test would be in cosmetics since it has similar functions to ceramides that are used in shampoo and wrinkle cream.

In short, MELs is an exceptional biosurfactant with several properties and applications that should be explored.

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8. Appendix

8.1 Appendix A

<u>Excluded substances</u>

The substances indicated below must not be included in the product formulation regardless of concentration:

- Alkyl phenol ethoxylates (APEOs) and another alkyl phenol derivatives;
- Atranol;
- Chloroatranol;
- Diethylenetriaminepentaacetic acid (DTPA);
- Ethylenediaminetetraacetic acid (EDTA) and its salts;
- Formaldehyde and its releasers (e.g. 2-bromo-2-nitropropane-1,3-diol, 5-bromo-5-nitro-1,3- dioxane, sodium hydroxyl methyl glycinate, diazolidinylurea), with the exception of impurities of formaldehyde in surfactants based on polyalkoxy chemistry up to a concentration of 0,010% weight by weight in the ingoing substance;
- Glutaraldehyde;
- Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC);
- Microplastics;
- Nanosilver;
- Nitromusks and polycyclic musks;
- Phosphates (except in the case of industrial and institutional laundry detergents and industrial and institutional dishwasher detergents);
- Per-fluorinated alkylates;
- Quaternary ammonium salts not readily biodegradable;
- Reactive chlorine compounds;
- Rhodamine B;
- Sodium hydroxyl methyl glycinate;
- Triclosan;
- 3-iodo-2-propynyl butylcarbamate;
- Aromatic hydrocarbons (only in the case of hard surface cleaners);
- Halogenated hydrocarbons (only in the case of hard surface cleaners);
- Fragrances (only for professional hand dishwashing detergents products).

<u>Restricted substances</u>

The substances listed below shall not be included in the product formulation above the concentrations indicated:

- 2-methyl-2H-isothiazol-3-one: 0,0015% weight by weight;
- 1,2-Benzisothiazol-3(2H)-one: 0,0050 % weight by weight;
- 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one: 0,0015% weight by weight.

The total phosphorus (P) content calculated as elemental P shall be limited to:

Table A.1- Limits for total P content of each product category.

Product Category				Total P content
LD (g/kg of laundry)	Laundry detergents			0,04
	Stain removers			0,005
IILD (g/kg of laundry)	Light soil			0,50
	Medium soil			1,00
	Heavy soil			1,50
DD (g/wash)	Dishwasher detergents			0,20
	Rinse aids			0,030
IIDD (g/l of washing solution)	Pre-soaks	Water hardness (mmol/ $CaCO_3$/l)	Soft (<1,5)	0,08
			Medium (1,5-2,5)	0,08
			Hard (>2,5)	0,08
	Dishwasher detergents		Soft (<1,5)	0,15
			Medium (1,5-2,5)	0,30
			Hard (>2,5)	0,50
	Rinse aids		Soft (<1,5)	0,02
			Medium (1,5-2,5)	0,02
			Hard (>2,5)	0,02
	Multi-component system		Soft (<1,5)	0,17
			Medium (1,5-2,5)	0,32
			Hard (>2,5)	0,52
HSC (g/l of RTU product or cleaning solution)	All-purpose cleaners		RTU	0,02
			Undiluted	0,02
	Kitchen cleaners		RTU	1,00
			Undiluted	1,00
	Window cleaners		RTU	0,00
			Undiluted	0,00
	Sanitary cleaners		RTU	1,00
			Undiluted	1,00
HDD (g/l of washing water)	Hand dishwashing detergent			0,08

Fragrance substances subject to the declaration requirement provided in Regulation (EC) No 648/2004[8] shall not be present in quantities ≥ 0.010 % weight by weight per substance.

Only in the case of hard surface cleaners, VOCs (VOCs mean any organic compound having a boiling point lower than 150 °C) shall not be present above the limits specified below.

Table A.2- VOC limits for each product type of hard surface cleaners.

Product type		VOC limit
RTU (g/l of RTU product)	All-purpose cleaners	30
	Kitchen cleaners	60
	Window cleaners	100
	Sanitary cleaners	60
Undiluted (g/l of cleaning solution)	All-purpose cleaners	30
	Kitchen cleaners	60
	Window cleaners	100
	Sanitary cleaners	60

b. Hazardous substances

(i) Final product

The final product shall not be classified and labelled as being acutely toxic, a specific target organ toxicant, a respiratory or skin sensitizer, carcinogenic, mutagenic or toxic for reproduction, or hazardous to the aquatic environment, as defined in Annex I to Regulation (EC) No 1272/2008 and in accordance with the list in Table 18.

(ii) Ingoing substances

The product shall not contain ingoing substances at a concentration limit at or above 0,010% weight by weight in the final product that meet the criteria for classification as toxic, hazardous to the aquatic environment, respiratory or skin sensitizers, carcinogenic, mutagenic or toxic for reproduction in accordance with Annex I to Regulation (EC) No 1272/2008 and in accordance with the list inTable 18. Where stricter, the generic or specific concentration limits determined in accordance with Article 10 of Regulation (EC)No1272/2008 shall take precedence.

Table A.3 - Restricted hazard classifications and their categorization.

Acute toxicity	
Categories 1 and 2	**Category 3**
H300 Fatal if swallowed	H301 Toxic if swallowed
H310 Fatal in contact with skin	H311 Toxic in contact with skin
H330 Fatal if inhaled	H331 Toxic if inhaled
H304 May be fatal if swallowed and enters airways	EUH070 Toxic by eye contact
Specific target organ toxicity	
Category 1	**Category 2**
H370 Causes damage to organs	H371 May cause damage to organs
H372 Causes damage to organs through prolonged or repeated exposure	H373 May cause damage to organs through prolonged or repeated exposure
Respiratory and skin sensitization	
Category 1A/1	**Category 1B**
H317 May cause allergic skin reaction	H317 May cause allergic skin reaction
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled	H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled
Carcinogenic, mutagenic or toxic for reproduction	
Categories 1A and 1B	**Category 2**
H340 May cause genetic defects	H341 Suspected of causing genetic defects
H350 May cause cancer	H351 Suspected of causing cancer
H350i May cause cancer by inhalation	
H360F May damage fertility	H361f Suspected of damaging fertility
H360D May damage the unborn child	H361d Suspected of damaging the unborn child
H360FD May damage fertility. May damage the unborn child	H361fd Suspected of damaging fertility. Suspected of damaging the unborn child
H360Fd May damage fertility. Suspected of damaging the unborn child	H362 May cause harm to breast fed children
H360Df May damage the unborn child. Suspected of damaging fertility	
Hazardous to the aquatic environment	
Categories 1 and 2	**Categories 3 and 4**
H400 Very toxic to aquatic life	H412 Harmful to aquatic life with long-lasting effects
H410 Very toxic to aquatic life with long-lasting effects	H413 May cause long-lasting effects to aquatic life
H411 Toxic to aquatic life with long-lasting effects	
Hazardous to the ozone layer	
H420 Hazardous to the ozone layer	

Substances and mixtures included in Table 19 are exempted from point (b) (ii) of this Criterion.

Table A.4 - Derogated substances.

Product Catergory	Substance	Hazard statement
ALL PRODUCT CATEGORIES	Surfactants	H400 Very toxic to aquatic life
		H412 Harmful to aquatic life with long-lasting effects
	Enzymes(*)	H317 May cause allergic skin reaction
		H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled
	NTA as an impurity in MGDA and GLDA (**)	H351 Suspected of causing cancer
LD, IILD, DD, IIDD and HDD	Subtilisin	H400 Very toxic to aquatic life
		H411 Toxic to aquatic life with long-lasting effects
LD and IILD	ε-phthalaimido-peroxy-hexaoic acid (PAP) used as bleaching agent at max concentration of 0,6 g/kg of laundry	H400 Very toxic to aquatic life
		H412 Harmful to aquatic life with long-lasting effects
IILD	Peracetic acid/hydrogen peroxide used as bleaching agent	H400 Very toxic to aquatic life
		H410 Very toxic to aquatic life with long-lasting effects
		H412 Harmful to aquatic life with long-lasting effects
(*) Including stabilisers and other auxiliary substances in the preparations (**) In concentrations lower than 0,2 % in the raw material as long as the total concentration in the final product is lower than 0,10 %.		

The sub-criterion "Substances of very high concern" (SVHCs) shall apply to all ingoing substances regardless of their concentration in the product, the updated list of SVHCs is available on the European Chemicals Agency website.

e. Preservatives

(i) The product may only include preservatives in order to preserve the product, and in the appropriate dosage for this purpose alone. This does not refer to surfactants which may also have biocidal properties.

(ii) The product may contain preservatives if they are not bio-accumulating. A preservative is considered not bio-accumulating if the BCF is < 100 or log K_{ow} is < 3. If both the BCF and log K_{ow} values are available, the highest measured BCF value shall be used.

(iii) It is prohibited to claim or suggest on the packaging or by any other communication that the product has an antimicrobial or disinfecting effect.

f. Colouring agents

(i) If both BCF and K_{ow} values are available, the highest measured BCF value shall be used.

(ii) If a colouring agent is approved for use in food, it is not necessary to submit documentation of bio-accumulation potential, just provide the proper documentation to the CB to ensure that the colouring agent is approved for food use.

8.2 Appendix B

CMC calculation for hand dishwashing formulations.

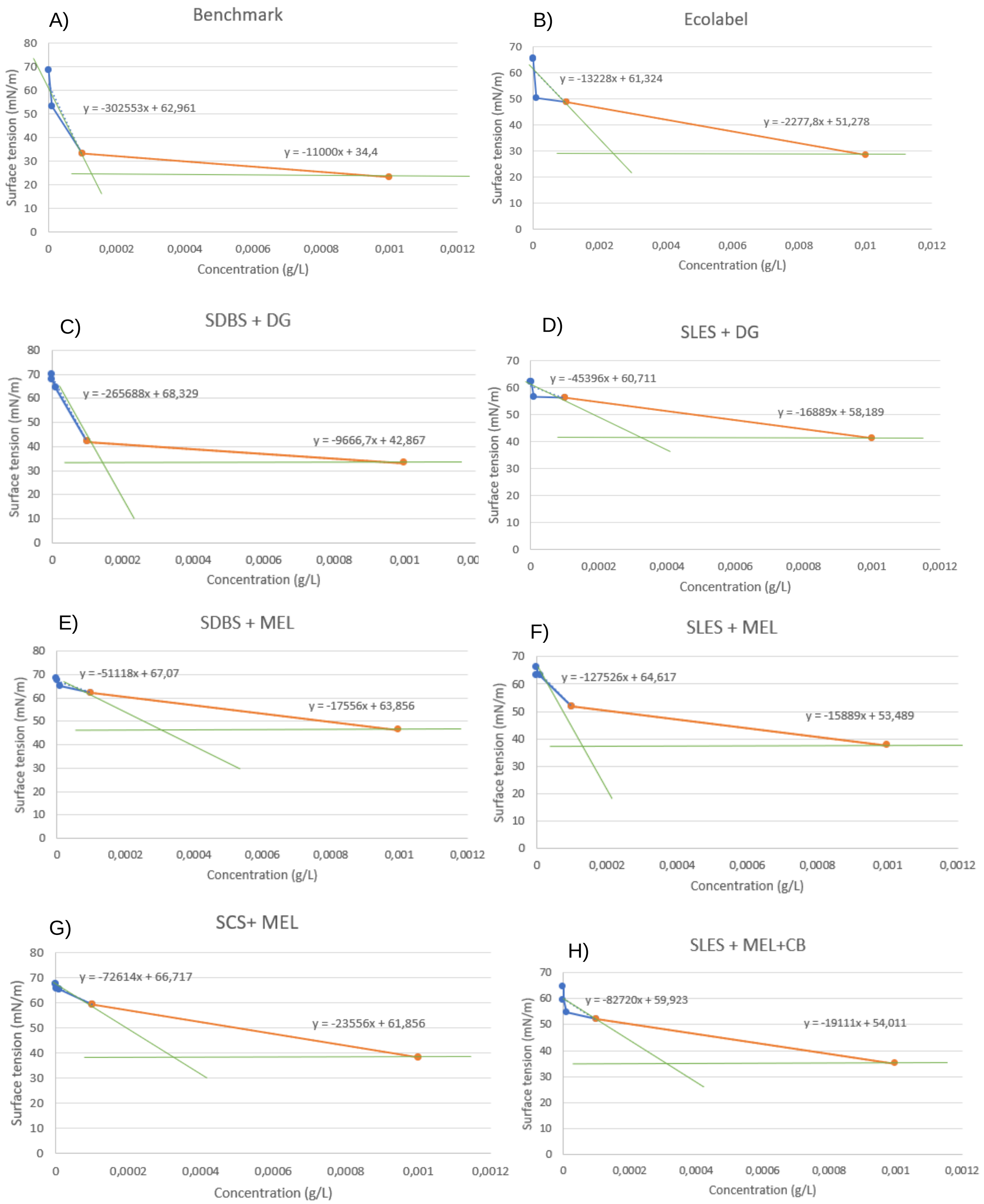


Figure B.1 - Graphs used to calculate the CMC for each hand dishwashing detergent formulation. The blue and orange represent the surface tension values as a function of concentration.

A) Benchmark; B) Ecolabel; C) SDBS+DG; D) SLES+DG; E) SDBS+MELs; F) SLES+MELs; G) SCS+MELs; H) SLES+MEL+CB.

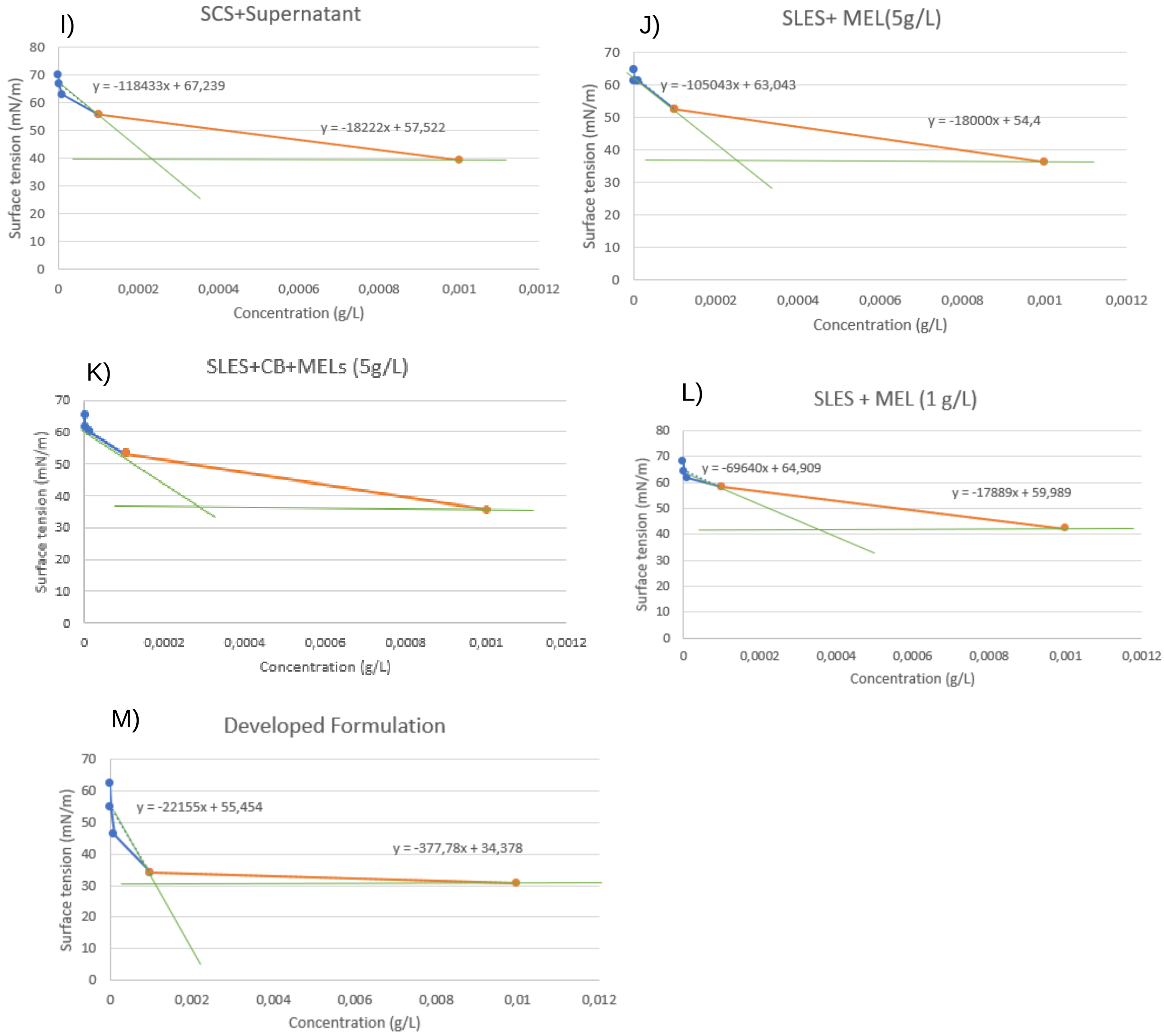


Figure B.2 - Graphs used to calculate the CMC for each hand dishwashing detergent formulation. The blue and orange represent the surface tension values as a function of concentration.

I) SCS+Supernatant; J) SLES+MEL (5 g/L); K) SLES+CB+MELs (5 g/L); L) SLES + MELs (1 g/L); M) Developed Formulation

8.3 Appendix C

CMC calculation for glass cleaner formulations.

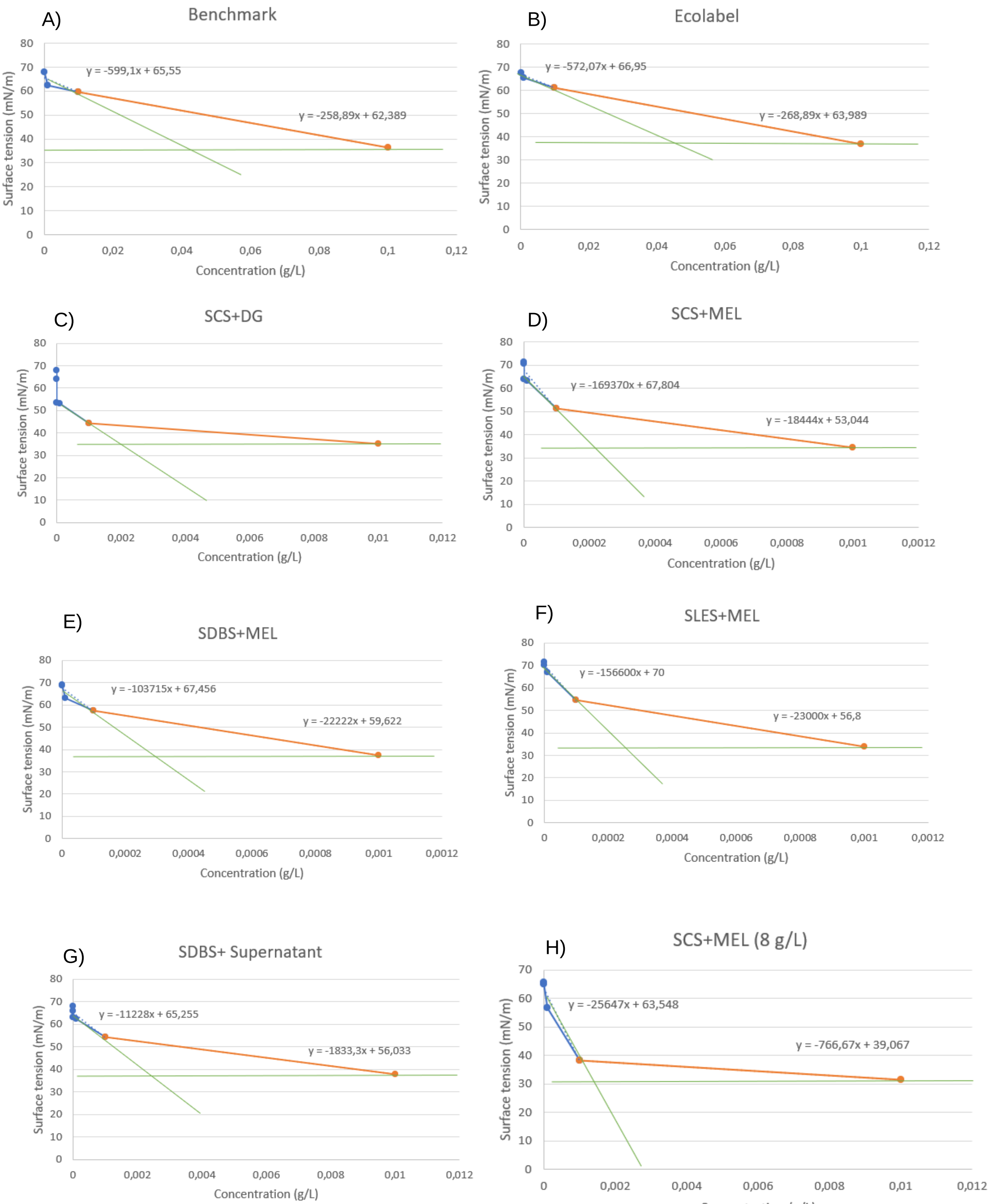


Figure C.1 - Graphs used to calculate the CMC for each glass cleaner formulation. The blue and orange represent the surface tension values as a function of concentration.

A) Benchmark; B) Ecolabel; C) SCS+DG; D) SCS+MELs; E) SDBS+MELs; F) SLES+MELs; G) SDBS+Supernatant; H) SCS+MELs (8 g/L).

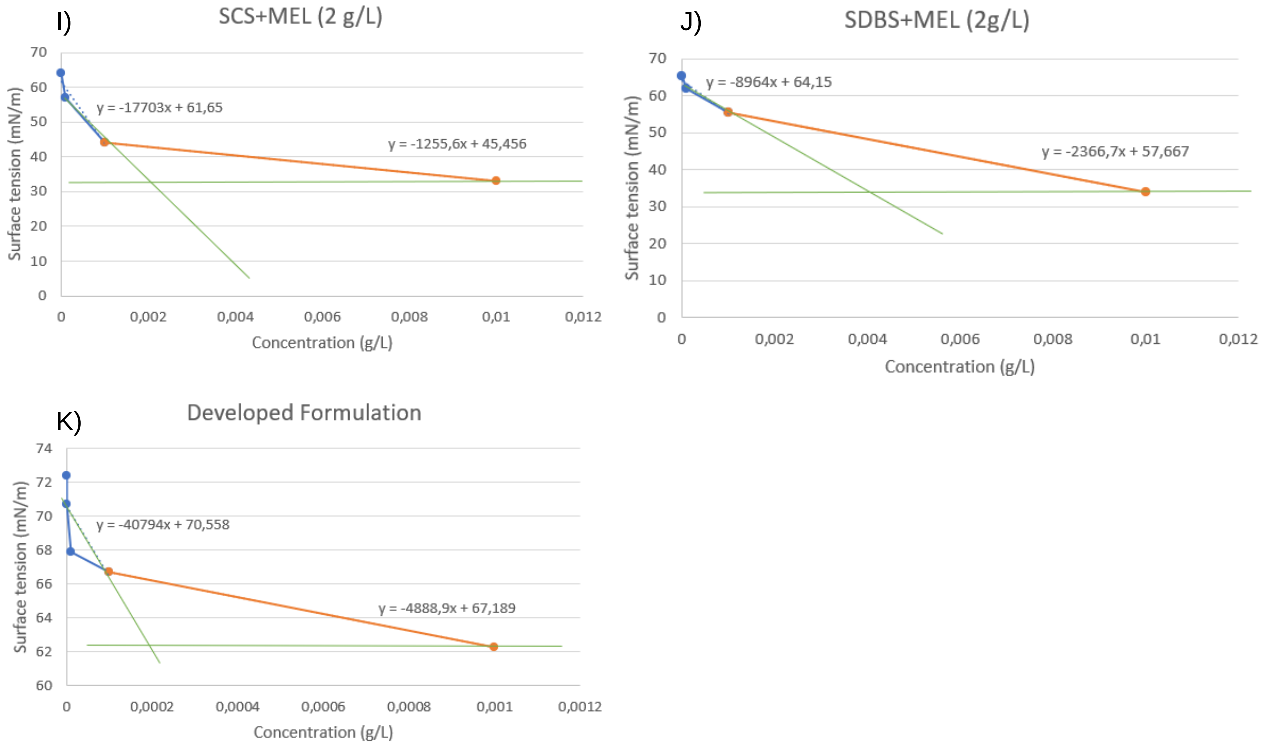


Figure C.2 - Graphs used to calculate the CMC for each glass cleaner formulation. The blue and orange represent the surface tension values as a function of concentration.

I)SCS+MELs (2 g/L); J) SDBS+MELs (2 g/L); K) Developed Formulation.

8.4 Appendix D

Others soils used in glass cleaner performance test.

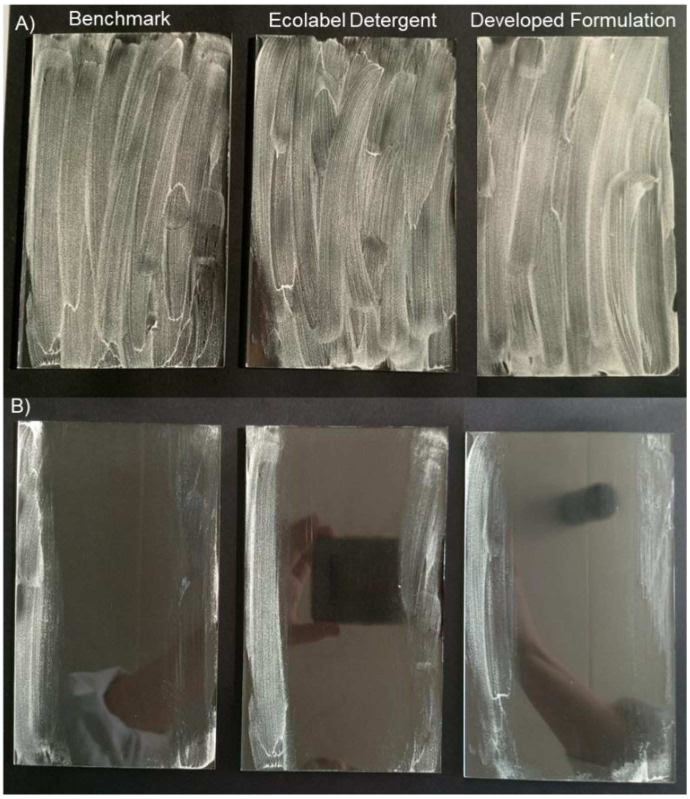


Figure D.1 - Performance test of glass cleaner using the cream gel as soil. Each glass tile corresponds to the benchmark, ecolabel certified detergent and the developed detergent, respectively. A) Appearance of the tiles at the beginning of the test. B) Appearance of the tiles at the end of the test.

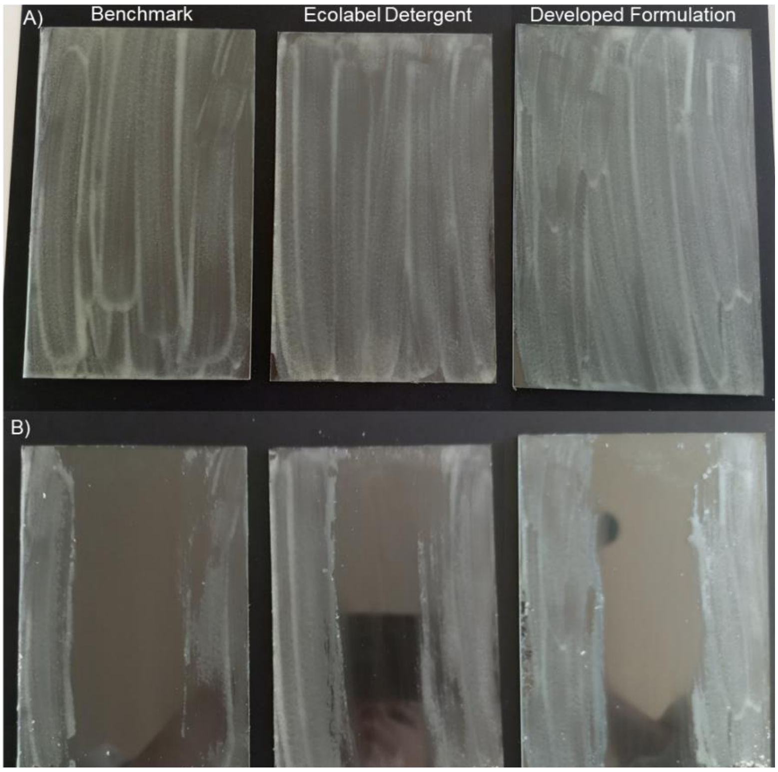


Figure D.2 - Performance test of glass cleaner using hair tonic (40%), flour (20%) and water (40%) mixture as soil. Each glass tile corresponds to the benchmark, ecolabel certified detergent and the developed detergent, respectively. A) Appearance of the tiles at the beginning of the test, B) Appearance of the tiles at the end of the test.

8.5 Appendix E

Questionnaire – Hand dishwashing detergent comparison, Answers

<u>Classification scale</u>

Classification	1	2	3	4	5
Definition	Very bad	Bad	Fair	Good	Very good

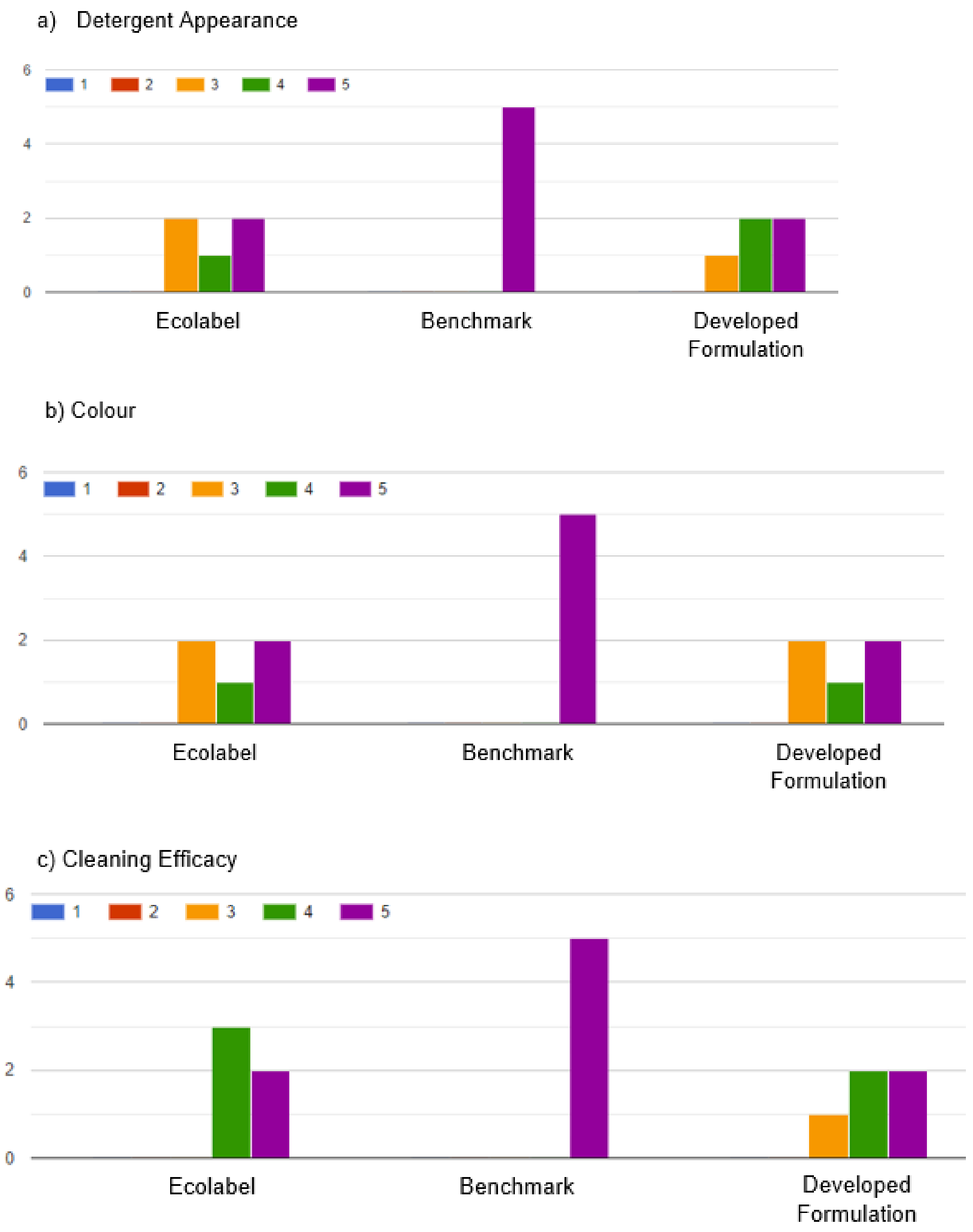


Figure E.1 - Results of the questionnaire conducted to compare the developed hand dishwashing detergent with the benchmark and with an ecolabel certified detergent.

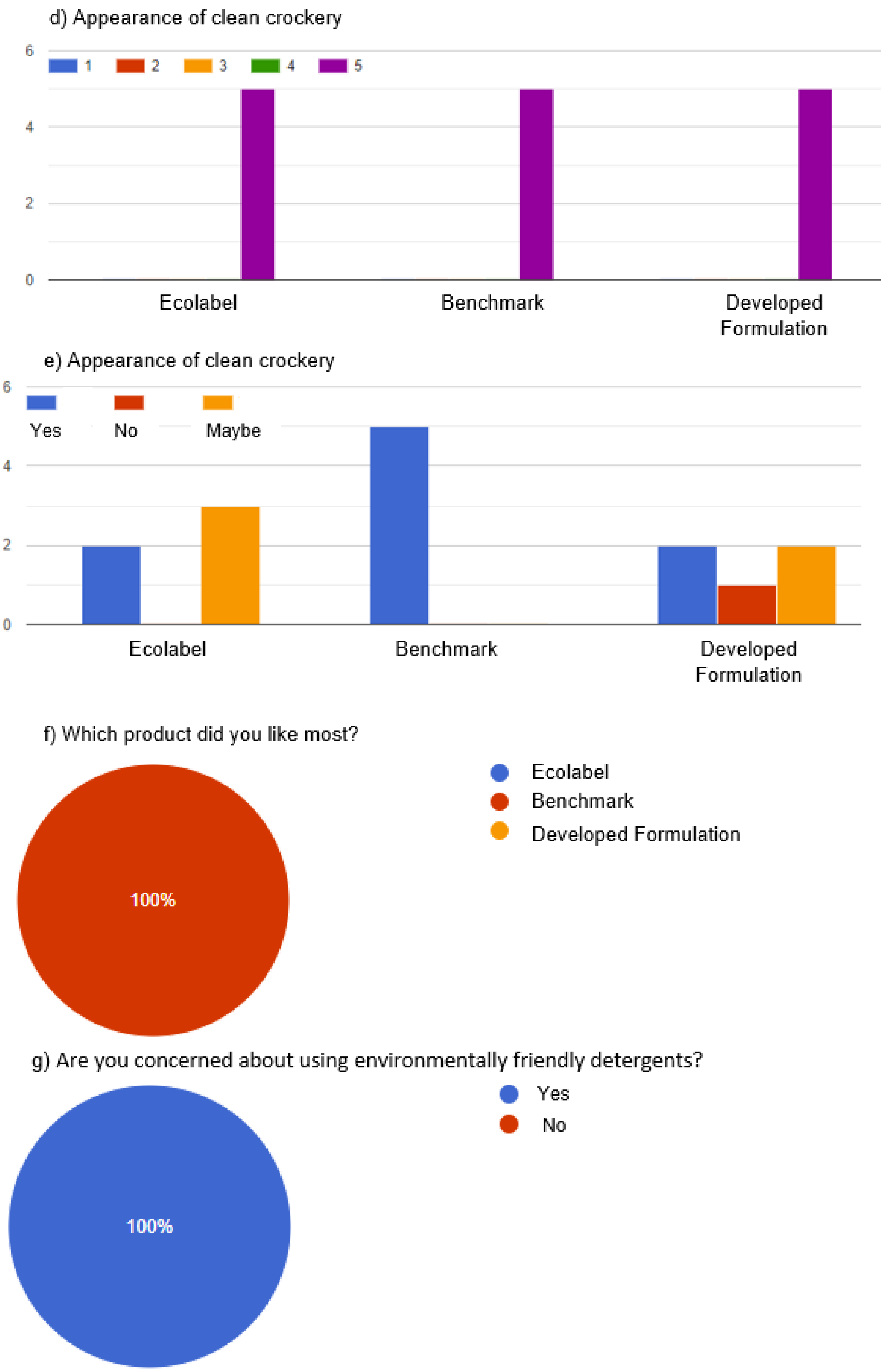


Figure E.2 - Results of the questionnaire conducted to compare the developed hand dishwashing detergent with the benchmark and with an ecolabel certified detergent (continuation).

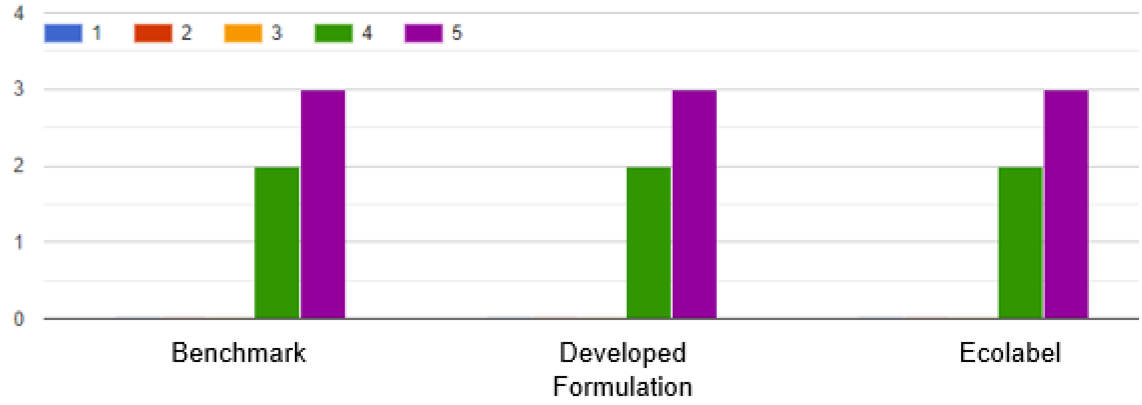
8.6 Appendix F

Questionnaire – Glass cleaner detergent comparison, Answers

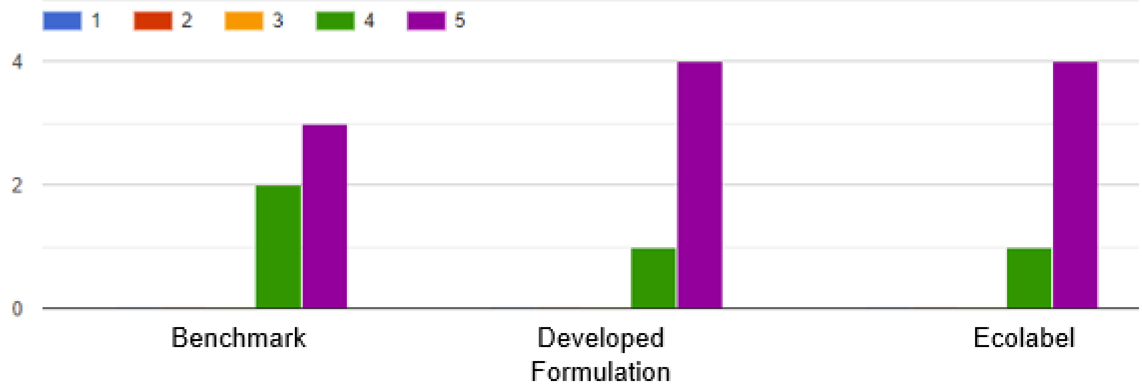
Classification scale

Classification	1	2	3	4	5
Definition	Very bad	Bad	Fair	Good	Very good

a) Detergent Appearance



b) Colour



c) Cleaning Efficacy

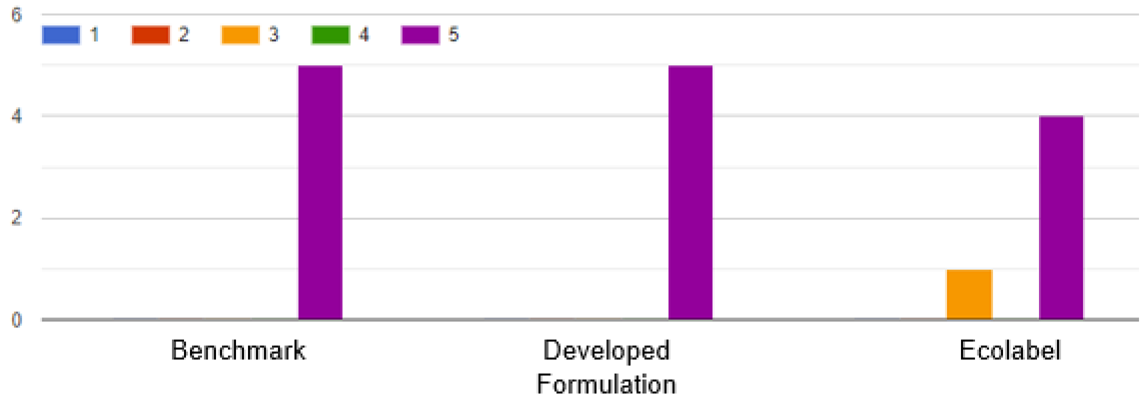


Figure F.1 - Results of the questionnaire conducted to compare the developed glass cleaner detergent with the benchmark and with an ecolabel certified detergent.

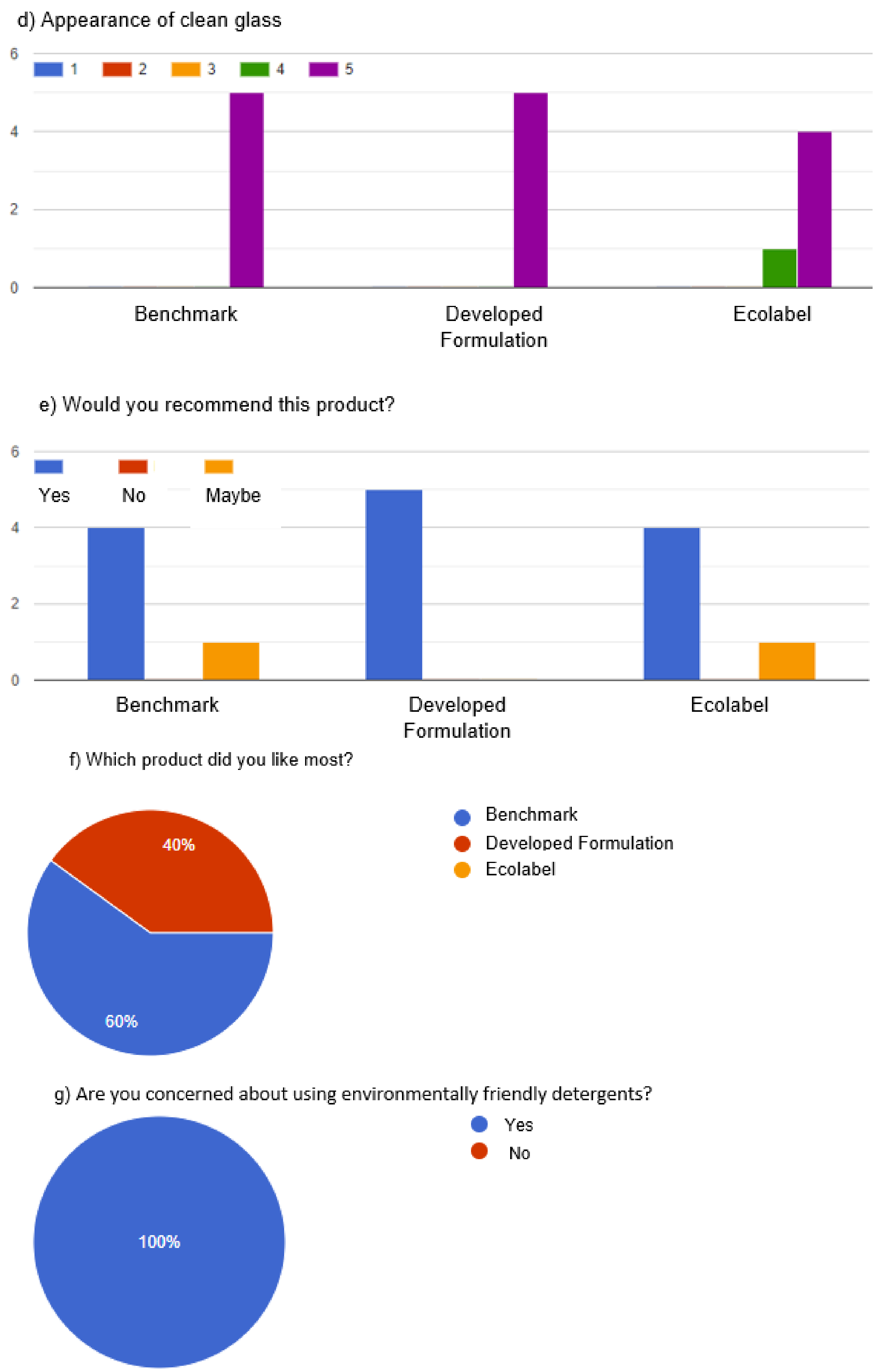


Figure F.2 - Results of the questionnaire conducted to compare the developed glass cleaner detergent with the benchmark and with an ecolabel certified detergent (continuation).