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Optimization of Different Builders in Formulation of Protease Detergent using Response Surface Methodology Approach

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DECLARATION

I declare that this thesis entitled "Optimization of different builders in formulation of protease detergents using Response Surface Methodology Approach" is the results of my own research except as cited in the references.

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Optimization of Different Builders in Formulations of Protease Detergent using Response Surface Methodology Approach

ABSTRACT

Proteases present in detergents efficiently break down protein residues from blood, grass, and egg, converting them into soluble peptides that can be easily washed away by the detergent. Optimize detergent formulations through Response Surface Methodology with varied enzyme and builder concentrations to enhance cleaning efficacy and efficiency. The objective of this study is to determine the most effective detergent builders based on the washing performance evaluations and to optimize and select the optimal detergent formulation with the chosen builders through washing performance assessments using Response Surface Methodology. The study assessed detergent effectiveness on cotton fabric stained with chicken blood, grass, and egg yolk. Seven formulations were tested, including controls with and without EDTA, and distilled water. Five formulations with varied builders (sodium tetraborate, sodium carbonate, sodium citrate, sodium silicate, and citric acid) were evaluated. The most efficient builder was selected, and its concentration, along with enzyme, was optimized using Response Surface Methodology. Stain removal was measured using the CIELAB system. This study showed that citric acid and sodium silicate were the most effective builders among all the six builders. From the response surface methodology analysis revealed that enzyme concentration consistently influenced stain removal, with sodium silicate and citric acid effects varying by stain type. In conclusion, our study highlights the significant role of builders in improving stain removal, recommending further optimization of detergent formulations for enhanced performance.

Keywords: Protease Detergent, Builder, Response Surface Methodology, effectiveness, washing test.

Optimumkan Pelbagai Pembina dalam Formulasi Deterjen Protease

menggunakan Pendekatan *Response Surface Methodology*

ABSTRAK

Protease yang terdapat dalam detergen dengan efisen memecahkan sisa protein daripada darah, rumput dan telur, menukarnya kepada peptida larut yang boleh dihanyutkan dengan mudah oleh detergen. Optimumkan formulasi detergen melalui *Response Surface Methodology* dengan pelbagai kepekatan enzim dan pembina untuk meningkatkan keberkesanan dan kecekapan detergen. Objektif kajian ini adalah untuk menentukan pembina detergen yang paling berkesan berdasarkan penilaian prestasi pencucian dan untuk mengoptimumkan serta memilih formulasi detergen yang optimum dengan pembina yang dipilih melalui penilaian prestasi pencucian menggunakan *Response Surface Methodology (RSM).* Kajian itu menilai keberkesanan detergen pada kain kapas yang berlumuran dengan darah ayam, rumput dan kuning telur. Tujuh formulasi telah diuji, termasuk tiga kawalan dengan dan tanpa EDTA, dan air suling. Lima formulasi dengan pembina pelbagai (natrium tetraborat, natrium karbonat, natrium sitrat, natrium silikat, dan asid sitrik) telah dinilai. Pembina yang paling efisen telah dipilih, dan kepekatannya, bersama-sama enzim, telah dioptimumkan menggunakan *Response Surface Methodology (RSM)*. Penyingkiran kotoran diukur menggunakan sistem CIELAB. Kajian ini menunjukkan bahawa asid sitrik dan natrium silikat adalah pembina yang paling berkesan antara kesemua enam pembina. Daripada analisis *Response Surface Methodology (RSM)* mendedahkan bahawa kepekatan enzim secara konsisten mempengaruhi penyingkiran noda, dengan kesan natrium silikat dan asid sitrik berbeza-beza mengikut jenis noda. Kesimpulannya, kajian kami menyerlahkan peranan penting pembina dalam meningkatkan penyingkiran noda, mengesyorkan pengoptimuman lanjut formula detergen untuk prestasi yang dipertingkatkan.

Kata Kunci: Deterjen Protease, Pembina, Response Surface Methodology, keberkesanan, ujian pencucian.

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Detergents are frequently used for cleaning and laundering, and they typically include a variety of components detergent additives such builders, enzymes, and surfactants. Nowadays, a variety of detergents, including liquid detergents, powder detergent, gels, and bars, use enzyme based detergent formulations. The potential of enzymes to improve the cleaning effectiveness of detergents, lower the usage of harsh chemicals, and increase environmental sustainability has led to an increase in the application of enzymes in laundry detergents. The development of enzymatic detergent should be based on economic and environmental conditions. Proteases found in detergents are easily able to hydrolyze the proteinaceous residue of blood, grass, sweat, and egg to create soluble peptides that are then simply eliminated by detergents (Keshwani et al., 2015).

The efficiency of enzymes in detergents is significantly influenced by the quality of the water used (Singh et al., 2021). The hardness of the water has a significant impact on the surfactants and builder efficiency. Due to eco-toxicity, large concentrations of surfactants in detergents considerably raise the biological demand in water and create an enormous burden on sewage systems and the environment. Detergent builders are usually combined with surfactants in detergent composition to reduce the number of surfactants present (Gurkok, 2019). Detergent builders should comply with a wide range of criteria including calcium sequestration capability, alkalinity, buffer capacity, dispersive power, and other concerns in terms of economic and environment (Pan et al., 2013).

Builders are the second – most crucial component in detergent because of its ability to increase or build upon the cleansing effectiveness of the surfactants. Builders

are used to reduce the number of calcium and Magnesium ions present in hard water as well as the number of surfactants in the detergent formulation. Builders plays an important role in water softening by binding the minerals in hard water, preventing water from ionizing and aiding surfactants in concentrating on removing soil from clothes which improves the effectiveness of the surfactants (Koohsaryan et al., 2020).

1.2 Problem Statement

There is a less understanding of how specific detergent components, such as EDTA, sodium tetraborate, sodium carbonate, sodium citrate, sodium silicate, and citric acid, interact when combined with protease enzyme detergents, and how their concentrations can be systematically optimized to improve overall detergent performance. The performance of various builders varies; therefore, it is essential to know which builder works most effectively with protease detergents. Furthermore, there is a need to evaluate the cleaning performance of these detergent formulations on diverse stains like blood, grass, and egg yolk which applied to cotton fabric. There has been limited research on optimizing detergent formulations utilizing Response Surface Methodology (RSM) with variable concentrations of different builders and enzymes. This study aims to examine the combined impacts of builder and enzyme concentrations on detergent effectiveness using a comprehensive RSM methodology.

1.3 Objectives

The objective of this study are;

1. To determine the most effective detergent builders based on washing performance evaluations.

2. To determine optimal detergent formulation with the chosen builders through washing performance assessments using Response Surface Methodology.

1.4 Scope of Study

The scope of this study on the effect of using different builders on the effectiveness of protease detergent would include the comparison of various builders and stains. The builders used in this study are ethylenediaminetetraacetic acid (EDTA), sodium tetraborate, sodium carbonate, sodium citrate, sodium silicate, and citric acid. The objective of this study is to evaluate how these builders affect the detergent's protease activity and overall cleaning effectiveness. In aiming to analyze the most effective builder concentration for maximum cleaning effectiveness and optimize the detergent formulation using Response Surface Methodology (RSM). With a focus on protease enzyme-based detergents, the study would be carried out through laboratory tests and offer insights into the role of various builders in the efficiency of enzymatic detergents. The study's conclusions will help in the creation of detergent compositions that are more effective and efficient and have better cleaning capabilities.

1.5 Significances of Study

Builders in detergent are known for their ability to soften water by binding the minerals in hard water, preventing water hardness ions and enhancing the effectiveness of surfactants. The use of six different types of builders has different cleaning effectiveness on the stain. The potential findings of this study include comparing the cleaning abilities of enzymatic detergents with various types of stains as well as improving the composition of enzymatic detergent by determining the most efficient builder type and concentration. This study's contribution to the detergent industry is its best knowledge of how to formulate protease enzyme detergents with effective builders, which results in the development of improved detergents. Furthermore, the application of RSM in this study shows the efficiency of statistical modelling in optimizing detergent formulation. Overall, this research has the potential to increase the understanding of detergent builders and how it affects the detergent's effectiveness.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Humans have used enzymes practically since the beginning of human history. Since then, the enzyme market was established and has seen numerous stages of growth. Enzymes are known as proteins biocatalysts that are crucial for metabolic and biochemical processes. Since they can replace the use of high temperatures, extreme pH values, and organic solvents while also providing high substrate specificity, low toxicity, product purity, reduced environmental impact, and ease of activity termination, the use of enzymes are used in wide range of industrial processes. Under normal circumstances, enzyme biocatalysts are remarkably effective because to its high activity, biodegradable nature, and selective ability (Aruna et al., 2023b). The main source of enzymes are microbes, since it can be genetically modified on bacteria's cells to increase the production of enzymes, and it also can be cultivated in huge amount in a short period of time (Anbu et al., 2017).

The industrial enzyme business will expand gradually, mostly as an outcome of new application areas, cheaper enzymes produced with increased production efficiency, novel enzymes discovered through screening programs, and engineering features of conventional enzymes (Sanchez & Demain, 2017). The total worth of the overall worldwide enzymes market was estimated to be US$12.46 billion in 2022, and the CAGR from 2022 to 2030 is predicted to be 6.42%. Industrial enzymes used in the production of detergents, textiles and food industry account for 56% of the overall market (Morilla et al., 2023). Since enzyme-based processes are precise, quick, and frequently more cost-effective than traditional ones in terms of raw materials, energy, chemicals, and water, it has been adopted by a wide range of industries in the recent years (Jegannathan & Nielsen, 2013).

2.2 Protease

Protease makes up one-third of all enzymes that are manufactured by companies worldwide (Aruna et al., 2023a) . Proteases are a class of enzymes which are often referred to as proteolytic enzymes, that convert larger proteins into smaller peptides or amino acids. Proteins and polypeptides include peptide bonds, which are hydrolyzed by protease. Proteases are necessary for optimal metabolic processes in cells, particularly during the mitochondrial process, in physiological situations. The size, content, and structure of significant proteins are all regulated by proteases, which are essential for a few biochemical activities (Quirós et al., 2015). These enzymes are categorized according to their origin, purpose, and mechanism of action. The detergent and pharmaceutical sectors then the food industry are the ones that employ them most frequently. Protease accounts for 60% of the industrial enzymes available today. A compound annual growth rate (CAGR) of 6.1% by 2024 and the market size for protease enzyme has been projected for the to exceed $3 billion USD (Raveendran et al., 2018).

2.2.1 Source of Protease

Protease can be obtained from several sources, such as microbes, plants, and animals. The most widely used enzyme sources are microbial enzymes, which are produced by fungi and bacteria. It is preferred due to several benefits it must provide. In addition, the extraction of enzyme from animal and plant sources is restricted by environmental variations, moral concerns, and the labor-intensive procedure (Vn et al., 2013). The cost of producing microorganisms is often cheaper and the composition of enzyme derived from microbes is easier to predict and control. Unlikermore, unlike microorganisms, tissues from plants and animals contain more potentially dangerous substances. Enzymes produced by microbes are more active and stable than plant and animal-based enzyme (Anbu et al., 2017).

One significant source of protease is from plants is papaya. An enzyme called papain which is known as protease is found in the papaya's latex. This enzyme is essential in many commercial applications such as food, pharmaceuticals, and textile industry. It is often used as a meat tenderizer since it softens the fibers of tough meat and its tenderness. Due to its proteolytic action, which allows it to hydrolyze peptide links in proteins, papain has drawn a lot of interest

for a variety of protein-based applications because of its wide substrate specificity. Additionally, bromelain is also a plant enzyme which is derived from pineapples. It has strong protein-dissolving abilities and effectively eliminates stains made of proteins (Vn et al., 2013).

Proteases found in animals have been extensively explored and used in a variety of industries such as pharmaceuticals, biotechnology, and the food industry. Rennin, chymotrypsin, pancreatic trypsin, pepsin are some of the proteases that are derived from animals which play an important role in digestive process. These enzymes are often extracted from animal tissues or organs such as pancreases, stomach, and intestine. Animal's stomach contains pepsin (protease), specifically in gastric juice. In addition, animal pancreas commonly includes the protease trypsin and chymotrypsin. It helps the small intestine digest proteins. These enzymes have a long history of application in biotechnological processes including protein purification and sequencing, peptide synthesis, and as medical treatments (Gurumallesh et al., 2019).

Protease derived from microbial sources, such as bacteria and fungus, have drawn a lot of interest because of its various enzymatic activity and possible industrial uses. Proteases from microbial sources offer several benefits over those from animal and plant sources, such as ease of production, high stability, and the capacity to function in a variety of pH and temperature environments. Numerous industries, including those in food, pharmaceuticals, leather, detergent, and bioremediation, have found use for microbial proteases. Microbial proteases are used as additives in the detergent industry to improve the effectiveness of laundry detergent by removing protein-based stains (Singh et al., 2017).

Due to the high production capacity and catalytic activity, alkaline protease generated by bacteria is more commercially significant in the leather, silk industries and food processing. These proteases are recognized by their strong activity at alkaline pH levels between 8-12 and the temperature 50^{o}C - 70^{o}C. The best source of alkaline protease for usage as a commercial enzyme in detergent composition is alkaline protease from Bacillus species (Deng et al., 2010).

2.2.2 Classification of Protease

Protease can be categorized according to the origin such as microbial, animal or plant, catalytic mechanism (exopeptidases or endopeptidases), ideal pH (acid, neutral or alkaline protease) and

catalytic sites. Serine protease, cysteine protease, aspartic protease, and metalloprotease are four major categories into which proteases belong based on their active sites, catalytic residues, and three-dimensional structures. Alkaline serine proteases are popular among the numerous kinds of microbial protease because they are often active from neutral to alkaline pH (Gurkok, 2019). Additionally, they breakdown protein-based stains on clothing including blood, milk, gravy, and egg yolk (Deng et al., 2010).

Serine proteases Serine residues are found in the active sites of serine proteases, which are enzymes that are part of the broader class of proteases. They are present in many different types of organisms, such as fungi, bacteria, plants, and animals. Based on their structural characteristics, catalytic processes, and biological activities, serine proteases may be further divided into subfamilies. Proteases that are like chymotrypsin, trypsin, and elastase are examples of serine protease subfamilies. The pH range where serine proteases are active varies depending on the enzyme, with some being active in acidic circumstances, others at neutral pH, and alkaline serine proteases being active at higher pH levels (Hedstrom, 2002).

All living things contain cysteine proteases in them. Cysteine proteases carry out a variety of activities in addition to their primary functions in catabolism and protein processing. The very first cysteine protease to be isolated and characterized is from Carica papaya was papain in 1937 (Liu et al., 2018). The family of cysteine proteases that is most prevalent includes papain and cathepsins. Lysosomal cathepsins are a major class of cysteine proteases found in mammals. Exopeptidases and endopeptidases are the two general categories used to describe the cysteine proteases family (Verma et al., 2016). The ideal conditions for cysteine proteases are pH between 2 to 3 and temperature between 40ºC to 55ºC (Alagarsamy et al., 2006).

The pepsin family of proteolytic enzymes includes aspartic proteases (EC3.4.23), which often work in acidic conditions and have a common catalytic mechanism. With a molecular mass ranging from 30 to 45 kDa and a variety of functions, aspartic proteases are peptidases. Pepstatin significantly inhibits aspartic proteases, which are active in the pH range of 3 to 5 and are acidic in nature. These proteases function best at temperatures between 40 ºC and 55 ºC. These proteases are mostly obtained from Aspergillus spp, animal tissue (stomach), Mucor, Rhizopus, and Penicillium (Alagarsamy et al., 2006).

By cleaving the peptide bonds in proteins, metalloproteases are a family of enzymes that are essential to many biological processes. The catalytic process is made possible by these enzymes' use of a metal ion, usually zinc, in their active sites. A family of hydrolases known as metalloproteases breaks down peptide bonds by using a water molecule that has been activated by complexing with metal ions. These proteases typically function at pH values between 5 and 7, temperatures between 65 °C and 85 °C, and have molecular masses between 19 and 37 kDa (Alagarsamy et al., 2006).

2.2.3 Industrial Applications and Market Value of Protease

Application of enzymes in industry are becoming quite widespread as primarily as an outcome of new application areas, cheaper enzymes produced with increased production efficiency, novel enzymes are discovered via screening program and engineering features of conventional enzymes (Sanchez & Demain, 2017). One of the most used enzymes for industry is protease, which is widely employed in many fields, including food, detergent, pharmaceuticals, textile, and leather.

Protease is used in the food industry in the process of dairy processing, baking, protein hydrolysis, flavour improvement, and meat tenderization. As an example, proteases aids in the breakdown of collagen and connective tissue in meat resulting in soft meat (Abril et al., 2023). Proteases are also widely used in the biotechnology and pharmaceutical industries. Protease helps to produce pure and high-quality therapeutic proteins by removing undesirable impurities during protein purification procedures (Sánchez-Trasviña et al., 2021). In addition, by protease also contribute to the discovery and development of new drugs and utilized in wound healing (Whittam et al., 2016). Protease enzyme is also used in detergent industries as it helps to remove protein- based stains like blood, grass, and food residues. It removes the stains and increases the cleaning effectiveness of detergent. Proteases are increasingly in demand in the food processing and its uses in pharmaceutical and biotechnology industries are developing (Gurumallesh et al., 2019).

The market value of protease has been steadily growing due to increasing demand across various industries. The annual sales of protease range between $1.5 and $ 1.8 billion, accounting

for around 60% of the global enzyme market. Among these industries, protease is primarily used in the production of detergents, which generate sales of roughly $1 billion (Reddy et al., 2022). Alkaline protease has seen a significant increase in use as an industry catalyst in the past few years. The creation of new protease enzymes with enhanced features is also being fueled by developments in enzyme engineering and biotechnology, substantially extending its potential uses and market value (Aruna et al., 2023b).

2.3 Protease Mechanism of Action in Removing Protein- Based Stain

Proteases are frequently used to catalyze different organic changes and are typically engineered to function under physiological settings, although the focus of biocatalysts is the efficient use of proteases as process catalysts in specific circumstances (Aruna et al., 2023b). The hydrolysis of peptide bonds is catalyzed by proteases, enzymes that are essential for the breakdown of proteins. Their mode of action is crucial for getting rid of protein-based stains. Protein-based stains, such as those made of blood, sweat or food, that are attached to surfaces and create chemical connections with them when they meet them. The protein molecules must be broken down to move these stains. Protein-based stains can only be removed by breaking proteins into tiny molecules, which is done by protease enzymes. This stain is broken down because of these enzyme's precise targeting and cleavage of peptide bonds in protein molecules. This level to which protease enzymes are selective for various protein substrates and identify certain amino acid sequences varies (Naeem et al., 2022).

2.4 Detergent Formulation

Detergent formulations are compositions of chemicals that are utilized for cleaning a variety of surfaces and materials. It is used to clean dirt, stains and other impurities off form various surfaces including fabrics, floors, dishes, and others. A detergent formulation is often made up of a variety of chemicals that combine to improve cleaning performance. Detergent in the form of powder has been around for a while. The use of liquid detergent, however, is continually growing. Detergent that contains enzymes are biodegradability, low toxicity, non-corrosiveness,

environmentally friendly, enhanced improvement characteristics, as well as increased potency and stability in many formulations (Gurkok, 2019).

2.4.1 Composition of Detergent

Detergent's primary components are surfactants, builders, and fillers. Surfactant, the surface-active substance present in detergent, dampens the fabric so it helps in cleaning it. Surfactants make up around 15%-40% of the overall detergent composition and are therefore the 13 most important component in laundry and cleaning products for home. Commercial formulations include additional components including enzymes, stabilizers, bleaching agents, dispersion agents, and other minor additions such as fragrance, dyes, optical brighteners (Gaur et al., 2023). This substance combination can lessen the surface tension, soften water, remove stain, add fragrance to the clothes, and extend the shelf life of the detergent. Depending on the specified usage, detergent's particular formulations might be different, but generally always aim to efficiently remove dirt from clothes and surfaces.

2.4.2 Builders

Builders play a significant role in detergent formulations as they enhance the cleaning efficiency of surfactants. The main functions of builders include softening water, preventing water hardness, improving soil removal, increasing surfactant effectiveness, providing alkalinity for better cleansing, and suspending soils to prevent redeposition. One of the main builder's main functions is water softening. Calcium and magnesium ions are among the elements in hard water that might cause difficulty during washing. To stop them from interacting with the surfactants, builders bind these minerals found in hard water. Builders ensure that the surfactants can more efficiently remove dirt from clothes by softening the water (Koohsaryan et al., 2020).

Additionally, builders also help in preventing water hardness ions from interfering with the cleaning action of the detergent. These ions will combine with surfactants 15 to generate insoluble complexes that limit stain removal. These hardness ions adverse impacts on the cleaning process are avoided by builders by sequestering or chelating the ions. Furthermore, builders help focus surfactants on dirt removal from clothing. Builders help to optimize the

surfactant's effectiveness by preventing it from being excessively diluted by the water. This guarantees that the surfactants will interact with the stains and makes it easier to remove them (Yu et al., 2008). Moreover, builders help disperse and suspend soils to prevent them from re-depositing on clothing. They aid in maintaining the loosened dirt and grime in suspension, enabling simple rinsing throughout the washing process. This aids in preserving the materials cleanliness and preventing in re-soling (Koohsaryan et al., 2020).

2.4.3 Classifications of builders

Laundry detergents include detergent builders as they increase cleaning effectiveness by enhancing surfactant effectiveness and managing water hardness. Builders may be divided into many groups according to their chemical structure and mechanism of operation. Each builder has unique roles and uses in detergent compositions are shown by this categorization. There are three types of builders such as organic, inorganic and polymer builders.

Organic builders are made of organic components and are often biodegradable. By binding and sequestering the calcium and magnesium ions found in hard water, it is efficient in reducing water hardness. Inorganic builders are generally salts or minerals that help in regulating water hardness and enhancing detergent effectiveness. Several examples of inorganic builders are sodium carbonate (soda ash), sodium silicate and sodium tripolyphosphate (STPP). Calcium and magnesium ions are prevented by inorganic builders from interfering with the efficiency of the detergent by precipitating or converting 16 them into insoluble forms. Lastly, polymer builders are high-molecule-weight chemicals that may sequester metal ions and improve detergent performance (Yu et al., 2008).

2.4.4 Builders Mechanism of Action

Ion exchange, precipitation, and sequestration are few techniques used by builders to soften water. To make hardness ions ineffective at hindering the cleaning process, sequestration involves the formation of compounds with ions. Precipitation occurs when the building chemicals interact with the hardness ions, resulting in the formation of insoluble precipitates that

are simple to remove. The process of ion exchange involves changing out the calcium and magnesium ions for sodium ions, further lowering the hardness of the water (Caracciolo, 2016).

2.5 Characteristic of Detergent Compatible – Protease

Detergent- compatible proteases are a particular type of enzymes that are essential in detergent formulations because it help to remove protein-based stains. Proteases have been specifically developed or designed to endure harsh detergent solutions and function at their best when combined with surfactants, builder, and other detergent components. It is crucial to comprehend the properties of detergent-compatible proteases to use it effectively in laundry and cleaning products. The production of detergent-compatible proteases is greatly affected by several factors, including initial pH, incubation time, incubation temperature, inoculum size and carbon and nitrogen sources. Optimizing these factors is crucial for enhancing the yield of the enzyme. Therefore it is necessary to optimize the media components and cultural parameters to ensure the production of detergent- compatible proteases in sufficient quantities (Niyonzima & More, 2015).

CHAPTER 3

MATERIALS AND METHODS

3.1 Apparatus

Beaker, spatula, micropipette, micropipette tips microcentrifuge tubes, rubber gloves, face mask, hotplate, media bottle, magnetic stirrer, pH meter, measuring balance, measuring cylinder, stirring rod, cuvette.

3.2 Instruments

Spectrophotometer, centrifuge, water bath, colourimeter

3.3 Chemical and Reagents

Sodium hydroxide, Tris-HCl, Azo casein, Tween 80, Polyethylene glycol 600, Thermostable alkaline protease 50a, sodium tetraborate, sodium carbonate, sodium silicate, sodium citrate, EDTA (ethylenediaminetetraacetic acid), citric acid, Trichloroacetic acid, Bovine serum albumin (BSA), phosphate buffer solution (PBS), Bradford reagent.

3.4 Methods

3.4.1 Determination of Protease Assay using Azocasein

The azocasein substrate solution, freshly prepared on the same day, involved dissolving azocasein in 0.1 M Tris-HCl buffer (pH 8.9). This solution was placed in a 2 ml microcentrifuge tube and preincubated in a water bath shaker at 70°C for 5 minutes. The control solution was created by dissolving azocasein in distilled water. Subsequently, 0.1 ml of thermostable alkaline protease 50a was added to each microcentrifuge tube, including the control, and the control mixture was mixed with 1 ml of trichloroacetic acid (TCA). The reaction mixture was then incubated at 70°C for 30 minutes. Following incubation, 1.0 ml of 10% Trichloroacetic acid (TCA) was added to the reaction mixture and left at room temperature for 10 minutes. After centrifugation at 13,000 rpm for 10 minutes, 1ml of the supernatant was mixed with 1 ml of sodium hydroxide (NaOH). The absorbance was measured at 450 nm, with distilled water serving as the blank. This entire process was conducted in triplicate to ensure the reliability and accuracy of the results.

3.4.2 Determination of Protein content Concentration based on Standard Curve

First, the PBS buffer and BSA protein standard were prepared according to Table 3.1. The concentration of BSA for each tube was determined based on the given concentration of BSA stock, the volume of BSA, and the volume of PBS using the formula $M_1V_1=M_2V_2$. The prepared solutions were then vortexed for 3-5 seconds and allowed to stand for about 1 minute. Next, 1 ml of Bradford reagent was pipetted into each cuvette. The absorbance was read at 595 nm. To conduct the Bradford assay, the Bradford reagent was first prepared. Then, 1 ml of the protein sample was combined with 1 ml of the reagent and mixed thoroughly. The sample was incubated at room temperature for 10 minutes. The protein concentration was detected spectrophotometrically at a wavelength of 595 nm using the Bradford method (Ibrahim et al., 2020).

3.4.3 Assay protein sample

A volume of 0.1 ml of thermostable alkaline protease 50 a was added to 1.0 ml of Bradford reagent, and the mixture was vortexed for 3-5 seconds. After a 5-minutes, the absorbance at 595 nm was measured. The protein content was calculated by referring to a standard curve that had been created with known based on method 3.4.2. (Ibrahim et al., 2020).

3.4.4 Liquid Detergent Formulation

The liquid detergent was formulated as in Table 3.2. Water was then added to the beaker, and once the water had heated up, the PEG 600 was added, followed by the builder, sodium carbonate. Tween 80, thermostable alkaline protease 50 a, and rose oil were then added to the solution and agitated for 5 minutes at 400 rpm (Ibrahim et al., 2020). A similar process was performed to prepare the remaining 6 formulations mentioned in Table 3.2. The control (negative) is made with the same formula but without a builder.

Table 3.1 Liquid Detergent Formulations

Components	**Composition concentration**	**Control Formulation**		**Substitution**
		Positive	Negative	
Surfactant	7.5%	Tween 80		-
Builder	2%	EDTA	None	- Citric acid - Sodium tetraborate - Sodium carbonate - Sodium citrate - Sodium silicate
Enzyme	1%	Thermostable alkaline protease 50a		-
Stabilizer	4.5 %	PEG 600		-
Fragrance	0.1 %	Rose oil		-
Solvent	Made up to desired volume up to 50 ml	Distilled water		-

3.4.5 Liquid Detergent Performance Analysis

The effectiveness of the protease detergent containing different types of builders was determined by how effectively it removes stains like chicken blood, egg yolk and grass from the same type of fabric which is cotton has similar size which is 7× 7cm. Following the application of stains to the fabric the clean fabric was stained with 1.0 ml of each stain, the stained material was left at room temperature for a duration of one month to ensure the formation of stubborn stains. All the stained fabric was soaked for 10 minutes before undergoing a washing test. The comparison between the fabric with different types of stain was observed visually. The control is a clean fabric without stain. To evaluate the color variations before and after washing, color measurements for cleaning performance are utilized using CIELAB colorimetry (Dalen et al., 2008).

3.4.6 Experimental Design using RSM

The appropriate ranges for the concentrations of the best builders based on the usage levels and compatibility with the detergent formulation are determined. Box-Behnken design was used in this experiment. After preparing detergent formulations with various builders according with the experimental procedure, followed by stain removal test on various stain which has standardized water temperature and duration. Response surface methodology (RSM) was used to optimize the detergent component concentration. To determine the ideal builders and other factors to maximize the stain removing efficiency of the detergent. RSM can assist in identifying the ideal values or ranges for the builder parameters as well as the relative significance of each variable. The variables in this study are concentration of different builder, surfactant and enzyme as a factor and colour changes as response variable (Madiwale et al., 2015).

Table 3.2 Factors and ranges for BBD study concentration of detergent ingredients.

RSM	Lower limit (%)	Average (%)	Higher limit (%)
Builder 1	1	2 %	3 %
Builder 2	1	2 %	3 %
Enzyme	0.5	1 %	1.5 %

Note: The table illustrates the lower limit, average, and higher limit percentages for each factor, representing various concentrations of builders and enzymes utilized in detergent formulation.

3.4.7 Liquid Detergent Formulation based on RSM and ANOVA analysis.

The liquid detergent was formulated by following Table 3.4. Water was added to the beaker, and once the water had heated up, the PEG 600 was added, followed by the builder. Tween 80, Thermostable alkaline protease 50 a, and rose oil were then added to the solution and agitated for 5 minutes at 400 rpm (Ibrahim et al., 2020). A similar process was performed to prepare the remaining formulations. The fabric staining and washing test was conducted based on method 3.4.6. The comparison between the fabric with different types of stain was observed visually. To evaluate the color variations before and after washing, color measurements for cleaning performance are utilized using CIELAB colorimetry. The analysis of variance (ANOVA) was conducted to assess the statistical significance of the regression model used to fit the experimental data on the stain removal (İnan et al., 2020).

Table 3.3 Liquid Detergent formulations utilizing Response Surface Methodology

Components	Composition concentration	Control Formulation
Surfactant	7.5 %	Tween 80
Builder	1 – 3 %	Citric acid Sodium carbonate
Enzyme	0.5 -1.5 %	Thermostable alkaline protease 50a
Stabilizer	4.5 %	PEG 4000
Fragrance	0.1 %	Rose oil
Solvent	desired volume up to 50 ml	Distilled water

CHAPTER 4

RESULTS AND DISCUSSION

4.1 ` Protease assay

The production of crude thermostable alkaline protease 50a was achieved at 114.33 U/ml of enzyme activity using azocasein assay. The total protein content and total activity of the thermostable alkaline protease 50a were found to be 568.0994 mg and 45732 U, respectively with the specific activity of 80.50 U/mg. In this study, the Bradford assay was employed to measure the protein concentration of thermostable alkaline protease 50a, a protease enzyme. The Bradford assay is quicker, simpler, and more sensitive than the Lowry technique. It is also less susceptible to interference from common reagents and non-protein components in biological samples (Kielkopf et al., 2020).

The experiment was carried out using bovine serum albumin (BSA) as a standard protein with known quantities ranging from 5ug/mL to 100ug/mL, and its absorbance measurements at 595nm were utilised to create a standard curve. In addition, the protein concentration of thermostable alkaline protease 50a, an unknown protein sample, was measured using the Bradford method. The Bradford protein test is a dye-binding assay that uses the differential colour change of a dye to respond to varied protein concentrations (Becker et al., 1996). The unknown protein concentration in 50a protease sample was estimated by substituting the average Abs595 reading into the y-value of the BSA standard curve function (y=0.0513x).

4.2 Liquid detergent formulations and performance analysis

The detergent formulation was developed to eliminate various types of stains, including blood, grass, and egg yolk, from cotton fabric. The detergent formulations are composed with detergent additives such as surfactant, builder, enzyme, stabilizer, fragrance, and solvent (distilled water). These detergent formulations were employed with 5 different builders: citric acid, sodium tetraborate, sodium carbonate, sodium citrate, and sodium silicate. The positive control utilized EDTA as a builder, while two negative controls were employed. The first negative control consisted of detergent with only enzyme and without a builder, and the second negative control involved the use of distilled water.

Stains can occur on everyday clothes, therefore knowing how to remove stubborn stains from clothing is essential (Washizu & Ishihara, 2008). This study was done to determine the effectiveness of the detergent composition protease enzyme, which contains different kinds of builders. This detergent was tested to see how well it removed blood, grass, and egg yolk stains from cotton cloth. The stains were purposefully left on the cloth for a month to replicate stubborn stains that may develop in real life. Before the stain removal process, the fabrics' visual colour was assessed using a colour meter to determine hue, chroma, and lightness L* The L* value indicates the brightness of a color on a scale from 0 to 100, with 0 being the darkest (black) and 100 being the lightest (white) (Becker, 2016).

The washing test was conducted, the comparison between the fabric with chicken blood stain was observed visually as shown in Figure 4.1, grass stain in figure 4.2 and egg yolk stain figure 4.3 after the washing test. Based on the visual observations, it can be concluded that the blood stain was still visible on all the cloths, but it was less noticeable on the cloth treated with the detergent containing builders, specifically sodium silicate and citric acid. The stain was also less visible in the detergent that only used enzymes. Next is grass stain which can be noticed clearly in all the fabric, but it is less visible the fabric treated with the detergent containing builders, specifically EDTA, sodium silicate and citric acid. The egg yolk stain was mostly invisible in all the fabric but to obtain a more precise result to evaluate the color variations after the washing test, color measurements for cleaning performance were utilized using CIELAB colorimetry.

Based on the data provided on Figure 4.4, the lightness values of chicken blood stains before washing tests ranged from approximately 21.59 to 37.86. After the washing test, these values notably increased, ranging from approximately 48.71 to 74.5. Similarly, for grass stains, the lightness values before washing ranged from around 35.98 to 55.09, after washing, the lightness values increased, ranging from approximately 49.2 to 69.1. For egg yolk stains, the lightness values before washing tests ranged from 50.3 to 65.48, while after washing, these values increased to a range of approximately 86.74 to 96.4. These findings suggest that washing significantly increases the lightness values of all types of stains, indicating a successful removal of the stains from the fabric surfaces.

The fabrics' visual colour was assessed using a colour meter to determine hue, chroma, and lightness. The L* value for before and after washing test obtained is shown in figure 4.4. To determine the best stain remover among EDTA, sodium silicate, citric acid, sodium carbonate, sodium citrate, and sodium tetraborate, the difference of stain removal by subtracting the L* value obtained after washing from the L* value obtained before washing. Based on table 1.3 (appendix), the stain removal difference value is shown. It can be concluded that the value difference for sodium silicate and citric acid is the highest. So, sodium silicate and citric acid is chosen as the most effective builders.

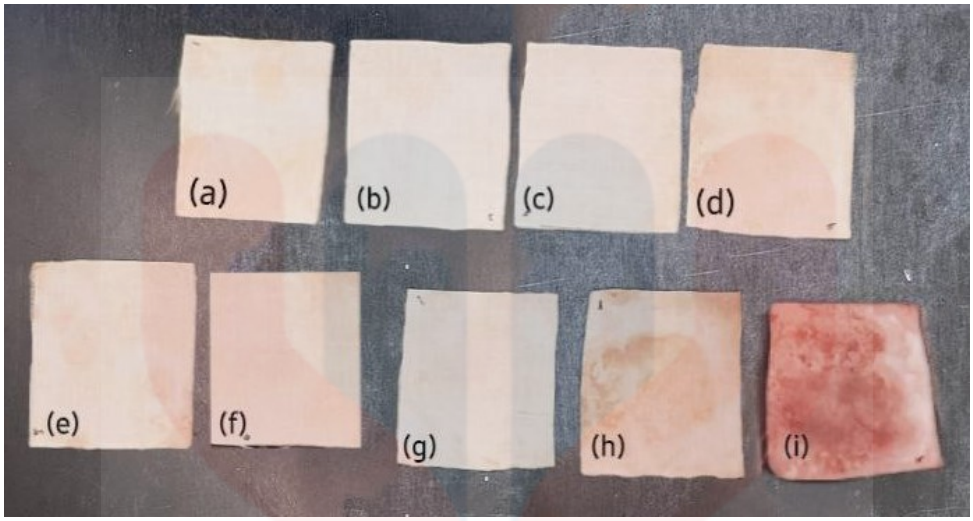

Figure 4.1 Washing performance of chicken blood stain on cotton fabric with formulated detergent using different builders.

Note: (a) EDTA as positive control, (b) Sodium silicate, (c) citric acid, (d) Sodium carbonate, (e) sodium citrate, (f) Sodium tetraborate, (g) without builder as negative control (h) Distilled water and (i) chicken blood stain before washing test

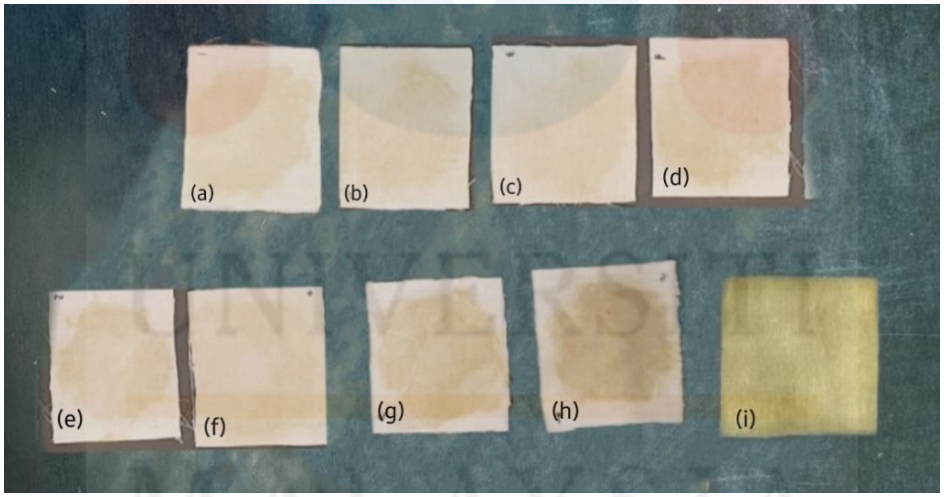

Figure 4.2 Washing performance of grass stain on cotton fabric with formulated detergent using different builders.

Note: (a) EDTA as positive control, (b) Sodium silicate, (c) citric acid, (d) Sodium carbonate, (e) sodium citrate, (f) Sodium tetraborate, (g) without builder as negative control (h) Distilled water and (i) grass stain before washing test

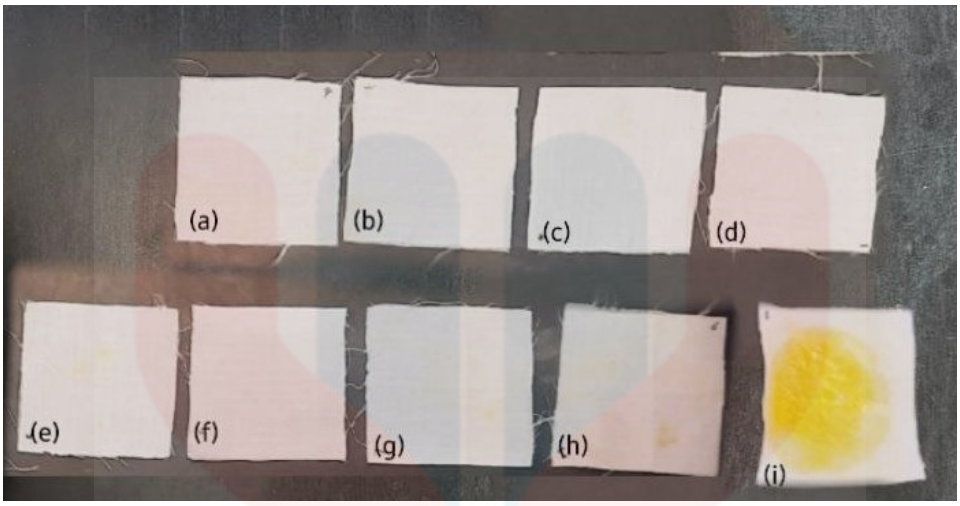

Figure 4.3 Washing performance of egg yolk stain on cotton fabric with formulated detergent using different builders.

Note: (a) EDTA as positive control, (b) Sodium silicate, (c) citric acid, (d) Sodium carbonate, (e) sodium citrate, (f) Sodium tetraborate, (g) without builder as negative control (h) Distilled water and (i) egg yolk before washing test

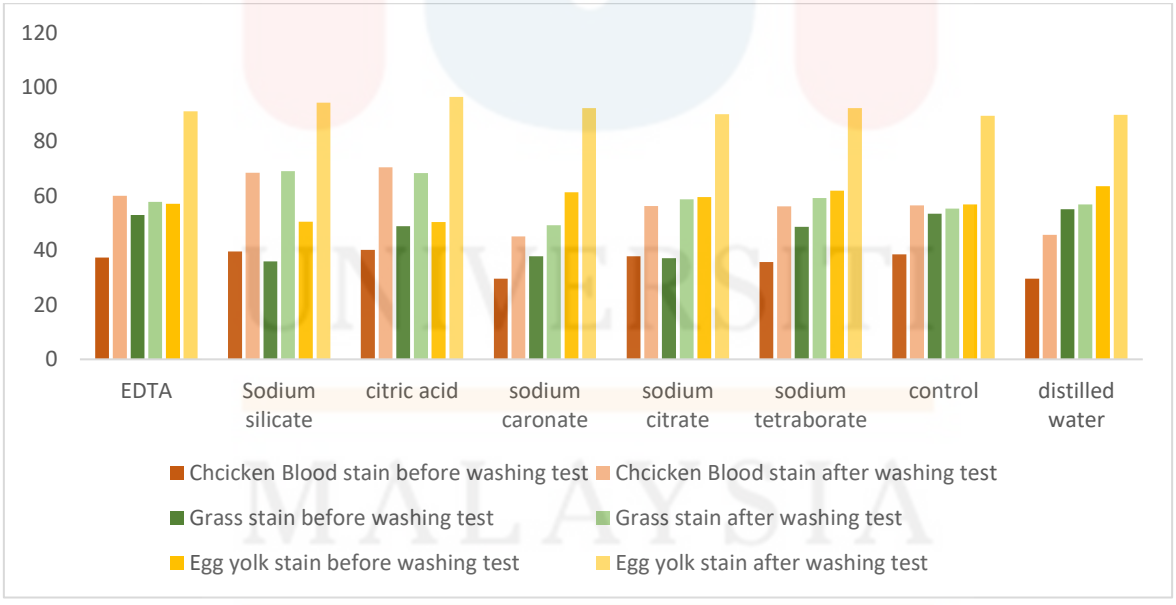

Figure 4.4 Indicates the L* value of the stained fabric (chicken blood, grass, and egg yolk) before and after the washing performance.

4.3 Liquid detergent formulation based on RSM performance and analysis.

The detergent component content was changed using response surface methodology (RSM). RSM generates an empirical polynomial model that approximates the actual response surface throughout a factor area. A Box-Behnken Design (BBD) was utilised to assess the impact of various components. The BBD algorithm is effective for optimising processes and fitting quadratic surfaces (Madiwale et al., 2015). The three optimum parameters included amounts of a factor 1 (sodium silicate), factor 2 (citric acid), and factor 3 which is enzyme. The response studied was the chicken blood stain removal percentage (%), grass stain removal percentage (%) and egg yolk stain removal percentage (%) which indicates detergent performance.

The detergent formulations utilized, as depicted in methods Table 3.3, are composed of surfactant, builders, enzyme, stabilizer, fragrance, and solvent (distilled water). These detergent formulations were employed with 2 different builders which is citric acid and sodium silicate which was the most effective. The stain, which is chicken blood, grass, egg yolk stain was on 17 fabrics cotton fabrics. The fabric was left at room temperature for 1 month to create stubborn stain. The use of enzymes in detergent additives is both economical and ecologically beneficial. Enzymes may break down a variety of stains while washing fabrics. Water can be used to break down protein peptide bonds. Additives such as proteases can help remove stains completely. Enzymes are necessary because stains are not entirely eliminated if a detergent lacks enzymes (Tian et al., 2022).

The washing test was conducted, all the stained fabric was soaked for 10 minutes before washing it with room temperature tap water and it was left to dry at room temperature for 1 day. The comparison between the fabric with different types of stains was observed visually in Figure 4.5 Chicken blood stain, Figure 4.6 Grass stain and Figure 4.7 Egg yolk stain. In these figures, the cotton fabric was labeled from (a) to (q). This labeling system corresponds to the experimental runs conducted according to the Response Surface Methodology (RSM) outlined in Table 4.1. Each label, such as (a) to (q), denotes a specific experimental condition, with (a) representing run 1 and subsequent labels representing subsequent runs. Additionally, the cotton fabric labeled as (r) represents the condition of the fabric with stains before undergoing the washing test.

Based on Figure 4.5, illustrating the washing performance of chicken blood stains on cotton fabric using a formulated detergent with varying concentrations of builders and enzymes, it can be observed that some blood stains are slightly visible, while others are not. In Figure 4.6, depicting the washing performance of grass stains on cotton fabric with formulated detergent using different concentrations of builders and enzymes, it is evident that some fabric samples show clearly visible grass stains, while others appear clean. Figure 4.7 displays the washing performance of egg yolk stains on cotton fabric with formulated detergent using different concentrations of builders and enzymes, showing that all fabric samples are clean. However, to accurately evaluate color variations after the washing test, color measurements for cleaning performance were assessed using CIELAB colorimetry.

The reading of the lightness L* value of the stained fabric (chicken blood, grass, and egg yolk) before and after the washing performance was recorded. This data is shown in table 1.4 and table 1.5 (appendix). The difference of stain removal is calculated by subtracting the L* value obtained after washing test from the L* value obtained before washing test and dividing it with L* value obtained after washing test. The difference was recorded as response, after finding out the stain removal percentage of chicken blood, grass, and egg yolk stain along with the range used for factor 1, factor 2 and factor 3 in table 4.1 RSM detergent formulation and response (stain removal percentage).

In the conducted experiment, the range of Response 1, which measures the effectiveness of stain removal for chicken blood stains, spans from 41.91% to 64.92%. This indicates a considerable variability in the performance of the stain removal method across different trials. Furthermore, Response 2, representing grass stain removal efficiency, exhibits a range from 30.21% to 43.46%. This variability underscores the need to carefully consider the factors influencing the efficacy of the stain removal process. Similarly, Response 3, which gauges the removal of egg yolk stains, shows a range from 24.90% to 45.91%. These ranges highlight the diverse outcomes observed in the experiment, suggesting that multiple factors contribute to the overall effectiveness of stain removal.

Table 4.1 RSM detergent formulation and response (stain removal percentage)

Std	Run	Factor 1 A: Sodium silicate	Factor 2 B: Citric acid	Factor 3 C: Enzyme	Response 1 Chicken blood Stain removal (%)	Response 2 Grass Stain removal (%)	Response 3 Egg yolk Stain removal (%)
1	8	1	1	1	58.09	32.49	42.29
2	4	3	1	1	49.3	36.17	33.97
3	1	1	3	1	54.06	35.61	43.67
4	12	3	3	1	64.92	43.46	45.58
5	15	1	2	0.5	61.53	32.29	37.05
6	17	3	2	0.5	62.08	43.25	45.91
7	10	1	2	1.5	51.14	33.05	39.75
8	5	3	2	1.5	56.36	43.1	36.76
9	13	2	1	0.5	45.49	33.1	36.67
10	9	2	3	0.5	61.03	37.05	41.54
11	14	2	1	1.5	64.57	33.75	44.75
12	11	2	3	1.5	57.44	43.22	37.75
13	3	2	2	1	41.91	35.06	39.21
14	16	2	2	1	50.71	35.54	33.37
15	2	2	2	1	52.87	32.6	24.90
16	7	2	2	1	45.48	36.44	37.34
17	6	2	2	1	56.48	30.21	37.06

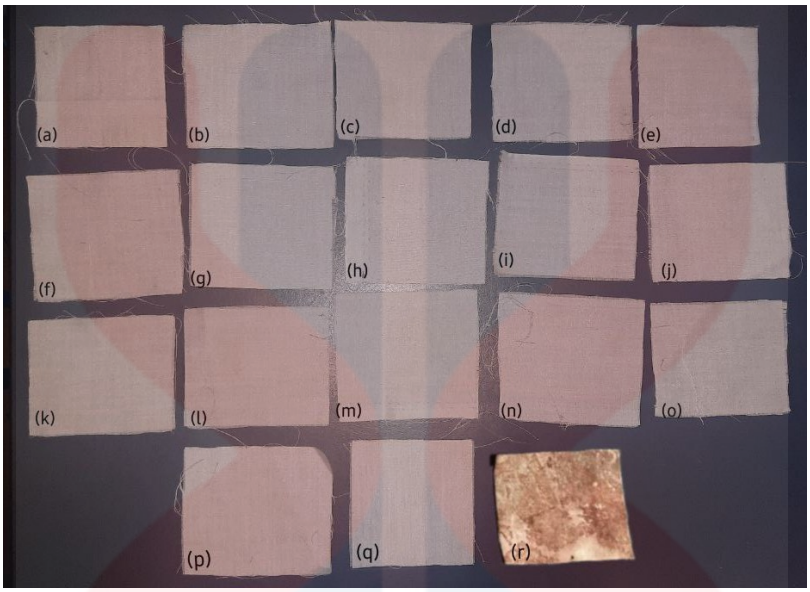

Figure 4.5 Washing performance of chicken blood stain on cotton fabric with formulated detergent using different concentrations of builders and enzyme.

Note: (a) to (q) is based on RSM run on Table 4.1 r) chicken blood stain before washing test

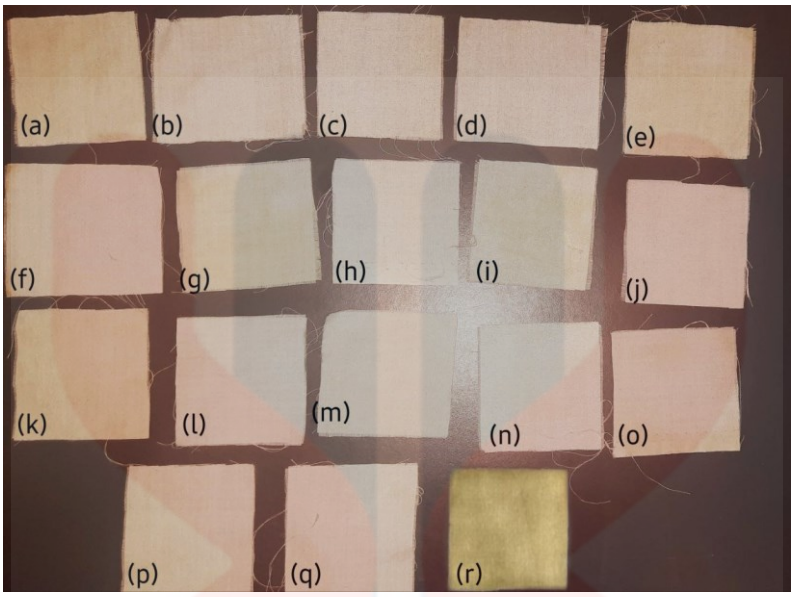

Figure 4.6 Washing performance of grass stain on cotton fabric with formulated detergent using different concentrations of builders and enzyme.

Note: (a) to (q) is based on RSM run on Table 4.1 r) grass stain before washing test

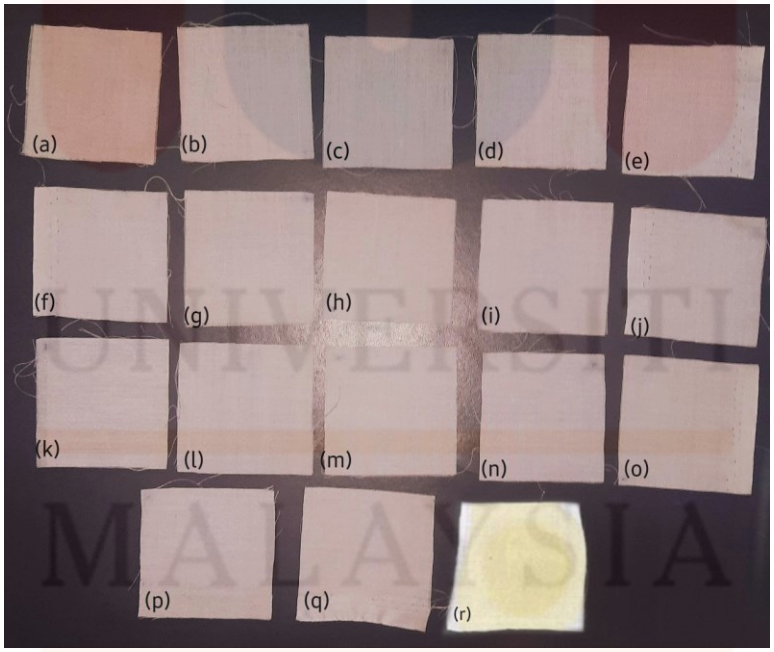

Figure 4.7 Washing performance of egg yolk stain on cotton fabric with formulated detergent using different concentrations of builders and enzyme.

Note: (a) to (q) is based on RSM run on Table 4.1 r) egg yolk stain before washing test

4.4 Response surface Methodology analysis

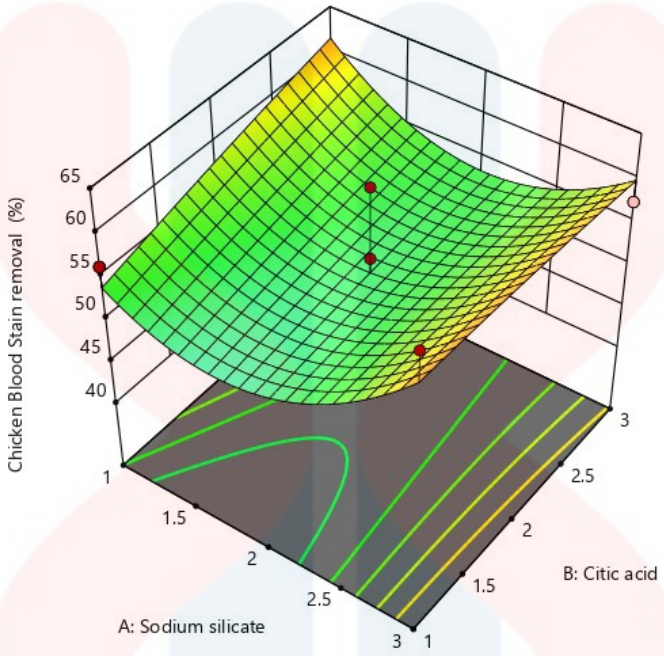

Figure 4.8 Effect of sodium silicate and citric acid on chicken blood stain removal

Table 4.2 ANOVA results for chicken blood stain removal percentage (%)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	510.69	9	56.74	1.58	0.2799	not significant
A-Sodium silicate	18.06	1	18.06	0.5027	0.5012	
B-Citic acid	17.08	1	17.08	0.4755	0.5127	
C-Enzyme	211.05	1	211.05	5.87	0.0458	
AB	21.21	1	21.21	0.5903	0.4674	
AC	2.42	1	2.42	0.0673	0.8028	
BC	26.42	1	26.42	0.7354	0.4195	
A^2	209.21	1	209.21	5.82	0.0466	
B^2	0.2814	1	0.2814	0.0078	0.9320	
C^2	9.27	1	9.27	0.2579	0.6271	
Residual	251.47	7	35.92			
Lack of Fit	95.93	3	31.98	0.8223	0.5458	not significant
Pure Error	155.54	4	38.89			
Cor Total	762.15	16				

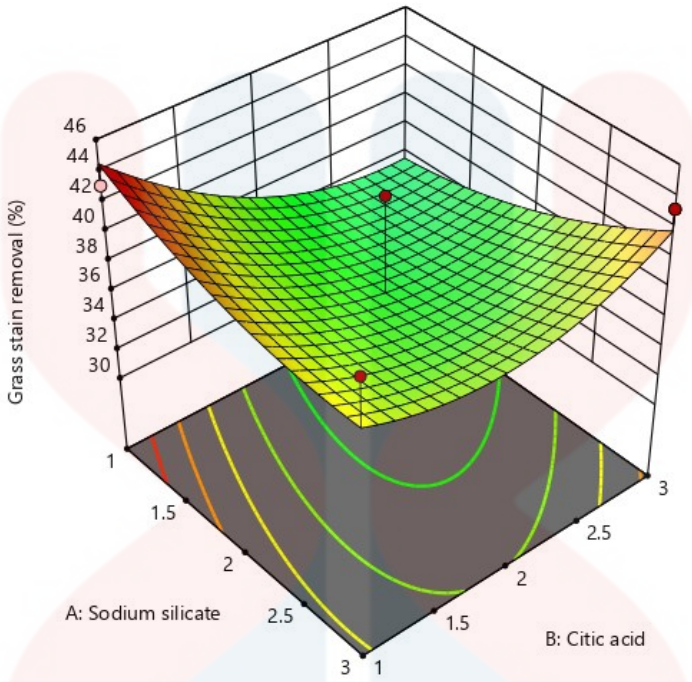

Figure 4.9 Effect of sodium silicate and citric acid on grass stain removal

Table 4.3 ANOVA results for grass stain removal percentage (%)

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	196.45	9	21.83	1.40	0.3358	not significant
A-Sodium silicate	1.94	1	1.94	0.1245	0.7346	
B-Citic acid	26.64	1	26.64	1.71	0.2323	
C-Enzyme	7.33	1	7.33	0.4706	0.5148	
AB	26.88	1	26.88	1.73	0.2304	
AC	1.40	1	1.40	0.0901	0.7728	
BC	0.0072	1	0.0072	0.0005	0.9834	
A^2	5.76	1	5.76	0.3694	0.5625	
B^2	26.19	1	26.19	1.68	0.2359	
C^2	106.77	1	106.77	6.85	0.0345	
Residual	109.09	7	15.58			
Lack of Fit	51.53	3	17.18	1.19	0.4183	not significant
Pure Error	57.55	4	14.39			
Cor Total	305.53	16				

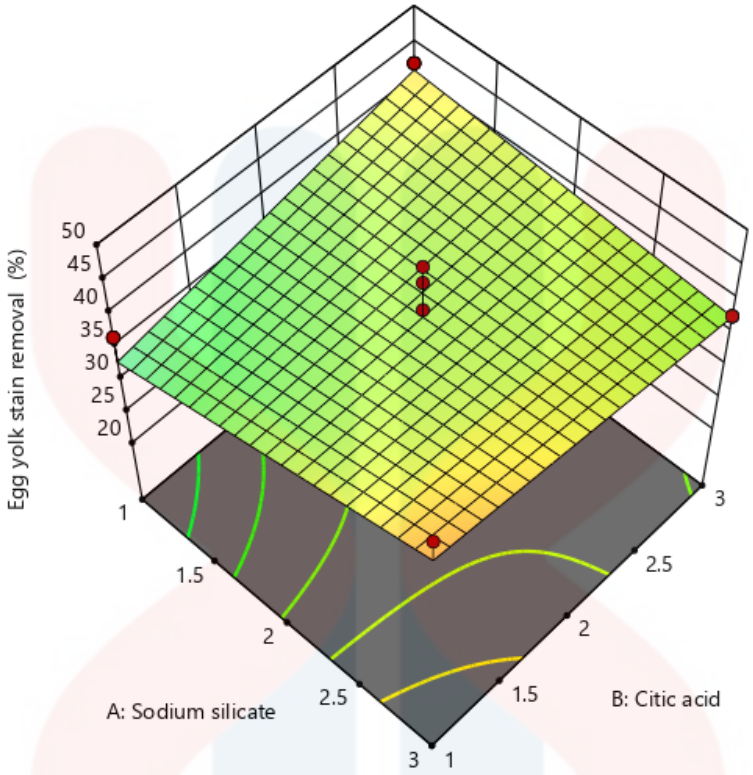

Figure 4.10 Effect of sodium silicate and citric acid on egg yolk stain removal

Table 4.4 ANOVA results for egg yolk stain removal percentage (%)

Sources	Sum of squares	df	Mean square	F-value	p-value	
Model	230.08	6	38.35	1.82	0.1911	not significant
A-Sodium silicate	17.85	1	17.85	0.8495	0.3784	
B-Citic acid	5.35	1	5.35	0.2544	0.6249	
C-Enzyme	44.51	1	44.51	2.12	0.1762	
AB	44.62	1	44.62	2.12	0.1757	
AC	69.31	1	69.31	3.30	0.0994	
BC	48.44	1	48.44	2.31	0.1599	
Residual	210.13	10	21.01			
Lack of Fit	118.31	6	19.72	0.8591	0.5875	not significant
Pure Error	91.81	4	22.95			
Cor Total	440.20	16				

The effect of significant factors influencing stain removal percentage on detergent performance was analysed using the response surface plots provided. Figure 4.8 indicates that the two main elements affecting the elimination of chicken blood stain are A^2 (squared term of sodium silicate) and C (enzyme). The response surface plot for this model would most likely indicate a significant rise in stain removal % as enzyme concentration increased, as well as a quadratic influence on sodium silicate concentration. Based on table 4.2 which shows the ANOVA results for chicken blood stain removal percentage (%). The Model F-value of 1.58 in the table indicates that the model for chicken blood stain removal was not statistically significant. However, variables C (Enzyme) and A^2 (Squared term of Sodium silicate) were significant with p-values of 0.0458 and 0.0466, respectively. These data imply that enzyme concentration and the quadratic action of sodium silicate are major factors in stain elimination. The Lack of Fit was likewise not significant, suggesting that the model was well-fitted to the data.

In Figure 4.9, only C^2 (Squared term of Enzyme) was found as a significant component for grass stain removal. The response surface plot shows a substantial link between enzyme concentration and stain clearance %, potentially indicating a quadratic impact. In Table 4.3 which indicate the ANOVA results for grass stain removal percentage (%), the model for grass stain removal also lacked overall significance, with a Model F-value of 1.40. The squared term of Enzyme (C^2) was significant with a p-value of 0.0345, showing its involvement in stain removal. The Lack of Fit was likewise not statistically significant, indicating that the model effectively matches the data.

Figure 4.10 shows that there are no major parameters for removing egg yolk stains. As a result, the response surface plot may have very flat contours, indicating that none of the parameters examined had a substantial influence on stain removal %. Table 4.4 presented the ANOVA results for egg yolk stain removal percentage (%), the model for egg yolk stain removal that did not show overall significance, with a Model F-value of 1.82. None of the model terms were determined to be significant, as evidenced by p-values that above 0.05. However, the Lack of Fit was not significant, suggesting that the model fitted the data satisfactorily despite the absence of significance in the individual variables.

When comparing the impacts of the key components across stain kinds, enzyme concentration constantly plays an important role, the effects of sodium silicate and citric acid may vary depending on the stain type. This emphasises the significance of adjusting detergent formulas to specific stain types for best results.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

In conclusion, our study focused on the development and analysis of liquid detergent formulations with various builders to enhance stain removal performance, particularly for blood, grass, and egg yolk stains on cotton fabric. The formulated detergents, incorporating different surfactants, enzymes, stabilizers, fragrances, and solvents, were systematically tested with diverse builders, including citric acid, sodium tetraborate, sodium carbonate, sodium citrate, and sodium silicate. The visual assessment of stained fabrics before and after the washing process, as well as colorimetric measurements, provided valuable insights into the effectiveness of these formulations.

The results indicate that sodium silicate and citric acid, among the chosen builders, played a crucial role in improving stain removal for blood, grass, and egg yolk. The ANOVA results underscored the significant impact of these builders on stain removal percentages, with sodium silicate and citric acid consistently demonstrating noteworthy effects across all three stain types. However, it's important to note that the lack of fit test for grass stain suggests the need for further refinement in the model to better capture the complexities associated with grass stain removal.

5.2 Recommendation

Based on our findings, we recommend further exploration and optimization of detergent formulations, particularly focusing on the synergistic effects of sodium silicate and citric acid in stain removal. Fine-tuning the concentration of these builders and exploring additional components could lead to improved detergent performance. Additionally, the application of response surface methodology (RSM) allowed us to investigate the impact of various components on stain removal percentages, highlighting the potential for further optimization using this approach. Furthermore, it is essential to conduct additional studies to evaluate the environmental and economic aspects of the developed detergent formulations, ensuring sustainability and cost-effectiveness.

Real-life testing scenarios with a broader range of stains and fabrics could provide more comprehensive insights into the applicability and efficacy of the formulated detergents. Collaboration with industry partners for scale-up and production feasibility studies would also be beneficial for transitioning these formulations from the laboratory to practical, consumer-friendly products. In summary, while our study provides valuable insights into the formulation and performance analysis of liquid detergents, ongoing research and collaborative efforts are necessary to refine and advance detergent technology, addressing the evolving needs of consumers for effective and sustainable stain removal solutions.

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APPENDIX A

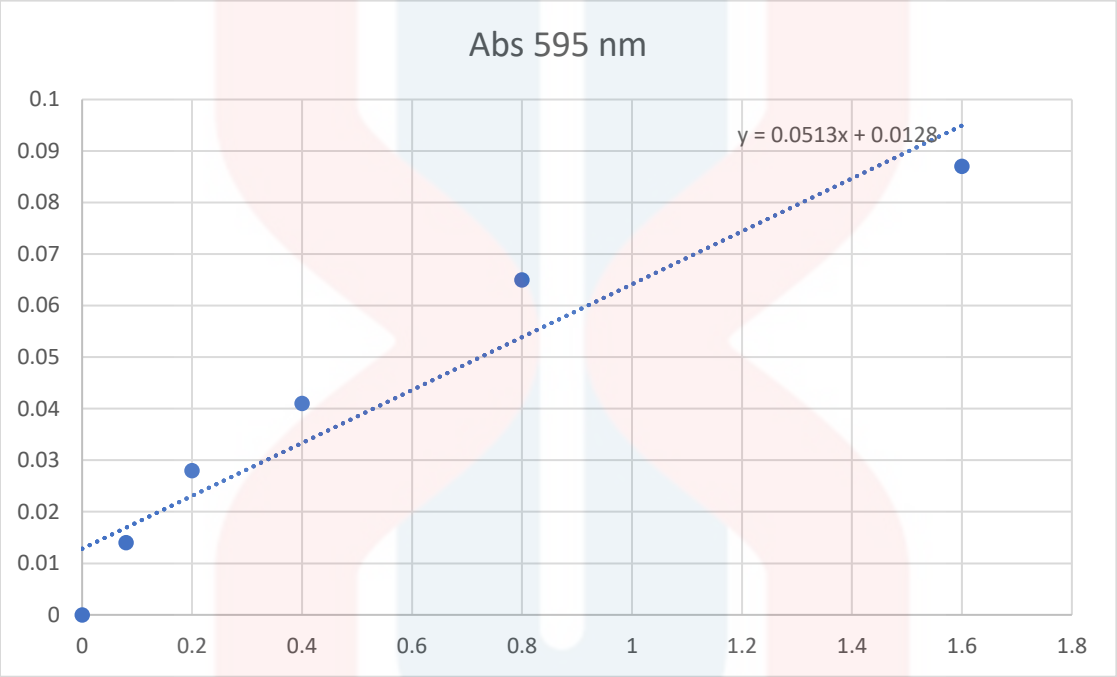

Figure 1.1 The standard curve for the Bradford Assay

APPENDIX B

Figure 1.2 Chicken Blood Stain on cotton fabric

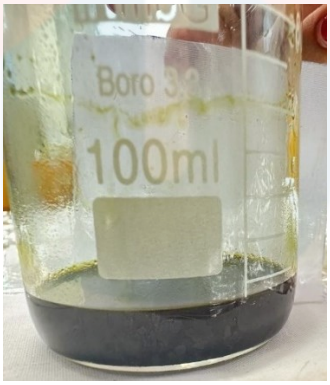

Figure 1.3 Grass stain

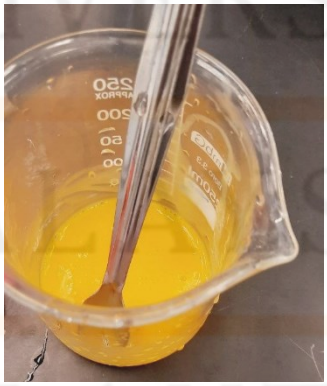

Figure 1.4 Egg yolk

APPENDIX C

Table 1.1 Reading of L* lightness by the colour meter of the stained fabrics before the washing test performed using the CIELAB system.

Detergent formulation	**Chicken Blood stain**	**Grass stain**	Egg yolk stain
Positive control (EDTA)	37.36	53.03	57.11
Sodium silicate	31.61	35.98	60.52
citric acid	29.18	48.87	65.48
sodium carbonate	21.59	37.8	50.3
sodium citrate	37.86	37.16	59.6
sodium tetraborate	24.71	48.66	50.91
Negative Control (without builder)	28.61	53.46	56.91
distilled water	29.6	55.09	51.57

Table 1.2 Reading of L* lightness by the colour meter of the stained fabrics after the washing test performed using the CIELAB system.

Detergent formulation	**Chicken Blood stain**	**Grass stain**	Egg yolk stain
Positive control (EDTA)	65.11	57.8	91.1
Sodium silicate	71.54	69.1	95
citric acid	74.5	68.45	96.4
sodium carbonate	59.15	49.2	92.3
sodium citrate	60.27	58.75	90
sodium tetraborate	61.17	59.25	92.3
Negative Control (without builder)	68.54	55.34	89.47
distilled water	48.71	56.9	86.74

Table 1.3 The L* value difference of the stained fabric (chicken blood, grass, and egg yolk) before and after the washing performance.

Detergent	Chicken blood stain removal percentage %	Grass stain removal percentage %	Egg yolk stain removal percentage %
EDTA	60.85	27.79	59.52
Sodium silicate	72.9	80.92	86.09
citric acid	75.11	83.7	90.83
sodium caronate	52.75	30.26	50.49
sodium citrate	48.81	58.14	50.34
sodium tetraborate	57.07	21.74	49.17
control	46.28	36.52	57.04
distilled water	54.7	26.22	41

APPENDIX D

Table 1.4 Reading of L* lightness by the colour meter of the stained fabrics before the washing test performed using the CIELAB system.

RUN	Chicken blood stain	Grass stain	Egg yolk stain
1	24.6	30.87	50.58
2	31.15	34.56	59.11
3	28.63	42.5	52.43
4	23.65	33.76	51.75
5	21.25	32.3	55.67
6	24.15	37.1	51.15
7	29.7	41.1	53.8
8	30.35	40.58	59.10
9	29.92	39.95	56.45
10	25.7	37.54	54.22
11	20.47	42.3	50.09
12	29.07	35.7	58.37
13	33.28	48.75	52.90
14	28.76	33.85	57.45
15	25.8	34.8	63.20
16	30.04	39.11	58.37
17	24.55	40.99	56.4

Table 1.5 Reading of the L* lightness by the colour meter of the stained fabrics after the washing test performed using the CIELAB system.

RUN	Chicken blood stain	Grass stain	Egg yolk stain
1	58.7	45.73	87.66
2	61.45	54.15	89.52
3	62.33	66.01	93.09
4	67.41	59.71	95.10
5	55.25	47.71	88.43
6	63.7	65.38	94.57
7	60.78	61.39	89.3
8	69.55	71.32	93.45
9	54.89	59.73	89.14
10	65.95	59.64	92.75
11	57.78	63.85	90.67
12	68.30	62.88	93.77
13	57.29	75.08	87.03
14	58.35	52.52	86.23
15	54.75	51.63	84.16
16	55.10	61.54	93.15
17	56.42	58.73	89.62