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Effect of Protease in Commercialized Detergent Powder on Blood Removal Efficiency

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Abstract: Blood is one of the most dangerous things to clean up because most diseases spread through blood. The problem that motivates this research is that commercial detergents on the market today are difficult to completely remove the bloodstain on the cloth. The goals of this study are to determine the efficacy of blood removal detergent powder under various raw material compositions and temperatures. To test the effectiveness of removing blood stains, two brands of commercialised detergent powder were used. The protease concentration in the detergent powder was varied from 0% to 10% in this experiment. The washing temperatures were set to 27 °C, 40 °C, 60 °C, and 80 °C. The detergent powder's effectiveness was tested using chicken blood. The results show that commercialised detergent powder X with a 10% protease addition and a washing temperature of 27 °C were the best conditions.

Keywords: Detergent Powder, Blood Stain, Protease

1. Introduction

Blood is one of the most dangerous things to clean up. Human immunodeficiency virus infection and acquired immunodeficiency syndrome (HIV/AIDS), Hepatitis B and C, methicillin-resistant Staphylococcus aureus (MRSA), and other communicable diseases will all be transmitted through blood. Because some bloodborne viruses can survive outside the body for days and cause infection, dried blood can be hazardous at times. The Hepatitis B virus, for example, can survive in dried blood for up to a week, and the Hepatitis C virus can survive for up to four days (Sean, 2018).

Hepatitis C, for example, is usually spread through contact with infected blood. Although the active virus may be present in dried blood, transmission requires the virus to enter another person's bloodstream. If one of the people inadvertently touches the dried blood with his wounded hand, he will become infected. Researchers discovered in 2013 that the hepatitis C virus can survive and remain infectious outside the body for up to 6 weeks at different temperatures. There is a risk of infection if a

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person with hepatitis C spills a drop of blood on a common surface, such as a desk or door handle. You may become infected if you lose more blood (Sissons, 2020).

The bacteria and viruses in the blood stain will infect humans. To avoid infection, the blood stain should be completely clean. The detergent powder must be used to completely clean it. The blood stain cannot be removed simply by using water. The detergent is required to remove the blood stain. This is because the detergent's main ingredients and supplementary materials, such as enzymes, are capable of removing the blood stain (Bouassida et al., 2018).

A detergent is a chemical agent used to separate and remove dirt and grease (Woodford, 2020). Laundry detergent powders (LDP) are commonly made up of surfactants, builders, bleaching chemicals, enzymes, and fillers. Surfactants, or surface-active agents, are essential among these ingredients because their cleaning ability has long been a driving force in detergent development (Siwayanan et al., 2015).

When blood comes into contact with oxygen, the haemoglobin in it causes it to clot. Because of haemoglobin's clotting ability, blood stains on clothing are impossible to remove. The problem of blood removal stains is especially severe in hospitals. Patients' bodies are covered in blood when they arrive at the hospital. When the patient lies down on the sheet or sits in the chair, those areas may become stained with blood. Furthermore, the patient's blood will stain the clothing of the emergency personnel. Typically, the staff will not clean it right away due to a lack of time. After a long time, the blood will have dried and will be difficult to remove (Mayntz, 2021).

There are several reasons why detergent cannot completely remove blood stains, including the raw material used, the temperature of the water, and the method used to wash the cloths. The temperature of the water has an impact on the washing performance. The removal of stains by various protease enzymes was discovered to be most effective at temperatures ranging from 50 to 75 °C (Paul et al., 2014). It demonstrates that when cleaning a bloodstain on a cloth, the temperature of the water must be within the range. It is inconvenient for customers because they frequently wash their clothes with tap water. The temperature of tap water ranges from 25 °C to 27 °C. This is due to the fact that the temperature of the water is affected by the weather. Water temperature is affected by the weather temperature due to heat transformation between water and air (Ahmad et al., 2013). Therefore, the purpose of this research is to determine the efficacy of blood removal detergent powder under various raw material compositions and temperatures.

2. Literature Review

2.1 Blood Stain Remover/Removal

According to Nuiplot (2020), Emran et al. (2020), and Bindhu & Singh (2014), blood stain removal is an activity that involves removing blood stains from cloth or fabrics using detergent and additives. This activity aids in completely removing the blood stain from the cloth. The bloodstain is most commonly found in hospitals, primarily in patients, menstruating women, mischievous children, and the elderly. Blood stains on clothes take a long time to remove and pose health risks, such as the risk of contamination from blood and the condition of using unsanitary items (Nuiplot, 2020). There are several supplements that can be used to help remove bloodstains. There are various types of enzymes, the most important of which are bacterial enzymes. Enzymes have been widely used in the detergent industry due to their high activity and stability in the high alkaline range. The pH range of detergents is typically 9.0 - 12.0. (Haddar et al., 2009). Enzymes are becoming increasingly popular as detergent additives due to their wide range of applications and distinct properties. The ability of enzymes can aid in the removal of bloodstains on fabric. Enzymes are environmentally friendly and cost-effective detergent additives (Al-Ghanayem & Joseph, 2020). During fabric washing, enzymes can break down various types of

stains. By passing water through peptide bonds, it can break down protein peptide bonds. Proteases, when used as an additive, can aid in the complete removal of stains. Enzymes are important because stains cannot be completely removed if a detergent lacks enzyme (Niyonzima & More, 2015a). Alkaline proteases, according to Mothe & Sultanpuram (2016), are important enzymes in many commercial applications, particularly in the laundry industry. Proteases are one of the three major types of commercial enzymes, accounting for approximately 60% of all enzyme sales worldwide. Proteases are classified as endoenzymes or exoenzymes based on whether they work on protein substrates (Geethanjali & Anitha, 2011). By incorporating proteases into the detergent powder, bloodstains can be removed. It is capable of completely removing the blood stain (Annamalai et al., 2014).

2.2 Mechanism of Blood Stain Removal

Bilayer solubilisation by detergents occurs in three stages. Figure 1 depicts the mechanism for removing a blood stain from a cloth (Das et al., 2014). Proteases are enzymes that hydrolyse the peptide bonds in proteins. Protein polymers are made up of amino acids, which are the basic building blocks. Peptide chains are formed by linking amino acids together. A peptide bond is a link between two amino acids. Proteases that can break peptide bonds with water are classified as exoproteases and endoproteases. Exoproteases cleave peptide chains from either end of a single amino acid. Endoproteases are enzymes that break down the peptide bonds in a protein chain. This mode of attack typically produces smaller polypeptides and peptides as a result of hydrolysis (Smith & Olson, 2002).

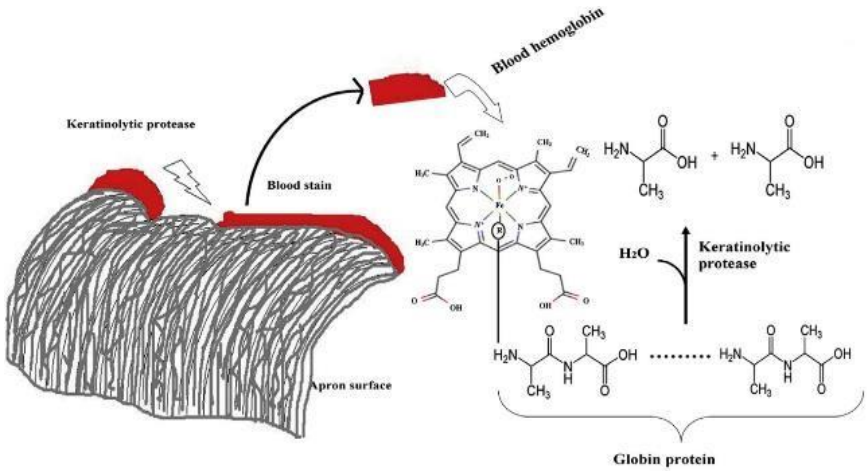

Figure 1: Mechanism of blood stain removal (Das et al., 2014)

2.3 Market Value of Detergent Powder

The global laundry detergents market is expected to grow at a CAGR of 2.88 percent between the years 2021 and 2026. The rapid advancement of technology is providing a competitive advantage and hastening the development of the laundry care industry. This is due to an increase in consumer demand for fragranced laundry and household cleaning products, as well as allergenic or green products. Because detergents are easily accessible and purchased at supermarkets and grocery stores all over the world, the laundry care market is dominated by offline markets. Nonetheless, online purchases of home care products such as laundry care products have increased significantly in recent years as potential customers began to purchase these products online. The primary market trend is rising demand for detergent powder. The growing importance of healthy lifestyles, as well as the concerns of many individual citizens about living in a clean environment free of bacteria and viruses, microbes, dirt, and soil, is driving up demand for detergent powder. The majority of customers are looking for detergents with flavour and aroma, which forces vendors to differentiate their products. Furthermore, a rise in household consumption and expansion in the real estate sector due to an increase in residential units are influencing global demand for laundry detergent goods. As a result of these factors, vendors effectively

use certain online platforms and influential e-commerce sites to market their goods, increasing the popularity and reachability of their products (Mordor Intelligence, 2021). The market value is increasing year by year, rising from 109.6 billion USD in 2018 to 181.39 billion USD in 2026. Top retailers such as Procter & Gamble and Church & Dwight Co., Inc. were especially prevalent in 2016, accounting for more than 67 percent of the market. Brand innovation in terms of formula and packaging is essential for revenue growth. From 2020 to 2027, the global soap and detergent market will be segmented by geography (Grand View Research, 2017).

2.4 Previous Studies on Blood Stain

Zhang et al. (2019) used a halotolerant metalloprotease from the marine bacterium Vibrio sp. LA-05 as a detergent additive to further remove the bloodstain from the fabric. As an additive in the detergent, the crude halotolerant metalloprotease can completely remove the blood stain on the cloth after 30 minutes at temperatures ranging from 15 °C to 45 °C. To remove the blood stain, Bhange et al. (2016) used a protease, amylase, and biosurfactant mixture as an additive in the detergent. The results show that among the three flasks, the blood-stained cloth treated with a detergent supplemented with protease, amylase, and biosurfactant removed the most blood. The bloodstain on the cloth was completely removed. Das et al. (2014) conducted the experiment using human blood as well. The apron was stained with blood. In this experiment, an enzyme and detergent (NirmaTM) mixture were used. In terms of stain removal, the detergent augmented with crude enzyme outperformed the detergent alone. Paul et al. (2014) conducted an experiment that was nearly identical to Das et al (2014). However, Das et al. (2014) use an apron to conduct the experiment, whereas Paul et al. (2014) used clean cotton cloth pieces measuring 4 cm x 6 cm. Paul et al. (2014) are also conducting the experiment with human blood and detergent containing crude keratinase enzyme. The detergent, in combination with crude keratinase and commercial protease, thoroughly cleans the bloodstain fabric, removing blood stains more effectively than the detergent alone. Sathishkumar et al. (2015) used a marine ascidian-associated bacterium, Virgibacillus halodenitrificans RSK CAS1, and marine waste as a substrate to refine and produce protease using response surface methodology. The most bloodstains were removed from a blood-stained cloth after it was washed with proteases enzymes and commercial detergent. Niyonzima & More (2015b) investigated the possibility of using a protease called Aspergillus terreus in detergent formulations to improve blood stain removal. The best results are obtained when stained cloth is washed with alkaline protease, super wheel, and tap water. Annamalai et al. (2013) performed a blood removal stain experiment with Bacillus halodurans CAS6 enzymes isolated from marine sediments. The same experiment was carried out by Annamalai et al. (2014), but the protease used was Bacillus alveayuensis CAS 5. The combination of enzymes and commercial detergent removed the blood stain in 25 minutes, according to the results. The aim of Mechri et al. (2017) is to use statistical methods to optimise the culture and nutritional conditions of the immersed Lysinibacillus fusiformis strain C250R for the production of serine alkaline proteases in the submerged fermentation process. The blood stains can be removed by using a detergent containing enzymes at 60 °C and pH 9 - 11. The blood removal stain experiment was carried out by Saleem et al. (2012) using Bacillus cereus proteases. The Bacillus cereus protease was confirmed to be capable of removing the blood stain using only the protease.

3. Research Methodology

3.1 Preparation of Detergents

In this experiment, two types of detergent powder are used: commercialised detergent powder X and commercialised detergent powder Y. Proteases were added to the detergent to observe its capability to remove blood stains from the cloth. The detergent powder was 10 g for each mixture, and the proteases were added in different beakers with 0.1 g (1 wt %), 0.2 g (2 wt %), 0.5 g (5 wt %), and 1 g (10 wt %) to mix with the detergent powder.

3.2 Preparation of Blood-Stained Cloth

In this study, cotton cloth was used. The cotton clothes measure 4 cm in length and 4 cm in width. Subsequently, 5 mL of chicken blood was stained on a clean fabric cloth, dried with a hair dryer, and left for 8 hours.

3.3 Washing Process

The stained fabric was cleaned in a 500 mL beaker containing 400 mL of tap water and detergent powder mixtures. Every 5 minutes, the clothes were removed for visual inspection and a photo was taken. After washing for 15 minutes, the cotton cloth was dried with a hair dryer. It was then visually inspected once more. Aside from that, the washing temperature was varied between 27 °C, 40 °C, 60 °C, and 80 °C.

4. Results and Discussion

4.1 Mixture of Commercialised Detergent Powder and Protease

Figure 2 depicts the results of blood stain removal after 5-15 minutes using a mixture of commercialised detergent powder X with varying wt % of protease. It is clear that the blood was quickly removed after only 5 minutes of washing. Only a small amount of blood was removed by the detergent powder mixture after 10 to 15 minutes. It has also been discovered that increasing the wt % of protease will improve the blood removal process. This can be demonstrated by looking at the colour of a cotton cloth after it has been dried with a hair dryer. When compared to the other samples, the sample without protease showed the most yellowish colour.

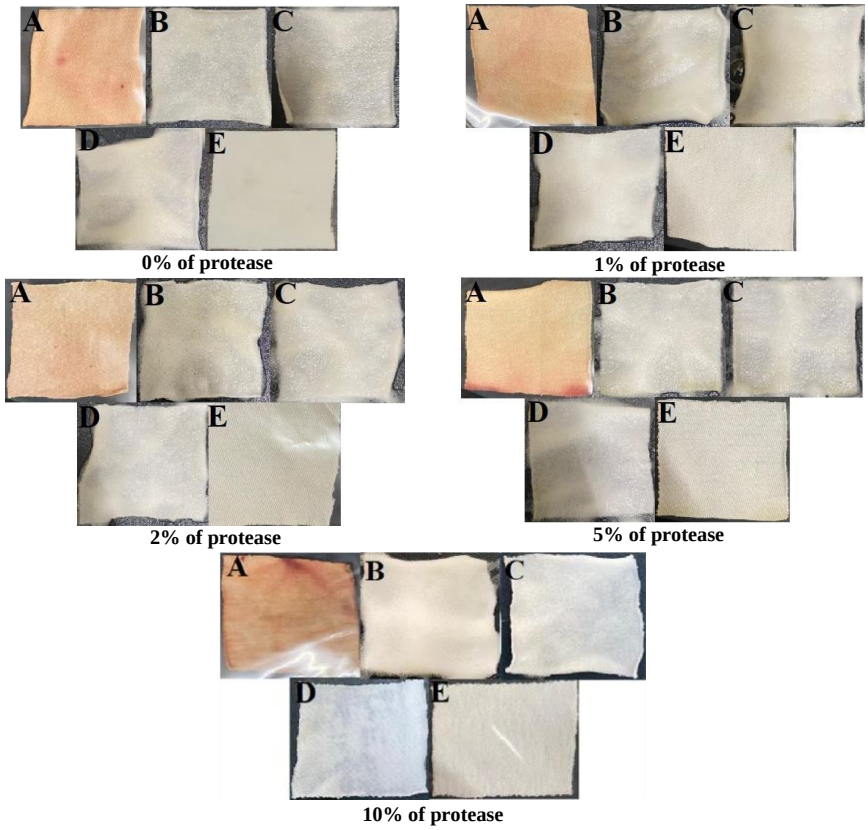

Figure 2: Visual inspection of cotton cloth for mixture of commercialised detergent X with varying wt % of protease at room temperature (A) Initial (B) after 5 minutes (C) after 10 minutes (D) after 15 minutes (E) dried sample

Based on Figure 3, a similar pattern can be seen for cotton cloth washed with commercialised detergent powder Y containing varying amounts of protease. It can be concluded that the higher the concentration of protease used to remove the blood stain from the cotton cloth, the more effective it is. This is due to the presence of protein in the blood, and protease has the ability to break down protein peptide bonds. As a result, the blood stain is easily removed. (Smith & Olson, 2002). Aside from that, it can be revealed that commercialised detergent powder X has greater blood removal capability than Y. This is due to the fact that samples, which was washed with commercialised detergent powder Y, had a more yellowish colour than X.

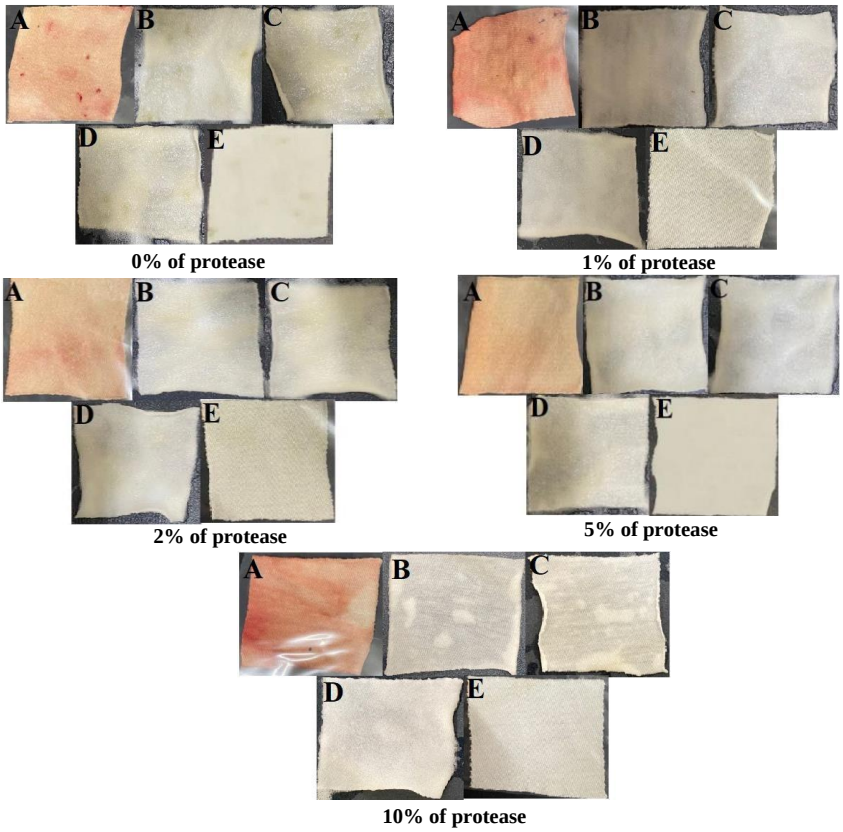

Figure 3: Visual inspection of cotton cloth for mixture of commercialised detergent Y with varying wt % of protease at room temperature (A) Initial (B) after 5 minutes (C) after 10 minutes (D) after 15 minutes (E) dried sample

Protein, according to Novozymes (2021), is made up of long chains of bonded amino acids, which is why detergents have a difficult time removing it. Protease enzymes attack protein stains by breaking down protein molecules into shorter chains of amino acids that can be easily removed from the cloth during washing. Because detergents cannot remove the blood stain, protease is required to remove the stain on the cotton cloth. The detergent powder was mixed with protease before being poured into 400 mL of water. Protein peptide bonds will be hydrolysed by the protease. Amino acids, which are the basic building blocks of protein polymers, are used to create them. Peptide chains are formed by connecting amino acids. A peptide bond connects two amino acids. Exoproteases and endoproteases are types of proteases that can break peptide bonds with water. Exoproteases are enzymes that cleave peptide chains from either end of a single amino acid. Endoproteases are enzymes that degrade peptide bonds in protein chains. As a result of hydrolysis, this type of assault frequently results in smaller polypeptides and peptides (Smith & Olson, 2002).

In comparison to Zhang et al. (2019), Sathishkumar et al. (2015), and Annamalai et al. (2013), this experiment used the shortest time to remove the blood stain, which was 15 minutes. Zhang et al. (2019) wash the stained cloth for 30 minutes and the blood stain is removed. Sathishkumar et al. (2015)

incubated the cotton cloth for 30 minutes, washing it every 5 minutes with distilled water, but the blood stain was not completely removed. Annamalai et al. (2013) removed the blood stain from the stained cotton fabric in 25 minutes. This demonstrates that different detergent brands and protease types produce different results. Table 1 compares the current findings to other researchers.

Table 1: Comparison on the effect of protease with previous researchers

Author	Enzyme	Formulation	Optimum condition for removing blood
Zhang et al., 2019	Halotolerant metalloprotease	15 mL of tap water, 5 mg/mL of liquid detergent, 490 U/mL of diluted liquid detergents that included crude protease.	Liby, Bluemoon, and Walch liquid detergents were tested for 30 minutes at 15 - 45 °C with this crude halotolerant metalloprotease.
Sathishkumar et al., 2015	*Virgibacillus halodenitrificans* RSK CAS1 protease	100 mL distilled water, 1 mL of commercial detergent, 3 mL of purified enzyme.	Blood-stained cloth washed at 50 °C for 30 minutes with protease enzyme and commercial detergent.
Annamalai et al., 2013	*Bacillus halodurans* CAS6	100 mL of distilled water, 1 mL of 5 mg/mL commercial detergent, and 2 mL of 500 U/mL refined enzymes.	Within 25 minutes, a commercial detergent containing enzymes had removed the blood stain.

4.2 Effect of Washing Temperature

The best result after testing the effectiveness through the detergent powder composition is 10 wt % of protease. This composition is tested at four different temperatures: 27 °C, 40 °C, 60 °C, and 80 °C. Every 5 minutes, the cotton cloth is removed from the washing machine to be visually inspected. Figure 4 depicts the results of all samples tested at various temperatures. The results show that the cotton cloth washed with 27 °C water removed the most bloodstains and had the whitest colour when compared to the others. The results show that the protease is more effective when washing the cotton cloth with room temperature water and is ineffective when washing the cotton cloth with 80 °C water.

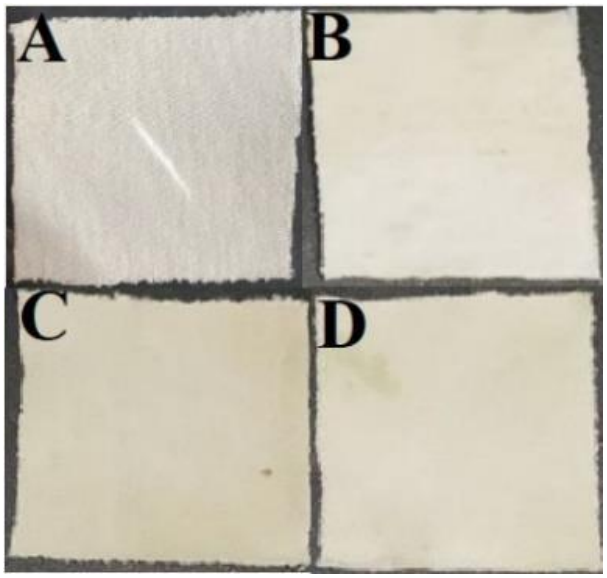

Figure 4: Visual inspection of cotton cloth for mixture of commercialised detergent X with 10 wt % of protease at various temperature (A) 27 °C (B) 40 °C (C) 60 °C (D) 80 °C after 15 minutes

This experiment yielded the best results at 27 ℃. Sathishkumar et al. (2015) performed the experiment at 50 ℃, but the blood stain on the cotton cloth was not completely removed. According to Mechri et al. (2017) and Paul et al. (2014), the best results were obtained at 60 ℃ and 50 ℃. In comparison to other researchers, this experiment has a lower temperature of 27 ℃ or room temperature. This is due to the fact that different types and compositions of enzyme were added to the detergent powder. Table 2 compares the effect of temperature with the findings of other researchers.

Table 2: Comparison on the effect of temperature with previous researchers

Author	Enzyme	Formulation	Optimum condition for removing blood
Mechri et al., 2017	Serine alkaline protease (*Lysinibacillus fusiformis* strain C250R)	Tested at different temperature: 30 – 90 ℃	Blood stains can be removed in 60 minutes by using a detergent containing enzymes at 60 ℃ and pH 9-11.
Paul et al., 2014	Crude kerantinase enzyme	The experiment was tested under 25 ℃, 50 ℃, and 75 ℃	Blood stains was completed removed by combination of crude keratinase and commercial protease at pH 9 and temperature of 50 ℃ for 30 minutes.
Sathishkumar et al., 2015	*Virgibacillus halodenitrifica ns* RSK CAS1 protease	Tested at 50 ℃ only	Blood-stained was completely removed at 50 ℃ for 30 minutes with protease enzyme and commercial detergent.

5. Conclusion

In conclusion, the addition of protease to commercialised detergent powder improves the efficacy of removing blood stains. This is primarily due to the presence of protein in the blood, and protease is capable of breaking down protein peptide bonds. After only 5 minutes of washing, the blood was quickly removed. After 10 to 15 minutes, the detergent powder mixture removed only a small amount of blood. Furthermore, the amount of protease used increases the efficiency of blood stain removal. The optimum blood stain removal parameter is 10% protease at a temperature of 27 ºC.

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