Evaluation of enzymes in laundry detergents as effective stain removers

[1]A. M. Sylaja, [2]Sadaf Kalam, [3]Naga Vedika and [4]Rajeshwari Kandadi
[1,2] Lecturers, [3,4] Students
[1,2,3,4] Department of Biochemistry, St. Ann's College for Women, Mehdipatnam, Hyderabad- 500028, Telangana, India.

ABSTRACT
Enzymes are the most important functional components of detergents contributing to development and improvement of commercial detergents. Laundry detergents principally comprise of four major classes of enzymes, viz., proteases, lipases, amylases, and cellulases. Each enzyme catalyses specific reactions on the stains, thereby enhancing washing performance and efficacy of the detergent. The present research work is a laboratory exercise to explore the stain removing capacity and cleaning efficiency of locally available laundry detergents. This discussion aims at comparing different brands of detergents, containing various enzymes with known composition with those which do not contain enzymes having unknown composition.

INDEX TERMS- Detergents, enzymes, stains, known composition, unknown composition, stain removing capacity

I.INTRODUCTION

Over the past four decades, enzymes have played an important role in the development and advancement of an array of detergent products. Before the advent of modern detergent formulations, soap and sodium carbonate were the major detergent ingredients, and the cleaning action in laundering could be credited to only the mechanical action [1]. In this direction, presently, enzymes have become the integral functional components in detergents contributing to efficient, mild eco friendly cleaning of laundry. Enzymes as are derived from renewable sources, prove to be environmentally safe and have garnered adequate attention in detergent industry [2].

Laundry stains and dirt come in various forms including proteins, starches and lipids. The deliberate use of detergents in water at high temperatures along with vigorous mixing, removes most type of stains and dirt but leads to shortening of clothing life and other materials. Introduction of detergents with enzyme formulations have been a major breakthrough in detergent research and development [3]. Use of enzymes allows lower temperatures to be employed with shorter periods of agitation with only a preliminary period of soaking. Generally, enzyme detergents remove protein from clothes soiled with blood, milk, sweat, etc. far more effectively than non-enzyme detergents [4]. Among the enzymes, proteases have dominated the detergent market, whereas, there has been enhancement in employing amylases and lipases for the removal of starchy and fatty stains [5,6]. There has been a recent entry of cellulases, in the detergent market, and it's mode of action is to directly act on the fabric rather than to degrade particular stains. Various patents too have been filed for various enzymes in detergents [7].

Enzymes have been used in surprisingly small amounts in most detergents encapsulated in dust-free granulates [8]. Once released from its granulated form, the enzyme must withstand pH values between 8.0 and 10.5 and must retain activity upto 60˚C. Microbial sources have contributed significantly to cheaper enzyme production. Several species of *Bacillus* effectively produce these enzymes, including proteases like Alcalase, from *B. licheniformis*, Esperase, from an alkalophilic strain of a *B. licheniformis* and Savinase, from an alkalophilic strain of *B. amyloliquefaciens*. *B. licheniformis* supply Maxatase, which works between 10-65˚C and pH 7-10.5 [9,10]. Savinase and Esperase are active up to pH 11 and 12, respectively. Suitable enzyme formulations are been marketed by detergent manufacturers in powder as well as liquid forms for domestic use.In developed countries, the use of enzymes based detergent formulations has become very common [11]. Although, detergent industry is the largest industry with 25-30% of total sales, research targeting the comparative analysis and efficacy of various detergent brands has rarely been published.

With this background we have formulated our present research work to conduct a comparative study of different brands of detergents, containing various enzymes with those which do not contain enzymes.

II. MATERIALS AND METHODS

1. Detergents- The following detergents were used in order to identify their mode of action.
They were further classified into two groups based upon their composition.
(A). Detergents that contain enzymes (Brands)
1. A1; 2. A2; 3. A3; 4. A4; 5. A5
(B). Detergents with unknown composition (Brands)
1. B1; 2. B2; 3. B3; 4. B4; 5. B5

2. STAINS
The stains employed while performing the experiment were-

1. Pomegranate- The seeds of the fruit were taken and smashed on the cloth pieces to form the stain.
2. Pickle- A homemade pickle was taken and applied to the cloth pieces.
3. Ink- A standard quality ink was applied on to the cloth pieces and were dried.
4. Peanut butter- A readymade peanut butter available in the market was applied on to the cloth pieces.

5. Vermillion (kumkum)-A packet of vermilion was mixed with some water to form a paste and was applied to the cloth pieces.
6. Nail polish- A yellow color (any color of choice) nail polish was applied to the cloth pieces.
7. Black dye- A black dye available in the market was mixed with water and the paste was applied to the cloth pieces.
8. Egg yolk- Egg yolk from some eggs was collected and was applied on to the cloth pieces.
9. Soy sauce- The soy sauce available in the market was applied to the cloth pieces.
10. Grease- Grease was applied to the cloth pieces and dried. All the stained cloth pieces were dried completely before washing

3.CLOTH

The protocols followed for evaluating detergent performance were according to the methodology followed by [12,13]. A regular cloth piece (white) was taken and cut into uniform size (10cm x 10cm). A circle was drawn with a marker in order to differentiate the stained portion from the unstained portion. Various stains were applied on the cloth pieces in triplicates (Fig. 1). Stain removing capacity of different detergents was achieved by comparing the two categories of enzymes *viz*., (A) Detergents with enzymes and (B) Detergents with unknown composition. A total of 10 different controls were prepared by applying the stain on measured size of cloth. Beakers (200 ml) were taken and filled with 100mL of water. To each beaker 1 spatula of detergent (weighing, 1.20g approximately) was added and mixed thoroughly. The dried stained cloth pieces were soaked in the beakers for about 10 minutes (Fig. 2). After 10 minutes, the cloth pieces were handwashed, dried and preserved for observation (Fig. 3, 4)

III. RESULTS AND DISCUSSION

As depicted in Fig 5 and tables- 1.1, 1.2, 1.3, 1.4 and 1.5; the following observations were recorded for the detergents containing enzymes, indicating their stain removal efficiency-A1 detergent, exhibited 100% efficiency by removing 5 different stains, 95% of two stains, 90% of two different stains and least efficiency towards one stain. A2 detergent showed 100% efficiency by removing four different stains, 95% of two stains, 90%, 80%, 70%,50%, of other stains. A3 detergent was able to remove 100% egg yolk stain, 95%, 90% each of one stain respectively,80% of five stains, 75% of nail polish stain and 50% of Black dye stain. A4 detergent could remove two stains 100% efficiently, 95% of soy sauce, 90% of four different stains, 85%, 60%, 50% one of each stain respectively.A5 detergent could remove five different stains 100% efficiently, 95% of one stain,90%, 85%, 80% of each one stain respectively.

With regards to detergents with unknown composition, as shown in Fig 6 and tables- 2.1, 2.2, 2.3, 2.4 and 2.5, the following observations were recorded indicating their stain removal capacity- B1 detergent could effectively remove six different stains with 100% efficiency, one stain 90% efficiently, 80% of two stains, one stain upto 60%. B2 detergent could remove 100% efficiently three different stains, 95% , 90% of each two stains, 75%, 70%, 60% of each one stain respectively. B3 detergent could remove two different stains with 100% efficiency, 90% of two stains, 80% and 50% of three stains each respectively. B4 detergent could remove two stains 100%, 70%, 60% efficiently 95%, 90% , 75%, 50% of each one stain. B5 detergent could remove three stains 100% effectively, 95%, 85%, 80%, 70%, 50% of one each stain respectively and 60% of two stains.

Thus, A1 detergent was more effective than the other detergents with enzymes. It could remove most of the tough stains like nail polish, dye, ink, and pomegranate with 100% efficiency which other detergents could not remove. Whereas, among the detergents of unknown composition, B1 was found to be effective as it could remove most of the stains like pomegranate, peanut butter, Vermillion (kumkum), egg yolk, soya sauce and grease 100% effectively.

Experiments with locally available detergents targeting their stability and compatibility with various enzymes have been conducted by several researchers [9,10, 11, 12, 13]. Our results were in complete agreement with observations of other researchers.

Future prospects

Although, enzymes in detergents have provided new developments in detergent industries. Still, there is a requirement for environmentally safe laundry detergents with smaller quantities of organic and inorganic chemical ingredients. In addition to this core research into detergents with lesser requirement of water, energy and maximal washing performance at lower temperatures would lead to development of effective, laundry detergents. We need to explore eco-friendly alternatives which can be employed in cleaning the laundry efficiently without the use of hazardous and toxic chemicals.

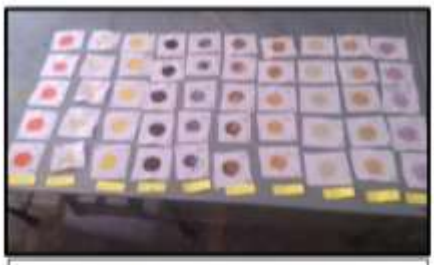

Fig. 1. Application of various stains -:1- Pomegranate juice, 2-Pickle, 3-Ink, 4-Peanut, 5-Vermilion. 6-Nail polish,7- Black dye, 8-egg yolk, 9-Soy sauce, 10-Grease

Fig. 2.Stained cloths soaked in different detergents.

Fig. 3.Stained cloths, treated with detergents having known composition,containing enzymes, dried and segregated.

Fig. 4. Stained cloths, treated with detergents having unknown composition, dried and segregated.

Table 1.1 STAIN REMOVING CAPACITY OF A1 DETERGENT

	% of stain removed	Stain applied
A1 DETERGENT	100%	Pomegranate, peanut butter, Vermillion, egg yolk, soy sauce
	95%	Pickle, Grease
	90%	Nail polish, Black dye
	70%	Ink

Table 1.2 STAIN REMOVING CAPACITY OF A2 DETERGENT

	% of stain removed	Stain applied
A2 DETERGENT	100%	Pomegranate, egg yolk, soy sauce, Grease
	95%	Peanut butter, vermillion
	90%	Nail polish
	80%	Black dye
	70%	Pickle
	50%	Ink

Table 1.3 STAIN REMOVING CAPACITY OF A3DETERGENT

	% of stain removed	Stain applied
A3 DETERGENT	100%	Egg yolk
	95%	Soy sauce
	90%	Peanut butter
	80%	Pomegranate, pickle, Ink, vermilion, Grease
	75%	Nail polish
	50%	Black dye

Table 1.4 STAIN REMOVING CAPACITY OF A4 DETERGENT

	% of stain removed	Stain applied
A4 DETERGENT	100%	Peanut butter, egg yolk
	95%	Soy sauce
	90%	Pomegranate, pickle, ink, vermilion
	85% 60% 50%	Grease Black dye Nail polish

Table1.5 STAIN REMOVING CAPACITY OF A5 DETERGENT

	% of stain removed	Stain applied
A5 DETERGENT	100%	Pickle, peanut butter, egg yolk, soy sauce
	95% 90%	Vermilion ,pomegranate Grease
	85% 80% 60%	Black dye Nail polish Ink

Table 2.1 STAIN REMOVING CAPACITY OF B1 DETERGENT

	% of stain removed	Stain applied
B1 DETERGENT	100%	Pomegranate, peanut butter, vermilion , egg yolk, soy sauce, grease
	90%	Pickle
	80% 60%	Nail polish, black dye Ink

Table 2.2 STAIN REMOVING CAPACITY OF B2 DETERGENT

	% of stain removed	Stain applied
B2 DETERGENT	100%	Peanut butter, egg yolk, soy sauce
	95%	Grease, pomegranate
	90%	Pickle, vermilion
	75% 70% 60%	Nail polish Black dye Ink

Table 2.3 STAIN REMOVING CAPACITY OF B3 DETERGENT

	% of stain removed	Stain applied
B3 DETERGENT	100%	Egg yolk, soy sauce
	90%	Peanut butter, grease
	80%	Pomegranate, pickle, vermilion
	50%	Ink, nail polish, black dye

Table 2.4 STAIN REMOVING CAPACITY OF B4 DETERGENT

	% of stain removed	Stain applied
B4 DETERGENT	100%	Egg yolk, soy sauce
	95% 90%	Peanut butter Grease
	75% 70%	Vermilion Pomegranate, pickle
	60%	Nail polish, black dye
	50%	Ink

Table 2.5 STAIN REMOVING CAPACITY OF B5 DETERGEN

	% of stain removed	Stain applied
B5 DETERGENT	100%	Egg yolk, soy sauce, grease
	95%	Peanut butter
	85% 80% 70% 60%	Pickle Pomegranate Nail polish Vermilion, Black dye
	50%	Ink

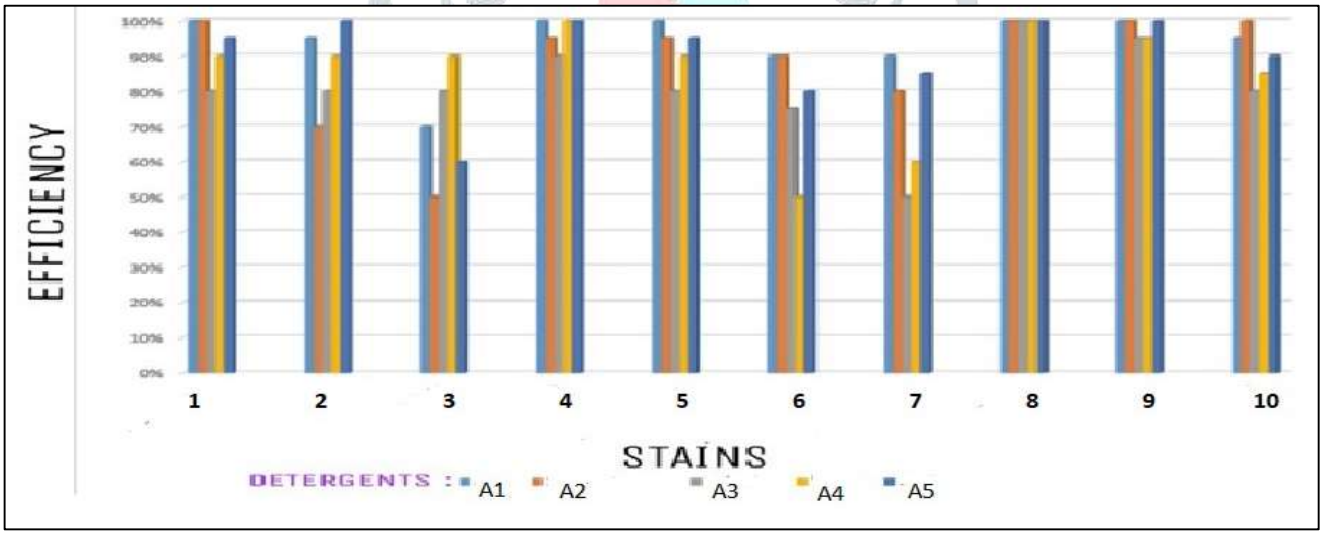

Fig. 5 STAIN REMOVING CAPACITY OF DETERGENTS WITH ENZYMES

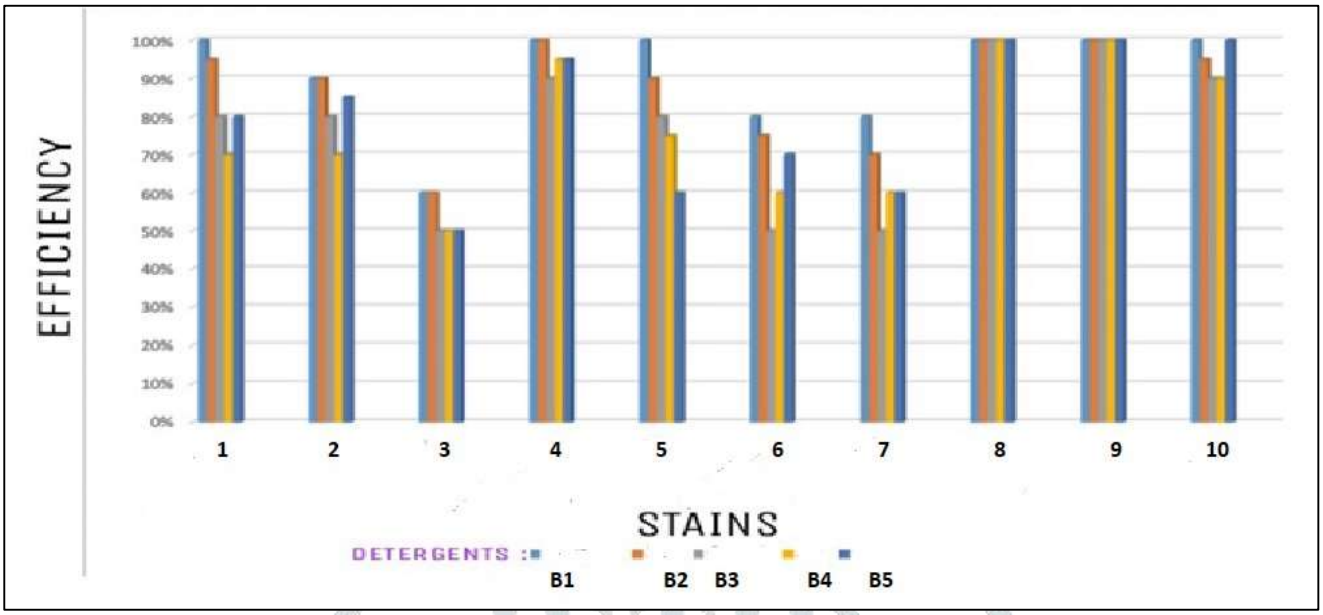

Fig. 6 STAIN REMOVING CAPACITY OF DETERGENTS WITH ENZYMES

Stains -:1-Pomegranate juice, 2-Pickle, 3-Ink, 4-Peanut, 5-Vermilion. 6-Nail polish, 7- Black dye, 8-Egg yolk, 9-Soy sauce, 10-Grease

IV. ACKNOWLEDGEMENT

The authors are thankful to Principal Sister Amrutha, St. Ann's College for women, for providing the necessary laboratory facility and for financial assistance.

V. REFERENCES

1. Olsen, H. S. and Falholt, P. (1998). The role of enzymes in modern detergency. Journal of Surfactants and Detergents, 1(4), 555-567.
2. Saperas, N. and Fonfría-Subirós, E. (2011). Proteolytic enzymes in detergents: evidence of their presence through activity measurements based on electrophoresis. Journal of chemical education, 88(12): 1702-1706.
3. Hasan, F. Shah, A. A. Javed, S. and Hameed, A. (2010). Enzymes used in detergents: lipases. African Journal of Biotechnology, 9(31), 4836-4844.
4. Kumar, C. G. Malik, R. K. and Tiwari, M. P. (1998). Novel enzyme-based detergents: an Indian perspective. Current Science, 75(12), 1312-1318.
5. Houston, J.H. (1997). In: Enzymes in Detergency, edited by J.H. van Ee,O. Misset, and E.J. Baas, Marcel Dekker Inc., New York, 1997,Surfactant Science Series, Vol. 69, pp. 11–21.
6. Showell, M.S. (1998). In: Powdered Detergents, edited by M.S. Showell, and E.J. Baas, Marcel Dekker Inc., New York, 1998, Surfactant Science Series, Vol. 71, pp. 1–19.
7. Maurer, K. H. Weiss, A. Paech, C. G. Goddette, D. W. Christianson, T. M. Tang, M. R. and Wilson, C. R. (2001). U.S. Patent No. 6,197,589. Washington, DC: U.S. Patent and Trademark Office.
8. Eriksen, N (1996).In: Industrial Enzymology; Godfrey, T., West, S., Edited by Macmillan Press LTD: London, pp 187-200.
9. Kiran, K. K. and Chandra, T. S. (2008). Production of surfactant and detergent-stable, halophilic, and alkali tolerant alpha-amylase by a moderately halophilic *Bacillus* sp. strain TSCVKK. Applied Microbiology and Biotechnology, 77(5):1023-1031.
10. Schallmey, M. Singh, A. and Ward, O. P. (2004). Developments in the use of Bacillus species for industrial production. Canadian Journal Microbiology, vol. 50, n. 1, p. 1-17.
11. Kuddus, M. and Ramteke, P.W. (2009). Cold-active extracellular alkaline protease from an alkaliphilic *Stenotrophomonas maltophilia*: production of enzyme and its industrial applications. Canadian Journal of Microbiology, 55 pp. 1294-1301
12. Ahmed, M., Rehman,R., Siddique, A. et al. (2016). Production, purification and characterization of detergent, stable, halotolerant alkaline protease for eco-friendly application in detergents' industry. International Journal of Biosciences. 8(2):47-65.
13. Alonso, L. Chaidez, A and Hannah, L. et al. (2017). Investigation of Cleaning Power: An Experiment to Practice Experimental Design. Journal of Laboratory Chemical Education. 5(4): 67-78.