IJARSCT

ISSN (Online) 2581-9429

IJARSCT
Impact Factor: 6.252

International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)

Volume 2, Issue 3, December 2022

Extraction of Lipase Enzymes from Animals and Birds' Fats and its Activity on Margarine

Mr. Kunal Thakur[1] and Mr. Udaybhan Yadav[2]

Assistant Professor, Department of Microbiology, ZSCT's Thakur Shyamnarayan Degree College, Kandivali, Mumbai[1]

Coordinator, ZSCT's Thakur Shyamnarayan Degree College, Kandivali, Mumbai[2]

Abstract: *Lipases are extremely adaptable enzymes that have caught the interest of numerous industrial processes. Animal, vegetable, and microbial sources of lipase are all possible. Long chain triglycerides are hydrolyzed by microbial lipases. The lipase enzymes' microbial origins are theoretically flexible and capable, and they also have a wide range of industrial uses in the production of modified molecules. The hydrolysis, esterification and alcoholysis reactions were all catalyzed by the special lipase (triacylglycerol acyl hydrolase) enzymes. Since immobilization has improved the performance of microbial lipases, they are now suited for a variety of reactions and required to add scent to the immobilization processes. The immobilization method and carrier type affect the immobilized enzymes. When choosing a carrier, factors including biocompatibility, chemical and thermal stability, insolubility during reactions, ease of rejuvenation and reusability, as well as with lipases serving as a multifunctional biological catalyst, it is now possible to meet the demands of a number of industries, including those that produce biodiesel, foods and beverages, leather, textiles, detergents, and medicines and medical products. This paper discusses microbiological sources of lipases, techniques of immobilization that boost output and market profitability, and logistical considerations that lessen risk to the environment and the user.*

Keywords: Lipase, Immobilization, Fats, Triglycerides

I. INTRODUCTION

In comparison to lipases derived from plants or animals, microbial lipases are more desirable because of the wide range of catalytic actions they can perform, the high yield production, ease of genetic manipulation, lack of seasonal changes, and regular supply more stability safer and more convenient and the growth rate of microorganisms very high in economically media [1]. The bacterial isolates offer higher activities such as neutral or alkaline pH optima and the thermostability associated with yeasts [2]. Bacterial strains such as *Pseudomonas alcaligenes, P. aeruginosa, P. fragi, P. fluorescens BJ-10, Bacillus subtilis, B. nealsonii S2MT* and some species of fungi are *Penicillium expansum, Trichoderma, Penicillium chrysogenum; Aspergillus niger*produces lipases in higher quantities [3]. The market is expanding because microbial lipases have greater advantages over animal and plant lipases. Due to their numerous applications in food processing, microbial sources are expected to be in high demand in the near future [4]. Lipase was successfully substituted for harsh chlorine bleach thanks to the development of industrial microbial lipases, which also helped to reduce sewage and industrial contamination of freshwater. Due to its stability, ease of handling, and convenience in packing and transportation, microbial lipases in the form of powder are anticipated to dominate the microbial lipase markets [5]. These have broad applications in a variety of other industries, including agrochemicals, dairy, food and beverage, animal feed, cleaning, biofuel, pharmaceuticals, textile cosmetic, perfumery, flavor industry, fine chemical production, esters and amino acid derivatives, and biocatalytic resolution [6].

There are several cutting-edge purification techniques available to obtain consistent amounts of lipase from a wide range of bacteria and fungi [7]. Depending on the purity assumed for food use, multiple processes are typically included in the purification of lipases. Cells are liberated during the fermentation process after the extracellular microbial lipases from the culture broth are removed by centrifugation or filtering [8,9]. The cell-free culture broth is concentrated using ammonium sulfate precipitation, ultrafiltration, or extraction with organic solvents [133]. About 80% of the sample was purified using precipitation processes, followed by 60% ammonium sulfate and 35% ethanol utilizing affinity chromatography and gel filtration. The final step in gel filtering results in a homogeneous product.

II. METHODOLOGY

All chemicals were purchased from S. D fine chemicals and were of analytical grade.

2.1 Sample Collection and Isolation of Pure Cultures

A Fat sample was collected in a container from an animal (Mutton) and bird (Chicken). The fat sample was kept open for 1 week in open air to allow the occurrence of microbial and fungal activity at room temperature.

After 1 week the sample was suspended in sterile saline solution and then the saline suspension of the sample was plated on a Nutrient agar plate.

1st plate: Animal fat saline suspension

2nd plate: Bird fat saline suspension

2.2 Enrichment of Extracted Organisms

The organisms were plated into the NA (Nutrient agar) plates, and kept for incubation for 24hrs at 37°C.

The 24 hours old culture was resuspended into the Nutrient Broth for next 24 hours as an incubation process and used for lipase extraction.

2.3 Lipase Extraction

Using the NaOH the isolated organisms were subjected to cell lysis, and the matter was taken as density gradient centrifugation at 6000rpm and the enzyme was extracted.

2.4 Estimation of Extraction Lipase by KI (Potassium Iodide) Method

Requirements

1. Lipid (cooking oil can be used)
2. Acetic acid
3. Chloroform
4. Potassium iodide
5. Sodium thiosulfate
6. Starch

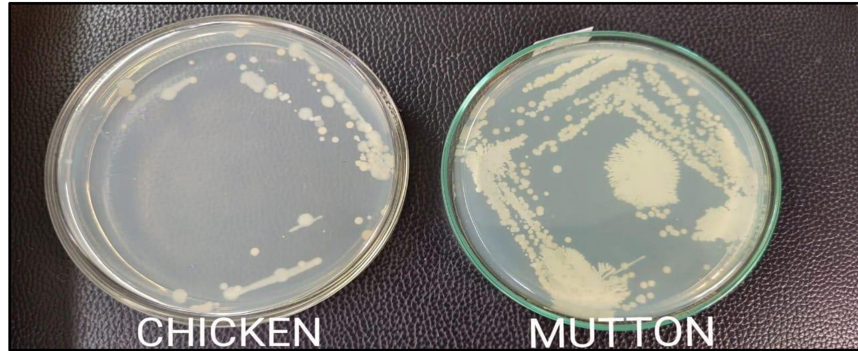
Rancidity is brought about by the action of air (oxidative rancidity) or by microorganism (ketonic rancidity) in oil. In the oxidative rancidity oxygen is taken up by the fat with the formation of peroxide. The degree of peroxide formation and the time taken for the development of rancidity differ among oil.

PV is an important quality control tool and measures the amount of hydroperoxides that are formed in the oil during lipid oxidation. Lipid oxidation occurs adjacent to double bound in unsaturated lipids, creating hydroperoxides. These peroxides, however, are very reactive and may actually decrease during the storage of the lipid (or food containing lipids). Therefore, a very high or very low PV provides little information about the quality of the lipid unless the storage history of the sample is known or if the samples are tested periodically over time (i.e. Once a week for 6 months).

The peroxide value is determined by measuring the amount of iodine liberated from a saturated solution of potassium iodide solution by a lipid dissolved in a solution of acetic acid and chloroform.

- Weight a known amount of lipid into an Erlenmeyer flask (5 g, record exact amount). Conduct in duplicate.
- In the hood, dissolve the lipid in 30 mL of a solution of acetic acid and chloroform (3 parts acetic acid and 2 parts chloroform, this will be pre-prepared for you).
- Mix to dissolve lipid, add 0.5 mL of saturated KI (potassium iodide), and return to the lab for the titration. Allow mixture to stand for 1 minute, and mix occasionally.
- Add 30 mL of water to the solution and gently mix.
- Slowly titrate the liberated iodine with a standard solution of sodium thiosulfate (commercial stock solution will be provided) while stirring until the pale-yellow color is nearly gone.
- When the pale-yellow color begins to disappear, add 0.5 mL of starch solution (1%) to help free any color is gone.
- Record volume of sodium thiosulfate. S x N x 1000 %PV = Weight of lipid Where, S = mL of sodium

thiosulfate and N = normality of the sodium thiosulfate. Note: A bank should also be set at the same time.



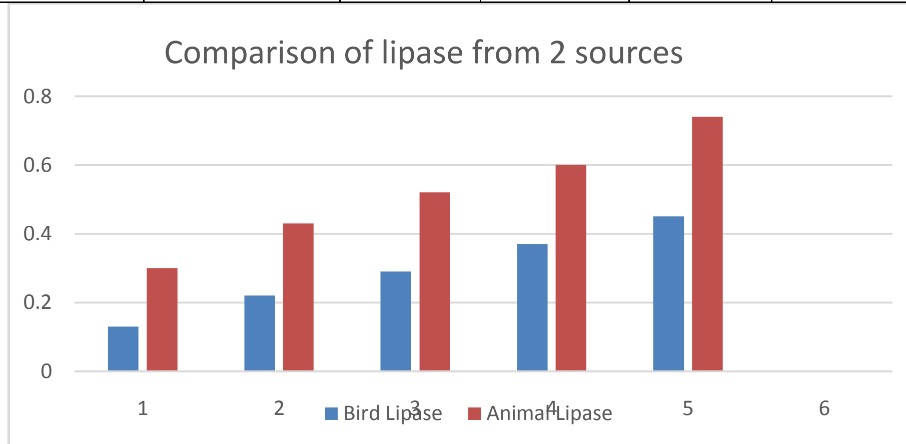
2.5 Observation Table

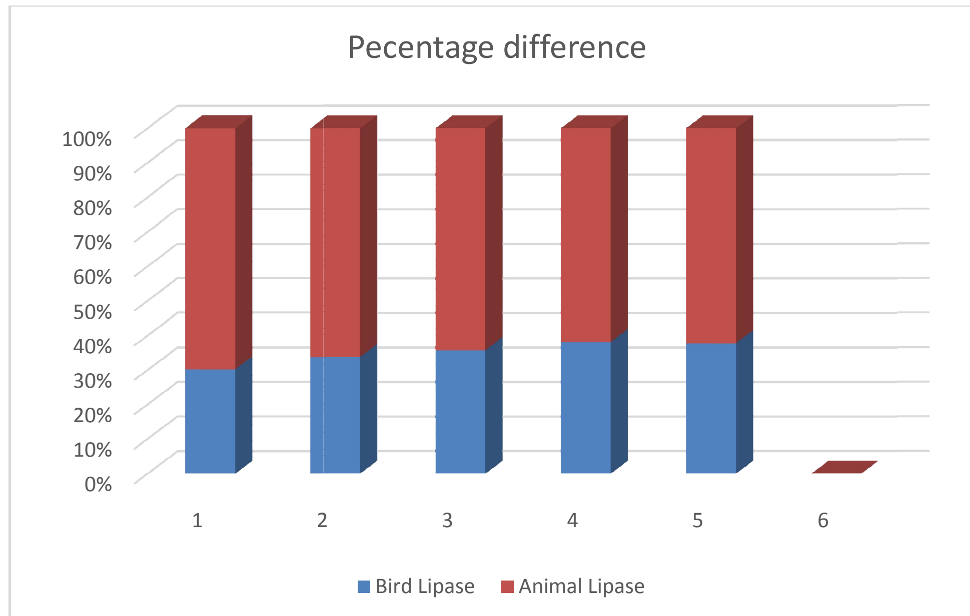
Animal source

Sr. No	Lipid (stock) (ml)	Diluent(D/W) (ml)	Enzyme (ml)		KI solution (ml)		OD at 540 nm
1	0.2	0.8	1		2	Add 2 drops of Starch Indicator with 1 ml $Na_2S_2O_3$	0.3
2	0.4	0.6	1	Incubate at 37^0C for 2 hours	2		0.43
3	0.6	0.4	1		2		0.52
4	0.8	0.2	1		2		0.60
5	1.0	0.0	1		2		0.74
6	Blank	1.0	1		2		0.0

Bird Source

Sr. No	Lipid (stock) (ml)	Diluent(D/W) (ml)	Enzyme (ml)		KI solution (ml)		OD at 540 nm
1	0.2	0.8	1		2	Add 2 drops of Starch Indicator with 1 ml $Na_2S_2O_3$	0.13
2	0.4	0.6	1	Incubate at 37^0C for 2 hours	2		0.22
3	0.6	0.4	1		2		0.29
4	0.8	0.2	1		2		0.37
5	1.0	0.0	1		2		0.45
6	Blank	1.0	1		2		0.0





III. RESULT

The more lipase producer's were extracted from the Animal source as compared with the Bird source.

IV. CONCLUSION

In comparison to lipases derived from plants or animals, microbial lipases are more desirable because of the wide range of catalytic actions they can perform, as from the comparison the more quantity of lipases can be obtained from the Animals than the Birds. Also, the structural components are valid for these differences.

REFERENCES

[1]. Oterholm, A. and Ordal, Z.J. (1965) Improved methods for detection of microbial lipolysis. J. Dairy Sci. 49, 1281-1284.

[2]. Fryer, T.F., Lawrence, R.C. and Reiter, B. (1966) Methods for isolation and enumeration of lipolytic organisms. J. Dairy Sci. 50, 477-484.

[3]. Mourney, A. and Kilbertus, G. (1976) Simple media containing stabilised tributyrin for demonstrating lipolytic bacteria in food and soils. J. Appl. Bacteriol. 40, 47-51.

[4]. Kouker, G. and Jaeger, K. E. (1987) Specific and sensitive Plate Assay for Bacterial Lipases. Appl. Environmental . Microbiol. 53, 211-213.

[5]. Karnetova, J., Mateju, J., Rezanka, T., Prochazka, P., Nohynek, M. and Rokos, J. (1984) Estimation of Lipase activity by the diffusion Plate method. Folia Microbiol. 29, 346-347.

[6]. Brockerhoff, H. and Jensen R.G. (1974) Lipolytic Enzymes. Academic Press, New York.

[7]. Jensen, R. G. (1983) Detection and determination of lipases (acylglycerolhydrolyases) activity from various sources. Lipids 18, 650-657.

[8]. Alford, J.A. and Steinle, E.E. (1967) A doubled layered plate method for the detection of microbial lipolysis. J. Appl. Bacteriol. 30, 488-494.

[9]. Lawrence, R. C., Fryer, T. E and Reiter, B. (1967) Rapid method for quantitative estimation of microbial lipases. Nature (London) 191, 1264-1265.