SUMMARY

Waste animal blood and blood components may include liquid waste, products of blood, items saturated and/or dripping with animal blood that are now caked, as well as dried animal blood including, serum, biochemicals and bedding of animals that were known to have been exposed to infections agents during research (including in veterinary hospitals) production of biological or testing pharmaceuticals. Therefore, the present study aims at the use animal blood as a raw materials for production of alkalophilic thermostable microbial protease and lipase for application in Bio-detergent technology as well as production of fibrinogen from blood.

This study includes three parts; isolation and identification of proteolytic and biolytic bacteria from waste animal blood, study of some environmental and nutritional parameters controlling the enzymes productivity by the selected bacteria. Second part, production of protease and lipase enzymes and study the enzyme stability. Third part; enzymes application in biodetergent technology and fibrinogen production from animal blood.

To achieve these aims :

Fifty bacterial isolates were isolated from animal blood. These isolates were purified and selected to first screening program to evaluate their proteolytic and lipolytic activities. Out of these ten isolates gave the highest proteolytic and lipolytic productivities on synthetic media. The most proteolytic and lipolytic bacterial isolates were allowed to grown on animal blood wastes as sheep source media for production of alkaline enzymes for application in biodetergent.

Identification of the two bacteria was carried out to the genera and species level where *Bacillus* was identified as *B. cereus* and *Pseudomonas* was identified as *Ps. fluorescens*. As well as identification *Ps. fluorescens* was carried out to strain level where *Ps. fluorescens* was identified as Egyptian strain based on 16s rRNA and lipase genes with expected 1154 and 1525 bp sizes. As well as conformed by nucleotide and amino acids sequencing and compared with other strains recorded in Gene Bank.

Factors affecting for hydrolytic enzyme productivities, produced by *B. cereus* and *Ps. fluorescens* were investigated. The effect of different incubation temperatures and pH were the first parameters controlling protease and lipase productivities by *B. cereus* and *Ps. fluorscens* EG srains. The previous studies revealed that to two bacterial isolates exhibited highest enzyme productivities. So all the parameters were conducting using these conditions. The performed parameters were different incubation temperatures initial pH values substrate concentrations carbon sources, nitrogen sources, inocula sizes, incubation periods, metal ions vitamins, LA concentrations, amino acids, static and shaking conditions, and use bottle capacities on two alkaline enzymes productivities.

Protease and lipase produced by *B. cereus* and *Ps. fluorescents* growing on animal blood wastes under solid state fermentation ere selected due to their high productivity for further investigation.

The purification of the enzymes included preparation of cell free filtrate applying precipitation by ammonium sulfate dialysis (against tap water and sucrose respectively) and perform once of sephadex followed by thin layer chromatographic technique.

Purified enzymes increase of specific activities, where the specific activities were 1900.38 and 8.80 U/mg^{-1} protein corresponding to 310.38 and 18.90 fld purification for purified protease and lipase produced by *B. cereus* and *Ps. fluorescens*.

The amino acid composition of two alkaline purified enzymes preparation was carried out. The results obtained emphasized that acidic amino acids recorded a high quantity in all purified enzymes, the quantities of the all other detected amino acids varied greatly with the type of amino acid. On the other hand was not methionine and tyrosine in the lipase enzyme detected.

Enzymes stability and factors affecting on the purified enzyme activities produced by *B. cereus* and *Ps. fluorescents* were investigated. A summary of results as following :-

The purified protease and lipase are stable at the maximum temperature with activities at 30ºC pH for maximal enzyme activities 9 and 7. The purified enzymes exhibits good stability with chlorine. The optimum incubation temperature 60ºC and pH were 9 and 7 respectively. The enzyme activities increased gradually with the increase of time up to 36 h incubation of the reaction mixture and concentration of both enzyme and substrate with …. % and … % of gelatin and substrate with …% and …% gelatin and tributyrine exhibited maximal activity of protease an lipase respectively. The effect of different metallic ions and oxidizing agents and surfactants on purified enzymes activities recorded that 15 ul/ml of chlorid. Oxidizing agents and surfactants showed that enzymes not only stable but enhanced maximal activity by certain agents e.g. between 20 for protease and triton X 100 for lipase. Compatibility of purified enzymes with different commercial

detergents reported that protease and lipase are instable in presence cholorax and cholorine but are able to restore and enhance protease and lipase activity kin the presence of calcium chloride.

The wash performing analysis of the present enzymes revealed that, it could effectively removed a variety of stains such as blood, chocolate, mango, strawberry, salad when treated at 30ºC and 50ºC for 15 min, when alkalophilic crude or purified enzymes whereas added separately or in combination with or without detergent (Rubso) as an Egyptian product.

This study describes a purification protocol of animal fibrinogen that gives a consumable and highly clottable fibrinogen, some characteristics of animal and human fibrinogen are compared. Fibrinogen was purified from ammonium sulfate precipitation followed by ultra filtration the clottability of the purified animal fibrinogen was 90%. The α, β and Y chains of animal fibrinogen were homogenous with molecular 90-110; 55 and 50 KDa respectively.