

**UNIVERSITI TEKNOLOGI MARA**

**OPTIMISING BROMELAIN  
MILK CLOTTING ACTIVITY  
FROM JOSAPINE PINEAPPLE  
PEEL EXTRACT FOR SOFT  
CHEESE PRODUCTION**

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## ABSTRACT

During canning and juice processing of pineapple, pineapple peel usually discharged. Discharged of pineapple peel during this production will produce a lot of waste. In industrial practices, pineapple waste is either used as animal feed or disposed to the soil as waste. Pineapple peel contain valuable natural enzyme which is bromelain. Bromelain is enzyme which believed to have many benefits and very promising to the development of food and pharmaceutical industries. The purpose of this study is to determine the milk clotting and physicochemical properties of soft cheese made using bromelain extracted from pineapple peel variety Josapine. Purified bromelain powder from pineapple peel was produced through purification process. These include extraction from pineapple peel using purified water as a medium extraction, purification by ammonium sulphate precipitation, desalting using continuous diafiltrator and followed by freeze drying. Each step was found to produce different effect on bromelain activity, protein content, specific enzyme activity and purification level. It was found that it is best to store the bromelain powder at frozen temperature (-20°C) because its bromelain activity could only loss 10.34% up to one month storage time for maturity index 2. Thus, maturity index 2 was chosen for optimisation step. The optimisation condition for milk clotting activity of bromelain during the production of soft cheese using Response Surface Methodology (RSM) was found with bromelain concentration of 0.53%, incubation temperature of 54.2°C and incubation time of 2.33 hours. It was found that the rheological properties of soft cheese made from pineapple peel and bromelain standard shows a non-Newtonian Pseudoplastic behaviour (shear-thinning). Meanwhile, for the proteolysis of soft cheese using SDS PAGE found that the molecular weight for  $\alpha$ ,  $\beta$  and  $\kappa$  casein was around 30 kDa. In conclusion, the bromelain enzyme extracted from pineapple peels variety Josapine has a potential to be utilized as milk clotting enzymes especially in the production of soft cheese.

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# CHAPTER ONE

## INTRODUCTION

### 1.1 Research Background

Data of studies on commercial production of cheese shows that most of the available cheese in the market are manufactured through the addition of rennet from bovine sources. Rennet, a proteolytic enzyme, is an important element in making cheese as it assists in separating milk into curds and whey. The enzyme used in cheese production comes from the extract of the ruminant stomach. The enzyme, known as rennet, is primarily obtained from a calf's stomach. However, the halal status of the rennet is often doubted. For example, Parmesan cheese is the traditionally made from the rennet enzyme (Vegetarian Times, 2023) and the price for this type of cheese is RM 21.10 for 80 gram (Galbani grated Parmesan cheese) which is expensive (Myweekend Plan, 2023).

Growing demand on Halal-certified cheese among Muslim consumers worldwide must be fulfilled. This warrants comprehensive and rigorous studies to find suitable plant-based enzymes that can be useful for cheese production as a coagulant to cater the increasing need for it worldwide. Bromelain is reported to be an alternative source for the production of cheese and is foreseen to be a suitable rennet enzyme replacement in cheese production in Malaysia. Furthermore, the use of plant-based enzyme in cheese manufacturing promotes the greater acceptability by vegetarians and may improve their nutritional intake (Komansilan *et al.*, 2021). Enzyme used in halal certified cheese include fermentation-produced rennet and vegetarian-friendly rennet. The sources of this type of enzyme usually come from bacteria, fungi, or yeasts (Yvonne Maffei, 2024; Halal Research Council, 2022; Tillamook, 2024).

Pineapple, or *Ananas comosus*, is a perennial plant from the Bromeliaceae family. The pineapple is an edible fruit and contains vitamins, enzymes, and antioxidants such as vitamin C, bromelain enzyme and manganese, respectively. The pineapple fruit is believed to be originated from tropical and subtropical America, and the fruit has come to be spread elsewhere, whereby the fruit can now be found to grow throughout the tropical and subtropical regions of the world. In Malaysia, the pineapple is a major fruit and the nation is ranked the ninth country in the world in terms of