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Gene Expression Analysis of Fruit Bromelain in Ripening of *Ananas comosus* Cultivar MD 2

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Abstract. Fruit bromelain is a proteolytic enzyme harbouring cysteine catalytic residue found abundantly in pineapple fruit. The expression of cysteine proteases is usually regulated during fruit ripening. In the present study, we aimed to study the expression and proteolytic activity level of fruit bromelain during the ripening stage of *A. comosus* cultivar MD 2. The gene expression of fruit bromelain was investigated via relative gene expression analysis using qPCR while the proteolytic activity of fruit bromelain was analysed via enzymatic assay using casein as a substrate. The qPCR analysis revealed that the expression of fruit bromelain was down-regulated 10-fold in ripe pineapple fruits. Besides that, the unripe pineapple fruits [1.9101 ± 0.0831 U/mL] had a higher proteolytic activity than the ripe MD 2 pineapple fruits [1.1333 ± 0.0896 U/mL]. This result showed that the function of fruit bromelain may be related to the protection of young pineapple fruits during the fruit development stage.

Introduction

Ananans comosus or more commonly known as pineapple contains a large amount of sulfhydryl proteolytic enzymes, particularly bromelain. In contrast to stem bromelain (EC 3.4.22.32) concentrated in pineapple stem, fruit bromelain (EC 3.4.22.33) is ubiquitously found in pineapple fruit. Bromelain is classified in the papain superfamily (clan CA, family C1) in the MEROPS database. Owing to their unique cysteine thiol catalytic triad (cysteine-histidine-asparagine), protein hydrolysis occurs by cleaving the glycyl, alanyl and leucyl bonds of the substrates [1]. In addition, bromelain has received high attention in various industrial and therapeutic applications due to its unique properties and advanced commercial values [2].

Information regarding the expression and function of CA1 proteases is still fragmentary. Until now, it has been found that plant cysteine proteinase usually have higher activities in unripe fruits [3]. For instance, higher papain activity has been found in green papaya but reduced to trace amount during maturation [4]. This is also applied to actinidain in kiwifruit. The mRNA level [5], activity and concentration [6] of actinidain keeps increasing until the harvest stage. The differences in CA1 proteinases during the ripening stage might be associated with the defensive function of the protein in plants against pathogens [7].

Pineapple plants are vulnerable to attack by insects and fungus such as *Cyanophora paradoxa*, *Diaspis bromeliea*, mealybugs, etc. which affects the quality of pineapple fruits [8], [9]. MD 2 is a hybrid cultivar which has a bright gold colour, higher vitamin C, sweeter taste, lower fibre and acidity, thinner skin, and longer shelf life [8]. Since fruit bromelain has showed its pathogen resistance potential as observed in transgenic *Arabidopsis thaliana* [10] and *Brassica rapa* [11], we postulate that pineapples fruits should have a higher fruit bromelain expression in its unripe stage to avoid early destruction by its predators and pathogens. To the best of our knowledge, no study has

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