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Enzymatic retting of *Piper nigrum* L. using commercial Pectinase (Peelzyme)

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ABSTRACT

White peppers produced from *Piper nigrum* L. retted with different concentration of commercial pectinase (PeelZyme) and blanching treatment in hot water were compared. The effects of these treatments on surface morphology and piperine content of white berries was studied. PeelZyme at the concentration of 500 ppm successfully produced white berries after 5 days. However, white berries retted with PeelZyme at the concentration of 500 ppm without blanching gave the best surface morphology but there was a reduction in the piperine content by 3.04%. Blanching in hot water resulted in reduction of surface quality but an increase of piperine content up to 40% was obtained.

Keywords: PeelZyme, piperine, morphology, white berries

INTRODUCTION

White pepper is a value-added form of black pepper (*Piper nigrum* L.), where the dark particles, sharp pungent aroma and flavour are undersirable. There is a growing worldwide demand for white pepper due to its mild flavor, pungent aroma and light colour. In 2013, the price of white pepper was quoted at US$7,166/tonne for black pepper and at US$9,300/tonne for white pepper in overseas market. These growing demand for white pepper showed a considerable expansion in the production of white pepper should be done.

Currently, white pepper are produced from fully ripe fruits by soaking the pepper berries in running water for 12 to 14 days. During retting, the pericarp gets rotten, removed by rubbing and deskinned berries are washed and sun dried. This traditional technique is time-consuming, which took almost one month and also requiring a high labour work that limits the productivity of white pepper. White berries may be produced via steaming and mechanical decortication but does not yield the same aroma and flavor.

Improved conventional retting employing commercial pectinase enzyme, PeelZyme aimed to shorten the retting period and improved the end product quality. Therefore, this study was undertaken to evaluate the effect of concentration of PeelZyme and blanching in hot water towards the morphology and piperine content of white pepper produced

METHODOLOGY

Experimental Design: The enzyme used in these experiments was PeelZyme (brand Novo Nordisk from Denmark). The enzyme was added into 0.05 M sodium acetate buffer at pH 5 at final concentration of 500, 1000, and 4000 ppm. In total, 3 g of green fresh pepper that still attached to its spikes were added into 30 ml of the enzyme mixture with the ratio of 1:10. The berries were prepared in three ways before retting; soaked in 70% ethanol, blanched in 80^{o}C water for 1 minute and blanched in boiling water for 3 minutes. The blanched berries were cooled to the ambient temperature before added into the enzyme mixture.

Pectinase Enzymatic Assay: Pectinase activity was determined via dinitrosalicylic acid (DNS) method using galacturonic acid as standard. Reaction mixture contained 200 µl of culture supernatant and 800 µl of 1% pectin was solvated in sodium acetate buffer (0.05 M, pH 4.5) and was incubated at 37^{o}C for 20 minutes. Then, 1 ml of 3,5-dinitrosalicylic acid was added into reaction mixture and then boiled for 10 minutes in order to develop colour. Subsequently, 1 ml of Rochelle salt was added and then allowed to cool. Reducing glucose released in the enzymatic reaction was determined by recording the absorbance reading at 575 nm. One unit of the enzyme activity was defined as the activity produced 1 µmol of galacturonic acid per minute.

Stereo Microscopy: Samples were dried and viewed at fixed magnification of 16X using Olympus SZX7 Zoom Stereo Microscope (Edmund Optics Inc., USA).

Scanning Electron Microscopy: The surfaces of berries were studied using a Jeol JSM 639OLV Scanning Electron Microscope (JEOL USA, Inc., USA) with an accelerating voltage of 10kV. The fibers were sputtered with Au/Pd for 5 minutes prior to imaging.