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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 80ºC 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 37ºC 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.

Piperine Extraction: White berries weighing of 0.3 g was grinded with pestle and mortar. Piperine was extracted with ethyl acetate using a Soxhlet apparatus for 3 hours. The solution was filtered, dried and diluted with dimethyl chloromethane for GCMS analysis.

GC-MS Analysis: GC-MS analysis of the piperine extract was performed using a Shimadzu system comprising an auto-sampler and a gas chromatograph interfaced to a mass spectrometer (GC-MS). The injection mode was split less. For the detection of GC-MS ion, an electron ionization system with ionizing energy of 70 eV was used. Helium gas was used as the carrier gas at constant flow rate of 1.2 ml/min and an inject ion volume of 1µl was employed. The ion-source temperature was 250°C and the oven temperature was programmed from 50°C for 2 minutes with an increase of 20°C/min, to 300°C for 2 mins.

RESULT AND DISCUSSION

PeelZyme has shown to be able to perform pepper retting. In the present study, pepper retting was carried out using different concentration of PeelZyme (500 ppm, 1000 ppm, and 4000 ppm). Full removal of the pericarp was observed on day 5 and the white berries were dried at room temperature (27 -29°C) for 2 to 3 days. Smooth and wrinkled dried berries were observed. In Fig. 1, white berries retted using 500 ppm of PeelZyme (Fig. 1b) showed smooth surface and comparable to the surface of the white berries retted via conventional method (Fig. 1a). In contrast, wrinkled and darkened retted berries are observed when 1000 and 4000 ppm of PeelZyme was employed (Fig. 1c and 1d). Smooth surface and round shape of white berries indicated perfect retting while wrinkled surface and deformed shape of the berries might happened due to weakened strength of the fibre indicating over retting.

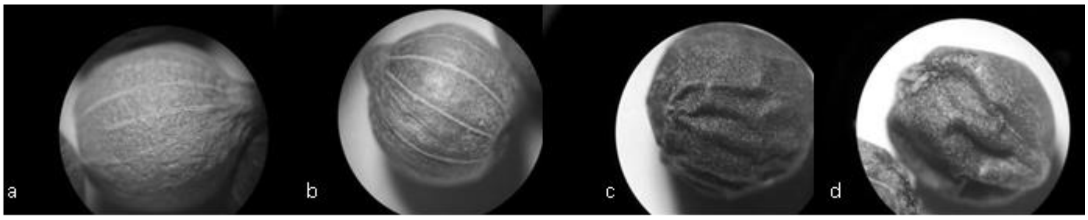

Fig. 1. Surface of white berries under stereo microscope: a) White berries retted via conventional method, b) White berries retted using 500 ppm PeelZyme, c) White berries retted using 1000 ppm PeelZyme and d) White berries retted using 4000 ppm PeelZyme.

From the observation, PeelZyme at the concentration of 500 ppm was found to be able to ret the green berries and produced smooth retted white berries within 5 days. This contradicted to manufacturer's recommendation as PeelZyme at the concentration of 4000 ppm will require 1 to 2 h only to fully ret the green pepper berries. Unfortunately, no full decortication was obtained from day 1 until day 4 of the pepper retting. This might be due to the type of berries used. White pepper can be produced from ripe or matured green berries or dried black pepper, whereas black pepper was produced from the unripe fruits and green berries.

It has been reported that cellulase enzyme successfully produced white pepper from black pepper after 12 days, 3 days and 7 days, respectively. On the other hand, it was found that PeelZyme fully retted green berries within 1 day. This indicated that the differences of chemical compounds exist in each type of berries might require different enzymes to ret fully. PeelZyme at the concentration of 500 ppm was chosen to be employed in the next step of pepper retting trial and the ability to use PeelZyme at the lowest concentration of 500 ppm to ret green berries is the first to be reported.

In our present study, pectinase activity of PeelZyme under retting condition for 5 days was observed. All trials showed that pectinase activity increased along the retting days until it reached to the highest peak on day 3 and decreased after day 3 (Fig. 2). The highest pectinase activity on day 3 was 2.964 ± 0.41, 2.606 ± 0.72, 1.946 ± 0.22, 1.939 ± 0.38 and 1.175 ± 0.22 U/mL representing 500, 4000 and 1000 ppm of PeelZyme, blanching A and blanching B, respectively. These values of pectinase enzymatic activity is important as this could be a standard for others to obtain or improve their pepper retting process.

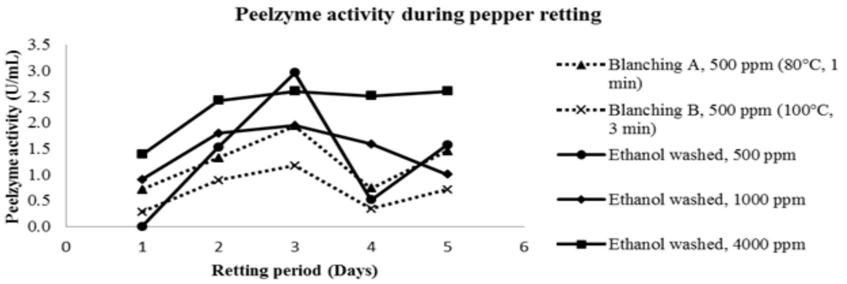

Fig. 2 PeelZyme enzymatic activity during pepper retting.

Two blanching conditions blanching A and blanching B were employed; at 80°C for 1 min and 100°C for 3 min, respectively. It was observed that the enzyme activity of blanching A and blanching B showed lower pectinase activity compared to ethanol washed berries using the same concentration of PeelZyme at 500 ppm. Nevertheless, blanching B showed lower pectinase activity than blanching A. This could be due to the fewer barriers or disruption of substrate for pectinase activity after boiling as blanching crushed the exocarp of the green berries to allow enzyme penetration and solubilization of pectic substances and caused cellular collapse and tissue softening.

The retting period to produce white berries was not drastically shorten for blanched berries, which was similar to that reported by Rosnah and Chan as Viscozyme and Celluclast enzymes required 7 to 15 days to fully soften the pericarp of threshed and blanched green pepper berries. In contrast, blanching of green berries had shortened the retting period as when PeelZyme was used at the concentration of 4000 ppm, 2000 ppm, and 1000 ppm, as it took 1, 2 and 3 days, respectively, to remove the mesocarp of threshed and blanched green berries. Additionally, hot water blanching had reduced contaminants, increased drying rate, improved colour of the pepper berries and removed browning defect on the retted and brightened white berries. However, no difference was observed between brightness or colour of blanched and non-blanched white berries (data not shown) by naked eyes but contaminations by microorganism growth were hindered in all retting conditions.

The PeelZyme enzymatic activity was followed by scanning election microscopy analysis and piperine percentage of the retted berries. Fig. 3b and 3c showed satisfactory smooth surface compared to Fig. 3a but Fig. 3p and 3q were not as smooth as commercially processed white berries (Fig. 3o) when zoomed at 1000X magnification. Fig. 3r and 3s showed smooth surface as commercial white berries (Fig. 3o) but the round shape of the berries were deformed due to over-retting. Surface appearance of blanched berries in Fig. 3f and 3g were inferior compared to those in Fig. 3a, b and c indicating blanching caused skin damage. Similarly, many studies reported the correlation between blanching and microstructural changes, and also suggested that the lower the blanching temperature the lower the damage. In Fig. 3, the images of f and g showed obvious striated surface, while others were absent because the characteristic striations fractured might be due to the mechanical effects of blanching in hot water.

Piperine is the major constituent responsible for the pungent taste of pepper and also found to be ranged from 4 to 10 per cent in pepper but only represents crude piperine. Thus the content of pure piperine is lower. In this study, piperine from green berries was the positive control and represented relatively 100% as the total piperine content prior treatments. Compared to fresh green berries, there was a loss in piperine content in white berries retted at 500 ppm of PeelZyme whereas increase of piperine content in blanched berries as showed in Table 1.

Table.1.Piperine Content of Green Berries and White Berries

Pepper Berries	Piperine Content (%)
Green berries (non retted)	100.00
White berries (500 ppm of PeelZyme)	96.96
White berries (500 ppm of PeelZyme and blanched at 80°C for 1 min)	148.18
White berries (500 ppm of PeelZyme and blanched at 100°C for 3 min)	144.24

CONCLUSION

The enzymatic retting improved the conventional retting technique with the addition of PeelZyme to remove the pericarp of pepper berries and thus has been able to shorten the retting time. PeelZyme at the concentration of 500 ppm successfully produced white berries after 5 days. PeelZyme exhibited highest pectinase activity on day 3 (2.964 ± 0.41 U/mL) at the concentration of 500 ppm without blanching treatment. 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 re duction of surface quality but there was an increase of piperine content up to 40%. White berries retted with PeelZyme at 500 ppm without blanching was considered as the best white berries produced in this study.

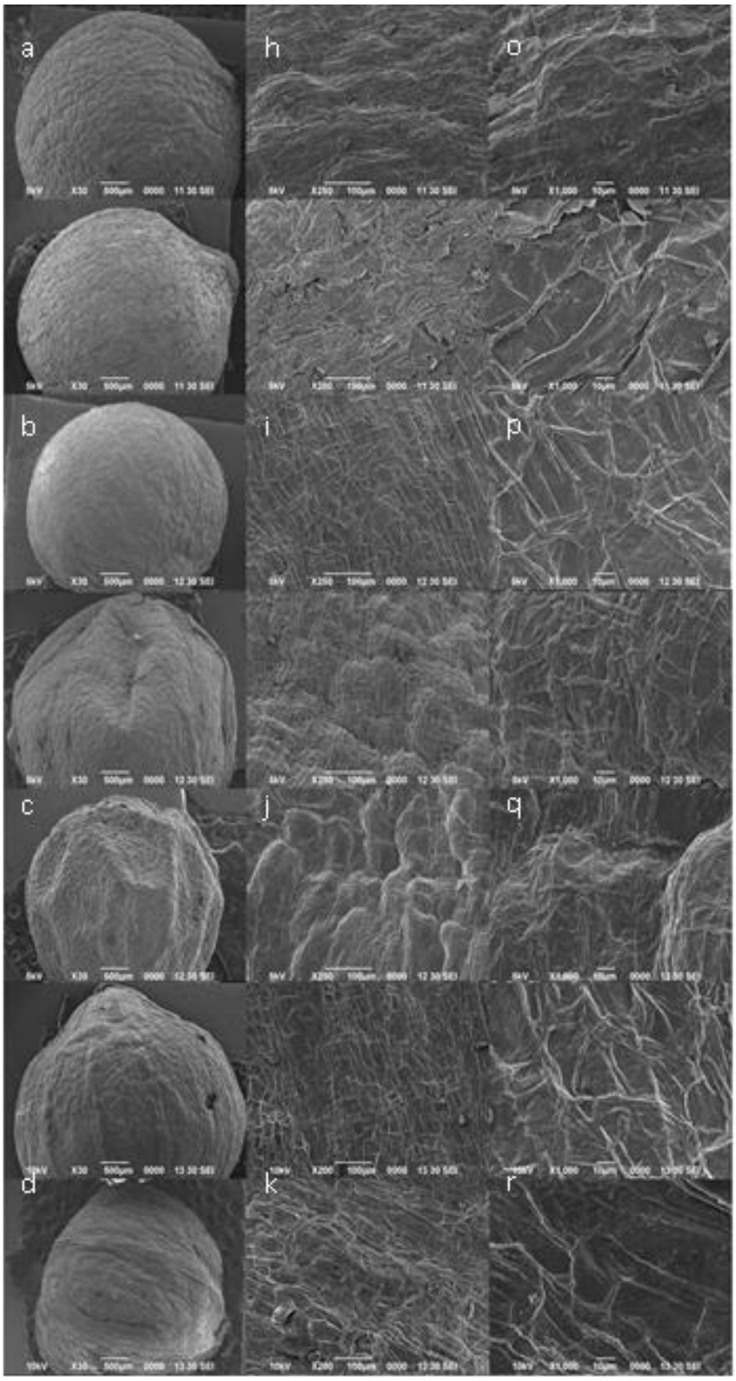

Fig. 3 Scanning electron micrograph (SEM) of white berries retted under various conditions at 30X, 250X and 1000X, (a) to (g), (h) to (n) and (o) to (u), respectively. (a) Commercial white berries; (b) White berries retted via conventional method; (c) White berries retted using 500 ppm PeelZyme; (d) White berries retted using 1000 ppm PeelZyme; (e) White berries retted using 4000 ppm PeelZyme; (f) White berries retted under blanching A (80ºC for 1 minute): (g) White berries retted under blanching (100ºC for 3 minutes)

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