

Extraction and Evaluation of Pectin Methyltransferase from *Morinda citrifolia*

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ABSTRACT

Morinda citrifolia Linn. (Noni) is used in traditional medicine. Pectinmethyltransferases (PME) is an enzyme that is responsible for the degradation of pectin. The current study was aimed to extract PME from *M. citrifolia* (MPME) and compared with commercial PME, and studied the temperature mediated inactivation of PME. The results revealed that the optimum time, pH, and temperature of MPME was found as 5 min, pH 7, and 30°C, respectively. The pretreatment of *M. citrifolia* for 5 min at 100°C can significantly prevent the methanol formation. Further detailed study on the purification and characterization of MPME and the influence of pretreatment of *M. citrifolia* on methanol formation under progress.

INTRODUCTION

Pectinmethyltransferases (PME), an enzyme that acts on pectin, and commonly present in plants and produced by the plant pathogenic fungus and bacteria. PME reacts with pectin, a major compound of plant cell wall, and leads to demethoxylated pectin chains and methanol. PME also catalyze the polygalacturonase activity, which resulted in the loss of texture and fast ripening of fruits that affect the industrial and market value (Rexova-Benkova and Markovic, 1976; Verlent *et al.*, 2004). *Morinda citrifolia*, comes under the family of Rubiaceae and commonly known as noni, has been used in traditional medicine and food. The reports suggested that the noni is a reservoir of nutrients such as scopoletin, potassium, vitamin C, terpenoids, alkaloids, anthraquinones, β -sitosterol, carotene, vitamin A, flavone glycosides, alizarin, acubin, L-asperuloside, linoleic, octanoic, caproic, caprylic, and ursolic acids, etc.

(Mian-Ying *et al.*, 2002; Chaiyasut *et al.*, 2013). The bioactivities like antimicrobial, antihypertension, anti-inflammatory, anticancer, antiulcer were reported in *M. citrifolia*, and noni has been active against constipation, and autoimmune diseases (Wang and Su, 2001; Wang *et al.*, 2002; Kamiya *et al.*, 2004; Chaiyasut *et al.*, 2013). The fermented plant beverages (FPB) are one of the functional food, which provides some necessary vital nutritional supplements for the prevention of diseases, and for the betterment of life. The FPB has quality assurances like chemical contents, microbial safety, and side effects. The amount of alcohol in FPB is one of the strict regulations to be followed by the food industries. The presence of indigenous PME in the raw material affects the methanol content in FPB.

Thus, the current study was aimed to extract PME from *M. citrifolia* (MPME) and study the enzyme activity with different conditions and compared the activity with commercial PME (CPME). Moreover, the effect of thermal treatment on the activity of crude PME extract of *M. citrifolia*. The present work was an initial attempt to extract and study the PME of noni, which further helps to prevent the methanol contamination in FPBs.

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MATERIALS AND METHODS

The pectin methyl esterase (PME) of *Aspergillus niger* (Pectinex[®] Yield MASH), here after denoted as commercial PME (CPME), was purchased from Novozymes.

Extraction of PME from *Morinda citrifolia* L.

About 33 g of clean, fresh, unripened and chopped *M. citrifolia* was blended with 100 ml of 30 mM phosphate buffer pH 7.5, and sieved through cheesecloth and centrifuged at 6,000 rpm for 30 min. The supernatant was collected and the pH up to 7.5 was adjusted using 0.25 M NaOH. Then the supernatant was subjected to ammonium sulfate (30, 50, 70, and 80 % saturation) precipitation for overnight at 10 °C. After incubation; the suspension was centrifuged at 10,000 rpm for 30 min. The pellet was collected and dissolved in 5 ml of 30 mM phosphate buffer.

Optimization of conditions

About 2 ml of 0.5% w/v pectin, 1.95 ml of phosphate buffer (pH 2, 3, 4, 5, 6, and 7) and 500 µl of CPME (1U) or MPME were mixed and incubated at 20, 30, 40, 50, 60, and 72 °C for 1 to 10 min. Followed by, the samples were collected after every one min and determined the methanol content. All experiments were performed in duplicate.

Determination of methanol

After the particular reaction time, the solution was pressed filtered through 0.45 µm membrane filter (Whatman, EU) and mixed with an internal standard (100 ppm n-butanol). The methanol quantification was done as detailed in a previous study (Chaiyavat *et al.*, 2017) using gas chromatography (GC-14B, Shimadzu, Japan) with Carbowax-20M polyethylene glycol capillary column (30 m × 0.53 mm). The flow rate of carrier gas nitrogen was set at 40 ml/min. The temperatures at injector port, column oven, and detector were set at 180, 38, and 260 °C, respectively, and 5 µl of samples were injected by splitless injection.

Pretreatment of *M. citrifolia* and reaction

M. citrifolia fruit was crushed and preheated at 70 and 100 °C for 5 min. 0.2 g of crushed sample, 650 µl of phosphate buffer and 125 µl of MPME extract solution were mixed and incubated for 30 min. The reaction setup with only treated *M. citrifolia* fruit or MPME extract was used as a control.

Statistical analysis

The experiments were performed in triplicates. The results were represented as a mean ± SD. Statistical analysis was executed using one way - analysis of variance (SPSS version 17 statistical software [Chicago, SPSS Inc., U.S.A]).

RESULTS AND DISCUSSION

The optimum time, pH, and temperature of Pectinex[®] Yield MASH (CPME) was verified by measuring the methanol

content in the reaction of PME on pectin by GC and found that 5 min, pH 5, and 60 °C was optimum conditions for CPME activity (Fig. 1a, b, c).

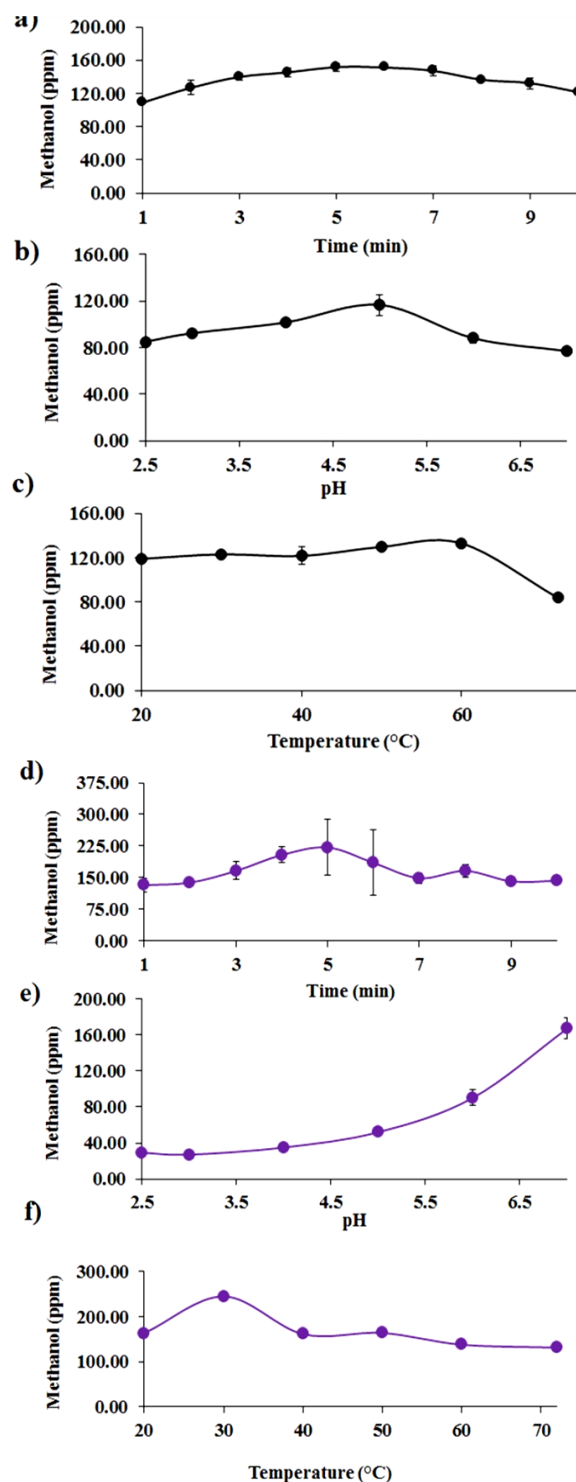


Fig. 1: Activity of commercial PME (a, b, c), and PME from *M. citrifolia* (d, e, f) at different conditions.

The crude PME of *M. citrifolia* (MPME) was extracted and precipitated with ammonium sulfate. The concentration of ammonium sulfate influences the precipitation of active PME in

the crude extract. The results revealed that about 70% of ammonium sulfate efficiently precipitated the MPME (Fig. 2).

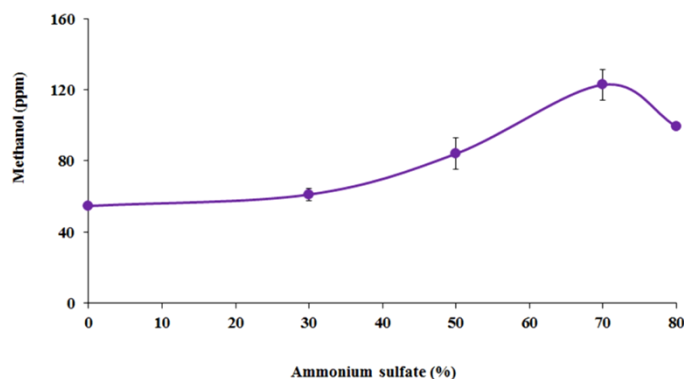


Fig. 2: The impact of ammonium sulfate concentration on the recovery of PME from *M. citrifolia*. The quality of the PME was represented in terms of methanol production.

The optimum time, pH, and temperature of MPME was found as 5 min, pH 7, and 30°C, respectively (Fig. 1d, e, f). The optimum pH and the temperature were drastically varied from MPME to CPME. CPME was originally isolated from a fungus, so apparently the optimum conditions for enzyme activity have variations compared to plant PME.

The small and middle scale FPB production units majorly depend on the natural fermentation, rarely they use starter culture and some ingredients to prevent the undesirable chemical reactions, and are carried out in natural condition especially at room temperature.

The room temperature of the tropical countries falls anything between 28 to 32 °C. The results of the present study proved that the optimum temperature of MPME was 30°C, which primes a new dimension that the FPBs made from *M. citrifolia* are certainly occurred with methanol due to the optimum activity of indigenous PME.

To address this issue, the influence of temperature pretreatment of *M. citrifolia* on methanol production was studied. About 929.3 ± 80.3 , 333.8 ± 19.6 , and 24.8 ± 1.8 ppm of methanol content was observed in the reaction with untreated, 70, and 100 °C pretreated *M. citrifolia*, respectively. The results suggested that the temperature treatment significantly inactivates the PME, which was further confirmed by the reaction with pretreated *M. citrifolia* and crude MPME (Fig. 3).

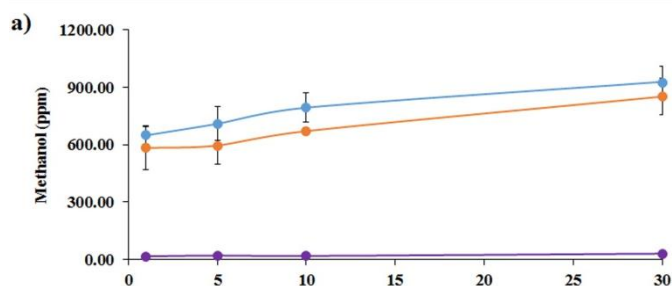


Fig. 3: continued.

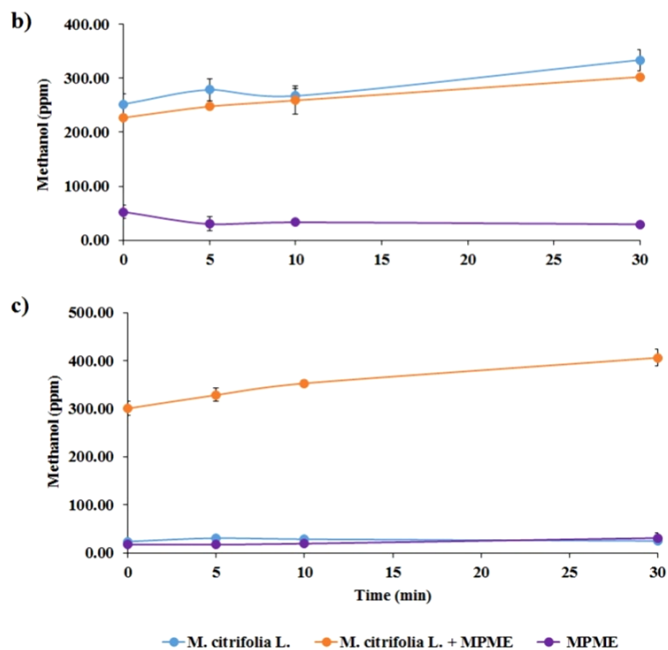


Fig. 3: The influence of pretreatment of *M. citrifolia* on PME activity. a) Untreated samples, b) boiled *M. citrifolia* at 70 °C for 5 min, c) boiled *M. citrifolia* at 100 °C for 5 min.

CONCLUSION

The study primarily attempts to extract PME from *M. citrifolia* and studied the optimum conditions for its activity concerning the methanol production. The primary study on MPME revealed that the indigenous PME is the major reaction for the methanol formation during the production of FPB in natural conditions. Moreover, the pretreatment of *M. citrifolia* for 5 min at 100°C can significantly prevent the methanol formation has been demonstrated.

Further, a detailed study on the purification and characterization of MPME and the influence of pretreatment of *M. citrifolia* on methanol formation and other nutraceuticals changes during fermentation process are required to produce functional FPBs in industrial scale. A potent functional food supplement can be an alternative candidate for the pharmacological application.

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Conflict of Interests: There are no conflicts of interest.

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