

Survey the Polyphenol Extraction from *Artocarpus Altilis* of Vietnam by Enzymatic Method

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Abstract:

Polyphenols has potential applications as antioxidant component that obtained good application in the food processing, medicine and cosmetic. *Artocarpus altilis* is a very common plant in Asian country and in Vietnam. The leaves of this plant contain high polyphenols. The polyphenols extraction was surveyed, with pectinase and cellulase using. The experiment was conducted according to a central composite design, with the following variables: with pectinase, solvent pH (pH-P, 3.0–5.0), the concentration of pectinase (C-P, 0.1–0.3%), solvent temperature (ST-P, 30–50 °C) and extraction time (ET-P, 20–40 min); with cellulase, solvent pH (pH-C, 4.0–6.0), the concentration of cellulase (C-C, 0.1–0.3%), solvent temperature (ST-C, 40–60 °C) and extraction time (ET-C, 20–40 min).

Artocarpus altilis were ground with the solvent, vacuum filtered, centrifuge in 20 min with 6000 rpm and evaluated for polyphenol contents. A highly significant model was obtained for polyphenol extraction efficiency (PEE), which was positively affected by ST-P (ST-C) and C-P (C-C), and inversely affected by pH. The most extraction conditions with pectinase were pH, 4.0; C-P, 0.1%; ST-P, 40 °C and ET-P, 25 min; at such condition, 68.607 mg/g raw material was extracted. The most extraction conditions with cellulase were pH, 5.0; C-C, 0.2%; ST-C, 50 °C and ET-C, 25 min; at such condition, 131.927 mg/g raw material was extracted.

Keywords: *Artocarpus altilis*, polyphenols, pectinase, cellulase, antioxidant

1. Introduction

Sake (*Artocarpus altilis*) is under the Moraceae, grown in the tropical area of Southeast Asia and the Pacific Islands. Sake is the big trees with the high about 15 - 20m [1], [3], [7].

Pradhan et al. (2013) [5] have published about the presence of phenolic compounds and terpenoids in sake via using ethyl acetate and methanol to extract.

Polyphenols are known as an antioxidant, found in onions, cocoa, tea, apples,... According to the American Cancer Society, polyphenols help

prevent the risk of cancer, high blood pressure, heart disease and diabetes. By chromatography and spectroscopic methods, scientists have isolated and identified the compounds in sake, such as flavonoids, triterpene, chalcone, stilbene ... [2]. Flavonoids are an important group of polyphenol compounds and biological activity. Therefore, the study of extraction of phenolic compounds with biological activity in food applications will be highly beneficial.

Some recent studies have also published polyphenol extraction by using pectinase and cellulase. [4].

In this study, we extracted polyphenols from the leaf of sake by using pectinase and cellulase.

2. Materials and Methods

Materials: Sake leaves that were used in this study were collected from Vinh Loc Industrial Park, Binh Chanh, Vietnam

Basic extraction: Material was extracted in water with enzyme pectinase/cellulase, solution was vacuum filtered, centrifugal in 20 min with 6000rpm/min.

Survey the effect of pectinase enzyme on the extraction: four surveys were done: extraction temperature (30, 35, 40, 45, 50°C); extraction time (20, 25, 30, 35, 40 minutes); pH (3, 3.5, 4, 4.5, 5); enzyme concentrations (0.1, 0.15, 0.2, 0.25, 0.3%).

Survey the effect of cellulase enzyme on the extraction: four surveys were done: extraction temperature (40, 45, 50, 55, 60°C); extraction time (20, 25, 30, 35, 40 minutes); pH (4, 4.5, 5, 5.5, 6); enzyme concentrations (0.1, 0.15, 0.2, 0.25, 0.3%)

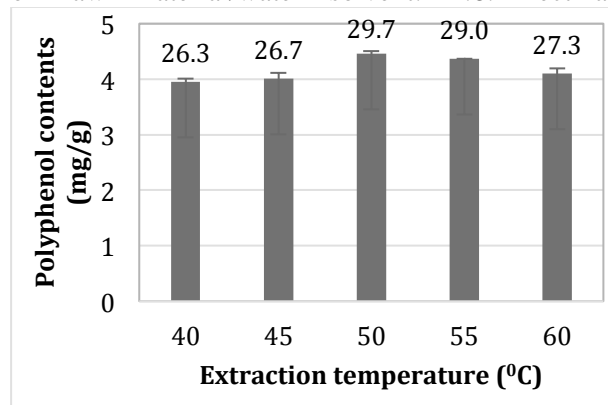
Methods of analysis: the quantification of total polyphenols by the Folin - Denis method [6]

Data analysis: All experiments were repeated 3 times. Data is processed by ANOVA - one way using statistical software Statgraphics Centurion XV to test differences between pairs of samples with a significance level of 5%.

3. Results and Discussion

3.1. Effect of temperature on the extraction

Fixed time of 20 min, pH 4.5 (pectinase), pH 5 (cellulase), 0.15% enzyme concentration, the ratio of raw material/water solvent: 1:8. Pectinase



(a)

enzyme changes in temperature: 30°C, 35°C, 40°C, 45°C, 50°C (enzyme cellulase: 40°C, 45°C, 50°C, 55°C, 60°C). The results was shown in Figure 1.

The velocity of the enzyme reaction will increase with temperature, but it increases only to a certain limit.

The highest polyphenol content at 40°C when using the enzyme pectinase and 50°C with cellulase enzyme.

3.2. Effect of the extracted time on the extraction:

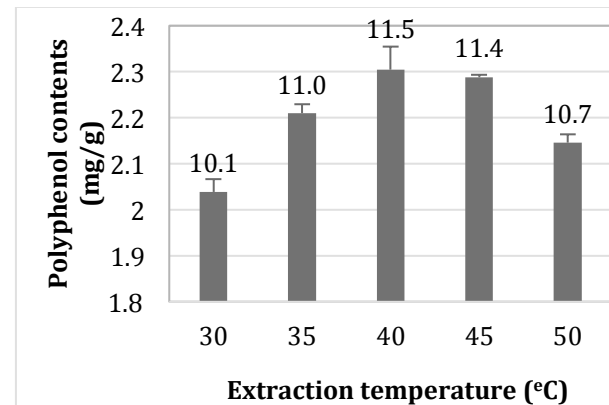
Fixed pH 4.5 (pectinase), pH 5 (cellulase), 0:15% enzyme concentration, the ratio of raw material/water solvent: 1:8, temperature extract of pectinase enzyme 40°C (50°C cellulase enzyme). Changing time of 20 minutes, 25 minutes, 30 minutes, 35 minutes, 40 minutes. The results were shown in Figure 2.

Increasing extraction time, the reaction rate increases. Polyphenol content increased slightly from 25 to 30 minutes. Polyphenols are only stable in acid environment, and unstable in base environment. So there should be appropriate extraction time for enzyme reaction.

3.3. Effect of the extracted pH on the extraction

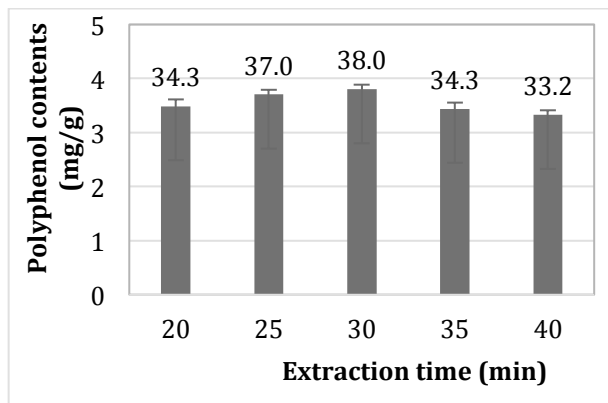
Fixed time of 25 minutes, 0:15% concentration of enzyme, the ratio of raw material/water solvent: 1:8, temperature extract of pectinase enzyme 40°C (50°C cellulase enzyme), pH changes: 3; 3.5; 4; 4.5; 5 (cellulase: 4, 4.5, 5, 5.5, 6). The results were shown in Figure 3.

pH affects to reaction of the enzyme and durability. Polyphenol content between different pH values is not much difference. The highest polyphenol content at pH 4 (enzyme pectinase), pH 5 (enzyme cellulase).

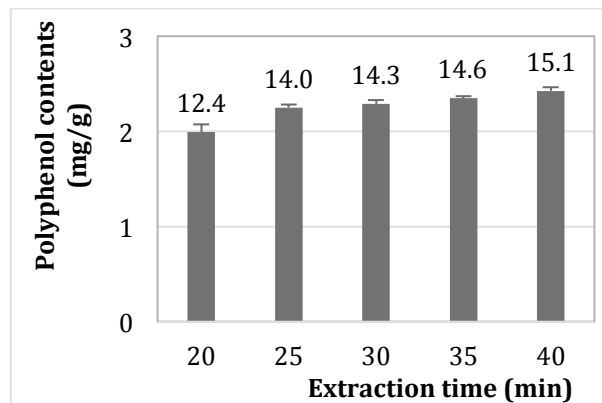


(b)

Fig 1. Effect of temperature to extract polyphenol. a) enzyme cellulase; b) enzyme pectinase)

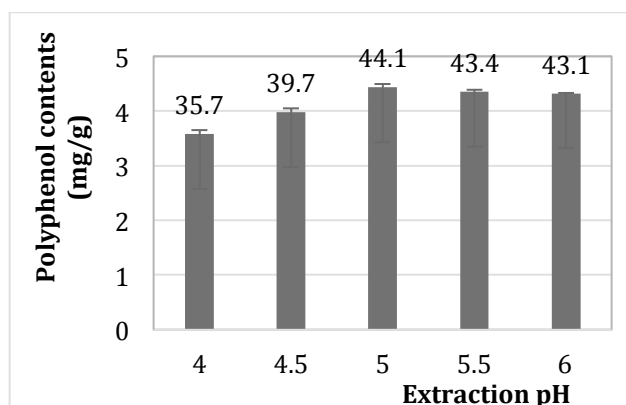


(a)

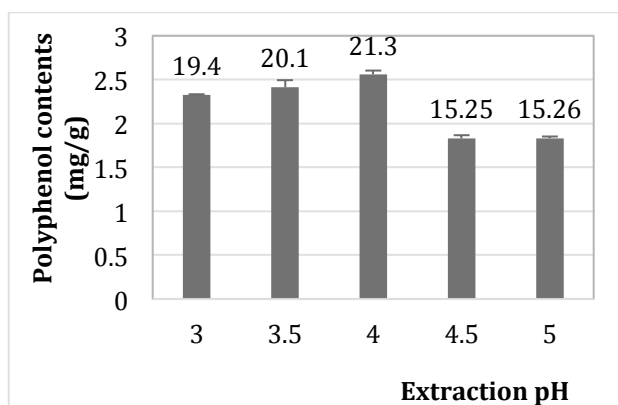


(b)

Fig 2. Effect of extraction time to extract polyphenol. a) enzyme cellulase; b) enzyme pectinase)



(a)



(b)

Fig 3. Effect of pH to extract polyphenol. a) enzyme cellulase; b) enzyme pectinase)

3.5. Effect of the concentration of enzyme on the extraction

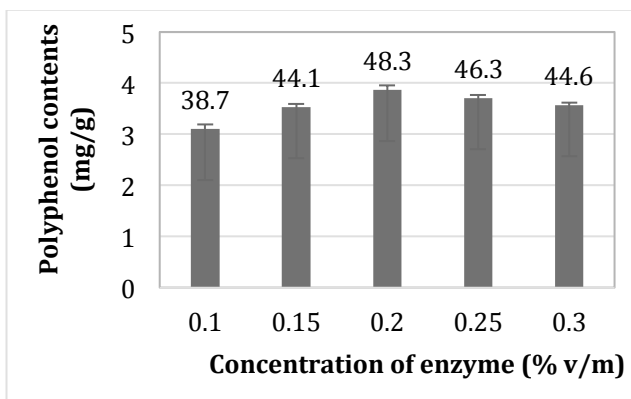
Fixed time of 25 minutes, pH 4 (pectinase), pH 5 (cellulase), the ratio of raw material/water solvent: 1:8, temperature extract of enzyme pectinase 40⁰C (50⁰C enzyme cellulase), Changing enzyme concentration of 0.1%, 0.15%, 0.2%, 0.25%, 0.3%. The results was shown in Figure 4.

Extractable polyphenol content corresponding to the enzyme concentration is not much difference. Increasing concentrations of 0.1 - 0.2%, the concentration of polyphenols increased and decreased slightly at the next level.

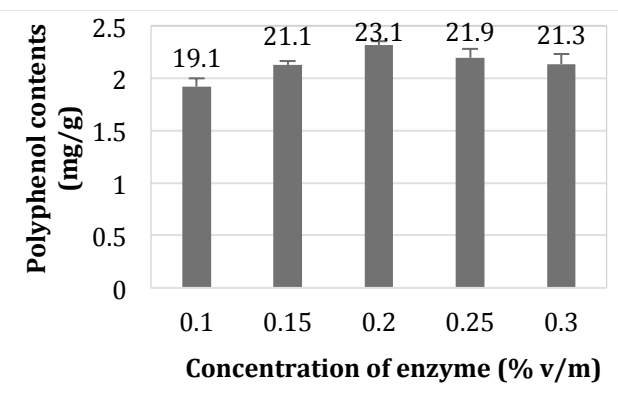
When excess substrate, the reaction rate increased with increasing concentrations of enzyme but when the concentration of enzyme saturation with substrate concentration, reaction rate did not change or not increased with increasing enzyme concentration.

3.6. Effect of the extracted time on the extraction

Total 4 times extraction using pectinase enzyme, polyphenol content obtained is 68.607mg/g, and when using enzyme cellulase with 4 times extraction, polyphenol content obtained is 131.927mg/g.



(a)



(b)

Fig 4. Effect of concentration of enzyme to extract polyphenol. a) enzyme cellulase; b) enzyme pectinase)

4. Conclusion

In this study we found that temperature, time, pH, enzyme concentration are the factors that is affecting the extraction process.

The extraction method using enzyme pectinase after 4 times extraction, total obtained polyphenol content is 68.607mg/g at 40^oC temperature, time of 25 minutes, the ratio of raw material/water solvent 1:8, pH 4, concentration of enzyme 0.2%. And when using enzyme cellulase after 4 times extraction, the total obtained polyphenol content is 131.927mg/g at 50^oC temperature, time of 25 minutes, the ratio of raw material/water solvent 1:8, pH 5, concentration of enzyme 0.2%.

So using cellulase in this case will give more affection on the polyphenol extraction from sake leaves. The researched method is easy to apply in the industry. The product can be used in food industry

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