The effect of soaking time on mucilage removal from the coffee bean using pectinase enzyme

Fitri¹, A B Tawali² and A Laga²

¹Agricultural Science Study Program, Graduate School, Hasanuddin University, Jl. Perintis Kemerdekaan Km 10, Tamalanrea, Makassar 90245
²Food Science and Technology Study Program, Department of Agricultural Technology, Faculty of Agriculture, Hasanuddin University, Jl. Perintis Kemerdekaan Km.10, Tamalanrea, Makassar 90245

E-mail: fitrihamzah@outlook.com

Abstract. The perfect coffee fermentation process is characterized by the release of mucilage that covers the coffee beans. The mucilage that doesn't come off entirely from the coffee beans can affect the taste of the coffee. The mucilage, which is composed of pectin, can be removed using the pectinase enzyme. This study aims to determine the optimal soaking time required to remove the mucilage from the coffee beans. Coffee beans are soaked in a liquid containing the pectinase enzyme for 1, 2, 3, and 4 hours. The results showed that soaking coffee beans for 4 hours in the pectinase enzyme was the best contact time in releasing the coffee bean mucilage. Soaking coffee beans for 4 hours in the pectinase enzyme produced a pectin yield of 0.25%, total dissolved solids 1.05°Brix, pH 6.55, and total sugar 15.67%.

1. Introduction

Coffee is one type of plantation crop that is widely cultivated in Indonesia and throughout the world. That plant becomes one of the crops that has a high selling value in international trade [1,2]. Quality greatly affects the selling value of a coffee bean, where the quality of aroma and taste are the most important factors that affect consumer acceptance of a coffee bean [2].

The post-harvest processing of coffee is an important factor affecting the quality of taste and aroma of coffee beans. Generally, there are three post-harvest processing methods for coffee beans: dry-processed, wet-processed, and semi-dry (pulped natural) processing [3]. In the dry process, cherry coffee is dried directly under the sun until it reaches a moisture content of about 10% - 12% [4]. In the wet process, the pulp from the cherry coffee is removed mechanically. After that, the coffee beans are soaked in water for a few days and then dried. Most of the Arabica coffee is processed using a wet process [3,5]. And in the semi-dry process, the skin is exfoliated mechanically, and the coffee beans are dried directly without going through a soaking process [3,4].

During the wet process, the coffee beans undergo fermentation. Fermentation is a chemical process that causes changes in the molecules contained in coffee beans. Fermentation of coffee is essential to the removal of mucilage from parchment coffee. The perfect fermentation process is characterized by the release of mucilage from the coffee beans. The mucilage still attached to the parchment can prolong the drying time of the coffee beans. Besides, mucilage that is not removed properly can cause mould growth during the storage process, which will reduce the quality of the coffee beans [6].
Coffee mucilage is a thin layer that sticks to the coffee beans' walls composed of polysaccharides. In the fermentation process, mucilage is broken down in coffee pulping stations and coffee plants in the field by microorganisms that occur naturally. Bacteria, yeast, and fungi break down sugars and pectins within the mucilage, producing acids and alcohol [7]. Besides, through the fermentation process, the mucilage in the coffee beans can also be removed by adding enzymes that can degrade the polysaccharides that compose mucilage, for example, the pectinase enzyme. Several factors can affect the enzyme's work in degrading the substrate, one of which is the contact time between the enzyme and the substrate. Therefore, this study was conducted to see the effect of the soaking time of coffee beans on the pectinase enzyme on the release of coffee beans mucilage.

2. Material and methods

2.1. Material
The sample used in this study was coffee beans that came from the Arabica type. The pectinase enzyme used is a commercial pectinase enzyme.

2.2. Methods
2.2.1. Preparation of pectinase enzyme solution. The commercial pectinase enzyme was weighed as much as 0.083 grams and dissolved in a citrate buffer pH 4.5 to obtain a pectinase enzyme solution with 50 units/gram activity.

2.2.2. Determination of pectin yield. Each coffee bean is weighed as much as 40 g, then put into a round bottom flask, 1000 mL of 0.01 M HCl solution was put into a round bottom flask, and the extraction process was carried out at 60°C. After that, the extraction process results are filtered using filter paper to separate the extracted filtrate from the residue. The resulting extracted filtrate is added with 96% alcohol solution (1:1) and left to stand for one night. The resulting precipitate is then filtered with filter paper and washed with 96% alcohol. The acid-free sediment is then placed in a porcelain dish and dried in an oven at 60 – 62°C for ± 3 hours. The dried pectin was then weighed using an analytical balance to determine the dry pectin weight.

2.2.3. Analysis of total dissolved solids. Total dissolved solids were measured with a hand refractometer.

2.2.4. Total sugar analysis using the luff schoorl method [8]. The sample of coffee beans was weighed 3 grams and added 100 ml of 3% HCl. The samples were heated for 3 hours. Transfer to an Erlenmeyer or beaker glass. Add 30% NaOH dropwise while stirring, then measure the pH to 5.5. Transfer it to a 250 ml measuring flask with the volume of distilled water, then homogenize it. Filter the filtrate. Save the filtrate into the bottle. Add 10 ml of Luff Schrool solution and 25 ml of distilled water and heated for 15 minutes. After cold. Add 10 ml of 20% KI solution and 15 ml of 25% H$_2$SO$_4$ slowly. Then drop as soon as possible 0.1 N thio solution with 0.5% starch as much as 5 ml as an indicator. The titration reacts when the new colour changes to a milky white colour. Make a blank test (35ml distilled water + 10 ml of Luff Schrool solution + a few grains of stones dipped in Erlenmeyer). Simmer for 10 minutes using an upright cooler. Bring to a boil quickly, then add 10 ml of 20% KI solution and 15 ml 25% H$_2$SO$_4$, titrate with 0.1 N Tio (use starch indicator)

2.2.5. Analysis of pH. Ground coffee beans and put in a 10 ml beaker. The pH of the coffee beans is then measured using a calibrated pH meter.
3. Results and discussion

3.1. Pectin yield
Chemically, coffee mucilage is composed of water, pectin, sugar, and organic acids. Where the total pectic substances contained in coffee mucilage reach 39% [9]. The yield of pectin in coffee beans after soaking in the pectinase enzyme solution can be seen in figure 1.

![Figure 1: Pectin yield of the coffee bean after soaking on pectinase.](image)

Based on the results, it can be seen that soaking coffee beans in the pectinase enzyme can degrade the pectin content, which is a constituent of coffee bean mucilage. The lowest pectin yield was obtained in soaking coffee beans for 4 hours (0.25%). The yield of pectin in coffee beans decreased with increasing soaking time.

Pectin is a complex polysaccharide that generally forms plant cell walls. In coffee plants, these molecules are present in the mucilage layer attached to the surface of the coffee bean and become the main constituent of the mucilage. Soaking coffee beans in the pectinase enzyme solution causes the pectin molecules to degrade so that the coffee mucilage breaks down and can be removed from the coffee beans. This is due to the ability of the pectinase enzyme to break down the α-1,4-glycoside bonds in pectin. This is in accordance with [10], which states that the pectinase enzyme is an enzyme capable of hydrolyzing the α-1,4-glycoside linkage in pectin and producing D-galacturonic compounds.

3.2. Total dissolved solids and total sugar
Total dissolved solids are the amount of all organic and inorganic in a solution [11]. Total dissolved solids are closely related to total sugar content. The total dissolved solids can show all the amounts of sugar that are solids in a solution. The total dissolved solids and the total sugar of coffee beans soaked in the pectinase enzyme solution can be seen in figure 2 and figure 3.

![Figure 2: Total dissolved solid of the coffee bean after soaking on pectinase.](image)
Figure 3. Total sugar of coffee bean after soaking on pectinase.

Based on figure 2 and figure 3, it can be seen that the total solids and total sugar content in coffee beans tend to increase with increasing soaking time in the pectinase enzyme. Total dissolved solids (1.05°Brix) and the highest sugar content (15.67%) were obtained from soaking coffee beans for 4 hours. When the coffee beans are soaked in the pectinase enzyme, this enzyme will hydrolyze the coffee mucilage's pectin compound. Apart from containing galacturonic acid, it also contains neutral sugars such as galactose, arabinose, and xylose. So that the hydrolysis of pectin compounds can increase total sugar levels and total dissolved solids in coffee beans. This is in accordance with [10], which states that some pectins are composed of repeated disaccharides that carry the branch chains of neutral sugars galactose, arabinose, and xylose.

3.3. pH

pH is the degree of acidity used to express the level of acidity and alkalinity of a solution. The pH level of coffee beans soaked in the pectinase enzyme can be seen in figure 4.

Figure 4. pH of the coffee bean after soaking on pectinase.

Based on the results, it can be seen that there is no significant change in the pH of coffee beans soaked in the pectinase solution. The mucilage removal process does not cause a change in the acidity of the coffee beans. Changes in acidity usually occur in fermented coffee beans. This is in accordance with [12], which states that during the fermentation process, the microflora will break down the molecules in the coffee beans and produce alcohol and organic acids, increasing the acidity of the coffee beans.
4. Conclusions
The addition of the pectinase enzyme to the coffee beans can help remove the mucilage from the surface of the coffee beans. The longer the soaking time, the more mucilage that can be broken down. The process of removing the coffee bean mucilage can also affect the total sugar and the total dissolved solids of the coffee beans.

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