Effect of maltodextrin addition on the preparation of lipase from Coconut haustorium

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Abstract. Lipase is a biocatalyst that hydrolyzes esters bonds such as triglycerides to produce free fatty acids and glycerol. Coconut haustorium is an unused source of lipase enzyme. One of the methods in the preparation of the lipase enzyme from coconut haustorium that ready to be used is by a drying process. However, the drying process can damage the structure of the enzyme and decreases its activity. Therefore, the maltodextrin is required to protect the structure of the enzyme during the drying process. This study aims to identify the effect of maltodextrin addition to the lipase enzyme preparation. This study started by shredding coconut haustorium to reduce the size. Then, the maltodextrin at concentrations 0%, 10%, and 20% was added to the coconut haustorium. The mix was then dried using a blower oven with a temperature of 40°C for 9 hours. The parameters tested in the study were protein content, enzyme activity, water content, and yield. The best treatment in the preparation of the enzyme lipase from coconut haustorium was the addition of 20% maltodextrin. This treatment gave the highest activity of 0.438 U/ml, protein content of 7.168 mg/ml, the specific activity of 0.058 u/mg protein, the water content of 18.96% and yield of 26.71%.

1. Introduction
An enzyme is a biocatalyst that has an essential role in industry and biotechnology. The specific nature of the enzymes makes many of them used in various industrial processes, both for food and non-food industry. More than 70% of the industries use enzymes as the catalysts [1]. Enzymes trading reaches 10-15% per year due to the increasing number of new enzymes and the type of application. One of the industrial enzymes is a hydrolytic enzyme such as lipase. The lipase enzyme works in the esterification process of triglycerides to produce free fatty acids and glycerol. In the food industry, the lipase enzymes are widely used in the dairy industry, oleochemical industry, meat and sausages processing, as well as alcoholic beverages.

The markets of enzymes in Indonesia in 2017 was up to 2500 tons and is expected to increase every year [2]. This significant value should encourage the self-reliance efforts for national scale enzyme production. One of the ways to anticipate the dependence on imports is by producing local Indonesian enzymes using the biological resources available, such as coconut haustorium.
Coconut haustorium, an embryo of a coconut shoot, has round in shape and stick to the flesh of the fruits. The coconut haustorium has high lipase activity. The embryo obtained from the coconut, which has been sprout for the past 30 days has a specific lipase activity by 0.0581 units/mg protein [3]. The lipase enzyme in coconut haustorium has been immobilized so it can be used repeatedly. One of the methods in preparation of lipase enzyme from coconut haustorium that ready to be used is the drying method. However, the drying process can damage the structure of the enzyme that decreases the activity of the enzyme. Therefore, the filler material, in the form of maltodextrin, is required to protect the enzyme's structure during the drying process. The aim of this study is to identify the effect of maltodextrin addition to the lipase enzyme activity of coconut haustorium.

2. Materials and Methods

2.1. Coconut haustorium preparation
The coconut haustorium were purchased from farmers in Enrekang Regency, South Sulawesi, Indonesia. The coconut haustorium was weighed, and its diameter was measured. Then, the coconut haustorium is shredded for size reduction. Maltodextrin with various concentrations (0, 10, and 20%) was then added. The use of 10 and 20 % maltodextrin were based on the solid weight of the coconut haustorium which around 15%, therefore the range of additional maltodextrin is close to its solid weight. The mix was then dried using a blower oven with a temperature of 40°C for 9 hours.

2.2. Sample analysis
The protein content, lipase activity, water content and yield were analyzed.

2.2.1. Protein content (lowry method)
The crude extract of the sample was dissolved in distilled water, and then taken 2 ml and reacted with 5 ml of C reagent and homogenized. Then it was left for 10 minutes at the room temperature. After that, 0.5 ml folin reagent was added and allowed to stand for 30 minutes at room temperature [4]. For control, the enzyme was replaced with distilled water; further treatment was the same as the sample. Then the absorbance was measured at a wavelength of 650 nm. The protein level was measured using a standard BSA (Bovine Serum Albumin).

2.2.2. Lipase activities (colorimetric method)
1.5 ml olive oil was added by 1 ml of phosphate buffer pH 7 and 1 g of the coconut embryo lipase. This mixture was incubated in a water bath shaker with the agitation 200 rpm, 45°C, for 60 minutes. The enzyme reaction in the emulsion system was stopped by adding 1 ml of HCl 6 N and 5 ml of isooctane, followed by mixing using vortex for 5 min. 4 ml of the upper layer containing the fatty acids was drawn off to a test tube and added with 1 ml of Cu(II) acetate reagent for analysis. Furthermore, the mixture was centrifuged at 4000 rpm for 10 minutes, then the absorbance measured with a UV-VIS spectrophotometer at a wavelength of 715 nm [5]. The control was made following the sample procedures, of which the enzyme solution was replaced by distilled water. The enzyme activity was measured using the standard curve of oleic acid.

The lipase enzyme activity was calculated in a unit. One unit per ml was defined as the number of ml of lipase enzyme required to produce 1 µmol of oleic acid per minute with olive oil as the substrate with the following Equation 1 and 2.

\[
U \ (\text{u/ml/min}) = \frac{\mu\text{mol oleic acid}}{\text{minute}}
\]  
(1)

\[
\mu\text{mol oleic acid} = M \times \text{ml solution} \times 1000
\]  
(2)
2.2.3. Water content
The porcelain was dried in an oven for 1 hour at 105°C, put in a desiccator for 15 minutes and weighed. The sample, 1-2 grams, was put in porcelain and dried in an oven at of 105°C until the constant weight obtained. Then it was put in the desiccator for 30 minutes before it weighed again [4] that was calculated using Equation 3.

\[
\text{\% Water Level} = \frac{\text{Weight of Dried Sample}}{\text{Weight of the sample}} \times 100\% \tag{3}
\]

2.2.4. Yield calculation
The yield of the lipase enzyme was calculated by weighing the initial weight of the raw material (coconut haustorium) and the weight of the coconut haustorium powder produced with the following Equation 4.

\[
\text{Yield} = \frac{\text{weight of the coconut embryo powder (gram)}}{\text{initial weight of the raw material (gram)}} \times 100\% \tag{4}
\]

3. Results and Discussion
The maltodextrin acts as a filler material and protective layer to keep or maintain the activity of enzymes during the drying process.

3.1 Enzyme activity
The average activity of lipase enzyme from coconut haustorium based on the concentration of maltodextrin added that is 0.166 – 0.438 U/ml (Figure 1). The results of the analysis of variance show that the addition of maltodextrin significantly affects the lipase activity (p<0.05).

![Figure 1](image_url)  
**Figure 1.** The effect of adding maltodextrin on the lipase enzyme activity.

The results indicate that the addition of maltodextrin can maintain enzyme activity during the drying process. The increase is made possible by the addition of maltodextrin as the filler material that can maintain the structure of the enzyme. Maltodextrin has good solubility, can form a film layer, has a low hygroscopicity, can inhibit crystallization and has a strong bonding effect [6]. The maltodextrin acts as a film layer that protects the structure of the enzyme when the drying process takes place so that damage to the structure of the protein enzyme can be minimized. Maltodextrin consists of glucose units which protect the structure of enzyme by binding water from both the material and the environment so that the dissolved oxygen can be reduced and also the oxidation process can be prevented.

3.2 Protein content
The average protein content based on the concentration of maltodextrin from 7.066 to 7.618 mg/ml. The result of the analysis of variance shows that the addition of maltodextrin in a variety of concentrations does not significantly affect the lipase enzyme protein content (p<0.05). The addition of maltodextrin by 0%, 10%, and 20% gives the protein content of 7.066 mg/ml, 7.495 mg/ml, and 7.618 mg/ml.
It can be seen that maltodextrin addition can increase the protein content. The result indicates that the use of maltodextrin has a significant role in protecting the protein content during the drying process. This is because the maltodextrin acts as an encapsulation agent that protects the functional compound such as protein. The use of maltodextrin can prevent the nutrient component release, protect essential compounds due to the extreme temperatures because maltodextrin can form the body and has a strong bonding effect on the coated compounds [7].

3.3 Specific activity
The average specific activity of the lipase enzyme based on the concentration of added maltodextrin ranges from 0.024 to 0.058 U/mg of protein (figure 2). The result of the analysis of variance at a significant level of 5% shows that the addition of maltodextrin significantly affects the specific activity of the lipase enzyme.

![Figure 2](image_url) The effect of adding maltodextrin on the specific activity of the lipase enzyme.

Figure 2 shows that the use of maltodextrin can increase the specific activity of the lipase enzyme. These results show the similar phenomenon of the enzyme purification, in which the specific activity of the enzyme will increase when the purification performed because the inhibiting factors such as contaminants which interfere the enzyme have been excluded. The basic principle is to separate the lipase enzyme from other components such as water molecules, other proteins, nucleic acids, and other small molecules using drying and adding the filler material. The use of maltodextrin can strengthen the hydrophobic interaction between the non-polar amino which causes the rigidity of the protein and the resistance to thermo-deactivation so that the stability can be maintained [8]

3.4 Water content
The water content of lipase enzyme from coconut haustorium at 0%, 10%, and 20% are 25.34%, 20.88%, and 18.96%, respectively. The results show that the increasing concentration of maltodextrin reduces the water content. This is possible by the nature of maltodextrin that able to bind water. The more maltodextrin is added, the faster the crystallization and evaporation of water so the coconut haustorium water content will be lower. In addition, the maltodextrin can increase the total solids of the dried material so the amount of water evaporated can be lower. Consequently, the increasing concentration of maltodextrin will reduce water content [9]. One of the properties of maltodextrin can bind free water in a material so that the increasing amount of maltodextrin added can reduce the water level. The results of the analysis of variance show that the addition of maltodextrin does not significantly affect the water content of the lipase enzyme at the 5% level of significance.

3.5 Yield
The treatment testing results of the maltodextrin addition to the yield of lipase from coconut haustorium obtained the average values ranging from 18.79 to 26.71% (Figure 3). The lowest yield is in the drying
treatment without the addition of maltodextrin amounted to 18.79%. The highest yield is in the drying treatment with the addition of 20% maltodextrin by 26.71%. The results of the analysis of variance show that the lipase enzyme preparation with the addition of maltodextrin significantly affects the yield of lipase from coconut haustorium (p<0.05).

![Figure 3. The Effect of adding maltodextrin on the yield of coconut embryo lipase enzyme.](image)

The figure shows that the yield of coconut haustorium increased along with the increasing concentration of maltodextrin used. This is because the yield is influenced by the number of ingredients added. The addition of maltodextrin caused the addition of the total solid material. The amount of yield also depends on the drying material [10]. The higher the dry matter content also gives the higher yield. The addition of maltodextrin in this study, besides maintaining the activity of lipase enzyme when dried, it also aims to reduce the loss of volume. Maltodextrin has a relatively low viscosity so that the use of it in the large quantities is still permitted. Besides as a filler material (*filler*), the use of maltodextrin can also increase the weight of the product produced.

4. Conclusions
The addition of maltodextrin increased the enzyme activity by 62.10%. The best treatment was the addition of maltodextrin by 20% based on the highest specific activity of 0.058 U/mg protein. That treatment also produced the activity of 0.438 U/ml, the protein content of 7.618 mg/ml, the water content of 18.96%, and the yield of 26.71%.

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