Submerged-Fermentation of *Brassica oleracea L. capitata* using *Lactobacillus plantarum* to Reduce Anti-Nutrient Compound

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Submerged-Fermentation of *Brassica oleracea L. capitata* using *Lactobacillus plantarum* to Reduce Anti-Nutrient Compound

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Abstract. Fermentation of cabbage (*Brassica oleracea L. capitata*) by *Lactobacillus plantarum*has been successfully conducted using submerged-fermentation technique. The aims of this study is to determine the correlation between tannins and total sugar in the fermentation of cabbage. Fermentation process is proposed to reduce the concentration of tannins as anti-nutrient compound in cabbage. The tannin concentrations are determined using spectrophotometric method. Tannaseenzyme produced by *L. plantarum* is hypothesised to be mainly responsible for the degradation of glucoside bonds, thus, reducing tannins contents. Total sugar contents were also investigated in this fermentation process. Fermentation process was carried out with variation of *L. plantarum*inoculum concentrations of 5%, 10%, 15%, and 20% (w/v). Results showed that the tannins value incabbage raw materials was 290.877 mg/g FW. After fermentation process the optimal inoculum concentration reduced tannins concentration to 75.938 mg/g FW, at 5% inoculum concentration and pH 6for 96 h at 25 ºC. The correlation between tannins and total sugar in cabbage fermentation was found inversely proportional.

Keywords: sub-merged fermentation, *Brassica oleracea L. capitata*, *L. plantarum*, tannins

1. Introduction

Vegetables are the fresh, edible and succulent parts of herbaceous plants. They contain appreciable amount of vitamins and mineralswhich are highly beneficial for the maintenance of health and prevention of disease. They also contain high amount of dietary fiber and minimal amount of protein [1]. *Brassica oleracae L. capitata* or cabbage is an herbaceous green leafy vegetable belonging to the Brassica genus [2]. *Brassica oleracaeL capitata* has been used in ancient times, both as food and medicine. However, cabbage also contains some anti-nutrient compounds, including tannins, phytic acid and oxalates. Tannins in cabbageis considered to be nutritionally undesirable because they form complexes with protein, starch and digestive enzymes and cause a reduction in nutritional values of food. Small-molecule tannins are suggested to have fewer anti-nutritional effects and can be more readily absorbed [3].

Fermentation is one of the oldest biotechnology methods, and is widely used process for cabbage [4]. Fermented food can be classified on the basis of the primary substrate used by the microbial agent.Many investigators have reported that fermentation can be effectively applied for reducing anti-nutrients [4].Submerged fermentation involves the growth of the microorganism as a suspension in a liquid medium in which various nutrients are either dissolved or suspended as particulate solids in many commercial media [5], such as pH, inoculum concentration, temperature has some effect for submerged fermentation in *Brassica*. Lactic acid bacteria especially *Lactobacillus plantarum* should play an important rule when tannins are present in food and intestines because it hydrolyzes tannins and decrease adsorption phenomenon on the cells [6]. *L. plantarum* is a lactic acid bacterial species that is most frequently encountered in the fermentation of plant materials where tannins are abundant. These plant fermentations include several food and feed products, e.g., olives, grape must, and a variety of vegetable fermentation products [7]. Among food lactic acid bacteria, strains from the *L. plantarum* group possess tannase activity Therefore, the study of reducing tannins as anti-nutrient seems to be a promising way to application in *Brassica* fermentation process. The present study reports the optimization of fermentation condition for reducing tannins as anti-nutrients using *L. plantarum*.

2. Materials and Methods

2.1. Chemicals and instrumentation

Culture of *Lactobacillus plantarum* was obtained from Department of Food Science and Technology, Brawijaya University, Malang, while *Brassica oleracae L. capitata* was obtained from Poncokusumo, Malang regency. All reagents of analytical or higher purity grade were purchased from Merck or Sigma-Aldrich and were used as received: HCl (37% aqueous solution), HNO_3 (trace pure, 65% w/w in H_2O), NaOH (99.9%), H_2SO_4(98%, d = 1.84 g/mL), ethanol (96%), $MgCl_2{\cdot}2H_2O$, KH_2PO_4, Na_2HPO_4, NaCl, NH_4Cl, CH_3COOH (100%; d =1.05 g/mL), $NaCH_3COON$, and pepton. Water was purified using distillation technique. The growth media for *L plantarum* were MRSA (*de Mann Rogosa and Sharpe Agar*) and MRSB (*de Mann Rogosa and Sharpe Broth*). Instruments used were UV-Vis spectrophotometer (1601-Shimadzu), and autoclave.

2.2. Sample preparation

White cabbage (around 100g) of FW (fresh weight) and the outer leaves were striped. The hard part was cut off. The leaves were shredded into smaller pieces and washed, dried for 10 min.

2.3. Inoculation and fermentation

Direct culture of *L. plantarum* was kept at 37°C and revitalized by 26 h growth in MRSBat anaerobic conditions. Fermentations were carried out with 100g cabbage in *L. plantarum* inoculum variations of 5%, 10%, 15% and 20% (w/v).The fermentation process also was conducted with pH variations of 4, 5, 6 and 7.The fermentation process was performed in triplicates, each for 96 h, at 25 ^{0}C.

2.4. Determination of total sugar content

A sample (0.05 g) was added with 5 mL of 80% ethanol to test tubes, placed in water bath, and heated for 1 h at 80^0 C. Then, 1 mL of the sample extract was taken in another set of test tubes and mixed with 1 mL each of 5% phenol and 5 mL of sulphuric acid. These was mixed using vortex. The absorbance of the sample was read at 485 nm wavelength on aspectrophotometer UV-Vis, using 80% ethanol solution as a blank solution.

2.5 Determination of tannins content

A sample (2.5 g) was placed in an extraction flask and 20 mL of distilled water was added. The mixture was heated for 10 min and filtered using Whatmanno 40 filter paper. These was then washed using 2 mL distilled water. Then, 400 µL of sample was transferred into the test tubes, 0.5 mL Follin-

Denis reagent and 1 mL of sodium carbonate were added. The absorbance of the sample was read at 760 nm wavelength on a spectrophotometer UV-Vis, using distilled water solution as a blank solution.

3. Results and Discussion

3.1. Effect of variations in the amount of L. plantarum inoculum

The effect of different concentrations of inoculum is shown in Figure 1. It is shown that optimum inoculum for this fermentation was achieved at inoculum concentration of 5%.

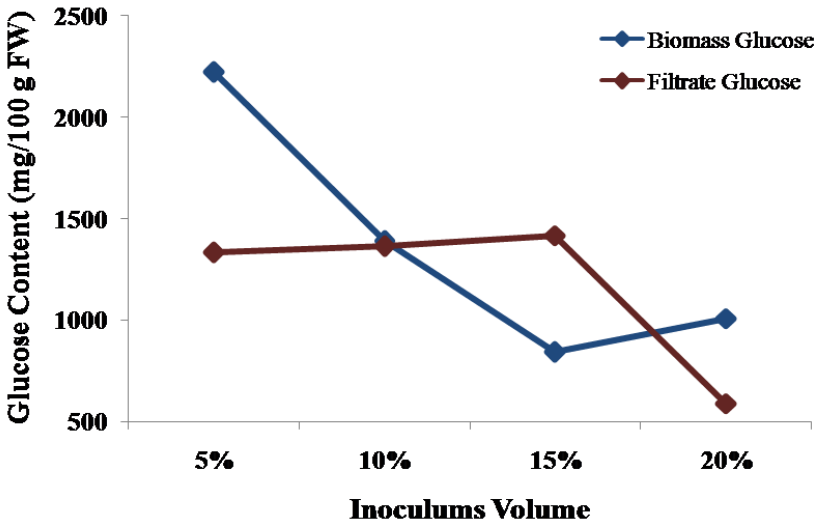

Figure 1.Correlation between biomass and filtrate in total sugarcontent using different variations of *L plantarum* inoculum

The relationship between total sugar contents in biomass and filtrate of fermentation products was shown inversely proportional. The highest sugar content was produced in the filtrate of fermentation products, using 5% inoculum concentration. In contrast, 5% inoculum concentration resulted in the lowest sugar content in the biomass of fermentation products. This fermentation process depends on the fermentation substrate that provide high sugar concentration**.** Another factor that contributes to increasing sugar in biomass is the nutrient of fermentation value [4]. Sugar in biomass is already available in a degradable form; and therefore *L.plantarum* cells can metabolize sugar directly. *L. plantarum* also able to produce invertase enzyme, that are catalysing the degradation of sucrose to glucose and fructose. The interactions between substrates and enzyme are using hydrogen bond[7]. The correlation between tannins product in biomass and filtrate showed in (Figure 2).

Figure 2 shows that the correlation of tannin contents in biomass and filtrate were directly proportional. Only in 20% inoculum the sugar was low because the microorganism was grown fastly. Based on Figures 1 and 2, there were inversely correlation between sugar and tannin in biomass after fermentation procees. Decreasing tannins as anti-nutrient was conducted using submerged fermentation. In the raw material, the content of tannins was 290.877 mg/g FW, this value decreased after fermentation, using 5% inoculum of *L. plantarum*. The tannin concentration decreased 75.938 mg/g FW. Tannins in cabbage sample exist as hydrolyzable tannins which consist of a polyhiydric alcohol esterified with gallic acid or derivatives of gallic acid [5]. Upon hydrolysis, tannins produce glucose as one of their products. Tannins also produce tannase enzymes. Tannase, when produced by submerged fermentation has been reported to be intracellular enzyme that produced by *L. plantarum*[6]. Tannase present in microorganism plays an active role in the decomposition and recycling of plants materials containing tannins[4].

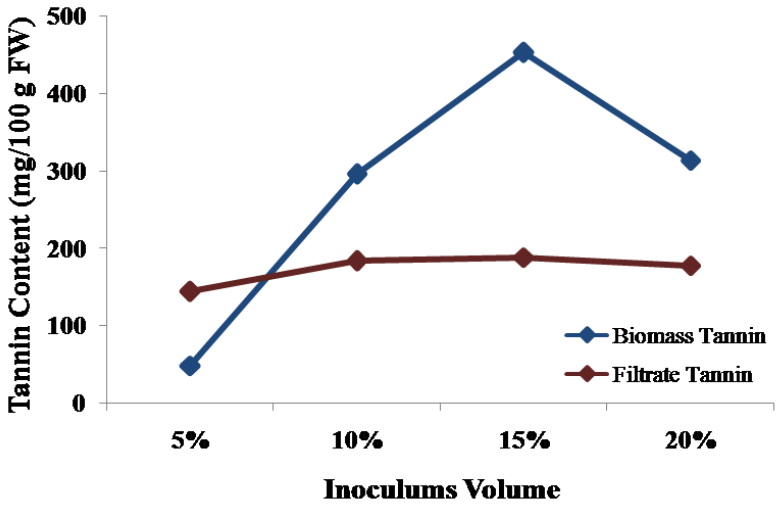

Figure 2.Correlation between biomass and filtrate in tannins content using different variations of *L plantarum* inoculum

3.2. Effect of pH variations in the fermentation process

Fermentation efficiency showed whether or not the fermentation was optimum, because with the optimum fermentation the maximum product will be obtained with little by product [2]. pH was a factor of fermentation efficiency for microorganism growth, metabolite production and decrease anti-nutrient especially tannins in *Brassica* during the fermentation process [2]. It showed that taninns was reduce after fermentation process. The optimum pH for the fermentation of *L. plantarum* was 6 in the 5% inoculum and reduced tannins content to 75.938 mg/g FW.This causes *L. plantarum* is able to develop in low oxygen levels for growth and can grow in the pH ranges of 3-6. Therefore, this condition can be called acidophil [2] (Figure 3).

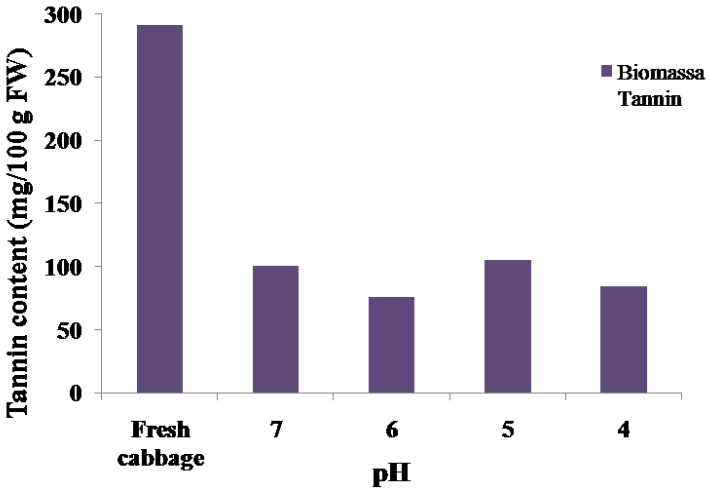

Figure 3.Effect of pH to Reduce Tannins

For this fermentation, the optimum pH is 6 for reducing tannins as anti-nutrient. *L. plantarum* produces tannase at pH 6 and it includes high-viability microorganisms to be used as a starter in fermentation. The tannase enzyme has a pH that allows it to carried out activities optimally, below or

above the optimum pH, the enzyme activity will decrease. pH plays important role in determining fermentation pathway [7]. Therefore, pH contributes to the quality of fermentation products.

4. Conclusion

This paper has demonstrated that fermentation in cabbage used *L. plantarum* has successfully conducted to reduce tannins contents in the cabbage. This is shown by the decrease of value of tannins in raw material, from 290.877 mg/g FW to 75.938 mg/g FW. The optimum conditions for the fermentation process were achieved at using 5% *L. plantarum* inoculum and at pH 6.

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