Utilization of Peanut Shell Waste (*Arachis hypogaea*) As a Growth Media for Probiotic Bacteria *Streptococcus thermophilus*

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Abstract

The increasing demand for peanut-based snacks has an impact on increasing peanut shell waste in Indonesia. The cellulose content in peanut shells is still relatively high, so it requires a long period for natural degradation. Though the waste can be converted and utilized in other forms, as a prebiotic. This far, inulin has been used as a prebiotic source. Products with inulin fortification have high prices, so we need to look for other cheaper ingredients. This research was conducted to test the ability of peanut shell waste extract as a prebiotic substitute material. To achieve this goal, peanut shells were extracted for cellulose. *Streptococcus thermophiles* probiotic bacterial growth test performed with a completely randomized design (CRD) pattern. The treatment factor used was crude extracts concentration of peanut shell waste. 0% was for the negative control, 20%, 40%, 60%, 80% and inulin were for positive control. Observation parameters in the research were the identification of colony morphology, gram staining, and growth curve analysis. The results of the study showed the crude extract of peanut shell waste affected *Streptococcus thermophiles* growth rate, even though it was not equivalent to the results of inulin treatment. The addition of coarse peel extract (40%) showed the most optimal growth curve pattern. The cellulose from peanut waste used as a carbon source for bacteria growth.

Keywords: Peanut shell, prebiotics, *Streptococcus thermophilus*

Introduction

Nowadays, the increasing product consumption of peanuts helps increase the abundance of peanut shells. This waste is organic waste that pollutes the surrounding environment, even though the cellulose content in peanut shell is still relatively high (47.19 %), while the content of lignin and hemicellulose were respectively by 30.57 % and 7.19 % (Oktasari, 2018). Besides the shell xylan fiber peanut at 6.3 % (Nathalia, 2011). High fiber content in peanut shells make it beneficial as prebiotics, food for probiotic bacteria (a natural micro flora in the body).

Currently, widely used prebiotic are inulin. This material is a type of prebiotic carbohydrate polymer group, synergizing with prebiotic minosa side chain groups. Inulin usually used as a nutrient for probiotic bacteria. Azhar (2009) found that the addition of inulin can help to increase the number of good microflora in the body. The biggest source of inulin found in the dahlia bulbs, but less in banana, garlic and wheat plants. Fewer sources of inulin make inulin prebiotic production limited. Besides, it makes the price of inulin fortification products more expensive. Utilization of high-fiber waste such as peanut shells can be used as an appropriate solution. A substance is called a prebiotic if it is not being hydrolyzed and not absorbed in the upper part of the digestive tract, selective substrates for one or a number of beneficial microflora in the colon and able to enrich the colon microflora to produce metabolite compounds that benefit for probiotic

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bacteria in the colon, such as *Bifidobacteria*, *Lactobacillus* (Murti et al, 2014) and *Streptococcus* (Daud, 2010). In this study, *Streptococcus thermophilus* bacteria, one of the starter microbes in probiotic beverage products was used. Although it has the potential as a prebiotic candidate, a study on the utilization of peanut shell waste has never been done. This study aims to examine the potential of peanut waste shell as a prebiotic. Testing the potential of crude extracts of peanut shell waste as an alternative candidate for prebiotics other than inulin is one of the ways to examine it, through the growth test of *Streptococcus thermophilus*, one of the probiotic bacteria.

Materials and Methods

The study adopted the experimental method of Witchencot et al (2010) with modification. Only a few parameters used by Witchencot et al. (2010) was used in this study. Overall, the research procedure was divided into three main stages: peanut shell preparation, peanut shell extraction, and probiotic bacterial growth test. The experimental design used a Completely Randomized Design (CRD). The study was conducted in April - July 2019 in the Laboratory of Faculty of Agriculture, Tidar University.

Tools and materials

The tools used in the study include ovens, basins, Erlenmeyer, beaker cups, funnels, Petri dish, test tubes, ose needles, gloves, microscopes, haemocytometers, glass preparations, drop pipettes, measuring cups, autoclaves, and other supporting equipment. The materials used in the experiment were peanut shell waste, aquadest, MRS broth media, inulin media, 70% alcohol, masks, and *Streptococcus thermophilus* strain FNCC 0040 obtained from the Food and Nutrition Laboratory of Gadjah Mada University.

Peanut Shell Preparation

Washing peanut shell used flowing distilled water. The purpose of washing was to remove dirt in the form of soil attached to the shell of peanuts. After washing the peanut shell, the first drying process was drying under the sun or air-dried for 24 hours. The second drying carried out using an oven at 60°C. Drying was done when it has got a constant weight of peanut shell. Peanut shell that has passed the drying process was then converted into powder (crude) to facilitate the extraction process.

Extraction and Formulation

The extraction process used water as a solvent. Extraction using water at room temperature done by mixing water and peanut shell powder in a ratio of 5:1. Stirring ran for 1 hour. The mixture was allowed to stand for 24 hours in a closed place. The mixture was filtered using a cotton filter and then followed by Wattman 42 filter paper, the frequency was three times filtering to a minimum filtrate residue (clear). The extract obtained was then mixed into the basal growth media according to the treatment level, the sterilization process carried out using an autoclave for 15 minutes at 121°C, pressurized 1 atm. The results of the

extraction stages were rough extracts of peanut shell combined with bacterial growth basal media (MRS Broth) according to the crude content of each treatment and were ready to be inoculated. The control treatment consisted of a negative control (KN) using basal medium and positive control (KP) with inulin. Each treatment was repeated 3 times. Growth media formulations are presented in Table 1 below.

Table 1. Formulation of Lactic Acid Bacteria

Codes	Types of bacteria	Types of treatment
SKN		100% MRSB
S.20		20% crude extract + 80% MRSB
S.40	*Streptococcus*	40% crude extract + 60% MRSB
S.60	*thermophilus*	60% crude extract + 40% MRSB
S.80		80% crude extract + 20% MRSB
SKP		100% inulin

Lactic Acid Bacteria Growth Test

Streptococcus thermophilus inoculums prepared by inoculation on growth media formulations (according to treatment) were incubated at 37°C, the colony was counted using a haemocytometer for 12 hours incubation period with 2 hours calculation intervals. Data from bacterial calculations on the haemocytometer were then calculated to get CFUs/ml (Colony Forming Units) and converted in the form of growth curves to obtain growth patterns with optimal logarithmic phases.

Results and Discussion

Observation of Bacterial Morphology and Gram Painting

The coloring technique divided bacteria based on basic differences in the structure of the cell wall. Gram-positive bacteria have thick mesh-like cell walls made of peptidoglycan (50-90% by weight of the cell envelope), while Gram-negative bacteria have a thinner layer of about 10% by weight of the cell envelope (Putri et al, 2018).

(a) (b)

Figure 1. Probiotic staining: (a) *S. thermophilus* culture, (b) Pure *S. thermophilus* (40 times magnification).

Staining results of isolates grown in prebiotic candidate media of rough extracts of peanut waste showed a type of gram-positive bacteria with purplish color. *S. thermophilus* has a spherical morphological structure with chain colonies (Takagi et al, 2014; Oliveira et al, 2012). The results of morphological observations (colonies or cells) and Gram staining showed that the bacteria grown were true *S. thermophilus* probiotic bacteria, and not a contaminant bacteria so that research can be proceed to the next stage.

Analysis of Lactic Acid Bacterial Growth Curves

According to Rohan et al (2016), the bacterial growth curve was divided into 4 stages: the initial phase (lag phase), the quadratic phase (log phase), stationary phase, and the declination phase (death phase).

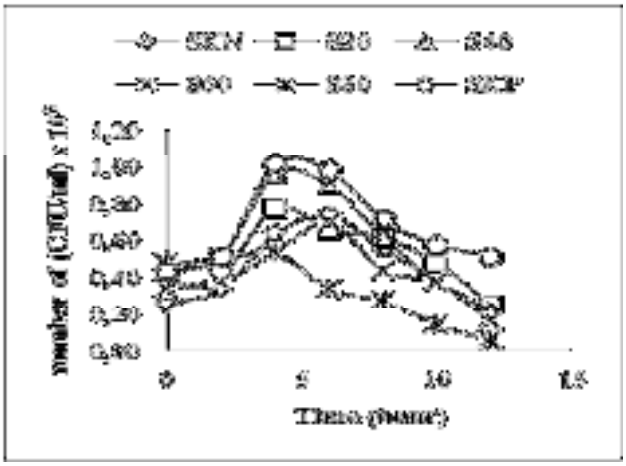

Figure 2. *Streptococcus thermophilus* LAB growth curve.

The results of the study showed that the initial phase (lag phase) occurred between the zero hours to fourth hours after inoculation. The lag phase is a phase when microbes are adjusting to the surrounding environmental conditions, in this case, a new growth medium (Middelbeek et al., 1992; Mangunwidjaja and Suryani, 1994; Yuliana, 2008). The adaptation phase is largely determined by the number of cells inoculated, the appropriate physiological and morphological conditions also the cultivation media needed. Oliveira et al (2012) explained that *S. thermophilus* which was inoculated from MRS media to inulin media experienced a lag phase (initial) at 0-3 hours after inoculation. The logarithmic phase (log phase) describes cells dividing at a constant rate, the mass doubling at the same rate, a constant metabolic activity, and a state of balanced growth. This phase is the right phase to take bacterial isolates as starter material (Sharah et al, 2015).

The logarithmic phase of *Streptococcus thermophilus* observed in the experiment ranged from 2 - 5 hours. Minj and Vij (2012) explained that the probiotic bacteria *S. thermophilus* ST144 had an optimal logarithmic growth phase at 3 - 6 hours in MRS media inoculation with the addition of 3% inulin. The experimental results showed that the optimal logarithmic

phase was reached when the optimum point of *S. thermophilus* of the optimal logarithmic phase was 1.02 x 10^5. *Streptococcus thermophilus* reached the optimum logarithmic point between the 4^{th} to the 6^{th} hours after inoculation. *Streptococcus thermophilus* bacteria which were inoculated on coarse peel extract media reached optimal logarithmic phase conditions at a concentration of 40% (S.40) with the number of bacteria (CFUs/ml) 9.6 x 10^5 between 6^{th} to 8^{th} hours. Coarse peanut extract was used by lactic acid bacteria as carbon material in cell metabolism. The carbon compound was obtained from cellulose content in peanut shells that are hydrolyzed together with basal media. When concentrations were above 40%, the carbon material contained in the media has a too high concentration that will inhibit the process of cell division.

The results showed that the stationary phase of *Streptococcus thermophilus* ranged from 6^{th} hour to 7^{th} hour. The stationary phase of bacteria is a steady growth phase which is characterized by a constant growth between living and dead bacteria, due to reduced nutrients and the formation of metabolic compounds that tend to be toxic to bacteria. This phase occurs the accumulation of metabolites as a result of cell metabolic activity (lactic acid) and nutrient content begins to run out, resulting in a competition of nutrients so that some cells die and others continue to grow so that the number of cells becomes relatively constant (Reiny, 2012). The stationary phase of lactic acid bacteria tends to be short because the results of metabolites in the form of lactic acid will be an inhibiting agent in bacterial cell division.

The death phase of *Streptococcus thermophilus* began after the 7th hour after incubation. The death phase occurs when the number of dead cells becomes more than the formation of new cells. Cells that are in a fixed phase will eventually die if it is not being transferred to other fresh media. The logarithmic form of the declining phase or death is a straight line that comes down, described by the number of living cells against time, the number of living bacteria decreases (Sharah et al, 2015). This research has different result with Pradipta *et al* (2017) which *Streptococcus thermophilus* incubated in PGY medium aged 24 hours has viability at 3,7 x 10^8 CFU/ml. Rohan *et al* (2016) explained that microbes in the phase of death are caused by several factors including the number of nutrients that have been used up, the age of microbial cells, and the presence of secondary metabolites from cell metabolic processes, including lactic acid, alcohol and so on.

According to the results of the growth curve analysis, the optimal growth curve was found in the positive control treatment using inulin growth media (SKP) both on *Streptococcus thermophilus* compared to other treatments. Oliveira et al (2012) in their experiments reported, inulin stimulates biomass growth and the result as the effect of fructose release from

hydrolysis and metabolism into additional carbon and energy sources on the kinematics of the growth of lactic acid bacteria. This is what drives the growth of logarithmic phase bacteria better than media without inulin. Although the treatment of adding crude extracts has not been able to show equivalent results to the treatment of inulin, the treatment of crude extracts of peanut waste shows the same graphic pattern so that it has the potential as an alternative prebiotic candidate, other than inulin for the growth media of *Streptococcus thermophilus*. Coarse peanut shell extract has the potential as a prebiotic preparation to meet the needs of prebiotics, both small scale or fermentative industry scale.

Conclusion

The use of coarse peel extract gives a different effect on the growth of probiotic bacteria with different levels of addition. Optimal growth of *Streptococcus thermophillus* reached when the formulation of the addition of peanut shells crude extract (S.40) was 40% although the results have not equivalent to inulin treatment.

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