

1 INTRODUCTION

1.1 Background of Research

Nowadays consumers are looking for functional food that can bring some beneficial effects for their body, either to improve human health or reduce the risk of some diseases. Probiotic food product is one of functional food that brings a lot of advantages for human body. Generally, probiotic food products are presented as beverage products. Probiotic beverages are beverage that has been fermented by probiotic bacteria like *Lactobacillus* or *Bifidobacterium*. Probiotics bacteria may produce many compounds that can inhibit the pathogen's growth such as lactic acid and acetic acid. These compounds can lower the pH and inhibit the growth of pathogens, but they also can be toxic to some microbes. Because of that, probiotics bacteria have many benefit for human health such as prevent lactose intolerant and milk allergy person from diarrhea, prevent constipation, lowered blood cholesterol, increase immune, and prevent cancer (Pundir *et al.*, 2013)

Probiotic beverages that have been commercialized are mostly milk based (yogurt and kefir), but nowadays, consumers like to consume some foods that made from healthy plant sources which have minimal cholesterol content. Vegetarian people also can not consume the probiotic beverages which is made from cow milk. Because of that, there are some research about making probiotic beverages using fruits and vegetables juice. Fruits and vegetables can be an ideal substrates for the culture of probiotics since they contain a lot of beneficial nutrient like minerals, vitamins, fibre, and antioxidants (Vasudha & Mishra, 2013).

“Kambas” Bitter melon is one of fruit family that is known as tropical or subtropical plant. It is widely cultivated in warm climate country such as South America, India, China, East Africa, and South Asia (Joseph & Jini, 2013). From the research about antioxidant properties of bitter melon which has been evaluated by Hamissou *et al.* (2013), it showed that bitter melon contains high concentration of antioxidants. Compared with other vegetables like carrot, tomato, celery, cabbage, green pepper, onion, and winter melon, bitter melon has richer antioxidants component (Ee-Shian *et al.*, 2015).

Fermentation is the method for making probiotic beverages. Fermentation can enhance the nutrient value of food through the biosynthesis of vitamin and amino acid by increasing the protein and fibre digestibility. To ferment food products, some microorganism is needed. Mostly, the microorganism that is used for fermenting food are yeast and lactic acid bacteria (LAB) (Magala *et al.*, 2015). LAB also have some properties of probiotics bacteria (Pundir *et al.*, 2013). Probiotics bacteria is defined as live microorganism in adequate amount that can give some health benefit for human body (FAO/WHO, 2001 *cit.* Patel, 2017). These bacteria can produce lactic acid and be able to reduce the pH and inhibit the growth of pathogens and also can be toxic to some microbes. Because of that, probiotics bacteria have many benefits for human health such as reduce lactose intolerance person from diarrhea, lower blood cholesterol, increase immune, and prevent cancer (Pundir *et al.*, 2013).

The research about the effect of lactic fermentation on antioxidant activity in herbal tea by Ibrahim *et al.* (2014) reported that fermentation of herbs using lactic acid bacteria can increase the antioxidant activity. The result showed that fermented herbal tea has a higher antioxidant activity than the fresh one (from 45% to 85%). Because of that, the fermentation of bitter melon juice perhaps can produce a probiotic beverage that has more functional properties because it contains higher concentration of antioxidant and some antimicrobial compounds.

Lactobacillus fermentum LLB3 isolated from bamboo shoot pickles has probiotic characteristics (Lindayani *et al.*, 2018). This LAB can be used for making probiotic beverages. The research about fermentation of “Kambas” bitter melon (*Momordica charantia* L.) juice using *Lactobacillus fermentum* LLB3 for making probiotic beverages is still limited. Therefore, this research is important because it examined the ability of “Kambas” bitter melon as media for lactic acid bacteria’s growth, the amount of *Lactobacillus fermentum* LLB3 during fermentation, and also the effect of “Kambas” bitter melon fermentation to its component properties (antioxidant, bacteriocins, and antimicrobial inhibitory activity) in two different time of fermentation (24 hours and 48 hours).

1.2 Literature Review

1.2.1 “Kambas” Bitter Melon (*Momordica charantia* L.)

Bitter melon (*Momordica charantia* L.) is known as tropical or subtropical plant that widely cultivated in warm climate country such as South America, India, China, East Africa, and South Asia (Joseph & Jini, 2013). Bitter melon is categorized as fruit that contains a lot of antioxidant and vitamin that gives a lot of benefits for human's health. Bitter melon has been used as medicine in Asian and African since the ancient time to treat some diseases like tumor, malaria, rheumatism, vaginal discharge, and controlling blood sugar level (Rani *et al.*, 2014). According to Altinterim (2012)'s research, bitter melon has ability to reduce blood glucose and enhance insulin sensitivity, therefore, it can be used as antidiabetic especially for diabetes mellitus type 2.

Based on the research done by Ee Shian *et al.* (2015), bitter melon is one over 1000 plants that has been recognized of their antioxidant and anti-diabetic potential. There are two types of bitter melon : “Kambas” bitter melon which has a long light green appearance and “Katak” bitter melon which has a small dark green appearance. The research about antioxidant properties of bitter melon found that the antioxidant component in bitter melon was 82.05% as effective as ascorbic acid for inhibiting the free radical DPPH compared with zucchini which only has 12.19% of effectiveness (Hamissou *et al.*, 2013). Previously study by Wu & Ng *.cit.* Ee-Shian *et al.*, (2015), conclude that the amount of antioxidants in the bitter melon are richer than common vegetables such as carrot, tomato, celery, cabbage, green pepper, onion, and winter melon. Bitter melon also has been evaluated for having better scavenging effect than vitamin E. Ozysaglam & Karakoca *cit.* Ee-Shian *et al.*, (2015) also summarized that the ripe bitter melon extract has the strongest antioxidant activity compared with the unripe one.

1.2.2 Lactic Acid Bacteria (LAB)

Lactic Acid Bacteria (LAB) is a group of Gram positive, non-spore forming, cocci or rods bacteria. This bacteria can produce lactic acid that can be used to make food product from fermentation of carbohydrates. LAB also has some properties of probiotics bacteria (Pundir *et*

al., 2013). Probiotics bacteria is defined as living microorganism in adequate amount that can give some health benefit for human body (FAO/WHO, 2001 *cit.* Patel, 2017). Commonly, bacteria that is considered as probiotics bacteria are member of genus *Lactobacillus* and *Bifidobacterium* (Kechagia *et al.*, 2013). Probiotics bacteria may produce many compounds that can inhibit the pathogen's growth such as lactic acid and acetic acid. These compounds can lower the pH and inhibit the growth of pathogens, but they also can be toxic to some microbes. Because of that, probiotics bacteria has many benefit for human health such as reduce lactose intolerance person from diarrhea, lower blood cholesterol, increase immune, and prevent cancer (Pundir *et al.*, 2013).

Lactobacillus fermentum is one of the species in genus *Lactobacillus*. In this research, LAB strain that were used is *Lactobacillus fermentum* LLB3 found in traditional fermented food which are pickle bamboo shoot (Lindayani *et al.*, 2018). *Lactobacillus fermentum* also included as bacterial strain that have been used for making probiotics food. This bacteria have high ferulic esterase activity that can give some health benefits such as reduce the progression of atherosclerotic lesion in hypercholesterolemic hamster (Omar *et al.*, 2013). Maximum growth of *Lactobacillus fermentum* is at pH 4 and 5. The growth will be suppressed at pH 2-3 (Tallapragada *et al.*, 2018). Besides that, *Lactobacillus fermentum* also has an ability to inhibit the growth of another microbial due to the production of bacteriocins that involves as antimicrobial substances (Bao *et al.*, 2010).

1.2.3 Fermentation of Lactic Acid Bacteria

Fermentation is one of the most ancient method in food processing. It can also be used for preservation process to increase the shelf life of food without using additives or preservation storage like refrigerator (Petre, 2012). Besides that, Fermentation also can enhance the nutrient value of food through the biosynthesis of vitamin and amino acid by increasing the protein and fibre digestibility and enhance food safety by reducing toxic compound and inhibiting food borne pathogens. To ferment food products, some microorganism is needed. Mostly, the microorganism used for fermenting food are yeast and lactic acid bacteria (LAB) (Magala *et al.*, 2015). There are two types of LAB fermentation, spontaneous fermentation and controlled fermentation. Spontaneous fermentation leads to variations in sensory properties which differ according to the quality of material, temperature, and environment

conditions. Various LAB will naturally grow and fermented the product. For controlled fermentation, environmental condition must be created in accordance with the characteristics of LAB bacterial growth (Ray & Montet, 2014). In the research about the effect of lactic fermentation on antioxidant activity in herbal tea by Ibrahim *et al.* (2014), it showed that fermented herbal tea has a higher antioxidant activity than the fresh one. The antioxidant activity is increasing from 45% to 85%.

LAB is used for making dairy product fermentation such as yogurt, cheese, buttermilk, and kefir. This type of bacteria was chosen because it has a fermentative ability to enhance food safety, increase functional properties of food, and give a lot of health benefits for human (Gessesse, 2015). The use of milk as the base ingredient is the most excellent carriers for probiotics bacteria. However, because the main ingredient of this fermentation is dairy product (milk), some consumers with high cholesterol and vegetarian have a limitation to consume this product. Because of that, there are some research about producing the fermentation beverage using fruit juice as the media or main ingredient. The characteristics of LAB is suitable to make fermentation juice because LAB has an ability to survive in wide range of pH and temperature. This criteria is really important because mostly fruit juice has a low pH value (below 5) (Khezri *et al.*, 2016).

1.2.4 Free Radical and Antioxidant for Human Health

Free radical is reactive oxygen species and reactive nitrogen species generated by our body in various endogenous systems. Oxidative stress is a condition that are caused by the destroy of the body's ability by free radical component. Free radicals thus adversely alter lipid, protein and DNA and trigger a number of human diseases (Lobo *et al.*, 2010). Antioxidant is a molecule that has a capability to slow or prevent the oxidation of other molecules. Cellular damage of free radical scavenging properties can be delayed or inhibited by antioxidant molecule. In other words, antioxidant also has some important rules in defense mechanism system such as blocking the production of free radicals, scavenging the oxidants, blocking the production of toxic metabolites, converting the oxidants into less toxic compounds, and repairing the molecular injury that caused by free radical (Kabel, 2014).

1.2.5 Bacteriocins

Bacteriocins are described as ribosomally synthesized small poly peptides that have bacteriostatic or bactericidal activity against other microorganism (Güllüce *et al.*, 2013), and are naturally an antibiotic peptides that can be produced by gram positive bacteria such as lactic acid bacteria (LAB). There are three groups of bacteriocins *i.e* class I (lantibiotics produced by *Lactococcus lactis*), class II (small heat non-lactibiotics produced by *Pediococcus sp.*), and class III (small heat labile protein produced by *Lactobacillus helveticus*) (Mortvedt *et al* in Mohammed *et al.*, 2013). Bacteriocins have been describes as growth inhibitory agent against some bacteria because they have an antimicrobial activity. The production of bacteriocins will be influenced by some factors such as temperature, pH, and source of nutrients (Yusuf *et al.*, 2013).

A large amount of bacteriocins have been isolated from LAB and some of them have been acquired a status as potential antimicrobial agent because they can be used as natural food preservatives and against pathogens bacteria. One of the most thoroughly studied bacteriocins is from *Lactococcus lactis* spesies. This bacteria is used in the fermented milk production that contain probiotics bacteria which give a lot of health benefits (Aly *et al.*, 2006). Devugst & Vandamme (1994) *cit.* Mohammed *et al.*, (2013) also reports that the first bacteriocins that commercially used as food preservatives is nisin produced by *Lactococcus lactis*. Besides that, bacteriocins that produced by *Lactobacillus acidophilus* also can be used for diarrhea treatment in children. The other bacteriocin of *Lactobacillus* has been reported to have a potential effect as natural food preservatives (Mohammed *et al.*, 2013).

Bacteriocins produce by LAB will inhibit another bacteria through forming pores in the cell membrane and remove the proton motive force. The cation side of bacteriocins will bind to the target cell surface and the C-terminal inside hydrophobic helical will half penetrates into the hydrophobic part of the target and apparently it binds to mannose and cause the leakage of the membrane. Besides that, bacteriocins can also bind the membrane protein. Between Gram-Positive and Gram-negative bacteria, Gram-Negative bacteria is more resistant to bacterocins compound because it is protected by the outer membrane of the cell (Yusuf *et al.*, 2013).

1.2.6 Probiotic Beverages

Probiotic beverages are beverage that has been fermented by probiotic bacteria such as *Lactobacillus* or *Bifidobacterium*. Nowadays, probiotic beverages are really common in the market. But, mostly probiotic beverages are milk based. So, consumers with lactose intolerance, high cholesterol, and vegetarian still hesitate to consume or can not consume this healthy product. Besides that, several regions in Indonesia do not have a lot of milk as the ingredient of probiotic beverage. Because of that, there are some research about making probiotic beverages using another main ingredients such as cereal, vegetable, or fruit juice (Vasudha & Mishra, 2013).

The research about non-dairy probiotic beverages has been reviewed by Vasudha & Mishra (2013). This research explained about another alternative main ingredient for making probiotic beverages are cereals, legumes, fruits, and vegetables. Fruits and vegetables can be ideal substrates for the culture of probiotics since they contain a lot of beneficial nutrient like minerals, vitamins, fibre, and antioxidants (Yoon *et al.*, 2004 *cit.* Vasudha & Mishra, 2013). The interest in producing probiotic drinks from fruit and vegetable as the based ingredients is because they have a fresh taste and are already known as healthy foods.

Some beverages can be known as probiotic beverages if they have total amount of probiotic bacteria $>10^6$ CFU/ml (Yerlikaya *et al.*, 2013). According to the research by Sheenan *et al.* (2007) *cit.* Vasudha & Mishra (2013), the addition of *Lactobacillus* and *Bifidobacterium* in orange and pineapple juice has a longer lifetime of probiotics bacteria than in the cranberry juice. In orange juice media, the probiotics bacteria can survive at above 7.0 log CFU/ml and in pineapple juice at level above 6.0 log CFU/ml for at least 12 weeks. In another study using beet juice, even though there are placed in cold storage, the strain of *Lactobacillus acidophilus* still remains at 10^6 - 10^8 CFU/ml. Some lactic acid bacteria also grow well in cabbage and carrot juice media. All of these result approve that fruit and vegetable media also can be used for making probiotic beverages.

1.3 Objectives

The objectives of this study are to examine the ability of “Kambas” bitter melon as media for lactic acid bacteria’s growth, the amount of *Lactobacillus fermentum* LLB3 during fermentation, and also the effect of fermentation on “Kambas” bitter melon properties such as antioxidant, bacteriocins, and antimicrobial inhibitory activity against pathogenic bacteria (*Escherichia coli* FNCC 0091, *Staphylococcus aureus* FNCC 0047, and *Salmonella typhimurium* FNCC 0187) in two different time of fermentation (24 hours and 48 hours).

