

Effects of Different Types of Plant-Based Milk and Animal-Based Milk Delivery Vehicle on Growth of *Lactobacillus Plantarum*

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Abstract

Probiotics has been discovered long time ago for its beneficial effect on health when consumed especially to the people who had allergy and gastrointestinal disease. This preliminary study was conducted to find out which vehicles can allow better growth of probiotic. *Lactobacillus plantarum* was used in this study as choice of probiotic to be cultured in the four types of milk. The pH value was recorded for determination of growths and metabolic activity of the probiotic. Results showed that *L. plantarum* in soy milk can grow and had a better metabolic activity in the cultured soy milk with pH 3.46 compared to others. The highest growth was recorded at optical density of 1.137 in soy milk at 560nm. This result showed that *L. plantarum* in soy milk multiply rapidly compared to other milks. As conclusion, soy milk has better development of probiotic as delivery vehicles compare to cow milk, goat milk and coconut milk.

Keywords

Lactobacillus plantarum, Probiotics, Optical Density, pH Value, Soy Milk, Delivery vehicles.

Introduction

Over the past 20 years, there were numerous studies on the effect of probiotics on the immune system. There was also evidence that certain strains of probiotic can enhance the acquired immune response (Toma & Pokrotnieks, 2006). In order to enhance the well-being of the host, probiotics are usually delivered in the supplements or foods. Probiotic has been applied on many things include milk, feed supplements, dairy products, pharmaceutical, fruit juices, chocolates and also meat products (Musa et al., 2009). Since the benefit of probiotic was discovered, the demand towards probiotic has increased tremendously among consumers who realize the important to stay healthy (Dianawati et al., 2016). In this research, animal-based milks used were cow milk and goat milk. Cow milk is the most popular nonhuman milk that commonly consumed by human (Wiley, 2011). This milk is rich with calcium and other important nutrient but long-term avoidance will associate with poor bone health (Black et al., 2002). In addition, cow milk is cheaper compared to goat milk and has the highest production in the world. Goat milk has lower acidity compared to cow milk (Arora & Joshi, 2013). Furthermore, when comparing to cow milk, goat milk has the highest content of crude protein, fat, ashes and total dry matter (Borkova & Snaselova, 1995). For the people who have allergic towards cow milk, goat milk is one of choice to replace cow milk due to its popular beneficial and therapeutic effect. There are many health benefits of soybean that has been discovered by researcher and one of it is the ability to reduce the cholesterol (Jooyandeh, 2011). It also one of an alternative to the patient who has problem with lactose intolerance and beneficial for vegetarians (Barnes, 2010). The aim of this study was to study the development of probiotic using different types of plant-based and animal-based milk as a delivery vehicle.

Methods

Materials and probiotic

Fresh cow milk, goat milk, coconut milk and soy milk were purchased from local market in required amount and processed according to Fatemeh et al. (2016). The milks were stored in cold room at temperature 0°C to 4°C. The MRS broth powder and bacteria strain *Lactobacillus plantarum* were supplied by Department of Food Technology, School of Industrial Technology, Universiti Sains Malaysia (USM) and sub cultured on MRS agar plate.

Preparation of MRS broth

A total of 5.2 g of MRS broth powder was mixed with 100 ml of distilled water. The mixture was mixed thoroughly until the powder dissolved completely. A total of 20 ml broth was transferred into universal bottles and autoclaved at 121°C for 15 minutes.

Preparation of culture

One colony of *Lactobacillus plantarum* from MRS plate was inoculated into 20 ml of MRS broth in universal bottle and incubated at 37°C for 24 h. After incubation, 1 ml of incubated MRS broth were pipetted into 100 ml of each bottles.

Determination of pH value

The pH meter was calibrated by using 5 and 7 pH buffer. After incubation, MRS broth was transferred into each milk, the pH meter was dipped into each milk and the reading was recorded for every two hours until reach 24 h. The pH meter was rinsed with sterile distilled water before collecting the data to avoid contamination and bias result (Aboulfazli et al., 2014).

Optical Density (OD)

Optical density was tested by using spectrophotometer. Sample was taken every 2h for 24 h and the optical density was recorded. Each milk was pipetted into cuvette by using sterile pipette tips and the reading was recorded and repeated three times for average reading (Fatemeh et al., 2016).

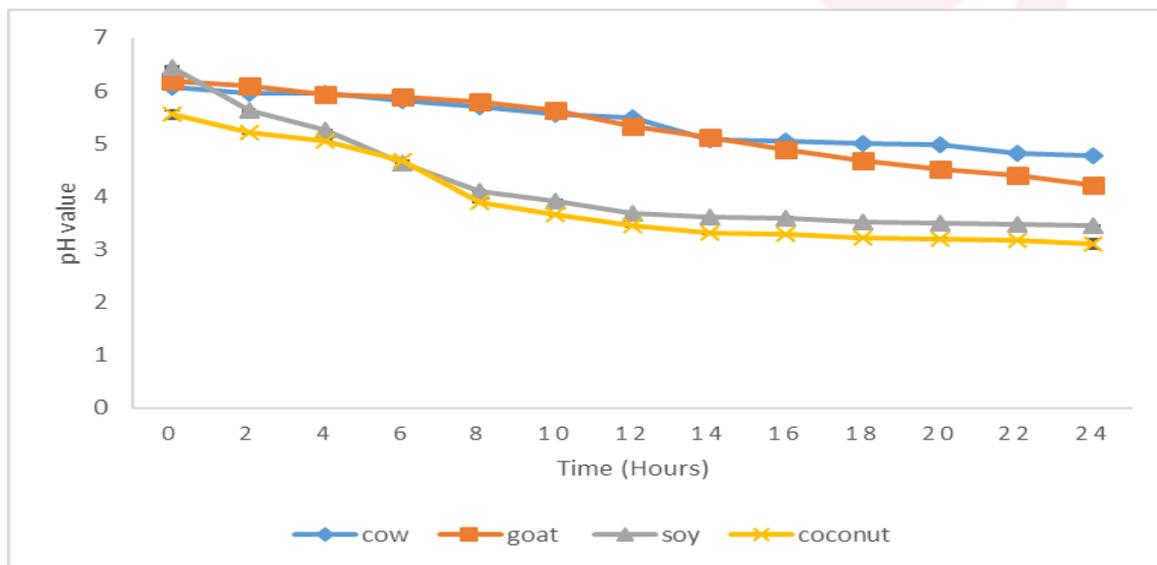
Statistical Analysis

All recorded data has been analyzed by using SPSS version 22 and Microsoft excel for plotting an accurate graph. The graph was plotted to determine the changes of the pH and bacterial growth in 24 h.

Results

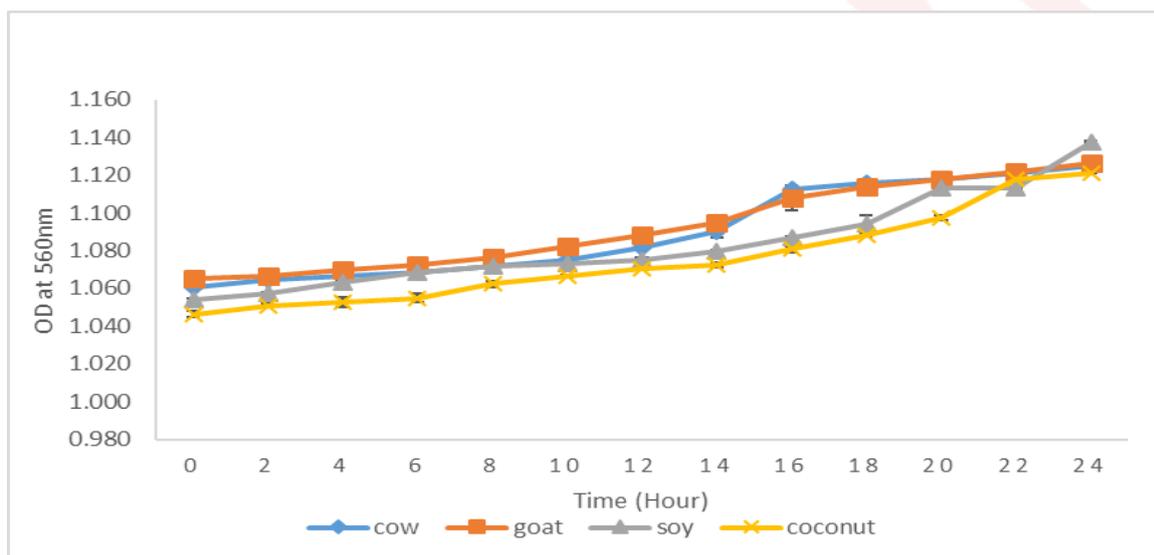
Results for the pH value changes were recorded in Figure 1 showing that the highest pH for bacteria growth was in soy milk and lowest in coconut milk.

Figure 1: The pH changes in four different milks vehicles inoculated with *L. plantarum* taken every 2 h for 24 h.



The Optical Density (OD) result shown that there were significant differences ($p < 0.05$) in growth of *L. plantarum* between each cultured milk. The highest growth was observed in soymilk and lowest was in coconut milk.

Figure 2: The bacterial growth at respective optical density in the four different milks vehicles inoculated with *L. plantarum* taken every 2 h for 24 h.



Discussion

The trend nowadays can be seen that people are interested in healthcare products and this has contribute to an increasing in demand towards functional dairy products like yogurts and fermented milks which potentially brings beneficial health effects. Most commercial probiotic strain that has been used in fermented milks were *Lactobacillus* and *Bifidobacterium*. These two genera were safe to be used in the production of fermented products and usually can be found as normal microbiota in gastrointestinal track of human (Shiby & Mishra, 2013). The probiotic used in this research was *Lactobacillus plantarum* which was locally isolated. *L. plantarum* is a non-starter lactic acid bacteria (NSLAB) which can tolerate with the environment and having high survival ability while giving protection to the host towards pathogenic bacteria such as *Listeria monocytogenes* that can cause infection called listeriosis (Wu et al., 2016). These bacteria able to improve the health status of human and increase in the absorption of nutrient in the human gut.

Thus, the aim of this study was to determine the pH value changes and the optical density of cultured fermented milks. Decrease in pH of cultured milks indicate the changes in bacterial population and increase in lactic acid concentration and metabolism. Lactic acid produced by probiotic caused increase of hydrogen ions in the milk which result in the reduction of pH readings from time to time. So, the decreases of pH in the cultured milks proves that there was active metabolism of probiotic inside the cultured milks and also the survival of the probiotic. As for the optical density (OD), the results were based on the turbidity of the milks and probiotic population by using spectrophotometer at 560nm. The higher the turbidity would suggest that there were increasing in the probiotic population inside the cultured milks. (Settachaimongkon et al., 2014)

For the determination of pH value in each milks the result was plotted and analyzed as shown in Figure 1. Graph shows that soy milk has the highest reduction of pH when compared to other milks which was from pH 6.44 to 3.46. Theoretically, the optimum pH for *L. plantarum* is 5.5 to 6.5 and the lowest growth at pH 3.3 and below (Aryani et al., 2016). Thus, the pH drop efficiently because of high metabolic activity of *L. plantarum* in soy milk. This proved that soy milk has the potential to be one of the best milk vehicle for the growth of *L. plantarum*. Soy milk contains high amino acid, fibre, polysaccharide, lecithin and unsaturated fat which helps in the probiotic growth. Lecithin in the soy milk act as emulsifier which give protection to the membrane protein of the soy and it also assist in the structure and taste of the fermented soy milks. The other components were mineral, vitamin, bioactive polyphenol such as isoflavones, saponin and phenolic acids. (Aboulfazli et al., 2014).

The pH reduction of soy milk drop to 3.46 which was acidic might makes people wonder about the safety of the consumption of cultured soy milk. This actually due to all of the cultured milks has been

incubated and not refrigerate. This had caused the probiotic to continuously active and converting sugars into lactic acid that result in continuous reduction of pH value. In order to avoid this from becoming very low, the cultured milks need to be refrigerated to maintain the stability of the milks and makes the probiotic become less active or even inactive (Saxelin, 2008). On top of that, no stabiliser and additive was added to the milks in this study because this study was focused more into differentiation of milks which can become a better vehicles as preliminary study.

Soy milk that has been inoculated with probiotic bacteria were able to produce beverage which was good and has multifunction properties. Besides that, this fermented milk is improved in nutritional value due to the fermentation process by probiotic and this product can be consumed by people who has lactose intolerance (Ghosh and Chattopadhyay, 2011). According to Martinez (2012) fermented soy milk also has antihypertensive and antioxidant properties thus any product from soy milk can become a prevention therapy towards cardiovascular related disease These previous findings also suggested that soy milk have a better health benefit after cultured with probiotics.

On the other hand, cow milk showed the lowest reduction of pH. This result showed that probiotic that was cultured in cow milk might have lower metabolism and growth. Even though cow milk has been claimed to have a good buffering capacity, however, the optical density of the cultured cow milk did not increase as much as soy milk. Besides that, cow milk has a factor that can accelerate the iron absorption in human body and contain low levels of ascorbic acid (Vanderhoof & Kleinman, 2015).

Based on Figure 2, the highest growth of *L. plantarum* was recorded in soy milk cultured with optical density of 1.137 (560 nm). The lowest growth of *L. plantarum* was recorded in the goat milk. Previous research showed that goat milk has a balance essential amino acid and the level of calcium was high. It also contains smaller size fat globules and showed lower allergenic properties compare to other milk. But, despite from these characteristics, it actually has lower folic acid and vitamin D and the amount of probiotic in goat milk is important to determine the beneficial health effect toward human (Tsend-ayusha & Yoon, 2013). Instead of that, Rysstad & Abrahamsen (1983) also stated that the manufacture of fermented goat's milk products such as set-style yoghurt faces a problem of over-acidification due to a low buffering capacity of goat's milk.

People preferences towards milk are generally based on several factor which include intolerance, bad taste, and lifestyle choice. If children do not consume milk, their dietary calcium intake will become lower and need to substitutes the milk with the mineral supplement or calcium-rich drinks (Woodside et al., 2016). Besides that, some people have a lactose intolerance which can trigger allergic reaction as well as other issues such as dietary preferences include vegetarians and other health issues. There are many probiotics product in the market are mainly based on animal products, therefore it is important to have probiotic based on plant source with a very good taste even it has been fermented so that probiotic drinks later on can grasp the interest of these people towards probiotic milk without any side effect (Lúcia et al., 2011). This will help a lot in people who need nutritional components which can be found only in milk compared to supplement which most of them were chemically synthesized. So, this study hit the target to have probiotic beverages based on plant source which was soy milk.

Conclusion

In conclusion, there was a significant different with p value less than 0.05 for the growth of *L. plantarum* between four types of delivery vehicles which were cow milk, goat milk, coconut milk, soy milk. Soy milk can be suggested as the best delivery vehicle of probiotic compared to other milks



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