

International Journal of Advance Tropical Food (IJATF)

Vol. 2, No. 1, Mei 2020, pp. 8-17

E-ISSN: 2715-5889, DOI: <http://dx.doi.org/10.26877/ijatf.v2i1.6095>.

Effect of Cryoprotectant Concentration on Starter Culture Viability Sinbiotic Yogurt with Freeze Dried Sweet Potato Extract Supplementation

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Abstract

Synbiotic yogurt with purple sweet potato extract supplementation as prebiotics and *Lactobacillus plantarum* Dad 13 isolated from buttermilk as probiotics has potential as functional food, but requires low storage temperatures. The freeze drying technique requires cryoprotectant as a protective material for products such as yogurt. The purpose of this study was to determine the effect of sucrose concentration on the level of viability of Lactic Acid Bacteria and *Lactobacillus plantarum*. This study used a Completely Randomized Design with one factor : concentration of sucrose as cryoprotectant: 0%, 2.5%, 5%, 7.5%, and was carried out in three replications. The results showed that the concentration of sucrose significantly affected the yield of freeze dried synbiotic yogurt, total Lactic Acid Bacteria (LAB) after freeze drying, and total *Lactobacillus plantarum* before and after freeze drying, but did not significantly total amount of LAB before freeze drying. The best treatment, shown in frozen dried synbiotic yogurt with a sucrose cryoprotectant concentration of 5%. The treatment has the following characteristics: yield, 14.797%, total Lactic Acid Bacteria 1.98×10^9 CFU / ml before freeze drying, 9.28×10^8 CFU / ml after freeze drying, total *Lactobacillus plantarum* 8.23×10^8 CFU / ml before freeze drying and 6.81×10^8 CFU / ml after freeze drying.

Keywords: synbiotic yogurt, freeze-dried, starter viability, cryoprotectant

How to Cite: Tari, A. I. N., Handayani, C. B., & Hartati, S. (2020). Effect of Cryoprotectant Concentration on Starter Culture Viability Sinbiotic Yogurt with Freeze Dried Sweet Potato Extract Supplementation. *International Journal of Advance Tropical Food*, 2(1),8-17. <http://dx.doi.org/10.26877/ijatf.v2i1.6095>.

INTRODUCTION

Together with the increasing public awareness on the importance of healthy foods, the consumer demands related to food qualifications have begun to shift. Consumers currently look for foods that contain not only a good nutritional composition with attractive appearance and taste but also certain physiological impacts for the body, such as its benefits to maintain the balance of intestine microbiota and immune system. The healthy consumption pattern can be achieved through the addition of synbiotic yogurt. The types of synbiotic yogurt include local probiotic-based yogurt and purple sweet potato extract-supplemented yogurt.

Tari et al. (2013) reported that purple sweet potato extract-supplemented yogurt made from commercial cultures and indigenous probiotics, such as *Streptococcus thermophilus* FNCC 0040, *Lactobacillus bulgaricus* FNCC0041, and

Lactobacillus plantarum Dad 13 by the ratio 1: 1: 0.5 had now been available completed with the physical properties (pH = 3.78, viscosity = 5.1987 cP, chromatic color = 18.559) and chemical properties (titrated acid content = 1.2733%, moisture content = 85.2664%, ash content = 0.8041%, reduction-sugar level = 3.3278%, dissolved protein content = 1.4782%, fat content = 0.08%, and anthocyanin level = 8.5315%). Tari et al (2014) also shared that indigenous probiotics, such as *Lactobacillus plantarum* Dad 13 supplemented to the purple sweet potato extract yogurt could act as a diarrhea-lowering agent in experimental animals. The discovery was indicated by the decreasing water content of the experimental animals' feces from 63.32% to 62.73%, in addition to the decreasing water content of the experimental animals' cecum from 83.31% to 35.13%. The indigenous probiotics are also able to reduce free radical components as indicated by the decreasing blood MDA level of the experimental animals from 4.23 mmol/ml to 1.52 mmol/ml and the decreasing liver MDA level at from 5.60 mmol/ml to 2.96 mmol/ml at the end of the study.

According to Spreer (1998), fermented milk products must be stored at the temperature less than 10°C to avoid the negative environmental effects. This low temperature storage is needed to inhibit the fermentation process, so that the number of microbes that remains, remains high. This result in high storage and distribution costs. One way to overcome this problem is by making synbiotic yogurt using the freeze dried method.

Davidson et al (1999) explained that the freeze-drying process could reduce the number of bacteria up to 1 log cycle, as the process allows the microbial death (sublethal). Therefore, the addition of protective substances (cryoprotectant) before the freeze-drying process is required to minimize the damage.

The coating material (cryoprotectant) which is commonly used as an encapsulant can be derived from gum, carbohydrates and protein, which is a material used to coat core material (bacteria) with specific purposes such as covering up unpleasant taste and odor, protection against environmental influences, increasing stability and preventing stability evaporation. This cryoprotectant material can be either carbohydrates or protein.

According to Rizqiati (2006), the use of protein as a cryoprotectant can maintain bacterial resistance while the use of carbohydrates as cryoprotectant can both improve microcapsules texture and maintain the resistance of probiotic bacteria. Chattopadhyay (2002) stated that sucrose could function as a protective material for bacteria. It is safe for consumption and able to increase sweetness. The addition of sucrose as a *cryoprotectant* functions to protect the structure and proteins in microbial cells (Morgan et al, 2006).

However, there have not yet been known the ability of sucrose as a cryoprotectant agent in lactate-fermentation drinks such as indigenous yogurt probiotics (local) with sweet potato supplementation through a freeze drying process to the viability of Lactic Acid Bacteria (LAB) and *Lactobacillus plantarum* Dad 13 and its characteristics. That is why this research needs to be done.

This research aims to determine the effect of sucrose concentration as cryoprotectant on yield, total Lactic Acid Bacteria (LAB) and total *Lactobacillus plantarum* Dad 13 in freeze dried lactate fermentation drinks.

RESEARCH METHOD

Research flowchart

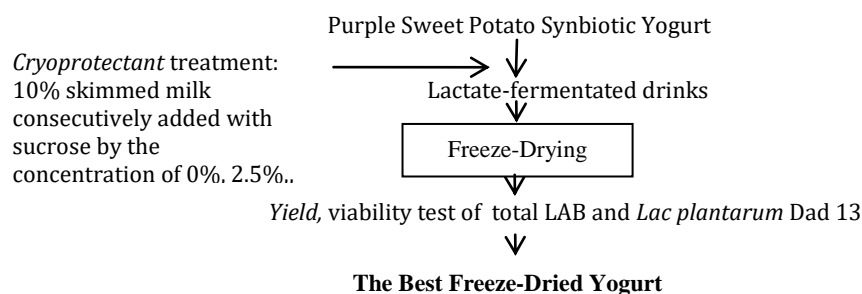


Figure 1. Research Flowchart

Materials and Instruments

The material used consist of Lactic Acid Bacterial cultures collection of FNCC (Food and Nutrition Culture Collection) from PAU Food and Nutrition UGM Yogyakarta. The bacterial cultures were *Streptococcus thermophilus* FNCC 0040, *Lactobacillus bulgaricus* FNCC 0041, and *Lactobacillus plantarum* Dad 13 as probiotic indigenous LAB cultures which were collected from UGM Researchers Association. The MRS (de Mann Rogosa Sharpe) Agar/Broth (Oxoid) used for maintenance of Lactic Acid Bacteria culture. LPS media is a selective media for the growth of *Lactobacillus plantarum* Dad13. Supporting chemicals such as 70% alcohol, spiritus, and distilled water are obtained from the Lab. Biology-Chemistry and Microbiology, Faculty of Agriculture, University of Bantara, Sukoharjo. Equipment used includes glassware (test tubes, Beaker cups, Erlenmeyers, and petridish), autoclaves (All America), incubators (Inko), Ovens (Binders), entkas.

Research Procedure

The stages of this research include making culture stock, making starter culture, making purple sweet potato extract, making probiotic yogurt by supplementing purple sweet potato extract and freezing drying (Figure 1).

Preparation of bacterial culture stocks

The Commercial bacteria (*Streptococcus thermophilus* FNCC 0040 and *Lactobacillus bulgaricus* FNCC 0041) and *Lactobacillus plantarum* Dad 13 as indigenous probiotic LAB culture in slant culture were taken 1 ose, then grown in a 10 ml of sterile liquid MRS. The cultures were then etched in a sterile agar slant and incubated at 37°C during 24 to 48 hours. The stocks of bacterial cultures in the agar slants were stored in a refrigerator at 2°C to 3°C and regenerated once every two weeks.

Preparation of purple sweet potato extract

The procedure of purple sweet potato extraction referred to the preliminary research conducted by Tari (2012). The procedure was begun by dice-cutting the sweet potatoes in a (5x5) cm cube size and extracting the cuts in a juicer. The product was then left to stand for 24 hours at a 4°C temperature, so the purple sweet potato starch would settle, while the filtrate would remain. The filtrate of the purple sweet potato extract would be taken to produce the yogurt.

Preparation of Starter Cultures

This stage included the preparation of 5 ml sterile liquid MRS media in three tubes. Each tube was then inoculated with the upright-shaped bacterial cultures, including *Streptococcus thermophilus* FNCC 0040, *Lactobacillus bulgaricus* FNCC 0041, and *Lactobacillus plantarum* Dad 13. All of the isolated cultures were incubated at a 37°C temperature for 24 hours. To produce the starter cultures, each 0.1 ml of cultures were then inoculated into 5 ml sterile skimmed milk and incubated at a 40°C temperature for 7 to 8 hours or at a 37°C temperature for 24 hours. From this phase, the total production of Lactic Acid Bacteria and *Lactobacillus plantarum* Dad 13 as the starter cultures reached by 10⁹ CFU/ml.

Preparation of purple sweet potato extract-supplemented yogurt

Fresh milk, skimmed milk (5% w/v), and purple sweet potato extract (10% v/v) were pasteurized at 72°C - 80°C for 30 minutes and cooled at 40°C - 45°C. Furthermore, inoculated using *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus plantarum* Dad 13 probiotic bacteria with ratio 1: 1: 0. aseptically at 40-45°C, as much as 5% (v / v). The product was then shaken to homogeneous. and incubated at a 40°C for 8 hours or a 30°C for 20 hours to produce purple sweet potato extract-supplemented yogurt.

Freeze-Drying process for lactate-fermented drink

Yogurt with purple sweet potato extract supplementation and probiotics (*Lactobacillus plantarum* Dad13) was added with sucrose (according to treatment) and skimmed milk (10% v/v), then stir aseptically until homogeneous. The mixture was put into freeze-dryer glass tubes and frozen at a -20°C temperature for 12 hours, then dried in a freeze-dryer for 10 hours.

Research design

The research used a Completely Randomized Design (CRD) of one treatment, namely cryoprotectant concentration in the form of sucrose (S), with different concentrations, including S₁= 0%, S₂= 2.5%, S₃=5%, and S₄= 7.5%. The study was conducted three replications, so that obtained 12 experimental units. The observed parameters were yield of freeze-dried purple sweet potato extract synbiotic yogurt, total viability of LAB (Lactic Acid Bacteria) before and after the freeze-drying treatment, viability of *Lactobacillus plantarum* Dad 13 before and after the freeze-drying treatment. The data were analyzed using the RAL One-Way ANOVA. If the treatment showed a real significance, then proceed with the DMRT test (Steel and Torie, 1995).

Observation parameter

The experiment tested the fermented products and dried products in order to find out: the yields of freeze-dried purple sweet potato extract synbiotic yogurt, the total Lactic Acid Bacteria (LAB) before and after the freeze-drying process, and the viability of *Lactobacillus plantarum* Dad 13. All of the tests utilized the Bacteriological Analytical Manual (BAM) method (2002) (FDA, 2001).

RESULTS AND DISCUSSION

Characteristics of freeze-dried purple sweet potato extract-supplemented synbiotic yogurt

The freeze-dried synbiotic yogurt supplementation of purple sweet potato extract was made from milk-based yogurt which was supplemented with purple sweet potato extract (as a prebiotic) and fermented using *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus plantarum* Dad 13 as local probiotics which were isolated from buttermilk and freeze-dried using a *cryoprotectant* in the form of 10% skimmed milk and various concentrations of sucrose ranging from 0%, 2.5%, 5% and 7.5%. The products were displayed in Figure 2.



Figure 2. Freeze-Dried Synbiotic Yogurt Supplementation of Purple Sweet Potato Extract S with Various Sucrose Concentrations

Yields of freeze-dried purple sweet potato extract-supplemented

The yields of freeze-dried synbiotic yogurt supplementation of purple sweet potato extract (SYSPSP) was the final product following the freeze-drying process. The content of yields (Y) is generated by the following formula:

$$= \frac{\text{SYSPSP after freeze drying}}{\text{SYSPSP before freeze drying}} \times 100\%$$

The analysis of the addition of 10% skimmed milk which was combined with various concentrations of sucrose as a cryoprotectant signified significantly different effects ($p < 0.05$) to the yields of the freeze-dried yogurt. The statistical test result is explained in Figure 3.

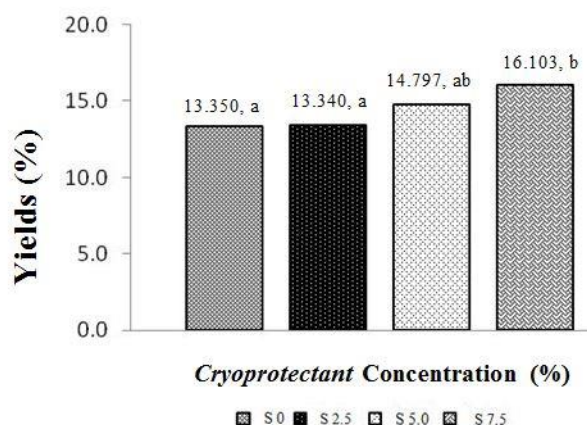


Figure 3. Impact of Various Sucrose Concentrations on Yields of Freeze-Dried Synbiotic Yogurt Supplementation of Purple Sweet Potato Extract. Numbers with the same alphabetical notations signify real indifferent results in DMRT test with an α -value = 0.05.

Figure 3 signifies that the higher sucrose concentration, the higher yield obtained of the freeze-dried synbiotic yogurt supplementatation of purple sweet potato. The condition was due to the combination of skimmed milk and sucrose to increase the volume and total of material solids. This research is relevant with Endang and Prasetyastuti (2010) who concluded that the increase of yields was affected by the large amount of cryoprotectant that resulted in a higher total of material solids. A high total of material solid correlates with a large quantity of yields.

The Total of Lactic Acid Bacteria (LAB)

The measurement of the total of LAB is an important parameter, as it closely relates to the amount of skimmed milk and sucrose as the cryoprotectant. Carvalho (2003) stated that a good protector in the freeze-drying process ought to be cryoprotective, easy to dry, able to form a good matrix to maintain cell stability, and easily rehydrated. Sucrose is one of the cryoprotectants that meets with these criteria. The effect of sucrose concentration as cryoprotectant on the total LAB is explained in Figure 4.

Figure 4 shows that the total of LAB before the freeze-drying process ranged from 12.93×10^8 to 39.77×10^8 CFU/ml or around 10^9 CFU/ml with no significant difference ($p > 0.05$) at various sucrose concentrations. After the freeze-drying process, the total of LAB decreased by 1 log cycle that varied from 2.75×10^8 to 9.28×10^8 CFU/ml with a significant difference ($p < 0.05$) at various sucrose concentrations.

The total of LAB at the 5% sucrose concentration as the cryoprotectant showed a significantly different number among other treatments. The reduction in the total of LAB at the 5% sucrose concentration before and after the freeze-drying process showed the smallest number which was less than 1 log cycle. It was suspected that

sucrose as the cryoprotectant could protect the structure and function of microbial cell proteins (Morgan et al., 2006). Additionally, sucrose could also function to strengthen the cell resistance to the freezing condition. The mechanism of cryoprotectant function in the reaction of cell preservation is marked by 1) the decreasing freezing point of the cryoprotectant medium, 2) the protective reaction of cell membranes, and 3) the suppressing rate of a high concentration effect ((Carvalho, et.al., 2003).

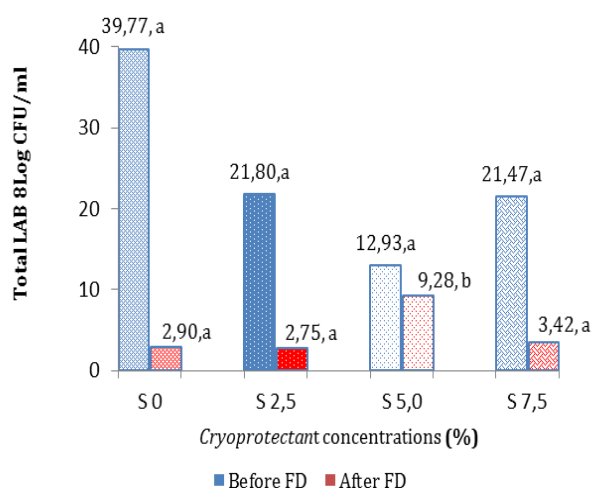


Figure 4. The Total of LAB in Synbiotic Yogurt Before and After Freeze-Drying Process. Numbers with the same alphabetical notations signify real indifferent results in DMRT test with an α -value = 0.05.

An over-high concentration of sucrose (7.5%) could lead to the osmotic imbalance inside and outside the bacterial cells. The condition would potentially spur bacterial lysis and cause bacterial death. Figure 4 signifies a lower total of LAB at the 7.5% sucrose concentration compared to the 5% sucrose concentration.

The Total of *Lactobacillus plantarum* Dad 13

The enumeration of *Lactobacillus plantarum* Dad 13 in the freeze-dried purple sweet potato extract-supplemented synbiotic yogurt utilized the LPSM (*Lactobacillus plantarum* Selective Medium). It is a simple medium which is sensitive to *L. plantarum* and other potentially probiotic *Lactobacillus* species (Miettinen et al., 1996; Coeuret et al., 2004). The medium contains a 4 μ g/ml concentration of ciprofloxacin, as an antibiotic that functions to inhibit most of the infectious bacteria, including the endogenous acid lactic bacteria that grow in MRS agar, however, has no adverse effect on the recovery of *L. plantarum*. The effect of sucrose concentration as the cryoprotectant on the total of *Lactobacillus plantarum* is explained in Figure 5.

Figure 5 shows that the total average of *L. plantarum* before the freeze-drying process ranged from 8.23×10^8 to 14.73×10^8 CFU/g or around 10^8 CFU/ml with a significant difference ($p < 0.05$) among various sucrose concentrations. After the freeze-drying process, the total average of *L. plantarum* following the addition of

cryoprotectant at various sucrose concentrations (0%, 2.5%, 5%, and 7.5%) ranged from 1.74×10^8 to 6.81×10^8 CFU/ml with a significant differences ($p < 0.05$) among various sucrose concentrations. At the control treatment ($S_1=0\%$), the total average of *L. plantarum* decreased by 1 log cycle following the freeze-drying phase. The total average of *L. plantarum* after the sucrose treatment at various concentrations from 2.5%, 5%, to 7.5% also decreased, however not by 1 log cycle.

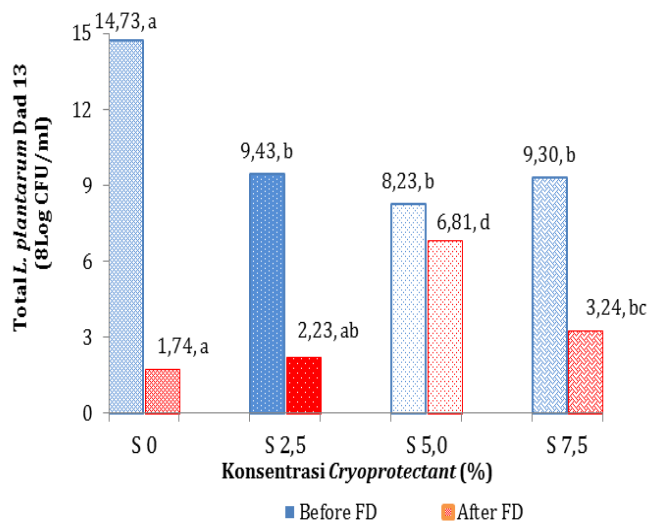


Figure 5. The Total of *L. plantarum* of Synbiotic Yogurt Before and After Freeze-Drying Process. Numbers with the same alphabetical notations signify real indifferent results in DMRT test with an α -value = 0.05.

Figure 5 indicates that the decreasing cell viability was probably due to the freeze-drying process. The freezing process caused the cell to lose its stability, thus it became easily damaged during the drying process. The osmotic shock was suspected as the main factor of the bacterial cell damage during the drying process, as signified by the membrane damage and displacement of hydrogen bonds that affected the hydrophilic macromolecule properties of the bacterial cells (Ray, 1996).

Another factor that affected the cell viability is the selection of cryoprotectant materials. According to Lin Lin and Hwang (1995), the use of two types of encapsulants from protein and carbohydrate matrices can produce a higher efficiency compared to the use of one type of encapsulant. It happens as the encapsulants can interact in forming granules, thus they can better overlay the encapsulated components.

The skimmed milk which contains lactose also provides good protection against the freeze-drying effects. The components of lactose in the form of glucose and galactose include simple sugars with a low molecular weight that results in easy entry into bacterial cells and provide protection from the two sides of the cell membranes during the freeze-drying process. The presence of sucrose as a cryoprotectant can protect the structures and functions of microbial cell proteins (Morgan et al., 2006), thus sucrose can improve cell resistance in freezing conditions.

In addition to the external factors, internal factors also affect viability. *Lactobacillus plantarum* bacteria are gram-positive bacteria. According to Fardiaz (1992), the gram-positive bacteria have a cell composition that consists of 90% peptidoglycan, lipids (1% to 4%), teichoic acid, and other components. The composition causes the gram-positive bacteria to be more resistant to physical and enzymatic treatments, such as the freeze-drying process than the gram-negative bacteria.

CONCLUSION

The various sucrose concentrations significantly affected the yields of the freeze-dried purple sweet potato extract-supplemented synbiotic yogurt, the total of Lactic Acid Bacteria (LAB), and the total of *Lactobacillus plantarum* before and after the freeze-drying process, however, indicated no significant effect on total quantity of LAB. The best treatment was shown in the freeze-dried purple sweet potato extract-supplemented synbiotic yogurt with a 5% of sucrose concentration as the cryoprotectant. The treatment using a 5% of sucrose concentration resulted the following characteristics: a 14.797% of yields, 1.98×10^9 CFU/ml of total Lactic Acid Bacteria (LAB) before the freeze-drying and a 9.28×10^8 CFU/ml after the freeze-drying, a 8.23×10^8 CFU/ml of total *Lactobacillus plantarum* before the freeze-drying and a 6.81×10^8 CFU/ml after the freeze-drying.

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