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Chemical Characteristics and Viability of Starter Cultures of Freeze –Dried Sweet Potato Extract –Supplemented Synbiotic Yogurt Agustina Intan Niken Tari1,*, Catur Budi Handayani1, Sri Hartati1, Damat Damat2, and Karina Stanev 3 1Department of Agricultural Product Technology, Faculty of Agriculture, University of Veteran Bangun Nusantara Sukoharjo, Jl. Letjend Sujono Humardani no.

1, Sukoharjo 57521, Central Java, Indonesia 2Department of Food Science and Technology, Faculty of Agriculture and Animal Science, University of Muhammadiyah Malang, Jl Raya Tlogomas No. 246, Malang 65144, East Java, Indonesia 3Department of Environmental Science, University of Latvia, Raina Blvd.19, Riga LV-1050, Latvia Abstract. The research aimed to determine the sucrose concentration as a cryoprotectant to obtain the chemical properties and the v iability of Lactic Acid Bacteria (LAB) and Lactobasillus plantarum in the synbiotic yogurt.

It adopted a one –factor Completely Randomized Design (CRD) by including sucrose in concentrations from 0 %, 2.5 %, 5 % to 7.5 %, three replications. The results showed various sucrose concentrations significantly affected the yi elds of the purple sweet potato extract – supplemented synbiotic yogurt, reduction –sugar level, the total of LAB, and the total of L.

plantarum before and after the freeze –drying process, no significant impact on the moisture con tent and total quanti ty of LAB. The best treatment of the synbiotic yogurt was induced through the addition of sucrose with a concentration of 5 % as a cryoprotectant. The treatment signified the following characteristics: 14.797 % of yields, 7.51 % of water content, 14.59 % of reduction –sugar level, 1.98 × 109 CFU mL –1 of total LAB before the freeze –drying and 9.28 × 108 CFU mL –1 after the freeze –drying,

8.23 × 108 CFU mL –1 of total L.

plantarum before the freeze–drying and 6.81 × 108 CFU mL –1 after the freeze–drying. Key words: Healthy consumption, fermented drink, functional food, free radical, sucrose as cryoprotectant. 1 Introduction Together with the increasing public awareness on the importance of healthy foods, the consumer demands related to food q ualifications 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 *Corresponding author: intanniken@gmail.com E3S Web of Conferences 226, 00006 (2021) https://doi.org/10.1051/e3sconf/202122600006 ICoN BEAT 2019 to maintain the b alance of intestine microbiota and immune system.

The healthy consumption pattern can be achieved through the addition of synbiotic yogurt. The types of symbiotic yogurt include local probiotic –based yogurt and purple sweet potato extract–supplemented yogurt. Tari et al. [1] Reported that purple sweet potato extract–supplemented yogurt made from commercial cultures and indigenous probiotics, such as Streptococcus thermophilus FNCC 0040, Lactobacillus bulgaricus FNCC 0041, and Lactobacillus plantarum Dad 13 by the ratio 1: 1: 0.5

had nowbeen available completed with the physical properties (pH = 3.78, viscosity = 5.198 7 cP, chromatic color = 18.559) and chemical properties (titrated acid content = 1.273 3 %, moisture content = 85.266 4 %, ash content = 0.804 1 %, reduction sugar level = 3.327 8 %, dissolved protein content = 1.478 2 %, fat content = 0.08 %, and anthocyanin level = 8.531 5 %). Tari et al.

[2] also shared that indigenous probiotics, such as L. plantarum Dad 13 supplemented to th e 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 expe rimental animals from 4.23 mmol mL–1 to 1.52 mmol mL–1 and the decreasing liver MDA level at from 5.60 mmol mL–1 to 2.96 mmol mL–1 at the end of the study. According to [3], fermented milk products must be stored at a temperature of less than 10 °C to avoid negative environmental impacts.

The storage at low temperature functions to inhibit the fermenta tion process thus will

maintain a high number of microbes. This treatment, however, will result in high storage and distribution costs deal with the challenges, freeze–dried synbiotic yogurt could offer the solution. Cruz [4] explained that the freeze –drying process could red uce the number of bacteria by 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 common use of coating material (cryoprotectant) derives from encapsulants, such as gum, carbohydrates, and protein to coat the core materials (bacteria) with specific purposes, such as to cover up the bad taste and odor, protect against the environmental influences, increase the stability, and prevent evaporation.

This cryoprotectant material can be either carbohydrates or protein. According to [5] the use of protein as a cryoprotectant can maintain the bac terial resistance, while the use of carbohydrates can both improve microcapsule texture and maintain the bacterial resistance. Bhat [6] 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 function to protect the structure and proteins in microbial cells [7]. However, there have yet been available any relevant studies that proved the function of sucrose as a cryoprotectant agent in la ctate–fermented drinks such as indigenous probiotic yogurt (local) produced from sweet potato supplementation through a freeze –drying process to the viability of Lactic Acid Bacteria (LAB) and L.

plantarum Dad 13, in addition to its characteristics. The notion underlies this research background. This research aims to signify the effect of sucrose concentration as cryoprotectant on the yields, water content, reduction –sugar level, total LAB , and total L. plantarum Dad 13 of the freeze–dried lactate–fermented drinks. 2 E3S Web of Conferences 226, 00006 (2021) https://doi.org/10.1051/e3sconf/202122600006 ICoN BEAT 2019 to maintain the b alance of intestine microbiota and immune system.

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probiotic yogurt (local) produced from sweet potato supplementation through a freeze –drying process to the viability of Lactic Acid Bacteria (LAB) and L.

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Materials The research used a number of materials, which consisted of LAB cultures from the Universitas Gadjah Mada (UGM) Yogyakarta , Indonesia FNCC (Food and Nutrition Culture Collection). The bacterial cultures were i dentified in upright shapes, including S. thermophilus FNCC 0040, L. bulgaricus FNCC 0041, and L. plantarum Dad 13 as probiotic indigenous LAB cultures which were collected from UGM Researchers Association.

The MRS (de Mann Rogosa Sharpe) Agar/Broth (Oxoid) media was utilized for the LAB culture maintenance. Meanwhile, the LPS media was utilized as the selective media for the L. plantarum Dad 13 growth. Other supp orting chemical substances were 70 % of alcohol, methylated spirit, and distilled water that we re obtained from the Biological –Chemistry and Microbiology Laboratory, Faculty of Agriculture, University of Veteran Bangun Nusantara Sukoharjo , Central Java, Indonesia.

The research utilized several instruments, including glasswares (test tubes, beaker cu ps, Erlenmeyer flasks, and petri dish), autoclaves (All America), an incubator, an oven (binder), and a laminar airflow (LAF). 2.2 Research procedure The research procedures ranged from the preparation of bacterial culture stocks, starter culture, purple sweet potato extract, and probiotic yogurt through supplementation of purple sweet potato extract, and freeze –drying process. 2.2.1 Preparation of bacterial culture stocks The commercial bacteria (S.

thermophilus FNCC 0040 and L. bulgaricus FNCC 0041) and L. plantarum Dad 13 as indigenous probiotic LAB cultures in the form of agar slants were taken one ose and grown in a 10 mL sterile liquid MRS. The cultures were then etched in a sterile agar slant and incubated at 37 °C during 24 h to 48 h.

The stocks of bacterial cultures in the agar slants were stored in a refrigerator at 2 °C to 3 °C and regenerated once every 2 wk. 2.2.2 Preparation of purple sweet potato extract The procedure of purple sweet potato extraction referred to the preliminary research conducted by [8]. The procedure was begun by dice –cutting the sweet potatoes in a 5 cm × 5 cm cube size and extracting the cuts in a juicer.

The product was then left to stand for 24 h at a 4 °C temperature, so the purple sweet potato starch would settle, whil e the filtrate would remain. The filtrate of the purple sweet potato extract would be taken to produce theyogurt. 2.2.3 Preparation of starter cultures This stage included the preparation of 5 m L sterile liquid MRS media in three tubes.

Each tube was then inocu lated with the upright –shaped bacterial cultures, including S. thermophilus FNCC 0040, L. bulgaricus FNCC 0041, and L. plantarum Dad 13. All of the isolated cultures were incubated at a 37 °C temperature for 24 h. To produce the starter cultures, every 0.1 mL of the cultures were then inoculated into 5 m L sterile skimmed milk 3 E3S Web of Conferences 226, 00006 (2021) https://doi.org/10.1051/e3sconf/202122600006 ICoN BEAT 2019 and incubated at a 40 °C temperature for 7 h to 8 h or at a 37 °C temperature for 24 h. From this phase, the total production of LAB and L.

plantarum Dad 13 as the starter cultures reached by 109 CFU mL –1. 2.2.4 Preparation of purple sweet potato extract – supplemented yogurt Fresh milk, skimmed milk (5 % w v –1), and purple sweet potato extract (10 % v v –1) were pasteurized at the temperature ranged from 72 °C to 80 °C for 30 min and cooled at the temperature ranged from 40 °C to 45 °C.

The product was aseptically inoculated with 5 % (v v –1) S. thermophilus, L. bulgaricus, and L. plantarum Dad 13 probio tic bacteria by the ratio 1:1: 0.5 at the temperature ranging from 40 °C to 45 °C. The produc t was then shaken until it reached a homogeneous state and incubated at a 40 °C temperature for 8 h or at a 30 °C temperature for 20 h to produce purple sweet potato extract –supplemented yogurt. 2.2.5

Freeze – drying process for lactate – fermented drink The yogurt produced from the supplementation of purple sweet potato extract and probiotics (L. plantarum Dad 13) was added with sucrose (consecutively based on the treatment) and skimmed milk (10 % v v –1). The yogurt was then aseptically stirred until it reached a homogeneous state.

The mixture was put into freeze –dryer glass tubes and frozen at a –20°C temperature for 12 h. The product was dried in a freeze –dryer for 10 h. 2.2.6 Research design The research used a Completely Randomized Design (CRD) through a single treatme nt by including sucrose (S) as a cryoprotectant with the variations of concentration percentage, including S1= 0 %, S2= 2.5 %, S3= 5 %, and S 4= 7.5 %.

The study was conducted by three times of replications, thus 12 experimental units were produced. The obs ervation parameters consisted of the viability of L. plantarum Dad 13 before and after the freeze –drying treatment, total viability of LAB before and after the freeze –drying treatment, moisture content, solubility, and reduction –sugar level. The data were analyzed using the RAL One –Way ANOVA.

The treatment would proceed to the DMRT test, if the result showed a real significance [9]. 2.2.7 Observation parameter The experiment tested the fermented products and dried products in order to find out: The yields of fr eeze–dried purple sweet potato extract synbiotic yogurt, the result of moisture content test through a thermogravimetric method [10], the result of reduction –sugar level test through the Nelson Somogyi method [11], the total LAB before and after the freeze –drying process, and the viability of L. plantarum Dad 13.

All of the tests utilized the Bacteriological Analytical Manual (BAM) method (2002). 3 Result and discussions 3.1 Yields of purple sweet potato extract –supplemented synbiotic yogurt The yields of purple sweet potato extract –supplemented synbiotic yogurt (PSPE –SSY) was the final product following the freeze –drying process. The content of yields (Y) was 4 E3S Web of Conferences 226, 00006 (2021) https://doi.org/10.1051/e3sconf/202122600006 ICoN BEAT 2019 and incubated at a 40 °C temperature for 7 h to 8 h or at a 37 °C temperature for 24 h. From this phase, the total production of LAB and L.

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The content of yields (Y) was generated by the formula (1): (1) The addition of 10 % skimmed milk as a cryoprotectant combined with various concentrations of sucrose gave a significant effect ($p < 0.05$) to the yields of the freeze –dried yogurt. The statistical test result was explained in Figure 1. Fig. 1. Impact of various sucrose concentrations on yields of freeze –dried purple sweet potato extract –supplemented synbiotic yogurt Figure 1 signifies that a higher sucrose concentration resulted in a higher quantity of the freeze –dried synbiotic yogurt yields.

The condition was due to the combination of skimmed milk and sucrose to increase the volume and total of material solids. This research was relevant to [11] 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. 3.2

Moisture content The moisture content of the final product s became an important parameter in making instant products. The combination of 10 % skimmed milk and various concentrations of sucrose as a cryoprotectant indicated no impact on the moisture content of the freeze –dried purple sweet potato extract –supplemented synbiotic yogurt ($p > 0.05$) as shown in Figure 2.

Such a condition was possible due to the small interval of sucrose concentration used as a cryoprotectant, thus offered less impact on the moisture content of th e product. The use of sucrose was cryoprotective as a drying medium, as it can form porous structures to facilitate the release of the product moisture during the freeze –drying pr ocess and become the medium at the rehydration process [12].

Y Yield =PSPE -SSY after freeze drying PSPE -SSY after freeze drying ×100 % 5 E3S Web of Conferences 226, 00006 (2021) https://doi.org/10.1051/e3sconf/202122600006 ICoN BEAT 2019 Fig. 2. Impact of various sucrose concentrations on moisture content of freeze –dried purple sweet potato extract –supplemented synbiotic yogurt. 3.3

Reduction –sugar level A reduction –sugar is a group of monosaccharides and disaccharides that have free –reducing groups, such as glucose, fructose, lactose, galactose, and maltose [13]. Figure 3 showed that the concentration of the cryoprotectant made from combination of 10 % skimmed milk and various sucr ose concentrations ha d a sign ificantly different impact ($p < 0.05$) on the reduction –sugar of the freeze –dried purple sweet potato extract –supplemented synbiotic yogurt. Fig. 3.

Impact of various sucrose concentrations on reduction –sugar level of freeze dried purple sweet potato extract –supplemented synbiotic yogurt The decreasing reduction –sugar level was considered due to its use as the energy source for the bacterial cell growth and propagation, in addition to the formation of bacterial metabolites dur ing the freeze –drying process. Such a condition was concluded from the total average of LAB that ranged from 12.93 × 108 CFU mL –1 to 39.77 × 108 CFU mL –1 or around 10 9 CFU mL –1 before the freeze –drying process and decreased by 1 log cycle ranging from 2.75 × 108 CFU mL –1 to 9.28 × 108 CFU mL –1 after the freeze –drying. The total average of L.

plantarum before the freeze –drying process ranged from 6 E3S Web of Conferences 226, 00006 (2021) https://doi.org/10.1051/e3sconf/202122600006 ICoN BEAT 2019 Fig. 2. Impact of various sucrose concentrations on moisture content of freeze –dried purple sweet potato extract –supplemented synbiotic yogurt. 3.3 Reduction –sugar level A reduction –sugar is a group of monosaccharides and disaccharides that have free –reducing groups, such as glucose, fructose, lactose, galactose, and maltose [13].

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The data confirmed the findings shared by [14] that lactic acid bacteria utilized sugar as the energy source for its growth and produced lactic acid during the fermentation process. 3.4 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. Li et al.

[15] 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 meet with these criteria. The effec t of sucrose concentration as cryoprotectant on the total LAB was explained in Figure 4. .

Remarks:Numbers with the same alphabetical notations signify real indifferent results in DMRT test with an ? –value = 0.05 Fig. 4. The total of LAB in synbiotic yogurt before and after freeze –drying process Figure 4 showed that the total of LAB before the freeze –drying process ranged from 12.93 × 108 CFU mL –1 to 39.77 × 108 CFU mL –1 or around 10 9 CFU mL –1 with no significant difference (p > 0.05) at various sucrose c oncentrations.

After the freeze –drying process, the total of LAB decreased by 1 log cycle that varied from 2.75 × 108 CFU mL –1 to 9.28 × 108 CFU mL –1 with a significant difference (p < 0.05) at various sucrose concentrations. The total of LAB at the 5 % su crose

concentration as the cryoprotectant showed a significantly different number among other treatments.

The reduction in the total of LAB at the 5 % sucrose concentrati on before and after the freeze –drying process showed the smallest number which was les s than 1 log cycle. It was suspected that sucrose as the cryoprotectant could protect the structure and function of microbial cell proteins [7]. 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, i) the decreasing freezing point of the cryoprotectant medium, ii) the protective reaction of cell membranes, and iii) the suppressing rate of a high concentration effect [15]. 7 E3S Web of Conferences 226, 00006 (2021) https://doi.org/10.1051/e3sconf/202122600006 ICoN BEAT 2019 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. 3.5 The total of L. plantarum Dad 13 The enumeration of L. plantarum Dad 13 in the freeze –dried purple sweet potato extract –supplemented synbiotic yogurt utilized the LPSM (L. plantarum Selective Medium).

It is a simple medium which is sensitive to L. plantarum and other potentially probiotic Lactobacillus species [15,16]. Te edm ns 4μ mL –1 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 L. plantarum is explained in Figure 5. Figure 5 showed that the total average of L. plantarum before the freeze –drying process ranged from 8.23 × 108 CFU g –1 to 14.73 × 108 CFU g –1 or around 10 8 CFU mL g –1 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 concent rations (0 %, 2.5 %, 5 %, and 7.5 %) ranged from 1.74 × 108 CFU mL –1 to 6.81 × 108 CFU mL g –1 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

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The osmotic shock was suspected as the main factor of the bacterial cell damage during the drying process, as signified by t he membrane damage and displacement of hydrogen bonds that affected the hydrophilic 8 E3S Web of Conferences 226, 00006 (2021) https://doi.org/10.1051/e3sconf/202122600006 ICoN BEAT 2019 An over –high concentration of sucrose (7.5 %) could lead to the osmotic imbalance inside and outside the bacterial cells.

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The osmotic shock was suspected as the main factor of the bacterial cell damage during the drying process, as signified by t he membrane damage and displacement of hydrogen bonds that affected the hydrophilic macromolecule properties of the bacterial cells [17]. Another factor that affected the cell viability was the selection of cryoprotectant materials.

According to [18], 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 intobacterial cells and protect the two sides of the cell membranes during the freeze –drying process. The presence of sucrose as a cryoprotectant could protect the structures and functions of microbial cell proteins [7], thus sucrose could improve cell resistance in freezingconditions. In addition to the external factors, internal factors also affect viability. L. plantarum bacteria are gram –positive bacteria.

According to [19], the g –positive bacteria have a cell composition that consists of 90 % peptidoglycan, lipids (1 % to 4 %), teichoic acid, and other components. The composition caused the gram –positive bacteria to be more resistant to physical and enzymatic treatments, such as the freeze –drying process than the gram –negative bacteria.

4 Conclusions The various sucrose concentrations significantly affected the yields of the freeze –dried purple sweet potato extract –supplemented synbiotic yogurt, reduction –sugar level, the total of LAB, and the total of L. plantarum before and after the freeze –drying process. However, there were indicated no significant effect s on the moisture

content and 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 concentr ation as the cryoprotectant. The treatment using a 5 % of sucrose concentration resulted in the following characteristics: A 14.797 % of yields, a 7.51 % of water content, a 14.59 % of reduction –sugar level, a 1.98 × 109 CFU mL –1 of total LAB before the fr eeze–drying and a 9.28 × 108 CFU mL –1 after the freeze –drying, a 8.23 × 108 CFU mL –1 of total L. plantarum before the freeze –drying and a 6.81 × 108 CFU mL –1 after the freeze –drying.

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