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EFFICIENCY OF VACUUM DRIED METHOD ON PHYSICAL, ORGANOLEPTIC AND VIABILITY PROPERTIES OF LACTIC ACID BACTERIA SYNBIOTICS

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ABSTRACT: Vacuum drying storage is a more efficient storage method for synbiotic feeds, compared to fresh storage. The current study aimed to examine the effect of vacuum drying on the physical, organoleptic, and microbiological qualities of synbiotics made from cabbage and Chinese cabbage greens. The study was conducted using a completely randomized design with a 5x3 factorial pattern with two replications consisting of two factors, namely five levels of drying time (24, 48, 72, 96, and 120 hours) and three levels of storage time (4, 8 and 16 weeks). The variables observed were physical-organoleptic quality in water content, color, odor, and texture, and microbiological quality in the form of total lactic acid bacteria. The results showed no interaction between the two treatments in terms of vacuum drying method, drying time, and storage time. The recommended treatment is drying for 48 hours, as evidenced by the moisture content factor supporting the viability of the lactic acid bacteria and maintaining the sensory properties. This study suggests a more efficient storage method of synbiotics for food applications.

Keywords: Lactic Acid Bacteria, Orgoleptic, Storage, Synbiotic, Vacuum Drying.

INTRODUCTION

The use of antibiotic growth promoters (AGP) can enhance livestock growth by suppressing bacterial catabolism and improving intestinal flora. However, the consequential issues of resistance and water pollution are potential threats to food security and public health (Corpet, 2000). The use of AGP has been banned since 2005 in the European Union (Wu et al., 2020). In Indonesia, the ban on the use of AGP officially began in January 2018 (Prasetyo et al., 2020). One of the alternative products to replace AGP is the provision of feed additives with synbiotics, which are mixed products of probiotics and prebiotics (Hartono et al., 2016). Fermented vegetable extracts in cabbage and Chinese cabbage meet the standards of probiotics since they contain *Lactobacillus Brevis, Lactobacillus Plantarum, Saccharomyces cerevisiae,* and *Rhizopus Oryza* (Utama et al., 2018). The lactic acid bacteria (LAB) content in the fermented vegetable extract is 2.1 x 10^{10} CFU/ml (Utama et al., 2013).

In order to enhance the function of Lactic acid bacteria in animals, it is necessary to supplement the diet with prebiotics. The prebiotics provide essential nutrients to the bacteria, allowing them to function more effectively as probiotics (Andriani et al., 2017). Among the prebiotics suitable for livestock are fructooligosaccharide (FOS), galactooligosaccharide (GOS), mannan oligosaccharides (MOS), and inulin (Allahdo et al., 2018). Cassava waste and soybean meal can serve as prebiotics due to their distinct properties. Cassava waste contains high carbohydrates in the form of polysaccharides, acting as a substrate for LAB. Soybean meal contains feed fiber Jenin soybean oligosaccharides (SOS) which is used as nutrition for LAB (Renschler et al., 2020). Moreover, amino acids and high protein in soybean meal make it a valuable nutritional provider for LAB (Wu et al., 2020).

Synbiotics made from cabbage and fermented Chinese cabbage greens are prone to quality deterioration during prolonged storage due to their high moisture content. Therefore, implementing a method to extend the shelf life while maintaining the quality, is of utmost importance (Solihin et al., 2015). Common techniques for the long-term preservation of bacterial cultures involve various drying applications, such as ordinary ovens, vacuum ovens, spray drying, drying with fluid flows, freeze-drying, and sublimating liquids from frozen products known as lyophilization (Soro-Yao et al., 2014). Regular drying ovens are frequently used; however, spray drying, which typically involves high temperatures ranging from 110 to 180°C at the inlet and 85 to 105°C at the outlet during culture preservation, may potentially lead to damage of synbiotic products.

While the use of vacuum drying in synbiotic feeds has been studied by Yang et al. (2020) and Goh et al. (2022), it has not been widely applied in Indonesia despite its ability to operate at lower temperatures and pressures. The

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fundamental principle of vacuum drying involves optimizing the vacuum pressure in the drying room to facilitate drying at low temperatures. This drying is used to obtain products with high quality, minimize the waste of odor and active and volatile substances (volatile), and minimize nutrient damage to ingredients, such as protein denaturation, browning (browning of ingredients), and enzymatic reactions (Widyanti et al., 2019).

Synbiotics made from fermented cabbage and mustard greens can maintain their properties when stored fresh with a temperature range of 5-10°C. However, this method is considered less efficient since it requires a large container, susceptibility to damage, and substantial energy demands to maintain ideal environmental conditions. The dry storage method is an effective choice as it can overcome these problems and ensure that the synbiotic remains in optimal condition. Drying can extend shelf life, decrease the costs of logistics due to the decrease in water activity, and provide unique physical properties as a dried product (Omolola et al., 2017). The drying process will reduce the water content, resulting in extended preservation and shelf life. However, this process also causes various changes in the physical, chemical, sensory and nutritional properties of the products. Therefore, it is important to consider the potential impact of the drying process on the overall quality and characteristics of the product (Khan et al., 2016). The quality of the dried products is affected by the drying method and physicochemical changes (Ramírez, et al., 2011). Sensory evaluation, an integral part of the quality evaluation (Noorbakhsh et al., 2013), is initiated after the samples are stored (Zhu et al., 2022). Preserving the characteristics of probiotics during the drying process is of utmost importance, considering the numerous benefits they provide. The viability and activity of LAB must be carefully considered as they should be abundant and viable at the time of consumption. Moreover, the survival of these microorganisms is critical to maintaining the product functionality (Sfakianakis and Tzia, 2004).

Given the above studies, it is evident that vacuum drying does not cause significant damage to the active compound of the dried material. Therefore, observations conducted on the odor, color, and texture of the synbiotic in the current study aimed to demonstrate that the chemical reactions leading to changes in color and texture during the drying process do not significantly affect the active compound. In addition, the study also confirmed that the LAB of the synbiotics dried using the vacuum drying method could remain viable even after storage.

MATERIALS AND METHODS

Materials

The cabbage, Chinese cabbage, glutinous rice tuning, chili, garlic, molasses, cassava waste, soybean meal, NaCl, and MRS were purchased from the local market, city of Semarang-Indonesia. The tools used were blenders (Miyako®, Indonesia), basins, jars, sieves, spoons, plastic sealers, spatulas, label paper, trays, digital scales (SF-400, Indonesia), beaker glass (Boro3.3, Germany), sealers (Arashi AIS-300, Indonesia), autoclave (Autoclave All American 75X, U.S.A), oven binder (ED 53 UL, U.S.A), petri dish (Pyrex 3160-120, U.S.A), analytical scales (balance kern ABJ-220, Germany), silica gel (Merck, Germany), desiccator (Normax, Portugal), vacuum machine (Araki, Indonesia), Thermohygrometer (HTC-2®, Switzerland), litmus paper (Merck®, Germany), the vacuum tube (GP, China), and petri dish (Iwaki®, Indonesia).

Method

The prerequisite to performing a completely randomized design (CRD) is that the materials and components used in the experiment are relatively homogeneous, except for the effect of the treatment given to the object (Steel and Torrie, 1980). The materials used in the current study had relatively consistent LAB content and organoleptic physical characteristics. This homogeneity facilitated the evaluation of the effect of drying and storage treatments on the object under study. Therefore, the experimental design used in this study was a CRD with a factorial pattern of 5 x 3 with two factors. The first factor (F1) was drying time at five levels, of 24, 48, 72, 96, and 120 hours and the second factor was storage time using three levels, namely 4, 8, and 16 weeks. The treatment combinations were as follows:

CA1.0 = Fermented vegetable liquid extract + carrier 100%
CA2.0 = Fermented vegetable liquid extract + chilli + carrier 100%
PA1.0 = Fermented vegetable solid extract + carrier 50%
PA2.0 = Fermented vegetable liquid extract + chilli + carrier 50%
CA1.1 = Fermented vegetable liquid extract + carrier 100% + MRS 1 ml
CA2.1 = Fermented vegetable liquid extract + chilli + carrier 100% + MRS 1 ml
PA1.1 = Fermented vegetable solid extract + carrier 50% + MRS 1 ml
PA2.1 = Fermented vegetable liquid extract + chilli + carrier 50% + MRS 1 ml

Each sample was repeated according to the drying time and storage time level. In order to maintain the functional properties of synbiotics, it is necessary to consider the sensory properties that change during drying and the viability of LAB (Khan and Karim, 2016; Sfakianakis and Tzia, 2004). Therefore, the parameters observed to ensure product quality included color, odor, texture, physical form, and microbiological content in the form of total LAB. The determination of

total microbial LAB was conducted following the methodology outlined in a study by Sulistiyanto et al. (2019). The research method consisted of four stages of activity, including the fermentation, mixing fermented extracts with prebiotics, drying process, and storage.

Fermentation of vegetable

The fermentation process for the fermented vegetable mixture followed the procedure outlined by Sulistiyanto et al. (2019). To create a fermented mixture of cabbage, white mustard greens, garlic, and chilies, all ingredients were thoroughly washed, cut into small pieces, and finely ground. The finely ground mixture was weighed according to a specific formula and homogenized. Then, the homogeneous mixture was placed into fermenter containers and tightly sealed to create an anaerobic environment. A hole in the lid of the fermenter tube was connected to a hose and thermometer, which was covered with plasticine to maintain an aerobic atmosphere and monitor changes in temperature and pressure in the fermentors. The fermentation process lasted 5 days, with the mixture kept in an anaerobic environment at room temperature. After the fermentation process, the liquid and solid extracts were separated through mechanical extraction.

The soybean meal and cassava waste were sterilized first by autoclaving at 121°C for 15 minutes before being used as prebiotics. In the next step, the cassava waste and soybean meal were homogenized with 60% of soybean meal and 40% of cassava waste. The extracted liquid and solids were supplemented with prebiotics according to the treatment and then placed in a petri dish to be dried.

The petri dish was filled and the sample was then dried in the drying stage using vacuum drying. The vacuum engine was turned on one time per 24 hours, with the duration of the engine running for 15 minutes. In the storage stage, the dried samples were put into a vacuum tube to be stored according to the treatment for 4, 8, and 16 weeks.

Testing parameters

During the analysis stage, comprehensive testing was conducted, including organoleptic quality evaluation in terms of color, odor, and texture, as well as microbiological assessment considering LAB and water content analysis. Physical-organoleptic testing was conducted by distributing questionnaires among 15 semi-trained panelists, who were selected from adept student who worked in the Feed Technology Laboratory but were not members of the Team. These judges were chosen based on their excellent health, normal sense of smell and taste, and absence of color blindness, ensuring they possessed the necessary skills for accurate assessments. To guarantee unbiased and precise evaluations, the judges underwent thorough training, including in-depth instruction on the assessment of the subtle nuances of odor, color, and texture, which was conducted three times to ensure that the judges were fully prepared for the task. The normal values, according to Sulistiyanto et al. (2019), Kurniawan (2020), and Anggraeni et al. (2021), are as follows:

- Color index on a scale ranging from 1 to 3 as 1 = dark brown, 2 = light brown, 3 = cream
- Texture index on a scale ranging from 1 to 3 as 1 = lumpy and smooth, 2 = slightly lumpy and smooth, 3 = no lumps and smooth
- Odor index on a scale ranging from 1 to 3 as 1 = rotten, 2 = odorless, 3 = acid

Data processing was performed using descriptive methods. The research data were arranged in tabular form, facilitating the arrangement of the data and then interpreted according to the existing observations. For the analysis of moisture content, a drying method was employed using an oven set at 105°C for 4 hours. The total LAB count was carried out using the total plate count (TPC) method (Nadliroh et al., 2019).

The total plate count can be calculated by = colonies × 1/dilution factor × $1/\sum$inoculum.

Data analysis

The obtained data were analyzed using analysis of variance (ANOVA) through SPSS Statistic 26 (IBM, 2018). In cases where the analysis indicated a significant effect, further analysis was carried out through Duncan's difference test at the 5% significance level. For the graphical presentation of the data, Figure Pad Prims 9.4.1 was used.

RESULTS AND DISCUSSIONS

Synbiotic physical-organileptic test

The organoleptic values of synbiotics dried by the vacuum drying method are presented in Table 1. Physical-organoleptic testing is one way to test synbiotic quality using the five senses and can be measured quantitatively. The observed physical-organoleptic features include color, texture, and odor. The quantitative data was carried out by a scoring method involving 15 semi-trained panelists. As can be seen in Table 1, extended drying resulted in a lighter color, smoother texture with fewer lumps, and a drier, sour odor in the synbiotic. Organoleptic test before and after storage shown there was not change the synbiotics' color, texture, and odor.

Color

The results of the ANOVA test showed that the interaction of drying time had a significant effect on synbiotic color ($P<0.05$). The analysis of data in Table 1 revealed that synbiotics dried by the vacuum drying method affected synbiotic color. Synbiotics dried for 24 hours were dark brown, while synbiotics dried for 120 hours were cream, changing color from dark to light as the length of synbiotic drying increased. The change of color in synbiotics was due to the water content, meaning that a decrease in the water content correlated with a brighter synbiotic color. According to Bora et al. (2018), water content significantly affects a product's color. Horváth (2016) stated that adding color to a product is influenced by several factors, including its particle size, oil content, and water content, as well as the content of color agents. The light color represents the high quality of synbiotics, while the brownish color indicates the lower quality synbiotics (Utama et al., 2020). Changes in color may result from the presence of mold and causing the color to shift from green to dark brown. Recognizing color as an important quality attribute, Rather and Rajain (2019) emphasize that it plays a crucial role in the product's overall attractiveness.

Texture

The results of the ANOVA test showed that the interaction of drying time had a significant effect on the synbiotic texture ($P<0.05$). The scores ranged from the lowest at 1.00 to the highest at 3.00 (Table 1). There was a noticeable change in texture from clumping to non-clumping, corresponding to the increased duration of synbiotic drying. The texture in the sample dried for 24 and 48 hours had a lumpy and smooth texture due to the high moisture content in synbiotics, making them prone to clumping. The moisture content can play a significant role in synbiotic texture. According to Zainuddin et al. (2014), moisture content can affect the physical properties and texture of the feed. The higher moisture content tends to promote quicker coagulation of powder products, while lower moisture content enhances smoothness and reduces clumping. The clumping observed in synbiotics due to high water content suggests a potential limitation in shelf life. An increase in the moisture content of a powder product leads to clumping (caking), which is a sign of poor quality and safety (Kurniawan, 2020).

Table 1 - Organoleptic score of dry synbiotics at different drying time

Parameter	Sample	Drying Time				
		24 Hours	48 Hours	72 Hours	96 Hours	120 Hours
Colour	CA 1.0	1±0	1.67±0.49	2.67±0.49	3±0	3±0
	CA 1.1	1±0	1±0	2±0	3±0	3±0
	CA 2.0	1±0	1.67±0.49	2±0	3±0	3±0
	CA 2.1	1±0	1±0	2±0	2.67±0.49	3±0
	PA 1.0	1±0	1±0	2±0	2.67±0.49	3±0
	PA 1.1	1±0	1±0	2±0	2.33±0.49	3±0
	PA 2.0	1±0	1±0	2±0	2.33±0.49	3±0
	PA 2.1	1±0	1±0	2±0	3±0.00	3±0
	Average	1±0	1.17±0.31	2.08±0.24	2.75±0.30	3±0
Texture	CA 1.0	1±0	1.60±0.50	2.60±0.50	3 ±0	3±0
	CA 1.1	1±0	1.00±0	2±0	3±0	3±0
	CA 2.0	1±0	1.73±0.46	2±0	3±0	3±0
	CA 2.1	1±0	1±0	2±0	2.60±0.51	3±0
	PA 1.0	1±0	1±0	2±0	2.60±0.51	3±0
	PA 1.1	1±0	1±0	2±0	2.70±0.46	3±0
	PA 2.0	1±0	1±0	2±0	2.47±0.51	3±0
	PA 2.1	1±0	1±0	2±0	3±0.00	3±0
	Average	1±0	1.17±0.31	2.08±0.21	2.78±0.26	3±0
Odour	CA 1.0	3±0	3±0	3±0	3±0	3±0
	CA 1.1	3±0	3±0	3±0	3±0	3±0
	CA 2.0	3±0	3±0	3±0	3±0	3±0
	CA 2.1	3±0	3±0	3±0	3±0	3±0
	PA 1.0	3±0	3±0	3±0	3±0	3±0
	PA 1.1	3±0	3±0	3±0	3±0	3±0
	PA 2.0	3±0	3±0	3±0	3±0	3±0
	PA 2.1	3±0	3±0	3±0	3±0	3±0
	Average	3±0	3±0	3±0	3±0	3±0

CA 1.0= Fermented vegetable liquid extract+carrier 100%; CA 2.0= Fermented vegetable liquid extract +chilli+ carrier 100%; PA 1.0= Fermented vegetable solid extract+carrier 50%; PA 2.0= Fermented vegetable liquid extract + chilli+carrier 50%; CA1.1= Fermented vegetable liquid extract+carrier100%+MRS 1 ml; CA2.1= Fermented vegetable liquid extract+chilli+carrier 100%+MRS 1 ml; PA1.1= Fermented vegetable solid extract+carrier 50%+MRS 1 ml; PA2.1= Fermented vegetable liquid extract+chilli+carrier 50%+MRS 1 ml

Odor

The results of the ANOVA test showed that there was no significant interaction between drying time and synbiotic odors ($P<0.05$). The odor values obtained were all 3.00, indicating a sour odor. The process of vacuum drying has been proven to be effective in maintaining the favor and odor of synbiotics. This method ensures that the aroma of synbiotics remains consistent throughout all stages of treatment. Hence, vacuum drying is considered a reliable and efficient for preserving the distinctive odor of synbiotics. According to Zhang et al. (2019), vacuum drying produces products with an odor similar to fresh products. Odor is often used to determine the quality of the products produced as good or bad. The resulting odor indicates the level of microorganisms contained in synbiotics, with a sour odor suggesting a lower level of microorganisms (Utama et al., 2020). According to Kurniawan et al. (2020), changes in odor are also caused by bacteria that change complex compounds to be more straightforward.

The sour odor in synbiotics comes from the fermentation process that produces lactic acid bacteria. Gonzalez et al. (2011) stated that the acid odor is caused by the presence of lactic acid, acetaldehyde, propionic acid, butyric acid, and other volatile compounds produced by starter cultures due to fermentation. Setiarto et al. (2017) added that the odor of acid comes from the activity and growth of LAB, which decomposes lactose into lactic acid, resulting in a decrease in the pH value. A good synbiotic odor is an odor that resembles the raw materials used in its production, presenting a fresh and non-rancid aroma (Utama et al., 2020).

Synbiotic moisture test

The ANOVA test showed an interaction between drying time and synbiotic moisture content ($P<0.005$). The average values ranged from 48.4 to 8.2 (Table 1). The findings indicated that the decrease in water content was accompanied by an increase in the drying time of the synbiotic (Figure 1). The moisture content was tested using proximate analysis, revealing optimal moisture content of 12% for well-dried synbiotics. The high level of water content (above 15%) led to a decrease in synbiotic quality by rendering it susceptible to bacterial and fungal contamination, while also facilitating coagulation of synbiotic texture. As indicated in Figure 1, the duration of drying was inversely correlated with synbiotic moisture content. The synbiotic moisture content after storage remained stable.

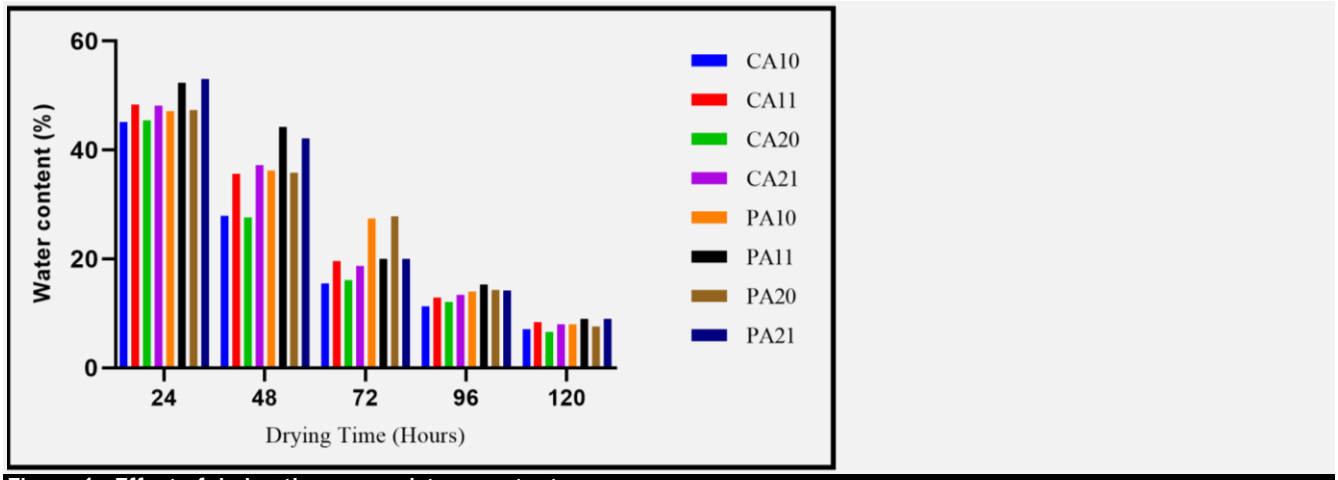

Figure 1 - Effect of drying time on moisture content

Figure 1 shows an overall decline in moisture content levels throughout the drying process. Longer drying time resulted in lower moisture content, with a notable reduction to 7.2% in CA10 and CA20 samples during 120-hour drying. According to Balzarini et al. (2018), the drying time is the time used in reducing the water content of the material; the longer the drying time, the more water evaporates from the dried material, so the water content obtained is lower. The decrease in water content for each synbiotic can be seen in Figure 1, and this occurs depending on the level of the water content of each synbiotic. According to Wirawan et al. (2020), the composition of water in feed ingredients, such as free water and bound water, can affect the material's drying rate or duration.

The grinding process before 24 hours was a constant rate phase, where in this phase, the drying process of the moisture content rate in the material moved to the surface of the material. Between 24 and 96 hours, there was a sharp decrease as substantial water evaporation occurred on the surface, resulting in dryness. After 96 hours, the moisture content decreased slowly indicating that the moisture content in the material was already low. Xu et al. (2022) stated that the vacuum drying process is divided into three different phases, the first is the initial transient period and the continued constant rate period, where the water extract evaporates and exits the material to form a layer of water on the surface of the porous medium, in the period of the rate of decline where the loss of moisture and water extracts (turbid particles and macromolecules) gradually becomes a porous medium. The remaining bound water diffuses as a layer on the material's surface. The grinding process before 48 hours was at a constant rate phase, where in this phase, the drying process of the

moisture content rate in the material moved to the surface of the material. The vacuum drying process was carried out by applying air pressure to the synbiotics, facilitating the diffusible removal of water towards the material's surface and subsequent conversion from the synbiotic surface to free air. According to Balzarini et al. (2018), vacuum drying is an alternative method for processing heat-sensitive products by maintaining air pressure so that the material releases moisture in the drying chamber. The longer the drying time and the pressure applied to the synbiotics result in greater moisture release, leading to lower water content. Xu et al. (2022) emphasize that an increase in drying time and temperature contributes to increased evaporation of water molecules from the dried material and lower water content in the final product.

Microbiological (*Lactic acid* bacteria validity) test

The validity test was conducted to determine the LAB's viability during drying and shelf-life. The results of the analysis indicated that the interaction of drying time with storage time did not yield a significant difference in LAB viability ($P<0.05$). Since the study aimed to find out which group had the highest LAB, further analysis was carried out using Duncan's test. Based on the results of further tests using Duncan's test (Table 2), it is known that simultaneously drying time and storage time had no significant effect on LAB. However, it has been found that drying time and storage time individually have a significant effect on LAB. Based on the results of the analysis, the best sample was shown in CA1.1 with the highest LAB value. This can be related to the water content and growth phase of LAB. Lower water content leads to increased water evaporation, resulting in a lower water activity value and suppressing the growth rate of microorganisms. Rolfe et al. (2012) proposed that the LAB growth phase comprises four phases. The first phase is the lag phase, during which bacterial growth is slow as they activate to the environmental conditions. The second phase is the exponential phase, where bacterial growth occurs at a rapid rate. The third phase is the stationary phase, where bacterial growth ceases due to the depletion of nutrients in the media and energy reserves. Finally, the fourth phase is the death phase, where the bacteria population decreases over time.

Table 2 - Results of dry synbiotic lactic acid bacteria at different drying and storage times

Storage time	Sample	Drying time (Hours) —— × 10^6 CFU/ml ——					Average
		24	48	72	96	120	
4 weeks	CA 1.0	0	6.25±7.42	1.0±7.64	5.00±3.27	0.26±0.35	10.39±25.31[a]
	CA 1.1	44.85±62.44	113.50±77.07	10.0±1.41	18.50±2.97	92.15±35.14	
	CA 2.0	0.50±0.71	2.00±1.41	0	0	0	
	CA 2.1	5.10±7.21	0.50±0.71	2.50±5.94	0	0.25±0.35	
	PA 1.0	0.53±0.74	0.29±0.29	0.50±0.16	1.50±1.17	1.75±0.35	
	PA 1.1	62.04±77.73	4.75±5.30	1.00±0	0	1.00±0	
	PA 2.0	0	0	0	0	0	
	PA 2.1	0.05±0.07	38.75±54.80	1.00±0.70	0	0.25±0.35	
8 weeks	CA 1.0	2.45±3.46	1.77±2.45	0.50±0.28	0.50±0.35	5.40±0.57	6.26±14.25[ab]
	CA 1.1	43.15±6.58	8.50±2.83	0	0	12.50±17.68	
	CA 2.0	0.25±0.35	5.00±7.07	0	0	0.01±0.01	
	CA 2.1	32.20±41.44	42.70±59.82	0	0	0.06±0.08	
	PA 1.0	1.00±1.41	1.20±1.13	1.00±0.70	0	0	
	PA 1.1	32.85±14.35	58.20±61.94	0	0	0.25±0.35	
	PA 2.0	0	0.75±1.06	0	0	0	
	PA 2.1	0	0.25±0.35	0	0	0	
16 weeks	CA 1.0	3.50±0	0	1.00±0	0	0.50±0	1.18±2.18[b]
	CA 1.1	4.00±0	0	0.50±0	0	0	
	CA 2.0	8.00±0	0.50±0	0	0	0	
	CA 2.1	5.00±0	0	0	0	0.50±0	
	PA 1.0	1.50±0	0	1.00±0	0	5.50±0	
	PA 1.1	0.50±0	0	0.50±0	0.50±0	0.00±0	
	PA 2.0	0.50±0	0	9.00±0	0	1.00±0	
	PA 2.1	1.00±0	0	2.00±0	0	0.50±0	
Average		10.37±17.95[a]	11.87±26.69[a]	1.31±2.61[b]	1.08±3.86[b]	5.08±18.76[ab]	

[a.b.] Means within a column with different superscripts differ significantly ($P<0.05$); CA 1.0= Fermented vegetable liquid extract+carrier 100%; CA 2.0= Fermented vegetable liquid extract+chilli+carrier 100%; PA 1.0= Fermented vegetable solid extract+carrier 50%; PA 2.0= Fermented vegetable liquid extract+chilli+carrier 50%; CA1.1= Fermented vegetable liquid extract+carrier100%+MRS 1 ml; CA2.1= Fermented vegetable liquid extract+chilli+carrier 100%+MRS 1 ml; PA1.1= Fermented vegetable solid extract+carrier 50%+MRS 1 ml; PA2.1= Fermented vegetable liquid extract+chilli+carrier 50%+MRS 1 ml

The results of the current study indicated that vacuum drying was less effective for synbiotic drying since the longer the drying process, the lower the water content and activity of water, leading to a lower LAB growth rate. The LAB value significantly decreased during the drying period from 48 hours to 96 hours due to the pressure exerted by the vacuum machine, resulting in a reduced water activity value. Consequently, bacterial cells were damaged and there was a decrease in LAB in synbiotics. As mentioned by Juniawati et al. (2019), when the capsule wall is not strong enough to withstand the pressure of the microcapsule particles, the wall breaks, and the particles collapse so that the cell is damaged. Damage to the bacterial membrane in the vacuum process is due to dehydration, thereby reducing the production of lactic acid bacteria (Romano et al., 2021).

The most optimal storage of synbiotics is four weeks of storage as the water content of the dried synbiotic for 48 hours is an average of 35.9%. Therefore, higher water content results in the shorter shelf life of the synbiotic. Utama et al. (2020) reported that the storage of a product can be affected by the water content of the product. Nurhidajah et al. (2020) added that high and low water content affect the shelf life meaning that lower water content results in longer shelf life.

CONCLUSION

Based on the experiment results, it can be concluded that using the 48-hour vacuum drying method is effective in preserving synbiotics in dry form without affecting their organoleptic and physical properties or the viability of lactic acid bacteria. Further research is required to investigate the impact of using synbiotic products dried through the vacuum method on the growth and health of animals.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' Contribution

B. Sulistiyanto is responsible for coordinating research activities, data processing, and finalization of scientific articles; C.S. UTAMA provide suggestions and finalization of scientific articles; K.U. Albab is responsible for research, preparation of tools and materials, research data processing.

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Competing interests

The authors declare that they have no competing interests.

Animal Ethical regulation

The treatment of experimental animals in accordance with the "Guidelines for the Care and Use of Animal Laboratory" from the Diponegoro University. All procedures carried out in this study involving animals have been in accordance with ethical standards and approved by the Feed Technology Laboratory, Faculty of Animal and Agriculture Sciences, Diponegoro University.

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