

**DETERMINATION OF SHELF LIFE USING
ACCELERATED SHELF LIFE TEST (ASLT) METHOD
ON INSTANT POWDER PROBIOTIC ORANGE SWEET POTATO FREEZE DRY**

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ABSTRACT

Probiotic beverages produced using freeze-drying techniques are commonly developed to extend shelf life. In this study, instant probiotic powder made from orange sweet potato was prepared using the freeze-drying process at -56°C with the addition of 20% maltodextrin as a coating material. This study aimed to determine the shelf life of instant orange sweet potato probiotic powder using the Accelerated Shelf Life Testing (ASLT) method based on the Arrhenius equation model. The instant orange sweet potato probiotic powder was stored at temperatures of 4°C , 28°C , and 40°C for 56 days. The parameters observed every seven days were moisture content, pH, total acidity, and total lactic acid bacteria (LAB). The experimental results showed a linear relationship between the increase in moisture content and pH, and the decrease in total acidity and total LAB count over storage time at each temperature. The estimated shelf life of the instant orange sweet potato probiotic powder stored at 4°C , 28°C , and 40°C was 49, 45, and 43 days based on moisture content; 34, 42, and 46 days based on pH; 212, 207, and 206 days based on total acidity; and 236, 306, and 344 days based on total LAB, respectively. The shelf life of instant probiotic orange sweet potato powder can be determined using the ASLT method, with variations depending on the quality parameters evaluated.

Keywords: Shelf Life, Accelerated Shelf Life Testing, Orange Sweet Potato, Freeze Drying, Instant Drink Powder

INTRODUCTION

Probiotics are live microorganisms that, when consumed in adequate amounts, confer health benefits, particularly by

maintaining the balance of intestinal microflora and enhancing the immune system. In recent decades, the demand for functional food products, including

probiotics, has increased in line with growing consumer awareness of health and disease prevention. One of the innovations in probiotic product development is the powder formulation, which offers advantages in terms of stability, ease of distribution, and longer shelf life compared to liquid products or fresh yogurt (Daysita *et al.*, 2024).

Orange sweet potato (*Ipomoea batatas*) is a food material rich in fiber, vitamins, and antioxidants such as β -carotene, which functions as a natural prebiotic. The combination of probiotics and prebiotics in powder formulations provides synergistic effects in supporting digestive health. Furthermore, orange sweet potato has high potential as a substrate for probiotic products because it can support the growth of lactic acid bacteria during the fermentation process (Yulia, Rubiyanti and Yuliana, 2024).

Although probiotic powders offer several advantages, a major challenge lies in maintaining the viability of probiotic bacteria during storage. The stability of these microorganisms is influenced by factors such as temperature, humidity, and product moisture content. Therefore, determining the shelf life of probiotic powder is essential to ensure that the microorganisms remain viable and effective until consumption (Chaturvedi and Chakraborty, 2024).

Drying techniques, such as freeze drying or spray drying, as well as the addition of carrier materials like maltodextrin, have been widely applied to extend shelf life and improve product stability (Gullifa *et al.*, 2023). The shelf life of probiotic products is generally determined by evaluating bacterial viability under various storage conditions over a specific period. Viability is assessed based on the number of surviving colonies during storage. In addition, the use of maltodextrin as a carrier material in powder formulations can enhance the stability of probiotics (Vivek, Mishra and Pradhan, 2021).

This study aims to determine the shelf life of orange sweet potato-based probiotic powder by evaluating changes in bacterial viability and the physicochemical properties of the product during storage under different environmental conditions. The findings of this research are expected to contribute to the development of more stable and high-quality probiotic products.

MATERIALS AND METHODS

Materials

The materials used were Orange sweet potato taken and determined at the Plant Taxonomy Laboratory Department of Biology UNPAD, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria

from starter culture, aluminum foil, filter paper, spiritus, Nutrient agar media (Merck), MRS Agar media (Merck), 70% Ethanol, fresh milk, sucrose, skimmed milk, aquadest and other materials.

Tools

The tools used are autoclave (Labtech), incubator (Memment®), digital scale (MRC), blender (Cosmos®), measuring cup (pyrex), petri dish, test tube (pyrex), digital pH meter (Hanna), thermometer, colony counter (MRC), bunsen, ose needle, hot plate, spatula, oven (Memment®), desiccator (Iwaki®), biosafety cabinet (Biobase®), freeze dryer (Biobase®), viscometer and other glassware.

Method

1. Preparation of Orange Sweet Potato Probiotic Beverage

The preparation of orange sweet potato probiotic beverage began with the pasteurization of a mixture of orange sweet potato juice and fresh milk at a ratio of 2:1 for 15 minutes at 90°C, followed by cooling to 40°C. The mixture was then inoculated with 3% lactic acid bacteria (LAB) at a ratio of 1:1 and incubated at 37°C for 24 hours (Yulia, Rubiyanti and Yuliana, 2024).

2. Orange Sweet Potato Probiotic Instant Powder

The resulting liquid probiotic was dried using the freeze-drying method with the

addition of 20% maltodextrin as a carrier material to produce a probiotic yogurt powder. One gram of the resulting powder was packed in aluminum foil with a thickness of 90 µm and hermetically sealed. The packaged samples were stored at temperatures of 4°C, 28°C, and 40°C for 56 days (Yulia, Rubiyanti and Yuliana, 2024).

3. Probiotic Cell Viability Test During Storage

The viability of probiotic cells in the orange sweet potato probiotic instant powder was determined using the Total Plate Count (TPC) method with slight modifications. The test was performed every 10 days for samples stored at 4°C and 30°C, and every three days for samples stored at 37°C. A total of 1 g of probiotic powder was dissolved in 9 mL of 0.85% NaCl solution (10^1 dilution). Then, 1 mL of the suspension was transferred into 9 mL of 0.85% NaCl to obtain a 10^2 dilution, and serial dilutions were continued as required. From the last three dilutions, 1 mL of each solution was inoculated onto MRS agar medium and incubated at 37°C for 48 hours. The resulting colonies were counted using a colony counter and expressed as CFU/g. The total number of colonies was calculated using the following formula: (Miskiyah and Yuanita, 2020).

$$SI = \frac{\sum C}{n_1 + 0.1n_2} \times d$$

Where:

SI = number of colonies (CFU/g)

$\sum C$ = total colonies from all plates,

n1 = number of plates at the first dilution,

n2 = number of plates at the second dilution,

d = lowest calculated dilution factor.

4. Moisture Content

The moisture content was determined gravimetrically. The weighing cup was first oven-dried, cooled in a desiccator, and weighed to obtain the empty weight (A). Approximately 2 g of sample was weighed into the cup and oven-dried at 105°C until a constant weight was achieved.⁷ The moisture content was calculated using the following equation:

$$\text{Moisture content (\%)} = \frac{B - C}{B - A} \times 100\%$$

Where:

A = weight of empty cup (g)

B = weight of cup + sample before drying (g)

C = weight of cup + sample after drying (g)

5. Estimation of Shelf Life Using the ASLT Method

Shelf life estimation was conducted using the Accelerated Shelf-Life Testing (ASLT) method.⁸ The probiotic instant powder was packaged in aluminum foil (90 μm thickness, 2×3 cm) and stored at 4°C,

28°C, and 40°C. The critical parameter observed was the viability of probiotic cells, measured every 7 days for 56 days. The decrease in cell viability was analyzed using zero-order and first-order reaction kinetics. A graph showing the relationship between the rate constant (k) and time (t) was constructed to determine the reaction order. The regression equation with the higher determination coefficient (R^2) was selected. Each equation consisted of an intercept value (a), representing the initial viability (day 0), and a slope value (b), representing the reaction rate constant (k_0). The dependence of the degradation rate constant (k) on temperature (T) was described by the Arrhenius equation as follows:

$$\ln k = \ln k_0 - \frac{E_a}{RT}$$

Where:

k = rate constant of degradation

k_0 = pre-exponential factor

E_a = activation energy

T = storage temperature (K)

R = gas constant (8.314 J·mol⁻¹·K⁻¹)

Shelf life (TS) was calculated based on the following kinetic with zero-order reaction and first-order reaction.

Data analysis

The data obtained were analyzed using the the SPSS 26 application with a completely randomized design (RAL). Data

in this study in the form of numerical variables were tested using one-way ANOVA followed by Duncan's post hoc test (Analysis Of Variance) with a confidence level of 95% ($\alpha < 0.05$).

RESULTS AND DISCUSSION

The preparation of instant orange sweet potato probiotic powder began with the production of an orange sweet potato probiotic beverage using *Lactobacillus bulgaricus* and *Streptococcus thermophilus* strains. The beverage was then processed into instant powder through freeze-drying with the addition of 20% maltodextrin as a binder. The resulting powder is presented in Figure 1.



Figure 1. Instant probiotic powder from fermented orange sweet potato

The organoleptic evaluation of the instant orange sweet potato probiotic powder showed that the product had a slightly granular texture with a bright yellow–orange color. This appearance is attributed to the natural orange color of the sweet potato yogurt powder and the white base color of maltodextrin. The taste was slightly sweet

due to the presence of sucrose. The difference between maltodextrin and sweet potato yogurt powder did not significantly affect the overall taste of the probiotic drink powder, as maltodextrin does not increase sweetness. The aroma of the instant powder was characterized by the distinctive scent of sweet potatoes combined with the metabolic products of lactic acid bacteria generated during fermentation.

The results of the moisture content analysis of the probiotic powder showed values ranging from 5.33% to 14.11% during 17 days of storage at 4°C, 5.33% to 10.93% during 32 days of storage at 28°C, and 5.33% to 7.19% during 42 days of storage at 40°C. The shelf-life estimation of the instant orange sweet potato probiotic powder followed a zero-order reaction model, as indicated by a higher R^2 value compared to the first-order model at each storage temperature. The moisture content profile during storage is presented in Figure 2.

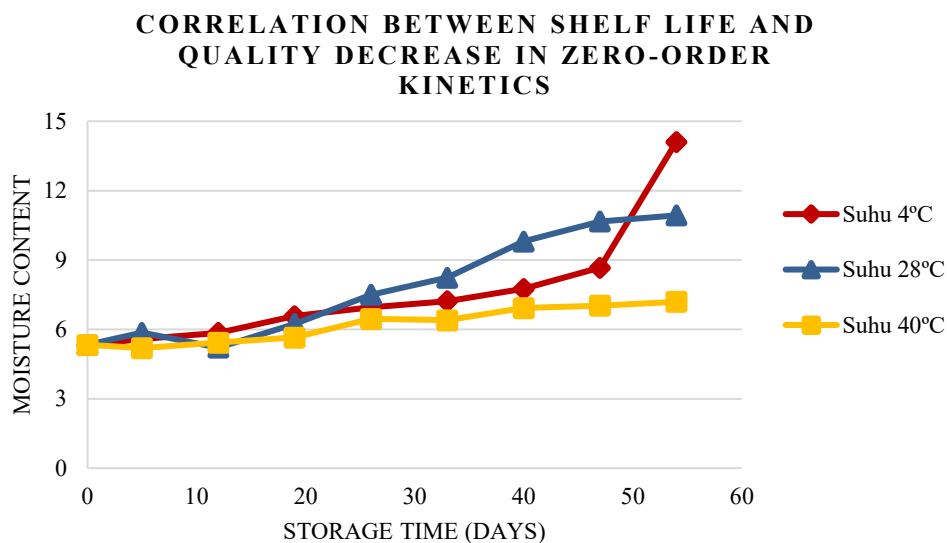


Figure 2. Changes in moisture content during storage

Moisture content has a direct relationship with the physical and organoleptic stability of a product. Storage under low-humidity conditions can help maintain stable moisture levels. Moisture content is a critical parameter influencing product stability during storage. The observed moisture content range is consistent with typical values for microencapsulated products obtained through spray drying, which is 2–6% (Pradipta, 2018). High moisture content tends to accelerate chemical reactions such as hydrolysis and oxidation, and increase microbial activity, thereby affecting product quality during storage. Conversely, low moisture content helps extend shelf life by minimizing the risk of microbiological and chemical degradation (Estiasih, Waziroh and Fibrianto, 2022).

The acidity values of the probiotic powder ranged from 3.96 to 4.69 during 34 days of storage at 4°C, 3.99 to 4.90 during 42 days of storage at 28°C, and 4.16 to 4.76 during 46 days of storage at 40°C. The shelf-life estimation of the instant orange sweet potato probiotic powder followed a zero-order reaction model, as indicated by the higher R² values compared to the first-order model at each storage temperature. The acidity profile during storage is presented in Figure 3.

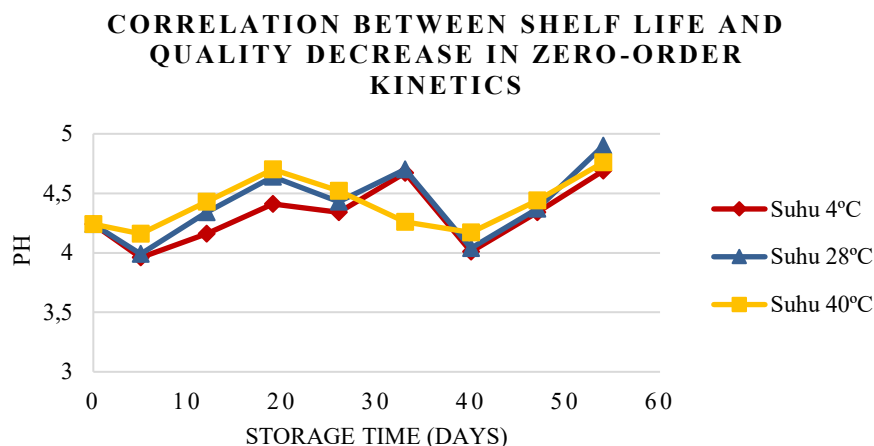


Figure 3. Changes in pH values during storage

Metabolic processes, including the fermentation of lactose into lactic acid, are strongly influenced by temperature (Gänzle, 2015). At higher temperatures, the metabolic activity of microorganisms increases, resulting in a faster production of lactic acid and consequently higher acidity values. Conversely, storage at low temperatures slows down enzymatic activity and microbial growth, leading to more stable and lower acidity levels (Rossi *et al.*, 2021). In addition, low storage temperatures reduce the rate of lactose degradation and the formation of organic acids, thereby contributing to the stability of product quality during storage (Bottiroli *et al.*, 2021).

The initial decrease in pH is caused by the production of lactic acid by lactic acid bacteria during carbohydrate metabolism. Over time, the pH gradually increases due to a reduction in lactic acid production. This occurs as the sugar substrate becomes

depleted and the increasingly acidic environment leads to the decline or death of most lactic acid bacteria (Ferdaus *et al.*, 2017).

Total titratable acidity (TAT) in probiotic powder is measured as an indicator of lactic acid production during lactose fermentation by lactic acid bacteria. The TAT of probiotic powder during storage at 4°C ranged from 1.31% to 1.48% over 212 days; at 28°C it ranged from 1.29% to 1.48% over 207 days; and at 40°C it ranged from 1.31% to 1.48% over 206 days. Lactic acid production is the main metabolic product of lactic acid bacteria (LAB), such as *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, during yogurt fermentation. In general, TAT values show a negative correlation with pH values. After a storage period of up to 212 days, the TAT value of the orange sweet potato probiotic powder remained within the maximum limit

specified by the Indonesian National Standard (SNI) for yogurt, which is 0.5–2.0%

(Nasional, 2009). The TAT profile during storage is presented in Figure 4.

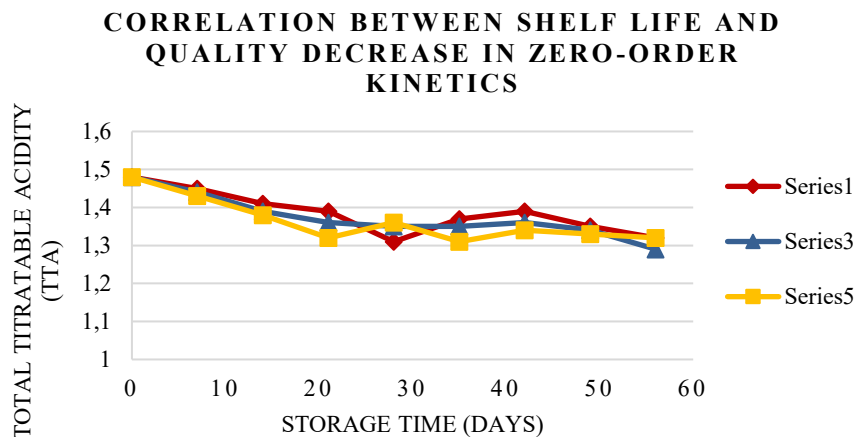


Figure 4. Changes in total titratable acidity (TTA) during storage

The total lactic acid bacteria (LAB) count in the probiotic powder during storage at 4°C ranged from 2.25×10^7 to 2.36×10^7 CFU/g over a period of 796 days. The high stability observed at low temperatures is attributed to the slowdown of bacterial metabolic activity due to decreased enzymatic activity, thereby minimizing cell damage and maintaining cell viability. Low-temperature storage is commonly applied to extend the shelf life of probiotic products, as it helps protect bacterial membrane structures and proteins from degradation (Dianawati, Mishra and Shah, 2016).

At 28°C, the total lactic acid bacteria (LAB) count ranged from 2.24×10^7 to 2.35×10^7 CFU/g over 610 days of storage. Although bacterial metabolism is more active at this temperature compared to 4°C, the probiotic powder formulation

demonstrated good stability. The use of protective agents such as maltodextrin helps maintain bacterial viability during storage by protecting cells from environmental stress (Sukarminah *et al.*, 2017).

At 40°C, the LAB count decreased to a range of 2.20×10^7 to 2.35×10^7 CFU/g after 542 days of storage. High temperatures increase the risk of protein denaturation, cell membrane damage, and the formation of reactive free radicals, which accelerate the decline in bacterial viability (Putri, Kusnadi and Saparianti, 2021). However, the optimized formulation of this product enables the bacteria to survive at elevated temperatures for a relatively long period, indicating its potential for application in tropical climates. The LAB viability profile during storage is presented in Figure 5.

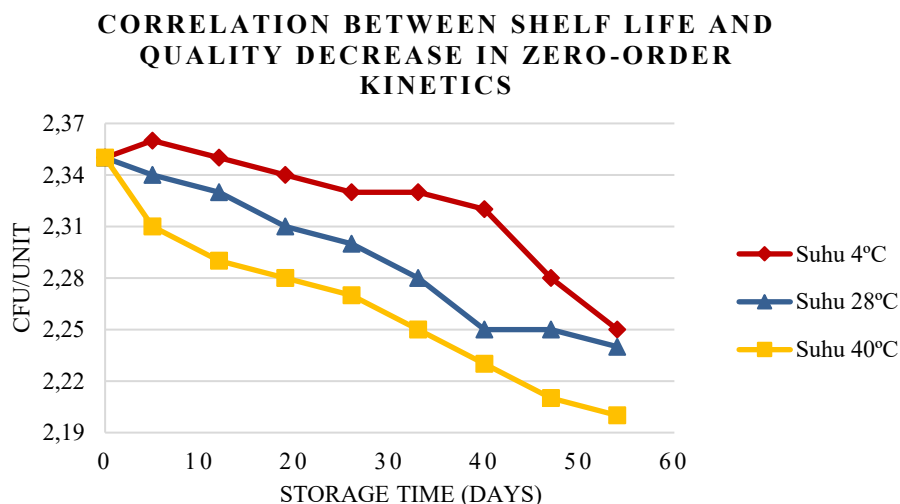


Figure 5. Changes in total lactic acid bacteria (LAB) during storage

Table 1. Estimation of the shelf life of yogurt

Temperature		Value k	Shelf Life	
°C	°K		Day	Month
4	277	0,0024	212	7.06
28	301	0,0025	207	6.90
40	313	0,0025	206	6.86

The stability of lactic acid bacteria (LAB) viability in probiotic instant powder is strongly influenced by storage temperature and duration. This study demonstrated that the total LAB count in the probiotic powder exhibited high stability under various storage conditions, maintaining levels above the minimum effectiveness threshold of 10^6 CFU/g as recommended by FAO/WHO. Shelf-life estimation was carried out by identifying the critical parameter with the highest R^2 value. From this equation, the k value at each storage temperature was determined and subsequently used to calculate the product's shelf life based on its

reaction order. Based on total LAB calculations, the probiotic powder had an estimated shelf life of approximately 207 days at room temperature (25°C). Storage at lower temperatures, such as in a refrigerator (4°C), extended the shelf life to 212 days (approximately 30.3 weeks). Although a gradual decline in LAB viability was observed during storage, the bacterial count remained within the range of 2.20×10^7 to 2.35×10^7 CFU/mL until the end of the observation period. These findings indicate that the orange sweet potato probiotic powder effectively supports LAB viability,

which is essential for maintaining its functional health benefits upon consumption.

CONCLUSION

The shelf life of orange sweet potato probiotic powder can be estimated based on the critical parameter of the fastest quality deterioration using the Arrhenius model, with the equations $y = -0.0024x + 1.4486$; $y = -0.0027x + 1.4276$; and $y = -0.0024x + 1.4053$, and a determination coefficient (R^2) of 0.0474. The estimated shelf life of synbiotic yogurt powder at room temperature is 207 days. Under cold storage conditions (4°C), the shelf life extends to 212 days, whereas at high temperatures (40°C), it decreases to 206 days. These findings highlight the importance of temperature control in maintaining product stability and prolonging shelf life.

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