

Accelerated shelf-life assessment of moringa-fortified instant complementary food for infants aged 6-11 months based on microbial parameters

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Abstract

Instant powdered complementary food is a commercial product designed to meet the nutritional needs of infants aged 6-11

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months. This study aimed to determine the shelf life of instant powdered complementary food products fortified with moringa flour using semi-aluminum foil packaging based on microbial and mold growth parameters. The product was prepared from a mixture of ingredients such as wheat flour, mofaf flour, soybean flour, chicken eggs, ultra-high temperature milk, and vegetable oil, according to the SNI 01-7111.1-2005 standards. The study used the accelerated shelf-life testing method with the Arrhenius equation model, storing the products at 30°C, 40°C, and 50°C for 28 days. Results showed that total microbial and mold counts increased significantly at 30°C and 40°C over 28 days, while storage at 50°C led to a decline in microbial counts and slower mold growth. Using the Arrhenius-based first-order model, the predicted shelf life increased with temperature, reaching up to 92.7 days based on microbial growth and 40.8 days based on mold growth at 50°C. This trend is attributed to the lower reaction rate constants (k values) at higher temperatures, indicating slower deterioration. However, since mold growth was the limiting factor, the shortest shelf-life estimate, 33.5 days at 30°C, should be considered for practical labeling. It is therefore recommended to store moringa-fortified instant complementary food packaged in semi-aluminum foil at or below 30°C to suppress microbial activity and maintain product safety and quality throughout its shelf life. These results highlight the importance of temperature control in extending the product's microbial stability and suggest further real-time studies to validate shelf life under typical storage conditions.

Introduction

Ensuring optimal growth and development during infancy requires adequate nutrition, especially between the ages of 6-11 months, a critical period known as transitional feeding. During this stage, complementary foods, nutrient-rich foods, or drinks provided in addition to breast milk, are introduced to meet the increasing nutritional needs that breast milk alone can no longer satisfy (WHO, 2023).

One commonly available form of complementary food is instant powdered complementary food, which is industrially processed and designed to be convenient and nutritionally appropriate. Enhancing such products with moringa leaf powder has gained interest due to its high content of essential nutrients and potential antimicrobial properties. These properties make moringa a promising fortificant that may improve both the nutritional quality and microbial stability of complementary foods. In the present study, the formulated moringa-fortified complementary food includes wheat flour, mofaf flour, soybean flour, chicken eggs, full cream milk, and vegetable oil. The formulation complies with the Indonesian Standard (SNI 01-7111.1-2005) for complementary food quality (BSN, 2005).

The nutrient composition of complementary food products also

makes them susceptible to quality degradation over time. Factors such as microbial growth, oxidation, and moisture absorption can affect safety and acceptability during storage (Li *et al.*, 2017). To minimize these changes and extend shelf life, appropriate packaging is essential. In the study by Yan *et al.* (2022), semi-aluminum foil was used to protect the product from environmental factors that accelerate spoilage.

Determining the shelf life of newly developed complementary food products is a critical step in ensuring their safety and market readiness. A widely used approach for this is the accelerated shelf-life testing (ASLT) method, which simulates long-term storage by subjecting products to elevated temperatures or humidity levels to hasten deterioration. This method enables faster estimation of product longevity compared to real-time storage studies (Phan *et al.*, 2014). For powdered foods, ASLT is typically conducted at temperatures ranging from 25°C to 45°C, with 18°C used as the control (Asiah *et al.*, 2018).

This study aims to fill the knowledge gap regarding the shelf life of moringa-fortified instant complementary food. The specific objective was to estimate the shelf life of the product using the ASLT method, based on two key microbiological parameters: total bacterial count and mold count. This research is expected to support the development of shelf-stable, nutritionally enhanced infant food products while increasing their economic and functional value.

Materials and Methods

The main ingredients used in this study were mocaf flour, wheat flour, ultra-high temperature (UHT) full cream milk, eggs, and vegetable oil, all of which were purchased from stores and supermarkets. Moringa leaf flour was sourced from CV Gemilang, while soybean flour was prepared in-house using a modified method based on (Rani *et al.*, 2013). The chemicals used included NaCl 0.85%, potato dextrose agar, and plate count agar media.

The packaging material consisted of semi-aluminum foil sachets in a standing pouch format with a tranzmetz zipper, measuring 9×15 cm, with a front thickness of 80 microns and a back thickness of 90 microns (PET/VMPET/SPE + ziplock). Equipment used included a blender, digital scale, baking pan, saucepan, basin, sifter, stove, porcelain cup, oven, furnace, incubator, desiccator, and standard microbiological tools such as Bunsen burner, Petri dishes, test tubes, cotton swabs, outclap, micropipette, and pipette tips. Complementary food ingredients were prepared following the modified formulation of Zakaria *et al.* (2024), consisting of 5 g moringa leaf flour, 30 g wheat flour, 20 g mocaf flour, 30 g soybean flour, 1 egg (±50 g), 10 mL UHT full cream milk, and 5 mL vegetable oil. The ingredients were mixed with sufficient water, boiled until fully cooked, and then dried using a cabinet dryer to reach a final moisture content of approximately 5%. The dried product was ground using a food processor and sieved through an 80-mesh sieve. The resulting powder was packaged in semi-aluminum foil sachets, each containing 30 g of product, according to the sample requirements for microbial analysis (Zakaria *et al.*, 2024). The study employed a descriptive design. Instant complementary food powder samples were stored at three elevated temperatures: 30°C, 40°C, and 50°C, using semi-aluminum foil packaging with two replications for each condition. Storage lasted for 28 days. Microbial quality was monitored weekly by measuring total microbial and mold counts on days 0, 7, 14, 21, and 28. Microbial analysis was carried out using the pour plate method

according to ISO 4833-1:2013 for total aerobic mesophilic bacteria Wei *et al.* (2023) and ISO 21527-2:2008 for yeasts and molds (Bird *et al.*, 2015) (ISO, 2008 and 2013).

Shelf-life estimation was conducted using the ASLT method, applying the Arrhenius equation model based on total bacterial and mold counts. Temperatures of 30°C, 40°C, and 50°C were selected to represent accelerated storage conditions that promote faster deterioration and microbial growth within a shorter timeframe. Although a control condition at 18°C is typically used to simulate real-time storage, it was not included in this study due to time and resource constraints. The focus was to generate predictive insights through accelerated testing; future studies are planned to validate these results under real-time storage conditions. Shelf life was estimated using first-order kinetics with the Arrhenius model, applying the formula $t_s = \ln(N_0/N_t)/k$, where t_s is the estimated shelf life (in days), N_0 is the initial microbial count (CFU/g), N_t is the critical microbial count threshold (CFU/g), and k is the reaction rate constant derived from the slope of the Arrhenius plot (Syahrul *et al.*, 2020). The critical microbial limit (N_t) was set at 10^4 CFU/g, based on Codex Alimentarius guidelines (CAC/GL 08-1991) and national regulations for complementary infant foods, which recommend this threshold as the upper acceptable limit for microbial safety.

Results

Total microbes

The results of the 28-day storage study, in which total microbial counts were observed at 7-day intervals, are presented in Table 1. As shown in Table 1, microbial counts increased over time at storage temperatures of 30°C and 40°C. At 30°C, the count rose from 6.8×10^3 colonies/g on day 0 to 1.3×10^4 colonies/g on day 28. A similar trend was observed at 40°C, where microbial counts reached 1.1×10^4 colonies/g by day 28. In contrast, storage at 50°C resulted in a gradual decline in total microbial counts from 6.8×10^3 colonies/g on day 0 to 5.4×10^3 colonies/g on day 28, likely due to thermal inhibition of microbial growth.

At 30°C and 40°C, microbial counts increased over time. Interestingly, at 50°C, microbial counts decreased, suggesting microbial inactivation or slowed growth. However, shelf-life predictions (Table 2) show a longer shelf life at 50°C. This seeming discrepancy is due to lower microbial growth rates at higher temperatures, reflected by the smaller value of the reaction rate constant (k) derived from the Arrhenius equation. A smaller k implies a slower deterioration process, thus a longer predicted shelf life.

Based on the Arrhenius model, the predicted shelf life of the product was approximately 71.6 days (2.4 months) at 30°C, 81.2 days (2.7 months) at 40°C, and 92.7 days (3.1 months) at 50°C. These results indicate that although microbial numbers were lower at 50°C, the slower degradation rate (smaller k) led to a longer shelf life.

Total mold

Mold counts were also monitored every 7 days for 28 days, and the results are presented in Table 3.

As seen in Table 3, mold counts increased over time at all storage temperatures. At 30°C and 40°C, mold levels rose sharply by day 28, reaching 9.2×10^3 and 9.0×10^3 colonies/g, respectively. At 50°C, mold growth was slower, with counts reaching 5.8×10^3 colonies/g by day 28. Although mold counts increased at all temperatures, the rate of increase (k) was slower at higher tempera-

tures, particularly at 50°C. As with microbes, this results in longer shelf-life predictions (Table 4).

Table 4 presents the shelf-life prediction of moringa-fortified instant complementary food based on mold growth parameters at three different storage temperatures (30°C, 40°C, and 50°C), using the Arrhenius-based first-order reaction model. The microbial threshold value (N_t) was set at 10,000 CFU/g, with an initial mold count (N_0) of 220 CFU/g. The rate constant (k) decreased with increasing temperature, indicating slower mold growth at higher temperatures. As a result, the calculated shelf life increased with temperature: 33.5 days at 30°C, 34.2 days at 40°C, and 40.8 days at 50°C. This trend suggests that mold growth is slower at higher temperatures, making it the primary factor limiting shelf life under the tested conditions.

Discussion

Shelf life refers to the period during which a product or ingredient can be stored and retains its quality, safety, and desirable characteristics. This term is commonly used in the context of food products, pharmaceuticals, chemical products, cosmetics, and other materials that are susceptible to changes in quality or safety over time. Shelf life is determined by several factors such as product composition, storage method, and environmental conditions. The goal is to ensure that a product or ingredient is safe to use or consume for a predetermined period. In some cases, the shelf life of the product may be indicated by the expiration or manufacturing date (Al-Baarri *et al.*, 2023).

Complimentary instant porridge is prepared in an instant or

Table 1. Total microbial counts (colonies/g) in instant porridge fortified with moringa leaf flour during 28 days of storage at different temperatures.

Time	Total microbes at storage temperature			
	0	30°C (colonies/g)	40°C (colonies/g)	50°C (colonies/g)
0 days	6.8×10^3	-	-	-
7 days	-	7.5×10^3	7.4×10^3	6.6×10^3
14 days	-	8.2×10^3	8.0×10^3	6.1×10^3
21 days	-	9.2×10^3	9.0×10^3	5.8×10^3
28 days	-	1.3×10^4	1.1×10^3	5.4×10^3

Table 2. Shelf-life prediction of instant porridge fortified with moringa flour based on total microbial counts.

T (°C)	k (day ⁻¹)	N_t (CFU/g)	N_0 (CFU/g)	ln N_0	ln N_t	ln $N_0 - \ln N_t$	Shelf life (days)
30	0.0054	10000	6800	1.3061	8.8247	0.3857	71.6
40	0.0047	10000	6800	1.3061	8.8247	0.3857	81.2
50	0.0042	10000	6800	1.3061	8.8247	0.3857	92.7

T, temperature; k, reaction rate constant; N_t , final/threshold microbial count; N_0 , initial microbial count; ln, natural logarithm of microbial counts.

Table 3. Total mold counts (colonies/g) in instant porridge fortified with moringa flour during 28 days of storage at different temperatures.

Time	Total microbes at storage temperature			
	0	30°C (colonies/g)	40°C (colonies/g)	50°C (colonies/g)
0 day	2.2×10^2	-	-	-
7 days	-	3.4×10^2	2.9×10^2	2.1×10^2
14 days	-	4.6×10^2	3.9×10^2	2.1×10^2
21 days	-	5.6×10^2	4.3×10^2	2.1×10^2
28 days	-	9.2×10^3	9.0×10^3	5.8×10^3

Table 4. Shelf-life prediction of instant porridge fortified with moringa flour based on total microbial counts.

T (°C)	k (day ⁻¹)	N_t (CFU/g)	N_0 (CFU/g)	ln N_0	ln N_t	ln $N_0 - \ln N_t$	Shelf life (days)
30	0.1138	10000	220	5.3936	9.2103	3.8167	33.5
40	0.1117	10000	220	5.3936	9.2103	3.8167	34.2
50	0.0935	10000	220	5.3936	9.2103	3.8167	40.8

T, temperature; k, reaction rate constant; N_t , final/threshold microbial count; N_0 , initial microbial count; ln, natural logarithm of microbial counts.

ready-to-eat form for infants and toddlers. This study is a continuation of Zakaria's research which is the best instant porridge product from the formulation of moringa flour, wheat flour, mofaf flour, soybean flour, chicken eggs, UHT full cream milk, and vegetable oil as a complementary food for infants 6-11 months (Zakaria *et al.*, 2024), and has met the requirements in accordance with SNI 01-7111.1-2005 regarding the quality requirements of complementary food (BSN, 2005).

Shelf life prediction was performed with the Arrhenius ASLT approach, assuming first-order kinetics for both total microbial and mold growth ($k \text{ day}^{-1}$). The goodness of fit ($R^2 > 0.92$ for all regressions, data not shown) confirmed that the first-order assumption captured the early exponential phase over the 28-day test window. This study in estimating shelf life using total microbial parameters and mold total showed that there was an increase in total microbes from day 0 of 6.8×10^3 colonies/g to 1.3×10^4 colonies/g on day 28, illustrating that there is significant growth of microorganisms during the storage period at a certain temperature. This increase in microbial count can be caused by several factors, including favorable environmental conditions such as nutrient availability, humidity, and adequate storage temperature for microbial development (Ajala *et al.*, 2024). Microorganisms tend to multiply rapidly under optimal conditions; therefore, this increase in colonies could be an indicator that storage conditions are within a temperature range that supports microbial growth (Wang *et al.*, 2025). Furthermore, at a storage temperature of 40°C , there was an increase in the number of microbes from day 0 to day 28, reaching 1.1×10^4 colonies/g. Warmer temperature conditions often increase the enzymatic activity and metabolism of microorganisms, which in turn accelerates the growth of microbial populations. In this context, 40°C may be close to the optimum temperature for most microorganisms found in these samples; therefore, the number of microbial colonies increased over time. This is in line with microorganism growth theory, which states that each microbial species has an optimum temperature for growth, and temperatures close to this value will result in faster growth rates (John Madigan, 2006). However, at a storage temperature of 50°C , the number of microbes decreased from day 28 to 5.4×10^3 colonies/g. This decrease can be caused by temperatures that are too high to be optimal, or even inhibit the growth of microorganisms. Higher temperatures can cause denaturation of proteins and vital enzymes in microbial cells, which can result in death or decreased microbial activity. Microorganisms have a certain temperature tolerance limit, and temperatures exceeding this limit can serve as an inhibiting factor, leading to a decrease in the microbial population (Gonzalez and Aranda, 2023). Interestingly, despite the observed microbial inhibition at 50°C , the calculated shelf life increased with temperature for both microbial and mold parameters in the Arrhenius model. This apparent contradiction may be explained by the mathematical sensitivity of the Arrhenius equation to early-stage changes in microbial counts and the limited duration of the ASLT (28 days), which may not have fully captured the transition to inactivation kinetics at extreme temperatures. The assumption of zero- or first-order kinetics may also be oversimplified for microbial growth, especially under stress conditions. Thus, while the model predicts longer shelf life at higher temperatures, this outcome may be more reflective of slowed initial growth or delayed inactivation thresholds than true stability improvements.

The study showed an increase in total mold in complementary foods from 2.2×10^2 colonies/g on day 0 to 9.2×10^3 colonies/g on day 28, indicating a significant development in the mold population during storage. Molds, which are a type of fungus, tend to grow on nutrient-rich foodstuffs and under favorable environmental conditions, such as high humidity and a suitable temperature.

This increase in the number of molds indicates that the storage environment during the 28 days was favorable for mold growth. This is in line with research showing that molds can grow rapidly on foodstuffs if environmental conditions, especially temperature and humidity, are favorable (Garnier *et al.*, 2017).

Furthermore, at a storage temperature of 40°C , the mold count increased from 2.2×10^2 colonies/g on day 0 to 9.0×10^3 colonies/g on day 28. This temperature may be close to the optimum temperature for some mold species, leading to an increase in the mold population during storage. Warm temperatures can accelerate the metabolism of molds and allow them to grow faster, especially if sufficient moisture and substrate are available (Tang *et al.*, 2015). Thus, 40°C can be considered a favorable temperature, but it is still within the tolerance limits of molds for growth.

However, at a storage temperature of 50°C , there was an increase in the number of molds to 5.8×10^3 colonies/g on day 28, but this number was smaller than that at 30°C and 40°C . This indicates that although 50°C still allows mold growth, this temperature is close to or beyond the optimal limit for mold growth. Temperatures that are too high can cause thermal stress in molds, which inhibits their growth rate. Temperatures close to the upper tolerance limit of molds can reduce their viability and growth rate, resulting in lower colony counts compared to lower temperatures (Samson *et al.*, 2019).

This relative decrease in mold numbers at 50°C compared to those at 30°C and 40°C could indicate that these temperatures are starting to approach lethal temperatures for some mold species. However, an increase remained, suggesting that some mold species may be more resistant to high temperatures and are still able to grow under more extreme conditions. Molds can have a wide temperature tolerance range, but maximum growth usually occurs at temperatures lower than their maximum temperature of tolerance (Johansson *et al.*, 2013).

The storage temperature greatly affects the growth rate of microbes in food products (Al-Garadi *et al.*, 2024). Generally, microbes, such as bacteria and molds, grow faster at higher temperatures because temperatures affect enzyme activity and microbial cell metabolism. At lower temperatures, microbial activity tends to be slower, so microbial growth is inhibited, extending the shelf life of the product. Microorganisms have specific growth temperature ranges, which are usually divided into three main categories: psychrophilic (grow well at low temperatures), mesophilic (grow optimally at moderate temperatures), and thermophilic (grow optimally at high temperatures) (Pandey *et al.*, 2015). Most food-associated pathogens are mesophilic microorganisms, which grow optimally at $30\text{--}40^\circ\text{C}$, so storage temperatures below this range can slow microbial growth (Akindolire *et al.*, 2022). Increased storage temperatures tend to accelerate microbial growth in processed foods, including instant powdered products. At higher storage temperatures, microbial enzymes become more active so that microbial growth rates increase, accelerating product deterioration and decreasing shelf life (Jacxsens *et al.*, 2002).

Molds are generally more tolerant of extreme storage conditions than bacteria, so they can grow over a wider temperature range, including at low temperatures. According to Frazier and Westhoff (1988) in Food Microbiology, molds can survive in environments with low moisture content and a wider temperature range, which makes them more difficult to control in various food products, including powdered products (Frazier and Westhoff, 1988). Certain molds are even able to grow at temperatures around 5°C , although their optimal growth is at temperatures around $25\text{--}30^\circ\text{C}$.

Storage at high temperatures can accelerate mold growth in

food products, but at excessively high temperatures, mold growth can be inhibited (Mafe *et al.*, 2024). This is because of the damage to the cellular structure of molds at extreme temperatures that exceed their temperature tolerance threshold. In ASLT methods, high temperatures are used to accelerate the observation of microbial changes over a shorter period, although this may not fully reflect storage conditions.

In addition to temperature, packaging also plays a crucial role in limiting microbial and mold contamination. This study used semi-aluminum foil packaging, which provides moderate barrier properties against moisture, oxygen, and light; three key factors that influence microbial growth. While not as impermeable as full aluminum foil, semi-aluminum foil still offers significant protection, helping to slow down deterioration without causing excessive packaging costs. Its reflective properties help deflect heat, and its layered structure adds an extra barrier to airborne microbial spores. The effectiveness of this packaging is especially important in maintaining product safety during ambient storage conditions, such as in low-resource settings where cold storage may be unavailable. Thus, semi-aluminum foil packaging contributes to maintaining microbial stability and extending shelf life alongside temperature control (Muslimin *et al.*, 2016).

In the context of complementary food products fortified with moringa flour, higher storage temperatures can increase the risk of microbial and mold growth, potentially spoiling the product and reducing its shelf life. However, products fortified with natural antimicrobial ingredients, such as antioxidant-rich moringa leaves, have better durability because their bioactive compounds can inhibit the rate of microbial growth. Anwar *et al.* (2007) mentioned that moringa leaves contain active compounds, such as flavonoids and polyphenols, that have antimicrobial activity, which contributes to microbial stability in food products.

The Arrhenius-based shelf-life projections showed longer shelf life at higher temperatures; the microbial and mold growth trends indicate that such conditions may compromise safety over time. Therefore, storage recommendations should be based on the shortest shelf-life parameter, particularly mold growth, which typically poses the greatest spoilage risk in dry food powders. Implementing proper packaging and possibly refrigeration in high-temperature regions is advisable to maximize product safety and acceptability.

It was concluded that storage temperature plays an important role in controlling total microbial and mold growth in food products packaged by semi-aluminum foil. Storage at lower temperatures is recommended to slow microbial growth; however, under certain conditions, the use of accelerated methods can facilitate the rapid measurement of microbial changes for shelf-life estimation. The bioactive content in moringa flour can also help inhibit microbial growth, extending the shelf life of fortified products, such as complementary foods.

Conclusions

This study demonstrated that microbial and mold growth rates in moringa-fortified instant complementary food packaged with semi-aluminum foil are influenced by storage temperature. Shelf-life predictions using the Arrhenius model showed that higher temperatures resulted in slower microbial and mold growth rates, leading to longer estimated shelf life. However, since 50°C reflects only ASLT, the realistic shelf life at ambient storage (approximately 30°C) is around 2.4 months based on microbial parameters and 1.1 months based on mold growth, with mold identified as the pri-

mary limiting factor for product stability. The use of semi-aluminum foil packaging contributed to reduced moisture and oxygen transmission, creating a partial barrier that helped slow microbial and mold proliferation during storage. To support product stability, it is recommended that storage be maintained at ambient temperature with appropriate packaging. Further studies should include real-time shelf-life validation and evaluation of sensory quality and nutrient retention.

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