



Torch Ginger Flower Powder as a Natural Preservative for Frozen Vegan Oyster Mushroom Meatballs: Shelf-Life Prediction Using the Arrhenius Model

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Abstract

Frozen vegan oyster mushroom meatballs are a promising plant-based alternative; however, their shelf life is limited by microbial deterioration during storage. This study investigated the combined use of torch ginger flower powder and xanthan gum to improve the stability of frozen vegan oyster mushroom meatballs and to predict shelf life using the Accelerated Shelf-Life Testing (ASLT) and the Arrhenius model. Samples were formulated with 3 concentrations of torch ginger flower powder (0%, 0.5%, and 0.7%) and 2 concentrations of xanthan gum (0.3% and 0.5%), then stored at -18 ± 2 °C (freezer), 4 ± 1 °C (chiller), and 27 ± 2 °C (room temperature). Quality evaluations were conducted on days 0, 7, 14, 21, and 28 of storage. Total plate count (TPC), mold and yeast count, and total phenolic content were evaluated during storage. Higher concentrations of torch ginger flower powder and xanthan gum significantly suppressed microbial growth and better preserved phenolic compounds, particularly under chiller and freezer storage. Among the evaluated quality parameters, TPC showed the greatest deterioration and was selected as the critical parameter for shelf-life prediction. The formulation designated as K2P2 (0.7% torch ginger flower powder and 0.5% xanthan gum) exhibited the greatest storage stability, with a predicted shelf-life of 61.7 days during freezer storage, compared with 36.5 days for the control formulation. These findings demonstrate the potential of torch ginger flower powder as a natural preservative for improving the microbial stability and shelf life of frozen vegan oyster mushroom meatballs.

Keywords: Accelerated Shelf-Life Testing; microbial count; phenolic compounds; plant-based meat analog; xanthan gum

INTRODUCTION

Public awareness of healthy eating patterns in Indonesia has increased in recent years, along with growing attention to health, sustainability, and food safety (Rizkyanto and Ulfah, 2025). This condition has encouraged consumers to seek food products that are safer, more nutritious, and more in line with current plant-based food innovations in Indonesia (Putra and Alversia, 2023). The development of

vegetarian and vegan communities has also contributed to the wider adoption of plant-based diets and has encouraged research on meat analog products (Lubis et al., 2024). This trend provides an opportunity to develop food products that support environmentally friendly agriculture, food security, improved nutrition, and public health (Kraak and Aschemann-Witzel, 2024).

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Among plant-based products, vegan meatballs have potential as an alternative food because they can resemble the texture and sensory characteristics of animal-based meatballs (Permatasari et al., 2024). White oyster mushroom (*Pleurotus ostreatus*) is suitable as a raw material because it has a fibrous structure, contains bioactive compounds, is relatively affordable, and supports the development of sustainable plant-based foods (Setyawan et al., 2023). However, vegan oyster mushroom meatballs still have limitations, especially related to quality deterioration during storage (Fetriyuna et al., 2025).

One of the main challenges in storing vegan oyster mushroom meatballs is microbiological deterioration, which is closely related to their high moisture content and the perishable nature of plant-based ingredients (Nurman and Heriawita, 2022). Cold storage can help slow microbial growth, but it cannot completely stop chemical and biochemical changes, such as oxidation, pH changes, and texture degradation (Abel et al., 2022). Therefore, the use of natural preservative ingredients and stabilizing agents is needed to help maintain product quality and extend shelf life (Putra and Alversia, 2023).

Several studies have attempted to extend the shelf life of meatball products through the application of natural plant extracts, edible coatings, hydrocolloids, modified-atmosphere packaging, and other preservation technologies. These approaches have shown varying degrees of effectiveness in suppressing microbial growth, delaying quality deterioration, and maintaining the physicochemical properties of meatballs during storage (Meng et al., 2022). Similar preservation strategies have also been widely applied to meat and meat products, including synthetic preservatives, modified atmosphere packaging, and plant-derived preservatives. Recently, increasing consumer demand for clean-label foods has accelerated the development of natural preservatives, because they are considered safer, environmentally friendly, and capable of inhibiting microbial growth while maintaining product quality (Olvera-Aguirre et al., 2023).

Compared with many other plant-derived preservatives, torch ginger flower is abundant in Southeast Asia, economically affordable, and rich in phenolic compounds, flavonoids, anthocyanins, and essential oils that exhibit strong antioxidant

and antimicrobial activities. Torch ginger flower powder is a natural functional ingredient with potential as a preservative because it contains bioactive compounds, especially phenolics and flavonoids. These compounds are commonly associated with antioxidant activity and may also help inhibit the growth of spoilage microorganisms during food storage (Septiani and Fadhila, 2025). In vegan oyster mushroom meatballs, the addition of torch ginger flower powder is expected to help slow microbiological deterioration and maintain product quality during storage. Xanthan gum was also added in this study as a stabilizing agent to improve product consistency and matrix stability (Rahman et al., 2022).

Previous studies have applied Accelerated Shelf-Life Testing (ASLT) combined with the Arrhenius model to estimate the shelf life of various processed foods, including meatball products, cookies, food bars, and other food products (Sumartini et al., 2024). ASLT is particularly suitable for products whose quality deterioration is influenced by storage temperature, because quality changes can be described using deterioration kinetics and the Arrhenius equation (Pramono et al., 2024). This approach has been successfully used to evaluate several quality parameters, including microbial growth, mold and yeast growth, moisture content, lipid oxidation, texture changes, and degradation of bioactive compounds (Ardyanti et al., 2024).

Previous studies have also demonstrated the potential of torch ginger flower powder as a natural preservative due to its high content of phenolic compounds and flavonoids, with antimicrobial and antioxidant activities. Meanwhile, hydrocolloids such as xanthan gum contribute to the structural stability of plant-based meat analog systems (Br Girsang et al., 2022). However, although natural preservatives and hydrocolloids have been widely investigated separately, studies evaluating the combined application of torch ginger flower powder and xanthan gum in frozen vegan oyster mushroom meatballs remain limited. Furthermore, information regarding shelf-life prediction based on microbial deterioration using the ASLT-Arrhenius approach under different storage temperatures for this product is still limited (Olvera-Aguirre et al., 2023). Therefore, researchers hypothesized that the combined

incorporation of torch ginger flower powder and xanthan gum would better maintain the microbiological quality of frozen vegan oyster mushroom meatballs by suppressing microbial growth. Consequently, this would extend the product's shelf life compared with formulations without torch ginger flower powder.

MATERIALS AND METHOD

Materials and equipment

This research was conducted from December 2025 to May 2026 at Telkom University Purwokerto, Indonesia. Vegan meatball preparation was carried out in the Food Process Engineering Laboratory, while chemical and microbiological analyses were performed in the Basic Science Laboratory and the Microbiology Laboratory, respectively.

The main materials used were fresh white oyster mushrooms (*P. ostreatus*) obtained from Pasar Wage, Purwokerto, and fresh torch ginger flowers (*Etlingera elatior*) obtained from Pasar Manis, Purwokerto. Other ingredients included tapioca flour (Rose Brand, PT Budi Starch & Sweetener Tbk., Indonesia), wheat flour (Rose Brand, PT Budi Starch & Sweetener Tbk., Indonesia), isolated soy protein (commercial grade, Indonesia), carrageenan (commercial grade, Indonesia), sodium tripolyphosphate (commercial grade, Indonesia), xanthan gum (commercial grade, Indonesia), salt (Daun Meja®, PT Susanti Megah, Indonesia), garlic, and ice.

The microbiological media used were plate count agar (Merck KGaA, Darmstadt, Germany) and potato dextrose agar (Merck KGaA, Darmstadt, Germany). The main equipment included a vacuum sealer (Zeelea, China), freezer (Sharp Corporation, Osaka, Japan), refrigerator or chiller (Sharp Corporation, Osaka, Japan), incubator (Mettler GmbH + Co. KG, Schwabach, Germany), colony counter (IS-One, Indonesia), and UV-Vis spectrophotometer (Range Scanning Spectro 150).

Raw material handling

Fresh white oyster mushrooms were sorted to remove damaged, discolored, or deteriorated portions. The mushrooms were then washed under running potable water and drained for 5 minutes before processing (Castellanos-Reyes et al., 2021). Fresh torch ginger flowers were sorted,

washed under running potable water, and drained before being processed into powder.

All utensils, containers, cutting boards, and work surfaces were thoroughly cleaned with detergent and potable water before meatball preparation. The raw materials were processed within 1 hour after purchase. Before processing, the mushrooms and torch ginger flowers were stored at 4 ± 2 °C for approximately 5 hours (Včelik et al., 2026). Commercially available ice cubes were added during batter preparation to maintain a low mixing temperature.

Torch ginger flower powder preparation

Fresh torch ginger flowers were washed, sliced into pieces approximately 3 to 5 mm thick, and dried in a cabinet dryer at 60 °C for 12 hours. The dried flowers were ground using a blender and sieved through an 80-mesh sieve to obtain a uniform powder. The powder was stored in an airtight polyethylene container at room temperature until use (Santika and Batubara, 2024).

Experimental design

The experiment was arranged using a factorial randomized block design consisting of 2 factors. The first factor was the concentration of torch ginger flower powder, namely K0 = 0%, K1 = 0.5%, and K2 = 0.7% w/w. The second factor was the concentration of xanthan gum, namely P1 = 0.3% and P2 = 0.5% w/w. The combination of these factors produced 6 treatments: K0P1 (Control), K1P1, K2P1, K0P2, K1P2, and K2P2. Each treatment was prepared in 4 replications.

Vegan meatball preparation

The formulation of vegan oyster mushroom meatballs with different concentrations of torch ginger flower powder and xanthan gum is presented in Table 1. Fresh white oyster mushrooms were blended using a blender for approximately 2 to 3 minutes. The blended mushrooms were then manually mixed with tapioca flour, wheat flour, isolated soy protein, carrageenan, sodium tripolyphosphate, salt, garlic, ice, torch ginger flower powder, and xanthan gum according to the formulation assigned to each treatment until a homogeneous dough was obtained. The dough was manually shaped into meatballs weighing approximately 10 g each, with a diameter of approximately 4 cm. The meatballs were boiled in water at 100 °C

Table 1. Percentage composition of frozen vegan oyster mushroom meatball formulations (% w/w)

Ingredient	K0P1	K0P2	K1P1	K1P2	K2P1	K2P2
White oyster mushroom	69.54	69.40	69.20	69.06	69.06	68.92
Tapioca flour	8.69	8.68	8.65	8.63	8.63	8.62
Wheat flour	8.69	8.68	8.65	8.63	8.63	8.62
Soy isolate protein	8.69	8.68	8.65	8.63	8.63	8.62
Torch ginger flower powder	0.00	0.00	0.49	0.49	0.69	0.69
Xanthan gum	0.30	0.49	0.29	0.49	0.29	0.49
Salt	1.74	1.74	1.73	1.73	1.73	1.72
Garlic	0.87	0.87	0.86	0.86	0.86	0.86
Sodium tripolyphosphate	0.43	0.43	0.43	0.43	0.43	0.43
Carrageenan	0.17	0.17	0.17	0.17	0.17	0.17
Ice	0.87	0.87	0.86	0.86	0.86	0.86
Total (%)	100.00	100.00	100.00	100.00	100.00	100.00

until they floated on the water surface, indicating they were fully cooked. The cooked meatballs were then drained and cooled at room temperature for 15 to 25 minutes before being vacuum-packaged.

Vacuum packaging

Ten meatballs were placed in polyethylene (PE) vacuum pouches measuring 12 cm × 20 cm, with a thickness of 80 µm. The samples were vacuum-packaged using a Zeelea vacuum sealer (Gao et al., 2023). The vacuum packaging process was performed at a vacuum pressure of approximately -60 kPa using the standard automatic mode, with each vacuum and heat-sealing cycle requiring approximately 30 to 60 seconds to complete.

Storage conditions and observation schedule

The vacuum-packed samples were stored in a freezer (-18±2 °C), a chiller (4±1 °C), and at room temperature (27±2 °C) for 28 days. Observations were conducted at 7-day intervals on days 0, 7, 14, 21, and 28 (Hastuti et al., 2023). Freezer storage was considered the recommended condition because the products were developed as frozen, vacuum-packed vegan meatballs. Chiller storage refers to refrigerated storage following partial thawing or a temporary cold-chain deviation. Room-temperature storage was selected as an accelerated temperature-abuse condition that could occur during handling, distribution, temporary storage, or interruption of the cold chain (Van der Sman, 2020).

The use of 4 °C and 27 °C was therefore intended to accelerate measurable quality deterioration relative to frozen storage. These

accelerated storage conditions enabled the determination of deterioration kinetics within a practical experimental period while maintaining the relevance of the Arrhenius shelf-life prediction model. Nevertheless, these temperatures were not considered recommended storage conditions for the final frozen product (Aprialdi et al., 2024).

Quality observations

Product quality was evaluated periodically during storage to monitor microbiological stability and changes in bioactive compounds under different storage temperatures. The selected quality parameters included total plate count (TPC), mold and yeast count, and total phenolic content. These parameters were chosen because they represent the microbiological safety, fungal stability, and antioxidant-related quality of the frozen vegan oyster mushroom meatballs throughout storage.

Total plate count (TPC)

TPC analysis was performed by aseptically homogenizing 25 g of the sample with 225 ml of sterile physiological NaCl solution. Serial dilutions were prepared up to 10⁻⁵. Appropriate dilutions were plated on plate count agar using the pour-plate method and incubated at 37 °C for 48 hours. The colonies were counted using a colony counter, and the results were expressed as colony-forming units per gram and converted into log CFU g⁻¹ (Shofa et al., 2024).

Mold and yeast count

Mold and yeast analysis was conducted by homogenizing 25 g of the sample with 225 ml of sterile physiological NaCl solution. Serial dilutions were prepared up to 10⁻³. Appropriate

dilutions were plated on potato dextrose agar and incubated at 25 to 28 °C for 5 days. The colonies were counted using a colony counter, and the results were expressed as colony-forming units per gram and converted into log CFU g⁻¹ (Shofa et al., 2024).

Total phenolic content

Total phenolic content was determined to evaluate the stability of phenolic compounds during storage, as these compounds are responsible for the antioxidant and antimicrobial properties of torch ginger flower powder. Monitoring changes in total phenolic content also provides information on the retention of bioactive compounds under different storage temperatures. Total phenolic content was determined using the Folin–Ciocalteu method, with gallic acid as the standard. Absorbance was measured at 731 nm using a UV–Vis spectrophotometer, and the results were expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE g⁻¹) (Dotulong et al., 2023).

Shelf-life prediction using the Arrhenius model

Shelf life was predicted using the Arrhenius approach by fitting the observed quality changes to zero-order and first-order kinetic models (Elfiana et al., 2024). For the zero-order model, the quality parameter was plotted against storage time. In contrast, for the first-order model, the natural logarithm of the quality parameter was plotted against storage time.

The reaction order was selected by comparing the mean coefficients of determination (R^2) values of the zero-order and first-order models for each quality parameter and formulation across all storage temperatures. The model with the higher and more consistent R^2 value was selected as the most appropriate deterioration model.

The reaction rate constant (k) was obtained from the slope of the selected kinetic model. The natural logarithm of k ($\ln k$) was subsequently plotted against the reciprocal of absolute temperature ($1/T$) to establish the Arrhenius equation. The activation energy (E_a) was calculated using Equation 1.

$$E_a = -\text{slope} \times R \quad (1)$$

Where E_a is the activation energy, the slope is obtained from the relationship between $\ln k$ and $1/T$, and R is the universal gas constant of 1.986 cal mol⁻¹·K⁻¹.

The critical quality parameter was selected by considering the appropriateness of the kinetic model, its sensitivity to storage temperature, and its relevance to microbiological quality and product deterioration. To date, no specific Indonesian National Standard (SNI) has been established for vegan meatballs. Although SNI 3818:2014 specifies a microbiological limit for conventional meatballs, this criterion could not be directly applied in the present study because the initial TPC of all formulations exceeded the specified limit on day 0. Therefore, the terminal quality value for shelf-life prediction was defined as the experimentally observed TPC at the end of storage. This endpoint was selected to enable kinetic modeling and comparative evaluation of the relative storage stability among formulations under identical experimental conditions, rather than to establish a regulatory shelf-life limit.

Shelf life was defined as the time required for the TPC to reach the experimentally established terminal quality value. The terminal value was derived from the final microbial count observed under accelerated storage conditions and was used solely as the endpoint for Arrhenius modeling. Accordingly, the predicted shelf life should be interpreted as a comparative estimate of product stability under the experimental conditions, rather than as the commercial shelf life or regulatory expiration date of the product.

Because a specific microbiological standard for vegan oyster mushroom meatballs was not available, the microbiological limit for the closest comparable processed or ready-to-eat product category was used as a reference. Therefore, the predicted shelf life represents an estimate based on microbiological quality rather than an absolute regulatory expiration date.

Statistical analysis

The data were presented as mean±standard deviation. The effects of torch ginger flower powder concentration and xanthan gum concentration were evaluated using two-way analysis of variance (ANOVA) at a significance level of $\alpha = 0.05$. When a significant main effect or interaction was detected, Tukey's honestly significant difference test was performed as a post hoc multiple-comparison test at $p < 0.05$. Statistical analyses were conducted using IBM SPSS Statistics version 27 (IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

Changes in TPC during storage

TPC was analyzed to evaluate the microbiological deterioration of vegan oyster mushroom meatballs during storage at different temperatures, as presented in Table 2. TPC is a widely used indicator of overall microbial quality because it reflects changes in the total viable bacterial population during storage. Monitoring TPC provides important information on the effectiveness of torch ginger flower powder and xanthan gum in suppressing microbial growth under different storage conditions. In addition, TPC was selected as the critical quality parameter for shelf-life prediction using the Arrhenius model because microbial deterioration was considered the primary factor limiting product quality and storage stability.

The ANOVA results showed that torch ginger flower powder concentration, xanthan gum concentration, and storage time significantly affected TPC values under freezer, chiller, and room temperature storage conditions. The interaction between formulation and storage time was also significant in most storage conditions, indicating that TPC changes during storage were influenced by the combination of formulation treatment and storage duration.

TPC increased throughout storage in all formulations at room, chiller, and freezer temperatures. The initial TPC values were

relatively high, indicating that microbial populations were already present at the beginning of storage. However, this study focused on comparing deterioration behavior among formulations rather than evaluating commercial product quality. The relatively high initial microbial load may be associated with the high moisture content of oyster mushrooms and the absence of synthetic preservatives (do Carmo Silva et al., 2020). Consequently, TPC was considered an important parameter for monitoring microbiological deterioration during storage (Oulahal and Degraeve, 2022).

The increase in TPC was greatest at room temperature because higher temperatures provided favorable conditions for microbial metabolism, enzyme activity, and cell division, thereby accelerating microbial growth and quality deterioration (Aprialdi et al., 2024). In contrast, chiller and frozen storage reduced microbial proliferation by slowing metabolic activity, while frozen storage further restricted water mobility, making microbial growth less favorable (Roos, 2021). This finding agrees with Pramono et al. (2024), who reported that increasing storage temperature accelerated quality deterioration and shortened the shelf life of meatball products, as evaluated using the ASLT–Arrhenius approach.

Among the formulations, K0P1 showed the highest TPC values, while K2P2 generally showed the lowest during storage. This result indicates that the addition of torch ginger flower

Table 2. TPC of vegan meatballs during storage

Storage	Day	K0P1	K0P2	K1P1	K1P2	K2P1	K2P2
Room	0	6.97±0.01 ^a	6.88±0.01 ^a	6.78±0.01 ^a	6.69±0.02 ^a	6.59±0.02 ^a	6.47±0.03 ^a
	7	7.23±0.01 ^b	7.18±0.01 ^b	7.10±0.01 ^b	7.01±0.02 ^b	6.90±0.02 ^b	6.77±0.03 ^b
	14	7.35±0.01 ^c	7.33±0.01 ^c	7.27±0.01 ^c	7.19±0.01 ^c	7.08±0.02 ^c	6.96±0.02 ^c
	21	7.39±0.01 ^d	7.37±0.01 ^d	7.35±0.01 ^d	7.30±0.01 ^d	7.20±0.01 ^d	7.09±0.02 ^d
	28	7.40±0.00 ^d	7.39±0.01 ^c	7.38±0.01 ^c	7.35±0.01 ^c	7.28±0.01 ^c	7.20±0.01 ^c
Chiller	0	6.97±0.01 ^a	6.88±0.01 ^a	6.78±0.01 ^a	6.69±0.02 ^a	6.59±0.02 ^a	6.47±0.03 ^a
	7	7.11±0.01 ^b	7.05±0.01 ^b	6.93±0.02 ^b	6.82±0.02 ^b	6.71±0.03 ^b	6.58±0.03 ^b
	14	7.25±0.01 ^c	7.17±0.01 ^c	7.07±0.01 ^c	6.98±0.02 ^c	6.86±0.02 ^c	6.74±0.02 ^c
	21	7.36±0.01 ^d	7.30±0.01 ^d	7.21±0.01 ^d	7.11±0.02 ^d	7.00±0.02 ^d	6.89±0.02 ^d
	28	7.39±0.00 ^c	7.37±0.01 ^c	7.31±0.01 ^c	7.23±0.01 ^c	7.13±0.01 ^c	7.02±0.02 ^c
Freezer	0	6.97±0.01 ^a	6.88±0.01 ^a	6.78±0.01 ^a	6.69±0.02 ^a	6.59±0.02 ^a	6.47±0.03 ^a
	7	7.04±0.01 ^b	6.95±0.01 ^b	6.84±0.02 ^b	6.73±0.02 ^b	6.63±0.02 ^b	6.54±0.03 ^b
	14	7.10±0.01 ^c	7.02±0.01 ^c	6.91±0.02 ^c	6.80±0.02 ^c	6.70±0.02 ^c	6.60±0.02 ^c
	21	7.20±0.01 ^d	7.12±0.01 ^d	7.00±0.02 ^d	6.90±0.02 ^d	6.79±0.02 ^d	6.69±0.03 ^d
	28	7.28±0.01 ^c	7.20±0.01 ^c	7.09±0.01 ^c	6.99±0.02 ^c	6.89±0.02 ^c	6.79±0.02 ^c

Note: Room = 27 °C; Chiller = 4 °C; Freezer = -18 °C. Values are expressed as mean±standard deviation (log CFU g⁻¹). Means followed by different superscript letters within the same row differ significantly according to Tukey's HSD post hoc test ($p < 0.05$)

powder and xanthan gum tended to improve the microbiological stability of vegan oyster mushroom meatballs. The lower TPC observed in K2P2 could be attributed to the higher concentration of torch ginger flower powder, which contains phenolic compounds, flavonoids, terpenoids, and saponins with antimicrobial activity. These bioactive compounds may disrupt microbial cell membranes, increase membrane permeability, interfere with enzyme activity, and inhibit microbial metabolism, thereby reducing microbial proliferation during storage (Oulahal and Degraeve, 2022).

In addition, xanthan gum contributed to the stability of the food matrix by improving water-binding capacity and limiting moisture migration, which may indirectly reduce conditions favorable for microbial growth. The combined effect of these ingredients likely resulted in the superior microbiological stability observed in K2P2 (Asase and Glukhareva, 2024). Similar antimicrobial mechanisms of plant-derived phenolic compounds have also been reported by Yu et al. (2021), who demonstrated that phenolic compounds inhibit bacterial growth by damaging cytoplasmic membranes and causing leakage of intracellular components.

Changes in mold and yeast count during storage

Mold and yeast counts were analyzed to evaluate fungal growth during storage,

as presented in Table 3. This parameter complements the TPC by providing information on fungal contamination, which may affect product quality and shelf life. Changes in mold and yeast counts were used to assess the effectiveness of torch ginger flower powder and xanthan gum in maintaining microbiological stability during storage.

The ANOVA results showed that torch ginger flower powder concentration and storage time significantly affected mold and yeast count under freezer, chiller, and room-temperature storage conditions ($p < 0.05$). Xanthan gum concentration significantly affected mold and yeast count only under room-temperature storage, but not under freezer and chiller conditions. The interaction between torch ginger flower powder and xanthan gum was significant under freezer storage. In contrast, the interaction between torch ginger flower powder and storage time was significant under room temperature storage.

Mold and yeast count increased during storage in all formulations, with the highest increase generally observed at room temperature. Freezer storage showed the lowest fungal growth, indicating that low temperature was effective in slowing microbial activity. Similar use of mold and yeast growth as a shelf-life-related parameter was reported by Safira and Yulistiani (2025), who applied the ASLT-Arrhenius model to predict shelf life based on mold and yeast growth and E_a .

Table 3. Mold and yeast on vegan meatballs during storage

Storage	Day	K0P1	K0P2	K1P1	K1P2	K2P1	K2P2
Room	0	6.23±0.20 ^a	6.19±0.10 ^a	6.02±0.00 ^a	6.00±0.00 ^a	6.00±0.00 ^a	6.00±0.00 ^a
	7	6.67±0.10 ^b	6.72±0.10 ^b	6.43±0.10 ^b	6.51±0.00 ^b	6.28±0.20 ^b	6.33±0.10 ^b
	14	6.87±0.10 ^c	6.93±0.10 ^c	6.63±0.00 ^c	6.72±0.00 ^c	6.47±0.10 ^{bc}	6.51±0.00 ^c
	21	7.04±0.10 ^d	7.11±0.10 ^d	6.79±0.00 ^d	6.89±0.00 ^d	6.64±0.20 ^{cd}	6.68±0.00 ^d
	28	7.15±0.00 ^d	7.16±0.00 ^d	6.94±0.00 ^c	7.02±0.00 ^c	6.78±0.20 ^d	6.82±0.00 ^c
Chiller	0	6.23±0.20 ^a	6.19±0.10 ^a	6.02±0.00 ^a	6.00±0.00 ^a	6.00±0.00 ^a	6.00±0.00 ^a
	7	6.36±0.10 ^{ab}	6.35±0.10 ^b	6.18±0.10 ^b	6.17±0.10 ^{ab}	6.03±0.00 ^a	6.01±0.00 ^a
	14	6.49±0.10 ^{bc}	6.50±0.10 ^{bc}	6.36±0.10 ^c	6.36±0.10 ^{bc}	6.18±0.10 ^{ab}	6.19±0.10 ^b
	21	6.61±0.10 ^{cd}	6.63±0.10 ^{cd}	6.52±0.10 ^d	6.50±0.20 ^{cd}	6.32±0.10 ^{bc}	6.36±0.10 ^c
	28	6.72±0.01 ^d	6.75±0.01 ^d	6.65±0.10 ^c	6.60±0.20 ^d	6.44±0.20 ^c	6.50±0.00 ^d
Freezer	0	6.23±0.20 ^a	6.19±0.10 ^a	6.02±0.00 ^a	6.00±0.00 ^a	6.00±0.00 ^a	6.00±0.00 ^a
	7	6.28±0.20 ^{ab}	6.28±0.10 ^{ab}	6.10±0.10 ^{ab}	6.05±0.00 ^{ab}	6.00±0.00 ^a	6.02±0.00 ^a
	14	6.35±0.10 ^{ab}	6.37±0.10 ^{bc}	6.16±0.10 ^{bc}	6.09±0.10 ^b	6.00±0.00 ^a	6.07±0.00 ^b
	21	6.37±0.10 ^{ab}	6.41±0.10 ^{bc}	6.19±0.10 ^{bc}	6.15±0.00 ^c	6.01±0.00 ^a	6.12±0.00 ^c
	28	6.44±0.00 ^b	6.45±0.10 ^c	6.25±0.10 ^c	6.18±0.00 ^c	6.07±0.00 ^b	6.18±0.00 ^d

Note: Room = 27 °C; Chiller = 4 °C; Freezer = -18 °C. Values are expressed as mean±standard deviation (log CFU g⁻¹). Means followed by different superscript letters within the same row differ significantly according to Tukey's HSD post hoc test ($p < 0.05$)

Formulations with higher torch ginger flower powder and xanthan gum tended to show lower mold and yeast counts than the control. Overall, K2P2 showed better microbiological stability, supporting the TPC results.

Changes in total phenolic content during storage

Total phenolic content was analyzed to evaluate the stability of bioactive compounds during storage, as presented in Table 4. Total phenolics are important bioactive constituents of torch ginger flower powder that contribute to its antioxidant and antimicrobial properties. Changes in total phenolic content during storage provide an indication of the retention of these compounds and their stability under different storage temperatures.

The ANOVA results showed that torch ginger flower powder concentration, xanthan gum concentration, and storage time significantly affected total phenolic content under freezer, chiller, and room temperature storage conditions ($p < 0.001$). The interaction between torch ginger flower powder and xanthan gum was also significant under all storage temperatures, whereas the three-way interaction was significant only under room temperature storage. Total phenolic content generally decreased during storage, indicating that phenolic compounds were gradually degraded or transformed over time. Differences in initial phenolic values among

storage treatments may be influenced by sample variability and handling conditions during sequential analysis (Wen et al., 2022). Therefore, the interpretation of total phenolic content focused on the overall decreasing trend and phenolic retention during storage rather than direct comparison of initial values among storage treatments.

This finding is in line with Saarniit et al. (2023), who reported that phenolic compounds may change or decrease during storage depending on product matrix, storage conditions, and exposure to degradation factors. Formulations with higher torch ginger flower powder, especially K2P2, showed higher total phenolic content than the control, indicating its contribution as a source of bioactive compounds. Although total phenolic content decreased during storage, formulations containing torch ginger flower powder maintained higher phenolic retention, suggesting better chemical stability.

Kinetic model and Arrhenius analysis

Kinetic analysis was performed to determine the reaction model that best described changes in TPC during storage. The R^2 of the zero-order (0) and first-order (1) kinetic models were compared for each formulation and storage condition. The model with the higher R^2 value was selected to determine the k , which was subsequently used for Arrhenius analysis and shelf-life prediction (García et al., 2022).

Table 4. Total phenolic content of vegan meatballs during storage

Storage	Day	K0P1	K0P2	K1P1	K1P2	K2P1	K2P2
Room	0	1.72±0.25 ^a	2.46±0.06 ^a	2.53±0.10 ^a	2.66±0.11 ^a	3.04±0.24 ^a	3.43±0.19 ^a
	7	1.49±0.00 ^b	2.31±0.10 ^b	2.49±0.13 ^b	2.61±0.13 ^a	2.79±0.17 ^a	3.27±0.21 ^a
	14	0.85±0.04 ^c	1.26±0.07 ^c	1.32±0.03 ^c	1.47±0.03 ^b	1.82±0.06 ^b	1.90±0.04 ^b
	21	0.57±0.16 ^d	0.71±0.09 ^d	0.80±0.10 ^d	0.95±0.10 ^c	1.32±0.13 ^c	1.41±0.10 ^c
	28	0.39±0.09 ^d	0.55±0.04 ^c	0.57±0.03 ^d	0.74±0.05 ^d	0.96±0.32 ^d	1.03±0.35 ^d
Chiller	0	0.49±0.19 ^a	0.59±0.01 ^a	0.60±0.01 ^a	0.62±0.11 ^a	0.69±0.01 ^a	0.73±0.06 ^a
	7	0.37±0.04 ^{ab}	0.44±0.07 ^b	0.46±0.06 ^b	0.43±0.03 ^b	0.52±0.04 ^b	0.61±0.03 ^b
	14	0.32±0.03 ^b	0.38±0.00 ^b	0.39±0.00 ^c	0.40±0.03 ^b	0.47±0.00 ^c	0.55±0.01 ^c
	21	0.16±0.04 ^c	0.27±0.03 ^c	0.29±0.04 ^d	0.38±0.04 ^b	0.44±0.02 ^c	0.51±0.00 ^c
	28	0.13±0.01 ^d	0.21±0.05 ^d	0.22±0.04 ^c	0.25±0.03 ^c	0.26±0.02 ^d	0.45±0.02 ^d
Freezer	0	0.46±0.06 ^a	0.61±0.03 ^a	0.62±0.02 ^a	0.65±0.02 ^a	0.67±0.02 ^a	0.93±0.06 ^a
	7	0.41±0.10 ^a	0.50±0.10 ^b	0.44±0.14 ^{ab}	0.55±0.08 ^b	0.59±0.07 ^b	0.83±0.01 ^b
	14	0.26±0.10 ^b	0.27±0.09 ^c	0.40±0.02 ^b	0.42±0.02 ^c	0.53±0.04 ^b	0.75±0.06 ^b
	21	0.14±0.01 ^c	0.20±0.02 ^{cd}	0.36±0.16 ^b	0.33±0.03 ^d	0.43±0.02 ^c	0.49±0.08 ^c
	28	0.13±0.01 ^c	0.17±0.03 ^d	0.31±0.20 ^b	0.25±0.07 ^c	0.35±0.02 ^d	0.36±0.04 ^d

Note: Room = 27 °C; Chiller = 4 °C; Freezer = -18 °C. Values are expressed as mean±standard deviation in mg GAE g⁻¹. Means followed by different superscript letters within the same row differ significantly according to Tukey's HSD post hoc test ($p < 0.05$)

As shown in Table 5, the zero-order model generally exhibited higher R^2 values than the first-order model across most formulations and storage conditions. Therefore, the zero-order model was selected for subsequent analyses. These results indicate that the increase in TPC occurred at a relatively constant rate during storage. Although slightly higher R^2 values for the first-order model were observed under some storage conditions, particularly during frozen storage, the differences were minimal and did not change the overall conclusion that the zero-order model provided the best overall fit (Chen et al., 2026).

Selecting an appropriate kinetic model is essential because it determines the k used in the Arrhenius equation for shelf-life prediction. The zero-order model assumes a constant deterioration rate over time, making it suitable for describing the increase in TPC observed in vegan oyster mushroom meatballs during storage. Consequently, the zero-order model provides a reliable basis for predicting microbiological quality changes and estimating product shelf life (Du et al., 2022).

The effect of storage temperature on the rate of deterioration in microbiological quality of vegan oyster mushroom meatballs was evaluated using the Arrhenius model. The k values obtained from the selected zero-order kinetic

model were transformed into $\ln k$ and plotted against the reciprocal $1/T$ to establish the Arrhenius equation. This relationship was subsequently used to determine E_a and to describe the temperature dependence of TPC deterioration in vegan oyster mushroom meatballs. The calculated k , Arrhenius equations, R^2 , and E_a values are presented in Table 6, while the corresponding Arrhenius plots are shown in Figure 1. The Arrhenius model is widely applied in food shelf-life studies because it quantitatively describes the influence of temperature on deterioration kinetics and provides a reliable basis for shelf-life prediction.

In general, lower storage temperatures resulted in lower k values, indicating slower microbial deterioration. The R^2 values (0.5749 to 0.9545) show that the Arrhenius model adequately described the temperature dependence of TPC deterioration. The relatively low R^2 value observed for K0P1 suggests that temperature alone did not fully explain the deterioration kinetics of this formulation.

Formulation K2P2 exhibited the highest E_a (2,785.17 cal mol⁻¹), indicating that a greater amount of energy was required to accelerate microbial deterioration, which suggests better stability during storage (Alessandro Del Nobile and Conte, 2023). E_a reflects the sensitivity of deterioration kinetics to temperature changes.

Table 5. Comparison of R^2 values of zero-order and first-order kinetic models for TPC

Formulation	Storage	Time (days)	R^2 Order 0	R^2 Order 1	Fit order
K0P1	Room	0-28	0.7924	0.7877	0
	Chiller	0-28	0.9617	0.9594	0
	Freezer	0-28	0.9966	0.9972	1
K0P2	Room	0-28	0.8073	0.8019	0
	Chiller	0-28	0.9802	0.9777	0
	Freezer	0-28	0.9963	0.9969	1
K1P1	Room	0-28	0.8583	0.8514	0
	Chiller	0-28	0.9961	0.9948	0
	Freezer	0-28	0.9911	0.9922	1
K1P2	Room	0-28	0.9022	0.8948	0
	Chiller	0-28	0.9979	0.9970	0
	Freezer	0-28	0.9808	0.9820	1
K2P1	Room	0-28	0.9326	0.9256	0
	Chiller	0-28	0.9990	0.9990	0
	Freezer	0-28	0.9797	0.9813	1
K2P2	Room	0-28	0.9538	0.9472	0
	Chiller	0-28	0.9978	0.9977	0
	Freezer	0-28	0.9889	0.9902	1

Note: Room = 27 °C; Chiller = 4 °C; Freezer = -18 °C; 0 = Zero-order; 1 = First-order

Table 6. Reaction kinetics and Arrhenius parameters for TPC deterioration

Formulation	k room	ln k room	k chiller	ln k chiller	k freezer	ln k freezer	Arrhenius equation	R ²	E _a (cal mol ⁻¹)
KOP1	0.0145	-4.2336	0.0157	-4.1541	0.0111	-4.5008	Y = -467.95x - 2.6014	0.5749	929.35
KOP2	0.0174	-4.0513	0.0176	-4.0399	0.0117	-4.4482	Y = -687.93x - 1.6883	0.7581	1,366.23
K1P1	0.0205	-3.8873	0.0192	-3.9528	0.0112	-4.4918	Y = -1,042.3x - 0.3359	0.8550	2,070.01
K1P2	0.0231	-3.7679	0.0196	-3.9322	0.0112	-4.4918	Y = -1,242.4x + 0.4357	0.9281	2,467.41
K2P1	0.0241	-3.7255	0.0197	-3.9271	0.0109	-4.5190	Y = -1,360.3x + 0.8694	0.9423	2,701.56
K2P2	0.0254	-3.6730	0.0202	-3.9021	0.0112	-4.4918	Y = -1,402.4x + 1.0569	0.9545	2,785.17

Note: k = reaction rate constant; ln k = natural logarithm of k; E_a = activation energy

Formulations with higher E_a require greater thermal energy for deterioration to accelerate, indicating that the deterioration process is more temperature-dependent. Therefore, the higher E_a of K2P2 suggests that temperature had a stronger influence on its deterioration kinetics than on formulations with lower E_a .

Figure 1 presents the Arrhenius plots generated from the relationship between $\ln k$ and the reciprocal of $1/T$. The linear relationships observed for all formulations confirm that the Arrhenius model appropriately described the effect of storage temperature on microbial deterioration. Similar linear relationships have been widely reported in food deterioration kinetics, indicating that deterioration rate constants generally increase exponentially with increasing storage temperature according to the Arrhenius equation (Hidayati et al., 2021).

The steeper slope observed in formulation K2P2 corresponded to its higher E_a , suggesting improved stability against microbial deterioration during storage. This improvement is likely associated with the combined antimicrobial activity of torch ginger flower powder, which contains phenolic compounds capable of inhibiting microbial growth. Furthermore, the stabilizing function of xanthan gum improves water retention and matrix integrity, thereby limiting conditions favorable for microbial proliferation (Olvera-Aguirre et al., 2023).

Overall, the kinetic and Arrhenius analyses demonstrated that storage temperature significantly influenced the rate of microbiological deterioration of vegan oyster mushroom meatballs. The Arrhenius model adequately described the temperature dependence of microbial deterioration and provided the kinetic parameters required for subsequent shelf-life prediction, consistent with previous studies applying the ASLT–Arrhenius approach to food products (Aprialdi et al., 2024).

Shelf-life prediction and best formulation

Shelf-life prediction was performed using TPC as the critical quality parameter because it directly reflects microbiological deterioration (Hidayati et al., 2021). The prediction was based on the selected zero-order kinetic model combined with the Arrhenius equation to estimate the time required for TPC to reach the terminal quality value (Aprialdi et al., 2024). The predicted shelf

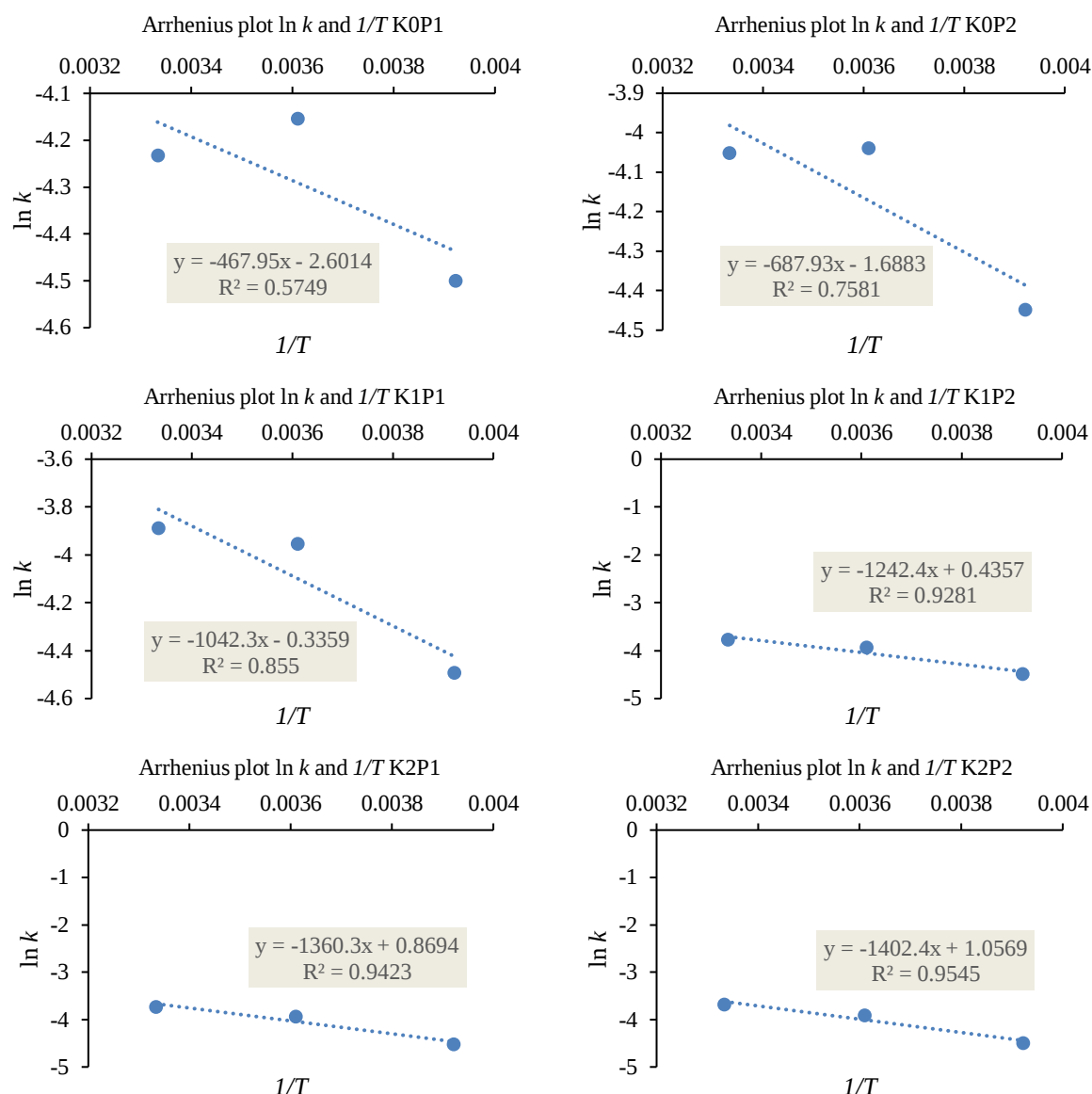


Figure 1. Arrhenius plots showing the relationship between $\ln k$ and reciprocal absolute temperature ($1/T$) for TPC deterioration of vegan oyster mushroom meatballs under different formulations

life, k , and $\ln k$ values for all formulations are presented in Table 7.

As shown in Table 7, the predicted shelf life varied among formulations and storage temperatures. Freezer storage resulted in the longest shelf life, whereas room temperature storage produced the shortest shelf life because higher temperatures accelerated microbial growth. This observation is consistent with the Arrhenius principle, in which increasing temperature accelerates deterioration reactions and increases microbial growth rates, thereby shortening product shelf life (Hidayati et al., 2021).

In general, formulations containing higher concentrations of torch ginger flower powder and xanthan gum exhibited longer shelf lives than the control, indicating improved microbiological stability. Among the formulations, K2P2 showed the best storage stability, with predicted shelf lives of 27.0, 39.8, and 61.7 days at room, chiller, and frozen storage, respectively. The Arrhenius model for K2P2 yielded the equation $\ln k = -1402.4(1/T) + 1.0569$ with an R^2 value of 0.9545, indicating a good fit between the k and storage temperature. The decrease in k with decreasing storage temperature indicates that microbial deterioration proceeded more slowly under refrigerated and

Table 7. Predicted shelf life of vegan oyster mushroom meatballs based on the terminal TPC value

Formulation	Storage	$\ln k$	k	Shelf-life (days)
K0P1	Room	-4.1612	0.0156	27.7
	Chiller	-4.2908	0.0137	31.6
	Freezer	-4.4365	0.0118	36.5
K0P2	Room	-3.9814	0.0187	27.1
	Chiller	-4.1718	0.0154	33.1
	Freezer	-4.3861	0.0124	41.0
K1P1	Room	-3.8102	0.0221	26.9
	Chiller	-4.0987	0.0166	35.9
	Freezer	-4.4234	0.0120	49.7
K1P2	Room	-3.7056	0.0246	27.1
	Chiller	-4.0495	0.0174	38.2
	Freezer	-4.4365	0.0118	56.3
K2P1	Room	-3.6649	0.0256	27.0
	Chiller	-4.0414	0.0176	39.3
	Freezer	-4.4651	0.0115	60.1
K2P2	Room	-3.6178	0.0268	27.0
	Chiller	-4.0059	0.0182	39.8
	Freezer	-4.4427	0.0118	61.7

Note: k = reaction rate constant; $\ln k$ = natural logarithm of k ; Room = 27 °C; Chiller = 4 °C; Freezer = -18 °C

frozen conditions. Similar temperature-dependent deterioration kinetics have been reported in previous ASLT–Arrhenius studies on food products (Pertiwi and Ginting, 2017).

Although frozen storage produced the longest predicted shelf life, the estimated shelf life remained relatively short compared with that of many commercial frozen food products. This may be associated with the relatively high initial microbial load of the samples, the absence of synthetic preservatives, and the use of the experimentally observed terminal TPC value as the microbiological endpoint for kinetic modeling (do Carmo Silva et al., 2020). Therefore, the predicted shelf-life values should be interpreted as comparative estimates among formulations under the experimental conditions rather than as commercial shelf-life claims. The superior shelf life of K2P2 is attributed to the combined antimicrobial activity of torch ginger flower powder and the matrix-stabilizing effect of xanthan gum, which together slowed microbiological deterioration during storage (Fetriyuna et al., 2025).

Study limitations

This study has several limitations. A primary limitation is the relatively high initial microbial load of the samples, which may have influenced the predicted shelf life. Although all samples were

prepared using the same processing procedures and hygienic conditions, improvements in raw material sanitation and processing hygiene could further reduce the initial microbial count and provide a more accurate estimation of shelf life. Future studies should optimize sanitation procedures and evaluate their effects on microbial quality and shelf-life prediction.

Furthermore, shelf-life prediction was based solely on microbiological deterioration without sensory evaluation. In addition, the relatively high initial microbial load and the use of microbiological criteria as the shelf-life endpoint may have influenced the predicted shelf-life values. Future studies should include sensory evaluation and consumer acceptability to complement microbiological shelf-life prediction.

Another limitation of this study is that the terminal quality value was determined from the experimental endpoint because the initial TPC exceeded the microbiological limit specified for conventional meatballs. Consequently, the predicted shelf life should be interpreted as a relative comparison among formulations rather than as an absolute commercial shelf-life estimate. Future studies should reduce the initial microbial load and adopt an externally established microbiological criterion for shelf-life prediction.

CONCLUSIONS

The deterioration of TPC was best described by the zero-order kinetic model and was therefore selected for Arrhenius-based shelf-life prediction. The addition of torch ginger flower powder and xanthan gum improved the physicochemical characteristics, microbiological quality, and predicted shelf life of frozen vegan oyster mushroom meatballs. Among the formulations, K2P2 (0.7% torch ginger flower powder and 0.5% xanthan gum) showed the best overall performance because it provided the greatest microbiological stability. This resulted in the longest predicted shelf lives of 27.0, 39.8, and 61.7 days at room, chiller, and frozen storage, respectively. TPC was identified as the critical parameter for shelf-life prediction using the ASLT–Arrhenius model. These findings demonstrate the potential of combining torch ginger flower powder and xanthan gum as a natural preservation strategy for vegan meatballs. Future studies should include organoleptic evaluation to complement microbiological shelf-life assessment.

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DECLARATION OF GENERATIVE AI USE

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist with language editing, improvement of academic writing, and refinement of manuscript structure. The AI tool was also used to support the organization of ideas and drafting of text. All AI-assisted outputs were critically reviewed, revised, and verified by the authors. The authors take full responsibility for the accuracy, originality, and integrity of the final manuscript.

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