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Research Article

Physicochemical properties, antioxidant activity, and shelf-life assessment of spicy candied mulberry chew developed from pomace

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Abstract

Mulberry pomace shows promise as a functional ingredient for dietary applications. This study aimed to assess the physicochemical characteristics, antioxidant activity, sensory acceptability, and shelf-life of the formulated spicy candied mulberry chew. Shelf life was determined using accelerated shelf-life testing (ASLT) at 25°C, 35°C, and 45°C to predict shelf life of the product when stored at 37°C. The developed spicy candied chew (75% mulberry pomace and 25% fresh mulberries) exhibited a_w of 0.5505 ± 0.01 , TSS of 67.57 ± 0.69 °Brix, pH of 3.84 ± 0.06 , and TTA of $0.83 \pm 0.02\%$. It also contained a total phenolic content of 2.37 ± 0.07 mg GAE.g⁻¹ and antioxidant activities of 3.81 ± 0.18 and 2.05 ± 0.25 mg Trolox eq.g⁻¹, as measured by ABTS and FRAP, respectively. Microbial analysis revealed that *Salmonella* spp. were not detected in 25 g samples, *Staphylococcus aureus* was <10 CFU.g⁻¹, and *Escherichia coli* was <3 MPN.g⁻¹. Sensory evaluation indicates that positive consumer acceptance ranged from 7.0–7.8 (n = 100). Shelf-life estimation of the product stored at 37°C, based on total plate count using the ASLT method with kinetic reaction models (Q_{10} and Q_1 values), was approximately 24 days. The findings indicate that the product provides added value as a functional food and promotes sustainable utilization of fruit-processing by-products.

Keywords

accelerated shelf-life testing, fruit by-product, functional food, microbial quality, sensory evaluation

Abbreviations

ABTS – 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid); a_w – water activity; FRAP – ferric ion reducing antioxidant power; MP – mulberry pomace; TSS – total soluble solids; TTA – total titratable acidity

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Introduction

Mulberries (*Morus* spp.), members of the *Moraceae* family, have been cultivated for centuries across many regions globally, including Asia, Europe, Africa, and the Americas (Krishna et al. 2020). The three main species, black (*M. nigra* L.), white (*M. alba* L.), and red (*M. rubra* L.), contain high concentrations of bioactive compounds. These compounds include anthocyanins, phenolics, flavonoids, polysaccharides, and other functional components (Kattil et al. 2024). Among these, phenolic compounds extracted from mulberries have been linked to several health benefits, including anti-inflammatory, anti-diabetic, cholesterol-lowering, anti-proliferative, and antioxidant properties (Turan et al. 2017). The high anthocyanin content contributes to specific health-promoting properties, such as antioxidant, anti-inflammatory, and anti-cancer activities, which may help in the treatment of chronic illnesses (Rohela et al. 2020). Additionally, mulberries are an excellent source of dietary fiber, which is important for regulating blood sugar levels by promoting a more balanced and effective insulin response (Boraste and Raut 2025). While mulberries are usually consumed fresh, their rich phytochemical composition and health-promoting qualities have led to their frequent processing into popular products, including juices, jams, syrups, jellies, and wines (Jan et al. 2021).

Mulberry pomace (MP), the primary by-product of mulberry juice extraction, can account for up to 40% of the fruit's dry weight (Garg et al. 2019). MP is rich in dietary fiber and phenolic compounds that have significant antioxidant activity. However, it is often discarded or used as compost or animal feed. MP is primarily composed of anthocyanins, including cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside. In addition, MP is a good source of pectin and contains polysaccharides primarily constituted of arabinose, galactose, and glucose. The polysaccharides have anti-inflammatory, antioxidant, and prebiotic properties (Ai et al. 2021). These bioactive properties possess a strong potential as a functional food ingredient that can enhance both nutritional value and health benefits in food products. Mulberry polysaccharides can improve gastrointestinal health by controlling intestinal microorganisms by increasing the amount of Bacteroidetes and decreasing that of Firmicutes

(Chen et al. 2016). Recent research has increasingly focused on incorporating fruit pomace into a variety of food products in order to optimize its potential as a functional food ingredient. Numerous studies have demonstrated that dietary fiber and phenolic compounds found in MP can enhance the nutritional quality of food such as bread, cookies, pasta, and chocolate (Kim et al. 2013; Jeon et al. 2013; Ta et al. 2023). However, its application in chewable products has not been addressed in the literature.

Functional foods have grown in popularity as consumer awareness of their health benefits continues to grow. Confectionery products are very popular, particularly chewy candies (Jukniene et al. 2025). Chewable products are of interest because they offer the convenience of portability, provide an effective dosage of bioactive substances, and are easy to enjoy while having a long shelf life. Spicy candied mulberry chew, known as Mhon Yee, is a traditional Thai product made from ripe mulberries. This product can be developed as a value-added functional food due to its antioxidant content, nutritional value, and traditional Thai preservation methods. Therefore, MP, rich in polyphenols, anthocyanins, and antioxidant activity, represents a promising ingredient for functional food development. Processing MP into a spicy candied mulberry chew offers a sustainable approach to enhance the value of this by-product. This strategy not only reduces food processing waste but also adds economic and nutritional values. With increasing consumer demand for safe and high-quality food products, it is essential to assess product quality and estimate shelf-life following product development. Shelf-life studies under normal storage conditions can be time-consuming. Therefore, accelerated shelf-life tests (ASLT) is typically utilized to address this problem. ASLT employs a variety of kinetic models to evaluate how temperature variations affect product quality (Jedermann et al. 2009).

In our previous studies, incorporating 25% fresh mulberries into 75% mulberry pomace was found to optimize the texture and minimize the graininess of the pomace paste. Therefore, this ratio was selected for the production of spicy candied chew. Thus, this study aimed to develop and characterize a spicy chew based on mulberry pomace and evaluate its physicochemical characteristics, antioxidant activity, sensory attributes, microbial qualities, and

shelf life under tropical conditions using ASLT. The product was stored and tested for about 10 weeks at various temperatures (25°C, 35°C, and 45°C), and the data obtained from ASLT was analyzed using kinetic reaction models, including Q_{10} and Q_1 values.

Materials and Methods

Materials. The mulberry pomace from the Chiang Mai 60 variety, obtained as a by-product of juice extraction, was supplied by Patsiri Co., Ltd., located in Ban Mai Santi, Makluea Mai Subdistrict, Sung Noen District, Nakhon Ratchasima Province, Thailand. All other ingredients, including brown sugar (Mitr Phol brand), glucose syrup (Fancy Carp brand), salt (Prungthip brand), and roasted dried chilli powder (Makro brand), were obtained from local suppliers in Nakhon Ratchasima, Thailand. The food-grade citric acid was provided from Hannong Chemicals Inc., Korea.

Chemical properties. Fresh mulberries and mulberry pomace were analyzed for moisture, protein, and ash content using the [AOAC standard method \(2000\)](#).

The pH, total titratable acidity (TTA), and total soluble solid (TSS) determination. Five grams of the sample were combined with 45 mL of deionized water, homogenized (IKA T25 Digital Ultra Turrax Staufen, Germany), and centrifuged at 3,500 rpm for 5 min (HERMLE Labortechnik GmbH centrifuge Wehingen, Germany). The supernatant was subsequently collected for the determination of pH, TTA, and TSS. The pH was measured using a digital pH meter (ATX224, Shimadzu, Tokyo, Japan). The TSS was measured using a hand refractometer (Master-500, Atago, Tokyo, Japan). The TTA was quantified by titrating with 0.01 N NaOH ([AOAC, 2000](#)). TTA was calculated as % citric acid equivalent, using the following equation:

$$\text{TTA (\% citric acid)} = \left[\frac{V_{\text{NaOH}} \times N_{\text{NaOH}} \times 0.064}{\text{sample weight (g)}} \right] \times 100 \quad (1)$$

Where: V_{NaOH} is a volume of NaOH solution, mL; N_{NaOH} is Normality of the standardized NaOH solution; 0.064 is milliequivalent weight of citric acid

Preparation of spicy candied chew from mulberry pomace. In previous work, fresh

mulberries were incorporated into the pomace in different proportions (5-25%) to enhance the texture and reduce the grainy feel of the mulberry pomace paste. The result indicated that incorporating 25% fresh mulberries into 75% mulberry pomace was optimal for producing mulberry pomace paste. Therefore, the spicy candied chew was formulated using this ratio. The components of the spicy candied chew are presented in Table 1. Initially, fresh mulberries were blended until a fine consistency was achieved. Mulberry pomace and water were then added to the blender and processed until the mixture was completely smooth. This mixture was transferred to a heated brass pan and stirred until it became sticky and almost dry. Brown sugar, citric acid, and salt were incorporated, and the mixture was stirred continuously until thickened. Roasted dried chilli powder was subsequently added, and the mixture was stirred until it reached a dry and sticky consistency. The spicy candied chew was then shaped into round balls weighing 3-5 g, placed on a tray and dried in an oven at 55°C for 4 h. After cooling, the dried spicy candied chews were coated with brown sugar and packaged in polyethylene (PE) plastic bags, as shown in Fig.1.

Table 1. Ingredients of the spicy candied mulberry chew made from mulberry pomace and fresh mulberries with a ratio of 75%:25% (w/w)

Ingredients	% by weight	Contents, g
Mulberry pomace	29.7	1,500
Fresh mulberries	9.9	500
Water	19.8	1,000
Brown sugar	19.8	1,000
Glucose syrup	19.8	1,000
Salt	0.5	25
Citric acid	0.4	20
Roasted dried chilli powder	0.2	9
Total	100	5,054

Physicochemical properties of spicy candied chew. The a_w of the sample was evaluated using a water activity meter (AQUA LAB 4TE dew point, Pullman, WA, USA). The moisture content, pH,

TTA, and TSS were determined using the procedures previously outlined (AOAC 2000).

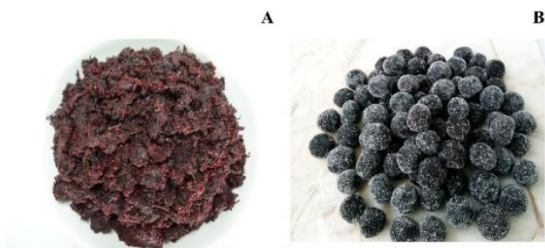


Figure 1. Mulberry pomace (A) and spicy candied chews made from mulberry pomace (B)

Total phenolic content and antioxidant activity determination of spicy candied chew. A 5 g portion of spicy candied chew was mixed with 25 mL of deionized water. The mixture was homogenized and centrifuged at 8,000×g for 10 min. The resulting supernatant was collected for the determination of total phenolic content and antioxidant activity following the method outlined by Khongla et al. (2024).

Total phenolic content. The folin-ciocalteu method was used to evaluate the total phenolic content (TPC), following the procedure outlined by Musika et al. (2022). Briefly, 100 µL of the sample was mixed with 2 mL of 2% Na₂CO₃. Subsequently, 100 µL of folin-ciocalteu reagent (diluted 1:1 v/v in methanol) was incorporated into the mixture. This mixture was incubated at room temperature for 30 min, after which the absorbance was measured at 750 nm. TPC was expressed as milligrams of gallic acid equivalents per gram (mg GAE eq.g⁻¹) of sample.

Antioxidant activity. The ABTS radical scavenging activity and the ferric reducing antioxidant power (FRAP) of the sample was determined using the method described by Khongla et al. (2024). For the ABTS assay, a 20 µL of the sample was added to 1,980 µL of ABTS solution and allowed to stand in the dark for 5 min. The absorbance was then measured at 734 nm. Results were reported as milligrams of Trolox equivalents per gram (mg Trolox eq.g⁻¹) of sample. For FRAP assay, a 100 µL aliquot of the sample was mixed with 1 mL of FRAP reagent. The mixture was incubated at 37°C for 15 min, and absorbance was measured at 593 nm. Results were expressed as

milligrams of Trolox equivalents per gram (mg Trolox eq.g⁻¹) of sample.

Microbiological analysis of spicy candied chew. Microbiological analysis included determination of total plate count, *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli*, and yeast and mold counts, performed in accordance with the United States Food and Drug Administration (U.S. FDA 1998) Biological Analytical Manual (BAM).

Sensory evaluation of developed spicy candied chew. A sensory evaluation was conducted with 100 untrained panellists using a 9-point hedonic scale to assess the acceptability based on appearance, color, flavor, texture, acidity, sweetness, and overall preference. The study protocol was approved by the ethics committee of Rajamangala University of Technology Isan, Nakhon Ratchasima University (Approval no. HEC-01-67-076).

Shelf life evaluation of spicy candied chew using accelerated testing. The shelf life of the spicy candied chew was evaluated using accelerated shelf life testing (ASLT), with total plate count values used as the quality index. The shelf life of the product stored at 37°C was estimated through ASLT by employing kinetic reaction models, including Q₁₀, and Q₁ values. Samples (25 g each) were packed in PET/PE laminated bags (size 10 × 15 cm) and stored under three different conditions: 25°C, 35°C, and 45°C at 80% relative humidity, with 20 bags allocated to each condition. Samples were randomly collected at least six times over a period of ten weeks for shelf life analysis. The product was considered expired when the total plate count exceeded 1 × 10⁶ CFU.g⁻¹ (critical value) for spicy candies fruits and vegetable, as specified by the Thai Community Product Standard (TCPS 521/2015). To determine the reaction order deterioration, data from at least 6 to 7 sample points were analyzed. Total plate count values were plotted against storage period and fitted to kinetic models (zero-order, first-order, and second-order). The change in total plate count values was best described by first order kinetics, as indicated by the highest R² value. Therefore, linear regression was carried out using the first-order kinetic equation (Eq.2).

$$\ln(A_t - A_0) = kt \quad (2)$$

Where A_t is the total plate count value at time t, A₀ is the total plate count value at starting time (t = 0),

k is the reaction rate constant (slope), and t is the storage period (days).

The linear regression equations derived from the graph plotting based on Eq.2, shown in Eq.3 and Eq.4, were used to calculate the shelf life at 25°C (θ_{ST}) and 35°C (θ_{ST+10}), respectively. However, the reaction rate constant ($k = 0.0273$) at 45°C was lower than those at 25°C and 35°C. When k values ($\ln k$) derived from the three temperatures were plotted with $1/T$ (temperature in °Kelvin) following the Arrhenius model, no significant correlation was observed between temperature and rate of degradation ($R^2 = 0.3352$). Therefore, only the equations derived from 25°C and 35°C were used for shelf life and Q_{10} calculation, with the 45°C data excluded due to poor model fit.

$$\text{At } 25^\circ\text{C}; \quad y = 0.0807x + 0.4193 \quad (3)$$

$$\text{At } 35^\circ\text{C}; \quad y = 0.1702x + 0.2935 \quad (4)$$

Where, y is the natural logarithm (\ln) of the total plate count [\ln critical (A/A_0)] and x is the shelf life (θ).

The Q_{10} -value, which describes the effect of temperature on the reaction rate and is defined as the relationship of the reaction rates at $(T+10)$ and T , was calculated as described by Labuza (1982) (Eq. 5). To estimate the shelf life of spicy candied chew stored at 37°C, Q_{10} and Q_1 values were applied to predict the shelf life of the product when the temperature difference between the predicted temperature and temperature T (ΔT) were not equal to 10°C ($\Delta T \neq 10$) (Eqs. 6, and 7).

$$Q_{10} = \frac{k_{(T+10)}}{k_{(T)}} \quad (5)$$

$$Q_1 = Q_{10}^{0.1} \quad (6)$$

$$Q_1^{\Delta T} = \frac{\theta_{(ST)}}{\theta_{(ST+\Delta T)}} \quad (7)$$

Where:

$\theta_{S(T)}$ = shelf life at the initial temperature (days)

$\theta_{(ST+\Delta T)}$ = shelf life at temperature $T + \Delta T$ (days)

Q_{10} = ratio of reaction rates (k)

at temperatures $T+10$ and T

Q_1 = ratio of reaction rates at temperatures differing by 1°C

ΔT = temperature difference between predicted and T temperature

Statistical analysis. The experiments were conducted in triplicate ($n = 3$), and the results were expressed as mean \pm standard deviation. Data analysis was performed using analysis of variance (ANOVA) at a 95% confidence level. Duncan's Multiple Range Test (DMRT) was employed for mean comparisons, with significance determined at $p \leq 0.05$. SPSS software (version 17, SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Results and Discussion

Chemical characteristics of fresh mulberries and mulberry pomace. The chemical composition of fresh mulberries and mulberry pomace is summarized in Table 2. Fresh mulberries had a pH of 4.02 ± 0.03 , whereas the pomace exhibited a higher pH of 5.21 ± 0.02 . This increase may be attributed to chemical reactions occurring during storage or processing, where a decrease in organic acids, such as malic and citric acids, leads to higher pH levels. The pH values of various mulberry fruit varieties have been observed to range between 3.70–3.86 (Parida et al. 2020) and 3.79–6.27 (Gozlekci et al. 2015). Fresh mulberries showed a total soluble solids (TSS) concentration of $8.83 \pm 0.23 \text{ g} \cdot 100 \text{ g}^{-1}$, whereas the pomace exhibited no detectable solids. Kattil et al. (2024) reported a TSS range of 12.11–20.10%, while Babazadeh Darjazi and Jaimand (2023) observed values between 12.40–16.00%. In terms of acidity, fresh mulberries exhibited a total titratable acidity (TTA) of $0.57 \pm 0.02\%$ (as malic acid) and $0.64 \pm 0.02\%$ (as tartaric acid), whereas the pomace displayed significantly lower acidity levels of $0.05 \pm 0.00\%$ and $0.06 \pm 0.00\%$, respectively. The TTA values for various mulberry species were reported to be 0.01–0.68% (Kattil et al. 2024), and 0.10–1.73% (Gozlekci et al. 2015). The Brix/acid ratio of fresh mulberries was consequently higher, reaching 13.89 ± 0.26 for tartaric acid and 15.55 ± 0.29 for malic acid. The pomace exhibited no measurable Brix/acid ratio due to the absence of soluble particles. Mulberries may contain different levels of sugar and acid depending on the species, genotype, production methods, and environmental conditions. The higher values of TSS, TTA, and Brix/acid ratio in fresh mulberries compared to pomace can be attributed to the composition and distribution of

soluble chemicals within the fruit. Fresh mulberries possess intact cellular structures that retain sugars, organic acids, and other soluble solids (Makhoul et al. 2017). During processing, the loss of juice and

the retention of insoluble fibers in the pomace decrease TSS and TTA, thus reducing the Brix/acid ratio.

Table 2. Chemical composition of fresh mulberries and mulberry pomace

Chemical composition	Fresh mulberries	Mulberry pomace
pH*	4.02±0.03	5.21±0.02
Total soluble solids, g.100 g ⁻¹ sample*	8.83±0.23	0.00
Total titratable acidity, % malic acid*	0.57±0.02	0.05±0.00
Total titratable acidity, % tartaric acid*	0.64±0.02	0.06±0.00
°Brix/acid ratio, % malic acid*	15.55±0.29	0.00
°Brix/acid ratio, % tartaric acid*	13.89±0.26	0.00
Protein content, %*	1.06±0.01	2.49±0.09
Ash content, %*	0.63±0.06	1.14±0.05
Moisture content, %*	86.32±0.76	76.82±0.58

*indicates a statistically significant difference according to an independent T-test ($p \leq 0.05$).

Mulberry pomace exhibited higher protein content (2.49±0.09%) compared to fresh mulberries (1.06±0.00%), which aligns with previous research findings (Ta et al. 2023; Kattil et al. 2024). This difference may be attributed to variations in species, genotype, maturity stage, and environmental conditions. The ash concentration was also higher in pomace (1.14±0.05%) than in fresh fruit (0.63±0.06%). Ta et al. (2023) reported an ash content of mulberry pomace of 2.81±0.05%, with differences in ash concentration likely resulting from variations in soil conditions, cultivar, and processing techniques. The moisture level was significantly lower in pomace (76.82±0.58%) compared to fresh mulberries (86.32±0.76%), probably due to water loss during juice extraction. According to previous research, the moisture content of black mulberries ranged from 82.95-86.74%, white mulberries ranged from 62.20-74.62%, and red mulberries ranged from around 74.6% (Kattil et al. 2024), emphasizing that varietal differences and processing techniques play key roles in moisture retention. These results suggest that mulberry pomace possesses considerable nutritional value, highlighting its potential for application in food product development.

Physicochemical properties of spicy candied chew. The physicochemical properties of the spicy

candied chew are detailed in Table 3. The product had a low a_w of approximately 0.6, which inhibits microbial growth, indicating satisfactory microbiological stability and potentially a long shelf life (TCPS 521/2015). The product showed an a_w of 0.5505±0.01, which is safe. Additionally, the product had pH of 3.84±0.06, TSS of 67.57±0.69% °Brix, TTA of 0.83±0.02%, and a °Brix/acid ratio of 81.45±2.62. Gummy candies enhanced with mountain germander extract had an a_w value of 0.687-0.793, while the pH value varied from 3.54-3.91; products with a pH between 5-7 provide a higher probability of contamination by pathogenic microorganisms (Vojvodic Cebin et al. 2024). The pH values of apple and apricot vinegar gummy candy were 4.37±0.02 and 4.36±0.02, respectively (Szekelyhidi et al. 2024). Functional candy with banana and ginger pulp replaced showed a_w of 0.622±0.01, pH of 6.78±0.07, TSS of 84.10±0.14%, and TTA of 0.255±0.002% (Yadav et al. 2021). Variations in physicochemical qualities may result from differences in product type, fruit-based applications, mixing efficiency, and heating duration. Louarme and Billaud (2012) reported that, in fruit processing, longer heating durations and higher temperatures led to the formation of degradation products, including 5-HMF and furfural. These products, in turn, promoted

increased acid formation and the breakdown of sugars. The spicy mulberry chew produced in this study demonstrates a sweet-sour-spicy flavor profile. It exhibited a low a_w , indicating favorable microbiological safety. The pH was reduced (3.84 ± 0.06), typical of concentrated sugar-based product, which reduces pathogenic bacteria contamination. In addition, Han et al. (2025) found

that increasing sourness can decrease or change spiciness depending on acid and capsaicin concentrations. Sourness enhances the spicy feeling due to shared trigeminal stimulation, while sweetness balances the spiciness in chewable confectionary. Although the product has a low pH, its high Brix/acid (81.45 ± 2.62) ratio balances the overall taste.

Table 3. Physicochemical properties spicy candied chew

Sample	a_w	pH	a_w	pH	°Brix/Acid ratio
Spicy candied chew	0.5505 ± 0.01	3.84 ± 0.06	67.57 ± 0.69	0.83 ± 0.02	81.45 ± 2.62

Total phenolic content of spicy candied chew.

Natural antioxidants, such as phenolic compounds, enhance the oxidative stability of food and provide numerous health benefits. Plant-based dietary products are abundant in polyphenols, which include antioxidant and nutraceutical characteristics. Table 4 demonstrated the total phenolic content (TPC) of the product with a value of 2.37 ± 0.07 mg GAE.g⁻¹, which is higher than that reported by Szekelyhidi et al. (2024), who found TPC values of 1.37 ± 0.05 mg GAE.g⁻¹ for apple and 1.46 ± 0.09 mg GAE.g⁻¹ for apricot in fruit vinegar gummy candy. Similarly, the functional candy made from banana pulp supplemented with ginger pulp had a TPC ranging from 3.97-8.59 mg GAE.g⁻¹ (Yadav et al. 2021); the type of fruit incorporation and the product type significantly influence the quantity of TPC. Additionally, the most abundant phenolic component in mulberry fruits was caffeoylquinic acids. The compounds included cryptochlorogenic acid, chlorogenic acid, neochlorogenic acid, and 1,5-dicaffeoylquinic acid (Li et al. 2023). The mechanisms of phenolic compounds act as an antioxidant by interacting with various free radicals through hydrogen atom transfer, single electron transfer, sequential proton loss electron transfer, and chelation of transition metals. Numerous food products include phenolic compounds which possess several health benefits such as antibacterial, antihyperlipidemic, anticancer, antioxidants, cardioprotective, neuroprotective, and antidiabetic properties (Zeb 2020).

Antioxidant activity of spicy candied chew. The ABTS assay quantifies the antioxidant capacity of a

sample by measuring the residual concentration of the radical cation chromophore 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate) after reaction with an antioxidant, whereas the ferric reducing antioxidant power (FRAP) assay assesses antioxidant capacity based on the integrated reducing ability of the sample. The antioxidant activity of the product is illustrated in Table 4. The product exhibited notable antioxidant activity, with ABTS and FRAP values of 3.81 ± 0.18 and 2.05 ± 0.25 mg Trolox eq.g⁻¹, respectively. These findings indicate that the spicy candied chew retains considerable antioxidant potential, likely attributed to the presence of phenolic compounds, including anthocyanins, flavonoids, and phenolic acids found in mulberries. Several studies have shown that increasing the amount of dried mulberry pomace leads to higher levels of phenolics and antioxidant activities (Jeon et al. 2013; Du et al. 2023). Additionally, previous studies have highlighted the potential of fruit and vegetable by-products as functional ingredients rich in bioactive compounds and antioxidants, making them suitable for food applications. Apple pomace, orange peel, and banana peel possess substantial quantities of vitamin C, flavonoids, and phenolic compounds, which enhance their potent antioxidant activities (Hernández-Carranza et al. 2016). These findings suggest that mulberry pomace could effectively improve the nutritional and health-promoting properties of functional foods.

Microbial quality of spicy candied chew. The microbial quality of the spicy candied chew is detailed in Table 5. The total plate count averaged 5.8×10^4 CFU.g⁻¹, while the yeast and mold counts

averaged 1.7×10^2 CFU.g⁻¹. Pathogenic microorganisms, including *Staphylococcus aureus* and *Escherichia coli*, were found to be below 10 CFU.g⁻¹ and 3 MPN.g⁻¹, respectively.

Table 4. Total phenolic content and antioxidant properties of the spicy candied chew

Sample	TPC, mg GAE.g ⁻¹	ABTS, mg Trolox eq.g ⁻¹	FRAP, mg Trolox eq.g ⁻¹
Spicy candied chew	2.37±0.07	3.81±0.18	2.05±0.25

In addition, no *Salmonella* spp. was found in 25 g samples. These findings indicate that the spicy candied chew meets the acceptable microbiological quality criteria as specified by the Thai Community Product Standard (TCPS 521/2015). There was no contamination found during microbial analyses of chewable or functional candies (Yadav et al. 2021; Vergara et al. 2022). Previous research demonstrated that mulberry has antimicrobial

activity against *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Proteus mirabilis*, and *Staphylococcus aureus* (Miljkovic et al. 2018). Similarly, mulberry fruit extracts also exhibited antibacterial properties against *Vibrio cholerae*, *Salmonella* Typhi, *Escherichia coli*, *Shigella dysenteriae*, and *Staphylococcus aureus* (Suriyaprom et al. 2021). Consequently, the spicy candied chew is safe for human consumption.

Table 5. Initial microbiological quality of spicy candied chew

Quality parameters	Spicy candied chew	Thai Community Product Standard (TCPS 521/2015)
Total plate count	5.8×10^4 CFU.g ⁻¹	$< 1 \times 10^6$ CFU.g ⁻¹
Yeasts and molds	1.7×10^2 CFU.g ⁻¹	$< 1 \times 10^3$ CFU.g ⁻¹
<i>Staphylococcus aureus</i>	< 10 CFU.g ⁻¹	< 10 CFU.g ⁻¹
<i>Escherichia coli</i>	< 3 MPN.g ⁻¹	< 3 MPN.g ⁻¹
<i>Salmonella</i> spp.	Not detected in 25 g	Not detected in 25 g

Sensory evaluation of spicy candied chew. The sensory evaluation of the spicy candied chew was conducted with 100 panellists, as shown in Table 6. The scores for appearance, color, flavor, texture, and overall acceptability ranged from 7.6-7.8,

corresponding to ratings between “like moderately” and “like very much”. Additionally, attributes including acidity and sweetness also received positive ratings (7.0-7.5), indicating positive consumer acceptance of the product.

Table 6. Sensory evaluation of spicy candied chew

Appearance	Color	Flavor	Texture	Acidity	Sweetness	Overall acceptability
7.7±0.9	7.6±1.0	7.6±0.9	7.7±0.9	7.0±0.8	7.5±1.0	7.8±0.7

N = 100; Preference Score: 1 = Dislike extremely; 2 = Dislike very much; 3 = Dislike moderately; 4 = Dislike slightly; 5 = Neither like nor dislike; 6 = Like slightly; 7 = Like moderately; 8 = Like very much; 9 = Like extremely

Shelf-life estimation of spicy candied chew. Changes in the total plate count of spicy candied chew during storage at 25°C, 35°C, and 45°C are shown in Table 7. The microbial load tended to increase with an increase in the storage period, with the initial number of bacteria averaging 5,833

CFU.g⁻¹ sample. At the end of storage at 25°C (47 days), the total plate count reached 271,515 CFU.g⁻¹ sample. At 35°C, the microbial load of the product exceeded the critical value (1×10^6 CFU.g⁻¹) after 33 days of storage. However, the microbial load of the product stored at 45°C was lower than that observed

at 25°C and 35°C. Mesophilic microbes generally grow between 20°C and 45°C, with an optimal growth temperature ranging from 30°C to 39°C (Mohanty and Meena 2022). At temperatures above 39°C, the growth of mesophilic microbes decreases,

which may explain the lower total plate count observed at 45°C compared to 25°C and 35°C. Therefore, data from 45°C were excluded from the shelf life and Q₁₀ calculations, as previously noted.

Table 7. Changes in the total plate count of spicy candied chew during storage at 25°C, 35°C, and 45°C

Storage period, days	Total plate count, CFU.g ⁻¹ sample		
	Temp. 25°C	Temp. 35°C	Temp. 45°C
0	5,833	5,833	5,833
5	17,906	23,364	8,567
12	27,667	228,182	8,700
19	37,061	330,909	8,533
26	-	353,939	8,967
33	-	2,500,000	7,533
36	217,879	-	-
40	256,364	-	-
43	-	-	21,455
47	271,515	-	22,758

Total plate count of 1×10⁶ CFU.g⁻¹ (critical value) was used as a marker for product expiration.

The total plate count obtained at 25°C and 35°C (Table 7) were plotted against the storage period to determine the reaction order, and the results are present in Fig.2. The results indicated that the relationship between the microbial load and storage period followed first-order kinetics, as the linear

regression (R²) was close to 1 when the natural logarithm (Ln) of the total plate count was plotted against the storage period (t) (Fig.2B). Therefore, the regression equation from first-order reaction was used to calculate the shelf life of spicy candied chew at 25°C and 35°C, following Eqs. (3) and (4).

$$\begin{aligned}
 \text{At } 25^{\circ}\text{C}; \quad y &= 0.0807x + 0.4193 \\
 \text{Ln critical (A/A}_0) &= \text{Ln}(1000000/5833) = 0.0807x + 0.4193 \\
 x &= [\text{Ln}(1000000/5833) - 0.4193]/0.0807 \\
 x &= 58.55 \text{ days} \\
 \text{At } 35^{\circ}\text{C}; \quad y &= 0.1702x + 0.2935 \\
 \text{Ln critical (A/A}_0) &= \text{Ln}(1000000/5833) = 0.1702x + 0.2935 \\
 x &= [\text{Ln}(1000000/5833) - 0.2935]/0.1702 \\
 x &= 28.50 \text{ days}
 \end{aligned}$$

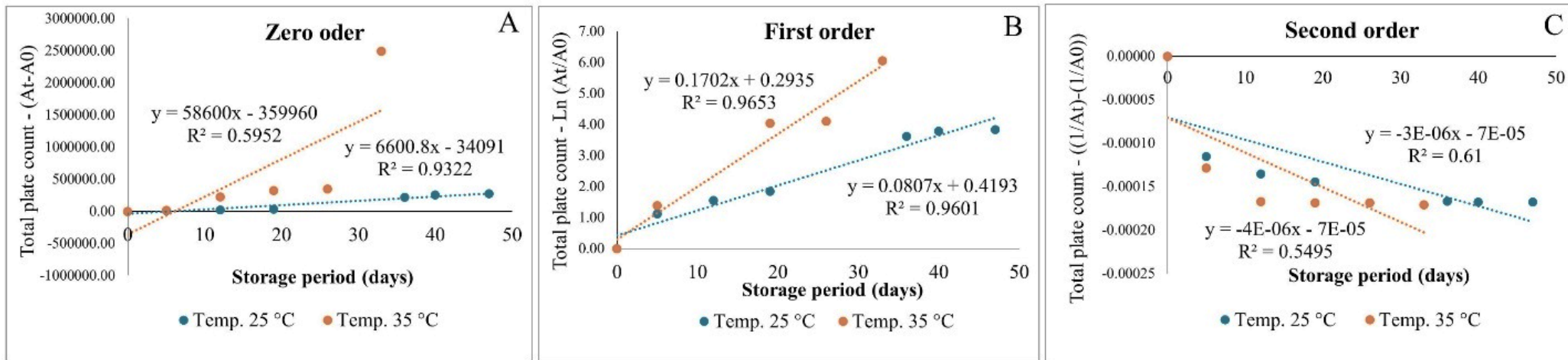


Figure 2. Reaction order for the spicy candied chew stored at 25°C and 35°C: (A) Zero-order, (B) First-order, and (C) Second-order models.

The results from the calculation revealed that the expected expiration dates of the product stored at 25°C and 35°C were 58.55 days and 28.50 days, respectively (Table 8). From these plots, k values (slope) were obtained as 0.0807 and 0.1702 for 25°C and 35°C, respectively, and were used to calculate the Q₁₀-value using equation (Eq. 5);

$$Q_{10} = \frac{0.1702}{0.0807} = 2.11$$

Then, Q₁ value was calculated using Q₁₀ value, as shown in equation (Eq.6);

$$Q_1 = 2.11^{0.1} = 1.08$$

The shelf life of the spicy candied chew stored at 37°C (θ_(ST+ΔT)) was calculated using (Eq.7);

$$Q_1^{\Delta T} = \frac{\theta_{(ST)}}{\theta_{(ST+\Delta T)}}$$

$$1.08^{(37-35)} = \frac{\theta_{(35)}}{\theta_{(37)}}$$

$$\theta_{(37)} = \frac{28.50}{1.08^2} = 24.43 \text{ Days}$$

These results conclude that the predicted shelf life of spicy candied chew in PET/PE laminated bags (size 10 × 15 cm) is approximately 24 days when stored at 37°C (representing the ambient temperature in Thailand) (Table 8). All parameter values derived from ASLT method using kinetic reaction models, Q₁₀, and Q₁, are summarized in Table 8. Sulaiman et al. (2014) found that the shelf-life prediction of nutmeg candy utilizing the ASLT method, based on moisture content, indicated that the candy can be stored for 50.89 days at 35°C. Additionally, sugar palm fruit jam had a shelf life at 28°C for 40 days in packaging pouches and 93 days in jar bottles based on color parameters (Anggraini et al. 2019). The variation in shelf life could result from variances in product type, temperature, packaging, and the criteria applied for determining expiration dates. Natural preservatives, an effective approach to extend shelf life and improve the quality of food products, such as polyphenols, essential oils, and plant extracts, exhibited antibacterial and antioxidant properties. According to research, fruit and vegetables rich in phenolics can extend shelf life and enhance microbiological stability (Bag et al. 2022; Giri et al. 2024).

Table 8. Regression equations, R-square values, reaction rate constants (k), Q₁₀, Q₁ and predicted shelf life of spicy candied chew stored at different temperatures

Temp., °C	Regression equation (First order)	R-square	k	Q ₁₀ ,	Q ₁ ,	Shelf-life, days
25	y = 0.0807x + 0.4193	0.9601	0.0807	2.11	1.08	58.55
35	y = 0.1702x + 0.2935	0.9653	0.1702			28.50
Expected 37						24.43

x, shelf life (days); y, total plate count - Ln (A_t/A₀); x, shelf life (θ.); k, reaction rate constant

Conclusions

Mulberry pomace exhibits significant potential for use as a functional ingredient for value-added food products. The developed spicy candied chew from mulberry pomace possessed desirable physicochemical properties, including high total phenolic content and antioxidant properties. Microbial quality confirmed the product's safety, while sensory evaluation confirmed its acceptability. Accelerated shelf life testing

demonstrated that the product remained microbiologically acceptable for approximately 24 days when stored at 37°C. These findings highlight the potential of mulberry pomace as a valuable ingredient for developing functional and appealing food products.

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Author Contributions

Conceptualization, S.M., P.Y., and C.K.; methodology, S.M., P.Y., and C.K.; formal analysis S.M. and C.K.; writing-original draft preparation, S.M. and C.K.; review and editing, S.M., P.Y., and C.K.; All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The sensory evaluation involving human participants was performed according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Rajamangala University of Technology Isan, Nakhon Ratchasima University (Approval No. HEC-01-67-076, dated 3 December 2024).

Informed Consent Statement

All participants provided informed consent prior to sensory evaluation. The study protocol and consent procedure were approved by the Institutional Review Board of Rajamangala University of Technology Isan, Nakhon Ratchasima University.

Data Availability Statement

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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