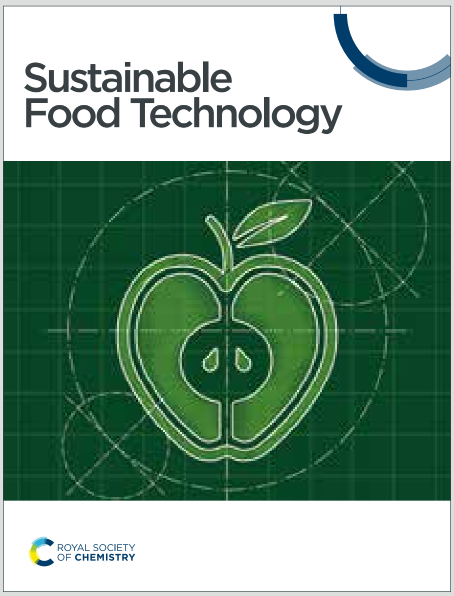
Sustainable Food Technology

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: S. Chheng, S. Jafari, D. Mishra and K. Assatarakul, *Sustainable Food Technol.*, 2025, DOI: 10.1039/D5FB00227C.



ROYAL SOCIETY
OF CHEMISTRY

View Article Online
DOI: 10.1039/D5FB00227C

Sustainability Spotlight Statement

This research showcases *Sesbania javanica* (Sesban flower) extract as a sustainable, plant-based alternative to synthetic additives in gel-based foods. By enhancing antioxidant activity, inhibiting microbial growth, and extending shelf life, this underutilized botanical offers a clean-label solution that reduces chemical preservative use. Its multifunctionality not only improves product quality but also supports biodiversity, promotes local resource utilization, and contributes to a more resilient and environmentally responsible food system.

View Article Online
DOI: 10.1039/D5FB00227C

Sesban Flower Extract as a Natural Functional Ingredient: Effects on Texture, Antioxidant Activity, and Shelf- Life Stability of Jelly Formulation

To be submitted to **Sustainable Food Technology**

Running title: Antioxidant and Microbial Stability of Jelly with Sesban Extract

Sochannet Chheng[1,2], Saeid Jafari[1], Dharmendra Mishra[3], and Kitipong Assatarakul[1]*

[1]Department of Food Technology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, 10330

[2]Department of Food Chemical Engineering, Kampong Speu Institute of Technology, Kampong Speu 050601, Cambodia

[3]Department of Food Science, Purdue University, West Lafayette, IN 47907, USA

*To whom correspondence should be addressed.

E-mail: Kitipong.A@chula.ac.th (K. Assatarakul). Tel.: 02-218-5328

View Article Online
DOI: 10.1039/D5FB00227C

Abstract

This study highlights the potential of *Sesbania javanica* Miq. (Sesban flower) extract (SFE) as a sustainable, plant-based ingredient to enhance the functional and preservation qualities of carrageenan-based jelly. Jelly formulations were prepared with varying SFE concentrations (1%, 3%, and 5%) and evaluated over 30 days of refrigerated storage at 4°C. The 5% SFE jelly exhibited the highest levels of bioactive compounds, including total phenolics (9.63 ± 0.29 mg GAE/g dw) and flavonoids (5.27 ± 0.28 mg QE/g dw), and significantly greater antioxidant activity (DPPH: 14.04 ± 0.20 µM Trolox/g dw; FRAP: 8.81 ± 1.53 µM Trolox/g dw) compared to the control (TPC: 1.22 ± 0.42 mg GAE/g dw; TFC: 0.89 ± 0.03 mg QE/g dw; DPPH: 2.48 ± 0.95 µM Trolox/g dw; FRAP: 1.17 ± 0.94 µM Trolox/g dw). Textural analysis showed reduced hardness (3.26 ± 0.58 N in 3% SFE jelly) while maintaining springiness and cohesiveness. Importantly, the 3% and 5% SFE jellies inhibited microbial growth throughout storage, whereas the control spoiled by day 24. Color stability was influenced by SFE, with ΔE reaching 11.29 ± 0.52 in the 5% jelly at day 30. These findings underscore SFE's multifunctionality as a natural ingredient supporting antioxidant protection, textural modification, and microbial stability in gel-based foods, contributing to sustainable food product development.

Keywords: *Sesbania javanica*, Functional jelly, Antioxidant activity, Plant-based ingredients, Natural preservatives, Phenolic compounds

View Article Online
DOI: 10.1039/D5FB00227C

1.0 Introduction

In recent years, the growing awareness of health and wellness has driven consumers to seek food products that offer not just nourishment but also health benefits. This shift has spurred significant interest in the incorporation of natural bioactive compounds into food formulations. Among these compounds, plant extracts have emerged as promising sources of antioxidants, which are vital in combating oxidative stress, a contributing factor to various chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions [1, 2].

Carrageenan, a sulphated polysaccharide derived from red seaweed, is a critical gelling agent widely used in jelly formulations due to its ability to create elastic and cohesive gels at low concentrations [3]. Its interactions with other food ingredients can influence texture, water-holding capacity, and stability, making it central to the quality of gel-based products.

Sesbania javanica Miq., commonly known as Sesban flower, is a leguminous plant native to Southeast Asia, traditionally used in both culinary and medicinal applications. The flowers are rich in bioactive compounds such as flavonoids, phenolic acids, and polysaccharides, which have been reported to exhibit antioxidant, antimicrobial, and anti-inflammatory properties [4, 5]. Despite these attributes, its utilization as a functional ingredient in gel-based food systems has not been fully explored.

Jelly is a widely consumed confectionery product admired for its appealing texture and vibrant colors. However, traditional jelly formulations often lack significant nutritional value and are susceptible to microbial spoilage, leading to concerns about shelf life and health impacts among consumers [6]. Although the bioactive properties of *Sesbania javanica* (Sesban flower) extract (SFE) have been well documented, its application in gel-based food systems such as jelly remains largely unexplored. Incorporating SFE into jelly formulations offers the potential to

View Article Online
DOI: 10.1039/D5FB00227C

enhance nutritional content, improve sensory attributes, and extend shelf life, representing an innovative strategy for functional food development.

Despite existing research on plant extracts in functional foods, no prior study has systematically evaluated how *Sesbania javanica* extract affects the antioxidant capacity, texture, color stability, and microbial safety of gel-based products like carrageenan jellies. This study uniquely fills this gap by investigating the multifunctional role of SFE in enhancing physicochemical quality and shelf stability, contributing new insights to the development of sustainable, plant-based food products.

This study aims to explore the effects of varying concentrations of optimized SFE on the quality and shelf life of jelly. Specifically, we seek to identify the optimal concentration of the extract that maximizes antioxidant potential while maintaining desirable sensory properties in the jelly. Furthermore, this research assessed the stability of the jelly over a 30-day period at 4°C, providing valuable insights into the application of bioactive extracts in food products.

Through this research, we aspire to contribute to the advancement of functional food development, offering innovative solutions that align with consumer preferences for healthier and more nutritious options.

2.0 Materials and methods

2.1 Chemicals and reagents

All chemicals, reagents, and solvents used in this study were of analytical grade. The jelly gelling agent was carrageenan powder derived from *Gracilaria fisheri*, supplied by KC Krungthepchemi Co., Ltd. (Thailand; F013CG). Other materials included ferric chloride, methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), sourced from Ajax Finechem (Australia). Aluminum chloride was

View Article Online
DOI: 10.1039/D5FB00227C

obtained from Ajax Finechem (New Zealand). Ethanol, Folin-Ciocalteu reagent, gallic acid, glacial acetic acid, hydrochloric acid, quercetin, sodium carbonate (Na_2CO_3), and tripyridyltriazine (TPTZ) were purchased from Merck (Germany). Sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$) was also acquired from Ajax Finechem (Australia).

Plate Count Agar (PCA; Oxoid, UK) was used for total plate counts. Potato Dextrose Agar (PDA; Merck, Germany) was employed for yeast and mold enumeration. Salmonella Shigella Agar (SSA; Merck, Germany) was used for detecting *Salmonella* spp., and Mannitol Salt Agar (MSA; HiMedia, India) was utilized for *Staphylococcus aureus* analysis. All media were prepared and sterilized according to the manufacturers' instructions.

2.2 Sesban flower extract (SFE)

Sesban (*Sesbania javanica* Miq.) flowers were purchased from a local market in Bangkok, Thailand. After cleaning, drying and grinding, the flowers were extracted using an ultrasound-assisted extraction (UAE) method optimized by response surface methodology (RSM) technique using a Box-Behnken design in our previous study [7]. The extraction was carried out in an ultrasonic bath (Elmasonic E 70 H, Elma Schmidbauer GmbH, Singen, Germany), operated at a frequency of 50/60 Hz with an output power of 520 W, at 40°C for 20 minutes using 70% ethanol as the solvent. The resulting extract was centrifuged and evaporated using rotary evaporator (BÜCHI, Switzerland), re-dissolved in disstiled water, and stored at 4°C until further use.

2.3 Jelly preparation

The jelly formulations were prepared with varying concentrations of SFE (0%, 1%, 3%, and 5%) to investigate the impact of the extract on the jelly's quality and stability over a 30-day

View Article Online
DOI: 10.1039/D5FB00227C

period. These concentrations were chosen based on preliminary studies, which indicated that higher concentrations (>5%) resulted in unsatisfactory color attributes, such as excessive pigmentation, while lower concentrations (<1%) provided limited enhancement in antioxidant properties. Furthermore, selecting these concentrations aimed to balance the efficient utilization of the extract with its functional benefits. Jelly was prepared following a method adapted from [8], with modifications. Briefly, carrageenan powder (1.5% w/w) sourced from *Gracilaria fisheri* (KC Krungthepchemi Co., Ltd., Thailand; F013CG) was dissolved together with 5% sugar and 0.1% citric acid in distilled water. The mixture was heated to boiling (90–95°C) under continuous stirring until completely dissolved. The solution was then cooled to approximately 50°C, at which point the appropriate concentration of SFE was added, ensuring thorough mixing. The jelly mixtures were poured into molds and refrigerated at 4°C for 24 hours to set. The control jelly contained no extract, while the experimental formulations (denoted as J1%, J3%, and J5%) incorporated increasing amounts of SFE. The detailed composition for each formulation is shown in Table 1. Jellies were analyzed for physicochemical, antioxidant, and microbiological properties over storage at 4°C, sampled on days 0, 6, 12, 18, 24, and 30. The carrageenan concentration of 1.5% was chosen based on the manufacturer's recommended range (0.2–3.0% w/w) for achieving suitable gel firmness and texture.

Table 1. Formulations of jelly.

Ingredient	Formula (%)			
	Control	J1%	J3%	J5%
Water	93.40	92.40	90.40	88.40
Carrageenan	1.50	1.50	1.50	1.50
Sugar	5.00	5.00	5.00	5.00
Citric acid	0.10	0.10	0.10	0.10

View Article Online
DOI: 10.1039/D5FB00227C

SFE	0.00	1.00	3.00	5.00

SFE: Sesban flower extract

2.4 Color

Color was assessed using a colorimeter (Minolta CR-400 color meter, Osaka, Japan) with silicone photocells detector, illuminant D65 and 10^{o} standard observer, and aperture diameter of 8 mm, measuring L* (lightness), a* (red-green), and b* (yellow-blue) values over the 30-day period.

2.5 Water activity (a_w) and moisture content

Water activity (a_w) was measured using a water activity meter (Aqualab 3TE, USA) at 25°C to monitor the potential for microbial growth and its effect on jelly texture. The moisture content was determined using moisture analyzer (Mettler Toledo, Greifensee, Switzerland) in triplicate.

2.6 pH and total dissolved solids (°Brix) measurements

The 2g of jelly sample were homogenized in 18 mL of distilled water and centrifuged at 4,000 rpm for 10 min. The pH of the jelly samples was measured using a digital pH meter (Mettler Toledo FE20-Kit FiveEasy™ Benchtop pH Meter, Switzerland). pH changes were monitored over the 30-day period to assess the potential impact on taste, texture, and microbial stability. The soluble solids content of the jelly was measured using a digital refractometer (HI96803, Africa). The results were expressed as degrees Brix (°Bx).

2.7 Texture analysis

View Article Online
DOI: 10.1039/D5FB00227C

Texture properties, including firmness, springiness, cohesiveness, gumminess, and chewiness, were analyzed using a texture analyzer TA.XTplus Texture Analyzer (Stable Micro Systems, England). Texture Profile Analysis (TPA) tests were performed on jelly samples using a cylindrical probe (P/100) with a diameter of 100 mm. The pre-test speed was set at 1 mm/s, the test speed and post-test speed were both 5 mm/s. Each jelly sample was cut into uniform cubes of $1 \times 1 \times 1$ cm^3. The samples were compressed twice (double compression cycle) to 50% of their original height to obtain TPA parameters, simulating the mastication process. Measurements were performed in triplicate at room temperature (~25°C).

2.8 Determination of bioactive compounds

2.8.1 Bioactive compounds extraction

The jelly extraction for bioactive extraction was done according to the previous method study [9, 10] with a slight modification. Jelly (5 g) dissolved in 25 mL of methanol/water solution (8:2) with 1% (0.25 ml) of 37% HCl. The dispersion was heated at 55°C on a hot plate and mixed with magnetic stirrer for 30 min and then centrifuged at 15,000×*g* for 10 min. The supernatant was collected for the determination of bioactive compounds.

2.8.2 Determination of total phenolic compound content

Total phenolic compound content (TPC) was determined following to Folin-Ciocalteu method as previously described by [7] with slightly adjustment. Briefly, 30 µL of properly diluted extract solution was added 1.2 mL distilled water and mixed with 2 ml of Folin-Ciocalteu reagent, which was pre-diluted, 10 times, with distilled water. After standing for 5 min at room temperature, 1.5 ml of (7.5% w/v) sodium carbonate solution was added. The solutions were mixed and allowed to stand for 1 h at room temperature. Subsequently, the absorbance was

View Article Online
DOI: 10.1039/D5FB00227C

measured at 765 nm, using a UV–visible spectrophotometer (GENE-SYSTM 20 Visible, Thermo Fisher Scientific, USA). A calibration curve was prepared, using a standard solution of gallic acid (0-150 mg/L). Results were reported as mg gallic acid equivalents per gram dry basis (mg GAE/g db.) using the gallic acid standard curve equation ($y = 0.0034x - 0.0124$, $R^2 = 0.99$).

2.8.3 Determination of total flavonoid content

The analysis of total flavonoid (TFC) was conducted using aluminum chloride colorimetric method according to the procedure outlined by [11] with slight adjustments. Briefly, 1 mL of each diluted extract was mixed with 1 mL of 2% $AlCl_3$ methanol solution. After a 30-min incubation period, the absorbance was measured at 430 nm using spectrophotometer (Thermo Fisher Scientific, GENESYSTM 20 Visible, U.S.A). Flavonoid contents were calculated from a calibration curve ($y = 0.0069x + 0.032$, $R^2 = 0.99$) of quercetin (0-160 mg/L) and expressed as milligrams of quercetin equivalent per gram of dry basis (mg QE/g db.).

2.8.4 Antioxidant activity

Antioxidant activity of the jelly samples was analyzed on extracts obtained according to Section 2.8.1. Thus, all measurements represent the antioxidant capacity of SFE incorporated into the jelly matrix and subsequently extracted.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed following the method of [12], with modifications to validate the protocol for jelly matrices. A DPPH stock solution was prepared by dissolving 0.024 g of DPPH in methanol and adjusting the volume to 100 mL. Before use, the DPPH solution's absorbance was measured and adjusted to approximately 1.1 at 515 nm. For analysis, 0.2 mL of jelly extract was mixed with 2 mL of the prepared DPPH solution, vortexed, and incubated in the dark at room temperature for 30 minutes. The

View Article Online
DOI: 10.1039/D5FB00227C

absorbance was measured at 515 nm using a spectrophotometer (Thermo Fisher Scientific, GENESYS™ 20 Visible, USA) against methanol as a blank. The initial absorbance of the DPPH solution ($A_{initial}$), and the absorbance of the sample (A_{final}) were measured after incubation. The difference in absorbance (A_{diff}) was calculated as:

$$A_{diff} = A_{final} - A_{initial} \quad [1]$$

Antioxidant activity was determined from a Trolox calibration curve ($y = 0.0016x + 0.0312$, $R^2 = 1$) and expressed as µM Trolox equivalents per gram dry weight. The method was previously tested to confirm suitable linearity and matrix compatibility for jelly extracts.

The ferric reducing antioxidant power (FRAP) assay followed [13], with validation for jelly matrices. The FRAP reagent was pre-warmed to 37°C for 6 minutes, after which 2.85 mL of FRAP reagent was mixed with 0.15 mL of jelly extract, vortexed, and incubated in darkness at room temperature for 30 min. Absorbance was measured at 593 nm against distilled water as a blank. A_{diff} was calculated as:

$$A_{diff} = A_{final} - A_{initial} \quad [2]$$

Antioxidant capacity was determined from a Trolox calibration curve ($y = 0.0009x – 0.0203$, $R^2 = 0.9945$) and expressed as µM Trolox equivalents per gram dry weight. Validation experiments confirmed the assay's linearity and reproducibility for jelly extracts.

2.9 Microbiological analysis

Jelly sample (10g) was aseptically weighted into the sterile bag and homogenized with 90 mL of sterile water for 3 min. Serial dilution required for sample plating were prepared in 9 mL of sterile water. Pour plating method were prepared using the following media and culture condition. Plate count agar for total plate count, potato dextrose agar for yeast and mold counts, salmonella shigella agar for *Salmonella* spp., and mannitol salt agar for *Staphylococcus aureus*,

View Article Online
DOI: 10.1039/D5FB00227C

and then incubated at 37°C for 24 h, 30°C for 48 h, 37°C for 24 h, and 37°C for 48 h, respectively.

2.10 Data analysis

The results were expressed as mean ± standard deviation (SD) in triplicate. Statistical analyses were conducted using SPSS 29.0 software (SPSS Inc., IL, USA). Statistical analysis was conducted using ANOVA followed by Tukey's post hoc test to determine significant differences between treatments. A p-value of less than 0.05 was considered statistically significant.

3. Results and discussion

3.1 Physical properties

3.1.1 pH stability during storage

The initial pH values of all jelly formulations ranged from 3.91 to 4.06 (Table 2), consistent with typical acidified gel-based products. Over the 30-day refrigerated storage period (Fig. 1a), only minor pH fluctuations were observed. The fluctuations were likely due to gradual acidification caused by enzymatic or oxidative reactions producing organic acids in the jelly matrix [14]. The control jelly exhibited a gradual decrease, reaching approximately 3.70 by day 30, whereas the SFE-enriched formulations (J1%, J3%, and J5%) maintained significantly more stable pH values throughout the study ($P<0.05$). Similar pH stability trends have been reported in jellies incorporating hibiscus and rosemary extracts, where plant polyphenols contributed to maintaining acidity levels during storage [8]. Compared to those systems, SFE-containing jellies in our study-maintained pH within an even narrower range, suggesting potential superior buffering capacity.

View Article Online
DOI: 10.1039/D5FB00227C

The observed pH stability in SFE-containing jellies could be partly attributed to antioxidant activity mitigating oxidative processes that otherwise lead to acidification in food systems. Although polyphenolic compounds have been reported to help stabilize internal pH indirectly by scavenging reactive oxygen species and reducing the formation of acidic byproducts [15], there are currently no published studies that directly quantify or confirm a specific buffering capacity for Sesban flower extract itself. Therefore, the notion of SFE exerting a direct pH-buffering effect in jelly remains a hypothesis based on its biochemical profile rather than a proven mechanism.

Maintaining a stable pH is critical for preserving jelly quality, as it directly affects microbial growth, gelling behavior, color, and flavor profile. The reduced pH drift in the SFE formulations thus reinforces the potential multifunctional role of Sesban extract in improving the physicochemical and microbial stability of acidified food products [14].

3.1.2 Water activity (a_w)

The initial water activity (a_w) values across all jelly formulations were high, ranging from 0.988 to 0.994 (Table 2), reflecting the moisture-rich nature of the gel matrix. Over the 30-day storage period (Fig. 1b), a_w values increased slightly in all samples, approaching 0.999 by day 30. This upward trend was most prominent in the control jelly, while the SFE-enriched formulations (J1%, J3%, and J5%) exhibited significantly more stable a_w values over time ($P<0.05$). Water activity increased slightly during storage, possibly as a result of water redistribution from bound to free states within the gel network, a common phenomenon in gel-based products [16]. Comparable studies using rosemary or hibiscus extracts in gels also noted reduced water activity fluctuations, attributed to interactions between polyphenols and the gel

View Article Online
DOI: 10.1039/D5FB00227C

matrix [17]. Our findings align with these observations, indicating that SFE performs similarly in limiting water mobility.

Table 2. Initial physicochemical properties and texture attributes of jelly formulations with varying concentrations of Sesban flower extract (SFE).

Properties	Control	J1%	J3%	J5%
Lightness (L*)	41.14 ± 0.66^{a}	38.24 ± 0.04^{b}	35.25 ± 0.04^{c}	36.48 ± 0.25^{bc}
Greenness (a*)	1.08 ± 0.07^{a}	1.11 ± 0.03^{a}	1.09 ± 0.04^{a}	0.86 ± 0.19^{a}
Yellowness (b*)	-0.84 ± 0.01^{d}	0.40 ± 0.56^{c}	2.13 ± 0.19^{b}	6.35 ± 0.06^{a}
pH	3.91 ± 0.05^{a}	3.92 ± 0.03^{a}	3.92 ± 0.01^{a}	4.06 ± 0.05^{a}
Water activity (a_w)	0.988 ± 0.02^{a}	0.993 ± 0.02^{a}	0.994 ± 0.02^{a}	0.992 ± 0.02^{a}
Moisture (%)	88.54 ± 0.47^{a}	89.66 ± 0.35^{a}	89.87 ± 0.33^{a}	89.08 ± 0.09^{a}
Brix (^{o}Brix)	0.80 ± 0.02^{ab}	0.60 ± 0.02^{bc}	0.70 ± 0.02^{abc}	0.80 ± 0.02^{ab}
Hardness (N)	11.05 ± 0.06^{a}	6.36 ± 0.16^{b}	3.26 ± 0.58^{c}	6.78 ± 1.04^{b}
Springiness (mm)	0.35 ± 0.01^{a}	0.45 ± 0.17^{a}	0.34 ± 0.03^{a}	0.37 ± 0.05^{a}
Cohesiveness	0.03 ± 0.00^{a}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.04 ± 0.02^{a}
Gumminess	0.33 ± 0.01^{a}	0.22 ± 0.07^{a}	0.11 ± 0.05^{a}	0.28 ± 0.18^{a}
Chewiness	0.12 ± 0.00^{a}	0.09 ± 0.01^{a}	0.04 ± 0.01^{a}	0.11 ± 0.08^{a}

Control: Jelly with no SFE, data presented in average ± deviation. Different letters show significant difference among data (p <0.05).

Although high a_w values are generally associated with a greater risk of microbial proliferation, the presence of SFE may help mitigate this risk through its antimicrobial activity. Furthermore, the relatively smaller increase in a_w observed in the SFE-containing samples suggests possible interactions between the extract's bioactive compounds and the gel matrix, potentially enhancing water binding and limiting free water availability. However, no direct experimental analyses were performed in this study to confirm these interactions.

View Article Online
DOI: 10.1039/D5FB00227C

The observed a_w stability could also be influenced by the hydrocolloids used in the jelly, particularly carrageenan, which is known for its excellent water-binding capacity and ability to reduce water mobility in gel matrices [16]. It is hypothesized that phenolic compounds in SFE might form hydrogen bonds or other interactions with water molecules or the carrageenan network, contributing to improved water retention. Future studies using analytical techniques such as differential scanning calorimetry (DSC) or nuclear magnetic resonance (NMR) spectroscopy could help elucidate these molecular interactions and confirm the mechanisms underlying water activity control in SFE-enriched jelly systems.

Maintaining lower a_w variability during storage is important for ensuring microbial stability, texture preservation, and extended shelf life, particularly in high-moisture products with minimal or no synthetic preservatives [18].

3.1.3 Moisture content

The initial moisture content of the jelly formulations ranged from approximately 88% to 90% (Fig. 1c), typical of high-moisture gel-based products. Over the 30-day storage period, minor fluctuations were observed across all formulations, which is expected due to internal water redistribution. Fluctuations in moisture content reflect minor evaporation losses and internal water migration, processes typical during refrigerated storage of gel-based foods [19]. However, the control formulation exhibited a significant decrease in moisture content by day 30 ($P<0.05$), suggesting moisture loss likely due to syneresis or evaporation.

In contrast, formulations containing Sesban flower extract (SFE) retained significantly higher moisture levels throughout storage. This suggests that SFE contributes to improved water-holding capacity within the gel matrix, likely due to interactions between polyphenolic compounds and the hydrocolloid network. Several studies have demonstrated that phenolic

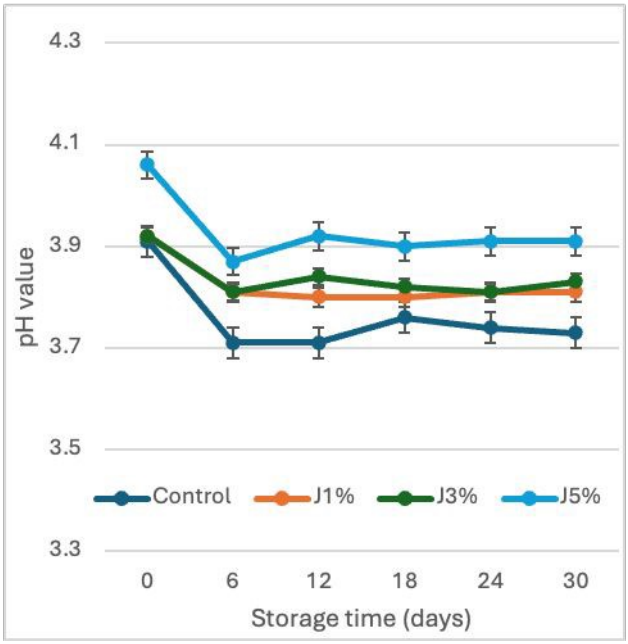
View Article Online
DOI: 10.1039/D5FB00227C

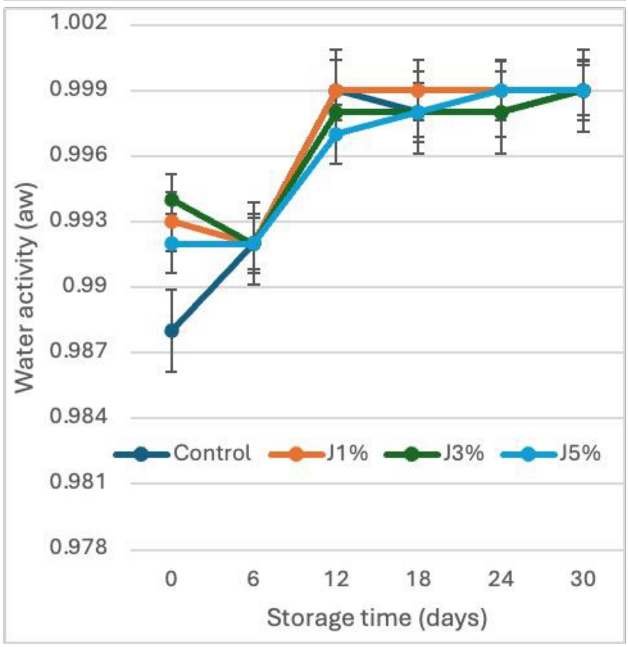
compounds can interact with polysaccharides (such as carrageenan) through hydrogen bonding, hydrophobic interactions, and even covalent linkages, which helps reinforce the gel structure and reduce water mobility [20-23]. These interactions can create a denser gel matrix, trap water molecules and mitigating moisture loss during storage.

Furthermore, plant-derived polysaccharides and bioactive compounds in SFE may act synergistically with carrageenan to enhance gel cohesiveness and minimize water migration. For instance, phenolic compounds have been shown to bind water molecules directly through multiple hydroxyl groups, forming hydration shells that improve water retention in food matrices [24, 25]. Such interactions contribute to maintaining desirable texture, preventing structural degradation, and extending product shelf life.

Overall, the better moisture retention observed in SFE-treated jellies highlights the multifunctional role of Sesban flower extract in improving both the physicochemical stability and sensory attributes of gel-based food products.

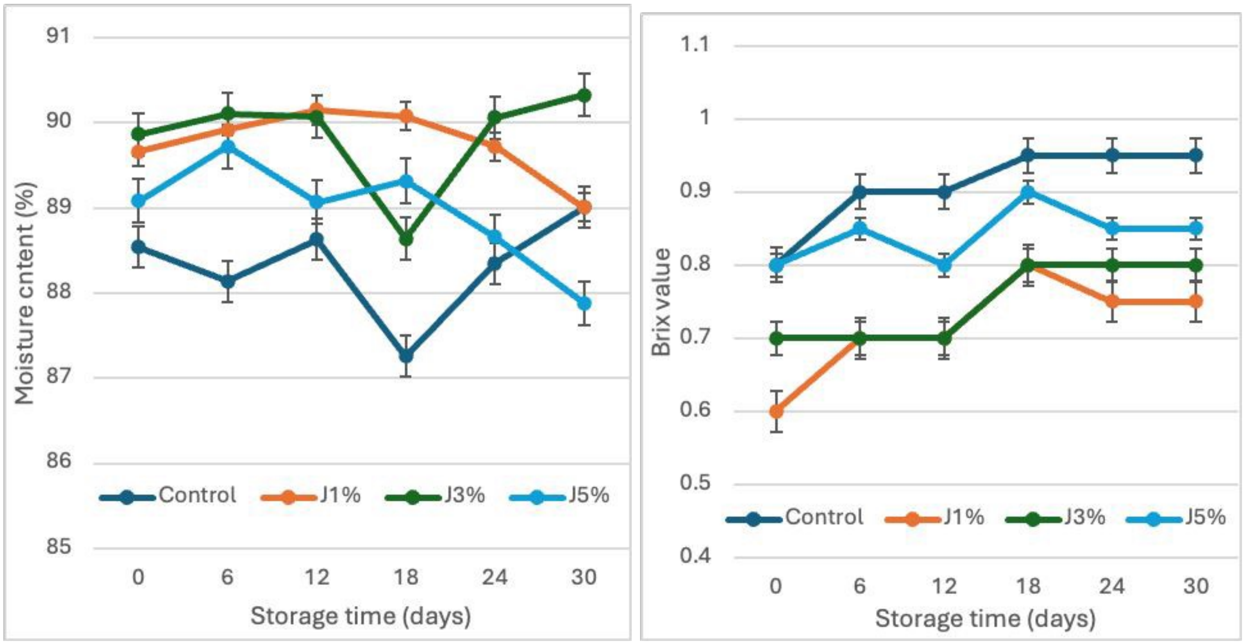
View Article Online
DOI: 10.1039/D5FB00227C





(a) (b)

View Article Online
DOI: 10.1039/D5FB00227C



(c) (d)

Fig. 1 Changes in (a) pH, (b) water activity (a_w), (c) moisture content, and (d) °Brix of jelly formulations containing varying concentrations of Sesban flower extract (SFE) (Control, J1%, J3%, J5%) during 30 days of refrigerated storage at 4°C. Values are means ± SD (n = 3). Different letters at each time point indicate significant differences among formulations ($P < 0.05$).

3.1.4 Total dissolved solid (°Brix)

Brix values, representing total dissolved solids, exhibited a gradual increase in all formulations over the 30-day storage period (Fig. 1d). The control sample reached approximately 0.95 °Brix by day 30, suggesting a slight concentration of soluble solids due to moisture loss. In contrast, formulations containing Sesban flower extract (J1%, J3%, and J5%) maintained significantly more stable Brix values over time ($P<0.05$), indicating better control of water loss and soluble solid retention.

The increase in Brix observed in the control may be attributed to syneresis or evaporation, which concentrates sugars and other dissolved compounds as water is lost. However, the greater Brix stability in SFE-enriched samples suggests that polyphenolic compounds in the extract may interact with the gel matrix, enhancing water-binding capacity and

View Article Online
DOI: 10.1039/D5FB00227C

reducing water migration [26-28]. Such interactions can form a denser gel network that restricts the mobility of water molecules, helping to maintain equilibrium between moisture and dissolved solids.

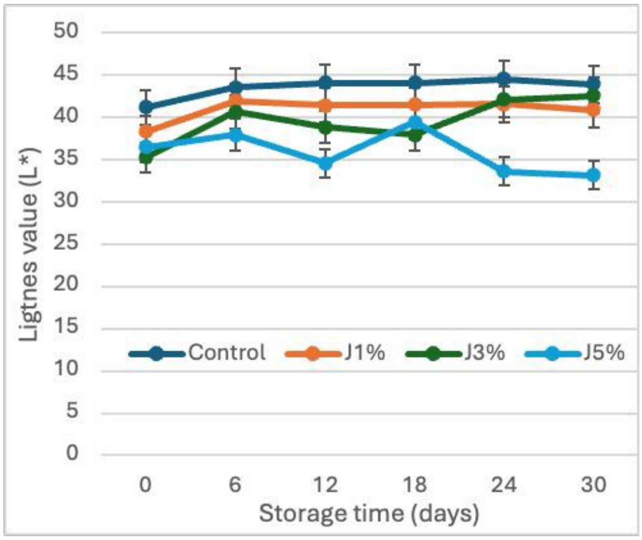
Phenolic compounds have also been shown to bind water molecules through hydrogen bonding, thereby reducing free water evaporation and maintaining equilibrium between moisture and dissolved solids [17, 29]. This stabilization not only preserves sweetness and consistency but also contributes to maintaining overall product quality during storage.

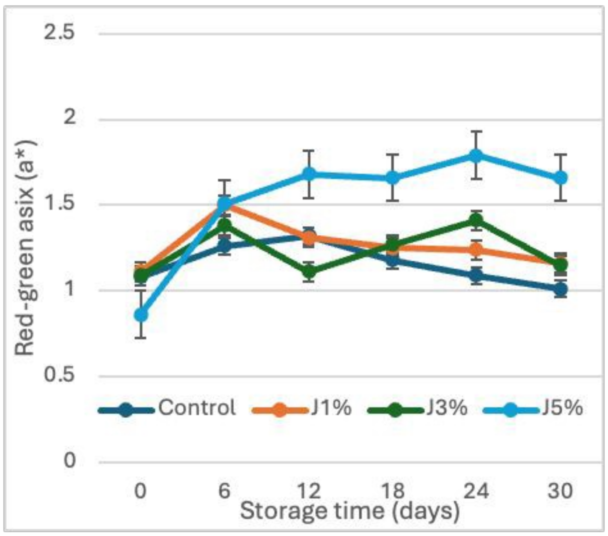
Collectively, the results indicate that the incorporation of SFE enhances the physicochemical stability of jelly, further supporting its role in improving shelf-life performance and functional quality.

3.1.5 Color stability (L, a**, b*, ΔE)

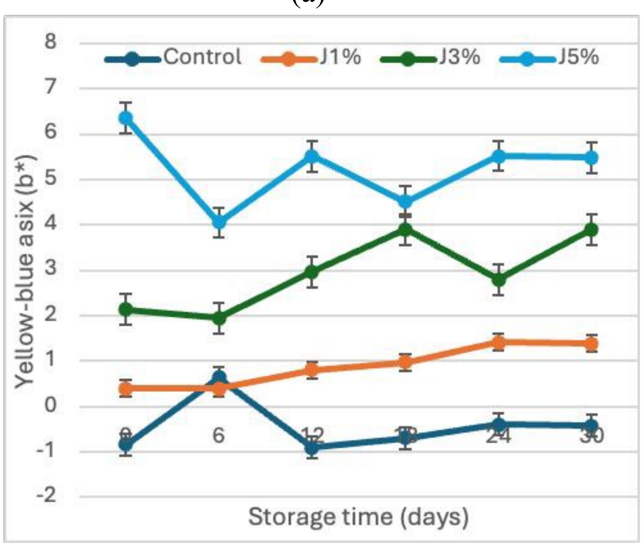
The initial color parameters (L*, a*, b*) for each formulation (Table 2) varied significantly, with J5% exhibiting the highest b* (yellow-blue) values, indicating a strong yellowish tint contributed by the extract. Over the storage period, fluctuations in L* and b* values were observed across all formulations, with the control sample generally maintaining the most stable color profile (Fig. 2). The reduction in lightness (L*) and increase in yellowness (b*) in higher extract concentrations may be attributed to pigment oxidation or degradation under storage, as is common in products rich in natural phenolics [30].

View Article Online
DOI: 10.1039/D5FB00227C





(a) (b)



(c)

View Article Online
DOI: 10.1039/D5FB00227C

Fig. 2 Changes in color parameters (L*, a*, b*) of jelly formulations with varying concentrations of SFE during 30 days of storage at 4°C. Values are means ± SD (n = 3). Different letters at each time point indicate significant differences among samples (P < 0.05).

Formulations with higher SFE levels (e.g. J3% and J5%) showed greater stability in greenness (a*) values compared to the control, suggesting that bioactive compounds such as flavonoids may help preserve color integrity by acting as natural antioxidants [31-33].

Table 3. Color difference (ΔE) values of jelly formulations during 30 days of storage.

Time (Day)	Color change (ΔE)			
	Control	(J1%)	(J3%)	(J5%)
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
6	2.64 ± 0.18[a]	3.71 ± 0.20[b]	6.03 ± 0.25[c]	4.82 ± 0.28[d]
12	2.91 ± 0.19[a]	3.63 ± 0.22[b]	7.19 ± 0.32[c]	6.91 ± 0.30[d]
18	3.02 ± 0.21[a]	4.28 ± 0.25[b]	6.48 ± 0.34[c]	6.21 ± 0.35[c]
24	3.36 ± 0.24[a]	4.30 ± 0.28[b]	8.40 ± 0.39[c]	9.53 ± 0.42[d]
30	4.24 ± 0.31[a]	6.66 ± 0.36[b]	8.98 ± 0.46[c]	11.29 ± 0.52[d]

Different superscripts indicate significant differences between values in the same row (p<0.05).

The ΔE values, representing overall color difference, increased over storage for all samples, with J3% and J5% showing higher ΔE values than the control (Table 3). While elevated ΔE values indicate more pronounced color changes, the practical significance of these changes depends on perceptual thresholds. According to [34], a ΔE value below 1.5 is generally undetectable to the human eye, whereas values between 1.5 and 3.0 are noticeable but often acceptable, and values above 3.0 indicate obvious color differences that may impact consumer acceptance. In the present study, the ΔE values for the SFE formulations exceeded 3.0 after storage, implying noticeable color changes. However, these changes may still be acceptable if

View Article Online
DOI: 10.1039/D5FB00227C

the yellowish hue is perceived positively as a signal of natural ingredients or functional properties, as has been documented in consumer studies on plant extract-enriched jellies [8, 17, 35].

Therefore, while the ΔE values indicate measurable color differences, the consumer perception of such color shifts remains context-dependent, influenced by expectations for natural coloration in functional foods. Further sensory studies would be beneficial to determine acceptable ΔE thresholds specific to jelly products containing SFE.

3.2 Texture profile of jelly formulations

3.2.1 Firmness (hardness)

The initial firmness of the jelly formulations differed significantly ($P<0.05$) depending on the concentration of Sesban flower extract (SFE). The control sample had the highest hardness value (11.05 ± 0.06 N), while formulations with SFE showed progressively lower values, with J3% exhibiting the lowest initial firmness (3.26 ± 0.58 N) (Table 2). This inverse relationship suggests that the inclusion of SFE contributes to a softer gel structure. Over the 30-day storage period, firmness gradually decreased across all formulations (Fig. 3a), likely due to gel relaxation or internal moisture redistribution. The control jelly, despite its higher initial firmness, also showed notable softening by day 30.

The reduction in firmness with increasing SFE concentration is likely attributable to interactions between phenolic and polysaccharide compounds in the extract and the gelling matrix. These bioactive constituents may interfere with carrageenan's network formation by competing for water or disrupting hydrogen bonding, resulting in a less rigid gel structure [36, 37]. Additionally, phenolic compounds may bind weakly within the gel matrix, leading to lower gel strength and increased softness.

View Article Online
DOI: 10.1039/D5FB00227C

Similar reductions in firmness following the incorporation of plant extracts into gel systems have been reported by [38] and [8], supporting the present findings. Their studies noted that natural extracts tend to disrupt polymeric gel formation, producing softer textures, a desirable trait in products aimed at children or elderly consumers.

Beyond its bioactive and antioxidant functions, SFE appears to play a textural role in product development. The softer texture observed at higher extract concentrations may enhance consumer acceptability by providing a more pleasant mouthfeel, underscoring the multifunctional potential of SFE in developing functional gel-based foods.

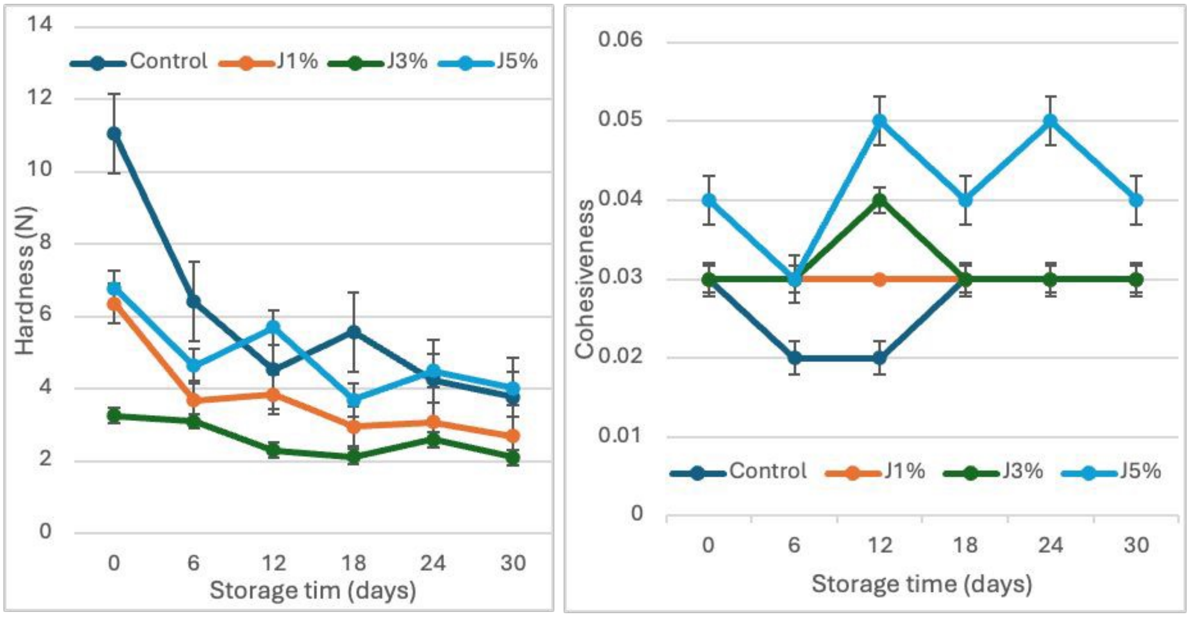
3.2.2 Springiness

Springiness, which reflects the jelly's ability to recover its original shape after deformation, showed minimal variation across all formulations (Table 2). No significant differences ($P>0.05$) were observed between the control and SFE-enriched samples, indicating that the addition of Sesban flower extract did not adversely affect this textural attribute.

The consistency of springiness values suggests that while SFE may alter firmness and reduce gel strength, it does not compromise the product's elastic behavior. This implies that the integrity of the gel's three-dimensional network remains intact despite the presence of extract compounds. Such resilience is crucial for maintaining a desirable bite and chewiness, especially in gel-based products expected to hold their form during handling and consumption.

These results are consistent with prior findings that certain plant-based ingredients may modify structural firmness without significantly disrupting spring-like behavior [39]. The preservation of springiness supports the notion that SFE can be incorporated into jelly formulations without negatively impacting the tactile and functional qualities expected by consumers.

View Article Online
DOI: 10.1039/D5FB00227C



(a) (b)

Fig. 3 Changes in texture profile attributes of jelly samples formulated with SFE during 30 days of storage at 4°C: (a) firmness (N), and (b) cohesiveness. Values are means ± SD (n = 3). Different letters indicate significant differences among samples at the same time point ($P < 0.05$).

3.2.3 Cohesiveness

Cohesiveness, defined as the degree to which a gelled product holds together under repeated stress, showed comparable initial values across all jelly formulations, ranging from 0.03 to 0.04 (Table 2). However, during storage, a marked increase in cohesiveness was observed in formulations with higher concentrations of Sesban flower extract (SFE), particularly in J5%, with peak values occurring around days 6 and 18 (Fig. 3b). The observed fluctuations in cohesiveness during storage could result from temporary rearrangements in the gel network as moisture redistributes and bioactive compounds interact dynamically with carrageenan chains [40].

This enhancement may be attributed to interactions between bioactive components in the extract, especially phenolic compounds and the carrageenan gel network. These compounds may

View Article Online
DOI: 10.1039/D5FB00227C

contribute to additional cross-linking or reinforce structural integrity within the matrix, improving its ability to recover from mechanical stress and resist fragmentation [40].

The increased cohesiveness in SFE-enriched formulations suggests a stabilizing effect of the extract on the internal gel structure. This is particularly valuable during extended storage, where maintaining gel uniformity and texture is essential for consumer satisfaction. These findings underscore the multifunctionality of SFE, not only enhancing antioxidant and antimicrobial properties but also contributing positively to the mechanical strength and structural resilience of jelly formulations.

3.2.4 Gumminess and chewiness

Gumminess and chewiness values were significantly higher ($P<0.05$) in the control jelly compared to the SFE-enriched formulations (Table 2). The control exhibited a gumminess value of 0.33 ± 0.01, while SFE-containing formulations ranged from 0.11 ± 0.05 (J3%) to 0.28 ± 0.18 (J5%). Similarly, chewiness values declined from 0.12 ± 0.00 in the control to 0.04 ± 0.01 (J3%) and 0.11 ± 0.08 (J5%), indicating that the addition of SFE resulted in a softer texture that required less effort to chew.

These reductions align with the observed decrease in firmness and suggest that SFE interferes with gel network development, thereby weakening the structural density of the jelly. During storage, gumminess and chewiness values declined slightly across all formulations, but SFE-enriched samples consistently maintained significantly lower values than the control (Fig. 3c, 3d). This trend supports the hypothesis that bioactive compounds in SFE modulate the gel structure throughout the storage period.

Similar observations were reported by [41], who found that incorporating natural plant extracts into pectin-based jellies reduced gel rigidity and chewiness. The softening effect of SFE

View Article Online
DOI: 10.1039/D5FB00227C

is likely attributed to phenolic and polysaccharide components that disrupt carrageenan's ability to form a compact gel network [42]. These compounds may reduce cross-linking density, resulting in a more pliable and less gummy texture.

The observed decrease in firmness and gumminess in jellies with higher SFE concentrations could potentially align with consumer preferences for softer gel textures, which are often perceived as more palatable, especially for specific populations such as children and the elderly [38, 41]. However, this study did not include sensory evaluation to directly confirm consumer preference. Future work should incorporate sensory testing to validate whether the modified texture profiles of SFE-enriched jellies are indeed preferred by consumers.

3.3 Total phenolic content (TPC)

The initial total phenolic content (TPC) of the jelly formulations varied significantly ($P<0.05$), with the control sample having the lowest value (1.22 ± 0.42 mg GAE/g dw), compared to formulations containing Sesban flower extract (Table 4). Among the extract-enriched samples, J5% exhibited the highest TPC (9.63 ± 0.29 mg GAE/g dw), followed by J3% (5.08 ± 0.68 mg GAE/g dw) and J1% (2.32 ± 0.10 mg GAE/g dw), confirming a dose-dependent contribution of SFE to the phenolic enrichment of the jelly.

During refrigerated storage, TPC declined across all formulations (Fig. 4a). The control showed the steepest reduction, retaining only 0.74 ± 0.21 mg GAE/g dw by day 30. In contrast, J5% and J3% maintained significantly higher levels (6.12 ± 0.45 and 3.85 ± 0.37 mg GAE/g dw, respectively). These results suggest that higher SFE concentrations mitigate oxidative degradation, likely due to the stabilizing effect of its polyphenolic constituents.

The degradation of phenolics during storage is a well-established process driven by oxidation and environmental stressors such as light, oxygen, and pH [43]. However, SFE's

View Article Online
DOI: 10.1039/D5FB00227C

phenolic profile potentially rich in flavonols and hydroxycinnamic acids may offer antioxidant synergy that delays this loss. Phenolics can act both as radical scavengers and as stabilizers by interacting with gelling agents, forming complexes that resist oxidative breakdown [44].

These findings highlight the functional potential of SFE not only as a phenolic source but also as a stabilizer in gel-based products. The results align with previous studies reporting phenolic retention and antioxidant stability in food systems enriched with plant extracts like *Moringa oleifera* and *Rosmarinus officinalis* [8, 41].

Table 4. Initial antioxidant properties of different jelly formulations: TPC, TFC, DPPH, and FRAP values

Formulation	TPC (mg GAE/g dw.)	TFC (mg QE/g dw.)	DPPH (µM Trolox/g dw.)	FRAP (µM Trolox/g dw.)
Control	1.22 ± 0.42[c]	0.89 ± 0.03[c]	2.48 ± 0.95[d]	1.17 ± 0.94[b]
J1%	2.32 ± 0.10[c]	1.50 ± 0.18[c]	7.45 ± 0.02[c]	1.61 ± 0.08[b]
J3%	5.08 ± 0.68[b]	3.21 ± 0.38[b]	11.01 ± 0.29[b]	4.11 ± 0.08[b]
J5%	9.63 ± 0.29[a]	5.27 ± 0.28[a]	14.04 ± 0.20[a]	8.81 ± 1.53[a]

Data presented in average ± deviation. Different letters show significant difference among data ($p<0.05$).

3.4 Total flavonoid content (TFC)

The total flavonoid content (TFC) of the jelly formulations showed significant differences (P<0.05) among the tested samples, reflecting the impact of Sesban flower extract (SFE) concentrations (Table 4). The control sample had the lowest initial TFC (0.89 ± 0.03 mg QE/g dw), while J5% showed the highest (5.27 ± 0.28 mg QE/g dw), followed by J3% (3.21 ± 0.38 mg QE/g dw) and J1% (1.50 ± 0.18 mg QE/g dw). This clear dose-dependent trend demonstrates the contribution of SFE to increasing flavonoid content in the jelly matrix.

View Article Online
DOI: 10.1039/D5FB00227C

Throughout the 30-day storage period, TFC values declined across all formulations (Fig. 4b), likely due to oxidative degradation, a common fate of flavonoids in aqueous and semi-solid food systems. By day 30, the control retained only 0.51 ± 0.02 mg QE/g dw, whereas J5% and J3% retained significantly higher levels (3.92 ± 0.31 and 2.48 ± 0.25 mg QE/g dw, respectively; P<0.05). The higher retention rates in SFE formulations indicate that the extract's antioxidant environment may offer protection against oxidation, preserving flavonoid integrity.

Flavonoids are particularly sensitive to environmental factors such as light, oxygen, and temperature. However, when co-existing with other phenolic compounds as in SFE, they may form synergistic antioxidant systems. Such synergy could enhance radical scavenging efficiency and delay the oxidation cascade, as previously noted in studies using polyphenol-rich plant extracts [45, 46]. Furthermore, flavonoid–gel matrix interactions may stabilize the compounds via hydrophobic binding or hydrogen bonding, limiting molecular mobility and degradation.

These findings underscore the functional potential of SFE not only as a flavonoid source but also as a stabilizing system that preserves bioactivity during shelf life. They are consistent with results from other flavonoid-enriched gel-based systems, such as those formulated with hibiscus, dandelion, or rosemary extracts [8, 17].

3.5 DPPH radical scavenging activity

The DPPH radical scavenging activity, a widely used indicator of antioxidant potential, was significantly enhanced (P<0.05) in jelly formulations containing Sesban flower extract (SFE), compared to the control (Table 4). The control showed the lowest initial activity (2.48 ± 0.95 µM Trolox/g dw), while J5% exhibited the highest (14.04 ± 0.20 µM Trolox/g dw), followed by J3% (11.01 ± 0.29 µM Trolox/g dw) and J1% (7.45 ± 0.02 µM Trolox/g dw). These

View Article Online
DOI: 10.1039/D5FB00227C

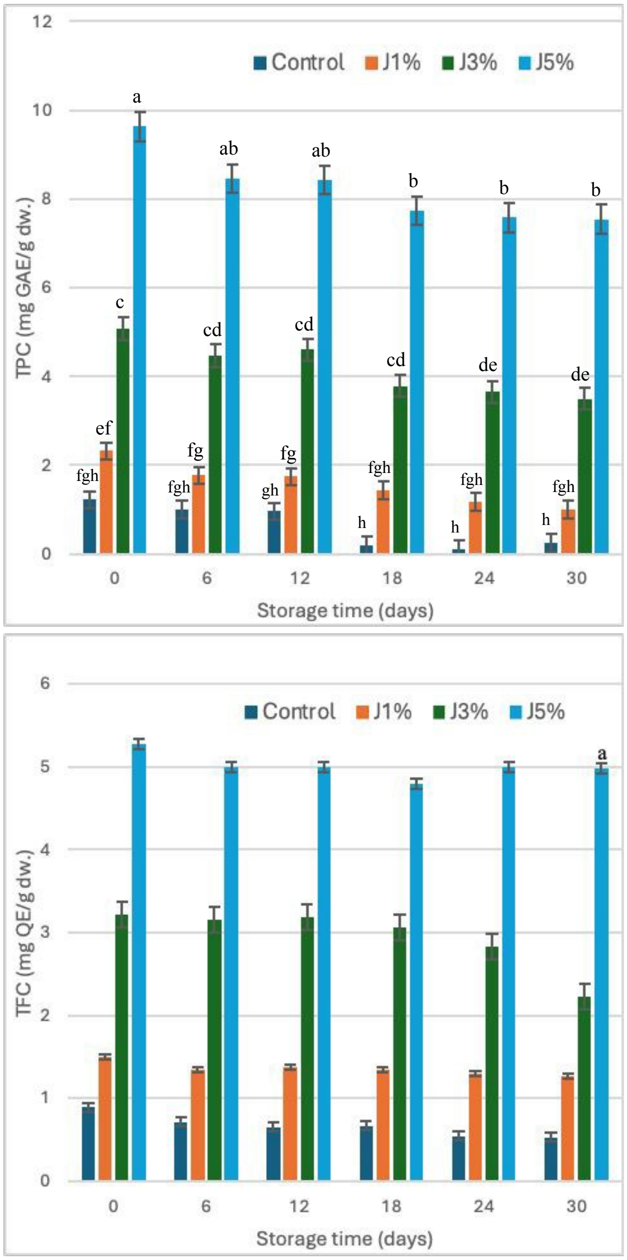
findings confirm a dose-dependent enhancement in free radical scavenging capacity imparted by the SFE.

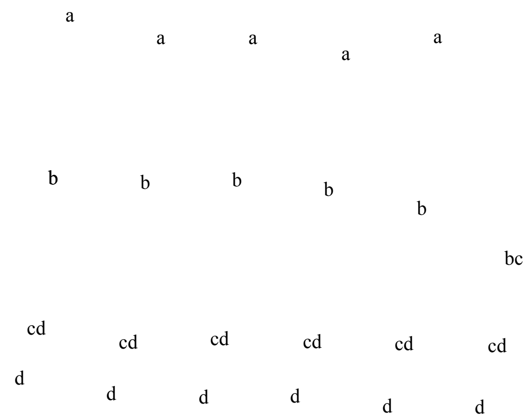
During 30-day storage, a gradual decline in DPPH activity was observed across all formulations (Fig. 4c). The control experienced the steepest reduction, retaining only 0.92 ± 0.05 µM Trolox/g dw by day 30. In contrast, J5% and J3% retained significantly higher activities (9.26 ± 0.38 and 6.83 ± 0.22 µM Trolox/g dw, respectively), suggesting better antioxidant preservation in extract-containing jellies.

This trend correlates with the observed decreases in TPC and TFC over time, supporting the role of phenolic and flavonoid compounds as key contributors to antioxidant activity in plant-based foods [47]. The superior retention of DPPH activity in J5% and J3% may result from both the quantity and quality of phenolics in the extract, which actively neutralize free radicals and interrupt oxidative chain reactions [45].

Additionally, the presence of synergistic bioactive compounds in SFE could enhance antioxidant effectiveness through multiple mechanisms including electron donation, hydrogen transfer, and metal ion chelation, thereby extending the extract's protective effect during storage [46]. Similar observations have been reported in jelly systems fortified with other antioxidant-rich extracts such as hibiscus and dandelion [8, 17], further validating the multifunctional role of SFE in improving oxidative stability in gel-based food products.

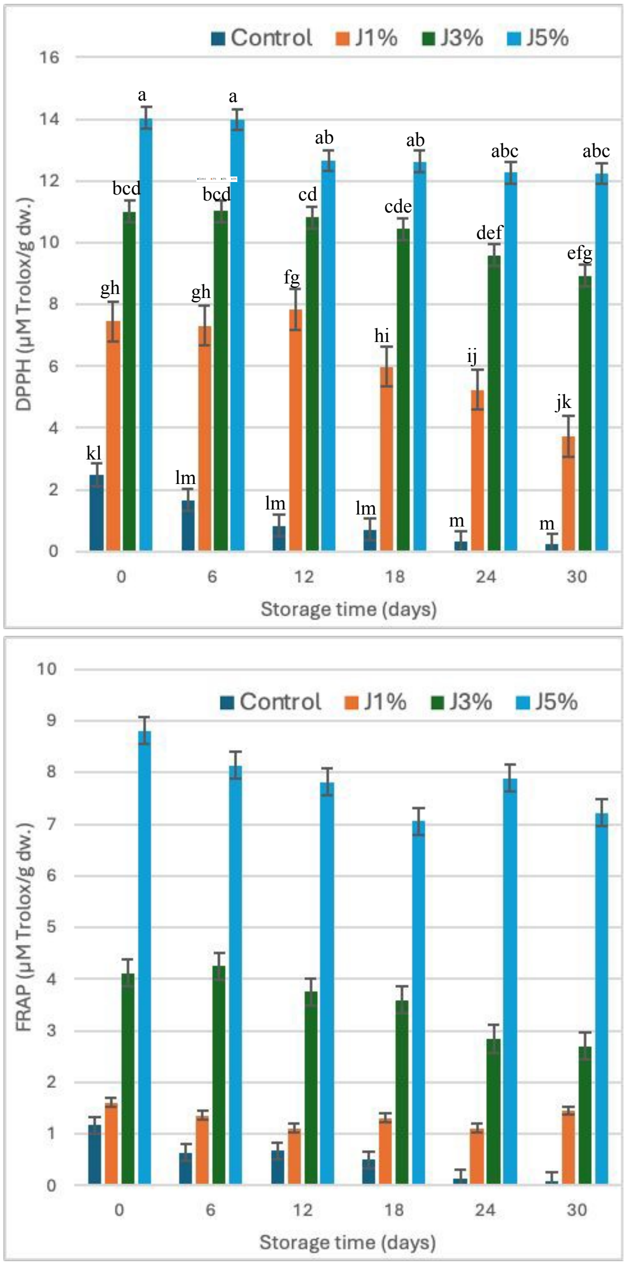
View Article Online
DOI: 10.1039/D5FB00227C





(a) (b)

View Article Online
DOI: 10.1039/D5FB00227C



(c) (d)

Fig. 4 Changes in antioxidant properties of jelly samples containing SFE during 30 days of storage at 4°C: (a) total phenolic content (TPC), (b) total flavonoid content (TFC), (c) DPPH radical scavenging activity, and (d) FRAP reducing power. Values are means ± SD (n = 3). Different letters indicate significant differences among formulations at each time point ($P < 0.05$).

3.6 Ferric reducing antioxidant power (FRAP)

View Article Online
DOI: 10.1039/D5FB00227C

The ferric reducing antioxidant power (FRAP), which reflects the ability of bioactive compounds to reduce Fe^{3+} to Fe^{2+}, was significantly higher ($P<0.05$) in jelly formulations containing Sesban flower extract (SFE) compared to the control (Table 4). Among the treatments, J5% showed the highest initial FRAP value (8.81 ± 1.53 µM Trolox/g dw), followed by J3% (4.11 ± 0.08 µM Trolox/g dw) and J1% (1.61 ± 0.08 µM Trolox/g dw), while the control had the lowest value (1.17 ± 0.94 µM Trolox/g dw).

Over 30 days of refrigerated storage, FRAP values declined across all formulations (Fig. 4d). The control exhibited the sharpest reduction, retaining only 0.45 ± 0.02 µM Trolox/g dw by day 30. In contrast, J5% and J3% retained significantly higher FRAP activities (6.02 ± 0.25 and 3.25 ± 0.18 µM Trolox/g dw, respectively), indicating greater stability of reducing agents in SFE-enriched samples.

The FRAP assay is strongly influenced by the presence of reducing compounds such as phenolic acids, flavonoids, and other polyphenols, which donate electrons to neutralize oxidizing species [48]. The sustained FRAP values in SFE-enriched jellies suggest that these compounds not only enhance initial antioxidant capacity but also resist oxidative degradation during storage. The protective effect is likely reinforced by synergistic interactions among different polyphenols in the extract.

Previous studies have demonstrated similar trends, where the incorporation of phenolic-rich plant extracts improved reducing power and extended antioxidant functionality in gel-based systems enriched with natural extract such as dandelion, hibiscus, or rosemary [17, 49, 50]. This is attributed to their dual action: directly scavenging free radicals and maintaining redox balance by stabilizing the food matrix [46].

In summary, the inclusion of Sesban flower extract at higher concentrations significantly boosts and sustains FRAP values during storage, confirming its role as a potent natural

View Article Online
DOI: 10.1039/D5FB00227C

antioxidant. These results further support its application as a functional ingredient for enhancing the oxidative stability, nutritional value, and shelf life of jelly and other soft-textured food products.

It is important to note that antioxidant measurements in jelly matrices can be affected by matrix effects, including gel network entrapment of compounds, water activity variations, and interactions with hydrocolloids such as carrageenan. Both DPPH and FRAP assays were selected because they are widely validated and commonly applied in semi-solid food matrices, including gel-based systems [12, 50]. While DPPH primarily measures free-radical scavenging ability through hydrogen atom donation, FRAP quantifies reducing power via electron transfer mechanisms. Together, these assays provide complementary insights into the antioxidant potential of complex food matrices like jelly.

However, it should be acknowledged that neither assay fully replicates *in vivo* antioxidant behavior, and results can be influenced by sample solubility and diffusion limitations inherent in gel systems. To minimize these limitations, our study included an extraction step from the jelly matrix prior to assay performance, following optimized protocols adapted for gel-based foods [9, 10]. Despite these precautions, some variability inherent to gel systems may remain. Future investigations might benefit from applying kinetic modeling approaches, such as zero-order or first-order degradation kinetics, to standardize antioxidant decay rates and enable more robust comparisons of antioxidant retention over storage. Such models could correct for matrix effects and enhance the interpretation of antioxidant stability data in gel-based food products, as suggested by [48] and [49].

In addition, it is relevant to note which specific phenolic compounds might be responsible for the observed antioxidant and antimicrobial effects of SFE. Although this study focused on functional properties, our earlier work [7] identified isorhamnetin, kaempferol, chalcones,

View Article Online
DOI: 10.1039/D5FB00227C

flavanones, and caftaric acid as key phenolic constituents in *Sesbania javanica* flower extract. These compounds are known for potent antioxidant activity via mechanisms such as hydrogen atom donation, radical scavenging, and metal ion chelation [44, 46]. Moreover, flavanones, isorhamnetin, caftaric acid, chalcones, and kaempferol contribute to antimicrobial effects by disrupting microbial cell membranes, inhibiting bacterial enzymes, and interfering with quorum sensing pathways [51, 52]. Their presence likely underpins both the elevated antioxidant values and the microbiological stability observed in SFE-enriched jelly formulations. Future studies should aim to quantify these specific compounds within the jelly matrix to better correlate individual bioactives with functional outcomes.

3.7 Microbiological quality

3.7.1 Total plate count

The total plate count (TPC) remained undetectable (ND) in all jelly formulations during the first 12 days of storage (Table 5). In the control sample, microbial growth was first observed on day 18, increasing to $4.50 \times 10^2 \pm 2.12$ CFU/g by day 24. At this stage, visible spoilage and mold formation were present, and the sample was no longer suitable for microbiological analysis on day 30 (recorded as "NDS": Not Determined due to Spoilage). In contrast, J1% exhibited detectable microbial growth only at day 30, reaching $5.50 \times 10^2 \pm 0.71$ CFU/g, while both J3% and J5% maintained undetectable microbial counts throughout the 30-day storage period.

These findings highlight the antimicrobial potential of Sesban flower extract, especially at concentrations of 3% and 5%. The ability of these formulations to inhibit microbial proliferation suggests that SFE contains bioactive compounds capable of extending product shelf life by delaying microbial spoilage. Flavonoids and phenolic acids, abundant in SFE, are known

View Article Online
DOI: 10.1039/D5FB00227C

to exert antimicrobial effects by disrupting bacterial cell walls, altering membrane permeability, and inhibiting enzymatic activity essential for microbial growth [51, 53].

Notably, in many commercial jelly products, synthetic preservatives such as potassium sorbate or sodium benzoate are commonly used to inhibit microbial growth at levels up to 1000 ppm, from Codex Alimentarius, 2023 [54]. Although this study did not directly benchmark SFE against specific preservatives, the absence of microbial growth in J3% and J5% throughout 30 days is comparable to performance reported for chemically preserved gels. Furthermore, food safety standards typically allow aerobic plate counts up to 10^4 CFU/g in non-pathogenic confectionery products [55]. The counts observed in the control and J1% remained well below this threshold, indicating microbiological acceptability, though SFE offers additional protection by keeping counts undetectable.

Additionally, phenolic compounds may chelate essential minerals or disrupt microbial quorum sensing pathways, further reducing microbial colonization and growth. The dose-dependent inhibition observed here supports prior findings on the antimicrobial efficacy of phenolic-rich plant extracts in perishable food systems. Overall, the results suggest that incorporating SFE at sufficient concentrations may eliminate the need for synthetic preservatives while maintaining microbiological safety.

Table 5. Microbiological quality of of jelly formulations during 30 days of cold storage.

Formulation	Time (days)	Total plate count (CFU/g)	Yeast & Mold (CFU/g)	*Salmonella* spp.	*Staphylococcus aureus*
Control	0	ND	ND	ND	ND
	6	ND	ND	ND	ND
	12	ND	ND	ND	ND
	18	$1.50\times10^2 \pm 0.71$	ND	ND	ND

View Article Online
DOI: 10.1039/D5FB00227C

	24	$4.50×10^2 ± 2.12$	$5.00×10^2 ± 2.83$	ND	ND
	30	*NDS*	*NDS*	*NDS*	*NDS*
J1%	0	ND	ND	ND	ND
	6	ND	ND	ND	ND
	12	ND	ND	ND	ND
	18	ND	ND	ND	ND
	24	$1.50×10^2 ± 0.71$	$2.50×10^2 ± 0.71$	ND	ND
	30	$5.50×10^2 ± 0.71$	$6.50×10^2 ± 0.71$	ND	ND
J3%	0-30	ND	ND	ND	ND
J5%	0-30	ND	ND	ND	ND

ND: Not Detected; *NDS*: Not Determined due to visible spoilage and mold, as evidenced by changes in color, texture, and odor, making further microbiological analysis impractical. No specific microbial threshold was defined; visible spoilage alone triggered termination of further testing.

3.7.2 Yeast and mold count

Yeast and mold were undetectable in all jelly formulations up to day 18 of storage. By day 24, the control sample exhibited a yeast and mold count of $5.00 × 10^2 ± 2.83$ CFU/g, indicating early fungal contamination. In J1%, fungal growth was first detected at day 30, reaching $6.50 × 10^2 ± 0.71$ CFU/g. In contrast, both J3% and J5% showed no detectable fungal growth throughout the entire 30-day storage period, indicating strong resistance to spoilage.

The absence of yeast and mold in the higher extract concentrations (J3% and J5%) suggests that Sesban flower extract exerts effective antifungal activity when incorporated above a certain threshold. Phenolic compounds and flavonoids in the extract are known to suppress fungal proliferation by disrupting spore germination, increasing membrane permeability, and interfering with key metabolic and enzymatic pathways [56]. For context, regulatory standards typically regard yeast and mold counts below 10^3 CFU/g as acceptable in confectionery products

View Article Online
DOI: 10.1039/D5FB00227C

(FDA, 2022). The observed counts remained well below this limit, and the complete inhibition in J3% and J5% formulations demonstrate SFE's potential to match or exceed the efficacy of synthetic antifungal agents like sorbates and benzoates.

These results support the hypothesis that SFE can serve as a natural antifungal preservative in gel-based food products, particularly at 3% and 5% concentrations. The dose-dependent inhibition observed aligns with previous reports on the antifungal potential of phenolic-rich plant extracts in controlling mold growth in minimally processed foods. Therefore, the inclusion of SFE not only improves antioxidant performance but also provides microbiological protection, offering a clean-label strategy for extending shelf life.

3.7.3 Salmonella spp. *and Staphylococcus aureus*

Salmonella spp. and *Staphylococcus aureus* were not detected (ND) in any of the jelly formulations, including the control, throughout the 30-day storage period. This consistent absence indicates that the formulation process, hygienic handling, and storage conditions were effective in minimizing contamination by these common foodborne pathogens. It also affirms the safety of the jelly product under refrigerated storage conditions.

Notably, the inclusion of Sesban flower extract in the formulations may have contributed additional antibacterial protection. Phenolic compounds, which are abundant in SFE, have been reported to exhibit strong antibacterial effects by compromising bacterial membrane integrity, inhibiting nucleic acid synthesis, and disrupting enzymatic systems necessary for microbial survival [52]. Although not directly benchmarked against synthetic antimicrobials, the absence of pathogens in all SFE-containing formulations suggests comparable efficacy in preventing foodborne hazards.

View Article Online
DOI: 10.1039/D5FB00227C

Overall, the microbiological analysis confirms that SFE, particularly at higher concentrations, not only delays spoilage organisms like total plate count and fungi but may also contribute to a hostile environment for pathogenic bacteria. Similar microbial inhibitory effects have been reported in jellies containing rosemary and oregano extracts, though our results indicate that SFE at 3% and 5% achieved complete microbial inhibition over 30 days, which compares favorably to those systems [52]. These results highlight the potential of Sesban flower extract as a natural antimicrobial agent for enhancing food safety and extending shelf life without reliance on synthetic preservatives.

4. Conclusions

This study demonstrates the potential of *Sesbania javanica* (Sesban flower) extract (SFE) as a sustainable, multifunctional ingredient for enhancing the quality, antioxidant capacity, and microbial safety of jelly formulations. The incorporation of SFE, particularly at 3% and 5% concentrations, not only improved shelf life and product stability but also offered a natural alternative to synthetic preservatives, aligning with consumer demand for clean-label and eco-friendly food products. Moreover, utilizing SFE leverages a locally available, underutilized botanical resource, contributing to biodiversity utilization and sustainability.

However, several limitations remain. This study was conducted at laboratory scale, and scalability for industrial production has not yet been assessed. Consumer acceptability and sensory evaluation were not performed, leaving the impact of SFE on taste, color perception, and overall consumer preference unverified. Additionally, the stability of bioactive compounds under commercial processing conditions requires further investigation.

Future research should therefore focus on evaluating sensory properties and consumer acceptance of SFE-enriched jellies, assessing the scalability and cost-effectiveness of SFE

View Article Online
DOI: 10.1039/D5FB00227C

production, characterizing the specific bioactive compounds responsible for antioxidant and antimicrobial effects, and testing the stability and efficacy of SFE under industrial manufacturing and storage conditions. These investigations will support the broader application of SFE as a natural functional ingredient, promoting innovation and sustainability in the food industry.

Data availability

The data in the current study are available from the corresponding author upon reasonable request.

Ethics declaration

This research did not involve the use of human participants, human data or tissue, or animals. Accordingly, ethical approval was not required.

Funding

This research project was supported by The Second Century Fund (C2F), Chulalongkorn University, Thailand and funded by the 90th Anniversary of Chulalongkorn University Scholarship under the Ratchadapisek Somphot Endowment Fund (GCUGR1125671047M).

Acknowledgement

The author (SC) would like to thank Second Century Fund (C2F), Chulalongkorn University for providing the PhD Scholarship.

Authors Contribution

View Article Online
DOI: 10.1039/D5FB00227C

Sochannet Chheng: Data curation, Formal analysis, Investigation, Methodology, and Writing – original draft and Writing – review & editing. Saeid Jafari: Data curation and Writing – original draft. Dharmendra K. Mishra: Supervision and Writing – original draft. Kitipong Assatarakul: Conceptualization, Data curation, Funding acquisition, Project administration, Supervision, Writing – original draft and Writing – review & editing.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. A. Moure, J. M. Cruz, D. Franco, J. M. Domı́nguez, J. Sineiro, H. Domı́nguez, M. a. J. Núñez and J. C. Parajó, *Food chemistry*, 2001, **72**, 145-171.
2. D. Krishnaiah, R. Sarbatly and R. Nithyanandam, *Food and bioproducts processing*, 2011, **89**, 217-233.
3. J. Necas and L. Bartosikova, *Veterinarni medicina*, 2013, **58**, 187-205.
4. O. Brahim Mahamat, S. Younes, B. B. Otchom, S. Franzel, A.-D. Ouchar Mahamat Hidjazi and E. i. Soumaya, *The Scientific World Journal*, 2024, **2024**, 1225999.
5. N. P. Minh, *Journal of Pure & Applied Microbiology*, 2021, **15**.
6. E. Teixeira-Lemos, A. R. Almeida, B. Vouga, C. Morais, I. Correia, P. Pereira and R. P. Guiné, *Open Agriculture*, 2021, **6**, 466-478.
7. S. Chheng, M. Fikry, S. Jafari, S. K. Mehta, D. K. Mishra and K. Assatarakul, *ES Food & Agroforestry*, 2025, DOI: 10.30919/faf1434.
8. T. T. Nguyen, I. F. Olawuyi, J. J. Park and W. Y. Lee, *Journal of Food Measurement and Characterization*, 2022, 1-13.
9. R. R. do Nascimento, T. C. Pimentel, S. Garcia and S. H. Prudencio, *Food Bioscience*, 2023, **56**, 103128.
10. A. A. Masri, F. I. Bakar, M. Abidin and N. H. Malik, *Tropical Journal of Natural Product Research*, 2023, **7**.
11. M. Fikry, S. Jafari, K. A. Shiekh, I. Kijpatanasilp, S. Chheng and K. Assatarakul, *Food and Bioprocess Technology*, 2024, 1-18.
12. K. Thaipong, U. Boonprakob, K. Crosby, L. Cisneros-Zevallos and D. H. Byrne, *Journal of Food Composition and Analysis*, 2006, **19**, 669-675.
13. S. Chheng, M. Fikry, S. Jafari, D. K. Mishra and K. Assatarakul, *Journal of Stored Products Research*, 2025, **112**, 102660.
14. Y. Liu, C. K. Lim, Z. Shen, P. K. Lee and T. Nah, *Atmospheric Chemistry and Physics Discussions*, 2022, **2022**, 1-29.

View Article Online
DOI: 10.1039/D5FB00227C

15. S. Elahi and H. Fujikawa, *Journal of food science*, 2019, **84**, 3233-3240.
16. Y. Y. Zhao, F. H. Cao, X. J. Li, D. D. Mu, X. Y. Zhong, S. T. Jiang, Z. Zheng and S. Z. Luo, *International Journal of Food Science & Technology*, 2020, **55**, 785-794.
17. S. C. de Moura, C. L. Berling, A. O. Garcia, M. B. Queiroz, I. D. Alvim and M. D. Hubinger, *Food Research International*, 2019, **121**, 542-552.
18. Ernawati, H. Suryadi and A. Mun'im, 2021.
19. D. N. Afifah, F. Ayustaningwarno, A. Rahmawati, D. N. Cantikatmaka, N. Wigati, E. R. Noer, N. Widyastuti, H. S. Wijayanti, D. N. Sugianto and Y. P. A. Ningrum, *Scientific Reports*, 2023, **13**, 15161.
20. J. Pan, C. Li, J. Liu, Z. Jiao, Q. Zhang, Z. Lv, W. Yang, D. Chen and H. Liu, *Foods*, 2024, **13**, 3896.
21. F. Zhu, *Critical reviews in food science and nutrition*, 2018, **58**, 1808-1831.
22. Z. J. Lee, C. Xie, K. Ng and H. Suleria, 2025.
23. Y. Wang, J. Liu, F. Chen and G. Zhao, *Journal of Agricultural and Food Chemistry*, 2013, **61**, 4533-4538.
24. F. Herzyk, D. Piłakowska-Pietras and M. Korzeniowska, *Foods*, 2024, **13**, 1713.
25. A. Capuzzo, M. E. Maffei and A. Occhipinti, *Molecules*, 2013, **18**, 7194-7238.
26. M. Maisto, E. Schiano, E. Novellino, V. Piccolo, F. Iannuzzo, E. Salviati, V. Summa, G. Annunziata and G. C. Tenore, *Foods*, 2022, **11**, 1453.
27. H. Tang, A. D. Covington and R. Hancock, *Biopolymers: Original Research on Biomolecules*, 2003, **70**, 403-413.
28. S. Tang, X. Yang, C. Wang and C. Wang, *Molecules*, 2025, **30**, 1674.
29. Ł. Sęczyk, U. Gawlik-Dziki and M. Świeca, *Antioxidants*, 2021, **10**, 1825.
30. R. Pandiselvam, S. Mitharwal, P. Rani, M. A. Shanker, A. Kumar, R. Aslam, Y. T. Barut, A. Kothakota, S. Rustagi and D. Bhati, *Current Research in Food Science*, 2023, **6**, 100529.
31. E. M. Altuner and Ö. Tokuşoğlu, *International journal of food science & technology*, 2013, **48**, 1991-1997.
32. C. d. S. Araújo, W. C. Vimercati, L. L. Macedo, S. H. Saraiva, L. J. Q. Teixeira, J. M. G. da Costa and C. J. Pimenta, *Journal of Food Science*, 2022, **87**, 4056-4067.
33. J. D. Everette, Q. M. Bryant, A. M. Green, Y. A. Abbey, G. W. Wangila and R. B. Walker, *Journal of agricultural and food chemistry*, 2010, **58**, 8139-8144.
34. R. D. Paravina, R. Ghinea, L. J. Herrera, A. D. Bona, C. Igiel, M. Linninger, M. Sakai, H. Takahashi, E. Tashkandi and M. d. Mar Perez, *Journal of Esthetic and Restorative Dentistry*, 2015, **27**, S1-S9.
35. M. Spinei and M. Oroian, *Foods*, 2023, **13**, 98.
36. P. S. Widyawati, T. I. P. Suseno, A. I. Widjajaseputra, T. E. W. Widyastuti, V. W. Moeljadi and S. Tandiono, *Molecules*, 2022, **27**, 5062.
37. F. Zhu, *Trends in Food Science & Technology*, 2015, **43**, 129-143.
38. K. M. Akelom, T. Y. Bisetegn and T. Y. Bereka, *Heliyon*, 2022, **8**.
39. K. Kaewpetch, S. Yolsuriyan, T. Disayathanoowat, P. Phokasem, T. Jannu, G. Renaldi and R. S. Samakradhamrongthai, *Gels*, 2024, **10**, 282.
40. I. Faber, L. Pouvreau, A. J. van der Goot and J. Keppler, *Food Hydrocolloids*, 2024, **154**, 110123.
41. M. Aydın and D. Arslan, *Taiwanese Journal of Agricultural Chemistry & Food Science*, 2022, **60**.
42. N. Yusof, I. Jaswir, P. Jamal and M. S. Jami, *Malaysian Journal of Fundamental and Applied Sciences*, 2019, **15**, 604-608.
43. M. Mrázková, D. Sumczynski and J. Orsavová, *Antioxidants*, 2023, **12**, 962.
44. J. M. Lü, P. H. Lin, Q. Yao and C. Chen, *Journal of cellular and molecular medicine*, 2010, **14**, 840-860.
45. S. H. Hassanpour and A. Doroudi, *Avicenna journal of phytomedicine*, 2023, **13**, 354.
46. P.-G. Pietta, *Journal of Natural Products*, 2000, **63**, 1035-1042.

View Article Online
DOI: 10.1039/D5FB00227C

47. J.-S. Kim and J.-H. Lee, *Preventive Nutrition and Food Science*, 2020, **25**, 84.
48. R. Amarowicz and R. B. Pegg, in *Advances in food and nutrition research*, Elsevier, 2019, vol. 90, pp. 1-81.
49. P. Chaudhary, P. Janmeda, A. O. Docea, B. Yeskaliyeva, A. F. Abdull Razis, B. Modu, D. Calina and J. Sharifi-Rad, *Frontiers in chemistry*, 2023, **11**, 1158198.
50. Q. D. Nguyen, T. T. Dang, T. V. L. Nguyen, T. T. D. Nguyen and N. N. Nguyen, *Food science & nutrition*, 2022, **10**, 191-203.
51. I. Górniak, R. Bartoszewski and J. Króliczewski, *Phytochemistry reviews*, 2019, **18**, 241-272.
52. N. Oulahal and P. Degraeve, *Frontiers in Microbiology*, 2022, **12**, 753518.
53. K. Ecevit, A. A. Barros, J. M. Silva and R. L. Reis, *Future Pharmacology*, 2022, **2**, 460-498.
54. N. D. Fortin, in *Research handbook on international food law*, Edward Elgar Publishing, 2023, pp. 227-242.
55. I. C. o. M. S. f. Foods, *Microorganisms in foods 8: Use of data for assessing process control and product acceptance*, Springer Science & Business Media, 2011.
56. W. Mączka, M. Twardawska, M. Grabarczyk and K. Wińska, *Antibiotics*, 2023, **12**, 824.

View Article Online
DOI: 10.1039/D5FB00227C

Data availability Statement

The data in the current study are available from the corresponding author upon reasonable request.