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Storage quality assessment of a sheet snack produced from liang leaves (*Gnetum gnemon* var. *tenerum*)

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ORIGINAL ARTICLE

Abstract

This study investigates a novel leaf sheet snack from liang, an indigenous vegetable in Southern Thailand. The prepared snack has high protein, fiber, calcium, and vitamins A and B_2. Storage conditions and packaging impact its shelf life. Based on the calculation, laminated aluminum foil extended shelf life to 1 year at 30°C and relative humidity (RH) of 60%. Moisture absorption during storage led to physicochemical changes, including reduced vitamin content, while total polyphenols, flavonoids, and antioxidant activity remained stable. Microbiological quality was acceptable over a period of 12 weeks but high RH during storage deteriorated sensory attributes. Liang leaves showed promise of being a functional food ingredient.

Keywords: antioxidant; liang leaf; packaging material; shelf life; vegetable sheet snack

Introduction

Liang (*Gnetum gnemon* var. *tenerum*), a leafy vegetable found in Southern Thailand, belongs to the *Gnetum* family and is recognized as a rich source of essential amino acids, dietary fiber, vitamins, and minerals, as well as for its anti-diabetic properties (Anisong *et al*., 2022; Siripongvutikorn *et. al*., 2023; Suksanga *et al*., 2022). The bioactive compounds in liang leaves possess antioxidant, antidiabetic, and anti-inflammatory properties, contributing various health benefits (Anisong *et al*., 2022; Suksanga *et al*., 2022). However, fresh vegetables are a typically delicacy material containing high moisture, with optimum pH (5–6), and harbor microbial load, which results in a short shelf life and difficulties in transport and commercial storage. Perishable characteristics of vegetables lead to deteriorate of health benefits compounds therefore people have a high risk of not having enough phytochemicals for health benefit. The increasing prevalence of cancer in younger age group (<50 years) is associated with factors, such as inadequate sleep, insufficient exercise, alcohol consumption, intake of processed or unhealthy food, and smoking. These factors contribute to conditions, such as overweight, hypertension, obesity, and cancer (AlOudat *et al*., 2021; *The Harvard Gazette*, 2022).

Healthy diet, regular exercise, and a sound environment are important for good health. Consumption of unhealthy diet high in sugar, saturated fat, and salt increases the risk of non-communicable diseases (NCDs), such as diabetes, cancer, hypertension, and heart disease whereas a healthy diet can help prevent these conditions (Bergman *et al*., 2022). Thus, consumers now prefer nutritious foods with health benefits that are convenient to eat, particularly

healthy snacks. Ready-to-eat snacks, such as dried vegetable sheets or leathers made from vegetable paste mixed with seasonings, are gaining popularity in the international market. These snacks offer high nutritional value, good taste, and convenience, and are easier to transport and store because of their light weight, low water activity (a_w), and low moisture content, which leads to a longer shelf life (Jiang *et al*., 2021).

Incorporating liang leaves into snacks offers a healthier alternative to conventional snacks, contributing to better overall dietary patterns and potentially reducing the risk of diet-related diseases. In addition, developing snacks from indigenous plants, such as liang, is not only consistent with growing consumer demand for health but also meets the concept of sustainable food systems. The Food and Agriculture Organization of the United Nations (FAO, n.a.) defines sustainable food systems as those that deliver food security and nutrition in such a way that the economic, social, and environmental bases for generating food security and nutrition for future generations are not compromised. This includes promoting local food production, minimizing the environmental impact, ensuring economic viability, and supporting social equity and health. Pretreatment techniques are used as well to enhance the quality of dried vegetables. Blanching is one of the most popular pretreatment procedures for maintaining or improving color and texture. Fruits and vegetables are blanched in hot water or with chemical solutions, such as sodium chloride (Saencom *et al*., 2011), calcium chloride (Severini *et al*., 2003), and magnesium carbonate (Maharaj and Sankat, 1996). The main purpose of blanching with hot water and/or chemical solution is to prevent enzymatic browning reaction (Wibowo *et al*., 2019) and reduce microbial contamination (Vandeweyer *et al.*, 2017). Different texture-modifying agents, such as agar, carrageenan, and glycerin, are added as well at concentrations of 0.5–1% (w/w) to improve the texture of dried vegetable sheets (Dumrongratkul *et al*., 2005). This study aimed to develop a nutritious alternative product to dissolve conventional unhealthy snacks by using the rich nutritional profile of liang leaves. Additionally, this study evaluated the changes in quality during storage, which is essential for ensuring product stability, safety, and nutritional value over time. This research also identified potential areas for improvement in product, storage conditions, and packaging.

Materials and Methods

Preparation of sheet snacks

A flowchart of sheet snack preparation process is shown in Figure 1. Liang leaves (*Gnetum gnemon* var. *tenerum*) of intermediate edible stage (Pae Salat) (Figure 1A) were purchased from a local farm. The stems were detached and leaves without stems were washed with chlorinated water at 100 ppm for 15 min to clean and remove debris. The leaves were washed twice with running tap water to control chlorine residue (<1 ppm as a safety regulation) and drained on a basket for 10 min, with thickness of overlay leaves being not more than 1 cm. Liang leaves were blanched with brine water (NaCl 1%) for 3 min at 100°C and then immediately cooled with cold water. Excess water was drained.

Seasoning was prepared by mixing xanthan gum, water, sugar, salt, oyster sauce, and sesame oil in an undeclared amount because of petty patent and entrepreneur requirements. Then, the mixture of seasoning and blanched leaves was blended in specified proportions into a paste before spreading onto a Teflon sheet in a flat mold of 10-cm wide, 15-cm long, and 0.5-cm thick. The paste was then dried at 70°C for 5 h in a hot air oven to obtain a dried product with a moisture content of less than 8% (Figure 1B). The sheet snack was cut in half, packaged, and heat-sealed in laminated aluminum foil and nylon/linear low-density polyethylene (LLDPE) pouches with two pieces per pouch. The packaged samples were used to study quality changes during 12-week (W) storage at 30°C and relative humidity (RH) 60% and 90%. The treatments investigated were laminated aluminum foil packaging stored at RH 60% (A60), laminated aluminum foil packaging stored at RH 90% (A90), nylon/LLDPE packaging stored at RH 60% (N60), and nylon/LLDPE packaging stored at RH 90% (N90).

Nutrition facts

Products at the beginning and after storage for 12 weeks were analyzed for nutritional value by the Centre of Measurement and Standard with Accreditation, Faculty of Science, Prince of Songkla University (ISO/EIC 17025:2017). Moisture, protein, fat, ash, fiber, sugar, saturated fatty acid, cholesterol, and mineral (sodium, calcium, and iron) contents were determined according to Association of Official Analytical Chemist (AOAC, 2019), while carbohydrate content was determined according to Ellefson (1993). Vitamins A, B_1, and B_2 were determined according to the methods described by Visuthi (1994), Woollard and Indyk (2002), and Wehling and Wetzel (1984), respectively. The energy value was determined according to the method described by Sullivan (1993).

Moisture and water activity

Moisture content and a_w were determined using the oven method (AOAC, 2000) and a_w analyzer (METER-AQUALAB PRE, Decagon Devices Inc., Washington, USA), respectively.

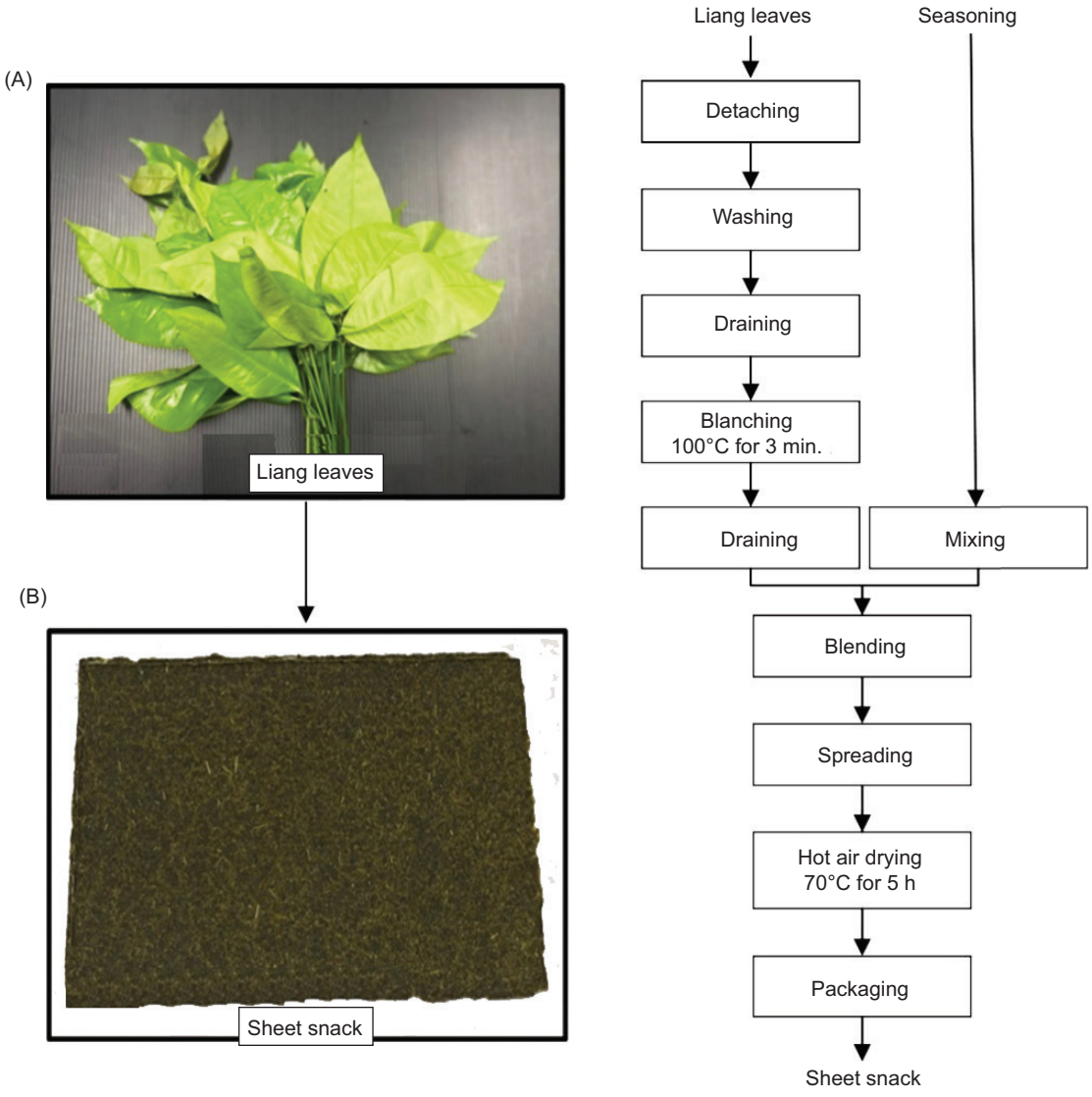

Figure 1. Sheet snack preparation process.

Water vapor transmission rate and permeability coefficient

The water vapor transmission rate (WVTR) of the films was determined following the standard cup gravimetric method, according to the American Society for Testing and Materials (ASTM) standard E96-80. Aluminium cups containing the films were secured with a silicone ring and stored in a humidity-controlled chamber at 25°C and RH 50%. The cups were weighed daily periodically for 7 days using an analytical balance. The WVTR was determined from the slope of a plot drawn between storage time and weight gain and the exposed area of films. The permeability coefficient (P) was calculated using Equation (1):

$$P = \frac{WVTR \times L}{\Delta p}, \quad (1)$$

where:

WVTR = water vapor transmission rate,

L = film thickness, and

Δp = water vapor pressure difference across the film.

Moisture content change

The moisture content was evaluated by determining moisture gain during storage under two different conditions: 30°C and RH 60%, and 30°C and RH 90%. Weight measurements were taken every 2 weeks for a period of 12 weeks, during which the samples were stored in their tested packaging. The moisture content of the packaged product was calculated using Equation (2) at each measurement interval:

$$\text{Mt}\ (\%) = \left[\frac{Wt \times (1 + Mi)}{Wi} - 1\right] \times 100, \quad (2)$$

where:

Mt = product moisture content at time *t*,
Mi = initial moisture content of the sample,
Wi = initial weight of the sample, and
Wt = weight of the sample at time *t*.

Shelf life estimation of sheet snack

The shelf life of a sheet snack can be mathematically estimated based on the water vapor permeation of packaging film and storage conditions. Approximately 3 g of each sample was stored in a humidity-controlled chamber at 30°C and RH 75%. The samples were evaluated every 1 h for sensory crispness for 3–4 days until the products reached an unacceptable crispness level. The critical moisture content and water activity of the samples were measured. The shelf life of the sheet snack was estimated based on Equation (3):

$$t = \frac{q \times L}{A \times P \times \Delta p}, \quad (3)$$

where:

q = quantity of absorbed moisture of product to reach critical moisture content,
L = film thickness,
A = film area,
P = permeability coefficient of the film,
Δp = water vapor pressure difference across the film.

$$\mathrm{p} = \frac{1}{2} \times ps \times ((a_w o - a_w i) + (a_w o - a_w c)), \quad (4)$$

where:

Δp = water vapor pressure difference across the film.
ps = saturated water vapor pressure at storage temperature,
a_wo = water activity of storage conditions,
a_wi = initial water activity of the sample, and
a_wc = critical water activity of the sample.

pH and Brix value

pH and Brix were measured using a pH meter (Sartorius-Sartorius AG, Docu-pH+ Meter, Goettingen, Germany) and a refractometer (Atago, Pen Refractometer, Tokyo, Japan), respectively.

Color change

Changes in colors were determined by Commission Internationale de l'Eclairage (CIE) L*, a*, and b* color space using a colorimeter (ColorFlex EZ; Hunter Associates Laboratory Inc., Virginia, USA) and expressed as ΔE, as shown in Equation (5).

$$\Delta \mathrm{E} = \sqrt{(\Delta \mathrm{L})^2 + (\Delta \mathrm{a})^2 + (\Delta \mathrm{b})^2} \quad (5)$$

where:

Standard = freshly produced sample,
ΔE = color difference between the standard
ΔL = difference between lightness (L*) and the standard
Δa = difference between redness–greenness (a*) and the standard, and
Δb = difference between yellowness–blueness (b*) and the standard.

Total polyphenols content, total flavonoid content, and antioxidant activity

Total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity were reported as standard equivalent units per assay, and values of the product during storage were compared to the value of freshly produced product using Equation (6) (Jayakody *et al*., 2021):

$$\text{Relative value (\%)} = \frac{\text{value}}{\text{initial}} \times 100 \quad (6)$$

where:

relative value (%) = percentage value of the initial value,
value = value of the product during specific storage period, and
initial = value of freshly produced product.

Sample preparation and extraction

The samples were mixed with 90% ethanol (v/v) in a ratio of 1:10 and stirred in the dark at 25°C for 24 h. The mixture was then separated by vacuum suction using a Buchner funnel and centrifuged at 17,700 ×g at 4°C for 15 min. Evaporator was used to vaporize ethanol to obtain a concentrated sample.

Total phenolic content

Total phenolic content was determined using the method described by Singleton and Rossi (1965) with some modifications. Briefly, 20 µL of the sample extract was added to a 96-well plate, followed by 100 µL of 10% Folin reagent (v/v). After incubation in the dark at 30°C for 6 min, 7.5% Na_2CO_3 (w/v) was added, and the mixture was incubated for another 30 min. The absorbance of the mixture was measured at 765 nm using gallic acid, Trolox, and

L-ascorbic acid as standards at concentrations of 0–100 μg/mL (R^2 = 0.999), 0–500 μg/mL (R^2 = 0.999), and 0–200 μg/mL (R^2 = 0.999), respectively.

Total flavonoid content

Total flavonoid content was determined using the method described by Ha *et al.* (2020) with some modifications. Briefly, 100 μL of the sample extract was mixed with 100 μL of 2% $AlCl_3$ (w/v) and incubated in the dark at 30°C for 60 min. Absorbance of the mixture was measured at 420 nm using quercetin and rutin as standards at concentrations of 0–20 μg/mL (R^2 = 0.999) and 0–80 μg/mL (R^2 = 0.998), respectively.

2,2-Diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity was determined using the method described by Brand-Williams *et al*. (1955) with some modifications. First, 100 μL of the sample extract was mixed with 100 μL of 0.2-mM DPPH in 95% ethanol. After kept in the dark for 30 min at 30°C, the absorbance of the mixture was measured at 517 nm using gallic acid, Trolox, and L-ascorbic acid as standards at concentrations of 0–2.5 μg/mL (R^2 = 0.998), 0–12 μg/mL (R^2 = 0.998), and 0–14 μg/mL (R^2 = 0.997), respectively.

2,2-Azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging activity

The ABTS assay was performed as described by Arnao *et al.* (2001). ABTS radicals were generated by incubating 7.4 mM of ABTS solution in the dark at 30°C for 12 h. The radical solution was then diluted to obtain an absorbance of 1.1 ± 0.02 at 734 nm. Subsequently, 20 μL of the sample extract was mixed with 280 μL of the radical solution and incubated in the dark for 2 h at 30°C. The absorbance of the mixture was measured at 734 nm using gallic acid, Trolox, and L-ascorbic acid as standards at concentrations of 0–21 μg/mL (R^2 = 0.998), 0–110 μg/mL (R^2 = 0.999), and 0–110 μg/mL (R^2 = 0.999), respectively.

Ferric reducing antioxidant power (FRAP)

The FRAP assay was performed using the method described by Benzie and Strain (1996). A freshly prepared FRAP solution containing 300-mM acetate buffer (pH 3.6) and 10-mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40-mM HCl and 20-mM $FeCl_3$ $6H_2O$ (10:1:1) was warmed at 37°C for 30 min. Next, 15 μL of the sample extract was mixed with 285 μL of FRAP solution and incubated for 30 min at 37°C. The absorbance of the mixture was measured at 593 nm using gallic acid, Trolox, L-ascorbic acid, and $FeSO_4$ as standards at concentrations of 0–12 μg/mL (R^2 = 0.999), 0–100 μg/mL (R^2 = 0.999), 0–100 μg/mL (R^2 = 0.999), and 0–90 μg/mL (R^2 = 0.999), respectively.

Microbiological quality

Critical microbial types, such as total viable count (TVC), coliform bacteria, *Escherichia coli*, and yeast and mold (YM), were evaluated for microbial safety by the Center of Measurement and Standard Accreditation, Faculty of Science, Prince of Songkla University (ISO/IEC 17025:2017).

Sensory evaluation

Eight attributes of samples, such as appearance, color, odor, texture, flavor, taste, overall acceptability, and percentage of consumer acceptance, were evaluated using a 9-point hedonic scale (1 to 9) by 50 untrained panelists with the ethical guidelines outlined by the PSU Human Research Ethics Committee (reference No. 2023-005-1-1).

Statistical analysis

All quality parameters, except sensory tests, were performed using a completely randomized design (CRD) whereas the sensorial score was determined using a randomized complete block design. Differences in mean values and variations were tested using one-way ANOVA with Tukey's test.

Results and Discussion

Nutrition facts

According to the nutrition claim based on the Ministry of Public Health (1998), the developed sheet snack was classified as having high levels of protein, fiber, calcium, vitamin A, and vitamin B_2 (Table 1). The Ministry of Public Health (1998) also recommends that the daily dietary intake for humans should include 50 g of protein, 25 g of fiber, 4.8 mg of vitamin A, 1.7 mg of vitamin B_2, 800 mg of calcium, and 15 mg of iron. Consuming a 24-g portion of the sheet snack would provide 7.12% of protein, 16.11% of fiber, 13.40% of vitamin A, 8.19% of vitamin B_2, 7.17% of calcium, and 5.44% of iron of the recommended daily intake (RDI). The sheet snack had a relatively high protein and dietary fiber contents.

Table 1. Nutrition facts of freshly produced sheet snacks and those after 12-week storage (per 100 g/wet basis).

Parameter	Treatments					Nutrition claims per 100-g sample (high level)*
	F	A60	A90	N60	N90	
Moisture content (g)	3.56	4.27	4.14	14.46	16.65	–
Protein (g)	14.83	14.45	14.52	12.92	11.81	10
Carbohydrate (g)	64.78	64.00	63.77	56.46	57.40	–
Dietary fiber (g)	16.78	20.97	20.14	20.93	22.86	6
Sugar (g)	12.33	28.03	21.92	20.54	20.68	–
Total fat (g)	9.52	9.74	10.01	9.56	8.27	–
Saturated fatty acid (g)	1.63	1.79	1.81	1.74	1.48	–
Cholesterol (mg)	4.44	5.19	4.92	6.18	5.72	–
Ash content (g)	7.31	7.54	7.56	6.6	5.87	–
Vitamin A (mg)	2.68	0.98	0.76	0.79	0.89	1.44
Vitamin B_1 (mg)	ND	ND	ND	ND	ND	0.45
Vitamin B_2 (mg)	0.58	ND	ND	ND	ND	0.51
Sodium (g)	1.78	1.97	2.03	1.74	1.53	–
Calcium (mg)	238.94	191.19	196.79	181.14	165.79	240
Iron (mg)	3.40	2.43	1.90	1.52	1.23	4.5
Total energy (kcal)	404.12	401.46	403.25	363.56	351.27	–
Energy from fat (kcal)	85.68	87.66	90.09	86.04	74.43	–

Notes: F: freshly produced sheet snack; A60: laminated aluminum foil packaging stored at RH 60%; A90: laminated aluminum foil packaging stored at RH 90%; N60: nylon/LLDPE packaging stored at RH 60%; N90: nylon/LLDPE packaging stored at RH 90%; ND: not detected; *high level: a nutrition claim that describes high levels of a nutrient contained in the food following the notification of the Ministry of Public Health (1998); "-" indicates not regulated.

Siripongvutikorn *et al*. (2023) reported that liang leaves are a good source of fiber, which was approximately 40% on dry basis. By contrast, potato chips generally have low protein and fiber contents. Khalil *et al.* (2023) found that the protein content of potato chips ranged from 5.43% to 9.25%, which was significantly lower than sheet snacks. The high fiber content of sheet snacks was also superior to potato chips, which typically contain less than 3 g of fiber per 100 g (Miller *et al.*, 1998).

Potato chips are known for their high-fat content, which leads to negative health outcomes if consumed in excess. Khalil *et al*. (2023) reported that the fat content of potato chips ranged from 34.47% to 36.63%, depending on cultivars. By comparison, sheet snacks had lower fat content, ranging from 8.27% to 10.01%. Sheet snacks also contained notable amounts of micronutrients, such as vitamin A, calcium, and iron. Potato chips are not typically considered a significant source of vitamins and minerals, suggesting that sheet snacks may be a healthier alternative to conventional snacks in terms of nutrition. Sheet snacks are unfried products that may have lower acrylamide levels than potato chips, making them a potentially safer choice for consumers. During 12-week storage, energy, protein, and carbohydrate contents were reduced in N60 and N90, with increased moisture content. The increase in dietary fiber and sugar content was unexpected, possibly due to hydrolysis or the formation of new compounds, including xylan and homogalacturonan (Lin *et al*., 2022; Schäfer *et al*., 2017).

The moisture content determined by the standard method using the oven method exhibited gradual moisture absorption in sheet snacks, which can be attributed to the hygroscopic properties of dried foods, leading to an increase in water activity and moisture content. This facilitates non-enzymatic and chemical reactions, such as Maillard browning between proteins and reducing sugars (Wong *et al*., 2015). This can contribute to protein loss and changes in color and flavor over time. The gradual breakdown of complex carbohydrates into simpler sugars can also lead to a decrease in the carbohydrate content of snacks (Jan *et al*., 2022). The combined loss of protein and carbohydrate contents due to degradation processes can result in a decrease in the energy content of the product.

Vitamin A was reduced to less than 40% whereas vitamin B_2 was not detected after 12-week storage. This result concurred with results of Yang *et al.* (2021), who reported that 43–44% of vitamin A remained after 1 month when stored at room temperature. Vitamin A can be degraded by exposure to oxygen, ultraviolet light,

acidity, temperature, moisture, and impurities (Dhakal and He, 2020). By using nylon/LLDPE as a packaging material, vitamin A content is preserved in a better manner than using laminated aluminum foil packaging. Vitamins A (retinol) and provitamin A (β-carotene) exhibit antioxidant activity (Dottore *et al*., 2018), while vitamins C and E are more potent antioxidants than vitamin A (El-Senousey *et al*., 2018).

Oxygen is a major contributor to nutrient degradation (Fata *et al*., 2018). The free transmission of oxygen and soluble oxygen in water can lead to the degradation of oxidation-susceptible nutrients, particularly antioxidants. Vitamin C is the most sensitive nutrient to oxidation (Giannakourou and Taoukis, 2021) and may help prevent the oxidation of vitamin A. Reduced amount of compounds indicates self-degradation and/or the ability to protect other compounds. Overall, sheet snacks packaged in laminated aluminum foil (A60 and A90) showed better preservation of moisture and nutrient contents, particularly for protein, dietary fiber, and vitamin A, compared to those packaged in nylon/LLDPE (N60 and N90). The results suggest that laminated aluminum foil was an effective barrier in maintaining the nutritional quality of sheet snacks.

Moisture content and water activity

A sharp increase in moisture content, determined by the oven method, and a_w in nylon/LLDPE packaging was observed throughout the storage period whereas in laminated aluminum foil packaging, these parameters remained constant (Figures 2 and 3). As expected, both moisture content and a_w were higher at 90% RH than at 60% RH, even in heat-sealed packaging, indicating that high RH conditions and packaging materials played an important role.

Water reabsorption is a common problem in dried foods. If a_w reaches 0.6–0.7, then yeasts and molds grow faster (Hyun *et al*., 2018), leading to undesired characteristics, such as softness, sogginess, and rubbery (Le *et al*., 2021). The results revealed that a_w of N90 exceeded 0.6 and did not meet the community product standard for dried seaweed (Thai Industrial Standard Institute, 2014). In addition, some hyphae of mold were observed in N90 after storage for 10 weeks. A higher moisture content and a_w of the sheet snacks packaged in nylon/LLDPE packaging were observed because water vapor permeation of this packaging material was higher than that in the laminated aluminum foil packaging.

Water vapor transmission rate (WVTR) and permeability coefficient (P)

Permeability coefficient is an intrinsic property of a material that allows gas permeation whereas WVTR is a measure of gas flow through a specific material under given conditions (Wang *et al*., 2018a). As shown in Table 2, the laminated aluminum foil film exhibited significantly lower WVTR and P than the nylon/LLDPE film,

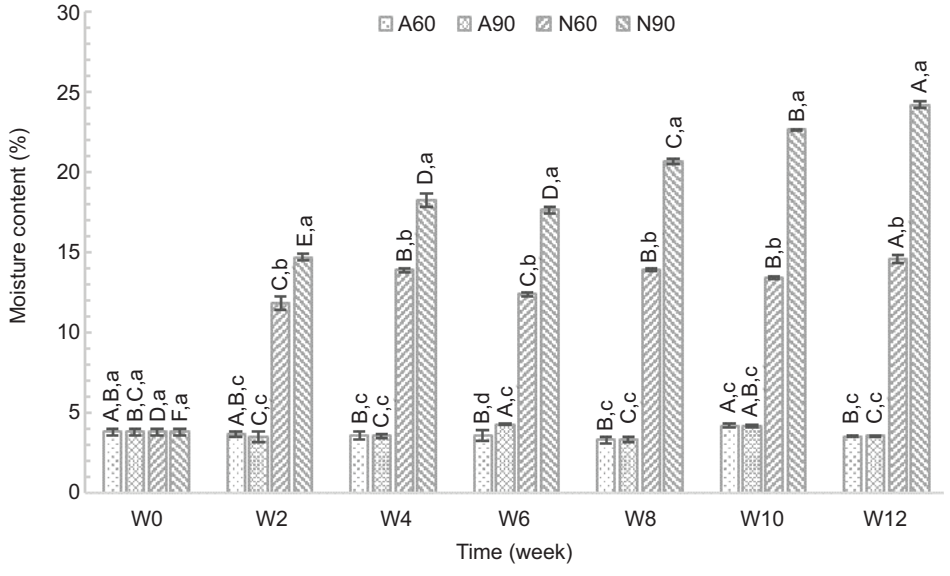

Figure 2. Moisture content of sheet snacks during 12-week storage (n = 3). Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments on each day ($p < 0.05$). A60: laminated aluminum foil packaging stored at RH 60%; A90: laminated aluminum foil packaging stored at RH 90%; N60: nylon/LLDPE packaging stored at RH 60%; N90: nylon/LLDPE packaging stored at RH 90%.

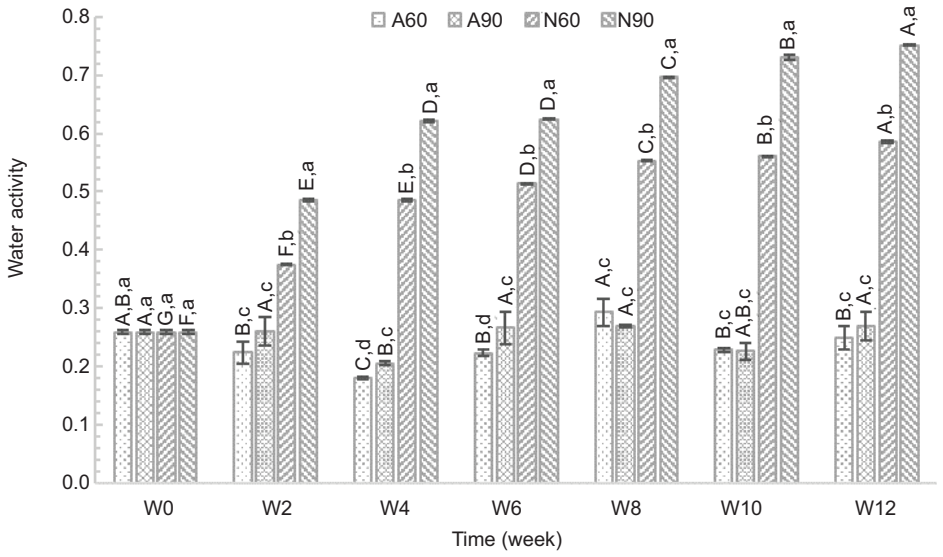

Figure 3. Water activity of sheet snacks during 12-week storage (n = 3). Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments on each day ($p < 0.05$). A60: laminated aluminum foil packaging stored at RH 60%; A90: laminated aluminum foil packaging stored at RH 90%; N60: nylon/LLDPE packaging stored at RH 60%; N90: nylon/LLDPE packaging stored at RH 90%.

indicating that the former provided higher barrier resistance to moisture transfer, suggesting that food products packaged in laminated aluminum foil films experienced slower moisture uptake from the surrounding environment, leading to prolonging shelf life. Conversely, higher WVTR and P values of nylon/LLDPE film implied greater permeability to water vapors, resulting in accelerated moisture transfer into packaged food, thereby reducing its shelf life. These findings aligned with previous research, highlighting the critical role of packaging materials in determining the moisture barrier properties and shelf life of food products. Han *et al.* (2018) and Robertson *et al*. (2020) emphasized the importance of selecting packaging materials with low WVTR and P values to minimize the moisture-related deterioration of food products, thereby extending their shelf life, while Lee and Yam (2019) described the adverse effects of high WVTR films on the quality and stability of packaged food items because of increased moisture absorption.

Table 2. Moisture barrier properties of pouches used for packaging sheet snacks.

Packaging film	Thickness (μm)	WVTR (g/day × cm^2)	P (g × μm/day × cm^2 × mmHg)
Laminated aluminum foil	93.0	3.22×10^{-6}	2.52×10^{-5}
Nylon/LLDPE	77.6	7.39×10^{-5}	4.83×10^{-4}

Notes: WVTR: water vapor transmission rate; P: permeability coefficient.

Change in moisture content

A stability study of sheet snacks packaged in laminated aluminum foil (A60, A90) and nylon/LLDPE (N60 and N90) was conducted at 60% RH and 90% RH at 30°C, and weight gain over time was monitored regularly. The moisture content shown in Figure 4 is derived from the storage stability study, where the moisture content was calculated periodically during the storage period using Equation (2). The results suggest that sheet snacks absorbed moisture from the surrounding environment because of their hygroscopic properties. The rate of absorption of moisture was faster at 90% RH than at 60% RH because more moisture was available for sheet snacks to absorb at higher RH. As expected, higher RH increased the moisture uptake of sheet snacks, leading to higher moisture content in the samples that prompted them to absorb moisture from their surroundings.

Increased moisture content accelerates various spoilage reactions, such as enzymatic browning, microbial growth, and textural changes, leading to a shorter shelf life (Al-Muhtaseb *et al*., 2002). The moisture content of samples packaged in nylon/LLDPE (N60 and N90) reached a critical point within 3 weeks and 2 weeks, respectively (Figure 4) whereas samples packaged in laminated aluminum foil (A60, A90) showed only a slight

increase in moisture content. The moisture content of both samples was 3.7% after 12 weeks of storage, with only a 0.15% increase from week 0. Thus, type of packaging film had a significant impact on the moisture content of the product. Sheet snacks packaged in laminated aluminum foil (A60 and A90) had a slower rate of moisture absorption than those packaged in nylon/LLDPE (N60 and N90) because laminated aluminum foil has a very high water vapor permeability barrier coefficient (P), compared to nylon/LLDPE, which has a high barrier coefficient (Wang *et al*., 2018a). Consequently, packaging in laminated aluminum foil and storing it under storage conditions with a lower RH can effectively extend the shelf life of sheet snacks.

Shelf life estimation of sheet snacks

Sheet snacks are perishable moisture-sensitive food items. As such, the moisture content is commonly used as an indicator of shelf life. Table 3 lists the parameters used to calculate the shelf life. Samples stored at 30°C and RH 60% had the longest shelf life whereas samples stored at 30°C and RH 90% had the shortest shelf life, corresponding to the changes in moisture content. Using laminated aluminum foil as packaging material extended the shelf life by up to 363 days or 1 year at RH 60%. The shelf life was reduced by 55% when stored at RH 90%. By contrast, samples packaged in nylon/LLDPE had shorter shelf lives of only 16 and 7 days, respectively, and the moisture content reached a critical point when stored at RH 60% and RH 90%, respectively, because of inferior moisture barrier. Consequently, storage in conditions with RH levels not exceeding 60%, combined with the use of laminated aluminum foil packaging, prevented the ingress of moisture from the environment and resulted in a shelf life of up to 1 year.

pH value

The pH values of all treatments during storage for 12 weeks are shown in Figure 5. Decrease in pH after storage for 2 weeks, especially in case of N90, indicated high acid

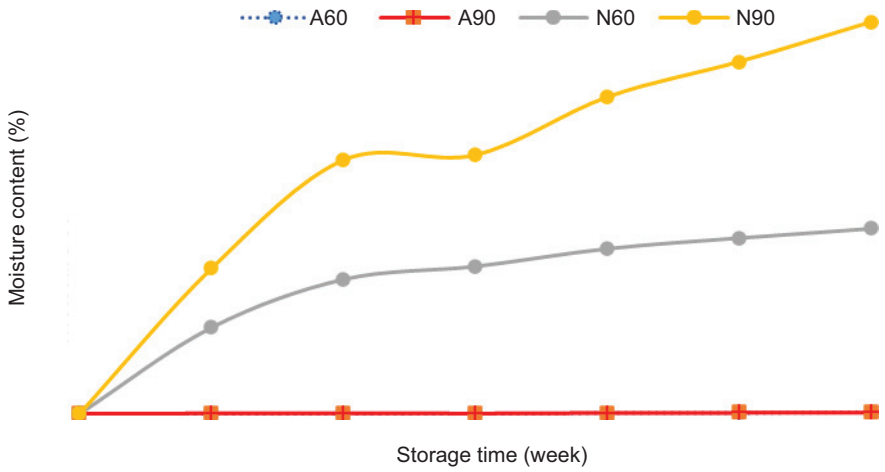

Figure 4. Moisture content of packaged sheet snacks during 12-week storage. A60: laminated aluminum foil packaging stored at RH 60%; A90: laminated aluminum foil packaging stored at RH 90%; N60: nylon/LLDPE packaging stored at RH 60%; N90: nylon/LLDPE packaging stored at RH 90%. Note: The line representing A60 is shown with blue circles and a dashed line, while the line for A90 is depicted with red squares and a solid line.

Table 3. Parameters used for estimation and calculation of shelf life.

Sample	Mi (%)	Mc (%)	a_wi	a_wc	a_wo	*ps* (mmHg)	Area (cm^2)	*Wi* (g)	Shelf life (days)
A60	3.56	11.77	0.26	0.44	0.6	31.824	315	3	363
A90	3.56	11.77	0.26	0.44	0.9	31.824	315	3	165
N60	3.56	11.77	0.26	0.44	0.6	31.824	315	3	16
N90	3.56	11.77	0.26	0.44	0.9	31.824	315	3	7

Notes: Mi: initial moisture of sample; Mc: critical moisture of sample; a_wi: initial water activity of sample; a_wc: critical water activity of sample; a_wo: water activity during storage condition; *ps*: saturated vapor pressure at storage temperature; *Wi*: initial weight of sample.

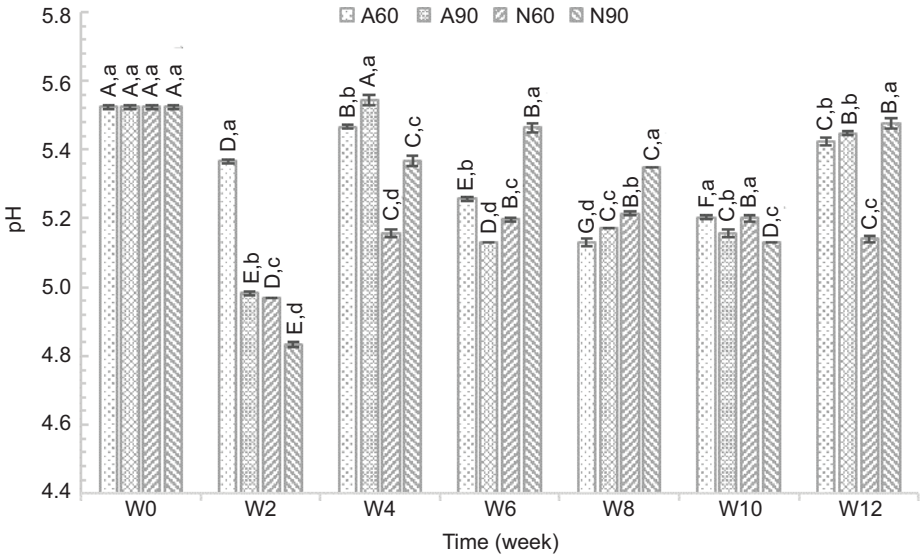

Figure 5. pH values of sheet snacks during 12-week storage (n = 3). Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments on each day ($p < 0.05$). A60: laminated aluminum foil packaging stored at RH 60%; A90: laminated aluminum foil packaging stored at RH 90%; N60: nylon/LLDPE packaging stored at RH 60%; N90: nylon/LLDPE packaging stored at RH 90%.

production during storage under high RH conditions. Higher moisture content and a_w values are associated with higher chances of hydrolysis and microbial action, respectively. Moisture is essential for chemical reactions and for living cells. Hydrolysis of many complex molecules, such as pectin and polyphenols, in most plants liberates smaller compounds, such as pectinic acid, pectic acid, and phenolic acid, resulting in acidic conditions, as indicated by lower pH (Li *et al*., 2020; Liu and Kokare, 2017). However, pH of all treatments fluctuated during storage due to deterioration because of both oxidation effect and liberation effect to form other compounds, as explained previously.

Brix value

The Brix values of sheet snacks are shown in Figure 6. The Brix value indicates the total soluble solids (gram of soluble solids/100 g of solution) (Zoecklein *et al*., 2010). This value decreased after storage for 2 weeks, related to pH changes. Some soluble solids, such as sugar and phenolic acids, are utilized in chemical reactions and/or microbial growth. During storage, the Brix values of the products packaged in nylon/LLDPE packaging (N60 and N90) were higher than those of the products packaged in laminated aluminum foil packaging (A60 and A90). This result supported that a higher a_w leads to a higher rate of hydrolysis and subsequently increases total soluble solids when smaller compounds are generated (FAO, 2003; Hameed *et al*., 2019). Interestingly, a significant reduction in pH and Brix values at 2 weeks in products packaged in laminated aluminum foil packaging, particularly A60, was noticed where a_w was low. This result suggested that some strong chemical reactions might have occurred during storage. This could be attributed to several factors. For example, some cross linking has occurred between polyphenols and soluble solid compounds, such as protein, sugar, minerals, etc. Therefore, further investigation is needed to understand this phenomenon.

Color change

The L^*, a^*, and b^* color space values of the front side (not in contact with Teflon sheet) were 22.53, -0.97, and 11.95, respectively, while the L^*, a^*, and b^* values of the back side (in contact with Teflon sheet) were 21.69, -0.07, and 15.74, respectively. The ΔE value is the color difference between the sample and a freshly produced snack. ΔE of the front side of sheet snacks increased gradually with storage time (Figure 7A). The front side ΔE values of samples packed in laminated aluminum foil packaging were higher than those packed in nylon/LLDPE packaging. In general, the backside ΔE values (Figure 7B) of all samples were higher than the frontside ΔE values. Higher ΔE values indicate a large color difference whereas lower ΔE values indicate a small color difference (Gordon, 2022). Unexpectedly, these results indicated that longer storage resulted in higher ΔE, particularly for products packed in laminated aluminum foil packaging with high RH storage conditions.

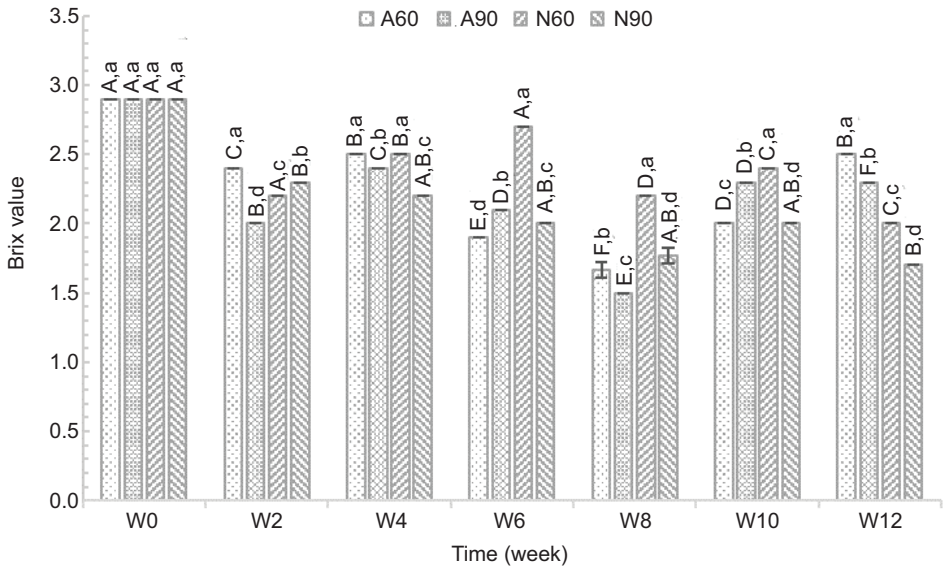

Figure 6. Brix values of sheet snacks during 12-week storage (n = 3). Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments on each day ($p < 0.05$). A60: laminated aluminum foil packaging stored at RH 60%; A90: laminated aluminum foil packaging stored at RH 90%; N60: nylon/LLDPE packaging stored at RH 60%; N90: nylon/LLDPE packaging stored at RH 90%.

Total phenolic content, total flavonoid content, and antioxidant activity

Total phenolic content and total flavonoid content

TPC and TFC of sheet snacks during 12 weeks of storage are shown in Figures 8 and 9, respectively, with changes in TPC and TFC during storage shown in Tables 4 and 5, respectively. TPC and TFC of sheet snacks on day 0 were lower than recorded in *Ulvas intestinalis* Iran seaweeds, with TPC ranging from 1.26 to 5.08 mg GAE/g and TFC ranging from 8.05 to 33.09 mg rutin equivalent (RE)/g, depending on species (Farasat *et al*., 2014). Raw material is an important factor that affects nutritional value and other biological activities. TPC and TFC of all sheet snacks decreased after storage for 2 weeks. A significant decrease in TPC during the first period of storage at 4°C, which remained constant throughout storage, was observed in litchi pericarp (Deng *et al*., 2018), while the TPC of rambutan fruit decreased during 16 weeks of storage at 4°C (Thitilertdecha, 2022). The decrease in TPC and TFC during 2 weeks of storage was due to oxidation or the formation of fewer antioxidant polymers with lower antioxidant capacity (Zhang *et al*., 2021). Changes in TFC showed a trend similar to the Brix value, which decreased after storage for 2 weeks while maintaining a higher RH. With higher water absorption and increased hydrolysis, TFC was liberated or loosened from the product structure, making extraction easier. Fermentation and enzyme hydrolysis led to an increase in TPC and TFC, including quercetin and kaempferol, as confirmed by Wang *et al*. (2018b). Both fermentation and enzyme hydrolysis enhance antioxidant activity (Wang *et al*., 2018b). Interestingly, TPC changed with no trend and exhibited notable fluctuations, with TPC at some weeks exceeding 100%, compared to the freshly produced sample at week 0. The increase in TPC is attributed to hydrolysis or fermentation process during storage, which enhances the release of polyphenolic compounds and other antioxidant compounds from samples.

Antioxidant activity (DPPH, ABTS, and FRAP) assays

The antioxidant activities of sheet snacks are shown in Figures 10–12, with the changes in antioxidant activity during storage shown in Tables 6–8. Sheet snacks exhibited the highest antioxidant activity on day 0. A reduced antioxidant activity was observed after storage for 2 weeks. Polyphenol and flavonoid compounds are major antioxidants produced by plants (Sulaiman and Balachandran, 2012). Reduced TPC and TFC resulted in lower antioxidant activity after 2 weeks of storage. Reduced antioxidants during 2 weeks of storage could be due to vitamin C degradation, in which fresh liang leaves contained approximately 2.71 mg/DW (data not shown). Reduced vitamin C by 50% was observed in broccoli, green beans, and peas after storage for 7 days at 4°C (Balan *et al*. 2016). A slight change was observed throughout storage; however, antioxidants generally remained constant. There was no difference between treatments based on DPPH and FRAP assays whereas a significant decrease in FRAP was observed in treatments packaged in nylon/LLDPE pouches, with moisture absorption leading to antioxidant degradation.

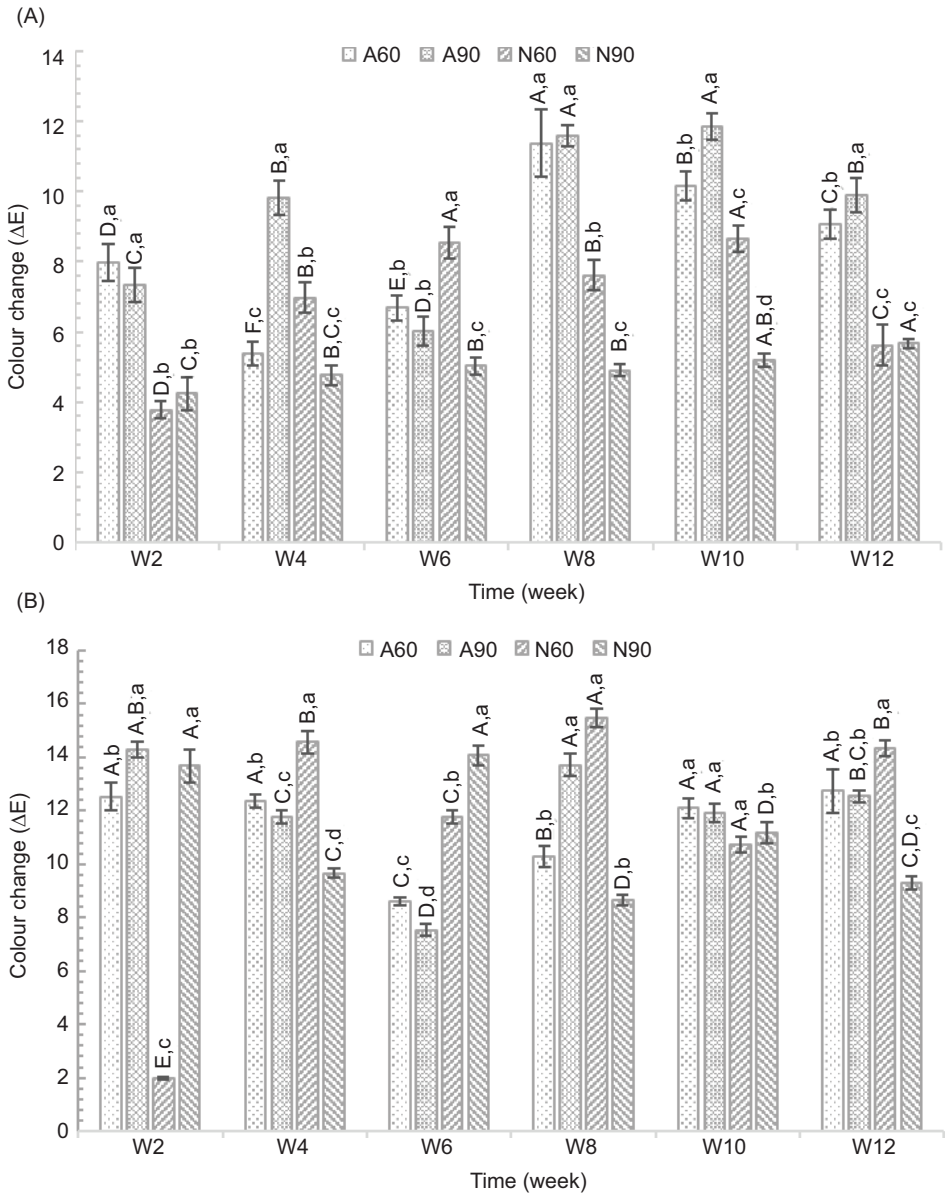

Figure 7. Color change of sheet snacks during 12-week storage (n = 3). Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments on each day ($p < 0.05$): (A) front side (not contact with Teflon sheet); (B) back side (contact with Teflon sheet). A60: laminated aluminum foil packaging stored at RH 60%; A90: laminated aluminum foil packaging stored at RH 90%; N60: nylon/LLDPE packaging stored at RH 60%; N90: nylon/LLDPE packaging stored at RH 90%.

Reduced antioxidant stability of α-tocopherol in corn oil with increasing RH was reported by Wongklom and Moonsin (2018). The highest antioxidant activity of sheet snacks was observed in ABTS assay, followed by FRAP and DPPH assays. High antioxidant levels in ABTS assay indicated polar antioxidant compounds in liang leaves, with hydrogen donors or electron transfer capability to any radical, including metal ion status (Fe^{+3} to Fe^{2+}), as determined by FRAP assay.

Microbiological quality

The microbiological qualities of sheet snacks are shown in Table 9. Despite the washing and sanitization treatment carried out on liang leaves before the preparation process of sheet snacks, microbial load in freshly produced sheet snack (F) remained high. The hot air-drying step at 70°C reduced moisture content and a_w but did not reduce microbial load. Siripongvutikorn *et al*. (2023)

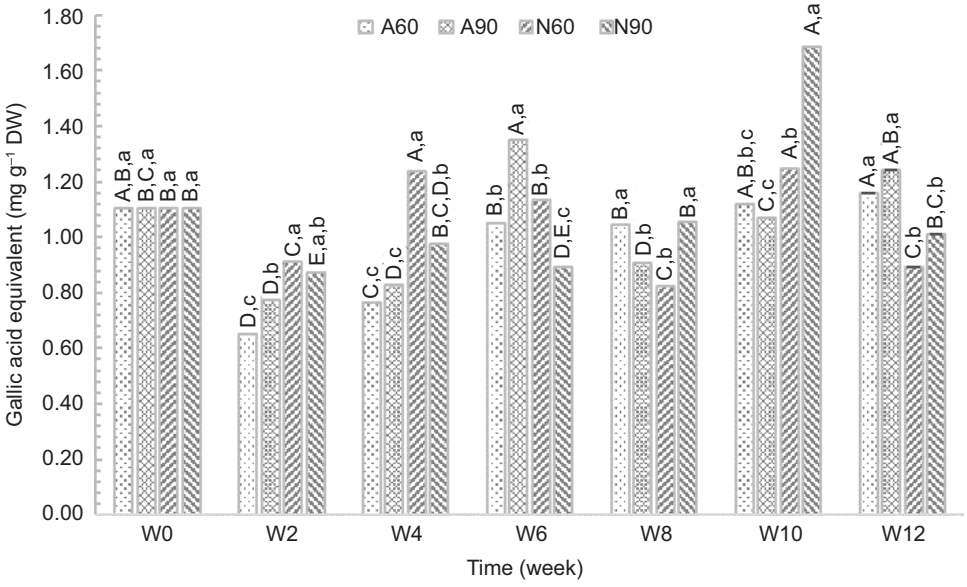

Figure 8. Total phenolic content (TPC) of sheet snacks during 12-week storage (n = 3). Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments on each day ($p < 0.05$). A60: laminated aluminum foil packaging stored at RH 60%; A90: laminated aluminum foil packaging stored at RH 90%; N60: nylon/LLDPE packaging stored at RH 60%; N90: nylon/LLDPE packaging stored at RH 90%.

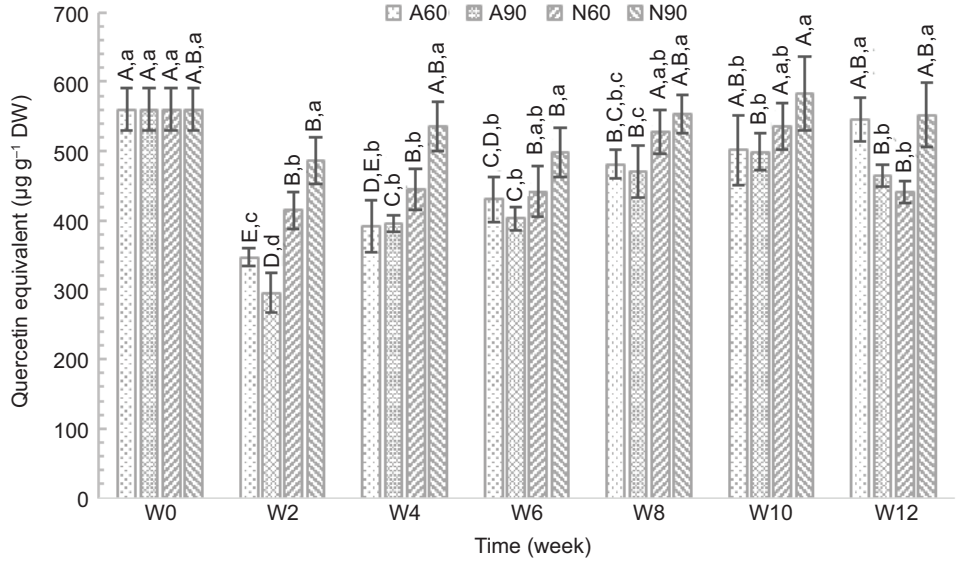

Figure 9. Total flavonoid content (TFC) of sheet snacks during 12-week storage (n = 3). Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments on each day ($p < 0.05$). A60: laminated aluminum foil packaging stored at RH 60%; A90: laminated aluminum foil packaging stored at RH 90%; N60: nylon/LLDPE packaging stored at RH 60%; N90: nylon/LLDPE packaging stored at RH 90%.

found that the TVC of fresh liang leaves exceeded 7 cfu/g, while washing by soaking in a chlorinated solution indicated that it was not sufficiently strong to decontaminate and reduce microbial load (Rosberg *et al.*, 2021). The initial high microbial load was probably due to the use of untreated water from well ponds used for irrigation. The TVC values varied between samples, but both packaging materials demonstrated effective preservation of microbiological quality, with low TVC and YM values observed throughout the storage period. However, fluctuations in TVC and YM values suggested that factors such as the initial microbial load, moisture content, water activity, and packaging material also influenced dynamics of microbial growth. Further investigations into these

Table 4. Percentage of total phenolic content (TPC) of sheet snacks during 12-week storage, compared to freshly produced sheet snacks at week 0 (as 100%).

Condition/time (weeks)	A60	A90	N60	N90
2	58.80 ± 5.15^{D,c}	69.99 ± 2.22^{C,b}	82.39 ± 3.62^{C,a}	78.91 ± 4.94^{C,a,b}
4	69.17 ± 2.31^{C,c}	75.02 ± 4.85^{C,c}	111.92 ± 2.08^{A,a}	88.49 ± 4.69^{B,C,b}
6	95.14 ± .73^{B,b}	122.17 ± 6.61^{A,a}	102.37 ± 3.93^{B,b}	80.87 ± 4.28^{C,c}
8	94.30 ± 2.97^{B,a}	81.84 ± 5.84^{C,b}	74.63 ± 0.92^{C,b}	95.39 ± 3.49^{B,a}
10	101.19 ± 3.56^{A,B,b,c}	96.88 ± 3.00^{B,c}	112.93 ± 5.80^{A,b}	152.65 ± 8.99^{A,a}
12	104.85 ± 5.48^{A,a}	112.21 ± 8.19^{A,a}	80.54 ± 3.04^{B,b}	91.54 ± 4.00^{B,C,b}

Notes: n = 3. Different uppercase superscript letters indicate significant differences within the same treatment. Different lowercase superscript letters indicate significant differences between weekly treatments ($p < 0.05$). A60: aluminum packaging with storage at RH 60%; A90: aluminum packaging with storage at RH 90%; N60: nylon packaging with storage at RH 60%; N90: nylon packaging with storage at RH 90%.

Table 5. Percentage of total flavonoid content (TFC) in sheet snacks during 12-week storage, compared to freshly produced sheet snacks at week 0 (as 100%).

Condition/time	A60	A90	N60	N90
2	61.98 ± 2.12^{D,c}	54.89 ± 1.56^{C,d}	74.61 ± 5.26^{B,b}	89.35 ± 2.91^{B,a}
4	72.19 ± 4.87^{C,D,b}	70.64 ± 2.21^{B,b}	81.11 ± 4.38^{B,b}	97.97 ± 5.04^{A,B,a}
6	79.43 ± 0.90^{B,C,b}	72.92 ± 2.30^{B,b}	81.55 ± 2.71^{B,a,b}	91.77 ± 1.53^{B,a}
8	85.95 ± 4.42^{A,B,b,c}	86.64 ± 3.51^{A,c}	96.50 ± 2.13^{A,a,b}	99.66 ± 5.38^{A,B,a}
10	92.77 ± 6.10^{A,b}	91.07 ± 1.45^{A,b}	97.95 ± 3.16^{A,a,b}	107.78 ± 5.64^{A,a}
12	99.41 ± 3.47^{A,a}	83.82 ± 1.97^{A,b}	79.62 ± 2.08^{B,b}	101.89 ± 3.86^{A,B,a}

Notes: n = 3. Different uppercase superscript letters indicate significant differences within the same treatment. Different lowercase superscript letters indicate significant differences between weekly treatments ($p < 0.05$). A60: aluminum packaging with storage at RH 60%; A90: aluminum packaging with storage at RH 90%; N60: nylon packaging with storage at RH 60%; N90: nylon packaging with storage at RH 90%.

factors are necessary to ensure the consistent microbiological quality of sheet snacks.

Sensory evaluation

Table 10 presents the sensory scores of sheet snacks stored for 2 weeks and 12 weeks, evaluating attributes, such as color, odor, taste, texture, and overall acceptance. In general, A60 and A90 exhibited higher sensory scores across all attributes, compared to N60 and N90. Reduced scores for texture, flavor, taste, overall acceptability, and consumer acceptance of N60 and N90 were noted by panelists after 2-week storage. During 10-week storage, the N90 sample was excluded from sensory evaluation because of observed hyphae, which indicated potential spoilage. While microbial quality parameters did not exceed acceptable standard limits, a visible sign of mold growth, that is the presence of hyphae, caused a significant concern regarding product's safety. It is known that mold growth is shown by hyphae after germination of a spore, while bacteria grow by binary fission. Therefore, signs of bacteria and mold growth may be different in colony forming unit (CFU/g) and consumer perception.

Results revealed that both high a_w and high fiber content in sheet snacks after 12-week storage provided and allowed mold due to cellulase enzymes (Naher *et al.*, 2021). A noticeable decline in sensory scores for all samples was observed after 12 weeks. Scores for A60 and A90 decreased but remained relatively high than those for N60 and N90. The decrease in sensory attributes is more pronounced in N samples, with N60 and N90 showing the lowest scores after 12 weeks, particularly in texture and overall acceptance. This decline is visually shown in Figures 13 and 14, which show sensory attributes over time, highlighting the effective performance of laminated aluminum foil in maintaining their sensory qualities. A decrease in the sensory characteristics of sheet snacks was caused by reabsorption of water moisture in a high RH environment.

The principle of dried food is to extend shelf life by decreasing a_w and subsequently reducing chemical

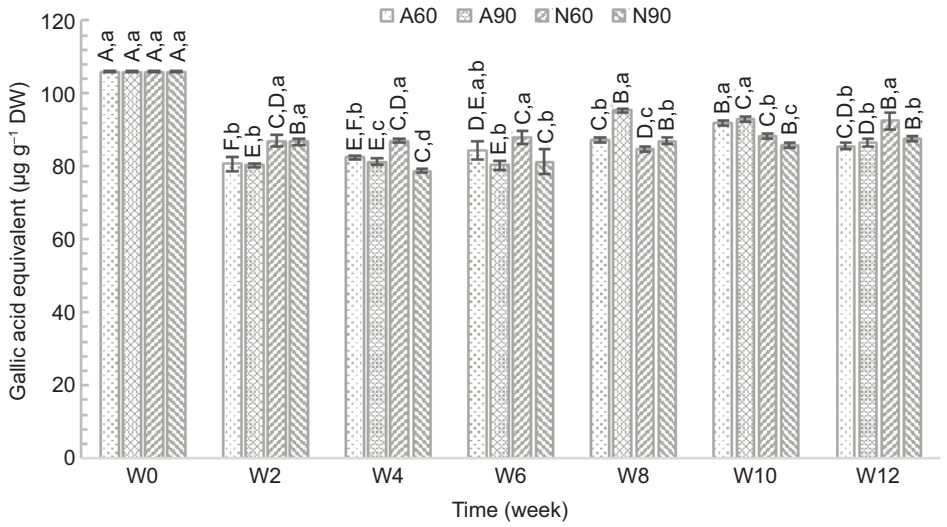

Figure 10. DPPH of sheet snacks during 12-week storage (n = 3). Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments on each day ($p < 0.05$). A60: laminated aluminum foil packaging stored at RH 60%; A90: laminated aluminum foil packaging stored at RH 90%; N60: nylon/LLDPE packaging stored at RH 60%; N90: nylon/LLDPE packaging stored at RH 90%.

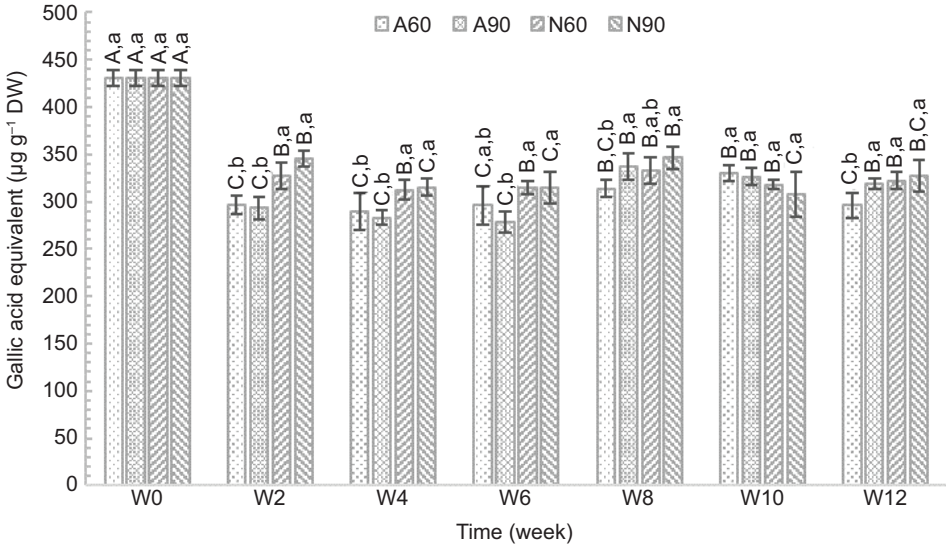

Figure 11. ABTS of sheet snacks during 12-week storage (n = 3). Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments on each day ($p < 0.05$). A60: laminated aluminum foil packaging stored at RH 60%; A90: laminated aluminum foil packaging stored at RH 90%; N60: nylon/LLDPE packaging stored at RH 60%; N90: nylon/LLDPE packaging stored at RH 90%.

reactions and microorganism activity (Prabhakar and Mallika, 2014). Dried food requires storage at low RH in a cool environment. Increasing a_w in dried food leads to the growth of microorganism, chemical reactions, and physical changes (Allen, 2018; Ciesarova *et al.*, 2006). Higher RH in N90 caused greater changes in sensory scores than in N60. A further increase in a_w by water absorption led to sticky texture, and unpleasant taste and flavor, resulting in lower overall acceptability and consumer acceptance. The panelists perceived a bitter taste and sticky texture in N60 and N90 after storage for 4 weeks. High RH (80%) led to high water reabsorption, leading to increased rancidity, bitter taste, chewiness, and no crispiness found in English walnuts (Mitcham *et al.*, 2022). Therefore, A60 and A90 samples offered a better retention of sensory qualities over time.

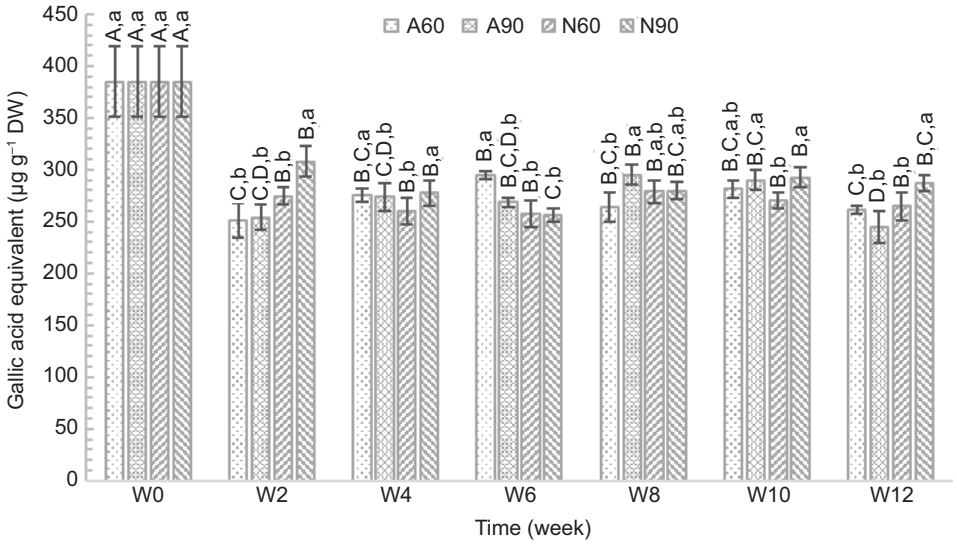

Figure 12. FRAP values of sheet snacks during 12-week storage (n = 3). Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments on each day ($p < 0.05$). A60: laminated aluminum foil packaging stored at RH 60%; A90; laminated aluminum foil packaging stored at RH 90%; N60: nylon/LLDPE packaging stored at RH 60%; N90: nylon/LLDPE packaging stored at RH 90%.

Table 6. Proportion of DPPH in sheet snacks during 12-week storage, compared to freshly produced sheet snacks at week 0 (as 100%).

Condition/time (weeks)	A60	A90	N60	N90
2	$75.60 \pm 1.44^{E,b}$	$75.87 \pm 0.55^{D,b}$	$82.36 \pm 1.79^{B,C,a}$	$81.81 \pm 0.90^{A,a}$
4	$77.91 \pm 0.62^{D,E,b}$	$76.77 \pm 1.07^{D,c}$	$82.27 \pm 0.43^{B,C,a}$	$74.55 \pm 0.28^{B,d}$
6	$78.67 \pm 1.49^{C,D,a,b}$	$76.33 \pm 0.90^{D,b}$	$82.37 \pm 1.08^{B,a}$	$75.45 \pm 1.90^{B,b}$
8	$82.49 \pm 0.49^{B,b}$	$90.19 \pm 0.49^{A,a}$	$79.97 \pm 0.71^{C,c}$	$82.43 \pm 0.71^{A,b}$
10	$87.04 \pm 0.58^{A,a,b}$	$87.63 \pm 0.61^{B,a}$	$83.48 \pm 0.86^{B,b}$	$81.02 \pm 0.91^{A,c}$
12	$81.15 \pm 0.40^{B,C,b}$	$81.86 \pm 0.89^{C,b}$	$86.57 \pm 1.94^{A,a}$	$83.06 \pm 0.55^{A,b}$

Notes: n = 3. Different uppercase superscript letters indicate significant differences within the same treatment. Different lowercase superscript letters indicate significant differences between weekly treatments ($p < 0.05$). A60: aluminum packaging with storage at RH 60%; A90: aluminum packaging with storage at RH 90%; N60: nylon packaging with storage at RH 60%; N90: nylon packaging with storage at RH 90%.

Table 7. Proportion of ABTS in sheet snacks during 12-week storage, compared to freshly produced sheet snacks at week 0 (as 100%).

Condition/time (week)	A60	A90	N60	N90
2	$69.07 \pm 2.63^{B,b}$	$67.47 \pm 2.78^{B,b}$	$74.85 \pm 2.40^{A,a}$	$79.70 \pm 1.94^{A,B,a}$
4	$66.87 \pm 4.98^{B,b}$	$65.05 \pm 1.06^{B,b}$	$72.09 \pm 2.52^{A,a}$	$72.53 \pm 1.90^{B,C,a}$
6	$67.84 \pm 4.81^{B,a,b}$	$64.60 \pm 2.91^{B,b}$	$73.07 \pm 1.98^{A,a}$	$73.47 \pm 4.33^{C,a}$
8	$71.96 \pm 1.15^{A,B,b}$	$77.03 \pm 1.57^{A,a}$	$76.39 \pm 3.14^{A,a,b}$	$80.37 \pm 3.16^{A,a}$
10	$76.50 \pm 2.20^{A,a}$	$75.08 \pm 1.37^{A,a}$	$73.73 \pm 1.31^{A,a}$	$69.91 \pm 4.86^{C,a}$
12	$68.06 \pm 2.96^{B,b}$	$73.66 \pm 1.14^{A,a}$	$74.05 \pm 1.13^{A,a}$	$77.29 \pm 3.03^{A,B,C,a}$

Notes: n = 3. Different uppercase superscript letters indicate significant differences within the same treatment. Different lowercase superscript letters indicate significant differences between weekly treatments ($p < 0.05$). A60: aluminum packaging with storage at RH 60%; A90: aluminum packaging with storage at RH 90%; N60: nylon packaging with storage at RH 60%; N90: nylon packaging with storage at RH 90%.

Table 8. Proportion of FRAP in sheet snacks during 12-week storage, compared to freshly produced sheet snacks at week 0 (as 100%).

Condition/time (weeks)	A60	A90	N60	N90
2	$63.78 \pm 3.14^{C,b}$	$65.71 \pm 3.53^{C,D,b}$	$70.68 \pm 1.98^{A,b}$	$78.83 \pm 3.63^{A,a}$
4	$71.28 \pm 1.63^{A,B,a}$	$71.35 \pm 4.06^{A,B,C,a}$	$66.52 \pm 3.40^{A,a}$	$71.00 \pm 1.94^{B,a}$
6	$76.35 \pm 0.93^{A,a}$	$69.33 \pm 1.12^{B,C,D,b}$	$65.92 \pm 3.04^{A,b}$	$66.49 \pm 1.90^{C,b}$
8	$68.78 \pm 4.28^{B,C,a}$	$77.40 \pm 2.27^{A,a}$	$71.33 \pm 1.70^{A,a,b}$	$73.20 \pm 2.20^{B,a,b}$
10	$73.34 \pm 2.32^{A,B,a,b}$	$75.10 \pm 2.92^{A,B,a}$	$69.73 \pm 1.93^{A,b}$	$76.91 \pm 1.49^{A,B,a}$
12	$68.03 \pm 0.99^{B,C,b}$	$62.88 \pm 4.54^{D,b}$	$69.42 \pm 3.73^{A,b}$	$73.66 \pm 1.02^{B,a}$

Notes: n = 3. Different uppercase superscript letters indicate significant differences within the same treatment. Different lowercase superscript letters indicate significant differences between weekly treatments ($p < 0.05$). A60: aluminum packaging with storage at RH 60%; A90: aluminum packaging with storage at RH 90%; N60: nylon packaging with storage at RH 60%; N90: nylon packaging with storage at RH 90%.

Table 9. Microbiological quality of sheet snacks after storage for 12 weeks.

Condition/time	TVC (log cfu/g)	YM (log cfu/g)	*E. coli* (MPN/g)	*S. aureus* (per 0.1 g)	*Salmonella* spp. (per 25 g)
F	5.81	1.00	<3	ND	ND
W6A60	6.41	2.92	<3	ND	ND
W6A90	6.18	1.00	<3	ND	ND
W6N60	2.87	3.48	<3	ND	ND
W6N90	1.18	1.00	<3	ND	ND
W12A60	4.08	1.00	<3	ND	ND
W12A90	1.54	1.00	<3	ND	ND
W12N60	5.32	1.00	<3	ND	ND
W12N90	1.18	1.00	<3	ND	ND

Notes: F: freshly produced sample; W6A60: laminated aluminum foil packaging stored at RH 60% for 6 weeks; W6A90: laminated aluminum foil packaging stored at RH 90% for 6 weeks; W6N60: nylon/LLDPE packaging stored at RH 60% for 6 weeks; W6N90: nylon/LLDPE packaging stored at RH 90% for 6 weeks; W12A60: laminated aluminum foil packaging stored at RH 60% for 12 weeks; W12A90: laminated aluminum foil packaging stored at RH 90% for 12 weeks; W12N60: nylon/LLDPE packaging stored at RH 60% for 12 weeks; W12N90: nylon/LLDPE packaging stored at RH 90% for 12 weeks.

Packaging materials play a crucial role as moisture barriers, with laminated aluminum foil performing better than nylon/LLDPE in minimizing moisture transfer and extending product shelf life. Sheet snacks packed in laminated aluminum foil packaging had a shelf life of up to 1 year under storage conditions of 30°C and RH 60%. The sensory evaluation highlighted the importance of storage conditions, with higher RH resulting in accelerated deterioration of texture, flavor, and overall acceptability.

The microbiological quality assessment indicated that microbial survival was influenced by a high initial microbial load. These findings emphasized the importance of selecting optimal packaging materials and storage conditions to maintain nutritional quality, sensory attributes, and microbial safety of sheet snacks. Further research is required to optimize preparation process parameters and enhance the shelf life and quality of the sheet snacks to ensure greater potential as a nutritious and tasty food product.

Declaration of Competing Interest

The authors declared no conflict of competing interest.

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Table 10. Sensory scores of sheet snacks stored for 2 and 12 weeks (n = 50).

Attributes	Time (weeks)	Control (freshly produced sample)	A60	A90	N60	N90
Appearance	2	7.20 ± 1.25^{a}	7.38 ± 1.09^{a}	6.84 ± 1.35^{a}	7.38 ± 1.10^{a}	7.46 ± 0.99^{a}
	12	7.62 ± 0.83^{a}	7.52 ± 1.18^{a,b}	7.18 ± 1.27^{a,b}	6.16 ± 1.54^{b}	-^{c}
Color	2	7.14 ± 1.11^{ab}	7.58 ± 0.86^{a}	6.84 ± 1.40^{b}	7.26 ± 1.07^{a,b}	7.34 ± 0.94^{a,b}
	12	7.44 ± 0.95^{ab}	7.42 ± 1.25^{a}	6.88 ± 1.24^{a,b}	5.74 ± 1.44^{b}	-^{c}
Odor	2	7.02 ± 1.02^{a}	7.10 ± 0.93^{a}	6.66 ± 1.29^{a}	6.86 ± 1.18^{a}	6.67 ± 1.20^{a}
	12	7.10 ± 1.05^{a}	6.76 ± 1.36^{a}	6.61± 1.02^{a}	6.14 ± 1.18^{a}	-^{b}
Texture	2	7.26 ± 0.99^{a}	7.48 ± 1.09^{a}	7.16 ± 1.33^{a}	4.66 ± 1.95^{b}	4.66 ± 2.00^{b}
	12	7.48 ± 1.13^{a}	7.42 ± 1.33^{a}	7.46 ± 0.91^{a}	3.78 ± 1.76^{b}	-^{c}
Flavor	2	7.00 ± 1.11^{a}	7.16 ± 1.00^{a}	6.62 ± 1.41^{a,b}	6.22 ± 1.23^{b}	6.22 ± 1.34^{b}
	12	7.06 ± 1.11^{a}	6.96 ± 1.32^{a}	6.72 ± 1.34^{a}	6.04 ± 1.24^{a}	-^{b}
Taste	2	7.12 ± 1.08^{a}	7.26 ± 1.07^{a}	6.66 ± 1.38^{a,b}	6.00 ± 1.43^{b}	6.04 ± 1.34^{b}
	12	7.06 ± 1.11^{a}	7.24 ± 1.19^{a}	6.82 ± 1.41^{a}	6.04 ± 1.31^{a}	-^{b}
Overall acceptability	W2	7.08 ± 1.01^{a}	7.42 ± 0.84^{a}	6.90 ± 1.33^{a}	5.56 ± 1.59^{b}	5.48 ± 1.66^{b}
	12	7.26 ± 1.16^{a}	7.38 ± 1.14^{a}	6.94 ± 1.36^{a}	4.72 ± 1.5^{b}	-^{c}
Percentage of acceptance	2	94.00	96.00	88.00	44.00	44.00
	12	98.00	96.00	94.00	24.00	-

Notes: n = 50 panelists. Different lowercase superscript letters indicate significant differences between treatments within the same period ($p < 0.05$). A60: laminated aluminum foil packaging stored at RH 60%; A90: laminated aluminum foil packaging stored at RH 90%; N60: nylon/LLDPE packaging stored at RH 60%; N90: nylon/LLDPE packaging stored at RH 90%; "-" indicates not determined due to hyphae observed in some sheet samples.

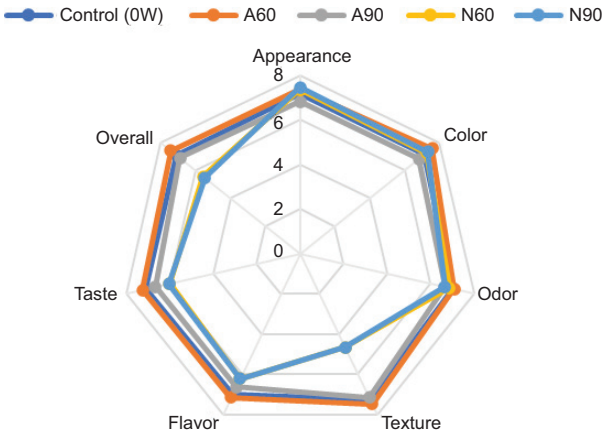

Figure 13. Web diagram of sheet snacks' sensory scores after 2-week storage, compared to freshly produced sheet snacks (control: 0 week).

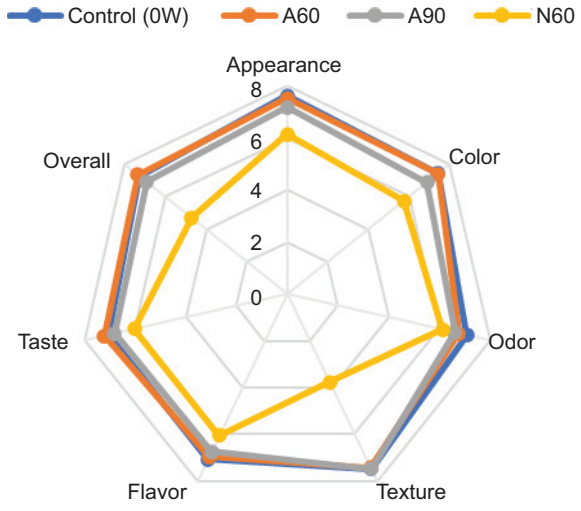

Figure 14. Web diagram of sheet snacks sensory scores after 12-week storage, compared to freshly produced sheet snacks (control: 0 week). Note: No data for N90 at 12 weeks because the sample was discarded due to mold growth.

Conclusions

The development and storage of sheet snacks revealed several key findings. Sheet snacks demonstrated promising nutritional attributes, with significantly higher levels of protein, fiber, calcium, vitamin A, and vitamin B_2, although these values varied, compared to the literature data for seaweed snacks. The nutritional content decreased over storage time, particularly with respect to the levels of vitamins A and B_2. Reduced TPC, TFC, and antioxidant activities were observed after 2-week storage but remained constant for up to 12 weeks.

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Author Contributions

W. Usawakesmanee supervised and acquired funding. S. Siripongvutikorn, W. Usawakesmanee and S. Pisuchpen prepared methodology and conceptualization, validated data, and reviewed and edited original article. N. Khatcharin and C. Rujirapong conducted formal analysis, investigation. C. Rujirapong prepared original draft.

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