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Quality changes during storage in Thai indigenous leafy vegetable, Liang leaves (*Gnetum gnemon* var. *tenerum*) after different preparation methods

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Abstract

Liang or *Gnetum gnemon* var. *tenerum*, an indigenous southern vegetable has recently attracted increasing interest due to its high nutritional value, creamy taste and lack of smell. The leaves with or without stem are washed with chlorinated water at 100 ppm for 15 min, stored at 4°C and investigated for physiochemical, chemical and sensory evaluations over time. Total phenolic and flavonoid contents were higher in treatments with stems ($P < 0.05$). Washing significantly increased moisture content and water activity (a_w) in all treatments ($P < 0.05$). In addition, washing resulted in significantly higher DPPH and ABTS activity ($P < 0.05$). However, washing and stem detachment had no effect on sensory and physicochemical qualities. The sensory score of the 8-days stored sample was comparable to the fresh one (Day 0).

Keywords: antioxidant; *Gnetum gnemon*; preparation; quality; stem; washing

Introduction

Liang or *Gnetum gnemon* var. *tenerum*, a common signature southern vegetable, has the potential to become a new economic plant with less or free from pesticide residue. Generally, Liang is grown in backyards or as a fence plant by people in Southern Thailand. Liang has a creamy, umami taste with less green flavour and is often grown as an intercrop between various economic plants such as rubber, palm oil, durian and orchard plants to maximise the use of space and increase income. The *tenerum* shrub variety is grown in Thailand, whereas in Malaysia and Indonesia, the *gnemon* variety is grown as a tree (Anisong *et al*., 2022). Preliminary tests showed that the *tenerum* variety produced leaves containing essential amino acids with high health benefits including antioxidant, antidiabetic, anti-inflammatory, anti-breast cancer and gut microbiota–enhancing effects (Suksanga *et al*., 2022) due to high protein and phytochemical compounds such as chlorophyll, beta-carotene, phenolic compounds, flavonoids and dietary fibre, both soluble and insoluble. After harvesting, the leaves are usually bunched with rubber bands or packaged in open bags for transport to local or fresh markets (Figure 1), while in supermarkets, packaging in sealed bags is usually applied (Figure 2). To serve the new generation of people who live busy lives, ready-to-cook or minimal process ingredients are required to meet their needs. In supermarkets, ready-to-cook leafy vegetables are usually packaged as leaves without stems.

Liang leaves are eaten as a fresh vegetable or as a side dish with spicy foods. The leaves are also cooked and used in recipes for various menus (Suksanga *et al*., 2022). Liang

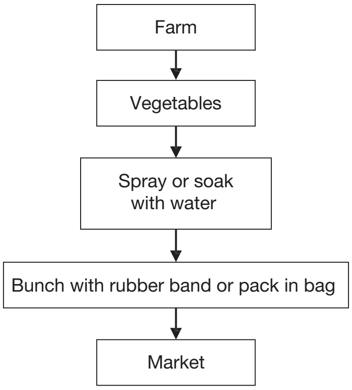

Figure 1. Flowchart of vegetable preparation from farm to market.

Figure 2. Liang leaves bunched with a rubber band (A) and sealed in a plastic bag (B).

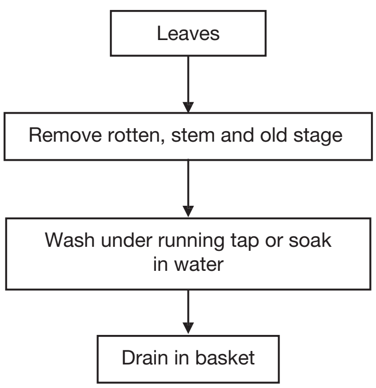

Figure 3. Flowchart of the conventional washing process.

leaves are typically washed before cooking to ensure hygiene and safety (Figure 3). Gardeners and merchants generally spray water on vegetables or soak them to remove dirt. This reduces plant temperature and controls weight loss. Low temperature and appropriate humidity (60–70%) could slow down the leaf deterioration rate after harvesting (de Frias *et al.*, 2018). However, each preparation process causes cumulative physical damage (Mulaosmanovic *et al.*, 2021), leading to chemical and microbiological spoilage (Ariffin *et al.*, 2017). Wounds also increase biochemical and chemical reactions owing to the liberation of substrates and enzymes from damaged or injured plant cells (Leveau and Lindow, 2001). Injured leaves lead to lower quality and shortened product shelf life (Ariffin *et al.*, 2017). Physically damaged cells or wounds enhance both organic and inorganic nutrient release that accelerates microbial growth and chemical reactions (Aruscavage *et al.*, 2008, 2010).

Washing also increases moisture content, a_w level as well as physical damage due to excessive forces during washing and draining (FAO and WHO, 2003; Mulaosmanovic *et al.*, 2021). However, no scientific information is available on the preparation (washing and stem detachment) of leafy vegetables, particularly Liang. This is of significant interest for a new S-curve for Liang because of its consumer palatability, low chemical or pesticide content and high nutritional value. Therefore, quality changes in Liang due to the preparation process represent is of utter importance. This study focused on the effect of stem detachment and washing on the physical, chemical and sensory qualities and antioxidant activity to develop a proper method for Liang leaf preparation in current commercial markets.

Materials and Methods

Leaf preparation and sampling

Young Liang or *Gnetum gnemon* var. *tenerum* leaves (pae-salat) were collected from a farm. To avoid injury from weight and dense packing, 2 kg of bulky Liang was packed into a low-density polyethylene bag (LDPE) and sent to the laboratory within 24 h, as shown in Figure 4. Tropical leafy vegetables are usually stored above 4°C to avoid chilling injury. The sample was checked for visual damage, and old, torn and rotten leaves were removed before the stem was detached following hygienic practice by wearing sanitation gloves. Leaves with and without stems were soaked in chlorinated water at 100 ppm for 15 min, washed twice with running tap water to remove the chlorine residue and drained in a basket for 10 min with a controlled thickness of leaves overlay at not more than 1 cm. The Liang leaves were divided into four groups as follows: no washing with stem (NWS), no washing without stem (NWNS), washing with stem (WS) and washing without stem (WNS) (see Figure 5).

Figure 4. Liang leaves received from the farm (A) Liang leaves packed in LDPE and (B) Liang leaves.

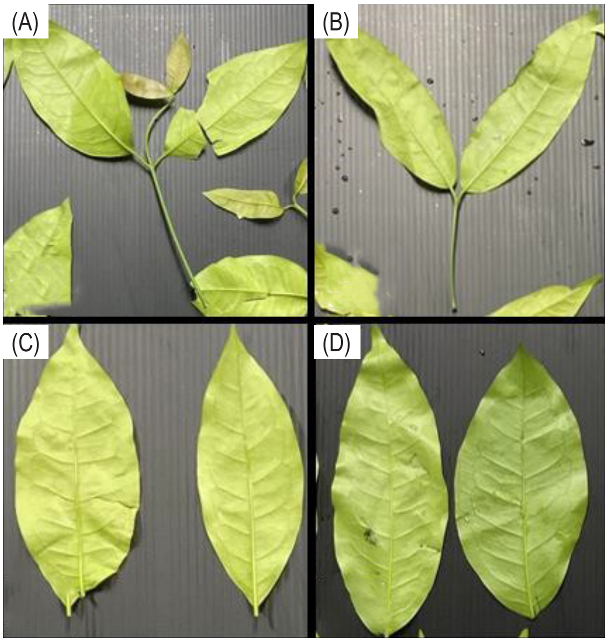

Figure 5. The groups of Liang leaves used in this study including (A) no washing with stem (NWS); (B) no washing without stem (NWNS); (C) washing with stem (WS) and (D) washing without stem (WNS).

Finally, all four groups were stored at 4°C for 8 days, as presented in Figure 6. On Days 0, 4, 5, 6, 7 and 8, samples were collected for physical, chemical, quality and sensory evaluation.

Physiochemical and chemical quality determination

Colour change

Colour changes were determined by CIE L*, a* and b* using a colorimeter (ColorFlex EZ, Hunter Associates Laboratory Inc., Virginia, USA) as described by Lee *et al.* (2022) and expressed as ΔE, as shown in Equation 2.

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

Where,

ΔE = colour difference between standard (fresh produce)
ΔL = difference between lightness (L*) standard (fresh produce)
Δa = difference between redness-greenness (a*) standard (fresh produce)
Δb = difference between yellowness-blueness (b*) standard (fresh produce)

pH, moisture content and a_w

The pH, moisture content and a_w were measured using a pH meter (Sartorius- Sartorius AG, Docu-pH+ Meter, Goettingen, Germany), oven method (AOAC, 2000), and an a_w analyser (Aqualab Pre., Decagon Devices Inc., Washington, USA) at predetermined times.

Brix value

Brix values were measured using a refractometer (Atago, Pen refractometer, Tokyo, Japan) (Thakulla *et al.*, 2021).

Chlorophyll content

Chlorophyll content was measured by the colorimetric method at 400–700 nm, as described in AOAC Methods 940.03 662 and 646 (AOAC, 2000) for chlorophyll a and b, respectively.

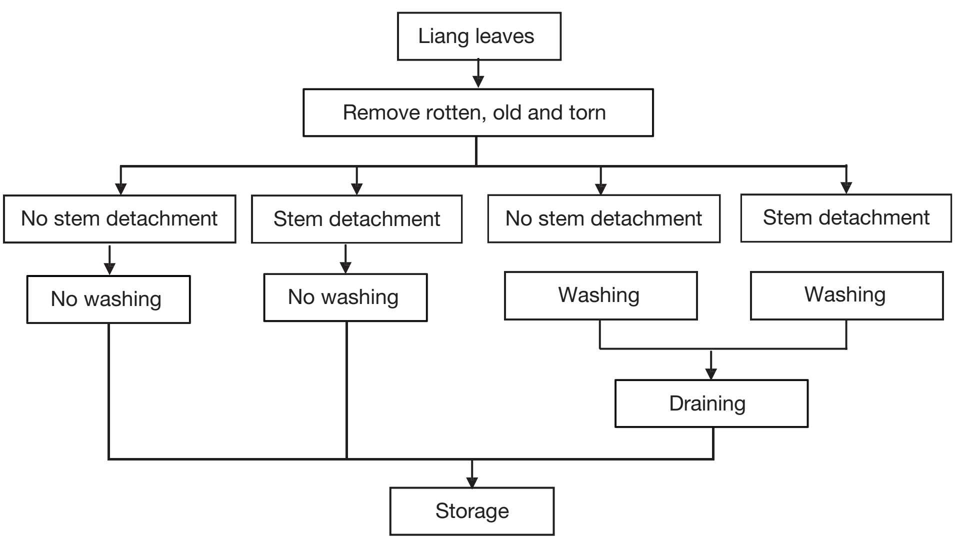

Figure 6. Flowchart of Liang leaves preparation.

Fibre content

Fibre content was determined as described in AOAC Method 2009.01.

Total phenolic content, total flavonoid content and antioxidant activity

Sample preparation and extraction

Sample was extracted using the method described by Srisook *et al.* (2021) with some modifications, such as ethanol 90% for 24 h instead of 95% ethanol for 5 days. Liang leaves and 90% ethanol (v/v) at a ratio of 1:10 were mixed 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 4°C for 15 min at 12,000 rpm. An evaporator was used to vaporise the ethanol and to obtain a concentrated sample.

Total phenolic content (TPC) determination

TPC was determined using the method described by Singleton and Rossi (1965) with some modifications. Briefly, 20 µl of sample extract was added to 96-well plates followed by 100 µl of 10% Folin reagent (v/v). After incubation in the dark at 30°C for 6 min, 7.5% Na2CO3 (anhydrous) (w/v) was added, and the mixture was incubated for another 30 min. The absorbance was measured at 765 nm using a microplate reader (Varioskan LUX, Thermo Scientific, Singapore). TPC content was reported as mg gallic acid equivalent/g DW using gallic acid as the standard at a concentration of 50–170 µg/ml ($R^2 = 0.999$).

Total flavonoid content (TFC) determination

TFC was determined using the method described by Ha *et al.* (2020) with some modifications. Briefly, 100 µl of sample extract was mixed with 100 µl 2% AlCl3·6H2O (w/v) and incubated in the dark at 30°C for 60 min. The absorbance of the mixture was then measured at 420 nm and reported as mg quercetin equivalent/g DW using quercetin as the standard at a concentration of 10–50 µg/ml (R2 = 0.9963).

DPPH radical scavenging activity

2,2-Diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity was determined using the method described by Brand-Williams *et al.* (1995) with some modifications. First, 100 µl of sample extract was mixed with 100 µl 0.2 mM DPPH in 95% ethanol. The sample was incubated in the dark for 30 min at 30°C. Finally, the absorbance of the mixture was measured at 517 nm and reported as µg gallic acid equivalent/g DW using gallic acid as the standard at a concentration of 0.5–3.5 µg/ml ($R^2 = 0.9959$).

ABTS radical scavenging activity

2,2-Azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) assay was determined as described by Arnao *et al.* (2001). ABTS radical was generated by incubating 7.4 mM of ABTS solution in 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. Then, 20 µl of sample extract was mixed with 280 µl of radical solution and kept in the dark for 2 h at 30°C. The absorbance of the mixture was measured at 734 nm and reported as mg gallic acid equivalent g DW using gallic acid as the standard at a concentration of 5–30 µg/ml ($R^2 = 0.998$).

Ferric reducing antioxidant power (FRAP) assay

The ferric–reducing antioxidant power (FRAP) assay was determined using the method of Benzie and Strain (1996). A freshly prepared FRAP solution containing 300 mM acetate buffer pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mM HCl and 20 mM FeCl3·6H2O (ratio 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 and reported as µg gallic acid equivalent/g DW using gallic acid as the standard at a concentration of 6–30 µg/ml ($R^2 = 0.9981$).

Sensory evaluation

Before the sensory evaluation, all samples were steamed for 3 min, and eight attributes including appearance, colour, texture, odour, flavour, taste, overall acceptability and consumer acceptance were evaluated using a 9-point hedonic scale by 50 untrained panelists.

Statistical analysis

All quality parameters, except for sensory tests, were assessed using a completely randomized design (CRD), whereas the sensorial score was determined using a randomized complete block design (RCBD). Differences in mean values were tested using ANOVA with specific differences between groups or treatments assessed by Tukey's test.

Results and Discussion

Physicochemical changes

Moisture and a_w

Moisture content of all no washing treatments (NWS and NWNS) decreased at fourth day of storage, whereas the moisture content of all washing treatments (WS and WNS) increased and was significantly higher than that of the no washing treatments (Figure 7). At fourth day of storage, the a_w of the no washing treatments (NWS and NWNS) remained constant, whereas the a_w of the washing treatments (WS and WNS) increased (Figure 8). Throughout the study, the moisture content of WNS treatments was significantly higher than WS treatments because stem detachment in WNS treatments resulted in an increased surface area, particularly at the end of the petiole, leading to higher water uptake and weight retention. Using LDPE plastic bags also prevented moisture loss, with moisture content reducing slightly until reaching equilibrium during 5 days of storage.

a_w of WS and WNS were higher than those of NWS and NWNS, indicating the effect of picking up water, as described in the moisture content determination. Thus, washing resulted in an increase in a_w and moisture content. Results showed the drawbacks of washing in terms of an increase in a_w, an important parameter for microbial spoilage and biochemical reactions which lead to faster product deterioration.

Brix value

The Brix value is a measure of the soluble solids content of a solution (Zoecklein *et al.*, 2010). Brix values of all treatments increased at fourth day of storage (Figure 9). An increase in Brix value indicates an increase in water-soluble substances such as sugar, soluble fibre, amino acids, salt and organic acids or a decrease in water in the solution system such as evaporation and respiration (Kusumiyati *et al.*, 2020). Therefore, the increase in Brix value was related to the higher water-soluble solids retained in the leaf samples.

The unwashed samples (NWS, NWNS) exhibited higher Brix values than the washed samples (WS, WNS) during storage for 5–8 days. Washing increased the water uptake of the samples. Brix values gradually decreased after storage for 4 days because of the higher utilisation of soluble solids particularly sugar, weak acids and minerals from microbial growth and biochemical reactions over time. Decrease in Brix value by microbial utilisation of soluble solids is also observed in yogurt, wort and beer (Adadi *et al.*, 2017; Kim and Han, 2019). The sharp increase in Brix values on eighth day of storage indicated an increase in soluble solid compounds due to a softening process by microbial and biochemical autolyses. Results revealed that the utilisation and production of such compounds occurred naturally, in parallel, as a result of the reaction. Therefore, when the former was lower than the latter, the increment increased rather than decreasing.

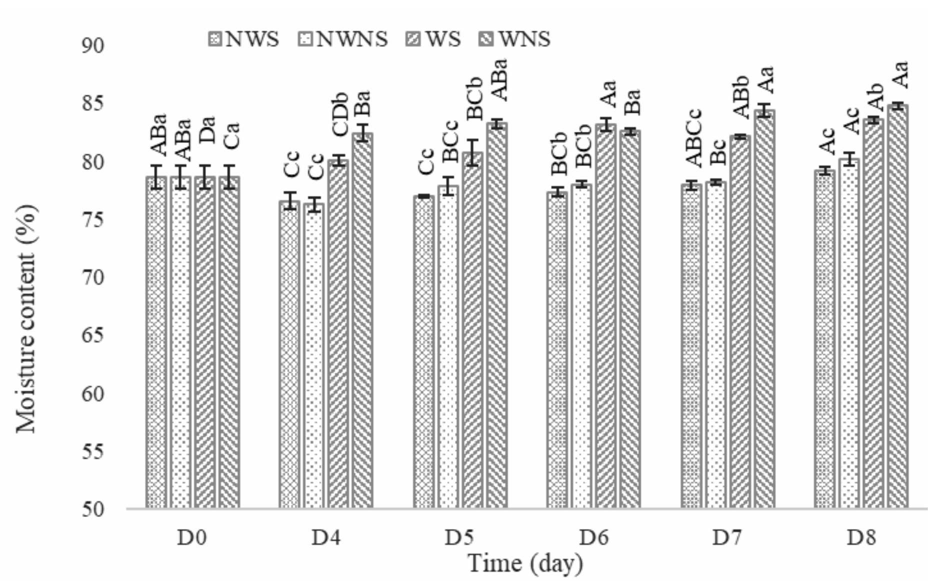

Figure 7. Moisture content of Liang or *Gnetum gnemon* var. *tenerum* leaves after storage at 4°C for 8 days. Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments within each day (P < 0.05). NWS means no washing with the stem; NWNS means no washing without the stem; WS means washing with the stem; WNS means washing without the stem.

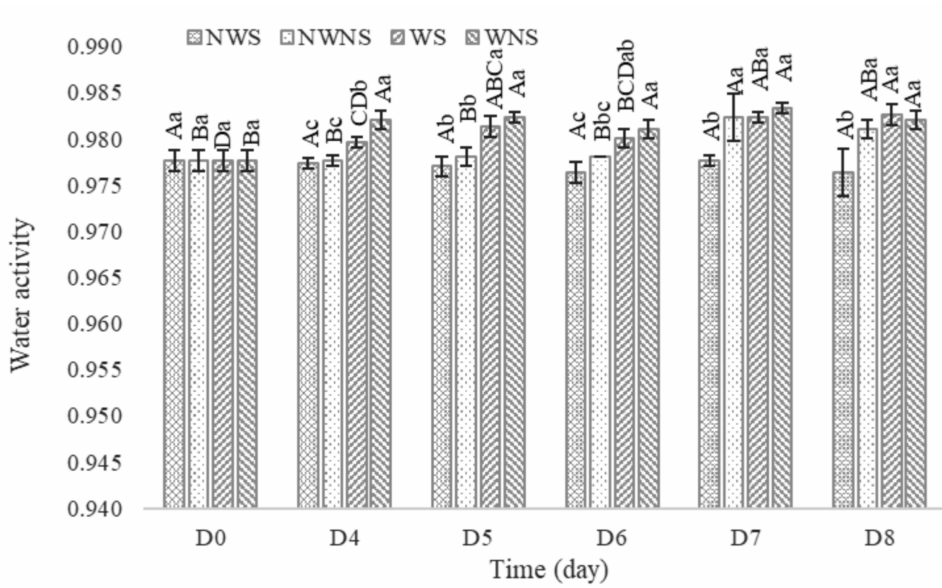

Figure 8. **The water activity of Liang or *Gnetum gnemon* var. *tenerum* leaves after storage at 4°C for 8 days. Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments within each day ($P < 0.05$). NWS means no washing with the stem; NWNS means no washing without the stem; WS means washing with the stem; WNS means washing without the stem.**

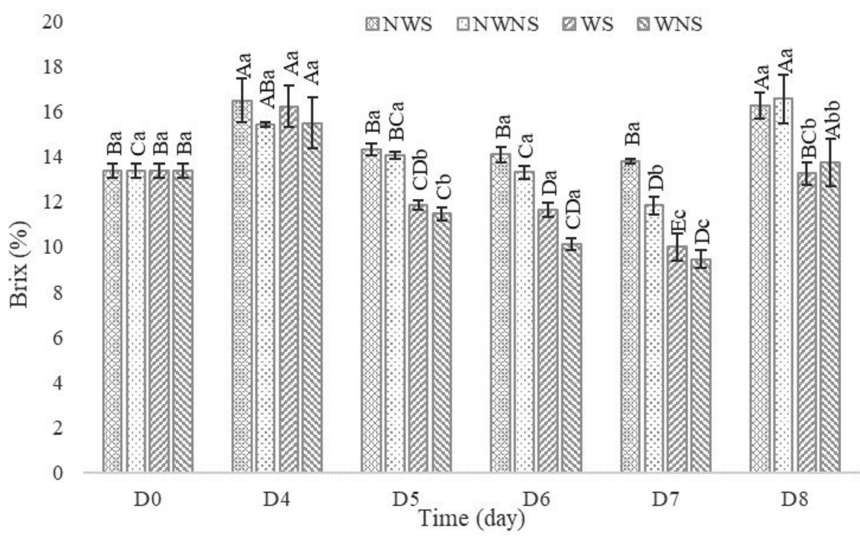

Figure 9. **Brix values of Liang or *Gnetum gnemon* var. *tenerum* leaves after storage at 4°C for 8 days. Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments within each day ($P < 0.05$). NWS means no washing with the stem; NWNS means no washing without the stem; WS means washing with the stem; WNS means washing without the stem.**

pH value

The initial pH of the fresh leaf samples was 6.20 ± 0.03 (Figure 10), which is similar to the pH of approximately 6 as reported by Anisong *et al.* (2022). At the fourth day of storage, the pH of all treatments significantly decreased, indicating three features including reduction of basic compounds, increase in acid and decrease in buffering capacity. It is recognised that a buffer can resist changes in pH if it is sufficient to bind with added protons and the starting pH of the solution (Bobulescu, 2020). While acidic compounds can reduce pH by providing hydrogen ions (H+), basic compounds increase pH by providing hydroxide ions (OH−) (Bartee *et al.*, ND). Consequently, pH gradually increased until Day 7 of storage. At eighth day of storage, a decrease in the

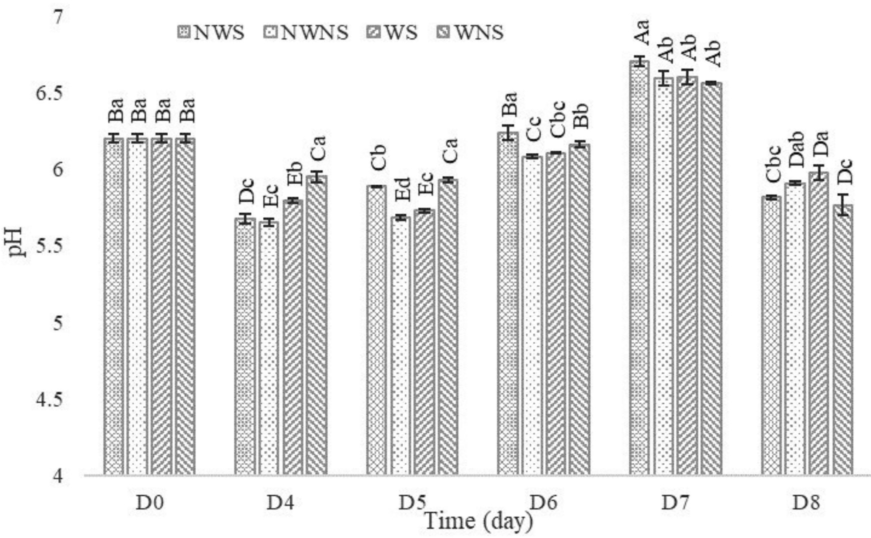

Figure 10. pH of Liang or *Gnetum gnemon* var. *tenerum* leaves after storage at 4°C for 8 days. Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments within each day (P < 0.05). NWS means no washing with the stem; NWNS means no washing without the stem; WS means washing with the stem; WNS means washing without the stem.

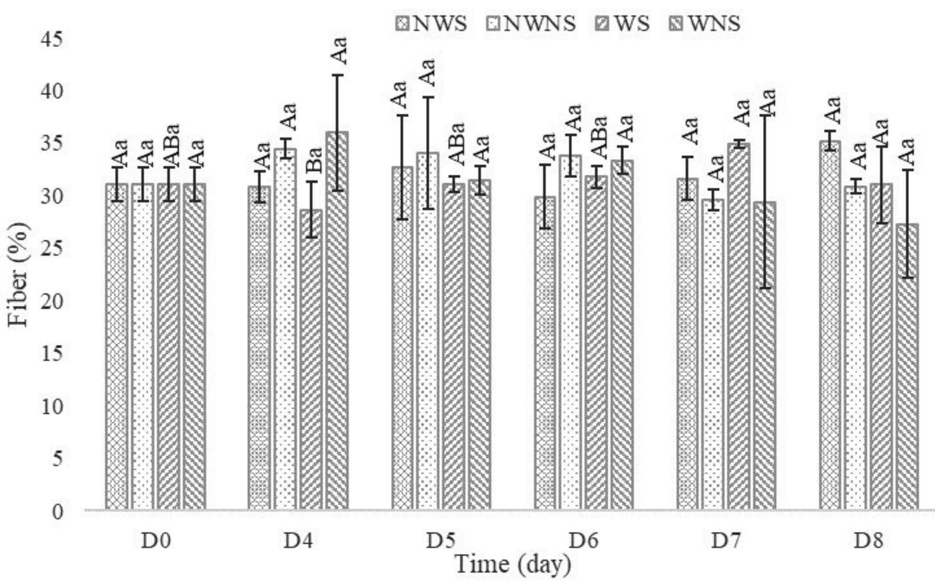

Figure 11. Fibre of Liang or *Gnetum gnemon* var. *tenerum* leaves after storage at 4°C for 8 days. Different uppercase letters indicate significant differences within the same treatment group. Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments within each day (P < 0.05). NWS means no washing with the stem; NWNS means no washing without the stem; WS means washing with the stem; WNS means washing without the stem.

pH of all treatments was observed. The reduction in pH in all treatments resulted in an unpleasant organic smell that was similar to the smell of sweet, fermented sticky rice (Khao mak), which may be due to fermentation by yeast and lactic acid bacteria (Mongkontanawat and Lertnimitmongkol, 2015) as well as anaerobic respiration (Toro and Manuel, 2015).

Fibre content

The fibre content of Liang leaves is shown in Figure 11. The total dietary fibre content was approximately 31% (DW), which was slightly lower than the 36.3% DW as reported by Anisong *et al.* (2022). The fibre content remained constant during storage. The experimental results corresponded to the fibre content of walnuts stored at 4°C (Zhang *et al.*, 2017). During storage, the texture became tougher but the fibre content remained the same. Therefore, the total fibre content was not a good parameter for determining quality changes in Liang leaves, with soluble and insoluble fibres being better options. Details on parameters such as reducing and non-reducing sugar and cellulose contents require further investigation.

Colour change

ΔE values of Liang leaves are shown in Figure 12. The colour of the adaxial and abaxial surfaces and ground leaves in all treatments significantly changed during storage. After storage for 4 days, ΔE of the upper side of the treatments without stem (NWNS and WNS) was higher than that of treatments with stem (NWS and WS). ΔE of the adaxial surface of the WNS treatment was the highest, followed by that of WS, indicating that washing had a greater effect on ΔE at 7 and 8 days of storage. Excess water or high moisture content and mechanical injury induced by washing and stem detachment led to biochemical reactions and increased microbial functions (Mulaosmanovic *et al.*, 2021). Interestingly, ΔE values of the lower side of the stem detachment treatments (NWNS and WNS) were significantly higher than those of stem treatments (WS and NWS). Washing also resulted in a higher ΔE than no washing, suggesting that washing and stem detachment played significant roles in leaf colour.

The ΔE values of ground WS and WNS gradually increased, while those of the others remained constant during storage. The highest ΔE in the ground sample was found in the NWS treatment, while the adaxial and abaxial surfaces under the WNS treatment had the highest ΔE, indicating that grinding affected the ΔE value of the leaves. The difference between the ΔE values of the non-ground sample (adaxial and abaxial surfaces) and the ground sample was caused by the water after the samples were ground. Therefore, the colour quality of stored leaves could be preserved using ground samples.

Chlorophyll content

At fourth day of storage, changes in chlorophyll content in the NWS treatment remained constant but not in the other treatments (NWNS, NWS and WS). See Table 1. Chlorophyll content in the NWS treatment decreased, whereas it increased in the WS treatment at eighth day of storage. The oscillation of chlorophyll content in this study may be due to the plant cell metabolism during storage (Mei *et al.*, 2022). Larrinaga *et al.* (2019) confirmed the oscillation of chlorophyll content in rocket leaves stored in dark at 4°C with a relative humidity of 65 ± 4.5% for 2 days, depending on the day–night cycle (circadian regulation). Interestingly, the chlorophyll content of Liang leaves in this study was higher than kale, which is considered as a high chlorophyll vegetable, at 136.18–172.10 mg/100 g (Lal, 2014). These results indicated that Liang leaves could be used as an alternative source of chlorophyll.

The results for total chlorophyll (Chl a/b) revealed that the chlorophyll a (Chl a) content in all treatments was higher than that of chlorophyll b (Chl b). The Chl a/b ratio in this study was 1.2, which is lower than that of most plant types (generally higher than 2) including trees, shrubs, herbs, conifers, broad-leaves, evergreen and deciduous (Li *et al.*, 2018). The low Chl a/b ratio was due to oxidative stress, as Chl a is more prone to oxidative damage than Chl b (Kasajima, 2019). The Chl a/b ratio of healthy rice leaves was recorded at 3.5, and decreased to 1.5 after subjection to oxidative stress (Kasajima, 2019). Shade plants generally produce more chlorophyll to increase photosynthesis efficiency because of the absorption of blue light in a low-light environment (Beneragana and Goto, 2010). This result was supported by Herrera *et al.* (2022), who reported that plants respond to shade by increasing the production of light-harvesting complexes by increasing Chl b. Shade-tolerant plants respond to low-light environments in diverse ways than normal plants by decreasing the Chl a/b ratio. Farmers usually plant Liang as an intercrop between rubber trees, and the plants adapt to the shaded environment by increasing the total chlorophyll content and Chl b, leading to a low Chl a/b ratio.

TPC, TFC and antioxidant activity

TPC and TFC

Phenolic compounds are produced by chloroplasts to protect cells from damage caused by reactive oxygen species (ROS), a by-product of photosynthesis (Zhang *et al.*, 2018). Leaves are major photosynthetic organs, and green-leaf plants contain an abundance of phenolic compounds (Zhang *et al.*, 2018). The TPC of Liang leaves at Day 0 was 4.32 mg/GAE DW lower than Malaysia Liang leaves which were 8.70 mg/GAE DW as reported by Wazir *et al.* (2011). The TPC and TFC values of Liang leaves are presented in Figures 13 and 14, respectively. Fluctuations in the TPC and TFC in each treatment were observed during storage. The results in this experiment concurred with TPC values in other vegetables and fruits after storage at 4°C, indicating that active plants or plant parts try to remain homeostatic for survival until the end of life (Hubert *et al.*, 2017; Kim, 2015). Cold storage

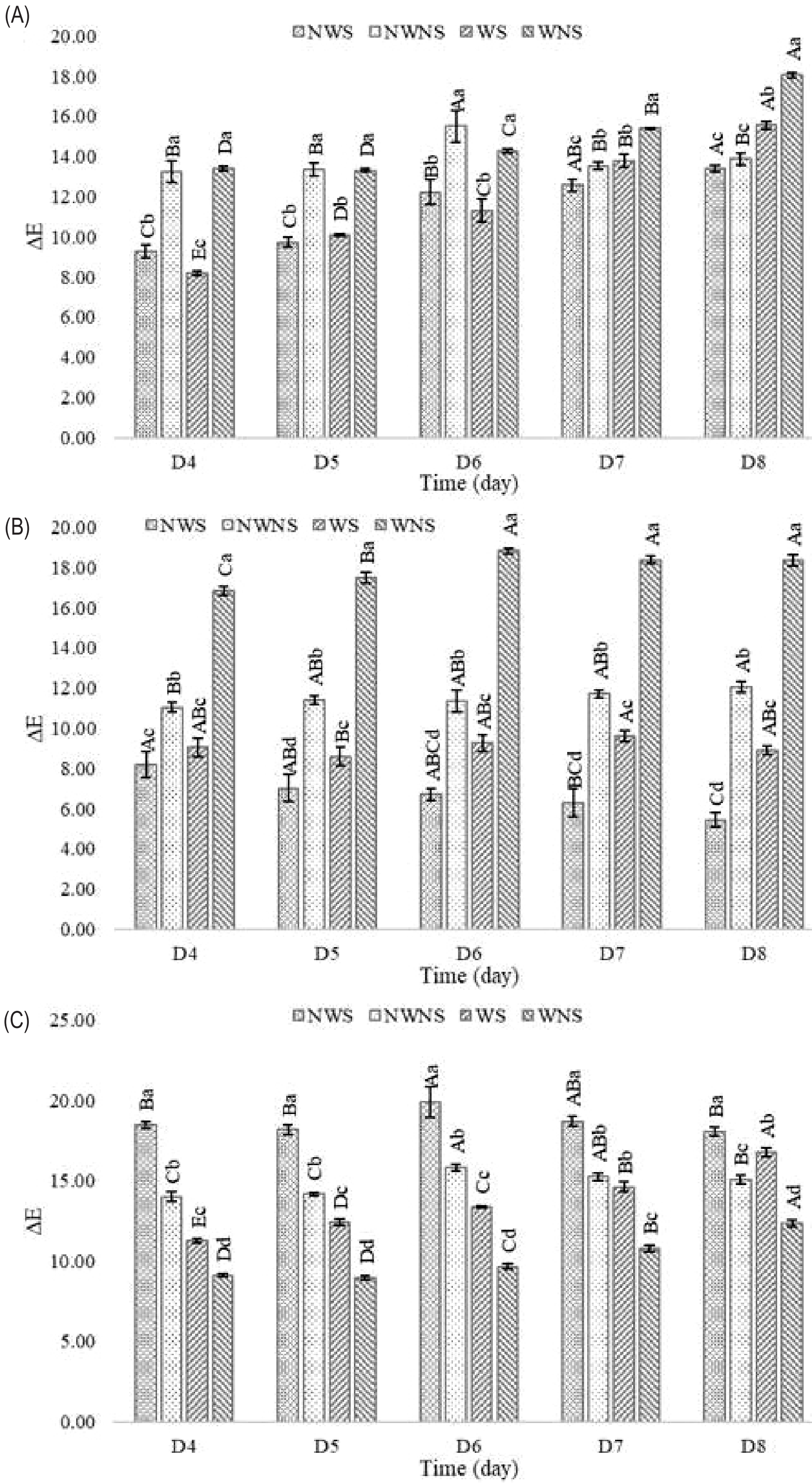

Figure 12. Colour difference (△E) of adaxial surface (A), abaxial surface (B) and ground leaves (C) of *Gnetum gnemon* var. *tenerum* leaves after storage at temperature 4°C for 8 days. Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments within each day ($P < 0.05$). NWS means no washing with the stem; NWNS means no washing without the stem; WS means washing with the stem; WNS means washing without the stem.

Table 1. Total chlorophyll (Chl a+b), chlorophyll a (Chl a), chlorophyll b (Chl b) and the ratio of chlorophyll a to b (Chl a/b) of Liang or *Gnetum gnemon* var. *tenerum* leaves after storage at 4°C for 8 days.

Time	Treatment	Chl a + b (mg/g DW)	Chl a (mg/g DW)	Chl b (mg/g DW)	Chl a/b
D0	NWS	226.28 ± 22.25ABa	114.55 ± 7.46Ba	101.53 ± 6.71Ba	1.13 ± 0.10Aa
	NWNS	226.28 ± 22.25Aa	114.55 ± 7.46Aa	101.53 ± 6.71Aa	1.13 ± 0.10Aa
	WS	226.28 ± 22.25Aa	114.55 ± 7.46Aa	101.53 ± 6.71Aa	1.13 ± 0.10Ba
	WNS	226.28 ± 22.25Aa	114.55 ± 7.46Ba	101.53 ± 6.71Aa	1.13 ± 0.10Aa
D4	NWS	254.46 ± 9.96Aa	138.41 ± 5.48Aa	116.05 ± 4.49Aa	1.19 ± 0.00Ac
	NWNS	188.16 ± 2.60Bb	103.61 ± 1.35ABb	84.55 ± 1.26Bb	1.23 ± 0.00Aab
	WS	172.60 ± 10.57Bb	95.01 ± 6.28Bb	77.60 ± 4.29Bb	1.22 ± 0.01ABb
	WNS	177.26 ± 1.47Bb	98.31 ± 1.00Cb	78.95 ± 0.47Bb	1.25 ± 0.01Aa
D8	NWS	200.07 ± 2.55Bb	110.67 ± 1.38Bb	89.40 ± 1.19Cb	1.24 ± 0.00Ab
	NWNS	181.50 ± 5.16Bc	101.41 ± 2.82ABc	80.10 ± 2.45Bc	1.27 ± 0.02Aa
	WS	177.03 ± 6.56Bc	99.44 ± 4.01ABc	77.59 ± 2.56Bc	1.28 ± 0.01Aa
	WNS	229.74 ± 5.79Aa	126.56 ± 3.41Aa	103.18 ± 2.40Aa	1.23 ± 0.01Ab

Remarks: NWS means no washing with the stem; NWNS means no washing without the stem; WS means washing with the stem; WNS means washing without the stem. Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments within each day (P < 0.05).

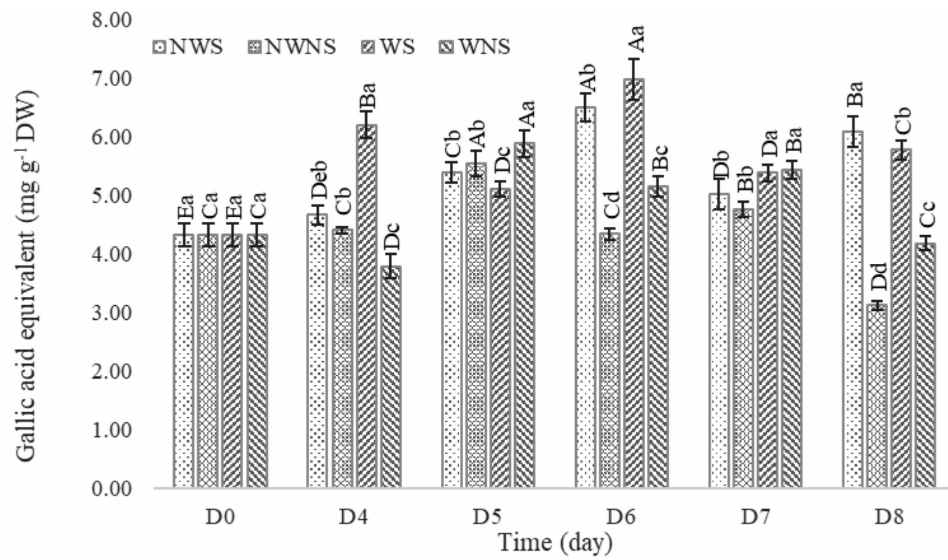

Figure 13. Total phenolic content of Liang or *Gnetum gnemon* var. *tenerum* leaves after storage at 4°C for 8 days. Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments within each day (P < 0.05). NWS: no washing with the stem; NWNS: no washing without the stem; WS: washing with the stem; WNS: washing without the stem.

induced phenolic production (polyphenolic phytoalexins) (Hubert *et al.*, 2017). A decrease in TPC and TFC during 8 days of storage with stem detachment (NWNS and WNS) indicated a reduced availability of nutrients to produce phenolic compounds, in contrast to treatments with stems (NWS and WS) which provided nutrients from the stems to the leaves. An increase in phenolic compounds production in wounded, stressed plants was observed in sweet potato roots (Dovene *et al.*, 2019). Stem detachment can also injure plant cells as a result of wounds, requiring more energy and a curing agent to treat the damaged cells.

The TPC content of all treatments increased or remained constant compared with that of the control (Day 0). The TFC content of the NWS and WS treatments was equal to that of the control (Day 0), whereas NWNS (second bar) and WNS (last bar) during storage for 8 days were lower on Day 0, indicating a loss of nutrition supply from the stems. Detaching the stems can be linked

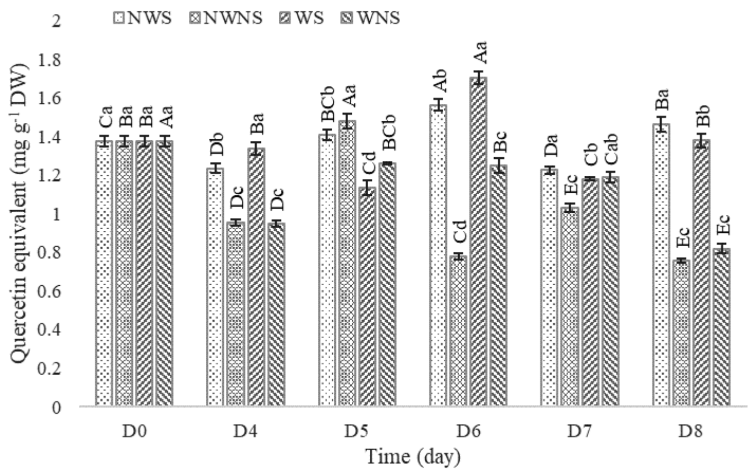

Figure 14. Total flavonoid content of Liang or *Gnetum gnemon* var. *tenerum* leaves after storage at 4°C for 8 days. Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments within each day ($P < 0.05$). NWS means no washing with the stem; NWNS means no washing without the stem; WS means washing with the stem; WNS means washing without the stem.

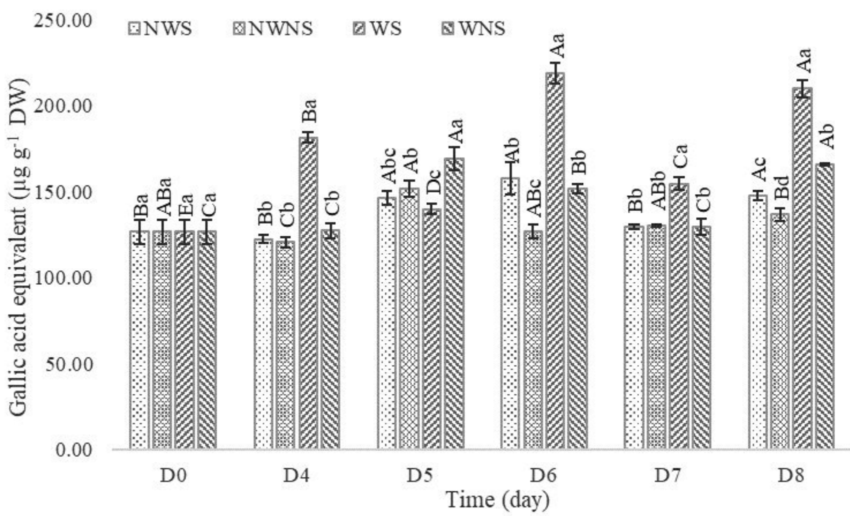

Figure 15. DPPH of Liang or *Gnetum gnemon* var. *tenerum* leaves after storage at 4°C for 8 days. Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments within each day ($P < 0.05$). NWS means no washing with the stem; NWNS means no washing without the stem; WS means washing with the stem; WNS means washing without the stem.

to the cutting of organs of living things; this not only causes injury but also requires more energy for recovery. Antioxidant compounds could be used to alleviate stress and control wounds (Comino-Sanz *et al.*, 2021).

Antioxidant activity (DPPH, ABTS and FRAP)

DPPH and ABTS assays are used to stabilize 2,2-diphenyl-1-picrylhydrazyl (DPPH) or 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) to measure the ability of hydrogen and electron transfer of antioxidants instead of very short-lived natural radicals such as hydroxyl (HO·), lipid alkyl (L·) and lipid peroxyl (LOO·) (Munteanu and Apetriel, 2021; Yeo and Shahidi, 2019). Ferric ion–reducing antioxidant power (FRAP) is a method for determining the electron transfer ability of antioxidants by reducing the colourless complex ferric ion (Fe^{3+}) to blue ferrous complex (Fe^{2+}) in an acidic environment (pH 3.6) (Munteanu and Apetriel, 2021). Generally, the results revealed that WS treatment had the highest antioxidant capacity, followed by NWS and WNS treatments (Figures 15–17). Remarkably, NWS offered antioxidant capacity comparable to WNS treatments, even

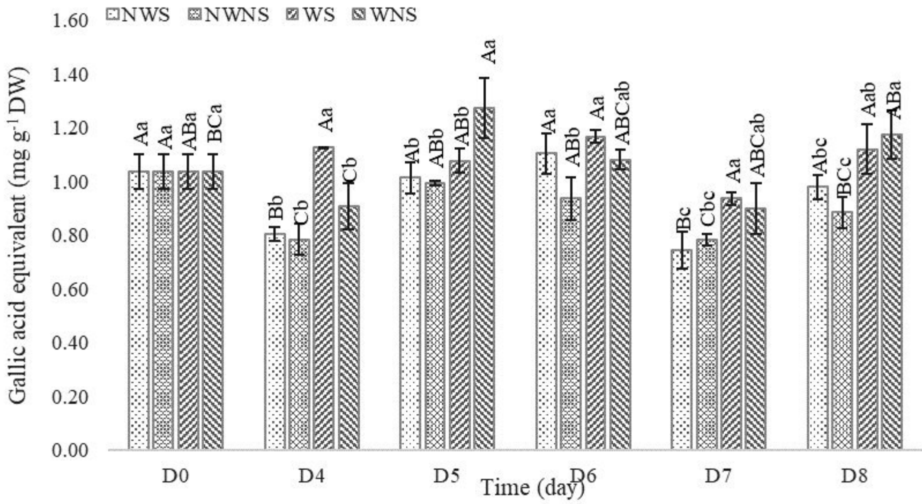

Figure 16. ABTS of Liang or *Gnetum gnemon* var. *tenerum* leaves after storage at 4°C for 8 days. Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments within each day ($P < 0.05$). NWS means no washing with the stem; NWNS means no washing without the stem; WS means washing with the stem; WNS means washing without the stem.

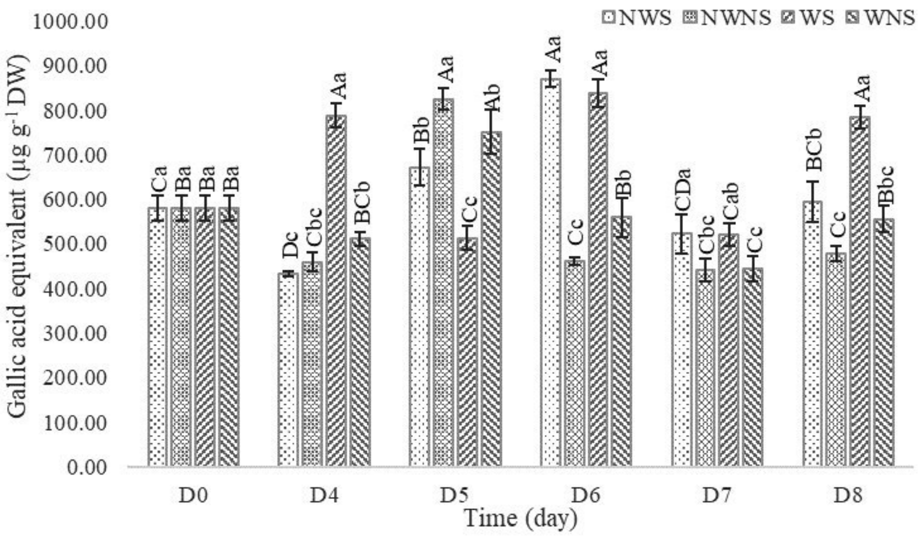

Figure 17. FRAP of Liang or *Gnetum gnemon* var. *tenerum* leaves after storage at 4°C for 8 days. Different uppercase letters indicate significant differences within the same treatment group. Different lowercase letters indicate significant differences between treatments within each day ($P < 0.05$). NWS means no washing with the stem; NWNS means no washing without the stem; WS means washing with the stem; WNS means washing without the stem.

though NWS contained lower TPC and TFC. The results indicated that injury from washing induced different groups of phenolic compounds with higher antioxidant ability in Liang leaves during storage (Pratyusha, 2021).

The ABTS assay exhibited the highest antioxidant capacity, followed by FRAP and DPPH assays. The higher ABTS value compared to DPPH indicated strong polarity by donating electrons and H+. Pongsetkul *et al.* (2023) also confirmed relation of ABTS assay and hydrogen donating capability of antioxidant in aqueous phase. As mentioned above, the FRAP value indicates the reducing power of the antioxidants to metal ions. The antioxidant activity in plants is generated by phenolic compounds and also other compounds such as vitamin C and pigments such as chlorophyll and carotenoids (Sarker *et al.*, 2020), therefore explaining why ABTS radical scavenging in this study was not well related to phenolic compounds because carotenoid, chlorophyll and vitamin C contained in Liang leaves were 3706 µg/100 g DW (Anisong *et al.*, 2022), 226.28 ± 22.25 mg/g DW and 2.71–5.25 mg/100 g DW (data in process for publication), respectively.

Sensory evaluation

The sensory qualities of treatments during storage for 8 days at 4°C are shown in Table 2. All treatments were comparable to those of fresh Liang leaves (Day 0). The odour, texture and flavour of NWS treatments remained stable during the storage period. The sensory scores showed that the panelists accepted all treatments after 8 days of storage, even though there was an unpleasant organic smell starting on Day 5 of storage when the bag was opened. However, after cooking, the off-odour of

Table 2. Sensory scores of Liang or *Gnetum gnemon* var. *tenerum* leaves after storage at 4°C for 8 days.

Attributes	Condition	Time					
		Day 0	Day 4	Day 5	Day 6	Day 7	Day 8
Appearance	NWS	7.52 ± 0.97^{ABa}	7.30 ± 1.13^{Ba}	7.79 ± 0.74^{Aa}	7.45 ± 0.97^{ABa}	7.52 ± 1.00^{ABa}	7.79 ± 0.82^{Aa}
	NWNS	7.73 ± 0.87^{Aa}	7.87 ± 0.86^{Aa}	7.57 ± 1.01^{Aa}	7.67 ± 0.92^{Aa}	7.67 ± 0.88^{Aa}	7.57 ± 0.86^{Aa}
	WS	7.40 ± 0.98^{Aa}	7.51 ± 0.95^{Aa}	7.43 ± 0.98^{Aa}	7.60 ± 0.88^{Aa}	7.71 ± 0.99^{Aa}	7.43 ± 1.07^{Aa}
	WNS	7.83 ± 0.79^{Aa}	7.63 ± 1.16^{Aa}	7.77 ± 0.82^{Aa}	7.37 ± 1.25^{Aa}	7.57 ± 0.90^{Aa}	7.77 ± 0.86^{Aa}
Color	NWS	7.52 ± 1.09^{Aa}	7.36 ± 0.99^{Aa}	7.73 ± 0.88^{Aa}	7.45 ± 0.90^{Aa}	7.45 ± 0.94^{Aa}	7.73 ± 0.80^{Aa}
	NWNS	7.57 ± 0.94^{Aa}	7.77 ± 0.77^{Aa}	7.60 ± 0.81^{Aa}	7.50 ± 0.94^{Aa}	7.43 ± 0.90^{Aa}	7.47 ± 0.97^{Aa}
	WS	7.31 ± 0.99^{Aa}	7.54 ± 0.89^{Aa}	7.54 ± 0.89^{Aa}	7.57 ± 0.92^{Aa}	7.63 ± 0.97^{Aa}	7.66 ± 0.94^{Aa}
	WNS	7.63 ± 0.85^{Aa}	7.70 ± 1.02^{Aa}	7.53 ± 0.94^{Aa}	7.30 ± 1.09^{Aa}	7.70 ± 0.84^{Aa}	7.63 ± 0.89^{Aa}
Odor	NWS	7.21 ± 1.17^{Aa}	7.15 ± 1.12^{Aa}	7.28 ± 0.96^{Aa}	7.48 ± 0.80^{Aa}	7.39 ± 1.06^{Aa}	7.45 ± 1.03^{Aa}
	NWNS	7.23 ± 0.97^{Aa}	7.63 ± 0.89^{Aa}	7.20 ± 0.92^{Aa}	7.30 ± 0.99^{Aa}	7.10 ± 1.24^{Aa}	7.23 ± 1.04^{Aa}
	WS	7.26 ± 1.22^{Aa}	7.43 ± 1.24^{Aa}	7.17 ± 0.89^{Aa}	7.17 ± 1.04^{Aa}	7.20 ± 0.99^{Aa}	6.94 ± 1.21^{Aa}
	WNS	7.50 ± 0.97^{ABa}	7.53 ± 0.82^{Aa}	7.33 ± 0.92^{ABCa}	7.03 ± 1.25^{BCa}	7.17 ± 0.99^{ABCa}	6.97 ± 1.33^{Ca}
Odor	NWS	7.21 ± 1.17^{Aa}	7.15 ± 1.12^{Aa}	7.28 ± 0.96^{Aa}	7.48 ± 0.80^{Aa}	7.39 ± 1.06^{Aa}	7.45 ± 1.03^{Aa}
	NWNS	7.23 ± 0.97^{Aa}	7.63 ± 0.89^{Aa}	7.20 ± 0.92^{Aa}	7.30 ± 0.99^{Aa}	7.10 ± 1.24^{Aa}	7.23 ± 1.04^{Aa}
	WS	7.26 ± 1.22^{Aa}	7.43 ± 1.24^{Aa}	7.17 ± 0.89^{Aa}	7.17 ± 1.04^{Aa}	7.20 ± 0.99^{Aa}	6.94 ± 1.21^{Aa}
	WNS	7.50 ± 0.97^{ABa}	7.53 ± 0.82^{Aa}	7.33 ± 0.92^{ABCa}	7.03 ± 1.25^{BCa}	7.17 ± 0.99^{ABCa}	6.97 ± 1.33^{Ca}
Texture	NWS	7.45 ± 1.23^{Aa}	7.00 ± 1.12^{Aa}	7.30 ± 1.33^{Aa}	7.61 ± 0.90^{Aa}	7.15 ± 1.39^{Aa}	7.55 ± 1.03^{Aa}
	NWNS	7.47 ± 0.82^{ABa}	7.67 ± 0.88^{Aa}	7.30 ± 0.92^{ABa}	7.30 ± 1.09^{ABa}	7.00 ± 1.17^{Ba}	7.40 ± 0.97^{ABa}
	WS	7.37 ± 1.06^{Aa}	740 ± 1.35^{Aa}	7.11 ± 1.13^{Aa}	7.17 ± 1.20^{Aa}	7.29 ± 1.05^{Aa}	7.20 ± 1.05^{Aa}
	WNS	7.50 ± 0.97^{Aa}	7.57 ± 0.94^{Aa}	7.47 ± 0.86^{Aa}	7.17 ± 1.23^{Aa}	7.47 ± 1.14^{Aa}	7.10 ± 1.37^{Aa}
Flavor	NWS	7.00 ± 1.22^{Aa}	7.15 ± 1.25^{Aa}	7.30 ± 0.98^{Aa}	7.45 ± 1.06^{Aa}	7.18 ± 1.13^{Aa}	7.33 ± 0.96^{Aa}
	NWNS	7.23 ± 1.01^{Aa}	7.50 ± 1.25^{Aa}	7.13 ± 1.43^{Aa}	7.10 ± 1.03^{Aa}	7.10 ± 1.24^{Aa}	7.07 ± 1.26^{Aa}
	WS	7.11 ± 1.21^{Aa}	7.23 ± 1.24^{Aa}	7.03 ± 1.04^{Aa}	7.00 ± 1.06^{Aa}	7.20 ± 1.02^{Aa}	6.91 ± 1.15^{Aa}
	WNS	7.37 ± 1.07^{ABa}	7.63 ± 0.81^{Aa}	7.30 ± 0.88^{ABa}	7.07 ± 1.23^{ABa}	7.23 ± 0.94^{AB}	7.03 ± 1.13^{Ba}
Taste	NWS	7.06 ± 1.14^{Aa}	6.91 ± 1.33^{Aa}	7.27 ± 0.88^{Aa}	7.18 ± 1.33^{Aa}	7.12 ± 1.32^{Aa}	7.21 ± 1.05^{Aa}
	NWNS	7.20 ± 1.00^{Aa}	7.40 ± 1.25^{Aa}	7.00 ± 1.58^{Aa}	7.30 ± 0.99^{Aa}	6.80 ± 1.42^{Aa}	6.87 ± 1.41^{Aa}
	WS	7.26 ± 1.38^{Aa}	7.26 ± 1.34^{Aa}	7.06 ± 1.11^{Aa}	6.97 ± 1.12^{Aa}	7.14 ± 1.26^{Aa}	6.91 ± 1.25^{Aa}
	WNS	7.37 ± 1.00^{Aa}	7.30 ± 1.39^{Aa}	7.20 ± 0.89^{Aa}	6.97 ± 1.19^{Aa}	7.10 ± 0.96^{Aa}	7.17 ± 1.12^{Aa}
Overall	NWS	7.12 ± 1.19^{Aa}	6.94 ± 1.25^{Aa}	7.15 ± 1.30^{Aa}	7.30 ± 1.05^{Aa}	7.03 ± 1.26^{Aa}	7.55 ± 0.90^{Aa}
	NWNS	7.37 ± 0.93^{Aa}	7.50 ± 1.20^{Aa}	7.07 ± 1.36^{Aa}	7.23 ± 1.01^{Aa}	6.87 ± 1.36^{Aa}	7.13 ± 1.20^{Aa}
	WS	7.17 ± 1.29^{Aa}	7.26 ± 1.15^{Aa}	7.14 ± 1.00^{Aa}	6.97 ± 1.15^{Aa}	7.14 ± 1.14^{Aa}	7.03 ± 1.12^{Aa}
	WNS	7.40 ± 1.04^{Aa}	7.57 ± 0.68^{Aa}	7.37 ± 0.96^{Aa}	7.20 ± 1.27^{Aa}	7.30 ± 0.95^{Aa}	7.20 ± 1.16^{Aa}
Acceptance	NWS	75.76^{Fd}	87.88^{Cc}	81.82^{Dd}	90.91^{Bc}	75.76^{Ec}	93.94^{Aa}
	NWNS	90.00^{Bc}	93.33^{Ab}	86.67^{Cb}	83.33^{Dd}	93.33^{Aa}	86.67^{Cd}
	WS	94.29^{Aa}	82.86^{Dd}	85.71^{Cc}	85.71^{Cc}	88.57^{Bb}	88.57^{Bc}
	WNS	93.33^{Cb}	100^{Aa}	96.67^{Ba}	100.00^{Aa}	93.33^{Ca}	90.00^{Db}

Remarks: NWS means no washing with the stem; NWNS means no washing without the stem; WS means washing with the stem; WNS means washing without the stem. Different uppercase letters indicate significant differences within the same treatment group (column). Different lowercase letters indicate significant differences between treatments within each day (row) ($P < 0.05$).

Liang leaves dissipated. An increase in organic acids and off-odour in vegetables by microbes were also confirmed by Jacxsens *et al*. (2003).

Conclusions

The physicochemical, chemical and sensory qualities of Liang leaves after washing with chlorinated water at 100 ppm for 15 min and detaching the stem were determined. Washing increased the moisture content and a_w in Liang leaves and led to lower soluble solid content. Stem detachment and washing of Liang leaves did not affect the amount of fibre and chlorophyll after storage at 4°C for 8 days. Liang leaves without stems contained lower TPC and TFC contents; however, washing increased antioxidant capacity based on the DPPH and ABTS methods, while stem detachment did not cause significant differences. Liang leaves stored at 4°C for 5 days produced an acid-like odour during storage, but this unpleasant odour dissipated after steaming. Liang leaves maintained acceptable quality when stored at 4°C for at least 8 days, and microbial quality should be further examined in future studies. This study provides a starting point for the future development of commercial ready-to-eat and ready-to-cook Liang leaf products. However, postharvest techniques and storage conditions require more detailed investigations to extend the shelf life of Liang leaves.

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