Songklanakarin J. Sci. Technol.
47 (3), 227–234, May – Jun. 2025

http://www.sjst.psu.ac.th

Original Article

Effects of incubation duration and temperature on sprout development and active compound contents in shallot sprout

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Received: 7 November 2024; Revised: 18 February 2025; Accepted: 21 April 2025

Abstract

One effective way to enhance the value of shallots is by producing shallot sprouts. This research aimed to determine the optimal duration and temperature conditions for producing shallot sprout with highest development and active compound content. A CRD with three replications was used. Dry shallots were incubated at 5 ± 2°C, 10 ± 2°C and 25 ± 2°C. Sprout development and active compound content were assessed at 2, 4, 6 and 8 weeks after incubation (WAI). The optimal germination conditions for shallot sprouts are 5°C for 4–6 WAI, with 6 WAI being ideal for preserving bioactive compounds and maximizing antioxidant activity. While 5°C is the most suitable temperature for overall sprout development, 10°C serves as a secondary option for promoting higher biomass accumulation. These conditions enhance shoot and root growth, maintain high levels of phenolic and flavonoid, sustain strong antioxidant activity, and minimize mold contamination, resulting in high-quality shallot sprouts with an extended shelf life.

Keywords: active compounds, germination, incubation time, shallot, temperature

1. Introduction

Shallots (*Allium ascalonicum* L.) are a popular economic crop that Thai people commonly consume (Phuengphai, Satchawan, Tongnunui, Jamnongkan, & Wattanakornsiri, 2022). They serve both as medicinal plants and culinary spices. Shallot leaves contain active compounds such as quercetin, kaempferol and spiraeoside, while only quercetin is found in the bulb (Tendaj & Mysiak, 2010; Yasurin, 2015). Shallots possess various medicinal properties, including antibacterial and antifungal effect, cholesterol lowering ability, blood pressure reduction, and potential inhibition of cancer cells (Phuengphai *et al*., 2022). In Thailand, shallots can be grown year-round, especially during the cold season. However, their price fluctuates due to market uncertainty. During the harvesting period, the price of shallots tends to drop as large quantities of shallot enter the market simultaneously. Additionally, post-harvest spoilage is a common issue. To solve these problems, shallot can be processed into higher-value products such as fried shallot or food ingredients. One interesting way to increase the value of shallots is the production of shallot sprout. Germination is a critical stage in a plant's life cycle, during which various physiological and biochemical changes occur. The temperature and duration of germination significantly impact both the germination process and the presence of bioactive compounds. Shallot sprout production is expected to add value to shallots, similar to rice sprout production, which has been shown to nearly double the value of regular rice (Institute of Food Research and Product Development [IFRPD] Kasetsart University, 2013). Shallot sprouts are produced by keeping dry shallots at a controlled temperature until they develop leaves approximately 3-5 cm long. These shallot sprouts can be consumed as fresh vegetables or used as ingredients in various dishes. The optimal temperature and

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incubation duration significantly affect the germination rate and the concentration of active compounds in various plants, including shallots. Morrall, Boyd, Taylor, and Van Der Walt (1986) reported that germination temperatures of 24°C and 28°C were ideal for developing diastatic power, free α-amino nitrogen and extract in sorghum malting, while higher temperatures and prolonged germination led to progressively poorer outcome. Similarly, Rezaie, Mansoori, Kafi, and Bannayan (2012) studied Persian shallot (*Allium altissimum*) germination at ten different temperatures (6, 9, 12, 15, 18, 20, 22, 24, 26 and 28°C), finding the highest emergence rate at 18°C, with a sharp decline at higher temperatures. In addition, Ichikawa, Ide, and Ono (2006) found that storing garlic at 4°C significantly enhanced the conversion of the γ-glutamyl peptides into sulfoxides (alliin and isoalliin). In contrast, storage at 23°C led to a marked decrease in the γ-glutamyl peptides and increase in cycloalliin Moreover, Buakum and Promchote (2021) reported that the best germination conditions for shallots involved storing them in 7x10 cm zip-lock bags (one bulb per bag, without additional air holes) and placing them in a clear container. Shallots weighing approximately 1.0 g per bulb exhibited better germination rate at 10 ± 2ºC compared to 5 ± 2 and 25 ± 2ºC after 1-4 weeks of incubation (Buakum and Promchote, 2021). Although Buakum and Promchote (2021) studied shallot spout germination at different temperatures, the active compounds contents of shallot sprouts have not yet been investigated. Therefore, this research aimed to determine the optimal duration and temperature conditions for maximizing shallot sprout development and active compound content.

2. Materials and Methods

2.1 Experimental design and treatments

The experiment was conducted at the Central Laboratory, Faculty of Agriculture, Ubon Ratchathani University. A completely randomized design (CRD) with three replications was used. Dry shallots, harvested at three months (Srisaket accession) (Figure 1A), were incubated at 5 ± 2°C, 10 ± 2°C and 25 ± 2°C. For the 5 ± 2°C and 10 ± 2°C treatments, the shallots were incubated in glass-front refrigerators set to the desired temperature. For 25 ± 2°C treatments, the shallots were incubated in the same room where the glass-front refrigerators were installed, with an air conditioner maintaining the temperature. Shallots in all treatments were exposed to the same fluorescent light. Data were collected at 2, 4, 6 and 8 weeks after incubation (WAI).

2.2 Incubation method

Bulbs of dry shallot, approximately 2 g in weight without fungal contamination, were selected. After removing the outer 2-3 scales, the shallots were soaked in 50 ppm chorine solution for 10 min and rinsed twice with clean water, and then left to dry. For incubation, the shallots were stored in a zip-lock bags of size 15x23 cm, one bulb per bag, without additional air holes. The bag was then placed in an airtight clear box made of polyethylene terephthalate (16 x 22 x 8 cm), following a modified method from Buakum and Promchote (2021). This experiment used larger zip-lock bags (15x23 cm) than those in the previous study by Buakum and Promchote (2021) (7x10 cm) because the shallots were incubated for a longer duration. Each box contained twelve bags. There was no water added to the shallot bags, and light condition was not controlled (Figure 1B).

(A) (B)

Figure 1. Dry Shallots (*Allium ascalonicum* L., Srisaket accession) (A) that were stored in a zip locked bag inside an airtight clear box (B)

2.3 Data collection

Data were collected at 2, 4, 6 and 8 WAI. Relative humidity was measured both inside and outside the box during the experiment using a hygro-thermometer (Digicon TH-03A). Leaf lengths and leaf numbers were recorded. The fresh weights of bulbs, shoots and roots were recorded. The samples were then dried in an oven at 80°C for 48 hours, and their dry weights were determined (Hunt, 1978). The shallot shoots were freeze-dried using a freeze dryer (Christ, Alpha 1-4 Ldplus, USA) and subsequently subjected to the extraction.

2.4 Sample extraction

Sample extraction processes were conducted as follows. Briefly, the freeze-dried shallot shoots (7.5 g) were extracted in 92.5 mL of 70% ethanol at room temperature for 24 hours using a shaker. The extract was then filtered through three layers of sheet cloth and Whatman No.1 filter paper. Then the solvent was evaporated to dryness using a rotary vacuum evaporator (Heidolph, Germany). The obtained extracts were further dried at room temperature and stored in an airtight bottle at 4ºC. Before use, the residue was dissolved in 10 mL of ethanol.

2.5 Total phenolic content

Determination of total phenol content was carried out by the Folin-Ciocalteu test according to Werawattanachi, Kaewamatawong, and Junlatat (2015). The extract (200 µL) was mixed with 1.0 mL of Folin–Ciocalteu's reagent and 0.8 mL of sodium carbonate (7.5%, w/v). The sample was kept in the dark at room temperature for 30 min. The absorbance of the reaction mixture was measured at 765 nm against blank. The standard curve of Gallic acid (0.002-0.012 mg/mL) was determined. The total phenolic content was expressed as mg gallic acid equivalents/100 g of extract.

2.6 Total flavonoid content

Total flavonoid content was measured using the aluminum chloride colorimetric method, following the

procedure of Werawattanachi *et al*. (2015). Briefly, 0.5 mL of sample, 1.5 mL of methanol, 0.1 ml of aluminum chloride solution, 0.1 mL potassium acetate solution, and 2.8 mL distilled water were added and mixed well. The prepared solution was filtered through Whatman No.1 filter paper before measuring absorbance at 415 nm. Quercetin, at a concentration of 6.25-100 ug/mL, was used as the reference standard.

2.7 Quercetin content analysis

Quercetin content was analyzed using Ultra High Performance Liquid Chromatography (UHPLC) according to the method of Careri, Corradini, Elviri, Nicoletti, and Zagnoni (2003) and Pudzianowska *et al*. (2012) with modifications. Briefly, the extract was filtered with a Supelco Iso-Disc syringe-tip filter unit with a PTFE membrane (diameter: 25 mm, pore size: 0.2 μm). The analysis was carried out using a UHPLC system (DIONEX, Ultimate 3000, Thermo Fisher Scientific) equipped with pump, degasser, photo diode array (PDA) detector and auto-sampler. Chromatographic separation was conducted using a C18 column (4.6 x 250 mm, 5 μm) as the stationary phase with column oven temperature of 30 °C. The mobile phase comprised 0.05% (V/V) formic acid aqueous solution and acetonitrile. The injection volume was 10 μL with a flow rate of 0.6 mL/min. UV detection was performed at a wavelength of 370 nm.

2.8 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity

Free radical scavenging activity of the sample was measured using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity, following the method of Yen and Wu (1999). Briefly, 4 mL of the extract was added to 1 mL of 0.2 mM DPPH solution. The mixture was then incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm against a blank using a UV-VIS spectrophotometer (UVmini-1240, Shimadzu). The DPPH radical scavenging activity was expressed as μM of Trolox equivalents per mg extract (μM TE/mg extract)

2.9 2, 2'-azino-bis (3-ethylbenzothiazoline-6 sulfonic acid) diammonium salt (ABTS radical scavenging activity)

ABTS radical scavenging activity was analyzed using a method modified from Re *et al*. (1999). The ABTS stock solution was prepared by mixing a 7.4 mM ABTS solution with a 2.6 mM potassium persulfate solution in 10 mM phosphate buffer (pH 7.4) and then kept in the dark for 16 hrs. The ABTS working solution was prepared by diluting the ABTS stock solution in a 10 mM phosphate buffer (pH 7.4) to achieve an absorbance of 0.7±0.02 at a wavelength of 734 nm. Ten microliters of extract (1 mg/mL) were mixed with 1 mL of ABTS working solution. Then the reaction mixtures were kept in the dark for 6 min and absorbance was measured at 734 nm. The ABTS radical scavenging activity was expressed as μM of Trolox equivalents per mg of extract (μM TE/mg extract).

2.10 Vitamin C

Vitamin C was determined using the titrimetric method with a 2,6-dichlorophenolindophenol reagent according to the method of Moo-Huchin *et al*. (2015). Two milliliters of crude extract were mixed with 5 mL of a 3 % oxalic acid solution. This solution was titrated with 0.02% 2,6-dichloro-phenolindophenol solution. The endpoint was considered to be reached when the solution attained a pink color which persisted for 15 sec. The calibration of the 2,6-dichlorophenolindophenol solution was performed with 0.05% ascorbic acid solution. Results were expressed as mg of ascorbic acid equivalents per 100 g of extract.

2.11 Yeast and molds

Yeasts and molds in shallot bulb were determined according to Association of Official Analytical Chemists (1984) using the spread plate method on potato dextrose agar. The sample (25 g) was weighted and mixed with 0.1% peptone water to achieve a 10^{-1} dilution and then homogenized for 2 min. A series of 10-fold dilutions (10^{-1} to 10^{-6}) was prepared. Each dilution (0.1 mL) of sample was aseptically pipetted on agar plate and the inoculum was spread using a sterile bent glass rod. Each dilution was plated in triplicate. The samples were incubated at 25 °C for 5-7 days. The results were reported as CFU/g.

2.12 Statistical analysis

Each sample analysis was performed in triplicate. Experimental results are reported as mean values. The results were analyzed using analysis of variance (ANOVA) and the least significant difference (LSD) test. Significance was accepted at $p < 0.05$. Calculations were performed using STATISTIX–8.

3. Results

3.1 Relative humidity

Relative humidity outside and inside the incubation box was significantly higher at 5°C than at 10 and 25°C, at 2, 4, 6 and 8 WAI. At 5°C, the relative humidity outside and inside incubation boxes ranged from 65.7% to 91.3% and from 77.0% to 94.0%, respectively. At 10 and 25°C, the relative humidity outside and inside the incubation box were similar at 2 and 6 WAI, but at 4 and 8 WAI, the relative humidities outside and inside the incubation box at 10°C were higher than at 25°C (Figure 2).

3.2 Sprout development

Shoot fresh weight of shallot sprout at different temperatures was not significantly different at 2, 4 and 6 WAI. At 8 WAI, shallot sprout had the highest shoot fresh weight at 5 and 10°C. Meanwhile, shoot fresh weight at 25°C was 0 g/bulb because most shallots had rotted by 8 WAI. Root fresh weight at 5 and 10°C were higher than at 25°C at 2 and 4 WAI. At 6 and 8 WAI, root fresh weights were not

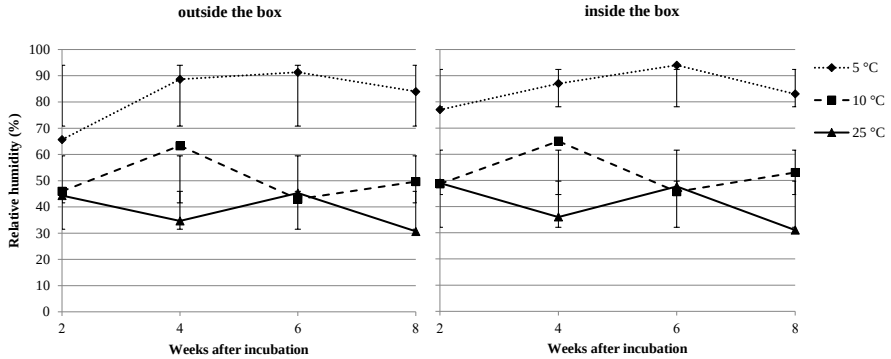

Figure 2. Relative humidities outside and inside the incubation box for shallot sprouts, at 5, 10 and 25°C for 8 weeks

significantly different. Bulb fresh weight at 5 and 10°C was higher than at 25°C at 8 WAI but not significantly different at 2, 4 and 6 WAI (Figure 3).

Shoot fresh weight increased over time, reaching its highest at 8 WAI for 5 and 10°C. However, root fresh weight decreased over time. At 25°C, shoot fresh weight was highest at 4 WAI but decreased afterward because the shallots had rotted (Figure 3). It was noted that there is no data on shoot fresh weight at 2 WAI for 10 °C.

The shoot dry weight of shallot sprouts was not significantly different at 2, 4 and 6 WAI. At 8 WAI, the shoot dry weight at 10°C was 26.4% greater than at 5°C. Shoot dry weight at 25°C was 0 g/bulb because most shallots had rotted by 8 WAI. Root dry weights of 5°C and 10°C were higher than 25°C at 2 and 4 WAI but not significantly different at 6 and 8 WAI. Bulb dry weights at 5°C and 10°C were higher than at 25°C at 8 WAI but were not significantly different at 2, 4 and 6 WAI (Figure 4).

Shoot dry weight increased over time at every temperature except 25°C. On the other hand, root dry weight and bulb dry weight decreased over time (Figure 4).

Leaf length and the number of leaves were not significantly different at 2, 4 and 6 WAI. However, at 8 WAI, both leaf length and the number of leaves in shallot sprout

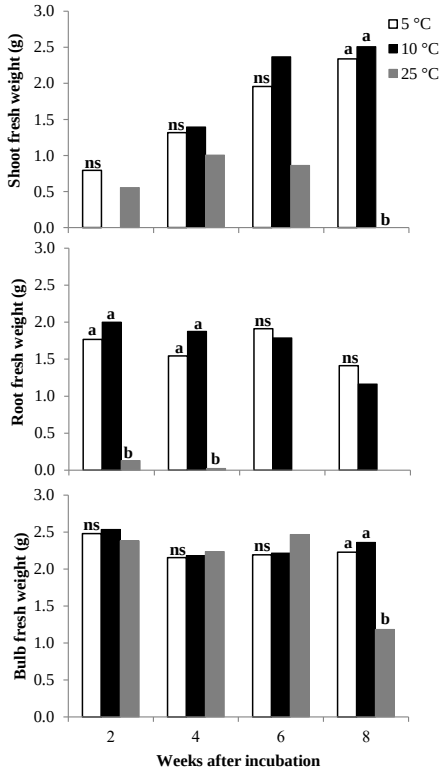

Figure 3. Shoot fresh weight (g/bulb), root fresh weight (g/bulb), and bulb fresh weight (g/bulb) of shallot sprouts at 5, 10 and 25°C for 8 weeks
ns, non-significant; bars with the same letter are not significantly different based on LSD (P≤0.05).

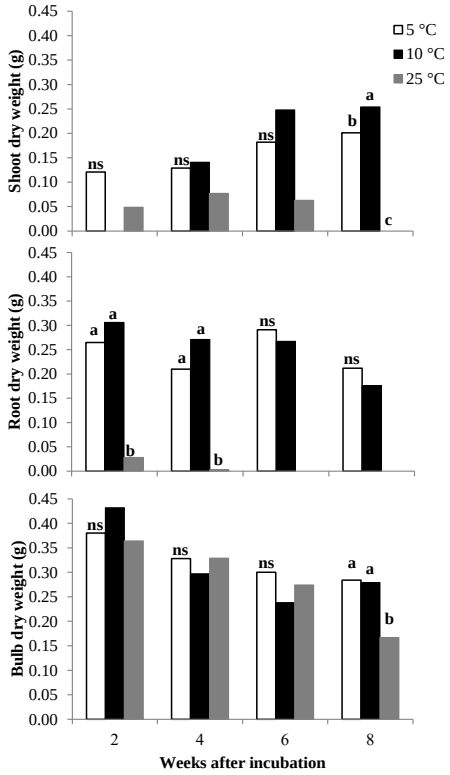

Figure 4. Shoot dry weight (g/bulb), root dry weight (g/bulb), and bulb dry weight (g/bulb) of shallot sprouts at 5, 10 and 25°C for 8 weeks
ns, non-significant; bars with the same letter are not significantly different based on LSD (P≤0.05).

showed significant differences. Leave length at 5°C was 86% and 505% higher than at 10 and 25°C, respectively. Similarly, the number of leaves at 5°C was 68% and 523% higher than at 10 and 25°C, respectively. Leaf length and the number of leaves increased over time at every temperature except 25°C (Figure 5).

3.3 Active compounds and antioxidant activity of shallot sprout

The active compound content and antioxidant activity of shallot sprout at 25°C could not be measured at 6 and 8 WAI due to the deterioration of the shallots. The total phenolic content of shallot sprout was not significantly different at 2, 4 and 6 WAI. However, at 8 WAI, the total phenolic content at 5°C was 9.2% greater than at 10°C. The highest total phenolic content was observed at 4 WAI. At 5°C, total phenolic contents were highest at 4 and 8 WAI (30.8 mgGAE/100 g crude extract). At 10 and 25°C, the highest total phenolic content was recorded at 4 WAI (34.8 and 34.0 mgGAE/100 g crude extract, respectively) (Table 1).

Total flavonoid contents at 5°C were higher than at the other temperatures at 2, 6 and 8 WAI but did not differ significantly at 4 WAI. The highest total flavonoid content was observed at 4 WAI, while the total flavonoid content peaked at 2 WAI (18.1 mg Quercetin/g extract). At 10 and 25°C, the highest total flavonoid content was recorded at 4 WAI (14.4 and 18.1 mgCE/g extract, respectively) (Table 1).

The antioxidant activity, as determined by DPPH assay, was higher at 5°C than at the other temperatures at 6 WAI but did not differ significantly at 2, 4 and 8 WAI. The highest DPPH was observed at 4 WAI. At 5°C, DPPH reached its maximum at 6 WAI (5.98 µM equivalent Trolox/mg extract). At 10 and 25°C, the highest DPPH was observed at 4 WAI (6.07 and 6.20 µM equivalent Trolox/mg extract, respectively) (Table 1).

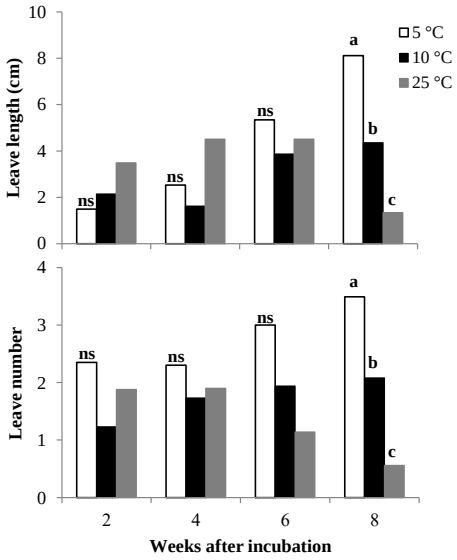

Figure 5. Leaf length and the number of leaves of shallot sprouts at 5, 10 and 25°C for 8 weeks
ns, non-significant; bars with the same letter are not significantly different based on LSD (P≤0.05).

Table 1. Total phenolic content, total flavonoid content, and antioxidant activity by DPPH and ABTS assays, of shallot sprouts at 5, 10 and 25°C for 8 weeks

Temperature	Weeks after incubation			
	2	4	6	8
Total phenolic content (mgGAE/100 g of extract)				
5	24.0	30.8	30.8	29.5[a]
10	27.1	34.8	25.6	27.0[b]
25	27.9	34.0	NA	NA
F-test	ns	ns	ns	*
C.V. (%)	7.11	11.89	15.56	6.12
Total flavonoid content (mg Quercetin/g of extract)				
5	18.1[a]	18.0	17.8[a]	16.0[a]
10	14.3[b]	14.5	11.5[b]	8.1[b]
25	13.5[b]	18.1	NA	NA
F-test	**	ns	*	**
C.V. (%)	6.81	11.78	23.89	17.28
DPPH (µM equivalent Trolox/mg extract)				
5	5.28	5.50	5.98[a]	5.65
10	5.25	6.07	5.20[b]	5.60
25	4.75	6.20	NA	NA
F-test	ns	ns	*	ns
C.V. (%)	4.33	7.09	10.66	3.76
ABTS (µM equivalent Trolox/mg extract)				
5	8.66[b]	8.64[b]	8.58[b]	8.63[b]
10	8.70[a]	8.68[a]	8.72[a]	8.72[a]
25	8.67[b]	8.70[a]	NA	NA
F-test	**	*	**	**
C.V. (%)	0.07	0.25	0.57	0.38

NA, not available

The antioxidant activity measured by the ABTS assay was highest at 10°C across all sampling points (2, 4, 6 and 8 WAI). The duration of germination did not appear to affect ABTS activity in shallot sprouts. At 5°C, ABTS activity was highest at 2 WAI (8.66 µM equivalent Trolox/mg extract). At 10°C, ABTS peaked at 6 and 8 WAI (8.72 µM equivalent Trolox/mg extract). At 25°C, the highest ABTS activity was observed at 4 WAI (8.70 µM equivalent Trolox/mg extract) (Table 1).

Vitamin C content was highest at 4 WAI. At 5°C, the maximum Vitamin C content was recorded at 6 WAI (2.12 mg/100 g). At 10°C, the highest Vitamin C content was observed at 4 WAI (2.57 mg/100 g). At 25°C, Vitamin C peaked at 2 and 4 WAI (2.20 mg/100 g) (Table 2).

At 2 and 4 WAI, shallot sprouts at 25°C exhibited the highest quercetin content (32.1 and 22.6 mg/L, respectively). At 6 and 8 WAI, the highest quercetin content was recorded at 5°C (13.5 and 12.0 mg/L, respectively). At 5°C, quercetin content reached its peak at 6 WAI (13.5 mg/L). At 10 and 25°C, the highest quercetin content was observed at 2 WAI (18.2 and 32.1 mg/L, respectively) (Table 2).

Mold growth at 25°C was lowest at 2 and 6 WAI. At 4 and 8 WAI, the lowest mold growth was observed at 5°C. The amount of mold increased over time at all temperatures except 5°C at 6 and 8 WAI (Table 2).

4. Discussion

Shallots incubated at 10°C exhibited the highest growth, as indicated by significantly greater shoot fresh

Table 2. Vitamin C, Quercetin and amount of mold of shallot sprouts at 5, 10 and 25°C for 8 weeks

Temperature	Weeks after incubation			
	2	4	6	8
Vitamin C (mg/100 g of extract)				
5	1.71	1.83	2.12	1.79
10	2.08	2.57	1.71	2.12
25	2.20	2.20	NA	NA
F-test	ns	ns	ns	ns
C.V. (%)	14.35	20.57	28.42	23.29
Quercetin (mg Quercetin/g of extract)				
5	12.8^{b}	13.4^{b}	13.5^{a}	12.0^{a}
10	18.2^{b}	10.5^{b}	0.8^{b}	1.3^{b}
25	32.1^{a}	22.6^{a}	NA	NA
F-test	*	*	**	**
C.V. (%)	24.42	13.63	14.49	9.75
Mould (cfu/ml)				
5	$1.07x10^{5}$	$1.56x10^{6}$	$2.26x10^{6}$	$2.26x10^{6}$
10	$1.52x10^{5}$	$1.90x10^{6}$	$3.46x10^{6}$	$4.79x10^{6}$
25	$1.11x10^{4}$	$1.71x10^{6}$	$2.05x10^{6}$	$3.89x10^{6}$

NA, not available

weight and shoot dry weight, at 8 WAI. Despite the absence of additional water in the incubation bags, the shallots were able to sprout by utilizing stored nutrient and moisture within the bulb, a finding consistent by Kusmali *et al*. (2020). In contrast, shallots incubated at 25°C exhibited signs of decay as early as at 6 WAI. The susceptibility of shallots to decay at a higher temperature aligns with previous research, which suggests that optimal storage conditions for shallots are 0–2°C with a relative humidity of 60-70%. At humidity levels exceeding 70% and temperatures between 14°C and 32°C, shallots tend to sprout, develop roots, and decay more rapidly (College of Agricultural Sciences, 2010). Similarly, Razaie *et al*. (2012) reported that temperature significantly ($P < 0.05$) influenced the emergence rate of Persian shallot (*Allium altissimum*). The highest emergence rate was obtained under 18°C, whereas increasing the temperature in the growth environment resulted in sharp decline in Perian shallot emergence rates. The findings of this study are also consistent with those of Purwanto and Nugraha (2017), who reported a higher incidence of bulb disorders in shallots stored at room temperature compared to those stored at 5 and 0°C. Additionally, Niam *et al*. (2019) reported that the optimum temperature for shallot germination is around 18-24°C, while leaf development is suitable at air temperatures of 20-25°C. These results highlight the critical role of temperature control in optimizing shallot sprouting and minimizing post-harvest losses.

Temperature had a significant impact on the growth and biomass accumulation of shallot sprouts. Lower temperatures (5°C and 10°C) promote shoot and root fresh weight accumulation by providing optimal conditions for nutrient mobilization, water retention, and controlled metabolic activity. In contrast, at 25°C, shoot and root fresh weight were significantly lower, likely due to increased respiration, higher water loss, and potential heat stress. Additionally, the retention of bulb fresh weight at 25°C suggested that less energy was allocated for sprout growth. These results emphasize the importance of temperature control in shallot sprout cultivation to maximize biomass production.

Germination temperature and duration had significant impact on the levels of total phenolic content, vitamin C, flavonoid, and quercetin in shallots. The highest total phenolic and vitamin C levels were observed at 6 WAI and 4 WAI, respectively, under 5 and 10 or 25°C, although the differences were not statistically significant. The increase of phenolic compounds during germination of shallots might be attributed the activation or synthesis of starch hydrolase and proteinase, which facilitate cell wall softening and then subsequently release bound phenolic compounds (Duenas *et al*., 2016). These findings are consistent with the study by Tang *et al*. (2021), which reported that total phenolic content and total flavonoid content in germinated three-color cultivars of highland barley were higher than that of non-germinated. Additionally, in germinated purple corn, total phenolic compound levels increased as germination duration and temperature increased up to 63 hours and 26°C, respectively. Vitamin C levels in shallots also showed a slight increase during germination, likely due to the reactivation of biosynthetic pathway in plant tissues (Wheeler, et. al, 1998). Phytochemical compounds of shallots were influenced by the germination conditions, particularly time and temperature. Conversely, quercetin and flavonoid contents decreased with prolonged germination. The degradation of quercetin followed a pattern similar to that of flavonoid reduction, as it belongs to the flavonoid group of polyphenols (Singh et al, 2021). These results agree with the findings of James *et al*. (2020) who reported that fermentation and germination decreased the total flavonoid content of selected lesser legumes over time. Duenas *et al*. (2016) suggested that the microbial enzyme **ß**-glucosidase, particularly from *Lactiplantibacillus plantarum*, hydrolyzes phenolics and flavonoids, contributing to their degradation The activities can lead to either an increase or a decrease in flavonoid content, depending on specific germination conditions (James, *et al*. 2020). In contrast, Ademiluyi and Oboh (2011) found that the total flavonoid content of Bambara groundnut increased during fermentation, likely due to the liberation of bound flavonoid compounds. However, in this study, flavonoid content in shallot sprouts significantly decreased with longer germination duration and higher temperature ($p<0.05$). These results indicate that incubation duration and temperature play a crucial role in influencing flavonoid content, with extended germination leading to flavonoid degradation rather than accumulation

The results indicated a high correlation between the bioactive compound contents of shallots and their antioxidant activity. At 2 and 4 WAI, shallot sprouts incubated at 10 and 25°C exhibited higher antioxidant activity, as measured by the ABTS assay and quercetin content, compared to those at 5°C.However, by 6 WAI, shallot sprout at 5°C demonstrated higher antioxidant activity, as determined by DPPH assay and active compound retention, in comparison to 10 and 25°C. This trend is reflected in the total phenolic content, total flavonoid content, and quercetin levels. The initial high quercetin content at 2 WAI in shallots incubated at 25°C may be attributed to increased metabolic activity, which enhanced flavonoid biosynthesis in response to temperature stress (Singh *et al*., 2021). However, quercetin levels subsequently declined, particularly at 25°C and 10°C, likely due to heat-induced degradation and enzymatic oxidation of flavonoids

(Duenas *et al*., 2016). In contrast, quercetin degradation was slower at 5°C, consistent with the finding that a lower storage temperature reduces enzymatic activity, thereby slowing flavonoid oxidation (James *et al*., 2020). Similar findings were reported by Ademiluyi and Oboh (2011), who observed flavonoid degradation during prolonged germination, with high temperatures accelerating this process. The observed decrease in quercetin content over time, particularly at higher temperatures, aligns with studies on other Allium species. Research on onion (*Allium cepa*) varieties demonstrated a significant reduction in quercetin levels during storage at high temperatures, primarily due to thermal degradation and oxidation reactions (Sharma and Lee, 2015; Yoo *et al*., 2013). This finding suggests that lower storage temperatures are more effective in preserving quercetin content in Allium species.

The increase in antioxidant activity (DPPH and ABTS) during early germination is associated with the biosynthesis of polyphenols, flavonoids, and vitamin C, which serve as free radical scavengers (Admiluyi and Oboh, 2011). However, the subsequent decline in antioxidant activity at later stages may be attributed to oxidation degradation of phenolic and flavonoid compounds, reducing their radical scavenging capacity (James *et al*., 2020). Polyphenols are known to function as hydrogen-donating antioxidants, playing a crucial role in free radical neutralization. Taraseviciene *et al*. (2019) reported a significant correlation between the total phenol content and the DPPH and ABTS radical scavenging activities in edible seeds. A significant positive correlation was found between the total phenol and the DPPH radical scavenging capacity in broccoli seed (r=0.996) and sunflower seeds (r=0.942) as well as between the total phenol and ABTS radical scavenging capacity in wheat and radish seeds. This highlights the role of germination in enhancing bioactive compounds and nutrient bioavailability in shallots.

The findings further confirm that temperature and germination duration significantly influence the accumulation and stability of phenolic compounds, flavonoids, and antioxidant activity in shallot sprouts. Shallots incubated at 5°C and 10°C retained higher bioactive compound levels over time, whereas 25°C resulted in a faster degradation. These results emphasize the importance of optimizing germination temperature to maximize the bioactive compound retention and antioxidant potential in shallot sprouts.

Moreover, shallot sprout at 5°C exhibited the lowest mold at 8 WAI compared to other temperature conditions. Purwanto and Nugraha (2017) reported volatile reducing substances (sulfur compounds and aldehydes) were significantly lost in peeled shallots stored at 0 and 5°C. Additionally, Benkeblia and Selselet–Attou (1999) found that changes in oligosaccharides and glucose were linked to the sprouting stage of onion (*Allium cepa* L.). Therefore, the germination temperature at 5 °C could effectively inhibit mold growth and suppressed fungal proliferation, thereby extending shelf life of shallot sprouts.

5. Conclusions

The optimal germination conditions for shallot sprouts are 5°C for 4–6 WAI, with 6 WAI being ideal for preserving bioactive compounds and maximizing antioxidant activity. While 5°C is the most suitable temperature for overall sprout development, 10°C serves as a secondary option for promoting higher biomass accumulation. These conditions enhance shoot and root growth, maintain high levels of phenolics and flavonoids, sustain strong antioxidant activity, and minimize mold contamination, resulting in high-quality shallot sprouts with an extended shelf life. Shallot sprouts serve as a nutritious and health-conscious food alternative. Moreover, scaling up production can increase the value of shallots, offering economic benefits to farmers. Challenges for large-scale production include standardizing growth conditions, using controlled environments, maintaining strict hygiene protocols, and implementing automated monitoring to ensure consistent quality and efficient production.

Acknowledgements

The authors are grateful for the financial support of the research project “Characteristics and active components in germinated shallot with different production temperature and period” from Ubon Ratchathani University under the National Science, Research and Innovation Fund.

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