บทความวิจัย

EFFECT OF LIGHT AND DARK CONDITIONS ON QUALITY CHANGES OF MUNG BEAN SPROUT DURING STORAGE

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ABSTRACT

Mung bean sprouts are an ingredient in many Thai dishes. However, this kind of component is easily perishable. Thus, the present research aimed to find the optimum condition for storing mung bean sprouts under light and dark circumstances. Mung bean seeds were soaked overnight, placed on a plastic tray that liner with three layers of tissue paper, and kept in the dark for 24 hours. Then, 50 grams of sprout was packed in a polyethylene plastic Ziploc bag, placed in a corrugated paper box in the absence or presence of light, and kept at 4°C; 90±2%RH and 25°C; 65±5%RH. It was revealed that the hypocotyl lightness (L* value) of the sprout that stored in the absence of light at 4°C had a bit changed. Though the low-temperature condition induced browning, keeping in the dark condition delayed the browning process and fibrousness in sprout hypocotyl. Nevertheless, light maintained more crispness at 4°C better than 25°C. Therefore, to extend mung bean sprout quality, it should be packed in opaque packaging and stored at 4°C.

Keywords: mung bean, packaging, seed, shelf-life, storage

Introduction

A leguminous seed contains a high amount of protein and dietary fiber. Mung bean sprouts are enriched with antioxidants widely consumed worldwide (López-Amoróse et al., 2006). Seed germination to sprout is one of the most effective methods to improve the quality of legumes. There was a significant increase in crude protein concentration, total dietary fiber content, and GABA (γ-aminobutyric acid) amounts in mung bean during seed germination (Tiansawang et al., 2016). The

germination process is influenced by external factors, particularly water soaking and germinating time in the absence of a light condition.

During storage period, most freshly plant parts continue their living processes: anabolism and catabolism. The rate of metabolism was dependent on the environmental stress factors such as high temperature and water stress. This reactive stress was related to the redox-reaction that emitted more reactive oxygen species (ROS), particularly superoxide anion ($O_2^{\bullet-}$). Plant discriminated the harmful $O_2^{\bullet-}$ to hydrogen peroxide (H_2O_2), a non-reactive ROS form by superoxide dismutase (SOD). H_2O_2 will be transformed into the harmful substances (hydroxyl radical; $OH^{\bullet}$) to caused membrane lipid peroxidation via Fenton reaction in the presence of Fe^{2+} (Noichinda et al., 2018; Chuenchom et al., 2021).

After harvest, the sprout is packed in a transparent plastic bag and places on the supermarket shelf display. However, bean sprout is highly perishable and inherently short shelf-life. Modified atmosphere packaging combined with cold storage was beneficial for extending the shelf-life of fresh sprouts (Varoquaux et al., 1996). Sprout generally remains stable at 5°C and reaches the lower limit of marketability after 3-4 days. This rapid loss of quality is due to wilting, softening, and browning of hypocotyls (Tajiri, 1979; Tonprasong et al., 2002). Under this condition, light and low temperature are necessary to prolong sprout quality and shelf life. Light exposure showed a prominent effect on the growth and quality of bean sprouts (Tajiri, 1985). Accordingly, the objective of this research was to evaluate the mung bean sprout quality changing in the presence and absence of light to achieve the best condition for extended shelf-life.

Methods

The uniform mung bean (*Vigna radiate* roxb) seeds were soaked in clean tap water until seed coat swelling (12 hours) at room temperature (30±3°C). The floating seeds were discarded while the sink ones were placed on a plastic tray (28x40x5 cm) that liner with 3 layers (0.066x3 mm thick) of tissue paper. Seeds were laid on tissue paper at the distance of 1.5 cm between each other, then covered with one layer of tissue paper. Clean tap water (80 ml) was poured on the covered tissue paper. This plastic tray was placed in an opaque plastic box (36x48x23 cm) and covered the plastic box with a bathroom towel (35x70 cm) for 24 hours at room temperature (30±3°C). Fifty grams of one-day germinating mung bean sprout was packed in a polyethylene plastic Ziploc bag (Big C®, 25x37.5 cm; 0.253 mm thickness). The packaged bean sprout was placed in a corrugated paper box (32x48x28 cm) that liner with aluminum foil. The box was illuminated at 30 µmole m^{-2} s^{-1} by a white fluorescent light bulb (Silvania®, 7 watts). After that, these corrugated paper boxes (with or without a light bulb) were divided into 2 groups: group one was kept at 4°C; 90±2%RH and group two was

kept at 25ºC; 65±5%RH. During storage, sprouts were randomly sampled to determine physical and chemical properties as described below:

L* a* b*

L* a* b* values of sprout hypocotyl color were measured by HunterLab (ColorFlex® EZ).

Sprout crispness

The crispness of sprout hypocotyl was measured using a texture analyzer (TA-XT 21, Stable Microsystems, UK) with a 2 mm spherical plunger, 2 mm distance depth, and 1.0 $mm \cdot s^{-1}$ test speed, and the maximum force was recorded in the Newton unit.

Phenolic content

The phenolics extraction and determination were followed by Singleton & Rossi (1965) method. The 5 g of sprout hypocotyl mixed with 5 ml of 80% ethanol was homogenated by homogenizer and centrifuged at 1200 rpm for 20 min. The supernatant (1 ml) was mixed in 5 ml of Folin-Ciocalteus using a vortex mixer. Subsequently, 4 ml of 7.5% sodium carbonate was added into a mixture solution and placed in a water bath (30ºC) for 1 h. The absorbance of the extracted solution was measured by spectrophotometer (Model SP-830) at 760 nm, and phenolic content was calculated from the absorbance of gallic acid standard.

Browning intensity

Browning intensity was measured according to Baloch et al. (1973) method. The 1 g of sprout hypocotyl was ground in 50 ml of 1.5% acetic acid and filtered with Whatman filter paper No. 1. Then, the filtered solution (250 ml) was acidified with 1.5% acetic acid, then measured by spectrophotometer at 420 nm.

Peroxidase (POD) activity

POD extraction was done by the following method. The 3 g of hypocotyl was ground in 20 mg polyvinylpyrrolidone, 6 ml of 0.2 M potassium phosphate buffer pH 7, and centrifuged at 5000g for 10 min. The supernatant was used as a crude enzyme for activity assay (Morita et al., 1988) which 1 ml of crude POD was mixed in 1.5 ml of 0.2 M potassium phosphate buffer pH 7 and 0.43 mM H_2O_2, 2.5 mM 4-aminoantipyrine, and 17 mM phenol. The absorbance of the reaction mixture was measured at 510 nm by a spectrophotometer.

Fiber content

Fiber content was determined by the following method. Hypocotyl (1 g) was dried in an oven at 130ºC for 1 h, then boiled in 0.128% H_2SO_4 for 30 min and filtered with a glass wool filter. The filtered residue was washed twice with clean tap water, boiled in 0.23% KOH for 30 min and washed again with clean tap water. After that, the residue was dried in an oven at 130ºC for 2 h, and calculated the % dried weight of dried residue as a crude fiber.

Polyphenoloxidase (PPO) activity

PPO extraction and assay were performed along with Coseteng and Lee (1987) method. The 5 g hypocotyl was ground in 20 ml of 0.2 M potassium phosphate buffer pH7 and 0.005 M L-cysteine hydrochloride, then centrifuged at 12000 rpm for 40 min. The supernatant was used as a crude PPO for activity assay. A crude enzyme (0.2 ml) was added into 2.8 ml of 0.01 M sodium acetate buffer (pH 5) and 1 ml of 0.5 M pyrocatechol. Next, the absorbance of the reaction mixture was measured at 420 nm by a spectrophotometer.

Protein content

Protein determination using 0.125% Coomassie Brilliant Blue was carried out according to Bradford (1976) method.

Statistical analysis

Means (from 4 replications each) of sprout hypocotyl crispness, phenolic content, browning intensity, POD activity, fiber content, and PPO activity were compared using Duncan's new multiple range test (DMRT) at $P < .05$.

Results and Discussions

L* a* b* value

The lightness (L*) of mung bean sprout hypocotyl stored at 25°C decreased nearly twice times faster than at 4°C under light exposure while there was not much changed when stored in the dark condition (Table 1). Moreover, light also stimulated green color developing in sprout hypocotyl as indicated by minus value of a* but this alteration was reduced when storage at 4°C (**Table 1**). However, under light condition, hypocotyl of all treatments showed slightly yellowing as indicated by zone '+' value of b*. The germinating bean sprout is a complete plantlet consisted of epicotyl, cotyledons, hypocotyl, and radicle. For survival, the leaf and stem of mung bean sprout noticeably developed photosynthetic pigment (chlorophyll) to synthesize carbohydrates from carbon dioxide and water under light conditions by a photosynthetic process as indicated by the minus value of a*. This finding was similar to DeEll et al. (2000) since they found better freshness and whiter hypocotyl in stored mung bean sprouts at the lower temperature.

Table 1 The hypocotyl color (L* a* b*) value of mung bean sprout storage at 4 and 25°C under light and dark conditions

Temperature (°C)	Storage (Days)	Light			Dark		
		L*	a*	b*	L*	a*	b*
4	0	60	-1	8	60	-1	8
	1	58	-1	9	55	1	9
	2	57	2.5	28	50	1	25
	3	55	3.8	30	48	2	35
25	0	60	-1	8	60	-1	8
	1	50	-4	15	53	-1	9
	2	40	-4.2	16	50	0	25
	3	29	-4.5	17	48	-2	30

Hypocotyl crispness

Bean sprout crispness was usually considered at the hypocotyl part. In this experiment, hypocotyl crispness of mung bean sprout rapidly declined during storage under both light and without light exposure at 25°C on the first day (Figure 1a). However, it seemed that the hypocotyl crispness slightly changed during storage under light exposure at 4°C. The higher temperature appears to affect the crispness of mung bean sprout hypocotyl as it observably dropped off during storage at 25°C more than 4°C (Figure 1a). Nevertheless, under light exposure, the sprout hypocotyl lost its crispness to some extent due to fiber content, and its synthesizing enzyme (POD) activity was still high in stored sprouts under light conditions (Figure 1b and 1c), which might influence the hardness property of hypocotyl texture. So, storage at 25°C might have had a water loss more significant than 4°C, resulting in reduced cell growth and reduced crispness.

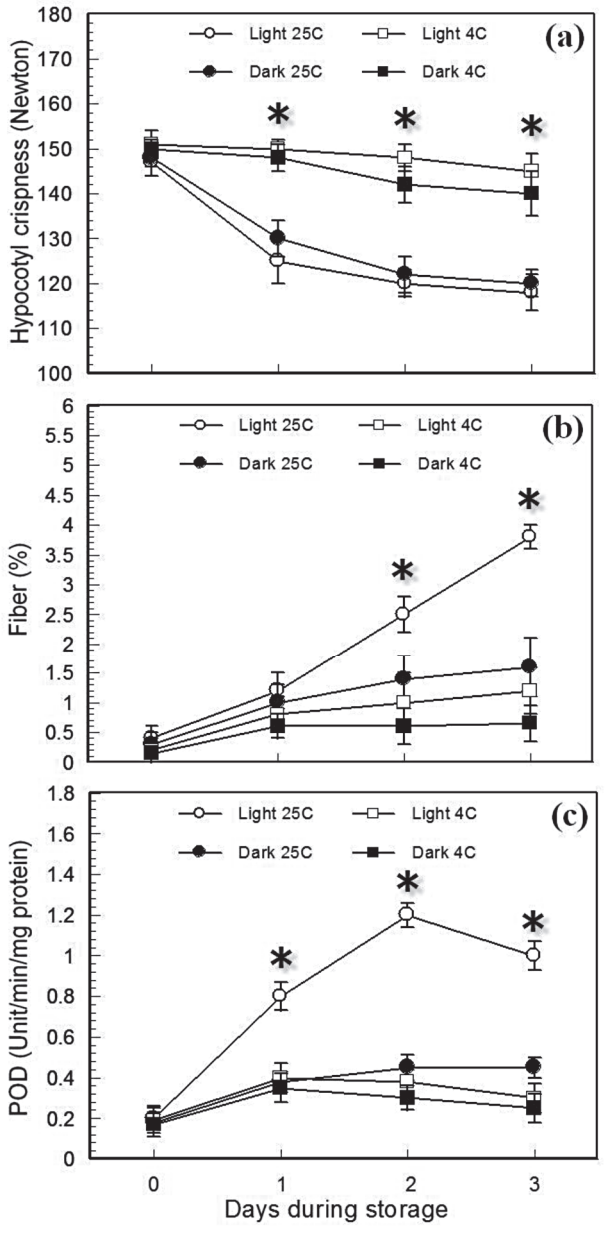

Figure 1 Mung bean sprout hypocotyl crispness and its relative enzyme. Data from each storage day with an asterisk indicated a statistically significant difference at $P < 0.05$.

Fiber content

Fiber content rapidly increased in mung bean sprout hypocotyl during storage under light condition at 25°C (Figure 1b) while its accumulation rate was delayed at 4°C or in the dark. Fiber is polymerized of phenol intermediate substances and H_2O_2 by peroxidase (POD) localizing in the

secondary wall. This type of sclerenchymatous tissue sets up the texture toughness in hypocotyls of a mung bean sprout. When light exposure was available during storage, the photosynthesis in mung bean sprout occurred to generate glucose that was utilized in cellular respiration and forming cellulose or fiber. As the rate of POD activity in bean sprouts under light exposure was higher than in the dark condition (Figure 1c), fiber content rose in sprout hypocotyl during storage under light exposure at 25°C (Figure 1b). This incident could occur because POD used H_2O_2 and phenol intermediates to connect monolignol-cell walls that strengthened the structure in many plants (Huang et al., 2013). Nevertheless, at 4°C, POD activity in mung bean sprout hypocotyl went up slightly, which indicated that low-temperature storage conditions might slow the metabolic processes of this enzyme.

POD activity

POD activity in mung bean sprout hypocotyl slightly increased during storage under light and dark condition at 4°C (Figure 1c). However, its activity rapidly increased during storage under light condition at 25°C. Light can inhibit the elongation of plant seedlings. Under light exposure, hypocotyl was relatively short, thick, and elongated slowly. Auxin plays a vital role in promoting hypocotyl elongation, and light causes growth inhibition by reducing auxin availability in seedling tissue (Iino, 1982; Jones et al., 1991). POD activity had an inverse relationship with sprout hypocotyl growth (Zheng & Van Huystee, 1992). POD plays a crucial function in plant physiological responses, including auxin catabolism (Savitsky et al., 1999), and lignification (Mäder & Füssl, 1982). Auxin was degraded in vitro by cationic POD in the presence of oxygen, whereas anionic POD was considered essential in the formation of lignin (Gazaryan et al., 1996). Besides, POD catalyzed the coupling reactions of monolignols using O_2 instead of H_2O_2 as electron acceptor (Sterjiades et al., 1993), and participated in lignin biosynthesis. The inhibition of mung bean sprout hypocotyl growth caused by light might be due to the degradation of endogenous auxin by cationic POD. The higher lignin level was correlated with the increased anionic POD activity in a light-treated sprout (Chen et al., 2002).

Browning intensity

For the Browning intensity of mung bean sprout hypocotyl, this symptom was lowest at 4°C under light conditions throughout the storage time (Figure 2a). It seemed that light condition delayed browning mechanism in sprout hypocotyl while dark with low-temperature condition stimulated browning intensity in sprout hypocotyl. Typically browning symptoms occurred in the plant after getting the mechanical injury in the presence of oxygen. At low-temperature injury, this circumstance could accelerate cell membrane damage and losing their permeability function by the induction of reactive oxygen species (ROS). Leakage of the cell membrane and tonoplast caused a browning reaction with the oxidation of *o*-diphenols to *o*-quinone by polyphenol oxidase (PPO). However, it is complicated to identify the browning symptom by the human eye at the early time of storage. A spectrophotometer must measure it.

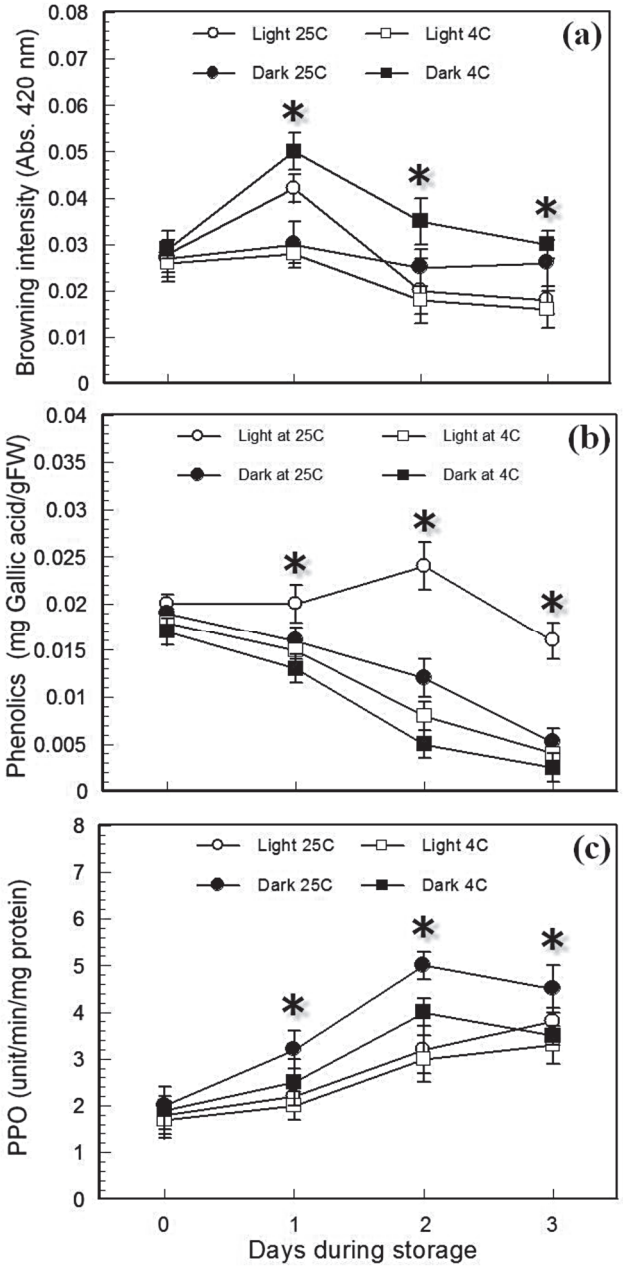

Figure 2 Mung bean sprout hypocotyl browning and its relative enzyme. Data from each storage day with an asterisk indicated a statistically significant difference at $P < 0.05$.

Phenolic content

In both storage conditions, phenolic content of mung bean sprout hypocotyl decreased continuously, but at 25°C under light exposure, phenolic content was higher than the other all over the experiment (Figure 2b). Nevertheless, under low temperature (4°C), together with the light condition, phenolic content was lower than the other during the experimental period. The phenolic compounds were utilized as a substrate for browning reaction by PPO in the presenting of oxygen. Under light and dark storage conditions, phenolic content in mung bean sprout hypocotyl is constantly reduced (Figure 2b). However, under light exposure at 25°C, this compound was still superior to other treatments. This result indicated that light exposure at 25°C possibly delayed the browning process in mung bean sprout hypocotyl via maintaining the phenolic compounds, which agree to Bakhshi & Arakawa (2006) found that light irradiation and temperature had a strong influence on the phenolics accumulation.

PPO activity

PPO activity in mung bean sprout hypocotyl increased in all treatments. However, under light exposure, this activity was retarded during the storage (Figure 2c). PPO, a copper-containing enzyme, plays an essential role in the browning reaction in several plants (Nagai & Suzuki, 2003). In this research, it appeared that PPO activity in mung bean sprout hypocotyl was induced by dark storage conditions, while its activity was retarded by light exposure during storage (Figure 2c). This outcome was related to the number of phenolic compounds, a substrate of PPO in the browning process, in a different direction. Under the dark condition, PPO had a high activity to reduce the phenolic compounds (Figure 2b and 2c) and induce the browning process in mung bean sprout hypocotyl (Figure 2a). Moreover, it was feasible that low temperature could induce stress, and mung bean sprouts subsequently released remarkable amounts of free radicals (ROS). Then, the sprouts need to produce antioxidants (via phenylpropanoid pathway) that can be used as precursors for many enzymes, both PPO for browning and POD for the occurrence of fibers.

Conclusions

This research focused on lighting during shelf-life, where lighting devices are already installed but not appropriate, and this beneficial effect of increased light could be applied as a postharvest management approach. Mung bean sprouts should be stored in an opaque packaging and placed on a shelf display at 4°C to maintain lightness (L* value). Although low-temperature storage conditions induced browning, low-temperature along with dark storage conditions delayed the browning process and fibrousness in mung bean sprout hypocotyls. However, light conditions maintained more hypocotyl crispness during storage at 4°C. Thus, the longer the mung bean sprouts are exposed to light while they are available for sale, they will maintain their quality longer.

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References

Bakhshi, D., & Arakawa, O. (2006). Induction of phenolic compounds biosynthesis with light irradiation in the flesh of red and yellow apples. **Journal of Applied Horticulture**, **8**(2), 101-104.

Baloch, A.K., Buckle, K.A., & Edwards, R.A. (1973). Measurement of non-enzymic browning of dehydrated carrot. **Journal of the Science of Food and Agriculture**, **24**, 389-398.

Bradford, M.M. (1976). A rapid and sensitive method for the contiquication of microgram quantities of protein utilizing the protein-dry binding. **Analytical Biochemistry**, **72**, 248-254.

Chen, Y., Shin, J., & Liu, Z. (2002). Effect of light on peroxidase and lignin synthesis in mungbean hypocotyls. **Plant Physiology and Biochemistry**, **40**, 33-39.

Chuenchom, P., Noichinda, S., Bodhipadma, K., Wongs-Aree, C., & Leung, D.W.M. (2021) Different chilling-induced symptoms and the underlying oxidative stress and antioxidative defense in the exocarp and mesocarp of immature sponge gourd (*Luffa cylindrica*) fruit. **Applied Science and Engineering Progress**, **14**(3), 348-359.

DeEll, J.R., Vigneault, C., Favre, F., Rennie, T.J., & Khanizadeh, S. (2000). Vacuum cooling and storage temperature influence the quality of stored mung bean sprouts. **HortScience**, **35**, 891-893.

Coseteng, M.Y., & Lee, C.Y. (1987). Changes in apple polyphenoloxidase and polyphenol concentrations in relation to degree of browning. **Journal of Food Science**, **52**, 985-989.

Gazaryan, I.G., Lagrimini, L.M., Ashby, G.A., & Thorneley, R.N.F. (1996). Mechanism of indole-3-acetic acid oxidation by plant peroxidase: Anaerobic stopped-flow spectrophotometric studies on horseradish and tobacco peroxides. **Biochemical Journal**, **313**, 841-847.

Huang, W., Liu, H., Zhang, H., Chen, Z., & Guo, Y. (2013). Ethylene-induced changes in lignification and cell wall degrading enzymes in the roots of mungbean (*Vigna radiate*) sprouts. **Plant Physiology and Biochemistry**, **73**, 412-419.

Lino, M. (1982). Inhibitory action of red light on the growth of maize mesocotyl: Evaluation of the auxin hypothesis. **Planta**, **156**, 388-395.

Jones, A.M., Cochran, D.S.M., Lamerson, P., Evans, M.L., & Cohen, D.J. (1991). Red light-regulated growth. I. Changes in the abundance of indole-acetic acid and a 22 kilodalton auxin binding protein in the maize mesocotyl. **Plant Physiology**, **97**, 352-358.

López-Amoróse, M.L., Hernández, T., & Estrella, I. (2006). Effect of germination on legume phenolic compounds and their antioxidant activity. **Journal of Food Composition and Analysis**, **19**, 277-283.

Mäder, M., & Füssl, R. (1982). Role of peroxidase in lignification of tobacco cells. **Plant Physiology**, **70**, 1132-1134.

Morita, Y., Yamashita, H., Mikami, B., Iwamoto, H., Aibara, S., Terada, M., & Minami, J. (1988). Purification, crystallization, and characterization of peroxidase from *Coprinus cinereus*. **Journal of Biochemistry**, **103**, 693-699.

Nagai, T., & Suzuki, N. (2003). Polyphenol oxidase from bean sprouts (*Glycine max* L.). **Journal of Food Science**, **68**, 16-20.

Noichinda, S., Bodhipadma, K., & Wongs-Aree, C. (2018). **Antioxidant potential and their changes during postharvest handling of tropical fruits**. In S. Pareek (Ed.), Novel Postharvest Treatments of Fresh Produce (633-662). Boca Raton: CRC Press.

Savitsky, P.K., Gazayan, I.G., Tishkov, V.I., Lagrimini, L.M., Ruzgas, T., & Gorton, L. 1999. Oxidation of indole-3-acetic acid by dioxygen catalysed by plant peroxidase: Specific for the enzyme structure. **Biochemical Journal**, **340**, 579-583.

Singleton, V.L., & Rossi, J.R. Jr. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. **American Journal of Enology and Viticulture**, **16**, 144-158.

Sterjiades, R., Dean, J.F.D., Gamble, G., Himmelsbach, D.S., & Eriksson, K.E.L. (1993). Extracellular laccase and peroxidase from sycamore maple (*Acer pseudoplatanus*) cell suspension cultures. **Planta**, **190**, 75-87.

Tajiri, T. (1979). Effect of storage temperature and its fluctuation on keeping quality of mungbean sprouts. **Nippon Shokuhin Kogyo Gakkaishi**, **26**, 18-24.

Tajiri, T. (1985). Improvement of bean sprouts cultivation by combined treatment with artificial sunlight lamp, ethylene and carbon dioxide. **Nippon Shokuhin Kogyo Gakkaishi**, **32**, 317-325.

Tiansawang, K., Luangpituksa, P., Varanyanond, W., & Hansawasdi, C. (2016). GABA (γ-aminobutyric acid) production, antioxidant activity in some germinated dietary seeds and the effect of cooking on their GABA content. **Food Science and Technology**, **36**(2), 313-321.

Tonprasong, C., Suthirawut, S., & Thanapase, V. (2002). Shelf-life extension of mungbean sprouts by modified atmosphere packaging. **Agricultural Science Journal**, **33**(6) (Suppl.), 200-203. (In Thai)

Varoquaux, P., Albernac, G., The, C.N., & Varoquaux, F. (1996). Modified atmosphere packaging of fresh beansprouts. **Journal of the Science of Food and Agriculture**, **70**, 224-230.

Zheng, X., & Van Huystee, R.B. (1992). Peroxidase-regulated elongation of segments from peanut hypocotyls. **Plant Science**, **81**, 47-56.