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Original Research Article

Application of Integrated Postharvest Technology for Maintaining the Quality of Methyl Bromide Fumigated Mangosteen Fruit

Kanlaya Sripong[1], Thanwalee Srinon[1], Apiradee Uthairatanakij[1], Sontaya Limmatvapirat[2] and Pongphen Jitareerat[1*]

[1]Department of Postharvest Technology, King Mongkut's University of Technology Thonburi, Bangkok, 10150 Bangkhuntein, Thailand, kanlaya.sripong@hotmail.com; thanwalee-srinon@hotmail.com; apiradee.uth@kmutt.ac.th; pongphen.jit@kmutt.ac.th

[2]Department of Pharmaceutical Technology, Silpakorn University, Nakhon Pathom, 73000 Mueang District, Thailand, sontaya@email.pharm.su.ac.th

*Corresponding author: Pongphen Jitareerat, Department of Postharvest Technology, King Mongkut's University of Technology Thonburi, Bangkok, 10150 Bangkhuntein, Thailand; pongphen.jit@kmutt.ac.th

Abstract: Methyl bromide fumigation aims to control insect infestation in mangosteen fruit before export which leads to the reduction of fruit quality. Thus, the objective of this study was to control the quality of methyl bromide fumigated mangosteen using integrated postharvest technology. The fruits were prepared by fumigating with 32 g m^{-3} for 2 hours, washed with tap water, and surfaced disinfestation with sodium hypochlorite. The prepared fruit samples were then further divided and treated with different methods as follows; 1) fruit were coated with ethanolic shellac-modified coconut oil (ES-MCO) and then packed in low-density polyethylene (LDPE) bag containing ethylene inhibitor (1-MCP sachet), 2) fruit were coated with ES-MCO and packed in LDPE bag, 3) fruit were treated with ES-MCO and packed in nylon net bag, and 4) fruit were non-coated and packed in nylon net bag (control). All fruit samples were kept at 13ºC for 20 days (as the shipment simulation) and then transferred to 25ºC for 2 days (as the shelf-life simulation). The results revealed that after 15 days of storage, the fruit treated with ES-MCO + LDPE bag + 1-MCP sachet showed the highest reduction of fruit rot disease and this treatment helped to delay the drying and browning of mangosteen calyx, chlorophyll degradation of the calyx, and reduce weight loss, the respiration rate and ethylene production. However, after storage for 20 days, the pericarp of mangosteen in fruit treated with ES-MCO + LDPE bag + 1-MCP sachet became hard; suggesting that the shelf-life was 15 days.

Keywords: 1-MCP sachet; coating substance; mangosteen; methyl bromide; packaging

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1. Introduction

Mangosteen (*Garcinia mangostana* L.) fruit is one of the economically important tropical fruits, and the demand for mangosteen fruit has gradually increased in both domestic and export markets. Thailand is one of the main producers and the largest exporter of mangosteen, with a production in 2021 was 336,860 tons and the volume of mangosteen fruit exported was 256,266 tons (Office of Agricultural Economics, 2021). However, the main problem with exporting mangosteen is the contamination of pests and insects including fruit flies, armoured scales, soft scales, mealybugs and ants (Syauqi *et al.*, 2020; Unahawutti *et al.*, 2014). Methyl bromide (MeBr) is therefore used as an insecticide fumigant. Some import countries specify that mangosteen from Thailand must be fumigated with MeBr at 32 g m^{-3} for 2 hours at 21°C as a quarantine treatment such as Australia and New Zealand (Ormking, 2017). However, MeBr is high in toxicity which can cause damage and loss of quality in some fruits (Torregrosa *et al*., 2021). For example, MeBr fumigation at 32 g m^{-3} for 3 hours at 21°C increased the decay and softening of blueberry (Ortiz *et al*., 2018). In the case of mangosteen fruit, it has been reported that MeBr fumigation at 32 g m^{-3} for 2 hours at 21°C caused the hardening of mangosteen pericarp within 14 days of storage at 15°C (Sirichai, 2013). The occurrence of pericarp hardening is caused by water loss (Jarimopas *et al*., 2009; Tac-an *et al*., 2021). In addition, mangosteen fruit has a short shelf life after harvesting because of its high respiration rate and ethylene production (Setyadjit & Setyabudi, 2022). Moreover, fruit rot disease is another cause of direct yield loss during transport, distribution and retail (Khewkhom *et al*., 2013). Thus, the application of postharvest technology for solving these problems is necessary.

Several postharvest technologies have been used to extend the shelf life of mangosteen, including coating the fruit surface and packing it in modified atmosphere packaging (MAP). Our research group, Thuong *et al*. (2015) reported that coating the fruit with ethanolic shellac-modified coconut oil (ES-MCO) and packing it in an low-density polyethylene (LDPE) bag could reduce fruit rot disease and extend the shelf life of mangosteen during storage at 13°C for 28 days. The efficiency of ES-MCO in extending the shelf life of mangosteen is due to ES-MCO which contains shellac wax and modified coconut oil (MCO). Shellac wax can prevent water loss by blocking the pores in the surface of the fruit and also provide a barrier to oxygen transfer resulting in the reduction of respiration rate and ethylene production (Accaseavorn *et al*., 2006; Soradech *et al*., 2017). Additionally, MCO has high antimicrobial activity (Ponphaiboon *et al.*, 2015; Subroto & Indiarto, 2020; Widianingrum *et al.*, 2019). However, the application of MAP packaging for extending the shelf life of fresh produces is based on the properties of films to allow oxygen and carbon dioxide transmission. Thuong *et al.* (2014) reported that LDPE bag was the proper packaging for delaying the senescence process and disease development of mangosteen during storage at low temperatures. In addition, postharvest treatment with 1-methylcyclopropene (1-MCP), which is an ethylene action inhibitor has been shown to extend the storage life of mangosteen

by delaying the calyx wilting, hardening of the pericarp and colour changes (Bayogan & Delgado, 2013; Piriyavinit *et al*., 2011).

In this work, our research group determined whether the integration of these treatments may be useful to alleviate the damage of mangosteen fruit after MeBr fumigation. Therefore, the purpose of this study was to investigate the combination effects of ES-MCO coating, LDPE bag and 1-MCP sachet on reducing the hardening of mangosteen pericarp, reducing fruit rot disease and prolonging the shelf life of MeBr fumigated mangosteen fruit.

2. Materials and Methods

2.1. Sample Preparation and Treatment

Mangosteen fruits at maturity stage 3 (reddish pink) were obtained from the commercial orchard in Nakhon Si Thammarat province, Thailand. The fruits with a similar size, colour and without any damage were selected. Then, the fruits were fumigated with MeBr at 32 g m^{-3} for 2 h at 25ºC. Afterwards, the fruits were washed with tap water, dipped in 200 mL L^{-1} sodium hypochlorite solution for 5 min and dried at the ambient temperature. Fruit were randomly grouped into four groups: 1) fruits were coated with ES-MCO and packed in LDPE bag containing 1-MCP sachet (EthylBloc ™, AgroFresh, Kent, USA) (ES-MCO + LDPE bag + 1-MCP sachet), 2) fruits were coated with ES-MCO and packed in LDPE bag (ES-MCO + LDPE bag), 3) fruits were coated with ES-MCO and packed in nylon net bag (ES-MCO + Net bag) and 4) fruits were non-coated and packed in nylon net bag (control). All treatments were kept in a cardboard box (8 bags per box) and stored at 13ºC for 20 days (as the shipment stimulation) and then transferred to 25ºC for 2 days (as the shelf-life stimulation). The fruits were randomised at five-day intervals during storage at 13ºC and every day during storage at 25ºC to evaluate the incidence and severity of fruit rot disease and the quality of the mangosteen fruits. Each treatment has four replications and one replication has eight fruits.

2.2. Determination of the Incidence and Severity of Fruit Rot Disease

Disease incidence was evaluated as a percentage of fruit showing fruit rot disease symptoms. The severity of disease was assessed by the extent of total decayed area on each fruit surface using a 0-5 score; 0 = no disease symptom, 1 = < 20% of disease symptom, 2 = 20–30% of disease symptom, 3 = 30.1–40% of disease symptom, 4 = 40.1–50% of disease symptom and 5 = > 50% of disease symptom on the fruit surface (Sripong *et al*., 2019).

2.3. Determination of the Quality of Mangosteen Fruit

The fresh weight of the fruit was determined on the initial day and at five-day intervals during storage using a digital balance (OHAUS, New Jersey, USA). Fruit weight loss was calculated using the differences in data between the initial and final indicated period and reported as a percentage.

The firmness of the mangosteen pericarp was conducted by using a TA-XT2 texture analyser (Stable micro-system, Surrey, England). The fruit was penetrated to a depth of 5 mm on the equatorial zone with a 5 mm diameter probe. The firmness value of each fruit was averaged and reported as Newton (N).

The browning and drying of mangosteen calyx were evaluated using a scale of 0–4 according to the appearance of the calyx; 0 = no browning and drying, 1 = slight browning and drying, 2 = moderate browning and drying, 3 = severe browning and drying in the calyx and 4 = extremely severe browning and drying in the calyx (Thuong *et al*., 2014).

2.4. Determination of the Total Chlorophyll Content in the Mangosteen Calyx

The content of total chlorophyll was determined according to Moran's (1982) method. The calyx (0.5 g) was mixed with N, N dimethylformamide (10 mL) and then incubated at 4°C overnight. Afterwards, the solution was filtered using Whatman paper (No. 1). The filtered solution was then measured at 663 and 645 nm by using spectrophotometrically (UV-1800, Shimadzu, Japan). The total chlorophyll content was calculated and expressed as mg g^{-1} FW using the following equation: Total chlorophyll = (20.2 × A_{645} nm + 8.02 × A_{663} nm) × dilution factor/ 1000.

2.5. Determination of Respiration Rate and Ethylene Production

Respiration rate and ethylene production were performed following the method of Pangaribuan (2006). Mangosteen fruits were sealed in a container and then kept at 13°C or 25°C for 2 h. Gas sample (1 mL) was taken from each container using a syringe and then injected into a Shimadzu gas chromatograph (Shimadzu, Kyoto, Japan). The respiration rate was expressed as mg kg^{-1} hr^{-1}, and the ethylene production was expressed as µL kg^{-1} hr^{-1}.

2.6 Statistical Analysis

All data were analysed as a one-way analysis of variance (ANOVA) by SAS software (SAS Institute, Cary, N.C., USA). The significant differences among treatments were compared using analysis of Duncan's multiple range test (DMRT).

3. Results and Discussions

3.1. Effects of ES-MCO Coating Combined with LDPE Bag and 1-MCP Sachet on Fruit Rot Disease of Methyl Bromide Fumigated Mangosteen Fruit

Fruit rot disease in all treatments was first detected on day 10 of storage and the highest incidence and severity of fruit rot were found in the control treatment. After 15 days of storage, the fruits treated with ES-MCO + LDPE bag + 1-MCP sachet showed the lowest disease incidence (56%) and severity (0.56 score), followed by fruits treated with ES-MCO + LDPE bag (63% and 0.63 scores) and ES-MCO + Net bag (66% and 0.80 scores), while

the control fruits showed the highest disease incidence (70%) and severity (0.83 scores). These results indicated that the combination treatments of ES-MCO + LDPE bag + 1-MCP sachet was the best treatment for reducing fruit rot disease. The results were attributed to the synergistic effects of each treatment. ES-MCO coating could be acted as a barrier against pathogenic infection, simultaneously a coating material includes MCO, which MCO is a very important source of monolaurin, and has high antifungal activity (Rihakova *et al*., 2002; Wang *et al*., 2020; Rozenbaum *et al*., 2019). Whereas the application of the LDPE bag on reducing disease was associated with gas inside the package, in which high CO_2 and low O_2 concentrations inside of LDPE bag were found in this study (data not shown). Moreover, the LDPE bag also acts as a barrier that could prevent the secondary infection of fungal pathogens and saprophytes from the environment. The application of 1-MCP sachet might be attributed to a direct effect by inhibiting spore germination and mycelium growth (Xu *et al*., 2017) and indirectly by delaying ripening and senescence processes of the fruit, which leads to delay in the reduction of antifungal compounds in fruit (Daulagala & Daundasekera 2015; Zhang *et al*., 2013). However, this study found that after storing the fruits for 20 days the disease incidence in all treatments developed to 100% (Figure 1A) and the severity of the disease was higher in the fruit treated with ES-MCO + LDPE bag and ES-MCO + LDPE bag + 1-MCP sachet than the control (Figure 1B). Increasing the disease severity in the fruit treated with ES-MCO + LDPE bag or ES-MCO + LDPE bag + 1-MCP sachet may cause the inside of the LDPE bag to contain high moisture, which is a favourable condition for fungal growth.

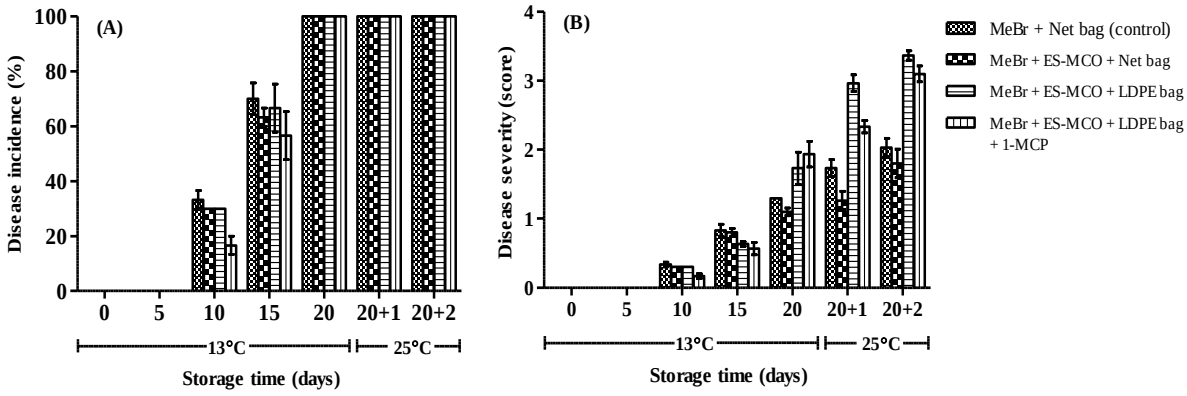

Figure 1. The combined effects of ES-MCO coating, LDPE bag and 1-MCP sachet on incidence (A) and severity (B) of fruit rot disease of MeBr fumigated mangosteen during storage at 13ºC for 20 days and 25ºC for 2 days. The fruits were non-coated and packed in net bags, coated with ES-MCO packed in net bags and coated with ES-MCO packed in LDPE bags were used as the controls.

3.2. Effects of ES-MCO Coating Combined with LDPE Bag and 1-MCP Sachet on the Quality of Methyl Bromide Fumigated Mangosteen Fruit

3.2.1. Browning of Calyx and Total Chlorophyll Content in the Calyx of Mangosteen Fruit

The appearance of the green calyx is an important characteristic that influences purchasing decisions. After harvesting, the calyx of mangosteen turned brown and shrive very fast, which was caused by water loss and chlorophyll degradation (Kaewsuksaeng *et al*., 2019). This study found that the calyx of mangosteen in the control treatment turned dark brown within 10 days of storage at 13ºC. The treatment of mangosteen fruits with ES-MCO + Net bag could not delay the browning of the calyx, where else fruits treated with ES-MCO + LDPE bag and ES-MCO + LDPE bag + 1-MCP sachet delayed the browning of the calyx for 15 days when compared with the control. On day 15 of storage, the fruits treated with ES-MCO + LDPE bag and ES-MCO + LDPE bag + 1-MCP sachet showed the lowest calyx browning score were 2.93 and 2.76, respectively, while the fruits treated with ES-MCO + Net bag had a 4 score as same as the control. This result suggested that LDPE bag was the main effect in delaying the calyx browning. The effect of the LDPE bag on delaying the calyx browning was related to maintaining a higher relative humidity within the package which minimises the water loss from the calyx (Pranamornkith, 1997; Vo *et al*., 2016). However, the calyx of mangosteen in all treatments developed into brown colour up to 100% after storage for 20 days (Figure 2A and Figure 3).

In addition, the browning of mangosteen calyx was associated with chlorophyll degradation. The present study found that total chlorophyll content in the control treatment sharply decreased during storage. Meanwhile, ES-MCO + LDPE bag and ES-MCO + LDPE bag + 1-MCP sachet treatments could delay the reduction of total chlorophyll, in which no significant difference was observed between both treatments. Whereas the fruit treated with ES-MCO + Net bag had low total chlorophyll content as same as the control. At the end of storage, the total chlorophyll content in ES-MCO + LDPE bag and ES-MCO + LDPE bag + 1-MCP sachet treatments were 1.13 and 1.23 mg g^{-1} FW, respectively, while the ES-MCO + Net bag and control treatments had only 0.68 and 0.39 mg g^{-1} FW, respectively (Figure 2B and Figure 3). The effect of ES-MCO + LDPE bag or ES-MCO + LDPE bag + 1-MCP sachet on delaying chlorophyll degradation was associated with high CO_2 concentration inside of the packaging, which can inhibit the activity of enzymes involved in chlorophyll degradation including chlorophyllase, pheophytinase, pheophorbidase and chlorophyll-degrading peroxidase (Li *et al*., 2019). Moreover, CO_2 has also reduced ethylene production, which resulted to maintain the green calyx of the fruit (Bender *et al*., 2020; Mu-bo et al., 2015; Piriyavinit *et al*., 2011).

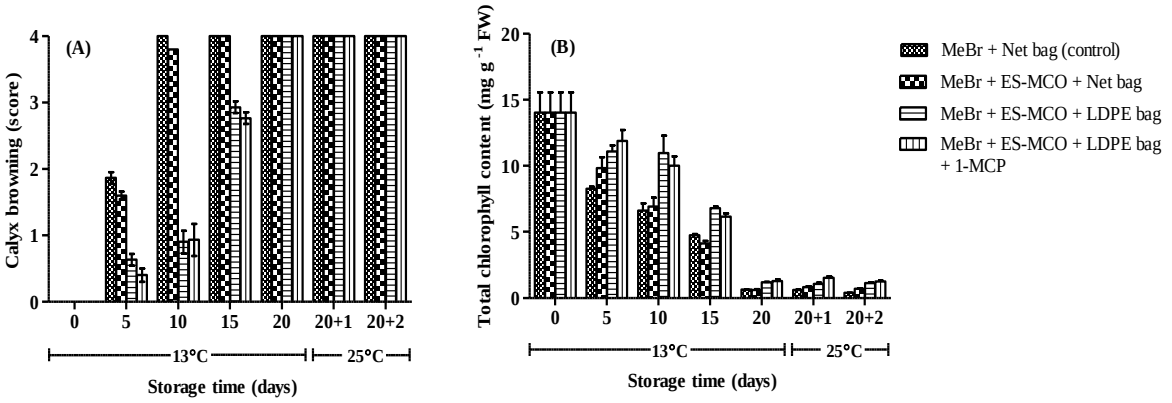

Figure 2. The combined effects of ES-MCO coating, LDPE bag and 1-MCP sachet on calyx browning (A) and total chlorophyll content in the calyx (B) of MeBr fumigated mangosteen during storage at 13ºC for 20 days and 25ºC for 2 days. The fruits were non-coated and packed in net bags, coated with ES-MCO packed in net bags and coated with ES-MCO packed in LDPE bags were used as the controls.

3.2.2. Weight Loss and Firmness of Mangosteen

The weight loss of the mangosteen fruits in the control treatment rapidly increased throughout the storage period. Coating with ES-MCO could reduce weight loss when compared with the control, especially when the fruits were coated with ES-MCO and then packed in LDPE bags, where the best effect was observed to reduce weight loss of mangosteen, regardless of the presence of 1-MCP sachet. On the last day of storage, the fruit treated with ES-MCO + LDPE bag and ES-MCO + LDPE bag + 1-MCP sachet lost only 0.70 and 0.82% in weight, while the fruit treated with ES-MCO + Net bag and the control were 4.87% and 6.12%, respectively (Figure 4A). This result indicated that treatments with ES-MCO + LDPE bag or ES-MCO + LDPE bag + 1-MCP sachet had the strongest effect on retarding weight loss of mangosteen fruit during storage. This may be caused by ES-MCO and LDPE bags which served as the barrier to prevent moisture loss. In addition, the application of coating and packaging to the reduction of weight loss was related to the reduction of the respiration rate of fruit (Kraśniewska *et al*., 2019).

Pericarp hardening is a prevalent problem in mangosteen, and several factors affect the occurrence of pericarp hardening (Dangcham & Ketsa, 2007; Workhwa & Teerachaichayut, 2015). Thavorn (2013) reported that MeBr fumigation is one of the causes of the pericarp hardening of mangosteen. The present study found that after fumigation and storage at 13°C the pericarp firmness in all treatments tended to maintain until 15 days and no significant differences among treatments. Thereafter, the firmness rapidly increased, especially in the fruit treated with ES-MCO + LDPE bag and ES-MCO + LDPE bag + 1-MCP sachet. By the end of storage, the fruits treated with ES-MCO + LDPE bag (83.2 N) and ES-MCO + LDPE bag + 1-MCP sachet (78.2 N) showed higher pericarp firmness than the fruit treated with ES-MCO + Net bag (43.87 N) and control (45.28 N) by approximately 2-fold (Figure 4B). This might be caused by the occurrence of fruit rot disease, which is the

most severe disease found in the fruit treated with ES-MCO + LDPE bag or ES-MCO + LDPE bag + 1-MCP sachet after storage for 20 days. In addition, high CO_2 concentration inside the LDPE bag might have affected pericarp tissue damage leading to the pericarp hardening. This finding has been confirmed by Assis *et al.* (2001) who reported that CO_2 concentration at 20% caused damage and increase lignin content in the pericarp, which causes the pericarp hardening of cherimoya fruit.

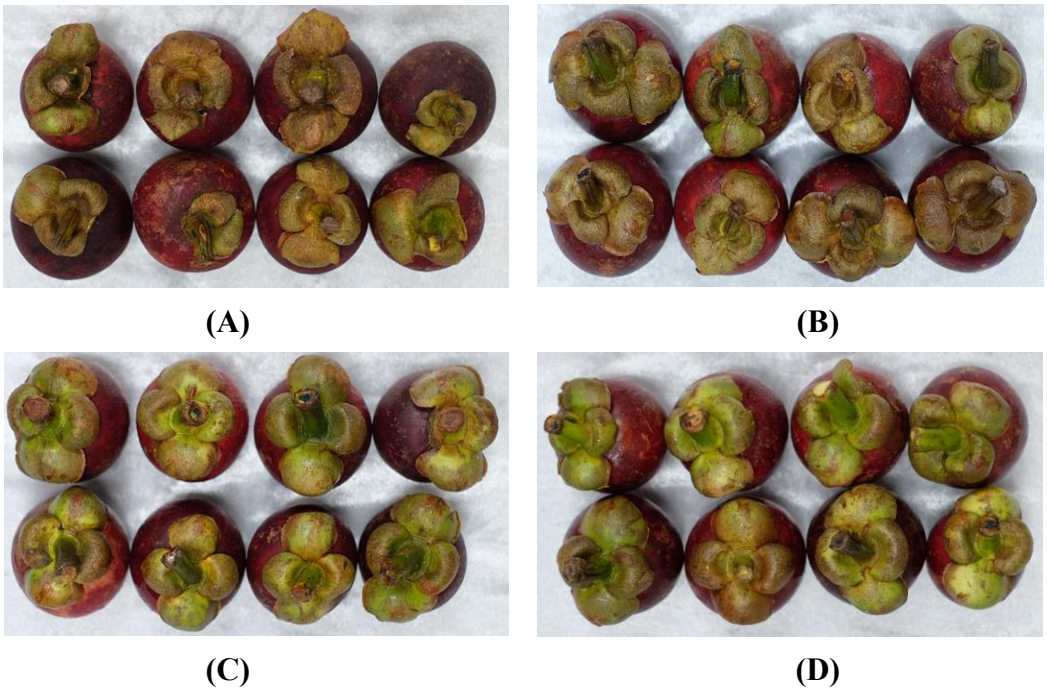

Figure 3. The appearances of MeBr fumigated mangosteen fruits according to the treatments of non-coated + net bag (A), coated with ES-MCO + net bag (B), coated with ES-MCO + LDPE bag (C) and coated with ES-MCO + LDPE bag + 1-MCP sachet (D) during storage at 13ºC for 15 days.

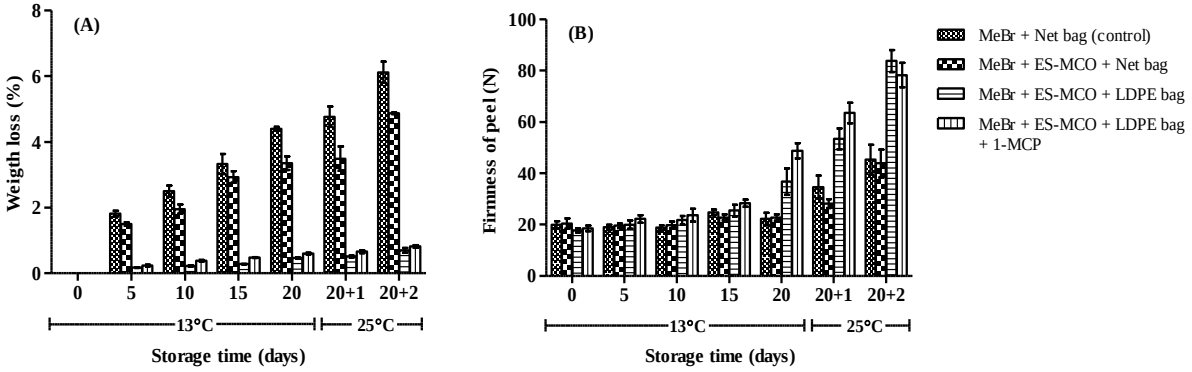

Figure 4. The combined effects of ES-MCO coating, LDPE bag and 1-MCP sachet on weight loss (A) and firmness (B) of MeBr fumigated mangosteen during storage at 13ºC for 20 days and 25ºC for 2 days. The fruits were non-coated and packed in net bags, coated with ES-MCO packed in net bags and coated with ES-MCO packed in LDPE bags were used as the controls.

3.2.3. Respiration Rate and Ethylene Production of Mangosteen Fruit

Mangosteen is a climacteric fruit, which has the characteristic of a sudden increase in ethylene production and respiration rate during ripening. This study found that at the beginning (day 0) the respiration rate in the control fruit was 143.3 mg CO_2 kg^{-1} hr^{-1}. In the initial treatments with ES-MCO + Net bag, ES-MCO + LDPE bag, and ES-MCO + LDPE bag + 1-MCP sachet, a reduction of the respiration rate was observed to 104.7, 100.6 and 96.5 mg CO_2 kg^{-1} hr^{-1}, respectively. Afterwards, the respiration rate in all fruits continued to decrease and remained stable until day 15 of storage, then sharply increased until the end of storage. However, ES-MCO + LDPE bag + 1-MCP sachet was the best treatment to reduce respiration rate (137.4 mg CO_2 kg^{-1} hr^{-1}), followed by ES-MCO + LDPE bag (202.9 mg CO_2 kg^{-1} hr^{-1}). Whereas the fruit treated with ES-MCO + Net bag (240.9 mg CO_2 kg^{-1} hr^{-1}) showed a high respiration rate similar to the control fruit (236.6 mg CO_2 kg^{-1} hr^{-1}) (Figure 5A). Ethylene production in all mangosteen treatments had a low concentration on the initial day (day 0) which was between 0.06 - 0.34 µl C_2H_4 kg^{-1} hr^{-1}. Then, the ethylene production in the control fruit rapidly increased and peaked at the end of storage with 10.92 µl C_2H_4 kg^{-1} hr^{-1}. Whereas the fruit treated with ES-MCO + LDPE bag + 1-MCP sachet showed the lowest ethylene production (0.01 C_2H_4 kg^{-1} hr^{-1}), followed by ES-MCO + LDPE bag (0.03 C_2H_4 kg^{-1} hr^{-1}) and ES-MCO + Net bag (2.77 C_2H_4 kg^{-1} hr^{-1}) (Figure 5B).

This result indicated that the application of ES-MCO + LDPE bag + 1-MCP sachet treatment was the most effective to reduce ethylene production and respiration rate. These findings may be related to the fact that 1-MCP is a competitive gaseous ethylene inhibitor that binds irreversibly to ethylene receptors, which can delay the senescence processes of many fruits (Blankenship & Dole 2003; Lv *et al.*, 2023). The effect of 1-MCP in reducing both ethylene production and respiration rate has been recorded in several fruits and vegetables such as mango, strawberry, plums, durian and cabbage (Dong *et al*., 2001; Li *et al*., 2020; Meng *et al*., 2019; Thongkum *et al.*, 2018; Tian *et al*., 2000). Moreover, wax coating and MAP packaging were related to the modified atmosphere inside of the package, which could be attributed to the high CO_2 and low O_2 concentration, thereby lowering the respiration rate and ethylene production of fruit (Maftoonazad *et al*., 2007; Ozturk *et al*., 2019).

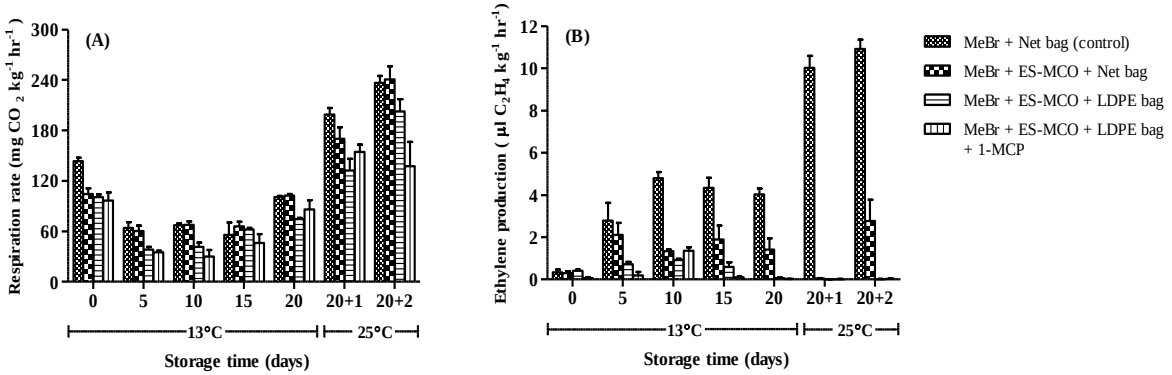

Figure 5. The combined effects of ES-MCO coating, LDPE bag and 1-MCP sachet on respiration rate (A) and ethylene production (B) of methyl bromide fumigated mangosteen during storage at 13ºC for 20 days and 25ºC for 2 days. The fruits were non-coated and packed in net bags, coated with ES-MCO packed in net bags and coated with ES-MCO packed in LDPE bags were used as the controls.

4. Conclusions

The findings in this study indicated that ES-MCO + LDPE bag + 1-MCP sachet treatment could reduce fruit rot disease and maintain the quality of MeBr fumigated mangosteen including delayed calyx browning and chlorophyll degradation, reduced weight loss, the respiration rate and ethylene production. Thus, the application of ES-MCO + LDPE bag + 1-MCP sachet may act as a method for prolonging the shelf life of MEBr fumigated mangosteen during storage at low temperatures.

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