Effects of moringa leaves (Moringa oleifera) extraction on quality changes and melanosis of giant freshwater prawn (Macrobrachium rosenbergii) during chilled storage

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Abstract
An aqueous extraction of moringa (Moringa oleifera) leaves were prepared as the edible coats for keeping the quality of the giant freshwater prawn (Macrobrachium rosenbergii). In addition, the antioxidant properties and activity; total phenolic content (TPC), total flavonoid contents (TFC), free radical scavenging activity (DPPH), and ferric reducing antioxidant power (FRAP) of moringa leaves were also determined. The phenolic compounds and antioxidant properties in the moringa leaves are low; 16.14 mgGAEg⁻¹ for TPC; 5.57 mgQEg⁻¹ for TFC; 1.36 mgTEg⁻¹ for DPPH; and 3.05 mgTEg⁻¹ for FRAP. The experiment was further conducted by coating the M. rosenbergii with moringa leaves extraction before chilled storage at 4°C for 15 days. Moringa leaves extraction was effectively reduced the microflora count in M. rosenbergii (P<0.05). Total volatile basis nitrogen (TVB-N) value showed a significant (P<0.05) lower amount in treated samples compared to the controls. Melanosis were obvious in controls compared to the treated samples. After 15 days of chilled storage, the sensory properties; taste, texture and odour of treated samples were acceptable by the panelists. Biopreservation of moringa leaves extraction significantly benefits in keeping the quality of M. rosenbergii.

Introduction
Giant freshwater prawn, Macrobrachium rosenbergii is native species to the Southeast Asia, northern Australia and Indo-Pacific region that had a great demand in national and international market (Reddy and Reddy, 2014). It is notable in market due to its culinary characteristics and the unique taste of their meat (FAO, 2014a). However, the qualities of M. rosenbergii easily to deteriorate due to bacteria and chemical action (Ali et al., 2010) and activity of digestive enzymes (Kirschnik et al., 2006). It is probably due to collagenolytic activity caused by the disintegration of the hepatopancreas (Lindner et al., 1988). The speed of this activity is related to storage temperature (New et al., 2010). In addition, the quality deterioration also associated with inappropriate post-harvest handling and preservation (Madrid and Phillips, 2000; Kirschnik et al., 2006).

Preservation is an important process for quality assurance in seafood industry. Natural products and their secondary metabolites are commonly used as antimicrobial and antioxidant biopreservatives (Anastasio et al., 2014; Palmieri et al., 2016; Mogosanu et al., 2017). Previous studies by Rathshiliva et al. (2014) documented that the leaves extraction of Moringa oleifera able to fight against bacterial infection and can act as a source of antioxidiant (Khalafalla et al., 2010). Moringa leaves contains unique nutritional qualities where it contains protein, vitamin A and C, also one of the well sources of minerals such as calcium, iron, manganese and copper (Rudrapra, 2014). Leaf extracts of M. oleifera were reported to exhibit antioxidant activity in vitro and in vivo due to abundant of phenolic acids and flavonoids (Atawodi et al., 2010). Chen and Verdes (2009) stated that M. oleifera is a good antimicrobial agent. Viera et al. (2010) emphasized that the extract of M. oleifera can act against Bacillus subtilis, Staphylococcus aureus and Vibrio cholera.

Therefore, this study is to investigate the effectiveness of the moringa leaves extraction on the quality changes and melanosis of giant freshwater prawn during 15 days of chilled storage. This study also reveals the antioxidant properties and the antibacterial activity of the moringa leaves extraction. These biopreservation technique may offers as a new alternative in preservation techniques, extended shelf life and enhanced safety by using the natural resources such as moringa leaves extract.

Materials and Methods
Sample collection and extraction
Mature moringa leaves were freshly collected and cleaned before dried in oven (Ecocell EC111, Germany) at 60°C for 24 hours. The extraction was prepared using methods by Porwal et al. (2012).

Determination of antioxidant properties and activity of M. oleifera leave extract
Total phenolic compounds (TPC) and total flavonoid compound (TFC) were determined according to Taga et al., (1984) and Chang et al., (2002), respectively. In addition, DPPH radical-scavenging activity and ferric reducing antioxidant power (FRAP) were determined according to method by Binsan et al. (2008) and Benzie and Strain (1996). The absorbance was measured by using spectrophotometer (UV Mini-1240 UV-VIS Spectrophotometer Shimadzu, Japan) and compared to the standard calibration curve accordingly to the method.

Sample preparation
M. rosenbergii were headed, peeled and soaked in 0.5 and 1.0% moringa leaves extract for 10 min at 4°C. Controls were left without coating. All samples were super-chilled in blast freezer (Inoxin Blast Freezer, USA) for 5 min before vacuum packed in polyethylene bags and stored at 4°C. Analysis were done at interval of five days during 15 days of chilled storage. All experiment were done in 3 replicates.

Microbiological analysis
Total bacterial count was determined using spread plate method on plate count agar using method by Linton et al. (2003); Karim et al. (2011). 10±0.1g of sample were collected and cleaned before dried in oven (Ecocell EC111, Germany) at 60°C for 24 hours. The extraction was prepared using methods by Porwal et al. (2012).
Table 1. Total phenolic content, total flavonoid content, radical scavenging activity and ferric reducing power of moringa leaves.

| Antioxidant properties and activity | Current studies | Ilyas et al. (2015) | Iqbal and Bhanger (2006) | Siddhuraju and Becker (2003) |
|------------------------------------|-----------------|---------------------|--------------------------|-----------------------------|
| Total phenolic content (mgGAEg⁻¹)  | 16.14±0.74      | 95.35±0.60          | 99.5 to 111.7            | 52.50 to 74.30              |
| Total flavonoid content (mgQEg⁻¹)  | 5.57±3.01       | 65.43±0.60          | 92.4 to 98.8             | 32.60 to 108.30             |
| Radical scavenging activity (DPPH) (mgTEg⁻¹) | 1.36±5.47     | 0.87±0.99           | -                        | 3070                        |
| Ferric antioxidant reducing power (FRAP) (mgTEg⁻¹) | 3.05±0.15    | -                   | -                        | 0.41 to 2.68                |
(Oreochromis niloticus) and silver cyprinid (Rasbora argentea) were effective to reduce the bacterial loads.

Onyuka et al. (2013) also confirmed that moringa extract contain antibacterial activity and can be used for fish preservation for longer time and safe for human consumption. A finding by Peixoto et al., (2011) emphasized moringa leaves has antibacterial effective against Staphylococcus aureus, Vibrio parahaemolyticus, Enterococcus faecalis and Aeromonas caviae. Viera et al. (2010) also documented that M. oleifera extract can act against Bacillus subtilis, Staphylococcus aureus and Vibrio cholera. Saadabi and Abu Zaid (2011) stated that the aqueous extract of moringa leaves were found able to inhibit pathogenic bacteria; Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Pseudomonas aeruginosa. A study by Fahey (2005) stated moringa contains 4- (4'-O-acetyl-a-L-rhamnopyranosyloxy) benzyl isothiocyanate, 4-(a-L-rhamnopyranosyloxy) benzyl isothiocyanate, niazimicin, benzyl isothiocyanate, and 4- (a-L-rhamnopyranosyloxy) benzyl glucosinolate that might act as a powerful antibacterial effect.

Total volatile basis nitrogen (TVB-N) value

M. rosenbergii coated with moringa extraction showed a significant (P<0.05) reducing effects on the TVB-N accumulation (Table 3). A higher concentration of moringa extraction (1.0%) showed more significantly (P<0.05) effective in reducing the TVB-N accumulation compared to a lower concentration (0.5%). TVB-N value showed significantly (P<0.05) increasing trend during the storage period (15 days) in all samples (Table 3). After 5 days stored in chilling temperature, untreated M. rosenbergii started to developed mild spoilage odors. Meanwhile, M. rosenbergii coated with 0.5% of moringa extraction were at the stage of the beginning of deterioration process. However, M. rosenbergii coated with 1.0% of moringa extract only started to produce spoilage odor after 10th day of storage period. TVB-N value were related to bacterial spoilage activity throughout the storage days (Cobb and Vanderzant, 1975) and associated with amino acid decarboxylase activity of microorganism during storage (Huss, 1995; Duman and Ospolat, 2014).

Shelf life prediction

The estimation shelf life of each treatments calculated from the response curve with curve constraints were taken as the bacteria count reach 10^7 CFUg^-1 (ICMSF, 1986) and TVB-N accumulation were at 35 mgN100g^-1 (Connell and Shewan, 1980). By using total bacteria count data, the shelf life of controls and samples coated at 0.5% reach of 10^7 CFUg^-1 at 8th day of storage for both treatments (Table 4). Meanwhile, samples coated with 1.0% of moringa extract, the shelf life were extended up to 9th day by using total bacteria count data. At limit of the acceptance TVB-N value (35 mgN100g^-2), the shelf life of controls was at 12th day of storage.

Table 2. Total bacterial count (log_{10}CFUg^-1) of Macrobrachium rosenbergii stored for 15 days in different concentration of moringa leaves extract.

| Storage | Control | 0.5% | 1.0% |
|---------|---------|------|------|
| Day 0   | 4.82±0.01^A | 4.65±0.05^A | 4.47±0.07^A |
| Day 5   | 6.47±0.02^B | 6.37±0.03^B | 6.32±0.02^B |
| Day 10  | 7.73±0.04^C | 7.52±0.09^C | 7.32±0.12^C |
| Day 15  | 8.86±0.04^D | 8.52±0.03^D | 8.38±0.13^D |

Table 3. Total volatile basis nitrogen value of Macrobrachium rosenbergii stored for 15 days in different concentration of moringa leaves extract.

| Storage | Control | 0.5% | 1.0% |
|---------|---------|------|------|
| Day 0   | 9.52±0.51^A | 6.05±0.67^A | 2.80±0.5^A |
| Day 5   | 15.68±1.94^A | 12.39±0.5^B | 9.86±0.39^B |
| Day 10  | 23.41±0.20^B | 19.04±1.94^C | 16.69±0.20^C |
| Day 15  | 38.08±3.83^C | 29.90±0.58^D | 25.76±1.94^D |

Table 4. Shelf life prediction of Macrobrachium rosenbergii stored for 15 days in different concentration of moringa leaves extract.

| Shelf life prediction | Control (day) | 0.5% prawn soaked moringa extract sample (day) | 1.0% prawn soaked moringa extract sample (day) |
|-----------------------|---------------|-----------------------------------------------|-----------------------------------------------|
| Total bacterial count  | 8             | 8                                             | 9                                             |
| Total volatile basis nitrogen | 12         | 16                                             | 18                                             |

Table 5. Sensory properties of GFP treated with different treatment.

| Storage time, days | Treatment | Melanosis Score | Taste | Texture | Odour |
|-------------------|-----------|-----------------|-------|---------|-------|
| 0                 | Control   | ND              | 4.00±0.00^a | 3.72±0.17^a | 3.83±0.13^a |
|                   | 1.25% SMS | ND              | 3.97±0.05^a | 3.59±0.08^a | 3.80±0.08^a |
|                   | 0.25% moringa extract | ND | 3.87±0.12^a | 3.77±0.12^a | 3.80±0.08^a |
|                   | 0.5% moringa extract | ND | 3.97±0.05^a | 3.77±0.19^a | 3.80±0.08^a |
| 15                | Control   | 10.00±0.00^a   | 2.30±0.08^a | 2.40±0.00^a | 2.30±0.22^a |
|                   | 1.25% SMS | 3.33±0.58^b   | 3.00±0.14^c | 3.80±0.08^a | 3.52±0.05^c |
|                   | 0.25% moringa extract | 8.67±1.53^a | 3.67±0.12^a | 3.47±0.05^c | 3.53±0.05^c |
|                   | 0.5% moringa extract | 8.67±1.53^a | 3.53±0.05^c | 3.13±0.12^d | 3.53±0.05^c |

Different letters in the same column indicate the significant differences (P<0.05).
chilled storage (Table 4). In case of samples coated at 0.5 and 1.0% moringa extract, TVB-N did not reach the value of 35 mgN\textperthousand100g\textperthousand1, the data were curve fitted by fitting to the linear regression as response curve to the TVB-N data. Therefore, the shelf life for samples coated with 0.5 and 1.0% were predicted and could extended up to 16\textperthousand6 and 18\textperthousand8, respectively (Table 4). The shelf life using TVB-N value had an extended day compared to the bacteria indicators. However, with a great consideration on the safety, microbial should be the dominant to estimate the shelf life.

Melanosis of Macrobranchium rosenbergii during iced storage

Initially, there were no melanosis formation in all samples. However, after 15 days of chilled storage, controls were significantly (P<0.05) had an obvious melanosis formation compared to other treatments (Table 5). In contrast, 1.25% SMS were significantly (P<0.05) retard the melanosis formation in M. rosenbergii during 15 days of storage. M. rosenbergii coated with moringa extract had severe melanosis formation. Edmonds (2006) documented the inhibition of melanosis activity in M. rosenbergii treated with 1.25% SMS are due to the bleaching effects and undergone discoloration. Sulphite are most widely and effectively used to prevent melanosis in crustaceans (Bono et al., 2012; Lopez-Cabellero et al., 2006; Nirmal and Benjakul, 2009). Inhibition of browning involves nucleophilic attack by sulphite ion in position 4 of the o-quinone with catechol as substrate to give 4-sulfocatechol after subsequent addition of hydrogen ion. Therefore, the quinone has been reduced in the reaction (Kim et al., 2000). Melanosis inhibitory in M. rosenbergii that treated with moringa extracts is caused by the extraction technique that decreases the content of ascorbic acid and the irreversibly oxidation characteristic that oxidase ascorbic acid to dehydroascorbic acid during the reduction process, thus allowing browning to occur upon its depletion (Marshall et al., 2000).

Sensory properties of M. rosenbergii during iced storage

Initially, all samples were extremely liked by the panelist. After 15 days of storage, the taste, texture and odor of the controls were significantly (P<0.05) unacceptable. Interestingly, the taste of M. rosenbergii treated with moringa extracts were significantly (P<0.05) preferred compared to the M. rosenbergii treated with 1.25% SMS. However, the texture of M. rosenbergii treated with 1.25% SMS were similar (P>0.05) even after 15 days of storage and significantly (P<0.05) desired compared to the M. rosenbergii treated with moringa extracts. But, there was no difference in odor likeness of M. rosenbergii treated with other treatments after 15 days of storage (P>0.05) (Table 5). Rotlant et al. (2002) stated the application of sulphites agents is to block the polyphenol oxidase activity and provide some partial bleaching to maintain an acceptable appearance. However, these are not necessarily meet the flavor acceptance by the consumers. Loizzo et al., (2012) stated sulphite-containing inhibitor may causing off-flavors in the applied product.

Conclusions

Moringa extracts at 1.0% could effectively control the bacterial growth and chemical quality changes for M. rosenbergii stored in chilling temperature. The shelf-life were prolonged up to 9th day. In addition, moringa extract also potentially to delay melanosis formation as it preserves the quality of the M. rosenbergii during iced storage. The common melanosis inhibitors, sodium metabisulphate has showed a better result inhibiting melanosis but leads to unacceptable taste to the consumers. Therefore, substitution of moringa extract are safe and potential in crustacean preservation agent.

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