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 were 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 Ratshilivha *et al*., (2014) documented that the leaves extraction of *Moringa oleifera* able to fight against bacterial infection and can act as a source of antioxidant (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 (Rudrappa, 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).

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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 superchilled in blast freezer (Irinox 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 experiments 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

homogenized with sterilized maximum recovery diluent (MRD). A serial samples dilution was prepared at appropriate dilution. Accurately, 0.1 mL of the dilution was spread on plate count agar (PCA) by using sterile glass spreader. All plates agar was incubated at 30°C for 48 hours. Plates with 30 to 300 colonies were selected and the total colonies were recorded. Total bacteria count was determined using formula:

Total bacteria per mL = number of colony x dilution factor

Determination of total volatile basis nitrogen (TVB-N)

Total volatile basis nitrogen (TVB-N) were determined using method from Karim *et al*. (2011). 100±0.1g of prawn samples were added to 200 mL of 7.5% trichloroacetic acid before homogenized for 1 minute. The homogenate was centrifuged at 2000*g* for 15 minutes and filtered through Whatman No. 1 filter paper. 25 mL of filtrate were pipetted into the Kjeldahl distillation tube followed by 5 mL of 10% sodium hydroxide. Steam-distillation were carried out using a vertical steam-distillation unit, and the distillate were collected into a beaker containing 10 mL of 4% (v/v) aqueous boric acid solution and 0.04 mL methyl red and bromocresol green indicator up to a final volume of 50 mL. The titration was allowed to run against aqueous 0.05 M sulphuric acid solution and it was shaken until pink color, neutralization was indicated and persists for 15 seconds. The quantity of TVB-N in mg from the volume of sulphuric acid (n mL) determined as below:

$$\text{TVB-N (mgN per 100g)} = n*16.8$$

Melanosis and sensory assessment

Giant freshwater prawn was dipped in 0.25 and 0.5% moringa extract, 1.25% sodium metabisulphate (SMS) as positive control, and distilled water as control at 4°C for 30 minutes. Samples were superchilled in blast freezer (Irinox Blast Freezer, USA) for 5 min and packed in (PE) vacuum pack (DZQ Vacuum Packer, China). Samples were kept in chilled temperature before analysed at initial and 15th days of storage.

Melanosis assessment were done according to method by Nirmal and Benjakul (2009). Melanosis of GFP were scored through visual inspection by 30 panelists using 10 point scoring test; 0=absent, 2=slight (20% shrimps surface affected); 4=moderate (40% of shrimps surface affected); 6=notable (60% of shrimps surface affected); 8=severe (80% of shrimps surface affected); 10=extremely heavy (100% of shrimp surface affected).

Sensory evaluation was done according to Nirmal and Benjakul (2009). All samples were placed on a stainless-steel tray covered with aluminum foil and steamed for 5 min. The cooked samples were evaluated by 30 panelists. Four-point hedonic scale were used to score the samples; 4=extremely like, 3=moderately like, 2=neither like nor dislike; 1=dislike. All panelists were asked to evaluate for taste, texture, and odor.

Statistical analysis

The entire experiment was replicated three times. The data were analyzed statistically using one-way ANOVA with post hoc Scheffe's test to compare the significant differences among groups at 0.05 level of probability. All statistical analysis were done using the IBM SPSS Statistic software (Version 20). The estimation shelf life of each treatment was fitted as the response curve with microbiology and chemical data. The microbial shelf life was taken as the time to reach 10^7 CFU g^{-1}, as recommended by International Commission on Microbiology Specification for Food (ICMSF, 1986). Meanwhile the chemical shelf life was taken as the time to reach 35 mgN $100g^{-1}$ for TVBN (Connell and Shewan, 1980).

Results and Discussion

Antioxidant properties and activity of *M. oleifera* leave extract

Total phenolic content (TPC) and total flavonoid content (TFC) in *M. oleifera* extraction were 16.14±0.74 mgGAEg^{-1} and 5.57±3.01 mgQEg^{-1}, respectively (Table 1). Ilyas *et al.* (2015) recently documented that TPC and TFC of *M. oleifera* collected from Pakistan were recorded at amount of 95.35±0.60 and 65.43±0.60 mgTEg^{-1}, respectively. Meanwhile, Iqbal and Bhanger (2006) showed the concentration of TPC and TFC; 99.50 to 111.7 and 92.40 to 98.8 mgTEg^{-1}, respectively. A slightly lower amount of antioxidant properties was found in moringa leaves collected from South Africa. The amount of TPC were recorded at 52.50 to 74.30 mgTEg^{-1}, meanwhile the TFC were recorded at 32.60 to 108.30 mgTEg^{-1} (Siddhuraju and Becker, 2003). Present studies recorded DPPH were 1.36±5.47 mgTEg^{-1} and FRAP were 3.05±0.15 mgTEg^{-1} (Table 1). FRAP value in *M. oleifera* collected from Pakistan showed a same range to the present studies; 0.41 to 2.68 mgTEg^{-1} (Table 1) (Siddhuraju and Becker, 2003). The antioxidant properties and activity of *M. oleifera* of present study were low compared to previous studies (Iqbal and Bhanger, 2006; Ilyas *et al.*, 2015). The variations of the TPC and TFC in samples could be due to the temperature and methods of extraction of polyphenolic compounds, degree of polarity of the solvents and geographical locations of the plants (Ilyas *et al.*, 2015). The differentiation also depending on the nature of the soil and season of cultivation (Ilyas *et al.*, 2015).

Total bacterial count

Edible coating of moringa leaves extract on *M. rosenbergii* were effectively reduced the total bacteria count ($P<0.05$) (Table 2). Coating at 1.0% of moringa leaves showed a significant ($P<0.05$) lower amount of total bacteria count compared to the samples coated with 0.5% leaves extraction. With regards to the storage, the total bacteria counts of controls were significantly ($P<0.05$) increased from 4.82 $\log_{10}$CFUg^{-1} on day 0 to 8.66 $\log_{10}$CFUg^{-1} at the end of storage day (Table 2). A significantly ($P<0.05$) similar trend of total bacteria count; an increasing count parallel with the duration of storage were found in both samples treated with 0.5 and 1.0% of moringa extract (Table 2). Previous study by Onyuka *et al*. (2013) documented that moringa extract at 80 µgmL^{-1} coated on tilapia

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 (*Rastrineobola 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 aeroginosa*. 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 day 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 Öspolat, 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^{-1}), the shelf life of controls was at 12th day of

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	$4.82\pm0.0^{la,A}$	$4.65\pm0.05^{b,A}$	$4.47\pm0.07^{c,A}$
Day 5	$6.47\pm0.02^{a,B}$	$6.37\pm0.03^{b,B}$	$6.32\pm0.02^{b,B}$
Day 10	$7.73\pm0.04^{a,C}$	$7.52\pm0.09^{a,C}$	$7.23\pm0.12^{b,C}$
Day 15	$8.66\pm0.04^{a,D}$	$8.52\pm0.03^{b,D}$	$8.38\pm0.13^{c,D}$

Different superscript (a, b, c) indicate significant difference (P<0.05) between treatment (controls, 0.5% and 1.0% concentration). Different superscript (A, B, C) indicate significant difference (P< 0.05) between the storage days.

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	$9.52\pm0.51^{a,A}$	$6.05\pm0.67^{b,A}$	$2.80\pm0.5^{c,A}$
Day 5	$15.68\pm1.94^{a,A}$	$12.99\pm0.5^{a,B}$	$9.86\pm0.39^{b,B}$
Day 10	$23.41\pm0.20^{a,B}$	$19.04\pm1.94^{b,C}$	$16.69\pm0.20^{c,C}$
Day 15	$38.08\pm3.88^{a,C}$	$29.90\pm0.58^{b,D}$	$25.76\pm1.94^{b,D}$

Different superscript (a, b, c) indicate significant difference (P<0.05) between treatment (controls, 0.5% and 1.0% concentration). Different superscript (A, B, C) indicate significant difference (P< 0.05) between the storage day.

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

Total bacterial count limit for human consumption at 10^7 CFUg^{-1}. Total volatile basis nitrogen limit for human consumption and prawn acceptability at 35 mg N 100g^{-1} sample

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}	3.73 ± 0.17^{a}	3.83 ± 0.13^{a}
	1.25% SMS	ND	3.97 ± 0.05^{a}	3.90 ± 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^{b}	2.40 ± 0.08^{b}	2.30 ± 0.22^{b}
	1.25% SMS	3.33 ± 0.58^{b}	3.00 ± 0.14^{c}	3.80 ± 0.08^{a}	3.53 ± 0.05^{c}
	0.25% moringa extract	8.67 ± 1.53^{a}	3.67 ± 0.12^{d}	3.47 ± 0.05^{c}	3.53 ± 0.05^{c}
	0.5% moringa extract	8.67 ± 1.53^{a}	3.53 ± 0.05^{d}	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 mgN100g^{-1}, 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 16th and 18th, 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 discolouration. 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 nuclephilic 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 (Marshal *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 necessary 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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