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EFFECTS OF GUAVA, *Psidium guajava* LEAVES EXTRACT COATING ON GIANT FRESHWATER PRAWN, *Macrobrachium rosenbergii* DURING CHILLED STORAGE

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Abstract: Guava, *Psidium guajava* leaf extract was tested for antioxidant properties and activity; total phenolic content, total flavonoid content (TFC), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP). Giant freshwater prawns, *Macrobrachium rosenbergii* were subjected to *P. guajava* leaf extract coating at concentrations of 0.5 and 1.0%. Controls were left untreated. All samples were individually vacuum packed before chilled storage at 4°C. Total bacteria count and total volatile basis nitrogen were analysed at five day interval during 15 days of chilled storage. The total phenolic content, TFC, DPPH and FRAP values were recorded at 383.67±15.22 mg GAE g^{-1}, 51.02±15.02 mg QE g^{-1}, 77.41±4.28 µM TE g^{-1} and 2.56±0.44 µM TE g^{-1}, respectively. *P. guajava* leaf extract glazing was significantly ($p<0.05$) effective in reducing the total bacteria accumulation in *M. rosenbergii.* Untreated *M. rosenbergii* reach the limit of acceptability on 7th day of chilled storage. Meanwhile the shelf-life of both samples coated with 0.5 and 1.0% were extended up to the 10th day of storage. TVBN value of treated samples showed a significantly ($p<0.05$) lower amount compared to the untreated samples. With regard to safety of food consumption, microbiology analysis are more reliable to reflect on the shelf life prediction of *M. rosenbergii* coated with *P. guajava* leaf extract.

Keywords: *Macrobrachium rosenbergii,* glazing, chilled storage, *Psidium guajava* leaf extract, food safety

Introduction

Giant freshwater prawn, *Macrobrachium rosenbergii* is an important crustacean species in many countries. High demand and mass commercialization on *M. rosenbergii* regulating the local and international trade. Fisheries products take several chains and time to reach consumers depending upon the marketing and delivery channels after harvesting. Thus, these may cause biodeterioration by microbial and enzyme activity that leads to economic losts. Application of synthetic antioxidants are commonly used by producers for food preservation and preclude undesirable rotting and deteriorations. Synthetic antioxidants play a significant role to reduce free radical damage and confer antiageing effects. However, the application of propylgallate, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Mona *et al.,* 2012) are proven to be harmful to consumers in long term. Scientific research found that BHT promotes the development of lung tumour (Malkinson, 1999) and had a significantly adverse effects on body weight (Meyer *et al.,* 1980). Meanwhile, BHA is documented to induce the formation of stomach tumour (National Toxicology Program, 1979; Kahl *et al.,* 1993). In contrast, natural antioxidants are not only safe but also do not present adverse carcinogenic consequences.

Flavonoids and polyphenols prevent the propagation of free radical reactions and delaying lipid oxidative rancidity (Duthie, 1993). *Psidium guajava* (Myrtaceae), guava leaves contain high antioxidant activity (Guo *et al.,* 2003) and would be a natural source of antioxidants (Ojan and Nihorimbere, 2004). Alkaloids, flavonoids, phenolic and tannins are important groups of phytochemicals (Amal *et al.,* 2009). Ojan and Nihorimbere (2004) documented that the total phenolic content from *P. guajava* leaf extract was recorded at 575.3±15.5 mg ml^{-1}. Kähkönen *et al.* (1999) also emphasized that polyphenolic compounds have remarkably high antioxidant

activity. Basically, flavonoids are structured by the flavan nucleus, which comprise 15 carbon atoms arranged in three rings. The structure and substitution alteration of the rings may affect the flavonoids antioxidant properties and phenoxyl radical stability (Wojdyło *et al.,* 2007).

According to Daglia (2012), the phenolic acids, and also small fragments of lignin, which can be found in the leaf extracts of *P. guajava,* could be potential antibacterial ingredients. It has been documented elsewhere (Chah *et al.,* 2006; Nair and Chanda, 2007) that the extract of *P. guajava* leaves has an antibacterial potential and effectively inhibit various bacteria; *Staphylococcus aureus, Staphylococcus mutatis, Pseudomonas aeruginosa, Salmonella enteritidis, Bacillus cereus, Proteus* spp., *Shigella* spp., and *Escherichia coli.* Garcia *et al.* (2002) added that extracts of *P. guajava* leaves are useful to inhibit spore formation and *Clostridium prefringens* type A. Previously, Oliver-Bever (1986) stated that guava leaves contain triterpenic acids and flavonoids; quercetin, avicularin and 3-L-4-pyranoside that have antibacterial action. Faharani (2008) emphasized that the aqueous extract of *P. guajava* leaves effectively inhibit the pathogenic bacteria, *Escherichia coli.* They added *P. guajava* leaves contain 9% tannins. Moila *et al.* (2014) documented that tannins benefit as antibacterial agents as they have phenol groups. In this study, giant freshwater prawns were soaked in two different concentrations (0.5% and 1.0%) of *P. guajava* leaf extract while non-soaked giant freshwater prawns are used as a control. The microbiology and biochemical quality of samples were analysed of intervals of five days during 15 days of chilled storage.

Materials and methods

P. guajava leaves extraction

Matured *P. guajava* leaves were freshly collected from Kota Bharu, Kelantan and brought to the laboratory for further analysis. Mature leaves were selected between the fifth and eighth tiers from the top of foliage where the leaves are fully developed. Fresh *P. guajava* leaves were weighted and dried in laboratory oven (Ecocell EC111, Germany) at 60°C for 24 h. Dried *P. guajava* leaves were weighted and grinded by using Waring laboratory blender into a powder. *P. guajava* leaves were extracted according to Porwal *et al.* (2012) with some modification. The aqueous extraction were done at a ratio of 1:30 of *P. guajava* leaf powder to distilled water (w/v). Approximately 30 ml of extraction were collected from steam distillation process. The leaf extraction solution of 0.5 and 1.0% (w/v/v) were prepared from the distillate solution. The extraction was stored at -20°C for further analysis.

Antioxidant properties and activity of P. guajava leave extract

Total phenol content

Total phenol compounds was determined by using Folin-Ciocalteu reagent assay with gallic acids as standard (Taga *et al.,* 1984). 100 µl *P. guajava* leaf extract was added to 2% sodium carbonate (Na_2CO_3) followed by 100 µl of 50% Folin-Ciocalteu reagent and left to stand for 30 min. The absorbance was measured at 750 nm using spectrophotometer (UV Mini-1240 UV-VIS Spectrophotometer Shimadzu, Japan).

Total flavonoid content

Total flavonoid compound (TFC) was determined according to Chang *et al.* (2002) with quercetin (QE) as standard. 1 ml of *P. guajava* extraction was added to 0.3 ml sodium nitrite, 0.3 ml aluminium chloride, and 2 ml of sodium hydroxide solution before making up to 10 ml with distilled water and left to stand for 10 min. The measurement of absorbance was recorded at 415 nm using spectrophotometer (UV Mini-1240 UV-VIS Spectrophotometer Shimadzu, Japan). TFC is expressed as quercetin (QE) in mg g^{-1} of sample.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical assay

DPPH radical scavenging activity were determined according to Binsan *et al.* (2008) with trolox as standard. 1.5 ml of *P. guajava* leaf

extract was added to 1.5 ml of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and left to stand for 30 min. The measurement of absorbance were recorded at 517 nm using spectrophotometer (UV Mini-1240 UV-VIS Spectrophotometer Shimadzu, Japan). The activity is expressed as l mol Trolox equivalents (TE) g^{-1} samples.

Ferric-reducing antioxidant power (FRAP) assay

FRAP assay was prepared as described by Benzie and Strain (1996) with trolox as standard. FRAP reagent contained 5 ml of a 10 $mmol^{-1}$ 2, 4, 6-tripyridyl-S-triazine (TPTZ) solution in 40 mmol L^{-1} hydrochloric acid (HCl) and 5 ml of 20 mmol L^{-1} ferric chloride ($FeCl_3$) and 50 mL of 0.3 mol L^{-1} acetate buffer (pH 3.6). 100 µL *P. guajava* leaf extract was mixed with 3 ml of FRAP reagent and left to stand for 10 min. The measurement of absorbance was recorded at 593 nm using the spectrophotometer (UV Mini-1240 UV-VIS Spectrophotometer Shimadzu, Japan). The activity was expressed as l mol Trolox equivalents (TE) g^{-1} samples.

Sample preparation

M. rosenbergii were headed, peeled and soaked accordingly in 0.5 and 1.0% *P. guajava* leaf extract for 10 min at 4°C. Meanwhile, the controls were left without coating. Samples were superchilled in a 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 being analysed at intervals of five days within 15 days of storage. The analysis were replicated three times.

Microbiological analysis

A total of 10±0.1 g of *M. rosenbergii* flesh was homogenised with 90 ml maximum recovery diluent (MRD). A serial dilution was prepared until appropriate dilution. Accurately, 0.1 ml of the dilution was spread on plate count agar (PCA) by using sterile glass spreader. The total bacterial counts were performed according to Linton *et al.* (2003) and Karim *et al.* (2011). Bacteria counts were expressed as log colony forming units per gram of samples (log10CFU g^{-1}).

Total volatile basis nitrogen (TVBN) analysis

Total volatile basis nitrogen (TVBN) was determined according to Malle and Tao (1987) with minor modification by Karim *et al.* (2011). *M. rosenbergii* flesh were mixed with trichloroacetic acid (TCA) at a ratio of 1:2 (w/v) and homogenised using blender (Waring Commercial Blender, USA) at speed 2. Samples were then centrifuged (Centrifuge 5430R Eppendorf AG, Hamburg, Germany) at 3000 rpm for 5 minutes and filtered through Whatman No.1 filter paper. 25 ml of samples were pipetted into the Kjeldahl distillation tube and 5 ml of 10% sodium hydroxide was added to the mixture. Steam distillation was performed using BUCHI Distillation Unit K-350, Switzerland. The distillate was titrated against 0.05 M sulphuric acid and until the colour turns pink.

Statistical analysis

All data were analysed using One-way Analysis of Variance (ANOVA). Significant differences among were determined using post hoc Turkey test at 0.05 level of probability. All statistical analysis were done using the IBM SPSS Statistics software version 20.

Results and discussion

Antioxidant properties and activity of P. guajava leaf extract

The total phenolic content, total flavonoid content (TFC), DPPH radical-scavenging and ferric-reducing antioxidant power (FRAP) values were recorded at 383.67±15.22 mg GAE g^{-1}, 51.02±15.02 mg QE g^{-1}, 77.41±4.28 µM TE g^{-1} and 2.56±0.44 µM TE g^{-1} respectively (Table 1). The total phenolic content in the current study is slightly lower compared to study of Qian and Nihorimbere (2004) recorded at 575.3±15.50 mg GAE g^{-1}. However, the current

study showed a higher amount of TFC compared to the study of Bedawey et al. (2010) recorded at 35.46±1.90 mg QE g^{-1}. The ring structure and substitution of flavonoids will influence the phenoxyl radical stability and the antioxidant properties (Wojdyło *et al.,* 2007). The principle of the FRAP method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous form in the presence of antioxidants. Previous studies by Tachakittirungrod *et al.* (2007) showed that FRAP content was 3.65±0.04 µM TE g^{-1} in *P. guajava* leaf extract.

Table 1: Antioxidant properties and activity of guava (Psidium guajava) leaves extract.

Antioxidant properties and activity	Guava leaves extract
Total phenolic content	383.67±15.22 mg GAE g^{-1}
Total flavonoid content	51.02±15.02 mg QE g^{-1}
DPPH	77.41±4.28 µM TE g^{-1}
FRAP	2.56±0.44 µM TE g^{-1}

All data shown as mean ± standard deviation.

Total bacterial count

M. rosenbergii coated with 1.0% *P. guajava* leaf extract showed a significantly ($p<0.05$) lower amount of total bacteria count among other treatments (Figure 1). Total bacteria count in samples coated with 1.0 % *P. guajava* leaf extract had a significantly ($p<0.05$) delayed bacterial accumulation within 15 storage days. At the 10th day of storage, the total bacteria count of non-soaked prawn were increased up to 7.43±0.03 $\log_{10}$ CFUg^{-1} and reached the limit of acceptability (7 $\log_{10}$ CFUg^{-1}). These indicates that *M. rosenbergii* were not in fresh condition and time spoilage had started.

According to Leitào and Rios (2000), the shelf-life of *M. rosenbergii* is approximately 10 days in 0°C storage. International Commission on Microbiological Specifications for Foods-ICMSF (ICMSF, 1986) stated that fresh seafood display total bacteria counts varying from 3.0 to 7.0 $\log_{10}$ CFUg^{-1} affecting the contamination levels with different conditions. However, *M. rosenbergii* soaked in 0.5% *P. guajava* leaf extract were detected to become spoiled in between day 10 and day 15 storage with total bacteria count of 8.10±0.02 log10 CFUg^{-1}. According to Jackson *et al.* (1997), the average values limit of 7.0 log10 CFUg^{-1} and above indicates organoleptic spoilage based on odour, colour and texture. Borch *et al.* (1996) reported that shelf-life are influenced by the number and types of bacteria at the beginning and during growth.

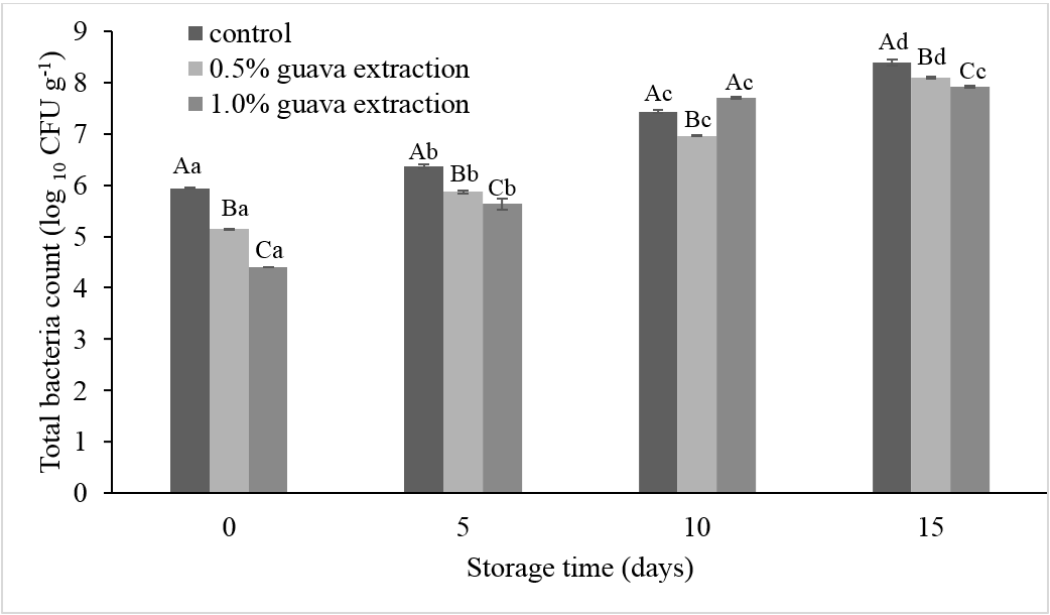

Figure 1: Total bacteria count of *M. rosenbergii* during storage at 4°C. All data shown as mean ± standard deviation.

Total volatile bases nitrogen (TVB-N) value

The initial value of TVB-N was 8.96±1.94 mgN 100g-^{1} and significantly ($p<0.05$) increased to 32.48±3.80 mgN 100g-^{1} after 15 days of storage for controls. However, TVB-N values was not significantly different ($p>0.05$) between day 5 and day 10. TVB-N values of *M. rosenbergii* soaked in 0.5% *P. guajava* leaf extract was initially 5.60±1.94 mgN 100g-^{1}, and steadily increased ($p<0.05$) to 23.52±3.36 mgN 100g-^{1} at 15th day storage. Meanwhile, *M. rosenbergii* treated with 1.0% guava leaf extract was 4.48±1.94 mgN 100g-^{1} at beginning of storage time and significantly ($p<0.05$) increased to 20.16±0.00 mgN 100g-^{1} on final day of storage (Table 3). Regardless to the storage day, TVBN value of *M. rosenbergii* treated at 0.5% *P. guajava* leaf extract was similar ($p>0.05$) to samples coated with 1.0% *P. guajava* leaf extract.

According to Begum *et al.* (2011) TVB-N value of *M. rosenbergii* without preservation (formalin) was up to 36.50 mgN 100g-^{1} after 10 days of storage. According to Leitão and Rios (2000), TVB-N content was recorded at 18.70 mgN 100g-^{1} in M. rosenbergii and increased up to 26.00 mgN 100g-^{1} after storing in ice for 10 days. On the other hand, result obtained for non-soaking *M. rosenbergii* was 32.48 ±3.88 mgN 100g-^{1} on day 15th storage and proved that more than acceptable level of TVB-N spoilage indicator. The acceptable level of TVB-N in fisheries product is below 30 mgN 100g-^{1} (Connell, 1975). In addition, Siddiqui et al. (2011) stated that the level of TVB-N is increased once spoilage has started including enzyme and microbiological processes. Therefore, TVB-N is suitable as indicator for index of spoilage.

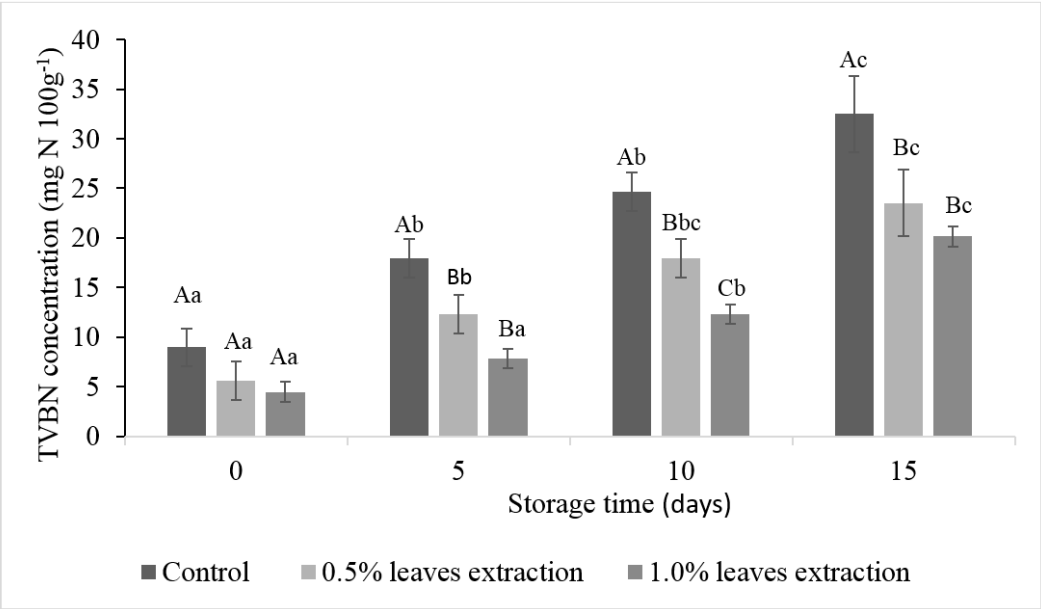

Figure 2: Total volatile bases nitrogen (TVB-N) value of freshwater prawn *(M. rosenbergii)* All data shown as mean ± standard deviation

Prediction of shelf-life

Microbial shelf life was taken as the time it takes to reach log10 7.0 CFUg-^{1}. TVB-N shelf life was taken as the time it takes to reach 30 mgN 100g-^{1}. The shelf life prediction for controls are at 7th day of storage and at 10th day of storage for M. rosenbergii soaked with 0.5% and 1.0% *P. guajava* leaf extract with regards to microbiology quality. With regards to TVB-N value, the shelf life of controls was predicted up to the 13th day of storage. Meanwhile, *M. rosenbergii* soaked with 0.5 and 1.0% of *P. guajava* leaf extract were predicted to have a shelf life of up to 20th and 25th day of storage, respectively (Table 4). *M. rosenbergii* were safe to eat before the 10th day of chilled storage at a soaking concentration of 0.5% P. guajava leaf extract.

Table 2: Prediction of shelf-life

	Microbial quality	TVB-N
Control	7th	13th
0.5% soaked with guava leaf extract	10th	20th
1.0 % soaked with guava leaf extract	10th	25th

Conclusion

P. guajava leaf extract contains antioxidant agent that is beneficial in keeping the quality of *M. rosenbergii* in chilled storage. *P. guajava* leaf extract at 1.0% concentration effectively delayed total bacteria growth and delayed the TVBN accumulation. Thus, it extends the shelf life of *M. rosenbergii* stored in chilled storage. TVB-N is a good indicator to determine the freshness for *M. rosenbergii*. The antioxidant and antimicrobial properties in *P. guajava* leaf extract was proved to preserve the freshness and prolong the shelf life of *M. rosenbergii* in chilled storage.

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