

## Capability of Amla Extract on Quality Retardation of Chilled Stored Indian White Prawn (*Fenneropenaeus Indicus* Milne Edwards, 1837)

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**ABSTRACT:** *Capability of amla extract was investigated on Indian white prawn (*Fenneropenaeus indicus*) stored at 4°C for 28 days. FTIR was done to obtain distinctive molecular components of analysed extract. Amla ethanolic extract was assigned to total phenolic content, total flavonoid content and PPO inhibition assay to evaluate its potentiality. Biochemical parameters, total viable count, melanosis formation and sensory evaluation were performed to investigate efficacy of amla and to determine shelf-life stability of chilled shrimp. Present study found amla extract to be potent to retard quality loss by lowering the increasing biochemical indices, microbial load and black spot formation as well as by maintaining sensory quality compare to untreated group ( $p < 0.05$ ).*

**KEYWORDS:** Indian white prawn, Amla extract, PPO inhibition, Quality, Black spot

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### INTRODUCTION

Shrimp is one of the most valuable and the most demanded product among all the seafood items because of its taste, aroma, and nutritional quality moreover its richness of polyunsaturated fatty acids (PUFA) such as omega-3 and omega-6 (Ackman RG, 1999). *Fenneropenaeus indicus* (Milne Edwards, 1837) is playing an important role in mariculture and coastal fisheries as well as in export market throughout the tropics and subtropics (MPEDA, 2015). However, shrimp is having a very short shelf life and main reasons behind this perishability are biochemical degradation followed by microbial deterioration. Although, melanosis is another most prominent problem of seafood industries as during chilled storage, black spots start forming on the cephalothorax and spreaded all over the body due to oxidisation of phenol to quinone by polyphenoloxidase (PPO) (Zamorano JP, et al. 2009) which makes the product unappealing to the customers. Due to the health concern issue, industries are now looking forward to the use of natural antioxidants as well as natural antimelanogenic agents because of their natural origin, hence regarded as safeguard and have good acceptance among consumers. However, *Phyllanthus emblica* Linn comes under the family Phyllanthaceae, otherwise known

as Amla. It is well known for its therapeutic efficacies in indigenous system of medicine as it comprises of a wide variety of phenolic compounds *viz.* phyllembelic, rutin, tannins, emblicol and curcuminoides and high amount of vitamin C loaded with different minerals (Kim et al. 2005). Different studies found that amla could lower the stress level by preventing lipid oxidation (Reddy, et al., 2010; Andican et al. 2005). Nevertheless, it is a great source of methyl gallate, mucic acid, aminoacids, phenolic glycosides, alkaloids, flavone glycosides, phyllanemblinins, phenolic acid, and carbohydrates (Zhang et al. 2000; Krishnaiah et al. 2009). So far any thorough study on amla fruit extract in inhibiting melanosis and quality retardation of chilled stored Indian white prawn has not been reported. Hence the main objective of this study was to investigate the ability of amla extracts on preventing quality loss and melanosis formation of Indian white prawn during chilled storage.

## **MATERIALS AND METHODS**

### **Chemicals and raw materials**

22 diphenyl 1 picryl hydroxyl hydrate (DPPH), Gallic acid, Ammonium molybdate, 2, 4, 6, tripyridyl 1, 3, 5 Follin ciocalteu reagent, Ascorbic acid, Nutrient agar were obtained from HiMedia and all other chemicals were of analytical grade reagents from Merck. Fresh amla fruit were purchased from local market of Kochi and Indian white prawn packed with ice in a ratio of 2:1 was brought from fish landing centre at Kalamukku, Kerala.

### **Preparation of Amla extract (AE)**

Amla extraction was done by the method of Ahmad et al. (1998) with slight modification. Dried amla fruit was crushed and grinded uniformly. 10g amla powder was then mixed with 50 ml of 95 % ethanol (aml powder: ethanol=1:5, w/v) and stirred continuously to obtain the maximum solvent dissolved constituents. Filtrate was then concentrated by using rotary evaporator for 20 min at 40°C under reduced pressure followed by two times re-extraction. Concentrated amla extract was further subjected to hot air oven for proper drying at 45°C for 12 h. It was then kept in the dark at 4°C.

### **Fourier Transform Infra Red Spectroscopy (FT-IR) analysis of amla**

Amla ethanolic extract was scanned in the range of 4000-400  $\text{cm}^{-1}$  with a resolution of  $4\text{cm}^{-1}$ . The FT-IR spectrum of sample was recorded in FT-IR spectrometer (Thermo Nicolet, Avatar 370) using DTGS detector and KBr beam splitter method. HATR assembly was used for convenience of the measurement. 2 mg of sample was used with the extraction solvent. The time required to finish the process was 20 s. Average of 3 scans were collected for analysis

### **Total phenolic content (TPC) estimation**

Estimation of total phenolic content present in the studied extracts were estimated spectrophotometric ally by using Folin-Ciocalteu reagent following the standard method reported by Macdonald et al. (2001) with slight modifications. Gallic acid was used in this study to obtain standard calibration curve. 1 ml of aliquots were added with 5ml Folin-Ciocalteu reagent and sodium carbonate (4 ml, 0.7M) followed by 30 min absorbance period. Absorbance values were read at 765 nm using spectrophotometer and the standard curve was

plotted. Phenolic content of both the extracts was expressed in mg/g GAE. The following formula was used to calculate the TPC,

$$T = CV/M$$

Where T = total phenolic contents (mg/g) in GAE; C = Gallic acid concentration obtain from mg/ml from standard curve; V = volume of extract taken (ml); M = weight of sample (g).

### Determination of Total flavonoid content (TFC)

Total flavonoid content was measured by the method of Patel et al. (2010) and Pallab et al. (2014) using aluminium chloride colorimetric assay. Extracts and standard quercetin solution of different concentration (100, 200, 400, 600, 800 µg/ml) was mixed at 1:1 ratio and 4ml of distilled water and 0.3 ml of 5 % sodium nitrite solution were added into each. 0.3 ml of 10 % aluminum chloride was added after an incubation of 5 minutes. At 6<sup>th</sup> minute, 2 ml of 1 M sodium hydroxide was added. Finally, volume was making up to 10 ml using distilled water and mixed properly. Orange yellowish color was developed. The absorbance was read at 510 nm spectrophotometer. Pure distilled water and quercetin were ran as blank and standard respectively. The samples were done in triplicates to minimize the error. The calibration curve was plotted using standard quercetin. The amount of total flavonoids of studied extracts was expressed as mgEq quercetin/ 100 g of dry mass.

### Inhibitory activity of Amla extract (AE) on PPO

100 µL of different concentrations of AE (100-500 µg/ mL) was mixed with 100 µL of crude PPO extract using the method of Simpson et al. (1987) and allowed to stand at room temperature for 30 min. After the incubation period, 400 µL of same phosphate buffer was added followed by 600 µL of 15mM L-DOPA pre incubated at 45°C. Reaction mixture was left for 3 min at 45°C and reading was taken at 475 nm. Control was used without sample. Relative activity was calculated based on the following formula:

$$\text{Relative activity (\%)} = \frac{\text{PPO activity in the presence of extracts} \times 100}{\text{PPO activity of control}}$$

### Shrimp sample preparation

Whole shrimp was divided into 2 slots *i.e.* amla treated slot (experimental) and control (without treatment). For amla treated slot, 50g/L amla ethanolic extract was used and soaked for 15 min. Shrimp was then drained or 3 min at 4°C. It was then packed separately in 12µ Polyester laminated with PE bags of 20×15cm dimension and 420 mm thickness and stored at 4°C. Samples were withdrawn every 3 days interval upto 28 days for biochemical, microbiological, sensory analysis and melanosis assessment.

### Evaluation of amla extract on the quality of chilled stored Indian white prawn

Determination of total volatile base Nitrogen content (TVB-N): TVB-N estimation was performed using Conway micro-diffusion method (Conway and Byrne, 1933).

Determination of Free fatty acid (FFA): Free fatty acid of samples was determined by the method of AOAC, 1975.

Determination of Peroxide value (PV): Peroxide value of samples was determined by the method of AOCS, 1989.

### **Microbiological analyses**

Aseptically 25g of minced shrimp sample was transferred to 225 mL of 0.85% physiological saline in a stomacher bag followed by 2 min uniform mixing in a stomacher blender. Further dilutions were obtained using same diluents. 0.1 mL of appropriate dilution was pipetted out and plated on pre poured nutrient agar plate and kept at 35°C for 48±2h for mesophilic bacterial counts following bacteriological analytical method (BAM, 2001).

### **Sensory evaluation**

Sensory evaluation was evaluated according to 9 point hedonic scale (Meilgaard MC, Carr, BT, Civille GV, 1999) where 9 represents the quality or like extremely and 1 represents the poorest quality or dislike extremely. Likewise, 7 point for moderate likeness, 5 for neutral likeness and 3 signified moderately disliking. Sensory quality was assessed by a 5 members expert panel chose from the department. All the treated sample lots and control slot were given specific code and each package contained ten numbers of shrimp.

### **Melanosis assessment**

Melanosis was assessed by melanosis scale obtained from Montero et al. (2001) where 0 represented absence of blackening; 2 represented blackening upto 20% of shrimp exoskeleton. Likewise, 4 score was assigned for moderate blackening or 20 to 40% of the shrimp body, 6 for 40 to 60% and 8 for severe (60-80% shrimp body) blackening and 10 was assigned for overall (80-100%) blackening. It was evaluated by the same panel assigned for sensory quality evaluation. All the treated sample lots and control slot were given specific code and each package contained ten numbers of shrimp.

### **Statistical analyses**

Each performed test was made in triplicate and performed in a completely randomised design (CRD). T-test was done using Graph pad prism 6 and SPSS package. *P* value less than 0.05 was considered as statistically significant.

## **RESULT AND DISCUSSION**

### **Fourier Transform Infra-Red Spectroscopy (FT-IR) analysis of green tea and amla**

In this present study, amla ethanolic extract was subjected to FT-IR analysis. **Fig 1** is showing the FT-IR spectral profile for amla. Main peaks range from 3278.53 cm<sup>-1</sup> to 766.71 cm<sup>-1</sup> for amla and are shown with extended marking. It was found that 3278.53, 1719.59, 1613.68, 1544.60, 1343.10, 1213.26, 1039.34, 918.96, 871.32, 812.71 and 766.71 cm<sup>-1</sup> main peaks for amla were clearly recorded in fingerprint region of FT-IR. In details, 3278.53 cm<sup>-1</sup> is due to O–H stretch, H–Bonded, which signifies the presence of Phenols, alcohols (Orcic et al. 2011).

From the spectral data of 1719.59, 1613.68, 1544.60  $\text{cm}^{-1}$  the possible interpretation is the presence of -CH symmetric /Asymmetric aliphatic bond, ketones / carbonyl groups, Phenyl ring and aromatic ring C-C stretching. Similarly, visual intensity estimates for the spectral band of 1343.10, 1213.26, 1039.34  $\text{cm}^{-1}$  is mainly due to C-O (stretching) – Ester, CO stretching and aromatic ring stretching. Possible reason of occurrence of spectral wavelength of 918.96, 871.32, 812.71 and 766.71  $\text{cm}^{-1}$  is because of HC=CH aromatic amides (Tatsuo et al. 1956).

### **Evaluation of Total phenolic content (TPC) and Total flavonoid content (TFC)**

The total phenolic and flavonoid content of amla ethanolic extract were found to be  $2.5151 \pm .036$  mg Eq GAE/g and  $348.1 \pm 2.669$  mgEq Quercetin/100g dry weight respectively. This finding is supporting the tatement of Shimoi et al. (1996) as their study revealed that polyphenolic compounds of amla fruit shows an important role in stabilizing lipid oxidation due to their potent antioxidant activity. Due to the important physiological functions of phenolic compounds, the total polyphenols content and flavonoid played an important role in preventing quality loss of shrimp due to lipid oxidation.

### **Effect of amla on PPO activity**

The effect of amla extract on inhibition of PPO from Indian white prawn showed an efficacy in dose dependent manner ( $p < 0.05$ ). It showed the highest ability as relative inhibition % at 500 uL followed by 100 uL and 300 uL ( $p < 0.05$ ). At 200 uL concentration, it showed less ability ( $p > 0.05$ ). Relative inhibition % of 100 uL to 400 uL was in a range of 20-60% whereas, 500 uL showed more than 60% relative inhibition ( $p < 0.05$ ). Nevertheless, present study found an encouraging increase for amla extract solution as ppo inhibition ( $p < 0.05$ ) which supports the previous finding of Janovitz-Klapp et al. (1990), where it was revealed that, interaction of enzyme with phenolic compound can prevent PPO activity effectively. Current result found that amla could be an alternative effective agent to control PPO effects on chilled stored shrimp.

### **Evaluation of effect of amla extract on the quality of chilled stored Indian white prawn**

#### **Total volatile base (TVB)**

The TVB value is used as an index of quality for deciding the state of freshness. A level of 35-40 mg TVB-N /100g of meat is usually regarded as the limit of acceptability, beyond which the fish can be regarded as spoiled (Lakshmanan Y. and Fung LC. 2000). TVB content of both the groups increased with storage time ( $p < 0.05$ ) is shown in **Fig 2**. At the beginning the initial TVB value was  $2.38 \pm 0.009$  mgN/100 g meat and it reached to  $34.39 \pm 0.04$  mgN/100 g meat in control slot whereas, treated group reached  $30.67 \pm 0.012$  mg N/100 g meat at 28<sup>th</sup> day. Control reached to an unacceptable condition whereas treated sample was showing better condition ( $p < 0.05$ ). Present study found a good correlation of TVB content with microbial load (**Fig.5**) as both the cases treated sample showed a lower value compare to untreated control group ( $p < 0.05$ ). With the increasing storage days all the samples showed an increasing trend but rate of increase varied with treatment significantly ( $p < 0.05$ ). Hence it could be a potential natural remedy to combat shrimp early spoilage problem.

#### **Free fatty acid (FFA)**

Lipid hydrolysis during storage period is one significant spoilage criteria which could be easily detectable by FFA assessment. Significant differences ( $P < 0.05$ ) in FFA concentrations were observed between the treatment group and control throughout storage period ( $p < 0.05$ ) (**Fig 3**). Present study found that lipid hydrolysis developed at a very slower rate in the samples treated with amla extract compare to control. As FFA increased from the initial value of  $0.017 \pm 0.001$  (expressed as percentage of oleic acid) to the final value of  $0.137 \pm 0.002$  for the control, and  $0.024 \pm 0.001$  to  $0.115 \pm 0.001$  for amla extract treated slot. Hence, amla treatment could be an effective way to prevent lipid hydrolysis in chilled stored shrimp product.

### **Peroxide value (PV)**

Hydroperoxides are odour- and flavour-less compound, so first it is difficult to interpret the sensory quality based on PV. Peroxides are unstable compounds, and they break down to aldehydes, ketones and alcohols that are volatile products causing off-flavour in products (Smith GM. *et al.* 1990). Peroxide values are the major chemical indices to measure the degree of oxidative rancidity at the later days of storage. In this study, the PV of oil extracted from Indian white prawn treated with amla extract and control both increased with storage days ( $p < 0.05$ ) (**Fig 4**) but there were a significant variation between control and amla treated samples ( $p < 0.05$ ). PV during study period increased from the initial value of  $0.81 \pm 0.004$  (expressed miliequivalents / kg of fat) to the final value of  $18.46 \pm 0.0004$  for the control. Whereas, amla treated sample reached to a significantly lower level of  $15.47 \pm 0.009$  ( $p < 0.05$ ). Amla treated slot revealed an encouraging result to control lipid oxidation.

### **Total viable count (TVC)**

During storage of shrimp in ice even under the best conditions where the temperature is maintained at  $0^{\circ}\text{C}$ , number of bacteria increased after a lag phase of 1 or 2 days, reaching maximum values of about  $10^7$  to  $10^8/\text{g}$  of muscle after 9 to 12 days (Gram LC. *et al.* 1990). Present study found a remarkable microbial load reduction in amla treated group compare to the control ( $p < 0.05$ ). Control group showed an increase of 8.32 log cfu/g from an initial microbial load of 4.47 log cfu/ml. Whereas treated group had an increase of 7.41 log cfu/ml from initial load of almost same as control group (**Fig 5**). However, this study found an interesting correlation between microbial load and TVB which were parallel increased in control and lowered in treated sample. In this study, changes in TVC during storage revealed the presence of a comparatively lower rate of microbial load in treated group. Sensory quality supports the microbial deterioration during the study as panellist found it unacceptable once it reached 6-7 log cfu/gm microbiologically.

### **Effect of amla extract on sensory quality of Indian white prawn at $4^{\circ}\text{C}$ refrigerated storage**

The changes in sensory quality of Indian white prawn treated with amla extract during 28 days of refrigerated storage study at  $4^{\circ}\text{C}$  is shown in **Fig 6**. At the first day, all samples had the score near about 9 in Hedonic scale and no differences in likeness (Appearance, Texture, Odour, Softness) were found between the samples ( $p > 0.05$ ). In general, the sensory scores of Indian white prawn showed a tendency to go down during ice storage. The rate of decrease of sensory scores was fastest in the control group for all the sampling days ( $p < 0.05$ ). The decrease of

sensory scores of studied species of AE treatment were significantly inhibited, compared with the control ( $p < 0.05$ ). Generally, end of shelf life is usually determined when essential sensory parameters such as off-odour and flavour becomes pungent or putrid caused mainly by microbial origin (Huss, 1974) and even appearance of shrimp becomes unaccepted from consumer point of view. Present study found treated group remained appealing till 20<sup>th</sup> day to the panellists as texture, odour, appearance were in accepted condition and score obtained 6-8 range for amla treated sample whereas control reached at 6-7 from 14<sup>th</sup> day onwards ( $p < 0.05$ ). Hence, present study found amla extract treated sample was more appealing to the panellists even after 14 days whereas, they found control as not up to the mark ( $p < 0.05$ ). It is suggested that amla can be used to treat chilled stored shrimp in order to retard the quality loss and retain its sensory quality during chilled storage.

### **Effect of amla on melanosis formation of Indian white prawn at 4°C refrigerated storage**

Melanosis score for Indian white prawn with amla extract during 28 days of refrigerated storage study at 4°C (**Fig 7**). On the 1<sup>st</sup> day all samples were found to have no melanosis. With the storage time, melanosis formation showed an increasing trend ( $p < 0.05$ ). Control slot attained the maximum score whereas, amla extract treated revealed significantly lower melanosis formation ( $p < 0.05$ ) during the storage period. Present study found that efficacy of amla extract to combat melanosis is encouraging ( $p < 0.05$ ). Amla extract at dose of 50gL<sup>-1</sup> found to be significant to reduce melanosis ( $p < 0.05$ ) compare to control. Similarly green tea too showed a promising result as an antimelanogenic agent. Study found that acceptable score for melanosis is 8 (Otwell WS. et al. 1992). Present study found control slot attained more than 8 score after 2 weeks of storage study Whereas, treated group was much slower to reach to that level ( $p < 0.05$ ).

### **CONCLUSION**

Present study showed Amla ethanolic extract is having a high level of phenolic and flavonoid present in it. Nevertheless the extract was found to have encouraging percentage of PPO inhibition ability. Present study found amla extract could delay the formation of melanosis in chilled stored (4°C) Indian white prawn during 28 days storage study. Even sensory quality was improved by its application. The increase biochemical and microbial indices were also significantly lowered in treated sample compared to control. Treatment with amla can be used as a PPO inhibitor too as it was found to inhibit PPO while assessed with L-DOPA substrate. Hence, it can be an alternative aid to combat melanosis and other quality loss in shrimp during post mortem handling and subsequent storage.

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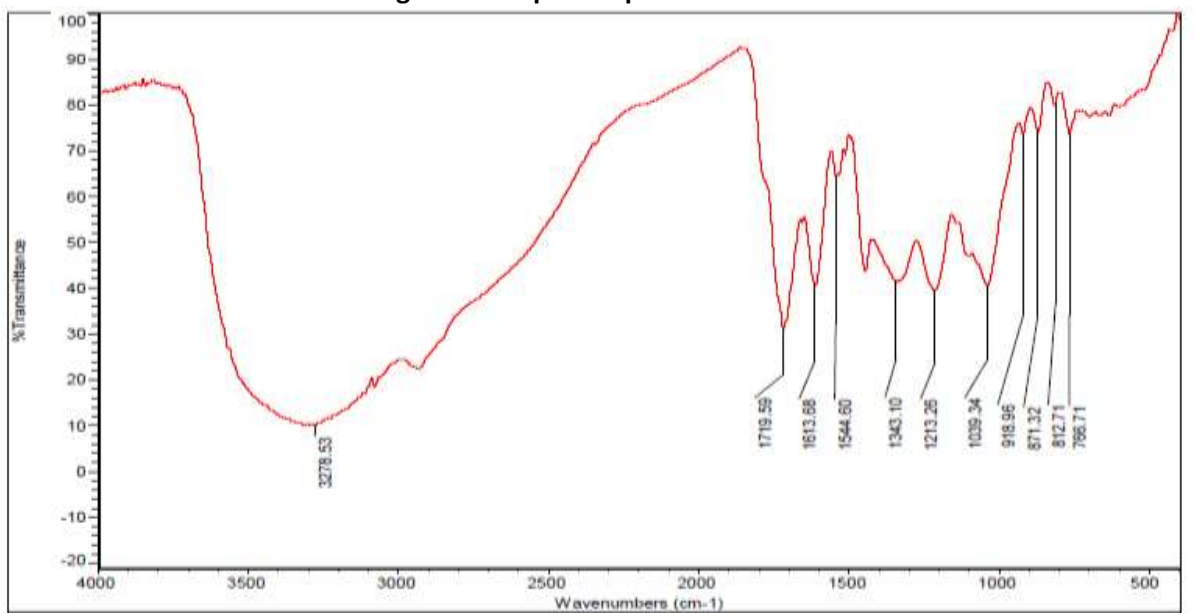
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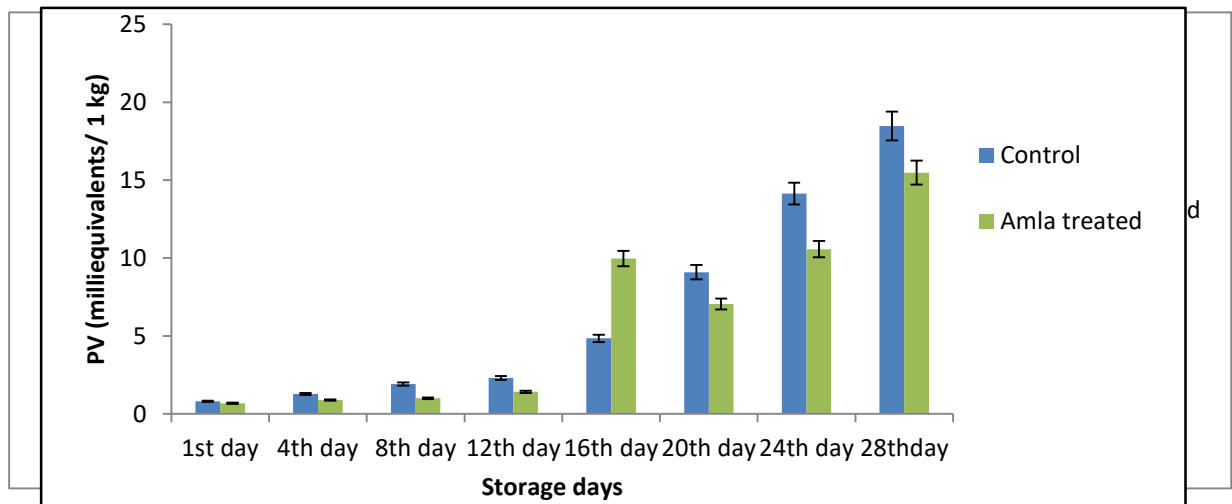
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## FIGURES

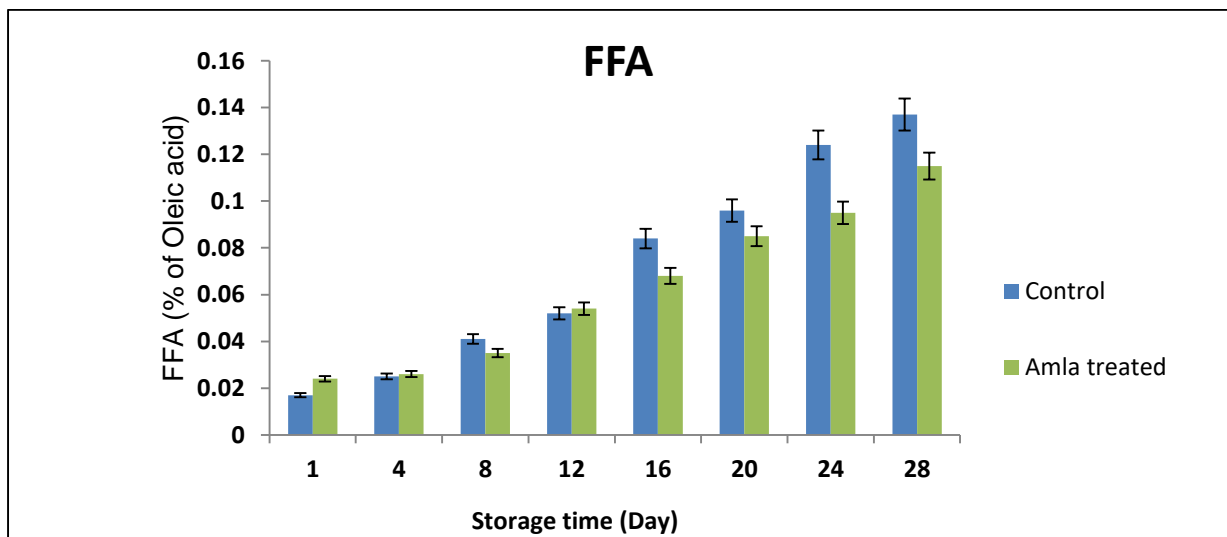
**Fig 1: FT-IR spectral profile for amla.**



**Fig 2: Total volatile base (TVB) content of Indian white prawn treated amla extract (AE) and control during 28 days of storage at 4°C. Bars represent the standard error (n=3).**

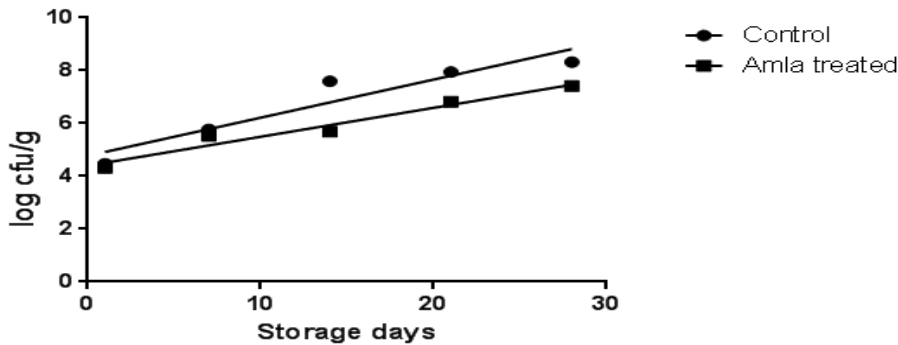


**Fig 3: Free fatty acid (FFA) content of Indian white prawn treated amla extract (AE) during 28 days of storage at 4°C. Bars represent the standard error (n=3).**

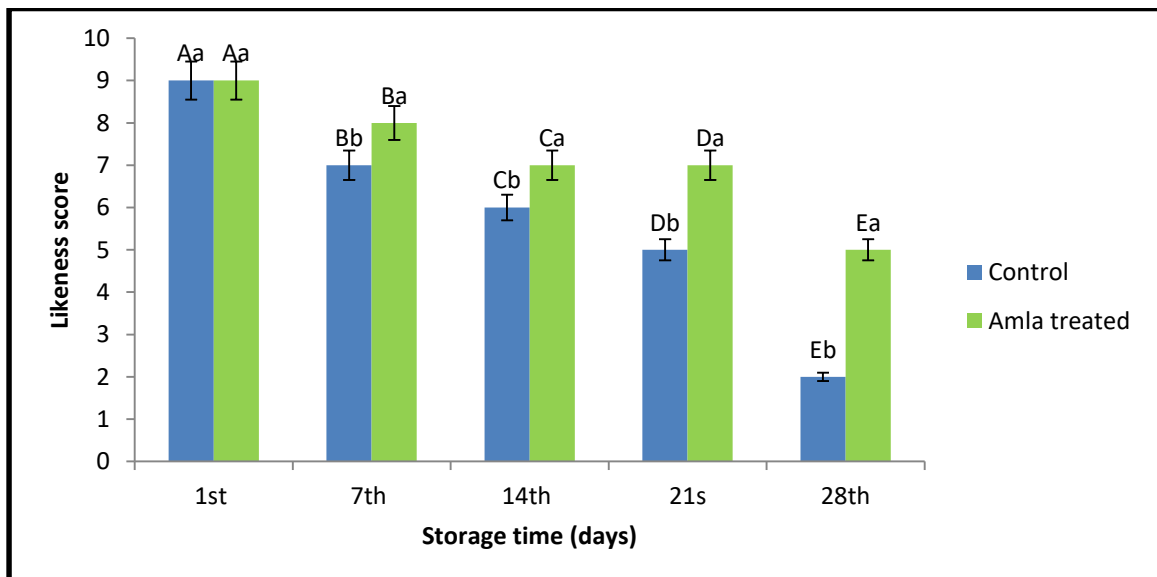


**Fig 4: Peroxide value (PV) content of Indian white prawn treated amla extract (AE) during 28 days of storage at 4°C. Bars represent the standard error (n=3).**

**Fig 5: Mesophilic bacteria count of Indian white prawn treated with amla extract (AE) and control during 28 days of storage at 4°C. Bars represent the standard error (n=3). Control showing increasing trend as compared to treated sample (p<0.05).**



**Fig 6: Sensory score of Indian white prawn control and amla extract treated sample during 28 days of refrigerated storage study at 4°C. Control showing quick unacceptability compared to treated sample (P < 0.05). Capital letters represents different days and in same day different letters depict significance variation between treatment and control.**



**Fig 7: Melanosis score of Indian white prawn with amla extract during 28 days of refrigerated storage study at 4°C Bars represent the standard error (n=3).**

