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The effect of Green tea on bacterial changes in white shrimp (*LithopenaeusVannamei*) refrigerated at a temperature of 4 ± 1 °C

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Abstract— Marine products form an important source of nutrients which are extensively used in human diets. Shrimp, a rich source of polyunsaturated acids and protein at a high percentage, is one the most important marine products. Green tea on the other hand contains a large quantity of antibacterial and antioxidant compounds which have been used to reduce putrescence in foodstuff. This research investigated the effect of various doses of green tea extract (200, 400, and 600 ppm) on the total bacterial load, psychrotopic and lactic acid bacteria and compared the results from the various doses because of the importance of the nutritional and economic value of shrimp. The results indicated that green tea extracts can increase shelflife between 2 to 5 days depending on the treatment type and extend the products use by date. In general the results indicated that different doses of green tea cause the increase in bacterial parameters in shrimp during refrigeration and the 600ppm is recommended as the best dose.

Keywords— bacterial load, green tea (Camellia Sinensis) extracts, quality, shrimp.

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I. Introduction

MARINE products play a significant role in food supply of the people of the world. The increase in demand for seafood as a valuable source of nutrients in recent years has resulted in the growth and development of the fishing industry, extraction of marine products, and remarkable progress in the fishing industry of most states containing coastlines near seas, gulfs, and open waters. Shrimp farming can be considered one of the most important industries in this area (Ojifard et al, 2000). Shrimp is one of the products enjoying a high consumption rates in the southern beaches of Iran especially Boushehr. These products are susceptible to putrescence because of their rich source of polyunsaturated acids and high protein content and preservation in unsuitable conditions can thereforecause the meat to putrefy and loose its quality. Undesirable changes in texture, colour, and aroma are some of the most important adverse changes that can occur (Nirmal and Benjakul, 2001). Cinnamon extract coating (Ojagh et al, 2012); rosemary extract (Etemadi et al, 2008), and thyme (Hamzeh&Rezaei, 2011) are some of the many measures taken to delay bacterial putrescence in the meat of the aquatic animals.

Green tea, whose antioxidant qualities are well-known, is one of the most recognised plant extract. For example the use of Tea polyphenols as an antioxidant in packaged mackerel under the Cololabissairavaccum was investigated and its effect on delaying the chemical and oxidization changes resulting in the extension of the shelf-life of the fish confirmed (Rezaei et al, 2011). Green tea leaves contain 36% of the polyphenols according to dry weight. Catechinsare the dominant group among polyphenols in tea and have been identified as the effective antioxidants countering oxygen radicals and bonding metallic ions (Ojagh et al, 2005)., This research aims to investigate the effect of the various doses of green tea extract on the magnitude of the bacterial parameters during refrigeration because of the importance of the nutritional and economic value of the shrimp and the benefits of green tea.

II. Materials and Methods

Sample Preparation

The samples were washed in cold water and grouped following purchase and transportation to the laboratory. They were then treated with 200, 400, 600 ppm extracts and were refrigerated together with a placebo sample for 15 days, and tested on 0, 5, 10, and 15 day periods.

ISSN (Online): 2305-0225
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Bacterial Analysis
The Ringer's solution was prepared in order to perform bacterial analysis and used as a diluent (National standard no. 8923-2). The culture media were prepared per instructions and samples were readied for bacterial analysis.

Preparation
10g of the sample was placed in special stomacher bags under sterile conditions and then 90ml of Ringer's solution was added. The bags are then placed in the stomacher machine and homogenization was performed for 10 minutes. The final solution had a -1 dilution level.

Table2: Culture Media Used in bacterial Analysis

Type of Bacterial Analysis	Culture Medium Used	Method of Analysis
Total Viable Count	Plate Count Agar	National standard No. 5272
Psychrotropic Bacterial	Plate Count Agar	National standard No. 5273
Lactic Acid Bacterial	MRS Agar	(Sallam, 2007)

Total Viable Count
1 ml of the desired diluted solution is poured in the marked sterile plate. 15ml of the solution in the Kant agar plate cooled to 45°C was then added. The plates were rotated in a figure of 8 formationin order to achieve uniform distribution of the micro – organisms and then laid horizontally on a cold surface and left to set. The plates were finally placed inverted in an incubator at a temperature of 30±1°C for 48 to 72 hours and a colony – counting device was subsequently used to conduct the enumeration.

Psychrotrophic count
1 ml of the desired diluted solution is poured in the marked sterile plate. 15ml of the solution in the Kant agar plate cooled to 45°C was then added. In order to achieve uniform distribution of the micro – organisms the plates were rotated in a figure of 8 formation and then laid horizontally on a cold surface and left to set. The plates were finally placed inverted in an incubator at a temperature of 6±1°C for 10 days and a colony – counting device was subsequently used to conduct the enumeration

Lactic acid bacterial count
1 ml of the desired diluted solution is poured in the marked sterile plate. 15ml of the solution in the Kant agar plate cooled to 45°C was then added. In order to achieve uniform distribution of the micro – organisms the plates were rotated in a figure of 8 formation and then laid horizontally on a cold surface and left to set. The set plates were subsequently placed in a vacuum jar and finally placed inverted in an incubator at a temperature of 37°C for 72 hours and a colony – counting device was subsequently used to conduct the enumeration

Statistical Analysis
Two-way analysis of variance in the form of a random factorial statistical model was used to analyse the data obtained and replicated three times. SPSS 18 was employed in order to investigate the existence or otherwise of any significant difference between treatments at a 5% confidence level (Zar, 1999).

III. Reslts and Discussion

Total Viable Count
The results of the measurement of the Total Viable Count during the refrigeration period are given in figure1. The results indicate rising trend in all samples. The *Total Viable Count* on the first day of refrigeration was 3.8 logarithms which experienced a significant rise in the placebo sample to 9.9 logarithms on day 15 ($P<0.05$). Comparison of the treatments indicates that no treatment experienced any significant change when compared to the placebo sample on day one. However these changes became significant on days 5, 10, and 15 such that the total bacterial load on day 15 on samples 200, 400, and 600ppm was 8.98, 8.5, and 8.1 respectively.

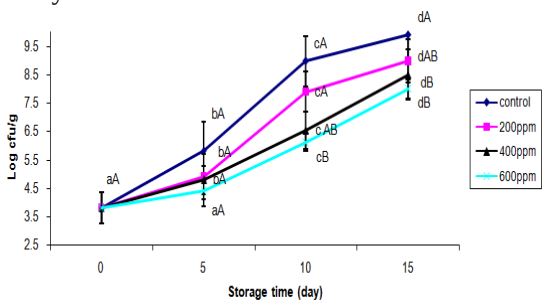

Fig1: Mean (± standard deviation) of the total viable count (colony logarithms per gram of meat) in shrimp for various doses of green tea extract in proportion to time (n=3) : Small and Capital letters signify significant differences ($P<0.05$) between preservation time and the concentration used)

Microrganisms are the most important cause of putrescent in food. The Total viable count as can be seen in figure 1 was less than 4 colony logarithm per gram which is indicative of

ISSN (Online): 2305-0225
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the high quality of the samples used (Hamzeh et al 2001). The rising trend in the total bacterial load was consistent with the placebo samples in other researches (Ojagh et al, 2004); Zolfaghari et al, 2011). Although the initial bacterial load in aquatic animals is dependent on factors such as the condition of the water, and the habitat temperature, the raw product can contain a high bacterial load which is dependent upon preservation and handling conditions (Hamzeh et al, 2011). The effect of the green tea extracts on the samples in this research was similar to that of the lactic acid bacteria in shellfish preserved in ice (Mohammadzadeh, 2013). The allowable limit for the total bacterial load that can grow is 10^6 colony logarithms per gram. According to this standard the placebo samples were fit for consumption for about 6 days(Zolfaghari et al, 2011).

Psychrotrophic count

The results of the measurement of the psychrotropic bacterial count indicated a rising trend in all samples. Statistical analysis indicated the existence of a significant rise between the initial total count (day 1) and the total count on the final refrigeration day (day 15) in all samples ($P<0.05$). The total bacterial count on day one was 2.2 logarithms which experienced a significant rise and reached 8.9 on day 15 ($P<0.05$). The comparison of the treatments was indicative of the fact that no significant change was observed in any of the extracts when compared with the placebo sampleson day one, however these changes became significant on days 5,10 and 15.

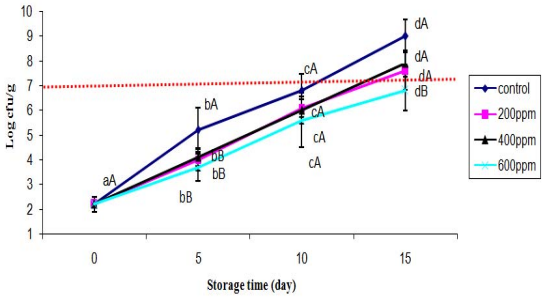

Fig2: Mean (± standard deviation) of the total psychrotropic bacterial count (colony logarithms per gram of meat) in shrimp for various doses of green tea extract in proportion to time (n=3).

Gram negative psychrotropic bacteria are among the dominant and principal bacterial groups in the meat of the aquatic animals and aid the widespread putrefaction of meat preserved in ambient and cold conditions. Lipase and phospholipase enzymes,which increases the free saturated acid are produced by these bacteria and especially by the pseudomonas species (Hamzeh et al, 2011; Etemadi et al, 2008). The rising trend in the psychrotropic bacteria was consistent with the placebo samples in other reseraches (Arashisar et al, 2004; rezaei et al, 2007). The highest limit for the number of psychrotropic bacteria in the aquatic animals is reported at 7 colony logarithms per gram (Arashisar et al, 2004). The effects of the green tea extracts on the samples under study were similar to that of the psychrotropic bacteria in the shrimp preserved in ice (Nirmal et al, 2011). According to this standard the preserved fillets were fit for human consumption until around day 10. The psychrotropic bacteria have a higher growth rate than other micro – organisms due to the favourable temperatures. Although the initial amount and its growth rate is highly dependent on the initial bacterial load and the preservation conditions (Pezashk et al, 2012).

Lactic Acid Bacteria

The results of the lactic acid bacterial count indicated a rising trend in all samples (Fig3). The total lactic acid bacterial count on the first day of refrigeration was 1.7 colony logarithms per gram which experienced a significant rise in the placebo sample until day 15 reaching 7 logarithms ($P<0.05$). Comparison of the treatments indicates that no treatment experienced any significant change when compared to the placebo sample on days1 and 5. However these changes became significant on days 10, and 15.

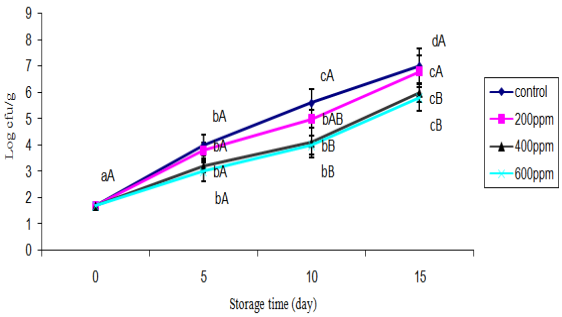

Fig3: Mean (± standard deviation) of the total lactic acid bacterial count (colony logarithms per gram of meat) in shrimp for various doses of green tea extract in proportion to time (n=3).

The lactic acid and psychrotropic bacteria count in meat indicate a better relationship with freshness in comparison with the total bacterial count (Ojagh et al, 2012). Lactic acid bacteria are a group of arbitrary anaerobic bacteria which are very effective in putrescence. This group of bacteria are multi – substance gram – positive bacteria and are capable of

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growing in relatively low pH (Khezri Ahmadabad et al, 2012; Etemadi et al, 2008).

According to figure3 the lactic acid bacterial count experienced a significant rise during the refrigeration period which was consistent with the rising trend in the lactic acid bacterial count in other research (Etemadi et al, 2008; Asghari et al, 2011). The effect of the green tea extract in the samples in this research was similar to the results obtained from lactic acid bacteria in shrimp preserved in ice (Nirmal et al, 2011)

IV. CONCLUSION

Putrescence in aquatic animals can be defined as an unfavourable change in qualitative properties which can be used as an indicator of the safety and nutritional value of the same. Bacterial putrescence is the most important example of putrescence in aquatic meat. The use of antibacterial and antioxidant bacterial activity is both beneficial and necessary in order to improve the quality and increase the shelf-life of the meat and the prevention of economic losses. This research investigated the effect of various doses of the green tea extracts (200, 400, and 600ppm) on bacterial parameters and compared the results. Bacterial analysis indicated that shelf – life can be extended by 2 to 5 days depending on the type of treatment. The results generally indicated that different doses of green tea extract result in the increase in bacterial parameters in shrimp during refrigeration. The 600ppm is recommended as the best dose.

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