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### Effect of pomegranate and dates molasses as anti *Vibrio Parahaemolyticus* on marinated shrimp

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#### ABSTRACT

Nowadays, natural antioxidant and antimicrobial compounds, especially of plant origin, have received increasing attention as food additives. Pomegranate (*Punicagranatum*) and dates molasses recently described as nature's power fruit are widely cultivated in the Mediterranean region. Pomegranate peel extract with an abundance of flavonoids and tannins has been shown to have a high antioxidant and antimicrobial activity. Marinades of pomegranate and dates molasses (1% and 2%) were evaluated for their antibacterial effects in peeled shrimp samples artificially inoculated with *V. parahemolyticus*. The samples were divided into eight equal groups (200 g each), and were inoculated by *V. parahemolyticus*  $10^7$  cfu/ml, separately. Initial count of *V. parahemolyticus* in peeled shrimp samples at zero hour was  $6.53 \pm 0.3$  log cfu/g. The results showed that the sensory analysis of all treated peeled shrimp and control one were acceptable for all judgment members either fresh or during storage. Pomegranate molasses 2% either with marinated solution or with distilled water recorded the highest reduction % of *V. parahemolyticus* count by 67.84 % and 83.15 at six days of refrigerating storage, respectively. While date molasses 2% with marinate showed moderate reduction percentage reach to 31.1 % but date molasses 2% with distilled water raise to 57.1% at fifth day of refrigerated storage. Results support the high efficacy of pomegranate molasses to control *V. parahemolyticus* growth, improved the sensory score and increase the safety of the peeled shrimp samples.

#### INTRODUCTION

Seafood including various species of fish, crustaceans, mollusks, and echinoderms, are excellent sources of protein, fat, vitamins, and minerals and are popular due to their delicacy with high nutritive value. However, the shelf-life of seafood is limited because of the high

contents of various nutrients, neutral pH, and high moisture content (Viji et al. 2017). Shrimp is one of the most commonly consumed types of seafood. It is a very nutritious healthy food. Shrimp is low in calories and rich in protein and healthy fats. It also contains a treasure of vitamins and minerals. On the negative side, it may be affected by many bacterial

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diseases which affect its health. Furthermore, it may be incriminated as a vector of foodborne illnesses that range from mild gastrointestinal upset to life-threatening diseases (**Fadel and El-Lamie 2019**).

*V. parahaemolyticus* is a globally disseminated, Gram-negative marine bacterium and one of the leading causes of seafood-borne gastroenteritis. Also, this bacterium has recently emerged as a shrimp pathogen and become a serious threat to the shrimp industry. *V. parahaemolyticus* encodes multiple virulence factors to enable efficient colonization of the host and disease, including adhesions, toxins, and types III and VI secretion systems (T3SS and T4SS) (**Santos et al. 2015**).

*V. parahaemolyticus* is a ubiquitous marine bacterium and also a human pathogen. This organism is frequently isolated from a variety of raw sea foods. Consumption of raw or undercooked seafood contaminated with *V. parahaemolyticus* is responsible for the development of acute gastroenteritis. Several virulence factors have been identified in this pathogen, of which the thermos table direct hemolysin (TDH), TDH-related hemolysin, and type III secretion system are considered very important (**Ramamurthy and Nair 2014**).

Shellfish of different origins are processed on the same process lines; this introduces the risk of cross contamination with microorganisms from one type of shellfish to another. The potential occurrence of spoilage and pathogenic bacteria in shellfish makes it important to evaluate if existing protocols for marinating are sufficient to inactivate or prevent growth of the microorganisms of concern (**Norhana et al. 2010**).

Recently, new trends in food processing to use the natural bioactive substances as antioxidants and antimicrobials to improve food hygienic quality and to safeguard human health. Plants are the cheapest and safer alternative sources of antimicrobials which may be added to food (**Lahucky et al. 2010**). There are various natural antioxidants, antimicrobials, sweeteners and coloring agents that are derived from

animals, plants and microorganisms. Bacteriocins, natamycin, reuterin from microbialsources; lysozyme, lactoperoxidase, lactoferrin from animal sources; polyphenols and essential oils from plant sources can be named as examples of natural preservatives (**Carcho et al. 2015**). Pomegranate molasses (PM) is a highly nutritive product since it is processed as concentrate especially the presence of high mineral contents of the fruit are in higher concentration of the molasses, makes it an exceptional nutritional property and it was found that to have a good antioxidant activity an important aspect important for human health (**Bigé et al. 2010**). Pomegranate molasses is a polyphenol-rich fruit juice with high antioxidant capacity and several studies suggested that pomegranate juice can exert anti-atherogenic, antioxidant, antihypertensive, and anti-inflammatory effects (**Sahebkar et al. 2017**).

Epidemiological studies have suggested that consumption of red fruit juices, such as grape, berry juices and pomegranate, correlates with reduced risk of coronary heart disease stroke, certain types of cancers and aging (**Malik and Mukhtar 2006**).

The date fruits and syrup were rich in phenolic compounds, so they are considered as antioxidants, anti-carcinogenic, anti-microbial, anti-mutagenic, anti-inflammatory agents, and they reduce the risk of cardiovascular disease. (**Baliga et al. 2011**). The antioxidant potential contribute to the bacteriostatic and bactericidal activity of date syrup pro-oxidants are known to cause physiochemical and structural changes to microorganisms that results in growth retardation (**Halliwell 2008**). Polyphenols of date have antioxidant activity (radical scavenging, and metal chelating activity) or pro-oxidant activity depending on environmental conditions, interaction, structural changes and exposure to microorganisms (**Yordi et al. 2012**). Marinades are solutions, including sugar, spices, oil, acids (from vinegar, fruit juice, wine) and they are used to improve tenderness, juiciness, flavor and aroma and to extend shelf life of meat, poultry, seafood (**Duman et al. 2012**).

The risk of *Vibrio* species infection will be more when sea foods are consumed insufficiently cooked and also when they are post-heat contaminated. The high frequency of *Vibrio* spp. in the samples may be due to the use of contaminated ice to cover the fish on the display bench or from long holding time on the display rack at the retail level without proper temperature control and mis-handling by fish sellers (Noordis et al. 2011). *V. parahaemolyticus* a Gram-negative non-spore-forming halophile that is curved or a straight rod. *Vibrio* is motile by a single flagellum and is aerobic or facultative anaerobic. This bacterium is a human pathogen that occurs naturally in marine and estuarine habitats (Ducan and Su 2005).

*V. parahaemolyticus* is a food borne pathogen which causes mild gastroenteritis in humans after consumption of raw or insufficiently treated seafood (Mikus et al. 2010). *Vibrio* species can be pathogenic to human and represent a possible health threat as a result of eating raw or undercooked seafood (Eyisi et al. 2013). In recent years, researchers have put much effort into searching natural preservatives that could inhibit the growth of bacteria and fungi in food and avoid negative health effects of chemical preservatives, which has prompted the food industry to find natural products used and developed as alternatives (Hassoun and Çoban 2017).

Therefore the objective of present study was to evaluate the antibacterial activity of pomegranate and dates molasses against *V. parahaemolyticus*.

## MATERIAL AND METHODS:-

### 1. Preparation of bacterial strain.

*V. parahaemolyticus* (NCTC 10885) pure strain was obtained from Reference Laboratory for Food Safety, Animal Health Research Institute (AHRI), Dokki, Egypt. The strain was maintained on tryptic soy agar slants containing 3% NaCl at 4°C. Directly, prior to the experiment, fresh microbial cultures were adjusted to 0.5 McFarland to be equivalent to about 10<sup>7</sup> cfu/ml (Shirazinejad and Ismail 2010) with tube dilution methods and considered as infective dose to be inoculated into peeled shrimp samples.

### 2. Preparation of shrimp Samples:

A grand total of 1600 g of peeled shrimp was purchased directly from local markets in Tanta, Gharbia governorate, Egypt. The samples were taken and transferred directly to the laboratory in an ice box under complete aseptic conditions without delay. The samples were divided into eight equal groups (200 g of each) and placed in aseptic polypropylene trays designed for disposable food packaging.

### 3. Preparation of Marinades:

Marinade solution consists of mixture of 5.9 g garlic powder, 9.23 g table salt, 0.5 g turmeric, 0.15 g hot chili powder, 0.5 g black pepper and 300 ml sterilized distilled water were mixed well according to Unalan et al. (2011).

### 4. Challenge trials:

All groups (200g of each) except (Control –ve) were inoculated with *V. parahaemolyticus* 10<sup>7</sup> cfu/ml (Shirazinejad and Ismail 2010) by pipetting the inoculums drop by drop as evenly as possible across the peeled shrimp samples and mixed well with a sterile glass rod for distribution of the inoculums and gentle rocking the samples immediately after inoculation. The inoculated shrimp samples were left for 30 min at room temperature to allow attachment and absorption of the inoculated bacteria (Dubal et al. 2004). Thus *V. Parahaemolyticus* in the inoculated sample was enumerated to get the initial load before addition of marinades (Terzi and Gucukoglu 2010).

The samples were divided into eight groups. These groups are arranged as follows:

**Control –Ve:** Untreated peeled shrimp samples were immersed in 300ml distilled water only.

**Control +Ve:** the samples were inoculated with 10<sup>7</sup> cfu/ml *V. parahaemolyticus* and immersed in 300ml marinated solution.

**Group I (PM1%):** the samples were inoculated with 10<sup>7</sup> cfu/ml *V. parahaemolyticus* and immersed in 300ml of marinade at a ratio of 1.5:1 (marinade: shrimp, ml/g), then adding pomegranate molasses (1% v/w).

**Group II (PM 2%):** as group I but adding pomegranate molasses (2% v/w).

**Group III (DM1%):** as group I

but adding date molasses (1% v/w).

**Group IV (DM2%):** as group I but adding dates molasses (2% v/w)

**Group V (P3):** infected samples immersed in 300ml sterilized distal water and pomegranate molasses by 2% without marinade solution.

**Group VI (D3):** as group V but with date's molasses 2% without marinade solution.

Marinades solution or sterilized distal water were covered all surface of the shrimp samples. The shrimp samples were gently agitated or pressed with a sterile spatula to ensure immersion in the marinade. All the trays were properly labeled, stored at 4°C.

### 5. Enumeration of *V.parahaemolyticus*.

*V.parahaemolyticus* were enumerated on its selective media Thiosulfate citrate bile salt sucrose agar (TCBS, Merck, Darmstadt, Germany) the TCBS plates were incubated at 35°C for 24 hrs. *V. parahaemolyticus* colonies are round (2-3 mm diameter) and bluish green on TCBS, until spoilage of the examined samples occurred. The above experiment was performed in triplicate (three times).

### 6. Sensory evaluation.

Sensory evaluation was carried out by a nine-member well trained panelist. Panel members were from Food Hygiene Department, Animal Health Research Institute, Tanta Branch. Panelists were asked to evaluate each group for color, texture, odor and overall acceptability on a 5-point hedonic scale according to **Pelin-Can and Arslan, (2011)**.

### 7. In vitro rapid antimicrobial evaluation.

In vitro study have demonstrated to evaluation the antibacterial activity of **pomegranate** and dates molasses against *V. parahaemolyticus* on Thiosulfate citrate bile salt sucrose agar (TCBS) and its reduction by well diffu-

sion method according to Clinical Laboratory Standards (**CLSI 2001**). Where 0.1ml pomegranate and dates molasses (1% and 2%) with marinade, 0.1 ml control +V and 0.1ml pomegranate and dates molasses 2% in sterilized distilled water were inoculated in well of (TCBS) media which was inoculated by *V. parahaemolyticus* spreading method.

### 8. Statistical analysis.

The data were analyzed and the mean and Standard Error (SE) were calculated by one-way ANOVA test according to Statistical Analysis System Users Guide SAS (2004) software and probability of ( $p < 0.05$ ).

**RESULTS:-**

Table (1) Effects of various concentrations of pomegranate and dates molasses on counts of *V.parahaemolyticus* (log CFU/g) in artificially inoculated peeled shrimp samples.

Duration / Groups	Zero day	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>rd</sup> day	5 <sup>rd</sup> day	6 <sup>rd</sup> day
Control -Ve	-	-	-	-	-	-	-
Control +Ve	6.1±0.3 <sup>a</sup>	5.3±0.24 <sup>a</sup>	5.45±0.3 <sup>a</sup>	-	-	-	-
Group I	5.13±0.2 <sup>b</sup>	4.7±0.12 <sup>ab</sup>	3.8±0.12 <sup>abc</sup>	3.19±0.36 <sup>cd</sup>	2.5±1.2 <sup>c</sup>	2.89±.06 <sup>a</sup>	-
Group II	5.1±0.1 <sup>b</sup>	3.9±0.05 <sup>c</sup>	3.3±0.17	2.1±1.05 <sup>d</sup>	1.72±0.15 <sup>d</sup>	1.69±0.15 <sup>c</sup>	2.1±0.1 <sup>a</sup>
Group III	5.5±0.3 <sup>ab</sup>	4.9±0.34 <sup>a</sup>	4.56±0.38 <sup>c</sup>	4.7±0.17 <sup>a</sup>	-	-	-
Group IV	5.2±0.2 <sup>ab</sup>	4.7±0.88 <sup>ab</sup>	4.3±0.41 <sup>abc</sup>	4.3±0.22 <sup>ab</sup>	4.5±0.23 <sup>a</sup>	-	-
Group V	5±0.1 <sup>b</sup>	3.8±0.38 <sup>c</sup>	3.8±0.25 <sup>b</sup>	3.2±0.23 <sup>cd</sup>	2.43±0.29 <sup>c</sup>	1.58±.06 <sup>c</sup>	1.1±.02 <sup>b</sup>
Group VI	5.2±0.2 <sup>ab</sup>	4.16±0.14 <sup>bc</sup>	4.1±0.36 <sup>abc</sup>	3.6±0.15 <sup>c</sup>	2.79±2.16 <sup>cb</sup>	2.08±.24 <sup>b</sup>	-

G -Ve – free from *V.parahaemolyticus*.

Initial load of *V.parahaemolyticus* at zero hr = 6.53 ±0.3 log cfu/g (without marinade solution).

Control +Ve at zero day reading represent *V.parahaemolyticus* count with marinade solution after one hour

The values represent Mean ± SE of three experiments.

Means within each column followed by different letters are significantly different ( $P < 0.05$ ).

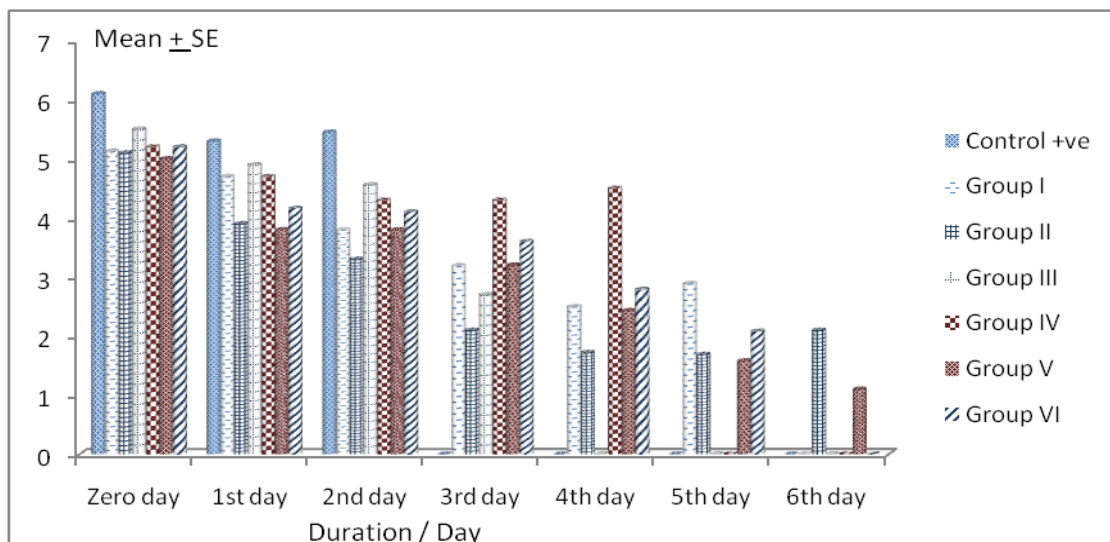
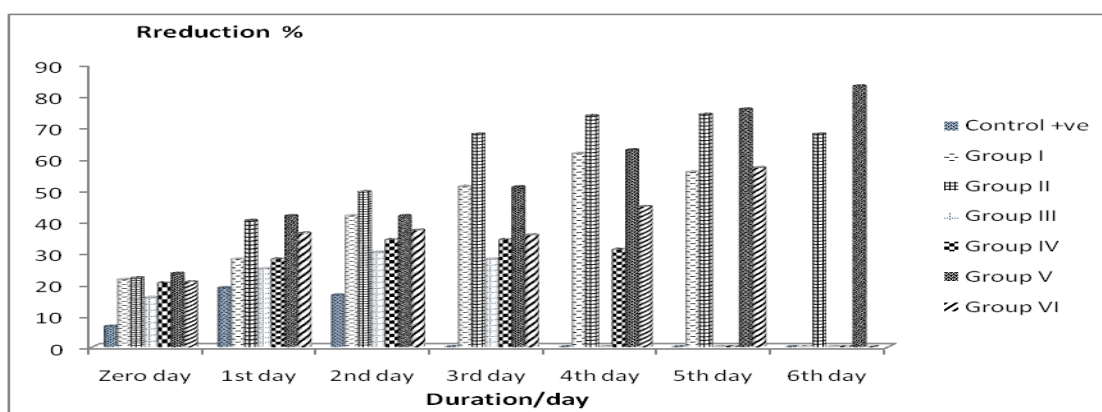


Fig (1) Effects of various concentrations of pomegranate and dates molasses on counts of *V.parahaemolyticus* (log CFU/g) in artificially inoculated peeled shrimp samples during refrigerated storage.

Table (2) Reduction percent of *V. parahaemolyticus* artificially inoculated into peeled shrimp samples treated with different marinades of pomegranate and dates molasses.

Groups/ Day	Zero day	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>rd</sup> day	5 <sup>rd</sup> day	6 <sup>rd</sup> day
	%	%	%	%	%	%	%
Group I	21.44	28.02	41.81	51.15	61.7	55.74	-
Group II	21.99	40.28	49.46	67.84	73.66	74.12	67.84
Group III	15.77	24.96	30.17	28.02	-	-	-
Group IV	20.37	28.02	34.15	34.15	31.1	-	-
Group V	23.43	41.8	41.81	51	62.79	75.80	83.15
Group VI	20.73	36.29	37.21	35.68	44.7	57.1	-

Fig (2) Reduction percent in *V. parahaemolyticus* artificially inoculated into peeled shrimp samples treated with different marinades of pomegranate and dates molasses.Table (3) Changes in sensory scores of marinated peeled shrimp samples artificially inoculated with *V. parahaemolyticus* during storage at 4°C.

Sensory Groups	Color			Texture			Odor		
	0day	3 <sup>rd</sup> day	5 <sup>rd</sup> day	0day	3 <sup>rd</sup> day	5 <sup>rd</sup> day	0 day	3 <sup>rd</sup> day	5 <sup>rd</sup> day
Control - Ve	5 <sup>a</sup>	-	-	5	-	-	4.7±0.17 <sup>b</sup>	-	-
Control +Ve	4.5±0.29 <sup>a</sup>	-	-	5 <sup>a</sup>	-	-	5 <sup>a</sup>	-	-
Group I	4.7±0.17 <sup>a</sup>	3.5±0.29 <sup>bc</sup>	2.5±0.29 <sup>bc</sup>	5 <sup>a</sup>	3.8±0.1 <sup>b</sup>	3 <sup>ab</sup>	5 <sup>a</sup>	4 <sup>ab</sup>	2.8±0.16 <sup>bc</sup>
Group II	4.8±0.17 <sup>a</sup>	3.8±0.17 <sup>ab</sup>	3 <sup>ab</sup>	5 <sup>a</sup>	4 <sup>ab</sup>	3.5±0.29 <sup>a</sup>	5 <sup>a</sup>	4.3±0.15 <sup>a</sup>	3.3±0.17 <sup>ab</sup>
Group III	4.3±0.33 <sup>a</sup>	3.2±0.17 <sup>cd</sup>	-	5 <sup>a</sup>	3.2±0.17 <sup>c</sup>	-	5 <sup>a</sup>	3.5±0.29 <sup>b</sup>	-
Group IV	4.3±0.33 <sup>a</sup>	2.8±0.17 <sup>d</sup>	2.3±0.17 <sup>c</sup>	5 <sup>a</sup>	3 <sup>c</sup>	2.1±0.17 <sup>c</sup>	5 <sup>a</sup>	3.7±0.17 <sup>b</sup>	2.5±0.29 <sup>c</sup>
Group V	5 <sup>a</sup>	4 <sup>a</sup>	3.2±0.17 <sup>a</sup>	5 <sup>a</sup>	4.1±0.17 <sup>a</sup>	3.5±0.29 <sup>a</sup>	5 <sup>a</sup>	4.5±0.29 <sup>a</sup>	3.8±0.17 <sup>a</sup>
Group VI	4.5±0.28 <sup>a</sup>	3.2±0.17 <sup>cd</sup>	2.5±0.29 <sup>bc</sup>	5 <sup>a</sup>	3 <sup>c</sup>	2.5±0.29 <sup>bc</sup>	5 <sup>a</sup>	3.5±0.29 <sup>b</sup>	2.3±0.3 <sup>c</sup>

The values represent Mean ± S.E of three experiments. Different letters in the same column within the same storage time indicate significant differences (P<0.05).



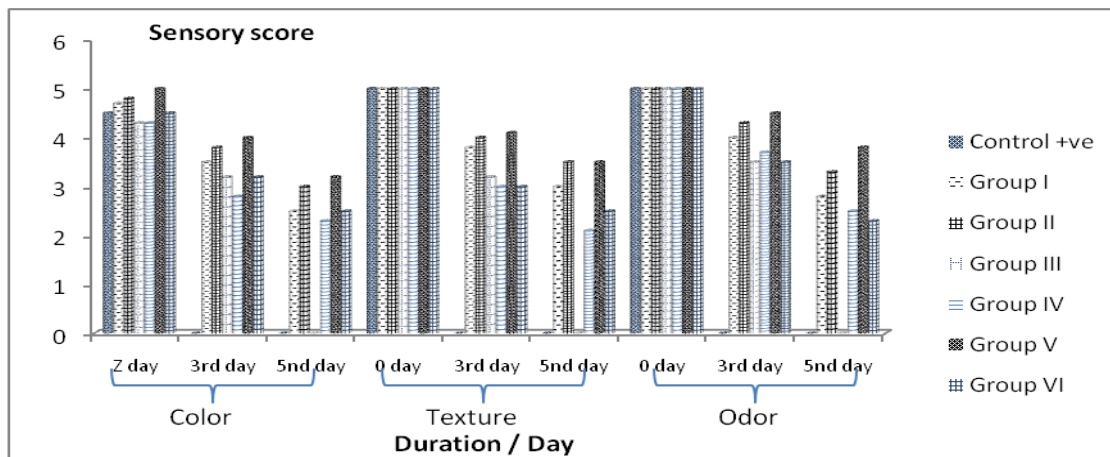
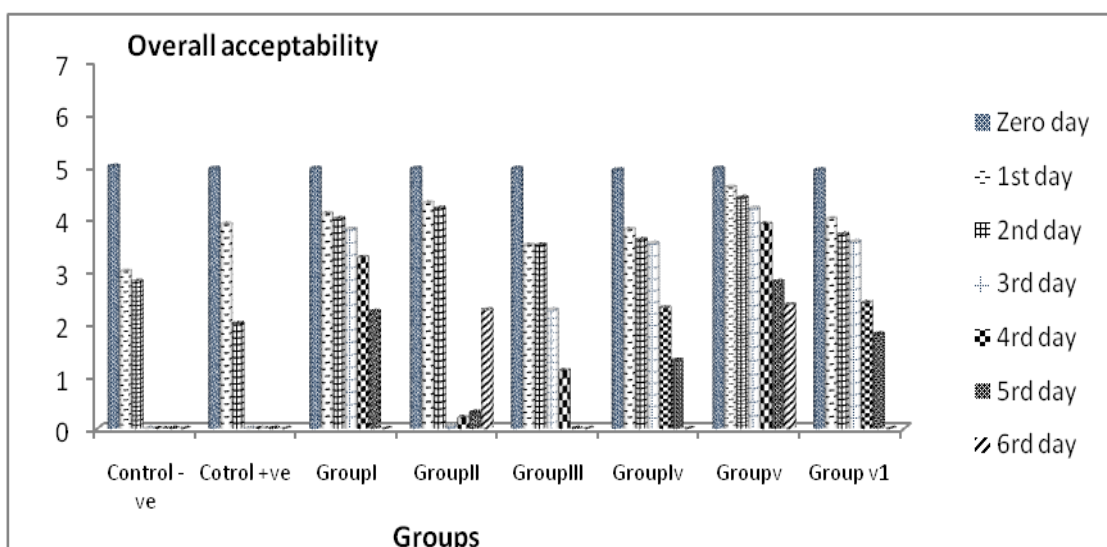


Fig (3) Changes in sensory scores of marinated peeled shrimp samples artificially inoculated with *V.parahaemolyticus* during storage at 4°C.

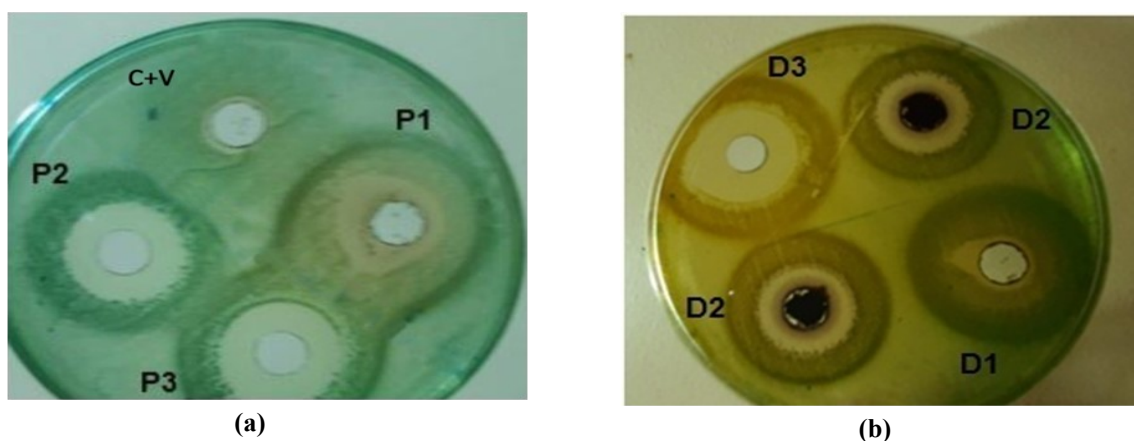
Table (4) Effect of different marinades (pomegranate and dates molasses) on overall acceptability of artificially inoculated peeled shrimp samples with *V.parahaemolyticus* during cold storage at 4 °C.

Groups	Zero day	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>rd</sup> day	5 <sup>rd</sup> day	6 <sup>rd</sup> day
Control -Ve	5	3.5±0.2	2.8±0.15	-	-	-	-
Control +ve	4.96±0.03	3.9±0.2	2.±0.18	-	-	-	-
Group I	4.96±0.03	4.1±0.18	4±0.17	3.8±0.16	3.26±0.14	2.23±.14	-
Group II	4.96±0.03	4.3±0.1	4.2±0.05	4±0.05	3.73±0.21	2.63±0.31	2.26±0. 14
Group III	4.96±0.03	3.5±0.23	3.5±0.08	2.26±0.18	1.1±0.05	-	-
Group IV	4.93±0.06	3.8±0.3	3.6±0.3	3.53±0.14	2.3±0.15	1.3±0.15	-
Group V	4.96±0.03	4.6±0.05	4.4±0.1	4.2±0.11	3.9±0.14	2.8±.15	2.36±0.18
GroupVI	4.93±0.06	4±0.3	3.7±0.26	3.56±0.06	2.4±0.08	1.8±.05	-

Fig (4) Effect of different marinades (pomegranate and dates molasses) on overall acceptability of artificially inoculated peeled shrimp samples with *V.parahaemolyticus* during cold storage at 4 °C.



5 = Very good, 4 = Good, 3 =Normal, 2 = Bad, 1= Very bad, - = Spoiled  
 The values represent Mean ± S.E of three experiments. (Pelin-Can and Arslan 2011).



Picture (1): *V. parahaemolyticus* on Thiosulfate citrate bile salt sucrose (TCBS) agar and its inhibition by well diffusion method where:

- (a) 0.1 ml of control +V with marinated solution only, 0.1 ml pomegranate molasses with marinade 1 % (PM1) and 2 % (PM2) and pomegranate 2% in sterilized distilled water (P3).  
 (b) 0.1 ml dates molasses with marinade 1% (DM1), 2% (DM2) and dates molasses 2% in sterilized distilled water (D3).

## DISCUSSION:-

*V. parahaemolyticus* is the leading bacterial cause of seafood-associated gastroenteritis worldwide. Moreover, infections and outbreaks caused by *V. parahaemolyticus* has kept increasing over the last two decades (Yang et al. 2019). So Plant extracts have been employed in seafood to maintain the quality, as well as to extend the shelf-life, by lowering microbial and chemical reactions (Besbes et al. 2016).

Results recorded in table (1) and figure (1) revealed *V. parahaemolyticus* counts (log cfu/g) in shrimp samples treated with different concentrations of pomegranate and date molasses. The control -Ve group was free *V. parahaemolyticus* and the initial count of *V. parahaemolyticus* after inoculation without addition of marinade was  $6.5 \pm 0.3$  log cfu/g.

Pomegranate molasses 2% highly reduced *V. parahaemolyticus* count that inoculated in peeled shrimp during storage in both G11 and GV as  $2.1 \pm 0.1$  and  $1.1 \pm 0.2$  log CFU/g with reduction percent reached to 67.84% and 83.15% after 6 days storage, respectively. While pomegranate molasses 1% moderately reduced *V. parahaemolyticus* count during storage in G1 to  $2.89 \pm 0.06$  log cfu/g, with reduction percent reached to 55.74% after 5 days storage,

so pomegranate molasses 2% had more antimicrobial activity than 1% and this agreed with Wu et al. (2016) who reported that when pomegranate peels extract (PPE) was applied on the shrimp, *V. Parahaemolyticus* count was 2 log less than the control group. Also results agree with Malviya et al. (2014) who tested the antibacterial activity of pomegranate peel extracts against four bacterial strains, *Staphylococcus aureus*, *Enterobacterogenes Salmonella typhi* and *Klebsiella pneumonia* and the extracts demonstrated remarkable antibacterial activities against all the tested bacterial strains, they also reported that *P. granatum* contains large amount of tannins (25%) and antibacterial activity may be indicative of the presence of secondary metabolites.

As showed in Table (2) Fig(2) Picture (1) the best results of *V. parahaemolyticus* reduction percentage in marinated shrimp samples were obtained by groups that marinated with PM (2%) and DM (2%) either in marinated solution or in distilled water at six and fifth day, respectively followed by that marinated with PM (1%) and DM(1%), and there is a significant difference between all groups ( $P \leq 0.05$ ). In the same side, PM (2%) either in marinade (GII) or in sterilized distilled water (GV) compete the growth of *V. Parahaemolyticus* in



shrimp samples till the six day of cold storage with high reduction percentage of 67.84 % and 83.15 %, respectively. This agreed with **Hemmat et al. (2018)** who reported that fish fillet samples treated with pomegranate peel extract recorded the highest reduction % in *V. parahae-molyticus* count by 76.30% after 5th day of cold storage, and **Elbagory et al. (2019)** who found that pomegranate peel extract 1% and 2% showed antibacterial activity by reduction % against *E.coli* which was 67.49% and 79.70%, respectively, all result in compared with Control -Ve and +Ve which spoiled early after the second day of cold storage, this indicated that marinate solution are used to improve tenderness to shrimp tissue with limited microbial effect and this were confirmed with PM2% that has the highest reduction percent.

In pomegranate molasses, tannins and other phenolic are the major bioactive compounds having antioxidant and antimicrobial activity. These antimicrobial constituents like phenols and flavonoids might be responsible for inhibitory action of PM against bacteria (**Reddy et al. 2007**).

The antimicrobial mechanisms of pomegranate molasses containing tannins can be summarized as follows: (i) the astringent property of the tannin may induce complication with enzymes or substrates. Many microbial enzymes in raw culture filtrates or in purified forms are inhibited when mixed with tannins. (ii) Tannin's toxicity may be related to its action on the membranes of the microorganisms. (iii) Complication of metal ions by tannins may account for tannin toxicity (**Chung et al. 1998**).

On the other hand marinated shrimp samples with date's molasses (DM1%) group III give mild reduction percentage 28.02 % in the third day and spoilage begin. Also date's molasses (DM2%) group IV give moderate reduction percent 31.1 % in fourth day of storage and spoilage begin, but shrimp samples with date's molasses 2% (D3) in sterilized distilled water, decrease count of bacteria to  $2.08 \pm 0.24$  and reduction percent raise up to 57.1 % at fifth day of cooled storage. This agree with **Hajer et al.(2016)** who use 15 mg/mL concentration of date surp resulting in antimicrobial activity in inhibiting *E. coli* and *S. aureus* and explained that the antimicrobial activity of date surp might be associated with the presence of

ant oxidative compounds that possess bioactive behavior. It was hypothesized that the phytochemical compounds present in date surp may be involved in red ox reactions mediated by the production of H<sub>2</sub>O<sub>2</sub> that results in bacterial inhibition.

Polyphenols are able to inhibit microorganisms and the antimicrobial activity of polyphenols is dependent on their chemical structure and environmental conditions (**Almajano et al. 2007**). Date molasses polyphenols are the major constituents contributing to DM's antibacterial activity (**Liu et al. 2013**). These observations could be the result of changes to the proteins and the bacteria as a result of the interaction with date surp and date molasses polyphenols, making it more susceptible to attack, and oxidative stress by the activation of several stress gene (**Brudzynski et al. 2012**).

**Basiri et al. (2015)** reported that live cells were still able to grow in marinated samples. In this condition, they were able to continue their activity more or less rapidly according to their ability to adapt to the medium during storage.

Increase reduction percentage of *V. parahae-molyticus* in pomegranate molasses (P3 2%) group V in sterilized distilled water than marinated pomegranate molasses (P2%) in group II and so on DM(2%) GVI than GIV may be attributed to that marinated solution make limited reduction 16.54 % in the second day and ripening to tissue of shrimps but the sample was spoiled and discard in the third day, also confirm that reduction actions were due to pomegranate molasses or dates molasses and marinated solution acts mainly to improve tenderness, juiciness, flavor, aroma and made ripening to shrimp tissue.

Pomegranate has therapeutic property due to the tannin rich, gallic acid, quercetin and phenolic acids which have antibacterial, antiviral, antifungal and antihelmenthic activity. The obtained results agreed with those obtained by **Howelland Souza (2013)** who reported that Punicagranatum extract has antibacterial activity against *Vibrio* spp. Polyphenols play an important role in protein precipitation and enzyme inhibition of microorganisms (**Fan et al. 2008**). The mechanism responsible for phenolic toxicity to microorganisms was related to reaction with sulphhydryl groups of proteins and

unavailability of substrates to microorganism (Naz et al. 2007).

The rapid microbial and biochemical reactions that occur in seafood immediately after death lead to changes in sensory and nutritional properties that reduce the shelf-life (Olatunde and Benjakul 2018).

Sensory scores of samples significantly decreased ( $p < 0.05$ ) throughout the storage period (Table 3) the appearance scores of samples in pomegranate molasses were significantly higher ( $p < 0.05$ ) than those found in date molasses. Darker appearance was observed for samples in date molasses than pomegranate molasses. However penetration of both date molasses and pomegranate to shrimp tissue caused darkening of shrimp tissue. In general consumers are accustomed to white color in marinated shrimp. For this reason, low scores for appearance of samples in both PM and DM were given by the panelists. However, higher ( $p < 0.05$ ) odor scores for samples in PM were found compared to samples in DM. PM also produced good texture with hard consistency along storage period, while DM cause ripening to the samples from the second day of storage of marinated shrimp. This result agreed with that obtained by Kanatt et al. (2010) who suggested that the addition of pomegranate extract on poultry products increase sensory acceptability and extended its shelf life, also Besbes et al. (2016) found that the shelf life of sardine fillets treated with 10% cactus fruit peel extract was extended to 12 days as compared to 7 days for the control (untreated sample).

Pomegranate fruit is considered to be a suitable fruit for processing and utilization due to its excellent flavor, color, physicochemical constitution and therapeutic properties (Dahham et al. 2010). Marinating process slows down the bacterial and enzymatic activity and provides best tenderness, textural and structural changes with a prolonged shelf life (Sallam et al. 2007).

#### CONCLUSION:-

From the above mentioned study, it can be concluded that Pomegranate and date molasses are safe, economic and effective in reducing *V. Parahaemolyticus* in peeled shrimp and offering addition "hurdle technology" to inhibit the

growth of *V. Parahaemolyticus* in peeled shrimp. Where, the effectiveness of Pomegranate and date molasses were uniform as the concentrations increased to 2% and it can be arranged in descending order as Pomegranate molasses (2%) date molasses (2%) Pomegranate molasses (1%) date molasses (1%). Such approach can have wide implications for improvement of food safety. Evaluation of *V. parahaemolyticus* stress responses in food systems, particularly raw shrimp, is a critical area of future research.

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