

## Impact of Using Organic Acids and Sodium Sulfite on the Quality of Unpeeled Shrimp

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**Abstract:** This study was done to evaluate the impact of using organic acid and sodium sulfite on the quality criteria of unpeeled shrimp. Total five kilograms of unpeeled shrimp were collected from retail markets at Giza city. Samples were divided into five lots each about one kilogram (approximately fifty samples for each). Samples were subjected to various treatments with (6% vinegar, 3% citric acid and 2% sodium sulfite). Treated samples were evaluated by sensory and bacterial examinations. Treated samples with 6% vinegar and 3 % citric acid improved the sensory quality and extended the shelf life of the samples stored at 0°C, also reduce the bacterial counts especially (APC, coliforms and fecal coliforms). Samples packed in crushed ice and those treated with 2% sodium sulfite (stored at 0°C for 4 days) showed deterioration changes (black color and abnormal odors) and also increasing pH values. The public health significance of isolated microorganism was also discussed.

**Key words:** Shrimp % Citric Acid % Sodium Sulfite % Vinegar % Coliforms % APC % Ph % Salmonella % *E. coli*

### INTRODUCTION

Shrimp are the most important items and exported marine among the range of sea foods in the global fishery trade [1]. Shrimp are highly susceptible to both microbiological and chemical deterioration due to its high water content, neutral pH and relatively large quantities of free amino acids and naturally presence of autolytic enzymes. The number and type of bacteria found on freshly caught shrimp is influenced by a number of factors such as water, temperature, harvesting area, type of sediment and size of shrimp [2]. Moreover, the number and type of bacteria in processed shrimp reflect the changes that have occurred in the initial flora and degree of contamination that have taken place during the course of handling [3]. After harvesting shrimps are protected from spoilage (especially bacterial spoilage) by either refrigeration or freezing. A more rapid pattern of shrimp deterioration was reported by Fonseka and Ranjini [3] as prawns spoiled within 12-16 hour at ambient temperature. Refrigeration is limited for the local production and consumption in near markets while freezing is proposed for long storage and exportation as freezing decrease the changes contributing to spoilage [4].

Maintain the quality of shrimp is important not only because it is a perishable product, but also because of the increasing demands for this product in markets [5].

The proper handling of shrimp between harvesting and marketing to the consumer is a crucial element in assuring microbiological quality of the finished product [6].

The quality deterioration of shrimp can be assessed by sensory evaluation [7], chemically by pH [8], as well as bacteriologically by enumerating spoilage microorganisms especially Coliforms, *Pseudomonas* [9], isolation and identification of pathogenic microorganisms [10].

Organic acids (OA) may provide the cornerstone of sanitizing formulation that can improve the microbial quality and safety of fish [11]. Preservation system consisting of citric acids and sodium sulfite can improve the quality of shrimp stored on ice [12]. Vinegar can be used as a preservative, because it reduces the thermal death time of microorganisms and either inhibits or kill microorganisms depending on the concentration used [13].

Therefore, the present study aimed to improving the quality and extending shelf life of the refrigerated raw shrimp using organic acids as citric acids, vinegar or Sodium sulfite.

## MATERIALS AND METHODS

**Collection of Samples:** Total five kilograms of fresh shrimps were collected from retail markets at Giza city. Whole shrimp samples were placed on ice and transported to the laboratory where they were rinsed with tap water and drained.

Shrimps were divided into five lots each about one kilograms (fifty sample for each) and were subjected to various treatments within one hour. The following treatment solutions were prepared: citric acids 3 % (w/v), vinegar 6% (v/v) and sodium sulfite 2% (w/v).

**Procedures:** Raw shrimp samples were collected to the following experimental trials:

- C Fresh unpeeled shrimp (raw)
- C Raw shrimps packed in crushed ice only.
- C Vinegar 6% for 10 minutes.
- C Citric acids 3% for 10 minutes.
- C Sodium sulfite 2% for 10 minutes.

Treated samples were drained on a sanitized perforated plate for two minutes at room temperature. Each treated sample was over wrapped, sealed and allocated in refrigerator at four C for examination at 2<sup>nd</sup> and 4<sup>th</sup> days of storage.

**Sensory Evaluation and pH:** The examinations were carried according to the specification given in E.S.S. (516/1993) [14] as following:

Appearance	Odor	Color	Texture
Clean and smooth mucous	Fresh odor (sea weed)	Normal "greyish pink"	Firm
Slimy mucous	Fishy to slightly ammoniacal		Soft
Sticky mucous	Strong ammoniacal	Abnormal "black"	Very soft

**Determination of pH:** According to the technique recommended by Duan *et al.* [15]. 10 grams of the sample were homogenized with 25 ml distilled water left for 10 min. and filtered. The pH was determined by using pH meter.

**Bacteriological Examination:** A sample of 10 grams were taken in a sterile polyethylene bag and pour in 90 ml of sterile Ringer solution in that bag and placed in a stomacher and blended for 2 minutes.

Serial decimal dilutions up to 10<sup>6</sup> were performed. Bacteriological analysis was performed according to the standard procedures for enumeration and identification of microorganisms as follows:

- C Aerobic plate count (APC), [16].
- C Enumeration of Aeromonas and Pseudomonas on (GSP, Merck, Art, 10230) Kielwein *et al.* [17].
- C *Staphylococcus aureus* count, [16].
- C Coliforms count, [16].
- C Fecal coliforms determined by APHA [16].
- C Isolation and identification of Salmonella and *E. coli*, [16].

**Statistical Analysis:** All data were analyzed using Statistical Analysis System [18]. Comparisons between treatments within each analysis were tested. Significance was determined by the F-test and least square means procedure. Main effects were considered significance at P#0.05.

## RESULTS AND DISCUSSION

Table 1 illustrates that sensory attributes of examined unpeeled shrimps samples. The appearance, odor, color, texture and overall acceptability are evaluated and recorded according to ESS 516-1993 [14]. Concerning the general appearance 48 (96%) of the 50 examined fresh unpeeled samples have clean feel and smooth touch, while 4% of the samples have slimy mucous feel. Appearance of iced unpeeled shrimp samples (control) at 2<sup>nd</sup> day of storage at 0°C showed 50% only are clean and smooth, while 50% of samples have either slimy or even sticky mucous feel. At 4<sup>th</sup> day of storage (90%) of iced unpeeled samples showed slimy and sticky mucous. The effect of different treatments with 6% vinegar, 3% citric acid and 2 % sodium sulfite on the appearance of shrimp samples revealed an improvement more than that the control samples especially at 4<sup>th</sup> day of storage. There is no significance difference in the appearance of the samples treated with vinegar 24% and that for the samples treated with 3% citric acid (30%) at the 4<sup>th</sup> day of storage at 0°C.

It is well assumed that odor is the most important evident of actual onset of spoilage of the shrimp [19]. It is evident that fresh shrimp, control samples and treated sample with vinegar and citric acid showed fresh odor at percent ranged from 90-100% at zero and 2<sup>nd</sup> day of storage. Meanwhile, control samples and samples treated with 2 % sodium sulfite subjected to deteriorative odor changes 70 - 90% at 4<sup>th</sup> day of storage at 0°C and became unfit for further analysis later on. On the other hand, citric acid and vinegar has remained acceptable and fresh shrimp odor at the 4<sup>th</sup> day of storage. Nearly similar results were obtained by Marshall and Kim [20]. Among the criteria used to evaluate shrimp organoleptically is the

Table 1: Sensory panel scores for fresh and treated shrimp

			Fresh shrimp		Shrimp in crushed ice (Control)		Shrimp treated with 6% vinegar		Shrimp treated with 3% citric acids		Shrimp treated with 2% sodium sulfite		Shrimp in crushed ice (Control)		Shrimp treated with 6% vinegar		Shrimp treated with 3% citric acids		Shrimp treated with 2% sodium sulfite	
			1 <sup>st</sup> day		2 <sup>nd</sup> day		2 <sup>nd</sup> day		2 <sup>nd</sup> day		2 <sup>nd</sup> day		4 <sup>th</sup> day		4 <sup>th</sup> day		4 <sup>th</sup> day		4 <sup>th</sup> day	
Parameter			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Appearance	Mucous	Slimy	2	4	10	20 <sup>a</sup>	5	10 <sup>b</sup>	7	14 <sup>c</sup>	15	30 <sup>d</sup>	20	40 <sup>e</sup>	30	60 <sup>b</sup>	25	50 <sup>e</sup>	20	40 <sup>e</sup>
		Sticky	0	0	15	30 <sup>a</sup>	0	0 <sup>a</sup>	0	0 <sup>a</sup>	10	20 <sup>c</sup>	25	50 <sup>e</sup>	8	16 <sup>b</sup>	10	20 <sup>e</sup>	20	40 <sup>e</sup>
		Clean and smooth	48	96	25	50 <sup>a</sup>	45	90 <sup>b</sup>	43	86 <sup>c</sup>	25	50 <sup>c</sup>	5	10 <sup>a</sup>	12	24 <sup>b</sup>	15	30 <sup>c</sup>	10	20 <sup>d</sup>
Odor	Fresh shrimp odor		50	100	45	90 <sup>a</sup>	45	90 <sup>a</sup>	45	90 <sup>a</sup>	40	80 <sup>b</sup>	0	0 <sup>a</sup>	28	56 <sup>b</sup>	28	56 <sup>b</sup>	10	10 <sup>d</sup>
	Fishy to slightly ammoniacal		0	0	5	10 <sup>a</sup>	5	10 <sup>a</sup>	5	10 <sup>a</sup>	10	20 <sup>b</sup>	5	10 <sup>a</sup>	22	44 <sup>b</sup>	22	44 <sup>b</sup>	5	10 <sup>a</sup>
	Strong ammoniacal		0	0	0	0 <sup>a</sup>	0	0 <sup>a</sup>	0	0 <sup>a</sup>	0	0 <sup>a</sup>	45	90 <sup>a</sup>	0	0 <sup>a</sup>	0	0 <sup>a</sup>	35	70 <sup>c</sup>
Color	Normal "greyish pink"		49	98	35	70 <sup>a</sup>	40	80 <sup>b</sup>	40	80 <sup>b</sup>	30	60 <sup>c</sup>	2	4 <sup>a</sup>	22	44 <sup>b</sup>	22	44 <sup>b</sup>	8	16 <sup>c</sup>
	Abnormal "black"		1	2	15	30 <sup>a</sup>	10	20 <sup>b</sup>	10	20 <sup>b</sup>	20	40 <sup>c</sup>	48	96 <sup>c</sup>	28	56 <sup>b</sup>	28	56 <sup>b</sup>	42	84 <sup>c</sup>
Texture	Firm		48	96	40	80 <sup>a</sup>	40	80 <sup>a</sup>	45	90 <sup>b</sup>	40	80 <sup>c</sup>	0	0 <sup>a</sup>	35	70 <sup>b</sup>	35	70 <sup>b</sup>	0	0 <sup>a</sup>
	Soft		2	4	10	20 <sup>a</sup>	10	20 <sup>a</sup>	5	10 <sup>b</sup>	10	20 <sup>a</sup>	5	10 <sup>a</sup>	15	30 <sup>b</sup>	15	30 <sup>b</sup>	3	6 <sup>c</sup>
	Very soft		0	0	0	0 <sup>a</sup>	0	0 <sup>a</sup>	0	0 <sup>a</sup>	0	0 <sup>a</sup>	45	90 <sup>a</sup>	0	0 <sup>a</sup>	0	0 <sup>a</sup>	47	94 <sup>c</sup>
Overall acceptability	Accepted		49	98	25	50 <sup>a</sup>	40	80 <sup>b</sup>	38	76 <sup>c</sup>	32	64 <sup>d</sup>	5	10 <sup>a</sup>	40	80 <sup>b</sup>	40	80 <sup>b</sup>	10	20 <sup>c</sup>
	Rejected		1	2	25	50 <sup>a</sup>	10	20 <sup>b</sup>	12	24 <sup>c</sup>	18	36 <sup>d</sup>	45	90 <sup>a</sup>	10	20 <sup>b</sup>	10	20 <sup>b</sup>	40	80 <sup>c</sup>

(a-c): Means with different superscript within the same row differ significantly (P&lt;0.05)

color. Shrimp should be rejected if there is an evident of reddish color or black spots even if the odor is still acceptable [19]. Concerning the color (98%) of the fresh shrimp showed normal greyish pink color while 70 % of shrimp samples in crushed ice showed blackish coloration at the junction between the head and body at 2<sup>nd</sup> day at 0°C. blackish greyish discoloration reached about 96% and 84% of iced shrimp samples and samples treated with 2 % sodium sulfite at 4<sup>th</sup> day of storage at 0°C respectively. It is of interest to recognize that there is no significant difference between samples treated with 6% vinegar and 3% citric acid concerning the color at 4<sup>th</sup> day of storage. However, [9] stated that changes in color of shrimp treated with acetate didn't differ significantly from control during the course of storage in the respect, [19] recognized that odor is the most important criteria followed by color. Moreover [21] pointed out that shrimp stored on ice retained their characteristic fresh shrimp odor for 2 days with development of slight ammoniacal and fishy odor by the 4<sup>th</sup> day of storage 0°C. Table 1 also indicated that 96 % of fresh shrimp show firm texture while 100 % of iced shrimp samples and treated samples with 2 % sodium sulfite showed soft or very soft texture meanwhile the firm texture percent of the samples treated with 3% citric acid (70%) are significantly higher than for samples treated with 6% vinegar (50%) at 4<sup>th</sup> day of storage the overall acceptability indicated that 5 (10%) of the examined iced unpeeled shrimp sample are accepted while 30 (60%), 40 (80%) and 10(20%) for treated samples with vinegar, citric acid and sodium sulfite respectively. In conclusion, the results given in Table 1 indicating that

deteriorated control untreated samples and high percent with 2 % sodium sulfite show slime formation and pale, dull appearance developed black spots and prominent unmistakable ammoniacal odor organic acid treated sample could provide unpeeled shrimp a resistance against blackening coloration soft texture and some mucous formation.

Table 2 revealed that the statistical analysis of the pH of the examined samples. pH of fresh shrimp samples are 6.85 while for the iced shrimp samples and for the treated samples with sodium sulfite are ranged from 7.2 to 7.82 and 7.59 to 7.84 for samples stored at 2<sup>nd</sup> and 4<sup>th</sup> day at 0°C. Similar pH values were achieved by Lannelongue *et al.* [22] who recorded pH values of 7.2 and 8 respectively for iced shrimp stored at 0°C. concerning the vinegar and citric acid treated samples pH significantly declined to 6.08 and 6.4 respectively. At 2<sup>nd</sup> day of storage while during storage the pH increased steadily and reached a level of 6.34 and 6.47 at the 4<sup>th</sup> day of storage respectively. It is important to recognize that the achieved pH values in combination with sensory attributes indicated that the majority of the examined treated samples are well accepted. Such findings were in agreement with [19] who pointed that the result of sensory analysis were in parallel to that of pH values. In this concern [3] came to the conclusion that irrespective to the storage temperature and in ice the pH tended to increase from 6.7 to 7.2 and values over 7.3 seemed to be suitable to indicate spoilage, at the time [23] found that the mean initial pH value of shrimp stored at 4°C was 7.59.

Table 2: Mean values of bacterial load (log10 CFU/g) and pH values of fresh and treated shrimp

Parameter	Shrimp in		Shrimp treated		Shrimp treated		Shrimp in		Shrimp treated		Shrimp treated		Shrimp treated	
	Fresh shrimp		crushed ice		with 6% vinegar		with 3% citric acids		with 2% sodium sulfite		crushed ice		with 6% vinegar	
	1 <sup>st</sup> day	2 <sup>nd</sup> day	2 <sup>nd</sup> day	2 <sup>nd</sup> day	2 <sup>nd</sup> day	2 <sup>nd</sup> day	4 <sup>th</sup> day	4 <sup>th</sup> day	4 <sup>th</sup> day	4 <sup>th</sup> day	4 <sup>th</sup> day	4 <sup>th</sup> day	4 <sup>th</sup> day	4 <sup>th</sup> day
APC	5.70	6.30 <sup>a</sup>	4.69 <sup>b</sup>	4.75 <sup>b</sup>	5 <sup>b</sup>	<0.48 <sup>b</sup>	7.30 <sup>a</sup>	5.78 <sup>b</sup>	5.85 <sup>b</sup>	5.70 <sup>b</sup>	<0.48 <sup>b</sup>	2.31 <sup>a</sup>	<0.48 <sup>b</sup>	<0.48 <sup>b</sup>
Coliforms	1.50	2.07 <sup>a</sup>	<0.48 <sup>b</sup>	<0.48 <sup>b</sup>	<0.48 <sup>b</sup>	<0.48 <sup>b</sup>	2.63 <sup>a</sup>	<0.48 <sup>b</sup>	<0.48 <sup>b</sup>	<0.48 <sup>b</sup>	<0.48 <sup>b</sup>	3.47 <sup>a</sup>	2.95 <sup>b</sup>	3.47 <sup>a</sup>
Fecal coliform	1.90	1.90 <sup>a</sup>	<0.48 <sup>b</sup>	<0.48 <sup>b</sup>	<0.48 <sup>b</sup>	<0.48 <sup>b</sup>	3.47 <sup>a</sup>	2.30 <sup>b</sup>	2.95 <sup>b</sup>	3.47 <sup>a</sup>	3.70 <sup>a</sup>	3.47 <sup>a</sup>	3.70 <sup>a</sup>	3.70 <sup>a</sup>
Staph	2.25	3 <sup>a</sup>	2 <sup>b</sup>	2.30 <sup>b</sup>	3.30 <sup>a</sup>	3.30 <sup>a</sup>	3.47 <sup>a</sup>	2.30 <sup>b</sup>	2.95 <sup>b</sup>	3.47 <sup>a</sup>	3.70 <sup>a</sup>	3.47 <sup>a</sup>	3.70 <sup>a</sup>	3.70 <sup>a</sup>
Pseudomonas and Aeromonas	2.38	3 <sup>a</sup>	2.9 <sup>a</sup>	3 <sup>a</sup>	3 <sup>a</sup>	3 <sup>a</sup>	3.34 <sup>a</sup>	3 <sup>a</sup>	3.47 <sup>a</sup>	3.70 <sup>a</sup>	3.70 <sup>a</sup>	3.47 <sup>a</sup>	3.70 <sup>a</sup>	3.70 <sup>a</sup>
pH	6.85 <sup>bc</sup>	7.20 <sup>c,d</sup>	6.08 <sup>a</sup>	6.40 <sup>ab</sup>	7.59 <sup>c,d</sup>	7.59 <sup>c,d</sup>	7.82 <sup>d</sup>	6.34 <sup>ab</sup>	6.47 <sup>ab</sup>	7.84 <sup>d</sup>	7.84 <sup>d</sup>	6.47 <sup>ab</sup>	6.47 <sup>ab</sup>	7.84 <sup>d</sup>

(a-d): Means with different superscript within the same row differ significantly (P&lt;0.05)

Table 2 showed that the initial mean APC of the fresh peeled shrimp is 5.70 (log10 CFU/g) reflecting an acceptable quality meanwhile the mean value of APC of shrimp stored in crushed ice were 6.30 (log10 CFU/g) at the 2<sup>nd</sup> day of storage reached to 7.30 (log10 CFU/g) at the 4<sup>th</sup> storage. Regarding the permissible limit not exceed 5 (log10 CFU/g) specified by ESS 516/1993 [14] the samples were unaccepted while the sample meet the limit 6-7 (log10 CFU/g) given by ICSMF [24]. The achieved APC for iced shrimp seems to be quite similar to that achieved by Fonseka and Rattagol *et al.* [25, 26] while [27, 28] recorded that higher counts exceeded 7 (log10 CFU/g) than that achieved during the present study. Shrimp treated with 6% vinegar has lower APC compared to the control and other treatments, such reduction continuous till 4<sup>th</sup> day of storage 5.78 (log10 CFU/g). Nearly, similar results was achieved by Al-Dagal and Bazaraa [9] as the APC reduction of citric acid 3% treated sample is higher than that of sodium sulfite 2% treated samples from 2<sup>nd</sup> day and 4<sup>th</sup> day of storage.

The effect of citric acid and sodium sulfite on APC was confirmed by Sharma and Gokogen [29, 30] who stated that citric acid exhibit certain antimicrobial properties against bacteria and that citric acid dipping alone or in combination with sodium metabisulfite extended the shelf life of shrimp for two days longer.

Table 2 also illustrated the mean coliforms counts of the fresh and experimentally treated unpeeled shrimp samples. The mean coliforms (MPN) count of shrimp at zero time was 1.5 (log10 CFU/g). While, the mean counts in iced shrimp at 2<sup>nd</sup> day of storage reached to 2.07(log10 CFU/g) and at 4<sup>th</sup> day of storage reached 2.31(log10 CFU/g). Treated shrimp sample with 6% vinegar 3% citric acid and 2% sodium sulfite are could effectively reduce the coliforms to undetectable limit. [31] Proved that acetic acid 2% immersion was among the most effective treatments for total coliform count reduction in muscle food. It is clear that samples of shrimp packed in crushed ice at the 4<sup>th</sup> day of storage had higher fecal

coliform levels reached 2.63(log10 CFU/g). While, treated samples with vinegar, citric acid and sodium sulfite were free from fecal coliforms.

Mean values of *Pseudomonas* and *Aeromonas* count of the examined iced shrimp samples were 3 to 3.34 (log10 CFU/g) at 2<sup>nd</sup> and 4<sup>th</sup> day of storage at 0°C. samples treated with 6% vinegar and 3% citric acid had an effective reduction on *Pseudomonas* and *Aeromonas* counts at 2<sup>nd</sup> day of storage at 0°C. Considering *Pseudomonas* and *Aeromonas* counts draw attention to the genera and numbers of bacteria isolated from shrimp during storage at 0°C [32]. Regarding the mean values of *Staphylococcus* counts of the treated group with sodium sulfite are higher than other treated group with citric acid and vinegar.

While the count of *Staphylococcus* are 3 to 3.34 at 2<sup>nd</sup> day and 4<sup>th</sup> day of storage for the shrimp samples stored in ice at 0°C. These limits occupied with ESS 516/1993[14].

*E. coli* and salmonella couldn't be isolated from any of the examined samples either fresh or treated samples similarly results recorded by Mohamed-Hatha *et al.* and Haassan [33, 34].

## CONCLUSION

Approximately the most difficulties facing unpeeled shrimp are the short life and quality deterioration rather than food poisoning. From the present study, it should be concluded that the sensory evaluation of fresh shrimp was significantly accepted than frozen shrimp, so they must be maintained good storage at chilling condition while, storage of shrimp samples in crushed ice for 4 days showed different deteriorative changes and increasing pH value, so shrimp subjected to dipping in 6% vinegar and 3% citric acid solution for 10 minutes and stored at 0°C to increase the shelf life and improve the sensory quality treated sample with 2 % sodium sulfite showed some changes in color, odor and increasing pH value.

So, no storage of unpeeled shrimp should be offered for sale or used for further processing if have any degree of decomposition (black coloration and soft texture) or increasing pH using only of crushed ice to preserve shrimp at 0°C isn't enough so it should be used in combination with organic acids to extend shelf life and maintain accepted quality.

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