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The Effect of Temperature on Bacterial Load of Prawn

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ABSTRACT :

This study was carried out to evaluate the effect of heat and cold on the microorganisms existing in prawn. Roasting for 5 minutes, boiling for 10 minutes and storage at -18°C for 10, 20, 30 days were applied on raw prawn to study the effect of each treatment on the bacterial load.

The average counts of Mesophiles, and Psycrophiles, Enterobacteriaceae, Coliforms and Staphylococci were $1,3X10^7 \pm 2X10^4$, $6X10^4$, $3X10^4 \pm 10^2$, $2X10^2 \pm 20$ and $3X10^3 + 10^2$ organisms per gram raw prawn sample respectively, then reduced to $10^4 \pm 2X10^2$, $8X10^2 \pm 80$, $3X10^3 \pm 90$, less than 3 and $3X10^2 \pm 25$ organisms per gram roasted prawn in shell, respectively. Such counts were more reduced in roasted peeled samples. It is evident, therefore, that roasting had a more destructive effect on microbes when applied on raw peeled samples than those in shell.

Boiling reduced the average counts of mesophiles and psychrophiles, Enterobacteriaceae, Coliforms and staphylococci from $2X10^5 + 10^5$, $2X10^7 \pm 3X10^4$, $3X10 \pm 10$, $3X10^4 \pm 102$ organisms per gram raw prawn samples to $2X10^5 \pm 3X10^3$, $3X10^3 \pm 10^2$, less than $2X10^2$, less than 3 and $4X10^4 \pm 50$ organisms per gram boiled prawn samples, respectively.

Freezing storage for 30 days caused a reduction in the average counts of mesophiles, psychrophiles, Enterobacteriaceae, Coliforms and Staphylococci from $4X10^7 \pm 2X10^5$, $5X10^5 \pm 2X10^3$, $3X10^5 \pm 2X10^3$, $10^3 \pm 150$ and $3X10^3 \pm 2X10^2$ organisms per gram to $5X10^4 \pm 10^3$, $10^3 \pm 120$, $3X10^2 \pm 70$, 10 ± 2 and less than $2X10^2$ organisms per gram, respectively.

Key words : Bacterial load, Mesophiles, Psycrophiles, Enterobacteriaceae, Coliforms, Staphylococci.

INTRODUCTION

The effect of temperature either heat or cold on lowering bacterial population is of importance on shelf-life extension of prawn. Several data are available in the microbial quality of raw and frozen prawn (Green, 1949; Nickerson and Polkan, 1972, Foster et. al., 1977; Swaertzentruber et al., 1980; Alvarez, 1984 and Zuberi et. al., 1985). However microbial activity frequently causes extensive deterioration of quality characteristics particularly when counts reach levels of 10^5 to 10^7 organisms per gram (Vanderzant and Nickelson, 1971). Processing of of prawn by exposure to temperature need for the chosen of ideal methods for reduction of growth rate, inactivation and destruction of microorganisms in prawn. In this respect, Fitzgerald (1974), Surkiewcz et. al. (1967), Arroyo (1969) and Vanderzant et. al. (1973), stated that cold storage of shrimp caused reduction in microbial counts.

The aim of this work was to study the effect of heat treatments (roasting and boiling) as well as freezing on the bacterial load of prawn.

MATERIALS AND METHODS

Experiment I

Effect of roasting :

Fifteen prawn samples (prawn sample : 100 gram) were collected from Cairo and Giza markets. Five prawn samples

were used in each experiment. The experiment was repeated three times. Collected samples were transferred to the laboratory without delay.

A. Preparation of samples :

Each prawn sample was devided into three equal parts under aseptic conditions. The external skeleton was removed from the first part, five grams were homogenized with 45 ml 0.1% sterile peptone water in sterile blender to provide homogenate of 10^{-1} dilution, from which further decimal dilutions were prepared (ICMSF, 1978).

B. Bacteriological examination :

1. Aerobic plate count (APC) : The drop plate method recommended by ICMSF (1978) was used. Inoculated plates were incubated for 3 days at 25°C for enumeration of mesophilic count and for 10 days at 0°C for enumeration of psychrophilic count.

2. Total enterobacteriaceae count : The technique recommended by Gork (1976) was applied by using Violet Red Bile Glucose agar (VRBG agar). Inoculated plates were incubated at 37 °C for 24 hrs. Representive colonies were isolated and tested for Gram reaction.

3. Most probable number of Coliforms (MPN) : Presumptive coliforms were determined on Lauryl sulphate tryptose broth using a 3-tube most probable number procedure. Positive tubes were confirmed on brilliant green bile lactose broth. Growth from positive tube was streaked on eosin methylene blue plates. Typical colonies of E. Coli were tested for IMVIC reactions.

4. Staphylococci count : Plates of Baird-Parker's agar were inculated at 37 °C for 24 hrs. Suspected colonies were subjected to Gram stain reaction. Isolates were tested by mannitol germentation (Bailley and Scott, 1974), catalase test (Mac-Faddin, 1976) and coagulase test (Gruickshank et. al. 1969). Identification of Enterobactericeae organisms was carried out according to Finegold and Martin (1982).

The second part of each prawn sample was roasted in shell for 5 minutes then allowed to cool at room temperature, peeled and examined as the first part while the third part was peeled before being roasted for 5 minutes, then examined as the other two parts.

Experiment II :

Effect of boiling :

To study the effect of boiling on the bacteria of prawn, three groups of prawn samples, each group included 10 individuals, were collected from different markets at Cairo and Giza. From each group 5 raw samples were examined bacteriologically as in experiment I while the other five samples were boiled for 10 minutes before being examined.

Experiment III :

Effect of freezing :

Three different groups of prawn were collected from different markets at Cairo and Giza Governorates. Each group included 20 individuals, 5 raw prawn samples were examined bacteriologically as mentioned above while remaining 15 individuals were frozen at -18 ^{o}C which examined bacteriologically after 10, 20 and 30 days storage using five prawn each time.

RESULTS AND DISCUSSION

Effect of Roasting :

The average results of the three experiments (table 1) revealed that the APC at 25 ^{o}C (mesophiles) and 0 ^{o}C (psychrophiles), Enterobacteriaceae count, coliforms (MPN) and staphylococci count were 1.3 x $10^7 \pm 2 \times 10^4$, 6 x $10^4 \pm 10^2$, 3 x $10^4 \pm 10^2$, 2 x $10^2 \pm 20$ and 3 x $10^3 \pm 10^2$ organisms per gram in raw prawn samples. These counts were significantly reduced to $10^4 \pm 2 \times 10^2$, 8 x $10^2 \pm 80$, 3 x $10^3 \pm 90$, less than 3 and 3 x $10^2 \pm 25$ roasted prawn in shell. While in roasted peeled parts all counts were sharply reduced and reached to 3 x $10^2 \pm 70$, organisms per gram for mesophilic count and less

Table 1. Summarized table showing the effect of roasting on the bacterial load of prawn of the three experiments.

	APC 25 C Mesophiles	APC 0C Psychrophiles	Enterobacter-iaceae count	Coliform MPN	Staphylo-cocci count
Raw prawn	$1.3 \times 10^7 \pm 2 \times 10^4$	$6 \times 10^4 \pm 10^2$	$3 \times 10^4 \pm 10^2$	$2 \times 10^2 \pm 20$	$3 \times 10^3 \pm 10^2$
Roasted in shell	$10^4 \pm 2 \times 10^2$	$8 \times 10^2 \pm 80$	$3 \times 10^3 \pm 90$	<3	$3 \times 10^2 \pm 25$
Roasted peeled	$3 \times 10^2 \pm 70$	$< 2 \times 10^2$	$< 2 \times 10^2$	<3	$< 2 \times 10^2$

Table 2. The effect of roasting on bacteria existing in prawn.

	Group A						Group B						Group C					
	Raw		Roasted in Shell		Roasted peeled		Raw		Roasted in Shell		Roasted peeled		Raw		Roasted in Shell		Roasted peeled	
Isolated Organisms	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No	%	No	%	No	%
Arizona group	-	-	-	-	-	-	1	20	-	-	-	-	1	20	-	-	-	-
E. Coli	-	-	-	-	-	-	2	40	-	-	-	-	1	20	-	-	-	-
Enterobacter aerogenes	2	40	1	20	-	-	2	40	1	20	-	-	5	100	2	40	-	-
Hafina	1	20	-	-	-	-	-	-	-	-	-	-	1	20	-	-	-	-
Proteus mirabilis	1	20	-	-	-	-	-	-	-	-	-	-	1	20	-	-	-	-
Proteus vulgaris	-	-	-	-	-	-	1	20	-	-	-	-	-	-	-	-	-	-
Proteus morgani	1	20	-	-	-	-	1	20	-	-	-	-	1	20	-	-	-	-
Providencia alcalifacions	-	-	-	-	-	-	2	40	-	-	-	-	1	20	-	-	-	-
Shigella boydii	1	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Staph. aureus	1	20	-	-	-	-	-	-	-	-	-	-	1	20	-	-	-	-

than 10^2 for each psychrophilic, Enterobacteriaceae and staphylococci counts, while the most probable number of coliforms was less than 3 organisms per gram.

Roasting proved its efficacy in reducing bacterial load of prawn and this agrees with the finding reported by Ridly and Siabyj, (1978) and Greenwood et. al. (1985). The reduction in bacterial load was more significant in peeled parts than those in shell and this could be mainly attributed to the protection of muscles by the shell cover.

Effect of boiling :

The average results of the three experiments are given in table (3). The average counts of mesophiles and Psychrophiles were $8x10^8 \pm 10^5$, and $2x10^5 \pm 2x10^3$ organisms per gram, respectively in raw samples while in boiled samples were reduced to $2x10^5 \pm 3x10^3$ and $3x10^3 \pm 10^2$ organisms per gram. The mean counts of Enterobacteriaceae and coliforms were $2x10^7 \pm 3x10^4$ and 30 ± 10 organisms per gram raw samples while in boiled samples were less than $2x10^2$ and less than 3 organisms per gram, respectively.

The average count of staphylococci in raw samples was higher than in boiled samples, each constituting $3x10^4 \pm 10^2$ and $4x10^2 \pm 50$ organisms per gram.

None of the isolated organisms from boiled samples could be isolated from samples of the three groups (Table 4).

Boiling of prawn has proved to be a valuable manner in reducing bacterial contamination. This substantiate the findings reported by Olsen and Shelton (1973), Ridly and Slabyj (1978) and ICNSF (1980). Moreover, Greenwood et al. (1985) stated that cooking of prawn and shrimp may not necessarily result in total destruction of the bacterial population. Sadik et al. (1985) suggested that the existence of microorganisms in cooked products are attributed to either prolonged bolding time or cross contamination after cooking. These explained the high aerobic count (5.4×10^8) recorded in boiled prawn by Arroyo (1969).

Table 3. Summarized table showing the effect of boiling on the bacterial load in prawn.

	APC 25 C mesophiles	APC 0 C Psychrophiles	Enterobacter-iaceae count	Coliforms (MPN)	Staphylococci count
Raw prawn	$8 \times 10^8 \pm 10^5$	$2 \times 10^5 \pm 2 \times 10^3$	$2 \times 10^7 \pm 3 \times 10^4$	30 ± 10	$3 \times 10^4 \pm 10^2$
Boiled prawn	$2 \times 10^5 \pm 3 \times 10^3$	$3 \times 10^3 \pm 10^2$	$< 2 \times 10^2$	< 3	$4 \times 10^2 \pm 50$

Table 4. The effect of boiling on bacteria existing in prawn.

Isolated Organisms	Group A				Group B				Group C			
	Raw		Boiled		Raw		Boiled		Raw		Boiled	
	No	%	No	%	No	%	No	%	No	%	No	%
Arizona group	1	2	-	-	-	-	-	-	1	20	-	-
E. Coli	1	2	-	-	3	60	-	-	1	-	-	-
Enterobacter aerogens	2	40	-	-	-	-	-	-	-	20	-	-
Enterobacter agglomerans	1	20	-	-	3	60	-	-	-	-	-	-
Proteus rettegri	1	20	-	-	2	40	-	-	-	-	-	-
Proteus vulgaris	-	-	-	-	-	-	-	-	1	20	-	-
Providencia alcalifacines	1	20	-	-	-	-	-	-	1	20	-	-
Providencia stuartii	2	40	-	-	-	-	-	-	-	-	-	-
Shigella boydii	-	-	-	-	1	20	-	-	-	-	-	-
Shigella flexeneri	-	-	-	-	1	20	-	-	-	-	-	-
Staph. aureus	1	20	-	-	1	20	-	-	-	-	-	-

Table 5. Summarized table showing the effect of freezing on the bacterial load of prawn of the three experiments.

	Mesophilic count	Psychrophilic count	Enterobacter-iaceae count	Coliforms $(MPN)^F$	Staphylococci count
Raw samples	$4x10^7 \pm 2x10^5$	$5x10^5 \pm 10^3$	$3x10^5 \pm 2x10^3$	$10^3 \pm 150$	$3x10^4 \pm 10^2$
10 days Freezing	$3x10^6 \pm 4x10^4$	$3x10^4 \pm 2x10^2$	$4x10^3 \pm 180$	$10 + 2$	$4x10^3 \pm 170$
20 days Freezing	$2x10^5 \pm 3x10^3$	$6x10^3 \pm 10^2$	$4x10^2 \pm 60$	10 ± 3	70 ± 10
30 days Freezing	$5x10^4 \pm 10^3$	$10^3 \pm 120$	$3x10^2 \pm 70$	10 ± 2	$< 2x10^2$

Table 6. Isolated organisms in groups of prawn samples subjected to freezing isolated organisms as recorded on 0 , 10 , 20 and 30 days after freezing.

	Group A								Group B								Group C							
	0 day		10 days		20 days		30 days		0 day		10 days		20 days		30 days		0 day		10 days		20 days		30 days	
	No	%	No	%	No	%	No	%	No	%	O	%	No	%	No	%	No	%	No	%	No	%	No	%
Arizona group	-	-	-	-	-	-	-	-	1	20	1	20	-	-	-	-	-	-	-	-	-	-	-	-
E. Coli	-	-	-	-	-	-	-	-	1	20	-	-	-	-	-	-	1	20	-	-	-	-	-	-
Enterobacter aerogens	-	-	-	-	-	-	-	-	3	60	3	60	3	60	2	40	2	40	2	40	2	40	2	40
Enterobacter agglomerans	-	-	-	-	-	-	-	-	1	20	1	20	-	-	-	-	1	20	1	20	-	-	-	-
Proteus mirabilis	-	-	-	-	-	-	-	-	1	20	1	20	1	20	-	-	1	20	1	20	1	20	1	20
Prot. rettegri	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	20	1	20	-	-	-	-
Proteus vulgaris	-	-	-	-	-	-	-	-	1	20	1	20	-	-	-	-	-	-	-	-	-	-	-	-
Proteus morgani	-	-	-	-	-	-	-	-	1	20	1	20	1	20	1	20	-	-	-	-	-	-	-	-
Providencia alcalifaciens	-	-	-	-	-	-	-	-	1	20	1	20	1	20	1	20	1	20	1	20	-	-	-	-
Providencia stuartii	-	-	-	-	-	-	-	-	1	20	1	20	1	20	-	-	1	20	1	20	-	-	-	-
Shigella boydii	-	-	-	-	-	-	-	-	1	20	1	20	-	-	-	-	1	20	1	20	1	20	-	-
Shigella flexneri	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	20	1	20	-	-	-	-
Hafnia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	20	-	-	-	-	-	-
Staph. aureus	-	-	-	-	-	-	-	-	2	40	2	40	2	40	2	40	1	20	1	20	1	20	1	20

Effect of Freezing :

From table (5) it is evident that the mean counts of mesophiles and psychrophiles, Enterobacteriaceae, coliforms (MPN) and Staphylococci were $4x10^7 \pm 2x10^5$, $5x10^5 \pm 10^3$, $3x10^5 \pm 2x10^3$, $10^3 \pm 150$ and $3x10^4 \pm 10^2$ organisms per gram raw prawn samples, respectively. After 10 days storage at - 11 ^oC these average counts were reduced to $3x10^6 \pm 4x10^4$, $4x10^3 \pm 180$, 10 ± 2 and $4x10^3 \pm 170$ organisms per gram respectively. Moreover, counts were highly reduced to $2x10^5 \pm 3x10^3$, $6x10^3 \pm 10^2$, $4x10^2 \pm 60$, 10 ± 3 and 70 ± 10 organisms as well as to $5x10^4 \pm 10^3$, $10^3 \pm 120$, $3x10^2 \pm 70$, 10 ± 2 and less than $2x10^2$ organisms per gram after 20 and 30 days storage, respectively. These findings agree with many investigators (Fieger and Dubois, 1946; Fitzgerald, 1947; Christophersen, 1968 and Fennem et al., 1973).

Frozen storage of prawn frequently causes reduction in bacterial population; freezing can destroy or sublethally injury bacterial cells (Kereluk and Gunderson, 1959 and Moss and Speck 1966).

Table 6 revealed that freezing storage was highly effective against members of Enterobacteriaceae, specially E. coli which disappeared more quickly than other coliforms and these agree with Elliott and Michener (1964). While gram positive organisms (Staphylococci) were more resistant to freezing and these substantiates the findings reported by Jakson (1974) and ICMSF (1980).

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تأثير الحرارة على الحمل البكتيري في الجمبري

ملخص :

تم إجراء هذه الدراسة لتحديد مدى تأثير المعاملات الحرارية المختلفة على الميكروبات المتواجدة في الجمبري . ولذلك تم إجراء الشيء لمدة خمس دقائق والغليان لمدة عشر دقائق والحفظ عند - ١٨ م ، لمدة ١٠ ، ٢٠ ، ٣٠ يوما للجمبري الطازج . ودلت النتائج على أن متوسط العدد الكلي للبكتريا عند ٢٥م ، البتكتيريا المحبة للبرودة ، الميكروبات المعوية ، الميكروبات القولونية ، المكور العنقودي الذهبي كانت ٣ر١ * $١٠^{٧}$ ، ٩ * $١٠^{٤}$ ، ٣ * $١٠^{٤}$ ، ٢ * $١٠^{٢}$ ، ٣ * $١٠^{٣}$ ، / جرام من الجمبري الطازج التي إنخفضت الى $١٠^{٤}$ ، ٨ * $١٠^{٢}$ ، ٣ * $١٠^{٣}$ ، أقل من ٣ ، ٣ * $١٠^{٢}$ / جرام بعد شيه في قشرته على التوالي وإزداد إنخفاض اعداد الميكروبات سالبة الذكر في الجمبري منزوع القشرة ومن ذلك يتضح أن شي الجمبري بعد انتزاع قشرته كان له اثر كبير على قتل الميكروبات وبغليان الجمبري انخفض مستوى اعداد الميكروبات الهوائية عند ٢٥م ، المحبة للبرودة ، المعوية القولونية ، والمكور العنقودي الذهبي من ٨ * $١٠^{٨}$ ، ٢ * $١٠^{٥}$ ٢ * $١٠^{٧}$ ، ٣ * $١٠^{٤}$ ، ١٠ * ٣ / جرام طازج الى ٢ * $١٠^{٥}$ ، ٣ * $١٠^{٣}$ ، أقل من ٢ * $١٠^{٢}$ ، أقل من ٣ ، ٤ * $١٠^{٢}$ / جرام على التوالي .

لوحظ بعد حفظ الجمبري عند - ١٨ م لمدة شهر أن متوسط أعداد الميكروبات الهوائية عند ٢٥م ، ، المحبة للبرودة ، المعوية ، القولونية والمكور العنقودي قد إنخفضت من ٤ * $١٠^{٧}$ ، ٥ * $١٠^{٥}$ ، ٣ * $١٠^{٥}$ ، $١٠^{٣}$ ، ٣ * $١٠^{٤}$ / جرام جمبري طازج الى ٥ * $١٠^{٤}$ ، $١٠^{٣}$ ، ٣ * $١٠^{٢}$ ، ١٠ ، أقل من ٢ * $١٠^{٢}$ / جرام .