Bacteriological Features of Dressed Squabs at Refrigeration Temperatures

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Abstract

The microbiological quality of 90 domestic squabs dressed collected from retail markets within Ismailia city was investigated. A sample size was divided into 2 main groups (chilled group & frozen one) where the individual birds of each group were subjected to bacterial examination according the plane adopted. The initial mean counts for total aerobes, psychrotrophs and staphylococci were $4.3x10^3$, $5.9x10^3$ and $6.8x10^2$ cfu/cm^2 of chilled bird respectively; meanwhile Salmonella spp. could not be detected. At the end of chill storage, the mean counts for total aerobes, psychrotrophs and staphylococci were $4.3x10^7$, $4.7x10^6$ and $3.1x10^2$cfu/cm^2 respectively. As regard to the frozen samples, the mean counts for each of total aerobes, psychrotrophs and staphylococci were $5.3x10^3$, $5.3x10^4$ cfu/cm^2 and non of staphylococci respectively, such count at the end of frozen storage . Absence of the pathogenic bacteria was a characteristic of refrigeration advantage; meanwhile there was a significant increase in population of aerobic psychrotrophic microorganisms occurred on samples throughout the chilling storage period

Introduction

Rearing pigeons for meat is an accepted industry all over the world. Young pigeons bred for meat are known as squabs. Squabs have never flown and have never usually eaten anything except "pigeon milk". It is usually tender, tasty and be served in many occasion in Egypt. It is relatively fatty, has high energy value and contains the necessary concern and proportion of essential amino acids for human nutrition. *Elsayed et al. (1980)*

Pigeon carcasses easily exposed to the infection during processing; where inevitable microbial contamination with risk pathogens is expected, namely salmonellae, *Staphylococcus aureus* and *Clostridium perfringens*. Unsanitary measures practiced in the hatchery, pen, breeding & rearing system adopted have a role in the spread of infection. Once infected carcass reaches the slaughter area, it is difficult to perform an effective control measures to eradicate completely the possibilities of infection which transmitted from the infected to the non-infected bird. *Nassar and Abu El-Ela (2000)*

Chilling as a step in the final stages of processing of poultry has been a con-

troversial topic with regard to its effect on contamination of carcasses. In fact, since chilling methods and their subsequent effect vary, it is necessary to specify the type of chilling operation used in describing the bacterial loads. Chilling generally delays growth of psychrotrophic spoilage bacteria, and prolong shelf life. Also it retards growth of pathogens.

Fresh meat of squabs present ideal media for microbial growth, psychrotrophic organisms soon become established and favored when dressed squabs kept at refrigeration temperatures about 0°C up to 10°C; noticeable growth with large population expected within 1 to 2 weeks. As temperature is lowered, growth of microorganisms is decreased until it finally stops. Psychrotrophs vary in their ability to grow at different low temperatures, and their growth may be halted at temperatures above or below the freezing point of the meat substrate.

Bacteriological data concerning squab meat are very limited; therefore, the current study was conducted to follow out microbiological quality of squab meat from the slaughter time throughout the storage period at refrigeration temperatures.

Materials and Methods

Collection of Samples: A sample size of 90 squabs (*Columba livia domestica*) were purchased alive (unknown age) from different poultry markets within Ismailia city. The squabs were slaughtered and dressed under the regular market conditions. After complete processing, exhaustion of the retained heat at the end of dripping point; each individual carcass was double-bagged and shipped to the lab. in pre-cooled insulated containers with frozen packs. Samples were divided into 2 main groups (chilled group & frozen one); each group in turn subdivided into subgroups (each of 10). Chilled group was stored in chiller adjusted at 4°C ± 1; where the bacterial quality was evaluated at interval of 0 time, 3, 6 and 9 days, each interval was represented by only one of the subgroups. Frozen group was maintained at –10°C & evaluated where the bacterial indices were checked up at interval of 30, 45, 60, 75 and 90 days of frozen storage.

Samples Preparation was done according to *Thiessen (2000).* After determining the weight of each carcass, it was shaken for one minute within a sterile bag containing 200 ml of the diluents (0.1 % peptone water) providing the rinse fluid. One ml of rinse fluid was transferred to a series of sterile test tubes containing 9 ml of 0.1 % sterile peptone water and well mixed to prepare serial decimal dilution up to 10^{-7}, such dilutions used for the bacteriological investigations.

Bacteriological evaluation: From appropriate dilutions 0.1ml was placed on each of the following media; Plate Count Agar (Difco Co., Ltd.) for total aerobic count; another

plate of Plate Count Agar for total psychrotrophic count and Baird-Parker Agar (Oxoid, CM 275) with Egg Yolk-Tellurite Emulsion (Oxoid, SR 54) for total staphylococci count.

***Salmonella* screening:** 25 g from each sample was aseptically removed, diluted with 225 ml of buffered peptone water in sterile stomacher bag and homogenized in a stomacher (*LAB-BLENDER, 400*) for one minute to form the original homogenate, the Rappaport Vassiliadis enrichment broth (CM 669, Oxoid) was used and incubated at 42°C for 24 h followed by streaking on Xylose Lysin-Desoxycholate (XLD) agar (CM 469, Oxoid), then incubated at 37°C for 24 h as recommended by *Harvey and Price , 1981.* Suspected colonies were identified biochemically and serologically.

Identification of psychrotrophic microorganisms isolated: Biochemical tests were applied as recommended by *APHA (2002)* Data from carcass rinse fluids expressed as cfu / ml; was converted to counts per cm^2 by using the formula published in the FSIS / USDA microbial baseline survey ***(USDA, 1996)*** as follows:

$$\frac{\text{CFU / ml recovered x ml used to rinse the carcass}}{(0.87 \times w) + 635}$$

Where "w" is the weight of the carcass in grams.

Results and Discussion

In the last decades a noticeable attention was given towards squab's breeding, slaughtering, packing, freezing, distribution for both of the local and retail markets. *Abd El-Aziz et al. (2002).* Elsewhere squabs or even pigeons were still sold as live birds to be killed and inspected by housewife, restaurant cooker or butcher.

Results given in table (1) revealed that the incidence of the microbial populations recovered from chilled squab carcasses maintained at 4°C were 100% for each of aerobic bacteria, psychrotrophs and staphylococci meanwhile *Salmonella* entirely was failed to be detected in the present work. As regard as the frozen carcasses maintained at -10°C table (1) also show incidence attained to 100% for both of aerobic and psychrotrophic bacteria and lower rate for staphylococci (70%) whereas the *Salmonella* was absent.

Results given in table (2) revealed the mean values of the total counts for chilled samples stored at 4°C. As regard as total colony counts were initiated in the first group which represent zero time $4.3 \times 10^3 \pm 7.8 \times 10^2$. These counts were increased with extending the storage time to reach ultimate mean value of $4.3 \times 10^7 \pm 1.3 \times 10^6$ cfu / cm^2 after 9 day storage at 4°C. Several of the studies were conducted on the microbial features of pigeon's flesh where different rates of the total aerobic bacteria were recorded *(Casagrande Proietti et al., 1997; Nassar and Abu El-Ela, 2000; Jeffery et al., 2001 and Abd El-Aziz et al., 2002).* The mean value of total psychrotrophic counts in the first group was $5.9 \times 10^3 \pm 7.2 \times 10^2$, meanwhile the mean value raised up to 4.7×10^6

$\pm$ 6.3 x 10^5 for the last group maintained for 9 days. The results obtained indicated the occurrence of ascendant increasing of the rate of the psychrotrophic microorganisms with the extension of the storage time. Some are pathogenic or toxinogenic for human, the most common causative organisms in reports on aerobic spoilage of refrigerated foods are the psychrophilic gram-negative rods usually found on refrigerated meats stored in aerobic environments *(Greer, 1989 and Lambert et al., 1991)*. The mean values of total staphylococci counts were initiated by 6.8 x $10^2 \pm$ 0.96 x 10^2, meanwhile the staphylococci count was significantly decreased ($p < 0.05$) in both of the 6 & 9 day samples. The samples maintained for 9 days recorded a mean value of 3.1 x $10^2 \pm$ 0.60 x 10^2. The presence of *Staphylococcus aureus* in a food may originate from the skin, mouth or nose of workers during handling of the product. The presence of large numbers of staphylococci is, in general, a good indication that the sanitation and temperature control have been inadequate. *Kraft (1986)* stated that low temperatures normally do not favour growth or toxin production by the pathogen, but exceptions do exist. *Staphylococcus aureus* has been reported to grow at 6.7°C with toxin production at 18°C. *Casagrande Proietti et al. (1997)* recorded *Staphylococcus aureus* from squab carcasses at incidence of 2%; where the count ranged between 10^2 up to 1.4 x 10^3 cfu/cm^2.

Table (3) revealed the mean values of the total counts recovered from the frozen samples of the squab carcasses maintained at -10°C for a storage period extended up to 90 days. As regard the mean values of total colony count were slightly decreased throughout the storage period, where minimum growth temperatures for control of organisms in foods have been listed as about -10°C for bacteria, -12°C for yeasts, and -18°C for other fungi *Rey (1975)*. The mean values of total psychrotrophic count were increased throughout the storage period, although psychrotrophic microorganisms will not grow in frozen foods maintained at -17 to -20°C, they can grow and cause spoilage if the food is allowed to thaw briefly or stored at high temperatures; also quality loss due to the microbial activity may occur in such foods after prolonged storage period *Gilliland et al. (1984)*. The mean value of staphylococci count after one month storage recorded marked drop of the staphylococci population where the mean value recorded were 1.1 x $10^2 \pm$ 0.27 x 10^2.cfu/cm^2 . It is worthy to state that freezing has a lethal effect on the bacterial cells of the staphylococci where it entirely cannot detected after freezing storage for 45, 60, 75 and 90 day. The author reported about the effect of freezing on coagulase – positive staphylococci which were not markedly decreased in incidence by freezing, but were during prolonged storage.

Table (4) revealed the incidence of the identified psychrotrophic strains isolated from refrigerated squab carcasses. The present study recorded the presence of psychrotrophic strains at different rates consequently *Pseudomonas* spp. (20.2%); *Bacillus* spp. (17.3%); *Streptococcus* spp. (14.4%); *Aeromonas* sp. (11.5%); *Acinetobacter* spp. (9.6%); *Micrococcus* spp. (8.7%); *Alcaligenes* (7.7%); *Staphylococcus* spp. (5.8%); *Corynebacterium* spp. (2.9%) and finally the *Lactobacillus* was the lowest strain where it recorded at rate of (1.9%). It had been well documented about spoilage of refrigerated foods that *Pseudomonas* species are among the most common causative organisms *(Gill, 1986).* As regard the incidence of *Bacillus* species recovered in the present study, the results may attributed to the contamination of raw carcasses due to the traditional and the primitive steps adopted in processing. The lactic acid bacteria, pediococcus and *Streptococcus* have been implicated in development of off-flavors, and associated with surface slime *(Stamer, 1976).* Although Aeromonas hydrophila has been implicated in food-borne disease associated with refrigerated foods, it apparently does not always grow at 0°C, and may have only slow growth at 5°C. It has more recently been recognized as a primary pathogen, and may be of particular concern.*Kraft (1986). Acinetobacter* bacteria could be of more importance in spoilage when they were major components of a high density flora by enhancing the spoilage activity of other organisms when the protein degradation increased *Gill (1986),* meanwhile Micrococci type bacteria are non-pathogenic but are important in food spoilage.Pathogens that may grow between 5°C and 12°C are Salmonella, Staphylococcus aureus, Bacillus cereus and Vibrio parahemolyticus, temperatures in the range 5°C – 12°C are typical of mild abuse temperatures and are of particular concern because refrigerated foods may often be kept in that range if not even higher. *Lactobacillus species* (2 %) beside the unidentified strains of *Bacillus* species can cause undesirable changes in the organoleptic characteristics of the final dressed carcasses; owing to its metabolic activities, moreover it may include pathogenic members.

Table (1) Incidence of the Microbial Populations Recovered from Refrigerated Squab Carcasses

	Bacteriological groups															
	Aerobic				Psychrotrophic				Staphylococci				*Salmonella*			
Refrigerated carcasses	+ve	%	-ve	%	+ve	%	-ve	%	+ve	%	-ve	%	+ve	%	-ve	%
Chilled carcasses	40	100	0	0	40	100	0	0	40	100	0	0	0	0	40	100
Frozen carcasses	50	100	0	0	50	100	0	0	35	70	15	30	0	0	50	100

Table (2) Statistical Analytic Results for Bacteriological Evaluation of Chilled Squab Carcasses Maintained at 4□C

	Aerobic				Psychrotrophic				Staphylococci			
Storage/ days	Min.	Max.	Mean	S.E.	Min.	Max.	Mean	S.E.	Min.	Max.	Mean	S.E.
0	$9x10^2$	$2x10^4$	$4.3 x10^3$	$7.8 x10^2$	$2x10^2$	$9x10^4$	$5.9x10^3$	$7.2x10^2$	$1x10^2$	$1x10^3$	$6.8x10^2$	$0.96 x10^2$
3	$2x10^3$	$1.6x10^5$	$1.3 x10^4$	$6.3x10^2$	$4x10^3$	$1.8x10^4$	$1.2 x10^4$	$1.4 x10^3$	< 100	$9.5x10^2$	$4.4x10^2$	$1 x10^2$
6	$2.7x10^4$	$3.2x10^6$	$3 x10^6$	$4.8x10^4$	$8x10^4$	$3.6x10^5$	$2.4 x10^5$	$2.8 x10^4$	< 100	$7x10^2$	$3.5x10^2$	$0.73 x10^2$
9	$3.7x10^5$	$5.1x10^8$	$4.3 x10^7$	$1.3x10^6$	$1.5x10^5$	$8.4x10^6$	$4.7 x10^6$	$6.3 x10^5$	< 100	$6.5x10^2$	$3.1x10^2$	$0.60 x10^2$

Table (3) Statistical Analytic Results for Bacteriological Evaluation of Frozen Squab Carcasses Maintained at -10°C

	Aerobic				Psychrotrophic				Staphylococci			
Storage /days	Min.	Max.	Mean	S.E.	Min.	Max.	Mean	S.E.	Min.	Max	Mean	S.E.
30	$2x10^3$	$7x10^3$	$3.9 x10^3$	$4.7 x10^2$	$1.9x10^4$	$7x10^4$	$3.9x10^4$	$4.8 x10^3$	< 100	$2x10^2$	$1.1x10^2$	$0.27x10^2$
45	$2.2x10^3$	$7.2x10^3$	$4.1x10^3$	$4.6x10^2$	$2.2x10^4$	$7.5x10^4$	$4.1x10^4$	$4.9 x10^3$	ND	ND	ND	ND
60	$2.5x10^3$	$7.9x10^3$	$4.7x10^3$	$5.1x10^2$	$2.5x10^4$	$7.9x10^4$	$4.8x10^4$	$5.2 x10^3$	ND	ND	ND	ND
75	$3x10^3$	$8.6x10^3$	$5.1x10^3$	$5.8x10^2$	$3x10^4$	$9x10^4$	$5.5x10^4$	$6.6 x10^3$	ND	ND	ND	ND
90	$3.1x10^3$	$8.6x10^3$	$5.3x10^3$	$5.6x10^2$	$3.1x10^4$	$9.1x10^4$	$5.3x10^4$	$5.9 x10^3$	ND	ND	ND	ND

Table (4) Frequency distribution of the identified psychrotrophic strains isolated from refrigerated squab carcasses

Species	Frequency	Percentage
Pseudomonas spp.	21	20.2
Bacillus spp.	18	17.3
Streptococcus spp.	15	14.4
Aeromonas spp.	12	11.5
Acinetobacter	10	9.6
Micrococcus spp.	9	8.7
Alcaligenes	8	7.7
Staphylococcus spp.	6	5.8
Corynebacterium spp.	3	2.9
Lactobacillus	2	1.9
	104	100

References

Abd El-Aziz, A.S; Elmossalami, M.K. and El-Neklawy, E. (2002): Bacteriological characteristics of dressed young pigeon (squabs) *columba livia domesticus*. Food / Nahrung, 46: 51-53.

American Public Health Association; APHA (2002): Methods for the Microbiological Examination of Foods 4th Ed., APHA Technical Committee on Microbiological Methods for Foods. Washington, D.C., USA.

Casagrande Proietti, P; Galli, R; Cuteri, V; Coletti, M. and Tacconi, G. (1997): Indagini sulle caratteristiche microbiologiche di carcasse di colombi da carne. La Selezione Veterinaria, 8: 721-726.

Elsayed, W.A; Shehab, A.H; Mourad, F.E; El-Nahry, F.I. and Said, A.K. (1980): Biochemical and biological evaluation of pigeon meats: Effect of type, age and sex. Food / Nahrung, 24: 821-828.

Gill, C. (1986): The control of microbial spoilage in fresh meats. In: Advances in Meat Research; Meat & Poultry Microbiology. Pearson, A. and Dutson, T. (eds.), vol. 2, chap. 2, pp.: 49-88. Westport, CT: AVI Publishing Co., Inc. . .

Gilliland, S.E; Michener, H.D. and Kraft, A.A. (1984): Psychrotrophic Microorganisms. In: Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed., chap. 9, pp.: 135-140 , APHA, Washington, DC, USA.

Greer, G. (1989): Red meats, poultry and fish. In: Enzymes of Psychrotrophs in Raw Food. Mckellar, R. (ed.), chap. 11, pp.: 267-292, CRC Press, Boca Raton, FL.

Harvey, W. and Price, H. (1981): Comparasion of Selenite F, Muller Kauffmann Tetrethionate and Rappaport medium for *Salmonella* isolation from chicken giblets after pre-enrichment in buffered peptone water. J. Hyg. Camb; 87: 219.

Jeffrey, J.S; Atwill, E.R. and Hunter, A. (2001): Prevalence of *Campylobacter* and *Salmonella* at Squab (Young Pigeon) Processing Plant. J. Poult. Sci., 80:151-155.

Kraft, A.A. (1986): Psychrotrophic organisms. In "Advances in Meat Research,; Vol. 2. Meat and Poultry Microbiology," Pearson A.M. and Dutson, T.R. (eds.) chap.6, pp.: 191-207, AVI Publishing Co., Inc., Westport, Conn.

Lambert, A.D; Smith, J.P. and Dodds, K.L. (1991): Shelf life extension and microbiological safety of fresh meat-a review. J Food Microbiol., 8: 267-297.

Nassar, A.M. and Abu El-Ela, A. (2000): Prevalence of some food poisoning pathogens in squabs and wooden pigeons carcasses in Assiut governorate. Assiut Vet. Med. J. 43: 209-218.

Rey, C.R. (1975): Numerical taxonomy and changes in the bacterial flora on frozen chicken. Ph. D. dissertation, Iowa State University, Ames, IA., USA.

Stamer, J.R.(1976): Lactic acid bacteria. In: "Food Microbiology: Public Health and Spoilage Aspects", Defigueiredo, M.P. and Splittstoesser, D.F.(eds.). pp. 404. Avi. Pub. Co., Inc.Westport, Conn. .

Thiessen, G. (2000): Canadian microbiological baseline survey of chicken broiler and young turkey carcasses. Canadian Food Inspection Agency, A62-53, pp. 3-4.

USDA/FSIS; United States Department of Agriculture- Food Safety and Inspection Service (1996): Pathogen reduction; hazard analysis and critical control point (HACCP) system. Federal Register 61: 38806-38989

الصفة البكتيرية لذبائح فرخ الحمام عند درجات حرارة التبريد

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الملخص العربي

تم دراسة الحالة الميكروبيولوجية لذبائح فرخ الحمام أثناء الحفظ على درجات حرارة منخفضة من تبريد وتجميد.حيث تم تجميع عدد ٩٠ فرخ حمام من أسواق الدواجن فى مدينة الاسماعيلية ثم تم تقسيم العينات الى مجموعتين رئيسيتين (مجموعة مبردة & أخرى مجمدة). وقدر متوسط الحمل البكتيرى الأولي للعينات متمثلة في البكتيريا الهوائية, البكتيريا المحبة للبرودة و المكورات العنقودية كالآتى: ٤,٣ x ١٠ ، ٥,٩ x ١٠ & ٦,٨ x ١٠ خلية لكل سم٢ على التوالى فى حين انه لم يتم عزل ميكروب السالمونيلا على الاطلاق. وقدر متوسط العدد الميكروبى لكل من البكتيريا الهوائية, البكتيريا المحبة للبرودة و المكورات العنقودية لنفس العينات في نهاية فترة الحفظ بالتبريد كالآتى: ٤,٣ x ١٠ ، ٤,٧ x ١٠ & ٣,١ x ١٠ خلية لكل سم٢ على التوالى بينما فى نهاية فترة الحفظ بالتجميد قدر متوسط العدد الميكروبى لكل من البكتيريا الهوائيةو البكتيريا المحبة للبرودة كالآتى: ٥,٣ x ١٠ & ٥,٣ x ١٠ خلية لكل سم٢ على التوالى ولم يتم عزل المكورات العنقودية من العينات المجمدة. و يعتبر من مميزات حفظ الأطعمة بالتبريد عدم ظهور البكتيريا الضارة, على الرغم من وجود زيادة ملحوظة في الميكروبات المحبة للبرودة.