Dept. of Food Hygiene and Control,
Faculty of Vet. Med., Suez Canal University

POTENTIAL CONTRIBUTION OF YEAST IN SPOILAGE OF QUAIL MEAT

(With 6 Tables)

By

SOAD AHMED SOLIMAN ISMAIL

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خطـــوره الخمائـــر المحتمله على تلف لحوم السمـــان

سعاد أحمد سليمان إسماعيل

لقد أجريت هذه الدراسة لعزل وتصنيف الخمائر من عينات السمان الطازج و المجمد وكذلك لتعيين الدور الذي تقوم به هذه الخمائر في فساد السمان. تم جمع ١٠٠ عينة من السمان من أسـواق ومحـلات السوبر ماركت بمحافظة الإسماعيلية ٥٠ عينة من كل نوع وقد تبين من الفحـص أن العـدد الكلـي للخمائر في عينات السمان الطازج و المجمد كانت تتراوح من (١٠×٤^{٢}-١٠×٥^{٥}) ومـن (١٠×٦ -١٠×٣^{٣}) فـي عينات السمان الطازج و المجمد علي التوالـي بيـنما كـان متوسـط العدد الكلي للخمائر في نفس العينات ١٠×٥^{٣} و$١٠\times٦,٣^{٣}$ مسـتعمرة/جـرام. كما تم تصنيف الخمائر المعزولة من هذه العينات وكانت عبارة عن ستة أنـواع وهـي الكـانديدا والكريبـتوكوكاس وديباروميس ورودرتوريول او تريكوسبورون وياروبـا ولقد كانت ياروبا ليبولتيكا وكانديدا زيلانوديس وتريكوسبورون بوليولانز هي أكثر الأنواع تواجدا في عينات لحوم السمان حيث تم عزلها بنسبه (٣١,٨، ٢٣,٦ و٨,٣) (٣٧,٢ ، ١٠,٢ و٢٠,٣) علـي التوالـي وبـناءا على ذلك تم اختيار هذه الأنواع لحقن السمان بها وحفظـه عند درجه ٥° م لمده اسبوع وقد تم اخذ عينات يوميا لدراسة مظاهر التلف على السمان المحقون وذلك بالفحص الحسي بالاضافه إلى قياس نسبه المواد النيتروجينية الطيارة وكانـت أقـوي الأنواع هي ياروبا ليبوليتكا حيث ظهرت علامات الفساد بعد ثلاثه ايام من التخزيـن وكانت نسبه النيتروجين أعلى من الأنواع الاخري ولقد أتضح من هذه الدراسة إن الخمائـر تلعب دورا في تلف السمان. وقد وضعت التوصيات الضروريه لتقليل التلوث بتلك الميكروبات.

SUMMARY

This study was undertaken to determine the yeast population on fresh and frozen quail, and to assess its potential role in quail meat spoilage. One hundred samples 50 each of fresh and frozen quail carcasses were examined for total yeast counts, types and the frequency of the isolated yeast species. Populations of yeasts (cfu/gm) ranged from 4×10^2 to 5×10^5 and from 6×10 to 3×10^3 in fresh and frozen quail samples

respectively. The mean values were 4.6x 10^3 and 6.3 x 10^2 in fresh and frozen quail samples respectively. A total of 131 cultures of yeasts were isolated from the examined quail samples. The isolates were six genera *Candida, Cryptococcus. Debaromyces, Rhodotorula, Trichosporon* and *Yarrowia*. These genera were included 14 different species .*Yarrowia lipolytica, Candida zeylanoides* and *Trichosporon pullulans* were predominant making up (31.8,23.6, and 8.3%) and (37.2, 10.2 and 20.3%) from fresh and frozen quail samples respectively. The spoilage of inoculated quail samples with the predominant yeast species stored at 5^0C was assessed by detection of the organoleptic changes (odour, consistency and slime formation), measurement of the pH and determination of the total volatile nitrogen. *Y. lipolytica* caused spoilage with strong ammoniacal odour and softening after three days of storage. Moreover it produced the highest values of total volatile nitrogen among the tested strains (56.2mg/100gm). The obtained results suggested that yeasts may play a prominent role in the spoilage of quail meat.

Key words: *Quail meat, yeast, spoilage*

INTRODUCTION

Yeasts contribute a small, but permanent part of the natural microflora of meat, the ability of some yeast to grow at low temperatures enables them to proliferate in refrigerated meats (Barnes *et al.,* 1978). Furthermore yeast can utilize many substrates as carbohydrates, proteins and lipids (Peppler 1977), Proteolytic and lipolytic yeast species are widely distributed among the genera *Candida*, *Trichosporon,* and *Rhodotorula* (kobatake and kurata 1983; Ismail *et al.,* 2000).

Hence the generation time for a yeast population to double may approximate 2 hours under ideal conditions, low population of the right yeast species can increase fantastically and cause food spoilage (Walker 1977).

Although numerous studies have been reported on the bacterial flora or the bacterial spoilage of quail (Ghoneim *et al.,* 1980, Renu - kumavi and Mushtari –Begun 1991, Sousa and Oliveria - Lima 1993, Mostafa 1997, Saleh *et al.,* 2002) there are few reports concerning yeast flora. Noumain *et al.,* 1980 isolated yeast from 91% of the examined migrating birds. The yeast counts reported by Mustafa 2001 were 3.7× 10^5 and 1.3×10^6 from frozen and fresh quail respectively. A more recent survey of slaughtered quail at north Sinai governorates revealed mean yeast count of 5.4×10^3 and 5.5×10^2 for fresh and frozen quail

respectively (Mohsen, 2005). Little information is available on the yeast species isolated and identified from quail and also the role of yeast in spoilage of quail.

The present study was undertaken to determine the incidence of yeasts in quail and their potential contribution in the spoilage of quail meat.

MATERIALS and METHODS

Samples

One hundred samples 50 each of fresh and frozen quail were collected from supermarkets in Ismailia province and transferred to the laboratory where they were subjected to the following analysis.

Sample preparation

The samples were prepared according to the technique recommended by APHA (1992). Each sample (25 gm) was placed in a stomacher bag with 225ml of sterile 0.1% peptone water and pummeled using a stomacher (model 400, Seward Medical, London, UK) at medium speed for 2 minutes, to obtain a dilution of $10^{-1,}$ from which further 10^{th} fold serial dilutions were made up to $10^{-6.}$

1-Determination of total yeast count

The total yeast count was determined using tryptone glucose yeast extract agar supplemented with chloramphenicol (TGYC) as recommended by Deak and Beuchat (1996).

2-Identification of the isolated yeast

Three to five yeast colonies were picked from (TGYC) agar plates and streaked on potato dextrose agar slants (PH 5.6) and incubated at 25°c for 3-5 days. The isolates were held at 5°c until identified according to the simplified identification method recommended by Deak and Beuchat (1996) and Yarrow (1998).

3-Spoilage assessment

The predominant yeast species *Yarrowia, lipolytica Candida zeylanoides* and *Trichosporon pullulans* were chosen for this assessment.

A) Preparation of yeast inoculums

Five strains from each yeast species were used. The strains were sub cultured by loop inoculation in 10 ml volumes of tryptone glucose yeast extract broth and then incubated at 30°c for 24 hours. The cell suspension was prepared by combining 5 ml of each culture with two liters of sterile distilled water.

b) Inoculation and sampling

Forty samples of fresh quail meat approximately 50 gm each were autoclaved at 121°c for 20 minutes, then the sterilized samples were submerged in the yeast suspension and gently mixed for 5 minutes and then drained on a sterile wire screen, before placing in plastic bags, sealing and storing at 5°c for up to one week, the control group was submerged in sterile distilled water.

The following tests were performed daily up to one week to detect the spoilage:

1) Determination of pH values according to the method recommended by Dodge and Stadelmen (1960)

2) Sensory spoilage detection

The extent of spoilage (odor, consistency, slime formation) of each of the stored samples was observed by 3 laboratory panelists.

3) Total volatile base nitrogen determination

The total amounts of volatile basic nitrogen produced in inoculated samples were determined by Conway's microdiffusion technique recommended by Lees (1975)

RESULTS

Table 1: Yeast counts (cfu/ gm) of fresh and frozen quail samples (N=50 of each).

	Fresh quail	Frozen quail
Minimum	4×10^2	6×10
Maximum	5×10^5	3×10^3
Mean	4.6×10^3	6.3×10^2
±S.E	2×10^2	1.5×10

Table 2: Frequency distribution of yeast species identified from fresh and frozen quail samples.

Yeast Species	Fresh		Frozen	
	No.	%	No.	%
Candida Cateneulata	2	2.8	0	0
C. Parapsilosis	5	6.9	3	5.1
C. Zeylanoides	17	23.6	6	10.2
Cryptococcus albidus	2	2.8	2	3.4
Cryptococcus humicolus	2	2.8	4	6.8
C. hungaricus	3	4.2	0	0
C. laurentii	2	2.8	1	1.7
Debaromyces hansenii	2	2.8	2	3.4
Rhodotorula glutinis	3	4.2	3	5.1
R. mucilaginosa	1	1.4	2	3.4
R. rubra	2	2.8	2	3.4
Trichosporon pullulans	6	8.3	12	20.3
Tri. Cutaneum	2	2.8	0	0
Yarrowia lipolytica	23	31.8	22	37.2
Total	72	100	59	100

Table 3: Sensory spoilage of quail samples inoculated with yeast species and stored at 5°C for up to one week. (No. 10 of each).

	Control	C. zeylanoides	Tri. Pullulans	Y. Lipolytica
Zero day	A	A	A	A
1st day	A	A	A	A
2nd day	A	A	A	B
3rd day	A	A	A	C
4th day	A	A	B	C
5th day	A	B	C	
6th day	B	B		
7th day	B	B		

A: No Spoilage
B: Initial spoilage (mild softening of muscle, presence of an odour different from control.)
C: Spoiled (sever softening of muscular tissue, and presence of putrid odor)

Table 4: Total volatile base nitrogen amounts (mg/100gm) in quail samples inoculated with yeast species and stored at 5°C for one week.

	Control	C. zeylanoides	Tri. Pullulans	Y. Lipolytica
Zero day	12	13.00	15.00	15.83
1st day	12.25	13.28	17.00	18.50
2nd day	12.50	14.50	20.00	22.30
3rd day	13.00	15.00	25.80	26.00
4th day	14.00	16.23	28.00	35.00
5th day	15.00	18.00	32.00	38.00
6th day	16.00	21.00	38.00	43.20
7th day	22.25	28.00	43.00	56.20

Table 5: PH values of inoculated quail samples during storage at 5°C up to one week.

	Control	C. zeylanoides	Tri. Pullulans	Y. Lipolytica
Zero time	6.4	6.5	6.4	6.2
1st day	5.8	6.0	6.0	6.1
2nd day	6.0	6.3	6.5	6.6
3rd day	6.2	6. 5	6.7	6.8
4th day	6.2	6.5	6.9	7.0
5th day	6.3	6.7	7.0	7.4
6th day	6.4	6.8	7.1	7.6
7th day	6.6	6. 8	7.2	7.9

Table 6: Yeast count (cfu/gm) of the inoculated samples.

	Control	C. zeylanoides	Tri. Pullulans	Y. Lipolytica
Zero time	$<10^1$	$3x10^5$	$17x10^5$	$30x10^5$
7th day	45x10	$23x10^6$	$31x10^6$	$42x10^7$

DISCUSSION

Yeasts are ubiquitous in nature and are found to be as indigenous component of microflora associated with quail meat (Nouman *et al.*, 1980, El Dengawy and Nasar 2001, Mostafa 2001. The most common sources of yeast contamination originated mainly from feathers and feet of the birds.

1-Total yeast count.

The obtained results in table (1) revealed that the total yeast count / gm in the examined samples of fresh and frozen quail ranged from 4×10^2 to 5×10^5 and 60 to 3×10^3 respectively, with mean values of 4.6×10^3 and 6.3×10^2 in fresh and frozen quail respectively. Similar counts were reported by Mostafa (2001) and Mohsen (2005). The existence of yeast in quail meat indicates poor sanitary conditions prevailed during processing and handling of quail carcasses. Therefore more care during slaughtering and dressing of quail carcasses should be taken to minimize contamination.

2-Identified yeast

The frequency distribution of yeast species identified from fresh and frozen quail was shown in Table (2). A total of 131 strains of yeast were isolated from fresh and frozen quails and identified to consist of 14 species. *Yarrowia lipolytica, Candida zeylanoides* and *Trichosporum pullulans* were predominant making up 31.8, 23.6 and 8.3 % isolates from fresh quail and 37.2, 10.2, and 20.3% of the isolates from frozen quail samples. Diriye *et al.* (1993) reported that the yeast isolates from frozen chicken carcasses were *C. zeylanoides* 58.3%, *Y. lipolytica*16.7% and *R. rubra* 16.7%., *Cry. laurentii* and *C. zeylanoides* were recovered from frozen turkey carcasses (Barnes *et al.*, 1978). The persistence of *C. zeylanoides*, *Tri- Pullulans* and *Y. lipolytica* in this study is attributed to there ability to grow at low temperature.

Other yeast species detected in fresh and frozen quail with different percentages as shown in Table (2) of these species *C. parpsilosis*, *C. zeylanoides Tri-cutaneum. Tri-pullulans,* have shown to posses lipolytic activity as reported by Johansen *et al.* (1984) while *D. hansenii* and *R. rubra* show proteolytic activity (kobatake *et al.*, 1992), more over *Tri. pullulans* and *Y. lipolytica* have both proteolytic and lipolytic activity (Guerzoni *et al.*, 1992) *Y. lipolytica* was the most active proteolytic species (kobatake and kurata 1983, Ismail *et al.*, 2000).

Spoilage of quail inoculated with yeast species.

Signs of spoilage such as softening of muscles, presence of abnormal odour and slime formation were observed upon storage of inoculated samples at 5°c for up to one week while the control samples were stiff, has characteristic fresh odor of meat.

The results shown in Table (3) revealed that spoilage caused by *Candida zeylanoides* appeared on the 5th day of storage as an odour differ from control with small amount of slime on the 6th day, *Tri. pullulans* inoculated samples spoiled with weak putrid odour and small amount of slime on the 4th day at 5°c. The inoculated samples by *Y. lipolytica* spoiled with strong ammoniacal odour on the 3rd day and liquefying on the 4th day. It is clear from the obtained results that *Y. lipolytica* was the most active species and produce detectable spoilage earlier than the other 2 species and this may be attributed to that *Y. lipolytica* has strong proteolytic and Lipolytic activity also it is a psychotropic yeast that grow rapidly at 5°C. These results were in line with those reported by kobatake *et al.* (1988, 1992) and Ismail *et al.* (2000).

Since proteolytic, psychotropic yeasts have different spoilage patterns and widely distributed in fresh and frozen quail, it is necessary when considering microbial control of quail to give full consideration to spoilage yeasts.

Total volatile base nitrogen (T.V.B N.) production by yeast.

The obtained results in Table (4) revealed that, at 7th day of storage the higher values of T.V.B.N produced by *Y. lipolytica* (56.2 mg/100) followed by *Tri. Pullulans* (43.0 mg /100gm). The lowest amount produced by C. zeylanoides (28.0 mg/100gm), compared with 22.25mg/100gm in the control group. This may be attributed to the strong proteolytic activity of *Y. lipolytica*, while *C. zeylanoides* has only lipolytic activity (kobatake *et al.*, 1988, 1992, Ismail *et al.*, 2000). The acceptable limit of T.V.B.N. according to the Egyptian Standards Specifications (E.S.S.) 1996 not more than 20mg/100gm.

From the above mentioned results it can be concluded that psychrotrophic, proteolytic yeasts particularly *Tri. pullulans* and *Y. lipolytica* play a role in spoilage of quail meat and consequently control of yeast growth in quail meat is necessary.

pH values

The achieved results in Table (5) revealed that the pH values of the inoculated samples increased to (6.8, 7.2 and 7.9) in quail samples inoculated with *Candida zylanoides, Trichosporon pullulans* and

Yarrowia lipolytica respectivly after one week of storage at 5 ^{0}C while the pH of the control group was 6.6. The increase of the pH values may be probably the result of an increase of basic substances as amines and amonia produced by the degradation of protein by the yeast. Over all, it's clear that there is a proportional relationship among yeast growth, T.V.B.N. production, pH values and spoilage produced by the tested yeast species.

From the achieved results in this study, it could be concluded that yeasts are substantially represented in the total microbial ecology of quail meat; indicating poor sanitary conditions prevailed during handling of quail carcasses and recommended more care during slaughtering and dressing of carcasses to minimize yeast contamination. Since yeasts have a significant contribution to lipolytic and proteolytic changes in a food even in small count this fact highlights the major role of yeast in spoilage of quail meat causing undesirable changes as off odors, off flavors and slime formation particularly in certain conditions as low initial numbers of bacteria in relation to yeast or restricted bacterial growth. The study also demonstrate the need for qualitative and quantitative estimation of yeast when controlling the microbial quality of quail meat

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