

Mycological quality of chicken carcasses and extending shelf -life by using preservatives at refrigerated storage

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ABSREACT

The objective of the present study was to evaluate the mycological quality of chicken carcasses and trial for extend shelf life of fresh refrigerated chicken meat using some preservative. A total of 50 random samples of chicken carcasses was collected from student campus of Benha University. The samples were taken aseptically in polyethylene bags without undue delay; they were transferred to the laboratory in ice box and mycologically examined.

The results revealed that all the examined samples were physically accepted but had different scores vary from, excellent to good. The mean pH values of chicken carcasses were 6.0 ± 0.04 . The mean value of total fungal and yeast count (TFC /cm²) in examined chicken carcasses were $6.7 \times 10^2 \pm 1.1 \times 10^2$ and $2.9 \times 10^2 \pm 7.6 \times 10^1$, respectively. In the examined samples, 8 mould and yeast genera could be identified. The identified mould and yeast belonged to the following genera were *Aspergillus* spp. (*A. candidus*, *A. flavus*, *A. fumigatus*, *A. niger* and *A. ochraceus*), *Penicillium* spp. (*P. aethiopicum*, *P. citrinum*, *P. corylophilum*, *P. decumbence*, *P. griseofulvum* and *P. oxalicum*), *Cladosporium* spp., *Fusarium* spp., *Mucor species*, *candida* spp., *Rhodotorula* spp. and *Torulopsis* spp. The chicken carcasses were packed into plastic bags after sprayed with potassium sorbate (2% & 2.5%), soaked in H₂O₂ (0.1% & 0.5%) and sprayed with natamycin (0.1% and 0.2%). The samples were stored in refrigerator at 4°C and examined after preparation and after 5 days. The obtained result showed that natamycin (0.2%) cause high reduction percent in the total fungal and yeast count in chicken carcasses than other antifungal used.

Key word: chicken carcasses, preservative, mould, yeast

INTRODUCTION

Chicken meat considered an excellent source of high quality animal protein, containing good balanced essential amino acids and it's a good source of most B-complex vitamins, and also contributes significant percentage of a number of minerals including iron, copper, zinc, sodium, potassium and magnesium. On the other side, chicken meat is ease of cooking and serving and of low cost than red meat of cattle. Also, poultry fat content is almost lower than fat content of beef (Zhang *et al.*, 2001 and Akl 2002). Microscopic filamentous fungi often contaminate vegetal and animal products, becoming a source of diseases for human and slaughter animals (Saleem 2008). It is of great magnitude to prevent the growth of these toxic moulds in food items and interfere with the production of mycotoxin to ensure human safety (Davidson and Parish 1989). There is increased interest in development and use of preservatives to preserve meat quality for longer shelf life periods while maintaining food safety. The food preservatives restrict microbial activity, enzymatic, chemical and physical reaction that cause deterioration and spoilage of meat and meat products. Meat preservation works by lowering the amount of substances in meat that microbes prefer to grow on. In the food industry, potassium sorbate and hydrogen peroxide (H₂O₂) and natamycin are often used as preservatives. Potassium sorbate is a naturally occurring unsaturated fatty acid and is completely safe with regard to health and has the lowest allergenic potential of all food preservatives (Alrabadi *et al.*, 2013). Hydrogen peroxide (H₂O₂) is a colourless aqueous solution, it has the same chemical

structure of water, but with extra oxygen molecule, it acts as powerful effective safe oxidant. H₂O₂ is highly unstable and breaks down into water and single oxygen molecule, oxygen is stable only when the molecules are pairs (O₂) (**Black *et al.*, 2008**). Natamycin is an effective antimicrobial preservative against yeasts and moulds, exhibiting a wide spectrum of activity and effectiveness at very low concentration. Natamycin has strongcidal activity towards susceptible microorganisms and is particularly effective against fungi, which may produce mycotoxins (**Food Standards, 2004**).

The present study was planned aiming for mycological evaluation of chicken carcasses and extended shelf life of fresh refrigerated chicken meat by using potassium sorbate, H₂O₂ and natamycin.

MATERIAL AND METHODS

Collection of samples

A total of 50 random samples of chicken carcasses was collected from student campus of Benha University. The samples were taken aseptically in polyethylene bags without undue delay; they were transferred to the laboratory in ice box and mycologically examined.

Sensory evaluation

Sensory raw chicken meat quality evaluation was performed and evaluated by three experienced panel tasters using taste panel score expressed in hedonic scales included scoring of colour and flavour and texture of the collected samples were examined according to **Anna (1998)** by using the 9-point hedonic scale.

Table (1): The scale points for meat quality evaluation:

Score	Grade		Acceptability
1	Excellent	9	Acceptable
2	Very good	8	
3	Good	7	
4	Acceptable	6	Low rate of acceptability
5	Poor	< 6	First off odour and taste

Determination of pH

pH value was measured according to the technique recommended by **Allen *et al.* (1997)**

Fungal isolation and identification

The collected samples were prepared according to the technique recommended by **APHA (2002)**. Determination of total mould and yeast count according to (**ISO, 2008**). The isolated mould were identified according to macro and microscopic characteristics as described in (**Pitt and Hocking, 2009**). While yeast isolates identifications were performed by using rapid miniaturised system API 20 C AUX (bioMérieux, France). Some complementary tests used for final identification of the isolates as recommended by (**Kurtzman *et al.*, 2003**).

Antifungal activity

A grand total number of 21 broiler carcasses from poultry slaughter shop in Cairo Egypt was collected. It was expected to have a high degree of similarity after complete preparation (Slaughtering-scalding-defeathering-evisceration). The collected broiler carcasses were classified into 7 groups as follow:

- 1st group, 3 broiler carcasses were soaked in sterilized distal water as control.
- 2nd group, 3 broiler carcasses were sprayed with sterilized distal water containing Potassium sorbate (2%).
- 3rd group, 3 broiler carcasses were sprayed with sterilized distal water containing Potassium sorbate (2.5 %).
- 4th group, 3 broiler carcasses were soaked in sterilized distal water for 60 min containing aqueous solution of 0.1% hydrogen peroxide.

5th group, 3 broiler carcasses were soaked in sterilized distal water for 60 min containing aqueous solution of 0.5% hydrogen peroxide.

6th group, 3 broiler carcasses were sprayed by sterilized distal water containing natamycin (0.1%).

7th group, 3 broiler carcasses were sprayed by sterilized distal water containing natamycin (0.2%).

Both of treated and control samples were drained for 10 min. then packed into polyethylene bags, labelled and stored at 4 °C. Mycological analysis (total fungal and yeast) was conducted after preparation (zero time) and 5 days during storage using the serial dilution and pour plate technique (**Pitt and Hocking, 2009**).

Results and Discussion

It is evident from the results in table (2) that the mean value of colour and odour of the examined chicken carcasses samples were 7.8 ± 0.14 and 8.4 ± 0.12 , respectively. From above mentioned results, it is clear that all the examined samples were physically accepted but has different scores vary from, excellent to good. The abnormal appearance of some examined samples may be attributed to deteriorative changes and high microbial levels (**Gracey & Collins 1992 and Miller, 1994**). In addition, surface discoloration and offensive odour are an anomaly characteristic of incipient microbial spoilage and any organoleptically detectable spoilage is usually attributed to microbiological induction.

The obtained data recorded in table (3) revealed that the mean pH values of chicken carcasses 6.0 ± 0.04 . It is noticed that the mean pH value of the examined chicken carcasses come in agreement with that reported by **Mahmoud and Hamouda (2006)** and **Ali et al. (2015)** who recorded that the mean pH value of the skinned and non-skinned examined breast samples was 5.83 ± 0.01 , 6.29 ± 0.01 , 6.25 ± 0.01 and 5.82 ± 0.01 , 6.26 ± 0.01 , 6.68 ± 0.01 , respectively. While it was 5.79 ± 0.01 , 6.11 ± 0.01 , 6.84 ± 0.01 and 5.77 ± 0.01 , 6.14 ± 0.01 , 7.31 ± 0.01 for the skinned and non-skinned thigh samples, respectively. High figure of pH values of chicken carcasses was obtained by **Atya (2007)** who reported that the mean value of the pH value ranged from 6.5 to 7.5 with a mean value of 6.8.

Moreover, the results given in table (4) illustrated that the total fungal count (TFC /cm²) in examined chicken carcasse samples were 1×10^1 to 2.6×10^3 with mean value $6.7 \times 10^2 \pm 1.1 \times 10^2$. The obtained results were nearly similar to those reported by **Yehia (2003)** and **Altalhi and Albashan (2004)** who reported that the maximal mould counts for fresh chicken meat was 3.1×10^2 . Meanwhile, higher results were recorded by **Eldaly et al., (2002)**, **Agamy and Hegazy (2011)**, **EL-Kewaley et al. (2014)** and **Mohamed (2014)** who reported that the mean level of count was 1.7×10^4 with a minimum value of 5×10^2 to 8.8×10^4 cfu / cm² as a maximum value of the isolated mould from examined chicken carcasses samples.

The total fungal count is used as an index of the proper sanitation and high quality product. Moulds can assist in the putrefactive processes and in other cases they may impart a mouldy odour and taste of foodstuffs. In addition, mould can grow over an extremely wide range of temperature; therefore, one can find mould on particularly all foods at almost any temperature under which foods are held. Besides, mould can assist in the putrefactive processes and may produce toxic metabolites, namely mycotoxins, which are harmful to man and animal (**Algabry et al., 2010**).

The data obtained from table (5) declared that the examined samples were contaminated with many fungal genera. The predominant mould genera isolated from chicken carcasses were *Aspergillus* species (*A. candidus*, *A. flavus*, *A. fumigatus*, *A. niger* and *A. ochraceus*), *Penicillium* species (*P. aethiopicum*, *P. citrinum*, *P. corylophilum*, *P. decumbence*, *P. griseofulvum* and *P. oxalicum*), *Cladosporium* species, *Fusarium* species and *Mucor* species. Nearly similar isolates were recorded in chicken carcasses and meat carcasses by **Abd-Elrahman et al., (2013)**; **Hassan (2013)**; **Samaha (2013)**; **El-Diasty et al., (2013)** and **Mekled (2015)**.

Regarding the results recorded in the table (6) it is obvious that the *Candida* spp., *Rhodotorula* spp. and *Torulopsis* spp. were the most frequent yeast species isolated from the

examined chicken carcasse samples. Poultry spoilage is mainly restricted to the surface of the carcass, and the processing equipment is the major source of contamination. Also reported that the presence of high and diverse populations of yeasts in the trachea of chickens, already adapted to the habitat, and they may contribute to the spoilage of poultry meat after slaughtering (Deák, 2008).

Consequently, food manufacturers have developed food processing treatments that help preserve foods, by destroying the microorganisms that are present or by injuring them and thus preventing their growth. There are many sites within a microorganism cell that can become damaged when the microorganisms are subjected to these food processing treatments. These sites include the genetic material of the cell (DNA, RNA) and also the cell membrane different kinds of preservatives are used to prevent biodeterioration of food products (Stanojevic *et al.*, 2009). In the food industry, potassium sorbate and hydrogen peroxide (H_2O_2) and natamycin are often used as preservatives.

Table (7) illustrated that potassium sorbate (2 %), potassium sorbate (2.5 %), hydrogen peroxide (0.1%), hydrogen peroxide (0.5%), natamycin (0.1%) and natamycin (0.2%) could reduce mould and yeast count from $1.3 \times 10^3 \pm 2.0 \times 10^2$; $4.6 \times 10^2 \pm 3.6 \times 10$; $4.8 \times 10^3 \pm 1.8 \times 10$; $1.2 \times 10^3 \pm 2.6 \times 10^2$; $1.1 \times 10^3 \pm 2.3 \times 10^2$; $2.4 \times 10^2 \pm 2 \times 10$ and $1.1 \times 10^2 \pm 3.7 \times 10$ after one hours days, respectively. A very high reduction percent in the total mould and yeast count by using of natamycin (0.2%) and natamycin (0.1%) were 91.5 % and 81.5%, respectively. While, moderate reduction in the total mould and yeast count by using of potassium sorbate (2%), potassium sorbate (2.5%) were 63% and 64.4% and little reduction in the total mould and yeast count as a result of addition of hydrogen peroxide (0.1 %), hydrogen peroxide (0.5 %) were 7.9% and 15.4% into broiler carcasses (Table 8). Mostafa (2010) reported that hydrogen peroxide had a limited effect on the reduction of total mould and yeast count (9.53%). But mentioned that using hydrogen peroxide remove 97.30% from the total bacteria contaminating broiler carcasses and 86.3% of coliform and 94.9% from *Staphylococcus aureus*. As general, hydrogen peroxide can reduced 72.01% from the all microorganisms contaminating broiler carcasses in addition to its harmless effect as hydrogen peroxide breaks down in the chiller water producing water and free oxygen which is powerful oxidizing agent that have a great effect in killing the microorganisms.

Results achieved in table (9) declared that potassium sorbate (2 %), potassium sorbate (2.5 %), hydrogen peroxide (0.1%), hydrogen peroxide (0.5%), could reduce mould and yeast count from $1.1 \times 10^4 \pm 6.7 \times 10^3$; $9.2 \times 10^2 \pm 3.1 \times 10^2$; $7.8 \times 10^2 \pm 1.1 \times 10^2$; $1 \times 10^4 \pm 6 \times 10^3$; $9.4 \times 10^3 \pm 4.8 \times 10^3$, respectively. Meanwhile, in case of natamycin (0.1%) and natamycin (0.2%) the mould and yeast growth not detected. Natamycin is an effective antimicrobial preservative against yeasts and moulds, exhibiting a wide spectrum of activity and effectiveness at very low concentrations. Natamycin has strong cidal activity towards susceptible microorganisms and is particularly effective against fungi, which may produce mycotoxins and create public health hazard (El-Diasty *et al.*, 2009).

A highly reduction percent in the total mould and yeast count by using of natamycin (0.2%) and natamycin (0.1%) were 100 % and 100%, respectively. While, moderate reduction in the total mould and yeast count by using of potassium sorbate (2%), potassium sorbate (2.5%) were 49.7% and 52% and little reduction in the total mould and yeast count as a result of addition of hydrogen peroxide (0.1 %), hydrogen peroxide (0.5 %) were 9% and 17.7% into broiler carcasses (Table 10).

Conclusion

From the present study, it was concluded that a wide range of moulds and yeasts coming from different sources is introduced to the surfaces of meat which contain abundant nutrients and have a high water availability. The obtained result showed that natamycin (0.2%) cause high reduction percent in the total fungal and yeast count in chicken carcasses than other antifungal used.

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الملخص العربي

الجودة الفطرية لذبائح الدواجن ومد فترة الصلاحية باستخدام المواد الحافظة في درجة حرارة التبريد

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**قسم الفطريات بمعهد بحوث صحة الحيوان بالدقي

***المدينة الجامعية بجامعة بنها

في الدراسة الحالية تم جمع عدد ٥٠ عينة بطريقة عشوائية من الدواجن واللحوم الطازجة المستلمة بالمدن الجامعية بجامعة بنها . هذه العينات تم فحصها لتواجد الفطريات المختلفة بها بالإضافة الى ذلك تم تقييم التأثير المضاد للفطريات لمادة الناتاميسين وسوربات البوتاسيوم بالإضافة الى مادة فوق أكسيد الهيدروجين في الدواجن الطازجة. وقد أوضحت النتائج أن العدد الكلي للفطريات (مستعمرة / سم^٢) في عينات الدواجن: هي: ١ × ١٠ إلى ٢,٦ × ١٠^٢ بينما كان العدد الكلي للخمائر هي: ٢,٩ × ١٠ ± ١٠ × ١٠^٢).

وقد تم تصنيف أنواع الفطريات المعزولة الى جنس الأسبرجيليس ١ الى ٥ أنواع وكانت كالتالي (الأسبرجيليس فلافس ، الأسبرجيليس نيجر ، الأسبرجيليس فيوميغاتس ، الأسبرجيليس كانديدس ، الأسبرجيليس أوكراشيس) ، كانت أنواع البنسيليوم

المعزولة (بنسيليوم ديكمبنس ، بنسيليوم كوريلوفيلم وبنسيليوم أوكساليكوم وبنسيليوم جريسفيلم وبنسيليوم سترنم وبنسيليوم اثيوبكيم) ، جنس كلادوسبوريم ، جنس فيوزاريم وبنسيليوم ميوكور وبنسيليوم الكانديدا وبنسيليوم الودوترولا وبنسيليوم تويولبسييس. ولما كان للتلوث الفطري من تأثير سلبي سواء جودة لحوم الدواجن وزيادة فرص تعرضها للفساد بالإضافة إلى الآثار الصحية الناتجة عن تناول تلك لحوم الدواجن الملوثة بتلك الفطريات لذا كانت هناك عدة محاولات لتقليل التلوث الفطري في لحوم الدواجن وذلك عن طريق استخدام المواد الحافظة التي تساعد في حفظ الدواجن وتقليل التلوث الفطري لها ومن هذه المواد الناتاميسين ، سوربات البوتاسيوم و فوق أكسيد الهيدروجين. وقد أوضحت نتائج التجربة مايلي: تفوق مادة الناتاميسين بتركيز (٠,٢%) و تركيز (٠,١%) في خفض العدد الكلي للميكروبات حيث كانت نسبة الخفض ١٠٠% و ١٠٠% على التوالي حيث لم يتم عزل أى من الفطريات أو الخمائر بينما تلتها مادة سوربات البوتاسيوم (٢%) و (٢,٥%) حيث تم خفض الميكروبات من بنسب ٤٩,٧% و ٥٢% على التوالي فيما تبين من النتائج أن نسبة الخفض للعدد الكلي للفطريات عند استخدام مادة فوق أكسيد الهيدروجين بتركيز (٠,١%) و فوق أكسيد الهيدروجين بتركيز (٩%) كانت (١٧,٧%) و (٩%) على التوالي. وقد تم مناقشة تأثير تلك الفطريات والخمائر على صحة الانسان والاهمية الصحية للسموم الفطرية و أيضا تم مناقشة الأهمية الاقتصادية لكل من الفطريات والخمائر التي تم عزلها من عينات الدواجن.

Table (2): Statistical analytical values of sensory evaluation of the examined samples of chicken carcasses (N=50).

Parameter	Min.	Max.	Mean \pm SE
Colour	4.7	9.0	7.8 \pm 0.14
Odour	5.3	9.0	8.4 \pm 0.12

Table (3): Statistical analytical results for pH values of the examined sample

Examined samples	Min.	Max.	Mean \pm SE
Chicken carcasses	5.4	6.4	6.0 \pm 0.04

Table (4): statistical analytical results of total mould & yeast counts /cm2 in examined samples

	Min.	Max.	mean \pm SE.
Total mould count	1 \times 10 ¹	2.6 \times 10 ³	6.7 \times 10 ² \pm 1.1 \times 10 ²
Total yeast count	1 \times 10 ¹	2.8 \times 10 ³	2.9 \times 10 ² \pm 7.6 \times 10 ¹

Table (5): Incidence of mould species isolated from examined samples (N=50)

Mould species	Chicken carcasses	
	No.	%
Aspergillus species		
<i>A. candidus</i>	6	12
<i>A. flavus</i>	26	52
<i>A. fumigatus</i>	8	16
<i>A. niger</i>	6	12
<i>A. ochraceus</i>	4	8
Penicillium species		
<i>P. aethiopicum</i>	3	6
<i>P. citrinum</i>	3	6
<i>P. corylophilum</i>	3	6
<i>P. decumbence</i>	4	8
<i>P. griseofulvum</i>	3	6
<i>P. oxalicum</i>	2	4
<i>Cladosporium species</i>	2	4
<i>Fusarium species</i>	5	10
<i>Mucor species</i>	4	8

Table (6): Incidence of yeast species isolated from examined samples (N=50)

Yeast species	Chicken carcasses	
	No.	%
Candida spp.	20	40
Rhodotorula spp.	10	20
Torulopsis spp	3	6

Table (7): Statistical analytical results of total mould & yeast count /cm² on the swabs of control and treated surfaces after preparation.

Treatments	Min.	Max.	Mean \pm SE
Control (untreated)	6.3×10^2	2×10^3	$1.3 \times 10^3 \pm 2.0 \times 10^2$
Potassium sorbate (2%)	4.5×10^2	5×10^2	$4.8 \times 10^3 \pm 1.8 \times 10$
Potassium sorbate (2.5%)	3.9×10^2	5×10^2	$4.6 \times 10^2 \pm 3.6 \times 10$
Hydrogen peroxide (0.1 %)	7.2×10^2	1.6×10^3	$1.2 \times 10^3 \pm 2.6 \times 10^2$
Hydrogen peroxide (0.5 %)	6×10^2	1.3×10^3	$1.1 \times 10^3 \pm 2.3 \times 10^2$
Natamycin (0.1%)	2×10^2	2.7×10^2	$2.4 \times 10^2 \pm 2 \times 10$
Natamycin (0.2%)	5×10	1.8×10^2	$1.1 \times 10^2 \pm 3.7 \times 10$

Table (8): Reduction % of surface mould & yeast count of treated chicken carcasses after preparation

Treatment	Control (untreated)	Potassium sorbate (2%)	Potassium sorbate (2.5%)	Hydrogen peroxide (0.1 %)	Hydrogen peroxide (0.5 %)	Natamycin (0.1%)	Natamycin (0.2%)
Mean \pm SE	$1.3 \times 10^3 \pm 2.0 \times 10^2$	$4.8 \times 10^2 \pm 1.8 \times 10$	$4.6 \times 10^2 \pm 3.6 \times 10$	$1.2 \times 10^3 \pm 2.6 \times 10^2$	$1.1 \times 10^3 \pm 2.3 \times 10^2$	$2.4 \times 10^2 \pm 2 \times 10$	$1.1 \times 10^2 \pm 3.7 \times 10$
Reduction percentage	-	63%	64.4%	7.9%	15.4%	81.5%	91.5%

Table (9): Statistical analytical results of total mould & yeast count /cm² on the swabs of control and treated surfaces after 5 days.

Treatments	Min.	Max.	Mean \pm SE
Control (untreated)	1.2×10^3	2.5×10^4	$1.1 \times 10^4 \pm 6.7 \times 10^3$
Potassium sorbate (2%)	6.2×10^2	1.5×10^3	$9.2 \times 10^2 \pm 3.1 \times 10^2$
Potassium sorbate (2.5%)	4.5×10^2	1×10^3	$7.8 \times 10^2 \pm 1.1 \times 10^2$
Hydrogen peroxide (0.1 %)	1.3×10^3	2.2×10^4	$1 \times 10^4 \pm 6 \times 10^3$
Hydrogen peroxide (0.5 %)	1.3×10^3	1.8×10^4	$9.4 \times 10^3 \pm 4.8 \times 10^3$
Natamycin (0.1%)	ND	ND	ND
Natamycin (0.2%)	ND	ND	ND

Table (10): Reduction % of surface mould & yeast count of treated chicken carcasses at 5 day.

Treatment	Control (untreated)	Potassium sorbate (2%)	Potassium sorbate (2.5%)	Hydrogen peroxide (0.1 %)	Hydrogen peroxide (0.5 %)	Natamycin (0.1%)	Natamycin (0.2%)
Mean \pm SE	$1.3 \times 10^3 \pm 2.0 \times 10^2$	$9.2 \times 10^2 \pm 3.1 \times 10^2$	$7.8 \times 10^2 \pm 1.1 \times 10^2$	$1.0 \times 10^4 \pm 6 \times 10^3$	$9.4 \times 10^3 \pm 4.8 \times 10^3$	ND	ND
Reduction percentage	-	49.7%	52%	9%	17.7%	100%	100%

