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Incidence of Psychotropic bacteria in frozen chicken meat products with special reference to Pseudomonas species

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ABSTRACT

Total of hundred random samples of frozen chicken products represented by breast, thigh, nuggets and burger (25 of each) were collected from various supermarkets located in Menoufia government. To study the incidence of psychotropic bacteria with special reference to Pseudomonas species. The study revealed that mean values of Psychotropic count were $8.17\times10^3\pm 1.42\times10^3$, $1.95\times10^4\pm 2.06\times10^4$, $3.63\times10^4\pm 0.89\times10^4$ and $7.58\times10^4\pm 1.16\times10^4$ respectively while the mean value of Pseudomonas counts were $3.51\times10^3\pm 0.76\times10^3$, $6.29\times10^3\pm 1.12\times10^3$,$8.44\times10^3\pm 1.85\times10^3$, $1.71\times10^4\pm 0.36\times10^4$ respectively for examined frozen breast, thigh, nuggets and burger. It was obvious that 166 isolates were identified as *P. acidovorans*, *P. aeruginosa*, *P. alcaligenes*, *P. cepacia*, *P. fluorescens*, *P. fragi*, *P. proteolytica*, *P. putida*, *P. putrefaciens*, *P. stutzeri*, *P. vesicularis.* The prevalence of Pseudomonas were 6, 1, 33, 8, 47, 10, 19, 3, 22, 2 and 15 from examined samples, respectively, where the highest contaminated product was the chicken burger 54/166 (32.5%) Pseudomonas isolates and *Ps. Fluorescens* was the most detected isolate. The isolation of Pseudomonas species from food samples is highly significant Therefore, its presence should be prevented during earlier stages of food preparation.

1. INTRODUCTION

Chicken meat is considered a highly nutritive food with a relatively cheap price and low fat and cholesterol content, consumed worldwide. However, it is highly perishable, and its storage life is relatively short even refrigerated temperature (Mantilla et al., 2011). Chicken meat has a short shelf life because psychrotrophic bacteria causes spoilage or off-flavors even at cold storage conditions (Carrizosa et al., 2017). The spoilage of meat depends on pH level, availability of oxygen, biodiversity of bacterial groups, and storage temperature (Ercolini et al., 2010). These factors, in turn, are closely associated with the growth of spoilage bacteria. The abuse of temperature control and poor food handling could encourage the growth of microorganisms which leads to contamination and spoilage of food (Gour et al., 2014). Storage temperature, however, is the most important factor that affects the growth of bacteria present in chicken meat. Psychrotrophic bacteria can grow at refrigerated conditions, and temperature can affect various microbial growth parameters including maximum growth rate and total bacterial counts (Mataragas et al., 2006). Pseudomonas spp. is a major psychrotrophic bacterium that produces proteinase and its optimal pH is from 6.5 to 8.0. proteinase hydrolyses chicken protein and cause spoilage (Nowak et al., 2012). Pseudomonas spp. found everywhere and are isolated from a different of sources like drinking water, human beings, plants, and also from a diversity of foods.

Pseudomonas is an aerobic, Gram-negative bacterium that is commonly found in soil. It can grow well in a range of temperature levels, from 2 to 35 °C (Ercolini et al., 2010), and can be easily found in chilled food products, as well as food prepared at room temperature. In the food industry, various foods harbor very diverse *Pseudomonas* species. Most of the isolates have the ability to grow at a low temperature and are capable of secreting enzymes that can affect the overall quality of the food products including cold-stored food (Caldera et al., 2016).

Four species of Pseudomonas, namely, *P. fluorescens*, *P. lundensis*, *P. fragi*, and *P. viridiflava*, are the main cause of food spoilage because these organisms produce enzymes and form a biofilm, thus causing spoilage in refrigerated food (Rawat 2018). For instance, *P. fluorescens* has been associated with spoilage of chicken carcasses. When its population reaches 10^8 cfu/ml, it could cause the production of a strong foul smell (Wang et al., 2014). In addition, *P. fragi* is commonly known to spoil milk and meat (Ercolini et al., 2010). This could also lead to the production of odor and slime in food products. Reusing the ingredients stored at room temperature for few hours pose risk to consumers especially if they are immune compromised (Tsao et al., 2018). Apart from being a spoilage microorganism, Pseudomonas spp. could cause urinary and blood stream infection. This is due to the fact that they develop resistance to certain antibiotics (Golemi-Kotra et al., 2008). Pseudomonas species decreases the storage life of food products and consequently their quality by producing enzymes as proteolytic and lipolytic which are the primary

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reason of food spoilage during storage (Franzetti and Scarpellini, 2007).
Therefore, the current study was carried out to evaluate the incidence of Psychrotrophic bacteria with especial reference to Pseudomonas species.

2. MATERIAL AND METHODS

2.1 Collection of samples:
A total of 100 random samples of frozen chicken products (each weighting 250 gm) represented by breast, thigh, nuggets and burger (25 of each) were collected from different supermarkets located in Menoufia government at different periods of time. Each sample was kept individually in separate plastic bag and was taken directly to the laboratory in an insulated ice box under complete aseptic conditions without undue delay.
The collected samples were examined bacteriologically for determination of their contamination with psychrotrophic and Pseudomonas bacteria.

2.2. Preparation of samples (FDA, 2002):
Under complete aseptic conditions, 25grams of the sample were weighed and transferred into a sterile flask containing 225 ml of sterile peptone water (0.1%). The content of the flask was homogenized for 3 minutes at 14000 rpm then allowed to stand for 5 minutes at room temperature. One ml from the homogenate was transferred into a separate tube containing 9 ml of sterile peptone water (0.1%) from which tenfold serial dilutions was prepared. The prepared samples were subjected to the following examinations:
2.2.1. Determination of Psychrotrophic count (ISO, 2002)

2.2.2 Determination of Pseudomonas count (ISO, 2004)
Accurately, 0.1 ml of each sample homogenate was separately inoculated into duplicate Petri-dishes of Pseudomonas selective agar medium base (HiMedia) supplemented with glycerol and evenly spread. The inoculated plates were incubated at 25 °C for 48 hours after which all developed colonies (greenish yellow colonies) were enumerated. The average count was calculated and recorded.

2.2.3 Identification of isolated Pseudomonas species:
The suspected colonies were purified and subcultured on nutrient agar slopes and incubated at 37°C for 24 hours. The purified colonies were subjected for further identification including morphological and biological identification according to Macfaddin (2000).

2.3 Statistical Analysis:
All the obtained results were statistically analyzed using the analysis of variance (ANOVA test) according to Feldman et al. (2003).

3. RESULTS

3.1. *Psychrotrophic count*
The psychrotrophic count in examined samples of chicken meat products was recorded in table (1) and it was ranging from 2.9×10^3 to 3.1×10^4, 5.4×10^3 to 7.7×10^4, 9.0×10^3 to 1.2×10^5 and 1.1×10^4 to 4.6×10^5 with mean values of $8.17\times10^3\pm 1.42\times10^3$, $1.95\times10^4\pm 2.06\times10^4$, $3.63\times10^4\pm 0.89\times10^4$ and $7.58\times10^4\pm 1.16\times10^4$respectively for the examined frozen breast, thigh nuggets and burger.

Table (1) Statistical analytical results of psychrotrophic counts in the examined samples of chicken meat products (n=25).

Chicken meat products	Min	Max	Mean ± S.E*
Breast	2.9×10^3	3.1×10^4	$8.17\times10^3\pm 1.42\times10^3$
Thigh	5.4×10^3	7.7×10^4	$1.95\times10^4\pm 2.06\times10^4$
Nuggets	9.0×10^3	1.2×10^5	$3.63\times10^4\pm 0.89\times10^4$
Burger	1.1×10^4	4.6×10^5	$7.58\times10^4\pm 1.16\times10^4$

S.E* = standard error of mean

3.2. Pseudomonas counts
The results showed in table (2) manifested that the *Pseudomonas* counts (CFU/g) in the examined samples were varied from 1.0×10^2 to $9.3\times10^3$with mean value of $3.51\times10^3\pm 0.76\times10^3$ for breast,3.0×10^2 to 2.5×10^4 with mean value of $6.29\times10^3\pm 1.12\times10^3$ for thigh, 4.0×10^2 to 3.2×10^4 with mean value of $8.44\times10^3\pm 1.85\times10^3$ for nuggets and 4.0×10^2 to 6.1×10^4 with mean value of $1.71\times10^4\pm 0.36\times10^4$ for burger.

Table (2) Statistical analytical results of Pseudomonas counts in the examined samples of chicken meat products (n=25).

Chicken meat products	Min	Max	Mean ± S.E*
Breast	1.0×10^2	9.3×10^3	$3.51\times10^3\pm 0.76\times10^3$
Thigh	3.0×10^2	2.5×10^4	$6.29\times10^3\pm 1.12\times10^3$
Nuggets	4.0×10^2	3.2×10^4	$8.44\times10^3\pm 1.85\times10^3$
Burger	4.0×10^2	6.1×10^4	$1.71\times10^4\pm 0.36\times10^4$

S.E* = standard error of mean

3.3. Incidence of identified Pseudomonas species.
The identified species of Pseudomonas isolated from the examined samples of frozen chicken products was recorded in table (3) and its incidence rate showed that *Ps. alcaligenes, P. cepacia, P. fluorescens, P. fragi, P. proteolytica, P. putrefaciens, P. vesicularis*. were isolated from 4(16%), 1(4), 9(36%), 2(8%), 5(20%), 4(16%) and 2(8%). The examined samples of chicken breast, *P. acidovorans, P. alcaligenes, P. cepacia, P. fluorescens, P. fragi, P. proteolytica, P. putida, P. putrefaciens, P. vesicularis* were isolated from 1(4%), 7(28%), 2(8%), 11(44%), 4(16%), 3(12%), 1(4%), 7(28%) and 3(12%) from the examined samples of chicken thigh.

Table (3) Incidence of identified Pseudomonas species isolated from the examined samples of chicken meat products (n=25).

Pseudomonas spp.	Chicken meat products							
	Breast		Thigh		Nuggets		Burger	
	No	%	No	%	No	%	No	%
P. acidovorans	0	0	1	4	3	12	2	8
P. aeruginosa	0	0	0	0	0	0	1	4
P. alcaligenes	4	16	7	28	10	40	12	48
P. cepacia	1	4	2	8	2	8	3	12
P. fluorescens	9	36	11	44	12	48	15	60
P. fragi	2	8	4	16	1	4	3	12
P. proteolytica	5	20	3	12	5	20	6	24
P. putida	0	0	1	4	2	8	0	0
P. putrefaciens	4	16	7	28	3	12	8	32
P. stutzeri	0	0	0	0	1	4	1	4
P. vesicularis	2	8	3	12	6	24	4	16

* The percentages were calculated according to number of samples (n=25)

Whereas *P. acidovorans, P. alcaligenes, P. cepacia, P. fluorescens, P. fragi, P. proteolytica, P. vesicularis, P. putrefaciens, P. stutzeri, P. putida* were isolated from 3 (12%), 10 (40%), 2 (8%), 12 (48%), 1(4%), 5(20%), 2 (8%), 3 (12%), 1 (4%) and 6(24%) from the examined

samples of chicken nuggets and P. acidovorans, *P. aeruginosa, P. alcaligenes, P. cepacia, P. fluorescens, P. fragi, P. proteolytica, P. vesicularis, P. stutzeri, P. putrefaciens* were isolated from 2(8%), 1(4%), 12(48%), 3(12%), 15(60%), 3(12%), 6(24%), 8(32%), 1(4%) and 4(16%) from the examined samples of chicken burger.

4. DISCUSSION

The psychrotrophic counts have been always used as a general indicator of the potential shelf life of chicken (Capita et al., 2001). It is distinct from the results which demonstrated in table (1) that higher psychrotrophic counts were recorded by Morshdy et al. (2018). $2.8 \times 10^4 \pm 1.1 \times 10^4$ in frozen pane, Hassanien et al. (2016). $5.71 \times 10^6 \pm 1.44 \times 10^6$ and $4.59 \times 10^6 \pm 1.26 \times 10^6$ in frozen breast and thigh, Azab (2016). recorded that psychrotrophic count was $9.2 \times 10^6 \pm 12.49 \times 10^6$ and $8.5 \times 10^6 \pm 14.61 \times 10^6$ in breast and thigh and Abd EL-Magied et al. (2009). Found that the psychrotrophic count was $1.43 \times 10^5 \pm 0.37 \times 10^5$ /g in breast samples and $4.28 \times 10^6 \pm 0.38 \times 10^6$ /g in wings.

Relatively the same psychrotrophic count were recorded by Eid et al (2014). $11.5 \times 10^3 \pm 2.2 \times 10^3$ in chicken breast and El-kewaiey (2012). was $8.6 \times 10^4 \pm 1.5 \times 10^4$ in chicken nuggets comparatively with Morshdy et al. (2018) who recorded lower results of psychrotrophic count which was $1.9 \times 10^3 \pm 0.9 \times 10^3$ in chicken nuggets. And Dan et al. (2008). Who recorded that the mean value was 2.88±0.32 (log10) cfu/g. The contamination of chicken meat products with great number of psychrotrophic bacteria could be attributed to the neglected sanitary measures adapted during intensive preparation, processing, handling and packaging as well as cold storage. (Cenci et al. 1990).

The findings recorded in table (2) coincide with other studies that recorded relatively the same count of Pseudomonas species in chicken products that were 3.6×10^3 (Morshdy et al., 2018), 2.6×10^4 CFU/g (Abd El-Aziz, 2015), 2.7-3.8 (Bruckner et al., 2012) and 3.6 log cfu/g (Abu-ruwaida et al., 1994).

Hinton et al. (2007) stated that although psychrotrophs weren't isolated from broiler carcasses juts after washing with chlorinated water, Pseudomonas spices was the most prevalent isolated psychrotrophs from all carcasses refrigerated for 7 to 14 days. *Pseudomonas* spices are found everywhere and isolated from several sources like drinking water, plants, and human beings and also from a variety of foods. To achieve ideal storage life and sensory properties, the initial count of Pseudomonas species shouldn't exceed 100 cfu/g on chicken products under aerobic conditions (Mead, 2005). Scalding step of poultry can destroy Pseudomonas, but the subsequent processing steps may re-contaminate the product. Many studies indicated that the initial count of Pseudomonas is connecting directly with the storage life of the product at refrigeration temperatures and when the number of pseudomonas organism ranging from 10^7 to 10^8 cfu/g in food spoilage will occurs.

It was obvious from results recorded in table (3) that 166 Pseudomonas isolates were identified as *P. acidovorans, P. aeruginosa, P. alcaligenes, P. cepacia, P. fluorescens, P. fragi, P. proteolytica, P. putida, P. putrefaciens, P. stutzeri, P. vesicularis* with an incidence of 6, 1, 33, 8, 47, 10, 19, 3, 22, 2 and 15 from examined samples, respectively. The highest contaminated product was the chicken burger 56/166 (32.5%) of Pseudomonas isolates and *Ps. fluorescens*, was the most detected isolate. From this results, burger samples were recorded the highest contaminated product with psychrotrophic and Pseudomonas species which may be due to malpractices, excessive handling, poor hygienic quality of raw materials especially the added spices and unhygienic practices during production and storage.

Arnaut-Rollier et al. (1999) in a study on fresh and refrigerated chicken skin, reported the prevalence of 4 main Pseudomonas spp. including *Pseudomonas fragi, Pseudomonas lundensis, Pseudomonas fluorescens biovars* and an unidentified strain similar to *P. fluorescens biovars*, 16 different species of Pseudomonas were isolated from 12 samples of chicken meat and *Pseudomonas weihenstephanensis and Pseudomonas psychrophila* were the most abundant lee et al. (2017) and 11 isolates of Pseudomonas were isolated from uncooked chicken burger and were identified as *P.fragi* 8, *P. fluorescens* 1 and *P. chicorii* 2 Franzetti and Scarpellini (2007).

In contrast no *P. aeruginosa* were isolated from any of hundred chicken meat samples examined by Iroha et al. (2011).

Food spoilage is usually associated with *P. fluorescens, P. aeruginosa, P. fragi, and P. lundensis.* (Caldera et al., 2016) in addition to this, the ability of these spoilage bacteria to survive under refrigeration temperatures may cause difficulty during the storage of foods. (Bellés et al. 2017; Wang et al., 2017).

Presence of *Pseudomonas spp*. in food samples is of great significance as the organism is considered as a pathogenic bacterium for man and as an indicator of food quality, Yagoub (2009). Apart from being a spoilage microorganism, *Pseudomonas spp.* could cause urinary and blood stream infection, Golemi-Kotra (2008).

5. CONCULSION

The presence of these opportunistic bacteria should be prevented during earlier stages of food preparation. Additionally, during serving, temperature abuse will lead to spoilage of food leading to bad odor and taste, which is not palatable for customers that can affect sales and reputation of the food service establishments.

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