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BACTERIAL PROFILE OF VACUUM PACKED POULTRY ROAST IN ASSOCIATION WITH PH AND NITRITE CONTENT

By

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ABSTRACT

Consumer interest for poultry products has been increased in recent years. Chicken and turkey roast are recently considered as one of the most popular cured cooked poultry product. These products are commonly sold as sliced and vacuum-packed "ready to eat". Therefore, recontamination and/or inadequate vacuum process of such products may be occurred causing public health hazards. Consequently, a total of forty-two poultry roast (twenty-one for each chicken roast) produced by seven different meat processing plants (three samples each) were collected from different production batches for determination of its quality. All examined samples "chicken and turkey roast" represented higher aerobic mesophilic, anaerobic bacterial count than permissible limit established by Egyptian standard specification "ESS" 3493/2005 for cured cooked poultry products. Moreover, all examined roast samples "chicken and turkey had high lactic acid bacterial count; however, it is not established by ESS as quality indicator parameters for such products. There were significant ($P< 0.05$) differences in pH values among products produced by different processing plant. In addition to there were significant ($P< 0.05$) differences in residual nitrite content between the most of investigated samples. The turkey roast product produced by 1st processing plant revealed significant ($P< 0.05$) reduction in all investigated bacterial groups and significant ($P< 0.05$) elevation in both pH value and residual nitrite content than that of produced by other processing plants. On the other hand, the turkey roast produced by 6th processing had the highest bacterial load and the lowest pH value and residual nitrite content than other plants. This study indicated that there is relationship between bacterial load, pH value and residual nitrite content of vacuum-packed poultry products.

Key words:

Chicken, turkey, roast, residual nitrite, lactic acid bacilli

INTRODUCTION

The consumption of animal products including meat and meat products has increased globally **(Nam *et al.,* 2010).** The people prefer meat products than fresh meat because of the high cost of fresh meat moreover, most of processed meat products are ready -to - eat or need short time to prepare **(Tsang, 2002)**. In recent years, production of poultry meat products is increased to meet the changing consumer needs. Poultry meat is more homogeneous in composition, texture, and color than mammalian meat, making poultry easier to formulate into products **(Sams, 2001)**. Generally, meat and poultry products are perishable foods especially if they are not stored under proper conditions resulting in growth of spoilage and pathogenic bacteria **(Kakouri and Nychas, 1994)**. To overcome this problem, there are many methods, which can be used for extension shelf life of these products. Meat curing and using of a vacuum packaging are considered the most important issues for production of high quality cured meat products with long shelf life. Meat curing which includes the addition of salt, nitrite and sometimes nitrate to fresh meat, enables preservative effect by removing moisture, reducing the water activity of the meat **(Parthasarathy and Bryan, 2012)**. In addition, the nitrite retards production of *Clostridium botulinum* toxin and fat oxidation **(Sindelarand Milkowski, 2012)**. Furthermore, nitrite has an inhibitory action on the growth of other micro-organisms rather *Clostridium botulinum*, such as *Enterobacteriaceae* and *Bacillus thermosphacta* **(Nielsen, 1983)**. However, bacteriostatic action of nitrite, it has no any inhibitory effect on lactic acid bacterial growth **(Nielsen, 1983)** which responsible for spoilage of cured meat products **(Von Holy *et al.*,1991)**. Therefore, cured meat products should be vacuum packed where, vacuum packaging has a potent relationship with nitrite through creation of an anaerobic condition at the product surface which prevent the growth of spoilage bacteria including lactic acid one.The pH value of vacuum -packed cured meat products affects the keeping quality of such products because at high pH (above 6.0), the growth of several types of spoilage bacteria is favored **(Spooncer, 2014)**. Higher pH also has negative effect on bacteriostatic action of nitrite **(Dordevic *et al.*,1980)**, where high pH retards the reduction of nitrate into nitrite resulting in rapid product deterioration. That means there were complementary action between bacteriostatic effect of nitrite, vacuum packaging and pH value of cured meat products. Therefore, the aim of this work, study the bacterial profile "aerobic, anaerobic and lactic acid bacteria" of vacuum-packed poultry products in association with pH and nitrite content.

MATERIAL AND METHODS

1. Samples:

A total of forty-two poultry roast (twenty-one for each chicken roast) produced by seven different meat processing plants (three samples each) were collected from different production batches. Each sample was represented by three packages (500g each) from the same production date. Samples were transferred to the laboratories of Food Hygiene and Control Department, Faculty of Veterinary Medicine, Cairo University and investigated immediately. All samples were subjected to determination of nitrite content, pH value and enumeration of aerobic, anaerobic and lactic acid bacteria.

2. Investigations:

2.1. Bacteriological examination

2.1.1Preparation of sample homogenate

Sample homogenate was prepared by homogenizing ten grams from each sample in 90 ml of 1/4 Ringer's solution (Oxoid BR 52) for intermitted 1.5 minutes using stomacher (Lab blender 400, Sweard lab. Model No. AB 6021). From the original homogenate, tenfold decimal dilution was prepared using the same diluents **(APHA, 1992)**. The following bacteriological investigations were performed:

2.1.1.Enemuration of mesophilic aerobic bacterial count

One hundred µl from each dilution of the previously prepared sample homogenate were aseptically spread over the dry surface of double sets of standard plate count agar plates (Oxoid, CM 463). Inoculated plates were incubated in an inverted position at 32°C for 48 hours for enumeration of aerobic mesophilic count **(Swanson *et al.*, 1992)**. The average count of the duplicate plates was enumerated and the mesophilic bacterial count/g was calculated.

2.1.2.Enemuration of mesophilic anaerobic bacterial count

One hundred µl from each dilution of the previously prepared sample homogenate were aseptically spread uniformly over the dry surface of duplicate plates of Reinforced Clostridial Medium "RCM" (Oxoid, CM 149). Inoculated plates were over lodged with an additional layer of about 10 ml of melted RCM (50-55ºC). After being solidified, all plates were incubated at 30-35ºC for 2 days in an anaerobic atmosphere produced by Oxoid Gas-pack system. The average count of the duplicate plates was enumerated and the anaerobic bacterial count/g was calculated **(Lake *et al*., 1992).**

2.1.3.Enemuration of lactic acid bacterial count

One hundred µl from each dilution of the previously prepared sample homogenates was spread aseptically on to the dried surface of double sets of deManRogosa Sharpe (Oxoid CM 0361) agar. Inoculated plates were incubated anaerobically at 30ºC for 48-72 hours. Colonies of lactic acid bacteria appeared white of uniform size on the surface of the agar or on the very bottom **(ISIRI, 1998).**

2.2. Determination of pH value.

Five grams from each sample were homogenized with 20 ml distilled water for 10-15 seconds, and the pH of the slurry was measured using digital pH meter (Lovibond Senso Direct) with a probe type electrode (Senso Direct Type 330) where three reading for each sample were obtained and the average was calculated. The meter was calibrated every two samples using two buffers 7.0 and 4.0 **(Honikel *et al.*, 1981)**.

2.3. Determination of nitrite content.

Reagents:

-NED reagent: 0.2 gm N- (1 Napthyl) ethylenediaminedihydrochloride in 150 ml, 15% (v/v) acetic acid.

-Sulphanilamide reagent: 0.5gm sulphanilamide in 150 ml 15% (v/v) acetic acid.

Nitrite standard solution:

-Stock solution: Dissolve l gm pure $NaNO_2$ in water and make up to one litter.

-Intermediate solution: Dilute 100 ml of stock solution to one litter with water.

-Working solution: Dilute 10 ml of intermediate solution to one litter with water.

-Filter paper:Filter approximately 40 ml water through each sheet.Add 4ml of Sulphanilamide reagent, mix, let to stand for 5 minutes, add 4 ml of NED reagent, mix and wait for 15 minutes. If any sheets are positive, do not use them.

-Stander curve: Add 10, 20, 30, 40 ml of nitrite working solution to 50ml flasks. Add 2.5 ml of sulphanilamide reagent and after 5 minutes add 2.5ml of NED reagent and proceed as above. Stander curve is straight line up to 1 ppm $NaNO_2$ in final solution.

Procedure:

Five grams of prepared sample were thoroughly homogenized with 40ml distilled water heated to 80°C, then enough hot water were added to bring volume to 300 ml. The fluid was transfered to steam bath and stand for 2 hours with frequent shaking, cooled to room temperature, diluted to volume with water, centrifuged and finally filtered. Two and half ml

sulphanilamide solution were added to an aliquot containing 5-50 µg $NaNO_2$ and mixed, then 2.5 ml NED reagent were added after 5 minutes, mixed and left for 15 minutes to develop color. The absorbance of the fluid was determined spectrophotometrically using Unico (1200 series, USA) spectrophotometer at 540 nm against blank of 45 ml water and 2.5 ml sulphanilamide reagent and 2.5 ml NED reagent **(AOAC, 2000).**

RESULTS

Table (1): Bacterial counts, pH value and residual nitrite content of Chicken roast produced by different processing plants.

Sample	Bacterial counts(log_{10} CFU/g)			pH	Residual nitrite "ppm'
	Aerobic bacterial	Anaerobic bacterial	Lactic acid bacilli		
I	5.74^{a} ±0.21	6.55^{a}±0.41	5.26^{a} ±0.19	6.06^{a}±0.01	19.00^{a}±2.31
II	9.31^{b}±0.01	9.23^{b}±0.07	7.79^{b} ±0.09	5.56^{b}±0.01	26.67^{b}±3.33
III	$8.88^{c,d}$±0.07	9.08^{b}±0.02	$8.25^{b,e}$±0.12	5.23^{c}±0.04	5.00^{c}±1.16
IV	5.95^{a} ±0.24	5.58^{c}±0.31	5.43^{a} ±0.23	6.17^{d}±0.01	5.67^{c}±.33
V	$9.09^{b,c}$±0.08	8.84^{b}±0.41	6.71^{d} ±0.49	5.37^{e}±0.01	$15.00^{a,d}$±3.06
VI	8.62^{d} ±0.06	8.67^{b}±0.04	9.20^{c} ±0.05	3.79^{f}±0.00	1.00^{c}±0.00
VII	$8.96^{b,d}$±0.12	9.24^{b}±0.11	$8.58^{c,e}$±0.08	5.06^{g}±0.02	$9.00^{c,d}$±1.00
Mean	8.08±0.32	8.17±0.32	7.32±0.33	5.32 ±0.16	11.62 ±1.98

*** a-g: Means with different superscripts differ significantly at P<0.05.**

Table (2): Bacterial counts, pH value and nitrite content of Turkey roast produced by different processing plants.

Sample	Bacterial counts (log_{10} CFU/g)			pH	Nitrite "ppm"
	Aerobic bacterial	Anaerobic bacterial	Lactic acid bacilli		
I	5.43^{a}±0.06	5.10^{a}±0.16	3.00^{a}±o.58	6.59^{a}±0.01	54.00^{a}±4.36
II	7.56^{b}±0.23	6.50^{b}±0.49	6.25^{b}±0.08	5.00^{b}±0.01	11.60^{b}±0.20
III	7.96^{c}±0.11	7.85^{c}±0.15	7.82^{c}±0.03	4.74^{c}±0.02	13.13^{b}±1.04
IV	$8.28^{c,d}$±0.04	$8.41^{c,d,e}$±0.18	7.91^{c}±0.13	5.71^{d}±0.02	$17.00^{b,c}$±0.58
V	7.57^{b}±0.08	$7.99^{c,d}$±0.09	7.87^{c}±0.06	5.42^{f}±0.01	19.00^{c}±0.58
VI	9.13^{e} ± 0.09	9.04^{e} ±0.08	8.52^{c}±0.02	4.91^{e}±0.01	2.67^{d}±1.20
VII	8.59^{d}±0.09	$8.61^{d,e}$±0.08	8.44^{c}±0.15	4.92^{e}±0.02	19.00^{c}±0.58
Mean	7.79±0.25	7.64±0.29	7.12±0.41	5.33±0.14	19.49±3.41

*** a-f: Means with different superscripts differ significantly at P<0.05.**

DISCUSSION

Chicken roast:

Results of bacteriological examination of chicken roast (Table 1) showed that, the examined samples were heavily contaminated with the different bacterial groups. The mean counts (log_{10} CFU/g) for the aerobic mesophilic, anaerobic and lactic acid bacilli bacteria were 8.08, 8.17and 7.32 respectively. The results also indicated the presence of significant difference ($P<0.05$) in the most of investigated bacterial groups between the different processing plants. ESS (3493/2005) regulations for heat treated poultry products requires a maximum of 10^4 and10^2 for aerobic mesophilc and anaerobic bacteria respectively. That mean all examined chicken roast samples exceed the permissible limit for aerobic and anaerobic bacterial count established by E.S.S. However, lactic acid bacilli bacteria were presented in high count in all samples, not established by ESS (3493/2005) regulations as quality indicator parameter. High count bacteria in chicken roast samples may be due to insufficient heat treatment during processing, using raw materials of high bacterial load, recontamination after processing **(Pérez-Rodriguez *et al.,* 2007)** and/ or insufficient vacuum packaging **(Borch *et al.,* 1996)**. The obtained results were in agreement with that of **Von Holy *et al*. (1991)** who reported that, the vacuumed cooked cured meat products had high lactic acid bacterial counts which referred to the combination of the microaerophilic conditions, the presence of curing salt and nitrite. The pH value of vacuum-packed meat products affects the keeping quality of such products **(Spooncer, 2014)**. Results of pH of chicken roast (Table 1) indicated that, the values ranged from 3.79 to 6.17 with an average value of 5.32. In general, there were significant ($P<0.05$) differences among the pH values of chicken roast samples produced by different processing plants, which could be referred to the differences in quality, quantity and type of added ingredients specially reducing agent. These differences also may be due to the differences in lactic acid bacterial counts of chicken roast samples among different processing plants. It is noted that, the product of sixth processing plant had the highest lactic acid bacterial count and lower pH value (Table 1). The obtained results were fixed with that of **Gardner (1983**); **Borch *et al.* (1991) and Blickstad and Molin (1983)** who established that lactic acid bacteria can grow in vacuumed products produce acids such as lactic acid, acetic acid and formic acid altering pH of the product into acidic side. (Table 1) showed that there were significant ($P<0.05$) differences in the residual nitrite content among the most of investigated samples with the product of 2nd processing plant had the highest value while the

product of sixth processing plant had the lowest value. The variations in residual nitrite content of chicken roast among different processing plant may reflect pH value, storage temperature, and length of storage, presence of reducing agents, heat treatment **(Honikel, 2008)** and bacterial load of the product. **Dordevic *et al.* (1980)** found that higher pH values of the product retard the loss of residual nitrite. Moreover, **Christiansen *et al.* (1978)** reported a reduction of residual nitrite (from 156 to 118 ppm) in cured pork immediately after cooking, with a further decline to 88 ppm after 3 days of storage at 27° C. From above results, it is obvious that, the product of 2nd processing plant had the highest lactic acid bacterial count and lowest values for pH and residual nitrite. These results indicated that there are relationship between lactic acid bacterial count, pH value and nitrite content of the vacuumed cured poultry product, where higher lactic acid bacterial count lead to formation of acidic media and consequently rapid reduction of nitrite into nitric oxide "low residual nitrite content". It is clear that, the residual nitrite of all chicken roast samples were very lower than limit established by **Brasil (2009)** "150 ppm". Moreover, **Sindelar and Milkowski (2012)** stated that the maximum permitted levels of nitrate and nitrite in processed meat products vary from country to other; according to Food and Drug Administration (FDA) regulations, level of sodium nitrate and sodium nitrite does not exceed 500 and 200 ppm in the finished product.

Turkey roast:

Table (2) showed that all examined turkey roast samples exceeded the permissible limit established by Ess (3493/2005) (not more than10^4,10^2 CFU/g) for aerobic mesophilic and anaerobic bacterial count respectively. And the mean counts ($\log_{10}$ CFU/g) for the aerobic mesophilic, anaerobic and lactic acid bacilli bacteria were 7.79, 7.64 and 7.12 respectively. Bacteriological analysis of turkey roast also represented that there were significant (P<0.05) differences in all investigated bacterial groups among the most of examined samples. The product of 1st processing plant had the lowest bacterial count while the product of 6th processing plant had the highest bacterial count. High bacterial counts of examined turkey roast samples may be referred to re-contamination after processing, inadequate good hygienic practices (GHPs) and good manufacturing practices (GMPs) in the processing plants **(Syne *et al.*, 2013)**. The mean pH value and residual nitrite "ppm" of turkey roast were 5.33 and 19.49 respectively. The product of 1st processing plant showed significantly (P<0.05) higher pH value and residual nitrite than other processing plant (Table 2). On the other hand,

the product of 1st processing plant had the lowest count (log10 CFU/g) for lactic acid bacteria. That mean there was opposite relationship between lactic acid bacterial count, pH and residual nitrite, where low lactic acid bacterial count produce low level of acid "high pH value" and consequently slowly reduction of nitrite to nitric oxide " high level of residual nitrite" **(Dordevic *et al.,* 1980)**. Similar to chicken roast all examined turkeyroast samples revealed lower nitrite level than permissible limit as discussed before.

CONCLUSION

All investigated roast samples had high bacteriological load and low residual nitrite. Form the present study we can conclude that there is relationship between bacterial count, pH value and residual nitrite content of vacuum poultry products. Where, high bacterial count mainly lactic acid bacilli lead to production of acids altering pH into acidic condition and consequently rapid reduction of nitrite into nitric oxide " low residual nitrite content" and vice versa.

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