

Effect of steaming and storage time on the microbial quality of duck and quail sausages

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ABSTRACT

The effects of steaming and storage time on the microbial quality of duck and quail sausages were investigated. Duck and quail sausages were either inoculated with or without pure cultures of *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes* during preparation and analyzed microbiologically for *Escherichia coli* and *Salmonella* spp., *Listeria monocytogenes*, total aerobic bacterial counts and coliforms on day 0, 3, 5, 7, and 10. Total aerobic bacterial counts for non-steamed inoculated and non-inoculated duck and quail sausages ranged from log 3.19 cfu/g in day 1 to too numerous to count in day 10. Coliform numbers for non-steamed inoculated and non-inoculated duck and quail sausages ranged from 4.9 MPN/g in day 1 to > 1100 MPN/g in day 10. Thus microbial load for non-steamed inoculated and non-inoculated duck and quail sausages increased with storage time. *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes* were not detected in steamed duck and quail sausages from day 0 to 10. Steaming of sausages at a temperature of 110 °C for 2.5 hours and to an internal temperature of 72 °C was effective to inactivate all the microorganisms investigated.

Keywords: Steaming; storage; duck sausage; quail sausage

Introduction

Sausages are popular and highly relished meat products produced and consumed worldwide. Different types of fresh, cooked and fermented sausages originated from specific countries, but are now available in other countries. For instance, we have UK style sausages, Frankfurter sausages mainly of Germany, Kielbassa (Polish sausage), Zampino sausage (Italy), Chorizo (Spain and Portugal), Andouille (France) and many more, all of which are enjoyed by consumers from different countries. Most of these sausages are made from beef, veal, pork, chicken, or lamb spiked with salt, breadcrumbs, spices and other ingredients, often stuffed into casings. Duck and quail meats and eggs like other poultry species are important source of protein, and contain some amounts of minerals, vitamins, fats and

carbohydrate (Anonymous, 2012a; Adzitey, 2012). Duck and quail meats also have potentials to be developed into new meat products (Panda and Singh, 1990; Huda et al., 2011). Pickled quail eggs are a specialty product and quail eggs have some medicinal properties playing a role in the control of chronic cholecystitis, asthma, and heart disease (Panda and Singh, 1990; Anonymous, 2012b). Duck and quail sausage appear to be unavailable in the market and the exploitation of this new product is essential to increase consumer taste, preference and choice. The presence of foodborne pathogens such as *Escherichia coli*, *Salmonella* species, *Listeria monocytogenes* and so on in ducks, quails or other food animals and food meat products have been reported (Adzitey et al., 2010; Akhtar et al., 2010; Frederick and Huda, 2011; Karahan et al., 2011; Adzitey

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et al., 2012a; Adzitey et al., 2012b; Adzitey et al., 2013; Shabana et al., 2013). Furthermore, the association between pathogenic bacteria in either fresh, cooked or fermented sausage is well established (Pond et al., 2001; Naim et al., 2004; Thévenot et al., 2005). Although most of these pathogens are destroyed during fermentation and cooking, recontamination can occur following poor handling (Adzitey and Huda, 2012). These pathogens have negative influence on the quality, safety and useful life of the prepared sausage. They also cause human illnesses some of which can lead to long term complications and even death. Consumer awareness of food safety issues have increased in recent times. Thus an understanding and establishing the effects of steaming on the microbial profile of sausages is vital. Information on the survival of pathogens in duck and quail sausage is very scarce. The study examines the level of total aerobic bacterial count and coliform contamination in duck and quail sausages during storage without prior steaming; and the effects of steaming and storage on the microbial quality (*Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes*) of duck and quail sausages.

Material and Methods

Sources of duck and quail meats: Duck meats were obtained from broiler Peking duck (*Anas platyrhynchos*) slaughtered at the age of about 16 weeks old. The frozen duck carcasses were supplied by a local farmer at Perak, Malaysia. While quail meats were obtained from broiler quail (*Coturnix japonica*) slaughtered at about 8 weeks old. Frozen quail carcasses were supplied by the Institute of Poultry Development, Johor Bahru, Malaysia. Preparation of duck and quail sausage: Duck and quail sausages were prepared

from minced duck/quail meats (75.00%), salt (2.50%), monosodium glutamate (MSG) (0.05%), sugar (1.00%), oil (5.00%), nitrite (0.02%), nitrate (0.02%), wheat flour (3.43%), spices (2.25%), and chilled water (10.00%). These ingredients were thoroughly mixed together in a mixer and stuffed into casings with a diameter of 2 cm using a stuffer. The stuffed sausages were linked to a length of 10 cm each (~25 g per sausage) and steamed cooked at 110 °C for 2.5 hours. Cooked sausages (with an internal temperature of 72 °C) were placed in cold water for about 12 to 15 min until the internal temperature was reduced to about 20-25 °C. The internal temperatures of the steamed cooked sausages were checked using thermocouples. After which the sausages were stored aerobically at 4 °C for 10 days during which microbiological analysis was carried out.

Inoculation of duck and quail meat product: Pure cultures of *Escherichia coli*, *Salmonella* species and *Listeria monocytogenes* were obtained from the Malaysian Institute for Medical Research. They were recovered in Tryptic Soy Broth (TSB) (incubated at 30 °C for 24 hours) and maintained on Tryptic Soy Agar (TSA) slant at 4 °C. Pure cultures were subcultured monthly by transferring a loopful of each colony into 90 ml of Tryptic Soy Broth and incubated at 30 °C for 24 hours. After 24 hours incubation 1 ml of each inoculated Tryptic Soy Broth was transferred into 9 ml buffered peptone water (BPW) such that the average cell concentration in the last dilution was 10⁶ cfu/ml. One (1) ml of buffered peptone water containing the pure cultures were injected into the sausages and mixed thoroughly during preparation.

Microbiological analysis: Microbial analysis for *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, coliforms and total aerobic bacterial count was done

according to the steps in Bacteriological Analytical Manual (1998). Microbiological analysis was carried out on fresh sausages in day 0, 3, 5, 7 and 10 for total aerobic bacteria and coliforms. Steamed sausages were analyzed on day 0, 3, 5, 7 and 10 for *Escherichia coli* *Salmonella* spp. and *Listeria monocytogenes*. Total aerobic bacterial counts were determined using Plate Count Agar (Merck), incubated at 37 °C for 48 hours; for *Salmonella* spp. on Rambach agar (Merck) and Xylose Lysine Deoxycholate Agar (Merck), incubated at 37 °C for 24 hours; *Listeria monocytogenes* on PALCAM agar (Merck), incubated at 35 °C for 24 - 48 hours; *Escherichia coli* on Eosin Methylene Blue Agar (Merck), incubated at 37 °C for 18 - 24 hours, and coliforms Lauryl Sulphate Tryptose broth (Merck) incubated at 35 °C for 48 hours using gas production. Presumptive colonies were confirmed biochemically using Gram stain, Oxidase, Catalase, Triple Sugar Iron and Lysine Iron Agar slants.

Statistical analysis: Analysis of Variance was conducted using Genstat Sixth Edition (Genstat Procedure Library Release PL14) and significant differences were separated using standard error of means at 5 % probability level. Total plate bacterial count was expressed in cfu/g (colony forming unit per gram) while coliform count was express in MPN/g (most probable number per gram).

Result

Table 1 shows the result for total aerobic bacterial count for both inoculated and non-inoculated duck and quail sausages before steamed cooked. The total aerobic bacterial plate count for inoculated/non-inoculated duck and quail sausages differed significantly ($P < 0.05$) at the beginning of the experiment. However, no significant difference ($P > 0.05$) was observed on day 3 and day 10. At day 5 and 7, non-

inoculated duck sausages and inoculated duck sausages, respectively were significantly higher than their counterparts (Table 1). Table 2 shows the number of coliforms observed for both inoculated and non-inoculated duck and quail sausages before steamed cooked. Total coliform counts for both inoculated/non-inoculated duck and quail sausages did not differ significantly ($P > 0.05$) from each other at the beginning of the experiment

Table 1: Total number of aerobic bacteria found on inoculation and non-inoculation duck and quail sausages from day 0 to day 10 before steamed cooked

Discussion

Total aerobic bacterial count for both inoculated and non-inoculated duck and quail sausages before steamed cooked is presented in Table 1. There was significant difference ($P < 0.05$) in the total aerobic bacterial plate count for inoculated/non-inoculated duck and quail sausages at the beginning of the experiment. Nevertheless, on day 3 and day 10 no significant difference ($P > 0.05$) was observed. At day 5 and 7, non-inoculated duck sausages and inoculated duck sausages, respectively were significantly higher ($P < 0.05$) than their counterparts. In general, total aerobic counts tended to increase from day 0 to day 10. At day 0, total aerobic bacteria count for inoculated (6.50 log cfu/g) and non-inoculated (6.49 log cfu/g) duck sausage were higher than acceptable limits ($< 1.0 \times 10^5$ cfu/g). Inoculated (6.37 log cfu/g) and non-inoculated (6.42 log cfu/g) quail sausages exceeded this range in day 3. Inoculation had no major effect on the number of total aerobic bacteria enumerated since both inoculated and non-inoculated duck and quail sausages had higher bacterial loads in turns. The results obtained indicated that microbes grow with increasing storage time when favorable conditions are provided. The unusual high

numbers observed at the beginning of the experiment could be due to the minced meat, spices and other ingredients which were already contaminated by some bacteria. It could also be due to cross contamination during processing from unhygienic processing equipments, from the hands of handlers or by contamination from the surrounding environment. The number of coliforms observed for both inoculated and non-inoculated duck and quail sausages before steamed cooked is presented in Table 2. Total coliform counts for both inoculated/non-inoculated duck and quail sausages were not significantly different ($P > 0.05$) from each other at the beginning of the experiment; contrarily to what was observed for total aerobic plate count at the beginning of the experiment. Significant difference ($P < 0.05$) occurred in day 3; although that was not the case for day 5 to day 10. Coliform numbers in both inoculated and non-inoculated duck and quail sausages exceeded the normal range (50 MPN/g for foods) on day 5 which is an improvement over the numbers of total aerobic bacteria present in both samples. Total coliform counts also increased with storage time. Inoculation did not have much influence on the results obtained. Nevertheless, the presence of coliforms can be an indication of faecal or non-faecal contamination of the duck/quail meats or other ingredients used to prepare the sausages.

Microbial numbers (total aerobic bacteria and coliform counts) might be used to evaluate the potential safety of food to a limited extent. Furthermore, the counts might indicate the effectiveness of sanitary procedures used and handling of the foods during processing and storage; and to determine the shelf life of processed meat products. Thus both non-steamed inoculated and non-inoculated fresh duck

and quail sausages had a shorter useable life. This is in agreement with findings made by George (1989) and Borch (1996). George (1989) reported that when microbial load reaches very high numbers such as 10^7 - 10^8 cfu/g, spoilage of food is eminent. Uncooked sausages have a shorter shelf life since raw ingredients carry high levels of microorganisms (Borch, 1996). Results obtained for both total aerobic bacteria and coliforms suggest that pathogens can survive and multiply in duck and quail sausages as in other sausages except that the microflora will differ depending on the ingredients, spices and the treatment conditions. Therefore, duck and quail sausages can be potential source of foodborne illnesses if expose to pathogens. Unlike the determination of total aerobic bacteria and coliforms, sausages were steamed cooked at 110 °C for 2.5 hours, allowed to cool and later stored at 4 °C before being analyzed on day 0, 3, 5 and 10. *Escherichia coli*, *Salmonella* spp. and *L. monocytogenes* were not detected in both cooked samples (inoculated/non-inoculated duck and quail sausages) after steam cooking at 110 °C for 2.5 hours. The temperature range within which these pathogens can survive is of particular interest. *Escherichia coli* have been shown to survive within a temperature range of 2.5-45 °C; *Salmonella* spp. within a temperature range of 5-45 °C; and *L. monocytogenes* between -20-45 °C (Tienungoon, 2000). Thus cooking to an internal temperature of 72 °C was enough to kill these pathogens. Although recontamination and/or cross contamination can re-occur after processing this was not observed meaning that, the sausages were handled hygienically after steaming. Duck and quail sausages can therefore be prepared and steamed-cooked to an internal temperature of 72 °C, and they will remain wholesome

for human consumption if handled hygienically. Deactivating these pathogens is of prime importance because of their association with foodborne illnesses and sometimes death. For instance Enterohemorrhagic *E. coli* 0157:H7 can cause diarrhea, abdominal pain, bloody diarrhea which can progress into hemolytic syndrome (one of the leading renal failure in children) (Nataro and Kaper, 1998). *Salmonella* causes salmonellosis which can lead into diarrhea, nausea, vomiting, abdominal pain, gastroenteritis and septicemia (Willford et al., 2007, Frederick and Huda, 2011). *Listeria monocytogenes* is associated with listeriosis, an illness that normally affects high risk individuals such as pregnant women, adults, babies, HIV and immunocompromised patients (Schlech, 2000). Pathogenic *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes* infections can lead to hospitalization and death in under severe conditions (Nataro and Kaper, 1998; Adzitey et al., 2012b; Noble et al. 2012; Scallen et al., 2012).

Conclusion: In conclusion, it is not unusual to find raw duck and quail sausages carrying some bacteria. Steam cooking of duck and quail sausages at 110 °C for 2.5 hours to an internal temperature of 72 °C, was effective in killing all *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes*. Measures such as effective heat treatment during cooking, careful handling and storage (at 4 ± 1 °C) of cooked duck and quail sausages would deactivate most foodborne pathogens, control microbial growth enhance the shelf life of duck and quail meatballs and sausages.

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Table 1: Total number of aerobic bacteria found on inoculation and non-inoculation duck and quail sausages from day 0 to day 10 before steamed cooked

Day	Quail Sausage		Duck Sausage		P	±SEM
	Inoculated (log cfu/g)	Non-inoculated (log cfu/g)	Inoculated (log cfu/g)	Non-inoculated (log cfu/g)		
0	3.19 ^a	4.11 ^b	6.50 ^c	6.49 ^c	<0.001	0.48
3	6.37 ^a	6.42 ^a	6.33 ^a	5.88 ^a	0.784	1.17
5	7.36 ^{ab}	7.08 ^a	7.40 ^{ab}	7.56 ^b	0.176	0.35
7	8.06 ^a	8.11 ^a	8.68 ^b	8.16 ^a	0.118	0.42
10	TNTC	TNTC	TNTC	TNTC	ND	ND

cfu/g: colony forming unit per gram; P: Probability; SEM: Standard error of means; TNTC: too numerous to count; ND: Not done; means within the same row with different superscript letters are different (P < 0.05) and vice versa.

Table 2: Number of coliforms found on inoculation and non-inoculation duck and quail sausages from day 0 to day 10 before steamed cooked

Day	Quail Sausage		Duck Sausage		P	±SEM
	Inoculated (MPN/g)	Non-inoculated (MPN/g)	Inoculated (MPN/g)	Non-inoculated (MPN/g)		
0	20.8 ^a	4.9 ^a	14.6 ^a	6.6 ^a	0.65	27.14
3	31.0 ^{bc}	7.8 ^a	21.5 ^b	41.0 ^c	0.02	12.06
5	93.0 ^a	112.0 ^{ab}	152.0 ^{ab}	210.0 ^b	0.23	98.20
7	225.0	>1100.0	1100.0	780.0	ND	ND
10	780.0	>1100.0	>1100.0	1100.0	ND	ND

cfu/g: colony forming unit per gram; P: Probability; SEM: Standard error of means; ND: Not done; Means within the same row with different superscript letters are different (P < 0.05) and vice versa.