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Effect of Temperature on the Growth and Survival of Pathogens in Duck and Quail Meatballs

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ABSTRACT: The effect of temperatures (pre-heating and heating) on the growth and survival of pathogens in duck and quail meatballs were investigated. The meatballs were inoculated with pure cultures of *Listeria monocytogenes*, *Salmonella* species, *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus* containing an average cell concentration of ~10^6 cfu/ml. Meatball samples were analyzed at 5 min intervals for 40 min, during two steps of cooking; pre-heating at 45 °C for 20 min and heating at 90 °C for further 20 min. Microbial load of the raw minced duck and quail meats, pre-heated and heated meatballs differed significantly ($P < 0.05$) from each other. Pre-heating at 45 °C for 20 min was not lethal to the pathogens examined. Heating at 90 °C for 20 min was enough to deactivate all pathogens in the duck and quail meatballs. This study suggests that measures such as hygienic precautions during preparation of meatball and heating at a temperature of ≥ 90 °C for 20 min or more will control microbial growth and provide wholesome and safe duck and quail meatballs for consumption.

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INTRODUCTION

Meat products are increasingly gaining much importance worldwide probable due to increase demand for ready-to-eat meals. Meatballs are popular meat products especially in Malaysia and other Asia countries. Variety of meatballs such as fish ball, chicken ball, beef ball, squid ball and prawn ball are available on the market; of which fish ball, chicken ball and beef ball are most commonly consumed by Malaysians. Duck and quail meat protein, iron, Vitamin A, Vitamin B1 and Vitamin B2 are comparable to that of chickens [1, 2] and when properly included as part of a well-balanced daily diet, can supply a substantial portion of the nutrients required by humans [3]. Duck and quail meatballs are not available in Malaysia, although duck and quail production as small sideline occupation is gradually growing in importance to that of the poultry meat industry.

Bacterial pathogens such as *Salmonella* species, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus* are associated with ducks, other food animals and meat products [4-11]. Microorganisms gain access into meatballs from the meat, spices and other ingredients, equipment, handlers during processing and the environment. Comminuting also increases the chances of microbial contamination of meat ball. While processing procedures or methods such as heat treatment [12] and gamma irradiation [13, 14] reduce microbial levels, recontamination can occur during post-processing, handling and storage of meat and meat products due to poor handling [15]. In Turkey, there have been a number human infections and intoxication cases due to consumption of raw meatball [16]. Consumers' awareness has increased in recent times for meat and meat products including meatballs that are microbiological safe. Thus, research into the microbial profile of meatballs, and the growth and survival of pathogens in meatballs is useful.

This study aims at determining the effects of pre-heating (45 °C for 20 min) and heating (90 °C for additional 20 min) on the growth and survival of *Salmonella* species, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus* in duck and quail meatballs. Duck and quail meat were used in this experiment because their meatball product is not yet available on the market -although there is the potential for these meats to be developed into several meat products- and their safety and hygienic status in terms of microbiology is not known.

MATERIALS AND METHODS

Sources of duck and quail meats

Frozen carcasses from broiler Peking ducks (*Anas platyrhynchos*) slaughtered at the age of 16 weeks were purchased from a local farmer at Perak, Malaysia, while frozen carcasses from broiler quail (*Coturnix japonica*) slaughtered at the age of 8 weeks were purchased from the Institute of Poultry Development, Johor Bahru,

Malaysia. Frozen carcasses were thawed and used for the experiment. The experiment was conducted at the Microbiology Laboratory of the School of Industrial Technology, Universiti Sains Malaysia, Malaysia.

Preparation of duck and quail meatballs

Each meatball composed (%) of minced duck/quail meat (65.00), starch (3.00), soy protein isolate (3.20), oil (10.00), salt (2.10), sugar (2.00), white pepper (0.30), garlic (0.30), ginger (0.30) and chilled water (13.80). These ingredients were thoroughly mixed together in a mixer. The blend was shaped into balls (~10 g) manually and set at 45 ^{o}C in warm water for 20 min to help retain the shape of the meatball. This was followed by cooking at 90 ^{o}C for 20 min.

Inoculation of duck and quail meatballs

Pure cultures of *Listeria monocytogenes*, *Salmonella* species, *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus* were obtained from the Malaysian Institute for Medical Research. They were recovered in Tryptic Soy Broth (TSB) (incubated at 30 °C for 24 h) 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 h. After 24 h 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 10^6 cfu/ml. One ml of buffered peptone water containing the pure cultures were injected into the meatballs and mixed thoroughly during preparation. Each inoculated sample (~10 g meatball inoculated with an average cell concentration of 10^6 cfu/ml) was analyzed microbiologically every 5 min for 40 min.

Microbiological analysis

Microbial analysis for *Listeria monocytogenes*, *Salmonella* species, *Escherichia coli*, *Bacillus cereus,* and *Staphylococcus aureus* were done according to Bacteriological Analytical Manual [17]. Enumeration was done by spread plate method, every 5 min interval for 40 min during pre-heating (at 45 ^{o}C for 20 min) and heating (at 90 ^{o}C for additional 20 min). *Salmonella* species enumerated on XLD (Xylose Lysine Deoxycholate Agar, Merck), incubated at 37 ^{o}C for 24 h; *Listeria monocytogenes* on ALOA (*Listeria* selon Ottaviani & Agosti Agar, Merck), incubated at 35 °C for 24-48 h; *Escherichia coli* on EMB agar (Eosin Methylene Blue Agar, Merck), incubated at 37 ^{o}C for 18-24 h; *Staphylococcus aureus* on BP (Baird Parker Agar, Merck), incubated at 37 ^{o}C for 24 h; and *Bacillus cereus* on MYP Agar (Mannitol Egg Yolk Polymyxin Agar, Oxoid), incubated at 37 ^{o}C for 24 h. Presumptive colonies were confirmed biochemically using Gram stain, oxidase, catalase, Triple Sugar Iron (TSI) and Lysine Iron Agar (LIA) slants.

Statistical analysis

Analysis of Variance was conducted using Genstat Sixth Edition (Genstat Procedure Library Release PL14) and significant differences were separated using ANOVA at 5% probability level. Microbial counts were expressed in log colony forming unit per gram (cfu/g).

RESULTS AND DISCUSSION

Table 1 shows the result for various levels of microbial load in the raw minced duck and quail meats during pre-heating at 45 ^{o}C for 20 min and heating at 90 ^{o}C for 20 min. Significant differences ($P < 0.05$) occurred between the raw minced meats, pre-heated and heated meatball samples. Microbial load (log cfu/g) in the raw minced meat were 4.61, 4.45 (*Escherichia coli*); 2.56, 3.22 (*Staphylococcus aureus*); 3.47, 3.2 (*Bacillus cereus*); 5.04, 4.26, 4.27 (*Salmonella* species); and 6.14, 6.36 (*L. monocytogenes*) for duck and quail meats, respectively. This indicates that these pathogens were present in the raw minced meats with the highest count being *L. monocytogenes*, followed by *Escherichia coli*, *Salmonella* species, *Bacillus cereus* and *Staphylococcus aureus*. Duck and quail meats might have been contaminated during processing of the live birds from the gastrointestinal tract, processing equipment, hands of handlers and/or at the selling points. Contamination may also have occurred during processing and mincing of the meats. Nichols et al. [18] found that pathogenic bacteria including *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* species in restaurants were transferred to the cooked foods by contaminated staffs' hands or dishes. Other researchers have also reported the presence of pathogens in raw minced or ground meats other than our findings with the duck and quail meats [19- 21]. Rose et al. [19] and Sorensen et al. [20] reported a prevalence rate of 7.5% and 1.3% respectively for *Salmonella* species in ground beef. Mrema et al. [21] sampled 122 minced meats in Botswana and found 20% to be positive for *Salmonella* species.

In an investigation conducted by Bohaychuk et al. [22] they found a shiga toxin (Stx) -producing *Escherichia coli* (STEC) O22: H8 in one raw ground beef sample. They also found *Salmonella* and *Campylobacter* species in 30 and 62%, respectively in raw chicken legs. *Listeria monocytogenes* was present in 52% of raw ground beef, 34% of raw chicken legs, and 24% of raw pork chops [22].

Table 1. Microbial load of raw minced duck and quail meats, and pre-heated (45°C at 20 min) and heated (90°C at 20 min) temperatures for duck and quail meatballs

Organism	Raw minced meat duck	Raw minced meat quail	Pre-heat duck meatball	Pre-heat quail meatball	Heat duck meatball	Heat quail meatball	*P*	±SEM
Salmonella sp. (cfu/g)	4.26a	4.27a	5.33b	4.73ab	0.00c	0.00c	< 0.001	0.634
S. aureus (cfu/g)	2.56a	3.22b	4.18c	4.01c	0.00d	0.00d	< 0.001	0.404
E. coli (cfu/g)	4.61b	4.45a	5.77c	5.76c	0.00d	0.00d	< 0.001	0.105
B. cereus (cfu/g)	3.47b	3.20a	3.35ab	3.34ab	0.00d	0.00d	< 0.001	0.154
L. monocytogenes (cfu/g)	6.14a	6.36a	6.83a	6.67a	0.00b	0.00b	< 0.001	1.214

cfu/g: colony forming unit per gram; *P*: Probability; SEM: Standard error of Means; Means within the same row with different superscript letters are different ($P < 0.05$) and vice versa.

Table 2. Microbial numbers found in duck and quail meatballs heated at 45 °C for 20 min

	***B. cereus* (cfu/g)**		***S. aureus* (cfu/g)**		***L. monocytogenes* (cfu/g)**		***Samonella* sp. (cfu/g)**		***E. coli* (cfu/g)**	
Time (min.)	Duck MB	Quail MB	Duck MB	Quail MB	Duck MB	Quail MB	Duck MB	Quail MB	Duck MB	Quail MB
0	3.68	3.77	4.46	4.65	7.46	7.01	6.01	5.87	6.24	6.05
5	3.48	3.60	3.81	4.62	7.33	6.97	5.74	5.38	5.98	5.68
10	3.35	3.47	4.63	4.46	6.81	6.91	5.89	4.94	5.97	5.45
15	3.37	3.43	4.54	4.16	7.07	6.77	5.36	4.82	5.84	5.33
20	3.35	3.34	4.18	4.00	6.83	6.67	5.33	4.73	5.77	5.25

cfu/g: colony forming unit per gram; min: minutes; MB: Meatball

Table 3. Microbial numbers found in duck and quail meatballs heated at 90 °C for 20 min

	***B. cereus* (cfu/g)**		***S. aureus* (cfu/g)**		***L. monocytogenes* (cfu/g)**		***Samonella* sp. (cfu/g)**		***E. coli* (cfu/g)**	
Time (min.)	Duck MB	Quail MB	Duck MB	Quail MB	Duck MB	Quail MB	Duck MB	Quail MB	Duck MB	Quail MB
5	ND	ND	3.91	3.6	6.70	6.49	3.88	3.63	ND	ND
10	ND	ND	3.85	ND	5.32	5.95	3.32	3.49	ND	ND
15	ND	ND	ND	ND	3.95	3.18	2.85	ND	ND	ND
20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

cfu/g: colony forming unit per gram; min: minutes; MB: Meatball; ND: Note detected

Pre-heating at 45 °C for 20 min did not have any significant effect ($P > 0.05$) on the reduction of microbial numbers (Tables 1 and 2). However, when the temperature was increased to 90 °C, *Bacillus aureus* (in both duck and quail meatballs), *Escherichia coli* (in both duck and quail meatball) and total bacterial count (in duck meatball) were not detected in the first 5 min as depicted in Table 3. After 15 min of heating at 90 °C only *L. monocytogenes* (in both duck and quail meatballs) and *Salmonella* species (in duck meatball) could be detected. Nevertheless, all these pathogens were destroyed when the temperature was increased to 90 °C at 20 min. Tavakoli and Riazipour [23] analyzed 54 grilled chickens and found 5.5% contaminated with *Escherichia coli.* They did not observe contamination by *Salmonella* species, *L. monocytogenes* and *Staphylococcus aureus*. The same researchers found 38.9% *Escherichia coli* contamination, 55.6% *Staphylococcus aureus* contamination and no (0%) contamination for *Salmonella* species and *L. monocytogenes* in grilled meat samples. Tokassian et al. [24] assessed the contamination level of cooked and raw meat samples of which 32 (6.8%) samples had *Salmonella* bacteria from which seven samples were from grilled ground meat. Fang et al. [25] evaluated 164 samples of prepared meat foods and found that 27.5%, 17.9%, 7.9% and 4.98% of the samples were contaminated by coliforms, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus* contaminations, respectively.

Our studies however showed that cooking duck and quail meat balls at a temperature of 90 °C for 20 min is enough to destroy all *L. monocytogenes, Escherichia coli, Salmonella* species*, Bacillus cereus, Staphylococcus aureus* and total aerobic counts. Furthermore, meat type did not have any significant effect ($P > 0.05$) on the microbial load. Furthermore, Putra et al. [26] found that generally no significant difference ($P > 0.05$) existed between pre-heating and heating of duck meatballs on the nutritional contents, physicochemical characteristics and sensory attributes of duck meatballs.

CONCLUSION

In conclusion, it is not unusual to find raw minced duck and quail meat contaminated by some pathogens. Heating at a temperature of 90 °C for 20 min is effective to destroy all *Listeria monocytogenes*, *Salmonella* species, *Escherichia coli*, *Bacillus cereus,* and *Staphylococcus aureus* in duck and quail meatball samples. However, in general, *Listeria monocytogenes* were the most resistant to the heat applied followed by *Salmonella* species, *Staphylococcus aureus,* and *Bacillus cereus* and *Escherichia coli* in this experiment. Measures such as low initial level of contamination, effective heat treatment during cooking, and careful handling of cooked meatballs would improve the microbiology quality and enhance the shelf life of duck and quail meatballs.

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