

Research Article

ASSESSMENT OF MICROBIAL LOAD OF SAUSAGES PREPARED FROM DIFFERENT COMBINATION OF SPENT DUCK AND SPENT HEN MEAT

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Received 11 May 2016, revised 27 October 2016

ABSTRACT: Aim of the present study was to assess the microbial load of sausages prepared from different combination of spent duck and spent hen meat. The combination are 100% spent duck (T1), 75%+ 25% spent duck and spent hen (T2), 50%+50% spent duck and spent hen (T3), 25%+75% spent duck and spent hen (T4) and 100% spent hen (T5). All the samples of different combination were subjected to total plate count (TPC), total psychrophilic count (TPSC) and total Coliform count (TCC). Mean of TPC for T1, T2, T3, T4 and T5 were 4.69, 4.62, 4.60, 4.49 and 4.46 log₁₀ CFU/gm respectively, while mean TPSC were 4.46, 4.46, 4.43, 4.36 and 4.36 log CFU/gm respectively. There were no significant ($p < 0.05$) difference between the different group of combination of sausages for TPS as well as TPSC but varies significantly ($p < 0.05$) from 14th day of storage in both cases. The coliform group of bacteria will not be detected in any combination of sausages. It is concluded that microbial load of sausage prepared from spent duck is high and it decreases as the percentage of duck meat decreases but, the upper limit of bacteria in each group of sausages is within limit and hence it is safe for human consumption.

Key Words: Sausage, Spent duck, Spent hen, TPC, TPSC, TCC.

INTRODUCTION

Meat is a very nutritive and protein rich commodity and it has tremendous role in overcome the malnutrition problem of our country. It is easily digestible and consumable to all age groups peoples. But the spent or culled poultry meat is very rare consume by humans

as such because tough in their texture, less juiciness, more fibrous and high content of collagen (Bailey 1984). Now a day's most of the young people like the different types of value added meat products and we can use this type of culled or spent birds meat in the production of different types of value added

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meat products. The nutritive value as well as proximate composition and their microbial load are within the permissible limits as per BIS specification (1969) and hence we can improve the economic status of farmers and also generate employment by production of value added products and overcome the protein deficiency in our country up to some extent.

MATERIALS AND METHODS

Preparation of different ratio of duck and spent hen sausage

The meat meant for sausage preparation was thoroughly screened for removing excess fat, tendon, etc. After adequate thawing in room temperature, meat was weighed, cut into small chunks and placed in the meat mincer (Stadler Ltd.). Mincing was done by 10mm diameter plate and 5mm diameter plates subsequently. The minced meat was than chopped in a bowl chopper (Stadler Ltd.) and the following recipe was added for preparing duck and spent hen meat sausage.

Different combination of duck and spent hen meat were used in 5 treatments T1, T2, T3, T4 and T5 as shown in table 1. Ingredients include prime and non-prime cuts of duck and spent hen meat, fat (Visceral fat & Skin), salt, STPP, nitrate & nitrite, sugar, spice mix, condiments, egg yolk, curd, corn flour, soya powder and

crushed ice.

Spice mixture is a combination of different spices in dry form and powdered separately and then mixed thoroughly. Spice mixture formula of Majhi (1973) with slight modification was followed. The condiment mixture prepared by blending peeled sliced Onion, garlic and ginger (in the ratio 2:1:1 respectively) in Bajaj grinder to the consistency of a fine paste.

Samples were prepared according to APHA (1992). 10 gm of meat sample was transferred to 90 ml of distilled water and serial dilutions (six dilutions) were prepared. A serial dilution of meat sample for inoculation was prepared according to ICMSF (1986). 1 gm of sample was aseptically weighed and transferred to a sterile mortar containing 9 ml of 0.1% sterile peptone water. The sample was homogenized for 2 minutes using a sterile pestle to make 10^{-1} dilution. Than 0.1% peptone water was used for making further dilutions. 1 ml of 10^{-1} dilution was mixed with 9 ml of 0.1% peptone water to obtain a 10^{-2} dilution and so on up to 10^{-8} . Preparation of samples and serial dilutions were made near the flame in a horizontal laminar flow apparatus observing all possible aseptic measures.

Total Plate Count (TPC)

It was determined by the APHA (1992)

Table 1. Formulation of Sausage with different ratio of spent duck and spent hen meat.

Species	Treatments				
	T1	T2	T3	T4	T5
Spent duck meat (%)	100	75	50	25	0
Spent hen meat (%)	0	25	50	75	100

Where, T1= 100% Duck, T2=75+25% Duck +Spent hen, T3=50+50% Duck +Spent hen, T4=75+25% Duck +Spent hen and T5=100% Spent hen.

Table 2. Estimation of values of TPC (cfu/gm) of Sausage stored at refrigeration temperature for different storage periods (Mean ± S.E).

Parameter	0 days	3 days	7days	14 days	21 days
T1	4.62±0.09 ^{Aa}	4.83±0.12 ^{Aa}	4.86±0.43 ^{aA}	5.38±0.14 ^{aB}	5.39±0.03 ^{aB}
T2	4.46±0.09 ^{aA}	4.56±0.18 ^{aA}	4.63±0.18 ^{aA}	4.75±0.23 ^{aA}	5.26±0.05 ^{aB}
T3	4.69±0.05 ^{aA}	4.76±0.09 ^{aA}	4.76±0.23 ^{aA}	4.68±0.25 ^{aA}	5.16±0.13 ^{aA}
T4	4.60±0.03 ^{aA}	4.84±0.04 ^{aAB}	4.78±0.09 ^{aAB}	4.76±0.23 ^{aAB}	5.13±0.13 ^{aB}
T5	4.49±0.12 ^{aA}	4.59±0.06 ^{aA}	4.69±0.05 ^{aA}	4.68±0.19 ^{aA}	5.14±0.04 ^{aB}

^{a-e} Mean±S.E. with different superscript in same column significantly differ at P <0.05.

^{A-D} Mean±S.E. with different superscript in same row significantly differ at P <0.05.

Table 3. Estimation of values of TPSC (cfu/gm) of Sausage stored at refrigeration temperature for different storage periods (Mean ± S.E).

Parameter	0 days	3 days	7days	14 days	21days
T1	4.46±0.06 ^{aA}	4.55±0.07 ^{aA}	4.60±0.15 ^{aAB}	4.88±0.02 ^{bC}	4.84±0.03 ^{cBC}
T2	4.46±0.08 ^{aA}	4.46±0.08 ^{aA}	4.53±0.12 ^{aA}	4.87±0.02 ^{bB}	4.82±0.04 ^{bcB}
T3	4.43±0.08 ^{aA}	4.50±0.10 ^{aA}	4.72±0.06 ^{aB}	4.90±0.03 ^{bB}	4.88±0.02 ^{cB}
T4	4.36±0.06 ^{aA}	4.46±0.09 ^{aA}	4.69±0.05 ^{aB}	4.83±0.02 ^{bB}	4.73±0.03 ^{abB}
T5	4.36±0.06 ^{aA}	4.36±0.06 ^{aA}	4.50±0.10 ^{aAB}	4.75±0.03 ^{aC}	4.67±0.03 ^{aBC}

^{a-e} Mean±S.E. with different superscript in same column significantly differ at P <0.05.

^{A-D} Mean±S.E. with different superscript in same row significantly differ at P <0.05.

Table 4. Total Coliform Count (TCC) cfu/gm of Sausage stored at refrigeration temperature for different storage periods (Mean ± S.E).

Parameter	0 days	3 days	7days	14 days	21 days
T1	ND	ND	ND	ND	ND
T2	ND	ND	ND	ND	ND
T3	ND	ND	ND	ND	ND
T4	ND	ND	ND	ND	ND
T5	ND	ND	ND	ND	ND

ND = Not detected.

method using plate count agar. 1 ml of appropriate dilution of sample was transferred aseptically to sterile petri plates in duplicate. The plates were then poured with 10-15ml melted plate count agar medium at 45°C. After solidification the Petri-plates were incubated at 37°C for 24-48 hrs. The colonies were counted by using colony counter. The average number of colonies was multiplied with dilution factor to obtain total count as colony forming unit (CFU) per g of the sample. This count was then converted to total plate count of log CFU/g of sample.

Total Psychrophilic Count (TPSC)

Psychrophilic counts of the samples were determined by the methods described by APHA (1992). Using Plate Count Agar, duplicate samples of 1ml volume of suitable dilutions was inoculated into the petridishes as described above. The plates were incubated at 4±1°C for 10-14 days and the colonies were counted and expressed as log c.f.u./g of sample.

Total Coliform Count (TCC)

The coliform count was determined as per the procedure described by APHA (1992). 1 ml of appropriate dilution of sample was transferred aseptically to sterile Petri plates in duplicate. The cultured plate was prepared by pouring 10-15 ml of melted violet red bile agar on sample. In order to ensure proper sample mixing the plates were rotated in clockwise and anticlockwise directions and allowed to set. These plates were incubated at 37°C for 18-24 hours. The average number of colonies was multiplied with dilution factor to obtain total count as colony forming unit (CFU) per gm of the sample. This count was then converted to coliform count of log CFU/g of sample.

RESULTS AND DISCUSSION

Mean ± S.E. values of total plate count (TPC) of different combination of sausages stored at refrigeration temperature for different storage periods are tabulated in Table 2.

The TPC of different group of sausages at refrigeration temperature increased gradually but not varied significantly ($p>0.05$) up to the 14th days except in T1, it vary significantly from 7th days of storage. While others group varies significantly ($P<0.05$) from 14th to 21st day of storage.

However, within groups all samples varied insignificantly ($p>0.05$) on each day of refrigeration throughout the storage periods. But higher ratios of duck meat in the formulation higher the TPC of the sausages (Biswas *et al.* 2006) However among these five groups of sausages S4 & S5 had minimum value of TPC throughout the storage periods.

Biswas *et al.* (2006) reported that the TPC value of duck sausages on 2nd day at ambient temperature ($\log 5.33 \pm 0.39$ c.f.u/g) was significantly ($p<0.01$) higher than that of 3rd day at chilling temperature ($\log 2.79 \pm 0.24$ c.f.u/g). A similar trend was also observed by Pangas *et al.* (1998) in refrigerated fried chicken gizzard bearing lower TPC ($\log 4.30$ c.f.u/g) than those at ambient ($\log 6.11$ c.f.u/g) storage on 7th day. Weiser *et al.* (1978) stated that optimum temperature (30 to 40°C) might cause a rapid multiplication of mesophilic organisms when compared to lower temperature. The TPC value of duck sausages did not show significant difference between 0 day and 3rd day at chilling storage. Rao *et al.* (1999) found that there was no significant change in TPC of smoked chicken sausages up to 1 week of chilling storage. A significant ($p<0.01$) increase in TPC of duck sausage was noticed on 7th day at chilling

storage. This increase in TPC of duck sausages with increase in storage periods may be due to multiplication of microorganisms during storage (Bawa *et al.* 1988).

Similar results have been reported by Kondaiah *et al.* (1988), Sahoo and Anjaneyulu (1997) for goat meat products. In the present study, high TPC values of duck sausages were noticed on 2nd day at ambient temperature and 21st day at chilling temperature ($\log 5.33 \pm 0.39$ cfu/g and $\log 5.62 \pm 0.31$ cfu/g respectively). The values observed in this experiment were far below the incipient spoilage level of $\log 6.70/g$ as indicated by Vohnoly and Holzaptel (1991).

Bhattacharyya *et al.* (2007) reported that Total plate count of sausage emulsion and cooked sausages did not vary significantly among these three (Broiler, spent hen & spent duck) types of sausages. TPC in sausage emulsion and cooked sausages up to 14th day of storage were within the permissible limit of specification of BIS (1969, 1992a), which specified that maximum level of aerobic plate count in uncooked and cooked sausages should not be more than 6 log cfu/g and 4 log cfu/g, respectively. The result shows that TPC increased with advancement of storage period significantly in all the sausages. Up to 3rd day, the difference was not significant and up to 14th day of refrigerated storage, both the sausages were acceptable according to BIS (1992a).

The Maximum Permissible Limits (MPL) for APC in sausage was 7 log₁₀ CFU/g (ICMSF, 1986). The TPC in the sausages of all groups in this study was well under the ICMSF recommended maximal permissible limits (MPL) for aerobic plate count (APC).

Mean \pm S.E. values of total Psychrophilic count (TPSC) of different combination of sausages stored at refrigeration temperature for

different storage periods are tabulated in Table 3.

The TPSC of different group of sausages at refrigeration temperature increased gradually but not varied significantly ($P > 0.05$) up to the 7th days except in T3 & T4. They varied significantly ($p < 0.05$) from 3rd to 7th day after did not varied significantly ($p > 0.05$) up to 21st days of storage. While others group varies significantly ($p < 0.05$) from 14th to 21st days of storage. This finding agreed with the reports of Kandeepan and Biswas (2007).

However, within groups all samples varied insignificantly ($p > 0.05$) on each day of refrigeration throughout the storage periods except on 14th and 21st day of storage. But higher ratios of duck meat in the formulation higher the TPSC of the sausages. However among these five groups of sausages T4 & T5 had minimum value of TPSC throughout the storage periods.

Biswas *et al.* (2006) reported that a significant ($p < 0.01$) increase in TPSC of duck sausage was noticed at ambient temperature, which might be due to growth of Psychrophilic organisms. Weiser *et al.* (1978) stated that the higher temperature limit for growth of psychrophilic organisms at 30°C and they also reported that the optimum temperature range for growth of psychrophilic organisms is 15°C to 20°C. in the present study, a significant ($p < 0.01$) increase in TPSC of duck sausages was noticed on 7th, 14th and 21st day of chilling storage as compared to that of 0 day. These results are similar to those of Pati *et al.* (1993).

Bhattacharyya *et al.* (2007) reported that TPSC increased with advancement of storage period significantly in all the sausages. Up to 7th day, the difference was not significant. The similar trend was observed by Bhojar *et al.*

(1997). The reason for lower value of TPSC in cooked sausage than that of emulsion was same as in the case of TPC. This might be due to increased enzyme activity of psychrotrops at low temperature hugely contributed to deterioration of meat quality (Kandeepan and Biswas 2007).

Mean \pm S.E. values of total Coliform count (TCC) of different combination of sausages stored at refrigeration temperature for different storage periods are tabulated in Table 4.

The freshly prepared and stored different combination of sausages were evaluated for the Coliform organism but TCC was nil in all groups throughout the refrigeration storage periods and within the group on each day of storage. The reason behind this observation might be the hygienic measures followed in different steps of processing of the sausage or may be due to death of organism during smoking (around 60°C) as the thermal death point of the coliform organism is 57°C. This result was in close agreement with the report of Sinhsamahapatra (2005), Hazarika (2000).

Biswas *et al.* (2006) reported that the TCC of duck sausage on 0, 3rd, 7th, 14th and 21st day at chilling storage were not at detectable levels. TCC of duck sausage on 2nd day at ambient temperature was $\log 14 \pm 3.62$ c.f.u/cm². Buxton and Fraser (1977) stated that the growth of coliform bacteria groups would occur over a temperature range of 20-44°C. Pal *et al.* (2003) noticed that no coliform could be detected up to 8th days in the cooked sausages prepared from vanaraja bird meat at chilling temperature.

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Assessment of microbial load of sausages prepared from different combination of ...

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***Cite this article as:** Kumar R, Biswas S, Singh V, Ram M (2016) Assessment of microbial load of sausages prepared from different combination of spent duck and spent hen meat. *Explor Anim Med Res* 6(2): 205-211.