

EFFECT OF TEMPERATURE AND PERIOD OF STORAGE ON THE BACTERIAL PROFILE OF KABAB*

E. Nanu¹, K.G. Narayan² and R. Yadava³

Department of Veterinary Public Health & Epidemiology,
Ranchi Veterinary College, Birsa Agricultural University,
Kanke, Ranchi 834007, BIHAR

In India, ready-to-eat pork products like Kabab, Salami, Trakker, Sausages etc. are being produced by 8 modern bacon factories and 144 pork processing centres in private sector under MFPO license. The demand for these highly perishable products is ever increasing. The composition and processing technique of these products differ from product to product and factory to factory. Bacterial profile also differ accordingly.

The present study was undertaken to assess the bacterial profile of Kabab** (Ranbac, Ranchi) and the effect of temperature and period of storage on it.

Materials and Methods

Collection of sample

One hundred and twenty Kabab samples were collected from the retail outlet of Ranchi Bacon Factory, Bihar. Each sample consisted of 200 g packed in a polythene

bag. Twenty samples were collected from each batch and were brought to the laboratory within 15 minutes. Samples from each batch were selected randomly and distributed as shown in figure 1. From each batch two samples were examined on the day of collection (0 day) and two samples each stored at 10°C, 4°C and -20°C were examined on day 4, 8 and 12 of storage.

Bacteriological examination

Preparation of sample

A ten fold dilution in NSS was made from minced sample. The types of bacteria enumerated, media used, volume of inoculum, method of inoculation, method of incubation, period and temperature of incubation followed according to various standard techniques are shown in Table 1. For enumerating each group of bacteria, from each sample 10² dilution and other selected dilutions were inoculated in duplicate plates or tubes.

* Part of Ph.D. thesis of the first author

Present address : 1. Professor, Dept. of VPH, College of Veterinary and Animal Sciences, Mannuthy, P.O. Thrissur, Kerala, Pin 680 651
2. Professor and Head; 3. Assistant Professor

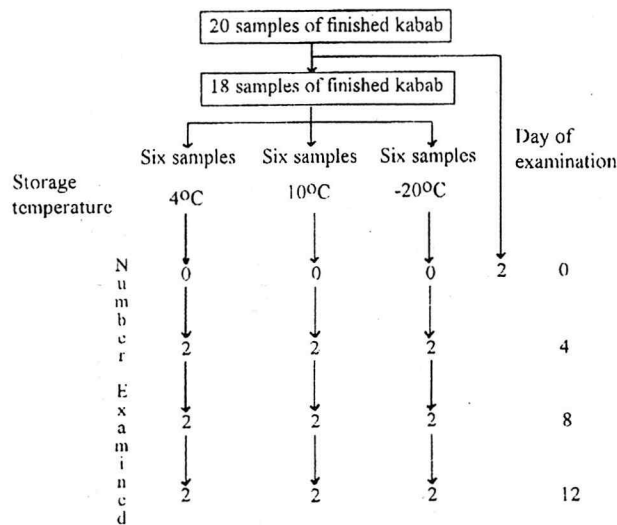
** Kabab is a deep fried ball of minced pork blended with spices and other ingredients

Table 1 Types of bacteria, types of media, method of inoculation, volume of inoculum, incubation temperature(s) and period of incubation used for different bacteria

Types of bacteria	Method of inoculation	Media used	Volume of inoculum (mL)	Incubation		References
				Temperature(s) (°C)	Period (Hours)	
Mesophilic	Pour plate	TGEA	1.0	37	24	Am. Pub. Hlth. Assoc. (1976)
Psychrophilic	Pour plate	TGEA	1.0	7	120	Am. Pub. Hlth. Assoc. (1976)
Coliform	Spread plate	VRBA	0.1	37 and 44	24	Nordic Committee on Food Analysis (1966)
Faecal streptococci	Spread plate	SBA	0.1	37	48	Nordic Committee on Food Analysis (1968)
<i>Staphylococcus aureus</i>	Spread plate	TPEYS	0.1	37	24	Leninger (1976)
Sulphite reducing clostridia	Agar tube TSCA	EY free	1.0	46	24 (water bath)	Hauschild and Hilscheimer (1974)

TGEA = Tryptone glucose yeast extract agar; VRBA - violet red bile agar; SBA = Slanetz and Bartley agar; TPEYA = Tellurite polymyxin egg yolk agar; TSCA = Tryptone sulphite cycloserine agar

Fig. 1. Distribution and storage of a batch of kabab samples to be examined under conditions simulating to marketing



Isolation of Salmonella : 2 x 25 g from each of the minced samples were weighed on a sterile butter paper, transferred into two different conical flasks containing 75 ml sterile nutrient broth and was incubated at 37°C for 24 hours. To each of these flasks, 75 ml double strength Muller Kauffman tetrathionate broth was added and was incubated at 37°C and 43°C. MacConkey's agar plates were streaked after 24 and 48 hours of incubation and the plates were incubated at 37°C for 24 hours. Suspected colonies were selected and subjected to further characterization as described by Edwards and Ewings (1962).

Results and Discussion

Of the 120 Kabab samples examined, none yielded coliforms growing at 37°C and 44°C, faecal streptococci and sulphite reducing clostridia in 10^{-2} dilution and above.

Salmonella was not detected in any sample examined by direct and enrichment technique. The mesophilic and psychophilic bacterial counts ranged between 0.05 to 1650.0 and 0.0 to 2.33 respectively.

The effect of storage of Kabab at -20°C, 4°C and 10°C and period of storage of 4, 8 and 12 days on counts of groups of bacteria is given in Table 2. It was found that the count of mesophilic bacteria at 20°C was significantly less than the count at 10°C ($P < 0.05$) irrespective of the period of storage. The difference between the mean counts at 4°C and 10°C and between 20°C and 4°C was not significant. On examination of the overall means, it was found that the mesophilic bacterial population did not increase significantly in four days. Storage at 20°C did not increase mesophilic bacteria significantly on 4, 8 and 12 days of storage.

Table 2 Mean value of counts of mesophilic bacteria in kabab stored at -20°C, 4°C and 10°C for 0, 4, 8 and 12 days and their interactions

Storage temperature (°C)	Period of storage in days				Overall mean
	0	4	8	12	
-20	135.28 ± 121.46	297.39 ± 169.10	421.88 ± 244.32	691.50 ± 327.06	386.51 ^A
4	135.28 ± 121.46 ^a	330.86 ± 160.55 ^a	7345.14 ± 4450.53 ^b	1941.58 ± 1108.95 ^{ab}	2438.21 ^{AB}
10	135.28 ± 121.46 ^a	407.39 ± 176.60 ^a	9955.81 ± 4844.74 ^b	5465.76 ± 2304.68 ^{ab}	3991.06 ^B
Overall mean	135.28 ^a	345.21 ^a	5907.61 ^b	2699.61 ^{ab}	
CD values	Temperature (A) 3377.98	Period (B) 2925.42	AxB 5850.84		

Values bearing same superscript (small letters for row and capital letter for column) did not differ significantly within the same row and same column

Analysis revealed that the effect of temperature was not significant, whereas, the effect of period of storage was significant ($P < 0.05$). The mean psychrophilic bacterial count of Kabab stored at 20°C, 4°C and 10°C for 0, 4, 8 and 12 days are presented in Table 3. Irrespective of the period of storage, temperature did not appear to increase the population of psychrophilic bacteria significantly. Similarly, four days of storage, irrespective of temperature at which Kabab was stored did not increase the number psychrophilic bacteria significantly. It was also observed that the psychrophilic bacteria did not increase significantly at any day of storage of Kabab at 20°C and also in four days of storage at 4°C and 10°C.

The number of staphylococci per gram of sample and also the number of sample positive for this bacterium were very low. At 20°C and 10°C the count became nil on 8 and 12 days. On day four of storage at these temperatures the staphylococcal count was reduced. The effect of the two factors viz., temperature and period of storage was not significant. There was no significant change in staphylococcal population at any storage temperature.

An attempt was made to determine the relationship between the growth of mesophilic bacteria and staphylococci during the storage studies. In general a non-significant and negative association between the two groups of bacteria was observed at all the temperatures and period of storage studied (Table 4). The association of two groups of bacteria was positive only at 10°C and 12 days but this was non-significant. A negative association suggested that with increase in population of mesophiles there

was a corresponding decrease in the population of staphylococci.

Growth curve of mesophilic and psychrophilic bacteria is presented in figure 2. The doubling time for psychrophilic bacteria at these temperatures was less compared to that for the mesophilic ones. Yet the generation time was very long as compared with the generation time for mesophilic bacteria at 37°C.

Fig.2. Growth curve of mesophilic and psychrophilic bacteria in kabab stored at 10, 4 and -20°C

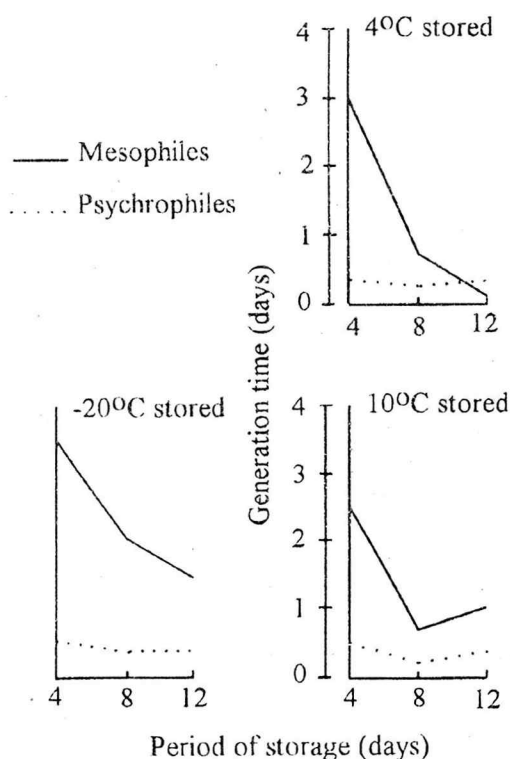


Table 3
Mean value of counts of psychrophilic bacteria in kabab stored at -20°C, 4°C and 10°C for 0, 4, 8 and 12 days and their interactions

Storage temperature (°C)	Period of storage in days				Overall mean
	0	4	8	12	
-20	0376 ± 0.21	167.25 ± 81.61	856.69 ± 370.87	256.69 ± 238.86	320.25
4	0.376 ± 0.21 ^a	367.81 ± 252.78 ^a	27750.25 ± 18069.71 ^b	3505.65 ± 1574.29 ^a	7906.02
10	0.376 ± 0.21	784.76 ± 298.00 ^a	34209.91 ± 24653.35 ^b	3666.70 ± 1767.26	9663.43
Overall mean	0.376 ± 0.21	439.94 ^a	20938.95 ^b	2476.34	
CD values	Temperature (A) 14684.22	Period (B) 12716.90	AxB 25433.83		

Values bearing same superscript did not differ significantly within the same row and same column

Table 4 Correlation coefficients between mesophilic bacteria and staphylococci in kabab samples stored at -20°C, 4°C and 10°C for 0, 4, 8 and 12 days

Storage period	Storage temperature (°C)		
	-20	4	10
0	-0.1794	-0.1794	-0.1898
4	-0.2315	-0.2232	-0.1751
8	*	-0.1338	-0.0830
12	*	-0.1383	0.1865

* Count being very low it could not be done

The bacteriological quality of fresh and stored Kabab compared well with the guide lines or standards recommended by Nebraska Department of Agriculture and North Dakota, Department of State Laboratory for similar heat treated products (Wehr, 1982).

This study brought out that fresh finished Kabab of good bacteriological quality and satisfactory hygienic status of production maintained a good keeping quality for 12 days at -20° C and for 4 days at 4° C and 10° C.

Summary

One hundred and twenty Kobab (Ranbac) samples were examined to assess the hygienic status of production, effect of storage temperatures (-20°C, 4°C and 10°C) and period of storage on bacterial profile of Kabab on day 4, 8 and 12 of storage. None of the samples yielded coliform count and sulphite reducing clostridial count. No sample yielded Salmonella in 2 x 25 g portion. The samples examined on 0 day yielded a mean mesophilic count of $135.28 \pm$

121.46×10^4 per gram. The mean count of psychrophiles and staphylococci in these samples were $0.376 \pm 0.021 \times 10^4$ and $0.0015 \pm 0.0007 \times 10^4$ per gram respectively. The mesophilic bacterial count at -20°C stored Kabab was significantly less than the count at 10°C ($P < 0.05$) irrespective of the period of storage. The psychrophilic bacterial count did not increase significantly at any day of storage at 4°C and 10°C. Staphylococcal count was reduced on day 4 of storage at 20°C, 4°C and 10°C. The count of Kabab stored at -20°C and 10°C became nil on day 8 and 12.

Acknowledgement

Financial assistance provided by ICAR to the first author as Senior Fellowship and sanction of a research scheme to the second author is gratefully acknowledged. The authors wish to thank the Dean, Faculty of Veterinary Science and Animal Husbandry and Director of Research, Birsa Agricultural University for providing facilities to carry out this work.

References

- Am. Pub. Hlth. Assoc. (1976). Compendium of methods for the Microbiological Examination of Foods. Speck, M.L. ed 1015 Eighteenth Street, New York, Washington DC 20036
- Edwards, P.R. and Ewings, W.H. (1962). Identification of Enterobacteriaceae, Burgeys Publishing Company, Minnesota
- Hauschild, A.A.W. and Hilscheimer, R. (1974). Enumeration of food-borne *Clostridium perfringens* in egg-yolk free tryptose-sulphite-cycloserine agar. Appl. Microbiol. 27: 521-526
- Leninger, H.V. (1976). Equipment, Media, Reagents, Routine tests and stains. In: Compendium of methods for the Microbiological Examination of Food. ed Speck, M.L. Am. Pub. Hlth. Assoc., 1015 Eighteenth Street, New York Washington, DC 20036
- Nordic Committee on Food Analysis (1966). Nr. 02 UDC 576. 851.48
- Nordic Committee on Food Analysis (1968). Nr. 68 UDC 576.581.21
- Wohr, H.M. (1982). Attitudes and policies of Governmental Agencies on Microbial criteria for Foods - an update, Fd. Technol. 45 - 54 and 98