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A STUDY OF THE MICROBIOLOGICAL ASPECTS OF COMMERCIAL AND DOMESTIC FROZEN BEEF AND BEEF PRODUCTS

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Frozen beef and beef products purchased from six selected stores of three different localities of the Lahore city were investigated for microbial contamination and compared with those frozen at -18^{o} in the laboratory for acceptable standards. Total viable counts and coliforms were examined. The results showed that some of the market meat products such as Shami Kabab and Samosa samples were contaminated with coliforms; the counts being higher than the values reported for the frozen meat and meat products. The observed microbial load in some of the market products could be attributed to (i) inadequate freezing techniques (ii) unhygienic handling practices and (iii) the contaminated spices and additives. The storage temperature of about -18^{o} was found quite suitable for storing beef and beef products for at least two months.

Key words: Beef, Freezing, Microbiology.

INTRODUCTION

Microbiological examination of frozen (-18^{o}) meat and meat products during storage is important for public health and safety. Meat and meat products may be contaminated by harmful microorganisms during various operations and treatments prior to freezing. Survival of microorganism during subsequent storage at freezing temperature may pose public health hazards. Freezing at about -20^{o} inhibits the growth of microorganisms (Poindexter; [10]) and also resutls in destroying about 40% of *Escherichia coli* (Warseck; [14]). Survey of the local frozen food markets has shown contamination of meat and meat products with pathogens which are potential hazard for public health. Microbiological count of fresh beef, obtained from retail shops, was found to be within permissible standards for the local retail market (Ehteshamuddin; [5]), Ahmad; [1]. Beef and beef products obtained from commercial storages have often shown the presence of coliforms and other organisms (Geopfert, [17]) making it imperative to undertake a systematic study to identify the extent and causes of contamination, in the frozen meat and meat products of local retail market.

The present study is a part of the continuing studies of the authors on microbiological quality of the animal sources of protein and their products, sold in the retail local markets in fresh as well as frozen states. Frozen storage of meat in freezer is becoming increasingly popular and, in some cases, it has become a necessity in the urban areas of Pakistan. This work examined the bacteriological quality of the domestic and commercial frozen beef and beef products, namely, Shami Kabab and Samosa to determine the effects of prevailing practices for storage of these precious sources of animal protein at household level.

MATERIALS AND METHODS

Materials. Nutrient agar (difco) ringer's solutions (pH 6.6) E.M.B. agar (difco)

Methods

(i) *Preparation of meat samples.* About 16 kg of boneless beef from the hind quarter of single animal tissue was obtained from local market. The meat was chopped to about 1½" x 1½" size. It was divided in 4 equal parts of 4 kg each.

One part was kept as such, without any further treatment, as control.

Second part was given salt treatment with 2% sodium chloride by rubbing salt on the meat pieces with hand.

To the third part 5% beef fat was added.

The fourth part was processed for the study of household thawing effect.

The four treatments were so designed to simulate household treatment of frozen meat and to monitor the effect of certain additives when added before freezing stored meat.

Each of these four parts was further sub-divided in 8 equal parts and packed separately in polythene bags, sealed and stored in deep freezer at about -15 to -18^{o}.

(ii) *Preparation of meat products.* Samosas and Shami Kababs were prepared using the minced beef of the same muscles of the same animal obtained separately. Each,

Samosas and Shami Kababs were prepared 128 in number. Half of each were fried in ghee on Tawa, (hot plate), whereas the other half was not fried and stored. Each half was equally distributed into 8 parts and packed in polythene bags and sealed. These samples were also stored in a domestic freezer at about −15 to -18^{o} for 2 months. One packet of each of the fried and non-fried samosas and Shami Kababs were taken out at fixed intervals of time, for microbiological examinations.

(iii) *Commercial market frozen samples.* Samples of meat, Shami Kababs and Samosas were purchased from selected shops of the three well-known retail markets of the city of Lahore (Gulberg, Muslim Town and Model Town). Samples were procured according to a planned experimental schedule and subjected to the microbiological examinations.

(iv) *Spices and additives used in meat products. Samples* of common spices and additives were obtained from three different localities of Lahore and subjected to microbiological examinations.

Microbiological method. The methods used for the microbial examination of the samples was plate count method in order to determine the total viable count and coliforms.

Before testing these samples, the samples were first blended in an electric mincer for 3 minutes.

Procedure (pour plate method)

Total viable count. Nutrient agar or E.M.B. Agar was melted and poured (15 ml) in tubes, cooled to 45^{o} in a waterbath. The petri-dishes were set out 4 per dilution. 10 gm of the sample was blended with 90 ml sterilized Ringer solution and 1:10, 1:100, 1:1000, 1:10000 dilutions were prepared. 15 ml melted, cooled, sterilized media was poured into each petri-dish, and allowed to solidify. One plate of the medium was run as a control. All the plates were incubated at 37^{o} for 48 hrs. Duplicate plates containing colonies were counted, results were averaged and reported as total count per gram of sample.

Coliform count. 1.0 ml of the sample (10^{1}-10^{4} dilutions) was transferred to the petri-dish and cooled E.M.B. medium (10-15 ml) was poured on it and was allowed to solidify. The duplicate plates were incubated at 37^{o} for 24 hrs. The number of colonies were counted and averaged. Number of coliforms per gm of sample was reported in this study as +ve of −ve only, for the presence or absence of coliforms.

RESULTS AND DISCUSSION

Commercial frozen beef and beef products. Bacteriological quality survey of frozen meat, shami kabab and Samosas from the three retail markets of Lahore is presented in Table 1. TVC for all of the samples from the retail shops were within the limits of 0.55×10^{5} to 3.3×10^{5}/gram. Values of APC obtained in similar studies (Poindexter; [10]) have been reported as 7.8×10^{6} for the initial unfrozen beef; 9.7×10^{6} for beef frozen at -20^{o} in laboratory deep freezer for 4 days; and 1.1×10^{7} for the same beef frozen at -20^{o} for 11 days. Several other workers also reported values of TVC for beef in this range (Westhoff, and Feldstein, [15]; Al-delaimy, and Stiles, [2]; Geopfert, [6]; Chambers, *et al.* [3]; Shoup and Oblinger, [12]; Summer, [13]; Ehteshamuddin *et al.* [5]; Ahmad *et al.* [1]; Seriven, and Sing, [11].

The effect of the duration of freezing at about -15^{o} is so gradual that it can only be significant over widely different storage periods (Kotula; [9]). Therefore, although the complete history of the samples of commercial frozen meat and meat products was not known, it was assumed that they had originated from meat of good bacteriological quality.

TVC were comparable to those reported earlier by Ehteshamuddin [5] and Ahmad [1] for fresh beef obtained from the local retail market of Lahore. Coliforms were not observed in any of the samples of these reported surveys. On the basis of observations coliforms were not expected to be present in the frozen beef samples. Therefore, positive results for coliform organisms in the commercially frozen beef from shop 1 of Gulberg, shop 2 of Muslim Town and shop 2 of Model Town strongly suggest that either sufficient care had not been exercised or the freezing techniques did not include initial rapid chilling to control microbial contamination, prior to freezing.

Sensitivity of coliforms to low temperature is well known (Warseck *et al.* [14]). A study by Poindexter [10] showed that in the samples of beef, the initial coliform (MPN) counts 392 reduced to 328 by freezing with dry ice ($.70^{o}$) for 48 hrs; to 285 by freezing at -20^{o} for 4 days; and to 270 by freezing at -20^{o} for 11 days. Other workers (Kotula; [9]) have also indicated that freezing at -15^{o} reduced pathogenic *C. jejuni*, similar to coliform by three orders of magnitude after three days. Thereafter the decrease became slow similar to the figures cited above.

The observed microbial contamination in the present commercial frozen beef must have occurred therefore, either during transportation or handling operations before the storage. It is however, presumed that chilling prior to storage would have reduced the MPN for coliforms within limits (Poindexter *et al.* [10]) and (Kotula *et al.* [9]).

Frozen beef. Effect of frozen storage on fresh and treated beef was studied over a period of 8 weeks, by freez-

ing fresh beef at -18^{O}. The initial TVC values were 12×10^5/gm and the coliforms were negative.

Similar to the observations of various authors mentioned above, the TVC decreased gradually with the duration of storage at -18^{O} from 12×10^5 to 2.2×10^5. Results for coliforms remained negative in all the samples from the initial to the last. These results indicate that if the initial sample be free from pathogenic contamination like coliforms, the meat can be stored safely in the freezer at -18^{O} without adverse microbial increase, for at least two months. The treated beef also behaved similarly for the TVC but the decrease in microbial number was more pronounced with the storage period. The appearance of coliforms in the 6th and 8th week of storage in the treated samples may be the result of contaminated salt or the methodology of salting.

Frozen beef products. For the meat products (Shami Kababs and Samosas) the microbial studies were complicated by the incorporation of spices, herbs etc. and the excessive handling operations during their preparations. Particular attention is needed to reduce the initial bacterial load from these sources. This is clearly shown by the positive coliform results in the commercial samples as well as in those prepared hygienically under laboratory controlled conditions.

The TVC and coliforms results on the commercial products are given in Table 1. No systematic trend is shown by these values with regard to storage period. The laboratory meat products i.e. Shami Kababs and Samosas were prepared with beef and selected spices, herbs and additives according to the customary practices. After determining the initial TVC and coliform (0-week), they were stored in freezer at -18^{O} for periods upto 8 weeks. Bacteriological results of these meat products at later storage periods are given in Table 3.

TVC for Shami Kababs and Samosas do not show systematic decrease with increasing periods of storage. This

Table 2. Bacteriological quality of Fresh Frozen Lab. stored meat.

Period of storage	pH		T V C/gram		Coliform Org./gram	
	Control	Treated*	Control	Treated*	Control	Treated
0 Week	5.3	5.3	12×10^5	7.5×10^5	–ve	ve
4th Week	5.2	5.4	4.7×10^5	1.9×10^5	–ve	–ve
6th Week	5.5	5.3	0.41×10^5	1.3×10^5	–ve	–ve
8th Week	5.6	5.32	2.2×10^5	1.0×10^5	ve	+ve

*Treated stand for 2% salted (NaCl) fresh lean beef.

Table 3. Bacteriological quality of Lab. stored frozen meat products.

Period of storage	T V C/gram		Coliform Org/gram	
	Kababs	Samosas	Kababs	Samosas
0 Week	1.6×10^5	7.0×10^5	–ve	+ve
4th Week	2.2×10^5	3.4×10^5	–ve	+ve
6th Week	$.034 \times 10^5$	1.3×10^5	–ve	–ve
8th Week	0.96×10^5	8.2×10^5	+ve	–ve

Table 1. Bacteriological quality survey of meat and meat products in the three retail markets of Lahore.

Name of locality		pH*	T V C/gram			Coliform Org./gram		
		Meat	Meat	Kababs	Samosas	Meat	Kababs	Samosas
Gulberg	Shop 1	5.3	8.0×10^5	8.5×10^5	5.8×10^5	+ve	+ve	+ve
	Shop 2	5.5	1.9×10^5	8.3×10^5	7.9×10^5	–ve	–ve	–ve
Muslim Town	Shop 1	5.5	55×10^5	7.8×10^5	1.2×10^5	–ve	–ve	–ve
	Shop 2	5.7	7.8×10^5	7.8×10^5	6.5×10^5	+ve	–ve	–ve
Model Town	Shop 1	5.57	60×10^5	6.2×10^5	14×10^5	–ve	–ve	–ve
	Shop 2	5.45	32×10^5	5.2×10^5	18×10^5	+ve	+ve	+ve

*The pH value was measured in case of meat not of the products

Table 4. Microbiological examination of ingredients used in meat products.

No.	Ingredients	*TVC/gm	*Coliform/gm	Remarks
1.	Chilli powder	$9.5x10^6$	–ve	Fungus observed
2.	Salt	$4.8x10^3$	–ve	Fungus observed
3.	Coriander powder	$1.7x10^4$	–ve	–
4.	White cumin	$6.7x10^5$	–ve	Fungus observed
5.	Black pepper powder	$7.1x10^4$	–ve	Fungus observed
6.	Clove	$7.3x10^3$	–ve	Fungus observed
7.	Cardamon large	$8.5x10^6$	–ve	Fungus observed
8.	Cinnamon	$8.5x10^3$	–ve	Fungus observed
9.	Black cumin	$7.5x10^5$	–ve	–
10.	Pomegranate seeds	$9.2x10^6$	–ve	Fungus observed

*Note: Average values of the ingredients from three different places.

leads to the conclusion that microbial contamination in this case is not of primary origin in beef, but is most probably due to spices/additives or handling operations.

The results of tests for coliform organisms in Shami Kababs and Samosas are also more or less of the same nature i.e. not systematic with respect to duration of storage. They may be explained on the basis of most probable contamination from spices, herbs and additives (Table 4). Baxter and Holzapfel [8] have reported such microbial contaminations originating from spices, herbs and additives. Among other microorganisms *E. coli* was also found in Rosemary by these workers.

The coliform contamination at 0 and 4th week in the case of Samosas and 8th week in case of Shami Kababs, is noteworthy. Although it is difficult to suggest a plausible explanation for coliform contamination in the frozen Kababs at the 8th week the method of preparation of Samosas itself, appears to be responsible for the pathogens appearing at the initial stage of storage. Samosa is a wrapped minced meat mixture with spices. The wrap is made with hand from dough made from fine wheat flour using edible fat.

Spices and additives. Some of the spices particularly chilli powder, cardamon large and pomegranate seeds were found to have high TVC/gm. No coliform was, however, detected in any ingredient. Fungus was observed in most of the ingredients (Table 4). Contamination in meat products therefore may be attributed to the spices and additives.

CONCLUSION

The important points brought to light by these studies are:

(1) Commercial frozen meat and meat products from the retail shops may contain pathogenic bacteria.

(2) Commercially and domestically frozen meat products (shami kababs and samosas) prepared with spices, herbs and additives may acquire bacterial load from these added ingredients or/and from the method of their customary preparations.

(3) The presence of coliforms at initial stages may be taken as indication of contamination due to unhygienic handling before freezing of meat and meat products.

(4) Salt treatment of meat before freezing reduces the bacterial load effectively during storage at -15°. However, the risk of secondary contamination increased during rubbing of salt on meat by hand.

Conventional domestic cooking in which internal temperature of the meat and meat products exceeds 70° for about 5-10 minutes ensures destruction of almost all pathogenic organisms, eliminating health risk to consumers. However, if under-cooked, the frozen beef and beef products remain potential hazard to public health.

Meat should, therefore, be frozen as soon as possible after slaughter. Delay for any reason may cause increase in bacterial load (Dack, [4]). Chilling the meat and meat products using liquid nitrogen or dry ice prior to frozen storage should be employed to minimize the risk of enhanced microbial growth.

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