Bangladesh Journal of Animal Science
Journal homepage: http://www.banglajol.info/index.php/BJAS

Effect of post-slaughter time and storage conditions on chemical and microbial changes in locally marketed beef

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Abstract

Fresh meat is commonly marketing at environmental temperature for long time in many developing countries including Bangladesh. The present study was conducted to assess whether elapsed time between slaughter and preservation and storage conditions influence the chemical and microbial changes of locally marketed beef. Meat samples were collected from local markets and divided into two groups, morning and evening beef. Morning beef was collected immediately after slaughtering from healthy cattle while evening one was collected 8 h after slaughtering. The samples were kept either in refrigerator (4^{o}C) or freezer (-20^{o}C). Refrigerated samples were stored for 7 days and analyzed on day1st, 3rd and 7th while frozen samples were stored for 90 days and analyzed on day 3rd, 45th and 90th. Results showed that there was a significant difference in chemical and microbial parameters between morning and evening beef ($p<0.01$ to 0.05). With respect to the advances of storage time, the dry matter, crude protein, ether extract and ash contents were increased in beef sample ($p<0.01$), indicating the moisture loss from meat time elapsed after slaughtering. Moreover, the coliform, yeast and mold counts were also increased with advance of storage time ($p<0.01$ to0.05),indicating the unhygienic conditions of slaughter house, equipment and water which is giving signal for the possible occurrence of food borne intoxication. In conclusion, we found that the quality of marketed beef degraded with the time elapsed before storage and storage temperature suggesting the importance of early preservation of meat at lower temperature. Our findings of increased number of microbial counts were also suggested the necessity to improve the hygienic conditions of slaughterhouse and equipment in developing countries like Bangladesh.

Key words: Beef, local market, microbial changes, storage conditions

 Bang. J. Anim. Sci. 2015. 44(1): 52-58

Introduction

Meat is one of the food items that spoils very quickly and makes it unacceptable to the consumers. Apart from physical damage and oxidation, other spoilage symptoms are ascribable to the undesired growth of microorganisms (Nortje and Naude 1981; Ercolini et al. 2006). Freezing is one of the important approaches for preserving meat from spoilage and widely used to store meat from months (Moore 1990) to years (Haygard et al. 1993). Numerous studies have been published on the effect of freezing and storage temperature on the quality of frozen beef (Boles and Swan 1996; Azad and Akhter 2005; Eneji et al. 2007; Pietrasik and Janz 2008; Dalia 2008). Results of these studies are not unanimous but it remains commonly assumed that increasing the rate of freezing and reducing the storage temperature will improve the quality shelf life of meat. However, storage even at low temperature can influence chemical and microbial properties of meat depends on the freezing rate (related to time-temperature), the conditions of frozen storage (duration, temperature and its fluctuation, exposure to light and/or air), and the thawing rate (Hanenian and Mittal 2004; Fernandez et al. 2007; Soyer et al. 2010). Therefore, the association between chemical changes and microbial development occurring from slaughtering to storage of meat is recognized as a potential means of revealing indicators of meat quality or freshness (Ercolini et al. 2006; Ukut et al. 2010).

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Fresh meat is commonly marketing at environmental temperature for long time in most of the developing countries including Bangladesh and consumers often store surplus meat for long period. Traditionally, the rural Bangladeshi was used curing agents to preserve the surplus meat. However, refrigeration and freezing approaches are become popular at recent days to preserve meat and meat products, although the continuous electricity supply is a major challenge. Moreover, many undesirable microbial and chemical changes might be occurred during long time marketing and storage as the animals are mostly slaughtered and marketed with minimal hygienic and/or unhygienic conditions. Although, the consumers and sellers are interested to know the degree of microbial and chemical changes occur in locally marketed beef, however, there is a paucity of information to address their queries. Therefore, the present study was conducted to investigate the effect of elapsed time between slaughter and storage as well as storage conditions on the chemical and microbial changes in locally marketed beef.

Materials and Methods

Immediate after slaughtering, boneless meat was obtained from healthy cattle slaughtered at local market and quickly transported to the laboratory. All visible fat and connective tissue from beef were trimmed off as far as possible with the help of clean knife. The meat was collected twice and divided into two groups: morning beef and evening beef. Morning beef was collected immediate after slaughtering and evening one was collected 8 h after slaughtered. Further, the meat samples were divided into two parts: one part was stored at 4^{o}C in refrigerator and the other part was stored at -20^{o}C in freezer.

Proximate analyses such as dry matter (DM), ether extract (EE), crude protein (CP) and ash contentsof beef were carried out according to the methods of AOAC (2005). All estimations were done in triplicate and the average value was taken for discussion.

For microbial assessment, standard plate, coliform, yeast and mold counts were undertaken. All the microbiological parameters of beef samples were determined as per the methods described by APHA (2001). Preparation of samples and serial dilution of meat were done near the flame in a horizontal laminar flow unit, observing all possible aseptic precautions. Sterile water was used as diluents for making serial dilutions. The number of colonies were multiplied with reciprocal of the dilution and expressed as log cfu/g.

After solidification of agar in a petridish, the plates were inverted and placed in an incubator operated at 38^{0}C for 48 hours. After incubation, the plates were taken out from the incubator and the plates having 30-300 colonies were selected for counting. Colonies were counted by a colony counter. The numbers of colonies were multiplied by the dilution factor and the standard plate count per gram of sample was recorded.

Violet Red Bile Agar (VRBA) was used for coliform count. A total of 41.5g of VRBA ingredients were in 1000 ml distilled water to dissolve the medium completely and poured into sterile petri dishes. Agar plates were examined and the number of coliform colonies was counted after 24 h of incubation at 35°C.

Potato Dextrose Agar (PDA) was used to enumerate the yeast and mold count. A total of 200g of previously peeled and sliced potato was taken in 1000 ml of distilled water and boiled for an hour. After boiling, stirring was done through clean cheesecloth. Twenty grams of commercial dextrose and 15g of agar were added to the potato infusion solution. For complete dissolution, the mixture was heated and dispensed into several 200 ml screw cap bottles and sterilized at 121^{0}C (6.795 kg pressure/sq. inch) for 20 minutes. The media was melted through boiling and around 2.5 ml of 10% tartaric acid was added per 100 ml of medium (45^{0}C) to lower down the pH value to 3.5±0.1. After solidification of agar, the plates were inverted and incubated at 25^{0}C for 3 days. Plates were examined and number of yeast and mold colonies were multiplied by the dilution factor and the counts per gram of sample were recorded.

Data were analyzed as per split plot design in Randomized Complete Block Design (RCBD) with the help of MSTAT, a statistical computer

package. In case of significant difference, mean comparison was done by LSD.

Results

The effect of time elapsed before storage on average values of chemical and microbial changes in beef are shown in Table 1. The mean DM(36.76±5.23 vs 38.45±4.91) and ash (5.98±1.85 vs 6.36±2.05) percentages were higher in evening beef than morning beef but it was not reach to significant level. However, the CP (28.70±2.76 vs 29.81±2.53) and EE (1.96± 0.43 vs 2.30±0.83) percentage were significantly higher in evening beef compared with morning one. The mean standard plate count in evening beef was significantly higher than morning beef (6.82±0.88 log cfu/g vs 7.34±1.04 log cfu/g) while no significant differences were observed on the mean values of coliform, yeast and mold count (cfu/g) between these groups of beef.

Table 1. Influence of time elapsed before storage on the chemical composition and microbial content of beef

Parameters	MB (Mean±SD)	EB (Mean±SD)	Sig. level
DM%	36.76±5.23	38.45±4.91	NS
CP%	28.70±2.76	29.81±2.53	*
EE%	1.96±0.43	2.30±0.83	**
Ash%	5.98±1.85	6.36±2.05	NS
SPC (log cfu/g)	6.82±0.88	7.34±1.04	**
Coliform (cfu/g)	11.17±4.31	11.94±3.93	NS
Yeast (cfu/g)	12.44±2.38	10.94±3.47	NS
Mold (cfu/g)	3.00±1.07	2.5±0.92	NS

*MB, morning beef; EB, evening beef; SD, standard deviation; DM, dry matter; CP, crude protein; EE, ether extract; SPC, standard plate count; cfu, colony forming unit; NS, non-significant; *, p<0.05; **, p<0.01*

The effect of storage temperature on chemical and microbial changes is shown in Table 2. The average mean values of DM, CP and ash were found similar in refrigerated and freeze beef while EE level was found significantly higher in the refrigerated samples (2.22±0.58 g/kg) than the samples kept in the freezer (2.04±0.63 g/kg). The mean value of coliform (5.50±2.01 log cfu/g vs 17.60±4.86 log cfu/g) and yeast (1.87±0.76 log cfu/g vs 21.51±3.21 log cfu/g) counts were also significantly higher in beef stored at 4^0C than -20^0C while the mold count did not differ significantly. On the other hand, significantly lowered value of standard plate count (7.74±1.03 log cfu/g vs 6.43±1.22 log cfu/g) was observed in refrigerated beef than frozen beef.

Table 2. Influence of storage temperature on the chemical composition and microbial content of beef

Parameters	Freezing (-20^0C) (Mean±SD)	Refrigeration (4^0C) (Mean±SD)	Sig. level
DM%	37.70±6.02	37.52±5.76	NS
CP%	29.28±2.34	29.24±3.41	NS
EE%	2.04±0.63	2.22±0.58	**
Ash %	6.32±1.87	6.01±1.48	NS
SPC (log cfu/g)	7.74±1.03	6.43±1.22	**
Coliform (cfu/g)	5.5±2.01	17.6±4.86	*
Yeast (cfu/g)	1.87±0.76	21.51±3.21	**
Mold (cfu/g)	2.16±0.93	3.31±0.85	NS

*SD, standard deviation; DM, dry matter; CP, crude protein; EE, ether extract; SPC, standard plate count; cfu, colony forming unit; NS, non-significant; *, p<0.05; **, p<0.01*

A significant time before storage × storage temperature interaction was observed in CP contents (Table 3). The evening beef preserved as freezing showed significantly higher amount of CP than morning beef. The increased level of EE was also observed in evening beef. However, other parameters of chemical composition and microbial contents had no effect on time before storage × storage temperature interaction.

Table 3. Interaction between beef samples and storage temperature on the chemical composition and microbial content of beef

Parameters	MB × -20^0C (Mean±SD)	MB × 4^0C (Mean±SD)	EB × -20^0C (Mean±SD)	EB × 4^0C (Mean±SD)
DM%	36.30±4.88	37.22±6.21	38.75±6.57	38.16±5.43
CP%	28.40^{c}±3.02	29.02^{bc}±2.84	30.10^{a}±2.73	29.53^{ab}±2.31
EE%	2.03±0.47	1.90±0.72	2.41±0.57	2.19±0.81
Ash%	5.80±1.87	6.16±1.42	6.23±2.18	6.48±2.50
SPC (log cfu/g)	6.18±1.23	7.47±1.41	6.70±1.35	8.00±1.53
Coliform (cfu/g)	16.33±4.03	6.00±2.31	18.88±4.57	5.00±2.01
Yeast (cfu/g)	23.53±4.31	1.73±0.80	19.88±4.37	2.00±0.45
Mold (cfu/g)	3.66±0.91	2.33±0.74	2.96±1.11	2.00±0.82

Mean with different superscript(s) in the same row differed significantly (p<0.05); MB, morning beef; EB, evening beef; ×, Interaction between; SD, standard deviation; DM, dry matter; CP, crude protein; EE, ether extract; SPC, standard plate count; cfu, colony forming unit; NS, non-significant

The results of chemical and microbial changes due to storage duration were presented in Table 4 and 5. The DM and ash contents of beef samples were significantly increased with the increases of storage time in both at 4ºC (Table 4) and -20ºC (Table 5). The CP and EE contents were also increased with the time of storage, however, the highest levels were observed at 3rd day of refrigeration or 45th day of freezing. The coliform, yeast and mold counts were significantly increased with the increase of storage time in both temperatures while SPC counts showed the opposite result.

Table 4. Influence of storage duration on the chemical composition and microbial content of beef kept in refrigerator (4^0C)

Parameters	Day 1 (Mean±SD)	Day 3 (Mean±SD)	Day 7 (Mean±SD)
DM%	35.77^{c}±6.42	37.83^{b}±5.76	39.34^{a}±5.32
CP%	28.41^{b}±2.74	29.90^{a}±2.81	29.52^{a}±1.93
EE%	1.83^{b}±0.41	2.18^{a}±0.52	2.10^{a}±0.37
Ash%	5.52^{b}±1.42	5.72^{b}±1.88	7.70^{a}±2.21
SPC (log cfu/g)	7.79^{a}±1.66	7.74^{b}±0.94	7.68^{c}±0.91
Coliform (cfu/g)	3.33^{c}±1.04	5.66^{b}±1.84	8.16^{a}±2.33
Yeast (cfu/g)	0.00^{b}±0.00	2.38^{a}±0.84	3.20^{b}±1.04
Mold (cfu/g)	0.33^{b}±0.06	2.50^{ab}±0.96	3.66^{a}±1.23

Mean with different superscript in the same row differed significantly (p<0.01); SD, standard deviation; DM, dry matter; CP, crude protein; EE, ether extract; SPC, standard plate count; cfu, colony forming unit

Table 5. Influence of storage duration on the chemical composition and microbial content of beef kept in freezer (-20^0C)

Parameters	Day 3 (Mean±SD)	Day 45 (Mean±SD)	Day 90 (Mean±SD)	Sig. level
DM%	32.43^{b}±5.13	40.07^{a}±6.31	39.98^{a}±5.57	**
CP%	28.23^{c}±2.78	30.18^{a}±2.10	29.32^{b}±3.11	**
EE%	1.85^{c}±0.42	2.47^{a}±0.64	2.34^{b}±0.87	**
Ash%	2.34^{b}±0.74	7.39^{a}±2.68	8.31^{a}±3.01	**
SPC (log cfu/g)	7.73^{a}±1.06	5.90^{b}±1.01	5.63^{c}±0.83	**
Coliform (cfu/g)	5.00^{b}±1.11	31.16^{a}±6.34	13.66^{b}±4.57	*
Yeast (cfu/g)	0.00^{c}±0.00	16.83^{b}±5.47	47.66^{a}±8.65	**
Mold (cfu/g)	0.00^{b}±0.00	5.5^{a}±2.21	1.83^{ab}±0.58	*

*Mean with different superscript in the same row vary significantly; SD, standard deviation; DM, dry matter; CP, crude protein; EE, ether extract; SPC, standard plate count; cfu, colony forming unit; NS, non-significant; *, p<0.05; **, P<0.01*

The results of the interactions between time before storage and duration of storage are shown in Table 6 (refrigeration) and 7 (freezing). A significant time before storage × storage duration and temperature interaction was noted for CP and EE % in freezing beef samples (Table 7). However, other parameters of chemical composition and microbial contents had no effect on time before storage × storage duration and temperature interaction.

Table 6. Interaction effect of beef samples and storage time on the chemical composition and microbial content of locally marketed beef in refrigerator (4^0C).

Parameters	MB × day 1 (Mean±SD)	MB × day 3 (Mean±SD)	MB × day 7 (Mean±SD)	EB × day 1 (Mean±SD)	EB × day 3 (Mean±SD)	EB × day 7 (Mean±SD)
DM%	34.83±4.87	37.41±5.21	39.02±5.57	36.71±6.11	38.25±5.73	39.66±7.10
CP%	27.90±2.45	29.76±1.88	29.41±2.21	28.92±2.67	30.05±3.08	29.62±2.58
EE%	1.64±0.38	2.04±0.47	2.00±0.31	2.01±0.56	2.32±0.80	2.21±0.73
Ash%	5.28±1.43	5.58±2.01	7.60±2.87	5.77±1.72	5.86±1.85	7.81±2.11
SPC (log cfu/g)	7.53±1.11	7.50±0.85	7.40±0.92	8.05±1.05	8.00±0.78	7.96±0.74
Coliform (cfu/g)	3.66±1.52	6.33±1.96	8.00±2.23	3.00±1.05	5.00±2.17	8.33±1.32
Yeast (cfu/g)	0.00±0.00	2.00±0.87	3.16±1.21	0.00±0.00	2.76±1.34	3.23±1.33
Mold (cfu/g)	0.66±0.07	2.66±0.86	3.66±0.92	0.00±0.00	2.33±0.84	3.66±1.11

Mean of different interaction in the same row did not differed significantly; MB, morning beef; EB, evening beef; ×, interaction between; SD, standard deviation; DM, dry matter; CP, crude protein; EE, ether extract; SPC, standard plate count; cfu, colony forming unit; NS, non-significant

Table 7. Interaction effect of beef samples and storage time on the chemical composition and microbial content of locally marketed beef in freezer (-20^{0}C).

Parameters	MB × day 3 (Mean±SD)	MB× day 45 (Mean±SD)	MB× day 90 (Mean±SD)	EB × day 3 (Mean±SD)	EB × day 45 (Mean±SD)	EB × day 90 (Mean±SD)	Sig. level
DM%	31.06±5.23	38.75±6.13	38.94±6.38	33.81±4.33	41.40±7.13	41.03±6.54	NS
CP%	27.27^{e}±2.08	29.24^{c}±1.78	28.67^{d}±3.12	29.19^{c}±2.78	31.13^{a}±3.21	29.97^{b}±3.45	*
EE%	1.57^{e}±0.31	2.31^{bc}±0.46	2.20^{cd}±0.51	2.13^{d}±0.43	2.63^{a}±0.48	2.48±0.51^{ab}	**
Ash%	2.20±0.95	7.15±2.31	8.06±2.78	2.48±0.84	7.63±2.86	8.56±1.87	NS
SPC (log cfu/g)	7.48±0.94	5.67±0.87	5.28±0.93	7.98±0.83	6.11±0.78	5.98±0.68	NS
Coliform (cfu/g)	5.00±1.86	18.33±4.74	10.66±3.48	5.00±1.73	44.00±5.34	16.66±3.21	NS
Yeast (cfu/g)	0.00±0.00	21.00±4.57	48.33±6.56	0.00±0.00	12.67±4.33	47.00±5.47	NS
Mold (cfu/g)	0.00±0.00	5.33±1.38	3.66±1.21	0.00±0.00	5.66±2.06	0.00±0.00	NS

*Mean with different superscript in the same row differed significantly; MB, morning beef; EB, evening beef; ×, interaction between; SD, standard deviation; DM, dry matter; CP, crude protein; EE, ether extract; SPC, standard plate count; cfu, colony forming unit; NS, non-significant; *, p<0.05; **, P<0.01*

Discussion

During storage, many reactions occur between different meat components and alter the quality of meat (Asghar et al., 1988). In fact, lipid and protein oxidation are thought to be the primary causes of the quality losses of preserved meat (Xiong 2000; Baron et al. 2007; Soyer et al. 2010). The present study was intended to follow whether prolong post-slaughter time and storage conditions alter the quality of locally marketed beef. As the results, it was found that increased level of DM and ash contents with advances of post-slaughter time (Table 1, 4 and 5) clearly indicated the moister loss from meat. In the present study, the higher CP content was also found in the evening beef compared to that of the morning beef (Table 1). The CP contents of refrigerated (Table 4) and frozen (Table 5) beefs were also increased with the advances of storage time. Therefore, the advances of time after slaughtering (either at marketing or storing), increased the protein level of meat. This might be due to higher level of DM and increase of myofibrillar proteins due to the osmosis and poor water holding capacity (Farouk et al. 2004; Konieczny et al. 2007). However, the lower level of protein was found at 90th day of frozen storage than that of the 45thdays (Table 5). The chemical changes induced during protein oxidation by oxygen radical species are responsible for many modifications, such as formation of protein polymers, loss of solubility, protein fragmentation or aggregation (Decker et al. 1993; Xiong 2000). These factors may be connected to the decrease of protein level at 90 days of storage. Although storage temperature has important role on sarcoplasmic and myofibrillar proteins solubility, numerically the difference was small and may not have a significant impact on the functionality of thawed meat (Soyer et al. 2010). In fact, the similar level of protein was found at 3 days of storage at 4°C or -20°C (Table 4 and 5, respectively). The similar average mean value of CP was also found in refrigerated and frozen beef (Table 2). These findings indicated that meat preserves either at 4°C for short time or -20°C for long time has very little effect on the overall protein level.

Lipid oxidations are responsible for the development of rancidity by the production of low molecular weight compounds that cause undesirable flavor and limiting the shelf life of food products (Frankel 1993). The mean EE value was increased in evening beef (Table 1) and beef stored either at 4°C or -20°C (Table 4 and 5). The mean EE value was increased after 45days of storage and decreased thereafter at 90 days of storage as observed in the case of protein content. Similar increase and thereafter decrease of lipid in meat during storage overtime has been described in previous reports (Teets and Were 2008; Soyer et al. 2010). Along with these reports, the present results suggested that to minimize changes in meat due to protein and lipid oxidation during frozen storage, it is important to freeze the meat at colder temperature as quickly as possible after slaughtering the animals.

Microbial activities are considered one of the major factors of meat and meat products that alters the quality of meat during storage. In the present study, we found that morning beef had lower SPC than the evening beef (Table 1). These data demonstrated the importance of temperature control during meat processing and marketing to prevent the outgrowth of pathogens. These also indicated the importance of proper sanitation and processing practices that prevent and reduce contamination of carcasses with pathogens. The mean coliform counts (cfu/g) of meat samples were significantly increased with advances of storage time either at 4°C or -20°C (Table 4 and 5). We also found the higher mean value of coliform counts in refrigerated beef than frozen beef (Table 2). The presence of high concentrations of coliforms in foods is indicative of failures during handling, storage or inadequate hygiene (Flores 2004). In fact, most of the slaughterhouses in Bangladesh still not maintain proper hygienic measure indicating the possible contamination of locally marketed beef during slaughtering, processing and marketing.

Although few cases, yeast growth is desired in association with meat products (Olesen and Stahnke 2000; Dura et al. 2004), but in most cases, it is undesirable and may cause spoilage of the product (Fleet 1992; Fadda et al. 2004). The mean value of yeast growth was higher if beef was stored at 4°C than -20°C(Table 2). Indeed, the growth of yeast was increased with the advances of storage time (Table 4 and 5). No mold was found in the meat samples on the initial day while it was significantly increased with the advances of storage time (Table 4 and 5). Increased number of yeast and molds in this study might be possible contamination of fresh meats by various ways like slaughtering and processing at unhygienic slaughterhouse, butcher's skin, mouth, or nose, poor environmental conditions due to dust and contamination of the water used during slaughtering (Sobukola et al. 2009). Our findings give indication that locally marketed meat may viable source of various diseases as coliform and other pathogens are increased with the advances of post-slaughter time. Therefore, it is necessary to improve the unhygienic conditions of slaughterhouse and equipment in developing countries. Moreover, meat handlers and sellers should be educated on proper personal and environmental hygiene and sanitation as well as educated on the adverse effect of contamination.

Conclusion

In conclusion, we found that the quality of marketed beef degraded with the time elapsed before storage and storage temperature. Although some changes occurred, locally marketed beef can be preserved as early as possible at 4^{o}C for short time or at -20^{o}C up to 90 days.

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