Summary & Conclusions
India is a major producer of buffalo meat with a total output of about 1.4 million tones, owing to a large population (~494.4 millions) of this animal. The production of buffalo meat in India contributes to about 50% of world production, and roughly 85% of it is being exported from India. However, the meat in this country is mostly produced from aged and unproductive animals and, therefore, it is usually fibrous and tough in texture. Grinding of this kind of meat leads to rapid formation of metmyoglobin with undesirable brown colour and oxidative rancidity, which severely affects the consumer acceptance.

Since, raw meat is highly perishable in nature, it demands immediate processing and preservation. Meat processed through common techniques as freeze dehydration or thermal processing is not only expensive but also adversely affects the quality of meat. These major limitations could be easily surmounted with the simple and inexpensive approach of hurdle technology, which relies on optimal combination of several preservation factors or hurdles. So far, more than 60 potential hurdles for food, which improves the stability and/or quality of products have been reported. In industrialized countries, the hurdle technology approach is currently of much interest for minimally processed food, which are mildly heated or fermented, and for underpinning the microbial stability and safety of foods coming from healthful foods requiring minimal packaging. In developing countries, the application of hurdle technology for foods that remain stable, safe and tasty if stored without refrigeration is of paramount importance. Much interest in intentional hurdle technology is also emerging for meat product in China and for dairy products in India.

For each stable and safe food, a certain set of hurdles is inherent, which differs in quality and intensity depending on particular product. In any case, the hurdles must keep the normal population of microorganisms in food under control. The initial population of microorganisms present in meat should not be able to leap over the
hurdles present during storage of meat; otherwise, the meat will spoil and even cause food poisoning.

The multi-target approach of food preservation involving the additive and/or synergistic effects of hurdles has prompted this study on development of two different hurdle-processed buffalo meat products namely pickles and powder. The study involves the effects of spices, condiments, acetic acid, sodium nitrite and potassium sorbate as hurdles in combination with pH and moisture content on pickling, powdering, packaging, quality attributes and stability of these two buffalo meat products.

The specific objectives of the present investigation were as listed below:

- Development of convenience buffalo meat products namely pickles and powder using a combination of selected hurdles viz. pH, moisture content, chemical preservatives, spices/condiments and packaging materials.

- Evaluation of microbiological, physico-chemical, textural and organoleptic characteristics of developed buffalo meat products.

- Evaluation of shelf stability of the developed products and changes in their characteristics, especially microbial characteristics during storage at ambient temperature in different packaging materials.

Keeping in view the above objectives, the effects of pickling medium and preservatives, materials of packaging, methods of packaging (in case of meat powder only) and period of storage were examined on microbiological, physico-chemical, textural and organoleptic characteristics of developed products and their shelf stability.

Pre-rigor meat consisting of round portions comprising mostly of semi-membranous, semi-tendinous bicep femoris and quadriceps muscles of male carcass of buffalo, slaughtered by ‘Halal’ method was used in the study. The animal was
slaughtered 2h before the sample collection. The meat samples were packed in HDPE bags and brought to laboratory within 10 minutes after separation from carcass and immediately stored in a deep freezer at 4 ± 1°C. The bicep was trimmed off and meat samples were cut into chunks of 2.5 to 3.5cm size before conducting further studies.

Although, the weight, sex, source, method of slaughtering and collection of different meat samples, procured on different days during experiments were tried to be kept same. Anticipating qualitative changes in meat, its proximate composition was experimentally determined. The average values for pH, moisture, protein, fat and ash contents and thiobarbituric acid (TBA) of fresh meat were determined at 5.89, 75%, 19.32%, 10.29%, 1.0% and 0.3 mg/kg, respectively.

For development of meat pickles, the similar recipe as recommended for preparation of vegetable pickles was used. The untreated control as well as the meat pickles treated with 2% each of cinnamon, clove, turmeric, garlic, 0.025 % of potassium sorbate and 0.02 % sodium nitrite were developed in soybean oil medium. However, the mustard (2% ) and acetic acid (6 %) treated meat pickles were developed in their own mediums.

The various microbiological characteristics viz. total plate count (TPC), coliform count, lipolytic count, acidophilic count and yeast and mold counts, physico-chemical characteristics (pH, moisture content, protein, ash content and thiobarbituric acid (TBA) values), textural characteristics (hardness, cohesiveness and gumminess) and organoleptic characteristics (colour, odour, texture, taste and palatability) of both the fresh and preserved meat pickles were determined following standard laboratory methods. Organoleptic evaluations of both products were carried out with the help of a six member panel of semi-trained panelists drawn from the laboratory/department using a nine point hedonic scale.
For storage and shelf life stability studies, the samples of all pickles developed in various mediums and treated with spices, acetic acid, potassium sorbate and sodium nitrite, were preserved in HDPE and glass jars by atmospheric packaging method. The packed samples were stored for 120 days at ambient conditions during March to September when the temperature ranged between 30 to 35 °C.

The data of all experimental studies were statistically analysed for variations among the mean values of each individual parameters. The two ways ANOVA for each quality parameter was carried out to find out any significant difference between the mean values.

For development of another hurdle processed meat product, “the meat powder”, raw buffalo meat was dehydrated and transformed into powder form. Dehydration of meat was carried out in a tray drier at 180°C to 60°C for 3 days. The dehydrated meat pieces were powdered in a grinder. For stability studies, the developed powder samples were packed in two different packaging materials namely combination film and autoclavable polythene. In this study, the hurdle parameters were incorporated also in the form of treatments. To increase the shelf life, the meat after dehydration was treated with clove, turmeric and potassium sorbate. Three other treatments used were related to packaging. All the samples were stored at ambient temperature during August to February when the atmospheric temperature ranged between 35°C to 12°C. The various microbiological, physico-chemical and sensory characteristics of preserved meat powder samples were evaluated by using standard techniques. To test the functional suitability of preserved meat powder, the powder was reconstituted and used for developing Shami-kababs. The sensory characteristics of developed kababs from preserved meat powder samples were also evaluated. All the data were statistically analyzed. The results are summarized as under:
5.1. **Product one: Hurdle processed meat pickles**

- Treatment of meat with certain natural products (spices and condiments) and synthetic chemicals as hurdles parameters during pickling resulted in differential reduction in pH in a time-dependent manner during 120 days of storage at ambient temperature. Spices used as natural preservatives, particularly the cinnamon, clove and garlic significantly reduced the pH values of pickles with in 20 days of storage vis-a-vis untreated control and synthetic preservatives, except acetic acid. Treatments with the various preservatives resisted the change in pH of meat pickle in the order as potassium sorbate > sodium nitrite > mustard > turmeric > garlic > clove > cinnamon > acetic acid. The low pH in acetic acid, cinnamon, clove and garlic treated meat pickles prevented the spoilage and extended the shelf life of the product by retarding microbial growth.

- Pickling treatments with natural products as preservatives have added to the protein content of meat as compared to control. The protein values of meat pickles increased in the order as clove > turmeric > cinnamon > garlic > mustard. The protein content of treated pickle remained unchanged upon storage, however, the decrease in protein content in control meat pickle was noticed during 120 days of storage. The results suggested the beneficial effects of spices and condiments on stability of meat pickles.

- Pickling treatments with natural and synthetic preservatives maintained the TBA number of meat during storage, however, it increased substantially as a function of time in case of control during storage at ambient temperature. Slight increase in TBA values was noticed in garlic, mustard, potassium sorbate, and sodium nitrite treated pickles. Nevertheless, the TBA values invariably lie with in the threshold limit of 1mg/kg at which lipid rancidity occurs. Comparative analysis revealed that the meat pickles packed in glass jars exhibit lower TBA values than the HDPE jars.
• The ash contents both in control and treated meat pickles with spices/condiments were found to increase significantly as compared to raw meat and did not change with storage. Insignificant effects of storage period and packaging material on ash content of pickles were observed.

• The effective concentrations of spices/condiment and synthetic chemicals used in the study were determined based on their MIC value against the pure cultures of common bacteria associated with meat spoilage. The MIC of the extracts of spices/condiments against *Staphylococcus aureus*, *Salmonella enteritidis*, *E.coli* and *Listeria monocytogenes* were determined to be 2%. However, the MIC of synthetic preservatives were determined to be 0.02%, 0.025% and 6% for sodium nitrite, potassium sorbate and acetic acid, respectively. Both the natural and synthetic products at these sub-lethal concentrations were applied as treatments to meat pickles.

• Pickling medium, preservatives, storage condition and time period have significantly influenced the microbial quality of meat pickles. Nevertheless, in all cases the pickles were in edible condition (hedonic rating 5.67 to 8.67) even after 120 days storage at ambient temperatures. Significant reduction in microbial population (TPC) was observed as a function of storage time in all treatments irrespective of packaging material. On the contrary, the TPC increased in untreated control and potassium sorbate treated meat pickles in both packaging materials.

• The efficacy of natural products (spices/condiments) for microbial control was observed to be in the order as clove > garlic > turmeric > cinnamon > mustard. Amongst the synthetic preservatives, the efficacy was in the order as sodium nitrite > acetic acid > potassium sorbate. Packaging of pickles in glass jar was found to be safer than HDPE jar for long term storage under ambient conditions.

• Treatment of meat with both the natural and synthetic preservatives significantly reduced the coliform counts in pickles. However, pickles treated with mustard and
potassium sorbate exhibited recurrence of *E.coli* growth after 20 days of storage at ambient temperature, which increased further with increase in storage period. Glass jar was found to be a better packaging material for long term storage of meat pickles. Cinnamon, clove, turmeric and garlic treatments exhibited effective anti-microbial activity, and suppressed the growth of coliforms during long term storage.

- Treatments with the natural products as preservatives resulted in significant decrease in *Staphylococcus* count in meat pickles after 20th days of storage. Amongst synthetic preservatives sodium nitrite and acetic acid suppressed the growth of *Staphylococcus* group of bacteria. However, potassium sorbate treatment increased the count during storage. Glass jar was suggested to be better a packaging material than HDPE jar.

- Treatment of meat pickles with clove, turmeric, cinnamon, effectively also checked the growth of proteolytic microorganisms. Moreover, all five natural products at 2% level viz. cinnamon, clove, turmeric, garlic and mustard inhibited the growth of lipolytic microorganisms. The synthetic chemicals viz. sodium nitrite, potassium sorbate and acetic acid also caused 100% inhibition of the growth of lipolytic bacteria up to 20 to 40 days. The lipolytic bacteria, however, reappeared upon prolonged storage at room temperature.

- Treatments of meat pickles with cinnamon, clove, turmeric, acetic acid and potassium sorbate were effective in controlling the growth of yeast and mold counts during 120 days storage. However, garlic in meat pickles was not effective in checking the growth of yeast and molds count.

- The pickles treated with all natural and synthetic preservatives resulted in the products, which were placed in ‘liked very much’ category based on sensory evaluation, as far as the colour is concerned. The sensory score, however, decreased during storage. Glass jar proved better than HDPE jar in retaining the colour of pickles during storage.
The odour scores of pickles increased significantly with storage period. All pickles except acetic acid treated pickles were rated as ‘liked very much’ on odour scores. Mustard and clove treated pickles were most liked while turmeric and acetic acid treated pickles were least liked. From odour score point of view also glass jar was better than HDPE jar for long term storage of pickles.

Invariably all the treatments significantly influenced the texture of pickles during storage at ambient temperature. The storage significantly improved the texture of all pickles. On the 120th day (at the end of storage) cinnamon, turmeric and mustard treated pickles had the highest score for texture which was liked very much. Acetic acid pickles, however, were slightly disliked. No significant effect of packaging material on textural qualities of meat pickles was observed. Treatment with clove and cinnamon and acetic acids significantly reduced the hardness of the pickles.

5.2. Product two: hurdle processed meat powder

The meat powder had average particle size of 0.26mm, moisture content of 4.0 to 4.2%, pH of 5.39 to 6.03, protein content of 67.1%, fat content of 11.77% and TBA number of 0.29 ± 0.07mg/kg and exhibited a shelf life of 120 days.

The moisture content, pH, fat content and ash content of meat powder did not significantly changed during storage upto 120 days at ambient temperature. The treatment of meat before drying and powdering had insignificant effect on pH and protein content of meat powder.

The two packaging materials used for meat powder namely combination film and autoclavable polyethylene also had insignificant effect on pH and protein content of meat powder.

Meat dehydration and powdering significantly increased the fat content of meat product. Treatment of meat powder with different preservatives after dehydration had
insignificant effects on fat content of powder as was the case with two packaging materials used for storage of meat powder.

- Treatments of meat after dehydration and powdering significantly increased the TBA number of meat powder during ambient storage, to extent that the product crossed the threshold level of rancidity, probably due to degradation of fatty acid.

- Meat powder treatments with clove and turmeric after dehydration and subsequent powdering completely checked the TPC of meat powder. The powder remained microbe free virtually with no detectable *Staphylococcus*, proteolytic or lipolytic bacteria throughout 120 days storage period. The bacterial count, however, increased in untreated control meat powder. Autoclavable polyethylene as packaging material in comparison to combination film was observed as inappropriate packaging material. Vacuum packaging was assessed to be better packaging alternative for maintaining low microbial count vis-à-vis modified atmosphere packaging of meat powder.

- Yeast and mold counts in untreated control meat powder have significantly increased during storage for 120 days. However, treatment with clove effectively controlled the growth of these microorganisms. Turmeric treated meat powder exhibited some yeast and mold growth after 60 days storage in the powder packed in autoclavable polythene. Potassium sorbate completely prevented the growth of yeast and mold in both packaging materials. As compared to CO₂ and N₂ flushing methods, the bacterial and fungal growth control was very good in vacuum packed meat powder.

- The developed meat powder was rated as ‘liked very much’ with respect to colour and odour. Treatments of meat significantly improved organoleptic characteristics. Vacuum packaging was the best method as compared to other methods of packaging.

- The Kababs developed from meat powder preserved for 120 days were rated as ‘most liked’ because of good organoleptic scores.
To the best of author's understanding, the present investigation is the first effort in global scenario for preparation, preservation and utilization of buffalo meat in powdered form. Such a product could be stored safely at ambient temperature for 120 days using spices/condiments as preservatives, maintaining the low TBA value and microbial counts. Also, this unique methods of meat preservation exhibits enormous potential of utilization of meat for a long duration and faster reconstitution to produce variety of meat products viz. kababs, patties and balls of varying dimension.

5.3. Conclusions

Based on above studies following conclusions are drawn:

Pickling of fresh buffalo meat and drying–cum–powdering of buffalo meat, using selected hurdle parameters viz pH, preservatives, dehydration, and packaging, etc. proved to be effective methods for controlling the meat microflora and preserving the meat for subsequent long term consumption.

The pickling medium (soybean/mustard oil), spices, organic acids and salts, packaging material and methods, storage temperatures, etc. create a distinctive microenvironment in and around the meat products, which inhibits the proliferation of the deleterious microflora responsible for meat spoilage. Pickling treatments and subsequent fermentation contribute to improvement in flavour and textural properties. The primary fermentation product lowers the meat pH and contributes to the stability of these pickles against food borne pathogens and other less desirable spoilage microorganisms.

Among the spices used in present study, the role of garlic, turmeric and clove in management of diabetes mellitus and lipid metabolism, and as anti-inflammatory agent, respectively are well documented. Development of meat pickle with these spices adds medicinal value to product as a bonus. These spices are also known to stimulate pancreatic digestive enzymes such as lipase, amylase and protease, which
may play a crucial role in digestion and reduction in food transit time in gastrointestinal tract.

Although, the spices/condiments used in present study were initially selected as hurdle parameters owing to their antimicrobial activity, to extend the shelf life of products (pickles and powder), however, the correlation of data with inherent characteristics of spices outspread their efficacy. The spices/condiments used in preparation/preservation of meat pickles and powder could exhibit multi-pronged benefits as (i) antimicrobial preservative, (ii) flavouring agent (iii) natural therapeutic agent with the host of beneficial physiological effects. Keeping in view many promising health beneficial effects, such food adjuncts could be regarded as ‘neutraceuticals’, which not only makes the food spicy but more healthy too.

Thus, both the hurdle processed meat products namely pickles and powder have the advantage of a longer shelf life (120 days), desirable organoleptic attributes, safe ingredients and low cost of processing. The longer shelf life of both the products at ambient temperature and good nutritive/medicinal values may add great convenience to many meat consumers. Both the products could also serve as protein supplement for the defense establishments, hotels/ restaurants/ fast food shops, and travelers etc. due to the logical advantages of ‘easy to pack’, ‘easy to cook’ and ‘ready to eat’ properties.