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Mohammed Nayeem
Department of Food Science & Technology, National Institute of Food Technology Entrepreneurship & Management (NIFTEM), Under Ministry of Food Processing Industries (MOFPI), Government of India, Kundli, Sonipat, Haryana, India

Komal Chauhan
Department of Food Science & Technology, National Institute of Food Technology Entrepreneurship & Management (NIFTEM), Under Ministry of Food Processing Industries (MOFPI), Government of India, Kundli, Sonipat, Haryana, India

MA Khan
Department of Post-Harvest Engineering & Technology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Mehjabeen Siddiqui
Department of Post-Harvest Engineering & Technology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Hamda Sidduqui
School of Biotechnology, Gautam Buddha University, Greater Noida, Uttar Pradesh, India

Correspondence
Mohammed Nayeem
Department of Food Science & Technology, National Institute of Food Technology Entrepreneurship & Management (NIFTEM), Under Ministry of Food Processing Industries (MOFPI), Government of India, Kundli, Sonipat, Haryana, India

Development and shelf life studies of buffalo meat sausages incorporated with foxtail millet (*Setaria italica*)

Mohammed Nayeem, Komal Chauhan, MA Khan, Mehjabeen Siddiqui and Hamda Sidduqui

Abstract
The aim of this work is to develop and investigate the shelf life of buffalo meat sausages fortified with foxtail millet (*Setaria italica*) at refrigerated storage temperature (0 °C). Studies on quality of sausage were done by analyzing different physico-chemical characteristics namely moisture content, ash content, pH, fat content, TBA number and microbial characteristic (total plate count and yeast & mould count) of the most appropriate combination of foxtail millet and meat, found with the help of Response Surface Methodology (RSM) software. The best combination as suggested by RSM was found to be 94.99 grams of meat mixed with 6.48 grams of foxtail millet. This was calculated so as to obtain the optimum range of moisture, ash, fat content and pH value. In total the four samples i.e. sausages prepared from whole meat, meat with foxtail millet, meat with foxtail millet plus sodium ascorbate and meat with foxtail millet plus tocopherol were taken for shelf life study. Sodium ascorbate (500ppm) and tocopherol (500ppm) were used as natural preservatives and hence the effect of such preservative on fortified sausages was also studied and was found that these assisted in improving the shelf life of the product. The product was acceptable for consumption even after 21 days of storage but total plate count, yeast and mould count and TBA number indicated the spoilage condition of sausage samples after 28 days. Therefore shelf life of the samples was found to be 21 days under refrigerated condition (0 °C). The study also provided evidences of increased dietary fibers and reduced fat content with high nutritional value in foxtail millet incorporated buffalo meat sausage, which can be considered as a functional food for healthy individuals and for patients of type 2 diabetes, cardiovascular diseases, obesity etc.

Keywords: Buffalo meat, Sausages, Foxtail millet, TBA number, Response Surface Methodology, Shelf life

Introduction
Principal components of buffalo meat besides water, are proteins that have all essential amino acids, fats and substantial amount of vitamins and minerals with a high degree of bioavailability. Lean buffalo meat has only 2g of fat per 100g and 24g of protein (Kandeepan, Biswas and Rajkumar, 2009) [24]. Buffalo meat is specifically valuable as a source of omega-3 fatty acids, vitamin B12, vitamin B6, niacin and bioavailable iron besides having appreciable amount of Zn and Se (Williams, 2007) [55]. Apart from the traditional nutrients which are essentially required for the human body, buffalo meat contains numerous bioactive substances that have been studied for their potential beneficial effects (Arihara K. 2006) [8]. Buffalo meat is one of the most abundant dietary source of taurine which is an important amino acid (Purchas 2004) [35]. Other bioactive compounds found in buffalo meat includes carnitine (Shimada L, Sakuma Y, Wakamatsu J 2004) [44], conjugate linoleic acid (Azain M 2003) [10], carnosine (Purchas 2004) [35], coenzyme Q10 or ubiquinone (Overvad K, Diamant B, Holm L 1999) [32], glutathione (Jones D, Coates R, Flagg E 1992) [23], caretine (Harris R, Nevill M, Harris D 2002) [22] and many others. Therefore meat may also be considered functional food to the extent that they contain numerous compounds thought to be functional.
Processed meats products, such as bacon, ham and sausage, are particularly unhealthy because of their high fat, preservative and salt content. Although buffalo meat sausages are one of the major items in the world yet it has certain drawbacks. The high fat and cholesterol content in meat may increase the risk for high cholesterol and heart diseases (O'Dea *et al*., 1990) [31]. Many epidemiological studies have demonstrated a relationship between a diet containing an excess of energy-dense food rich in fats and sugar are responsible for chronic diseases,

including colon cancer, obesity, cardiovascular diseases and several other disorders (Alexander *et al* 2009) [3]. Hence it becomes indispensible to fortify meat products and develop meat based functional food.

Buffalo meat products can be modified by fortifying high fibre sources to decrease the possibility of obesity, cardiovascular diseases and etc. This offers developer the opportunity to improve the nutritional and health enhancing properties. Various studies have already proven that foods having high dietary fibres have potential to reduce blood cholesterol, digestion problems, colon related cancer,(Andreasen, Landbo, Christensen, Hansen, & Meyer, 2001; Anderson *et al*., 2009; Cui, Nie, & Roberts, 2011; Estruch *et al*., 2009; Ajila and Prasada Rao; 2013; Thebaudin *et al*., 1997; Tungland & Meyer, 2002; Marlett *et al*., 2002) [6, 5, 16, 19, 2, 49, 52, 28], risk of diabetes mellitus (Type 2), coronary heart disease, blood pressure, obesity (Schweizer & W€ursch, 1986; Topping, 1991; Davidson & McDdonald, 1998; Schneeman, 1998; Terry *et al*., 2001;Wang, Rosell, & de Barber, 2002; Ferguson & Harris, 2003; Peters *et al*., 2003; Bingham *et al*., 2003; Cassidy, Bingham and Cummings, 1994; Viertanen and Aro, 1994; Ascherio and Willet, 1995; Kimm, 1995; Kim, 2000) [42, 51, 18, 43, 48, 54, 20, 33, 13, 14, 53, 9, 25, 26] and colon cancer (Theuwissen & Mensink, 2008; Wong & Jenkins, 2007; Gibson, Probert, Van Loo, Rastall, & Roberfroid, 2004) [50, 56, 21]. Fortification of good fibre sources with meat increases the cooking yield due to its water-binding and fat-binding properties and to improve texture (Cofrades *et al*., 2000) [15]. The possible ingredients which can be used for buffalo meat fortification are rice, wheat maize, sorghum and different types of millet.

Foxtail millet is a cheap source of fibre with high therapeutic index, it can probably be the best material for the fortification of buffalo meat. This millet is least allergic, easily digested and contains insoluble fibre which lowers triglycerides and contains high amount of lecithin, which is good for nervous system. It contains magnesium which reduces frequency of migraine attacks, lower high blood pressure, reduces risk of heart attacks (Prashant *et al.,* 2005; Xue *et al.,* 2008) [34, 57]. Foxtail millet reduces the risk of type 2 diabetic mellitus. Foxtail millets are high in phosphorus, a mineral that is an important source for nucleic acid, which are building blocks of genetic code.

One of the most common meat product consumed round the world is sausages. Sausages are a restructure food that is prepared by comminuted meat and is usually formed into a symmetrical shape. The word sausages means salted or meat preserved by salting. Presently, sausages making has developed as industry in many countries. This is a way to preserve the fresh meat that could not be consumed immediately. Thus sausages are among the most suitable products used for the fortification of buffalo meat.

Although in the last few years attempts have been made to fortify meat using various food products from dairy and vegetable origin, yet a research gap has been found where meat sausages can be fortified with cereals and millet based ingredients. The sausage incorporated with foxtail millet is an attempt to develop valuable meat product which retain all the health beneficial parameters of buffalo meat and minimize the risk of hyperlipidimia and hyperglycemia. The foxtail millet fortified meat sausage could be a novel product for health conscious consumer and shelf life could be made longer by giving suitable treatment of natural preservatives. Concentration of 500ppm is reported to have best result for the preservation of meat by sodium ascorbate and alpha tocopherol (Sahoo *et al*, 1997; Bao *et al* 2009) [41, 11]. Keeping this in view, the investigation was planned to develop buffalo meat sausages having suitable content of foxtail millet as optimized by RSM software and also to analyze its various quality parameters which affects the shelf life of the product.

Materials and Methods

Experimental design: The experimental work was carried out for the development and shelf life studies of meat sausages prepared from buffalo meat and fortified with foxtail millet. Studies were also conducted to examine the effect of different types of natural preservatives on physicochemical, microbiological, sensory quality and shelf life of buffalo meat sausage during refrigerated storage (0 ^{0}C). To study the effect on various parameters and for optimization of the experiment Response Surface Methodology (RSM) was used. The quality of buffalo meat sausage was evaluated on the basis of physico-chemical characteristics viz. pH, moisture content, ash content, fat content, thiobarbituric acid (TBA) number, microbial characteristics i.e. total plate count and yeast and mould count and sensory characteristics like color, flavor, texture, taste, juiciness, mouth feel and overall acceptability.

Procurement of Raw materials: Meat samples were collected from the local meat shop, Aligarh and were brought to the lab within 4 hours of slaughter. The cattle was kept in lairage for about 2 days prior to slaughtering by halal method. Meat samples from round portion (biceps femoris muscle) of 2.5 to 3.5 years aged female carcasses of good finish were obtained. Foxtail millet was procured from local market, Aligarh and was sieved, washed and brought to powdered form using mixer grinder in lab. The fibrous casing was procured from PRS technologies, New Delhi.

Preparation of buffalo meat Sausage: The meat chunks were washed; deboned and lean cuts were minced. A portion of the same was separated to serve as control (M). In the remaining portion foxtail millet powder was added and mixed thoroughly to serve as test samples. Thirteen different combinations of meat and foxtail millet mixture were prepared and quality evaluation was done as per the requirement of RSM software for the optimization of experiment. Once found the most suitable combination, three test samples viz. MF (Meat + Foxtail millet), MFA (Meat + Foxtail millet + Sodium Ascorbate) and MFT (Meat + Foxtail millet + α-tocopherol) were prepared, filled into casing and refrigerated. The shelf life of control and test samples were evaluated by analyzing TBA value, microbial parameters and sensory evaluation after every 7 days for a period of 28 days.

Quality Evaluation of Sausage: Estimation of moisture, ash and fat was done according to method given in AOAC manual while TBA number was measured by the method described by Strange *et al*., 1977. pH of the finely minced sausage samples were determined after homogenizing 20 gm of the sample with 100 ml distilled water using laboratory grinder (Inalsa Co., India). The pH of the suspension was recorded using reference and glass electrode portable type Digital pH meter (Model khera 6mk). Evaluation of microbiological parameters were determined by Total Plate Count and Yeast and mould count as explained in AOAC manual and sensory characteristics evaluation including color, flavor, texture, taste, juiciness and mouth feel of fresh buffalo meat sausages developed with incorporation of different levels of foxtail millet was done on nine hedonic rating tests.

Results and Discussion

Development of product using RSM (Response Surface Methodology): In order to get the suitable design from RSM to chose the best combination (of foxtail millet + meat) for the desired product, range of meat was kept at 80 to 95grams while that of foxtail millet was 5 to 20grams. With the obtained design, various factors/parameters i.e. moisture content, fat content, ash content, overall acceptability and pH of 13 different samples (with 3 replica of each) were calculated after performing the experiment. Table 1 shows experimental run of quality analysis of fresh sausage. Parameters for obtaining the best combination of meat and foxtail millet and the result for the same are shown in Table 2 and Table 3 respectively.

Table 1: Experimental run and values of proximate analysis at fresh condition

Code	Meat %	Foxtail millet %	Moisture Content	Fat content	Ash Content	pH	Overall acceptability
N-1	87.5	12.5	64.5	5.25	3.1	6.56	7.7
N-7	76.89	12.5	61.11	5.21	3.15	6.72	7.5
N-11	80	20	64.78	5.35	3.35	6.75	7
N-9	87.5	12.5	65.2	5.22	3.2	6.6	7.7
N-12	95	20	65.9	5.4	2.91	6.68	8.2
N-8	80	5	66.5	5.12	2.44	6.38	8.4
N-10	87.5	23.11	63.46	5.52	3.21	6.74	6.9
N-13	95	5	69	5.09	1.25	6.32	8.5
N-6	87.5	1.893	69.2	5	0.88	6.29	7.7
N-3	87.5	12.5	65.12	5.19	3.24	6.59	7.7
N-4	87.5	12.5	64.99	5.2	3.22	6.5	7.7
N-2	98.11	12.5	67.9	5.05	3.22	6.42	7.9
N-5	87.5	12.5	64.55	5.2	3.19	6.54	7.7

Optimization of experiments

Table 2: Present the criteria for optimization of meat and foxtail millet incorporation.

Name	Goal	Lower Limit	Upper Limit	Importance
Meat	in range	80	95	3
Foxtail millet	in range	5	20	3
Moisture cont.	maximize	65	67.5	3
Fat cont.	in range	5	5.5	3
Ash cont.	in range	2	3	3
pH	minimize	6.3	6.5	3
Overall acceptability	minimize	6.9	8.5	5

Table 3: Present the solutions for optimization of meat and foxtail millet incorporation.

S. No	Meat (g)	Foxtail millet (g)	Moisture content %	Fat content%	Ash content%	pH	Overall acceptability	Desirability
1	94.99	6.48	68.53	5.05	2.00	6.34	8.26	0.87
2	94.99	6.55	68.51	5.05	2.014	6.34	8.25	0.87
3	94.99	7.72	68.03	5.07	2.26	6.37	8.2	0.81

The best fit with the high desirability value of 0.87 as suggested by RSM software was having the proportion of meat and foxtail millet as 94.99g and 6.48g respectively as given against S. No. 1 in Table 3. Fortification of sausages was done on the basis of these values. A control sample was also prepared along with three fortified samples and subsequently their shelf life was studied.

Shelf life Studies

Effect on TBA number: Thiobarbituric acid (TBA) number is an important relevant characteristic of meat product that indicate the oxidation state and on later stage rancidity of the product. TBA number was determined as mg of malonaldehyde/kg. Malonaldehyde is produced as a result of fat oxidation and it react with TBA reagent to produce colored complex with an absorption max/min 530-532 nm. The red pigment produced is the reaction product obtained from condensation of two moles of TBA reagent with one mole of malonaldehyde. Result of TBA number of control and other samples have been presented in Fig. 1. For meat samples, TBA value of 0.5 or less is considered fit for consumption and no off flavor is detectable at this level (Tarladgis *et al*, 1960) [47]. Treatment of sodium ascorbate and tocopherol also controlled the increase of TBA number. It was found that there was continuous increase in TBA number during refrigerated storage of 28 days. This increase might be due to a concomitant increase of bacteria and oxidation of fatty acids and lipid peroxidation during storage. The criteria for spoilage of the sample were derived from TBA value as at the end of 28 days TBA value was found more than 0.5 for sample MF. However up to 21 days TBA value was below spoilage limit for all samples. These results were similar to Singh and Verma (2000) [45] in chicken meat patties extended with textured soya. A similar result was found by Altaf and Brahama (2011) [4]. They reported that the TBA value of ready-to-eat spiced buffalo meat product was increased during storage.

Effect on microbial activities

Effect on TPC: Total plate count (TPC) is one of the important microbiological parameter used to determine the shelf life of the product. Meat is rich in nutrition and therefore

it is center of all attraction for microorganism especially for bacteria due to their desirable pH for growth. Increased microbial population caused degradation of protein and fat into simpler compounds like fatty acid, amines, carbon dioxide and ammonia and sulphur dioxide (Ahmad *et al*., 2005) [1]. Therefore the sausage develops off flavor after complete spoilage. The total plate count of buffalo meat sausages increased significantly and progressively during the storage period. This might be due to the permissive temperature and relative availability of moisture and nutrients for the growth of the aerobes. The graphical representation of total plate count profile, during storage has been shown as in Fig 2 which represents no spoilage event after 28 days on this parameter. Ranken and Kill (1993) [39] described that the spoilage condition which was noticed when total plate count reached to total 10^7/g. A similar trend in the mean total plate count under refrigerated storage was also observed by Bhaskar Reddy *et al*., (2009) [12] in pork sausages incorporated with calcium milk co-precipitates and stored at refrigerated temperature, Murthy (1986) [29] in pork sausages, Nath *et al.*(1996) [30] in chicken patties and Rao *et al.,* (1999) [40] in smoked chicken sausages.

Effect on Yeast & Mould count: Yeast and mould count (log cfu/g) of buffalo meat sausage samples was evaluated before and during refrigerated storage at 0 °C. The yeast and mould count of all the samples was not detected in sausages in fresh condition. During refrigerated storage (0 ^{0}C) microbial count increased slowly in the first 2 weeks but thereafter rapid increase in the rate of growth was recorded. The graphical representation of yeast and mould count profile, during storage has been shown in Fig 3. Refrigerated storage consistently increased yeast and mold count of control and test samples. Cumaria *et al* (March 2003) [17] reported that when log cfu/g of yeast and mould count increases to 4.0 spoilage of food starts.

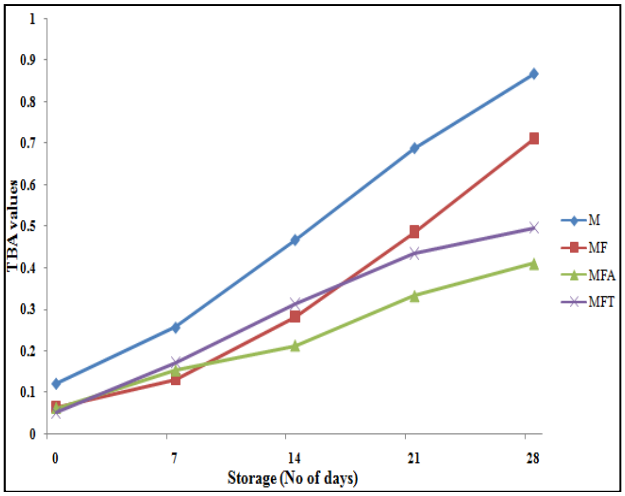

Fig 1: TBA value of control and various samples during storage period

Notes:-
M = Control (Buffalo meat sausage without foxtail millet)
MF = Meat + Foxtail Millet (94.99 g + 6.48 g)
MFA = Meat + Foxtail Millet + sodium ascorbate (500ppm)
MFT = Meat + Foxtail Millet + α-tocopherol (500ppm)
Values are means of three replicate

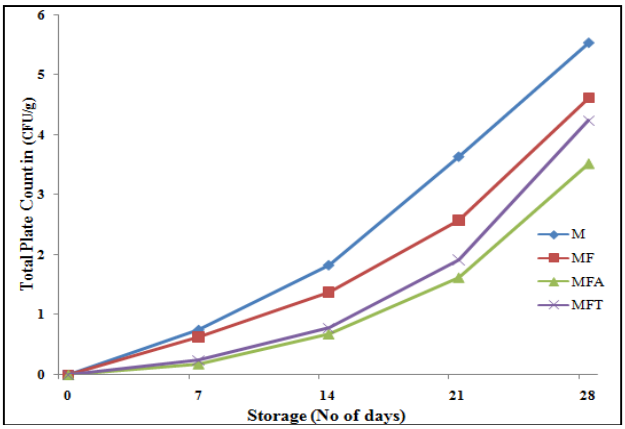

Fig 2: Total Plate Count of control and various samples during storage period

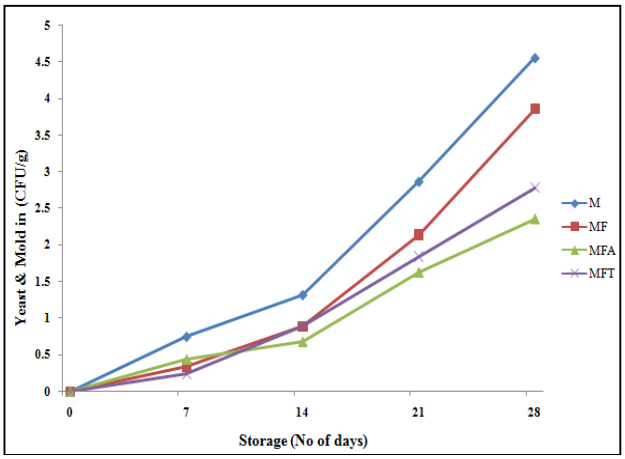

Fig 3: Yeast and Mold Count of control and various samples during storage period

Effect on sensory Characteristics: Change in the sensory attributes of fortified sausages with different preservatives was studied and has been presented in Fig 4. Sensory characteristics were measured in terms of color, odor, texture, taste and juiciness and overall acceptability.

The results shows that the samples treated with natural preservatives were awarded high score value for color when compared to control sample. This might be due to addition of light colored foxtail millet which reduced the red color of meat. It was observed from the score value of the samples those were incorporated with foxtail millet were found to be same. This leaves a scope for further studies to find ways to improve the sensory attributes of this neutraceutical.

All the sensory attributes showed a declining trend with advancement of storage. The score values of color, aroma, texture, taste, and juiciness constantly decreased during refrigerated storage but even after three weeks of refrigerated storage the product was acceptable for consumption.

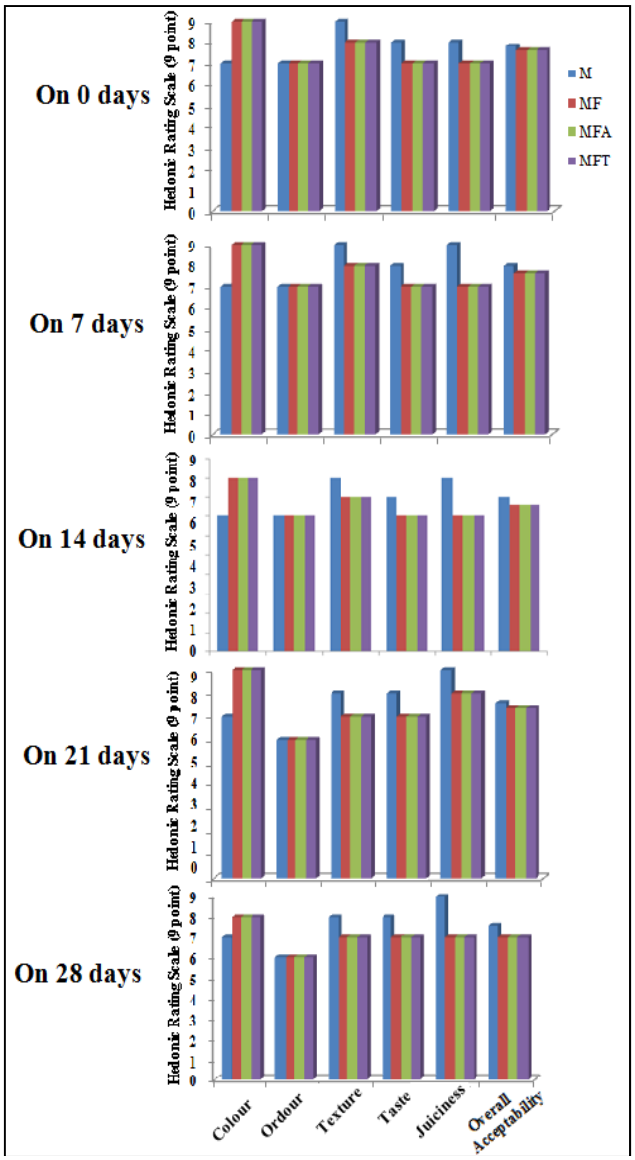

Fig 4: Oraganoleptic Attributes of control and various samples during storage period

Conclusion

The sausage incorporated with foxtail millet is an attempt to develop valuable meat product which retain all the health beneficial parameters of buffalo meat and minimize the risk of hyperlipidimia and hyperglycemia. Therefore foxtail incorporated sausage could be one of the best meat products for health conscious consumer for its reduced fat and cholesterol. In this study, it was found that too much buffalo meat consumption is unhealthy as it leads to various ailments including cardiovascular diseases. Millets grains are superior to major cereals with respect to protein, energy, vitamins and minerals beside they are rich source of dietary fibres.

The study has indicated that incorporation of foxtail millet brought considerable change in TBA value, microbiological (TPC and yeast and mould count) and sensory characteristic of buffalo meat sausage. Storage studies indicated that sample incorporated with foxtail millet had a bright yellow color even after 21 days of storage which was more accepted by the panel members compared to the control sample. Study also indicated that, sausages developed by adding foxtail millet which contained phytochemicals, when stored at refrigerated (0^0C) condition, remained palatable for more than 21 days, as compared to the sample prepared without such treatment. This was due to the presence of phytochemicals which were responsible for lowering the microbial load in the developed product. Such samples when treated with natural preservatives had extended shelf life.

Thus incorporation of foxtail millet in preparation of buffalo meat sausages improves the shelf life and nutritional and therapeutic index with negligible change in sensory characteristics of the developed meat product. However study also finds a research gap in analyzing the role and scope of phytochemicals in preserving meat products.

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