

## Application of Hurdle Technology for Preservation of Chicken Sausages at Ambient Temperature ( $37\pm 1^{\circ}\text{C}$ )

S. N. Rindhe, A. T. Shinde, B. A. Jadhav\* and V. H. Kumbhar

Department of Livestock Products Technology, College of Veterinary & Animal Sciences,

College of Food Technology, VNMKV, Parbhani (M.S) 431402, India

\*Corresponding Author E-mail: [jadhav.cft@rediffmail.com](mailto:jadhav.cft@rediffmail.com)

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### ABSTRACT

*Present study was aimed to develop acceptable shelf stable chicken sausages using textured soya protein as a humectants and lactic acid as a acidulent along with the use of Nisin to extend the shelf life of hurdle treated chicken sausages. Chicken sausage was prepared from emulsion of minced and chopped meat along with addition of non meat ingredients such as textured soya protein including spices and condiments. The emulsion was stuffed into artificial cellulose casing and cooked in autoclave without pressure for 20 min. to attain an internal core temperature of  $72 \pm 2^{\circ}\text{C}$ . Chicken sausages after cooling to room temperature were packed in high density polyethylene pouches and subsequently analyzed as per the experimental requirement. The shelf life of hurdle treated chicken sausages incorporated with or without Nisin was accessed at ambient temperature ( $37\pm 1^{\circ}\text{C}$ ). Application of different hurdles resulted in an initial reduction in all the sensory attributes as compared to control. Incorporation of Nisin did not have any significant effect on sensory quality of chicken sausages however overall sensory quality were slightly higher for chicken sausages incorporated with Nisin at 40 mg/kg. The sensory scores for all attributes declined with the progress of storage but Nisin incorporated hurdle treated chicken sausages were acceptable up to 6<sup>th</sup> day of ambient temperature storage ( $37\pm 1^{\circ}\text{C}$ ). During ambient temperature storage ( $37\pm 1^{\circ}\text{C}$ ) of chicken sausages the moisture, TBARS value and tyrosine value increased significantly ( $P<0.05$ ). Similarly total plate count increased significantly ( $P<0.05$ ) in all samples throughout storage and the increase was more pronounced in control sausages. Similar trend were observed for the yeast and mold growth but there absence in treatment II through ambient temperature storage ( $37\pm 1^{\circ}\text{C}$ ) could be attributed to addition of Nisin at higher level (40 mg/kg). Hence it is concluded that addition of Nisin at 40 mg/kg has beneficial effect to increase the shelf life of chicken sausages at ambient temperature storage ( $37\pm 1^{\circ}\text{C}$ ).*

**Key words:** Hurdle technology, Chicken Sausage, Nisin.

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## INTRODUCTION

We are passing through a crucial juncture of global insecurity against the right of everyone to have access to safe and nutritious food, consistent with the right to adequate food and the fundamental right of everyone to be free of hunger. India faces a major challenge in providing food security and nutritional security in the light of decreasing land holding, over exploitation of natural resources, declining productivity and population explosion. Poultry industry being an important wing of our Indian economy has been recognized as a potential tool to fight poverty and malnutrition with tremendous employment opportunities. The origin of poultry industry may be traced back to middle of the century where the birds were reared in the backyard to earn the supplementary income but during last three decades this enterprise has emerged in the form of an important organized sector. There has been an increased consumption of convenience foods due to changing life styles. In response to these changes, the food industry has developed a great variety of processed chicken products such as chicken sausages, nuggets, salami, patties and many of the researchers tried to produce value added products such as chicken sausages added with skin, fat, heart and gizzard<sup>18</sup>. Meat is highly perishable, having short shelf life and high value commodity which requires special attention to prevent it from microbial spoilage. Meat is a rich source of high quality proteins and micronutrients which makes it susceptible to microbial spoilage as well as unfit for human consumption within 16-18 hrs under prevailing Indian climatic conditions. Further the slaughter and handling practices as well as poor storage and transportation facilities adds to spoilage at a faster rate. Extension of shelf life of fresh and semi-processed meat products has been a focus for many research groups. In our country meat is consumed in fresh form contrary to western countries which necessitates the development of suitable preservative system to enhance the shelf life and acceptability under ambient temperature.

Fresh chicken meat and their semi-processed and/ processed products are quickly perishable due to high moisture and nutrient levels, which promote microbial growth and is

the primary factor associated with meat quality reduction, spoilage and economic loss. In order to prevent the microbial spoilage and to maintain the product quality from the time of production to consumption, fresh chicken or semi-processed products have to be preserved, mainly by refrigeration. Refrigerated storage and transport of meat products are common in developed countries, whereas in many developing countries like India, these refrigerated facilities are limited, especially in rural areas, leading to reduced meat consumption. Moreover, refrigeration will increase the cost of production. Shelf stable meat products both traditional and novel are storable without refrigeration and they comprise traditional as well as novel products. These products are stable and safe due to a combination of factors (hurdles) which inhibit microbial growth; hence hurdle technology can be employed successfully in development of shelf stable chicken products. To maximize the effectiveness of these hurdles in foods, it is necessary to adopt a systems approach (hurdle technology) to meet the challenge posed by the need for food preservation and to avoid the limitations of refrigeration as well as single chemical preservatives.

Hurdle technologies in developed countries are mostly used to improve food safety, stability, and quality. Hurdle technologies are proving to be more useful in developing countries like India (where electric interruption is one of the major problem) for the creation of novel foods, despite of minimal processing and which are stable at ambient temperature as well as for the modification of traditional intermediate moisture food (IMF) products to improve their taste and nutritional value by conversion to higher moisture foods.

## MATERIALS AND METHODS

### Chicken meat

Chicken meat required for the experiments were obtained from broilers of approx. 8 wks old slaughtered as per standard procedure at experimental abattoir of Livestock Products Technology Department, College of Veterinary & Animal Sciences, Parbhani. Meat was cut into cubes and ground using 10 mm plates in meat grinder (Stadler Pvt. Ltd., Mumbai). Ground meat was packed in LDPE

pouches and stored in deep freezer at  $-18 \pm 1^{\circ}\text{C}$  till subsequent use. Frozen meat was thawed at  $4 \pm 1^{\circ}\text{C}$  (in refrigerator) for 16 hrs before use.

### Spice mix

All the spice ingredients were cleaned to remove extraneous matter, dried in hot air

oven at  $60^{\circ}\text{C}$  for  $2\frac{1}{2}$  hr and then ground in a grinder using proportionate quantity to obtain dry spices mix for preparation of chicken sausage. Spice mixture so obtained was stored in airtight plastic container and used subsequently as per the suggested formulation.

**Table 1: Composition of spice mixture**

Sr.No	Spice Ingredient	Quantity (% by weight)
1.	Aniseed (Soanf)	10
2.	Black pepper (Kali mirch)	07
3.	Capsicum (Mirch powder)	12
4.	Caraway seed (Ajowan)	10
5.	Cardamom (Badi elaichi)	05
6.	Cinnamon (Dalchini)	05
7.	Cloves (Laung)	02
8.	Coriander powder (Dhania)	14
9.	Cumin seeds (Zeera)	15
10.	Dried ginger (Sunth)	15
11.	Turmeric (Haldi)	05
<b>Total</b>		<b>100</b>

### Formulation of chicken sausages

A typical formulation as suggested by Biswas *et al.*<sup>5</sup> with slight modification was used for

preparation of chicken sausage throughout the study.

**Table 2: Basic formulation of chicken sausage**

Sr. No.	Ingredients	Quantity (% by weight)
1.	Meat	74.50
2.	Chicken fat	14.00
3.	Common salt	01.80
4.	Sodium tripolyphosphate	0.05
5.	Monosodium glutamate	0.50
6.	Spice mix	01.50
7.	Condiment paste	03.63
8.	Sugar	01.00
9.	Refined wheat flour	03.00
10.	Sodium nitrite	0.02
<b>Total</b>		<b>100</b>

### Chicken sausages preparation

Meat emulsion was prepared using Bowl chopper (Stadler Pvt. Ltd., Mumbai) as per the procedure mentioned here under. About 4 kg batches were prepared, namely, 2980 g deboned meat, 560 g chicken fat, 145.20 g condiments mix, 120 g refined wheat flour, 60 g spices mix, 72 g salt, 40 g cane sugar, 2 g sodiumtripolyphosphate and 20 g monosodium glutamate. Also, sodium nitrite was added at 0.02 %. Spice mix was prepared

as per the formulation developed in the laboratory (Table 1). Onion and garlic were used in the ratio 3:1 as condiments. Different ingredients were kept at  $4 \pm 1^{\circ}\text{C}$  for 1.5 hrs before chopping to reduce the temperature rise during emulsification. To the ground chicken meat, salt, sugar, sodium nitrite and sodium tripoly phosphate were added and chopped for about 2 min. Condiments mix was then added and chopped again for 2 min (water/ice flakes were not added to reduce the water activity in

the sausages). Ground chicken fat was slowly incorporated while chopping which was continued till the fat was completely dispersed in the batter (3-4 min). Spice mix and refined wheat flour were added and chopping was continued for another 1 min to get a fine viscous emulsion. Emulsion was prepared in bowl chopper (Stadler Pvt. Ltd., Mumbai). Meat emulsions were then stuffed into 25 mm diameter artificial cellulose casings (Viskase Nojax, Viskase Co. Inc., Chicago, USA) using manual sausage filler (Stadler Pvt. Ltd., Mumbai) and linked manually at about 12 cm intervals. Cooking was done in a steam oven without pressure till the internal temperature reaches 75<sup>0</sup> C, as recorded by a digital probe thermometer. The sausages were cooled to room temperature and peeled off the casings. The temperature of the emulsion varied from 10-12<sup>0</sup>C.

### Experiment

About 4 kg meat emulsion was prepared as per the formulation mentioned above. Chicken sausages prepared by using TSP (4%) and emulsion pH (5.7) and incorporated with different levels of nisin (20 and 40 mg/ Kg) and packed in HDPE pouches were stored at ambient temperature (37±1<sup>0</sup>C) to assess its shelf life.

The samples were analyzed at regular interval of 2 days for various physico-chemical parameters viz., pH, moisture, TBARS, tyrosine value including sensory attributes and microbiological quality viz., total plate count (TPC) and yeast & mold count. The study was continued till the visual signs of spoilage and/or any off flavour were observed.

### Analytical Procedures

#### Sensory Evaluation

Standard sensory evaluation method using 8-point descriptive scale<sup>7</sup> was followed with modifications where 8= excellent, 1= extremely poor. The experienced panel (7 members) consisted of academic staff members and post-graduate students of Department of Livestock Products Technology, COVAS, Parbhani. Chicken sausages were warmed (40-45<sup>0</sup> C) using microwave oven for 1 min and served to the panelists. The panelists evaluated the samples for appearance, flavour, juiciness, texture and

overall palatability using a standard score sheet. Sensory evaluations were conducted between 3.00- 3.30 pm and filtered tap water was provided to the panelists for rinsing their mouth in between evaluation of different samples.

#### Physico-Chemical Characteristics

pH was determined by the method of Trout *et al.*<sup>21</sup>, using a digital pH meter (Model: LI 120, ELICO Pvt. Ltd., Hyderabad) equipped with a combined glass electrode. Moisture, fat and protein content of the sausages were determined as per standard procedures<sup>1</sup>. Tyrosine value was estimated by the procedure of Strange *et al.*<sup>17</sup>, with slight modifications. Tyrosine value was calculated as mg tyrosine per g of sample by referring to a standard graph which was prepared as per the procedure described by Pearson<sup>10</sup>. TBA value was determined by modified method as described by Strange *et al.*<sup>17</sup> with little modification in the technique. TBA reagent was prepared according to the method of Pearson<sup>10</sup> by dissolving 0.2883 g of Thiobarbituric acid in sufficient quantity of 90 % acetic acid with slight warming and the volume was made up to 100 ml.

#### Microbiological quality

The microbiological quality of chicken sausage was assessed on the basis of total plate count (TPC) and yeast & mold count as per the procedure of APHA<sup>2</sup>.

#### Statistical Analysis

The experiment was replicated a minimum of three times and the data generated for different quality characteristics were compiled and analyzed using randomized block design. The data were subjected to analysis of variance (one way ANOVA), least significant difference<sup>15</sup> and Duncan's multiple range tests<sup>16</sup> for comparing the means to find the effects between treatments for various parameters in different experiments. The smallest difference (D<sub>5%</sub>) for two means to be significantly different (p<0.05) is reported.

## RESULTS AND DISCUSSION

#### Sensory quality

Application of different hurdles resulted in an initial reduction in all the sensory attributes; however they maintain these attributes for

longer time as compared to control (Table 3). Evaluation of sensory quality of the control sausages was done only on the day of processing due to the development of off odour and sliminess on 3<sup>rd</sup> day.

The sensory scores for appearance of chicken sausages differed significantly ( $P<0.05$ ) as compared to control due to incorporation of hurdles which could be attributed to higher rate of conversion of myoglobin to brownish meat myoglobin and partly due to low emulsion pH resulted into the concentration of meat pigments as result of increase in moisture and fat loss. Addition of Nisin had no significant effect on their appearance. Thomas *et al.*<sup>20</sup> reported similar declining trend for appearance of shelf stable pork sausages during ambient temperature storage.

Incorporation of Nisin and application of hurdles significantly ( $P<0.05$ ) reduced the flavour of chicken sausages on the day of

processing. It may be due to the higher fat loss since the fat content of meat was reported to have significant ( $P<0.05$ ) effect on flavour<sup>9</sup>. The present findings are in agreement with that of Santamaria *et al.*<sup>13</sup> who have reported significant reduction in flavour of pamplona chchorizo sausages during ambient temperature storage which could be due to increased fat oxidation.

Addition of hurdle along with Nisin resulted in significant ( $P<0.05$ ) decrease in juiciness on the day of processing which may be due to humectant (TSP) and increased fat and moisture loss. The sensory scores for juiciness decreased significantly ( $P<0.05$ ) with storage which may be attributed to loss of moisture from the products during ambient temperature storage. Addition of Nisin at different levels had no effect on juiciness. Similar findings were recorded by Sengar<sup>14</sup> for shelf stable pork sandwich spread at ambient temperature storage.

**Table 3: Effect of incorporation of different level of nisin on the sensory attributes of hurdle treated chicken sausages during ambient temperature storage (37 ± 1°C)**

Type of product	Storage period (days)			
	0	2	4 <sup>A</sup>	6 <sup>A</sup>
<b>Appearance</b>				
Control	7.09±0.09 <sup>a1</sup>	ND	--	--
Treatment I	6.94±0.06 <sup>a2</sup>	6.78±0.13 <sup>b</sup>	6.62±0.07 <sup>c</sup>	6.39±0.17 <sup>d</sup>
Treatment II	6.94±0.06 <sup>a2</sup>	6.83±0.11 <sup>b</sup>	6.68±0.16 <sup>c</sup>	6.50±0.11 <sup>d</sup>
<b>Flavour</b>				
Control	7.00±0.13 <sup>a1</sup>	ND	--	--
Treatment I	6.83±0.02 <sup>a2</sup>	6.57±0.10 <sup>b</sup>	6.44±0.18 <sup>c</sup>	6.32±0.15 <sup>d</sup>
Treatment II	6.83±0.04 <sup>a2</sup>	6.61±0.11 <sup>b</sup>	6.50±0.15 <sup>c</sup>	6.37±0.24 <sup>d</sup>
<b>Juiciness</b>				
Control	7.17±0.13 <sup>a1</sup>	ND	--	--
Treatment I	7.06±0.17 <sup>a2</sup>	6.89±0.07 <sup>b</sup>	6.72±0.14 <sup>c</sup>	6.60±0.20 <sup>d</sup>
Treatment II	7.06±0.15 <sup>a2</sup>	6.94±0.05 <sup>b</sup>	6.76±0.11 <sup>c</sup>	6.66±0.11 <sup>d</sup>
<b>Texture</b>				
Control	7.11±0.11 <sup>a1</sup>	ND	--	--
Treatment I	7.00±0.24 <sup>a2</sup>	6.88±0.08 <sup>b</sup>	6.78±0.16 <sup>c</sup>	6.47±0.10 <sup>d</sup>
Treatment II	7.00±0.15 <sup>a2</sup>	6.93±0.06 <sup>b</sup>	6.83±0.13 <sup>c</sup>	6.56±0.24 <sup>d</sup>
<b>Overall palatability</b>				
Control	7.00±0.23 <sup>a</sup>	ND	--	--
Treatment I	6.89±0.11 <sup>a2</sup>	6.67±0.07 <sup>b</sup>	6.50±0.11 <sup>c</sup>	6.44±0.08 <sup>d</sup>
Treatment II	6.89±0.17 <sup>a2</sup>	6.72±0.15 <sup>b</sup>	6.56±0.12 <sup>c</sup>	6.51±0.14 <sup>d</sup>

ND- Not Detected

Control- No hurdles

Treatment I – Chicken sausages added with 4% TSP+ Nisin 20 mg/Kg

Treatment II- Chicken sausages added with 4% TSP+ Nisin 40 mg/Kg

Means with different superscripts (letters in the same row and numbers in the same column) indicate significant difference ( $P<0.05$ )

A- Data in the same column on day 4 and 6 was analyzed by paired t test.

B- Based on 8 point descriptive scale

The sensory scores for texture declined significantly ( $P < 0.05$ ) during ambient temperature storage ( $37 \pm 1^\circ\text{C}$ ) of 6 days. The texture score of hurdle treated chicken sausages were significantly ( $P < 0.05$ ) lower as compared to control on the day of processing. It may be due to increased denaturation of proteins and subsequent decrease in fat and water binding properties. Santamaria *et al.*<sup>13</sup> reported decrease in texture of hurdle treated sausages during ambient temperature storage which may be attributed to changes in disulphide bonds due to increased protein denaturation. Incorporation of Nisin did not have any significant effect on texture of chicken sausages. Present findings are in agreement with those reported by Thomas *et al.*<sup>20</sup> for shelf stable pork sausages.

Similar pattern was observed for overall palatability of hurdle treated chicken sausages as compared to other sensory attributes. The results indicating that the overall palatability of sausages was mostly influenced by their flavour and texture properties. Moreover, the scores for overall palatability were slightly higher for treatment

II (Nisin at 40 mg/kg) indicating that the overall quality was much better than that of control as well as treatment I (Nisin at 20 mg/kg). Similar findings were recorded by Thomas *et al.*<sup>20</sup> for shelf stable pork sausages.

#### Physicochemical properties

The pH of the control sausages increased by 0.43 units on day 2<sup>nd</sup> of storage, while that of treatment I and II showed only a slightly increase of 0.11 and 0.09 units respectively (Table 4). This may be due to higher microbial load on the control sausages (Table 4). Lawrie<sup>8</sup> and Bell & Shelef<sup>3</sup> reported that increase in spoilage organisms results in more protein degradation in meat products, which in turn increase their pH which may be attributed to the accumulation of more metabolites. However, the inhibitory action of the hurdles especially lower emulsion pH on microbial growth will contribute to the lower increase in pH on day 2 of the hurdle treated sausages as compared to control. Similar findings were reported by Karthikeyan *et al.*<sup>6</sup> for hurdle treated caprine keema at ambient temperature storage.

**Table 4: Effect of incorporation of different levels of nisin on physico-chemical characteristics of hurdle treated chicken sausages during ambient temperature storage ( $37 \pm 1^\circ\text{C}$ )**

Type of Product	Storage period (days)			
	0	2	4	6 <sup>A</sup>
	<b>pH</b>			
Control	6.35±0.05 <sup>b1</sup>	6.78±0.02 <sup>a1</sup>	SP	--
Treatment I	6.11±0.01 <sup>c2</sup>	6.22±0.02 <sup>a2</sup>	6.17±0.01 <sup>b</sup>	6.26±0.02 <sup>a</sup>
Treatment II	6.09±0.03 <sup>c2</sup>	6.18±0.01 <sup>b3</sup>	6.15±0.02 <sup>b</sup>	6.24±0.01 <sup>a</sup>
	<b>Moisture (%)</b>			
Control	59.18±0.03 <sup>d1</sup>	59.87±0.06 <sup>c1</sup>	59.94±0.03 <sup>b1</sup>	60.80±0.07 <sup>a1</sup>
Treatment I	60.25±0.07 <sup>d2</sup>	60.63±0.08 <sup>c2</sup>	61.43±0.06 <sup>b2</sup>	62.27±0.08 <sup>a2</sup>
Treatment II	60.25±0.03 <sup>d2</sup>	60.61±0.05 <sup>c2</sup>	61.38±0.09 <sup>b2</sup>	60.24±0.10 <sup>a2</sup>
	<b>TBARS value (mg malnoaldehyde /Kg)</b>			
Control	0.080±0.01 <sup>b2</sup>	0.587±0.02 <sup>a1</sup>	SP	--
Treatment I	0.097±0.01 <sup>d1</sup>	0.159±0.05 <sup>c2</sup>	0.136±0.08 <sup>b</sup>	0.289±0.05 <sup>a</sup>
Treatment II	0.094±0.05 <sup>d1</sup>	0.153±0.01 <sup>c2</sup>	0.214±0.06 <sup>b</sup>	0.280±0.04 <sup>a</sup>
	<b>Tyrosine value (mg/g)</b>			
Control	0.312±0.06 <sup>b3</sup>	0.806±0.02 <sup>a1</sup>	SP	--
Treatment I	0.339±0.02 <sup>d1</sup>	0.453±0.02 <sup>c2</sup>	0.513±0.02 <sup>b</sup>	0.627±0.01 <sup>a</sup>
Treatment II	0.349±0.01 <sup>d1</sup>	0.437±0.01 <sup>c2</sup>	0.497±0.04 <sup>b</sup>	0.623±0.02 <sup>a</sup>

SP- Spoiled

Control- No hurdles

Treatment I – chicken sausages added with 4% TSP+ Nisin 20 mg /Kg

Treatment II- chicken sausages added with 4% TSP+ Nisin 40 mg/Kg

Means with different superscripts (letters in the same row and numbers in the same column) indicate significant difference ( $P < 0.05$ )

A- Data in the same column on day 6 was analyzed by paired t test.

On day 4 the pH of hurdles treated sausages showed a decreasing trend as compared to day 2 which was significant ( $P<0.05$ ) in treatment I but not in treatment II. However pH increased significantly on day 6 in sausages from both treatment groups as compared to day 4 which may be due to the substantial increase in their microbial load, especially total plate count. The slightly lower pH values observed throughout the storage in treatment II as compared to treatment I, which may be due to their lower initial pH as a result of higher level of Nisin. Moisture content of control sausages as well as hurdle treated sausages incorporated with Nisin decreased significantly ( $P<0.05$ ) with the progress of ambient temperature storage ( $37\pm 1^{\circ}\text{C}$ ) of 6 days. Moisture content of both treatment I and II sausages were significantly ( $P<0.05$ ) higher than that of control but did not differ significantly ( $P<0.05$ ) among them during entire storage period which might be due to increase in hydrophilic and lipophilic groups that increase the protein-lipid and protein-water interactions (Pena-Ramos and Xiong, 2003). Results revealed that addition of Nisin had no effect on moisture content of sausages. Present findings are in agreement with that of Thomas *et al.*<sup>20</sup> for shelf stable pork sausages.

During storage TBARS value of control was observed to be significantly ( $P<0.05$ ) higher as compared to that of treatment I and II. On day 2, the TBARS value of control sausages increased very significantly ( $P<0.05$ ) as compared to hurdle treated sausages incorporated with different levels of Nisin (0.51 units vs. 0.06 units). TBARS value showed an increasing trend during subsequent storage but non significant difference was found among treatment I and II at any storage period. This indicates that addition of Nisin had no effect on TBARS value of the products. The significantly ( $P<0.05$ ) lower TBARS value of sausages from the treatment groups compared to controls on day 2 could be attributed to the presence of the hurdles viz. low emulsion pH and addition of humectant (TSP). These hurdles might have responsible for the

decrease in microbial load of treated sausages resulting in lower TBARS values, indicating positive correlation between microbial load and TBARS values which was also reported by Sahoo and Anjaneyulu<sup>12</sup> for ground buffalo meat. Present findings are in agreement with Thomas *et al.*<sup>20</sup> for shelf stable pork sausages.

Tyrosine value has been regarded as good index for meat protein break down (Pearson, 1968) and could be useful for the assessment of spoilage in meat and meat products. On the day of processing tyrosine value was significantly ( $P<0.05$ ) higher for the hurdle treated sausages added with Nisin. This could be due to denaturation and subsequent proteolysis of both meat and textured soya proteins during meat processing which might be accentuated by lactic acid<sup>19</sup>. On day 2, tyrosine values of the control sausages significantly ( $P<0.05$ ) increased from 0.312 to 0.806 mg/gm, the increase was markedly lower in hurdle treated sausages which may be due to increased proteolysis in the control sausages as a result of their increased microbial load (Table 4). Tyrosine values of treatment I and II did not differ significantly ( $P<0.05$ ) during entire ambient temperature storage ( $37\pm 1^{\circ}\text{C}$ ), indicating that addition of Nisin had no effect on tyrosine value of sausages. An increase in tyrosine value of hurdle treated goat meat keema at ambient temperature storage has been reported by Karthikeyan *et al.*<sup>6</sup>. Present findings are in agreement with those of Senger<sup>14</sup> for shelf stable pork sandwich spread and Thomas *et al.*<sup>20</sup> for shelf stable pork sausages.

#### Microbiological quality

Total plate count of hurdle treated chicken sausages incorporated with Nisin was significantly ( $P<0.05$ ) lower as compared to control (Table 5). Incorporation of Nisin at 40 mg/Kg resulted in further reduction of total plate count as compared to treatment I (20 mg/kg). Total plate count increased significantly ( $P<0.05$ ) in all samples throughout ambient temperature storage however the increase was more pronounced in control sausages. It reached about 6.78 log CFU/gm in the control sausages on day 2,

which is more than the incipient spoilage level of 6.70 log CFU/gm where as it was only about 6.23 log CFU/gm and 6.17 log CFU/gm in treatment I and treatment II respectively. It

may be due to addition of Nisin which retard the growth of spoilage as well as pathogenic organisms in food<sup>4</sup>.

**Table 5: Effect of incorporation of different levels of nisin on microbiological characteristics of hurdle treated chicken sausages during ambient temperature storage ( $37 \pm 1^\circ\text{C}$ )**

Type of Product	Storage period (days)			
	0	2	4	6 <sup>A</sup>
	<b>Total plate count (log cfu/g)</b>			
Control	4.21±0.06 <sup>b1</sup>	6.78±0.12 <sup>a1</sup>	SP	--
Treatment I	3.22±0.02 <sup>d2</sup>	3.93±0.09 <sup>c2</sup>	4.70±0.05 <sup>b1</sup>	6.23±0.08 <sup>a1</sup>
Treatment II	3.15±0.01 <sup>d2</sup>	3.83±0.03 <sup>c3</sup>	4.59±0.07 <sup>b2</sup>	6.17±0.02 <sup>a1</sup>
	<b>Yeast and molds count (log cfu/gm)</b>			
Control	1.36±0.05	1.97±0.12	SP	--
Treatment I	ND	1.41±0.16	1.97±0.12	2.21±0.11
Treatment II	ND	ND	ND	ND

ND- Non Detected SP- Spoilage

Control- No hurdles

Treatment I – Chicken sausages added with 4% TSP+ Nisin 20 mg/Kg

Treatment II- Chicken sausages added with 4% TSP+ nisin 40 mg/Kg

Means with different superscripts (letters in the same row and numbers in the same column) indicate significant difference (P<0.05)

A – Data in the same column on day 6 was analyzed by paired t test

Hurdles such as low emulsion pH, humectants and Nisin at 20 mg/kg (treatment I) were sufficient to inhibit yeast and mold growth up to day 2 but addition of Nisin at 40 mg/kg (treatment II) in hurdle treated chicken sausages inhibited yeast and mold growth throughout the 6 days. Although, yeast and molds were reported to be survived at lower emulsion pH and humectant level used in the present study, their absence in treatment II throughout ambient temperature storage could be attributed to addition of Nisin at higher level (40mg/kg). The overall palatability of hurdle treated chicken sausages was in the range of “very good” to “good” up to day 6. On the day 8<sup>th</sup>, the sliminess was observed on the surface of hurdle treated chicken sausages with detection of slight off flavour.

### CONCLUSION

Application of hurdles viz. humectant (Textured soy protein) @ 4% and low emulsion pH (5.70) adjusted with lactic acid retard the quality deterioration of chicken sausages during ambient temperature storage ( $37 \pm 1^\circ\text{C}$ ). Addition of Nisin @ 40 mg/kg with application of different hurdles to chicken

sausages retained good acceptability and product could be stored safely for 6 days at ambient temperature storage ( $37 \pm 1^\circ\text{C}$ ) which will be otherwise only about 1 (one) day.

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