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APPLICATION OF BACTERIOCIN IN MEAT PRESERVATION.

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ABSTRACT

Fresh meat is a highly perishable product due to its biological composition. Many interrelated factors influence the shelf life and freshness of meat such as holding temperature, atmospheric oxygen, endogenous enzymes, moisture, light and most importantly, micro-organisms. The effects of 2ml of nisin at different temperatures when tested with meat for preservation ability with viable cell counts of bacterial from Day 1 to Day 42. The increase in the concentration of nisin at higher storage temperature (-18 °C, 4°C and 20°C) and length of time (Day 1 to Day 42), the higher the viable microbial cells recorded. The total viable cell counts at dilution factor 10^{-3} for 2ml of nisin was 52 x 10^{-3} CFU/ml , 64 x 10^{-3} CFU/ml and 101 x 10^{-3} CFU/ml for -18 °C, 4°C and 20°C respectively. The results revealed that the increase in the volume of nisin at higher storage temperature and length of time, the higher the viable microbial cells recorded.

Keywords: Bacteriocin, Meat and Nisin

INTRODUCTION

Meat has long been considered as a highly desirable, nutritious and protein-rich food, but at the same time, unfortunately, it is also highly perishable because it provides the nutrients needed to support the growth of many types of microorganisms. Due to its unique biological and chemical nature, meat undergoes progressive deterioration from the time of slaughter until consumption (Kalalou *et al*., 2004). Microbial contamination of meat occurs primarily due to raw materials, grinding of meat which will spread exterior contamination essentially throughout the entire muscle mass, post processing handling, cross contamination and/or equipment, lack of refrigeration facilities, ambient temperatures above 20°C, lack of suitable transportation between the production and marketing areas and improper storage temperature (Olaoye and Ntuen, 2011).

Meat is defined as the flesh of animals used as food. The term 'fresh meat' includes meat from recently processed animals as well as vacuum-packed meat or meat packed in controlled-atmospheric gases, which has not undergone any treatment other than chilling to ensure preservation. The diverse nutrient composition of meat makes it an ideal environment for the growth and propagation of meat spoilage micro-organisms and common food-borne pathogens.

It is therefore essential that adequate preservation technologies are applied to maintain its safety and quality (Aymerich *et al.,* 2008). The processes used in meat preservation are principally concerned with inhibiting microbial spoilage, although other methods of preservation are sought to minimise other deteriorative changes such as colour and oxidative changes.

A number of interrelated factors influence the shelf life and keeping quality of meat, specifically holding temperature, atmospheric oxygen, endogenous enzymes, moisture (dehydration), light and, most importantly, micro-organisms. All of these factors, either alone or in combination, can result in detrimental changes in the colour (Faustmann and Cassens, 1990), odour, texture and flavour of meat. Although deterioration of meat can occur in the absence of micro-organisms (e.g., proteolysis, lipolysis and oxidation), microbial growth is by far the most important factor in relation to the keeping quality of fresh meat (Lambert *et al*, 1991). Traditionally, methods of meat preservation may be grouped into three broad categories based on control by temperature, by moisture and, more directly, by inhibitory processes (bactericidal and bacteriostatic, such as ionising radiation, packaging, etc.), although a particular method of preservation may involve several antimicrobial principles. Each control step may be regarded as a 'hurdle' against microbial proliferation, and combinations of processes (so-called hurdle technology (HT)) can be devised to achieve particular objectives in terms of both microbial and organoleptic quality (Lawrie and Ledward, 2006).

Microorganisms reside in and on the meat, or intentionally added to it. Initial contamination of fresh meat comes from the animal and the people slaughtering it with the equipment use. The nutrient environment is quite ideal for microbial growth, and the most easily employed barrier to growth of microorganisms is reduced temperature. With processed meat, the population of organisms present normally results from recontamination following heat processing.

The microbial situation is quite dynamic, with several species often present. The dominant organisms will increase in numbers and the weaker will decline. It has been known for some time that microorganisms produce antibiotics or similar compounds that in fact have preservative effects. These are known as bio preservatives, and current research is directed at explaining more about the preservative mechanism. The possibility of using the microorganisms present naturally in the meat to exert degree of preservation is quite acceptable to the consumer, especially in contrast to adding a chemical preservative.

Competition for survival among the mixed population of microorganisms normally present in meat can be strong and specific. Within meat, there are in fact microenvironments. Most notably there are different environments at the surface, near the surface and in the deep interior of meat. Clearly, for example, oxygen cannot penetrate completely into the depth of meat. In intact pieces of meat, there are different compositional areas such as island (or areas) of lipids. Additionally, the connective tissue framework and elongated tubular, cellular structure of muscle provide not only barrier but also channels for movement of substances and occasionally, microorganisms.

Finally, this environment is altered as microbial growth occurs and other postmortem changes proceed. Not only do chemical factors such as pH and nutrient availability change, but also products from microbial metabolism. Since meat derives from the live animal, it is appropriate to comment about the natural control of microorganism in the living animal, before it is converted to meat. The most effective and naturally occurring antimicrobial system is the mammalian

phagocytic cell. Not only do these cells migrate to the site of trouble by some chemotaxic response, but they are also extremely effective in damaging prokaryotic cells without harming eukaryotic host cells. The cell membrane of the organism to be attacked is obviously of key importance. Preservatives may damage it and alter the physiological function of the cell or they may change permeability of the cell membrane so that an agent can pass through and then act within the cell.

Food preservation methods.

Based on the mode of action, the major food preservation techniques can be categorized as;

i. Slowing down or inhibiting chemical deterioration and microbial growth
ii. Directly inactivating bacteria, yeasts. molds, or enzymes, and
iii. Avoiding decontamination before and after processing (Gould, 1995). A number of techniques or methods.

MATERIALS AND METHOD

Dairy sample was collected from yogurt bought from commercial stores in Kaduna.

1ml of yogurt was serially diluted and poured into plates on De Man, Rogosa Sharpe (MRS) agar and allowed to gel on the bench. It was then incubated at 37°C for 48 hrs. Each colony was picked and sub cultured into MRS broth tubes and labeled. The colonies were sub cultured into MRS agar plates. The pure isolates were characterized based on morphological, biochemical and physiological tests.Test for inhibitory quality of pure cultures of *lactococcus lactis* was carried out

using the indicator microorganisms (*Escherichia coli, Staphylococcus aureus, Clostridium spp* and *Pseudomonas aeroginosa*) was grown in NA for 24h at 37°C. Test for bacteriocin

production was also carried out to determine the inhibitory effect of free bacteriocin on test bacteria was determined by agar well diffusion method using the filtrate from the mixture and the supernatant alone. Mueller Hinton II agar plates was punched with 8 mm diameter sterile clubs. (Aslim *et al*., 2005).

THE PRESERVATION ABILITY OF BACTERIOCIN ON MEAT

2ml of nisin was tested on the meat to determine the preservative ability of the bacteriocin. Fresh meat purchased from a local supermarket in Barnawa, Kaduna was washed and then minced into small pieces and blended with distilled water to produce meat slurries (Rayman et al., 1981). The meat slurries were pipetted into tubes. 2ml of nisin was added to each test tube. The test tubes were placed in different temperatures to determine the shelf life and preservative ability of the nisin. The viable cell count were determined by inoculating serial dilutions of each of the samples on NA for the 42days. After 24hrs at 37°C of incubation, bacterial counts were determined using the Stuart Colony Counter (SC6 made in UK by Bobby Sterling ltd).

3.7. Statistical Analysis

Two-way analysis of variance, ANOVA, Tukey-Kramer Multiple Comparisons Test and T test are the statistical analysis carried out.

RESULTS

Preservation ability of Nisin at different concentrations and temperatures

The effects of 2ml of nisin at different temperatures when tested with meat for preservation ability were seen in Table 4 and 5 with viable cell counts of bacterial from Day 1 to Day 42. The increase in the concentration of nisin at higher storage temperature (-18°C, 4°C and 20°C) and length of time (Day 1 to Day 42), the higher the viable microbial cells recorded. Table 6 shows the appearance and odor of the preserved beef at different durations.

Table 4: Total viable count of bacteria in meat when preserved with Nisin at different temperatures and duration.

Dilution factor	Temp	Microbial cell counts (CFU/ml)													
		Day 1		Day 7		Day 14		Day 21		Day 28		Day 35		Day 42	
		Control	Nisin	Control	Nisin	Control	Nisin	Control	Nisin	Control	Nisin	Control	Nisin	Control	Nisin
10^{-1}	-18^{0}C	166	43	173	52	181	60	193	73	214	99	233	113	246	117
	4^{0}C	180	51	193	77	204	84	222	93	236	101	256	116	276	123
	20^{0}C	200	63	216	83	223	109	246	121	273	126	300	151	370	170
10^{-2}	-18^{0}C	162	24	172	42	179	56	182	71	193	88	196	103	203	112
	4^{0}C	173	32	181	65	183	72	186	76	190	90	223	110	233	120
	20^{0}C	183	50	199	73	205	97	219	101	223	117	234	133	270	146
10^{-3}	-18^{0}C	120	5	134	17	143	27	155	28	170	38	184	49	203	52
	4^{0}C	135	6	138	18	146	28	149	30	154	39	200	50	211	64
	20^{0}C	133	12	152	28	166	38	170	39	192	49	213	72	221	101
10^{-4}	-18^{0}C	104	4	127	15	133	20	139	25	139	34	142	39	157	42
	4^{0}C	114	7	120	16	125	27	133	38	145	49	156	52	170	59
	20^{0}C	116	10	126	19	130	29	142	49	147	63	169	84	290	90
10^{-5}	-18^{0}C	110	2	117	14	125	19	131	21	139	25	149	29	153	32
	4^{0}C	112	1	118	15	123	19	129	27	140	33	157	41	160	49
	20^{0}C	118	6	120	18	130	25	139	37	149	55	159	63	195	70

Figure 2 shows the number of colonies produced after treatment with 2ml of nisin and a dilution of 10^{-1} and temperatures -18°C, 4°C and 20°C. The duration was from day 1 to day 42 and during this time the number of colonies increased with time. At -18°C in day 1 the number of colonies of the microbial cells was 43 and increased to 117 on day 42. At 4°C in day 1 the number of colonies of the microbial cells was 51 and increased to 123 on day 42. At 20°C in day 1 the number of colonies of the microbial cells was 63 x CFU/ml and increased to 170 on day 42.

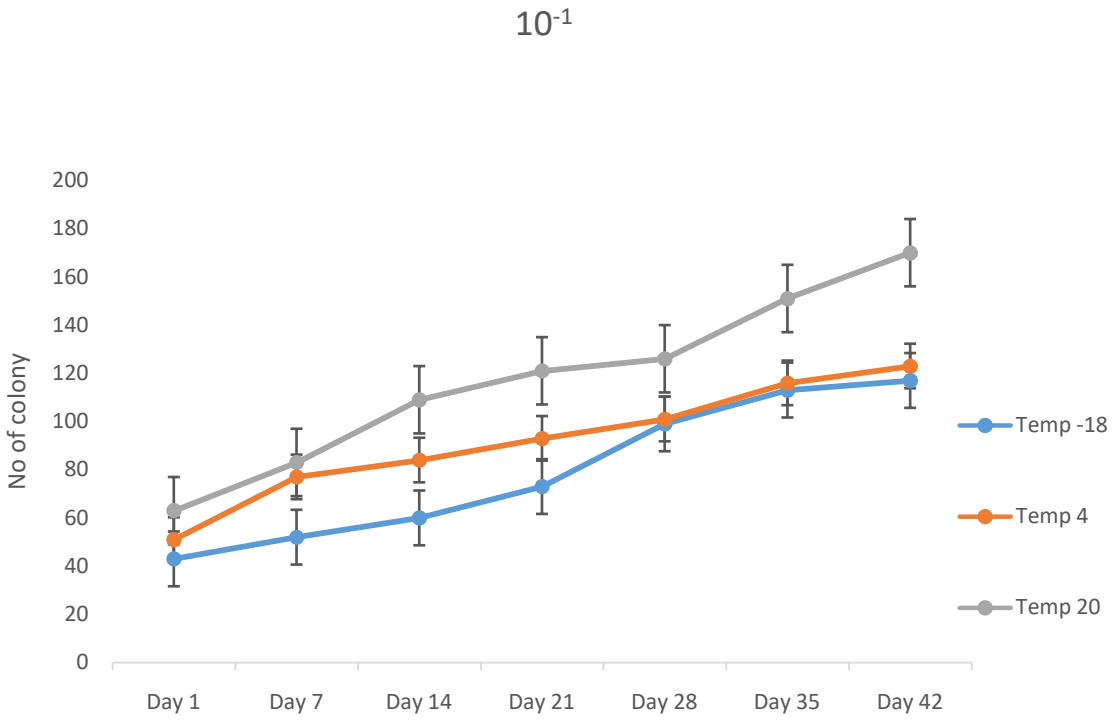

Fig 2: Number of colonies produced with respect to dilution factor 10^{-1} at 2ml of Nisin

Figure 3 shows the number of colonies produced after treatment with 2ml of nisin and a dilution of 10^{-2} and temperatures -18°C, 4°C and 20°C. The duration was from day 1 to day 42 and during this time the number of colonies increased with time. At -18°C in day 1 the number of colonies of the microbial cells was 24 and increased to 112 on day 42. At 4°C in day 1 the number of colonies of the microbial cells was 32 and increased to 120 on day 42. At 20°C in day 1 the number of colonies of the microbial cells was 50 and increased to 146 on day 42.

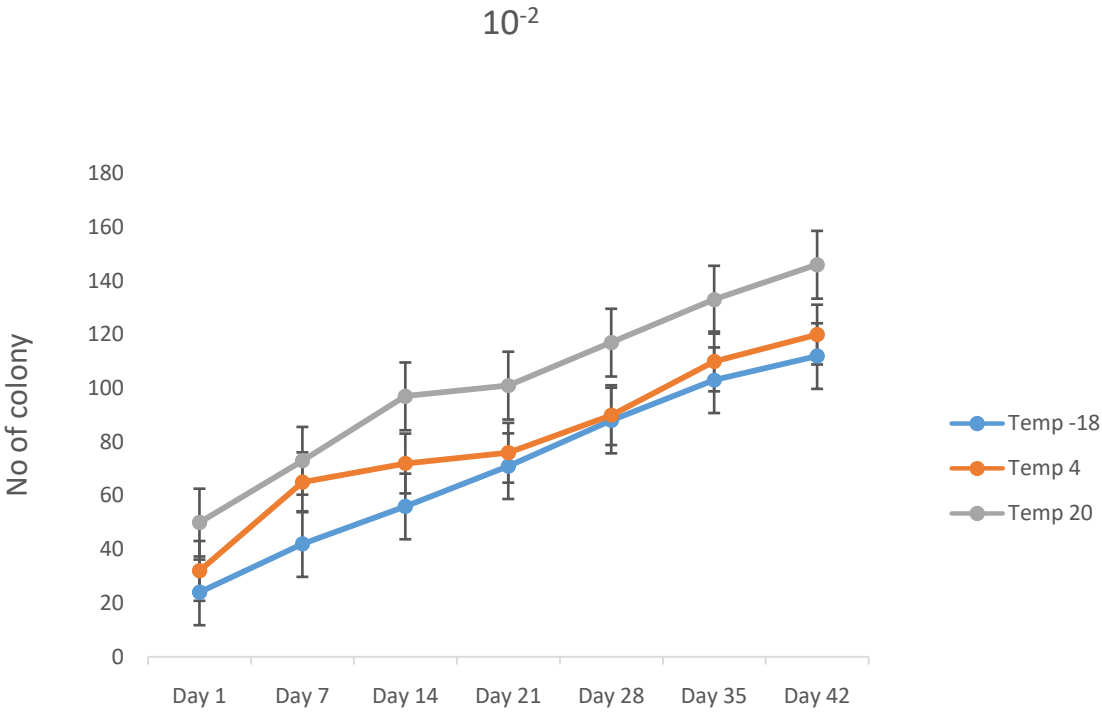

Fig 3 : Number of colonies produced with respect to dilution factor 10^{-2} at 2ml of Nisin

Figure 4 shows the number of colonies produced after treatment with 2ml of nisin and a dilution of 10^{-3} and temperatures -18°C, 4°C and 20°C. The duration was from day 1 to day 42 and during this time the number of colonies increased with time. At -18°C in day 1 the number of colonies of the microbial cells was 5 and increased to 52 on day 42. At 4°C in day 1 the number of colonies of the microbial cells was 6 and increased to 64 on day 42. At 20°C in day 1 the number of colonies of the microbial cells was 12 and increased to 101 on day 42.

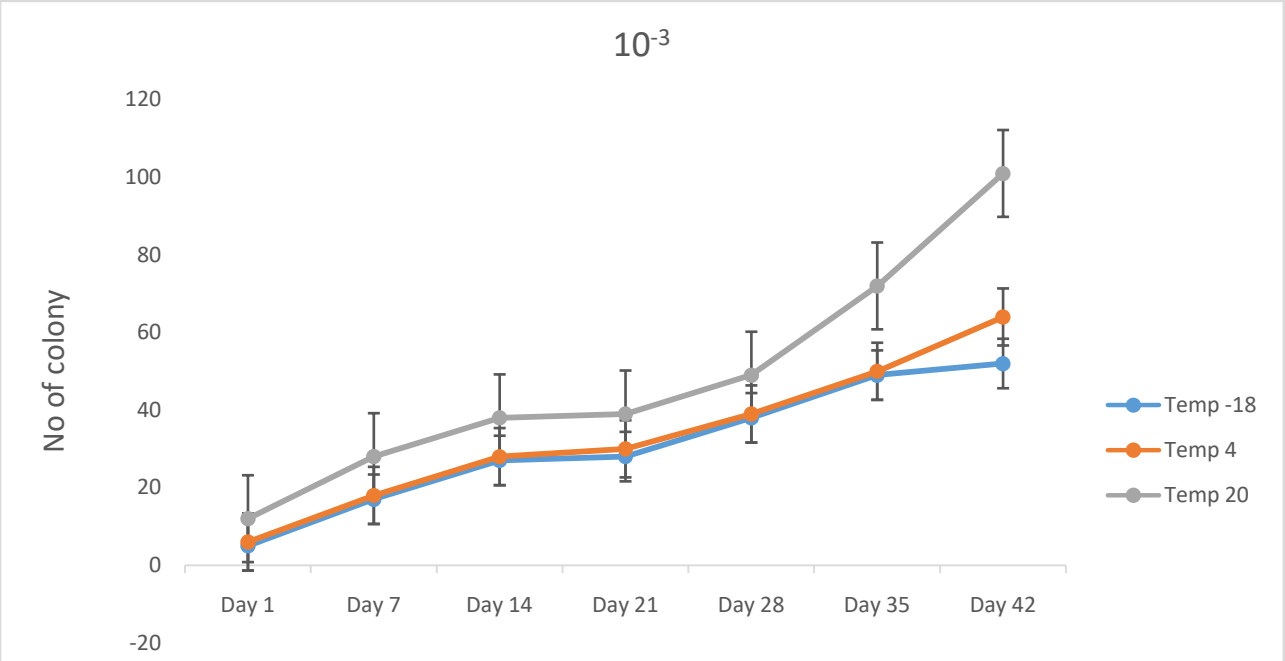

Fig 4 : Number of colonies produced with respect to dilution factor 10^{-3} at 2ml of Nisin

Figure 5 shows the number of colonies produced after treatment with 2ml of nisin and a dilution of 10^{-4} and temperatures -18°C, 4°C and 20°C. The duration was from day 1 to day 42 and during this time the number of colonies increased with time. At -18°C in day 1 the number of colonies of the microbial cells was 4 and increased to 42 on day 42. At 4°C in day 1 the number of colonies of the microbial cells was 7 and increased to 59 on day 42. At 20°C in day 1 the number of colonies of the microbial cells was 10 and increased to 90 on day 42.

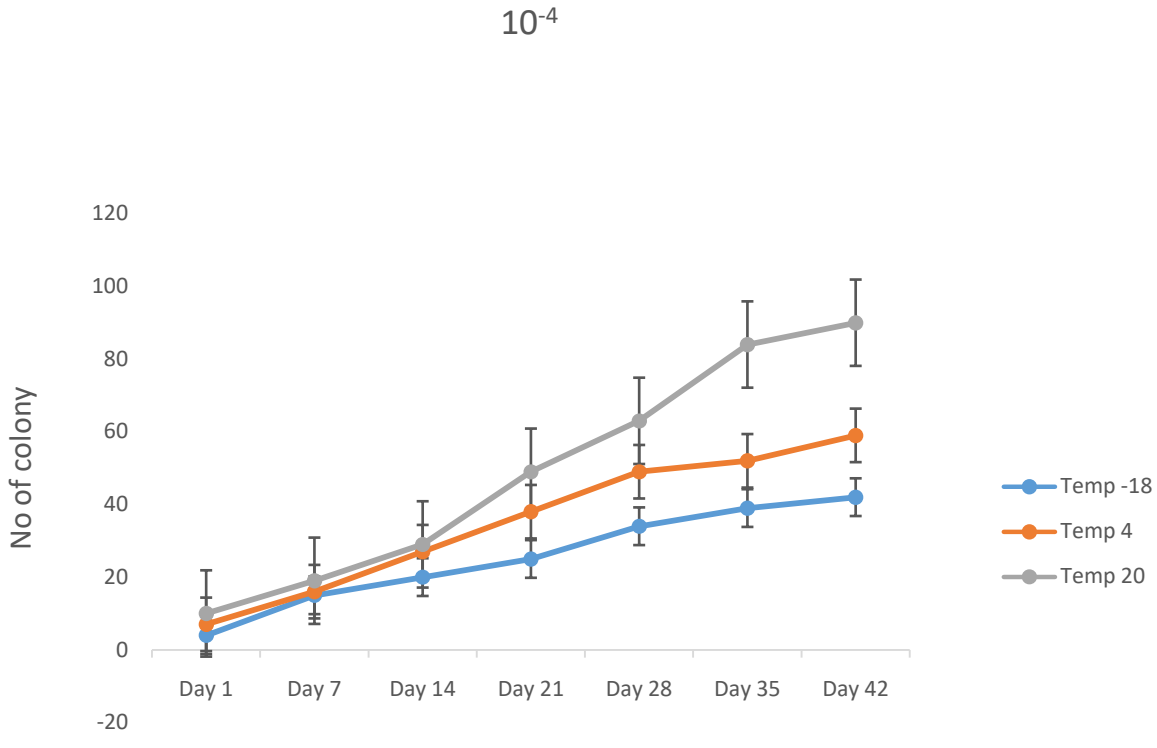

Fig 5 : Number of colonies produced with respect to dilution factor 10^{-4} at 2ml of Nisin

Figure 6 shows the number of colonies produced after treatment with 2ml of nisin and a dilution of 10^{-5} and temperatures -18°C, 4°C and 20°C. The duration was from day 1 to day 42 and during this time the number of colonies increased with time. At -18°C in day 1 the number of colonies of the microbial cells was 2 and increased to 32 on day 42. At 4°C in day 1 the number of colonies of the microbial cells was 1 and increased to 49 on day 42. At 20°C in day 1 the number of colonies of the microbial cells was 6 and increased to 70 on day 42.

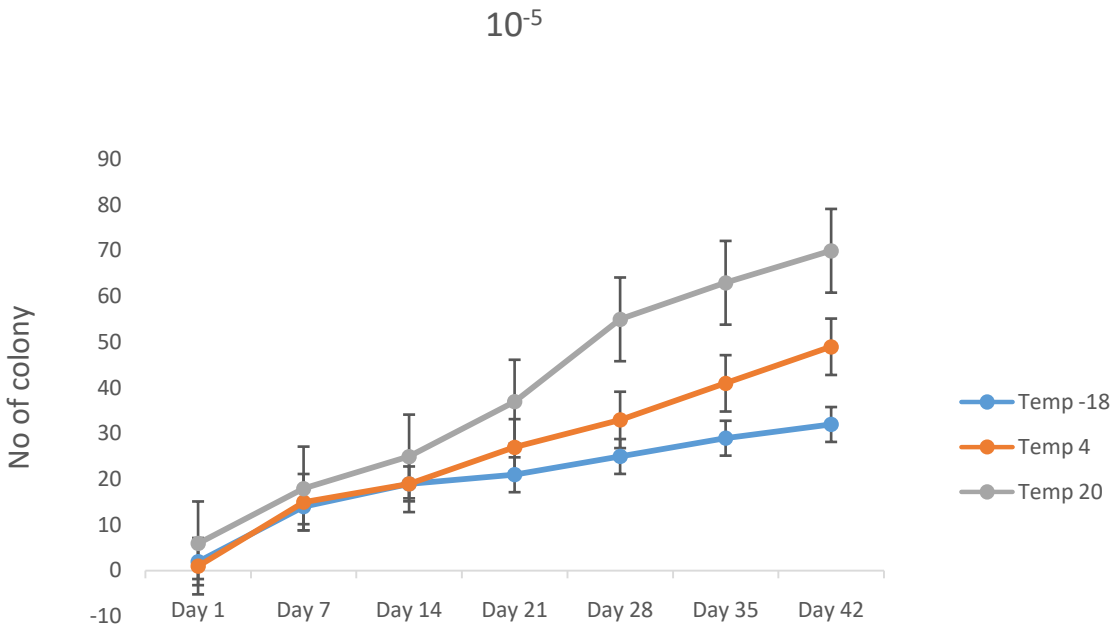

Fig 6 : Number of colonies produced with respect to dilution factor 10^{-5} at 2ml of Nisin

Table 6a shows that the meat stored at -18°C took about 28days to change colour from reddish to dark red and had a mild smell after 21 days and a pungent smell by 35 days after treatment and storage. While at 4°C the meat was reddish in the first 14 days but turned darkish red and foamy with mild smell on day 21, but became brown and foamy with pungent smell till day 42. At 20°C the meat's appearance was darkish red from day 14 and the smell was mild. But from day 28 the appearance was brown and foamy with pungent smell.

Table 6b shows that the meat stored at -18°C took about 21 days to change colour and appearance while at 4°C and 20°C it started on day 14

Table 6a. Showing the Appearance and Odor of the preserved beef at different temperatures and durations after preservation with nisin.

Temp	Duration (Days)	Appearance/color	Smell
-18^0C	1	Reddish	Normal
	14	Reddish	Normal
	21	Reddish	Mild
	28	Darkish red	Mild
	35	Brown and foamy	Pungent
	42	Brown and foamy	Pungent
4^0C	1	Reddish	Normal
	14	Reddish	Normal
	21	Darkish red and foamy	Mild
	28	Brown and foamy	Pungent
	35	Brown and foamy	Pungent
	42	Brown and foamy	Pungent
20^0C	1	Reddish	Normal
	14	Darkish red	Mild
	21	Darkish red and foamy	Pungent
	28	Brown and foamy	Pungent
	35	Brown and foamy	Pungent
	42	Brown and foamy	Pungent

Table 6b. Showing the Appearance and Odor of the preserved beef at different temperatures and durations after preservation without nisin.

Temp	Duration (Days)	Appearance/color	Smell
-18^0C	1	Reddish	Normal
	14	Reddish	Normal
	21	Darkish red and foamy	Mild
	28	Brown and foamy	Pungent
	35	Brown and foamy	Pungent
	42	Brown and foamy	Pungent
4^0C	1	Reddish	Normal
	14	Darkish red and foamy	Mild
	21	Darkish red and foamy	Mild
	28	Brown and foamy	Pungent
	35	Brown and foamy	Pungent
	42	Brown and foamy	Pungent
20^0C	1	Reddish	Normal
	14	Darkish red and foamy	Mild
	21	Brown and foamy	Pungent
	28	Brown and foamy	Pungent
	35	Brown and foamy	Pungent
	42	Brown and foamy	Pungent

4.4. Statistical analysis

At 10^{-1} dilution factor, the mean of control and Nisin were extremely significant with P-Value and T-Value of 0.0001 and 7.77 respectively. Also, there was significant difference in the mean of control and Nisin at 10^{-2} dilution factor, showing P-Value of 0.0001 and T-value of 8.43. The T-Value of the mean of control and Nisin at 10^{-3} dilution factor, is 11.56 which indicates that they are extremely significant. The control and Nisin was significantly different at 10^{-4} dilution factor, with T having a value of 7.54 and P-Value of 0.0001. At 10^{-5} dilution factor, the control and Nisin was significant with a T-Value of 10.93.

Analysis of variance show that the inhibitory effect of Nisin at different dilution factors differed extremely with a P-Value of 0.0001 (**Table 8**). As shown in **Table 7**, Pairwise comparison using Tukey's honestly significant difference test reveals that:

On day 1, Nisin at a dilution factor of 10^{-1} is significantly different from that of dilution factor of 10^{-3}, 10^{-4} and 10^{-5} while Nisin of dilution factor 10^{-2} differs significantly from that of 10^{-4} and 10^{5}.

On day 7, 10^{-1} dilution factor of Nisin differs from 10^{-3} and 10^{-4}. Dilution factor 10^{-5}. 10^{-2} differs significantly from10^{-3}, 10^{-4} and 10^{-5}.

On day 14, 10^{-1} dilution factor of Nisin differs from 10^{-3}, 10^{-4} and 10^{-5}.

On day 21, Nisin dilution factor of 10^{-1} differs from 10^{-3}, 10^{-4} and 10^{-5}.

On day 28, 10^{-1} dilution factor of Nisin differs from 10^{-3}, 10^{-4} and 10^{-5} while Nisin of dilution factor 10^{-2} differs significantly from that of 10^{-3}, 10^{-4} and10^{-5}.

On day 35, Nisin at dilution factor of 10^{-1} is significantly different from that of dilution factor of 10^{-3}, 10^{-4} and 10^{-5}.

As shown in **Table 9**, Analysis of variance at 0.01 level of significance, reveals that there is significant difference in the total viable cell count at (2ml and 0ml).

The data presented in **Table 10** is a result of Post Test (Tukey-Kramer Multiple Comparisons Test) carried out to further compare the relationship between the total viable cell counts. Interestingly, the viable cell counts between the control (0ml) and 2ml of nisin differed significantly.

There is no significant difference between the viable bacteria at temperature -18ºC, 4 ºC and 20ºC, with a P-Value and F-Value of 0.1671 and 1.979 respectively (**Table 11a**).

Two-way analysis of variance reveals that there is significant difference in the viable bacteria count at different temperatures and dilution factors, with a P-Value of 0.0001 (**Table 12**). The bacteria count at 20ºC were significantly different from -18ºC and 4 ºC (**Table 11b**).

Table 7: The mean and standard error of the total viable cell count at different dilution factor of Nisin

Values with the same letter in the superscript at the same row differ significantly at P<0.01 according to Tukey's Honestly Significant Different Test

	10^{-1}		10^{-2}		10^{-3}		10^{-4}		10^{-5}	
	Control	**Nisin**	**Control**	**Nisin**	**Control**	**Nisin**	**Control**	**Nisin**	**Control**	**Nisin**
ay	182±9.87	52±5.81^{abc}	172±6.06	35±7.68^{de}	126±3.79	8±2.19^{a}	111±3.71	7±1.73^{bd}	113±2.40	3±1.53^{c}
ay	194±12.14	71±9.49^{abc}	184±7.93	60±9.29^{def}	141±5.45	12±3.51^{ad}	124±2.19	17±1.20^{be}	118±0.88	16±1.2
ay	202±12.14	84±14.14^{abc}	189±8.08	75±11.9^{d}	152±7.21	31±3.51^{a}	129±2.33	25±2.73^{b}	126±2.08	21±2.0^{c}
ay	220±15.32	96±13.92^{abc}	196±11.72	83±9.28^{d}	158±6.25	32±3.38^{a}	138±2.65	37±6.93^{b}	133±3.05	28±4.6
ay	241±17.21	108±8.68^{abc}	202±10.53	98±9.35^{def}	172±11.01	42±3.51^{ad}	144±2.40	49±8.37^{be}	143±3.18	38±8.9
ay	263±19.65	127±12.19^{abc}	218±11.28	115±9.06^{d}	199±8.38	57±7.5^{a}	156±7.79	58±13.4^{b}	155±3.05	44±9.9
ay	297±37.35	137±16.76^{a}	235±19.37	126±10.3	212±5.20	72±14.7	206±42.3	64±14.0	169±12.99	50±10.9

Table 8: Analysis of variance of the inhibitory effect of Nisin at different dilution factors

Source of variation	Degree of freedom	Sum of squares	Mean square	F- value	P-Value
Between sample	3	27737	6934.2	10.918	0.0001
Within sample	30	19054	635.13		
Total	34	46791			

Table 9: ANOVA showing the relationship between mean total viable bacteria count at concentration 2ml and control.

Source of variation	Degree of freedom	Sum of squares	Mean square	F- ratio	1% F-limit
Between sample	3	104817	34939	83.679	4.72
Within sample	24	10021	417.54		
Total	27	114838			

Table 10: Tukey-Kramer Multiple Comparisons Test for the total viable cell at 2ml and control at dilution factor 10^{-3}. If the value of q is greater than 3.901 then the P value is less than 0.01.

Comparison	Mean difference	q-value	p value
Control vs 2ml	128.05	16.580	*** P<0.001

Table 11a: Total viable bacteria at different temperature

Duration (Day)	-18ºC	4 ºC	20 ºC
1	16	19	28
7	28	38	44
14	36	46	60
21	44	53	69
28	57	62	82
35	67	74	101
42	71	83	115
F-Value = 1.979	P-Value = 0.1671		

Table 11b: Mean total viable bacteria count at different temperature and Dilution factor.

Dilution factor	-18ºC	4 ºC	20 ºC
10^{-1}	80	92	118
10^{-2}	71	81	102
10^{-3}	31	34	48
10^{-4}	26	35	49
10^{-5}	20	26	39
Mean	45.6 ± 12.41^{a}	53.6 ± 13.63^{b}	71 ± 16.14^{ab}

Values with the same letter in the superscript at the same row differ significantly at P<0.01 according to Tukey's Honestly Significant Different Test

Table 12: Analysis of variance of the mean total viable bacteria count at different temperatures and Dilution factor.

Source of variation	Degree of freedom	Sum of squares	Mean square	F- Value	P-Value
Between sample	2	1715.2	857.60	42.316	0.0001
Within sample	4	11843	2960.8		
	8	162.13	20.267		
Total	14	13720			

CHAPTER FIVE

DISCUSSION

The effects of 2ml of nisin at different temperatures when tested with meat for preservation ability is dependent upon the storage temperature and length of storage. The meat stored at -18°C took about 28days to change colour from reddish to dark red and had a mild smell after 21 days and a pungent smell by 35 days after treatment and storage. While at 4°C the meat was reddish in the first 14 days but turned darkish red and foamy with mild smell on day 21, but became brown and foamy with pungent smell till day 42. At 20°C the meat's appearance was darkish red from day 14 and the smell was mild. But from day 28 the appearance was brown and foamy with pungent smell. According to Barbosa-Canovas *et al*., 1997 during this period of treatment, a large amount of energy is transferred to the food. However, this energy can trigger unwanted reactions, leading to undesirable organoleptic and nutritional effects. This may now lead t the change in appearance and odour.

When Nisin was used in the preservation of meat, the higher the storage temperature and length of time, the higher the viable microbial cells recorded. The results revealed that the longer the

duration or time the more the viable microbial cells recorded. On day 1 the number microbial cells recorded was 43 x 10^{-1} CFU/ml, 51 x 10^{-1} CFU/m and 63 x 10^{-1} CFU/ml for each temperatures -18^{0}C, 4^{0}C and 20^{0}C and on day 42 the number of microbial cells recorded 117 x 10^{-1} CFU/ml, 123 x 10^{-1} CFU/ml and 170 x 10^{-1} CFU/ml. This may be due to the bacterial population undergoing exponential growth. The number of new microbial cells appearing per unit time is proportional to the present population.

The higher the temperature used in the preservation of the meat slurries the higher the viable microbial cells recorded. It is possible this occurred because at low temperature tend to reduce growth rates which has led to refrigeration being instrumental in food preservation. Environmental conditions such as temperature influence rate of bacterial growth and this conditions tend to be relatively consistent between microbial cells. Microbial cells have optimal growth conditions they thrive, but once outside of those conditions the stress can result in either reduced or stalled growth, dormancy or death as observed in day 14 to day 21 at -18^{0}C and dilution factor 10^{-3}.

While the higher the dilution factor the less the total viable microbial cells recorded. The dilution factors 10^{-1} and 10^{-5} for temperatures -18^{0}C, 4^{0}C and 20^{0}C from day 1 to day 42 shows that at higher temperatures the total number of viable call counts increases with time. Dilution factor ($10^{-1)}$ for -18^{0}C is 43 x 10^{-1} CFU/ml on Day 1 and 117 x 10^{-1} CFU/ml for Day 42, at 4^{0}C is 51 x 10^{-1} CFU/ml on Day 1 and 123 x 10^{-1} CFU/ml on Day 42 and at 20^{0}C is 63 x 10^{-1} CFU/ml on Day 1 and 170 x 10^{-1} CFU/ml on Day 42.

Dilution factor (10^{-5}) for -18^{0}C is 2 x 10^{-5} CFU/ml on Day 1 and 32 x 10^{-5} CFU/ml for Day 42, at 4^{0}C is 1 x 10^{-5} CFU/ml on Day 1 and 49 x 10^{-5} CFU/ml on Day 42 and at 20^{0}C is 6 x 10^{-5} CFU/ml on Day 1 and 70 x 10^{-5} CFU/ml on Day 42. The decrease in the dilution factor leads to a decrease in the microbial cells in the meat. And this may be due to the fact that the viable cells that multiply via binary fission under controlled conditions decrease the average number of cells per CFU because many microorganisms are delicate and would suffer a decrease in the proportion of cells that are viable with each dilutions.

RECOMMENDATION

A synergistic effect clearly plays a role in preventing growth of pathogenic and spoilage microorganisms, extending the inhibitory activity spectrum to such intrinsically resistant bacteria as Gram-negative bacteria, improving the sensory, chemical and microbial qualities of food and ultimately, have a significant impact on food safety, shelf life extension and health requirements. Gram negative bacteria needs to be effectively inhibited or destroyed to prevent a wide range of spoilage in the food industries. Continued research on nisin needs to be carried out with increased stability and enhanced features, or extension of the antimicrobial spectrum to Gram-negative bacteria. The genomics will soon become an essential tool for exploring the antimicrobial potency of LAB and may yield characteristics that could be very rewarding especially with food safety.

There is also need to encourage the production of LAB for enhancing probiotic treatments. The bacteriocin production which is often proposed as a beneficial characteristic of probiotics that may contribute to host protection against gastrointestinal pathogens. Moreover, bacteriocin

production may facilitate the establishment of a strain in the competitive environment of the gut. So there is need for vast production of LAB in today's economy.

Lastly continued research must be carried out to help nisin production in large commercial scale by genetic engineering with all the associated consumer concerns. Therefore, these strains of *L. lactis* isolated from dairy sources may be included in the industrially important culture collection and may be recommended as starter culture for the manufacture of fermented foods and to provide safety against the microflora tested in the present investigation either as viable cells or purified form of antimicrobial agents.

CONCLUSION

In conclusion *Lactococcus lactis* was isolated from concentrated sour milk and characterized. Nisin was extracted from the *Lactococcus lactis* which was purified. From the study, -18^0C was the best temperature for storage of meat because at this temperature the number of viable cell counts was very low. When 2ml of nisin was inoculated in the meat slurry from 10^{-1} to 10^{-5} it shows that an increase in the dilution factor decreases the number of microorganism in the slurry and the preservation ability of the nisin was determined.

REFERENCES

Olaoye, O.A. and I.G. Ntuen, 2011. Spoilage and preservation of meat: a general appraisal and potential of lactic acid bacteria as biological preservatives. Review Paper. *Int. Res. J. Biotechnol*., 2: 33-46

Kalalou, I., M. Faid and A.T. Ahami, 2004. Extending the shelf life of fresh minced camel meat preservation of meat: a general appraisal and at ambient temperature by *Lactobacillus delbruekii subsp. delbruekii. Electronic J. Biotechnology*, 7: 246-251.

Barbosa-Canovas, G. V.; Pothakamury, U. H.; Palou, E.; Swanson,B. G. Nonthermal Preservation of Foods; Marcel Dekker: New York, 1997; p 304

Aslim B, Yuksekdag ZN, Sarikaya E, Beyatli, Y (2005). Determination ofthe bacteriocin-like substances produced by some lactic acid bacteria isolated from Turkish dairy products. Lebensm. Wiss. Technol., 38: 691-694

Aymerich, T., Picouet, P. A., & Monfort, J. M. 2008. Decontamination technologies for meat products. Meat Science, 78, 114−129.

Faustmann, C., and Cassens, R G. 1990. The biochemical basis for discoloration in fresh meat a: review. In: *Journal of Muscle Foods*. 1, (3):217-243.

Lambert, A. D., Smith, J. P., and Dodds, K. L. 1991. Shelf life extension and microbiological safety of fresh meat – a review. *Food Microbiology*, 8, 267−297.

Lawrie, R. A., and Ledward, D. A. 2006. Lawrie's Meat Science. Seventh English, edition ed. Cambridge England: Woodhead Publishing Limited.

Gould, G.W. 1995. Overview, In: *New methods of food preservation*. Blackie Academic and Professional, Glasgow.

Rayman M.K; Aris B; and Hurst A, 1981 Nisin: a Possible Alternative or Adjunct to Nitrite in the Preservation of Meats. *Applied And Environmental Microbiology*, p. 375-380 Vol. 41, No. 2