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.

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