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Effects of Chemical Hurdles and Packaging Materials on Microbial Load and Bacterial Distribution in Kilishi under Ambient Storage

[1]Abdullahi Nura,
[1]Department of Food Science and Technology,
Kano University of Science and Technology,
Wudil, Kano State Nigeria (P.M.B 3244)

[2]Araihu C. C. and [2]Abu J. O.
[2] Department of Food Science and Technology,
University of Agriculture Makurdi,
Benue State Nigeria (P.M.B 2373)

***Abstract* - The research work studied combine effects of chemical hurdles and packaging materials on microbial load and bacterial distribution in *Kilishi* under ambient storage (30±8^{0}C). Sucrose, citric acid and sodium benzoate were applied in different combination and concentrations. High Density Polyethylene (HDPE), aluminium foil and brown paper were used as packaging materials during twelve weeks ambient storage. The laboratory prepared samples were studied alongside a market sample which was collected from *Agadasawa* in Kano State Nigeria. Determination of total microbial load, and isolation and identification of bacterial group conducted at two weeks interval in the market sample and laboratory prepared samples. Increased in microbial counts was observed during storage with highest counts recorded in market sample throughout the storage time. Among the packaging materials, samples packaged in brown paper were found to have highest microbial load, and that packaged in HDPE were found to have least counts. *Streptococcus spp.*, *Staphylococcus spp.*, *Salmonella spp*., *Pseudomonas spp.* and *Bacillus spp.* were identified in all the samples. *Escherichia coli* was identified only in market sample. Growth of *Salmonella spp.* and *Staphylococcus spp.* was found to be subsiding during storage with total elimination occurred at week 8 and week 10 respectively from the treatment samples. Combination of 4% sucrose, 0.1% citric acid, 0.1% sodium benzoate and use of HDPE as packaging material provide best microbial quality.**

Key Words; Hurdle, Kilishi, Microorganism, Bacteria, Ambient storage

INTRODUCTION

Kilishi is a sun dried traditional meat product made principally from beef. It is an intermediate moisture or semidry meat product. The product appears to have developed as a means of preserving meat in the absence of refrigeration facilities by the early Fulani and Hausa herdsmen of Northern Nigeria and the Sahelian Africa. As a ready-to-eat convenience meat product, *Kilishi* possess an excellent shelf life (Isah and Okubanjo, 2012). Keeping quality of Kilishi is greatly affected by the season and location of production (Fonkem *et. al.*, 2010).

Food preservation implies putting microorganisms in a hostile environment in order to cause their death (Oladapo *et al.*, 2014). Preservative agents are required to ensure that manufactured foods remain safe and unspoiled (Brul and Coote, 1999). When food is to be stored for a prolonged period, use of preservatives is essential in order to maintain its quality and flavour. Their use prevents spoilage of foods due to the growth of bacteria and fungi. They also maintain the quality and consistency of the foods, along with its palatability and wholesomeness. Preservatives also maintain nutritional value, control appropriate pH and enhance flavour (Arora *et al.*, 2014).

Chemical preservatives may be injurious when used in higher concentrations. Arora *et al.* (2014) reported that at higher concentration benzoates can trigger allergies such as skin rashes, asthma and can also causing brain damage. He also reported that sodium chloride when used in high amount in meats and fish can lead to high blood pressure, kidney failure, stroke and heart attack.

External animal surfaces, as well as their feces and the environment, may serve as sources of contamination for carcasses during the slaughtering, dressing and cutting processes. Panagiotis *et al.* (2010) reported that majority of these microorganisms consist of nonpathogenic spoilage bacteria and indicator microorganisms, such as coliforms and *Escherichia coli* (at levels 10 to 10^7 cfu/cm^2 or higher). However, there is also potential for contamination by pathogenic microorganisms such as *Escherichia coli*, *Salmonella*, *Campylobacter spp*. etc. These presents challenging problems to the meat industry and low bacteriological quality raise concern about its potential for the transmission of foodborne infections. (Agwu and Chisom, 2014).

Joerg *et al.* (2007) reported that *Kilishi* suffers contamination from various sources, example; Slaughtering of animal and carcass processing take place under unhygienic conditions, meat transport and marketing are done without refrigeration and no protection against sun and dust. *Kilishi* production and storage under conditions free of microbial activity has been seen as a process usually difficult to achieve because of its nutritious nature that attract agents of microbiological spoilage (Ogbonnaya and Linus, 2009). *Kilishi* is preserved by drying which in traditional production is achieved through sun-drying. Drying is not lethal and many types of microorganisms may be recovered from dried foods, especially if poor-quality foods were used for drying and if proper practices were not followed in the drying steps (James, 2000). Fonkem *et al.* (2010) reported that some spices used in *Kilishi* production

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play a key role in inhibiting the growth and proliferation of some micro-organisms, this contradict the opinion of Ogbonnaya and Linus (2009) who reported that condiment used in the production of *Kilishi* can serve as a source of microbial contamination.

METHODOLOGY

Sample collection

Freshly prepared *Kilishi* was collected from *Agadasawa* in Kano metropolis, Kano State-Nigeria. To avoid contamination, the collected sample was wrapped in HDPE and aseptically transported to laboratory. The collected sample was divided in three, each of the portions was packaged into HDPE, aluminium foil and brown paper, and stored under ambient temperature (30 ± 8^0C) for period of twelve weeks. Samples were withdrawn from market and laboratory prepared samples and subjected to microbiological analyses at two weeks interval.

Procurement of raw material for Kilishi production

Beef was purchased from Kano central abattoir. Ginger, Cloves, Black Pepper, Hot Pepper, Sweet Pepper, onion, curry, salt, seasoning and peanut cake were purchased from *Kurmi* Market in Kano.

Production of Kilishi

Recipe for condiment production

The table below 2 provides recipe for the production of *Kilishi* condiment.

Table 1: Recipe for Production of *Kilishi* Condiment

Ingredients	Quantity (g)
Ginger	17.9
Cloves	1.3
Black Pepper	2.5
Hot Pepper	5.3
Sweet Pepper	11.0
Onion	12.5
Curry	3.7
Salt	23
Seasoning (Maggi)	53.5
Peanut cake	469.3

Source; Badau *et al.* (1997).

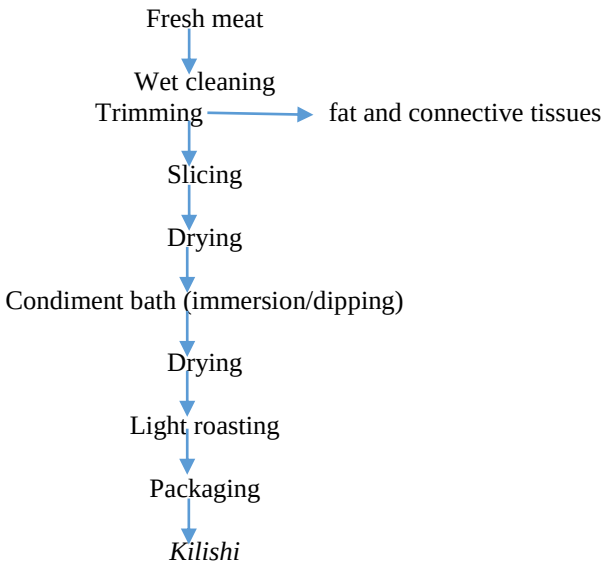

Figure 1: Tradition *Kilishi* Production Process

Source; Okonkwo *et al.* (2013)

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 08, August-2016

Table 2; Percentages of Hurdles and Condiment used in *Kilishi*

Sample Code	Sucrose	Citric	Sodium Acid	Condiment Benzoate	Meat
001	0.0	0.0	0.0	45.0	55
014	2.0	0.1	0.1	42.8	55
017	2.0	0.2	0.1	42.7	55
023	4.0	0.1	0.1	40.8	55

Microbiological analyses

Total microbial load was performed on nutrient agar using serial dilution method described by the American Public Health Association (APHA, 1992). Isolation and identification of bacterial groups was achieved through the following biochemical tests; gram reaction, coagulase test, acid production from sugar, methyl red test, voges proskauer test (Robert and Greenwood, 2003) motility test, indole test, catalase test, oxidase test, citrate test (Chessbrough, 2000). The results of biochemical tests were compared with characteristics of taxa described by Bergey's Manual for Determinative Bacteriology (Buchanan and Gribbons, 1974).

RESULTS

Table 3: Effect of hurdles and packaging materials on microbial load of *Kilishi* samples under ambient storage (30±8^{0}c)

Sample Code	Packaging Materials	Storage Time (weeks)						
		0	2	4	6	8	10	12
		Total Plate Count (×10^3 cfu/g)						
001	HDPE	2.40	2.48	2.51	2.58	2.66	2.72	2.76
	Aluminium foil	2.40	2.53	2.64	2.72	2.85	2.92	3.10
	Brown paper	2.40	2.60	2.74	2.80	2.98	3.20	3.38
014	HDPE	1.82	1.88	1.92	1.97	2.04	2.12	2.24
	Aluminium foil	1.82	1.90	1.97	2.12	2.18	2.26	2.32
	Brown paper	1.82	1.96	2.10	2.26	2.30	2.38	2.42
017	HDPE	1.77	1.82	1.88	1.94	1.96	2.10	2.25
	Aluminium foil	1.77	1.83	1.92	2.11	2.23	2.29	2.34
	Brown paper	1.77	1.89	2.10	2.22	2.28	2.40	2.60
023	HDPE	1.56	1.60	1.66	1.71	1.78	1.84	1.92
	Aluminium foil	1.56	1.63	1.70	1.79	1.85	1.90	1.97
	Brown paper	1.56	1.68	1.76	1.82	1.90	2.12	2.24
MS	HDPE	7.32	7.56	7.62	7.84	8.10	8.27	8.62
	Aluminium foil	7.32	7.60	7.69	7.98	8.22	8.96	9.26
	Brown paper	7.32	7.64	7.73	8.20	8.63	9.50	10.32

1

Table 4: bacterial distribution in *Kilishi* samples treated with different hurdles under ambient storage (30±8^{0}c) (Week 0)

Sample codes	001	014	017	023	MS
	%Hurdles				
Sucrose	0	2	2	4	0
Citric acid	0	0.1	0.2	0.1	0
Sodium benzoate	0	0.1	0.1	0.1	0
Bacterial group	Bacterial Distribution (%)				
Streptococcus spp.	20	25	25	15	15
Staphylococcus spp.	20	18	14	21	20
Salmonella spp.	08	06	06	04	14
Pseudomonas spp.	22	26	25	28	11
Bacillus spp.	30	25	30	32	30
E. coli	00	00	00	00	10
Total	100	100	100	100	100

Sample code	Sucrose (%)	Citric acid (%)	Sodium benzoate (%)
001	0	0	0
014	2	0.1	0.1
017	2	0.2	0.1
023	4	0.1	0.1

MS-Market sample

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 08, August-2016

Table 5: Bacterial distribution in *Kilishi* samples treated with different hurdles under ambient storage (30±8^{0}c) using various packaging materials (Week 2)

Sample codes		001	014	017	023	MS
		%Hurdles				
	Sucrose	0	2	2	4	0
	Citric acid	0	0.1	0.2	0.1	0
	Sodium benzoate	0	0.1	0.1	0.1	0
Packaging Material	Bacterial group	Bacterial Distribution (%)				
HDPE	*Streptococcus spp.*	22	27	20	12	15
	Staphylococcus spp.	18	16	13	19	15
	Salmonella spp.	12	06	05	05	15
	Pseudomonas spp.	20	25	28	32	18
	Bacillus spp.	28	26	34	32	27
	E. coli	00	00	00	00	10
	Total	100	100	100	100	100
Aluminium Foil	*Streptococcus spp.*	18	25	25	15	15
	Staphylococcus spp.	20	18	14	21	20
	Salmonella spp.	14	05	06	05	10
	Pseudomonas spp.	20	26	25	27	15
	Bacillus spp.	28	26	30	32	30
	E. coli	00	00	00	00	10
	Total	100	100	100	100	100
Brown Paper	*Streptococcus spp.*	23	26	25	16	15
	Staphylococcus spp.	15	17	14	20	20
	Salmonella spp.	16	06	06	04	11
	Pseudomonas spp.	20	24	27	30	15
	Bacillus spp.	26	27	28	30	27
	E. coli	00	00	00	00	12
	Total	100	100	100	100	100

Table 6: Bacterial distribution in *Kilishi* samples treated with different hurdles under ambient storage (30±8^{0}c) using various packaging materials (Week 4)

Sample codes		001	014	017	023	MS
		%Hurdles				
	Sucrose	0	2	2	4	0
	Citric acid	0	0.1	0.2	0.1	0
	Sodium benzoate	0	0.1	0.1	0.1	0
Packaging Material	Bacterial group	Bacterial Distribution (%)				
HDPE	Streptococcus spp.	20	25	20	13	10
	Staphylococcus spp.	20	18	15	20	15
	Salmonella spp.	14	05	05	05	15
	Pseudomonas spp.	22	30	30	31	22
	Bacillus spp.	24	22	30	31	28
	E. coli	00	00	00	00	00
	Total	100	100	100	100	100
Aluminium Foil	Streptococcus spp.	20	20	24	17	20
	Staphylococcus spp.	22	20	14	22	20
	Salmonella spp.	14	05	05	06	08
	Pseudomonas spp.	22	27	27	27	20
	Bacillus spp.	22	28	30	28	20
	E. coli	00	00	00	00	12
	Total	100	100	100	100	100
Brown Paper	Streptococcus spp.	22	24	22	16	15
	Staphylococcus spp.	17	17	17	18	15
	Salmonella spp.	15	06	05	04	10
	Pseudomonas spp.	18	30	26	30	25
	Bacillus spp.	28	23	30	32	23
	E. coli	00	00	00	00	12
	Total	100	100	100	100	100

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 08, August-2016

Table 7: Bacterial distribution in *Kilishi* samples treated with different hurdles under ambient storage (30±8^{0}c) using various packaging materials (Week 6)

Sample codes		001	014	017	023	MS
		%Hurdles				
	Sucrose	0	2	2	4	0
	Citric acid	0	0.1	0.2	0.1	0
	Sodium benzoate	0	0.1	0.1	0.1	0
Packaging Material	Bacterial group	Bacterial Distribution (%)				
HDPE	Streptococcus spp.	22	26	20	18	15
	Staphylococcus spp.	18	11	08	10	16
	Salmonella spp.	15	04	05	04	14
	Pseudomonas spp.	20	34	32	35	20
	Bacillus spp.	25	25	35	33	25
	E. coli	00	00	00	00	10
	Total	100	100	100	100	100
Aluminium Foil	Streptococcus spp.	22	24	28	25	22
	Staphylococcus spp.	22	10	03	08	18
	Salmonella spp.	12	10	03	08	10
	Pseudomonas spp.	20	30	31	30	20
	Bacillus spp.	24	32	34	32	18
	E. coli	00	00	00	00	12
	Total	100	100	100	100	100
Brown Paper	Streptococcus spp.	20	29	26	20	18
	Staphylococcus spp.	22	07	06	08	17
	Salmonella spp.	16	04	04	04	12
	Pseudomonas spp.	14	35	30	32	18
	Bacillus spp.	28	25	34	36	24
	E. coli	00	00	00	00	11
	Total	100	100	100	100	100

Table 8: Bacterial distribution in *Kilishi* samples treated with different hurdles under ambient storage (30±8^{0}c) using various packaging materials (Week 8)

Sample codes		001	014	017	023	MS
		%Hurdles				
	Sucrose	0	2	2	4	0
	Citric acid	0	0.1	0.2	0.1	0
	Sodium benzoate	0	0.1	0.1	0.1	0
Packaging Material	Bacterial group	Bacterial Distribution (%)				
HDPE	Streptococcus spp.	24	26	24	22	16
	Staphylococcus spp.	16	05	04	05	15
	Salmonella spp.	18	00	00	00	15
	Pseudomonas spp.	17	39	35	37	21
	Bacillus spp.	25	30	37	36	22
	E. coli	00	00	00	00	11
	Total	100	100	100	100	100
Aluminium Foil	Streptococcus spp.	23	22	24	25	22
	Staphylococcus spp.	22	10	00	00	16
	Salmonella spp.	14	00	00	00	12
	Pseudomonas spp.	19	38	38	39	23
	Bacillus spp.	22	30	38	36	15
	E. coli	00	00	00	00	12
	Total	100	100	100	100	100
Brown Paper	Streptococcus spp.	22	32	26	24	22
	Staphylococcus spp.	18	00	06	04	16
	Salmonella spp.	18	00	00	00	14
	Pseudomonas spp.	15	39	34	36	16
	Bacillus spp.	27	29	34	36	22
	E. coli	00	00	00	00	10
	Total	100	100	100	100	100

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 08, August-2016

Table 9: Bacterial distribution in Kilishi samples treated with different hurdles under ambient storage (30±8^{0}c) using various packaging materials (Week 10)

Sample codes		001	014	017	023	MS
		%Hurdles				
	Sucrose	0	2	2	4	0
	Citric acid	0	0.1	0.2	0.1	0
	Sodium benzoate	0	0.1	0.1	0.1	0
Packaging Material	Bacterial group	Bacterial Distribution (%)				
HDPE	Streptococcus spp.	22	27	24	24	18
	Staphylococcus spp.	18	00	00	00	16
	Salmonella spp.	18	00	00	00	13
	Pseudomonas spp.	20	41	40	40	24
	Bacillus spp.	22	32	36	36	19
	E. coli	00	00	00	00	10
	Total	100	100	100	100	100
Aluminium Foil	Streptococcus spp.	22	26	22	24	22
	Staphylococcus spp.	24	00	00	00	15
	Salmonella spp.	16	00	00	00	14
	Pseudomonas spp.	20	38	38	37	22
	Bacillus spp.	18	36	40	39	15
	E. coli	00	00	00	00	12
	Total	100	100	100	100	100
Brown Paper	Streptococcus spp.	24	30	24	25	22
	Staphylococcus spp.	16	00	00	00	15
	Salmonella spp.	20	00	00	00	15
	Pseudomonas spp.	16	35	36	35	18
	Bacillus spp.	24	35	40	40	20
	E. coli	00	00	00	00	10
	Total	100	100	100	100	100

Table 10: Bacterial distribution in *Kilishi* samples treated with different hurdles under ambient storage (30±8^{0}c) using various packaging materials (Week 12)

Sample codes		001	014	017	023	MS
		%Hurdles				
	Sucrose	0	2	2	4	0
	Citric acid	0	0.1	0.2	0.1	0
	Sodium benzoate	0	0.1	0.1	0.1	0
Packaging Material	Bacterial group	Bacterial Distribution (%)				
HDPE	Streptococcus spp.	22	24	22	24	18
	Staphylococcus spp.	20	00	00	00	15
	Salmonella spp.	20	00	00	00	14
	Pseudomonas spp.	18	40	38	38	24
	Bacillus spp.	20	36	40	38	18
	E. coli	00	00	00	00	11
	Total	100	100	100	100	100
Aluminium Foil	Streptococcus spp.	24	22	20	24	20
	Staphylococcus spp.	20	00	00	00	16
	Salmonella spp.	18	00	00	00	15
	Pseudomonas spp.	18	40	40	34	20
	Bacillus spp.	20	38	40	42	19
	E. coli	00	00	00	00	10
	Total	100	100	100	100	100
Brown Paper	Streptococcus spp.	22	25	20	20	20
	Staphylococcus spp.	20	00	00	00	14
	Salmonella spp.	18	00	00	00	17
	Pseudomonas spp.	18	35	35	30	18
	Bacillus spp.	22	40	45	50	21
	E. coli	00	00	00	00	10
	Total	100	100	100	100	100

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 08, August-2016

Stated in Table 3 above are the microbial loads in *Kilishi* samples treated with sucrose, citric acid and sodium benzoate at different levels and combinations and packaged into HDPE, aluminium foil and brown paper.

Sample 001 (control) and Market Sample (MS) contained no preservatives. Samples 014, 017 and 023 were treated with different hurdle combinations as stated in Table 2 above. At the start, the microbial loads for Sample 001, 014, 017 and 023 were found to be 2.40×10^3, 1.82×10^3, 1.77×10^3, 1.56×10^3 and 7.32×10^3 cfu/g respectively. Highest microbial counts were recorded in commercial sample throughout the storage time and least counts were recorded in Sample 023. Among the packaging materials used HDPE was found to be more efficient with low microbial counts in all the treatments. Hurdle pattern in Sample 023 with combination of HDPE was found to be the best treatment in retaining the microbial quality of *Kilishi*.

Table 4 to 10 above presented the percentage distribution of bacterial groups in *Kilishi* treated with sucrose, citric acid and sodium benzoate at different levels and combinations over twelve weeks ambient storage (30 ± 8^0C). Six bacterial groups were isolated from Market Sample these include; *Streptococcus spp., Staphylococcus spp., Salmonella spp., Pseudomonas spp., Bacillus spp. and Escherichia coli.* All the above groups with exception of *E. coli* were also found to be presence in laboratory prepared samples.

Bacterial successions were observed in all the treatments and the packaging system. Percentage distribution of *Pseudomonas spp.* and *Bacillus spp.* were found to be increasing in the treatment samples (014, 017 and 023) during storage. While that of *Streptococcus spp., Staphylococcus spp.* and *Salmonella spp*. were found to be increasing at the start then dropped drastically at different points (depending on the packaging material and hurdle combination) before the end of the storage time.

E. coli which serves as indicator organism for faecal contamination was only found to be presence in commercial sample and it is distribution was found to be fairly stable in all the packaging materials used during the twelve weeks ambient storage (30 ± 8^0C).

A graphical representations of the bacterial succession in different packaging materials were presented in Fig 2 to 4 below.

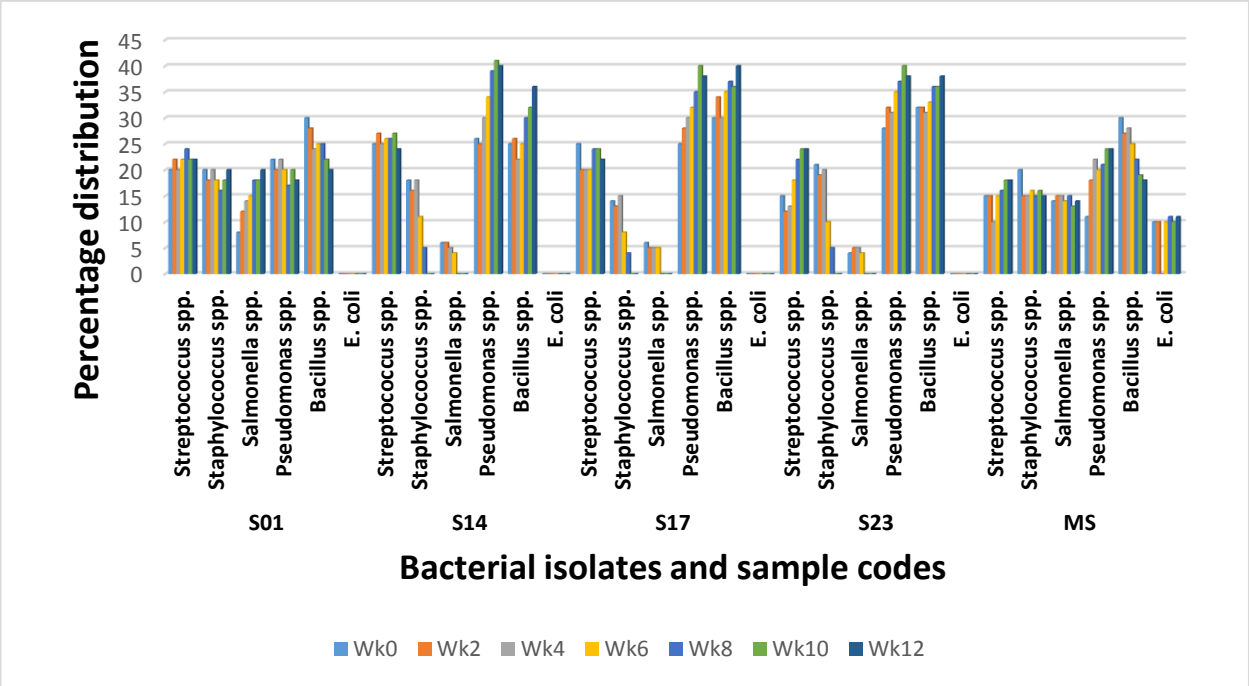

Fig 2: Bacterial succession in *Kilishi* samples packaged in HDPE over 12 weeks ambient storage (30 ± 8^0C)

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 08, August-2016

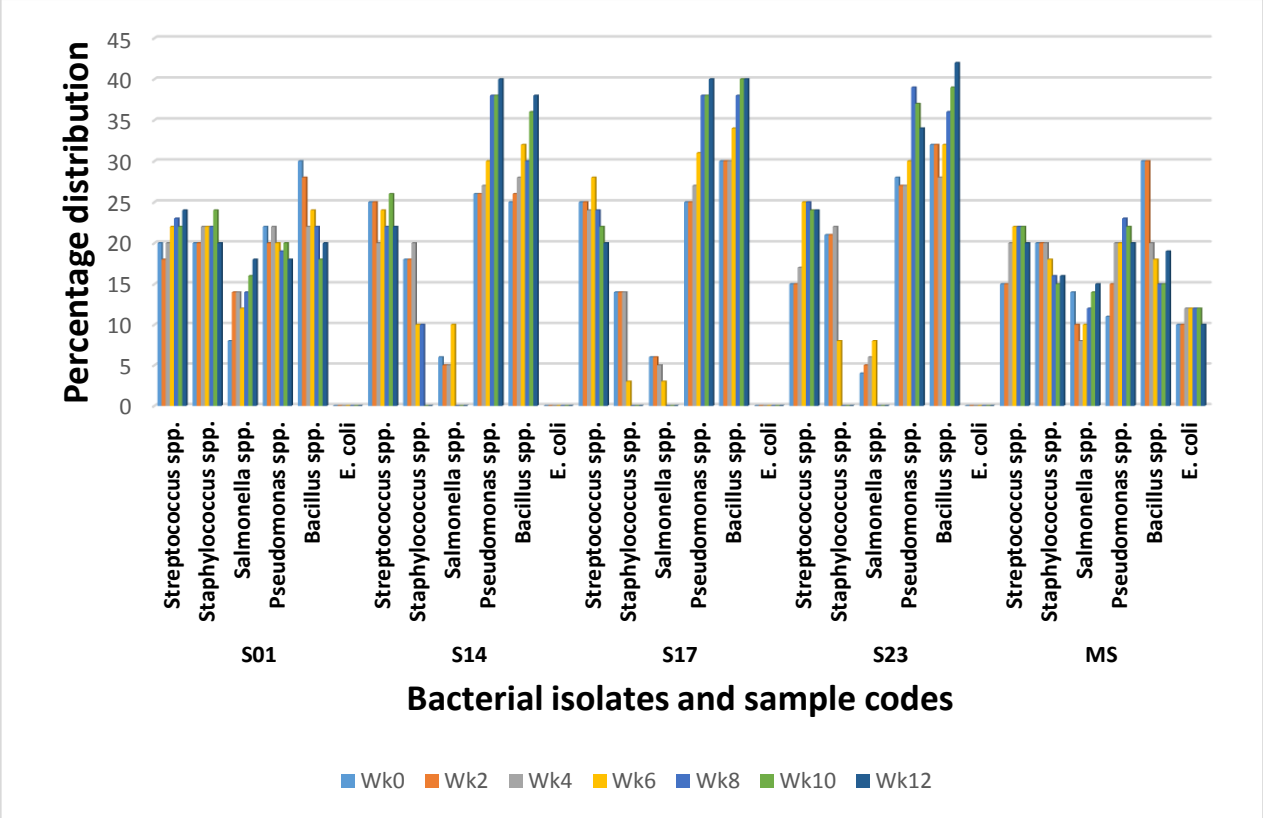

Fig 3: Bacterial succession in *Kilishi* samples packaged in Aluminium Foil over 12 weeks ambient storage (30 ± 8^0c)

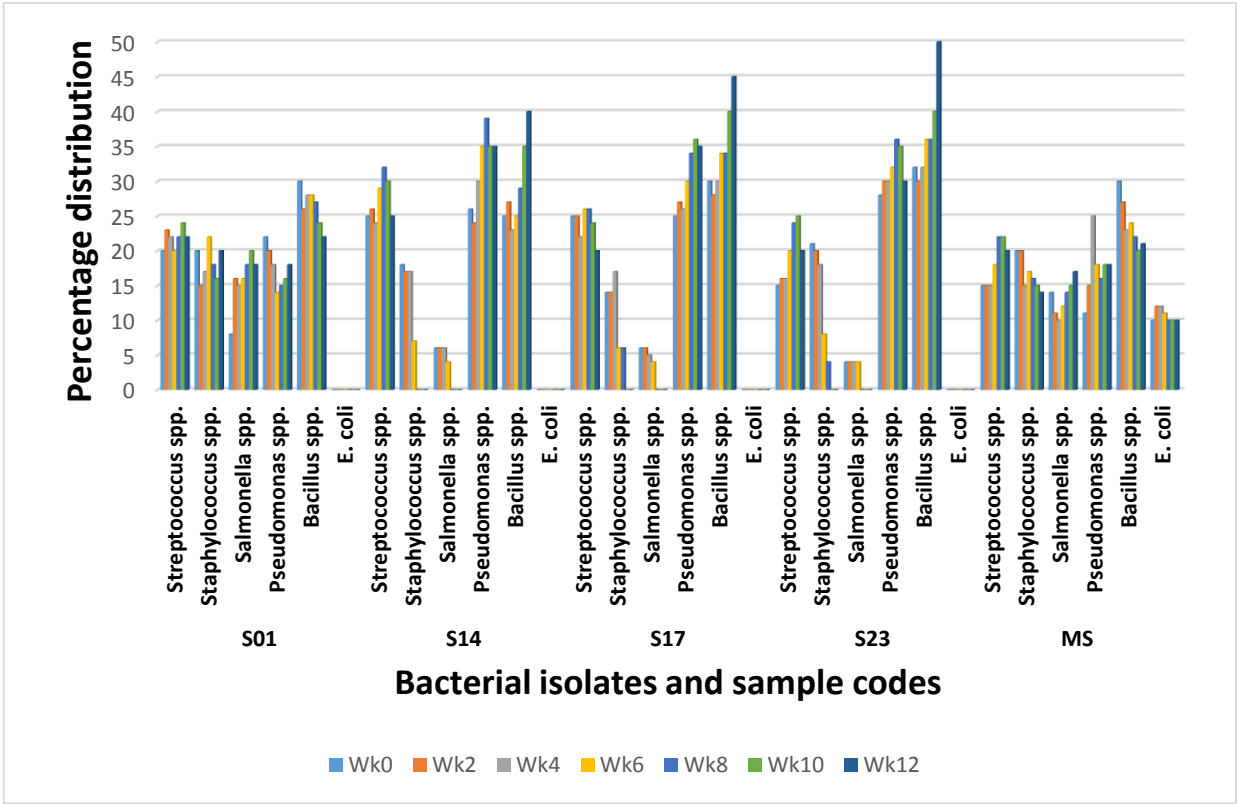

Fig 4: Bacterial succession in *Kilishi* samples packaged in Brown Paper over 12 weeks ambient storage (30 ± 8^0c)

International Journal of Engineering Research & Technology (IJERT)
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Vol. 5 Issue 08, August-2016

DISCUSSION

Microbial load

The microbial loads of *Kilishi* were found to be increasing in both commercial and laboratory prepared samples during ambient storage (30±8^{0}C). The microbial loads increased from 2.40×10^3 cfu/g to 3.38×10^3 cfu/g in sample 001, 1.82×10^3 cfu/g to 2.42×10^3 cfu/g in sample 014, 1.77×10^3 cfu/g to 2.60×10^3 cfu/g in sample 017, 1.56×10^3 cfu/g to 2.24×10^3 cfu/g in sample 023 and 7.32×10^3 cfu/g to 10.32×10^3 cfu/g in market sample. This is in agreement with finding of Jones *et al.* (2001) who reported increase in microbial counts and changes in chemical composition during ambient storage (31^{0}C) of *Kilishi* treated with potassium sorbet. The results contradict the finding of Ogbonnaya and Linus (2009) who reported decreased in microbial load during ambient storage of *Kilishi* treated with potassium sorbet.

Highest microbial counts were recorded in *Kilishi* samples packaged in brown paper in all the treatments throughout the storage period this agreed the finding of Jones *et al.* (2001) who reported significance difference between *Kilishi* samples packaged in brown paper and that packaged in polythene bag with highest counts recoded in samples packaged in brown paper. Moisture absorption characteristic of brown paper contribute to higher microbial loads in both laboratory and commercial samples during ambient storage (Okonkwo *et. al.*, 2013)

The microbial loads for market sample were found to be within the range reported by Ogbonnaya and Linus (2009) in freshly prepared commercial *Kilishi*. The results for microbial loads in the laboratory prepared samples were below the range reported by these researchers in laboratory prepared *Kilishi* treated with potassium sorbet, but above that reported by Okonkwo *et. al.*, (2013) in industrially produced *Kilishi*.

Commercial *Kilishi* samples collected from Port Harcourt (Okonkwo *et. al.*, 2013), FCT (Abuja) (Daminabo *et al.* 2013) and that collected from Calabar, (Odey *et al.* 2013) were found to have better microbial quality than that collected from Kano. The variation in the three Nigerian cities may result from variation in meat handling practices, *Kilishi* manufacturing process and ingredients, and also variation in environmental factors such as temperature and humidity in the locations. This match with finding of Fonkem *et al.* (2010) who reported variation in microbial load in *Kilishi* samples collected from three locations (Garoua, Maroua and Ngaoundere) in Cameroun.

Six bacterial groups were isolated from Market Sample these include; *Streptococcus spp., Staphylococcus spp., Salmonella spp., Pseudomonas spp., Bacillus spp. and Escherichia coli.* All the above groups with exception of *E. coli* were also found to be presence in laboratory prepared samples. These isolates were very similar to that reported by many researchers; Odey *et al.* (2013) isolated *Staphylococcus aureus, Escherichia coli, Streptococcus spp, Salmonella spp, Bacillus spp, Pseudomonas Spp* and *Proteus spp* from selected *Kilishi* samples collected from Calabar, Cross River State-Nigeria, the researchers also concluded that *Staphylococcus spp, Escherichia coli and Basillus spp* were the most frequently isolated organisms in *Kilishi*. Okonko *et. al.*, (2013) isolate *Bacillus* species, *Staphylococcus aureus* and *Escherichia coli* in *Kilishi* samples collected from Port Harcourt, Rivers State-Nigeria. Edema *et al.* (2008) isolated *Bacillus cereus, Staphylococcus aureus* and *Salmonella spp* in *Kilishi* samples collected from 6 selected cities within south western part of Nigeria. Fonkem *et al.* (2010) isolate *E. coli* and *Staphylococcus aureus* in Cameroonian *Kilishi.*

As the storage time progresses the condition in the treatment samples became critical for some bacterial species to survive. The growth of *Salmonella spp*, and *Staphylococcus spp* was arrested before the end of the twelve weeks ambient storage. Percentage distributions of this organisms were found to be decreasing during storage. At week 8, *Salmonella spp*, was totally eliminated from all the treatment samples and *Staphylococcus spp* was eliminated after 10 weeks. These revealed that the concentrations of the hurdles used in the treatment samples is beyond the range for optimal growth of these microorganisms and this result to their elimination. *Streptococcus spp., Bacillus spp.* and *Pseudomonas spp.* were found to succeed the growth *Salmonella spp*, and *Staphylococcus spp* during storage. Lee (2004) reported that hurdles used in food preservation could provide varying results depending on bacterial stress reactions such as the synthesis of protective proteins. These resistance organisms may likely synthesise these protective proteins during the storage time.

E. coli was found to be presences only in market sample throughout the storage time. The organism may source from unhygienic water used by local *Kilishi* producers during processing. Beney *et al.* (2003) reported that drying at lower temperatures enhances cell survival of *E. coli* after dehydration. Oladapo *et al.* (2014) reported that in an agar diffusion technique the minimum inhibitory concentration (MIC) of sodium benzoate and citric acid against growth of *Staphyloccocus aureus, Pseudomonas aeruginosa* and *E. coli* is 1.5mg/ml. Stanojevic *et al.* (2009) reported that combination of sodium nitrite and sodium benzoate at 10mg/ml of growth medium was found to eliminate *Bacillus spp* and *Staphylococcus aureus*. Same combination at 5mg/ml was found to eliminate *E. coli* and *Pseudomonas spp.* Bibek (2005) reported that the spores of *Bacillus spp* can withstand roasting. It was also reported by Leistner (2011) that the heat resistance of bacteria increases at low a_w.

There has been a debate concerning the acceptability limit for the total viable counts in ready-to-eat meat. London Health Protection Agency (2009) put $<10^6$cfu/g as satisfactory limit, and 10^6 to $<10^7$cfu/g as acceptable range. Public Health Laboratory Service (2000) put $<10^5$cfu/g as satisfactory limit, 10^5 to $<10^6$cfu/g as acceptable range and $>10^6$cfu/g as unsatisfactory limit. The limits set by both London Health Protection Agency (2009) and Public Health Laboratory Service (2000) render the all the samples acceptable for consumption twelve weeks after storage.

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CONCLUSION

Microbial loads in *Kilishi* samples treated with sucrose, citric acid and sodium benzoate were found to be increasing during ambient storage. Highest counts were recorded in samples packaged in brown paper and least counts were recorded in samples packaged in HDPE. Commercial sample recorded poor microbial quality compared to laboratory prepared samples. Sample with 4% sucrose, 0.1% citric acid and 0.1% sodium benzoate was found to be the best among the treatment samples. At the end of the twelve weeks storage the microbial loads were found to be within the acceptable limits set by London Health Protection Agency and Public Health Laboratory Service. The results of the research also revealed that combination of sucrose, citric acid and sodium benzoate has the potential of eliminating the growth of *Salmonella spp* and *Staphylococcus spp* in *Kilishi* during ambient storage. The packaging materials used in this research has no effect on the bacterial group in the treatment samples.

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