African Journal of Biotechnology Vol. 7 (20), pp. 3799-3803, 20 October, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Biopreservative activity of lactic acid bacteria on suya produced from poultry meat

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Accepted 27 August, 2008

The influence of lactic acid bacteria (LAB) isolated from poultry meat on the attributes of suya was investigated. *Lactobacillus plantarum* with the highest frequency of occurrence (90%) produced the highest amount of lactic acid (16.2 g/l) and inhibited all the indicator organisms with the exception of *Candida albicans* and *Proteus vulgaris*. Consequently, *L. plantarum* was chosen as the starter culture to inoculate pieces of poultry meat before (CB) and after (CA) grilling for suya production. Relatively low microbial counts (log cfu/g) of coliform (8.23), *Staphylococcus* (4.83), LAB (8.1) and yeast/mould (5.63) were observed for CA samples after six days of storage. Grilling at 80°C for 30 min gave the best suya attributes with crude protein content of 33.45%. The best packaging material was polyphenylchloride as compared to aluminum foil and newsprint.

Key words: Lactic acid bacteria, biopreservation, poultry meat, suya, shelf life.

INTRODUCTION

Suya is a traditional stick meat product that is commonly produced by the Hausas in West Africa from beef. It is produced from boneless meat hung on stick and spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting around a glowing charcoal fire (Alonge and Hiko, 1981). Beef is the major source of protein for most people in many parts of the world. In Nigeria there is increase in the cost as a result of demand. Also, traditionally prepared suya has been known to have a short shelf life due to the mode of handling during and after production. Many consumers today are concerned about the synthetic chemicals used as preservative in food, and there is a resulting trend towards less processed food (Yang and Ray, 1994). Alternatives include vacuum packing and refrigeration of food. These untreated foods can harbour dangerous pathogens, which can multiply under refrigeration. Treatment like ionizing radiation can destroy pathogens non-chemically, but may affect taste and do not protect food against post-treatment contamination. A solution to this dilemma is the use of antimicrobial metabolites of fermentative microorganisms (Ogunbanwo et al., 2004).

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Lactic acid bacteria (LAB) have been employed in the preservation of food materials for many centuries. In the meat industry, lactic acid bacteria are widely used as starter cultures for sausage fermentation (Liepe, 1983). The preservative activity of LAB is due to their ability to produce acid (low pH), hydrogen peroxide and bacteriocins (Ogunbanwo, 2005). The aim of this study is to determine the effect of LAB on the microbiological, biochemical and sensory property of suya produced from poultry meat.

MATERIALS AND METHODS

Sample collection

Poultry meat and suya spices including onion (*Alium cepa*) were purchased from a local market in Ibadan, Nigeria.

LAB was isolated from poultry meat by homogenizing 10 g meat in 90 ml of 0.1% peptone solution. The solution obtained was serially diluted and appropriate dilutions were plated on to de Mann Rogosa and Sharpe (MRS) Agar (de Man et al., 1960). Pure cultures of the isolates were obtained by repeated streaking of the LAB isolates on MRS agar. Characterization of the LAB isolates was carried out using API 50 CH strips according to manufacture's instructions (API system, Bio-merieux, France). Additional tests were performed where necessary and confirmation of identities was by reference to Bergey's Manual of Systematic Bacteriology (Kandler and Weiss, 1986). The pathogenic bacteria used as indicator organisms were obtained from the culture collection of

Medical Microbiology Laboratory, University College Hospital, Ibadan, Nigeria.

Determination of lactic acid, hydrogen peroxide and diacetyl production

For these measurements the test organisms were grown in MRS broth for 96 h and centrifuged at 3000 x g for 15 min. Lactic acid, hydrogen peroxide and diacetyl were determined according to the method of AOAC (1990).

***In vitro* inhibition**

The antimicrobial activity of the LAB isolated from poultry meat was determined using a well diffusion assay according to the method described by Schillinger and Lucke (1989). The indicator organisms employed were *Staphylococcus aureus*, *Pseudomonas* sp, *Candida albican, Escherichia coli* and *Proteus* v*ulgaris*.

The starter culture was selected based on the ability of the test isolate to produce antimicrobial compounds. The selected starter culture was propagated twice in MRS broth. The cells were then harvested by centrifugation at 3000 x g for 20 min. The cells were washed twice in normal saline to remove the medium components (Kalalou et al., 2004)

Preparation of suya from poultry meat

Suya was produced from poultry meat using a modified traditional method (Onilude et al., 2002). The meat was washed in 5% sterile saline water and cut into pieces of 25 g each. The meat pieces cured with ground pepper and salt were hung on stick (two pieces) and then divided into 3 parts. The first part was incubated with starter culture (10^7 cfu/g) (Kalalou et al., 2004), incubated at 35°C and then grilled (CB sample). The second part was first grilled, cooled and then inoculated (CA sample). The third part which was the control was not inoculated but grilled (CU sample). All the three samples were grilled at 80°C for 30, 40, 50 and 60 min. Groundnut oil was sprinkled during grilling to prevent burning. After grilling, the suya samples were allowed to cool down and then packaged using aluminum foil, newsprint and polyvinylchloride (PVC). They were stored at 20 and 35°C for 6 days; samples were removed at specific time interval for analysis.

Microbiological and biochemical analyses

Samples (10 g) were cut into small pieces and blended in 90 ml of saline water to make the initial dilution (10^{-1}). Serial dilutions up to 10^{-6} were then prepared (Kalalou et al., 2004). One milliliter of the appropriate dilutions was mixed with molten medium (45°C) using MRS (pH 5.5) for LAB, potato dextrose agar (PDA) for yeast/mould, MacConkey agar for coliforms and mannitol salt agar for *Staphylococcus*. The cultures were incubated for 48 h with the exception of yeast and mould (72 h).

For FFA determination 5 g of each suya sample was comminuted and the lipid extracted followed by alkali titration (AOAC, 1990). The Kjeldahl method was employed for determining crude protein (AOAC, 1990). The pH of the suya samples was determined with pye-unicam pH meter, model 291 mkz equipped with a glass electrode.

For the determination of thiobarbituric acid (TBA), suya samples (10 g) were blended with 15 ml of cold extracting solution containing 9% perchloric acid. The resulting slurries were transferred quantitatively to 100 ml volumetric flask and made up to 50 ml each with distilled water. The slurries were filtered through Whatman no. 2 filter paper. Fifty milliliter of each of the filtrates was transferred to test tubes and 5 ml of 0.02 N TBA reagent was added into each and mixed thoroughly. The tubes were kept in the dark for 17 h and the absorbance read at 530 nm with a spectrophotometer. TBA values were calculated from the standard solutions of tetraethoxy propane (AOAC, 1990).

Sensory evaluation

The suya samples were given to taste panelist comprising 10 students of The Polytechnic, Ibadan, Nigeria. Scores were allocated on appearance, texture, taste, flavour and general acceptability using 9 points hedonic scale, where 1 = dislike extremely and 9 = like extremely.

RESULTS

One hundred isolates of lactic acid bacteria (LAB) were obtained from poultry meat and were characterized to belong to three different species. These were *Lactobacillus plantarum*, *L. brevis* and *Leuconostoc mesenteroides*. *L. plantarum* had the highest percentage occurrence of 90% while *L. mesenteroides* and *L. brevis* occurrence were 5% each. The production of antimicrobial compounds by the LAB isolates was investigated. The results are presented in Table 1. The highest yield of lactic acid (16.2 g/l) was produced by *L. plantarum* while *L. brevis* had the least value (6.4 g/l) after 48 h of growth. The highest quantity of diacetyl was produced between 24 to 48 h. The peak of diacetyl produced was 1.93 g/l by *L. mesenteroides* while the lowest quantity (0.86 g/l) was produced by *L. brevis*. Moreover, hydrogen peroxide was produced to varying degree by the LAB isolates. *L. brevis* produced the highest quantity of hydrogen peroxide (0.024 g/l) while *L. plantarum* produced the lowest quantity (0.017 g/l) within 48 h of fermentation.

The antagonistic activity of the LAB isolates was tested against some pathogenic microorganisms (Table 2). *L. plantarum* inhibited all the indicator organisms with the exception of *C. albicans* and *P. vulgaris*. However, *L. brevis* inhibited only *S. aureus* and *E. coli*. Moreover, *L. mesenteroides* inhibited *S. aureus*, *P. aerigunosa* and *E. coli.* The diameters of the zone of inhibition were in the range of 8 and 20 mm. *L. plantarum* was selected as starter culture for the inoculation of poultry meat for suya production before and after grilling.

The microbial load of the suya samples was monitored during storage and the results are presented in Table 3. Suya samples inoculated after grilling (CA) showed a relatively low microbial count (log cfu/g) throughout the storage period. It has microbial count of 8.23 for coliform, 4.83 for S*taphylococcus*, 8.1 for LAB and 5.63 for yeast/mould at the end of six days storage. The uninoculated chicken suya has a high microbial load of 15.01 for coliform, 12.01 for S*taphylococcus*, 8.5 for LAB and 9.91 for yeast/mould by the same period of time.

Influence of grilling time on the quality attributes of the suya samples is presented in Table 4. 30 min was ob-

Table 1. Quantity of lactic acid, diacetyl and hydrogen peroxide (g/l) produced by the LAB isolates at different incubation peroids.

Isolate	Lactic acid				Diacetyl				Hydrogen peroxide			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Lactobacillus plantarum	4.00	16.20	14.28	12.20	1.62	1.72	1.53	1.29	0.017	0.022	0.019	0.015
Lactobacillus brevis	2.80	6.40	6.00	5.20	0.86	1.29	1.18	1.08	0.020	0.024	0.021	0.017
Leuconostoc mesenteroides	6.00	10.00	10.00	9.00	1.07	1.93	1.50	1.29	0.019	0.023	0.022	0.015

Table 2. Diameter of zones of inhibition of indicator organisms to antimicrobial substance produced by the LAB species.

LAB species	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Candida albicans*	*Escherichia coli*	*Proteus vulgaris*
Lactobacillus plantarum	20 mm	10 mm	-	15 mm	-
Lactobacillus brevis	10 mm	-	-	8 mm	-
Leuconostoc mesenteroides	17 mm	8 mm	-	10 mm	-

Table 3. Microbial load of suya samples during storage.

Storage period (days)	Sample	Coliform (log cfu/g)	*Staphylococcus* (log cfu/g)	LAB (log cfu/g)	Yeast/mould (log cfu/g)
1	CA	5.01	4.30	8.0	4.01
	CB	4.21	4.01	8.1	4.12
	CU	6.10	3.00	5.2	4.15
2	CA	3.21	2.40	8.2	6.12
	CB	6.02	4.02	8.2	4.83
	CU	8.01	3.53	5.6	4.26
3	CA	2.32	2.40	8.2	6.28
	CB	6.23	4.86	8.2	6.01
	CU	8.30	5.21	6.0	6.32
4	CA	4.13	2.57	8.2	6.29
	CB	8.20	5.00	8.2	5.70
	CU	8.74	7.12	6.5	6.58
5	CA	5.04	3.23	8.2	5.80
	CB	10.10	6.12	8.0	5.68
	CU	12.21	8.02	7.5	7.2
6	CA	8.23	4.83	8.1	5.63
	CB	12.48	6.84	8.2	4.8
	CU	15.01	12.01	8.0	9.91

CA = Suya samples inoculated with *Lactobacillus plantarum* after grilling; CB = suya samples inoculated with *L. Plantarum* before grilling; and CU = uninoculated suya samples.

served to be the best grilling time for CA samples with crude protein of 33.45%, TBA 0.68 mg maloaldehyde/kg, FFA 0.63 KOH/g lipid and aerobic plate count of 7.6 log cfu/g. For CB samples 40 min seemed to be the best grilling time with crude protein of 32.73%, TBA 1.08 mg maloaldehyde/kg, FFA 0.91 KOH/g lipid and aerobic plate count 8.0 log cfu/g. For the CU samples the grilling time of 30 min was the best with crude protein 26.85%, TBA 0.95 mg maloaldehyde/kg, FFA 0.84 KOH/g and aerobic plate count of 7.9 log cfu/g.

The influence of packaging materials, storage temperature and time on the quality attributes of suya samples are presented in Table 5. The suya stored in PVC at 35^{o}C had the highest crude protein of 33.05% by the sixth day of storage. The TBA and FFA for the same period were 0.63 mg maloaldehyde/kg and 0.51 KOH/g, respectively.

Table 4. Influence of grilling time (GT) on the quality attributes of suya samples produced from poultry meat.

Grilling time (min)	CA				CB				CU			
	CP	TBA	FFA	APC	CP	TBA	FFA	APC	CP	TBA	FFA	APC
30	33.45	0.68	0.63	7.6	32.23	0.90	0.77	7.8	26.85	0.95	0.84	7.9
40	29.66	0.74	0.98	7.4	32.73	1.08	0.91	8.0	26.50	1.17	1.05	8.0
50	28.88	0.56	0.91	7.6	30.54	0.74	0.70	8.1	26.39	1.31	1.19	8.1
60	27.83	0.43	0.49	8.0	28.53	0.61	0.63	8.2	25.81	1.49	1.26	8.2

CA = Suya samples inoculated with *Lactobacillus plantarum* after grilling; CB = suya samples inoculated with *L. Plantarum* before grilling; and CU = uninoculated suya samples.
CP, Crude protein (%); TBA, thiobarbituric acid (mg malonaldehyde/kg); FFA, free fatty acid (KOH/g lipid); APC, aerobic plate count (log cfu/g).
Values are means of duplicate readings.

Table 5. Effect of packaging materials, storage temperature and period on the quality attributes of suya sample inoculated after grilling (CA).

Storage temp. (^{o}C)	Storage period (days)	Aluminum foil				Newsprint				Polyvinylchloride			
		pH	CP	TBA	FFA	pH	CP	TBA	FFA	pH	CP	TBA	FFA
20	2	5.8	30.80	0.74	0.49	5.8	30.01	0.84	0.56	6.1	30.28	0.70	0.70
	4	5.8	31.50	0.84	0.77	5.8	30.63	0.93	0.70	5.9	30.98	0.79	0.91
	6	5.8	32.20	0.93	0.98	5.7	31.15	1.02	0.91	5.8	31.85	0.72	0.79
35	2	6.1	31.24	0.99	0.91	6.0	30.63	0.86	0.77	6.0	31.68	0.83	0.78
	4	6.0	30.29	1.04	1.12	6.0	31.06	0.99	0.91	6.0	32.20	0.81	0.73
	6	5.9	30.82	0.93	1.19	5.8	31.59	1.15	1.12	5.9	33.05	0.63	0.51

CP, Crude protein (%); TBA, thiobarbituric acid (mg malonaldehyde/kg); FFA, free fatty acid (KOH/g lipid).
Values are means of duplicate readings.

Table 6. Organoleptic properties of suya samples.

Sample	Appearance	Texture	Taste	Flavour	General acceptability
CA	4.4^{a}	4.1^{a}	3.8^{a}	3.6^{a}	3.8^{a}
CB	4.2^{a}	3.9^{a}	3.6^{a}	3.5^{a}	3.7^{b}
CU	3.8^{a}	3.9^{a}	3.4^{a}	3.2^{a}	3.5^{c}

CA = Suya samples inoculated with *Lactobacillus plantarum* after grilling; CB = suya samples inoculated with *L. Plantarum* before grilling; and CU = uninoculated suya samples.
Treatment means were subjected to comparison by Duncan multiple range test using Statistical Programme for Social Science (SPSS). Means with the same superscripts are not significantly different from one another at $p \leq 0.05$.

The pH range for all the samples was between 5.8 and 6.1. The suya samples were evaluated for sensory attributes as shown in Table 6. The CA samples were rated the best followed by CB while CU samples received the lowest scores.

DISCUSSION

Three different species of lactic acid bacteria were isolated form poultry meat. These were *L. plantarum, L. brevis* and *L. mesenteroides*. Jin et al. (1998) reported isolation of different strains of Lactobacillus which include *L. brevis, L. fermentum, L. acidophilus* and *L. cripatus* from chicken intestine. *L. plantarum* isolated from poultry meat showed the highest frequency of occurrence. Sanni et al. (1999) reported the dominance of *L. plantarum* among *Lactobacillus species* isolated from ogi, an indigenous fermented food. The LAB isolated in this work produced antimicrobial compounds to varying degrees. *L. plantarum* produced the highest amount of lactic acid. Ogunbanwo et al. (2004) obtained a similar result for *L. plantarum* isolated from fufu, a traditional fermented cassava product. The inhibitory activity of the LAB isolates against the indicator organisms was to a varying degree; *L. plantarum* showed the highest inhibitory activi-

ty while *L. brevis* had the least. This corresponds to the work of Vermeiren et al. (2004) who reported antibacterial activity of meat borne lactic acid bacteria among other things when they selected LAB strains as protective cultures for biopreservation of cooked meat products.

Suya samples inoculated after grilling had a low microbial count during the storage period as compared to those samples inoculated before grilling and the control. This is in agreement with the finding of Murthy et al. (1997) who preserved minced goat meat using *Lactococcus lactis var. lactic* biovar *diacetylactis*. Thirty minutes was the best grilling time for CA samples with the highest crude protein value (33.45%) whereas forty minutes was the best grilling time for the CB samples with crude protein value of 32.73%. This is similar to finding of Onilude et al. (2002) who reported the best grilling time of forty minutes for tsire (suya) inoculated with LAB after grilling. Lipolysis was observed in all the samples as determined by the values of TBA and FFA. Montel et al. (1993) made a similar observation in French dry sausages fermented with starter culture consisting of LAB and *Staphylococci*.

Polyvinylchloride appeared to be the best storage material at 35^{o}C having the highest crude protein value (33.05%) and reduced TBA and FFA values. Onilude et al. (2002) reported a similar result for tsire (suya). Based on the sensory attribute, CA samples were rated best followed by CB while CU samples were rated lowest. The ability of LAB to improve the sensory properties of dry fermented sausages was also reported by Erkkila et al. (2001). In conclusion, the inoculation of suya produced from poultry meat with lactic acid bacteria led to its improved nutritional qualities and extended shelf life.

ACKNOWNEDGEMENT

The authors thank Mr. B.M. Okanlawon of Department of Biomedical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria, for Technical Assistance.

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