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ANTIMICROBIAL ACTIVITY OF *OCIMUM GRATISSIMUM* EXTRACT ON *SUYA* (AN INTERMEDIATE MOISTURE MEAT) IN NIGERIA

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ABSTRACT

Matured leaves of *Ocimum gratissimum* were harvested and the extracts used to cure *Suya* (an intermediate moisture meat). *O. gratissimum* leaves were collected from Oyo state south west region of Nigeria, rinsed in distilled water and squeezed to extract the fluid. The meat used was *Semi membranosus* muscle from beef carcass, which was trimmed of all visible fat and connective tissues. The meat cut was sliced into sheets of 0.18cm-0.35cm thick and lengths of between 5.0cm-7.1cm. The study comprised five treatments with 10 replicates each in a completely randomized design. Treatment A (TA) served as the control (*Suya* without *O. gratissimum* extract(*OGE*), while treatment B (TB),treatment C (TC), treatment D (TD) and treatment E (TE) were *Suya* soaked in *OGE* for ½ hr, 1hr, l½ hrs and 2 hrs, respectively, before coating with *Suya* ingredients. A total of 50 sticks of *Suya* weighing from 38.10 - 59.30 grams of sliced meat per stick were prepared for each treatment. The meat on sticks was properly coated with *Suya* ingredients and arranged around glowing embers of charcoal. The morphological and biochemical characterization of aerobic bacteria, coliform and lactic acid isolates from the five treatments was carried out. At Day 0, isolates from samples of the five treatments include: Aerobic species of *Pseudomonas, Bacillus, Micrococcus,* and *Flavobacterium* species. Three Coliform species isolated were: *Proteus*, *Aeromonas* and *Enterobacter* species. The four Lactic acid bacteria isolated were *Pediococcus, Streptococcus*, *Lactobacillus* species and *Enterococcus feacalis*. The bacterial count on *Suya* meat soaked in *OGE* at different curing times of ½ hr, 1hr, 1½ hrs and 2hrs differed. The aerobic counts ($0.001x10^5$-$2.2x10^5$) were relatively low at the third and fifth days for TC, TD, and TE while for Lactic acid bacteria, the count reduced from $3.0x10^5$ in TA to $0.2x10^5$ in TE. Coliform counts of $6.0x10^5$ and $7.0x10^5$ recorded at day 7 for TA and TB were exceptionally high. *O. gratissimum* extract, used as a curing agent in *suya* production significantly reduced the aerobic count of treated meat, thus enhancing the keeping quality of the products.

Key words: *Suya*, *Ocimum gratissimum* Extract (*OGE*), Bacterial, antimicrobial

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INTRODUCTION

Ocimum gratissimum L is a popular fragrant culinary herb. Fresh basil leaves have a strong and characteristic aroma, not comparable to any other spice, slightly similar in pungency to cloves. It has an energy value of 23kcal, 2.65g carbohydrate, 3.15g protein, 0.64g total fat, and 1.60g dietary fibre per 100g of fresh leaves. It is rich in vitamins, electrolytes, minerals and phytonutrients [1]. The leaves and roots of the herb have several medicinal, culinary and other applications that are greatly beneficial to humans. *Ocimum* belongs to the Lamiaceae family. It is an important herbal medicinal plant in Nigerian communities and in sub-Saharan Africa. The leaves are rubbed between the palms and sniffed as a treatment for blocked nostrils [2]. They are also used for abdominal pains, sore eyes, and ear infections, coughs, barrenness, fever, convulsions, tooth gargle, regulation of menstruation and as a cure for prolapse of the rectum [3, 4, 5].

Several species and varieties of plants of the genus *Ocimum* have been reported to yield oil of diverse nature. *O. gratissimum* commonly known as basilica oils has been reported to contain some chemical compounds and active ingredients such as eugenol, linalol, methyl cinnamate, camphor and thymol [6, 7]. Various species of *Ocimum* have been reported for their numerous medical uses [8].

It is in line with this versatility of the plant that this work tried to explore the usefulness of *Ocimum gratissimum extract (OGE)* in *Suya* processing. *Suya* is an intermediate moisture dried meat product of West Africa that is easy to prepare and highly relished [9]. There are three types of *Suya* namely: *Tsire*, *Kilishi* and *Balangu* with *Tsire* being the most popular with consumers. *Tsire* is boneless meat pieces that are staked on slender wooden sticks and cooked by roasting using a glowing fire [10].

MATERIALS AND METHODS

Plant material: Fresh leaves of *Ocimum gratissimum* were collected from Abadina area of the University of Ibadan. The plant was identified at the herbarium unit of the Botany Department of the University of Ibadan.

Extract Preparation: Sixty kilograms of the fresh leaves were collected and for every 10kg of leaves squeezed, 200mls of extract was obtained. The leaves were washed in water, and then rinsed with distilled water prior to extraction. Extraction was water based. Washed leaves were slightly fine blended using a Kenwood blender, the fine blend was squeezed in a muslin cheese cloth to obtain the extract. A brownish green juice of 1200ml volume was obtained in all and kept in air-tight bottles in a refrigerator until ready for use the same day.

Experimental Design: The design of the experiment was a 5 x 4 factorial in a completely randomized design. There were ten replicates per treatment. A total of 50 samples were randomly allocated to the 5 treatments. Samples were obtained from these and used for the chemical and microbial analyses.

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Meat Preparation

Raw fresh meat was obtained from the slaughter slab of the Department of Animal Science of the University of Ibadan. Animals slaughtered were about 3years old. Beef used in this study was taken from the *Semi membranous* muscle of singed beef carcasses. The meat was trimmed of all visible bones, fat and connective tissues. It was cut into chunks of 12cm long and 6cm wide. The chunks were sliced into thin sheets of between 0.18cm and 0.35cm thickness in the same direction of the muscle fibre using a long knife with a very sharp blade. Meat allotted to each treatment was soaked in 200mls of extract except for the control treatment.

Ingredient Preparation

Spices and other ingredients were obtained from Bodija market in Ibadan, Oyo State. These ingredients were mixed together in this specific proportion to include defatted groundnut paste 52%, salt 8.5%, dried red pepper 10%, curry 5%, maggi 7.5%, groundnut oil 2%, other condiments 5% and ginger 10%. The *Suya* sticks were obtained from Sabo area in Ibadan, Oyo State.

Preparation of *Suya*

Labelled, weighed staked meats were spread on a flat tray for easy identification. A total of 50 sticks of meat were made for the treatments that comprised of Treatment A (control)-without *Ocimum* extract (*OGE*), Treatment B - meat soaked in *OGE* for ½ hr, Treatment C - meat soaked in *OGE* for 1hr, Treatment D - meat soaked in *OGE* for 1½ hrs and Treatment E -meat soaked in *OGE* for 2hrs. After soaking, all the staked meats were properly coated with *Suya* ingredients. The labelled meat sticks were then arranged around a glowing, smokeless fire made from charcoal that was medium hot (about 150 ^{0}C). The distance of the sticks of meat from the fire was 25.96 ± 2.31cm. The staked meats were allowed to stay around the fire for 25 minutes, turning at intervals and intermittently sprinkled with groundnut oil. Suya was ready at Medium doneness of 60 – 63 ^{0}C. Meat from all the sticks was exposed to the same degree of doneness because the distance from the heat source and cooking time were the same for all treatments. Meat samples from each treatment were stripped off the sticks, kept in transparent Polyvinly Chloride (PVC) bowls and refrigerated till needed. The weight of each *Suya* stick was determined after roasting and this was used in calculating the percentage cooking loss and the product yield and, samples from each of these treatments were taken for microbiological analysis.

MICROBIAL ANALYSIS

Preparation of Media

Four different culture media were used to carry out the bacteriological and mycological analysis. The Minimum Inhibitory Concentration (MIC) was determined as well as the Bactericidal / Bacteriostatic effects of the extract, the presence of specific microbes in the product was also established. Nutrient Agar (NA) was used for general microbial analysis, MacConkey Agar (MA) for coliform bacteria, Potato

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dextrose agar (PDA) for moulds and De Mann Rogosa and Sharpe (MRS) for lactic acid bacteria.

Preparation of Different Agar Media:

Nutrient Agar
Twenty eight grams of nutrient agar was suspended in 100ml distilled water using a water bath at 100 °C.

MacConkey Agar
Fifty two grams of weighed medium was dissolved in 100ml distilled water in a conical flask dipped in a water bath.

Potato Dextrose Agar (PDA)
Thirty nine grams of PDA was homogenized in 1 litre of distilled water using a water bath at 100 °C.

Stains Used
These included gram staining (crystal violet, logous iodine, safranin, ethanol), lactophenol cotton blue.

Isolation Technique – Serial Dilution
Isolations were made from the samples using the serial dilution methods of [11]. One gram of sample was mixed thoroughly in 9ml sterilized distilled water in McCartney bottle or test tube. A set of six bottles was assigned to each sample. Sterile 1ml pipettes were used to transfer 1ml sample mixture from bottle 1 through bottle 6 in succession mixing thoroughly each time the addition was made.

1ml of each sample dilution from the 5^{th} and 6^{th} dilution was discharged into the center of the different labelled petri dishes using a sterile pipette. About 15mls each of the previously sterilized Agar medium that had cooled to 45^0C was poured into pairs of labelled petri dishes for the last 2 dilutions (10^{-5} and 10^{-6}). Both medium and inoculums were rapidly and carefully mixed by a combination of to and fro then circular (clockwise and anticlockwise) movements for about 10 seconds, care was taken not to allow the medium to spill or touch the lid of the petri dish. The plates were left stationary and allowed to further cool and set. They were then turned over and incubated at 37^0C for 24 – 48 hrs except for the potato dextrose agar that lasted for 5 days, before readings were taken.

Isolation of Organisms on Nutrient Agar and Potatoes Dextrose Agar
This was done using the pour plate method. The plates containing the nutrient agar were allowed to stay overnight while that of potato dextrose agar was incubated for 3 days. Bacteria usually grow on nutrient agar while fungi grow on potatoes dextrose agar.

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MORPHOLOGICAL STUDIES

Colonies, which developed after incubation were examined for structural features such as elevation, size, surface form, degree of growth, opacity, edge, consistency, and pigmentation. Pure cultures of the microorganisms were obtained by repeated streaking on nutrient agar plates for bacteria and fungi isolates. Cellular characteristics of the pure culture of each isolated microorganism were examined under the microscope using the oil immersion objective after gram staining.

STATISTICAL ANALYSIS

Data obtained were subjected to analysis of variance (ANOVA) of [12]. The New Duncan multiple range test subjected treatment means to comparison

RESULTS

Results obtained from the five treatments: TA – the control treatment without *OGE*, TB - *Suya* soaked in *OGE* for ½ hr, TC – *Suya* soaked in *OGE* for 1 hr, TD – *suya* soaked in *OGE* for 1½ hrs and TE – *Suya* soaked in *OGE* for 2 hrs are shown in Figure 1, Tables 1 and 2.

The total bacteria count on *Suya* prepared with *Ocimum gratissimum extract is* shown in Figure 1. The association between the time of soaking in *OGE* and the number of days in storage significantly differed ($P<0.05$) for aerobic counts. The highest counts of 2.20×10^5 were observed on Day 5 for both TA and TC. There were significant differences ($P<0.05$) for Coliform counts. Coliform counts on Day 7 in TA (7.50×10^5) and TB (6.00×10^5) were significantly ($P<0.05$) higher than all other days of storage. The mean coliform counts significantly ($P<0.05$) increased in the control group with days of storage. Lactic acid bacteria counts increased with storage time for all the treatments except in TC with day 7 recording significantly ($P<0.05$) higher values amongst all treatment groups.

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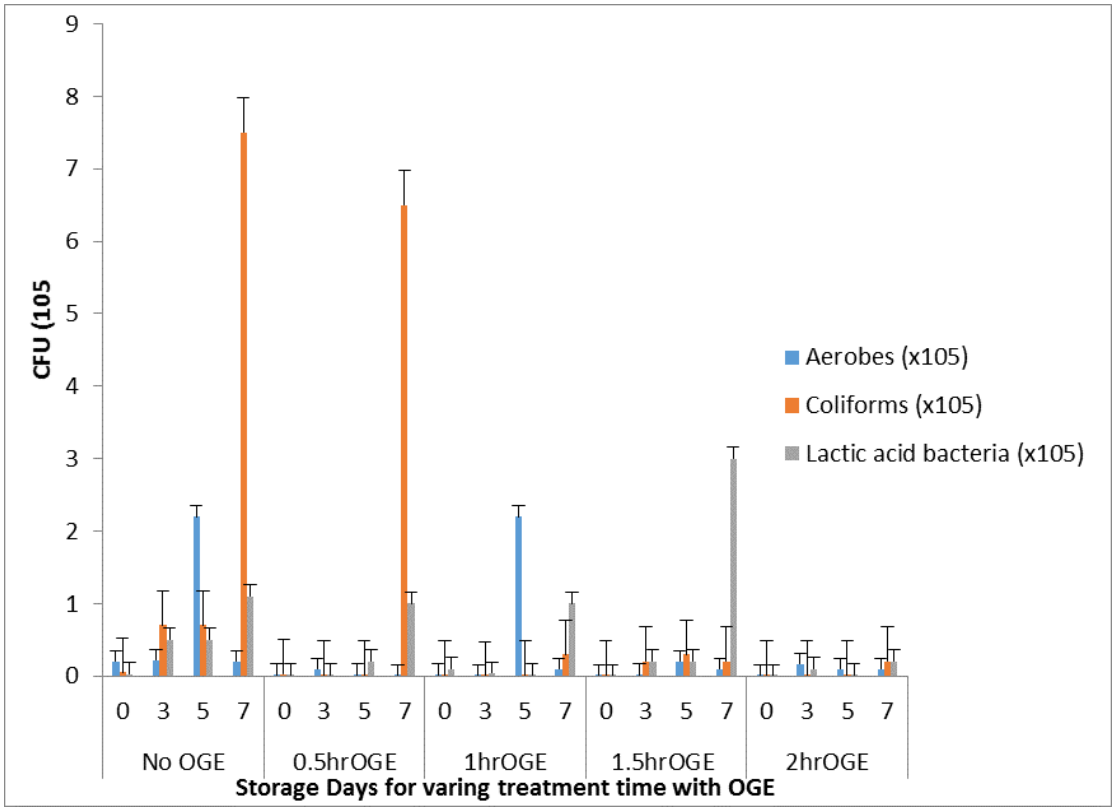

Figure 1: Total bacteria count on *Suya* prepared with *Ocimum gratissimum extract*

Table 1 shows the morphological and biochemical characterization of bacteria isolates from suya prepared with *Ocimum* extract. At Day 0, five Aerobic species were isolated from samples from the five treatments, namely: *Pseudomonas spp, Bacillus spp, Micrococcus Spp* and *Flavobacterium spp.*

Three Coliform species were also isolated from the five treatments namely: *Proteus spp*, *Aeromonas spp,* and *Enterobacter spp*.

Four Lactic acid bacteria were isolated from the five treatments namely*: Pediococcus spp, Streptococcus spp*, *Lactobacillus spp* and *Enterococcus feacalis*.

DISCUSSION

Suya, an intermediate moisture meat, and a delicacy savoured by many in the West African sub region is fast becoming a household name in other regions. It is a ready-to- eat snack that is usually consumed as soon as purchased. *Ocimum gratissimum* due to its antimicrobial properties was found in this study, to prevent meat spoilage, prolong shelflife and provide a natural alternative (or supplement) to chemical preservatives. Its antimicrobial components were found to work best when highly concentrated (Figure 1). The significantly lower count of microbes reported in this study among *O. gratissimum* treatments could be a result of the active chemotypes of *O. gratissimum* - eugenol as reported by Mshanu *et al.* [8, 13]. The compound (eugenol) has been demonstrated to have both antibacterial and antifungal properties [13, 14]. The *Ocimum* oil is dominated by eugenol, which accounts for 69 % of the oil

and methyl eugenol (13 %). Minor components include cisocimene (7.5 %), germacrene - D (4.3 %), transcaryophyllene (1.7 %) and pinene (1.10%). These eugenol imparts a clove-like fragrance which can be imparted to meat during processing in a number of ways.

The significantly high aerobic count observed in TA on day 5 is probably due to the microbes having reached the exponential phase where there is rapid increase in growth rate arising from the microbes been accustomed to the abundance of the nutrient supply [15]. The significant decline in Coliform count in treatments TC, TD and TE indicate that soaking meat in OGE from 1 – 2 hours had more impact on the keeping quality of suya so treated with *ocimum* oil. This is probably due to the antibacterial activity of OGE [13]. The trend observed for Lactic Acid Bacteria showed that increased storage time favoured its growth at ambient temperature up to day 7.

It has been reported by Nakamura *et al* [16] that the inhibition zones of *OGE* determined for six strains of Gram-positive or Gram-negative bacteria using the diffusion technique on solid media for *Proteus, Klebsiella, Escherichia, Salmonella, Staphylococcus* and *Shigella* showed inhibition zones ranging from 13 to 25 mm. *P. aeruginosa* was considered resistant since no inhibition zone was observed. The phenolic active ingredients present in *OGE* might have contributed to the relatively low microbial counts as the days of storage progressed.

The oil of O*cimum* has been found to have anti-infective functions by inhibiting many pathogenic bacteria like *Staphylococcus, Enterococci, Shigella* and *Pseudomonas* [1]. In another study where essential oil of *Ocimum* was evaluated for antimicrobial activity against path*oge*nic strains of Gram positive (*S. aureus, Bacillus spp*.) and Gram negative bacteria (*E. coli, P. aeruginosae, S. typhi, K. pneumoniae, P. mirabilis*) and a path*oge*nic fungus *C. albicans*, it was found to be active against all the bacterial strains [17]. The fungus, *C. albicans*, was highly susceptible to the essential oil. Other studies showed that the essential oils of four *Ocimum* species grown in Rwanda; *O. canum, O. gratissimum, O. trichodon* and *O. urticifolium*, displayed antimicrobial activity [18]. It has been reported that the volatile oil of this plant contains mostly phenols, particularly thymol [19] and that these are probably responsible for its reported antimicrobial actions.

CONCLUSION

It can be concluded from this study that *Ocimum gratissimum* and its extracts have antibacterial potentials and can possibly be used in the meat industry for large scale processing of meat and meat products. Further studies on its inclusion level in meat and food studies would better help to maximise its use. Although *O. gratissimum* appears safe to use in both food and as medicine, its level of inclusion in food processing has to be established to avoid its excessive use in meat processing so that the meat flavour will not be masked.

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Table 1: Morphological and biochemical characterization of bacteria isolates from suya prepared with *Ocimum* extract (Harvested period for Day 0)

Trts	CODE	Gram Rx	Cell Moph	Catalase	Oxidase	Casein Hyd	Gelatin Hyd	Methyl Red	Nitrate Redn	VP	Growth		Coag	Urease	Growth		SH	Growth NaCl
	Day(0)										60	30			3.9	9.2		
No OGE	A0	-	R	+	+	-	+	+	+	+	-	+	+	+	-	+	-	+
	C0	-	R	+	-	+	+	+	+	+	-	-	+	+	+	(+)	-	+
	L0	+	C	-	-	+	+	-	-	-	-	-	-	-	+	+	-	+
1/2hr OGE	A0	+	R	+	+	+	+	-	+	-	-	-	-	-	(+)	(+)	+	+
	C0	-	R	+	-	+	+	+	+	+	-	-	+	+	+	(+)	-	+
	L0	+	R	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
1.0hr OGE	A0	+	C	+	-	+	D	+	d	+	-	-	-	D	-	+	-	+
	C0	-	R	+	-	+	+	+	+	+	-	-	+	+	+	(+)	-	+
	L0	+	R	-	-	-	-	-	+	-	-	-	-	-	+	+	+	+
1.5hr OGE	A0	+	C	+	+	-	-	+	-	+	-	-	-	+	-	+	-	+
	C0	-	R	+	-	+	+	+	+	-	-	+		+	+	(+)	-	+
	L0	+	R	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
2hrs OGE	A0	+	R	+	+	+	+	+	+	-	-	-	-	-	(+)	(+)	+	+
	C0	-	R	+	-	+	+	+	+	+	-	-	+	+	+	(+)	-	+
	L0	+	C	-	-	+	+	-	-	-	-	-	-	-	+	+	-	+

A = Aerobes
C = Coliforms
L = Lactic acid bacteria

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Table 1) Contd: Morphological and biochemical characterization of bacteria isolates from suya prepared with *Ocimum* extract (Harvested period for Day 0)

DAY 0 (CONTD)

Trts No	Day (0)	Citrate Utilisation	Motility	Indole Test	Gluc	Fruc	Maltose	Lacto	Sucr	Galac	Xyl	Arab	Raff	Rham	Dulc	Mann	Probable identity
OGE	A0	+	+	-	+	-	+	+	+	+	-	-	-	-	(+)	(+)	*Psuedomonas putida*
	C0	+	+	-	+G	-	-	-	(+)	+	+	-	-	-	-	-	*Proteus mirabils*
	L0	+	-	-	+	+	+	+	+	+	+	+	+	-	+	d	*Pedicoccus acidilactis*
1/2hr OGE	A0	-	+	-	+G	-	+	+	+	+	+	+	+	+	-	+	*Bacilluslicheniformis*
	C0	+	+	+	+G	+	+	-	+	+	(+)	-	-	-	-	-	*Proteus vulgaris*
	L0	(+)	-	-	+	+	+	+	+	+	D	d	+	+	-	+	*Lactobacillus plataniun*
1hr OGE	AO	-	-	-	+	+	(+)	D	(+)	+	-	-	-	-	-	d	*Staphilococcus epidemis*
	C0	+	+	-	+G	+	-	-	(+)	+	+	-	-	-	-	-	*Proteus mirabilis*
	L0	(+)	-	-	+	+	+	D	d	+	-	+	w	-	-	+	*Lactobacillus plantenum*
1.5hr OGE	A0	-	-	-	+	+	+	-	+	+	+	+	-	+	-	+	*Pseudomonas putida*
	C0	+	+	-	+G	+	-	-	(+)	+	+	-	-	-	-	-	*Proteus mirabilis*
	L0	(+)	-	-	+	+	+	+	+	+	D	d	+	+	-	+	*pedicoccus acidilactis*
2hrs OGE	A0	+	+	-	+G	-	-	+	+	+	-	-	+	+	-	+	*Bacillus subtilis*
	C0	+	+	+	+G	+	+	-	+	+	(+)	-	-	-	-	-	*Proteus vulgaris*
	L0	+	-	-	+	+	+	+	+	+	+	+	+	-	+	d	*pedicoccus acidilactis*

A = Aerobes
C = Coliforms
L = Lactic acid bacteria

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Table 2: Morphological and biochemical characterization of bacteria isolates from suya prepared with *Ocimum* extract (Harvested period for Day 7)

DAY 7

Trts	Day(7)	Gram Rx	Cell Morph	Catalase	Oxidase	Casein Hyd	Gelatin Hyd	Methyl Red	Nitrate Redn	VP	Growth		Coag	Urease	Growth		SH	Growth NaCl
											60	30			3.9	9.2		
No OGE	A7	-	R	+	+	-	+	+	+	+	-	-	+	+	+	+	-	+
	C7	-	R	+	-	+	+	+	+	+	-	-	+	+	+	(+)	-	+
	L7	+	R	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
1/2hr OGE	A7	+	R	+	+	+	+	+	+	-	-	-	-	-	(+)	(+)	+	+
	C7	-	R	+	-	+	+	+	+	+	-	-	+	+	+	(+)	-	+
	L7	+	R	-	-	-	-	-	+	-	-	-	-	-	+	+	+	+
1hr OGE	A7	+	C	+	-	-	(+)	+	D	-	-	-	-	+	-	(+)	-	+
	C7	-	R	+	-	+	+	+	+	-	-	-	+	-	-	+	-	-
	L7	+	R	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
1.5hr OGE	A7	+	R	+	+	+	+	+	+	-	-	-	-	-	(+)	(+)	+	+
	C7	-	R	+	-	+	+	+	+	-	-	-	+	-	-	+	-	-
	L7	+	C	-	-	+	+	-	-	-	-	-	-	-	+	+	-	+
2hrs OGE	A7	-	R	+	+	-	+	+	+	+	-	+	+	+	-	+	-	+
	C7	-	R	+	-	-	((+))	-	+	-	-	-	+	d	-	+	-	-
	L7	+	C	-	-	+	+	-	-	-	-	-	-	-	+	+	-	+

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Table 2 Contd: Morphological and biochemical characterization of bacteria isolates of suya prepared with *Ocimum* extract (Harvested period for Day 7)

DAY7 (CONTD)										DAY 7 CONT											
Trts	Day(7)	Citrate Utilisation	Motility	Indole Test	Gluc	Fruc	Maltose	Lacto	Sucr	Galac	Xyl	Arab	**Prabable Identity**	Raff	Rham	Duic	Man n	Probable identity
No OGE	A7	+	+	-	+	+	-	+	+	-	+	+	*Pseudomanas cepacia*	-	-	(+)	(+)	pseudomonas cepacia
	C7	+	+	+	+G	+	+	-	+	+	(+)	-	*Proteus vulgaris*	-	-	-	-	proteus vulgaris
	L7	(+)	-	-	+	+	+	+	+	+	d	D	*Lactobacillus plantanium*	+	+	-	+	Lactobacillus plantanum
1/2hr OGE	A7	+	+	-	+G	-	-	+	+	+	-	-	*Bacillus subtilis*	+	+	-	+	Bacillus subtilis
	C7	+	+	-	+G	+	-	-	(+)	+	+	-	*Proteus mirabilis*	-	-	-	-	Aeromonas hydrophila
	L7	+	-	-	+	+	D	D	D	+	-	+	*Lactobacillus casei*	-	d	-	+	pediococcus acidilatis
1hr OGE	A7	-	-	-	+	(+)	D	+	D	+	d	-	*Micrococcus varias*	-	+	-	d	pseudomonas putida
	C7	-	+	+	+	+	+	(+)	D	d	+	-	*Aeromonas hydrophila*	D	-	-	+	Enterobacter aerogenes
	L7	-	-	-	+G	+	+	+	-	+	d	+	*Lactobacillus brevis*	+	-	w	-	pediococcus acidilatis
1.5hr OGE	A7	+	+	-	+G	-	-	+	+	+	-	-	*Bacillus subtilis*	+	+	-	+	Miccrococcus variais
	C7	-	+	+	+	+	+	(+)	D	d	+	-	*Aeromonas hydrophila*	D	-	-	+	Areomonas hydrophila
	L7	+	-	-	+	+	+	+	+	+	+	+	*Pediococcus acidilacti*	+	-	+	d	lactobacillus brevis
2hrs OGE	A7	+	+	-	+	-	+	+	+	+	-	-	*Pseudomonas putida*	-	-	(+)	(+)	Bacillus subtilis proteus
	C7	+	+	-	+	+	+	+	+	(+)	+	+	*Enterobacter earogenes*	D	(+)	+	d	mirabilis
	L7	+	-	-	+	+	+	+	+	+	+	+	*Pediococcus acidilacti*	+	-	+	d	lactobacillus casei

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KEY

+ = a positive reaction

- = a negative reaction

w = a weak reaction

d = a delayed reaction

(+) = a weakly positive reaction

Aerobes
Coliforms
Lactic acid bact

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