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The Effect of Temperature on the Antibacterial and Physicochemical Properties of Fermenting Beniseeds (*Sesamum indicum linn*)

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Research Article

The Effect of Temperature on the Antibacterial and Physicochemical Properties of Fermenting Beniseeds (*Sesamum indicum linn*)

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ABSTRACT

The effect of temperature on the antibacterial property of fermenting beniseed was assessed on six diarhoeagenic bacteria by keeping the fermenting seeds at 4^0C and 29^0C respectively. Both the liquor and the slurry were used in this study. The pH, total titratable acidity, microbial load and the microflora of the samples kept at the two different temperatures was determined daily for 7 days. The results showed that the liquor kept at 29^0C had its highest antibacterial activities after 24hrs of fermentation while the sample kept at 4^0C had its highest antibacterial activities after 72hrs of fermentation. The highest inhibitory effect was observed on *B.cereus* with zone diameter of 42mm by the sample kept at 29^0C and 36mm the sample kept at 4^0C. This result, when compared with standard commercial antibiotics showed that the fermenting liquor is more effective against four of the six bacteria used. The microbial load of the fermenting liquor increased from 2.4 x10^4 cfu/ml to 9.2x10^6cfu/ml in the sample kept at 29^0C and 2.4x10^4 cfu/ml to 2.8 x10^5 cfu/ml in the sample kept at 4^0C by day 4. Microorganisms found to be associated with the fermentation are *Lactobacillus acidophilus, Pediococcus cerevisiae* and *Leuconostoc mesenteroides.* The pH of the fermenting liquor decreased from 5.50 at day 0 to 4.90 by day 4 for the sample kept at 4^0C while it decreased to 3.80 by day 4 for the sample kept at 29^0C. The total titratable acidity for the sample kept at 4^0C was highest by day 1 with a value of 27.00 and lowest by day 6 with a value of 10.00 while the one kept at 29^0C recorded the highest value of 56.10 by day 1 and lowest value of 15.50 by day 4. Since the fermented liquor significantly inhibited the growth of the test organisms used, it is therefore suggested that in the absence of antibiotics, fermented beniseed liquor can be used to treat diarrhoea within 24hours in rural areas where they may not be quick access to conventional antibiotics and can be used up to seven days when preserved in refrigerator to combat diarrhoea caused by these organisms.

Keywords: Beniseeds, Fermentation, Temperature, Antibacterial, Physicochemicals.

INTRODUCTION

Diarrhea, a condition in which there is abnormal frequency in stooling which is watery and in some studies containing mucus and blood, is of great concern in the developing countries. This is so because diarrhoea is a common cause of morbidity of all ages especially among the pre-school children. Olorunfemi *et al*., (2006). Up till today, it is very difficult to treat diarrhoea because most of the antibiotics that are normally used also may induced diarrhea, which is known as antibiotic induced diarrhoea(Prescott *et al*.,2008). Not only that, most of the organisms causing infection have become resistant to most of these antibiotics. Oladunmoye (2006).

The concept of improving intestinal health using cheap and effective therapeutic agents is presently one of the avenues being exploited for possible treatment of diarrhea, especially antibiotic induced diarrhea. Beniseeds, which serves as food in various parts of the world, is known to have medicinal properties (Odugbemi, 2006). The plant belongs to the family pedaliaceae and is an annual crop that grows in tropical areas. The seeds are tiny, flat ovals and measuring about 3mm (Oshodi *et al.,* 2010). The plant root and leaves are used in treating migraine hypertension, ulcers, constipation, chicken pox and piles (Odugbemi, 2006). The Ebira people in Kogi State of Nigeria use it for treatment of intestinal disorder, for soup and roast/fry it for eating as snacks. Momoh *et al*. (2012) observed that fermented beniseeds have antibacteial properties. This research is focused on the evaluation of the effect of temperature on the antibacterial activities and physicochemical properties of the liquid fermentation of beniseed liquor and slurry against selected diarrhoergenic bacteria.

Materials and Methods

Sources of Materials

The seeds used were obtained from Okene central market, Okene in Kogi State. It was air dried before use.

Fermentation of Seed

500g of the seed was soaked in 1000ml of water for 3 days and grounded into a smooth paste. It was then filtered using muslin bag and the filtrate divided into two. Half was stored in refrigerator at 4^0C to study the effect of preservation and half kept at ambient temperature (29^0C). It was allowed to settle for 3 hrs before using the slurry and liquor for antibacterial sensitivity testing and isolation for day 0 to day 7.

Determination of total titratable acidity and pH.

The total titratable acidity was done using the method of Chris (2004), while the pH was determined using Jenway pH meter of 3015 model. This was first balanced using buffer solution of 4.00 and 9.00 before using it to determine the pH of the sample.

Test organisms

The test organisms, which are *Becilus cereus*, *E faecium*, *E. coli, Salmonella typhi*, *Shigella dysenteriae* and *Staphylococcus aureus* were obtained from the Microbiology department, University of Ibadan Teaching Hospital, Ibadan, Oyo State, Nigeria. The isolates were maintained throughout the period of study aseptically by sub-culturing it into freshly prepared nutrient agar medium.

Detection of antimicrobial activity

The six test organism were grown overnight in Nutrient both at 37^oC. The cell were then harvested and standardized from the stock culture using the method of Fawole and Oso (2004), and spectrophotometer (Unico 1100 RS series). One ml of the standard culture was pour plated with 20ml of the standard culture at 45^0C and allowed to set. Two wells were bored using diameter 4mm of sterilized cork borer and 0.4ml of the liquor was introduced into one well while the same volume of sterile distilled water was added to the other well to serve as control. The same process was used for the slurry. The plates were carefully incubated at 37^0C for 24hours in an incubator and the diameter of zones of inhibition measured. Standard antibiotics was also conducted on the test organism and the diameter of zones of inhibition were measured which were compared with the zones of inhibition from the fermented liquor and slurry.

Isolation and determination of microbial load

The microflora of the fermenting liquor and slurry were isolated daily, starting from day 0 to day 7. Also the microbial load of the preserved (refrigerated at 4^0C) and the unpreserved one (kept at ambient temperature of 29^0C) was determined to study the effect of temperature as a method of preservation all according to the method of Olutiola *et al*. (2000).

RESULT AND DISCUSSION

The result of this fermentation work showed that the fermenting sample at 29^0C can only be effectively used within day 0 and day 1, at most day 2 due to putrefactive smell from it. However, the preserved at 4^0C one was ok/effective through the seven days of fermentation. The antibacterial activity assay showed that the liquor has more antibacterial activity against the test organism than the slurry. It however showed high antibacterial property against *B. cereus*, followed by *Salmonella typhi* and *Shigella dysenteriae*, while its activity *E. coli*, *Staph. aureus* and *E. faecium* was averagely high. The antibacterial activity of the preserved sample was highest at day 3 and 4 while the unpreserved one had the highest value at day 1 (fig 2).

The result of the study of the effect of preservation by refrigeration shows that temperature has effect on the fermenting organism. The microbial load of the unpreserved sample increased from $2.4x10^4$ cfu/ml to $9.2x10^2$cfu/ml by day 4. The demented sample at 4^0C was more potent than commercial antibiotics, except against *E. coli* and *E. faecium* from the beginning of day 0 until the fermentation process was terminated. The isolation and identification of microflora associated with the fermentation process shows that *Lactobacillus acidophilus*, *Pediococcus cerevisiae* and *Leuconostoc mesenteriodes* were the bacteria involved (Table 1). The pH of both the preserved and unpreserved samples were acidic with both having a pH of 5.50 at day 0. However, the value kept decreasing at day 4, the pH of the unpreserved was 3.80 while the preserved at day 7 was 4.80 (fig 4). The total titratable acidity gradually decreased irregularly. The value obtained was however higher for the unpreserved sample throughout the analysis.

Table1: Standardized colony forming unit per ml of each organism suspension used.

Organism	Dilution powered	Cfu/ml	Spectrophotic reading	Standard cfu/ml
B. cereus	10^6	14	0.050	$1.4x10^7$
E. faecium	10^6	20	0.045	$2.0x10^7$
E. coli	10^6	25	0.043	$2.5x10^7$
Salmonella typhi	10^6	15	0.049	$2.0x10^7$
Shigella dysenteriae	10^6	15	0.051	$1.5x10^7$
Staphylococcus aureus	10^6	22	0.041	$2.2x10^7$

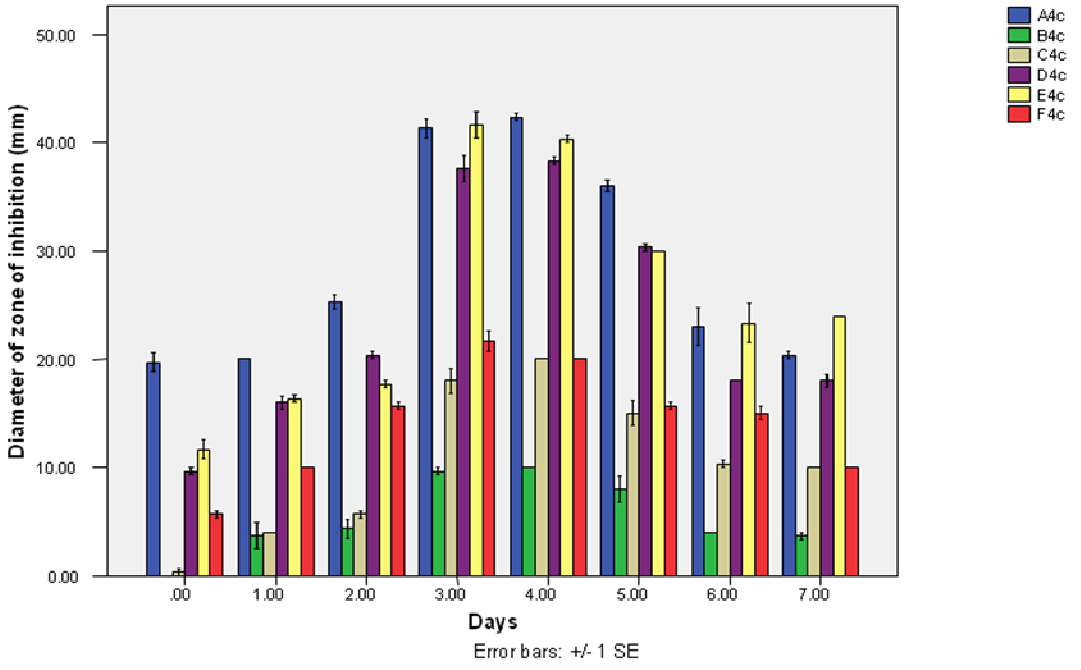

Fig 1: Diameter of Zones of inhibition (mm) of fermented liqour at 4^0C on the test organisms. Keys: A4c: *Bacillus cerueus,* B4c: *Enterobacter faecium,* C4c: *Escherichia coli,* D4c: *Salmonella typhi,* E4c: *Shigella dysenteriae,* F4c: *Staphylococcus aureus.*

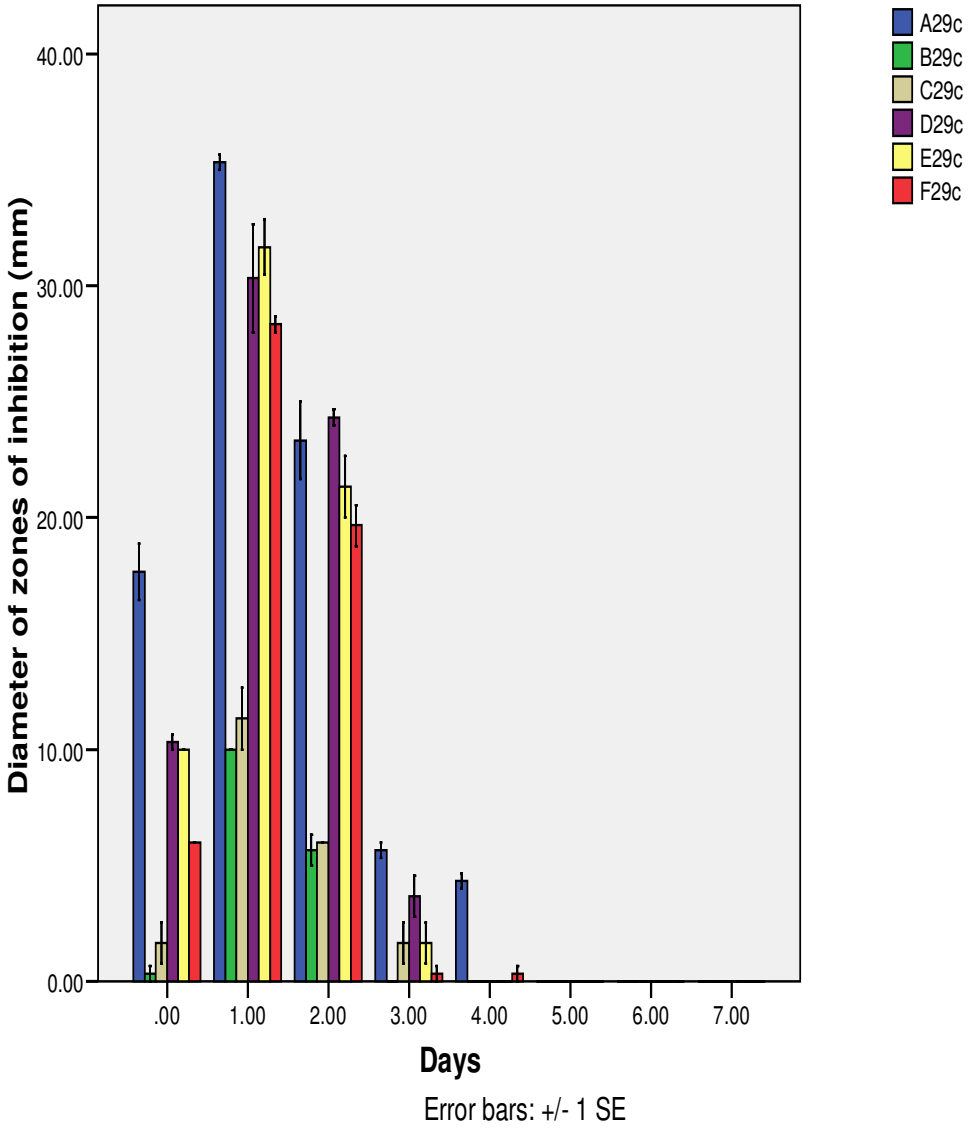

Fig 2: Diameter of Zones of inhibition (mm) of fermented liquor at 29^0C on the test organisms.

Keys: A29c: *Bacillus cerueus*
B29c: *Enterobacter faecium*
C29c: *Escherichia coli*
D29c: *Salmonella typhi*
E29c: *Shigella dysenteriae*
F29c: *Staphylococcus aureus*

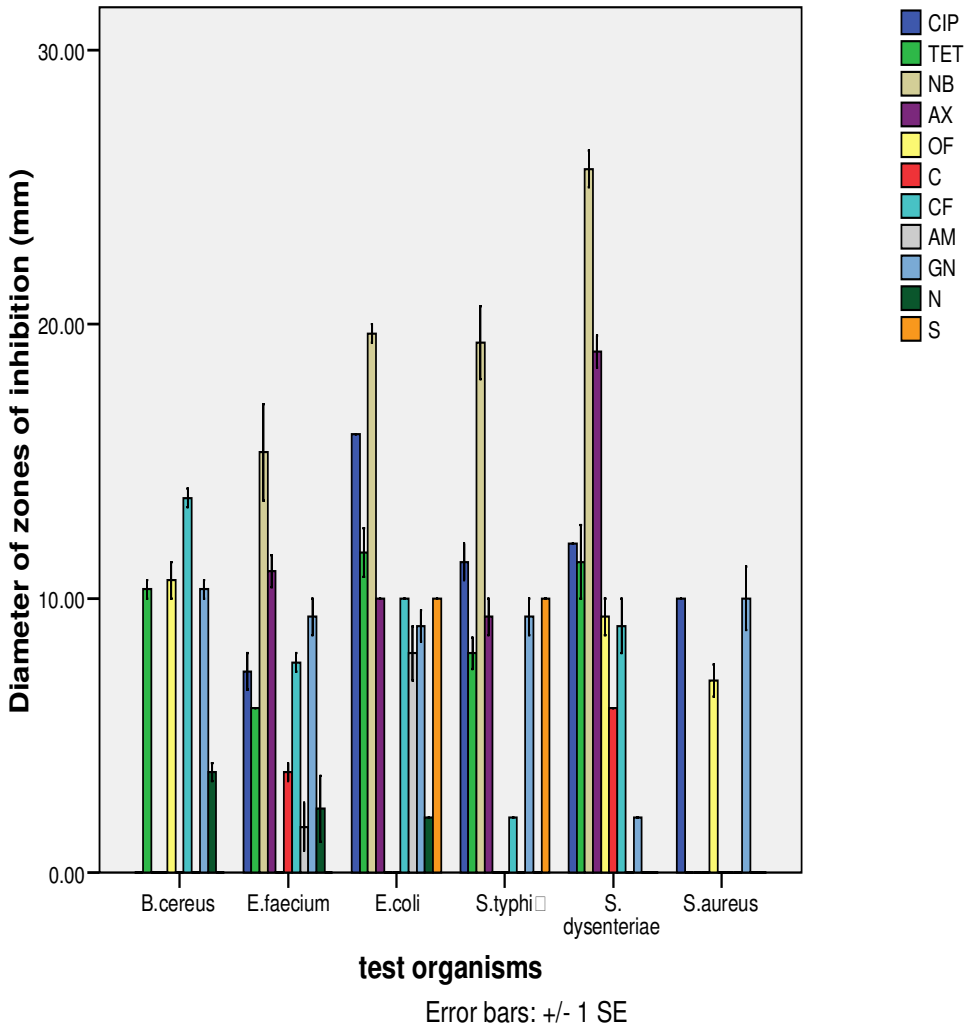

Fig 3: Diameter of Zones of inhibition (mm) of the test organisms by commercial antibiotics.

Keys: CIP: Ciprofloxacin
TET: Tetracyclin
NB: Norfloxacin
AX: Augmentin
OF: Tarivid
C: Cephalexin
CF: Cefalux
AM: Ampicillin
GN: Gentamycin
N: Nitrofurantoin
S: Streptomycin

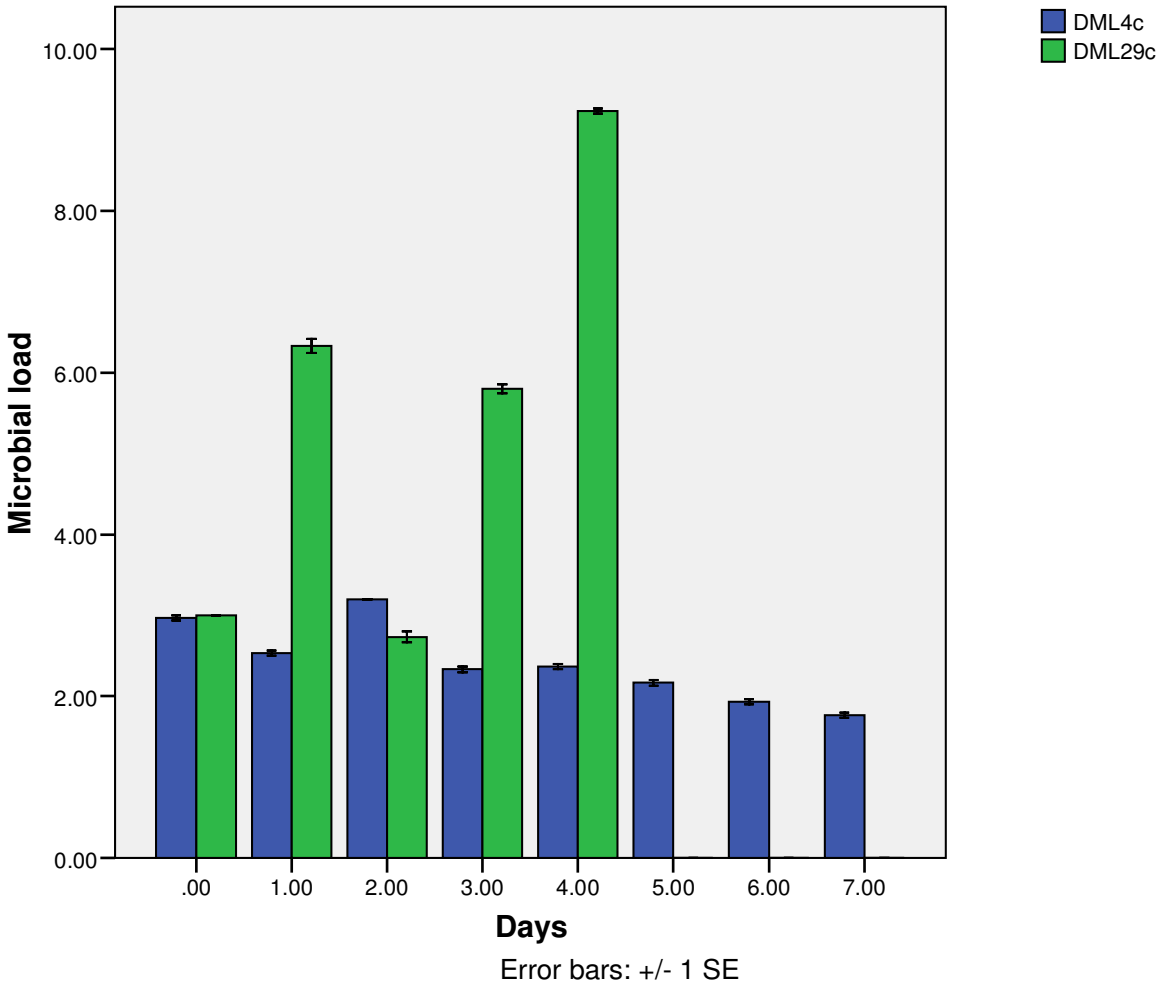

Fig 4: Daily microbial load of preserved and unpreserved samples of fermented beniseed liquor.

Key: DML4c- Daily microbial load at 4^0C
DML29c- Daily microbial load at 29^0C

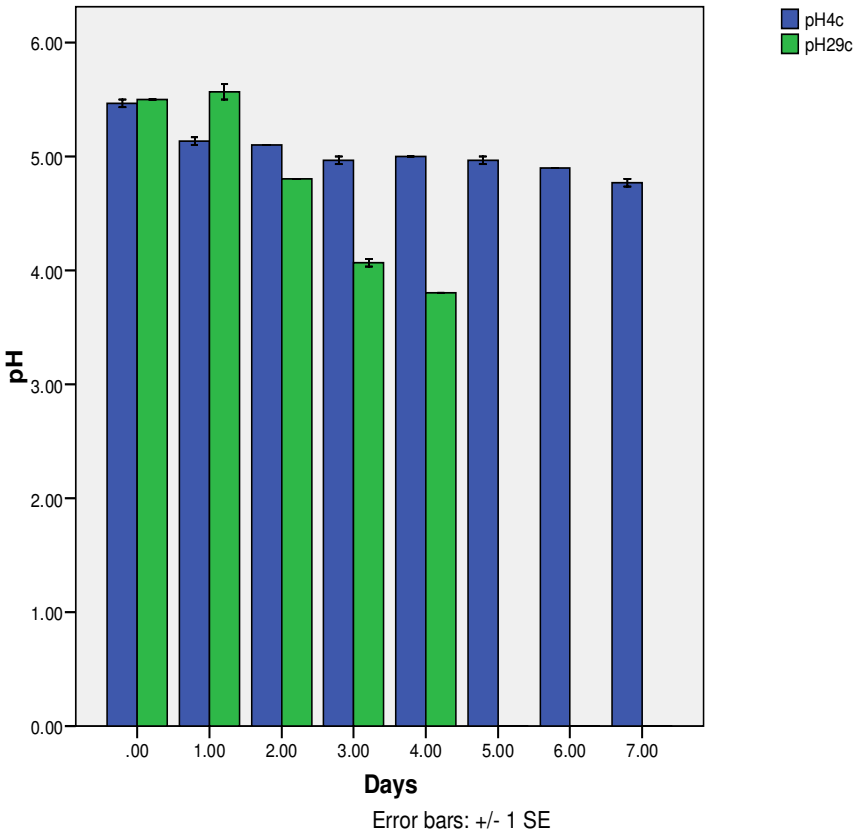

Fig 5: pH of fermenting beniseeds kept at 4^0C and 29^0C.

Key
pH4^0C: pH of fermented beniseed at 4^0C
pH 29^0C: pH of fermented beniseed at 29^0C

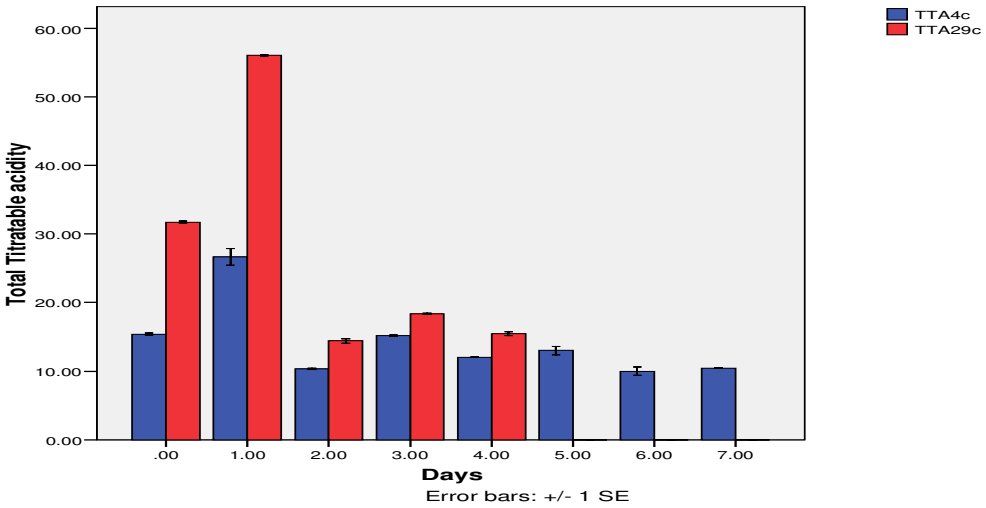

Fig 6: Total titratable acidity of fermented beniseed liquor kept at 4^0C and 29^0C.

Key: TTA4^0C: Total titratable acidity of fermented beniseed at 4^0C
TTA29^0C: Total titratable acidity of fermented beniseed at 29^0C

Table 2: Morphological and biochemical characteristics of isolates fermented beniseed.

Isolate Gram reaction	Morphology	Motility	Spore reaction	Oxidize	Catalase	Indole	H_2S	Glucose	Arabinose	Mannitol	Maltose	Sucrose	Galactose	Nitrate reduction	Raffinose	Lactose	Gas production	Identified Organism
IS1	Long rods	-	-	-	-	-	-	+	-	-	+	+	-	-	+	+	+	*Lactobacillus acidophilus*
IS3	Short rods	-	-	-	-	-	-	+	+	+	+	+	-	-	+	+	+	*Pediococcus earevisiae*
IS3	cocci	-	-	-	-	+	-	+	+	+	+	+	-	-	+	+	+	*Leuconostoc megenteroide*

Though, some of the antibiotics showed high antibacterial activity against the test organisms, none of the antibiotic inhibited all the organisms tested for. Since the fermented liquor was able to inhibit the entire organism tested for, it is therefore more effective. The high inhibition obtained from fermenting liquor may be attributed to the presence of metabolites such as organic acids, hydrogen peroxide and bacteriocin produced by lactic acid bacteria. The group to which all the three isolates belong. The antibacterial effect of lactic acid bacteria through its production of organic acids, hydrogen peroxide and bacteriocin has been well documented by Savadogo *et al.* (2004), Oyetayo and Osho (2004), and Olorunfemi *et al.*(2006). These substances inhibit growths of pathogenic bacteria and also alter the ecological balance of enteric commensals. The fermented beniseed liquor showed a decline in pH from 5.50 to 3.80 in the unpreserved sample and from 5.50 to 4.80 in the preserved sample. This is an indication that organic acids were actually produced in the beniseed liquor. Also the decrease in value of the titratable acidity as fermentation progresses equally supports the production of organic acids in the fermenting liquor. The results obtained in this study has shown that fermented beniseed liquor has inhibitory effect on the entire test organisms and that its inhibition was superior to that of all the antibiotics used against the organisms in this work. Though the actual substance responsible for the inhibition is yet to be determined, it is conceivable that when the substance is identified, it could be exploited in production of new drug for the treatment of bacterial diarrhoea. Since the fermented liquor significantly inhibited the growth of the test organisms used, it is therefore suggested that in the absence of antibiotics, fermented beniseed liquor can be used to treat diarrhoea within 24hours in rural areas where they may not be quick access to conventional antibiotics and can be used up to seven days when preserved in refrigerator to combat diarrhoea caused by these organisms.

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