International Journal of Advances in Engineering and Management (IJAEM)
Volume 7, Issue 02 Feb. 2025, pp: 242-248 www.ijaem.net ISSN: 2395-5252

Prevention of Milk Spoilage in Federal Polytechnic Offa Using Electrical Stimulation

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Date of Submission: 05-02-2025 Date of Acceptance: 15-02-2025

ABSTRACT

Electrical stimulation (ES) has served as a therapeutic modality accelerating the healing of wounds, particularly chronic wounds which have impaired healing due to complications from underlying pathology. This review explores how ES can serve as preventive means against the spoilage of liquid foods. Milk was used as the food sample in this research work. The spoilage microorganism was identified to be Bacillus cereus conventionally. In-vivo experiments investigating the effect of ES on the general mechanisms of preventing spoilage of milk was demonstrated using various voltages of 1.5, 3.0, 9.0 and 18.0. Significant reduction was observed with 9.0 and 18.0 while increase in growth of the spoilage bacteria was observed at 1.5 and 3.0 voltages. The growth and population of the spoilage microorganisms in the milk samples and Nutrient agar culture after 96 hours was observed reduced. Higher reduction in the microbial population in the milk sample media was directly proportional to increase in the voltage used for electrical stimulation. In conclusion, ES therapy can contribute to prevention of milk spoilage and increase in the shelf-life of left-over milk for future consumption. Prevention of microbial growth in the milk, potentially reduce the food poisoning associated with consumption of left-over milk. Therefore, financial burden associated with liquid food items spoilage management can be reduced by the adoption of electrical stimulation as a preventive measure.

KEYWORDS: food spoilage, electrical stimulation, in-vivo, pathology, therapy

I. INTRODUCTION

Food is an essential part of our daily life, and it plays a vital role in maintaining our health and well-being. However, food can easily spoil, leading to the growth of harmful microorganisms and the loss of its nutritional value, most especially milk (Bawcom et al., 2017). Milk is an easily perishable raw material. Contaminating bacteria may multiply rapidly and render it unsuitable for processing and/or unfit for human consumption (Jacob et al., 2019). Bacterial growth can be retarded by refrigeration, thereby slowing down the rate of deterioration (Sale et al.. 2017). Under certain conditions refrigeration may not be feasible due to economical and/or technical reasons. Difficulties in applying refrigeration are specially a problem for certain areas in countries setting up or expanding their milk production. In these situations, it would be beneficial to have access to a method, other than refrigeration, for retarding bacterial growth in raw milk during collection and transportation to the dairy processing plant according to Petrofsky et al., in 2018.

Preservation of food can be done by use of preservatives such as salt, sugar, and vinegar, can help prevent food spoilage by inhibiting the growth of microorganisms (Snezana et al., 2019). However, it is essential to use preservatives in moderation and follow the recommended guidelines to prevent adverse health effects, which is not properly followed in most of food industries. Other food preservative measures include prevention or delay of microbial decomposition through keeping out microorganisms (asepsis), removal of microorganisms, e.g., by filtration, hindering the growth and activity of microorganisms e.g., by low temperatures, drying,

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anaerobic conditions, or chemicals and killing the microorganisms e.g., by heat or radiation.

The extensive use of some of these methods such as chemical preservatives, heat etc. in food products can lead to the changes in their natural qualities. The inhibitory effect of ES on bacterial growth has been proposed as a mechanism to explain the useful effects of ES on food spoilage prevention. If we can control bacterial growth using ES, probability that we can control the major cause of food spoilage is high, easy, safe and cost effective (Shilpee et al. , 2018). The available research that has examined the capacity for ES to inhibit or destroy pathogens indicates that various parameters of ES (e.g., current type, current density, polarity, etc.) have been employed in the past to improve healing of wound (Ong, et al., 2017). Meanwhile, whether biophysical energies, such as ES, can be used as a treatment modality against spoilage microorganisms remains an open question.

Therefore, this research work employed the usage of voltage as a parameter of ES to inhibit the growth of microorganisms in milk (in-vivo) and NA agar broth (in-vitro).

STATEMENT OF PROBLEM

Bacterial contamination is one of the factors that enhance the process of spoilage of food. Bacteria produce enzymes that degrade the chemical components of food that brings about spoilage of food which is not economical friendly. However, the extensive use of chemical preservatives for the control of food spoilage has resulted to change in the natural quality of food products and series of allergy reactions in some consumers.

JUSTIFICATION

Recent studies have therefore, focused on the potential use of ES as a treatment modality against spoilage microorganisms in food. If the antibacterial effects of ES can be induced in food substance against spoilage microorganisms' activities, ES may prove to be a superior antimicrobial agent that would overcome some of the issues currently raised by most food preservatives.

AIM AND OBJECTIVE

Assessment of antimicrobial effect of ES on selected isolated indigenous spoilage organisms as test organisms.

The specific objectives were:

1. Isolation and identification of spoilage microorganism in the spoilt milk sample medium.
2. Setting up of electrical stimulating system using 3.0, 6.0, 9.0 and 12.0 voltages as a parameter of ES.
3. Examine the antimicrobial effect of ES on microbial growth in-vivo and in-vitro.

HYPOTHESIS

H: Electrical stimulation has antimicrobial effect on the growth of test organisms.

Ho: Electrical stimulation has no antimicrobial effect on the growth of test organisms.

II. MATERIALS AND METHODS

SOURCE OF RAW MATERIALS

Canned milk was bought from Owode market in Offa, Kwara state of Nigeria and was transported aseptically to the Microbiology laboratory in the Biological Sciences department of Federal Polytechnic Offa for the research bench-work.

MATERIALS

NA agar, Autoclave, Incubator, Hi-Tech battery cells of 3.0v, conducting wire with non-conducting coating, Mcathney bottles, Colony counting machine, Distilled water, Volt Meter, Petri dish, UV-Visible spectrophotometer, incubator, 70 % ethanol, weighing balance, conducting wire.

ISOLATION OF MICROORGANISMS FROM SPOILT MILK SAMPLE

Isolation medium used was NA agar (NA). The medium was prepared according to manufacturer instruction. The medium was sterilized at 121^0C for 15 minute and cooled to 45^0C. Liquid suspension of the sample was made by transferring 1ml of sample into 10ml of sterile distilled water and shaken adequately to dislodge and release microorganisms on the sample into the diluents. From the homogenate of the sample, suitable dilution in 10-fold (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, etc.) was prepared in sterile distilled water, two dilutions were chosen. Labelled duplicated set of Petri dishes were inoculated with 0.1ml aliquots from appropriate dilution of the sample. Some 10ml of molten NA, cooled at 45^0C was added to each of the Petri dishes accordingly and mixed carefully with its aliquot. The plates were allowed to set and inverted to avoid condensed water vapour on the inside of the plate cover (lid) from dripping, to disrupt the culture. This was avoided by drying plate, with the lid partly off, in 37^0C

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incubator for about 20 minutes so as to allow excess surface moisture to evaporate. The NA plates were incubated at 37^0C for 24hours (Palaniappan and Sastry, 2019).

MAINTENANCE OF PURE CULTURE

Following incubation of poured plates, colonies developed, the representative colonies were selected and streaked on sterile NA plates using a standard sterile wire loop and incubated at 37^0C for 24 hours. Result colonies were subculture on a fresh, sterile NA plates and isolate of pure cultures were obtained. Slopes were prepared in universal bottles to store the purified strains of bacteria (isolates) for later use. The sterile slope was inoculated with inocula for each pure culture of its bacterial strain and then incubated at 37^0C for 24 hours and then stored in refrigerator at 4-6^0C as stock culture for further experiment (Palaniappan and Sastry, 2019).

IDENTIFICATION OF SPOILAGE ORGANISMS OF MILK SAMPLE

Identification of the spoilage microorganism that was used for the study was done by conventional method. The microorganism isolates were subjected to the following biochemical tests which include catalase, citrate utilization, TSI agar, gelatin liquefaction, indole production, nitrate reduction, urease (Urea broth), Voges-proskauer, methyl red, oxidase and motility test and confirm their identity using Bergy's manual (Craven, et al., 2018).

SETTING UP ELECTRICAL STIMULATION SYSTEM

The waveforms of electrical stimulation (ES) used in this research were direct current (DC). DC stimulation with silver electrodes at the voltages of 1.5, 3.0, 9.0 and 18.0 for 96 h on the isolates in an in-vivo model in the milk sample and Nutrient agar broth were examined. Non-stimulated milk and inoculated Nutrient agar served as control. Voltage (V) refers to the electromotive force (EMF) which is capable of moving charged particles (ions across cell membranes in the microbial cells) that lie between two electrodes applied to the medium. The volt is a measure of electrical pressure (analogous to water pressure) and is the EMF (electron [or ion] moving force) that is needed to drive a current of 1 A through a resistance of 1 ohm. The relationship between voltage and amperage is expressed as Ohm's law, V=IR. To produce directed current flow, there was a source of free electrons from the ES device, conveyed to the food medium via conductive electrodes that were positioned to distribute the flow of a quantity of EF energy (charge) into the medium. With direct current (DC) and monophasic pulsed current (MPC), the two electrodes were polarized with regard to each other, with one being negative (cathode) and the other being positively charged (anode). The batteries were arranged in series to vary the voltage. Currents with polarity were used for the stimulation (Qin et al., 2018).

EXAMINE THE ANTIMICROBIAL EFFECT OF ES ON MICROBIAL GROWTH IN-VIVO AND IN-VITRO

The primary culture was made from the stock of Bacillus cereus by overnight incubation and secondary culture was then made. The B. cereus was standardized to 0.5 Mcfaland standard of 1.5×10^8 using UV-Visible spectrophotometer. Then, the standardized bacterial culture was taken and added to Nutrient agar broth. The cultured nutrient agar broth was put under electrical stimulation by immersion of the copper plates of the electrical set up system into the medium for in-vitro while milk solution was used for in-vivo. For the application of perpendicular electric field on bacteria, copper plates were kept parallel to each other while immersed in the sample (milk solution or cultured nutrient agar broth) contained in sterile conical flask. Sample was taken from the test medium at the interval of 24hrs for 5 days. Bacterial counting done and results were recorded at 0, 24, 48, 72 and 96hr of incubation (Fang, et al., 2016).

ENUMERATION OF MICROBIAL COLONIES

Bacterial count was done by direct viable cell count method with reference to Bendicho, et al., (2018). Counting the colony forming units requires the culturing of the bacteria of interest and counts the number of living cells, in contrast to the traditional microscopic counting which takes into account all the cells irrespective of them being dead or alive. Serial Dilution used to measure CFU, plated on nutrient agar plate. Dilution series were made till 10^4 dilution factors was reached and then grown on the plate. Colony counting was carried out using colony counting machine. The formula used for the CFU measurement is:

Viable cells/ml = (Average viable cells per square) × (Dilution factor) × 10^4.

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DATA ANALYSIS

The data gathered were processed using one way analysis of variance (ANOVA), SPSS 10.0. The level of significance was set at $P \leq 0.05$. Means were compared by Dunnet T- tests. Results were presented in tables and figures.

III. RESULTS AND DISCUSSION

Table1: Identification of isolate from non-stimulated (spoilt) milk

Biochemical tests	Reactions
Growth above 50^0C	-
Gram	+, rod
Catalase	+
Citrate utilization (Simmon's citrate Agar)	+
TSI Agar	G/A
Gelatin liquefaction (Nutrient Gelatin)	-
Starch hydrolysis	+
Indole Production	+
Nitrate Reduction	+
Urease (Urea Broth)	-
Voges-Proskaur	+
Methyl Red	+
Oxidase	+
Glucose	+
Mannitol	-
Arabinose	-
Xylose	-
Motility (SIM Medium)	+
Suspected organism	Bacillus cereus (Facultative aerobes)

Key: A/G- acid (yellow) and gas formation in butt of tube and acid (yellow) on slant surface

Figure 1: Setting-up circuit for electrical stimulation of milk

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Table 2: Enumeration of Bacillus cereus cells in stimulated freshly opened canned milk samples

In-vivo

Time (hr)	0	24	48	72	96
Stimulated freshly opened canned milk					
ES (Voltage)	Microbial population (cfu)				
1.5	1.0×10^1	3.5×10^2	2.7×10^6	4.2×10^9	numerous
3.0	1.0×10^1	2.9×10^2	2.0×10^4	1.1×10^8	0.3×10^{11}
9.0	1.0×10^1	1.0×10^1	-	-	-
18.0	1.0×10^1	-	-	-	-
Unstimulated freshly opened canned milk					
-	1.0×10^1	3.1×10^2	5.4×10^5	7.3×10^7	numerous

Table 3: Enumeration of Bacillus cereus cells in stimulated and unstimulated spoilt milk samples

In-vivo

Time (hr)	0	24	48	72	96
Stimulated spoilt milk					
ES(Voltage)	Microbial population (cfu)				
1.5	6.2×10^{11}	3.5×10^{13}	nemerous	numerous	numerous
3.0	6.2×10^{11}	2.9×10^{12}	4.2×10^{12}	3.3×10^{13}	numerous
9.0	6.2×10^{11}	3.0×10^9	7.5×10^6	2.4×10^3	3.0×10^2
18.0	6.2×10^{11}	1.0×10^7	3.0×10^4	3.0×10^2	3.0×10^1
Unstimulated spoilt milk					
-	6.0×10^{11}	2.1×10^{12}	5.4×10^{13}	numerous	numerous

Table 4: Enumeration of Bacillus cereus cells in stimulated and unstimulated cultured nutrient agar (NA) broth samples

In-vitro

Time (hr)	0	24	48	72	96
Stimulated NA broth culture					
ES (Voltage)	Microbial population (cfu)				
1.5	1.5×10^8	2.1×10^9	8.4×10^{10}	4.5×10^{11}	numerous
3.0	1.5×10^8	1.3×10^9	1.1×10^{10}	7.2×10^{10}	3.5×10^{11}
9.0	1.5×10^8	3.8×10^3	2.3×10^2	6.2×10^1	1.6×10^1
18.0	1.5×10^8	3.4×10^2	5.7×10^1	1.1×10^1	-
Unstimulated NA broth culture					
-	1.5×10^8	3.6×10^9	6.7×10^{11}	5.8×10^{13}	numerous

DISCUSSION

This research work provides evidence for useful effects of ES in terms of improvement and inhibition of bacterial growth as shown in Table 2, 3 and 4. The bacterial inhibitory action of ES is proportional to the increase in voltage and application time of the electric voltage as shown on Table 2, 3 and 4. This result indicates that the higher the voltage, the more severely the microbial cells are damaged and the higher the rate of content release. The electric voltage directly results in bacterial death by disruption of the integrity of the bacterial membrane or by electrolysis of molecules on the cell surface according to Jacob et al. 2019. Changes in pH were suggested as a possible mechanism for the indirect effects of ES (especially DC) on inhibition of bacterial growth. The production of toxic substances (•e.g., H_2O_2, oxidizing radicals, chlorine molecules) and galvanotaxic effects of ES might be other mechanisms for indirect antibacterial effects of ES (Knorr, et al., 2018). ES has a potential for bacteriostatic and bactericidal effects on in-vivo and in-vitro microorganism growth as shown in Table 2, 3 and 4. However, in the in-vivo and in-vitro condition, the antibacterial effects of ES are likely to result from electrolysis products.

ES is a new non-thermal processing technology for pasteurising liquid foods (Mertens and Knorr, 2018). The effects of ES on the viability

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of Bacillus cereus in simulated systems were systematically investigated in-vivo and in-vitro. Total number of colonies in the milk decreased from 1.0 cfu/ml to a sterile milk after ES of 9V and 18V at 5th day and these low levels remained stable over the 60-day storage period. The effect of direct voltage on the growth of bacteria has been reported in this work to be toxic which slows down the growth rate of bacteria and hence the lower number of colony formed in a given span of time.

However, the results reported in this research were observed at both lower applied voltages (≤3 volts) and higher applied voltages (≥ 9 volts, peak to peak). In this present study, it has been observed that lower applied voltage helps in growing bacteria (Rojas et al., 2017). It is to be noted that the results of this report are verified using the copper anode and cathode only. It would be interesting to check if the results hold good for other metals such as zinc, silver etc. as these materials are well known to have high conductivity activities. Further investigations are needed to answer this question and to understand the molecular mechanism of such an effect.

The pulsed current lead to a marked colony forming unit (CFU) reduction across three studied medium as shown in Table 2, 3 and 4. This is in accordance with the work done by Haughton in 2022 on efficacy of pulsed electric fields for the inactivation of indicator microorganisms and food borne pathogens in liquids and raw chicken. One mechanism behind this is that supplying electricity alters the pH of the bacteria's environment. This consequently damages the external membrane of bacteria, allowing an uncontrolled influx of solutes that ultimately kill the bacteria (Saif et al., 2017). Whilst the antibacterial effect of ES is beneficial for prevention of milk spoilage, supporting the potential use of ES in increasing the shelf-life of left-over milk to minimize the burden of milk spoilage according to Heinz et al. 2019.

IV. CONCLUSION

This research work showed how electrical stimulation (ES) influences the growth of microorganisms in milk at both low and high voltages for a specific period of time. Additionally, electrical stimulation (notably high voltages) significantly inhibits the growth of spoilage microorganisms in milk and by that prevents spoilage of milk when compared to control groups with no ES.

RECOMMENDATION

More studies need to be conducted to investigate the effect of ES using any other types of metal conductors as anode and cathode.

HIGHLIGHTS

- ES improves the growth of bacterial cells at low voltages of 1.5 and 3 and kills the bacterial cells at high voltages of 9, 18.
- The viable bacterial count in the ES-treated milk increases at low voltages and reduced at high voltages significantly in direct proportion to the increase in the period of exposure in both the in-vivo and in-vitro analysis.
- Milk spoilage is prevented by using ES at high voltages.

ACKNOWLEDGMENT

I am thankful to TETFUND and Federal Polytechnic Offa for providing me the financial and academic support for this research work.

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