Estimation of Electric field effect on E-coli in the preservation of milk

Ms.V.Gowrisree and Dr.K.Udayakumar

Abstract—**The threat of low socio economic status, poor sanitation and hygiene, inadequate preservative techniques aid in contamination and spurting of communicable disease causing microorganisms like bacteria and fungus. Electricity an essential and a common commodity has reached its tentacles also in the field of Food Technology and Preservation. Thus the study titled "Estimation of electric field effect on E.coli in the preservation of milk" was carried out to estimate the effect of various types of voltage on the bacterial cells of E.Coli in milk. E.Coli, a micro organism causes diarrhoea in immuno compromised patients and children through contamination of food products. In this study, 200 µl of E.Coli was inoculated into a sterilized Luria Broth medium and incubated at 37oC. On application of electric field at various magnitude ranging from 15kV/cm to 80kV/cm and with time duration of 1.2 to 100 µs at a constant number of impulses. It was found that the medium exhibited high inhibition in the growth rate of E.coli at an optimal voltage of 60kV/Cm. With this optimal voltage, the effect of E.Coli in milk was done and it was concluded that on application of high-intensified electric field between 20-60kV/Cm inhibits the growth rate of E.Coli in milk, thereby extending the shelf life of the milk.**

Key words—**E.Coli, PEF, (Pulse Electrical Field) Impulse voltage, inactivation of micro-organism.**

I. Introduction

India the second leading nation in agro production with rich fertile land, perennial water resources is deprived being a super power nation due to inadequate distribution of available resources.

Poor post harvesting methods, food technology causes wastage of essential products of basic commodities like food grains, diary etc. Hence, the present scenario of increased demand in one half of the country leading to poverty, draught etc. in contrast to wastage of food products in another part of the country prevails. This necessitates a novel food processing technology and redistribution of resources to the needy people.

The main purpose of food processing is food preservation, maintaining the high quality properties of food for as long as possible. Thermal methods, exposing foods to high temperature and overcooking mainly affect sensory (color, smell, texture and flavor) and nutritive (vitamins) attributes. Hence, different non-thermal methods have been explored throughout the past century, including high hydrostatic pressure, ultrasound, light pulses, magnetic field etc. Electrical pasteurization of milk was one of the earliest applications of electricity in food processing. The process involved passage of an AC electric current through milk generating heat responsible for bacterial death. (PALANIAPPAN, et al 1990) Transient arc discharges across the electrode gap in liquid food also found to destroy microorganism (Mizuno 1998). However, contamination of the treated liquid food from chemical products of electrolysis or disintegration of food particles by HV discharges was the major drawbacks. Using short duration and high intensity electric field pulses (PULSED ELECTRIC FIELDS - PEF) can minimize this drawback.

The use of high intensity pulsed electric fields (PEF) in food applications such as milk and fruit juices has gained much popularity and is claimed to represent a most promising non-thermal alternative to conventional pasteurization methods.

Supposed mechanisms of inactivation of bacteria cells with PEF is perforation of the cell membrane due to high voltages across the membrane or due to current concentration in the pores. In a cell membrane, protein channels and pores are present. The opening and closing of many channels constituted by proteins depends upon the trans-membrane voltage difference. When PEF is applied, many voltage-sensitive protein channels may open, and when the voltage difference reaches 150-500 nV, the lipid bilayer breaks down.

It is observed that when a cell membrane is exposed to an electric field larger than about 25kV/cm, irreversible electric breakdown of the cell membrane occurs. The extent of inactivation depends on the strength, duration and form of the electric pulses. Pulses with duration of 100-200 ns are reported to provide an efficient inactivation of micro-organisms.

Nevertheless, the results with PEF published in literature show large differences in lethal effects and levels of energy, under apparently similar conditions. The scatter in lethal effects may be due to different media, the condition of the bacteria, the treatment procedure or by the change with medium temperature of the susceptibility of the microorganisms to pulsed electric field.

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If taste and food qualities are not the important issues a more effective pulsed power method for inactivation of micro organisms is direct application of pulsed corona in the liquid. This pulsed corona method (PCOR) is based on the creation of inhomogeneous electric fields that are sufficiently high to generate corona discharges in the liquid. Discharge products such as radicals, ozone, aqueous electrons and UV are produced directly in the liquid to inactivate the bacteria.

Both PEF and PCOR require high voltages that are generated across a liquid load. To effectively apply high voltage to a conducting liquid, fast rise times and narrow pulses are needed to reduce the amount of dissipation. If the crucial effects to the liquid are limited to either high electric field or corona activity, it could be sufficient to maintain the pulse voltage only for a time that is of the order of the achievable rise time. Inactivation of microorganism by PEF has unique advantages including non-thermal or low heat treatment, no chemical additives and energy efficient.

Hence it is necessary to study the effect of pulsed electric field on bacteria under various magnitudes of voltages and pulse duration in food preservation. Thus, this initiates an objective to estimate the electric field effect on E-coli and to inactivate the bacterium E-coli inoculated in milk extending the shelf life period of the milk

II. METHODOLOGY

A. Estimation of electric field effect on E.coli

Luria Broth (LB) a bacterial medium of composition Tryptone 10gm, Yeast extract 5gm, NaCl 10gm and distilled water 1000ml was made with pH 7.3 to 7.5. After this LB medium was sterilized using an autoclaving machine for six hours.

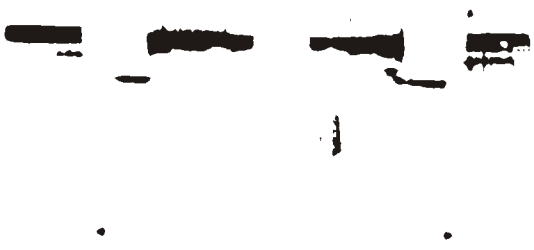

Fig. 1 Treatment chamber

The treatment chamber as shown in the Fig.1 was autoclaved and sealed. From a 2ml over night grown culture of DH5α (E-coli) with an Optical Density (OD) of 1.5 at 600nm.

200µl was taken and inoculated into a 200ml Luria Broth (LB) and incubated over night at 37°C in a water bath shaker for further experimental analysis.

The 200ml LB was equally distributed in sterile test tubes for further experiments. The OD of the culture was 0.548 at 600nm at zero hour, which is at the start of the experiment. These test tubes are taken and after pouring into a sterile container tested with impulse voltage of 15 - 70kV with number of impulses varying 40, 80, 120 and the pulse duration was within 1.2µs. to 100µs.

B. Testing procedure for inactivation of E.coli in milk:
The LB broth medium was prepared and autoclaved.

1. The treatment chamber was autoclaved and sealed.
2. The process of inoculation was performed inside a special chamber called the laminar blow chamber, which was completely sterile. Additional precaution was taken by wiping the containers and the test tubes with alcohol to prevent the entry of any external pathogen from the surroundings.
3. 100ml of LB broth was inoculated with 5µl of DH5α glycerol stock and was allowed to grow overnight. The following day, 25ml of culture was added to 475ml of milk. The dilution was adjusted so that $1*10^8$ organisms existed in the culture to be treated.
4. After the medium was transferred into the substrate (milk), the sample was poured into the testing unit. The electrodes were connected to the high voltage terminals.
5. After the application of the required field, the circuit was earthed and the treated milk was poured into the airtight falcon tubes for later analysis.

Using the experimental setup as shown in Fig.2 experiments were performed by varying the following variables. Voltage, the number of pulses and the distance between the electrodes. Samples were treated at 2kV, 4kV and 6kV for 30, 60, 90 and 120 pulses with the distance between the electrodes as 5mm and 10mm.The pulse duration was between 1.2µs. to 100µs.

Fig. 2 Experimental setup

The streak plate method as shown in Fig.3 was used to count the bacterial colonies after the conduction of experiments. The steps involved in the process are:

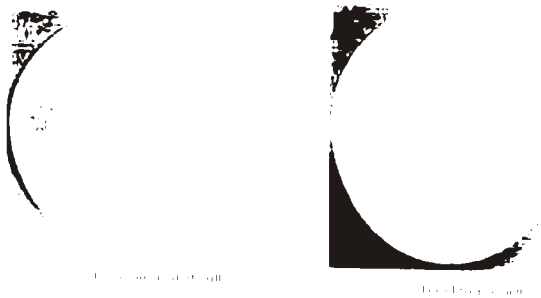

Fig. Streak plate

i. The inoculums was spread using a sterile loop, over a small area towards the edge of plate.
ii. The wire loop was sterilized and several streaks in the direction indicated were made.
iii. The wire loop was sterilized again and the procedure was repeated until complete.
iv. The plates were incubated at 37° overnight.

The plates are now ready with the bacterial colonies, which can be counted.

RESULTS AND DISCUSSIONS

The streak plate method was used to estimate the electric field effect on E.Coli. The loop full of LB medium inoculated with E.coli was streaked onto a plate and kept in an incubator at 37°C. temperature for growth. After 12 hours, the plate was analysed for contamination. If not contaminated, then the plate was viewed for growth by absorption method and compared with the reference samples. A Hitachi 1598 spectrophotometer was used to measure the absorbance at various times: zero hours, one hour, 6 hours and 24 hours and the results are plotted as shown in Fig4(a) and 4(b).

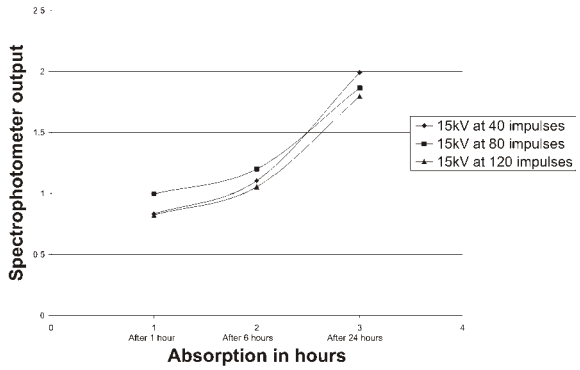

Fig.4(a) Inactivation of bacteria at constant magnitude of impulse varying number of pulses

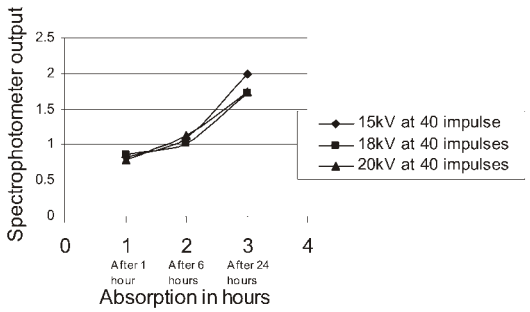

Fig.4(b) Inactivation of bacteria at constant number of pulses varying the voltage.

The bacterial surveillance in milk was estimated by using a bacterial colony count meter. The streaked plate with milk inoculated with E.Coli was incubated at 37°C and the bacterial growth was estimated by counting the bacteria present and compared with reference sample as shown in Fig 5(a) and 5(b).

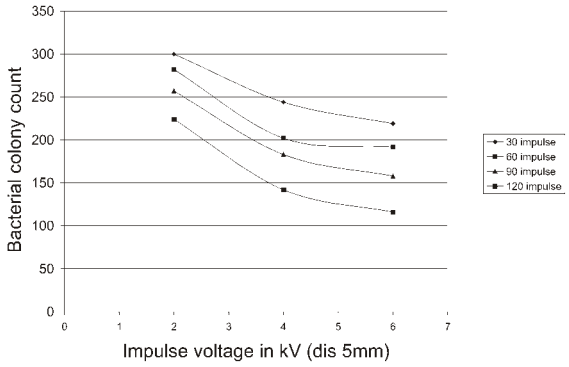

Fig. 5(a) Bacterial surveillance in milk by varying the number of pulses (Distance between the electrodes is 5 mm)

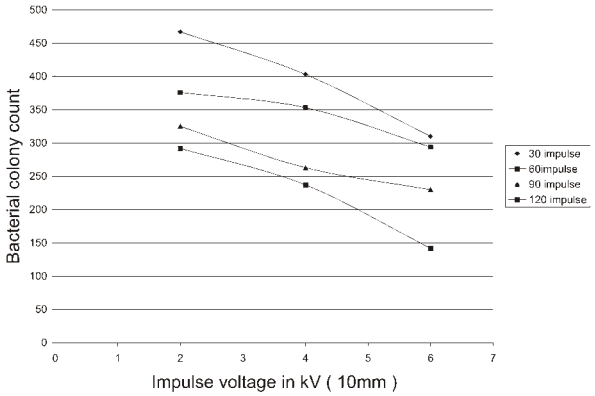

Fig. 5(a) Bacterial surveillance in milk by varying the number of pulses (Distance between the electrodes is 10 mm)

From the results, it is evident that there exists a threshold level for the field strength at around 15kV/cm below, which there is negligible inhibition. This exists even at over 150 impulses. There also exists a minimum value of threshold level for the number of impulses to be applied before which there is inhibition but this level varies with the magnitude of the field strength. There appears [Fig.5(a) and 5(b)] to be

an almost linear increase in the inhibition of the bacteria when there is an increase in the magnitude of the field strength. This increase in the inhibition also exists when there is an increase in the number of impulses though the slope or the rate of inhibition is less.

CONCLUSION

Hence, it can be concluded that for effective food processing either high electric field strength or more number of pulses need to be applied. At higher magnitude of voltage and higher number of impulses, the number of bacterial colonies decreases below harmful levels increasing the shelf life period of milk.

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