Non-thermal Pasteurization of Liquid Foods Using Non-thermal Plasma

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Abstract: A new non-thermal pasteurization technique-non-thermal plasma (NTP) was investigated. This study shows that NTP is capable of killing foodborne pathogens such as *Escherichia coli* O157: H7 and *Salmonellae* in liquid foods such as orange juice and milk. Five logs reduction in the bacteria counts was achieved under the experimental conditions. The NTP treatments were brief, and did not have noticeable impact on the samples as evidenced by the analytical data of vitamin C in orange juice and oxidation value in milk. The energy consumption rate was calculated as 1～2 J/mL, which is much lower than that for thermal and other non-thermal processes.

Key words: non-thermal plasma; non-thermal pasteurization; juice; milk; *Escherichia coli*; *Salmonellae*

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1 Introduction

Unpasteurized fruit juices have been associated with outbreaks of *E. coli* O157: H7 infection, *cryptosporidiosis*, and *salmonellosis*[1～7]. As recent as in 2000, 88 people in six western states of US were sickened by *salmonella* from unpasteurized orange juice. In 1999, a 16-month-old child in Colorado died of heart damage and kidney failure after drinking contaminated apple juice. Another case of contaminated apple juice occurred in 1996, resulting in the hospitalization of a 3.5-year-old Chicago girl for 24 days. The 1996 outbreaks of *E. coli* O157: H7 infection also due to consumption of unpasteurized apple cider in the Western United States caused illness in 66 persons and one death[1]. These outbreaks have raised serious concerns about the safety of consuming unpasteurized fruit juices. In some stage, production of the unpasteurized fruit juice has been suspended.[2]

In 1998, FDA adopted a regulation that forces fresh juice to either pasteurize their product or attach the label "WARNING: this product has not been pasteurized and, therefore, may contain harmful bacteria which can cause serious illness in children, the elderly and persons with weakened immune systems."[8] In 2001, FDA adopted the ruling to implement the Hazard Analysis and Critical Control Point (HAACP) procedures for the Safe and Sanitary Processing and Importing of Juice, effective February, 2002.[9] This puts a tremendous pressure on the fruit juice industry. Some companies have adopted a voluntary guideline for good practice in production of unpasteurized apple juice/cider.[10] However, pasteurization is the preferred choice in this life and death issue.

Unfortunately, current practical pasteurization method is based on thermal processes. Though some thermal processes such as the so-called "flash pasteurization" are very efficient, thermally pasteurized apple juice/cider cannot compare with the unpasteurized ones in terms of fresh flavor and aroma. One alternative is non-thermal pasteurization. Currently, there are several methods having the "non-thermal" claim: 1) pulse electric field (PEF), 2) irradiation, 3) UV light, 4) high pressure, and 5) freeze-thaw. These methods are either not true non-thermal process, or impractical due to high costs. Therefore, there is a need to seek other alternatives. One of such alternatives is non-thermal plasma (NTP).

NTP can be generated through electric discharge in gaseous volume. A simple NTP reactor may consist of two electrodes with a space (the discharge volume) and sometimes one or two dielectric layers in between and connected to a high voltage power supply. When a high voltage is applied to the electrodes, an electric field is generated across the space between the electrodes, which, if sufficiently high, causes electric

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discharge. Such electric discharge produces many energetic and highly reactive species that can react with chemical compounds in contact with them. The result of such chemical reactions or NTP induced chemical reactions may be decomposition of the chemical compounds.

NTP has been traditionally used for surface treatment and decomposition of polluting gases. Some researches have demonstrated that NTP is capable of killing air-borne pathogens and microbes on surfaces[11~13]. There are two fundamental questions regarding the application of NTP process in inactivation of pathogenic organisms in bulk liquid. First, can we effectively generate NTP species in bulk liquid? Second, can NTP species kill microorganisms in bulk water?

Information on the use of NTP in inactivation of microorganisms is limited in gas phase[11~14]. Research data on pasteurization of bulk liquids using NTP is almost nonexistent. In principle, NTP species are highly energetic, and are expected to interact with and cause damages to microorganisms in contact. There are however some research data showing that NTP destroyed air-borne microorganisms [12~15]. Birmingham and Hammerstrom[15] used ambient pressure non-thermal plasma to deactivate bacterial spores in gas and on surfaces. In their study, air contaminated with Bacillus globigi spore aerosols was sent through the annulus of a gas corona reactor system, which is a packed barrier discharge device. A 4 logs reduction in the spores was achieved after the treatment. In another experiment, the researchers placed contaminated materials such as steel, plastic, and cloth into the non-thermal plasma reactor, which resulted in significant bacterial reduction. The researchers showed through scanning electron microscopy that after plasma treatment, the cell walls of the bacterial spores were breached exposing the cellular contents while the remaining surface appeared folded and mottled. Montie[13] and co-workers developed the one atmosphere uniform glow discharge plasma (OAUGDP) system for sterilization of surfaces and materials. This system operates at atmospheric pressure in air and produces anti-microbial active species at room temperature. With this system, they were able to achieve 2~5 logs reduction in bacteria (*E. coli*, *S. aureus*, *Bacillus subtilus*, etc), yeast (*S. cerevisiae*, *C. albicans*), and viruses (*Bacteriophage phi* X 174), depending on the type of surface on which microbes were inoculated. The *D* values range from 2 seconds to 3 min. Spores and yeast seemed to require longer treatment time. Their transmission electron microscopy work indicated that for *E. coli*, the continuity of the cellular envelope was breached, and cellular content was released in the surrounding medium within 30 seconds of exposure to non-thermal plasma.

Though there is no direct evidence to show NTP's lethal effect on microorganisms present in bulk liquid, the fact that NTP kills air-borne microbes indicates the possibility of applying NTP process to deactivate pathogenic microbes in bulk liquid provided that we could establish contacts between the NTP species and microbes in bulk liquid.

As will be discussed later, NTP is generated through electric discharge in gas. Without sufficient gaseous phase in liquid, it is difficult to induce NTP in liquid. To solve this problem, the authors boldly proposed to artificially introduce gases in the form of bubbles into liquid, and apply electric field to the mixture of liquid and gas bubbles. This hypothesis was immediately proven valid after running a trial with a small NTP reactor. This small NTP reactor has become the basic model of the NTP systems for pasteurization of bulk liquid.

The killing mechanisms of NTP are not well established. However, there are some hypotheses. It is well documented that reactive oxygen species (ROS) such as oxygen radicals can produce profound effects on cells by reacting with various macromolecules[11]. NTP is predicted to contain these as well as many other more stable intermediates. Among the cellular macromolecules altered are membrane lipids, which are most vulnerable macromolecule of the cell[13], probably because of their location near the cell surface, and their sensitivity to ROS. When the cytoplasmic membrane lipids are altered, this will result in a massive release of macromolecules, and thus death of the cells. Various resistances to NTP exposure exhibited by different microbes are correlated to the protective outer polysaccharide layer structure (or thickness) of individual microbes [13]. For example, yeast and spores possess extremely thick polysaccharide cell walls and are thus the most resistant to NTP exposure [13].

The objectives of this study were to demonstrate the feasibility of NTP technology to kill pathogenic microorganisms, and to study key factors affecting the efficiency of the technology.

2 Materials and Methods

2.1 Experimental System

Fig. 1 shows that experimental system used in the study. Fig. 2 shows the five stages of the process. The experimental system consisted of following major parts: 1) raw sample receiving tank, 2) pump, 3) gas injection device, 4) NTP reactor, 5) pasteurized product tank, 6) gas source including oxygen generator, 7) power supply and control, and 8) liquid and gas flow control.

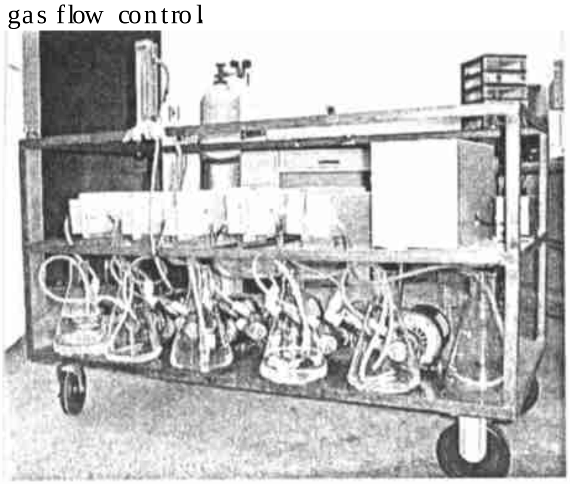
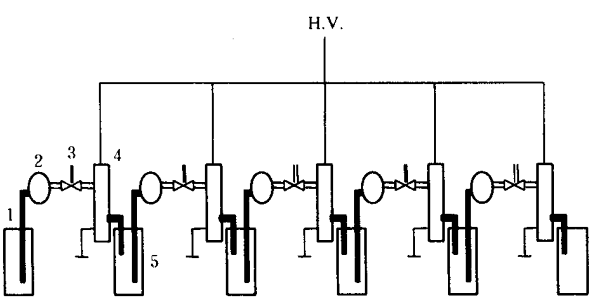


Fig. 1 The five-stage NTP liquid pasteurization system



1. raw sample receiving tank; 2. pump;
3. gas injection device; 4. NTP reactor;
5. pasteurized product tank

Fig. 2 Flow chart illustrating the five stages of the process

2.2 Sample Preparation

Orange juice, and milk were used as the liquid samples in the study. Sterilization of the samples was achieved by filtration (Corning Brand Bottle System Sterile, Corning NY 14831). Sterilized samples were inoculated with *E. coli* O157: H7 and *Salmonellae*. Prepared samples were treated within a few minutes.

2.3 Analysis

Control and treated samples were serially diluted in Peptone water (DIFCO Laboratories, MD, USA), and plated on Triptic Soy Agar (DIFCO Laboratories, MD, USA). Cells were incubated for 24 h at 35℃ and then counted. The plates with plasma treated were further incubated for a 24 h to check for possible cell injury since injured cells may not grow as quickly within 24 h of treatment to be counted, yet were still viable. Bacteria and spores inactivation are reported as the logarithm (base 10) of the microbial survival fraction, expressed as the ratio between surviving cell count (N) over initial cell (N_0). Vitamin C and oxidation value were also determined.

3 Results and Discussion

3.1 Effect of NTP Treatment on Bacteria

Most of our work was done on *Salmonella*, and some on *E. coli*. After testing with a single cell reactor, we designed and built a multi-cell system which consisted of 5 reactor cells arranged in serial. The number of the reaction cells to be used can be varied so that the effect of the number of cells that liquid sample passes can be examined. Fig. 3~4 show that this system can result in up to 5 logs *Salmonella* reduction.

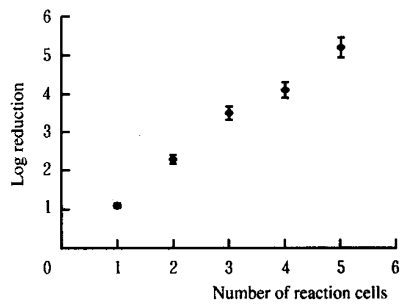


Fig. 3 Preliminary test result of the NTP liquid disinfection system with bacteria (20 kV, liquid flow rate at 30 galons/hour(113.55 L/h), air injection at 2 CFH).

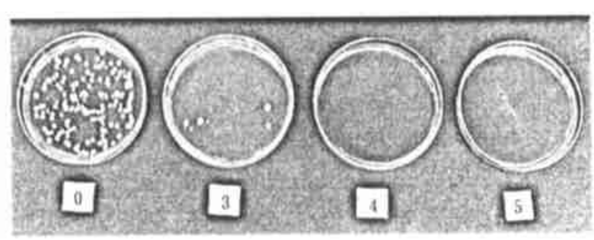


Fig. 4 Solid culture of *Salmonelae* samples subjected to NTP treatments. The numbers indicate the number of NTP reaction cells (arranged in serial shown in Fig. 1) in a particular test (20 kV, liquid flow rate at 10 galons/hour(37.85 L/h), air injection at 2 CFH 0.057 m^3/h)

Table 1 shows the importance of gas introduction in inactivation of *Salmonella* with the NTP system. Similar results were obtained for *E. coli* (Fig. 5). The order of increasing log reduction is no air, air, and oxygen. Mixing oxygen into the liquid stream is most effective. One possible explanation is that air has more nitrogen, which requires higher ionization energy than oxygen, and therefore the plasma concentration would be lower with air than with oxygen under the same discharge condition. The gas bubbles in the liquid stream could be regarded in some way as a "disinfectant" that needs to be activated by strong electrical fields (or "*electric-field-activated disinfectant*"). The quantity and quality of such "disinfectant" of course affect the efficiency of the process.

Table 1 Salmonella reduction by NTP treatment (The reactor had stripped electrodes, and was operated at 25 kV. The gap between electrodes was 10 mm, however the effective reaction volume had a gap of 7 mm)

Treatment	Log reduction
without air bubbles	～1
with air bubbles	3～4
with oxygen bubbles	> 5

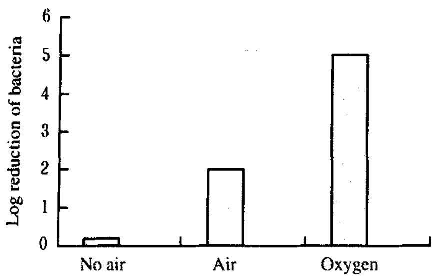


Fig. 5 Effect of gas introduction on reduction of *E. coli*

3.2 Chemical Effect of NTP Treatment

To investigate if NTP treatment affects the chemical properties of the samples, we measured the change in vitamin C (orange juice) or peroxide value (milk) after treatment. We believed that vitamin C and peroxide value are good indicators of oxidation caused by the treatment. Table 2 shows that the vitamin C values in orange juice dropped slightly (10%～15%) after NTP treatment, compared with total loss of vitamin C in thermally treated juice. These are very exciting data which indicate that NTP treatment does not cause much damage to active ingredients in foods. This could be a breakthrough in searching methods for disinfection of heat-sensitive materials such as fresh fruit juice, blood products, vaccines, and other biological fluids. It seems that the treatment with oxygen injection caused slightly more vitamin C loss than with air injection. The reason for that could be one or both of following: 1) oxygen caused more vigorous oxidative reactions than air during NTP treatment, and/or 2) residual oxygen in the sample after the NTP treatment continued to cause oxidation. If the second reason is true, degassing after treatment should reduce the loss due to post-treatment oxidation. Surprisingly, peroxide value was even reduced as a result of NTP treatment, probably due to measurement errors, or there were insufficient data points for meaningful interpretation of the result. The low oxidative effect of NTP process on food components may be associated with the low mean electron energies of NTP, which is about 20 eV, much lower than that of irradiation (a few hundred eV).

Table 2 Vitamin C and peroxide value as a function of NTP treatments

Vitamin C in juice/meq · (100 g)$^{-1}$			Peroxide value of milk/mg · kg^{-1}		
Original	With air	With O_2	Original	With air	With O_2
72.9	65.9	61.6	5.21	3.39	4.56

4 Conclusions

This research demonstrates that non-thermal plasma (NTP) is capable of killing foodborne pathogens such as *Escherichia coli* O157: H7 and *Salmonellae* in liquid foods such as orange juice and milk. Five logs reduction in the bacteria counts was achieved under the experimental conditions. The NTP treatments were brief, and did not have noticeable impact on the samples as evidenced by the analytical data of vitamin C in orange juice and peroxide value in milk. The energy efficiency was calculated as 1～2 J/mL energy input compared with 5～70 J/mL by PEF and 360 J/mL by high temperature short time (HTST), strongly suggesting that NTP is a technology that has the potential to compete or replace thermal pasteurization processes and some newly developed cold pasteurization processes such as pulsed electric field (PEF) and high hydraulic pressure (HHP) technologies.

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低温等离子体用于液体食品的低温杀菌

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摘 要: 介绍了一项新的非热(或低温)巴氏杀菌技术——低温等离子体(NTP)的初步研究结果。NTP一般由高压电场下的气体介质放电所诱发。该文作者的一项美国专利技术成功的在液体介质中诱发NTP。该研究表明这项新的NTP技术可以在常温下和极短的时间内杀死液体食品中的病源菌包括大肠杆菌O157: H7和沙门氏菌。实验中, NTP可使接种在橙汁和牛奶细菌总数降低5个对数值, 而对橙汁中的维生素C和牛奶的氧化值影响甚微。能量消耗估算为1～2 J/mL, 比之于热杀菌和其它低温杀菌技术能耗率要低得多。

关键词: 低温等离子体; 低温杀菌; 巴氏杀菌; 果汁; 牛奶; 大肠杆菌; 沙门氏菌