Non-thermal plasma pasteurization of milk using plasma technology (phase II)

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ABSTRACT

Phase II of the research developed and tested a pilot scale prototype system and conducted extensive experiments to generate the required data to petition FDA approval of the technology. Data generated during the study enhanced the scientific understanding of this novel process, and provided technical information on the effects of the treatment on inactivation of microbes, enzymes and quality of fresh milk. These data can also be used in commercial equipment and process development. The outcome of this study is designed to assess and confirm both technical and economical feasibilities, and speed up the potential commercialization of this innovative technology in the dairy industry.

BACKGROUND

Phase I of this project (funded by DMI, 2005-2006) proved our hypothesis that the non-thermal plasma-based dielectric barrier high intensity electric field can inactivate microbes in milk, non-thermally at atmospheric pressure on a lab scale, and also developed and tested a unique designed based on the hypothesis.

Phase II of this project further demonstrated the feasibility of this technology on a larger scale. Research focused processing variables and its on bacterial reduction and enzyme inactivation in milk. A a newly designed cylindrical high electric field and plasma reactor was used for this study.

The study was designed to answer several key technical questions important to the commercialization of the technology: (1) Can high electrical field be generated in the large scale treatment chamber with practically available power supplies? (2) Is it possible to obtain uniformly distributed electrical field within the treatment volume in the large scale treatment chamber? (3) What are the processing parameters to ensure optimal microbial kill and enzyme inactivation and minimum quality impact with continuous processing in larger scale?

During a previous study the effects on bacterial kill were examined using several different techniques such as various dielectric barrier materials, different reactor structures, several electrical field strength and frequency levels, and many types of liquid samples (water with different conductivity and milk with different dilutions). It was found that the bacterial kill was largely dependent upon the dielectric constant of the dielectrics, electrical field strength, and the conductivity of the samples. We also found that the electrical field strength can be greatly affected by the arrangement and structure of the electrodes.

Our data indicates that this new technology overcomes two major defects in traditional pulsed electrical field (PEF) process: the high cost of pulsed power supply, and electrode erosion and contamination. Based on the experimental data, and discussions with directors at DMI and the Midwest Dairy foods Research Center, and industry partners, we concluded that a reactor combined

plasma and high electrical field with innovative electrode configuration has a great potential of being accepted by the dairy and other food industry.

The goal of this project was to fully investigate the NTP-based pasteurization process and develop a pilot scale system for milk and milk-based beverage applications. The research was organized in two years: The first year focused on systematic studies, optimized the reactor and processes, and built a prototype demo system. The second year confirmed microorganism reduction, evaluated milk quality, conducted a shelf life stability study, and generated the necessary data to petition the FDA for approval of the technology.

OBJECTIVES

1. Develop and construct a high efficiency reactor with significantly increased electric field strength without a substantial increase in applied voltage.
2. Study the effect of treatments on microbial reduction and physical and chemical properties of milk samples.
3. Evaluate the shelf stability of treated milk samples.
4. Optimize the NTP-based pasteurization process.
5. Develop a mobile continuous prototype demonstration system.

MATERIALS AND METHODS

Develop and test high efficiency reactors

Preliminary studies of high efficiency reactors indicated that electrical field strength across the electrodes is essential to efficiently inactivate microorganisms and shorten the required treatment time. The electrical field strength can be increased using a higher applied voltage; however, this requires a higher voltage power supply and dielectric materials with higher breakdown strength. Unfortunately, both are impractible because they are either very expensive or unavailable.

Develop efficient dielectic layers

The electrical efficiency of dielectric layers was improved using high dielectric constant materials as the dielectric layers. Different reactors were constructed and used to test liquid model samples.

Effectiveness of microbial reduction, and physical and chemical properties of milk

Microbial reductions in test materials was tested with various NTP treatments using different reactors and processing conditions. The most resistant microbes of concern to NTP were identified.

Model liquid foods were prepared from sterilized distilled water with prescribed amounts of NaCl added to adjust the electrical conductivity. Samples were mainly for reactor and process development and verification.

Whole and skim milk were used as liquid samples to study the effects of the new Concentrated High Intensity Electric Field (CHIEF) treatments on bacterial reduction, enzyme, quality, and shelf stability. The microbiological and chemical properties of the milk samples were determined prior to and after CHIEF treatments. Pasteurized whole and skim milk were used in some experiments to control the bacterial inoculation and allow proper use of the indicator microorganism, E. coli.

Milk samples were treated using the CHIEF system under different processing conditions with combinations of voltage, frequency, treatment duration, flow rate and post-treatment storage. Microbial reduction and peroxidase activity were the measurables.

Shelf life stability of treated milk samples

Control and treated samples were stored at room temperature for up to 3 days, and at 6°C for up to 3 weeks. Physical and chemical properties, and microbial readings of the samples were determined and recorded periodically during storage.

Microbiological tests

Microbiological tests were conducted before and immediately after treatment, and during the shelf stability study at 6°C. Bacteria count of the raw samples and NTP treated samples were determined by 3M petrifilm counts.

Physical and chemical tests

A digital thermocouple (Omega Engineering, Inc, Stanford, CT, USA) was used to measure the temperature difference in the sample before and after treatments.

Process optimization

Data obtained from previously mentioned activities was used for process optimization. The developed schemes and models were used to optimize and control critical process parameters, maximize energy and cost efficiency, and minimize any quality impact.

Electrical properties measurement

Table 1. Bacterial count of *E. coli* O157:H7 inoculated into milk before and after treatment with CHIEF

Strain	Inoculum (log CFU/mL)	Post-treatment count (log CFU/mL)	Population reduction (log CFU/mL)
ATCC43890	5.76	< 2.00*	≥ 3.76
ATCC43895	5.94	< 2.00*	≥ 3.94
ATCC35150	5.4	< 2.00*	≥ 3.40
86-24	7.94	4.79	3.14
Mixture of ATCC43890, 43895, 35150, 86-24 and 3081	8.05	4.16	3.88

* Detection limit of microbiological method was 100 CFU/mL. (Data reported by Dr. Francisco Diez-Gonzalez, University of Minnesota, Department of Food Science and Nutrition)

A monitoring system consisting of special circuit was used to record the high voltage characteristics. This system included a high voltage probe (Tektronix Model P6015A, voltage-dividing ratio 1000:1, USA, Beaverton, OR), and a current monitor (Pearson Current Monitor Model 411, Pearson Electronics, Inc. Palo Alto, CA, U.S.A) connected to a 100MB digital oscilloscope (Fluke 105 Scopemeter, Everett, WA, USA). The probe, monitor, and oscilloscope recorded the high voltage and current profiles. An IBM compatible personal computer was programmed (Testpoint software, Keithley, Cleveland, Ohio, USA) to control the process parameters such as voltage and frequency, and to acquire data from the monitoring sensors/devices.

Pilot scale continuous prototype system

A mobile continuous prototype system with a capacity of 10 L/h was designed and constructed based on the information obtained from research activities. The system was used for process development and verification.

Data analysis

The SAS statistical package was used for statistical treatment of the data. Analysis of variance (ANOVA) established the presence or absence of significant difference in microbial reductions, enzyme activities, and oxidation values. Kinetic models were used to describe the relationships between microbial reductions, enzyme activities, chemical and physical properties, and equipment and process variables. All statistical analyses were carried out using Minitab 14.20.0.

RESULTS AND DISCUSSION

Based on theoretical calculationa and experimental measurements, a cylindrical concentrated high intensity electric field reactor was developed (CHIEF). The reactor uses quartz glass as dielectric materials that are able to withstand the required high voltage without electric breakdown of the materials.

Preliminary experiments with CHIEF show that a :S 3 log E. coli O157:H7 vegetative cell reduction was achieved with a one-pass treatment when the applied voltage was 35-40kV and the exit temperature was below 60°C, respectively (Table 1).

A greater bacterial reduction at higher electric field was due to increased electric field and a combination of electric field and temperature. There is a synergetic effect between the applied electric field and temperature. A temperature of 60°C, although below the usual pasteurization temperature, may cause stress reactions that results in bacteria exhaustion and reduced resistance to electric field treatment. Stress reaction and bacteria exhaustion are mechanisms proposed for the hurdle technology that consists of a series of minimal processes including mild heat treatment.

A second study was conducted with CHIEF to confirm reductions in bacterial and spore counts. Mixtures of 5 strains of E. coli O157:H7 were tested independently. Table 2 shows that bacterial inactivation ranged from 2 to 3.9 log CFU/mL after a single pass through the CHIEF apparatus. Salmonella appeared to be more sensitive and less variable to the CHIEF treatment than E. coli O157: H7 as their microbial reductions varied from 2.6 to 3.1 log CFU/mL.

Strains of a Gram-positive pathogen bacteria, Listeria

Table 2. Effect of a single-pass CHIEF treatment on viable count of pathogenic bacteria inoculated into skim milk

Bacteria	Serotype	Initial count (CFU/mL)	Final count (CFU/mL)	Reduction (CFU/mL)
E. coli O157:H7 (5 strain mixture)	EC	8.00	5.25	2.74
Salmonella (4 strain mixture)	S-N	7.93	4.86	3.07
	S-Tn	8.16	5.04	3.11
	S-Ty	8.07	5.14	2.93
Salmonella (5 strain mixture)	S	8.09	5.14	2.95
Listeria monocytogenes (5 strain mixture)	LM	7.91	5.16	2.74
Bacillus cereus 3 strain mixture)	BC	3.56	3.38	0.18

S-N= all *Salmonella* strains except Newport AM05104; S-Tn = all *Salmonella* strains except Tennessee; S-Ty1= all *Salmonella* strains except UK-1; S-Ty2 = all *Salmonella* strains except ATCC700804; S-Ty3= all *Salmonella* strains except ATCC14028

Table 3. Effect of a double-pass CHIEF treatment on viable count of pathogenic bacteria inoculated into skim milk

Bacteria	Serotype	Initial count (CFU/mL)	Final count (CFU/mL)	Reduction (CFU/mL)
E. coli O157:H7 *(5 strain mixture)*	ECD	7.87	3.51	4.36
Salmonella (5 strain mixture)	SD	7.99	2.44	5.55
L. monocytogenes (5 strain mixture)	LMD	8.18	3.44	4.73

Data were reported by Dr. Francisco Diez-Gonzalez, University of Minnesota, Department of Food Science and Nutrition

Table 4. Shelf stability and total aerobic bacteria of CHIEF treated whole milk stored at 4°C

Sample	0 day (cfu/mL)	5 day (cfu/mL)	10 day (cfu/mL)	15 day (cfu/mL)	20 day (cfu/mL)
Untreated milk	2.8	3.7	4.9	6.7	–
CHIEF treated	1.9	2.2	3.5	4.4	6.2

monocytogenes, were similarly sensitive than Salmonella with average reductions of 2.75 (± 0.25). However, the CHIEF treatment did not seem very effective in inactivating spores of B. cereus, as no more than 0.35 log CFU/ml spores were inactivated by a single pass.

Table 3 shows that a serial treatment of two consecutive passes through the CHIEF device appeared to have an additive effect on the extent of killing vegetative pathogenic strains. The final count of Salmonella was increased almost two-fold from 2.95 to 5.55 average log CFU/mL reduction. This enhanced inactivation, however, was smaller for Listeria and E. coli O157:H7 as the additional pass only increased 77% and 59% killing compared to the single-pass treatment.

Table 4 shows the shelf life stability of raw whole milk using the CHIEF system. Initial populations of aerobic microorganisms in fresh raw milk were approximately 2.9 log (cfu/mL). Nearly 1 log reduction was observed following the CHIEF treatment. Aerobic bacteria increased during the storage time. CHIEF-processed milk lasted up to 14 days before exceeding the established total bacterial limits (4.3 log units), while the untreated control milk have a shelf life about 7 days. Final data on pathogenic bacteria and spores shows the

average (± standard deviation) microbial inactivation, after subjecting milk samples to a single pass through the CHIEF device, were 2.74 (± 1.0), 2.95 (± 0.35), 2.75 (± 0.25), and 0.18 (± 0.15) for E. coli O157:H7, Salmonella, L. monocytogenes and Bacillus cereus, respectively.

When milk samples were pumped twice through the CHIEF apparatus, reductions of 4.36 (± 0.24), 5.55 (± 0.14), and 4.78 (± 0.78) E. coli O157:H7, Salmonella, and L. monocytogenes, respectively. These latter results clearly suggest that a CHIEF treatment has the potential to deliver similar microbial inactivation effectiveness as a standard HTST pasteurization system. Further work is needed to consistently observe reductions greater than 5 CFU/mL of pathogenic vegetative cells as well as spoilage organisms.

This study demonstrates that the CHIEF system can produce stable whole milk with a shelf life of 14 days, which is comparable to that of thermal pasteurized milk.

PATENTS OR INVENTIONS

Deng, Ruan, Chen, Lin, and Metzger. A method for non-thermal pasteurization of fresh milk. In patenting process

ABSTRACTS AND PUBLICATIONS

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