종 설

Pulsed Electric Fields : An Emerging Food Processing Technology-An Overview

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PEF 처리에 의한 식품의 가공

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적 요

PEF 기법은 안전하고 천연상태에 가까운 식품을 생산하기 위한 최신의 비열처리법이다. 이 기법은 영양가, 풍미, 물성 및 관능적 성질의 영향을 최소화 시키는 안전한 식품생산에 이용할 수 있다. 이 공정은 두 전극사이에 놓여지는 식품에 고압의 전기를(20～80kv/cm) 작용시킴으로써 실시된다. PEF 처리에 의해 미생물의 세포막이 분해되거나 불한정하게되어 미생물은 활력을 상실하게된다. PEF 공정에 의한 미생물의 비활성화 정도는 주로 전기장에서의 펄스강도와 처리시간에 의존한다. 미생물의 종류에 따라 상응하는 전기장의 강도와 처리시간이 요구된다. 처리효과는 미생물의 특성과 존재했던 배지에서의 생육단계에 의존한다. 효과적인 가공을 위해 여러종류의 공정조건이 적절하게 사용되어야 한다. PEF기법의 잠재적효용은 생물공학에서 식품저장에 이르기 까지 다양하다. 식품가공과 관련해서 이 기법은 특히 에너지효율면에서 경제적이며 처리되는 식품이 가공공정의 영향을 적게 받는다는 것이 이미 입증되었다. 이 기법을 식품가공에 응용하기위해 본 논문에서 개괄적으로 고찰한다.

(**Key words** : Pulsed electric fields, Minimal processing, Mechanism of microbial destruction, Non thermal methods, Safe foods)

Ⅰ INTRODUCTION

Consumers demand for fresh like products with little or no degradation of nutritional and organoleptic properties has led to the study of new techn ologies in food processing and preservation (Calderon-Miranda et al., 1999). Pulsed electric field(PEF) is one of such food processing technologies which are currently being investigated to inactivate microorganisms and certain enzymes (Bendicho et al., 2003). The growing awareness among consumers for safe, additive free, nutritious and shelf stable fresh like food products warrants to look for novel methods of food processing for obtaining safe foods with minimal effects on nutritional, flavor, rheological and sensory qualities of food products (Jayaprakasha et.al., 1997; Kaushik and Rajorhia, 2000). In this context, PEF is an effective non thermal processing technology which could be used to inactivate microorganisms in liquid food stuffs(Calderon-Miranda et al., 1999a, Min et al., 2003).

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Application of this technology for inactivation of microorganisms is one of the promising non thermal processing methods(Jeyamkondan et al., 1999). PEF process can be applied at modest temperatures at which no appreciable thermal damage occurs and the original taste, color, texture and the functionality of the products can be retained (Dunn, 1996). The knowledge of human health in relation to diet, the changing demography and economic prosperity have certain to have significant influence on the novel methods of food processing and food products development(Selmon, 1992; Jayaprakasha et al., 2000). In this direction, PEF is emerging as a novel minimal processing technology for food industry to obtain safe, nutritionally and organoleptically better foods.

Ⅱ PROCESSING BY PULSED ELECTRIC FIELDS

PEF processing involves the application of high voltage(typically 20～80 kV/cm) to foods placed between two electrodes at ambient, sub ambient or slightly above ambient temperature for less than one second. The PEF processor consists of voltage pulse generator switch which controls the discharge of the types of waves from the capacitor. The capacitor stores the high energy from DC source and release at high intensity to the chamber which contains the electrodes, which conduct the high intensity pulsed electric fields through the food article to be treated. The temperature of the two electrodes in the treatment chamber is controlled by a cooling device that recirculates cooling water through the electrodes (Anon, 2000). The mechanism of this process involves storing electric energy in a high energy density electrical storage capacitor and releasing it in short, high intensity pulses, generating high peak power. Such high peak power pulses of electric energy can be used to create intense pulse of high electric fields. The high intensity of these pulses results in unique bactericidal action(Dunn, 1996). Different laboratories have designed, pilot scale and industrial scale chambers and used for PEF treatment of foods consisting of batch and continuous types..

Ⅲ MECHANISM OF MICROBIAL INACTIVATION

The mode of microbial destruction by PEF processing has been postulated in terms of electric breakdown and electroporation. Inactivation of microorganisms exposed to high-voltage PEF is related to the electromechanical instability of the cell membrane(Jeyamkondan et al., 1999).

1. Electric breakdown

The inactivation of microorganisms in a food system by PEF treatment is considered to be the cause of dielectric breakdown of the cell membrane leading to altering the functionality of the cell membrane as a semi permeable barrier, resulting in loss of membrane functionality and inactivation of microorganisms(Calderon-Miranda et al., 1999). Electric breakdown may occur due to compression of bacterial membranes under high intensity electric fields. In a capacitor filled with a dielectric material of a low dielectric constant the typical dielectric constant of the bilayer structure of a cell membrane is two study of inactivation of *Lysteria innocua* by PEF treatment revealed that there was complete rupturing of the cell membrane. As a consequence, there was loss of cell membrane activity(Calderon-Miranda et al., 1999).

2. Electroporation

Electroporation is the phenomenon in which cell is exposed to high a voltage electric fields which temporarily destabilizes the lipid bilayer and proteins of cell membrane. An electrically perforated cell membrane becomes permeable to ions and small probe molecules. As a result, the native colloid osmotic pressure altered. The cell swells due to the influx of water and when cell volume approaches about 155% of the original volume, swelling leads to rupture of the cell membrane and release of the cytoplasm contents and eventual lysis of cell takes place(Vega et al 1996). PEF is related to electromechanical instability of cell membrane. Microorganisms in the presence of PEF suffer cell membrane damage (Calderon-Miranda et. al., 1999a), as PEF induces membrane permeabilization results in leakage of cell contents(Russell et. al., 2000). PEF treatment causes irreversible rupturing of cell membrane causing apparent leakage of intracellular contents, leading to cellular damage(Rowan et al., 2000).

Ⅳ FACTORS INFLUENCING PEF PROCESSING

Factors involved in PEF processing can be grouped into three categories, namely, processing factors, product factors and microbial factors.

1. Processing Factors

The extent of destruction of microorganisms in PEF processing depends mainly on the electric field strength of the pulses and treatment time. These are the two most important factors involved in PEF processing(Jeyamkondan et al., 1999). For each cell types, a specific critical electric field strength(EC) and specific critical treatment time(TC) are required depending on the cell characteristics and the type of the medium where they have been present(Grahl and Markl, 1996).

The microbial inactivation increases with an increase in the electric field intensity, above the critical trans membrane potential(Qin et al., 1998). The critical electric field increases with the trans membrane potential of the cell(Jeyamkondan et al., 1999). It also depends on the number of pulses and the pulse duration. An increase in any of these variables increases microbial inactivation(Sale and Hamilton 1997). Critical treatment time also depends on the electric field intensity applied. Above the critical electrical field, critical treatment time decreases with higher electric fields. Protease activity of *Bacillus subtilis* inoculated milk decreased with the increased treatment time, field strength and pulse repetition rate(Bendicho et al., 2003). The extent of inactivation of microorganisms also depends on the method of application of electric field pulses. Electric field pulses may be applied in the form of exponential decaying, square wave, oscillatory, bipolar, or instant reverse charges. Oscillatory pulses are the least efficient for microbial inactivation, and square wave pulses are more energy and lethal efficient than exponential decaying pulses. Bipolar pulses are more lethal than mono polar pulses. Bipolar pulses also offer the advantages of minimum energy utilization, reduced deposition of solids on the electrode surface, and decreased food electrolysis(Barbora et al., 1999). PEF treatment at moderate temperatures(about 50℃ to 60℃) has been shown to exhibit synergistic effects on the inactivation of micro-organisms(Jayaram et al., 1992). This may be due to the increase in the electrical conductivity of the solution at the higher temperature(Marquez et al., 1997). Additional effects of high treatment temperature are, changes in cell membrane fluidity and permeability, which increases the susceptibility of the cell due to mechanical disruption(Hulsheger et. al., 1981). Also, a low trans membrane potential decreases EC and therefore increases inactivation (Jeyamkondan, et. al.,1999). A progressive decrease in population of *Lysteria innocua* was observed with the increasing in the electric field intensity(30, 40, and 50 kV/cm) and the number of pulses applied(10.6, 21.3 and 32), with the greatest reduction being 2½cycles(Calderon- Miranda et. al., 1999). A hurdle effect that is as the electric field, number of pulses and nisin concentration increased, synergism was observed in the inactivation of *Lysteria innocua*, as a result of exposure to nisin after PEF treatment(Calderon-Miranda et.al., 1999a). Bacterial cell *Lactobacillus plantarum* were inactivated only above the critical values of 13kV × cm^{-} and 64 kJ × kg(− 1). Below these values cell damage was reversible. PEF can results in both reversible and irreversible cell damage depending on specific treatment conditions(Ulmer et al., 2002). In fresh apple cider samples inoculated with *E.coli 0157:H7* strain *0157:H7*, and treated with 10, 20, and 30 instant charge reversal pulses at electric field strength of 60,70, and 80 kV/cm, at 20, 30 and 42℃, cell death increased significantly with increased temperature and electric field strengths. A maximum of 5.35 log 10 CFU/ml($P < 0.05$) reduction in cell population was achieved in samples treated with 30 pulses and 80 kV/cm at 42℃. Overall, a combination of PEF and heat treatment was recommended as an effective pasteurization technique for reducing the number of viable *E.coli 0157:H7* cells in fresh apple cider to meet U.S Federal Drug Administration recommendations(Iu and Griffiths, 2001). Similarly, Liang et al.,(2002), observed a decreased population of *Salmonella typhimurium* with the increased pulse number and treatment temperature. A 5.9 log cycle reduction in count was observed in the freshly squeezed orange juice treated at 90 kV/cm, 50 pulses and 55℃. When the PEF treatment was carried out in orange juice in the presence of nisin(27.5 U/ml of orange juice) or lysosyme(2400U/ml) or a mixture of nisin(27.5 U/ml and lysosyme(690U/ml), cell viability loss increased by an additional 0.04 to 2.75 log cycles, indicating the hurdle effects.

2. Product factors

The PEF processing of food is influenced by some the inherent product characteristics such as conductivity, pH, ionic strength and type of food. Foods with higher electrical conductivity generate smaller peak electric fields across the treatment chamber and therefore are not feasible for PEF treatment(Barbora et al., 1999). An increase in conductivity was known to decrease in microbial inactivation rate as a result of increase in the differences between the conductivity of a medium and microbial cytoplasm resulting in increased flow of ionic substance across the membrane. Thus, the inactivation rate of micro-organisms increased with decreasing conductivity even with an application of equal input energy(Jayaram et al., 1992). The ionic strength and pH of the medium affects the cytoplasm when the elecrtroporation is completed as a result of PEF treatment depending on the type of micro-organisms. Acidic pH of the system is known to enhance the microbial inactivation(Dunn, 1996). In general, the combination of factors(hurdles) such as pH, ionic strength and antimicrobial compounds during PEF treatment would be an effective means to aid in the inactivation of micro-organisms with PEF(Iu et al., 2001; Liang et al., 2002). Protease activity of *Bacillus subtilis* inoculated milk decreased with the increased treatment time, field strength and pulse repetition rate. However, the extent of inactivation levels was higher in skim milk compared to the whole milk. A maximum inactivation(81%) was estimated in skim milk after an 866 micros treatment at 35.5 kV/cm and 111Hz. Promising results were obtained for inactivating of microbial enzymes by PEF(Bendicho et al., 2003).

3. Microbial factors

The extent of inactivation of microorganism during processing of foods by PEF depends on type of micro-organisms, concentration of microorganism and growth stage of microorganism in the food system. Gram positive bacteria are more resistant to PEF than Gram negative bacteria (Hulsheger et al., 1983). In general, yeasts are more sensitive to electric fields than bacteria due to their larger size, although at low electric fields they seem to be more resistant than Gram negative cells(Qin et al., 1995). The sensitivity of microorganisms to PEF varies with the type of organisms, for example *Salmonella typhimurium (CRA 1005)* was more sensitive than *Listeria monocytogenes(NCTC 11994*) (Russell et.al., 2000). The number of microorganisms in a food system may also have an effect on their inactivation. In general, logarithmic phase cells are more sensitive to stress than lag and stationary phase cells. When a high proportion of cells are undergoing division, the cell membrane is more susceptible to the applied electric field. The extent of destruction in the logarithmic phase is 30% greater than of those in stationary phase of growth**.** PEF treatment is an excellent process for inactivation of microorganisms in acid and thermo sensitive media, but not for complete disintegration of microbial cells(Grahl and Markl, 1996). They have observed four order magnitudes(99.99%) reduction in the counts of vegetative cells of bacteria and yeast in buffer solution and liquid stuffs. On the other hand, PEF treatment could not inactivate or kill endo ascospores. PEF is a promising technology for the inactivation of E.coli. A 5 log reduction in the counts of *E coli 0157:H7* and *E coli 8739* was observed by PEF treatment of apple juice at around 35℃ temperature(Everendilek et al., 1999). A progressive decrease in the population of L. innocua was observed in PEF treated skim milk with the greatest reduction being 2½ log cycles when electric field intensities of 30, 40 and 50 kV/cm and the number of pulses applied was 10.6, 21.3 and 32(Calderon-Miranda et al., 1999a). Microbial lipases are known to be heat resistance. Pasteurization of milk could destroy extra cellular lipase from *Pseudomonas fluorescens* only to an extent of 5 to 20 %, where as PEF treatment at 27.4 kV/cm of 80 pulses could destroy lipase to an extent of 62.1% in skim milk, indicating that PEF is more effective than the pasteurization treatment(Bendicho et al., 2002a).

Ⅴ EFFECT OF PEF TREATMENT ON FOOD COMPONENTS:

The effect of PEF treatment on some of the food components in liquid food stuff was studied by Grahl and Markl(1996). They observed a low or negligible destruction of whey proteins, enzymes and vitamins, and non significant destruction of taste of the food stuffs. PEF is a non thermal food preservation process where organoleptic and nutritional properties of the foods are maintained(Calderon-Miranda et al., 1999). PEF process can be applied at modest temperatures at which no appreciable thermal damage occurs and the original taste, color, texture and the functionality of the products can be retained(Dunn, 1996). Single strength orange juice treated by PEF at electric field strength of 35 kV/cm for 59 micros could able to retain its aroma compounds, color and Vitamin C and had a shelf life of > 16 weeks in glass and PET bottles at 4℃ storage temperature(Ayhan et al., 2001).

The effect PEF treatments at room or moderate temperature on water soluble and fat soluble vitamins were evaluated and compared with conventional and thermal treatments(Bendicho et al., 2002). Samples were subjected to PEF treatments up to 400 microns at field strengths from 18.3 to 27.1 kV/cm and to heat treatments up to 60 min at temperature from 50~90℃. No change in vitamin content of milk was observed after PEF treatments or heat treatments except for ascorbic acid. After 400 micros treatments at 22.6 kV/cm, the extent of retention of ascorbic acid was 93.4%. Whereas, milk retained only 49.7% ascorbic acid after low temperature pasteurization(63℃ for 30 min) and 86.7% in case of high temperature short time pasteurization(75℃ for 15 sec). Single strength orange juice was processed by PEF at electric field strength of 35kv/cm for 59 microns and packaged in sterilized bottles juice had very good shelf life up to 112 days without any adverse effect on any of the flavor, colour or any other sensory attributes of the orange juice(Ayhan, et al., 2002).

Ⅵ APPLICATION OF PEF PROCESSING.

The potential application of PEF technology is numerous ranging from Biotechnology, cell biology to food preservation. With respect to food industry, the application is mainly in processing of liquid foods such as fruit juices, milk, sauces, liquid egg, pumpable food such as fruit or vegetable purees and particulate solids. Some of the potential food processing applications of pulsed electric fields to acid or non-acid foods are outlined in the table* and the various other possible application of this technology based on literature survey has been summarized below.

Processing of liquid milk by PEF could extend the storage life of heat pasteurized milk. Effective treatment of raw milk by PEF has been accomplished with less flavor degradation at 55℃ with bacterial destruction levels in excess of those obtained with conventional time-temperature pasteurization conditions. Such treated milk may be possible to be used in manufacture dairy products such as cheese, butter and ice cream with improved flavor without affecting any of the physicochemical and sensory attributes. In case of egg products, dairy protein preparations and other food ingredients, electric pulses could be applied with the aim of reducing the microbial load without altering functional(thickening, gelling, etc.) or flavor properties. For processing of acid foods($pH < 4.5$) such as fruit juices, the U.S. FDA has accorded approval for evaluation of food pasteurization by PEF Technology.

Only pumpable fluids can be processed in continuous flow, but solid foods of moderate thickness(〈=30mm) can be efficiently subjected to electric pulses only in batch type treatment chambers. These solid foods need be homogenous enough to maintain a uniform electric field, and that their moisture content should be sufficient enough for microbial inactivation. Such application may be extended for processing small fruits or fruit pieces, gelled desserts, flat or sliced products such as fish slices, eggs, shell fish, shrimps; slices of meat, cured meat and many more food products.

Superior tasting fresh juices can be obtained by PEF treatments with lower bacterial count as compared to conventional pasteurization. Such treated orange juice, for example, tastes like fresh squeezed juice, but with no microorganisms which also avoids thermal pasteurization of juices. Heat sensitive emulsion products such as salad dressings can be pasteurized without breaking down the emulsion integrity. Similarly food ingredients and sensitive foods such as flavors,

Acid fluid foods	Non acid fluid foods
Orange juice / other citrus juices	Liquid egg
Apple juice(fresh juice / concentrate)	Liquid egg white
Tomato concentrate, ketchup	Soups / vegetable juices
Fruit purees	Vegetable purees
Salsa sauce, Mayonnaise	Skim milk / whole milk
Spaghetti sauce with meat pieces	Functional proteins preparation
Liquid yoghurt with or without fruits pieces	Spreads, fillings, cereal dough, syrups, honey
Jams	Oil / water emulsions
Light alcoholic beverages	Minced meat, fish mince, surimi
Wines, soft drinks	Effluents from food factories

* Barsotti et al., 1999

protein concentrates, and fermented products can be pasteurized more gently with this technology, allowing retention of the quality parameter and resulting in superior quality. Fruits and vegetables (apple, carrot, grapes) can also be processed with the objective of improving extraction or penetration of solutes besides apparently improving the sensory qualities of juices.

Ⅶ CONCLUSION

Pulsed electric fields is a novel, non thermal emerging food processing technology for obtaining safe and minimally processed foods. This technology has multifaceted applications in processing of various kinds of foods. With the improvement in the design fabrications, better understanding of mechanism of microbial destruction and the effects on the various food components in relation to the physicochemical, nutritional and sensory properties foods may help in commercial exploitation of this technology in various food processing applications. In the years to come, innovative developments in high voltage pulse technology may reduce the cost of pulse generation and make PEF processing competitive with thermal processing methods. A coordinated research in this direction by involving the food technologist, engineers, food chemist and microbiologist is necessary for effective implementation of this technology for safe and economical food processing

Ⅷ ABSTRACT

Pulsed electric fields(PEF) technology is one of the latest nonthermal methods of food processing for obtaining safe and minimally processed foods. This technology can be effectively explored for obtaining safe food with minimum effect on nutritional, flavor, rheological and sensory qualities of food products. The process involves the application of high voltage(typically 20～80 kV/cm) to foods placed between two electrodes. The mode of inactivation of microorganisms by PEF processing has been postulated in terms of electric breakdown and electroporation. The extent of destruction of microorganisms in PEF processing depends mainly on the electric field strength of the pulses and treatment time. For each cell types, a specific critical electric field strength and specific critical treatment time are required depending on the cell characteristics and the type and strength of the medium where they have been present. The effect also depends on the types of microorganisms and their phase of growth. A careful combination of processing parameters has to be selected for effective processing. The potential applications of PEF technology are numerous ranging from biotechnology to food preservation. With respect to food processing, it has already been established that, the technology is non-thermal in nature, economical and energy efficient, besides providing minimally processed foods. This article gives a brief overview of this technology for food processing applications

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