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Review

Opportunities and challenges in pulsed electric field processing of dairy products

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ABSTRACT

Consumer demand and current market conditions warrant investigation of dairy processing technologies that can deliver improved product quality and stability and reduced energy use during processing, without compromising product and process safety. One candidate technology for the extension of shelf-life in dairy products is pulsed electric field (PEF) processing. PEF is considered to be an effective, non-thermal intervention that appears to hold some promise. Research on the application of PEF to control spoilage and pathogenic microorganisms and enzyme systems in dairy products spans a wide array of processing equipment and reaction conditions. PEF has been reported to effectively reduce the numbers of both pathogens and spoilage organisms in milk; however, there is a high degree of variability between studies. The application of PEF in combination with lower temperature thermal processing can deliver comparable reductions in microbial load without significant detrimental effects to the sensory and physico-chemical properties of food products.

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1. Introduction

Pulsed electric field (PEF) processing involves the application of very short, high voltage pulses to a food product, which is placed between or passed through two electrodes. The application of PEF can disturb and perforate biological cell membranes (Gásková, Sigler, Janderová, & Plásek, 1996). Typically, several thousand volts per cm applied for 20–1000 μs are required for effective inactivation of microorganisms in food systems (Toepfl, Heinz, & Knorr, 2007). It is likely that the loss of cell membrane functionality through PEF is due to formation of hydrophilic pores in the membrane, as well as the forced opening of protein channels (Teissie, Prats, Soucaille, & Tocanne, 1985).

PEF in combination with sub-pasteurisation temperatures has the potential of achieving microbial inactivation levels in milk equivalent to conventional thermal pasteurisation (Shamsi, Versteeg, Sherkat, & Wan, 2008; Walkling-Ribeiro, Noci, Cronin, Lyng, & Morgan, 2009). With the growing interest in bioactive functional food ingredients from dairy sources, it is desirable to preserve the lactoperoxidase activity and other valuable heat-labile molecules during preservation processes for dairy products.

2. Principles of inactivation of microorganisms by PEF

The inactivation of vegetative microorganisms by PEF treatment is usually determined by the applied electrical field strength, treatment temperature and total treatment time, rather than to electrolysis or ohmic heating (Heinz, Alvarez, Angersbach, & Knorr, 2002). However, due to the multitude of variable parameters of this technology, a systematic study of the individual influences of parameters on desired and undesired reactions is often required. The sensitivity of microorganisms to PEF depends on cell characteristics (i.e., structure and size) and extrinsic system factors such as water activity, pH, soluble solids, and electrical conductivity (Saldana et al., 2009; Toepfl et al., 2007).

Early studies often suggested that PEF-induced microbial inactivation can be described by first-order reaction kinetics (Grahl & Markl, 1996; Reina, Jin, Zhang, & Yousef, 1998). However, this log-linearity was often observed in batch PEF systems and for moderate inactivation levels. More recent studies indicate that microbial inactivation kinetics often exhibit pronounced tailing during continuous PEF processing and under conditions that achieve higher microbial reduction (Saldaña, Álvarez, Condón, & Raso, 2013).

To date, there is no clear evidence of the molecular mechanisms involved in the rupture of the cell membrane and inactivation of microorganisms, exposed to an electric field. The two current hypotheses of electrical breakdown and osmotic imbalance are widely accepted and based on the same principles.

The electrical breakdown hypothesis considers the cell as a membrane-bound capacitor filled with a dielectric medium, which is surrounded by a medium with a higher electrical conductivity (Zimmermann, 1986). The membrane-bound intracellular milieu (cytoplasm) and surrounding medium (e.g., a food system) have a greater electrical conductivity than the membrane itself. The differences in electrical conductivity on either side of the membrane cause a transmembrane potential (TMP) that is generated through accumulation of free charges at the inner and outer surface of the membrane (Jeyamkondan, Jayas, & Holley, 1999). Application of an external electrical field causes movement of ions inside and, outside the cell to accumulate on the membrane (Teissie et al., 1985). Ions of opposing charge on either side of the membrane are attracted to each other, compressing the membrane and reducing its thickness. An electrical breakdown or pore formation is induced when the electrical field strength exceeds a critical threshold value of the TMP. According to Hamilton and Sale (1967), the critical TMP value is approximately 1 V. The electrical field strength required to achieve an electrical breakdown of the cell membrane depends on cell size, shape, orientation in the electric field, dielectric characteristics of the liquid food, cytoplasm and membrane, and the treatment temperature. Membrane pore formation is often reversible during mild PEF processing. However, membrane breakdown will occur when the ratio of pore size or quantity in relation to the membrane surface area becomes too large.

The alternative hypothesis proposes that microbial inactivation occurs due to the loss of cell membrane functionality through changes of its molecular order. It is considered that PEF primarily destabilises dipolar molecules such as phospholipids and proteins in the cell membrane (Teissie et al., 1985). Protein channels also depend on the TMP and their opening and closing potentials are 50 mV below the critical TMP (Tsong, 1991). Hence, protein channels may open and/or denature (forming irreversible pores) due to localised ohmic heating or electrical modification of protein structures during PEF processing.

3. Applications of PEF for extension of chill-stability of dairy products

A major factor in the deterioration of raw milk quality during storage and transportation is the growth of psychotropic microorganisms. The deterioration is not only due to the ability of psychrotrophs to grow in raw milk, but also to the production of heat-stable extracellular proteases, lipases and phospholipases that lead to the spoilage of raw milk. Among the psychrotrophs, *Pseudomonas* spp. constitute the predominant microorganisms that limit the shelf-life of raw milk under refrigeration conditions. The quality of raw milk is governed by hygiene at the time of collection and determines the initial level of psychrotrophs present in the raw milk. This, combined with external factors including the storage temperature (on farm, during transportation and in factory silo prior to processing), has a further impact on the growth of the psychrotrophs prior to processing.

PEF processing can be used to extend refrigerated shelf-life of raw milk prior to further downstream processing in the dairy factory. Studies conducted by Craven et al. (2008) indicated that PEF was very effective in the inactivation of *Pseudomonas* spp., with a shelf-life extension of milk of up to 8 days at 4 °C. PEF treatments (31 kV cm^{-1}, 19.2 μs) of sterile (UHT) skim milk with added spoilage *Pseudomonas* isolates showed little microbial inactivation at PEF treatment temperatures of 15 °C or 40 °C compared with 50 °C or 55 °C. The greatest inactivation (5 log_{10}) was achieved through PEF processing at 55 °C (outlet temperature) and a total electrical energy use of 139.4 kJ L^{-1}. This inactivation was not due to the heat treatment, as similar temperature profiles without PEF caused only minimal inactivation (0.2 log_{10}) of *Pseudomonas*. The total microbial shelf-life of the PEF-treated skim milk was 13 and 11 days for inoculation levels of 10^3 and 10^5 cfu mL^{-1}, respectively. These results clearly indicate that PEF is effective in the inactivation of *Pseudomonas*, the major spoilage bacteria of milk (Craven et al., 2008).

The effectiveness of PEF in inactivating *Pseudomonas* has also been shown by Michalac, Alvarez, Ji, and Zhang (2003). UHT-treated skim milk was inoculated with 10^5 cfu mL^{-1} of *Pseudomonas fluorescens*, *Lactococcus lactis*, or vegetative cells of *Bacillus cereus*, held at 4 °C for 24 h and brought up to 22 °C immediately prior to processing. A continuous PEF bench-scale system was used to deliver bipolar square wave pulses of 3.0 μs at 35 kV cm^{-1} for a