CHAPTER 5

EFFECT OF PEF ON ENZYMES AND FOOD CONSTITUENTS

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1. INTRODUCTION

Food quality is of key importance in food preservation, even more in the context of novel technologies. The search for alternative methods to preserve foods is driven by the trends in consumption patterns. Consumers nowadays demand healthier, fresher, and more natural foods, with high sensory and nutritional qualities, and at the same time with the highest degree of safety. Pulsed electric field technology aims to offer the consumer these high-quality foods.

Processing may cause detrimental changes in food constituents, such as proteins, with the consequent possible modifications in sensory and nutritional characteristics. PEF technology has been presented as advantageous in comparison to, for instance, heat treatments, because it kills microorganisms while better maintaining the original color, flavor, texture, and nutritional value of the unprocessed food. However, whereas a considerable amount of research papers have been published on the microbial aspects of food preservation by PEF, a lesser amount of information is available about the effect of this technology on food constituents and overall quality and acceptability.

In this chapter, the effect of PEF technology on food constituents will be reviewed. Especial emphasis is dedicated to the potential of PEF to inactivate enzymes which may be responsible of product spoilage during storage. Also, the available information about quality attributes of various PEF-treated products currently under study is presented. Finally, limitations of current studies and research needs for the future are analyzed.

2. EFFECT OF PEF ON ENZYME ACTIVITY

Enzymes are capable of a specific manipulation of all the molecules found in foods, and, therefore a wide variety of enzymes are used as supplements for several food processing operations. However, there are certain enzymes whose activities result in a deterioration of food quality, that have to be controlled in order to maintain quality and extend food shelf life. The effect of PEF on both types of enzymes has been studied, and data available is described next. Treatment conditions and media are detailed in Table 5.1.

Alkaline phosphatase: Alkaline phosphatase is a milk endogenous enzyme that is routinely used to assess the correct application of pasteurization treatments. There are several isoenzymes but

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Table 5.1. Effect on pulsed electric fields on enzymes.

Enzyme	Treatment conditions	Residual activity (%)	Treatment medium	Reference
Alkaline phosphatase	18.8 kV/cm; 400 μs pulse width; 70 pulses; batch system	40	Raw milk	Castro *et al.* (1994)
	21.5 kV/cm; batch system; exponential decay	100	Raw milk	Grahl and Märkl (1996)
	80 kV/cm; 2 μs pulse width; 30 pulses; batch system; exponential decay	95	Buffer pH 9.8	Ho *et al.* (1997)
	6.7–20 kV/cm; 2 μs pulse width; 200 pulses; batch system; square wave	100	Milk	Van Loey *et al.* (2002)
Glucose oxidase	50 kV/cm; 2 μs pulse width; 30 pulses; batch system; exponential decay	25	Buffer pH 5. 1	Ho *et al.* (1997)
Peroxidase				
From soybean	73 kV/cm; 2 μs pulse width; 30 pulses; batch system; exponential decay	73	Buffer pH 6.0	Ho *et al.* (1997)
From milk	21.5 kV/cm; batch system; exponential decay	100	Raw milk	Grahl and Märkl (1996)
From milk	19 kV/cm; 5 μs pulse width; 100 pulses batch system	100	Raw milk	Van Loey *et al.* (2002)
From horseradish	5–25 kV/cm; 1.5 μs pulse width; 207–1242 pulses; exponential decay	65.3–83.3	Buffer	Zhong *et al.* (2005)
Polyphenoloxidase				
From mushroom	50 kV/cm; 2 μs pulse width; 30 pulses; batch system; exponential decay	60	Buffer pH 6.5	Ho *et al.* (1997)
From apple	24 kV/cm; 20 μs pulse width; 300 pulses; batch system; bipolar exponential decay	3	Buffer pH 6.5	Giner *et al.* (1997)
From peach	24 kV/cm; 20 μs pulse width; 400 pulses; batch system; bipolar exponential decay	48	Not reported	Giner *et al.* (1999a)
From pear	22 kV/cm; 20 μs pulse width; 300 pulses; batch system; bipolar exponential decay	30	Not reported	Giner *et al.* (1999b)
From mushroom	10–20–30 kV/cm; 5–40 μs pulse width; 1–1000 pulses; batch system	79–100	Water; Buffer different pHs	Van Loey *et al.* (2002)
From apple	7–31 kV/cm; 1–40 μs pulse width; 1–1000 pulses; batch system	90–100	Buffer different pHs; apple juice	Van Loey *et al.* (2002)
Lipoxygenase				
From soybean	10–20–30 kV/cm; 5–40 μs pulse width; 1–1000 pulses; batch system	90–100	Distilled water	Van Loey *et al.* (2002)
From pea	2.5–20 kV/cm; 1 μs pulse width; 100–400 pulses; batch system	100	Pea juice	Van Loey *et al.* (2002)

(*continued*)

Table 5.1. (*Continued*)

Enzyme	Treatment conditions	Residual activity (%)	Treatment medium	Reference
Lipase				
From milk	21.5 kV/cm; batch system; exponential decay	40	Raw milk	Grahl and Märkl (1996)
From wheat germ	87 kV/cm; 2 μs pulse width; 30 pulses; batch system; exponential decay	15	Distilled water	Ho *et al.* (1997)
From *Ps. fluorescens*	27.4–37.3 kV/cm; 4 μs pulse width; 100 pulses; batch and continuous systems; exponential decay	38–87	Simulated milk ultrafiltrate	Bendicho *et al.* (2002a)
Lysozyme	87 kV/cm; 2 μs pulse width; 30 pulses; batch system; exponential decay	>100	Buffer pH 6.2	Ho *et al.* (1997)
α-amylase	80 kV/cm; 2 μs pulse width; 30 pulses; batch system; exponential decay	15	Buffer pH 7.0	Ho *et al.* (1997)
Lactate dehydrogenase	31.6 kV/cm; 0.96 μs pulse width; 200 pulses; batch system; exponential decay	100	Buffer pH 7.2	Barsotti *et al.* (2002)
Pectinmethylesterase				
From orange	35 kV/cm; 1.4 μs pulse width; treatment time 59 μs; continuous system; 60°C	12	Orange juice	Yeom *et al.* (2000a)
From orange	35 kV/cm; 1 μs pulse width; 1000 pulses; batch system	90–100	Orange juice	Van Loey *et al.* (2002)
From tomato	24 kV/cm; 20 μs pulse width; 400 pulses; batch system; exponential decay	6	NaCl solution	Giner *et al.* (2000)
From tomato	10–20–30 kV/cm; 5–40 μs pulse width; 1–1000 pulses; batch system	90–100	Distilled water	Van Loey *et al.* (2002)
Proteases				
Plasmin	30–45 kV/cm; 2 μs pulse width; 50 pulses; continuous system	10	Simulated milk ultrafiltrate	Vega-Mercado *et al.* (1995a)
Ps. fluorescens M3/6	14–61.6 kV/cm; 16–98 pulses	40; >100; 100 (depending on treatment medium)	Skim milk; casein solution	Vega-Mercado *et al.* (1995b, 1997)
Bacillus subtilis	16.4–27.4 kV/cm; 4 μs pulse width; 80 pulses; batch and continuous systems; exponential decay	87; 110–115; 100 (depending on treatment medium)	Simulated skimmed milk ultrafiltrate; milk	Bendicho *et al.* (2005)
Papain	20–50 kV/cm; 4 μs pulse width; 500 pulses; continuous system; square wave	100; 15 (after 24 h storage)	EDTA solution	Yeom *et al.* (1999)

the most abundant is constituted by two monomers and each dimmer is able to link five molecules of zinc (Belitz *et al.*, 2004). Inactivation of alkaline phosphatase by PEF has been studied because milk pasteurization is one of the possibilities to implement PEF to the food industry. Castro *et al.* (1994) investigated the effect of PEF in milk with different fat contents. PEF treatments applied were 70 pulses of 400 μs and field strengths varying between 18 and 22 kV/cm. These treatments were able to reduce enzyme activity to a 40% of the original. On the contrary, Grahl and Märkl (1996), Ho *et al.* (1997), and Van Loey *et al.* (2002) did not observe a significant enzyme inactivation in either milk or aqueous solutions. This difference could be due to the different electrical parameters (the main difference was pulse width) or to a poor control of temperature that could lead to underestimate the effect of temperature on alkaline phosphatase inactivation, as this enzyme is not particularly heat-resistant. For instance, Van Loey *et al.* (2002) observed that 8000 μs at 10 kV/cm resulted in a 74% inactivation of alkaline phosphatase in raw milk. However, the authors attributed this inactivation to a thermal effect due to an increase in temperature that went up to 70°C.

Oxidases: The effect of PEF treatments on some oxidoreductases has also been studied. One of the main characteristics of this group of enzymes is that their active site normally links a metal molecule that participates directly in the catalysis reaction. For example, polyphenoloxidases have several copper molecules, peroxidases have a heme group, and lipoxygenases contain a nonheme iron placed in its active site. On the other hand, glucose oxidase active center is much more complex, and in this case, a histidine residue seems to be important for redox reactions catalyzed by this enzyme (Belitz *et al.*, 2004). A priori, this kind of enzymes appear sensitive to PEF treatments because of the important implications of redox reactions in their activity, but then once again, results obtained are contradictory.

Glucose oxidase is an enzyme that is used in food industry as a technological tool. Their main uses are the oxidation of glucose to minimize browning and as an antioxidant through its oxygen removal ability. Ho *et al.* (1997) studied the effect of PEF on glucose oxidase obtained from *Aspergillus niger*. After 60 μs at 50 kV/cm (and higher field intensities) a reduction of enzyme activity to the 25% of the original was attained.

Peroxidases have also been studied. Ho *et al.* (1997) studied a peroxidase from soybean in phosphate buffer pH 6.0. Only a moderate reduction (to 70% of the original activity) could be achieved after 200 μs at 73 kV/cm. On the other hand, Grahl and Märkl (1996) studied the effect of PEF on lactoperoxidase in raw milk, and inactivation was negligible. Van Loey *et al.* (2002) obtained the same result: even after energy inputs as high as 500 kJ/kg, no decrease in lactoperoxidase activity was observed. Zhong *et al.* (2005) studied the effect of PEF on horseradish peroxidase. After 1821 μs at 22 kV/cm, only a reduction to the 65% of the original activity was observed. The authors also studied conformational changes using circular dichroism spectra analysis and fluorescence spectrum and noticed some conformational changes (mainly a loss in α-helix content) but without obtaining a clear conclusion.

Polyphenoloxidases (PPO), a group of enzymes, play an important role in quality food of plant origin because they are responsible of enzymatic browning, have also been studied. Ho *et al.* (1997) noticed an inactivation of mushroom PPO up to a 60% of the original activity after 60 μs at 50 kV/cm. Giner *et al.* (1997, 1999a,b) studied the effect of PEF on polyphenoloxidases from pear, peach, and apple. Experimental conditions used in their experiments were the following: exponential pulses in mono and bipolar mode, pulses of 20–100 μs and 3–24 kV/cm. No specification of temperature measurement and control were given although it is mentioned that temperature never exceed 25°C. Results obtained by this research group show an important effect of PEF in the inactivation of PPO. Apple PPO activity was reduced to a 3% of the original activity after 6000 μs at 24 kV/cm. Peach PPO activity could be reduced to a 30% by after 8000 μs at 24 kV/cm. For pear PPO a maximal reduction of 48% was noticed after 6000 μs at 22.3 kV/cm. On the contrary, no inactivation effect

of PEF on PPO was noticed by Van Loey et al. (2002). It has to be mentioned that this study is the most complete and important carried out about the effect of PEF treatments on enzymes relevant to food technology. Indeed, their work with oxidoreductases deserves to be mentioned apart. This research group tested the inactivation of peroxidase, polyphenoloxidase, and lipoxygenase using different field strengths (10, 20, and 30 kV/cm), two different pulses widths (5 and 40 μs), two pulse frequencies (1 and 100 Hz), and a number of pulses varying from 1 to 1000, depending on the field strength applied. Within these experimental conditions, they did not find an inactivation higher than 10%. There was only an exception, corresponding to long processing times applied with a low pulse frequency and a high number of pulses. Under these experimental conditions, an inactivation of 64% for lipoxygenase and 21% for polyphenoloxidase were noticed. In any case, the authors mentioned that the significant inactivation of these enzymes under these conditions was not caused by the high-voltage pulses but by an artifact, namely a small off-state current of the IGBT switch. The intensity of the off-state current varied with the voltage level charged on the capacitor. If treatment times are long, inactivation of these metal-containing enzymes would be due to electrochemical reactions with the electrodes surface rather than to the electric field.

Lysozyme: This enzyme is widely distributed and it is found in egg, many mammal tissues and secretions, in latex exudates of some plants, and in some fungi. It is an *N*-acetylmuramidase that hydrolyzes the cell wall of Gram-positive bacteria and therefore it has found application in food technology as a preservative (Belitz *et al.*, 2004). It consists of a peptide chain with 129 amino acids residues and four disulphide bonds. Ho *et al.* (1997) studied the effect of PEF treatments (60 μs at 87 kV/cm) on this enzyme and reported an activation of lysozyme (an increased activity) after PEF treatments.

Lipase: Grahl and Märkl (1996) studied the effect of PEF on lipase in raw milk and reported an inactivation to the 40% of the original activity. Ho *et al.* (1997) also studied the effect of PEF treatment on lipase from wheat germ. After 60 μs at 87 kV/cm, a reduction to a 15% of original activity could be achieved. Bendicho *et al.* (2002c) studied the effect on a commercial lipase from *Pseudomonas fluorescens*. In this case, lipase could be inactivated to a 38% of the original activity in batch treatments and only to an 87% of the original activity in a continuous system. The authors explain this difference on a basis of the difference in the applied voltage.

When working with enzymes from psychrotrophs, low-temperature-inactivation phenomenon has to be taken into account as well as the possibility of contamination with proteases (Owusu *et al.* 1991). Low-temperature-inactivation consists of a quite curious phenomenon: some of the exogenous enzymes from psychrotrophs (mainly proteases and lipases) inactivate faster at mild temperatures than they do at higher temperatures. There is not a clear explanation but proteolysis and conformational changes that lead to an unstable structure have been used to account for this experimental observation.

α-amylase: Ho *et al.* (1997) studied also an amylase from *Bacillus licheniformis*. This enzyme is used as a technological tool to hydrolyze 1.4, α-glucans. After 60 μs at 80 kV/cm, an inactivation to the 15% of the original activity was noticed.

Lactate dehydrogenase: Barsotti *et al.* (2002) studied the effect of PEF treatments on lactate dehydrogenase, a tetrameric enzyme that it is stabilized by electrostatic interactions. The main reason to study this enzyme was not its importance in food science but its sensitivity to treatments as freezing or high-pressure processing, probably due to a dissociation of the tetrameric structure. After 192 μs at 31.6 kV/cm and 30°C, no lactate dehydrogenase inactivation was observed. According to this result, it can be concluded that high-voltage pulses in the experimental conditions used are not able to break the electrostatic interactions that stabilize the tetramer.

Pectinmethylesterase: Pectinmethylesterase (PME) catalyzes the desterification of pectin molecules. De-esterified pectin molecules are able to interact through calcium bridges leading to a cloud loss and phase separation in juices. Stabilization of cloud in juices requires the inactivation

or inhibition of PME. Yeom *et al.* (2000a) investigated the effect of PEF on PME from Valencia oranges. The maximum inactivation achieved was to the 12% of the original activity after 59 μs at 35 kV/cm. These results disagree with those obtained by Van Loey *et al.* (2002), that reported that after 1000 μs at 35 kV/cm residual PME activity was about 90% of the original. In citrus, several PME isoenzymes, that differ in thermostability, have been found. Most of the problems of cloud stability are caused by the thermoresistant isoenzyme which is only about 2–15% of the total PME activity (Snir *et al.*, 1996), varying with cultivar, degree of ripening, and other factors. Juice cloud stability not only depends on total PME activity, but mainly in the inactivation PME fraction with clarifying capacity. According to this, the effectiveness of PEF treatment in cloud preservation will not be determined only by residual PME activity as will be discussed later. Giner *et al.* (2000) decided to investigate the inactivation on PME from tomato by PEF. A high inactivation was achieved (to a 6.2% of the original activity) after 8000 μs at 24 kV/cm.

Proteases: Most of the enzymes used so far to evaluate the effects of PEF on enzymatic activity have been proteases and the variety of results obtained is tremendous: inactivation, no effect, and even activation. Vega Mercado *et al.* (1995a) studied plasmin, an alkaline protease present in bovine milk. Their results were impressive: plasmin activity decreased to a 10% of the original activity after 100 μs at 30–45 kV/cm at 15°C. The same research group investigated the effect of PEF treatments on an extracellular protease from *Pseudomonas fluorescens* M3/6 (Vega-Mercado *et al.*, 1995b, 1997). With this enzyme results obtained varied depending on the treatment medium. Using TSB, a reduction of 80% of protease activity was achieved; if skimmed milk was used instead of TSB, an increase in proteolytic activity was noticed. When treatments were performed in casein–tris buffer, no protease inactivation was found. This variation in the results reported could perhaps be attributed to an autolysis phenomenon, as it will be discussed later. Yeom *et al.* (1999) investigated the effect of PEF on papain. The activity of papain did not change after PEF treatments, but a change in the behavior during storage depending on the PEF treatment applied was reported. The authors also studied conformational changes and the oxidation of the cysteine residue of the active site and concluded that the inactivation that papain suffers during storage after PEF treatments is due to a loss of the α-helix structure and not to an oxidation of cysteine active site. However, inactivation by proteolysis was not ruled out. Bendicho *et al.* (2005) studied PEF treatments on *Bacillus subtilis* protease. In their investigations, changes in enzymatic activity were only observed when PEF treatment was applied using a continuous flow device with coaxial electrodes. If treatment medium was SMUF (simulated milk ultrafiltrate), a slight inactivation was noticed. On the other hand, if PEF was carried out in milk an enhancement in proteolytic activity was found.

None of the previous works has taken into account autolysis to discuss results. Autolysis is one of the most important mechanisms that could inactivate a proteolytic enzyme and a global view of available data obtained PEF effects on proteases could give some clues about its possible role in the inactivation of proteolytic activity by PEF. First of all, in most of the proteases studied, reported results vary from inactivation to activation of the proteolytic enzyme, depending on treatment medium composition. The presence in PEF treatment medium of proteins could protect enzyme from autolysis acting as substrate for the proteases. Another factor to be taken into account is that, in the first PEF equipments, there was a possibility of a poor control or measurement of temperature. A higher temperature could lead to an increase in autolysis rate. Finally, another possibility is that PEF could originate small conformational changes leading to an enhanced proteolytical activity. Anyway, it is necessary to elucidate this possible mechanism in further investigations.

Summarizing the effects on enzymes: The observed effects of PEF on enzymes by different research groups seem to depend on several factors such as the enzyme, the PEF apparatus, PEF treatment conditions, and medium. One of the main problems is that there is not a standardization of PEF equipment, so the experimental conditions largely vary among the different studies, and in some of them there is a lack of details. This is the main reason explaining why it is very difficult to obtain

conclusions from results obtained at very different conditions of field strength, number of pulses, pulse width and shape, and batch or continuous systems. Another important factor that has to be taken into account is temperature control. Sometimes, the effect of temperature has not been considered and this is quite important if the enzymes are not specially thermoresistant (which is common in the experiments performed with PEF and enzymes) and a critical factor when working with proteases, because of the autolysis. It is possible to estimate a theoretical increase in temperature from the total energy input, assuming that all the electrical energy is dissipated as heat, but heat dissipation toward the environment depends mainly on the thermal properties of the materials used in the chamber, and also on its design. The main PEF parameter that seems to affect the stability of enzymes is pulse duration, even more that field strength.

Although there are some reports about the effect of PEF treatments on enzymes, further research is needed if this emerging technology has to be applied to preserve food. The first problem encountered is that, despite the diversity of results reported, it seems clear that the resistance of enzymes to PEF is higher than that showed by microorganisms. Moreover, whereas the mechanisms of vegetative cellular inactivation by PEF are more or less clear, or at least under study, for enzymes there is still a large number of questions without answers. As enzymes are proteins and some protein structures are stabilized by electrostatic interactions, it seems reasonable that PEF should somehow affect their stability, as will be discussed later. This would also apply to those enzymes that have metals as copper or iron in their active site, and therefore prone to be affected by redox reactions. Although some authors have reported changes in conformation, more research is needed to clarify the mechanisms through which enzymes could be activated or inactivated by PEF. Moreover, clarifying whether enzyme inactivation observed after some PEF treatments is not due to PEF itself, but to a thermal effect or electrochemical reactions is very important to fully exploit PEF technology for food preservation.

Another question still to sort out is whether the inactivation level reached is adequate for food preservation or not. Which is the inactivation level that we have to attain to preserve a food from enzymatic deleterious processes? This is a question that food scientists still have to answer in most cases. There is insufficient knowledge on the relationship between residual activity and quality deterioration. Anyway, contrary to microorganisms, enzymes do not multiply in food so the inactivation level required is lower for enzymes. But the inactivation of enzymes achieved with PEF treatments is not sufficient to control deleterious enzymes because in most of the studies, less than a decimal reduction is achieved. For example, it has been established (Eagerman and Rouse, 1976) that for cloud preservation in orange juice it is necessary to achieve two decimal reductions in PME activity (reduce the PME activity to 1% of the original). For PME inactivation by PEF, in the most intense conditions used and with the most sensitive isoenzyme, only a reduction to 12% of the original activity is noticed. So in this case, the inactivation effect, it is not sufficient to control enzymatic activities.

An additional drawback to implement PEF for controlling enzyme activity in foods is that most of the experiments have been performed in model systems. Their properties are very different from those of food systems as fruit juices, egg, or milk. Medium composition is very important because it determines both the parameters of PEF treatments and also the behavior of enzymes.

All these reasons indicate that still much research effort is needed to clarify the effects of PEF on enzymes under various experimental conditions.

3. EFFECT OF PEF ON FOOD CONSTITUENTS

As it has been reviewed in Chapter 4 of this book, it is well demonstrated that in several conditions, PEF is able to efficiently inactivate vegetative cells of microorganisms. However, there is a relative lack of knowledge about the effect of this emerging technology on food components. Next, the information about the effect of PEF on proteins, fats, vitamins, and pigments is presented and

discussed. However, it is noteworthy that the number of research works is scarce and results obtained are sometimes contradictory. A summary of the information available is presented in Table 5.2.

3.1. Proteins

Protein constituents of foods provide, in addition to nutritive value, a desirable textural quality. These properties are determined by protein structure and behavior. The native form of a protein is held together by a delicate balance of forces: hydrophobic, ionic, and van der Waals interactions, hydrogen and disulfide bonds. Each protein has a particular structure that is also maintained by different type of bonds. Native proteins (and enzymes) have at least, three structural levels that can be affected during food preservation treatments. The primary structure is determined by the specific amino acid sequence of the polypeptide chain. The secondary structure is related to the regular arrangements of the polypeptide chains in α-helix, β-sheet, and turns, and it is mainly maintained by hydrogen bonds. The tertiary structure refers to the arrangement of the secondary structure into globular units or domains. This level is stabilized by different types of bonds (hydrophobic, ionic, and van der Waals interactions, hydrogen and disulfide bonds) depending on the protein. Some proteins may also have an additional structural level, the quaternary structure, which is referred to the association of different monomeric subunits.

For protein denaturation and enzyme inactivation the first step that takes place is normally protein unfolding. This phenomenon is considered as reversible, and consists of a conformational change due to a modification of the balance of forces that maintains the native structure. Sometimes, the protein is able to recover its native structure, but in other cases, this structural change is followed by a rearrangement that leads to an inactive structure. After unfolding, some secondary events, that are highly specific for individual proteins, may take place. These events are either covalent changes, which result in chemically modified proteins, or noncovalent changes (Klibanov, 1983), which may lead to an incorrect folding (if they only affect to one molecule) or to aggregation (if several protein molecules are involved).

Protein structure is very dependent on environmental factors such as pH, presence of denaturating agents, soluble solids, or ionic strength, which can affect the delicate equilibrium that maintains the native structure. How PEF treatment can affect protein structure? It seems reasonable that the application of high-voltage pulsed electric fields could ionize some chemical groups and also break electrostatic interactions inside a polypeptide chain or between two monomeric units of protein. Anyway, the investigations performed till now are often descriptive and do not throw light on the possible mechanisms that could operate in protein modification by pulsed electric fields.

As the potential implementation of PEF in food preservation would be mainly related to liquid foods, it is necessary to study possible changes in proteins of liquid food systems as egg or milk. Available data about the effect of PEF on food proteins is mainly focused on egg white proteins (ovoalbumin and egg white) and β-lactoglobulin, the most abundant protein in cow's milk whey and its primary gelling agent.

Ovoalbumin is the major protein of egg white and also the main determinant of gelling properties of egg and its products. It is a phosphoglycoprotein with four thiol groups that are buried within the structure and that become exposed when the protein unfolds. Fernández-Díaz *et al.* (2000) studied the effect of exponential decay pulses (180 μs at 31.5 kV/cm) on ovoalbumin solutions. Their first observation was that ovoalbumin solutions (2% protein content) submitted to PEF treatments showed an increased reactivity of sulphydril groups. Nevertheless, this was a transient change because if ovoalbumin PEF-treated solutions were kept at 4°C during 30 min, thiol groups became less reactive again. According to this observation, PEF treatments would not induce permanent modifications in ovoalbumin. The authors also analyzed the fourth derivates of the UV spectra of native and PEF-treated ovoalbumin and spectra of both proteins were identical. As this method detects structural

changes in the environment of aromatic amino acid residues, this result suggests that no protein unfolding was induced by PEF treatments. A possible hypothesis given by the authors, which could explain why thiol groups became more reactive without changing the structure, is that PEF treatment increased the ionization of SH to S^-. As the thiol groups of ovoalbumin appear to be relatively close to the protein surface, their enhanced ionization could take place without important changes in the protein structure. Taking into account all the results obtained in this research work, it can be concluded that treatment conditions applied do not induce a significant unfolding or aggregation of ovoalbumin.

There are also some reports on the effect of PEF treatments on egg white. Jeantet *et al.* (1999) measured the surface hydrophobicity of egg white proteins and did not find any increase after PEF treatment, suggesting that no protein denaturation occurs. Fernández-Díaz *et al.* (2000) also found that PEF treatments did not induce significant changes in the gelling properties of dialyzed egg white.

Professor Cheftel's research group also studied the effect of PEF treatments on β-lactoglobulin solutions at different protein concentrations (Barsotti *et al.*, 2002). After the application of PEF treatments (up to 260 μs at 30 kV/cm) to β-lactoglobulin solutions with protein concentrations ranging from 2 to 12% w/v, no changes in the fourth derivate of the UV spectra were observed and no changes in PAGE and PAGE–SDS electrophoretic patterns were detected. PEF treatments on higher protein concentration β-lactoglobulin solutions (16.7%) did not induce changes in turbidity and viscosity. According to these results, it can be concluded that PEF treatments did not cause significant β-lactoglobulin unfolding or aggregation. Also, Ma *et al.* (1998) have reported no changes in the electrophoretic patterns of liquid whole egg treated at a selected electric field strength of 48 kV/cm and a maximum treatment time of 120 μs (temperature below 40°C). Even the most heat-sensitive proteins, such as the globulins responsible for the foaming ability of egg white, were unaffected.

Moreover, Li *et al.* (2005) have investigated the effect of processing on bovine immunoglobulin G secondary structure using circular dichroism spectrometry. PEF treatment at 41.1 kV/cm for 54 μs with bipolar pulses did not cause detectable changes in its secondary structure or its thermal stability.

Recently, a new approach to the application of PEF in food technology has emerged. PEF could be used to modify the relationship between structure/function of proteins. This new idea has its basis on the application of long length exponential pulses (ms instead of μs), less number of pulses, and a lower field strength. Perez and Pilosof (2004) studied the effect of PEF treatments on ovoalbumin and β-lactoglobulin. PEF conditions used were the following: exponential decay pulses of 2 ms, field strength was 12.5 kV/cm, and a number between 1 and 10 pulses. It has to be noted that conditions applied in this work are not sufficient to inactive microorganisms. Results obtained both for ovoalbumin and β-lactoglobulin show that PEF treatment can induce protein denaturation that leads to a partial aggregation. PEF treatment also affects the gelling properties of the proteins: enhancing gelation rate of β-lactoglobulin and decreasing that of egg white. The authors also proposed a model to explain the effects of PEF on proteins that involves several possible mechanisms: (1) a polarization of the protein molecule; (2) a dissociation of the quaternary structure by breaking noncovalent bonds; (3) changes in protein structure that lead to the exhibition of hydrophobic and thiol groups that were previously inside the protein core; and (4) if the duration of electric pulse was high enough, the formation of aggregates. Anyway, all the proposed mechanisms have to be proven and further research is needed to clarify this hypothesis.

Apart from the possible effect of long pulses at low electric field strengths described above, the results obtained so far strongly suggest that PEF could be applied to foods at intensities high enough to reduce microbial load without damaging the protein structure and functional properties.

3.2. Fats and Emulsions

Very few studies have been carried out to study the effect of PEF treatments on fats and fat-based foods. Only scattered data are available about the distribution of oil droplets in

Table 5.2. Effect of PEF treatments on foods.

Food	Treatment conditions	Characteristics studied	Comments	Reference
Milk	40 kV/cm, 40 μs, pulses 2 μs width	Physical and chemical properties (not specified)	Nd[a]	Qin *et al.* (1995)
		Sensory evaluation	No difference with heat-pasteurized milk	
	Nonspecified	Whey protein content	Nd[a]	Grahl and Märkl (1996)
		Vitamin A	Nd[a]	
		Vitamin C	90% loss	
		Sensory evaluation	Nd[a]	
	29 kV/cm, 200 μs, exponential decay pulses, 0.8 μs width	Fat globule size and distribution	Nd[a]	Barsotti *et al.* (2002)
	35 kV/cm, 188 μs, bipolar square wave pulses 2.9 μs width	Protein content	Nd[a]	Michalac *et al.* (2003)
		Total solids	Nd[a]	
		Color	Nd[a]	
		pH	Nd[a]	
		Particle size	Nd[a]	
		Density	Nd[a]	
		Electrical conductivity	Nd[a]	
	18.3–27.1 kV/cm, 400 μs, exponential decay pulses	Vitamin retention	Nd[a], except vit C (maximum loss, 30% approx.)	Bendicho *et al.* (2002b)
Yogurt-based products	30 kV/cm, 32 μs, square monopolar pulses 1.4 μs width + mild heat (60°C, 30 s)	Color (L, a, b)	Nd[a]	Yeom *et al.* (2004)
		pH	Nd[a]	
		°Brix	Nd[a]	
		Sensory evaluation	Nd[a]	
Orange juice	35 kV/cm, 59 μs	Pectinmethylesterase activity	90% decrease	Yeom *et al.* (2000a,b) and Ayhan *et al.* (2001, 2002)
		Vitamin C content	Nd[a]	
		Flavor compounds retention	Depending on the compound (100–130%)	
		Browning index	Nd[a]	
		Color (L, a, b)	Nd[a]	
		Particle size	Smaller	
		°Brix	Nd[a]	
		pH	Nd[a]	
	35 kV/cm, 60–87 μs	Flavor compounds retention	95–99%	Qiu *et al.* (1998)
		Vitamin C retention	96–95%	
	30 kV/cm, 240–480 μs, square waveform pulses, 2 μs width	Flavor compounds retention	97–91%	Jia *et al.* (1999)
	80 kV/cm, 40–60 μs, bipolar pulses, 2–3 μs width	Vitamin C retention	97.5%	Hodgins *et al.* (2002)
		Aroma compounds	Nd[a]	
		Pectinmethylesterase activity	92.7% reduction	
	40 kV/cm, 97 μs, pulses 2.6 μs width; maximum temperature 58°C for 5 s	Vitamin C retention	Nd[a]	Min *et al.* (2003a)
		Flavor compounds retention	98–81%, depending on the compound	
		Color (L, a, b)	Higher L value and hue angle	

(*continued*)

Table 5.2. (*Continued*)

Food	Treatment conditions	Characteristics studied	Comments	Reference
		Sensory evaluation	Same rating in color and appearance, but lower rating in texture, flavor, and overall acceptability than untreated control	
Tomato juice	40 kV/cm, 57 μs, bipolar square waveform pulses, 2 μs width; maximum temperature 53.5°C for 5 s	Flavor compounds retention	93–110%, depending on the compound	Min and Zhang (2003) and Min *et al.* (2003b)
		Ascorbic acid content	Nd[a]	
		Non-enzymatic browning degree	Lower than untreated control	
		Color (L, a, b)	Nd[a]	
		Lycopene content	Nd[a]	
		Particle size	Smaller	
		°Brix	Nd[a]	
		pH	Nd[a]	
		Viscosity	Nd[a]	
		Sensory evaluation	Preferred to thermally processed juice (92°C for 90 s)	
Cranberry juice	20–40 kV/cm, 50–150 μs, square waveform pulses, 2 μs width	Anthocyanin content	Nd[a]	Jin and Zhang (1999)
		Color (L, a, b)	Nd[a]	
		Aroma compounds retention	Nd[a]	
Apple juice	22–34 kV/cm, 166 μs, bipolar square waveform pulses, 4 μs width	Color (L, a, b)	Nd[a]	Evrendilek *et al.* (2000)
		Vitamin C retention	Nd[a]	
		Sensory evaluation	Slight preference for untreated juice	
	50–66 kV/cm, 2–16 pulses	Soluble solids	Nd[a]	Zárate-Rodríguez *et al.* (2000)
		pH	Nd[a]	
		Acidity	Nd[a]	
		Color	Lightening	
Fruit beverage	28 kV/cm, 100–600 μs, square waveform pulses, 2 μs width	Vitamin C retention	96–87%	Sharma *et al.* (1998)
		Color (L, a, b)	Nd[a]	
		Protein denaturation	6–7%	
		Viscosity	Nd[a]	
Horchata	20–35 kV/cm, 100–475 μs $T^a < 35°C$	pH	Nd[a]	Cortés *et al.* (2005)
		Total fat	Nd[a]	
		Peroxide index	Nd[a]	
		TBARS (thiobarbituric acid-reactive substances index)	Nd[a]	
		Formol index	Nd[a]	
IgG-enriched soymilk	Up to 41 kV/cm, 54 μs, bipolar square wave pulses	IgG activity	Nd[a]	Li *et al.* (2003)
		Color (L, a, b)	Nd[a]	
		Electric conductivity	Nd[a]	
		°Brix	Nd[a]	
		Viscosity	Nd[a]	
Liquid whole egg	25 kV/cm, 250 μs, bipolar square wave pulses, 2.12 μs width + mild heat (55°C, 3.5 min)	Viscosity	Nd[a]	Hermawan *et al.* (2004)
		Electrical conductivity	Nd[a]	
		Color (L, a, b)	Nd[a]	
		pH	Nd[a]	
		°Brix	Nd[a]	
	35 kV/cm, 20 μs, 2 μs width + 0.15% w/v citric acid	Sensory evaluation	No difference with commercial brand (scrambled eggs); preferred to commercial brand (overall appearance)	Qin *et al.* (1995)

Nd[a]: No difference with untreated control.

emulsions treated by PEF and fat oxidation degree. Barsotti *et al.* (2002) studied the effect of PEF treatments (200 μs, exponential decay pulses, at 29–32 kV/cm) on the fat globules size distribution of various oil-in-water emulsions, determined by Malvern laser granulometry. Despite the fact that the stability of protein-stabilized emulsions is partly charge-dependent, no marked modifications, nor in the size droplet distribution nor in the stability indexes could be detected. The only noticeable effect caused by PEF treatments was the rupture of fat globules aggregates into smaller droplets, in a similar way as anionic detergents such as SDS do. However, the extent of this droplet dispersion effect was very small. These conclusions were obtained in model oil-in-water emulsions stabilized by β-lactoglobulin, and further confirmed in food emulsions, specifically pasteurized half skimmed milk, pasteurized whole milk, and dairy cream (35% fat).

Also, the degree of lipid oxidation seems not to be affected by PEF. Cortés *et al.* (2005) have reported that TBARS index (thiobarbituric acid-reactive substances index) of horchata, which is a fat-rich vegetable beverage typical from Spain (more than 2% fat), did not vary with treatments of 100–475 μs at 20–35 kV/cm. The authors concluded that these treatments did not cause oxidation of fatty matter.

3.3. Vitamins

It is generally acknowledged that PEF treatments do not affect vitamin content and therefore PEF-treated foods should be nutritionally more complete than heat-treated foods. However, these assumptions have been made upon a limited number of results. From the scarce studies published up to date, it can be concluded that most vitamins are not affected by PEF treatments of such an intensity enough to attain a reasonable degree of microbial inactivation. The only vitamin that seems relatively more sensitive is ascorbic acid.

Early studies by Grahl and Märkl (1996) reported that high-energy input PEF treatments (>300 kJ/L) did not affect vitamin A content. However, they reported a 90% destruction of ascorbic acid (vitamin C). The experimental conditions applied were not specified. In any case, this is probably the only study reporting a high degree of loss of ascorbic acid. Bendicho *et al.* (2002a) investigated the retention of various water-soluble (thiamine, riboflavin, and ascorbic acid) and fat-soluble vitamins (cholecalciferol and tocopherol) in milk and simulated milk ultrafiltrate (SMUF) after PEF treatments of up to 400 μs at field strengths from 18.3 to 27.1 kV/cm. Also, the effect of the processing temperature was evaluated (20–25°C vs. 50–55°C). Thermal treatments at 63 and 75°C were also applied for comparison purposes. The content in fat-soluble vitamins and thiamine and riboflavin did not change with neither PEF treatments nor thermal treatments tested. According to results reported by Grahl and Märkl (1996), ascorbic acid content decreased after PEF treatment. However, the percentage of retention of this water-soluble vitamin was much higher, both in SMUF and in milk, than the 10% reported by Grahl and Märkl (1996). For instance, retention of ascorbic acid in milk was 93% after 400 μs at 22.6 kV/cm. This percentage of retention was higher than that determined by the same authors for both low (63°C, 30 min, 49.7% retained) or high (75°C, 15 s, 86.7% retained) heat pasteurization treatments. Under the more severe treatment conditions used in this study (27 kV/cm for 400 μs), ascorbic acid loss was approximately 20%. Similar experimental conditions have been proven to be effective to attain 2 log cycles inactivation of *Listeria monocytogenes* and 4 log cycles inactivation of *Salmonella dublin* in milk.

Loss of ascorbic acid increased exponentially with treatment time at any electric field strength tested, following first-order kinetics. However, there was not a relationship between loss of ascorbic acid and electric field strength. In other words, PEF treatments carried out at 18 kV/cm were more deleterious for this vitamin than treatments performed at 22 kV/cm. In any case, maximum loss of ascorbic acid reported was 30%.

An interesting observation is that ascorbic acid retention seemed to be higher in milk than in SMUF, indicating that protein content of milk may exert a protective effect on this vitamin.

The retention of ascorbic acid has also been studied in orange, apple, cranberry juice, and also a fruit-based drink. Most investigations have also reported either slight reductions or no reductions in the content of vitamin C (Evrendilek *et al.*, 2000; Yeom *et al.*, 2000a; Hodgins *et al.*, 2002; Min *et al.*, 2003a,b). Evrendilek *et al.* (2000) treated apple juice at 34 kV/cm for 166 μs (bipolar pulses of 4 μs length). Under these experimental conditions, they achieved a 4.5-log cycles reduction in the population of *E. coli* O157:H7, and no decrease in the ascorbic acid content. Also, Min *et al.* (2003b) analyzed ascorbic acid content of tomato juice processed at 40 kV/cm for 57 μs, and they concluded that there was no decrease due to PEF treatment.

3.4. Pigments

Color is one of the most important parameters determining food acceptability. It is well known that foods with altered color are rejected by the consumer. Perception of food color by the human eye depends on several factors, which include water content and distribution, occurrence of browning reactions, or light scattering due to fat globules, among others. One of the most relevant parameters in many foods of vegetable and animal origin determining color is the content in natural pigments. Type and nature of natural pigments is quite variable, and so is their stability to preservation procedures. Most investigations about color retention in PEF-treated foods use the Hunterlab measurement system, and scarce data are available on the stability of single pigments. However, data available on both water-soluble and fat-soluble pigments suggest that they are not significantly degraded during PEF treatments.

The concentration of anthocyanins in PEF-treated cranberry juice did not change after treatments of 50–150 μs at 20 and 40 kV/cm (Jin and Zhang, 1999). Min *et al.* (2003b) studied the effect of a PEF treatment (40 kV/cm, 57 μs) in tomato juice on lycopene concentration. Lycopene is a carotenoid pigment responsible for the red color in tomato. PEF treatment did not cause any decrease in the lycopene concentration of tomato samples. Also, the color (Hunter lab color parameters) of a nutraceutical beverage, containing annatto and turmeric as sole colorings, was not modified by a treatment of 28 kV/cm for 200 μs (Sharma *et al.*, 1998).

The influence of PEF treatments in color of various food products is described more extensively below in this chapter.

4. GENERATION OF NEW COMPOUNDS

The safety of PEF processing, specifically the possibility of the electric current generating harmful compounds, has raised some concern. Obviously, before new food preservation procedures get approval from the corresponding regulatory bodies, clear evidence of the safety of the processed foods has to be obtained.

Since the initial development of PEF technology, it has been questioned whether the lethal effect observed on microorganisms was due to the electric field itself or to compounds or ions generated by electrochemical reactions. It is known that many chemically active species can be produced by an electric discharge in the food and by electrode processes, which may decompose the chemical structure of liquids close to the electrode surfaces (electrolysis), eventually producing toxic chemical species, such as oxygen peroxide, hydroxyl radical, or chloride ions. If this was so, toxic species could increase the total lethality of the PEF process on microbes, which is desirable effect, but they

could also cause undesirable chemical reactions affecting quality and, more important, cause harmful effects on the consumers.

Some authors have investigated this topic. Hülsheger and Niemann (1980) suggested that hypochloric acid (HClO), generated from chloride in buffers under the action of an electric field, contributed to the lethality of PEF treatment, but Wouters *et al.* (1999) later demonstrated that the inactivation degree of *Listeria monocytogenes* in buffer systems containing chloride ions was not significantly higher than in buffers without chloride. They argued that the length of the pulse is a critical parameter controlling undesirable electrochemical reactions. In other words, if pulses are short enough, electrochemical reactions have little chance to take place (Morren *et al.*, 2003).

Studies available have shown that undesirable chemical species appear extensively when electric fields are applied in the form of high-voltage arc discharges and needle-shaped electrodes. This is not the case for PEF treatments in most laboratories and pilot-plant scale equipments worldwide. Stainless steel plate–plate geometry electrodes, short duration pulses, and bipolar pulses (Morren *et al.*, 2003) seem to be effective means in controlling the electrolysis reactions.

In summary, available data regarding the possible generation of toxic compounds by PEF treatments is inconclusive, and research in this direction is needed, although it appears that under adequate processing conditions the electrolysis phenomenon is minimum.

5. QUALITY OF PEF-PROCESSED FOODS

This section tries to summarize the information available about various quality parameters of PEF-processed foods, such as color, flavor retention, protein functionality, among others. Potential applications include mainly liquid or semisolid foods which can continuously flow between two electrodes. The use of PEF in solid foods has generally a different objective, which is not the production of a safe and stable food, but the extraction of components or acceleration of processes, and is dealt with in detail in other chapters of this book. Thus, this section is focused on the effects of PEF on constituents and quality parameters of liquid foods.

5.1. Milk

Milk was the first product proposed to be processed by PEF. Many studies have been focused on the inactivation of several pathogenic and spoilage microorganisms, as well as various enzymes of interest. Results regarding microbial inactivation clearly show that PEF could be an adequate alternative to heat pasteurization treatments, since it attains between 3 and 6 log cycles of destruction of most vegetative pathogenic species studied. Concerning enzyme deactivation, results are contradictory, as it has been described in Section 2 of this chapter. Nevertheless, information available suggests that enzyme inactivation in milk is lower than in buffer systems, and that insufficient inactivation of lipases and proteases could be expected. However, enzymatic activity of lipases and proteases, while is the most important factor determining shelf life of sterilized milks, play a minor role in pasteurized milk stability.

Although it is assumed that the small amount of heat generated during PEF should not cause detrimental changes in milk components and properties, little research effort has been done to prove it. There is a lack of documentation about the effect of PEF treatments under different experimental conditions on milk constituents, and conclusions exposed in this section have been taken from scattered investigations.

Qin *et al.* (1995) suggested that milk could be processed at 40 kV/cm for 80 μs, and a maximum temperature of 50°C, rendering a product with a shelf life of 2 weeks in refrigeration. According to

these authors, no apparent changes in the physical and chemical properties of milk were induced. Grahl and Märkl described in 1996 that several vegetative cells could be effectively inactivated by PEF treatments while some components such as vitamin A and whey protein did not undergo changes. Sensory evaluations showed no deterioration of milk. However, experimental conditions were not detailed. The lack of effect of PEF on milk properties was confirmed by Michalac *et al.* (2003). These authors used a PEF treatment consisting of 188 μs (bipolar pulses of 3 μs length) at 35 kV/cm in a continuous bench scale system. Maximum temperature attained by processed milk was 52°C. Color, particle size, total solids content, protein content, pH, electrical conductivity, viscosity, and density analysis showed no differences between PEF-treated and heat-pasteurized (73°C/30 s) milk. However, reduction of the natural microflora of milk was greater in heat-pasteurization (2.7 log vs. 1 log).

Also, distribution of fat globules in milk and cream does not change with PEF treatment, as it has been discussed previously (Barsotti *et al.*, 2002). Vitamins are also preserved after PEF treatments, except ascorbic acid, whose content decreases slightly (Bendicho *et al.*, 2002b). Finally, structural studies on β-lactoglobulin (Barsotti *et al.*, 2002) also support the view that no major modifications are caused by PEF treatments in proteins of milk.

5.2. Juices

Fruit juices are perhaps the most suitable products to be processed by PEF. Currently, juices have to be heat-treated to be commercialized either at refrigeration or at room temperature. Fruit juices can undergo changes in their quality due to microbiological growth, enzymatic activities, and also chemical reactions. Stability is generally assured by the combination of the heat treatment, the acidity of the product, and sometimes, refrigeration temperature. On the other hand, compounds responsible for flavor in juices are heat-sensitive, and thus heat-treated juices are perceived as significantly different from raw juices. For these reasons, PEF has been proposed as a possible advantageous alternative to current heat-processes. Unfortunately, due to the scarce efficacy of PEF against mold ascospores (Raso *et al.*, 1998) and some enzymes, PEF processing is viewed as an alternative to heat treatment for pasteurized, but not for sterilized juices. In other words, the obtained product would normally have to be kept under refrigeration.

Orange, cranberry, apple, and tomato juices have been processed by PEF in bench and/or pilot plant equipments (Qiu *et al.*, 1998; Jia *et al.*, 1999; Jin and Zhang, 1999; Evrendilek *et al.*, 2000, 2001; Yeom *et al.*, 2000a,b; Ayhan *et al.*, 2001, 2002; Hodgins *et al.*, 2002; Min and Zhang, 2003; Min *et al.*, 2003a,b).

Several authors have focused their investigations on orange juice because it is the most consumed one. Characteristics such as °Brix and soluble solids, acid content, flavor, color, nutritional content, or cloudiness are among those that determine citrus juices quality, together with microbiological quality. Very few systematic studies are available in which all the quality parameters are studied. Nevertheless, from studies published to date it can be concluded that any of the quality parameters of orange juice is negatively affected by PEF processes of intensity levels high enough to produce a microbiologically acceptable product. This means 5–6 decimal reductions in the natural flora and a shelf life longer than 3 months under refrigeration. Some particular parameters such as flavor or color seem even to be improved.

Yeom *et al.* (2000a) and Ayhan *et al.* (2001) performed a series of studies about various aspects of orange juice quality comparing untreated samples, PEF-treated samples (35 kV/cm for 59 μs, temperature under 60°C) and heat-treated samples (95°C for 30 s). They also analyzed the maintenance of the quality along the storage under several conditions of temperature and packaging materials. Parameters studied were pectinmethylesterase (PME) activity, vitamin C content, flavor

compounds content, browning index, color (L, a, and b values), particle size (Malvern granulometry), °Brix, and pH. Microbial load decreased from 10^3 cells/mL, mainly yeast, to less than 10 cells/mL. The processed juice kept these microbial counts for at least 110 days, either at 4°C or at 22°C. Simultaneously, PME activity was decreased by 88%. Other authors have reported similar inactivation percentages of PME in orange juice after 40–60 μs at 80 kV/cm (Hodgins *et al.*, 2002). Yeom *et al.* (2002) also reported that the inactivation level of PME treated by PEF in orange juice could not be solely attributed to the heat generated in the sample during processing, but mainly to the electric field itself. Whether a 90% PME average inactivation is enough for a long shelf life product under refrigeration is something that has yet to be determined, as it has been discussed above.

The content in ascorbic acid is almost not affected by PEF treatment. Initial losses, measured immediately after PEF treatment at various electric field strengths and total treatment times, ranged from 0 to 5% (Qiu *et al.*, 1998; Yeom *et al.*, 2000a; Hodgins *et al.*, 2002; Min *et al.*, 2003a). Decreases in the content of this vitamin are much more marked during storage than due to the treatment itself, especially if treated samples are stored in oxygen-permeable containers (Ayhan *et al.*, 2001).

PEF-treated orange juice maintains color attributes similar to those of untreated juices. Browning index (absorbance at 420 nm) and color parameters of PEF-treated juice are not significantly different from the untreated one (Ayahn *et al.*, 2001), and consistently better than those corresponding to heat-treated juices. In most cases L, a, and b parameters of PEF-treated orange juice have been found to be identical to untreated controls, and only occasionally, they have shown brighter and more yellowish color (Ayhan *et al.*, 2002), possibly due to the smaller particle size of PEF-treated juice.

Also, physicochemical parameters such as pH or °Brix were not modified by PEF processing as compared to untreated controls. Changes in pH and °Brix are mainly associated to microbial activity during storage.

Further attention has been dedicated to flavor compounds modification after PEF treatments. This is due to the great importance of flavor in orange juice consumers' acceptability. Orange juice flavor consists of more than 200 compounds of different chemical nature. The headspace content of some hydrophobic compounds involved in flavor has been found to remain similar or even increase after PEF processing (Ayhan *et al.*, 2002). This is the case for limonene, myrecene, valencene, and α-pinene, whose content increased by 18–32% after 59 μs at 35 kV/cm. The authors suggested that this increase was due to a release of these components from their hydrophobic environment by PEF. Polar compounds such as octanal, decanal, linalool, and ethyl butyrate remained unchanged. Also, little losses in flavor compounds, ranging from 0 to 11%, have been reported by several other authors (Qiu *et al.*, 1998; Jia *et al.*, 1999; Hodgins *et al.*, 2002; Min *et al.*, 2003). These losses could probably be minimized through a better control of treatment temperature, that occasionally reaches values close to 60°C in continuous-flow pilot plant equipments.

Cranberry juice is characterized by its special flavor and attractive color. Cranberry juice treated at 40 kV/cm for 150 μs had a microbial load similar to a heat-treated sample (90°C/90 s) (Jin and Zhang, 1999). However, retention of volatile compounds examined by headspace solid-phase microextraction gas chromatography has showed that PEF-treated samples could not be distinguished from untreated controls, indicating that PEF treatment does not alter flavor or aroma profile of cranberry juice. On the contrary, heat-treated samples had a significant altered flavor profile. Also, color of cranberry juice, which is mainly attributable to its anthocyanin content, was not modified by PEF. Anthocyanin pigments are particularly sensitive to heat treatments and oxidations. Whereas thermal treatment significantly reduced the anthocyanin content and modified juice color, PEF treatment did not cause any noticeable change (Jin and Zhang, 1999; Evrendilek *et al.*, 2001). In addition, the

concentration of the anthocyanin pigments decreased in a similar manner in untreated controls and in PEF-treated samples during storage at 4°C for 14 days.

A limited number of studies by Zhang and colleagues have focused on *tomato juice* PEF processing. Tomato juice acceptability is highly determined by flavor and color. With regards to flavor, in tomato juice there are more than 400 volatile compounds derived from fatty acids, amino acids, and carotenoids. Similarly to what it has been described for orange juice, PEF treatment either does not change or even increases the content of some flavor-related compounds (Min and Zhang, 2003). A PEF treatment of 40 kV/cm for 57 μs (maximum temperature 53.5°C) caused an increase in *trans*-2-hexenal to a 110% and in 2-isobutylthiazole to a 108%, with respect to the untreated control. The authors attributed this effect to a decreased particle size of tomato juice during PEF processing and a subsequent increased release of flavor compounds. Also in this case, nor decrease of ascorbic acid has been reported for PEF-treated tomato (Min *et al.*, 2003b), nor changes in °Brix, pH, and viscosity. The absence in viscosity changes has been attributed by the authors to the inactivation of the pectic enzymes during the hot break procedure employed before PEF treatment.

Tomato juice color is one of its most important quality attributes, and depends on factors such as the content in lycopene and the development of browning reactions, among others. PEF-treated tomato juice did not show differences in lycopene content and Hunter *a/b* ratio with respect to untreated juice (Min *et al.*, 2003b). In addition, brown color development was slower than in untreated juice. These data suggest that color of tomato juice is well preserved by PEF processing.

Finally, a similar trend has been observed for *apple juice*. Little, if any, changes in soluble solids, pH, acid content (Zárate-Rodríguez *et al.*, 2000), color, and ascorbic acid content (Evrendilek *et al.*, 2000) have been reported for PEF-treated apple juice.

Some research papers report sensory evaluation of PEF-treated juices, orange and tomato (Min and Zhang, 2003; Min *et al.* 2003a,b). Results from these sensory evaluations show that PEF-treated juices are preferred in terms of color, appearance, texture, flavor, and overall acceptability to heat-treated ones. However, these results, as well as other comparisons that have been mentioned along this chapter, have to be taken with caution since thermal treatments applied for comparison are often quite intense (90–92°C for 90 s). These heat treatments are close to those required for the production of stable products, and in fact, higher microbial stability and enzyme deactivation is reported, as compared to PEF treatments. More research is needed to determine the sensory acceptability of PEF-treated juices.

In summary, despite some aspects still need further research, fruit juices can be processed by PEF with substantial reduction of the natural microflora and endogenous enzymatic activity, without major changes in essential quality parameters such as color, flavor, cloudiness, and ascorbic acid content. This makes fruit juices one of the most attractive and suitable products to be processed by this technology.

5.3. Egg Products

Egg products, liquid whole egg, egg white, or egg yolk, are normally heat-treated and distributed frozen, dehydrated, or refrigerated. Heat pasteurization is required to guarantee the inactivation of pathogenic microorganisms such as *Salmonella*, which are common contaminants of these foods. The effects of heat on egg constituents are especially harmful because egg products are used as ingredients in the manufacturing of many other foods, due to their foaming, emulsifying, and gelling properties, among others. Therefore, the maintenance of their functional quality, besides their microbiological quality, is essential. Most of the functional properties of egg and egg products rely on their proteins, which are especially thermosensitive. Thus, nonthermal methods that guarantee the microbiological

safety and stability of egg and derivatives have been sought for years. PEF is one of the procedures that have been proposed. The first difficulty encountered for the application of PEF treatments on egg and egg derivatives is the high conductivity of these foods. Most PEF equipments currently available require samples of low conductivity to obtain electric field strengths of high intensity, therefore some of the research works published have been performed with egg products with reduced salt content, through dialysis or ultrafiltration.

As described before in this chapter, most authors have reported no protein coagulation after different PEF treatments in liquid whole egg or egg white (Jeantet *et al.*, 1999; Fernández-Díaz *et al.*, 2000; Ma *et al.*, 2001). With regards to other quality parameters of egg and egg products, very little is known. Qin *et al.* (1995) reported that liquid whole egg with 0.15% citric acid processed at 35 kV/cm for 20 μs and at a maximum temperature of 45°C was preferred over a commercial brand in an acceptance test. Also, scrambled eggs prepared with PEF-treated were not distinguished from a control in a triangle test. Hermawan *et al.* (2004) did not detect significant changes in the viscosity, °Brix, and color parameters (L, a, and b), between untreated liquid whole egg controls and samples treated by PEF (25 kV/cm, 250 μs) plus a following heat treatment at 55°C for 3.5 min. These treatment conditions were chosen to obtain a product with a long shelf life in refrigeration temperatures (more than 60 days) since PEF alone resulted insufficient. It is noteworthy that the combination of PEF processing with moderate heat treatments and/or antimicrobial substances has been suggested by various researchers as the most suitable alternative (Calderón-Miranda *et al.*, 1999; Hermawan *et al.*, 2004; Jeantet *et al.*, 2004) to completely assure the safety of the product with regards to pathogenic microorganisms such as *Salmonella* or *Listeria*.

From these data, it can be concluded that PEF processing is unlike to cause any detrimental effect either on egg protein functionality or color, viscosity, flavor characteristics, proven treatments are carried out at low temperatures. However, information available is scarce and more research effort is needed to fully characterize possible changes in egg product treated by PEF, especially in combination with moderate heat treatments.

5.4. Other Foods

Some other liquid foods and solid foods have been tested for PEF processing.

The maintenance of the immunoactivity of bovine IgG in soymilk subjected to PEF and thermal treatments was studied by Li *et al.* (2003). PEF treatments (41 kV/cm, 54 μs) resulted in no significant loss of antigen-binding activity, whereas thermal treatment yielded 86% decrease. It is noteworthy that both procedures (PEF and thermal treatment) were equivalent in terms of natural flora destruction (5.1–5.3 logs). No changes in color parameters (L, a, and b), electric conductivity, °Brix, or viscosity were detected in PEF-treated milk at various electric field strengths from 26.0 to 38.4 kV/cm, with respect to the untreated controls. This opens the possibility of producing functional foods processed by PEF with better characteristics than those of heat-pasteurized ones.

Also, the possible application of PEF to preserve horchata has been explored (Cortés *et al.*, 2005). Horchata is a beverage made up of tiger nut, which is characterized by a short shelf life of only 48 h. It is rich in starch; consequently it cannot be heated above 72°C to prevent gelation. Shelf life of horchata could be extended with either no fat oxidation or free amino acids release, and lower peroxidase activity than the untreated controls.

The effect of combined mild heat and PEF treatments (60°C/30 s plus 30 kV/cm/32 μs) on physical and sensory characteristics of high-viscosity yogurt-based products has been studied by Yeom *et al.* (2004). Color parameters, °Brix, and pH values of treated samples were identical to those of untreated controls. Sensory evaluation detected no changes in appearance, color, texture, flavor, and overall acceptability between untreated and treated samples.

6. CONCLUDING REMARKS

As it has been exposed along the chapter, there are some inconsistencies regarding the effect of PEF on food constituents, mainly on enzymatic inactivation, probably related to different experimental conditions among laboratories. Further research effort is required to clarify the degree and mechanisms of inactivation of enzymes by PEF before this technology can be implemented for food preservation purposes.

With regards to the effect of PEF treatments on quality parameters of liquid foods, and despite a wider range of experimental conditions and proper comparison with heat-treated and untreated foods would be desirable, it can be concluded that PEF, applied at treatment intensities to obtain pasteurized-like products, exerts a small impact, if any, on protein stability, fat globules distribution, vitamin content, color, flavor, general appearance, and most of the quality parameters of milk, juices, egg products, and some other foods. Juices and other particularly sensitive liquid food seem most adequate for PEF processing.

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