



Review

Feasibility of using pulsed electric fields to modify biomacromolecules: A review



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ABSTRACT

Background: The challenges encountered in the utilisation of biomacromolecules as functional ingredients can be overcome through modification of their structural elements. Recently, researchers have shown an increased interest in the usage of non-thermal or chemical free modification techniques to improve the stability and function of primary ingredients in the food, biomedical and pharmaceutical applications. This has led to the investigations of pulsed electric fields (PEF) technology as an alternative technique for enhancing modification of chemical reactions and microstructure of biomacromolecules.

Scope and approach: The goal of this paper was to conduct a systematic review on the effect of PEF on the functional properties of proteins, polysaccharides and their blends, focusing on the configurational and conformational modifications in the microstructure due to the application of micro/millisecond electric field.

Key findings and conclusions: PEF has potential to modify the microstructure and functional properties of biomacromolecules. PEF-induced modifications follow two primary mechanisms, i.e. electrochemical reactions and polarisation of the structural moieties. Critical PEF treatment intensity (E_c) is required for the onset of the microstructural changes in biomacromolecules. These changes are influenced by the settings and configuration of the PEF equipment, product characteristics (molecular weight, pH, conductivity) and system temperature. If properly managed, PEF treatment and subsequently the changes in molecular properties (i.e. molecular disintegration and network formation) could be tailored for the production of superior micro-/nano-structured products for various applications.

1. Introduction

Biomacromolecules (i.e. biological macromolecules) such as proteins, polysaccharides, organic polyoxoesters, oils, polythioesters, polyphenols among others (Ashogbon & Akintayo, 2014) have unique structural and functional characteristics which open various applications in food, biomedical and pharmaceutical industries. Proteins and polysaccharides are amphiphilic biomacromolecules that for the most part are responsible for the microstructure, stability, physicochemical and mechanical properties of biological materials and colloidal systems (Benichou, Aserin, & Garti, 2002; Fischer, 2013). Proteins possess the ability to adsorb into the oil-water interface (surface activity) to form unique networks and structures (Bouyer, Mekhloufi, Rosilio, Grossiord, & Agnely, 2012). The stability of these structures is maintained by polysaccharides, which also contribute to the water holding capacity (Sánchez-González, Arab-Tehrany, Cháfer, González-Martínez, & Chiralt, 2014; Walter, 1998). Protein-polysaccharides intermolecular

interactions, also known as supramolecular complexes, are essential in providing rheological and functional properties of colloidal systems due to their aggregation and gelation behaviour (BeMiller & Huber, 2015; Fischer, 2013; Rodríguez Patino & Pilosof, 2011).

Development of functional nano-/micro or macrostructures from the native form of biomacromolecules is still a challenge due to their weak structural stability (Bouyer et al., 2012; Turgeon, Beaulieu, Schmitt, & Sanchez, 2003). This weakness is prompted by poor water-macromolecules interaction, which causes phase separation due to variations in the application conditions (temperature, pH, moisture content) (Benichou et al., 2002; Fischer, 2013; Rhim & Perry, 2007). Therefore, there has been a growing interest in recent years to improve both the microstructure and functional properties of biomacromolecules (Ashogbon & Akintayo, 2014; Mirmoghtadaie, Shojaee Aliabadi, & Hosseini, 2016; Wang et al., 2016a; Zhu, 2015). Previous studies have presented three main categories of biomacromolecule modification techniques. (i) Thermal (heating, ohmic processing) (Zhu, 2015), (ii)

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Chemical (enzymes, acid, salt) (Carvalho, 2016) and (iii) Non-thermal/chemical free (irradiation, high hydrostatic pressure and ultrafiltration) (Mirmoghataie et al., 2016). However, it is also becoming increasingly necessary to reduce the time and costs associated with the modification process (Wang et al., 2016a). Besides, researchers are aiming at eliminating physical damage to biomacromolecules (Perez & Pilosof, 2004) as well as reducing undesired by-products that may require further purification (Polikovskiy et al., 2016; Puértolas, Luengo, Álvarez, & Raso, 2012).

Recent research has suggested that pulsed electric fields (PEF) processing could significantly alter the microstructure and macromolecular interactions of biomacromolecules (Hong, Chen, Zeng, & Han, 2016a; Ma, Yu, Zhang, & Wang, 2012). PEF processing involves the application of short pulses (μs – ms) of high voltage electric field to materials placed between two electrodes (Barbosa-Cánovas, Góngora-Nieto, Pothakamury, & Swanson, 1999). The energy dissipated during PEF can cause ionisation of functional groups in biomacromolecules (Wiktor et al., 2015). It can also break electrostatic interactions in macromolecular chains resulting in cleavage or coalescence of monomeric units (Fernandez-Diaz, Barsotti, Dumay, & Cheftel, 2000; Yao et al., 2011; Zhao & Yang, 2012). For example, during the PEF treatment of protein biomacromolecules, electric fields can interact with the dipole moments of peptides causing ionisation of the carboxylic ($-\text{COOH}$) and amino groups ($-\text{NH}_3^+$) (Li, Chen, & Mo, 2007). The presence of charged dipoles on similar proteins units leads to the development of electrostatic attractions, which causes unfolding and aggregation of proteins and subsequent disruption of the secondary structure (α -helix) (Liu, Zeng, Deng, Yu, & Yamasaki, 2011). However, little research has been conducted to understand the mechanism and functional properties of PEF-modified biomacromolecules systems.

Meanwhile, PEF-induced modification of the dipole chemical reactions and the resulting conformational changes in the state of biomacromolecules can provide an opportunity for incorporation and blending of several biomacromolecules (Hong, Zeng, Buckow, Han, & Wang, 2016b; Sun, Yu, Zeng, Yang, & Jia, 2011). For instance, Hong et al. (2016b) observed substitution of hydroxyl groups ($-\text{OH}$) by acetyl groups ($\text{O}=\text{C}-\text{CH}_3$) and an increase in granular hydrophobicity after PEF treatment of cassava starch-acetic anhydride system (specific energy (Q) = 42–117 kJ/L). Besides, Sun et al. (2011) reported a considerable increase in protein-polysaccharide inter-/intramolecular interactions through hydrogen bonding between dextran and whey protein isolate (WPI). The primary importance of PEF over conventional processing methods is the minimal increase in temperature during the treatment, which may permit the use of the technique in temperature-sensitive biomacromolecules (Polikovskiy et al., 2016). To this end, it is evident that PEF could provide an economically viable and acceptable method (Morales-de la Peña, Elez-Martínez, & Martín-Belloso, 2011) for modification of biomacromolecules. This review discusses the mechanisms involved in the PEF-induced modification of biomacromolecules with a focus on factors influencing the critical PEF treatment intensity. Further, the feasibility of using PEF as a modification technique was investigated, explicitly seeking to elucidate the effect of PEF on the structural elements of biomacromolecules, their conformation, configuration and functional properties.

2. Understanding the effect of PEF on modification of biomacromolecules

2.1. Pulsed electric fields system

Pulsed electric fields treatment can be carried out in a static or continuous flow-through the PEF chamber. The system is an assembly of two major components: a generator and a treatment unit. The essential components of a PEF power generator include a power supply, energy storage element, a switch and a pulse shaping and activating circuit. The treatment unit comprises of a treatment chamber and a

circulation pump (continuous system). The PEF treatment chamber consists of two electrodes held in a particular position by insulators that also form an enclosure containing food materials. The electrodes are separated by a distance (gap) (d) (cm), which depends on the type of the product and the intensity of processing required. The shape and size of the electrodes can be designed to suit specific food products (solid/liquid) and the treatment volume. Disc-shaped, round-edged electrodes help to minimise the enhancement of electric fields and reduce the possibility of a dielectric breakdown of fluid foods (Ho & Mittal, 2000).

PEF generates an electric field strength (kV/cm), which is the peak electrical potential difference (kV) between two electrodes divided by the distance between them (Barbosa-Cánovas et al., 1999; Zhang, Barbosa-Cánovas, & Swanson, 1995). As often observed in the literature sources, electric field strength and field intensity have been used interchangeably to describe the distribution of peak voltage between two electrodes (Yogesh, 2016; Zhao & Yang, 2012). Uniform electric fields are delivered using parallel plate electrodes with a gap sufficiently smaller than that of the electrode surface dimension. The two commonly generated pulse waveforms include square and exponentially decaying pulses in either monopolar or bipolar forms. Signals of voltage, current, frequency, and waveform are monitored and recorded via digital data acquisition systems such as an in-line oscilloscope (Ho & Mittal, 2000).

The effect of PEF treatment on structural and functional properties of biomacromolecules depends upon the ability of the electric field to increase the fluctuation of a system internal energy (Hu et al., 2014; Yan-Yan et al., 2014). The extra energy is absorbed by the carbon backbone of biological molecules inducing particle orientation effects and perturbations, which have potentials to modify the configuration of molecular chains (Tsuji, Yasunaga, Sano, & Ushio, 1976; Yan-Yan et al., 2014). Electric field strength (E), total specific energy (Q) and temperature (T) are the most critical processing parameters that effect modification of biomacromolecules. The effectiveness of a PEF treatment process depends on the characteristic of the pulse (pulse waveform, pulse width/ t_p , pulse number/ n , electric field strength/ E , and pulse repetition rate/ f) and the effective time of exposure to PEF conditions/ t_{exp} . Other technical aspects include the system configuration (batch or continuous) and the volume of the treatment chamber (flow rate (\dot{m}) in a continuous setup). Electrical properties of the product such as resistivity (R)/conductivity (σ), temperature, and pH are equally important (Aguiló-Aguayo, Soliva-Fortuny, & Martín-Belloso, 2010b; Morales-de la Peña et al., 2011).

2.2. Critical PEF treatment intensity

Researchers are increasingly becoming aware of PEF-induced modifications of characteristics and functional properties of biomacromolecules (Barsotti, Dumay, Mu, Fernandez Diaz, & Cheftel, 2001; Guan et al., 2010; Han, Zeng, Yu, Zhang, & Chen, 2009b; Han, Zeng, Zhang, & Yu, 2009a; Hong et al., 2016b; Polikovskiy et al., 2016). Applying PEF to modify biomacromolecules requires knowledge of the sufficient intensity necessary to cause expected changes as well as dielectric and electrical properties of the materials. Subsequently, various aspects of PEF such as optimum, maximum and critical PEF treatment intensity, joule heating among others applicable to the modification of biomacromolecules must be distinctly addressed (Zhang et al., 1995). Critical PEF treatment intensity (E_c) is the threshold required to initiate structural, conformational and functional changes to biomacromolecules (Barba et al., 2015; Hammadi & Veessler, 2009). E_c may include electric field strength, pulse number, pulse frequency, treatment time, pulse energy, total specific energy input, and treatment temperature (Jaeger, Meneses, & Knorr, 2014, pp. 239–244).

Significant modifications of biomacromolecules including electrochemical reactions, aggregation or disintegration processes of molecular structures begin to occur and progress at faster rates on attaining threshold PEF intensity (Barba et al., 2015; Morren, Roodenburg, & de

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