Trends in Food Science & Technology 55 (2016) 39–47

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: http://www.journals.elsevier.com/trends-in-food-science-and-technology

Review

Cold plasma interactions with enzymes in foods and model systems

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ARTICLE INFO

Article history:
Received 4 June 2016
Received in revised form
28 June 2016
Accepted 1 July 2016
Available online 5 July 2016

Keywords:
Ozone
Non-equilibrium
Peroxidase
Amylase
Lipase
Amino acid
Non-thermal

ABSTRACT

Background: The past decade has seen an increased interest in the application of non-equilibrium plasma for food processing. An important aspect of the interaction of chemical species of plasma with foods is the effect on enzymes, which play an important role in retaining the food quality and often serve as markers for processing efficiency.
Scope and approach: The present review covers a critical analysis of the current status of the relationship between plasma parameters and enzyme functionality, with an emphasis on the translation of this knowledge for food applications. The review provides a brief introduction to plasma technology, a summary of the enzyme inactivation studies, followed by a discussion of the mechanism and kinetics of inactivation, and finally, points at the future research needs.
Key findings and conclusion: Cold plasma inactivation of enzymes is primarily dependent on power input of the discharge, degree of exposure to reactive species, the mass transfer between the plasma-liquid phases, structural complexity and stability of the enzymes in their local environment. The mechanism of inactivation is primarily due to the loss of secondary structure due to breakdown of specific bonds or chemical modifications of the side chains by the action of the myriad of chemically active species constituting the plasma. Further research is required to understand the interactions of chemical species in plasma with proteins at a molecular level, coupled with better tools to monitor and control the plasma chemistry.

1. Introduction

To date, thermal processing remains the most widely employed pasteurisation and sterilisation method for the inactivation of micro-organisms and enzymes in foods. In order to overcome the issues related to the poor quality of thermally processed foods such as nutritional losses and adverse effects on organoleptic quality, research in food science over the last few decades have largely focused on development of non-thermal technologies. Several new approaches, including high pressure processing (HPP) (Eisenmenger & Reyes-De-Corcuera, 2009; Hendrickx, Ludikhuyze, Van den Broeck, & Weemaes, 1998), gamma irradiation (Farkas, 2006; Kuan, Bhat, Patras, & Karim, 2013), pulsed electric fields (PEF) (Van Loey, Verachtert, & Hendrickx, 2001; Zhao, Yang, & Zhang, 2012), ultraviolet light (Gómez-López, Ragaert, Debevere, & Devlieghere, 2007; Koutchma, 2009) and sonication (O'Donnell, Tiwari, Bourke, & Cullen, 2010) have been successfully evaluated for the inactivation of enzymes in a range of food products. However, some of these commercially viable methods are limited in practice due to associated adverse perceptions (e.g. irradiation and electron beams), high initial investments required and incompatibility with in-line treatments (e.g. HPP), incompatibility for treatment of whole solid foods (e.g. PEF), lack of developments in industrial scale equipment's for processing (e.g. ultrasound) and/or other constraints (Misra, Kadam, & Pankaj, 2011).

Non-equilibrium plasmas are indispensable in many industrial applications including material processing, electronics (Takayoshi et al., 2016), and polymer processing. Within recent years, the field of cold plasma applications has rapidly expanded into treatment of biomedical devices and biological materials (Miyamoto et al., 2016), including foods (Ishikawa & Hori, 2014). Cold plasma technology, which has recently drawn considerable attention of food scientists and researchers, shows potential for inactivation of a range of micro-organisms and enzymes (Ishikawa et al., 2012). The

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http://dx.doi.org/10.1016/j.tifs.2016.07.001
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application of cold plasma for microbiological decontamination of foods were reviewed by Misra, Tiwari, Raghavarao, and Cullen (2011), Niemira (2012), and Surowsky, Schlüter, and Knorr (2014). Besides decontamination, cold plasma obtained at atmospheric pressure has also shown promising potential for a number of innovative applications including surface hydrophobicity enhancement in biscuits (Misra, Sullivan, et al., 2014), modification of dough rheology and mixing properties (Misra, Kaur, et al., 2015), modulation of whey protein functionality (Segat, Misra, Cullen, & Innocente, 2015), technological performance of rice flour (Pal et al., 2016), enhancement of food grain (seed) germination (Chen et al., 2016), and enhancement of mass transfer (Kodama, Thawatchaipracha, & Sekiguchi, 2014). The uniqueness of non-equilibrium plasma processes is related to their multifaceted interaction with macromolecules, spanning across multiple time and length scales, emerging from the action of a myriad of chemically active species constituting the plasma.

The residual activity of many enzymes is detrimental to the quality of foods, resulting in effects such as browning, off-flavour and loss of vitamins. In certain cases, validation of processing conditions can only be achieved when the critical parameters of plasma processes assure inactivation of not only micro-organisms but also enzymes (Mastwijk & Groot, 2010). Through this review we provide a firm foundation for future investigation of cold plasma effects on food enzymes and the associated consequences on food quality. We discuss the concerned literature highlighting the effects of cold plasma on enzyme activity in various model and real food systems, the kinetics of inactivation, and the future research needs.

2. Cold plasma sources

Plasma is an ionised gas containing atoms or molecules in a metastable state with a roughly zero net electrical charge. Plasmas can be induced in any neutral gas by providing sufficient energy capable of causing ionisation of the gas. Within a general framework, plasmas can be divided into thermal plasma and those which are not; we refer to these as low-temperature plasma. In thermal plasmas, the temperature of all species (electrons, ions, neutral species) is the same, i.e. a thermodynamic equilibrium between electrons and other species exists. For the other class, often the term 'local thermal equilibrium' is used, which implies that the temperatures of all plasma species are the same in localized areas in the plasma (Bogaerts, Neyts, Gijbels, & van der Mullen, 2002). Low temperature plasma can be further subdivided into thermal plasma (quasi-equilibrium plasma), being in *local thermal equilibrium* state and non-thermal plasma (non-equilibrium plasma). Non-thermal plasma is also referred to as *cold plasma*. Earlier, cold plasmas were generated only under low-pressure conditions. However, recent advances in plasma physics and engineering have enabled generation of cold plasmas at atmospheric pressure, which has significantly boosted plasma research at the interface of life sciences (Segat, Misra, Cullen, & Innocente, 2016). The design and control of plasma sources operating at or near atmospheric pressure is of interest, both technically and commercially to the food industry because it does not require extreme processing conditions, such as high temperature and pressure (Misra, Pankaj, Frias, Keener, & Cullen, 2015).

The wide exploration of plasma applications for food is due among other things, to the advancements in plasma physics, offering the possibility of plasma generation at atmospheric pressure. The technologies used to obtain cold plasmas vary from the use of corona discharge (Chang, Lawless, & Yamamoto, 1991), microwaves (Leins et al., 2014) and radiofrequency waves to capacitive or inductive coupling methods or more commonly dielectric barrier discharges (DBDs) (Kogelschatz, 2003) at relatively lower frequencies, and the 1 atm uniform glow discharge plasma (OAUGDP) (Montie, Kelly-Wintenberg, & Roth, 2002). All these discharges are initiated and sustained through electron collision processes under the action of the specific electric or electromagnetic fields.

Among all the plasma sources, the DBD and plasma jet are the most widely explored configurations in food research, as these are simple in construction, easy to adopt and some configurations are commercially available. A DBD consists of two metal electrodes, in which at least one is coated with a dielectric layer, and a high potential difference is applied across the electrodes (see Fig. 1(a)). When the potential across the gap reaches the breakdown voltage the dielectric acts as a stabilizing material leading to the formation of a large number of micro-discharges. One can design a dielectric barrier discharge with different configurations, as desired. A new paradigm in the DBDs is the concept of *in-package* or so called, *encapsulated* plasma, where a plastic packaging material enclosing the food is used as the dielectric (Cullen et al., 2014; Misra, Keener, Bourke, Mosnier, & Cullen, 2014; Yong et al., 2015). A plasma jet is comprised of two concentric electrodes through which a gas (or mixture of gases) flow. The inner electrode is typically applied with a high voltage (100–250 V) at a high frequency (commonly at 13.56 MHz radio-frequency) causing ionisation of the gas (Schutze et al., 1998), which is directed through the nozzle on to the food surface located a few millimetres downstream (see Fig. 1(b)). As a general comment, the measurement and control of the actual food temperature during plasma treatment is an important aspect which needs special emphasis (especially when using plasma jets and microwave plasma) where temperatures may exceed 60 °C, and reliable temperature measurements for these systems has recently been studied (Knoerzer, Murphy, Fresewinkel, Sanguansri, & Coventry, 2012).

The unique 'one pot' generation of multiple chemically active species renders cold plasma as a novel, attractive anti-microbiological intervention and an attractive process for advantaged food chemistry. The reaction mechanisms resulting in the formation of active plasma chemical species include electronic impact processes (vibration, excitation, dissociation, attachment and ionisation), ion-ion neutralisation, ion-molecule reactions, Penning ionisation, quenching, three-body neutral recombination, and neutral chemistry, besides photoemission, photo-absorption and photo-ionisation. When nitrogen and oxygen molecules are present in the gas phase, energetic electrons collide with them along their trajectory and a cascade of linked reactions results in the formation of nitrogen oxides (NOx). In particular, in the presence of oxygen, hydroxyl radicals (•OH) and ozone (O_3) are created and they represent the most aggressive among other active chemical species. Hydroxyl radicals ($^{\bullet}$OH) may also help in formation of hydrogen peroxide (H_2O_2) and hydronium ions (H_3O^+) normally obtained in presence of water (gas humidity, surface moisture of food or aqueous phase of the sample). In addition to these species, ultraviolet (UV) radiation and charged particles may also be involved in the overall plasma effect (Misra, Tiwari, et al., 2011).

3. Inactivation effects

Cold plasma has been reported to inactivate a range of enzymes such as lysozyme (Takai, Kitano, Kuwabara, & Shiraki, 2012), polyphenol oxidase and peroxidase (Pankaj, Misra, & Cullen, 2013; Surowsky, Fischer, Schlueter, & Knorr, 2013; Tappi et al., 2014). Table 1 provides a summary of the salient results of studies regarding enzyme inactivation using cold plasma along with the process parameters employed. The differences in the observed levels of enzyme inactivation reported in literature are mainly attributable to the differences in the enzymes, plasma treatment