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Effect of atmospheric pressure cold plasma (ACP) on activity and structure of alkaline phosphatase

Annalisa Segat[a,b], *N.N. Misra*[b,c], *P.J. Cullen*[b,d], *Nadia Innocente*[a,*]

[a] *Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Udine, Italy*
[b] *School of Food Science & Environmental Health, Dublin Institute of Technology, Ireland*
[c] *GTECH, Research & Development, General Mills IPL, Mumbai, India*
[d] *School of Chemical Engineering, University of New South Wales, Sydney, Australia*

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ABSTRACT

Atmospheric cold plasma treatments (ACP) is a novel non-thermal processing technology with several emerging potential applications in food processing, including dairy. This study evaluates the effect of ACP on the activity and structure of alkaline phosphatase (ALP), an enzyme native to milk. ALP enzyme in solution was treated with ACP at three discreet high voltages (40, 50 and 60 kV) for durations ranging between 15 s and 5 min. Results demonstrated that the dielectric barrier discharge based plasma technology was able to inactivate the enzyme within a few seconds. Kinetic models, namely first order, Weibull and logistic models were fitted to the experimentally observed data and the model parameters were determined. The Weibull model was found to best describe the observed variance in residual activity for all the voltages applied. The dichroic spectra suggested that the enzyme was characterized by a predominance of α-helix structure, and the helical content showed a tendency to decrease with increase in treatment time and voltage. The maximum temperature recorded for most intense treatments was in the order of only 30 °C and no change in pH was noticed.

1. Introduction

Within recent years, nonthermal technologies have been widely studied for their efficacy in inactivating enzymes, either singularly or in combination with mild heat treatments. Particularly, several researchers have suggested that high pressure technology, ultrasound, ultraviolet (UV) light, pulsed electric field (PEF), and atmospheric pressure cold plasma (ACP) technology are capable of inactivating enzymes, both in model systems and in real food matrices (Ho et al., 1997; Huang et al., 2012; Innocente et al., 2014; Manzocco et al., 2009; Navarro et al., 2014; O'Donnell et al., 2010). ACP is relatively a new nonthermal technology which has been reported to efficiently inactivate microorganisms including bacteria, bacterial spores, fungi, and biofilms in model media and food systems (Hashizume et al., 2013, 2015; Ishikawa et al., 2012; Jahid et al., 2013; Misra et al., 2014d). Plasma consists of a plethora of chemically active species including electrons, positive and negative ions, neutrals, free radicals, excited or non-excited molecules, photons and atoms (Bárdos and Baránková, 2010; Misra et al., 2011; Pankaj et al., 2014). Based on the thermodynamic equilibrium between electrons and ions, plasmas can be classified into thermal and non-thermal (or "cold") plasmas. Thermal plasmas are characterized by a thermal equilibrium between ions and electrons, whereas cold plasmas exhibit strong non-equilibrium of temperature between ions and electrons. In cold plasma, cooling of ions and uncharged molecules is more effective than energy

* *Corresponding author at*: Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Via Sondrio, 2/A, 33100 Udine, Italy. Tel.: +39 0432558154; fax: +39 0432558130.
E-mail address: nadia.innocente@uniud.it (N. Innocente).
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transfer from electrons and the gas remains at low temperature (Fridman et al., 2008; Misra et al., 2014a). 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. ACP could be induced in a gas by subjecting the gas (or gas mixture) to an electric field, which in turn accelerates the charged particles leading to collisions with the heavy species (e.g., ions and neutrals). The cold plasma source for this study is based on a dielectric barrier discharge (DBD) phenomenon; details of the set-up are provided in an earlier publication (Moiseev et al., 2014).

In a recent study, ACP was investigated for its *Escherichia coli* inactivation efficacy in milk samples with different fat contents and it is was found that ACP treatment (between 1 and 20 min) significantly reduced the bacterial count in different types of milk (whole, semi-skimmed, and skimmed) without causing negative effects to pH and colour (Gurol et al., 2012). In a subsequent study, the same research group studied the chemical aspects of cold plasma treatment of milk (Korachi et al., 2015). While significant changes were not observed in the lipid composition of treated milk, changes in several volatile compounds were prominent. In yet another study, Cavalcante et al. (2013) evaluated ozone gas as a new preservation method for raw milk in combination with mild thermal treatment to reduce the microbial load and improve the shelf-life of milk during storage. These results suggest that cold plasma could be a potential alternative technology for milk decontamination (Cavalcante et al., 2013; Gurol et al., 2012; Korachi et al., 2015). In a recent study, Segat et al. (2015) investigated the main effects of ACP on model systems of whey protein isolate (WPI) solutions that can be employed as functional ingredients in different food formulations. The results showed remarkable changes in milk protein structure resulting in a modulated protein functionality. Particularly, the foaming and emulsifying capacities of whey proteins were found to have improved post-ACP treatments. Looking at these developments it appears that cold plasma could have potential applications in dairy sector, in general. Accordingly, in the present work we are interested in finding out if ACP can inactivate enzymes endogenous to milk.

Milk contains about 70 enzymes, which although minor, yet, are very important members of the milk protein system (Fox and Kelly, 2006). Some of them are significant for several reasons including technological relevance. Alkaline phosphatase (ALP, orthophosphoric mono-ester phosphohydrolase, EC 3.1.3.1) is an enzyme naturally present in blood and milk of all mammals (Fox and McSweeney, 1998). ALP enzyme has *z-value* similar to heat-resistant pathogens and its activity is routinely evaluated to measure the efficacy of pasteurization process in industrial milk processing (Rankin et al., 2010). ALP activity in pasteurized milk generally indicates inadequate pasteurization. Therefore, ALP represents a good model enzyme for evaluating the efficacy of ACP against inactivation of enzymes endogenous to milk. In addition, there are no studies in literature evaluating the effect of ACP on ALP. It is worth noting that while majority of studies have reported that cold plasma leads to inactivation of enzymes, this may not always be true. For example, Li et al. (2011) have reported an enhancement of lipase activity following cold plasma treatments. Based on these premises, the goal of the present study was to evaluate the effects of ACP on the activity of alkaline phosphatase (ALP). To keep the study simple and be able to perform circular dichroism spectroscopy for identifying structural changes, we conducted the studies in model buffered medium.

The specific objectives of this study were to: (1) find out if cold plasma can inactivate ALP in model liquids; (2) mathematically model the activity of ALP following ACP treatments; and (3) obtain mechanistic insights via CD spectroscopy and qualitatively determine structural changes, if any.

2. Materials and methods

2.1. Materials

The alkaline phosphatase (ALP) enzyme used was a commercial lyophilized powder from bovine intestinal mucosa (P7640, Sigma-Aldrich, Italy). The lyophilized powder was dissolved in phosphate buffer (50 mM, pH 6.8) at a concentration of 250 mg/L and can be stored at 5 °C for up to 5 days. For the experiments, an aliquot of this solution was diluted in phosphate buffer to give a final concentration of 0.38 mg/L; see c.f., Aguiar et al. (2012). All chemicals used were obtained from Sigma-Aldrich, Italy, unless explicitly specified.

2.2. Methods

2.2.1. Atmospheric cold plasma (ACP) treatment

The ACP source was based on a DBD plasma setup, further details regarding which can be found elsewhere (Misra et al., 2014b, 2014c). Briefly, it is comprised of two circular aluminium plate electrodes (outer diameter = 158 mm) over polypropylene (PP) dielectric layers (2 mm thickness) between which a polypropylene package, containing 15 mL of ALP enzyme solution in a petri plate was placed. ACP treatment was carried out at 40, 50, and 60 kV in atmospheric air for durations ranging from 15 s to 5 min, at room temperature. Immediately after treatment, the residual activity of enzyme was evaluated and the circular dichroism (CD) spectroscopy was performed.

2.2.2. Activity assay for alkaline phosphatase

The activity of ALP was determined according to FIL–IDF (1987) and Ludikhuyze et al. (2000). Untreated enzyme was used as a control. The residual activity (RA) was calculated according to the following equation:

$$\mathrm{RA}\,(\%) = \frac{A_t}{A_0} \times 100 \tag{1}$$

where, A_t is the residual activity of ALP solution at time t and A_0 is the activity of control. A_t was determined immediately after plasma treatment to avoid any effect of storage time.

2.2.3. ALP inactivation kinetics

Experimental data was modelled using three different inactivation kinetics equations viz. the first-order, Weibull and logistic equation. The first-order inactivation model is given by the following equation:

$$\mathrm{RA} = \mathrm{RA}_0 \times e^{-K_p \times t} \tag{2}$$

where, the inactivation rate constant K_p was obtained by least squares non-linear regression.

The Weibull model fitted to the data is given by:

$$\mathrm{RA}_t = \mathrm{RA}_0 \times e^{-(t/\alpha)^{\beta}} \tag{3}$$