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# Direct infusion of nitrite into meat batter by atmospheric pressure plasma treatment

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ABSTRACT

This study investigated the influence of direct atmospheric pressure plasma (APP) treatment on nitrite levels and physiochemical quality of meat batter during the mixing process. A compact APP system was developed for installation on top of a food mixer. Meat batter composed of pork, water and sodium chloride (80:20:1, w/w/w) was treated with APP during mixing. Plasma treatment gradually increased the temperature of meat batter over 60 min from 0.2 °C to 20 °C. Total aerobic bacterial count of meat batter was not influenced by plasma treatment for 30 min ($p > 0.05$). The nitrite level in meat batter increased steadily with increasing plasma treatment duration ($p < 0.05$), reaching 65.96 ppm at 30 min. Consequently, the CIE $a^*$- and $b^*$-values of cooked meat batter gradually increased and decreased, respectively, as the time of plasma treatment increased. According to the results, direct APP treatment can replace nitrite addition in cured meat processing.



## 1. Introduction

The process of curing improves sensorial quality, shelf life, and safety of processed meats by adding ingredients such as salt, sugar, spice, nitrite, and/or nitrate (Sebranek, 2009). Nitrite is an essential curing ingredient because of its multifunctional role. Nitrite develops color and flavor, inhibits lipid oxidation, and limits the growth of spoilage and pathogenic microorganisms including *Clostridium botulinum* in processed meats (Pegg & Shahidi, 2000).

Recently, the increase of consumer demand for natural and organic food has precipitated the development of natural curing processes for processed meat (Sebranek, Jackson-Davis, Myers, & Lavieri, 2012). For natural curing processes, natural nitrite sources such as vegetable juice concentrate containing nitrate with starter cultures that reduce nitrate to nitrite, or pre-converted (nitrate to nitrite) vegetable concentrate are used instead of chemical nitrite sources such as sodium nitrite (Sebranek et al., 2012; Parthasarathy & Bryan, 2012). However, a critical problem in the use of vegetable concentrates for curing has emerged. Jackson, Sullivan, Kulchaiyawat, Sebranek, and Dickson (2011) reported that the microbial safety of naturally cured meat products was lower than that of sodium nitrite cured meat products. The concentration of added and residual nitrite in processed meat is important for limiting microorganism growth (Jackson et al., 2011; Xi, Sullivan, Jackson, Zhou, & Sebranek, 2011). Nevertheless, the amount of nitrite added to processed meat by vegetable concentrates is lower than when sodium nitrite is used, due to the natural pigments and flavors they contain that cause undesirable sensory characteristics. Therefore, the optimal concentration of vegetable concentrate added into processed meats is about 0.3 g $kg^{-1}$ of formulation (Sindelar, Cordray, Sebranek, Love, & Ahn, 2007; Sebranek et al., 2012; Jung et al., 2015a). In addition, vegetable concentrates typically contain nitrite at a concentration of 15,000–20,000 mg $kg^{-1}$ (Sebranek et al., 2012). Therefore, the added concentration of nitrite into processed meat by vegetable concentrate is only about 45–60 mg $kg^{-1}$, which is not enough to assure microbial safety (Sebranek et al., 2012).

Plasma is ionized gas, literally defined as the fourth state of matter, along with solids, liquids, and gases (Thirumdas, Sarangapani, & Annapure, 2015). Plasma technology has been variously used in food processing for non-thermal sterilization, surface modification of packaging materials, increasing biological activity of natural compounds, and water purification (Foster, Sommers, Gucker, Blankson, & Adamovsky, 2012; Kim et al., 2014; Thirumdas et al., 2015; Jayasena et al., 2015; Kim et al., 2015; Yong et al., 2015). Recently, Jung et al. (2015a,b) suggested plasma treated water as a potential nitrite source for curing of processed meat as it contains nitrogen species such as nitrate and nitrite created by plasma-liquid interaction, without change in color or flavor. Therefore, we hypothesized that plasma treatment of meat batter could generate nitrite in meat batter by interaction of plasma with the liquid in meat batter. Furthermore, this process could

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sterilize meat batter, extending shelf life of the processed meat product and ensuring food safety.

In this study, the effect of direct atmospheric pressure plasma (APP) treatment on meat batter is investigated in terms of nitrite concentration, total aerobic bacterial count, and physicochemical quality of meat batter.

## 2. Materials and methods

### 2.1. Direct APP treatment

APP treatment was carried out using a dielectric barrier discharge (DBD) plasma system (Plasmapp PCS-20 N, Plasmapp Co., Daejeon, Korea). The system was composed of a main chamber for mixing, a plasma chamber, a plasma power supply, and a gas circulating module (Fig. 1). The main chamber stored a meat product and provided a sealed space, and plasma was discharged by the power supply in the plasma chamber, which was connected to the main chamber. The discharged gas was supplied into the main chamber to cure the meat product by the gas circulating module in which a diaphragm pump of the module received the activated gas from the main chamber and provided it into the plasma chamber. The plasma generator in the plasma chamber included 16 DBD modules spaced apart from each other, and the two plasma electrodes, which had different polarities, were coated with silver (Ag) on the opposite sides of the rectangular alumina ($Al_2O_3$) plate of each DBD module. The modules were supported by copper (Cu) blocks at both ends of the ceramic plate, and electrodes were connected in parallel to the plasma power supply whose input power and frequency were 550 W and 25 kHz, respectively. The power generated a strong electric field near the boundary lines of the electrodes, and the plasma was discharged from the lines on both sides of the module. Ambient gas consisting mainly of nitrogen and oxygen was excited by the discharged plasma to produce reactive nitrogen species (RNS) including nitrite ($NO_2$), which was supplied into the meat batter composed of pork, water and sodium chloride (80:20:1, w/w/w) in the main chamber.

### 2.2. Temperature

The temperature of meat batter was monitored during plasma treatment for 1 h. A cable probe (thermocouple type K) was attached to the bottom of the mixer and connected to a digital thermometer (YF-160A, Koang Yee Enterprise Co., Ltd., Taipei, Taiwan). The temperature of the meat batter was recorded at 1-min intervals.

### 2.3. Total aerobic bacterial counts

Samples were collected from three areas of meat batter at 5 min intervals during plasma treatment over 30 min. The collected meat batter (10 g) was blended with sterile saline (90 mL) for 2 min using a stomacher (BagMixer® 400; Interscience Ind., St. Nom, France). A series of decimal dilutions were prepared using sterile saline. Each dilution (0.1 mL) was spread in triplicate onto tryptic soy agar plates (Difco Laboratories, Detroit, USA). The plates were incubated at 37 °C for 48 h, and the microbial counts were expressed as log CFU/g.

### 2.4. Nitrite concentration

Samples were collected from three areas of meat batter at 5 min intervals during plasma treatment over 30 min. Nitrite concentration in meat batter was measured according to AOAC method 973.31 (AOAC, 1990) with modification. Collected meat batter (10 g) was thoroughly mixed with 150 mL of warm water (80 °C) in a 250-mL volumetric flask. Afterwards, 10 mL of 0.5 mol/L. NaOH was added. After mixing, 10 mL of 120 g/L. zinc sulfate was added into the flask and thoroughly mixed. The flask was heated for 20 min in a shaking water bath at 80 °C. After cooling in tap water for 10 min, 20 mL of 100 g/L. ammonium acetate (pH adjusted to 9.1 with 100 mL/L. ammonia water) was added into the flask and the mixture was diluted to a volume of 200 mL with deionized water. After mixing, the solution was filtered through Whatman No.4 filter paper (Whatman, Maidstone, England). A 20-mL volume of filtrate (sample solution) was transferred into a 25-mL volumetric flask, and then 1 mL of 30 mmol/L sulfanilamide in acid solution (HCl:water, 1:1, v/v) and 1 mL of 5 mmol/L N-(1-naphthyl)ethylenediamine dihydrochloride were added. The resultant solution was diluted to 25 mL with deionized water. The mixed solution was allowed to stand for 20 min for complete color development. The absorbance of the solution was measured at 540 nm on a spectrophotometer (DU®530, Beckman Instruments Inc., CA., USA) using water as a reference sample. Nitrite concentration of meat batter was calculated using a standard curve prepared from $NaNO_2$ absorbance readings (Sigma-Aldrich Co., St. Louis, MO., USA).

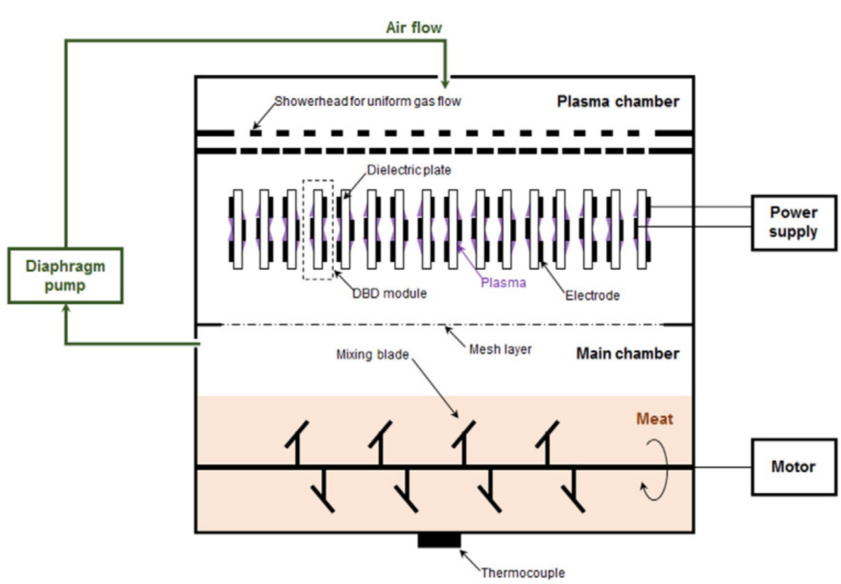


**Fig. 1.** Atmospheric pressure plasma system.