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Effect of dielectric barrier discharge plasma on background microflora and physicochemical properties of tiger nut milk

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ABSTRACT

The microbial and physicochemical properties of tiger nut milk were studied under different dielectric barrier discharge (DBD) plasma exposure time: 2, 4, 6, 8, and 12 min. Following the plasma treatment, a significant reduction in the microflora was observed at 2, 4, 6 and 8 min treatment time with 12 min reaching the undetectable limit. The treatment did not result in any significant change in soluble solids and fat contents. Significant reduction in pH was recorded after 8 and 12 min treatment, whereas heightened titratable acidity and lipid oxidation were noticed in all the treated samples. The protein content decreased significantly in all the treated samples, while peroxidase activity only decreased when the treatment time was extended beyond 4 min. The loss in enzyme activity was due to the oxidation effect plasma reactive species including atomic oxygen, ozone, and hydroxyl radicals, which might have oxidized the amino acid side chain. The findings presented here could be a prelude for the potential application of DBD plasma treatment of tiger nut milk in the food industry.

1. Introduction

Tiger nut (*Cyperus esculentus* L.) is a tuber crop grown in the soil. It is a traditional crop highly regarded for its vast nutritional and health values in Nigeria. The tuber is rich in fibre, starch, protein, fat, and vitamins. Its monounsaturated fatty acid rich oil made up about 23% of the tuber (Ezeh, Gordon, & Niranjan, 2016). Tiger nuts are cultivated in Africa, Asia and some part of Europe. In Nigeria, roasted tiger nuts with sugar are popularly served as local snacks. The low-acidic milk extracted from the tuber tastes sweet and also rich in fat, starch and other food contents like protein and vitamins. The composition of these constituents depended upon geographical location to which the tubers belong (Codina-Torrella, Guamis, & Trujillo, 2015; Codina-Torrella, Guamis, Ferragut, & Trujillo, 2017). The tiger nut milk (TNM) is a non-alcoholic beverage referred with different names depending on the production location. For instance, Spain called the TNM 'Horchata de chufa', in Africa, Ghana referred to it as 'Atadwe milk', and 'Kunun Aya' in Nigeria (Codina-Torrella et al., 2015; Cortés, Esteve, Frígola, & Torregrosa, 2005; Okyere & Odamtten, 2014). In Nigeria, apart from the street vending of TNM, it is also a special drink served on ceremonial occasions. Despite this popularity, the beverage can only be stored for 48 h under refrigeration (Nwobosi, Isu, & Agarry, 2013). The shelf-life is hampered by microbial changes caused by the background microorganisms and the physicochemical changes which occur during storage at temperatures above 8 °C. Lipid oxidation, enzyme activity, pH, starch and protein content are other quality parameters that are associated with a quality loss in TNM (Corrales, De Souza, Stahl, & Fernández, 2012; Selma, Salmerón, Valero, & Fernández, 2006). The initial aerobic bacteria density in the raw and pasteurized TNM were estimated in the ranges of 10^6 CFU/mL and 5×10^4 CFU/mL, respectively (Selma, Fernández, Valero, & Salmerón, 2003).

Thermal pasteurization adversely alters the organoleptic properties

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of foods such as colour, taste, aroma, and flavour (Roselló-Soto et al., 2018). In some food, a decrease in valuable nutritive contents, and the formation of toxic by-products were reported (Scholtz, Pazlarova, Souskova, Khun, & Julak, 2015). The thermal pasteurization of TNM at a temperature above 70° C has resulted in the gelatinizing of the TNM due to high starch content. In view of this, the strategy employed to curtail this problem was the removal of the starch from the product prior to the treatment. Consequently, the loss of aroma and flavour arises. This led to the addition of flavouring additives such as vanilla (Selma et al., 2006). Due to the aforementioned challenge, novel non-thermal technologies were harnessed to curtail these problems. They include gamma irradiation (Okyere & Odamtten, 2014), pulsed electric fields (Cortés et al., 2005) short wave ultraviolet light (UV−C) (Corrales et al., 2012), electrolyzed water (Xuan et al., 2017), and ultrahigh pressure homogenization (Codina-Torrella et al., 2017). Dielectric barrier discharge plasma (DBD plasma) is another nonthermal processing technology harnessed nowadays. This is due to its ability to minimally alter the quality and nutritional attributes of food products that are often impaired by excessive thermal treatments (Muhammad et al., 2018a). Additionally, the shelf-life of food has been extended considerably upon exposure to DBD plasma (Amini & Ghoranneviss, 2016; Liao et al., 2017a). DBD plasma is referred to as the fourth state of matter (after solid, liquid and gas) containing a variety of active electrically energized particles, such as electrons, ions, radicals, and metastable excited species that have sufficient energy to break covalent bonds to initiate some reactions to volatile compounds (Han et al., 2016; Mir, Shah, & Mir, 2016; Rød, Hansen, Leipold, & Knøchel, 2012). DBD plasma is generated at an atmospheric pressure between two electrodes and consists of various active agents. These reactive species may be one or more of the following: vibrationally and electronically excited oxygen O_2 and nitrogen N_2; reactive nitrogen species (RNS) such as atomic nitrogen N, excited nitrogen N_2, nitric oxide NO•; reactive oxygen species (ROS) such as atomic oxygen O, singlet oxygen $^1O_2$, superoxide anion O_2^- and ozone O_3. Depending on the plasma source, type, composition, and abundance of these active agents may vary substantially in accordance with plasma discharge source (Scholtz et al., 2015). On the other hand, many factors were reported to influence the microbial inactivation efficacy of DBD plasma. Liao et al. (2017a) and Fernández and Thompson (2012) categorized these factors as DBD processing parameters (processing time, mode of exposure, the intensity of the input power, gas type and flow rate); environmental factors (such as food matrix, pH and relative humidity); and microbial properties.

Numerous studies on the inactivation of microorganisms on different food matrix have been conducted and the potent effect of the plasma reactive species was reported. Laroussi and Leipold (2004) and Liao et al. (2018c) highlighted the crucial role played by O, OH, and NO_2 in the destruction of microorganisms through the cell membrane. The microbial safety of encapsulated DBD plasma treated milk was reported after 10 min of exposure. The quality attributes were reasonably intact while maintaining the microbial safety (Kim et al., 2015). Significant bacterial inactivation from 7.78 log CFU/mL to 3.63 log CFU/mL after 20 min of plasma application was attained in the milk without substantial change to pH and colour parameters (Gurol, Ekinci, Aslan, & Korachi, 2012). Tappi and co-authors reported OH and NO were responsible for 42% reduction of polyphenol oxidase activity during a 30-min treatment with low-frequency DBD using air as the process gas (Tappi et al., 2014). Numerous articles have reported the significant microbial safety and quality maintenance of DBD plasma on different types of food matrix and non-food media (Buβler, Steins, Ehlbeck, & Schlüter, 2015; Fernández & Thompson, 2012; Hertwig, Reineke, Ehlbeck, Knorr, & Schlüter, 2015; Khani, Shokri, & Khajeh, 2017). Detailed DBD plasma generation, application and the various role played by the plasma reactive species were discussed elsewhere (Mir et al., 2016; Misra, Schlüter, & Cullen, 2016; Surowsky, Schlüter, & Knorr, 2014).

To the best of our knowledge, there is no research reported concerning tiger nut milk treatment using DBD plasma. The aim of this work was to study the effect of DBD plasma on the microbial safety, physicochemical changes and peroxidase activity of TNM.

2. Materials and methods

2.1. Sample preparation

The tiger nut tubers were purchased from Beijing Sanlitun Technology, Limited, China. The dried tubers were sealed in polyethylene bags of 500 g package and kept at ambient condition, 23 ± 2 °C. The method used for the preparation of the TNM was similar to the traditional method used in Nigeria with some modifications. The tiger nuts were sorted to remove cracked tubers. About 200 g was washed and soaked for about 12 h using deionized water at ambient condition. The water was decanted, and the succulent tubers were subjected to wet milling for 3 min by adding deionized water (4:1, w: w). The milky extract was squeezed out from the paste through a muslin cloth. This was considered as our TNM sample, and it was stored in a refrigerated condition at 4° C until further analysis within 24 h.

2.2. DBD plasma treatment

DBD plasma was generated using atmospheric air as the process gas. The plasma equipment used was described elsewhere (Liao et al., 2017b). Briefly, is comprised of the reaction cell with aluminium electrodes of 50 mm diameter each, a glass dielectric barrier of 90 mm diameter (Fig. 1), voltage regulator and high-frequency power supply (CTP-2000K, Nanjing Suman Electronics Co., Ltd.). The device was operated at 30 V and a resonance balancing of 1.22 A. The distance between the powered electrode and sample surface was 6 mm 25 mL of TNM was poured into 150 mm stainless-steel dish to give a sample depth of 0.5 mm. This was treated with the DBD plasma for exposure times of 0, 2, 4, 6, 8, and 12 min under continuous stirring. The stirring was performed by subjecting the TNM to move in a circular manner in the dish using glass rod without distorting the plasma discharge. This was maintained throughout for each sample exposure at the preset treatment duration. The treatment was repeated for another 25 mL of TNM. This was combined to give an accumulated volume of 50 mL for each of the treated samples before the microbial and physicochemical analysis.

2.3. Microflora inactivation

For total bacteria count, 1 mL each of TNM sample was serially diluted with 0.85% sterile saline solution, and appropriate dilutions of 0.1 mL were spread on solidified Tryptone Soy Agar (TSA) (Qingdao Hope Bio-Technology Co., Ltd, China) plate in triplicates. The plates were incubated at 37° C for 24 h.

For moulds and yeast enumeration, 1 mL of TNM was serially diluted in 0.85% sterile saline and 0.1 mL of appropriate dilutions were plated in triplicates in Rose-Bengal agar (Qingdao Hope Bio-Technology Co., Ltd, China). Plates were incubated at 25 °C for 3 days. After the incubation, colonies were counted and results expressed as log CFU/mL of TNM.

2.4. Physicochemical properties

2.4.1. pH

The pH of the TNM was measured using a digital pH meter (PHS-550, Hangzhou Lohand Biological Co. Ltd, China). A beaker containing 10 mL of TNM sample was continuously stirred with a magnetic stirrer and pH was measured at 20° C. The pH meter was calibrated with standard solutions prior to carrying out each measurement (Abid et al., 2013).