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# Effect of low-pressure air plasma on the microbial load and physicochemical characteristics of dried laver

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ABSTRACT

Low-pressure air plasma (LPAP) has not been widely used for food products surface bio-decontamination. In the present study, LPAP was used as inactivating agent against surface microbial contaminants of sun-dried laver sheets. Microbial inactivation kinetics and physicochemical characteristics of LPAP-exposed laver sheets were determined. Also, the sensory properties of *kimbab* (rice rolled in laver), which was roll wrapped using plasma-treated dried laver, were evaluated. LPAP was generated at the vacuum pressure of 133.3 Pa or 1Torr, and a power density of 54.1 mW/cm$^3$. Upon LPAP-exposure, over 1-log reduction in viable cell count of aerobic bacteria was observed over a 20 min period. The microbial inactivation pattern fitted well to Singh-Heldman model or pseudo-first-order kinetics. Compared to plasma-unexposed laver, there were no changes in color characteristics, total phenolic content, and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of plasma-exposed laver. Furthermore, the use of LPAP-exposed laver sheets exerted minimal to no impact on sensory characteristics of *kimbab*.



## 1. Introduction

Plasma sterilization or bio-decontamination technology is emerging as an attractive substitute for chemical sterilization methods, which are known for their damaging effect, intrinsic toxicity and for leaving toxic residues on surfaces (Morent & De Geyter, 2011; Muranyi, Wunderlich, & Heise, 2007). Particularly, non-thermal plasma sterilization methods are more suitable for microbial decontamination of heat sensitive materials (Ehlbeck et al., 2011). Plasma can be generated over a wide range of temperature (~0 K–$10^8$ K) and pressure, by means of coupling energy to gaseous medium (Afshari & Hosseini, 2014).

Plasma sterilization is efficient with most gases such as $O_2$, $N_2$, air, $H_2$, halogens, $N_2O_3$, $H_2O_2$, $CO_2$, $SO_2$, $SF_6$, etc. (Ratner, Chilkotti, & Lopez, 1990). Plasma treatment can effectively inactivate a wide range of microorganisms including their spores (Feichtinger, Schulz, Walker, & Schumacher, 2003; Kelly-Wintenberg et al., 1999; Lee, Paek, Ju, & Lee, 2006). The plasma inactivation mechanism is still not completely understood. The working mode of plasma sterilization is more complex; it has been hypothesized that different plasma species (ions, electrons, radicals, UV light, vacuum UV, electric fields and metastables) attack chemically the microorganisms, and may create a synergistic effect by altering in-cell processes (Klampfl et al., 2012).

While generating low-pressure plasma, the discharge chamber is typically evacuated well below the atmospheric pressure (760 Torr or 101,325 Pa), and the overall exposure conditions remain at a low temperature. Although atmospheric plasmas are widely used for microbial inactivation, the lifetime of the reactive plasma species is much shorter under atmospheric conditions than that of low-pressure plasma (Shintani, Sakudo, Burke, & McDonnell, 2010).

There are numerous studies on microbial inactivation using low-pressure discharge plasmas (LPDPs). A 1 to 5 log reduction of aflatoxin producing fungal population from shelled and unshelled nuts in 5 min has been reported using low pressure cold plasma (LPCP), which was generated using air gases and sulfur hexafluoride as plasma gases (Basaran, Basaran-Akgul, & Oksuz, 2008). Low-pressure plasma has been used for inactivation of *Escherichia coli* (Mok & Song, 2010) and *Staphylococcus typhimurium* (Mok & Song, 2013). Sporicidal effect of LPDP using non-toxic gas on spores of *Geobacillus stearothermophilus* has been reported (Tamazawa, Tamazawa, & Shimauchi, 2012). Inactivation effect of low-

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pressure RF plasma (using $N_2$—$O_2$ and $SF_6$ gases) on *E. coli* O157:H7, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterobacter sakazakii* have been reported (Al-Mariri, Saloum, Mrad, Swied, & Alkhaled, 2013). Recently, our lab reported on the use of low-pressure air plasma (LPAP) for inactivation of foodborne bacterial pathogens on the surfaces of different packaging materials (Lee, Puligundla, & Mok, 2015).

Seaweed is directly used for culinary purposes in Asian countries. Phenolic content, flavonoids, chlorophyll, and carotenoids of seaweed may contribute to their high antioxidant potential (Lim, Cheung, Ooi, & Ang, 2002). Laver seaweed (*Porphyra*) is primarily prepared in the form of dried, roasted and seasoned products (Ganesan, Kumar, & Bhaskar, 2008). Raw, heated, roasted and seasoned laver sheets were reported to contain a substantial number of microorganisms (Park, Song, & Ha, 2014; Shibata & Anpo, 1983). Processing of laver decreases its microbial load. In a study, a gradual decrease of high initial aerobic plate counts (APC) of dried laver was reported upon commercial processing, from 4.4 to 7.8 log CFU/g to 1.3 to 5.9 log CFU/g in final products (Choi et al., 2014). Improperly processed and cross-contaminated laver consumption may have detrimental effects on human health. Pathogenic microbes are often found in *kimbab* rolled with contaminated dried laver, and many food-borne disease outbreaks are linked to consumption of such tainted products (Son et al., 2014).

Conventional drying systems are generally used for commercial drying of laver. Earlier, Shibata and Anpo (1983) have reported that laver sheets can be bio-decontaminated by placing them in an atmosphere of nitrogen or carbon dioxide gas, without any adverse effect on the quality of the laver sheets themselves. Emerging dielectric drying methods with microwave (MW) and radio frequency (RF) energy could also be used for decontamination during drying. However, non-uniformity is a major drawback of microwave drying (Yan et al., 2010). In addition, there is a possibility of tissue damage or undesirable changes in the food texture due to too rapid mass transport by microwave power (Nijhuis et al., 1998). These limitations can presumably be avoided by using cold plasma-based systems.

A uniform glow discharge of low-pressure plasma was successfully produced and used in our earlier works for inactivation of *E. coli* on shell egg surface (Mok & Song, 2010) and foodborne pathogens on packaging materials (Lee et al., 2015). Here, we propose that cold plasma treatment of conventionally dried laver may reduce the microbial load on it without affecting overall quality. Therefore, in this study, an attempt was made to inactivate the microbial contaminants on the surface of dried laver using LPAP. Also, possible changes in physicochemical characteristics of dried laver during plasma exposure was determined. In addition, we determined the sensory characteristics and microbial count of *kimbab,* a Korean rice roll with laver, which was prepared using LPAP-treated laver.

## 2. Materials and methods

### 2.1. Laver sample

Thin sheets (18 cm × 20 cm; 0.35 mm thickness) of sun-dried laver seaweed (*Porphyra*) were purchased from a local grocery store. Samples (n = 5) from different batches were sealed in polythene bags and stored in a dry place at room temperature (25 °C) until use. Experimentation was started in 48 h after sampling.

### 2.2. Detection & enumeration of laver microbial contaminants

Microbial contaminants of dried laver were detected and enumerated using selective growth media. Enumeration was done using the standard plate count method (KFDA., 2011). For 10 g of each powdered laver sample, which was taken in sample bag (3M® stomacher bag with lateral non-woven filter), and sterile saline solution (90 ml) was added. Then, the mixture was homogenized for 3 min in a paddle bag blender (Masticator, IUL, Barcelona, Spain) and filtered. Under sterile conditions, 1 ml aliquot from filtrate was removed from the stomacher bag and placed in an empty sterile petri plate (pour plate method) and poured in 15 ml melted agar which has been cooled to 45 °C, and incubated at 37 °C for 24 h. Plate count agar (PCA) for aerobic bacteria and selective agar media (Difco, Becton Dickinson and Co., Sparks, USA) including mannitol-egg yolk-polymyxin (MYP) agar for *Bacillus cereus*, potato dextrose agar (PDA) for molds, marine agar (MA) for marine bacteria, eosin-methyleneblue agar for *E. coli*, Baird—Parker agar for *Staphylococcus aureus*, xylose-lysine-deoxycholate (XLD) agar for *Salmonella* spp., thiosulfate-citrate-bile salts-sucrose (TCBS) agar for *Vibrio* spp., and Oxford Listeria selective agar supplemented with Oxford Listeria selective supplement (Merck, Darmstadt, Germany) for *Listeria monocytogenes* were used for detection and enumeration.

### 2.3. Low-pressure air plasma (LPAP) generation

Low-pressure plasma generating system (CUTE series, Femto Science Co. Ltd, Hwaseong, Korea) was used. A vacuum pump (Woosung Vacuum Co., Ltd, Incheon, Korea) with pumping speed of 12 $m^3$/h was used to reduce pressure inside the plasma generating chamber to 1 Torr. Tank containing plasma-generating gas (compressed air) was connected to the chamber, and gas flow was regulated by the adjustment of pressure adjusting valve in order to maintain the desired partial pressure of gas within the chamber. Compressed air was introduced into the chamber at a velocity of 350 ml/min. Plasma was generated at an optimal power density of 54.1 mW/$cm^3$ (Lee et al., 2015). However, the intensity of plasma can be regulated by varying the vacuum pressure and/or by varying the power density. About 50 mm gap was maintained between electrode and sample stage during plasma treatment.

### 2.4. Laver plasma treatment & determination of microbial inactivation

Laver sheets were cut into 2 cm × 2 cm squares, taken in petri plates, and treated with the plasma at predetermined time intervals (0, 5, 10, 15, and 20 min). Immediately after treatment, powdered samples (1 g for each treatment condition) were taken in individual stomacher sample bags, and sterile saline solution (9 ml to each bag) was added. Then, the samples were homogenized in a paddle bag blender for 3 min; and viable microbial counts were determined according to the aforementioned procedures.

### 2.5. Modeling of inactivation pattern

Modeling of microbial inactivation kinetics was conducted by using first-order and pseudo-first-order models, as described previously (Lee et al., 2015; Mok & Lee, 2012).

### 2.6. Physicochemical analysis

#### 2.6.1. Color measurement

Color characteristics of unexposed and plasma-exposed dried laver were determined using a Hunter lab colorimeter (Color difference meter, CR-200, Konica- Minolta Inc., Tokyo, Japan); and values are expressed in terms of the L (lightness), a (redness) and b (yellowness). Also, the color change (ΔE) was calculated.