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Research Article

Quality Evaluation of Rice Treated by High Hydrostatic Pressure and Atmospheric Pressure Plasma

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This study applied high hydrostatic pressure (HHP) and atmospheric pressure plasma (APP) treatments to rice and examined the effects of the treatments on the microbial contamination and physicochemical properties. The microbial population was 100% sterilized by HHP and reduced by up to 34% by APP. Color *a* values were increased by up to 285% and 33% in HHP and APP, respectively. HHP increased fructose (~8,256%) but decreased glucose, sucrose, and maltose (~97%, −100%, and −93%, respectively). APP only mildly modified sugar composition compared with HHP. Retrogradation factors were not changed remarkably by HHP or APP. In conclusion, HHP sterilized microorganisms, but the sterilization was accompanied by high modifications to color and sugar composition. APP had a lesser effect on the microbial population, but it only mildly changed the physicochemical properties of the rice. Therefore, application of either HHP or APP could be considered depending on the intended use of the rice.

1. Introduction

Rice (*Oryza sativa* L.) is a major staple crop cultivated in many countries including those of East Asia. Rice cultivation has increased steadily and rice production reached 753 million tons in 2016, of which 686 million tons was harvested in Asian countries [1]. In the food industry, rice utilization has been increasing due to its use as an ingredient in beverages, processed meats, puddings, salad dressings, and gluten-free breads [2]. However, physicochemical modifications are often accompanied by microbial contamination during storage and food processing, which affects the properties of the rice products [3]. Therefore, the importance of quality maintenance has increased with the growth of the rice industry.

Microbial contamination is the major risk factor for crop damage after harvest, thus various strategies have been proposed to prevent such damage [4]. Thermal treatment sterilization is a general method for microbial control, but it induces food color changes, protein denaturation, starch gelatinization, and loss of micronutrients [5]. Therefore, nonthermal treatments such as high hydrostatic pressure (HHP) and atmospheric pressure plasma (APP) treatments have been proposed as alternative strategies. HHP treatment improves storage stability and safety by minimizing damage to micromolecules, including pigments and vitamins. In contrast, macromolecules such as proteins are dissociated by HHP, thus pasteurizing microorganisms [6]. APP treatment creates partially ionized gas containing reactive oxygen species, reactive nitrogen species, and charged particles, as well as ultraviolet radiation [7, 8]. APP interacts with the cell wall and membrane of microorganisms, damaging nucleic acids and proteins. APP has been adapted to inhibit microbial contamination of fresh agricultural products such as cabbage and tomatoes [9].

In this study, we investigated the microbial population and physicochemical properties of rice from seven cultivars

grown in Korea after HHP and APP treatment. The results of this study should be useful for industrial applications through improved safety of rice for storage and processed foods.

2. Materials and Methods

2.1. Sample Preparation and HHP and APP Treatments. The rice cultivars used were the Dabo, Daebo, Sukwang, Sindongjin (Jeonbuk), Samkwang (Chungnam), Jinsumi (Chungbuk), and Haiami (Kyunggi) cultivars, which were grown during the 2016 growing season. The samples were stored in a refrigerator at 4°C until analysis.

The samples were subjected to HHP using a warm isostatic press pressure treatment system (WIP-L60-50-200, Ilshin Autoclave, Inc., Daejeon, Korea), with the temperature of the pressure chamber maintained at room temperature (20°C). A warm isostatic press is a reactor that applies isostatic pressure using water as the pressure medium without the use of heat or gas. It is composed of a high-pressure vessel, a high-pressure pump, a reservoir tank, a safety device, an alarm system, and a control system. The samples were transferred to a laminated aluminum foil film (Newpack, Seoul, Korea) and heat-sealed using vacuum packaging (chamber-type vacuum package, DP-901, Dew Pack Korea Machinery Co., Seoul, Korea). HHP was carried out immediately after germination to prevent enzyme inactivation. The packaged samples were subjected to a pressure of 300 MPa at 25°C for 30 min.

The plasma apparatus used in this study was used previously by Kim et al. [10]. Optimum conditions such as treatment time and input power of APP were established in a previous and preliminary study (data not shown). Briefly, air dielectric barrier discharge plasma source was constructed using a rectangular (parallelepiped) plastic container (137 × 104 × 53 mm). The actuator was made of copper electrodes, and a polytetrafluoroethylene sheet was attached to the inner walls of the container. A bipolar square-waveform voltage at 15 kHz was applied to one electrode, while the other electrode was grounded. The size of powered and grounded electrodes was 30 mm and 10 mm, respectively. Plasma was generated inside the container with an input power of 250 W. Each sample (15 g) was placed in a Petri dish at the bottom of the container, and the distance between the sample and the plasma generator was 20 mm. The sample was treated with the APP source for 20 min.

2.2. Microbial Analysis. The prepared sample (5 g) was mixed for 2 min in a sterile Stomacher bag containing 45 mL of sterile saline solution (0.85%) using a Stomacher Bag-Mixer 400 (Interscience Co., Saint Nom, France). Total plate count agar was prepared for counting of the total number of aerobic microbes (Difco Laboratories, Detroit, MI, USA). The plates were incubated at 37°C for 48 h, and the colony-forming units (CFUs) per gram were counted at a dilution of 30–300 CFU per plate.

2.3. pH. The pH was measured using a pH meter (Model 750; iSTEC, Seoul, Korea). About 1 g of each sample was added to 10 mL of distilled water and homogenized for 30 s. The pH was then measured. Calibration was performed using standard buffers provided by the manufacturer at pH 4, 7, and 10 at room temperature.

2.4. Sugar Content. The free sugar content was measured according to a modification of the method of Woo et al. [11], using fructose, glucose, maltose, and sucrose as standards for calibration curves. Samples were filtered through a 0.45 μm syringe filter (Millipore) and analyzed by high-performance liquid chromatography (HPLC) (Waters 2695; Waters, New Castle, DE, USA). The analytical column was for carbohydrates (4.6 × 150 mm, Waters), and the mobile phase was water-acetonitrile (25 : 75, v/v) at a flow rate of 1 mL/min. The injection volume was 20 μL, and the detector was an evaporative light scattering detector (Waters 2420). All samples were analyzed in triplicate.

2.5. Color. Each sample was poured into a Petri dish, and its color was evaluated using a color difference meter system (Spectrophotometer CM-3500d; Konica Minolta Sensing, Inc., Osaka, Japan). The Hunter color values, L^* (lightness), a^* (redness), and b^* (yellowness), were determined. The instrument was calibrated with a standard black and white plate before analysis. The Hunter values were monitored by a computerized system using SpectraMagic software (Konica Minolta Sensing, Inc.), and the measurements were performed in triplicate.

2.6. Thermodynamic Properties. The thermal behaviors of the samples were determined using differential scanning calorimetry (DSC) (Model Q1000 calorimeter, TA Instruments, Inc., New Castle, DE, USA). Each sample was weighed directly into a DSC pan and distilled water was added to obtain a flour-to-water ratio of 1 : 2.3 (w : w). The pan was then hermetically sealed and allowed to stand for 1 h prior to thermal analysis. Thermal scanning was undertaken from 4°C to 150°C at a heating rate of 5°C/min. The gelatinization onset (T_o), peak (T_p), and conclusion (T_c) temperatures and the transition enthalpy (ΔH) were determined from the peak area of the DSC endotherm.

2.7. Statistical Analysis. Data were presented as the mean ± SD. The Student's t-test was used to compare means between the control group and treatment group. If $p < 0.05$, the result was considered statistically significant.

3. Results and Discussion

3.1. Microbial Population in Rice after HHP and APP Treatments. We determined the effects of HHP and APP on the microbial population in rice from seven cultivars (Figure 1). In the untreated rice group, the microbial concentration was 4.08–4.11 log CFU/g, but APP decreased the population to 2.68–2.84 log CFU/g. The reduction compared to the nontreated rice was 31–34%. The HHP treatment sterilized the microbial contents in the rice from all cultivars

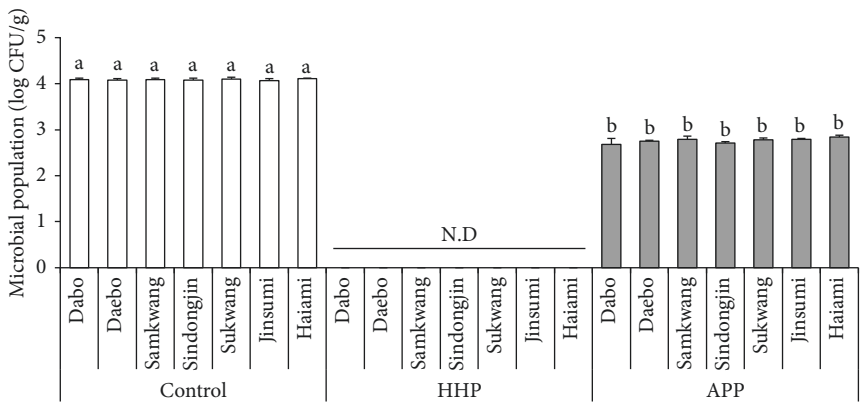


Figure 1: Microbial population (log CFU/g) of rice. Samples were treated by high hydrostatic pressure or atmospheric pressure plasma, and microbial populations were compared to control. Values with different superscripts are significantly different at $p < 0.05$ according to Tukey's multiple range tests within the same cultivar. ND, not detected.

(0 log CFU/g) and was more effective for microbial regulation than APP.

Microbial control is an important issue in food safety. Thermal treatment has been recognized as an effective and economical technique for sterilization; however, it is not suitable for preserving heat-unstable compounds. Therefore, nonthermal treatments for microbial control, including HHP and APP, have received considerable interest [12]. Previously, HHP was used for inactivation of microorganisms and enzymes in legumes and barley and also adapted for modification of allergenic protein in rice [13]. We also confirmed that HHP is effective for microbial inactivation in rice. APP using ionized gas with high kinetic energy has also been implemented for microbe sterilization. In contrast to HHP, APP displays a sterilization effect only against microorganisms on the surface of the material that can come in contact with the plasma gas. Therefore, APP is less effective at sterilizing the inner portion of the material, and this may explain its lower sterilization rate compared to that of HHP [14].

3.2. pH of Rice after HHP and APP Treatments. Microbial growth is affected by environmental factors, including pH [15]. We measured the pH of rice from the seven cultivars after the HHP and APP treatments (Figure 2). The pH in untreated rice was 6.44–6.56, and the pH after APP treatment was 6.39–6.59 which showed only minor changes. The pH of the rice after HHP treatment was 4.55–5.63, a statistically significant ($p < 0.05$) reduction of 13–27% compared with the untreated rice.

HHP treatment induces physical modifications of molecular structures that may alter the chemical compounds that affect pH [16]. However, pH of the rice treated with APP was slightly changed compared to the control, which is interpreted as a result of the low rate of physicochemical changes. Therefore, HHP regulates microorganism population by inducing physicochemical changes, while APP maintains original characteristics of rice and inhibits microbial growth on the surface.

3.3. Sugar Contents of Rice after HHP and APP Treatments. We analyzed the free sugar contents, which determine the quality of rice, by HPLC and expressed the results as the area of the peak (%, Figure 3). The fructose contents were 0.61–6.77% in untreated rice, 87.99–94.00% after HHP, and 0.47–6.94% after APP. The HHP treatment induced a significantly high percentage of fructose compared with both untreated and APP-treated rice. The glucose content was 7.23–19.68% in untreated rice, 0.57–1.51% in HHP-treated rice, and 5.86–19.15% in APP-treated rice. The glucose content was highest in the untreated rice, and the HHP-treated group showed the lowest level compared to the control group and the APP-treated rice ($p < 0.05$). In the sucrose content analysis, the peak areas were 8.73–18.74% for the control group, 0.73–4.28% for the HHP-treated rice, and 7.12–22.60% for the APP-treated rice. The HHP group showed a significantly lower sucrose level ($p < 0.05$) than the control group and the APP group. Lastly, maltose was 46.50–72.27% in the control group, 0.00–0.21% in the HHP group, and 56.46–78.02% in the APP group. The untreated rice contained maltose at a level similar to that of the APP group and higher than that of the HHP-treated rice ($p < 0.05$). To summarize these results, the HHP treatment increased the fructose level compared to the control, but the glucose, sucrose, and maltose levels were decreased significantly. In contrast, the APP treatment showed nonsignificant or minor changes compared with the untreated rice.

HHP preserves the primary structure of molecules and low molecular weight compounds such as vitamins, amino acids, and flavor molecules. However, the secondary and tertiary structures of macromolecules, including starch, can be destroyed [16, 17]. Therefore, the HHP treatment of the rice markedly increased the content of fructose, which has the lowest molecular weight of the sugars. In contrast, the levels of glucose, maltose, and sucrose were decreased by the HHP treatment. Plasma treatment produces reactive species known to react with amylose by depolymerizing, cross-linking, and binding with functional groups and thus modifying the starch structure [18]. However, the APP treatment in our study produced less modification than the

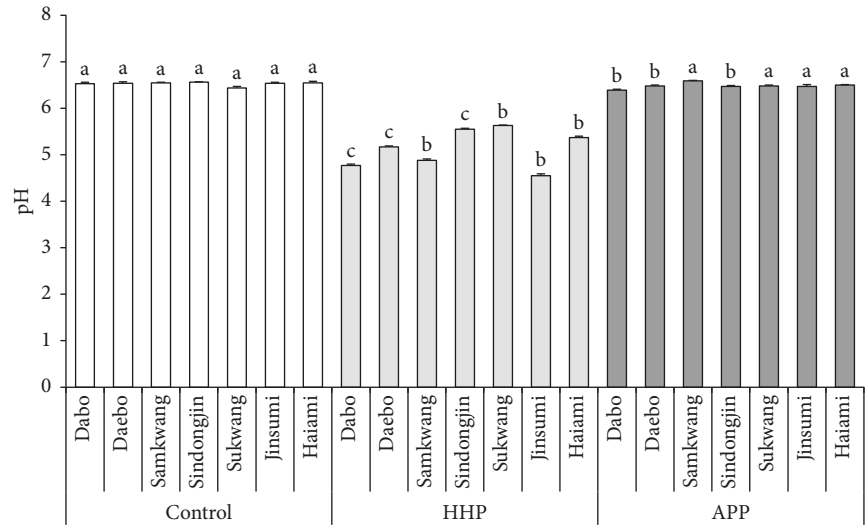
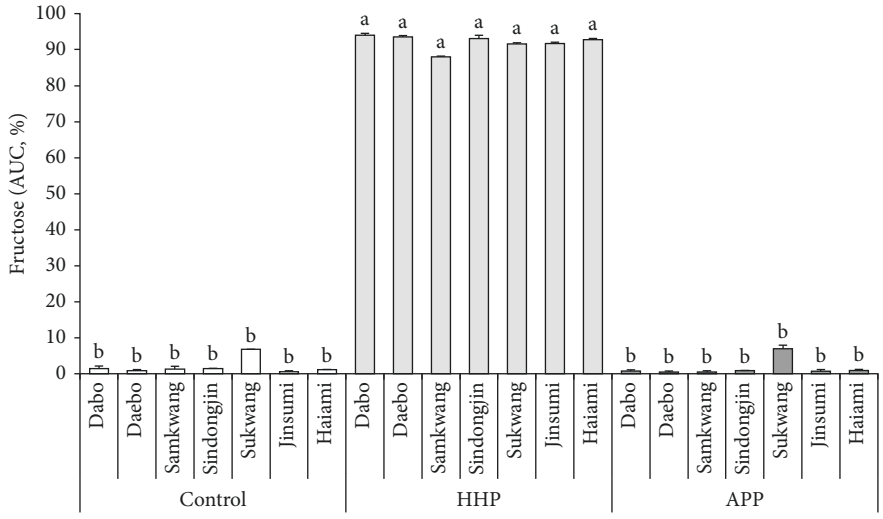


Figure 2: pH changes of rice. Samples were treated by high hydrostatic pressure or atmospheric pressure plasma, and pH changes were compared to control. Values with different superscripts are significantly different at $p < 0.05$ according to Tukey's multiple range tests within the same cultivar.



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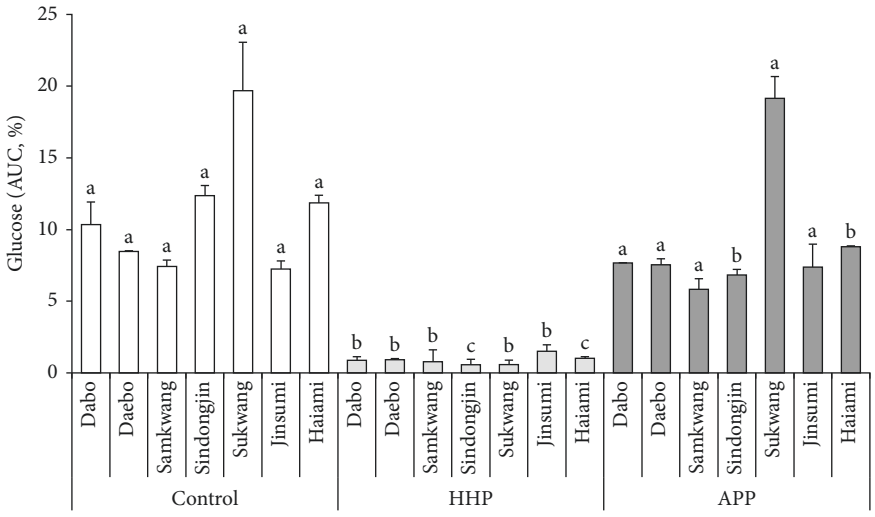

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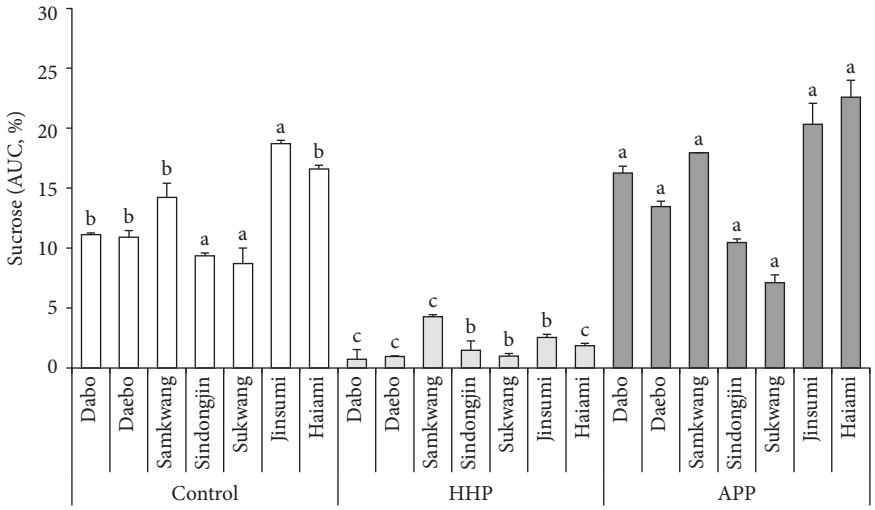

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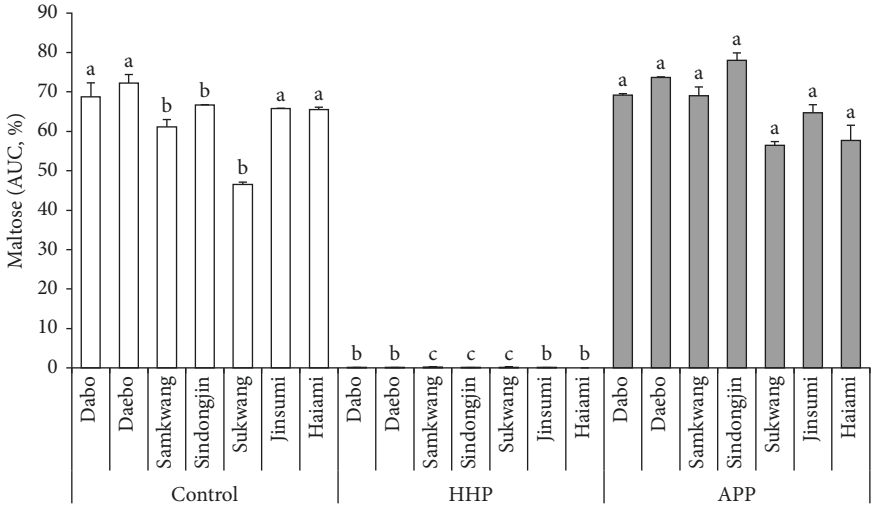
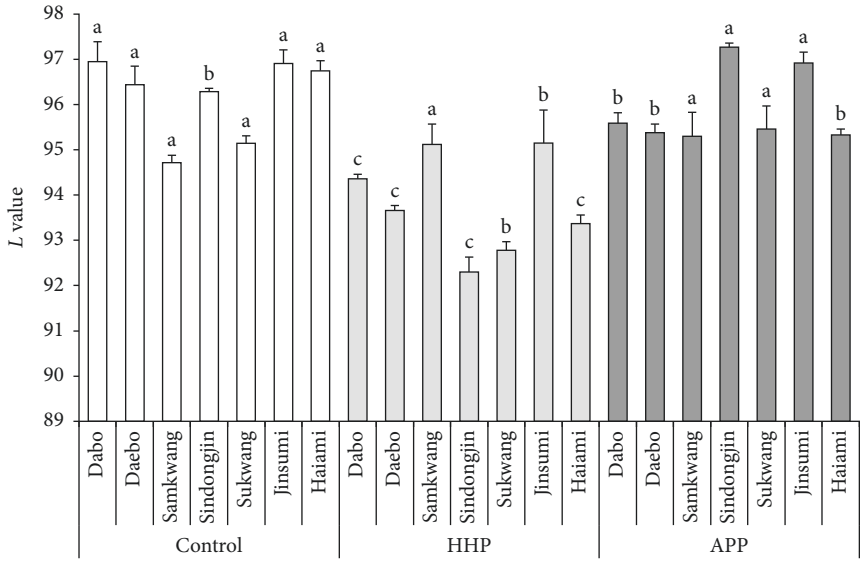


(d)

Figure 3: Sugar (fructose, glucose, sucrose, and maltose) contents of rice. Samples were treated by high hydrostatic pressure or atmospheric pressure plasma, and sugar contents were compared to control. Values with different superscripts are significantly different at $p < 0.05$ according to Tukey's multiple range tests within the same cultivar. AUC, area under the curve.



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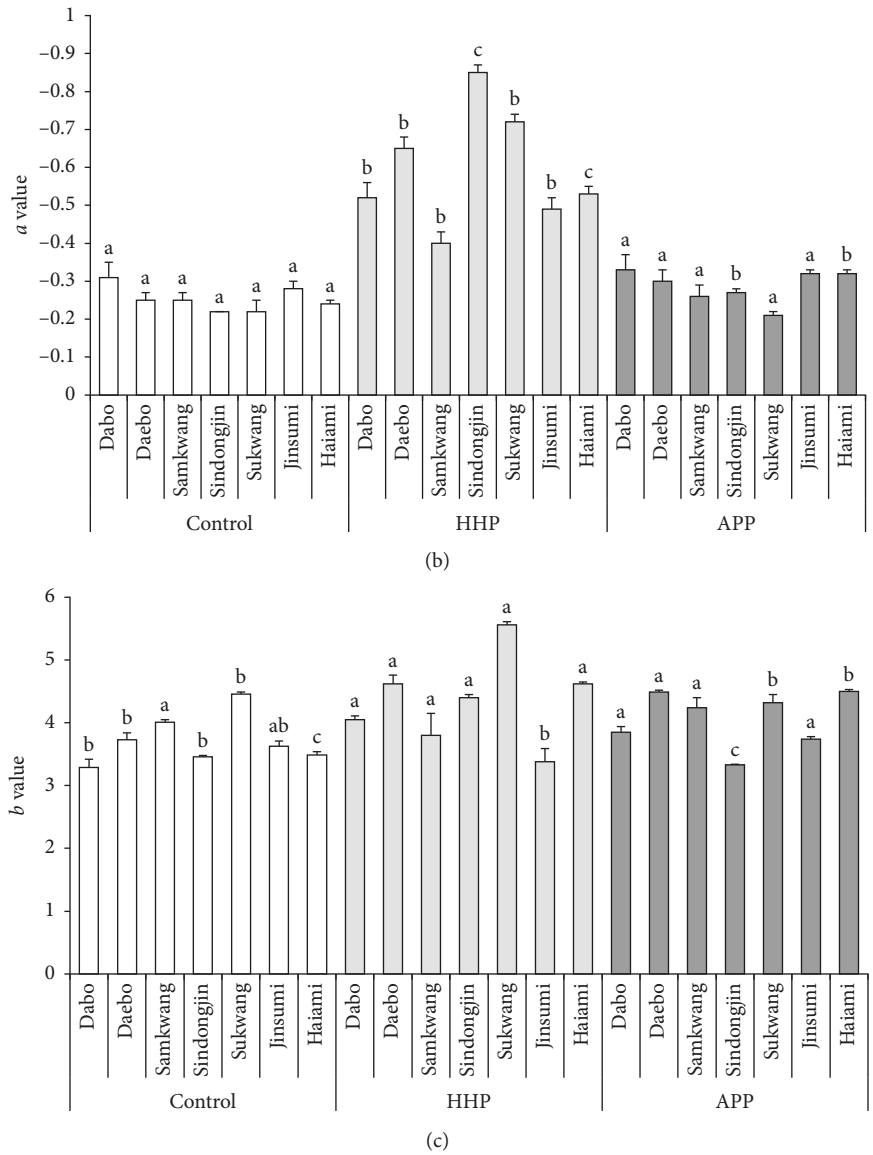
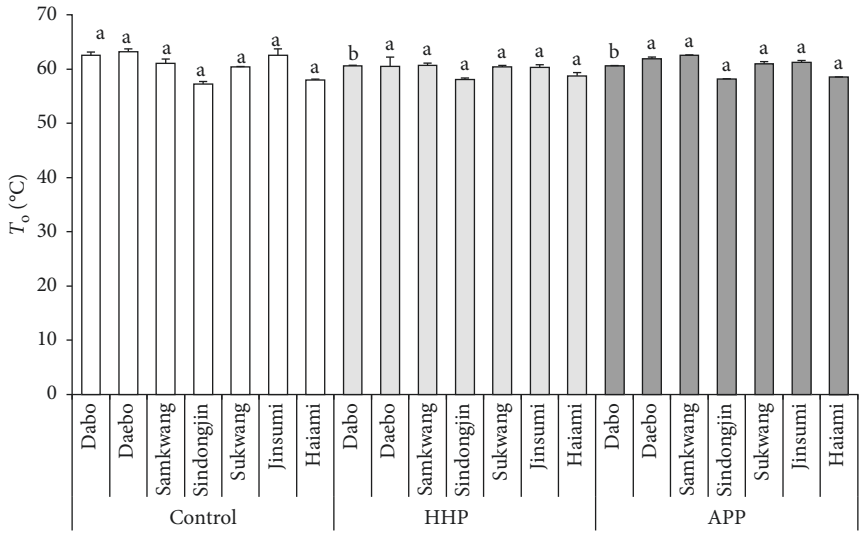


Figure 4: Colorimetry analysis of rice. Samples were treated by high hydrostatic pressure or atmospheric pressure plasma, and colors were compared to control. Values with different superscripts are significantly different at $p < 0.05$ according to Tukey's multiple range tests within the same cultivar.

HHP treatment, which may have been due to an insufficient energy level for interaction with starch molecules inside the material. Therefore, APP modified less sugar composition than HHP, which maintains nutritional value and taste similar to nontreated rice.

3.4. Rice Color after HHP and APP Treatments. We measured Hunter Lab values to confirm the effects of HHP and APP on the color of the rice (Figure 4). The brightness (L) value was 96.95–94.72 in the control, 95.15–92.30 in the HHP group, and 97.27–95.30 in the APP group. The Dabo, Daebo, Sindongjin, and Haiami cultivars showed significant differences compared to the control after HHP and APP, but the Sukwang and Jinsumi cultivars were significantly lower for only the HHP group ($p < 0.05$). The Samkwang cultivar showed no significant change in brightness with either HHP or APP. The a values, which indicate the degree of redness, were −0.22 to −0.31, −0.40 to −0.85, and −0.21 to −0.33 in the control, HHP, and APP groups, respectively. The APP-treated rice showed significantly low values in only the Sindongjin and Haiami cultivars. In the HHP-treated rice, the a value was decreased in every cultivar, indicating induction of greenness. The b value, indicating the degree of yellowness, was also measured. The ranges of the b value were 3.29–4.46, 3.38–5.56, and 3.33–4.50 in the control, HHP, and APP groups, respectively. The HHP treatment significantly increased the b value, except in the Samkwang and Jinsumi cultivars. The APP treatment increased the b value in the Dabo, Daebo, and Haiami cultivars. In conclusion, the HHP treatment produced a decrease in brightness and redness, and an increase in yellowness. The



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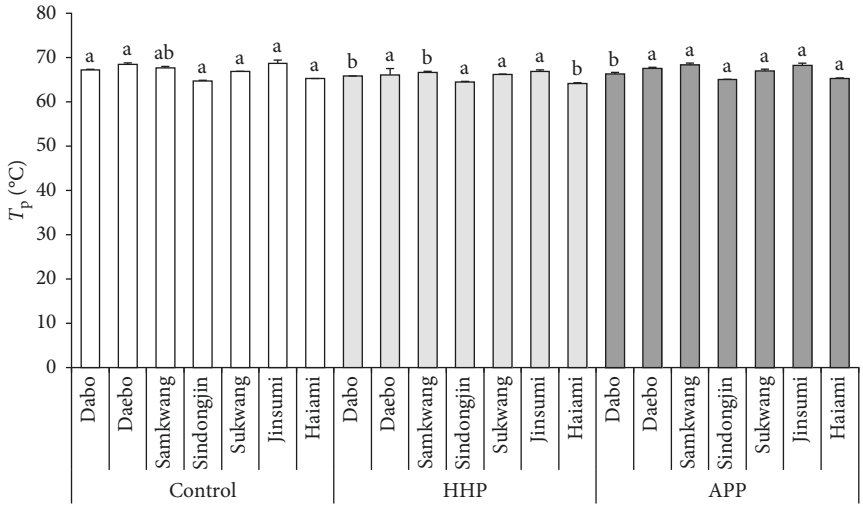

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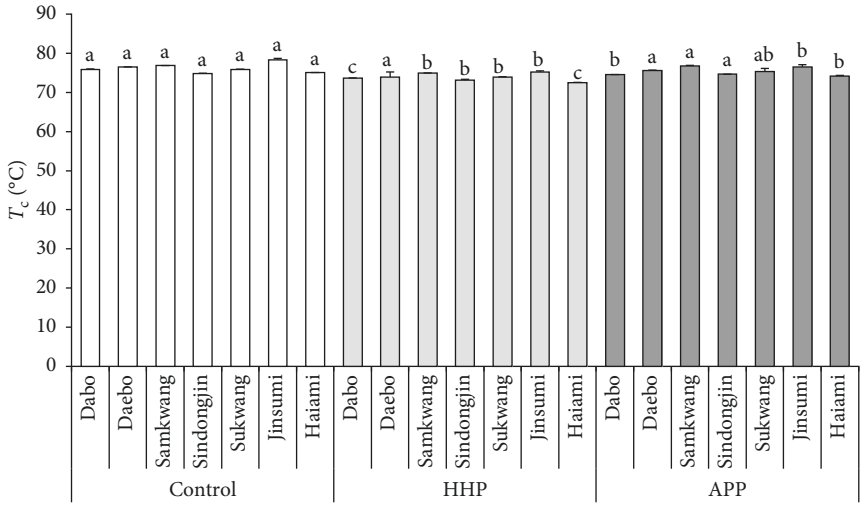

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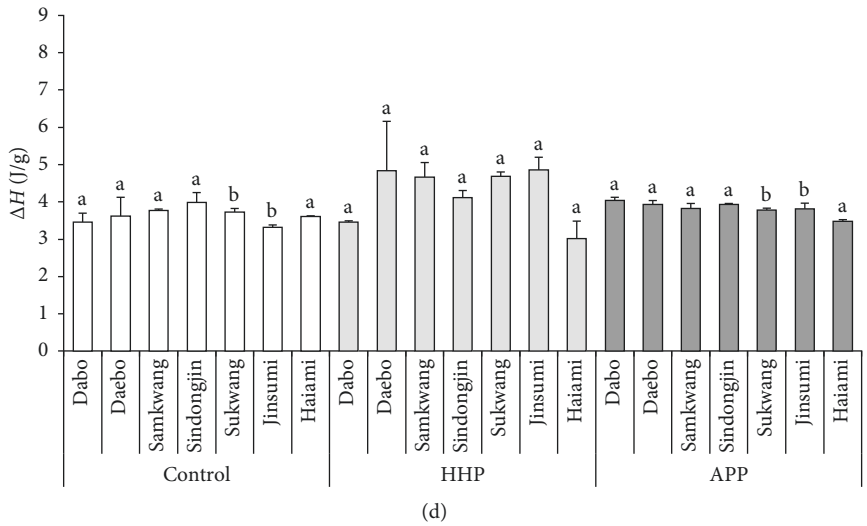

Figure 5: Differential scanning calorimetry (DSC) thermodynamic properties of rice. Samples were treated by high hydrostatic pressure or atmospheric pressure plasma, and DSC was compared to control. Values with different superscripts are significantly different at $p < 0.05$ according to Tukey's multiple range tests within the same cultivars.

APP treatment was similar to that of the control group or slightly altered in some varieties, but the change was less than that of the HHP treatment.

Overall, the color difference was highest for the *a* value after HHP treatment, and APP-treated samples were similar to the control. Previous studies that used HHP to treat food materials also showed changes in Hunter Lab values. However, the difference was less than thermal treatment because the color components are sensitive to temperature [19, 20]. Therefore, nonthermal treatments, especially APP, minimize color changes in foods with effective microbial inactivation.

3.5. DSC Thermodynamic Properties of Rice after HHP and APP Treatments. We analyzed starch retrogradation by DSC (Figure 5). The enthalpy change (dH) was determined by the energy transformation during the melting of recrystallized amylopectin, which is responsible for starch retrogradation [21]. There were no significant differences between the HHP or APP treatment groups compared to the control group, except for the HHP-treated Sukwang and Jinsumi cultivars (26% and 46% increases, respectively). The onset temperature (T_o), maximum peak temperature (T_p), and completion temperature (T_c) are dependent on the structure or degree of hydrogen bonding of the starch. These values predict the melting and destruction points of the amylose complex [21]. T_o value was decreased with HHP and APP treatment only in the Dabo variety. T_p values were significantly decreased in Dabo after HHP and APP and in Haiami after HHP. On the other hand, T_c was decreased significantly by HHP treatment (except for Daebo) and by APP treatment in Dabo, Jinsumi, and Haiami.

Starch retrogradation may occur following gelatinization, and HHP has been reported to induce damage to the starch structure by initiating gelatinization. The degree of retrogradation following gelatinization is lower in HHP than in thermal treatment due to the lower moisture content [22]. APP, which works by reactive energetic electrons, sometimes affects the inner portion of cereals, which results in damage to the interior organization of the grains [23]. However, the HHP and APP treatment procedures used in this study did not markedly alter the retrogradation factors of the rice. This may be due to different experimental factors such as time, pressure, and plasma intensity. Therefore, this result provides appropriate methods for microbial inactivation in rice with only minor changes to retrogradation factors.

In conclusion, The HHP and APP treatments effectively controlled the microbial population of the rice cultivars in this study. In particular, HHP powerfully sterilized microbial growth and produced remarkable changes in the physicochemical properties of the rice (pH, brightness, *a* value, and free sugar composition). In contrast, APP showed only a mild effect on rice qualities with an ~34% reduction in the microbial population. The results of this study could be applied in the rice processing industry and may provide a suitable method for both microbial regulation and maintenance of rice quality. The application of either HHP or APP could be considered depending on the intended use of the rice.

Abbreviations

APP: Atmospheric pressure plasma
CFU: Colony-forming unit
DBD: Dielectric barrier discharge
DSC: Differential scanning calorimetry
HHP: High hydrostatic pressure
HPLC: High-performance liquid chromatography.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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