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Effect of Plasma-Activated Water on the Microbial Decontamination and Food Quality of Thin Sheets of Bean Curd

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Abstract: Thin sheets of bean curd may serve as an excellent source of nutrition for microorganisms and are therefore prone to contamination, which can be harmful to public health. This study evaluated the influence of plasma-activated water (PAW) on the microbial load and food quality of thin sheets of bean curd. Treatment for 30 min with PAW that was activated for 90 s reduced the microbial count by 1.26 and 0.91 log_{10} CFU/g for total aerobic bacteria and total yeasts and molds on thin sheets of bean curd, respectively. The effect of PAW on microbial inactivation strongly depended on the activation time for PAW generation and the soaking time of the thin sheets of bean curd in PAW. Further, PAW could maintain total isoflavone content, sensory properties, and most of the textural properties of the thin sheets of bean curd. Although PAW treatments caused significant changes in color parameters of the thin sheets of bean curd, the appearance acceptance was not significantly influenced. This work highlights the potential application of PAW in the microbial decontamination of thin sheets of bean curd.

Keywords: plasma-activated water; thin sheets of bean curd; inactivation; food quality

1. Introduction

Thin sheets of bean curd are a kind of traditional bean product in Southeast Asia. They are a semi-dehydrated soybean product, produced by adding a coagulant to soy milk and then compressing it into thin sheets, and are used in a wide variety of popular dishes. They are rich in minerals, protein, and overall amino acid composition [1]. Moreover, the abundant isoflavones and lecithin act as functional ingredients for preventing osteoporosis, cardiovascular disease, as well as cancer [2]. However, due to their good nutrition, thin sheets of bean curd can be a vehicle for microorganisms, which are responsible for food spoilage and may present a health hazard to humans. Thus, it is necessary to control the contamination of thin sheets of bean curd.

Traditional technologies used for contamination control, such as additives, refrigeration, and steam sterilization, may reduce the sensorial characteristics and present some food safety issues [3]. However, nonthermal technologies, such as high-pressure, ultrasonic, and irradiation methods, have been utilized to control microbiological hazards and negative impacts on the intrinsic qualities of foods [4]. In recent decades, nonthermal plasma has been used as a new nonthermal technique that has exhibited enormous potential for applications in food industries [5]. Nonthermal plasma is produced by ionization of gas and is composed of charged particles, neutral particles, ultraviolet photons, and reactive species [6,7]. It has gained extensive attention considering its capacity for the inactivation of a wide range of microorganisms, including bacteria, bacterial spores, biofilms, viruses, and fungi [8,9]. Plasma-activated water (PAW), which refers to distilled water subjected to nonthermal plasma discharge [10], has been applied for the microbial decontamination of strawberries, grapes, button mushrooms, and mung bean sprouts [10–13]. The reactive oxygen species (ROS), high oxidation-reduction potential (ORP), and low pH of PAW are considered to play a key role in inactivating microorganisms [14,15]. These species in PAW are stable and can provide long-lasting effects [16]. However, there are few reports about the microbial decontamination effect of PAW for thin sheets of bean curd. Moreover, the influences of PAW on the intrinsic properties of thin sheets of bean curd also need to be investigated.

In this work, the microbial inactivation efficacy of PAW on thin sheets of bean curd was studied. The impacts of activation time and soaking time on the microbial inactivation efficacy of PAW were evaluated. Further, the color, textural properties, total isoflavone content, and sensory properties of thin sheets of bean curd treated by PAW were determined.

2. Materials and Methods

2.1. Generation of PAW

PAW was generated with an atmospheric pressure plasma jet system based on gliding arc discharge in air (Tonson Automation Equipment Co., Ltd., Shenzhen, China). The terminal of the plasma jet was above the water surface and the distance between them was kept at 3.5 cm. The PAW preparation was operated at a high working voltage (5 kV), a high-frequency sine wave inverter (40 kHz), and a high discharge power (750 W). Compressed air was used as the working gas with a flow rate of 30 L/min. Then, 300 mL of sterile distilled water (SDW) was activated by plasma for 30, 60, and 90 s to obtain PAW, respectively defined as PAW30, PAW60, and PAW90.

2.2. PAW Treatment

Thin sheets of bean curd manufactured in the same batch were selected from a local supermarket in Zhengzhou (Henan, China). Before PAW treatment, they were cut into pieces of about 2 × 30 cm with sterile knives. For the detection of each index mentioned below, the untreated and treated sample pairs were prepared from the same piece of thin sheets of bean curd for more effective comparisons.

The cut pieces of thin sheets of bean curd were firstly divided into four treatment groups and one control group to study the bactericidal effect and the influences on the physicochemical properties of these kinds of PAW. The untreated control was set to assess the initial population of microorganisms on the surface of thin sheets of bean curd and the inherent physicochemical properties. For the four treatments, samples (5 g) were immersed in SDW, PAW30, PAW60, and PAW90 with continuous agitation at 100 rpm for 20 min, separately. The four treatments are abbreviated here as SDW-20m, PAW30-20m, PAW60-20m, and PAW90-20m. Among them, the SDW-20m treatment was carried out to estimate the influence of the agitating process on the reduction of microorganisms on the thin sheets of bean curd.

Subsequently, to investigate the influence of immersing time on the bactericidal effect and physicochemical properties of thin sheets of bean curd, samples were immersed in PAW90 for 10, 20, and 30 min, as well as in SDW for 30 min, separately. For simplicity, they are correspondingly defined as PAW90-10m, PAW90-20m, PAW90-30m, and SDW-30m. The untreated sample was also used as a control to analyze the effects of the different treating times. After treatment, the bactericidal effect and physicochemical properties of the samples were analyzed immediately.

2.3. Microbial Enumeration

The numbers of total aerobic bacteria, as well as total yeasts and molds on the thin sheets of bean curd with different treatments, were detected. Briefly, 5 g of the sample was mixed with 45 mL of sterile saline (0.85% NaCl) and then vortexed at 200 rpm for 10 min. After the microorganisms attached to the thin sheets of bean curd were released into the saline, the collected solution was ten-fold serially diluted. Then, 100 µL of the dilutions was spread uniformly on plate count agar (PCA, Aobox Biotechnology Co., Ltd., Beijing, China), to count the total aerobic bacteria, and rose bengal medium (RBM, Aobox Biotechnology Co., Ltd., Beijing, China), to assess the total yeasts and molds. Subsequently, PCA plates were incubated at 37 °C for 48 h, and RBM plates were kept at 28 °C for 96 h. Finally, the number of colonies on the plates was counted and the results are represented as $\log_{10}$ CFU/g.

2.4. Color Measurement

A WSC-80C colorimeter (Beijing Optical Century Instrument Co., Ltd., Beijing, China) with a CIE system was used to determine the color parameters of the thin sheets of bean curd samples before and after different treatments. Specifically, the L^*, a^*, and b^* values were determined to express the degree of brightness, green/red, and blue/yellow of the sample, respectively. The total color difference represented as ΔE^* was calculated with Equation (1):

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (1)$$

where ΔL^*, Δa^*, and Δb^* represent the differences of L^*, a^*, and b^* values between the PAW-treated and control samples, respectively.

2.5. Textural Property Analysis

The texture attributes of treated and untreated thin sheets of bean curd were determined with a TA New Plus texture analyzer (ISENSO, USA). The samples were cut into disks (2 cm diameter) and placed on the nonlubricated flat plate. The texture properties were measured with a P/50 probe and the sample was compressed by 75%. The crosshead speed of the texture analyzer was set at 1.0 mm/s, whereas the test and post-test speeds were set at 0.8 mm/s. The compression force was 5.0 g. About seven measurements were taken for each sample.

2.6. Determination of Total Isoflavones

Thin sheets of bean curd were freeze-dried and ground into powder before measurement. The powder (2 g) was soaked in 30 mL of 70% (*v*/*v*) ethanol aqueous solution and kept at 70 °C for 2.5 h with continuous stirring. Then, after filtration, the filtrate was collected and diluted with 70% (*v*/*v*) ethanol to 100 mL. Subsequently, the content of extracted isoflavones was detected with three-wavelength spectrophotometry. The calibrated absorbance value (A) was calculated according to Equation (2):

$$A = A_{263\text{nm}} - \frac{(A_{283\text{nm}} + A_{243\text{nm}})}{2} \quad (2)$$

where $A_{263\text{nm}}$, $A_{283\text{nm}}$, and $A_{243\text{nm}}$ are the absorbance values at 263, 283, and 243 nm, respectively.

Genistein was used as a standard substance. A series of 70% (*v*/*v*) ethanol aqueous solutions with different contents of genistein were analyzed with three-wavelength spectrophotometry to obtain the standard curve. The content of total isoflavones in the extract was estimated from the standard curve by measuring the calibrated absorbance value. Yields of total isoflavones are expressed on a mass basis related to the dry materials of thin sheets of bean curd.

2.7. Sensory Test

The sensory attributes of appearance, flavor, brittleness, and overall acceptance of the thin sheets of bean curd with and without PAW treatment were assessed. A panel of 20 postgraduates was selected from the College of Food and Biological Engineering, Zhengzhou University of Light Industry, PR China. A consumption frequency of thin sheets of bean curd of more than once a week was set as the selection criterion. The panelists were told the essential requirements of the sensory test and were trained on sensory descriptors for fresh thin sheets of bean curd before participating in the sensory test. Each sample was put in a disposable white dish labeled with a random number and provided to the panelists. A nine-point hedonic scale was utilized to evaluate the attributes of the thin sheets of bean curd samples. The attributes of appearance, flavor, and overall acceptance were graded from 1 (extremely dislike) to 9 (extremely like), and brittleness was graded from 1 (extremely breakable) to 9 (extremely unbreakable) [17,18].

2.8. Statistical Analysis

Determinations were carried out at least in triplicate and values are presented as mean ± standard deviation (SD). An analysis of variance (ANOVA) was conducted to compare the results of different groups and significant differences were identified with a confidence level at $p < 0.05$.

3. Results and Discussion

3.1. Influence of PAW Activation Time on Inactivation Effect of Microorganisms

The numbers of surviving microorganisms on thin sheets of bean curd before and after treatments are shown in Figure 1. The initial bacteria count was 6.05 $\log_{10}$ CFU/g, while that of total yeasts and molds was 5.70 $\log_{10}$ CFU/g. As expected, there was no significant reduction of the total microorganism population on the thin sheets of bean curd after being subjected to soaking in SDW for 20 min. This means the agitating procedure does not have an impact on the inactivation of microorganisms on the thin sheets of bean curd.

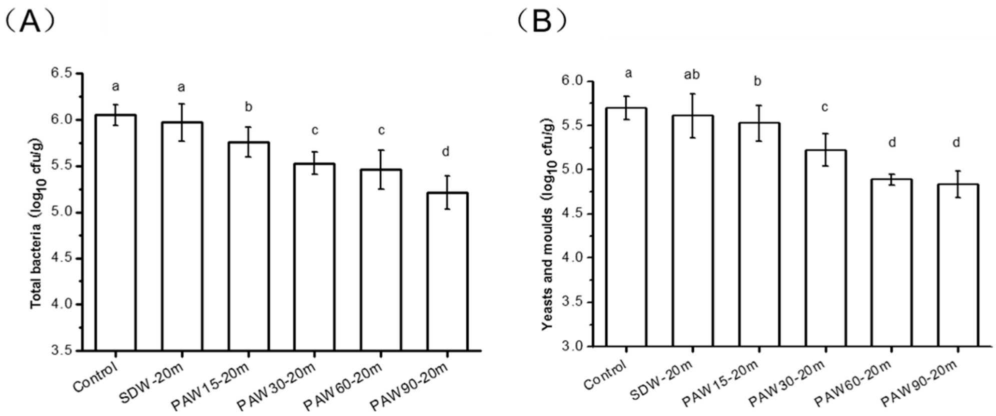

Figure 1. The numbers of surviving bacteria (**A**) and yeasts and molds (**B**) on thin sheets of bean curd before and after plasma-activated water (PAW) treatments with different activation times. Different letters in the bars indicate statistically significant differences ($p < 0.05$).

As shown in Figure 1A, after being treated with PAW15 for 20 min, 5.76 $\log_{10}$ CFU/g of bacteria was found on the thin sheets of bean curd, which was a significant reduction compared with the control group. For PAW30, the effect of bacteria inactivation was more obvious than that of PAW15. However, lengthening the PAW activation time to 60 s did not give rise to more significant inactivation, while, for a longer PAW activation time (PAW90), the bacteria number decreased to 5.21 $\log_{10}$ CFU/g and the inactivation effect of bacteria was the most significant. For total yeasts and molds, PAW15 showed poor inactivation effect and had no significant difference with SDW treatment. In contrast, the PAW60 and

PAW90 groups presented the best inactivation effects (Figure 1B). All of these results indicated that the plasma activation time for PAW generation had a positive effect on the inactivation of bacteria as well as total yeasts and molds. This may be attributed to the higher ORP and electrical conductivity values in the PAW activated for a longer time. The tendency was consistent with reports by Ma et al. [10] and Xu et al. [12].

3.2. Influence of Soaking Time on Inactivation Effect of Microorganisms

The inactivation efficiency of PAW was affected by the soaking time (Figure 2). Especially, SDW treatment for 30 min did not significantly change the population of bacteria as well as that of total yeasts and molds compared to the untreated group. The inactivation efficiency was improved along with extending the soaking time. An approximately 1.26 $\log_{10}$ CFU/g reduction of total bacteria and a 0.91 $\log_{10}$ CFU/g reduction of yeasts and molds on the thin sheets of bean curd were achieved after PAW90 treatment for 30 min. Therefore, the inactivation efficiency of PAW treatment was influenced by the soaking time in PAW, which is consistent with the conclusion of Xiang et al. [13]. According to a study by Guo et al. [11], an approximately 0.5 log reduction of *Saccharomyces cerevisiae* inoculated on grapes was achieved after PAW treatment for 30 min. The less-than-expected effects of PAW on the thin sheets of bean curd and grapes may be attributed to the nonsmooth morphology of their surfaces, which makes the microorganisms easy to stack [11]. A better microbial decontamination effect may be achieved when the synergistic treatment of PAW and other treatments (mild heating, nisin, etc.) is employed in the preservation of foods.

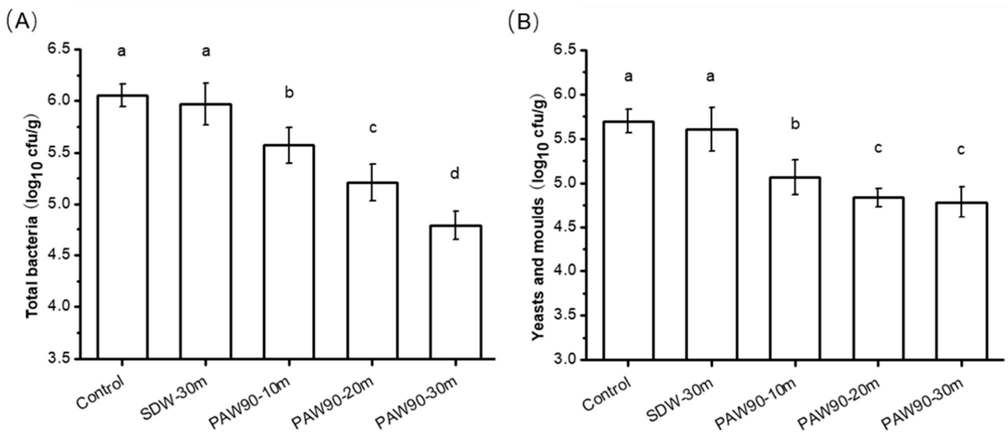

Figure 2. The numbers of surviving bacteria (**A**) and yeasts and molds (**B**) on thin sheets of bean curd before and after PAW treatments with different soaking times. Different letters in the bars indicate statistically significant differences ($p < 0.05$).

Our previous study showed that PAW activated by dielectric barrier discharge (DBD) plasma for 30 s had a lower pH value and higher ORP, electrical conductivity, ROS, and reactive nitrogen species (RNS) compared with SDW [13]. An acidic pH may inactivate the microbes by the interaction of a high level of protons and macromolecules [15,19]. ORP was perceived as contributing to the apoptosis and necrosis of microbes [20]. A considerable number of active ions may result in higher electrical conductivity. Further, it was reported that generated ROS and RNS in PAW could play an important role in the lethal effect on microbes [21]. These elements in PAW would interact with microbes more efficiently and yield better results over the soaking time.

3.3. Influence of PAW Treatments on Color of Thin Sheets of Bean Curd

Food color, which is a critical influence factor on food quality, often affects consumers' acceptability of food products. As shown in Table 1, L^* values (lightness) of PAW60-20m, PAW90-20m, and PAW90-30m were slightly higher than those of the other groups ($p < 0.05$). This indicated that longer PAW activation and soaking times would brighten the thin sheets of bean curd. The control, SDW-30m, and PAW90-10m groups displayed higher a^* values compared with other groups, meaning that soaking in PAW for a longer time would decrease the redness of the thin sheets of bean curd. Further, the mean b^* values of the soaking groups were significantly lower than that of the untreated group, which suggested that soaking in SDW or PAW could decrease the yellowness on the surface of thin sheets of bean curd. The changes in food color may be the result of interactions of active substances and water molecules with pigments in foods [22]. This decrease of a^* and b^* values induced by PAW was consistent with the changes of apple juice treated by plasma [23,24] but different than the changes of button mushroom [12]. This may be due to the different ingredients of various foods.

Table 1. Influence of plasma-activated water treatment on the color parameters of thin sheets of bean curd.

	L^*	a^*	b^*	ΔE^*
Control	77.44 ± 0.24 ^{b}	7.37 ± 0.04 ^{a}	36.18 ± 0.80 ^{a}	-
SDW-30m	77.96 ± 0.68 ^{b}	7.13 ± 0.25 ^{a}	33.98 ± 1.09 ^{bc}	2.39 ± 1.04 ^{b}
PAW15-20m	77.91 ± 0.53 ^{b}	6.88 ± 0.23 ^{b}	32.90 ± 0.89 ^{cd}	3.52 ± 1.14 ^{a}
PAW30-20m	77.90 ± 0.44 ^{b}	6.80 ± 0.23 ^{b}	32.40 ± 1.37 ^{d}	3.86 ± 1.40 ^{a}
PAW60-20m	78.95 ± 0.19 ^{a}	6.72 ± 0.17 ^{b}	32.88 ± 0.79 ^{cd}	3.69 ± 0.77 ^{a}
PAW90-10m	78.00 ± 0.89 ^{b}	7.27 ± 0.29 ^{a}	34.55 ± 0.92 ^{b}	1.97 ± 0.85 ^{b}
PAW90-20m	78.74 ± 0.26 ^{a}	6.77 ± 0.20 ^{b}	32.93 ± 0.84 ^{cd}	3.55 ± 0.88 ^{a}
PAW90-30m	78.71 ± 0.16 ^{a}	6.76 ± 0.20 ^{b}	32.70 ± 0.75 ^{d}	3.76 ± 0.74 ^{a}

Note: Data are presented as the mean ± standard deviation. Values in the same column with different letters differ significantly ($p < 0.05$).

The total color difference (ΔE^*) represents the difference between the target and reference colors in the L^* a^* b^* color space. As illustrated in Table 1, the ΔE^* values of the SDW-30m and PAW90-10m group samples were between 1.5 and 3.0, suggesting that the color differences were noticeable compared with the untreated sample. However, other PAW treatments generated well visible differences ($3.0 < \Delta E^* < 6.0$) in comparison with the fresh thin sheets of bean curd. The changes of the thin sheets of bean curd treated by PAW in terms of the total color difference were in accordance with those of PAW-treated strawberries and plasma-treated apple juice [10,25].

3.4. Influence of PAW on Textural Properties

As shown in Table 2, among the texture profile analysis (TPA) parameters of thin sheets of bean curd, springiness, cohesiveness, chewiness, and resilience showed no significant difference between the control and treatment groups. However, the thin sheets of bean curd in the control and PAW90-10m groups had lower hardness than the other groups. Notably, SDW, which has no abundant ROS and RNS, also hardened the thin sheets of bean curd through treatment for 30 min. Meanwhile, the hardness was not influenced by the contents of the active substances in different groups. This indicated that the hardness of thin sheets of bean curd might be related to the soaking time. The water ratio of gel is closely related to its textural properties. Due to soaking for a longer time, the water molecules, protein, and fat particles of thin sheets of bean curd may interact with each other and form a harder network.

Table 2. Influence of plasma-activated water treatment on the textural properties of thin sheets of bean curd.

	Springiness (g/s)	Cohesiveness	Chewiness (×1000)	Resilience	Hardness (kg)
Control	1.43 ± 0.20 [a]	0.93 ± 0.03 [a]	16.05 ± 2.53 [a]	0.85 ± 0.06 [a]	12.19 ± 1.35 [b]
SDW-30m	1.14 ± 0.43 [a]	0.94 ± 0.01 [a]	16.13 ± 1.70 [a]	0.89 ± 0.04 [a]	14.96 ± 2.09 [a]
PAW15-20m	1.19 ± 0.45 [a]	0.94 ± 0.01 [a]	17.57 ± 2.21 [a]	0.86 ± 0.03 [a]	15.79 ± 1.64 [a]
PAW30-20m	1.24 ± 0.58 [a]	0.93 ± 0.01 [a]	16.60 ± 1.07 [a]	0.85 ± 0.04 [a]	14.30 ± 0.78 [a]
PAW60-20m	1.03 ± 0.56 [a]	0.94 ± 0.01 [a]	14.30 ± 2.01 [a]	0.88 ± 0.03 [a]	13.93 ± 1.10 [a]
PAW90-10m	1.31 ± 0.56 [a]	0.93 ± 0.01 [a]	14.58 ± 1.62 [a]	0.85 ± 0.03 [a]	11.98 ± 0.84 [b]
PAW90-20m	1.56 ± 0.41 [a]	0.94 ± 0.01 [a]	16.13 ± 1.98 [a]	0.88 ± 0.03 [a]	14.70 ± 1.28 [a]
PAW90-30m	1.03 ± 0.54 [a]	0.94 ± 0.01 [a]	14.43 ± 1.87 [a]	0.89 ± 0.04 [a]	14.86 ± 1.06 [a]

Note: Data are presented as the mean ± standard deviation. Values in the same column with the same letters are not significantly different ($p > 0.05$).

3.5. Influence of PAW on Total Isoflavone Content

Isoflavones are a group of secondary metabolites found in soybean, which play a key role in lowering cholesterol levels and preventing cancer, osteoporosis, and cardiovascular disease [26–28]. Therefore, the total isoflavone content is an important food quality determinant of soybean products. As illustrated in Figure 3, the PAW-treated thin sheets of bean curd showed no significant difference with the untreated group in terms of total isoflavone content, which indicates that PAW treatment would not destroy the main functional ingredients of thin sheets of bean curd. This result is in agreement with the report by Guo et al. [11], who found that PAW treatment did not influence the anthocyanin content of grapes.

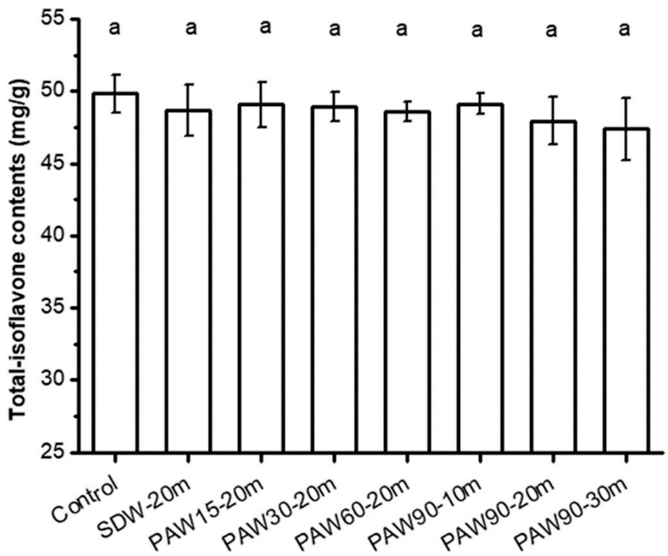

Figure 3. The total isoflavone content of the thin sheets of bean curd with and without treatments. The same letters in the bars indicate no significant difference with each other ($p > 0.05$).

3.6. Influence of PAW on Sensory Properties

The sensory properties of thin sheets of bean curd were evaluated immediately after different treatments. There was no significant difference among different groups with respect to the attributes of appearance, flavor, brittleness, as well as the overall acceptance of thin sheets of bean curd (Table 3). Although the total color differences were clearly visible from the PAW treatments according to the color measurements, the attribute of appearance acceptance was not significantly influenced. Therefore, this means that PAW treatment would not affect consumers' acceptance of thin sheets of bean curd. This is consistent with studies that found that PAW did not affect most sensory properties of mung bean sprouts, lettuce, and meat products [13,16,29].

Table 3. Influence of plasma-activated water treatment on the sensory characteristics of thin sheets of bean curd.

	Appearance	Flavor	Brittleness	Overall Acceptance
Control	5.08 ± 2.65 [a]	7.84 ± 1.22 [a]	6.82 ± 2.88 [a]	6.15 ± 2.00 [a]
SDW-30m	5.76 ± 2.35 [a]	7.04 ± 1.86 [a]	7.95 ± 0.80 [a]	6.82 ± 1.44 [a]
PAW15-20m	5.91 ± 1.91 [a]	7.02 ± 1.15 [a]	7.55 ± 1.20 [a]	6.46 ± 1.58 [a]
PAW30-20m	6.15 ± 2.25 [a]	7.18 ± 1.15 [a]	7.8 ± 1.86 [a]	6.51 ± 2.20 [a]
PAW60-20m	6.18 ± 2.25 [a]	6.65 ± 1.97 [a]	6.95 ± 2.30 [a]	6.19 ± 1.99 [a]
PAW90-10m	5.54 ± 2.79 [a]	6.54 ± 1.46 [a]	7.17 ± 1.54 [a]	6.05 ± 2.13 [a]
PAW90-20m	5.84 ± 2.20 [a]	6.45 ± 1.61 [a]	7.22 ± 1.44 [a]	6.66 ± 1.58 [a]
PAW90-30m	7.23 ± 1.97 [a]	6.71 ± 1.75 [a]	7.57 ± 1.82 [a]	7.15 ± 1.71 [a]

Note: Data are presented as the mean ± standard deviation. Values in the same column with the same letters are not significantly different ($p > 0.05$).

4. Conclusions

In this work, we demonstrated that bacteria, yeasts, and molds on thin sheets of bean curd can be significantly decontaminated by PAW treatment. The inactivation efficiency was related to PAW activation time and PAW soaking time. Moreover, PAW treatments had no significant influence on the total isoflavone content, sensory properties, and most of the textural properties of thin sheets of bean curd. PAW treatments caused some changes in the color parameters of thin sheets of bean curd, but they did not affect the appearance acceptance. Thus, PAW is a promising, cost-effective disinfectant for food sterilization. Further studies will focus on improving the inactivation efficiency of PAW and its applications in the food industry.

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