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Improving the quality of sunn pest (*Eurygaster integriceps*)-damaged wheat flour by atmospheric air cold plasma

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ABSTRACT

This study aimed to improve the quality of wheat flour affected by sunn bugs (*Eurygaster integriceps*) by examining the effects of cold plasma on its physicochemical and dough rheological properties. The sun-damaged wheat flour was treated with Surface Dielectric Barrier Discharge using atmospheric air cold plasma at different application times. The results demonstrated that modified sedimentation volumes increased with longer plasma treatment times and maximized in 60 s without causing sunn pest-damage. The alveograph tenacity (P) increased with plasma application, and as a result, the energy (W) reached approximately three times that of the application of 60 s. The results showed that cold plasma has a big potential for improving sunn pest-damage flour (SPDF) and dough properties. The plasma application caused this positive effect on dough properties at ≤ 60.0 s.

1. Introduction

Plasma, the fourth state of matter distinct from gas, is an ionized medium characterized by the loss of electrons from gas-phase atoms or molecules when sufficient energy is supplied, forming positively charged ions and free electrons. Plasmas are commonly found in natural phenomena such as stars, solar winds, and lightning, constituting about 99% of the universe. They can be categorized into two main types based on their energy densities and temperatures: thermal and cold plasmas. Thermal plasmas are typically found at high temperatures, often in the millions of Kelvins, and are primarily studied in fusion research and astrophysical events. On the other hand, cold plasmas operate at low temperatures, usually around room temperature, where electron temperatures are much higher than those of ion and neutral gases (Chu & Lu, 2013).

In laboratory settings, cold plasma production is achieved using various energy sources. Typically, gases such as helium, neon, and argon are ionized by applying high electrical voltages (Ilik & Akan, 2016). However, in industrial applications, atmospheric air cold plasmas, which utilize air as the gas source, offer cost and ease of production advantages. In atmospheric air cold plasmas, a wide variety of particles are generated, including electrons, ions such as O_2^+ (oxygen ion), N_2^+ (nitrogen ion), NO^+ (nitric oxide ion), O^+ (oxygen atom ion), N^+ (nitrogen atom ion), NO_2^- (nitrite), NO_3^- (nitrate), molecules such as O_2 (molecular oxygen), N_2 (molecular nitrogen), NO (nitric oxide), O_3 (ozone), N_2O (nitrous oxide), neutral atoms such as O (oxygen atom), N (nitrogen atom), radicals such as OH (hydroxyl radical), O (oxygen radical), NO (nitric oxide radical), NO_2 (nitrogen dioxide radical), excited species such as O* (excited oxygen atom), N* (excited nitrogen atom), O_2* (excited molecular oxygen), N_2* (excited molecular nitrogen), reactive oxygen species such as O_2^- (superoxide anion), O_3 (ozone), H_2O_2 (hydrogen peroxide), reactive nitrogen species such as NO_2 (nitrogen dioxide), N_2O (nitrous oxide), $ONOO^-$ (peroxynitrite), and ONOOH (peroxynitrous acid), as well as photons such as infrared, visible, and ultraviolet radiation. These species interact with the matter with which the cold plasma is in contact, leading to various surface and molecular structural changes. Plasmas have demonstrated efficacy in surface modification and coating processes, enhancing the functionality and durability of various materials in materials science and engineering (Akan et al., 2007; Musa et al., 2007). In biomedical applications, they have shown effectiveness in wound healing, blood coagulation, cancer/tumor treatment, and inactivation of bacteria, fungi, and viruses, as well as in the treatment of acne, eczema, venous ulcers, chronic leg ulcers, burn wounds, and skin regeneration (Akan et al., 2006; Fridman et al., 2008). The adverse effects of thermal treatments on foods, such as non-enzymatic browning, protein denaturation, changes in sensory properties, vitamin loss, and consumers' expectations for high-nutrient-content foods, have prompted researchers to explore non-thermal technologies. The efficacy of cold plasma technology has been

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demonstrated as an alternative tool for food disinfection, shelf-life extension, enzyme inactivation, toxin removal, and food packaging modifications (Barbhuiya et al., 2021; Asl et al., 2022). Cold plasma treatments have been observed to have minimal or no effect on various nutritional and sensory properties of different products (Saremnezhad et al., 2021). By effectively inactivating pathogens and spoilage microorganisms on food surfaces, cold plasma processes enhance food safety and extend shelf life. Additionally, they contribute to increased antioxidant activity in foods, slowing down oxidation processes to preserve freshness and quality. Furthermore, cold plasma treatments enhance surface disinfection of foods, thereby improving cleanliness and hygiene (Desai et al., 2024).

In the flour industry, AACP technology offers various advantages such as reducing the microbial load of flour, enzyme control, preserving nutritional value, improving functional properties, and extending shelf life. Its utilization enhances quality and safety in flour production while reducing the need for chemical additives. Further research is needed to better understand the effects of this technology on flour and baked goods, but existing findings are highly promising (Misra et al., 2015). Sunn pest (Eurygaster spp.) is a pre-harvest pest that causes occasional substantial loss of wheat both in yield and quality. The proteolytic enzymes that these insects release into the wheat grain cause the gluten proteins to break down. When damaged grains are used to make dough, the dough gets soft and sticky and sometimes disintegrates entirely. These doughs ultimately cause low-volume and poor-quality breads with defective texture and pore properties (Armstrong et al., 2019). Several methods are being used to minimize or improve sun-damaged flour's capability to be used for bread-making. These strategies include steam tempering, short-term microwave applications, using inhibitory substances (like potassium dihydrogen phosphate, sodium or calcium chloride, and sodium salicylate), and applying certain substances during the bread-making process (like bread additives like potassium bromate, L-ascorbic acid, vital gluten, DATEM, changing the conditions of the process, adding organic acids, and using sourdough) (Dizlek & Özer, 2021). These approaches can improve the rheological qualities of dough to some extent, but they cannot lead to broadly available advancements. The quality of the gluten mostly determines the viscoelastic qualities of the dough (Guzman et al., 2016). The amount of disulfide bonds (SS) and sulfhydryl groups (SH) in gluten is the main determining factor for the structure of the dough (Shewry & Jones, 2020). By oxidizing the thiol groups to disulfide bonds in sunn pest-damaged flour, the AACP approach is particularly successful at strengthening the gluten network and improving the dough's suitability for breadmaking (Tavakoli Lahijani et al., 2022). In this study, the possibility of the AACP approach to improve dough qualities with substantial sunn insect damage was examined.

2. Materials and Methods

2.1. Materials

The sunn pest-damaged wheat grains of the Ekiz bread wheat variety were provided by the Eskişehir Commodity Exchange. The Chopin CD1 laboratory flour mill (Chopin Technologies, Paris, France) was used to obtain refined white flour. The flour was stored in polyethylene bags and kept in a cold place (~ +10 °C) for further analysis.

2.2. Methods

Plasma treatment

In this study, a surface dielectric barrier discharge (SDBD) system was used as AACP. The SDBD plasma is produced in the lid of a closed container with a volume of 1 L (Figure 1). A 1.5-mm-thick copper cable was mounted 11 times in a zig-zag pattern on the inside of the lid to serve as the energy electrode. The distance of each mounting was 10 cm. Another 1.5 mm thick copper cable was laid in a zig-zag pattern on the outer part of the lid to serve as the ground electrode, opposite to the zig-zag copper cable inside. The thickness of the polypropylene lid is 2 mm. As the container's depth is 5 cm, AACP application on the flours can be accepted at a height of 4-5 cm. An alternative current (AC) high-voltage power supply with a voltage of 17.6 kV and a frequency of 12.5 kHz was used to generate cold plasma as micro-discharges (Akan & Durmuş, 2023). Since no additional gas is used in this treatment, purple-colored atmospheric air plasma is generated between the inside copper cable and lid when the system is energized (Figure 1)

The AACP was applied to the flours for different durations of 10, 45, and 60 s. Optical emission spectroscopy (OES) results from cold plasma systems provide information about the reactions and chemical types of plasma. OES measurements were taken from 1-2 mm to the high-voltage electrode. The optical emission spectra of the atmospheric air cold plasma generated as micro-discharges are given in Figure 2.

The spectrum was taken with the Ocean Optics USB2000+XR1-ES device. Figure 2 displays emission spectra of the SDBD micro-discharges generated in the air, showcasing strong bands of the second positive system (SPS) of N_2, including $C^3\Pi_u \rightarrow B^3\Pi_g$, and the first negative system (FNS) of N_2^+, including $B^2\Sigma_u^+ \rightarrow X^2\Sigma_g^+$, in the UV spectral range. The VIS-NIR range showed characteristic sequences of bands of the first positive system (FPS) of N_2, including $B^3\Pi_g \rightarrow A^3\Sigma_u^+$. Due to the atmospheric environment in which the SDBDs were generated, many nitrogen reactions may have occurred, leading to the presence of nitrogen ions and excited species in the micro-discharge plasmas. The atomic oxygen emission line was observed at 777 nm, and the NO molecule emission line was seen between wavelengths 200-300 nm, both of which exhibit significant chemical activities. SDBD plasmas are also known to produce high levels of ozone (Pekarek, 2012). When SDBDs have a plasma energy density between 800 and 1000 J/L, they generate more than 250 ppm of ozone (Pemen et al., 2009).

Physico-chemical analysis

Protein content and hardness of flours were determined using near-infrared spectroscopy (NIR 6500, Foss, Hillerød, Denmark), calibrated according to the American Association of Cereal Chemists (AACC) methods 46–19.01 and 55-31.01 (AACCI, 2010). The Single Kernel Characterization System (Perten SKCS4100) devices was used to measure the hardness values of the grain samples. The grains were then ground into flour, and the flour hardness value was established using calibration files in the NIR device. Therefore, using flour samples, the hardness value can be determined directly. The Zeleny sedimentation test was conducted according to the ICC Standard Method 116/1 (ICC, 2011). In the modified sedimentation test, as in the Zeleny sedimentation analysis, after bromphenol blue was added, the test tubes were incubated at 37 ºC for 2 h. After incubation, 25 mL of sedimentation test solution was added, and the sediment volume was read after exactly 5 min. Dough rheological properties were determined by using Alveo-Link (Chopin Technologies, Paris, France) as modified by the American Association of Cereal Chemists International (AACCI, 2010). The final water content (25 °C) was adjusted using the developed equations for 60 g of flour by Karaduman et al. (2023). After kneading for a total of 8 min with a mixer (Bastak Instruments, Ankara, Turkey), the dough was opened to the proper thickness with the standard mold of the device. The Alveo-AH test was applied to the dough samples after a rest of 20 min.

2.3. Statistical analyzes

The results were evaluated with the JMP statistical program (SAS Institute, 1998). An analysis of variance (ANOVA) was applied to the flour properties in a completely randomized design with three replications. The means were compared using a Tukey's HSD test ($P<0.05$).

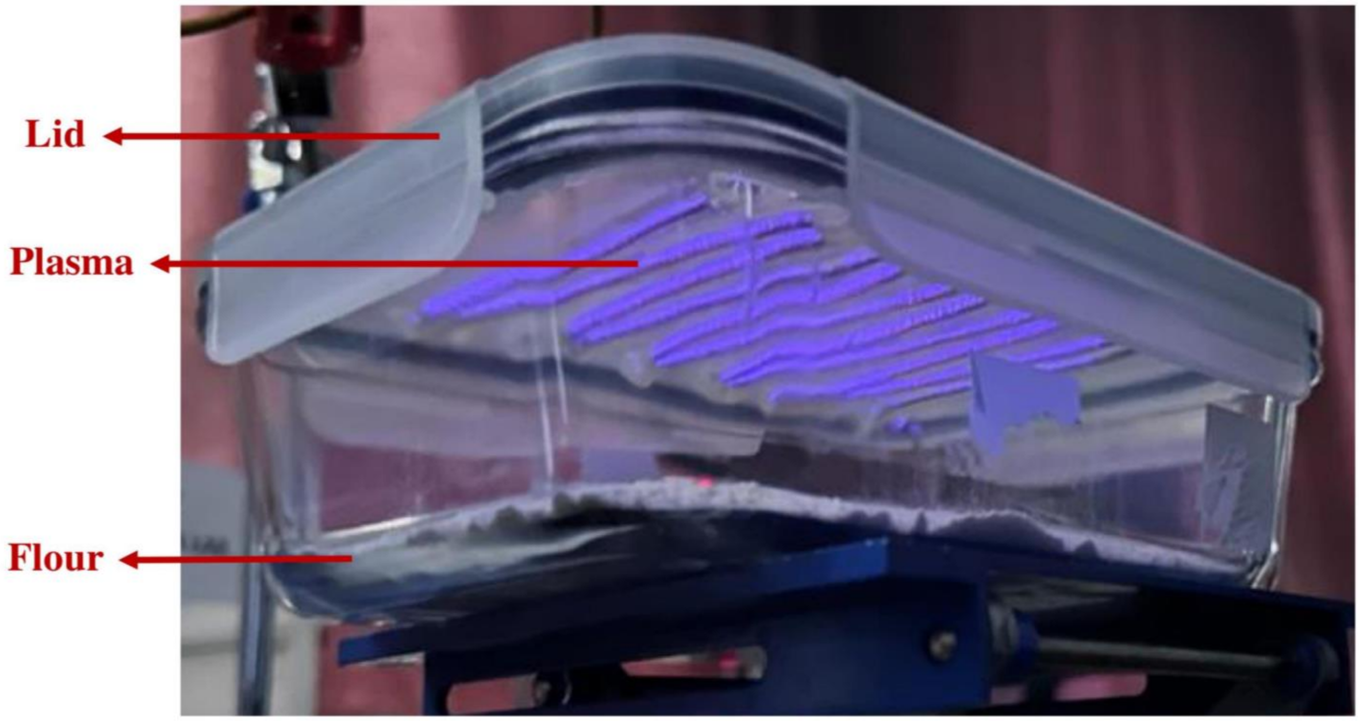

Figure 1. The system of application of AACP to the flour

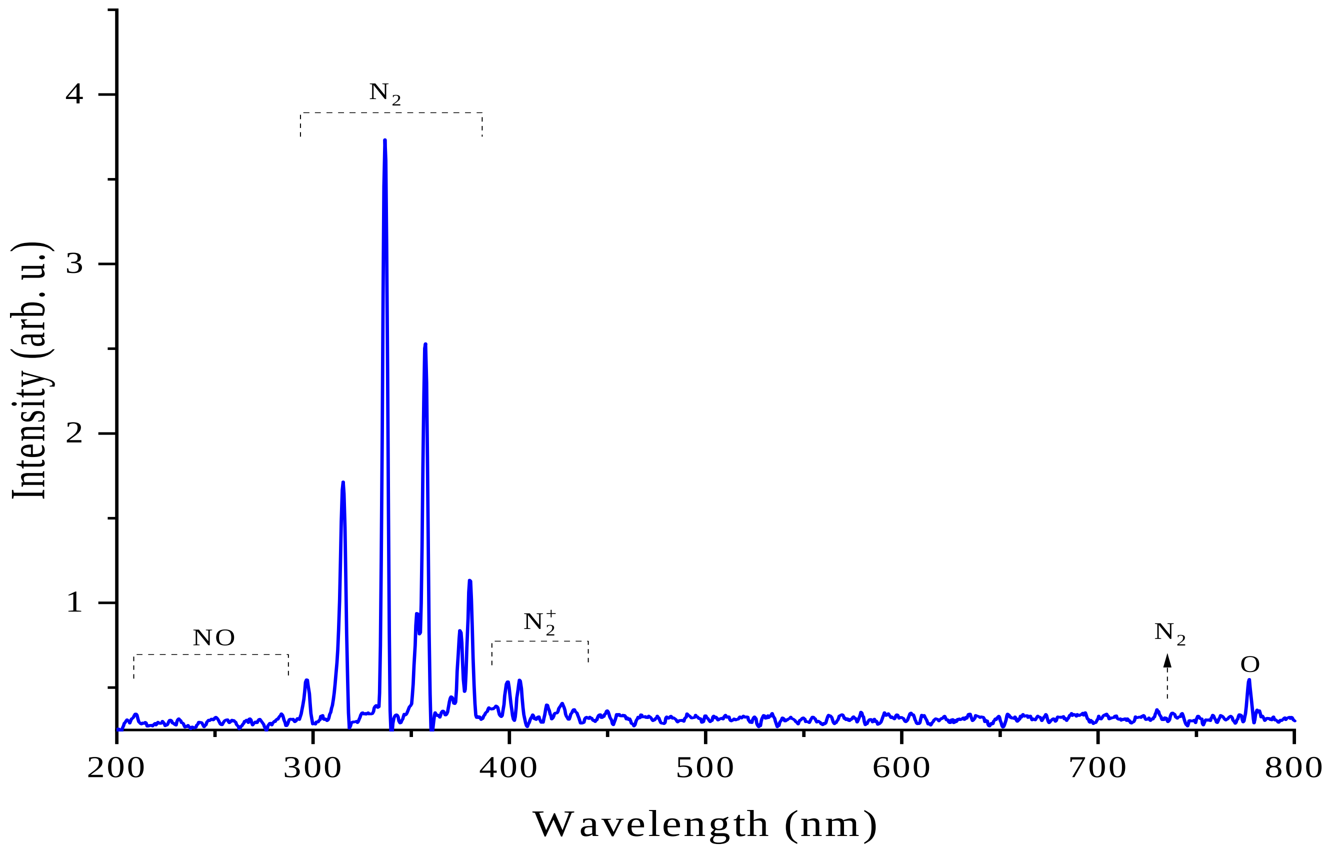

Figure 2. The optical emission spectrum in the atmospheric air SDBD

3. Results and Discussion

3.1. Proximate chemical composition

The physico-chemical properties (alveograph) of sunn pest-damaged wheat flour treated with different times of AACP are given in Table 1. Flour protein content is an important component in determining the final product quality (Shewry, 2024). In the study, the protein contents of the flours ranged from 11.17% to 11.40%, and as was normally expected, there was no variation between the flours when exposed to prolonged cold plasm. Ultimately, there should be positive developments in protein quality, which determines the final product quality rather than the protein content. Sedimentation tests can potentially help characterize the overall protein quality because of the ease of the measuring methods (Tömösközi et al., 2009). A high sedimentation volume indicates high gluten quality (Guzman et al., 2016). The average sedimentation volume of SPDF in the study was 34.50 mL, however, when the modified sedimentation test was used, it decreased to 12.50 mL because the sunn pest protease activity broke down the peptide linkages in the gluten. When the AACP was applied to SPDF for increasing periods, normal sedimentation volumes were close to each other, and modified sedimentation volumes increased linearly. After being treated with AACP for 60 s, it reached to a level where sunn pest-damaged does not occur (30.33 mL). One of the interesting findings in the study is that the hardness value increased with plasma application compared to the control flour at all application times. The degree of protein adherence to starch and the integrity of the protein matrix are related to hardness (Hrušková & Švec, 2009). The hardness of flour seems to be increased by the application of plasma, which strengthens the protein matrix and enhances especially intramolecular and intermolecular disulfide linkages in gluten.

The dough rheological properties (alveograph) of sunn pest-damaged wheat flour treated with different times of AACP are given in Table 2. Alveograph parameters are frequently used to determine the dough rheological properties of flours (Dubois et al., 2008). The length of the obtained curve between the start and end points represents its extensibility (A value), its height represents its elasticity (P value) (Jødal & Larsen, 2021), and its area represents the total energy required to explode (W value). The gluten balance value is obtained by dividing the P value by the L value (Guzman et al., 2016). A high energy (W) value and a P/L (gluten) of roughly 1.0-1.3 are required for viscoelastic dough having excellent gluten quality and high bakery quality (Jødal & Larsen, 2021). Once more, the breakdown of the gluten networks by the enzyme resulted in a considerable decrease in the tenacity (P), energy (W), and gluten balance (P/L) of the SPDF dough in the alveograph. The W value increased statistically in each AACP treatment, increasing from 51.67 10^{-4} *x* J to 137.67 10^{-4} *x* J in 60 s (r around 1.5 times in 10 s, twice in 45 s, and 3 times in 60 s). In the study, another important factor is that the gluten balance (P/L), which establishes the properties of flour at very close amounts of protein that are used for the production of the end product (Guzman et al., 2016), progressively increases from the mean level of soft wheat products (0.5) to the level that is necessary for producing bread (1.0-1.3). The AACP treatment strengthened the gluten network and enhanced the dough's ability for bread-making by oxidizing the thiol groups in SPDF to disulfide bonds (Tavakoli Lahijani et al., 2022).

The tenacity (P) values of the flours also increased from 30.0 mm to 70.0 mm with increasing plasma application time and the elasticity of gluten increased.

Table 1. The physico-chemical properties of sunn pest-damaged wheat flour treated with different AACP treated times

Plasma treatment time (s)	Protein content (%)	Flour hardness value (HI)	Sedimentation volume (mL)	Modified sedimentation volume (mL)
Control	11.38±0.04[a]	43.44±0.90[c]	34.50±0.50[a]	12.50±0.50[d]
10	11.17±0.07[b]	58.18±1.22[a]	31.67±0.29[b]	20.50±0.50[c]
45	11.27±0.05[ab]	54.61±1.11[b]	31.83±0.29[b]	25.67±0.58[b]
60	11.40±0.07[a]	55.27±0.59[b]	28.33±0.58[c]	30.33±0.29[a]
Mean	11.31	52.87	31.58	22.25
$LSD_{0.05}$	0.15**	2.54**	1.33**	1.39**

The means of flour properties in the same column marked with different lowercase letters are statistically different from each other (P<0.05). The significance between flour properties at the 1% level is indicated by two asterisks (**); Control: sunn pest-damaged flour (SPDF); HI: Hardness index

Table 2. The alveograph properties of sunn pest-damaged wheat flour treated with different AACP treated times

Plasma treatment time (s)	Alveograph tenacity (P, mm)	Alveograph extensibility (L, mm)	Alveograph energy (W, 10^{-4} *x* J)	Alveograph P/L value
Control	30.67±0.58[c]	57.33±0.58[b]	51.67±1.53[d]	0.535±0.009[d]
10	46.33±0.58[b]	51.67±0.58[c]	74.33±1.15[c]	0.897±0.011[b]
45	48.33±0.58[b]	68.00±1.00[a]	91.33±1.53[b]	0.711±0.005[c]
60	68.67±1.15[a]	53.00±0.00[c]	137.67±1.53[a]	1.296±0.022[a]
Mean	48.50	57.50	88.75	0.860
$LSD_{0.05}$	2.49**	1.56**	4.19**	0.039**

The means of flour properties in the same column marked with different lowercase letters are statistically different from each other (P<0.05). The significance between flour properties at the 1% level is indicated by two asterisks (**); Control: sunn pest-damaged flour (SPDF)

4. Discussion

Atmospheric pressure cold air plasma generates reactive species that can interact with the components of flour, leading to various chemical modifications. Flour primarily consists of starch (the main carbohydrate component, primarily composed of glucose polymers), proteins (includes glutenin and gliadin, which together form the gluten network), lipids (composed of phospholipids, triglycerides, and free fatty acids), and a small amount of minerals (present in small quantities as inorganic compounds). The reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced by plasma can react with the side chains of amino acids in proteins, causing oxidative modifications. This can lead to cross-linking, oxidation, or degradation of proteins. Amino acids such as methionine, cysteine, tyrosine, and tryptophan are particularly susceptible to these reactive species, resulting in structural changes in the proteins. When gluten proteins undergo oxidation, disulfide bonds may break or new ones may form, leading to alterations in the gluten network's elasticity and viscoelastic properties. Starch, a polymer of glucose units, can also be affected by ROS and RNS through depolymerization or oxidation, causing the breakdown of starch molecules. This impacts the gelatinization and water-holding capacity of starch. Lipids can undergo peroxidation due to ROS, resulting in oxidative degradation and the formation of free radicals. Lipid oxidation affects the shelf life and flavor of the flour. Additionally, electrons and anions (such as sulfide or oxygen anions) produced by AACP can interact with specific regions of proteins or starch, causing chemical modifications. Electrons can break specific bonds in proteins or starch chains or form new bonds. Ultraviolet (UV) radiation generated by AACP can induce photochemical reactions in proteins and starch, leading to photo-oxidation and structural changes. UV radiation can also damage the DNA of microorganisms, reducing the microbial load in the flour. These modifications can affect the rheological and functional properties of the flour. Changes in the gluten structure can alter the dough's elasticity, viscoelasticity, and consequently its behavior in bread-making processes (Thirumdas et al., 2012; Menkovska et al., 2014; Pankaj et al., 2014; Misra et al., 2016). The treatment of AACP with an oxidation-reduction potential increases the formation of disulfide bonds between glutenin proteins, hence enhancing the strength of the dough (Sandhu et al., 2011; Menkovska et al., 2014). In the study, gluten quality increased by encouraging the formation of gluten disulfide bonds by applying AACP to sunn pest-damaged flour. This situation was reflected in the sedimentation values, and even in 10 s application, the sedimentation value increased approximately twice. With 60 s of application, the sedimentation level with no sunn pest-damage was reached. It was thought that with plasma application, there was an improvement in the structure of the gluten protein, especially due to the development of intermolecular disulfide bonds, and the sedimentation values of the flour particles increased by absorbing more water. The increase in hardness value supported this, and the bonds increased by oxidation in the gluten structure strengthened the gluten and increased the hardness of the flour. The extent to which this improvement in the physicochemical properties of the flour is reflected in the properties of the dough is extremely critical before obtaining the product. In the study, the effect of AACP application on dough properties was determined by an alveograph, which is the most common test used for this purpose. The high alveograph energy (W) value is the basic parameter that determines the suitability of the dough for bread-making. The study showed that the alveograph energy value increased with each dose and that AACP application was very effective in strengthening the dough and improving its viscoelastic properties. The increase in the dough alveograph energy value sometimes occurs with a high tenacity (P) value, and the extensibility (L) value required for a volume increase in a bakery decreases. In other words, highly elastic gluten emerges, and the resulting breads are low in volume. This situation can be seen with the gluten balance (P/L value) on the alveograph. A high P/L value (>1.3) creates a highly elastic (tenacious) dough, while a small P/L value (<0.8) creates a highly extensible dough. With its high energy value, this ratio is desired to be between 1.0 and 1.3 in bread making. In our study, the positive improvement in P/L value with the increased energy value with plasma application and reaching optimum limits in 60 s were found valuable. As a result, plasma application improved the dough's rheological properties in a very positive way. The most important point in the study is that this positive effect on dough properties was obtained at ≤ 60.0 s with the plasma application system developed differently from the literature.

5. Conclusions

The results show that with increasing time of plasma treatment, modified sedimentation volumes increased and reached a level that did not cause any sunn pest-damage in the 60 s of treatment. The AACP application increased the tenacity (P) of the dough, which had intense sunn pest-damage. Thus, its energy value (W) increased approximately three times, and the gluten balance (P/L) reached the desired level in the application of 60 s. As a result, it was seen that the SPDF dough has gained better viscoelastic properties with the application of AACP.

Ethics statements

The authors have read and followed the ethical requirements for publication and confirm that the current work does not involve human subjects, animal experiments, or any data collected from social media platforms.

Author’ contributions

Arzu Akın: Collected the data, Investigation, Data curation, Formal analysis, Conceptualization, and Writing - original draft.

Yasar Karaduman: Supervision, Collected the data, Investigation, Data curation, Formal analysis, Conceptualization, and Writing - the original draft.

Tamer Akan: Collected the data, Investigation, Data curation, Formal analysis, Conceptualization, Writing - review & editing.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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