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Research Article

Effect of Radio Frequency Cold Plasma Treatment on Intermediate Wheatgrass (*Thinopyrum intermedium*) Flour and Dough Properties in Comparison to Hard and Soft Wheat (*Triticum aestivum* L.)

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Cold plasma is an emerging technology to improve microbiological safety as well as functionality of foods. This study compared the effect of radio frequency cold plasma on flour and dough properties of three members of the *Triticeae* tribe, soft as well as hard wheat (*Triticum aestivum* L.) and intermediate wheatgrass (*Thinopyrum intermedium*, IWG). These three flour types differ in their protein content and composition and were evaluated for their solubility, solvent retention capacity, starch damage, GlutoPeak and Farinograph profiles, and protein secondary structures. Plasma treatment resulted in dehydration of flours but did not change protein content or solubility. Farinograph water absorption increased for all flours after plasma treatment (from 56.5–61.1 before to 71.0–81.6%) and coincided with higher solvent retention capacity for water and sodium carbonate. Plasma treatment under our conditions was found to cause starch damage to the extent of 3.46–6.62% in all samples, explaining the higher solvent retention capacity for sodium carbonate. However, Farinograph properties were changed differently in each flour type: dough development time and stability time decreased for hard wheat and increased for soft wheat but remained unchanged in intermediate wheatgrass. GlutoPeak parameters were also affected differently: peak torque for intermediate wheatgrass increased from 32 to 39.5 GlutoPeak units but was not different for the other two flours. Soft wheat did not always aggregate after plasma treatment, i.e., did not aggregate within the measurement time. It was also the only flour where protein secondary structures were changed after plasma treatment, exhibiting an increase from 15.2 to 27.9% in β-turns and a decrease from 59.4 to 47.9% in β-sheets. While this could be indicative of a better hydrated gluten network, plasma-treated soft wheat was the only flour where viscoelastic properties were changed and extensibility decreased. Further research is warranted to elucidate molecular changes underlying these effects.

1. Introduction

Nonthermal plasma offers a multitude of application options for food scientists. Aside from increasing microbiological safety, it may also affect the functionality of food constituents such as starch and proteins [1]. For instance, nonthermal plasma treatment of starch has been reported to result in cross-linking [2] but also cleavage of glycosidic bonds [3]. As for the effect on protein, studies have reported changes in solubility [4, 5], secondary structure distribution, as well as other functional parameters such as emulsification properties [1]. Advantages of using nonthermal plasma include low losses of nutrients or sensory properties and its suitability for treatment of heat-sensitive materials [1]. Cold plasma has been proposed as a nonthermal treatment of flours to enhance functionality in wheat [6]. Previous research has reported oxidative changes in flour, which may modify dough properties [7]. Flours contain numerous components that may be affected by plasma treatment, most importantly the gluten-forming properties, starch, nonstarch polysaccharides, and lipids [8]. Studies often use different conditions of plasma treatment, and thus

a systematic evaluation of the effect of nonthermal plasma on main flour constituents, in dependence of treatment conditions such as carrier gas, is warranted. Misra et al. [9] reported that atmospheric pressure cold plasma treatment in the presence of air affected functional and structural parameters of soft and hard wheat flours. This change was related to proteins exhibiting a more ordered structure, increased dough strength, and modulation of the mixing behavior and viscoelastic properties.

Evaluating the effect of nonthermal plasma on different types of cereal flours is of interest due to their different suitability for certain products, e.g., hard wheat (HRW) for bread, soft wheat (SW) for cookies, crackers, cakes, or other products [10]. We have previously reported on chemical and functional characteristics of the intermediate wheatgrass (*Thinopyrum intermedium*, IWG) [11–13], a perennial crop with environmental benefits such as reduced nitrate leaching [14]. One limitation to its use as stand-alone flour is that it has poor gas-holding capability, due to being deficient in high-molecular-weight glutenins [12, 13].

Our overall aim for this study was to investigate the effect of radio frequency cold plasma treatment on flour and dough properties and to evaluate how protein properties, in particular gluten network formation in dough, were affected. Three *Triticum* genus members with different gluten-forming properties were contrasted: hard wheat usually has better viscoelastic properties and forms stronger gluten networks than SW [15]. Because bran to endosperm ratios are higher compared to annual crops such as hard wheat [16], IWG's total protein and insoluble dietary fiber contents are higher than annual crops, but its high dietary fiber and low glutenin contents negatively impact viscoelastic properties [11, 12].

The properties of dough systems as well as quality of products, especially when leavened, crucially depend on the formation of a strong gluten network [15]. We therefore evaluated changes in protein characteristics to assess the effect of nonthermal plasma on flour functionality.

2. Materials and Methods

2.1. Materials. Hard red wheat was graciously provided by Grain Millers Inc. (Eden Prairie, MN). Intermediate wheatgrass was grown in Rosemount, Minnesota, US, and obtained through the Department of Agronomy and Plant Genetics, University of Minnesota. Commercial soft wheat provided by Horizon Milling LLC (Mankato, MN, USA) was used. Samples were milled with a Quadrumat Junior mill (C. W. Brabender, South Hackensack, NJ, USA). Chemical reagents employed were of reagent grade or higher.

2.2. Radio Frequency Cold Plasma Treatment. About 10 g flour were weighed into 2 glass Petri dishes with inner diameters of 15 cm, spread out in thin layers (≈2 mm) as proposed previously [17], and subjected to radio frequency-generated cold plasma treatment based on conditions reported by Spencer and Gallimore [18] in a Plasma Etch PE75 (Plasma Etch, Carson City, NV, USA) operating at 120 W. Argon and carbon dioxide were used at flow rates of 10 and 25 cm^3/min, respectively. A cooling unit set to 25°C was used. Cycles began at a pressure of 0.6 atm. Samples were treated for 1 hour in two 30-minute cycles and stirred in between. The stirring step was implemented to achieve a more even treatment throughout the flour layer in the Petri dish.

2.3. Protein Content and Solubility. Protein content was quantified by Dumas procedure according to AACCI method 46-30.01 [19] on a TruSpec N (Leco 165 Corporation, St. Joseph, MI) calibrated with glycine. Protein solubility was assessed after sample extraction as described by Marengo et al. [20] except that sample amounts and extraction volume were downscaled by a factor of 10 to 50 mg and 1 mL, respectively.

2.4. Solvent Retention Capacity and Starch Damage. Solvent retention capacity profiles of each sample were assessed in duplicate through AACCI method 56-11.02 [19]. Starch damage was quantified with a Megazyme (Wicklow, Ireland) starch damage assay kit based on AACCI method 76-31.01 [19].

2.5. Farinograph Evaluation and Dough Extensibility and Resistance to Extension. A Brabender Farinograph (C. W. Brabender) equipped with a 50 g bowl was used to assess flours according to AACCI standard method 54-21.01 [19]. The parameters obtained included the time required to form an optimum dough (dough development time, DDT), the time for which this dough was stable (dough stability, DS), and the optimum amount of water required to form such a dough (Farinograph water absorption, FWA).

2.6. Assessment of Dough Extensibility and Resistance to Extension. For assessments of extensibility (mm) and resistance to extension (g), dough was prepared in a 10 g Farinograph bowl, sampled at the DDT according to the method described by Banjade et al. [16]. A TA.XT-Plus Texture Analyzer (Texture Technologies, Hamilton, MA) equipped with a Kieffer dough and gluten extensibility rig was used with Texture Exponent 32 version 6.0.6.0 software (Texture Technologies, Corp. Scarsdale, NY, USA). A total of 7 dough strips were tested from each dough replicate.

2.7. Protein Secondary Structures. Dough was prepared in the Farinograph, using the conditions described in Section 2.5, and sampled at the dough development time, as assessed by pretrials. Spectra of the dough were then recorded at least in triplicate on a Bruker Tensor 37ATR-FTIR spectrophotometer (Bruker Optics, Inc., Billerica, MA, USA) equipped with a horizontal multireflectance zinc selenide crystal accessory as described by Marti et al. [21], using OPUS 7.0 software. Protein secondary structures were calculated using second-derivative spectra of amide I regions (1600–1700 cm^{-1}), assigning 1620–1644 cm^{-1} as β-sheets,

1644–1652 cm^{-1} as random structures, 1652–1660 cm^{-1} as α-helix, and 1660–1685 cm^{-1} as β-turns.

2.8. GlutoPeak Analysis. The aggregation properties of flours before and after plasma treatment were assessed in duplicate on a Brabender GlutoPeak (C. W. Brabender) based on the method reported by Chandi and Seetharaman [22]. Flour moisture contents were measured on an Ohaus MB45 infrared balance on the day of the analysis. The time to reach peak maximum time (PMT, in s), the maximum torque (MT, in GlutoPeak units, GPU), and the aggregation energy (AE, in GPU) were determined using Brabender GlutoPeak v. 2.1.2 software.

2.9. Statistical Analysis. One-way analysis of variance (ANOVA) was performed in R (version 3.1.0) [23], two-way ANOVAs (with flour type and plasma treatment as factors), and paired *t*-tests (to differentiate flours before and after plasma treatment) in Excel (Microsoft, Redmond, VA). Differences among means were calculated with Tukey's honestly significant difference test, at $\alpha = 0.05$.

3. Results and Discussion

3.1. Protein Solubility. Protein solubility was evaluated in three media, i.e., buffer containing a low concentration of sodium chloride, the same buffer additionally containing 8 M urea, and buffer with 8 M urea and disulfide cleaving agent dithiothreitol (Figure 1). While the type of cereal and the solvent both had a significant effect on the solubility, the plasma treatment did not. Partly, our results are in contrast to Marti et al. [11], who reported that IWG flour had higher solubility in phosphate buffer than hard red wheat, in line with its reported higher contents of albumins and globulins [12]. These differences may be related to different flour samples used in our studies. In general, changes in protein solubility in buffers with different additives indicate how different protein fractions respond to a given treatment.

Plasma treatment did not significantly enhance or decrease protein solubility in any of the media used. Changes in protein solubility over processing can indicate if protein interaction and aggregation are affected by the treatment [20]. Adding dithiothreitol and/or urea solubilizes proteins that are either insoluble in water or dilute salt solutions or proteins that originally were soluble but became insoluble over the course of the treatment step [24]. Based on our data, plasma treatment did not alter protein interactions of any protein fraction, in any of the flour types, in a way that affected solubility.

3.2. Solvent Retention Capacity and Starch Damage. Solvent retention capacity (SRC) assessed flour swelling in four solvents which differ in their compatibility to the three main polymers, i.e., 5% lactic acid (La) to assess gluten swelling, 5% sodium carbonate (SC) to assess swelling due to starch damage, 50% sucrose (Su) to assess arabinoxylan-mediated swelling, and swelling in pure water (W), which is influenced by all three components [25, 26]. A change in the ability of a flour to swell in different solvents would indicate that the treatment affected the polymer targeted by the solvent. SRC was implemented to see if nonprotein polymers were being affected by plasma treatment. Before and after plasma treatment, HRW had the highest La-SRC, Su-SRC, and W-SRC (Table 1). SW had significantly higher La-SRC before and after plasma treatment than IWG, reflecting poor ability of IWG to form gluten networks [12] and lack of change from treatment. Overall, SRC values for HRW and SW were in the range of several previous studies [27–29], whereas to the best of our knowledge, no prior reports for IWG SRC have been published. The most notable observation in our sample set was that upon plasma treatment, the W-SRC of HRW and SW significantly increased (by 17 and 27%, respectively), whereas the change was not significant ($p = 0.056$) for IWG. In all three flours, the changes in W-SRC coincided with significantly ($p < 0.05$) increased SC-SRC; however, the flours were affected to a different degree. In untreated flours, the ranking for SC-SRC was IWG > HRW > SW, whereas after plasma treatment it changed to HRW > IWG > SW. While HRW and SW experienced an increase of ca. 30% for SC-SRC, it was only ca. 14% for IWG. In contrast, La-SRC and Su-SRC were not altered by plasma treatment, suggesting that gluten-forming proteins and arabinoxylans were not modified by plasma treatment, or at least not modified in a way to affect swelling behavior. Overall, the SRC results suggested that plasma treatment increased the level of starch damage of flours and that this also affected water absorption. Thus, starch damage levels were analyzed in addition to SRC measurements. While plasma treatment resulted in significantly ($p < 0.05$) higher starch damage levels in SW (from 3.34 in SW to 3.52% in SW + P) and HRW (from 6.03% in HRW to 6.62% in HRW + P), IWG's levels slightly, but significantly ($p < 0.05$) decreased (from 3.59% in IWG to 3.46% in IWG + P). Therefore, the influence of plasma treatment on parameters indicative for dough functionality may be higher for SW and HRW.

3.3. Protein Secondary Structures. The ratio of proteins folded into a certain conformational type has been demonstrated to reflect protein network formation in dough systems [30]. The viscoelastic properties of dough systems are influenced by β-turns and β-sheets [31], with the former being indicative of hydrated protein regions (referred to as "loops"), and the latter denoting regions of protein-protein interactions (referred to as "trains"). In our samples, the flour type significantly affected protein secondary structures: in hard and soft wheat, β-sheets represented the main conformational arrangement within the proteins, followed by β-turns (Table 2). Before plasma treatment, soft wheat flour had a significantly lower content of β-turns than the other two flours. IWG had a significantly lower content of β-sheets than the other flours before and after plasma treatment, in agreement with data from a study that compared whole IWG to hard red wheat [11]. No differences were observed among samples in random structures or

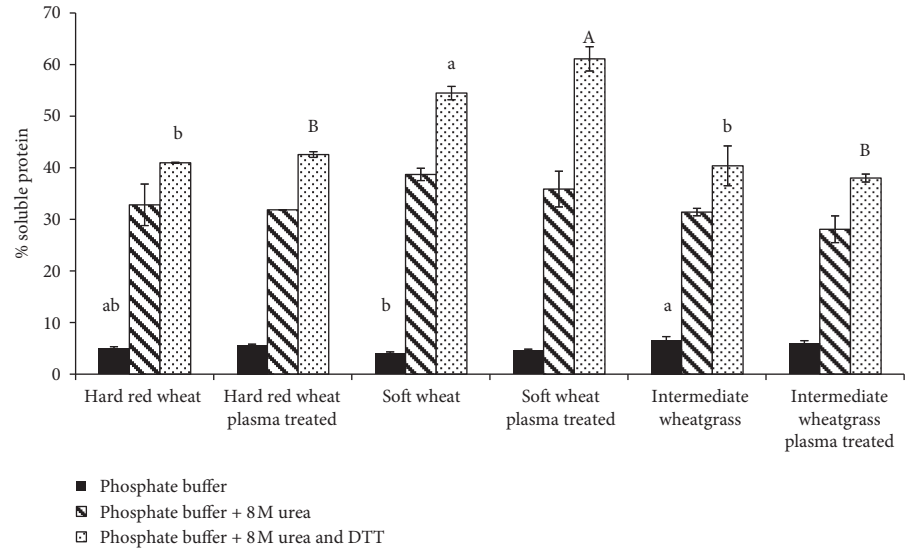

Figure 1: Protein solubility of hard red wheat, soft wheat, and intermediate wheatgrass flour in three solvents (pH 7 phosphate buffer containing 0.1 M sodium chloride, the same buffer with additional 8 M urea, and buffer with 8 M urea as well as 0.01 M dithiothreitol (DTT)). Error bars represent standard deviations, different lowercase letters represent differences in the solubility in the same solvent among samples before plasma treatment, and uppercase letters represent differences after plasma treatment, assessed via Tukey's HSD test ($p < 0.05$). Solubility in pH 7 phosphate buffer did not significantly differ in plasma-treated samples, and solubility in pH 7 phosphate buffer with 8 M urea did not significantly differ among any samples.

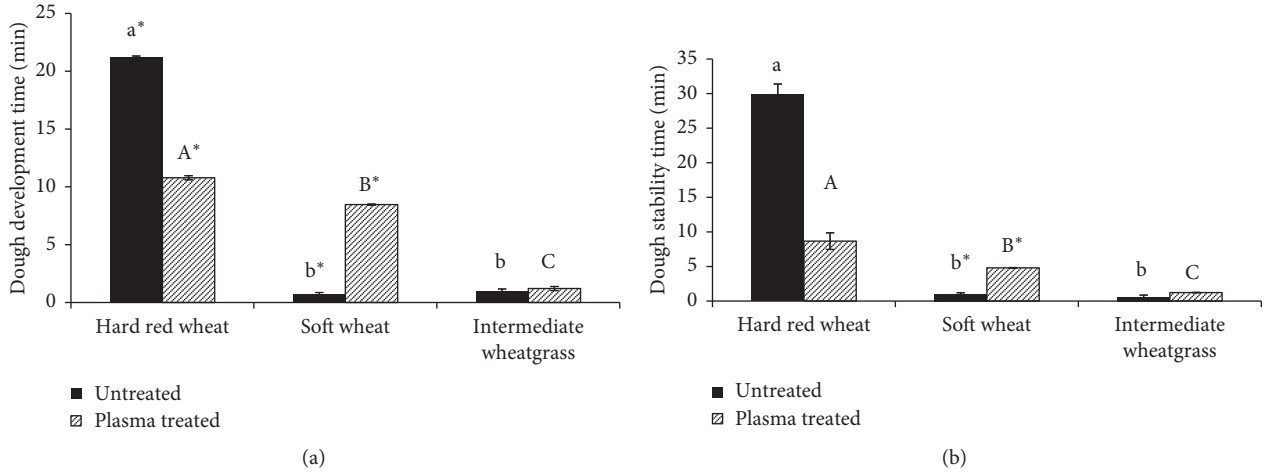

Figure 2: Dough development time (a) and stability (b) of hard red wheat, soft wheat, and intermediate wheatgrass flour. Error bars represent standard deviations ($n = 2$), and different lowercase and uppercase letters represent differences ($p < 0.05$) between flour types before and after plasma treatment (assessed by Tukey's HSD test), respectively. Asterisks represent differences ($p < 0.05$) within the same flour type due to plasma treatment (assessed by a paired t-test).

α-helices. Plasma treatment affected the samples differently: most notably, in soft wheat the proportion of β-turns significantly ($p < 0.05$) increased, while the proportion of β-sheets significantly decreased ($p < 0.05$). No significant changes were observed in IWG or hard wheat.

While Issarny et al. [29] did not find differences in protein conformations in dough from hard and soft Canadian wheat flour, Katyal et al. [32] reported that dough from extraordinarily soft wheat had fewer proteins with intermolecular β-sheet conformation than hard wheat, which could indicate fewer interactions among proteins. In our samples, the decrease in β-sheets observed for soft wheat due to plasma treatment could be indicative of lower ability for protein aggregation, which could impair the formation of networks capable of gas holding [33].

3.4. Farinograph and Viscoelastic Properties of Dough. Before plasma treatment, hard wheat had significantly ($p < 0.01$) higher DDT (Figure 2(a)) and DST (Figure 2(b)) than the other two flours, which did not significantly differ from each other. In general, flours with a stronger gluten

Table 1: Solvent retention capacity of flour types before and after plasma treatment.

Solvent	Hard red wheat	Intermediate wheatgrass	Soft wheat
Before plasma treatment			
Lactic acid	126.3^{a}	78.7^{c}	86.4^{b}
5% sodium carbonate	91.1^{b*}	98.1^{a*}	80.6^{c*}
50% sucrose	114.3^{a}	107.2^{b}	103.1^{c}
Water	79.1^{a*}	73.3^{b}	71.3^{b*}
After plasma treatment			
Lactic acid	121.3^{A}	79.2^{C}	88.6^{B}
5% sodium carbonate	120.0^{A*}	111.8^{B*}	104.7^{C*}
50% sucrose	140.5^{A}	115.3^{B}	108.7^{B}
Water	92.5^{A*}	84.1^{B}	90.9^{AB*}

Different letters indicate differences among values across rows, assessed via Tukey's HSD test ($p < 0.05$), i.e., between flour types for the same solvent. Lowercase letters were used to signify differences before plasma treatment and uppercase letters to distinguish flour types after plasma treatment. Asterisks denote significant differences (paired t-test, $p < 0.05$) in the proportion of a secondary structure type between untreated and plasma-treated flours of the same type.

Table 2: Proportional contribution of different secondary structures to overall protein secondary structure in hard red wheat, soft wheat, and intermediate wheatgrass before and after plasma treatment.

Protein secondary structure	Hard red wheat	Intermediate wheatgrass	Soft wheat
Before plasma treatment			
β-turns	29.8^{a}	40.4^{a}	15.2^{b*}
α-helices	7.4	16.0	6.0
Random	19.3	11.0	19.5
β-sheets	43.6^{b}	30.2^{b}	59.4^{a*}
After plasma treatment			
β-turns	29.9	39.1	27.9*
α-helices	3.7	9.6	8.8
Random	18.1	16.5	15.4
β-sheets	49.2^{A}	34.7^{B}	47.9^{A*}

No significant differences ($p < 0.05$) were detected among α-helices and random structures. Different letters represent differences among values across rows, assessed via Tukey's HSD test ($p < 0.05$); lowercase letters represent differences among flours before plasma treatment, and uppercase letters represent differences among flours after plasma treatment. Asterisks denote significant differences (paired t-test, $p < 0.05$) in the proportion of a secondary structure type between untreated and plasma-treated flours of the same type, i.e., differences across columns.

network are characterized by higher DDT and DST [34]. Plasma treatment affected the flours differently; it significantly ($p < 0.05$) decreased DDT for HRW and increased it for SW ($p < 0.01$) but did not affect it in IWG. While plasma-treated hard wheat flour still had higher DDT and DST values than the other two flours, plasma-treated soft wheat had significantly ($p < 0.05$) higher values for these parameters than IWG. Two-way ANOVAs for DDT, DST, and water absorption showed the same trend: there was a significant effect of sample type, plasma treatment, and their interaction on the dough properties. The FWA was significantly increased by plasma treatment (data not shown), presumably due to the higher starch damage in samples, in line with previous studies [35].

Previously, hard and soft wheat flour mixing properties have been reported to be affected by plasma treatment in a way that suggests formation of stronger dough systems, possibly due to disulfide linking of proteins when air was used during the treatment [9]. Bahrami et al. [7] reported an increase in high-molecular-weight proteins upon plasma treatment.

The extensibility and the resistance to extension were significantly higher for hard wheat than for SW and IWG before and after plasma treatment (Figure 3), signifying a better viscoelastic gluten network than the other two flours. In comparison to other studies, SW had lower resistance to extension, and extensibility [36]. Moreover, it was the only flour where extensibility decreased as a result of plasma treatment. Starch damage has previously been reported to decrease extensibility and could thus have been the reason for this change [35].

3.5. Protein Aggregation in the GlutoPeak. Before and after plasma treatment, HRW had significantly ($p < 0.01$) higher MT than the other two flours, which did not significantly differ from each other (Figure 4(a)). PMT (Figure 4(b)) was significantly ($p < 0.01$) affected by flour type, plasma treatment, and their interaction, while MT was only significantly ($p < 0.01$) affected by flour type and flour type × plasma treatment interactions, but not by plasma treatment. The MT and PMT of soft and hard wheat before plasma were in line with previous reports [22] that also had observed higher MT for HRW than for SW [36]. Protein aggregation did not vary before and after plasma treatment for HRW as its PMT and MT were not significantly different due to plasma treatment. HRW's AE was not significantly

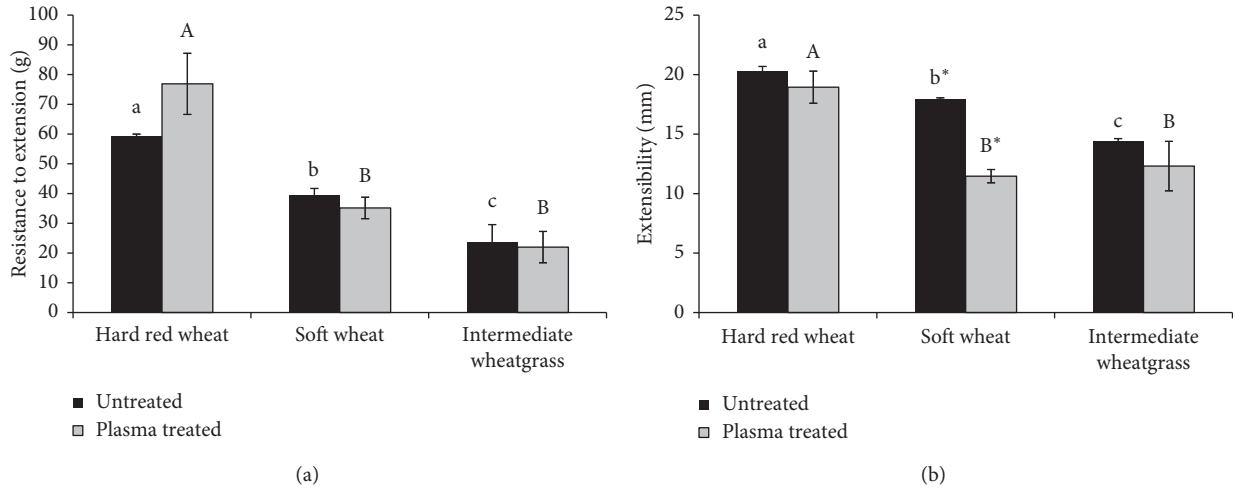

Figure 3: Extensibility and resistance to extension of dough ($n = 2$) prepared from hard red wheat, soft wheat, and intermediate wheatgrass flour before and after plasma treatment.

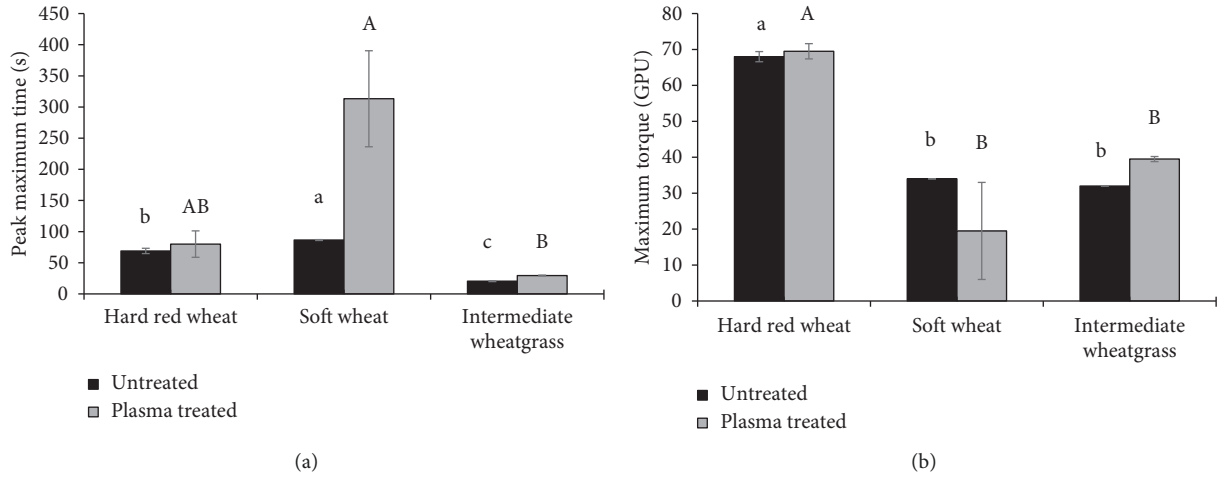

Figure 4: Peak maximum time (PMT) and maximum torque (MT) of hard red wheat, soft wheat, and intermediate wheatgrass flour before and after plasma treatment.

affected by plasma treatment and highest of all flours. In contrast, for IWG, AE significantly increased ($p < 0.05$) after plasma treatment from 678.0 ± 3.6 to 992.9 ± 26. Remarkably, after plasma treatment, SW flour exhibited no aggregation within the allotted time frame of the experiment. Thus, SW was uniquely affected by plasma treatment: while all flours exhibited higher SRC for water and SC (Table 1) and Farinograph water absorption, it was the only sample where this led to inhibition of gluten aggregation. Overall, plasma treatment under our experimental conditions had a negative influence on its properties. Higher levels of starch damage and water absorption are detrimental for the quality of products typically made with soft wheat, such as cookies [37, 38].

4. Conclusions

Dough rheology, secondary structure, and protein aggregation measurements showed that each flour had a different response to plasma treatment. With plasma treatment, SW had a longer period of stability in the Farinograph, no aggregation in the GlutoPeak, and an increase in β-turns at the expense of β-sheets. HRW had a shorter period of stability but no difference in protein aggregation or secondary structure as a result of plasma treatment. IWG had no change in dough stability or secondary structure, but it did show an increase in protein aggregation after plasma treatment. SW and HRW experienced a significant increase in starch damage, and all flours had higher water absorption. The different effects of plasma treatment on flour types present questions for future research to elucidate molecular mechanisms for these changes. Moreover, the functionality of the plasma-treated flours in food products needs to be evaluated.

Data Availability

All data are presented in figures and tables.

Conflicts of Interest

The authors declare no conflicts of interest.

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