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Enhancement of Wheat Flour and Dough Properties by Non-Thermal Plasma Treatment of Wheat Flour

Muhammad Jehanzaib Khan [1,*], Vojislav Jovicic [1,*], Ana Zbogar-Rasic [1] and Antonio Delgado [1,2]

[1] Institute of Fluid Mechanics (LSTM), Friedrich-Alexander-University Erlangen-Nuremberg (FAU), 91054 Erlangen, Germany
[2] German Engineering Research and Development Center LSTME Busan, Busan 46742, Korea
* Correspondence: muhammad.j.khan@fau.de (M.J.K.); vojislav.jovicic@fau.de (V.J.)

Abstract: Demand to improve food quality attributes without the use of chemicals has risen exponentially in the past few years. Non-thermal plasma (NTP) (also called 'cold plasma') is becoming increasingly popular for this purpose due to its unique low-temperature and non-chemical nature. In the present research, the concept of in situ dielectric barrier discharge (DBD) plasma treatment inside a rotational reactor for the direct treatment of wheat flour was experimentally analyzed. The primary research goal was to determine the effects of short-period NTP treatment of DBD type on flour and dough properties. For this purpose, the influence of different operating parameters was tested, i.e., treatment time, the amount of flour placed in the reactor and the environmental (air) temperature. Changes in the structural attributes of the most commonly used flours (type 550 and 1050) and their respective doughs were studied using a set of analytical techniques. Rheological analysis demonstrated the ability of NTP to significantly intensify the visco-elastic properties of dough produced from wheat flour type 550 that was treated for less than 180 s. This indicated that plasma treatment enhanced intermolecular disulphide bonds in gluten proteins, which resulted in stronger protein–starch network formations. However, longer treatment times did not result in a significant increase in the visco-elastic properties of wheat dough. The obtained results showed a 6–7% increase in flour hydration due to NTP treatment, which also makes a contribution to hydrogen bonding due to changes in the bonded and free water phase. Experimental findings further confirmed the dependence of NTP treatment efficiency on environmental air temperature.

Keywords: wheat flour; dough; rheology; non-thermal plasma; dielectric barrier discharge; wheat functionality; hydration properties; microstructure

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1. Introduction

Cereals and grains are abundantly used worldwide, constituting a major part of human food consumption. Cereals and their products are energy sources for humans and animals, and contain carbohydrates, proteins, fiber and vitamins (such as E and B), along with magnesium and zinc [1–3]. Grains are consumed either whole or in powder form, which forms exist in different granulations and have different chemical compositions [4]. Generally, a grain consists of three parts: (1) bran, i.e., the outer layer, mostly rich with dietary fiber; (2) germ, i.e., the inner layer, consisting of lipids and proteins; and (3) endosperm, i.e., the bulk of a kernel, full of starch, proteins and minerals [5]. A powder form, i.e., flour, is a blend of these parts in different proportions, leading to different flour classifications.

Plant-based foods are often subjected to thermal or chemical processing prior to consumption [6]. However, nowadays, the customer requirement to preserve nutrient ratios by reducing the treatment temperatures for preserving food quality and reducing the use of chemical treatment agents have urged food engineers to search for other, suitable, non-chemical food processing methods. Modern consumers demand rich organoleptic, additive-free food, environmentally friendly processing, freshness and high sensory and nutritional attributes [7,8]. On the other hand, insufficient processing of food products can lead to the proliferation of bacterial, parasitic and pathogenic infections in humans [9]. Food-borne illnesses have significantly increased in recent years due to consumption of food that has been inadequately processed and therefore correct storage is additionally important to prevent mould formation, pest infestation and grain germination [6]. Microorganisms and pathogens occur in grains stored in humid environments, in which bran absorbs a significant amount of water, thus lowering the concentration of gluten and starch in the kernel and ultimately affecting end product quality [5,10].

Conventional thermal processing measures for food, for boosting shelf life, hygiene and structural properties, can be very detrimental to food nutrient ratios and sensory and organoleptic qualities [11]. Currently employed sterilization and processing techniques, such as chemical preservation [12,13], addition of bio-preservatives [14], mild heat treatments [15], microwave processing [16], ultrasound processing [17], reduction of water activity [18], high hydrostatic pressure technology [19,20] and vacuum and hurdle techniques [21], provide limited benefits in comparison with their adverse alterations of food properties, such as shape, color, taste, smell, structure, nutrient degradation, formation of toxic byproducts, etc. [9,22].

Wheat is one of the most important and the world's leading staple crop, which is full of nutrients [3]. The main factors affecting the quality of wheat-based products are gluten proteins, which are responsible for the visco-elastic properties of end products [23]. Their properties affect the rheological behavior and the gas-holding capability of a dough, as well as the texture and final volume of baked products. Wheat kernels are composed of 70–75% starch, 10–14% proteins, with the remainder constituted by minerals [24]. Wheat proteins are divided into two groups, i.e., non-gluten and gluten proteins. Gluten proteins comprise around 80–85% of the total protein contents of wheat flour and are further divided into gliadins and glutenins. Gliadins are monomeric proteins, which are mainly responsible for viscous and extensibility behavior during dough network formation. Glutenins are polymeric or aggregative proteins which influence the elastic and cohesiveness properties of dough networks.

The number and distribution of sulphur–sulphur (S-S) bonds, which are formed due to the presence of cysteine residues in gliadins and glutenins, significantly influence dough properties. However, S-S bonds are formed differently in two protein types: in gliadins, they lead to the formation of intramolecular S-S bonds, while in glutenins intermolecular S-S bonds are formed [25]. The S-S bonds for dough network formation start to activate during the kneading process, as soon as the wheat flour is hydrated due to the addition of water. During the network formation, these S-S bonds are in the form of fibrils. Macrofibrils of protein aggregates are formed by the formation of intermolecular S-S bonds. These fibrils then transform to a continuous film, which finally forms a homogeneous and fine gluten matrix [26,27]. On the other hand, several low-molecular weight thiols, containing a free SH group, are present in wheat flour, and have strong dough-weakening effects. The tripeptide glutathione (GSH) has the biggest impact; it is present in reduced (GSH) and oxidized, glutathione disulfide (GSSG) forms. A free SH group reacts with glutenin and forms glutathionylated protein (PSSG), which negatively affects the formation of intermolecular S-S bonds.

Wheat flour is normally processed not only to improve hygiene, shelf life and texture-related properties, but also to minimize the negative effects of GSH and GSSH [28]. A common industrial practice is to use chemical additives, e.g., ascorbic acid (i.e., vitamin C) as a reducing agent, which is extensively used to improve wheat flour textural properties (e.g., elastic and viscous properties). Its oxidized form dehydroascorbic acid (DHA) is responsible for the removal of free SH groups in flour, hence preventing the glutathione to create PSSG. The reaction mechanism is shown in Equations (1) and (2). Ascorbic acid easily converts to DHA in the presence of an enzyme and is limited by the availability of atmospheric oxygen during kneading. DHA reacts with GSH to form ascorbic acid and

GSSG, thus limiting the formation of PSSG. As a result, the number of S-S intermolecular bonds increases.

$$\text{Ascorbic acid} + 0.5\ O_2 \rightleftharpoons \text{Dehydroascorbic Acid} + H_2O \quad (1)$$

$$\text{Dehydroascorbic Acid} + \text{GSH} \rightleftharpoons \text{Ascorbic acid} + \text{GSSG} \quad (2)$$

Although many other chemicals (KIO_3, $KbrO_3$, chlorine, etc.) and oxidizing bacteria have been used to modify wheat flour functionality, the food industry is moving towards restricting the use of chemicals, and therefore the need for non-chemical treatment methods increases. Ozone is an example of an oxidizing agent, acceptable for use in the food industry [29]. Mei et al. [30] and Desouky et al. [31] investigated the effects of ozone on both wheat flour and grain functionality and suggested it as a good alternative to chemicals for improving wheat flour characteristics. The key features that are responsible for the superiority of ozone over commonly used chemicals are its high oxidation potential and the fact that no residues are left after the treatment [32].

Non-thermal plasma (NTP) has emerged as a new and effective method in food processing that retains food quality better than conventional methods [33]. Corona discharge (CD) and dielectric barrier discharge (DBD) are two forms of atmospheric non-thermal plasmas that can produce abundant amounts of ozone together with some other active species [34]. In contrast to ozone generators, DBD and CD can produce ozone in situ and create a ground for a direct reaction between reactive oxygen species and wheat proteins. Plasma is a an overall neutral gas, mainly composed of positively and negatively charged particles, neutrons, ions and particles that are heavy and with high energies [35]. Plasma is the fourth state of matter and is abundantly present in the universe. It was first discovered in the 18th century by William Crookes and named by Irving Langmuir and Levy Tonks in 1929 [36]. Based on electron temperature and thermodynamic equilibrium, plasma can be classified as high-temperature (thermal) or low-temperature (non-thermal) plasma [37]. Ionization degree may vary from partial ionization, in the case of NTP, to full ionization, in the case of thermal plasma [38].

Thermal plasmas with high electron densities ($>10^{22}\ m^{-3}$) and temperatures (4000 to 20,000 K) can be produced by electric discharge to reach high current flow through a fully ionized gas [39]. Electron temperature in NTP is much higher compared to the temperature of the bulk gas molecules, leading to a much lower temperature of the overall plasma, in the range of 30–50 °C [7]. NTP is thus quite suitable for the treatment of living cells, tissues and heat-sensitive materials [38].

Numerous researchers ([4,34,40–45] have studied the interaction of DBD plasma with wheat flour and have found that ozone and active species ($H^\bullet$, $O^\bullet$, $OH^\bullet$, $HO_2{}^\bullet$) generated during gas ionization can promote structural changes in starch and protein macromolecules (e.g., promoting cross-linking, depolymerization between molecules and the formation of new functional groups) similar to the changes induced by the use of chemicals. Mishra et al. [46] studied the influence of NTP treatment on wheat flour structural properties and determined an increase in the elastic and viscous modulus of the prepared dough. Modifications to biological characteristics and surface properties were confirmed by Bahrami et al. [34]. They reported no change in the total aerobic bacterial count and non-starch lipids. No visible change in the total protein amount was reported; however, there was a trend towards higher-molecular weight fractions, which resulted in thicker dough.

NTP, which is produced in a gas phase, is rich in high-energy species and the contact between the surfaces of flour particles and plasma species is crucial for its structural changes. The reactor design for flour treatment with NTP is of vital importance, as it influences the uniform exposure to plasma species and thus allows for optimal effects of plasma treatment in terms of flour functionality [47].

This study introduces a novel concept of a cylindrical, rotational reactor for wheat flour treatment with in situ produced NTP. Ease of scaling up was considered as a key factor during the development of the concept. The research focus was on defining the optimal operating conditions (e.g., the minimal treatment time) to reach the maximum dough strength, as well as understanding the influence of reactor capacity and the influence of environmental air temperature change (often variable from day to day) on the efficiency of the NTP treatment process.

2. Materials and Methods

Germany's wheat flour type 550 (equivalent to all-purpose flour) and type 1050 (equivalent to high-gluten flour) were used in this study to investigate the effects of non-thermal plasma. The main difference between the two flour types lies in their protein contents. Flour analysis demonstrates protein contents of 11.10 g and 12.68 g per 100 g of flour in the types 550 and 1050, respectively. A change in the hydration properties and the microstructure of wheat flour was determined before and after the NTP treatment. The effects of NTP treatment in terms of change in dough strength were analyzed in detail by means of rheological analysis.

2.1. Experimental Setup

The experimental setup for a batch treatment of flour samples using NTP, as shown in Figure 1, consists of three parts: (1) a plasma generator (HVG 80-3000, company Diener, Germany) [48]; (2) a cylindrical, rotational reactor, for the treatment of flour samples with DBD plasma; and (3) a control and data acquisition system (DAQ) system.

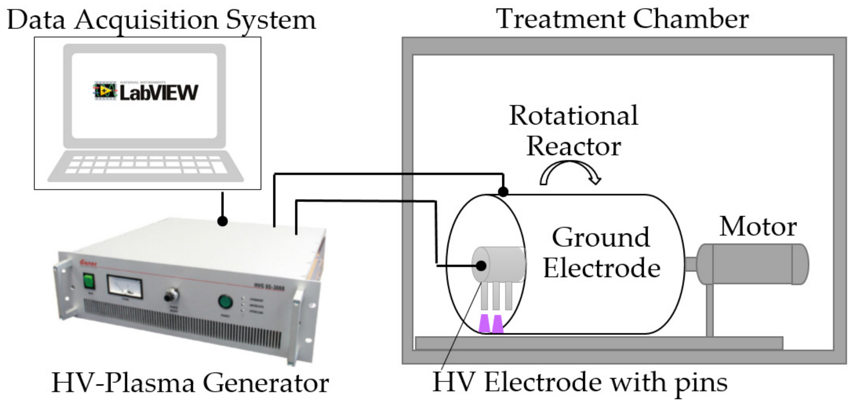


Figure 1. Experimental setup for Non-thermal plasma (NTP) treatment of wheat flour.

DBD plasma type was produced under atmospheric conditions using a high-voltage plasma generator (power: 120 W, frequency: 80 kHz, voltage: 7 kV). Air was used as plasma gas. The reactor has a capacity between 50 and 500 g of flour. It is designed as a rotational cylindrical vessel, made of stainless steel, and contains two concentric electrodes (Figure 2): (1) an outer (ground) electrode, which is in the form of a hollow, rotating cylinder (D = 150 mm, L = 300 mm, wall thickness = 2 mm) made of stainless steel and mounted axially on a rotating shaft. A dielectric barrier (thickness = 2 mm), made of Plexiglas (Polymethyl-methacrylate, PMMA), was attached to the inner side of the outer electrode. To ensure a uniform mixing during the NTP treatment, baffles (made of plastic, height = 30 mm and thickness = 3 mm) were placed on the inner wall of the outer cylinder. The outer electrode is connected to a motor, in order to provide its rotation; and (2) an inner (high-voltage) electrode (D = 50 mm, made of stainless steel), which is stationary and is positioned coaxially to the outer electrode. In order to reduce the electric power needed to induce DBD, a row of thin pins (made of aluminum, height = 43 mm and thickness = 5 mm) are attached to the high-voltage electrode, with a discharge gap of 2 mm between the pin tips and the dielectric layer (Figure 2). The reactor is closed with a cap from one side, and the other side is left open to the atmosphere.

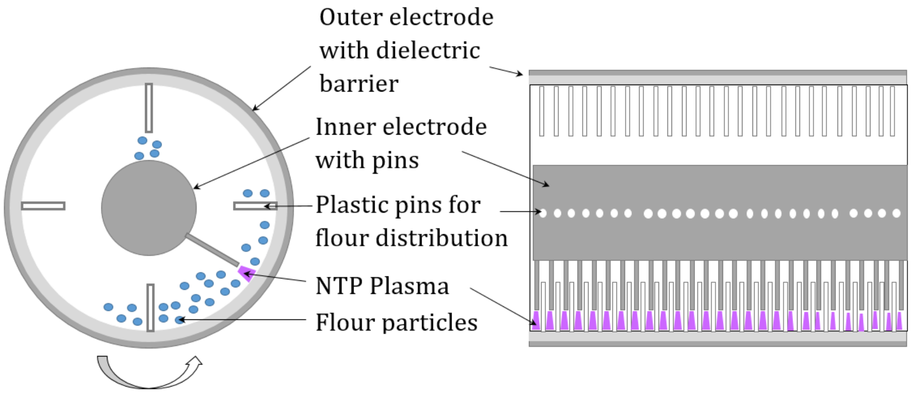


Figure 2. Test reactor for dielectric barrier discharge (DBD) plasma flour interaction.

At the beginning of each experiment, flour is added to the reactor and partially fills the annular gap between the electrodes. During the treatment, the reactor rotates at a constant speed of 10 RPM. Although the reactor is open to the environment, a low rotational speed was selected to avoid the formation of flour clouds that might lead to an explosive atmosphere. Since plasma-generated reactive species, including ozone, have very short half-lives, the described configuration enables a direct contact between these species and the target flour. After the treatment, flour samples were analyzed using a set of analytical techniques which will be described further in the text.

2.2. Dough Preparation

Doughs were prepared from untreated and treated flour samples in order to identify and compare the changes induced by the plasma treatment.

A standard approach to keep flour stable at room temperature for the purpose of packing and storing is to keep its moisture content at 14%. This standard moisture content may vary when flour is exposed to air, depending on its humidity. A compensation for this change in moisture content was used to modify the basic recipe. The correct amounts of flour and water for dough sample preparation were determined according to Equations (3) and (4), respectively.

$$\text{Flour mass (corrected)} = (100 - 14) \times M/(100 - x) \quad (3)$$

$$\text{Water mass (corrected)} = (\text{WAM} \times M) + (M - \text{corrected flour mass}) \quad (4)$$

where M (g) is the initial amount of flour (250 g), x (%) is the amount of moisture in flour (for this flour batch, determined to be 13.4%) and WAM (%) is the water-absorption capacity, for the default moisture content of 14%. Flour, water (25 °C) and salt (1 g) were mixed in a dough preparation machine (MUM48A1, Bosch). After 10 min of kneading, the dough was stored in a plastic bag to avoid drying and left to rest for 30 min at 25 °C. Afterwards, rheological analysis was performed to determine the dough's visco-elastic properties.

2.3. Analysis of Flour and Dough

2.3.1. Flour Hydration Properties

Water plays an important role in wheat dough strength due to the ability of protein matrixes to absorb and retain bound, capillary and physically entrapped water against gravity [49]. At lower hydration levels, water bonds flour proteins and starch granules via hydrogen bonds. Free water phase starts to appear at higher hydration levels (water fraction from 0.23 to 0.35), where chemical reactions (i.e., development of gluten matrix) occur during the dough development process [50]. Hence, small changes in both bonded and free water levels result in considerable variation in dough visco-elastic properties.

For this reason, the water-holding capacity (WHC) and the water-binding capacity (WBC) of untreated (control) and treated flour samples were measured and compared. The

WHC is the amount of water retained by flour particles without any stress conditions. For this purpose, 1 ± 0.05 g wheat flour was mixed with 10 mL of deionized water and left for 24 h. Afterwards, the supernatant was filtered and the wet wheat flour sample was weighed again. The WBC is the amount of water retained by flour after centrifugation. The samples were prepared according to the WHC procedure, and after 24 h centrifuged at 2000 revolutions for 10 min. Both WHC and WBC were measured according to the procedure described by Chaple et al. [41].

2.3.2. Dough Rheology

Rheological testing is a method commonly employed in the analysis of food-related substances; it is used to study the deformation and flow behavior (i.e., visco-elastic behavior) of test samples. Frequency and amplitude sweeps are two common practices used to study visco-elastic behavior. The former shows the influence of angular frequency on visco-elastic properties, while keeping the deformation amplitude constant. It is usually used to study frequency and time-dependent behavior within a non-destructive deformation range of materials. The latter demonstrates the influence of stress amplitude (at a constant frequency) on dough rheological properties.

The current investigation is based on an amplitude sweep study, by means of which G' (storage modulus), G'' (loss modulus), the linear visco-elastic region (LVE) and the yield and flow point can be determined. A rheometer (UDS-200, Anton Paar, Germany) [51] was used for the rheological study. A dough sample (10 g) was placed between the probe (MP31) and the bottom plate of the rheometer. Sandpaper (No. 60) was used on the probe and on the bottom plate, to avoid wall slip during the analysis. The frequency was set to 0.5 Hz and the amplitude gamma was set from 0.001 to 200% log to observe the visco-elastic region. The total number of measurement points was 40 and the time interval between each point was 20 s. Storage and loss moduli were plotted as functions of deformation on a log–log graph to study dough behavior.

2.3.3. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) is a technique that allows analysis of the microstructures of wheat dough samples. The development of a wheat dough microstructure is a complex process which depends on time, the addition of water and the input of mixing energy. The microstructure and the rheological properties of a dough are connected to the properties of the individual flour components, their behavior after the addition of water, the free water phase and the mobility of water [52].

The microstructural changes in the wheat flours and the respective doughs were followed by SEM (Auriga, Carl-Zeiss, Jena, Germany). The analysis was performed with an accelerating voltage of 1 kV and a working distance of 5 mm. Prior to SEM analysis, both flour and dough samples were placed in an oven at 30 °C for 24 h and then cooled in a desiccator in order to remove moisture from the samples without destroying their structures.

2.4. Experimental Procedure

In the scope of this research, the influence of process parameters, i.e., treatment time, treated flour amount and air temperature, on changes in wheat flour and dough was determined and quantified. In the first set of experiments, the influence of treatment time (1, 3 and 5 min) on dough functional properties was determined. For this purpose, wheat flour samples with a mass of 50 g were treated by NTP. In the second set of experiments, the effect of the treatment reactor design (i.e., reactor volume vs. mass of the treated flour) on dough strength was investigated. The reactor was filled with flour (50, 150, 250, 350 and 450 g) and treated for 3 min. The third set of experiments included the effects of NTP, produced at different air temperatures (10, 20, 30 and 40 °C), on dough strength (flour amount: 50 g, treatment time: 3 min).

3. Results and Discussion

3.1. Effects of Non-Thermal Plasma Treatment Time on Wheat Flour and Dough Properties

3.1.1. Hydration Properties of Flour

The variation in the moisture content of wheat flour samples with treatment time (1, 3 and 5 min) is shown in Figure 3. It was determined five times per sample, based on which the standard deviation was calculated. The obtained results showed a general trend towards a decrease in moisture content with respect to treatment duration. A low moisture content (i.e., 12–15%) keeps the flour stable at room temperature. A decrease in the moisture content of wheat flour is also connected to change in physically "bounded" water amount and has a decisive influence on rheological, physical and chemical properties. There are certain indications in the literature that a lowering of moisture content results in mechanical rigidity and the complex viscosity of a dough [53,54], which characteristics are directly related to the stronger visco-elastic properties of wheat dough.

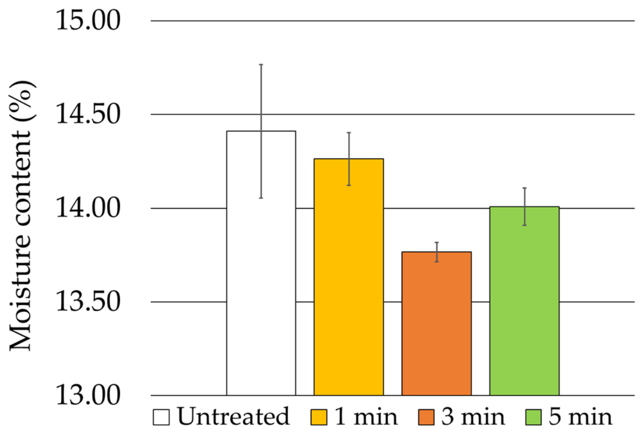


Figure 3. Change in the moisture content of wheat flour (type 550) as a function of NTP treatment time.

The dependence of the WHC on treatment time (1, 3 and 5 min), as shown in Figure 4a, was determined five times per sample, based on which the standard deviation was calculated. The WHC of the untreated sample was 168% ± 5% water retained per one gram of flour, which increased slowly with treatment time to reach its maximal value of 173% ± 4%, corresponding to 3 min of plasma treatment time. This corresponds to the finding shown in Figure 3, i.e., that as the moisture content decreases with treatment time, the water-holding ability of the flour increases. Still, no firm conclusion could be drawn at this point, as the standard deviation of this measurement was high compared to the observed change in WHC values.

The WBC dependence on treatment time, shown in Figure 4b, was also determined using five measurements per sample. The WBC level for untreated flour was measured to be 73% ± 3%. The maximum increase, just under 80% ± 1%, was observed for the NTP treatment of 3 min. Longer treatment resulted in decrease in WBC. Higher hydration values (WHC, WBC) were observed in the case of the untreated type 1050 flour (92% ± 1%) due to its higher protein content. However, no significant differences in the tendencies relating to hydration properties between the flour types 550 and 1050 were observed; thus, these were not separately presented.

Both the WHC and WBC of the tested wheat samples (types 550 and 1050) increased with plasma treatment time. The treatment time of three minutes resulted in a maximum enhancement (7% ± 3%) of the WBC of type 550 flour. It is postulated that this increase in WHC can be due to the hydrolytic depolymerisation of starch [41]. In addition, the surface modifications (increase in surface energy due to the addition or exchange of functional groups [55,56]) of flour particles due to the plasma treatment could be another important factor that influences the hydration properties of flour. It has also been reported in the literature that plasma treatment results in a decrease in water contact angle, leading to higher water permeability, which results in an increased affinity of flour particles towards water [57].

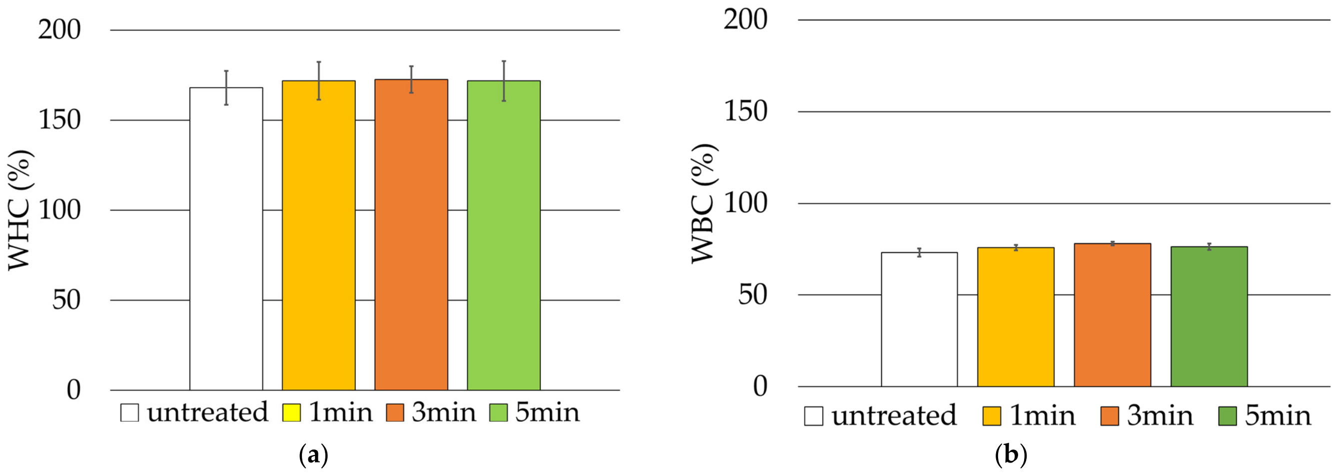


Figure 4. Change in the water-holding (**a**) and water-binding (**b**) capacity of wheat flour (type 550) as a function of NTP treatment time.

3.1.2. Visco-Elastic Properties of Dough

Changes in the functional properties of doughs produced with untreated and treated wheat flour were tested as a function of flour treatment duration. These properties were determined by rheological measurements, where the dependence of the storage and loss moduli (G′ and G″) of doughs were plotted against the induced deformation. The modulus G′ represents the elastic properties and the modulus G″ represents the viscous properties of a wheat dough. These two parameters, together with their combination, contribute to the functional properties of wheat dough, defining it as a visco-elastic material.

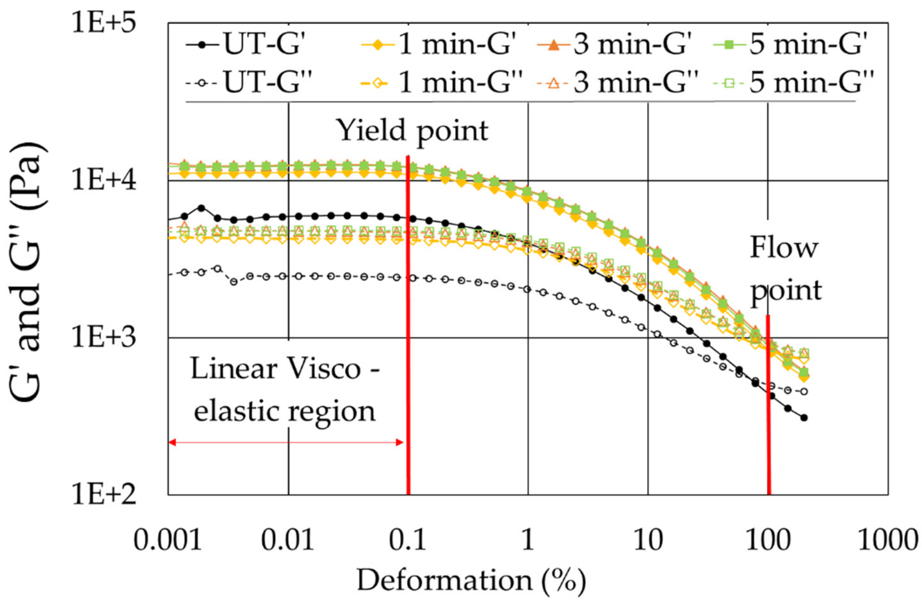
The change in the G′ and G″ values of doughs made from the two flour types (550 and 1050) as a function of deformation and treatment time on a logarithmic scale are presented in Figure 5a,b.

A rheological behavior diagram for wheat dough has three parts: (1) a linear visco-elastic region, where the elastic properties prevail. This lies between zero deformation and the deformation at which the yield point (i.e., the end of the elastic behavior and the start of plastic behavior) has been reached. This is the point at which the strain corresponds to a ~5% drop in the storage modulus [58]; (2) a plastic region, where the increasing deformation starts to permanently destroy the dough (or protein–starch) structure and cannot be regained. This is the region between the yield point and the flow point (i.e., the threshold of shear stress, above which solid material will start to flow); and (3) the region above the flow point, where a solid behaves as a liquid, since the deformation potential is so huge that all the forces responsible for holding the material structure are overruled.

Figure 5a,b shows that G′ is higher than G″, as is characteristic of visco-elastic materials. The experimental results demonstrate that the changes caused by the NTP treatment of flour, reflected by increases in the G′ and G″ values of the formed dough, initially increased as the treatment time was prolonged. This increase reached its maximum value for the treatment time of 3 min for both flour types, after which the values of G′ and G″ sank.

Figure 5a and Table 1 demonstrate that the NTP treatment showed its maximum potential for the type 550 flour, with a two-fold increase in the visco-elastic properties (G′ = 85–130% and G″ = 76–111%) for the treatment duration of 3 min. This finding corresponds to the maximum change in hydration properties of flour with the three minutes treatment, as shown in Figure 4. On the other hand, the moduli of the untreated high-protein flour (type 1050) were already significantly higher compared to those of the flour type 550, i.e., 200–400% for G′ and 200–330% for G″. Thus, the corresponding maximum changes in G′ and G″ in the plasma-treated samples of type 1050 were low to mild (G′ = 0–28% and G″ = 0–16%) (Figure 5b and Table 1). This finding also confirms the limited enhancement of the hydration properties of the type 1050 flour.

(**a**)



(**b**)

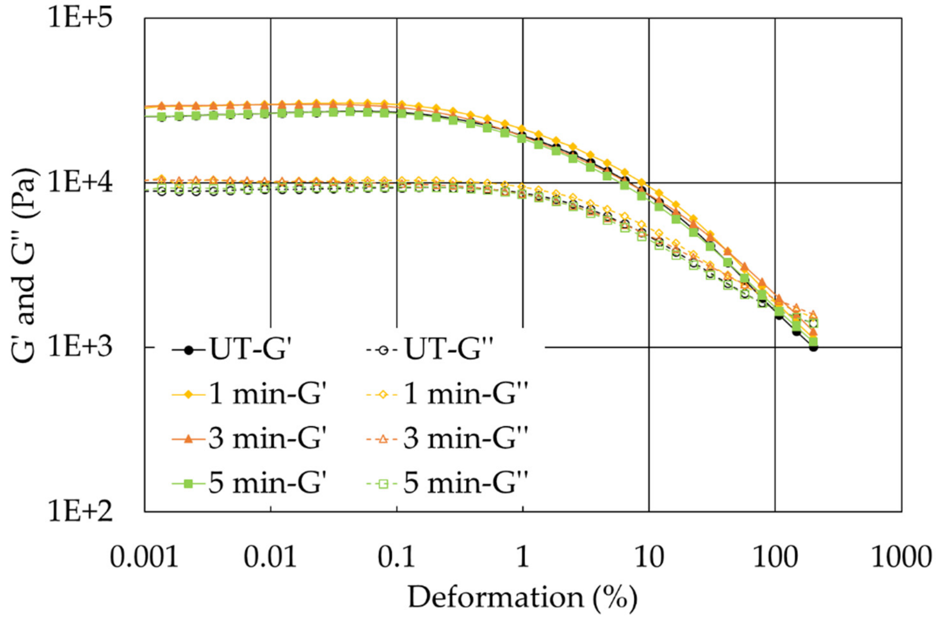


Figure 5. Change in the storage (G′) and loss (G″) moduli of wheat dough as a function of NTP treatment time: (**a**) for the flour type 550; (**b**) for the flour type 1050.

Table 1. Increase in G′ and G″ for the doughs produced with NTP-treated flour samples (presented as minimum-to-maximum increase) in comparison to doughs produced with untreated flour samples) as a function of treatment time.

Increase (%)	1 min		3 min		5 min	
Flour Type	**G′ (%)**	**G″ (%)**	**G′ (%)**	**G″ (%)**	**G′ (%)**	**G″ (%)**
550	67–105	57–88	85–130	76–111	81–123	72–110
1050	10–17	8–19	0–28	0–16	0–8	0–5

Analysis of variance (ANOVA) [59] was used in order to determine significant statistical differences between the samples. The obtained results for the type 550 flour ($p < 0.05$) (provided in the Supplementary Materials S1) confirmed the existence of significant statistical differences between untreated and treated samples, i.e., the effect of non-thermal plasma treatment on flour properties was statistically significant. However, in the case of type 1050, the obtained p-value was larger than 0.05. This implies that the effect of NTP treatment on type 1050 flour was not statistically significant, i.e., the influence of treatment cannot be established.

A general trend towards an increase in the yield points of the doughs produced with plasma-treated flour samples can be noticed, representing an increase in the linear visco-elastic (LVE) region. Increased elastic properties further indicate an increase in dough strength. The results presented in Figure 5a demonstrate that the flow point value increased by 51% and the yield point value by 75% when the flour type 550 was treated for 3 min in comparison to the untreated (control) sample. Chittrakorn et al. [60] explained an increase in dough strength in terms of an increase in the number of disulphide bonds within the protein matrix in the dough, i.e., the higher the number of disulphide bonds, the higher the storage and loss moduli. The properties of the dough made from flour type 1050 (Figure 5b) showed similar qualitative behaviors under the same plasma conditions; however, the quantitative change was much lower than for that made with type 550 flour.

Some other authors ([53,61,62]) have suggested that change in visco-elastic behavior could be connected to the hydration properties of wheat flour. The changes in moisture content, WHC and WBC, as shown in Figures 3 and 4, reflect the change in bonded and free water content levels, which directly affect gluten–starch and starch–starch bonding through hydrogen bonds. These bindings, enhanced by the plasma treatment, started to dominate the dough characteristics, making the treated samples stronger than the control samples.

Based on the presented results, it can be concluded that a plasma treatment time of 5 min will result in decreased structural strength of wheat dough, which could be connected to the weakening of disulphide bonds in the protein matrix and to the decrease in the WHC and WBC of wheat flour. Further, it can be concluded that NTP-induced intensification of visco-elastic properties is inversely proportional to the amount of protein present in the flour, i.e., with a higher protein content, the change in the visco-elastic properties of dough due to NTP is lower.

3.1.3. Wheat Dough Microstructure

Bechtel et al. [63] studied dough development with light and transmission electron microscopy and revealed that starch granules in dough are usually bigger particles, having an average size of 10–20 µm, while the protein components are in the form of sheets and fibrils which surround the starch particles. The gluten (protein) network structure is affected by gluten quantity, quality and the number of intermolecular disulphide bonds. Similar SEM analyses of starch and protein structures can be found in the literature [52,63,64].

The SEM images of doughs with untreated and treated (1, 3 and 5 min treatment time) flours (type 550) are shown in Figure 6. As claimed above, proteins create a smooth structure that surrounds the round starch particles. Further, two classes of starch granules can be noticed which differ with respect to their average diameters. It has been reported that type A starch granules (with a flattened shape and about 25 microns in diameter) contribute to malting yield, while type B granules (spherical in shape and about 6 microns in diameter) are responsible for water absorption due to their large surface-to-volume ratios [65].

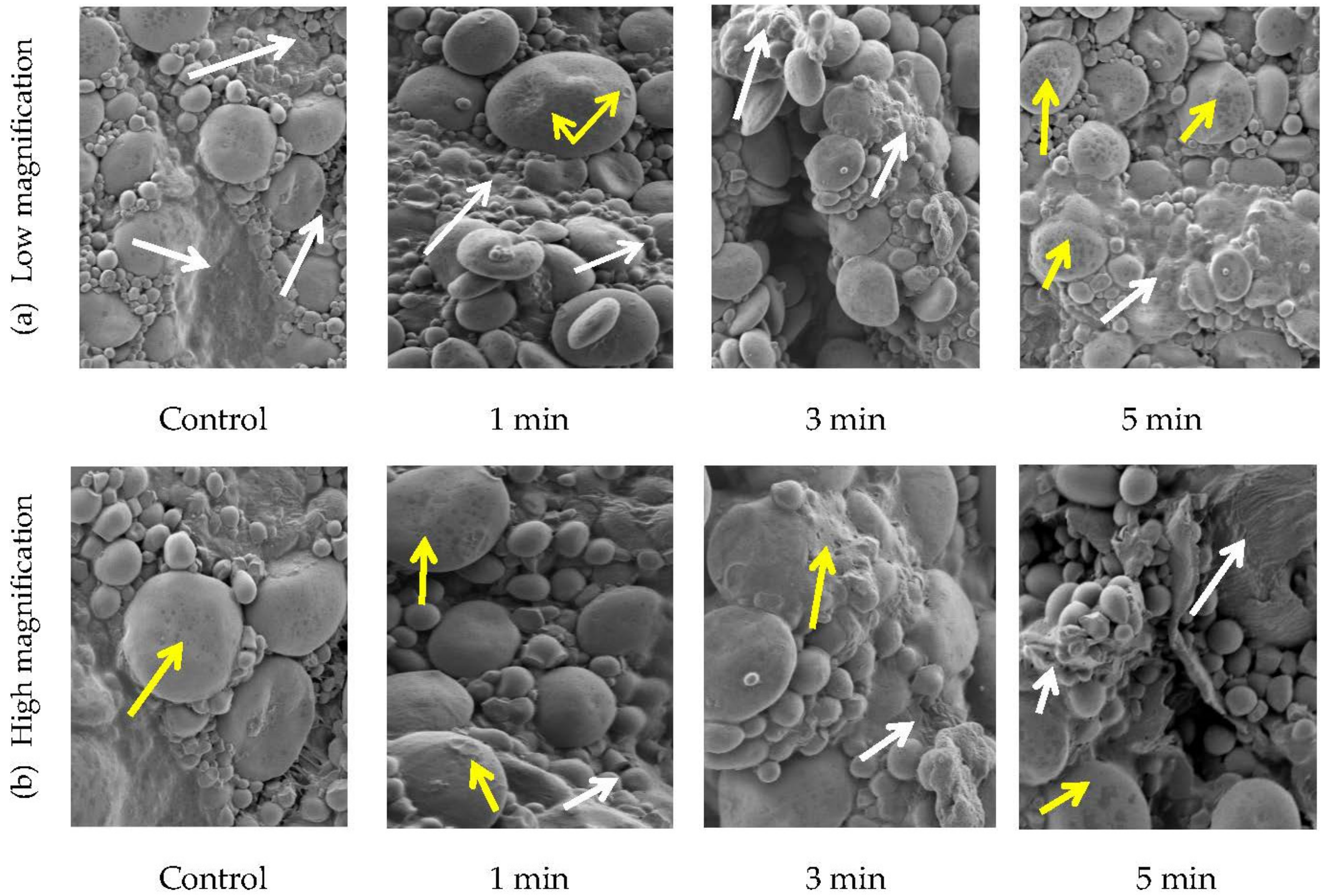


Figure 6. SEM Images of the wheat dough samples prepared with untreated and plasma-treated (1 min, 3 min, 5 min) flour under (**a**) low and (**b**) high magnifications (corresponding to 1000× and 2000× magnifications in the current SEM set-up). White arrows—gluten network; yellow arrows—changes to starch particles.

In the case of the control sample (Figure 6a), the gluten matrix was non-homogeneous, i.e., it was a discontinuous network in which large and small fragments of gluten could be observed (indicated by arrows). Starch granules are covered with thin gluten layers. However, the starch granules were not fully embedded in the protein matrix, which corresponds to the findings of Bajic et al. [66,67]. In comparison to that prepared with untreated flour, the SEM images of the dough prepared with plasma-treated flour (Figure 6a,b) showed a well-developed gluten matrix network in which starch particles were uniformly embedded, i.e., a well-developed gluten matrix could be observed. This resulted in a stronger dough, consistent with the rheological analysis presented in Figure 5.

The SEM images for increased treatment times showed only minor differences in the developed gluten network characteristics. However, when the treatment time was equal to 5 min, damage to starch granules was observed (Figure 6a,b), which led to the weakening of gluten networks. This negatively affects the storage and loss moduli of wheat dough [68,69], as confirmed by the results presented in Figure 5.

Comparison of the results presented in Figures 5 and 6 indicated an optimal plasma treatment time of 3 min to enhance dough rheological properties. If this time is exceeded, negative effects might occur as a result of the NTP treatment, leading to the weakening of dough networks.

SEM images for type 1050 samples are visually similar with respect to gluten and protein particles. However, no significant differences between the developed gluten networks of the NTP-treated samples were observed.

3.2. Effects of Non-Thermal Plasma-Treatment Reactor Filling on Wheat Flour and Dough Properties

As a next step, the role of flour mass in the plasma reactor on changes in flour properties was investigated. The reactor was filled with different amounts (50, 150, 250, 350 and 450 g) of wheat flour (type 550 and 1050), which are equivalent to 2, 6, 10, 13 and 17% occupancy of the reactor volume (flour density ρ = 593 kg/m^3 [70]).

3.2.1. Hydration Properties of Flour

Similar to the findings for moisture content, presented in Figure 3, the moisture content also remained close to 14% with varying flour masses and therefore the results are not discussed further here.

Figure 7a,b shows the dependence of WHC and WBC on wheat flour mass (type 550). Even though a stronger effect of NTP in a less full reactor would be expected, a general trend of increase in WHC and WBC values with increase in flour mass was observed. The WHC and WBC values generally increased for dough prepared with treated flour, with the maximal increase for the flour mass of 150 g, i.e., 6% filling of the reactor volume.

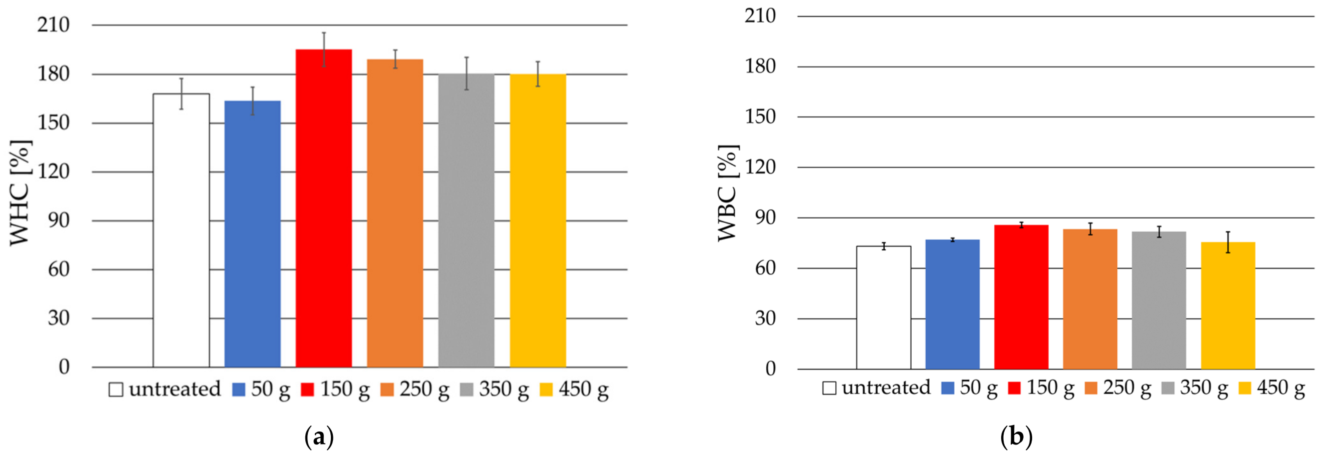


Figure 7. Effects of reactor filling on the WHC (**a**) and WBC (**b**) values of untreated and NTP-treated flour (type 550; treatment time: 3 min).

The above observation could be attributed to the fact that, during the flour treatment, a proportion of the flour particles stick to the reactor wall and to the HV pins. According to Chaple et al. [41], sample surface area exposed to plasma treatment is an important factor that influences the hydration properties of flours. At a lower flour mass (50 g, equivalent to 2% of reactor volume), a bigger share of the treated flour mass is immobilized and thus the treatment is not uniform. At a moderate mass (150–350 g, i.e., 6–13% of reactor volume), a smaller proportion of flour particles are 'glued' to the reactor surfaces and thus a more intensive mixing takes place, contributing to more effective contact between the plasma species and the flour particles. At higher mass (450 g, i.e., 17% of reactor volume), the contact between the flour mass and the plasma species decreases, hence contributing to a decrease in hydration properties. In the case of type 1050, the change in hydration properties lay within the standard deviation range, hence no conclusion could be drawn and the results are not shown here.

3.2.2. Visco-Elastic Properties of Dough

Equivalent to the analysis shown in Figure 5 and Table 1, Figure 8 and Table 2 describe the influence of flour mass (i.e., reactor filling) on wheat dough visco-elastic properties. Independent of the treated flour quantity, the results demonstrate an increase in visco-elastic properties of doughs made with treated flour samples in comparison to doughs prepared with untreated wheat flour.

(**a**) (**b**)

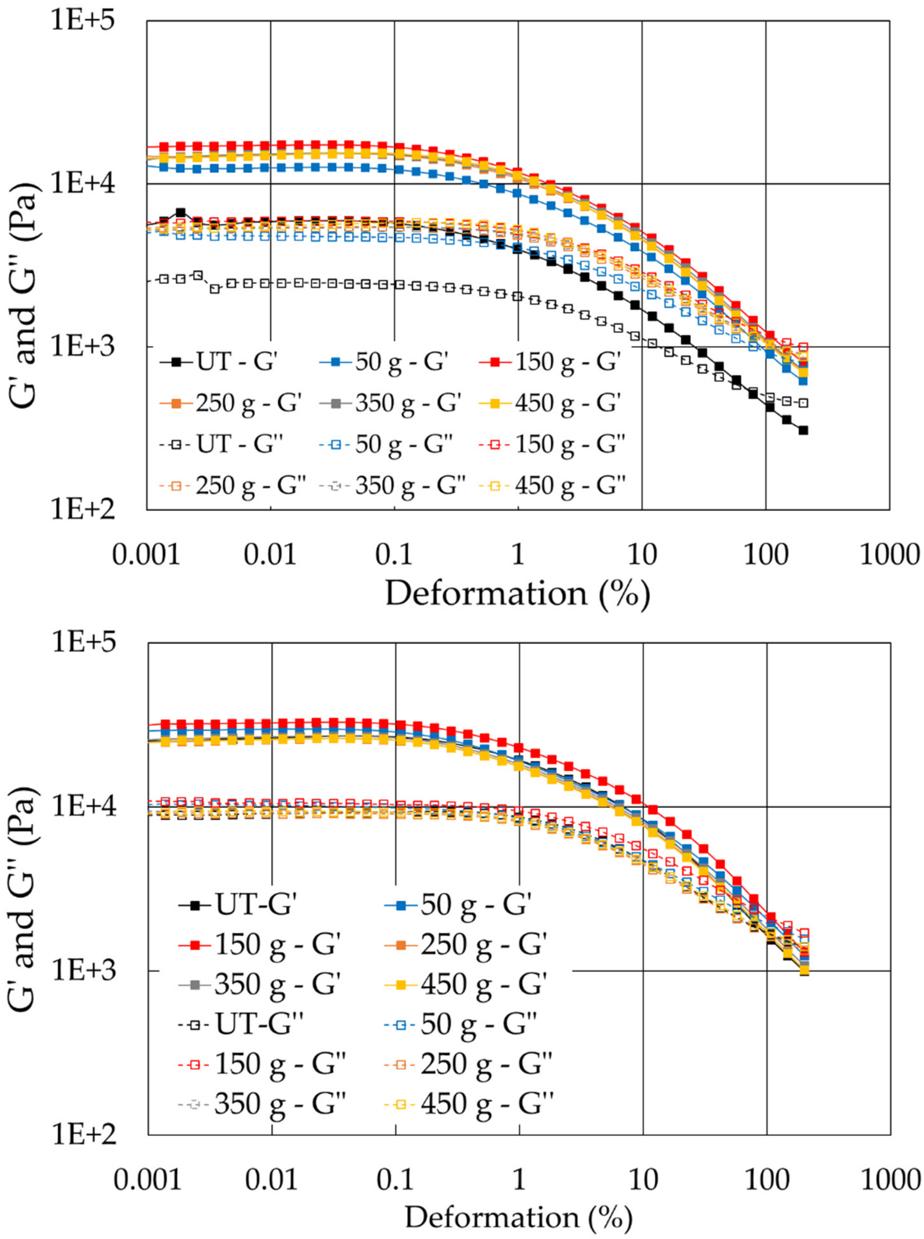


Figure 8. Change in the storage (G′) and loss (G″) moduli of wheat dough as a function of NTP treatment at different reactor fillings (50, 150, 250, 350 and 450g, at 10 RPM): (**a**) type 550; (**b**) type 1050.

Table 2. Increase in the G′ and G″ values of the doughs produced with plasma-treated flour samples (presented as minimum-to-maximum increase in comparison to doughs produced with untreated flour samples) as a function of reactor filling (i.e., flour mass).

Increase (%)	**50 g**		**150 g**		**250 g**		**350 g**		**450 g**	
Flour Type	**G′ (%)**	**G″ (%)**	**G′ (%)**	**G″ (%)**	**G′ (%)**	**G″ (%)**	**G′ (%)**	**G″ (%)**	**G′ (%)**	**G″ (%)**
550	85–130	76–111	155–203	114–158	119–172	94–141	119–184	91–150	115–183	93–159
1050	0–28	0–17	19–40	8–29	0–5	0–4	0–10	0–6	0–4	0–6

The experimental results demonstrate that when a too small (50 g) or a too large (450 g) amount of flour is placed inside the treatment reactor, the rheological properties of the formed dough are only mildly increased. G′ and G″ reach their maximum values with a flour mass of 150 g and then slowly decline again. Such a limitation is not observed in the case of rheological property enhancement using chemicals, such as Vitamin C or ascrobic acid, L-Cysteine and Azodicarbonamide (ADA) [71–73]. A few milligrams of such chemicals (e.g., 10–20 mg of ADA) per kilogram of wheat flour is enough to improve wheat flour properties [74]. Similar to the findings in Figure 5, the results shown in Figure 8 also confirm the previous findings about the limited influence of NTP treatment on the properties of dough made with flour type 1050. This can be connected to the changes in WHC and WBC values shown in Figure 7. It has also been reported that chemical improvers, e.g., vitamin C, also generally improve the visco-elasticity of low-gluten flour as compared with high-gluten flour [75]. Statistical analysis again confirmed *p*-values less than 0.05 in the case of type 550 and greater than 0.05 in the case of type 1050.

The presented results demonstrate the significance of flour particle–plasma species contact, which depends on the percentage of the reactor volume that is filled with flour. This factor can significantly limit the effect of NTP on flour functional properties. In order to achieve better effects of NTP treatment, the threshold for the reactor volume in this set-up is in a range of 6–13%.

3.3. Effects of Environmental Temperature during NTP Treatment on Dough Properties

Further, the effect of environmental air temperature on the NTP treatment of flour was investigated. A lower voltage demand for plasma formation and an earlier air breakdown due to increased air temperature was reported in the literature [76–78].

A flour sample (type 550, mass 50 g) was treated in the rotational reactor. The air temperature in the plasma reactor chamber was increased using an air heater in 10 °C steps from 10 °C to 40 °C while keeping the relative humidity at 50% ± 10%. The experimental results, shown in Figure 9, demonstrate the dependence of the visco-elastic properties of wheat dough produced at various air temperatures on the non-thermal plasma treatment. Statistical analysis (ANOVA) confirmed the existence of significant statistical differences between the untreated and treated samples, with *p*-values obtained less than 0.05. Independent of air temperature, the results demonstrated an increase in the visco-elastic properties of the dough produced with NTP-treated flour.

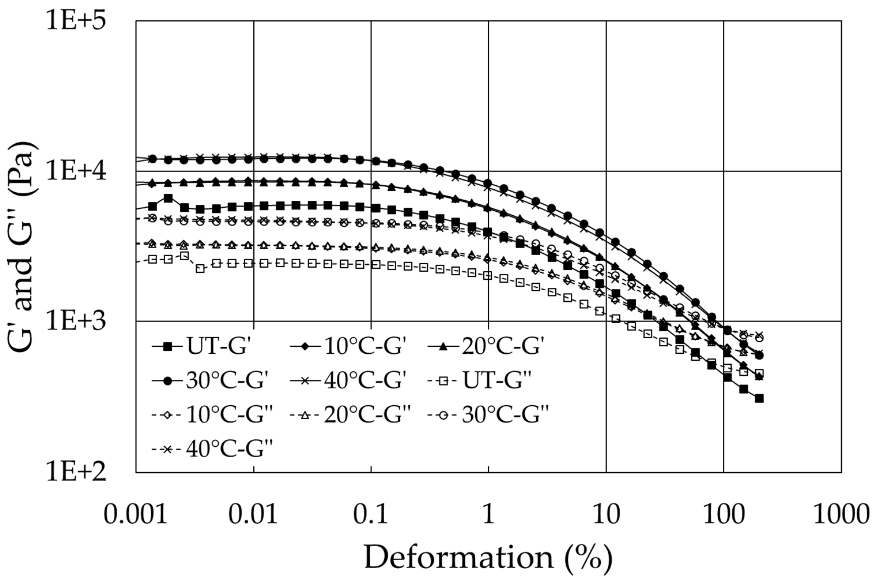


Figure 9. Change in the storage (G′) and loss (G″) moduli of wheat dough as a function of environmental (air) temperature.

The results presented in Table 3 demonstrate the change (in percentage) in visco-elastic properties (G′ and G″) of wheat doughs produced with plasma-treated flour samples at air temperatures between 10 °C and 40 °C in comparison to the control sample. When

NTP was generated at an air temperature up to 20 °C, the visco-elastic properties of dough increased maximally between 25 and 53%. When the air temperature increased to 30 °C, the change in visco-elastic properties almost doubled. A further increase in air temperature (i.e., 40 °C) did not further affect the values of visco-elastic properties significantly.

Table 3. Increase in G′ and G″ values for the doughs produced with plasma-treated flour samples (presented as minimum-to-maximum increase in comparison to doughs produced with untreated flour samples) as a function of environmental temperature.

Increase (%)	G′	G″
@ 10 °C vs. UT	25–53%	20–44%
@ 20 °C vs. UT	26–51%	17–43%
@ 30 °C vs. UT	80–121%	71–105%
@ 40 °C vs. UT	81–120%	75–110%

The breakdown voltage demand is strongly influenced by the gas temperature, as it is inversely proportional to the temperature of the gas, as shown in Equation (5) [78]:

$$U = (T_{r/}T_g) \times U_o \quad (5)$$

where U (V) and U_o (V) are the breakdown voltage at the desired gas temperature and gas breakdown voltage at room temperature, respectively; T_r is the ambient room temperature (300 K); and T_g is the gas temperature.

If the temperature of the gas is increased, the breakdown voltage demand decreases and vice versa. It has been reported that air warmed to a certain temperature impairs its insulating power and makes it dielectrically weak, resulting in the formation of the arc at longer distances [79]. This means that if the discharge gap and supply voltage is constant (as is the case here; i.e., if a 2 mm discharge gap and a constant maximum voltage of 7 kV is supplied to the system), then at lower temperatures (in this case, an air temperature of 10 °C) the gas is partially ionized, leading to the production of a limited number of active species. This limit is overruled by the increase in the gas temperature (in this case, 30 °C), as at that constant voltage and discharge gap, the gas is more easily ionized (decrease in air dielectric strength) due to higher temperatures. Further increase in temperature leads to a decrease in breakdown voltage demand; however, the number of active species stays constant, as the gas is already fully ionized at lower temperatures. This was the reason why a strong increase in visco-elastic properties in wheat flour was noticed as the air temperature was increased from 10 °C to 30 °C. However, no further increase in visco-elastic properties was detected when the air temperature was increased to 40 °C.

4. Conclusions

The key findings of the presented research are:

- Due to the non-thermal nature of the generated plasma, the moisture content of treated wheat flour (type 550 and 1050) does not change significantly and stays close to 14%.
- Three minutes was identified as an optimal NTP treatment time for all test cases, with which the changes in visco-elastic properties reached their maximum values.
- Increase in the hydration properties (WHC and WBC) of wheat flour with an increase in NTP treatment time was determined. A maximum increase in WBC value of approximately 6% was observed for the NTP treatment time of 3 min. Longer treatment times resulted in decreases in WBC values.
- Rheological analysis demonstrated the effectiveness of NTP in enhancing the visco-elastic properties of wheat dough. In the case of doughs formed using NTP-treated flour type 550, the visco-elastic properties, i.e., the storage (G') and loss (G'') moduli, increased, on average, by more than 100%. A maximum increase of 130% was detected for the dough made with flour treated with NTP for three minutes.

- For doughs produced with flour type 550 treated by NTP for three minutes, the yield and the flow point increased by 75 and 51%, respectively.
- Based on the rheological measurements, NTP treatment was found to be effective in enhancing the visco-elastic properties G' and G'' of the flour type 550 samples (the G' and G'' values more than doubled), while for the type 1050 samples its influence was less noticeable.
- The amount of treated flour, i.e., the extent of reactor volume filling, was identified as an important factor that significantly influences the effects of the NTP treatment of flour. Based on the hydration and rheological properties of the obtained doughs, it was concluded that for the presented reactor the optimal fill is in the range of 6 to 13% of the reactor volume.
- A wheat flour mass of 150 g (equivalent to 6% of the reactor volume) was identified as an optimal mass for the present geometry for which the maximum enhancement in rheological properties (type 550: $G' \geq 150\%$, $G'' \geq 200\%$; type 1050: $G' = 40\%$ and $G'' = 30\%$) was observed.
- Environmental (air) temperature has a significant effect on the visco-elastic properties of doughs produced with NTP-treated flour, indicating the importance of air temperature on plasma formation. The biggest difference (>100%) was noticed when the air temperature changed from 20 °C to 30 °C. Further increase did not contribute significantly to dough rheological properties.
- Analysis of variance (ANOVA) was performed in order to detect statistical differences among the samples due to NTP treatment. In the case of type 550 flour, the *p*-value was less than 0.05, confirming the existence of significant statistical difference and thus an influence of the non-thermal plasma treatment on flour properties. On the other hand, in the case of flour type 1050, the *p*-value was greater than 0.05, leading to the conclusion that no significant statistical difference existed between the untreated and treated samples.

NTP treatment has the potential to replace the use of chemicals in order to enhance the functional properties of wheat dough. However, as an outlook of this work, it is vital to investigate the effects of NTP on flour and dough structural network attributes, e.g., proteins, starch, microorganisms, color, etc., before further implementation of the treatment. Future scaling, involving such considerations as the processing efficiency, cost and control of the NTP process, could also be challenging. In the next phase of this research, the aim is to further study the effects of NTP on wheat network structural attributes in more detail.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app12167997/s1, manuscript-supplementary.

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