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Characterization and regeneration potential of vital wheat gluten treated with non-thermal plasma

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ABSTRACT

Non-thermal plasma (NTP) technology is an emerging physical process that has attracted the interest of researchers owing to its promising effects in food processing. This study aimed to determine the effect of NTP treatment at atmospheric pressure on vital wheat gluten (VG) properties. VG treated at powers of 50 W, 100 W, and 150 W and flow rates of 3, 5, and 8 L/min was characterized, and the empirical rheology of flours enriched with these VGs was determined. NTP promoted greater water absorption capacity, higher wet gluten content, and gluten index, and greater resistance to extension when 50 W and 5 L/min were applied. When power and flow rate were increased, resistance to extension decreased, and gluten extensibility increased. Under optimal VG treatment conditions, the rheological properties of the enriched wheat flours revealed an improvement in the development time and stability of dough. Based on the results obtained with the power and flow rate ranges tested herein, NTP can be used to modulate the functionality of VG.

1. Introduction

Variations in wheat flour technological quality affect bread properties and are related to wheat grain genotypic, environmental, and processing circumstances (Atwell, 2001). In addition, market demands for different types of breads, such as those enriched with dietary fiber, whole grains, and seeds, also reduce the technological potential of flour due to the dilution of native gluten (Barros et al., 2021). The technological properties of flour are a collection of physicochemical and rheological parameters that reflect how well flour dough performs during product manufacture (Orlotan et al., 2017). These properties are mainly governed by gluten and are related to the quantity and quality of its proteins (Wieser, 2007).

Gluten is essential for fabricating several products derived from wheat flour, especially bread; this is because the performance of the dough depends on its viscoelasticity (i.e., the hydration capacity and the elastic and extensible behavior of gluten) (Ortolan et al., 2017). These properties depend on the proportion of gliadins (extensibility) and glutenins (elasticity) present in the flour. Accordingly, during bread manufacture, after the addition of water and mechanical work, the gluten network is developed, and the gases from fermentation are retained in the dough and contribute to the shape and structure of bread (Wieser, 2007).

Wheat flour may not always present the desired baking characteristics for breads, usually due to its low gluten content and quality. As a result, often this flour must be improved using external agents. Flour improvers are agents added to flour for it to acquire ideal breadmaking characteristics. Oxidizing agents, emulsifiers, hydrocolloids, or enzymes are the most used enhancers. However, among improvers, the ingredient vital wheat gluten (VG) is often highlighted as it can increase the amount of gluten in the flour and enable the generation of bakery products of better quality (Ortolan and Steel, 2017). When added to wheat flour, VG generally improves dough strength, mixing tolerance, and handling. In addition, its viscoelastic properties provide expansion and gas retention abilities, increased volume, and improved texture and uniformity of the bread crumb. Furthermore, its water absorption capacity improves product yield, softness, and shelf life (Atwell, 2001).

In the industry, VG is produced by washing wheat flour dough with water. The soluble components, such as starch, are leached during washing, while the insoluble component, wet gluten, remains. Notably, components, such as starch, lipids, and minerals, are not completely removed during washing. After the washing step, wet gluten is dried at temperatures that should not exceed 70 °C, milled, packaged, and commercialized as VG. VG does not have the same quality as native gluten because of the alterations caused to gluten proteins during the VG

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Abbreviations

NTP	Non-thermal plasma
VG	Vital gluten
VGs	Vital glutens
WAC	Water absorption capacity
OAC	Oil absorption capacity
WG	Wet gluten
DG	Dry gluten
GI	Gluten index
WA	Water absorption
DT	Development time
S	Stability
MTI	Mixing tolerance index
Re	Resistance to extension
E	Extensibility

obtainment process (Ortolan and Steel, 2017).

In recent years, emerging technologies, including high hydrostatic pressure, pulsed light, ultraviolet, ultrasound, and non-thermal plasma (NTP), among others, have been tested as non-thermal physical methods to modulate the functionality of wheat flour (Barros et al., 2021). Of these technologies, NTP is a new technology that was initially used for surface disinfection and food decontamination (Misra et al., 2016). However, recent research has revealed its advantages relative to other methods employed to fortify wheat flour (Bahrami et al., 2016; Chaple et al., 2020; Held et al., 2019; Misra et al., 2015).

Plasma is defined as the fourth state of matter and is obtained when a gas or a mixture of gases receives a high charge of energy and is ionized or partially ionized. Plasma composition consists of chemically reactive species, such as molecules, atoms, positive and negative ions, free radicals, photons, metastable particles, and electrons. At atmospheric pressure, NTP is characterized by a net gas temperature close to ambient temperature (Misra et al., 2016).

In this study, a microwave-induced argon plasma system at atmospheric pressure was used to modify the properties of VG. The mechanism occurs via the bombardment of electrons, ions, and neutral charges (*neutral Ar, Ar^+, Ar^-*) in different excitation states owing to the different processes inside the reactor, such as excitation/deexcitation by electron impact, spontaneous light emission, radiation trapping, electron impact ionization, and metastable extinction due to diffusion to the walls. The concentration of electrons and their energy levels are dependent on the gas flow rate and the microwave power absorbed (Evdokimov et al., 2017). The NTP used in this study facilitates the treatment of dry foods such as flour, starch, grains and vital gluten. Microwave discharges are effective to ionize the gas, in addition to being easy to handle and have a large-diameter plasma discharge generated under low pressure (Clerici et al., 2019). The low oxidative effect of argon plasma is attractive to act on the three-dimensional structure of VG. Thus, the diffusivity of argon plasma in the structure of VG can trigger changes at structural levels (Ekezie et al., 2018), such as low oxidative reactions (Chaple et al., 2020).

The results of studies involving argon plasma in wheat flour are promising. Flours with increased hydration capacity (Chaple et al., 2020) and dough strengthening have been reported (Held et al., 2019). Therefore, this study aimed to evaluate the potential of NTP to improve the technological characteristics of VG isolated from wheat flour as a potential ingredient to strengthen wheat flour.

2. Experimental

2.1. Material

The wheat flour used for gluten extraction was purchased from Pastifício Selmi (Sumaré, SP, Brazil). This flour contained 13% moisture, 12% proteins, 1% lipids, 0.51% ash, and 71% carbohydrates. The weak wheat flour used for VG enrichment was kindly provided by Cooperativa Agrária (Agrária, Garapuava, PR, Brazil), and contained 13.97% moisture, 10.22% proteins, 3.04% lipids, 1.34% ash, and 71.43% carbohydrates, according to analysis through methods 44-15.02, 46-13.01, 30-25.00, and 08-01.01 of the AACCI (2010), and based on difference, respectively. The technological parameters of the weak flour used were: 29.48% wet gluten, 9.62% dry gluten, and 68.59% gluten index (GI), according to method 38-12.02 (AACCI, 2010); falling number of 301, 67 s, according to method 56–81.03 (AACCI, 2010); and 56% water absorption, 4.5 min development time, 5.8 min stability, and 58.67 BU (Brabender Units) mixing tolerance index according to method 54-21.02 (AACCI, 2010).

2.2. Methods

2.2.1. Extraction of gluten from wheat flour

Gluten was extracted as previously described by Kieffer et al. (2007), with modifications. Wheat flour (300 g) and NaCl solution (0.4 mol/L) (165 mL) were mixed in a farinograph (Brabender, Duisburg, Germany) mixing chamber for 2 min at 22 °C. After 10 min of rest, the dough was washed manually with distilled water (2 L) until a cohesive dough (gluten) was obtained. Portions of gluten (15 g) were then washed in a Glutomatic (Perten Instruments, Huddinge, Sweden) with distilled water (270 mL) for 5 min. The wet wheat gluten resulting from the process was subjected to lyophilization and subsequent milling, with particle size standardization at 60 mesh. After that, the gluten was stored in airtight jars and called VG. The leached wheat starch, resulting from the gluten extraction process, was dried, ground (60 mesh), and stored for future analysis. VG had 9.20% moisture, 71.02% proteins, 1.28% lipids, 0.62% ash, and 17.88% carbohydrates according to methods 44-15.02, 46-13.01, 30-25.00, and 08-01.01 of the AACCI (2010), and based on difference, respectively.

2.2.2. Non-thermal plasma (NTP) treatment of vital wheat gluten (VG)

The extracted VG was treated in the non-thermal plasma generation unit for indirect treatment (NTPGUIT), which belongs to the Institute of

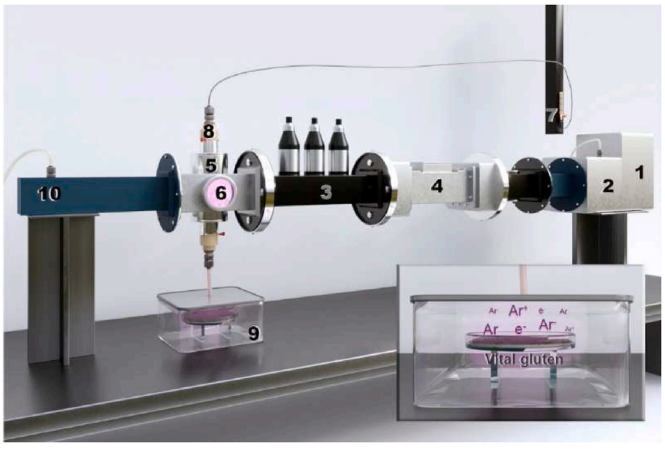

Fig. 1. Non-thermal plasma generation unit for indirect treatment (NTPGUIT). [1] high-voltage source, [2] microwave generator, [3] tuner, [4] transmitted and reflected power meter, [5] plasma generating reactor, [6] high voltage ignitor, [7] flowmeter, [8] gas conducting ducts, [9] sample conditioning container for non-continuous treatment, and [10] circulating water bath.

Food Technology (ITAL – Campinas, SP) (Fig. 1). The NTPGUIT works in a discontinuous single-mode system but can be adapted for continuous processes and is composed of elements for the generation and transmission of microwaves, with a frequency of 2.45 GHz × 1.9 kW. In addition, this unit has a gas supply system and an opening for introducing a high-voltage ignitor (1.5 kV). This ignitor is used for plasma formation in a specific accessory, which is connected through a conductive duct to a sample port, for the treatment of the food matrix, from the continuous post-discharge flow of the NTP generated (Clerici et al., 2019). Preliminary studies on VG (Barros et al., 2019; Barros and Steel, 2020) using this equipment in a continuous single-mode system revealed promising effects on the properties of this matrix.

The VG was placed in Petri dishes and left in a desiccator with saturated NaCl solution until equilibrium was reached (10.16% moisture content). A thin layer of VG (10 g) was added to a 90 × 15 mm (diameter x height) Petri dish and stirred with a spatula every 5 min to provide greater matrix contact with the post-discharge gas. The total exposure time was 10 min, and the ranges for power (P) and argon gas flow (V) were selected after preliminary tests; herein, the discharge temperature should not exceed 50 ± 5 °C, so as to be below the denaturation temperature of gluten (Ortolan and Steel, 2017). The ranges of variation P and V, and the respective samples are: Control: Untreated VG; P50V3: 50 W and 3 L/min; P50V5: 50 W and 5 L/min; P50V8: 50 W and 8 L/min; P100V3: 100 W and 3 L/min; P100V5: 100 W and 5 L/min; P100V8: 100 W and 8 L/min; P150V3: 150 W and 3 L/min; P150V5: 150 W and 5 L/min; and P150V8: 150 W and 8 L/min. All treatments were conducted at 45 ± 1% of relative humidity and 25 ± 2 °C. The treatments were performed in duplicate.

2.2.3. Characterization of vital wheat gluten (VG)

For the characterization of vital wheat gluten (VG), some analyses (moisture and pH, water absorption capacity, and oil absorption capacity) were made on gluten alone; others (gluten contents and gluten index, expansion test, extensibility test) required flour reconstitution by blending with wheat starch; and others yet (farinographic and extensographic analysis) evaluated its strengthening potential when mixed to weak wheat flour.

2.2.3.1. Moisture and pH. Moisture was determined using method 44-15.02 (AACCI, 2010), and pH was measured using a pH meter PHS3BW (Bel Engineering, Monza, Italy) after 1 g of VG was dispersed in 10 mL of deionized water. The analyses were performed in triplicate.

2.2.3.2. Water absorption capacity (WAC) and oil absorption capacity (OAC). The WAC of VG samples (Equation (1)) was determined using the method described by Sosulski1 (1962), with modifications. Briefly, the VG sample (500 mg) was dispersed in water (30.0 mL), mixed, shaken for 1 h, and centrifuged at 2000 rpm for 30 min, at 20 °C. Thereafter, the supernatant was discarded, and the remaining pellet was weighed. The OAC of the VG samples (Equation (2)) was determined according to Lin et al. (1974). Briefly, the VG sample (500 mg) was added to refined soybean oil (10.0 mL), stirred for 1 h, and then centrifuged at 2000 rpm for 30 min, at 20 °C. Then, the supernatant was discarded, and the remaining pellet was weighed. The analyses were performed in triplicate, and the results are expressed as percentages.

$$WAC: \ sediment\ weight / sample\ weight^{*}100 \quad (1)$$

$$OAC: \ sediment\ weight / sample\ weight^{*}100 \quad (2)$$

2.2.4. Gluten regeneration potential

To verify the regeneration potential of VG and its technological quality attributes, blends with native wheat starch (obtained from the gluten extraction process) were prepared in a planetary electric mixer model K45SS (KitchenAid™, Benton Harbor, United States), at 220 rpm, for 15 min, to derive blends containing 12 g/100 g of total protein. The blends were then stored in closed flasks at room temperature and protected from humidity. The nine treated VG blends with starch were compared to each other and to the untreated control VG blend. Each blend was produced in duplicate and identified according to the VG that generated it, as described in item *2.2.2.*

2.2.4.1. Gluten contents and gluten index. The contents of wet gluten (WG) and dry gluten (DG), and the gluten index (GI) were determined for the blends of VG mixed with starch through method 38-12.02 of the AACCI (2010). The analysis was performed in triplicate.

2.2.4.2. Expansion test. The expansion test was carried out by the baking wet gluten obtained from the blends in the Glutomatic, according to method 38-12.02 of the AACCI (2010), without the steps of centrifugation and drying. Once the gluten was removed from the equipment, it was placed in a rectangular aluminum pan (38 cm × 28 cm × 5 cm) and then in an electric convection oven, model HPE80 (Prática Technicook, Pouso Alegre, MG, Brazil), at 200 °C, for 20 min (Ortolan et al., 2018). The baked gluten balls were then allowed to cool at room temperature for approximately 1 h. Thereafter, the volume (v) was measured by the displacement of millet seeds and the mass (m) in an analytical scale, model B-TEC 500 (Tecnal, Piracicaba, SP, Brazil); the specific volume was calculated as the volume/mass ratio (v/m). The analysis was performed in triplicate.

2.2.4.3. Extensibility test. Gluten extensibility analyses were performed using a TA-XT2i texture analyzer (Stable Micro Systems, Surrey, UK), with a Kieffer accessory (Kieffer Dough & Gluten Extensibility Rig, A/KIE, Stable Micro Systems, UK). Briefly, approximately 2.5 g of wet gluten obtained from the Glutomatic was used. For this analysis, the test parameters were set at an elevation speed of 3.30 mm/s and a distance of 75 mm. Results were obtained for extensibility (Ex, mm) and resistance to extension (Re, g). The analysis was conducted on five replicates for each sample.

2.2.5. Rheological properties of weak wheat flour + vital wheat gluten (VG) mixes

Weak wheat flour was used for enrichment with VG until an equivalent of 12 g/100 g of total protein was obtained. The VG-flour blends were evaluated in triplicate by farinographic analysis (water absorption, dough development time, stability, and mixing tolerance index), according to method 54-21.02 of the AACCI (2010), using a model 827505 farinograph (Brabender, Duisburg, Germany), and extensographic analysis [resistance to extension (R, BU) and extensibility (E, mm)], according to method 54-10.01 (AACCI, 2010), in an extensograph, model 860703 (Brabender, Duisburg, Germany). The nine treated VG mixes with weak wheat flour were compared to each other and to the untreated control VG mix. Each mix was produced in duplicate and identified according to the VG that generated it, as described in item *2.2.2.*

2.3. Statistical analysis

All determinations were performed in at least triplicate for each analysis. Analysis of variance (ANOVA) was applied using Sisvar 5.6 (Ferreira, 2019), and the differences between the sample means were determined through the Scott Knott test ($p < 0.05$).

3. Results and discussion

3.1. Characterization of vital wheat gluten (VG)

The use of NTP to improve the technological properties of proteins has appeared in recent years (Barros et al., 2021). However, the influence of this technology on gluten is yet unknown, and we found no

studies reported in the literature regarding the use of NTP on VG. Therefore, to comprehend the modifications caused by this technology, we compared our results to research using other foods. Moisture content, pH, WAC, and OAC of VGs are shown in Table 1.

NTP treatment reduced the moisture content of all samples. The samples treated at high power and medium to high flow (P150V5 and P150V8) had lower moisture values (2.44%) than those treated at low power and low, medium, or high flow. Moisture reduction can occur through the interaction of active plasma species with free water present on the matrix surface (Chaiwat et al. (2016); Lambert et al. (2009)), driven by argon gas flow. Chaiwat et al. (2016) also observed moisture reduction from 13.5% to 6.2% in cassava starch with NTP treatment from 30 to 180 min. It is noteworthy that this study took 10 min for the treatment. Lambert et al. (2009) found that corn starch with an initial moisture content of 8% had moisture levels close to 1% at the end of the process. Moisture is a critical parameter for this study; the reduction of moisture can increase protein-protein interactions and reduce the hydration capacity of VG (Özeren et al., 2021). The temperature and relative humidity data inside the sample holder during 0, 5, and 10 min of VG treatment is available in the *Supplementary Material.*

The pH of the treated VGs was slightly reduced with an increase in power and flow (i.e., from 6.06 in the control to 5.96 in P150V8). The change in pH in the matrix treated with NTP is closely related to the dynamics of plasma chemistry (Segat et al., 2015). When plasma is induced in a noble gas and has ions and electrons in an excited state (Evdokimov et al., 2017), it can induce changes in VG. Kim et al. (2011) reported non-significant changes in bacon pH after helium plasma treatment. The reduction in pH observed in previous studies (Dong et al., 2017a; Segat et al., 2015) was explained by the time of action of the active species generated by atmospheric air plasma in contact with the sample during treatment.

For example, based on their studies on the effects of plasma with atmospheric air on the physicochemical and structural properties of powdered zein (corn protein), Dong et al. (2017a) reported that 7 min of 75 V treatment, by indirect exposure, increased the concentration of free sulfhydryl groups. According to the authors, low pH values can be attributed to two mechanisms: the production of free radicals combined with the exposure of new SH groups. VG contains cysteine-rich proteins, such as gliadin fractions (α, β, and γ-gliadin), high (HMW-GS), and low molecular weight (LMW-GS) glutenin fractions (Wieser, 2007). These fractions can be linked together in the VG network by disulfide bonds. Thus, after treatment with NTP, exposure of cysteine residues present in the VG network can reduce the pH.

Lowering the pH can affect gluten formation by moving away from its isoelectric point (6.20) (Majzoobi and Abedi, 2014). However, the treated VGs swelled and absorbed a greater amount of water (Table 1), while the OAC showed no statistical difference between the samples.

Treated VGs increased water absorption capacity (WAC) 9.41–17.18% compared to the control. WAC is an essential parameter for VG as it relates to hydration, capacity to form a viscoelastic network, and yield of the final product (Ortolan and Steel, 2017). VG has a lower WAC than native gluten in wheat flour, typically 1.3–1.5 fold its dry weight, whereas native gluten has a water absorption that is 2.5–3 fold its dry weight (Ortolan and Steel, 2017). Such a finding indicates that the plasma treatment influenced the hydration properties of VG.

Table 1
Characterization of vital wheat gluten treated with non-thermal plasma.

Samples	Moisture (%)	pH	WAC (%)	OAC (%)
Control	10.16 ± 0.48^{a}	6.06 ± 0.01^{a}	222.6 ± 2.6^{c}	250.4 ± 10.4^{ns}
P50V3	3.15 ± 0.19^{b}	6.05 ± 0.01^{a}	241.5 ± 2.3^{b}	249.8 ± 9.6^{ns}
P50V5	3.21 ± 0.12^{b}	6.03 ± 0.00^{b}	239.8 ± 3.9^{b}	248.0 ± 7.8^{ns}
P50V8	2.09 ± 0.07^{c}	6.03 ± 0.01^{b}	244.0 ± 6.6^{b}	247.8 ± 6.9^{ns}
P100V3	3.69 ± 0.30^{b}	6.02 ± 0.00^{b}	243.6 ± 4.6^{b}	252.8 ± 5.2^{ns}
P100V5	3.69 ± 0.30^{b}	6.01 ± 0.01^{c}	257.4 ± 2.9^{a}	256.5 ± 7.3^{ns}
P100V8	2.67 ± 0.08^{b}	5.99 ± 0.01^{d}	250.7 ± 6.5^{a}	254.4 ± 5.0^{ns}
P150V3	3.54 ± 0.26^{b}	5.97 ± 0.01^{e}	239.8 ± 2.9^{b}	247.2 ± 11.6^{ns}
P150V5	2.43 ± 0.18^{b}	5.97 ± 0.01^{e}	240.8 ± 2.4^{b}	251.9 ± 3.9^{ns}
P150V8	2.44 ± 0.19^{c}	5.95 ± 0.01^{f}	220.9 ± 19.3^{c}	263.7 ± 13.8^{ns}

Means of two experiments (analyzed in triplicate) followed by standard deviations. Different lowercase letters in the same column indicate a significant difference according to the Scott–Knott test ($p \leq 0.05$). Control: Untreated VG; P50V3: 50 W and 3 L/min; P50V5: 50 W and 5 L/min; P50V8: 50 W and 8 L/min; P100V3: 100 W and 3 L/min; P100V5: 100 W and 5 L/min; P100V8: 100 W and 8 L/min; P150V3: 150 W and 3 L/min; P150V5: 150 W and 5 L/min; and P150V8: 150 W and 8 L/min. WAC: water absorption capacity; OAC: oil absorption capacity.

3.2. Gluten regeneration potential

The wet (WG) and dry (DG) gluten contents and the gluten index (GI) are important parameters for assessing the quality and quantity of gluten. Samples P50V5 and P50V8 had the highest contents of WG and GI. The WG indicates the hydration capacity of gluten and is mainly related to the quantity and quality of its proteins. The GI is directly proportional to gluten quality and the elasticity of the network formed. GI is also a parameter that determines whether gluten quality is poor (GI < 30), normal (GI $= 30$–80), or strong (GI > 80) (Cubadda et al., 1992) for breadmaking.

It is possible to notice that the moisture content of the treated VGs decreased and the WG increased for the P50V5 and P50V8 samples, while for the P50V3, P100V3, and P100V5 samples, the WG did not differ statistically from the Control. It was expected that the WG content would decrease with decreasing moisture content. Studies report that NTP can affect the physicochemical properties of polymeric proteins by causing the formation of new groups containing oxygen or polar radicals, which are produced by the bombardment of extremely energetic ions (Mollakhalili-Meybodi et al., 2021) and increase the hydrophilicity of the protein.

According to recent research, NTP treatment can reduce protein particle size and boost surface charge, increasing protein-water interaction. Dong et al. (2017b) and Jiang et al. (2020) report that highly reactive species released by NTP treatment can increase protein interaction with water via the appearance of –SH groups that would be trapped in the protein network; it is worth noting that VG is rich in cysteine; however, these –SH groups are also prone to recombination forming S–S bonds and, as a result, affect the solubility and mechanical properties of proteins, as evidenced by the low WG values in samples P100V8, P150V3, P150V5, and P150V8.

Of the treated samples, only P50V5 and P50V8 had GI values above 30, indicating normal strength. The other samples had GI values below 30, indicative of weak gluten. These results are interesting because an increase in VG quality can promote desirable changes in dough development, such as greater water absorption, directly affecting the amount of this ingredient added to wheat flour. Images of the wet VGs are displayed in Fig. 2. Again, changes in the aspect of the treated glutens can be observed. Herein, samples P50V5 and P100V5 had a compact and tenacious appearance, while samples treated at 150 W were more flaccid and extensible.

The water absorbed by the treated VGs can affect the viscoelasticity of the network formed, as can the changes to the protein structure. The rheological properties of the VG samples were assessed using the Kieffer extensibility test (Table 2). The Re (gluten resistance to extension) is an indicator of gluten strength. Samples P50V5, P50V8, P100V3, and P100V5 were found to have higher Re values (up to 19.14% higher than the control) and low extensibility (Ex) (up to 30.74% lower than the control), indicating a stronger, more elastic gluten network.

Samples P150V5 and P150V8 had high extensibility, which indicates a more fragile network, indicating that samples treated under these conditions may influence the stability of the dough during the mixing step, also compromising the fermentation and baking steps. Extensibility, provided by gliadins, indicates the ability to stretch without breaking and refers to dough expansion during fermentation, but must

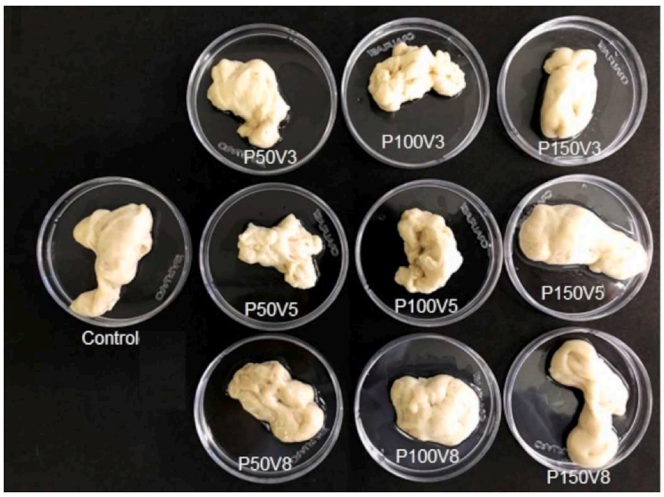

Fig. 2. Images of wet gluten after the Glutomatic test. Control: Untreated VG; P50V3: 50 W and 3 L/min; P50V5: 50 W and 5 L/min; P50V8: 50 W and 8 L/min; P100V3: 100 W and 3 L/min; P100V5: 100 W and 5 L/min; P100V8: 100 W and 8 L/min; P150V3: 150 W and 3 L/min; P150V5: 150 W and 5 L/min; and P150V8: 150 W and 8 L/min.

Table 2
Vital gluten regeneration potential.

Samples	Regeneration Capacity					
	WG (%)	DG (%)	GI (%)	Re (g)	Ex (cm)	SV (cm^3/g)
Control	55.6 ± 1.2^c	11.9 ± 0.4^a	29.4 ± 0.5^b	9.98 ± 0.81^c	10.3 ± 0.3^d	18.7 ± 0.3^b
P50V3	56.8 ± 1.3^c	11.7 ± 0.2^a	29.7 ± 0.4^b	10.03 ± 0.60^b	10.3 ± 0.3^d	18.9 ± 0.2^b
P50V5	75.0 ± 3.2^a	11.2 ± 0.5^b	34.5 ± 2.8^a	11.89 ± 0.75^a	7.1 ± 0.6^g	21.6 ± 0.5^a
P50V8	66.0 ± 3.8^b	11.6 ± 0.6^a	32.8 ± 0.4^a	11.52 ± 0.94^a	8.5 ± 0.6^f	21.2 ± 0.9^a
P100V3	56.1 ± 1.7^c	11.8 ± 0.2^a	29.3 ± 0.4^b	10.47 ± 0.43^b	9.4 ± 0.7^e	18.4 ± 0.6^b
P100V5	58.0 ± 0.6^c	11.3 ± 0.7^b	29.8 ± 0.7^b	10.49 ± 0.51^b	8.8 ± 0.6^f	20.8 ± 0.6^a
P100V8	35.0 ± 0.8^e	11.9 ± 0.3^a	28.1 ± 0.8^c	10.00 ± 0.40^c	12.4 ± 0.8^c	19.0 ± 0.8^b
P150V3	41.3 ± 2.3^d	11.1 ± 0.3^b	26.3 ± 0.6^c	9.25 ± 0.81^d	12.2 ± 0.7^c	17.6 ± 0.7^c
P150V5	33.3 ± 2.7^e	11.2 ± 0.9^b	26.4 ± 0.7^c	8.52 ± 0.58^e	14.5 ± 0.9^b	16.1 ± 0.7^d
P150V8	25.4 ± 1.3^f	10.9 ± 0.5^b	27.8 ± 0.4^c	6.67 ± 0.69^e	15.2 ± 0.8^a	15.3 ± 0.2^e

Means of two experiments (analyzed in triplicate) followed by standard deviations. Different lowercase letters in the same column indicate a significant difference according to the Scott–Knott test ($p \leq 0.05$). Control: Untreated VG; P50V3: 50 W and 3 L/min; P50V5: 50 W and 5 L/min; P50V8: 50 W and 8 L/min; P100V3: 100 W and 3 L/min; P100V5: 100 W and 5 L/min; P100V8: 100 W and 8 L/min; P150V3: 150 W and 3 L/min; P150V5: 150 W and 5 L/min; and P150V8: 150 W and 8 L/min. WG: wet gluten; DG: dry gluten; GI: gluten index; Re: gluten resistance to extension; Ex: gluten extensibility; SV: specific volume.

be balanced by elasticity, that is related to the capacity to retain CO_2 produced by yeast in breadmaking. P50V5 had the lowest extensibility (7.12 cm), while P150V8 had the highest extensibility (15.23 cm). Gluten denaturation can lead to low extensibility values (Atwell, 2001). High values of extensibility for VG could result in flours of low baking quality when enriched. The milder NTP treatment conditions led to desirable results. These results indicate that the effect of NTP on samples P50V5, P50V8, P100V3, and P100V5 could contribute to the strengthening of weak wheat flours.

Samples P50V5, P50V8, and P100V5 presented higher specific volumes (SVs) than the control in the expansion test (Fig. 3). The expansion test is a simple test that reflects the extent to which the gluten network structure supports biaxial expansion when there is an increase in the internal pressure of the gluten ball caused by the expansion of gases and water vapor in the oven (Ortolan et al., 2018). The greater the volume of the baked gluten ball, the greater the pressure it supports, result of a protein network of better viscoelastic quality (Ortolan et al., 2018). As shown in Fig. 3, sample P50V5 had the highest volume, indicating the ideal condition for treating VG with NTP.

The control gluten ball presented a thinner structure and greater sensitivity to touch (i.e. was more fragile); P50V5 presented a greater volume and a firmer structure than the control, while P150V8 presented a smaller volume and a rigid structure. These results indicate that NTP provided VG with better quality for baking when 50 W and 5 L/min were employed.

An enhanced WG was seen under mild NTP treatment circumstances, which reduced as treatment power increased. It appears that the partial unfolding at the beginning enhanced the exposure of the protein structure for improved interaction with water. But the increase in treatment power (150 W) showed that the proteins re-aggregated, possibly due to cross-linking by carboxyl and sulfhydryl groups (Dong et al., 2017b).

3.3. *Rheological properties of weak wheat flour + vital wheat gluten (VG) mixes*

Empirical rheology is the most effective approach to verify the effects of improvers on the performance of flour mixtures enriched with these ingredients and, thus, predict their performance in baking. The farinographic and extensographic analyses results are presented in Table 3.

The control sample showed a water absorption of 57%, development time (DT) of 5.32 min, stability (S) of 7.77 min, and mixing tolerance index (MTI) of 41.33 BU. These values indicate that the initial wheat flour used to add VG did not present ideal characteristics for bread production, justifying its enrichment. According to Atwell (2001), flour with good baking characteristics has high water absorption (>58%), high development times (>5 min), and high stability (>10 min). The samples of flours enriched with VGs had higher water absorption, with an average increase of 1.74%, except for sample P150V8. The DT reached 6.25 min for P50V5, indicating a delay in dough formation, while S only increased in samples P50V5 (8.48 min) and P100V5 (8.12 min). The MTI only increased significantly when high power and flow were used in the treatment of VG, showing a decrease in mixing tolerance for these samples. The increase is shown in WA, DT, and S by samples P50V5, P50V8, and P100V5 may represent better conditions for the reconstitution of VG to be applied in wheat flour.

The extensographic analysis results (Table 3) show that the resistance to extension (R) increased for P50V5, P50V8, and P100V5. Extensibility (E) was not significantly affected by the addition of VGs,

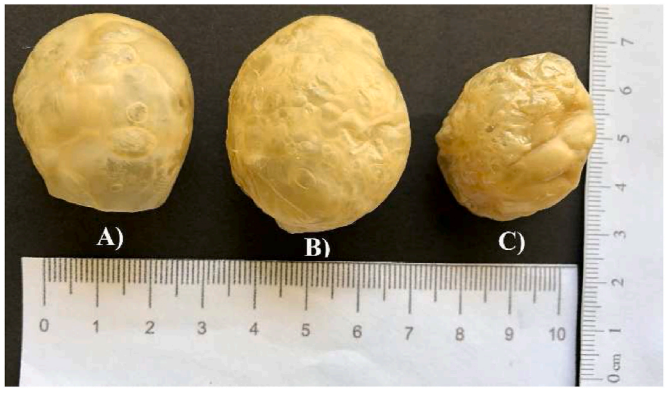

Fig. 3. Images of vital wheat gluten balls after the expansion test. A) Control: Untreated VG; B) P50V5: 50 W and 5 L/min; C) P150V8: 150 W and 8 L/min.

Table 3
Rheological parameters of weak wheat flour + vital wheat gluten (VG) mixes.

Samples	Farinograph			Extensograph			
	WA (%)	DT (min)	S (min)	MTI (BU)	R (BU)	E (mm)	R/E (BU/mm)
Control	57.0 ± 0.0^{c}	5.3 ± 0.3^{c}	7.8 ± 0.3^{b}	41 ± 4^{c}	457.3 ± 24.6^{c}	148.7 ± 4.3^{b}	3.1 ± 0.2^{b}
P50V3	57.6 ± 0.5^{b}	5.5 ± 0.3^{c}	8.1 ± 0.5^{a}	46 ± 3^{c}	443.2 ± 18.1^{d}	141.7 ± 2.8^{b}	3.1 ± 0.2^{b}
P50V5	58.0 ± 0.4^{a}	6.3 ± 0.1^{a}	8.5 ± 0.6^{a}	42 ± 6^{c}	551.3 ± 14.2^{a}	146.3 ± 9.3^{b}	3.9 ± 0.5^{a}
P50V8	58.1 ± 0.2^{a}	5.8 ± 0.5^{b}	7.7 ± 0.4^{b}	45 ± 2	545.5 ± 21.8^{a}	147.3 ± 7.2^{b}	3.7 ± 0.1^{a}
P100V3	58.2 ± 0.2^{a}	5.5 ± 0.3^{c}	7.8 ± 0.2^{b}	45 ± 3^{c}	488.7 ± 6.2^{b}	155.2 ± 9.2^{a}	3.2 ± 0.2^{b}
P100V5	58.1 ± 0.1^{a}	6.1 ± 0.1^{a}	8.1 ± 0.3^{a}	51 ± 2^{b}	542.3 ± 23.3^{a}	147.2 ± 5.9^{b}	3.7 ± 0.2^{a}
P100V8	58.1 ± 0.1^{a}	5.4 ± 0.3^{c}	7.7 ± 0.4^{b}	51 ± 3^{b}	472.0 ± 14.9^{c}	150.7 ± 6.7^{b}	3.1 ± 0.1^{b}
P150V3	58.0 ± 0.1^{a}	5.2 ± 0.2^{c}	7.0 ± 0.3^{c}	46 ± 4^{c}	488.3 ± 16.9^{b}	149.5 ± 3.1^{b}	3.3 ± 0.1^{b}
P150V5	58.0 ± 0.0^{a}	5.2 ± 0.1^{c}	6.7 ± 0.1^{c}	50 ± 2^{b}	425.7 ± 19.4^{d}	155.7 ± 5.4^{a}	2.7 ± 0.1^{c}
P150V8	57.1 ± 0.2^{c}	4.1 ± 0.2^{d}	4.6 ± 0.3^{d}	56 ± 3^{a}	391.7 ± 19.1^{e}	162.2 ± 1.9^{a}	2.4 ± 0.1^{d}

Means of two experiments (analyzed in triplicate) followed by standard deviations. Different lowercase letters in the same column indicate a significant difference according to the Scott–Knott test ($p \leq 0.05$). Control: Untreated VG; P50V3: 50 W and 3 L/min; P50V5: 50 W and 5 L/min; P50V8: 50 W and 8 L/min; P100V3: 100 W and 3 L/min; P100V5: 100 W and 5 L/min; P100V8: 100 W and 8 L/min; P150V3: 150 W and 3 L/min; P150V5: 150 W and 5 L/min; and P150V8: 150 W and 8 L/min. WA: water absorption, DT: development time, S: stability, MTI: mixing tolerance index, R: dough resistance to extension, E: dough extensibility, and R/E: resistance to extension and extensibility ratio; BU: Brabender Units.

except for P100V3, P150V5, and P150V8 which increased it, reaching 162.17 mm. These results are interesting because the extensibility of the dough in the extensograph did not corroborate with those of gluten (Table 2) obtained using the texture analyzer. When incorporating VG in wheat flour, different mixing behaviors can be obtained, as the level of denaturation of VG interferes with interactions with native gluten and other components of the flour. Gluten is a mechanically denatured network, and its incorporation into flour can reduce interactions with other constituents, which would not occur with native gluten, thereby providing non-expressive results. The farinographic and extensographic analyses showed that NTP treatment at low power and a flow rate of 5 L/min improved gluten strength, while high power and flow rate reduced its strength.

Similar results were found by Misra et al. (2015), who reported that atmospheric air plasma promoted a greater amount of disulfide bonds between the gluten protein subunits due to oxidation promoted by the plasma. Thus, wheat flour with higher DT and resistance (strength) was obtained. Bahrami et al. (2016) reported that atmospheric air plasma induced the formation of protein aggregates in wheat flour, leading to the formation of high molecular weight proteins, which provided stronger doughs. These studies used atmospheric air for plasma production to treat wheat flour. The plasma chemistry of each gas is unique, and highly oxidizing species, such as ROS and RNS, in atmospheric air should not be compared with the ions and electrons of argon, as the mechanism of action of each gas may not be the same. When Held et al. (2019) used NTP generated by radiofrequency at 120 W with argon and carbon dioxide, they did not observe any change in the content and solubility of gluten proteins. Accordingly, the researchers reported that starch is responsible for changes in the rheological properties of treated wheat flour.

Initial studies have already been carried out using the argon plasma proposed in this study. Barros et al. (2019) evaluated the effect of NTP on commercial vital gluten, aiming to obtain a better quality ingredient. The VG samples were added to weak wheat flour (8% protein content and $W = 239.69 \times 10^{-4}$ J) until a 12% protein concentration was obtained. The treated VG provided greater water absorption and dough strength (W), characteristics that can be considered positive for breadmaking. Barros and Steel (2020) evaluated the effect of NTP on VG by varying the argon gas flow (5 and 8 L/min) and power (50, 100, and 150 W). Treatments with a gas flow of 5 L/min and up to 100 W of power yielded VG with greater water absorption and higher WG, indicating greater ability to form a high quality viscoelastic network.

In this study, the NTP-induced alterations under ideal conditions on extracted gluten were found to promote more hydrated and cohesive gluten-reconstituted networks. Furthermore, rheological analysis of weak wheat flour enriched with these treated VGs showed the strengthening of the dough. However, on one side, more studies on the effects of NTP on VG structure are needed to understand the effects on functionality better. And on the other, the production of bread with treated VG-flour mixes is also necessary to verify if the impact of NTP observed on VG quality and dough are also observed after bread processing.

4. Conclusion

This study evaluated the effect of NTP application on the functionality of vital wheat gluten (VG). The results revealed improvements in the hydration and cohesion properties of VG when 50 W and 5 L/min were employed, and improvements in weak wheat flours enriched with VG treated under these conditions. Thus, NTP technology can be used to improve the technological properties of VGs. Under optimal conditions, VG can be used as an ingredient in various formulations of wheat derived products that require stronger flours. Overall, the results obtained herein are promising. However, further studies on the mechanism of action of non-thermal argon plasma on gluten structure and the baking performance of these treated VGs are necessary.

Author contributions

Jefferson Henrique Tiago Barros: Conceptualization, Investigation, Formal analysis, Writing - original draft/review & editing, Visualization; Flávio Martins Montenegro: Writing - review & editing, Visualization; Caroline Joy Steel: Conceptualization, Supervision, Funding acquisition, Writing - review & editing, Visualization.

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Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

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