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Effect of atmospheric cold plasma on the functional properties of whole wheat (*Triticum aestivum* L.) grain and wheat flour

Sonal Chaple^{a,b}, Chaitanya Sarangapani^b, John Jones^c, Edwin Carey^c, Lucie Causeret^d, Annaik Genson^d, Brendan Duffy^d, Paula Bourke^{a,b,e,*}

^a School of Biosystems and Food Engineering, University College Dublin, Belfield, Dublin 4, Ireland

^b Environmental Sustainability and Health Institute, Technological University Dublin, Ireland

^c School of Science and Computing, Tallaght Campus, Technological University Dublin, Ireland

^d Crest Technology Gateway, Technological University Dublin, Ireland

^e Institute for Global Food Security, School of Biological Sciences, Queens University Belfast, UK

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ABSTRACT

Atmospheric cold plasma (ACP) has emerged as a novel processing technology, with demonstrated efficiencies in microbial inactivation. However, studies on the effects of ACP and potential to modify the functional properties of foods are sparse. The objective of this study is to determine the effect of ACP on physico-chemical and functional properties of wheat flour. In this study, both whole wheat grains and wheat flour were subjected to a dielectric barrier discharge (DBD) contained plasma reactor for a range of treatment times (5–30 min) at 80 kV. Plasma treatment increased the flour hydration properties of wheat flour. Rapid visco-analyser results showed an increase in pasting and the final viscosities of wheat flour. The decrease in both endothermic enthalpies and crystallinity was attributed to the depolymerization of starch and plasma-induced changes. Overall DBD-ACP treatment can be tailored to develop a plasma process with potential to improve functionality of wheat flour.

1. Introduction

Wheat is a major dietary component worldwide. Wheat is mostly converted into flour and is consumed in the form of bread, biscuits, pasta products and other flour-based products. Processed wheat and bakery products are widely consumed, and the baking industry is dependent on the properties of flour. Several oxidising agents and enzymes are added to the flour which acts as a flour improver, dough conditioner or bleaching agents. Some FDA approved food additives such as potassium bromate-E924 (Li, Tsiami, & Schofield, 2000); (Nakamura et al., 2004)), chlorine oxide-E926 and chlorine-E925 (Joye, Lagrain, & Delcour, 2009), azodicarbonamide-E927 (Becalski, Lau, Lewis, & Seaman, 2004), ascorbic acid-E300 (Li et al., 2000; Miličević, Selimović, Oručević, Hadžić, & Ačkar, 2011) and glucose oxidase-E1102 (P. A. Caballero, Gómez, & Rosell, 2007; Dunnewind & Vliet, 2002; Rasiah, Sutton, Low, Lin, & Gerrard, 2005; Rosell, Wang, Aja, Bean, & Lookhart, 2003) are often used to improve flour functionality. Some disadvantages of chemical oxidising agents include the potential to incorporate toxicological effects in the food chain, whilst enzymes are expensive and can modify structures. These modifications also lead to the incorporation of

label amendments, e.g. bleached flour or bromated flour. Various non-thermal technologies have been investigated to improve the quality of bread flour. High-pressure processing (HPP) improved the functionality of wheat flour; however, increases in dough hardness, adverse changes to the protein structures of dough were associated with pressure above 50 MPa (Bárceñas, Altamirano-Fortoul, & Rosell, 2010). With the application of ohmic heating, bread exhibited better properties in specific volume, relative elasticity and porosity. Gamma radiation increased amylose fractions, oxidation of starch and decreased the intrinsic viscosity, which in-turn affected the final product quality (Abd Allah, Foda, & El Saadany, 1974).

There is a need for a novel technology that could improve rheological and functional properties of wheat flour without affecting nutritional quality. Recently cold plasma has emerged as a novel food processing technology. Plasma, as the fourth state of matter, is primarily composed of photons, ions and free electrons as well as atoms in their fundamental or excited states with a net neutral charge (S. Pankaj, Wan, Colonna, & Keener, 2017). Dielectric barrier discharge (DBD) is a widely investigated ACP approach because of its safety, flexibility in the use of atmospheric air for discharge generation and low energy input

* Corresponding author at: School of Biosystems Engineering, UCD, Ireland.
E-mail address: paula.bourke@ucd.ie (P. Bourke).

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(Sarangapani et al., 2016). Cold plasma can generate reactive species such as molecular oxygen and ozone, which are also the most common and universal oxidising agent used for conditioning wheat flour, as it is one of the strongest oxidising agents with a zero residue effect (Joye et al., 2009).

Several studies have reported on the efficacy of cold plasma for enhanced seed germination, microbial inactivation and shelf-life extension of fresh produce (Dobrin, Magureanu, Mandache, & Ionita, 2015; Rifna et al., 2019; Jiafeng et al., 2014; Ling et al., 2014; Sera, Spatenka, Serý, Vrchotova, & Hrusková, 2010; Volin, Denes, Young, & Park, 2000; Ziuzina, Patil, Cullen, Keener, & Bourke, 2013; Misra et al., 2014). Our previous studies on wheat grains and flour exploited application of cold plasma technology as a medium for cereal grain decontamination, enhancing seed germination and pesticide degradation (Los et al., 2018; Los, Ziuzina, Boehm, Cullen, & Bourke, 2017; Los, Ziuzina, Boehm, Cullen, & Bourke, 2019; Sarangapani, Misra, et al., 2016). Los et al., 2019 observed a decrease in the water contact angle of wheat grain for direct treatment indicating an increase in water permeability of grain, the morphological analysis suggested no impact of plasma on the external surface of the grain. Sarangapani, Misra, et al., 2016 found that DBD plasma can effectively degrade commonly used agricultural pesticides like dichlorvos, malathion, endosulfan. Hence, it can help in mitigating the chemical residues present in the crop after the degradation of pesticides.

Few holistic studies have been reported on the functionality of wheat flour. Misra et al. (2015) found that both strong and weak wheat flour categories were effected by contained ACP-DBD treatment at 60 and 70 kV for 5 and 10 min. The author observed improvement in dough strength and optimum mixing time due to formation of disulphide linkages, increase in viscoelastic properties of strong flour, improvement of the secondary protein structure of weak wheat flour. However, Held et al. (2019) observed no change in protein content and solubility when both soft and hard wheat flour was treated by radiofrequency-generated cold plasma operated at 120 W with argon and carbon dioxide. Menkovska, Mangova, and Dimitrov (2014) investigated the effect of cold plasma on the bread making quality of flour without the use of additives, and reported some enhanced flour properties when treated in counter flow exchange (concentration of 1000 ppm at 2.5 l/min) for 30 and 40 min. The current study compares the effects of cold plasma treatment when applied to wheat either in the form of whole wheat grains or in pre-ground wheat flour, to ascertain the impacts on the functional properties at different product stages. Hence, we investigate the effect of cold plasma on the interaction of water with starch, protein, thermal properties, crystalline structure and apparent viscosity of plasma treated wheat grain and plasma treated flour.

2. Materials and methods

2.1. Materials

Wheat grains (*Triticum aestivum*) were purchased from a local retailer and were of Turkish origin. All experimental chemicals were procured from Merck, Ireland. All chemicals and reagents used were of analytical

grade.

2.2. Plasma treatment of wheat grain and wheat flour

2.2.1. Preparation of samples

Whole wheat grains were crushed with a blender into a wheat flour, which was then treated with ACP, and this sample is termed Plasma Treated Flour (PTF) as shown in Fig. 1. Henceforth, plasma treated flour for 5, 10, 20 and 30 mins will be termed as PTF-5, PTF-10, PTF-20 and PTF-30 respectively. Whole wheat grains were also treated with ACP, and then grounded post plasma treatment for analysis. The flour resulting from grinding the plasma treated whole grains was termed as Plasma Treated Wheat Grain Flour (PTWGF) as shown in Fig. 1. Plasma treated whole wheat grain ground to flour treated for 5, 10, 20 and 30 mins will be termed as PTWGF-5, PTWGF-10, PTWGF-20, PTWGF-30 respectively. All treated and control samples were passed through BS No.250 mesh sieve (British Sieve Standards) to obtain a uniform particle size. All samples were stored in air-tight containers until further analysis.

2.2.2. Treatment of samples

A high voltage atmospheric air plasma reactor, based on a dielectric barrier discharge (DBD) design was used in this study and described previously by Sarangapani et al., 2017 Briefly, plasma is generated between two circular aluminium plate electrodes (outer diameter = 158 mm) using two thick acrylic dielectric layers (Fig. 2). The applied voltage to the electrode was provided by a step-up transformer (Phenix Technologies, Inc., MD, USA) with input of 230 V, 50 Hz from the mains supply. Five grams of each sample was weighed separately in a petri dish and placed in polypropylene boxes which were then sealed within a packaging film (Cryovac, Ireland). All samples were subjected to plasma treatment using an output discharge voltage of 80 kV, with treatment duration (0–30 min) as the variable process parameter. The treated samples were stored at room temperature for 24 h post treatment storage time (Ziuzina et al., 2013).

2.3. Flour hydration properties

The water holding capacity (WHC) is the amount of water retained by a sample when not exposed to any stress. The WHC of samples was done according to the method described by Jadhav and Annapure (2013). Wheat flour (1.0 ± 0.05 g) was mixed with deionized water (10 ml) and kept at room temperature for 24 h. After 24 h, the supernatant was decanted, and the sample was weighed again. WHC was expressed as grams of water retained per gram of solid.

The water binding capacity (WBC) is the amount of water retained by a sample post centrifugation, and was determined as described in Quinton and Kennedy (2002). Samples (1.0 ± 0.05 g) were mixed with deionized water (10 ml) and centrifuged at 2000 g for 10 min. WBC was expressed as grams of water retained per gram of solid.

Oil holding capacity (OHC), was determined following the method of de Hera, Gomez, and Rosell (2013), with slight modifications. Samples (100.0 ± 0.2 mg) was mixed with 1.0 ml of vegetable oil. The mixture

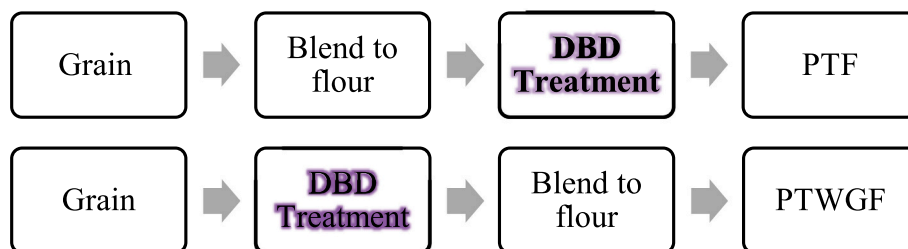


Fig. 1. Sample preparation to generate either Plasma Treated Flour (PTF) or Plasma Treated Whole Grain Flour (PTWGF).

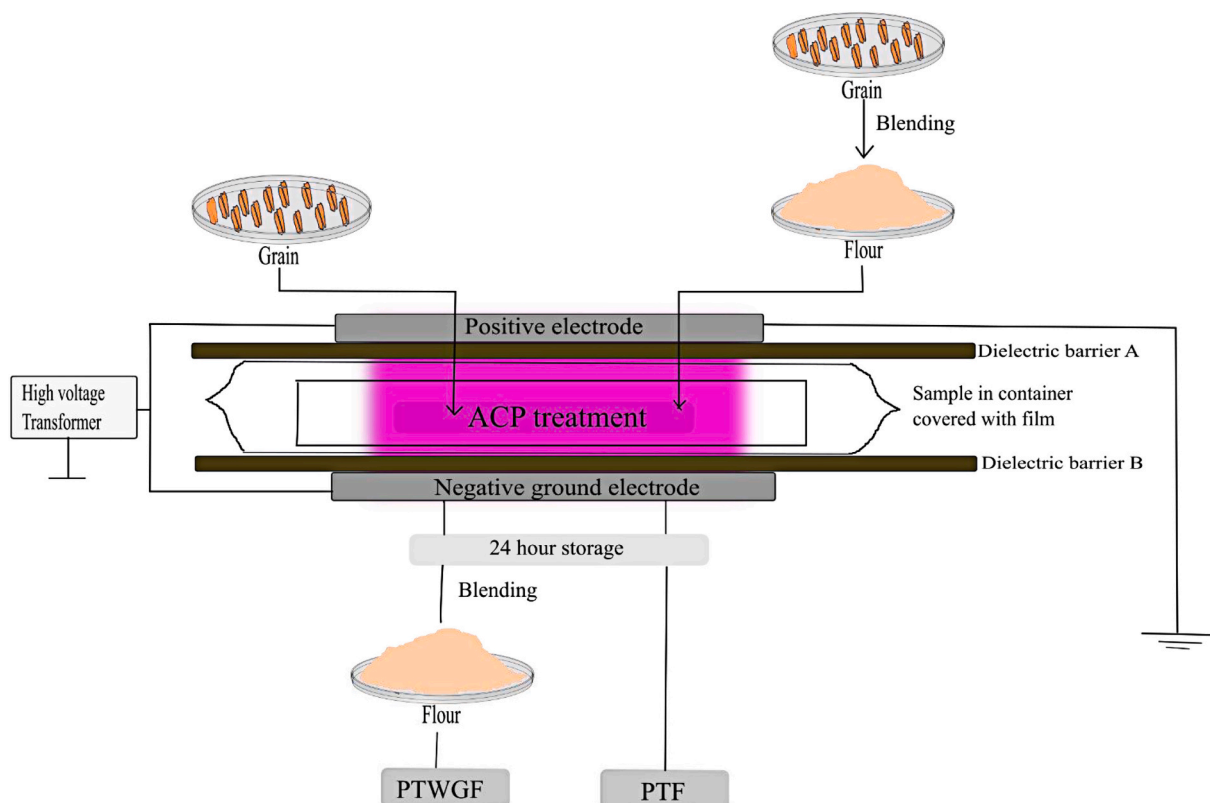


Fig. 2. Schematic of the experimental set-up for generation of plasma using DBD contained reactor.

was stirred for 1 min with a wire rod to disperse the sample in vegetable oil. After 30 min in the vortex mixer, tubes were centrifuged at 3000g and 4 °C for 10 min. The supernatant was removed with a pipette, tubes were inverted for 30 min to drain the oil, and the residue weighed (W_r). The oil absorption capacity was expressed as grams of oil bound per gram of the sample on dry basis. OHC was calculated by Eq. (1).

$$\text{OHC}(\text{g/g}) = \frac{W_r}{W_i} \quad (1)$$

where, W_i was the sample weight (g).

2.4. Gel hydration properties

Water absorption index (WAI) or swelling capacity and water solubility index (WSI) of wheat flour were determined following the method of Toyokawa, Rubenthaler, Powers, and Schanus (1989). Briefly, flour (50.0 mg \pm 0.1 mg) sample was dispersed in 1.0 ml of distilled water in an Eppendorf tube using a wire rod and cooked at 90 °C for 10 min in a water bath.

The cooked paste was cooled in an ice water bath for 10 min and then centrifuged at 3000g at 4 °C for 10 min. The supernatant was decanted into an evaporating dish and the weight of dry solids was recovered by evaporating the supernatant at 105 °C until constant weight was reached. Four replicates were made for each sample. Residues (W_r) and dried supernatants (W_s) were weighed and WSI or swelling capacity, solubility index and swelling power (SP) were calculated as follows.

$$\text{WAI}(\text{g/g}) = \frac{W_r}{W_i} \quad (2)$$

$$\text{WSI}(\text{g}/100\text{mg}) = \frac{W_s}{W_i} \times 100 \quad (3)$$

$$\text{SP}(\text{g/g}) = \frac{W_r}{W_i - W_s} \quad (4)$$

where W_i was the sample weight (g).

2.5. Instrumental color

Color was measured using CIELAB hunter lab colorimeter (Hunter, 1958) at room temperature (21 \pm 1 °C). The observation angle was 10°, equal to the perception of a human observer, and illuminant D65 was used (daylight source), following the CIE recommendations. For calibration, a white (X-78.8, Y-83.5, Z-89.8) and black reference standard tile was used. A 2.5-in. glass sample measuring cup was filled with flour or grain for color measurement. Color parameters obtained were L^* for lightness from black (0) to white (100), a^* from green (–) to red (+) and b^* from blue (–) to yellow (+). The whiteness index of flour was calculated by Eq. 5 (Lin, Liu, Yu, Lin, & Mau, 2009).

$$\text{Whiteness Index} = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (5)$$

2.6. Differential scanning calorimetry (DSC)

Thermal analysis was carried out using a Differential Scanning Calorimeter Q2000 TA Instruments (England, United Kingdom), calibrated with Indium as a standard. Wheat flour (6 \pm 0.1 mg) was weighed in Tzero Aluminium Hermetic pans and 10 ml of deionized water was added. The pans were sealed with Tzero Aluminium Hermetic lids. An empty Tzero pan with lid was run as a reference with each sample. The reference and sample pan were heated from 20 °C to 100 °C with a temperature ramp of 10 °C per minute. TA Universal Analysis software was used for analysis of the thermograph. The thermograph includes onset temperature (T_o), as the intersection point of baseline and curvature tangent at the start of the crystallization/melting peak. This gives the beginning of melting or crystallization peak for flour. Peak temperature (T_p) depicts the point at which the largest deviation from the virtual baseline of the heat flow signal is measured and it gives

temperature at the maximum/minimum of thermal change. The offset temperature (T_c) marks the intersection point of the baseline and curvature tangent at the end of the melting/crystallization peak. The enthalpy of the thermogram was estimated in terms of (J/g), depending upon the amount of heat supplied or withdrawn from the sample.

2.7. Fourier transfer infrared analysis (FTIR)

FTIR spectroscopy was performed using Spectrum GX FT-IR (Perkin Elmer, Dublin, Ireland) with an attenuated total reflectance (ATR). The samples were measured in the wavelengths range of 4000 to 400 cm^{-1} with 64 scans per sample. The background was collected before each measurement.

2.8. X-ray diffraction (XRD)

Diffraction patterns were obtained with an X-ray powder diffraction (XRD) Siemens D500 diffractometer. The X-ray generator was a Kristalloflex model operated at 40 kV and 40 mA, and the scanning angle was $2\theta = 4^\circ$ to 45° with a scanning rate of 0.5/min. Crystallinity (%) was defined as the percentage ratio of diffraction peak area to total diffraction area (Sarangapani, Misra, et al., 2016).

2.9. Rapid visco analysis (RVA)

The pasting properties of the flour were studied using a Rapid Visco Analyser (RVA, Newport Scientific, Warriewood, Australia) following the RVA General Pasting Method (Newport Scientific Pty. Ltd., 1998).

2.10. Statistical analysis

Statistical analysis was performed using SPSS software (IBM statistical analysis Version 25.0) and the results were analysed using one-way ANOVA. The significance among the samples was compared at $p \leq 0.05$ by Duncan's multiple range test post-hoc comparison. All the tests were performed in triplicate and the average of the tests are represented.

3. Results and discussion

3.1. Flour hydration properties

The effect of plasma treatment on flour hydration properties are shown in Fig. 3. There was a significant difference ($p < 0.05$) in flour hydration properties between control and cold plasma treated flours. The oil holding capacity (OHC) of PTF increased with treatment time;

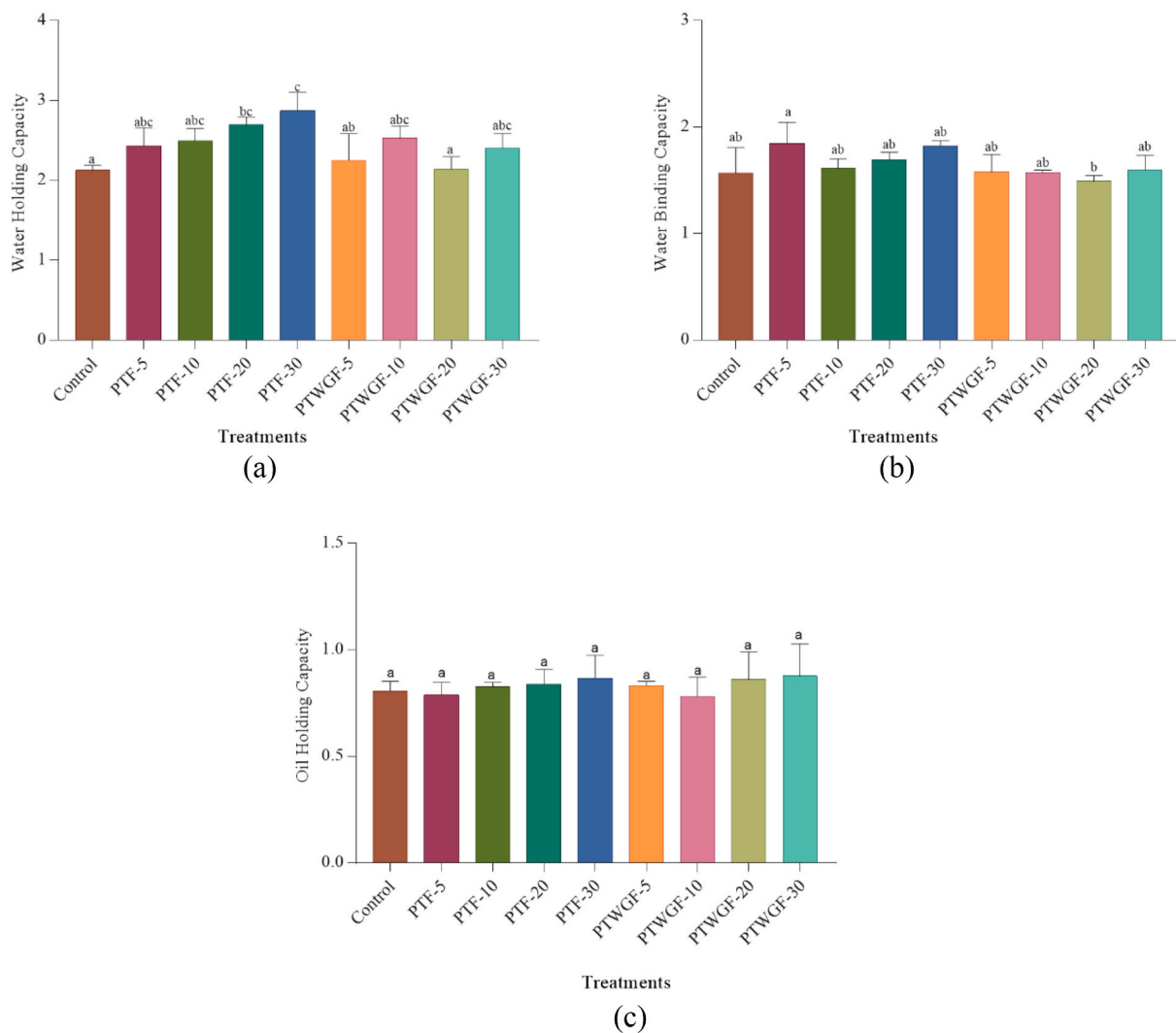


Fig. 3. Effect of plasma treatment on flour hydration properties of wheat flour and grain (a) water binding property (b) water holding capacity (c) oil absorption capacity where, control is untreated flour; PTF-30, PTF-20, PTF-10, PTF-5 are flour treated for 30, 20, 10 and 5 min respectively; PTWGF-30, PTWGF-20, PTWGF-10, PTWGF-5 are grain treated for 30, 20, 10 and 5 min respectively. All column bars are expressed as mean and error bars stating \pm standard deviations. Means with different superscript letters differ significantly ($p < 0.05$).

OHC of control and PTF-30 are 0.807 ± 0.036 g/g and 0.86 ± 0.08 g/g respectively. However, the OHC for plasma treated wheat grains flour (PTWGF) was unaffected by treatment up to 30 min. Zayas (2012) reported that the protein content, the amount of nonpolar amino acids, and the bulk density of the protein powder influenced the OHC of wheat flour. The insignificant changes in OHC may indicate that there are insignificant modifications in the protein structures of wheat flour and grain due to plasma treatment. Water Holding Capacity (WHC) indicates the ability of a molecule to absorb and retain water against gravity and includes bound water, hydrodynamic water, capillary water, and physically entrapped water (Damodaran, 2017). As shown in Fig. 3.a, the WHC of all PTF samples increased with treatment time. A significant difference ($p < 0.05$) was found between control (2.07 ± 0.04 g/g) and PTF-30 (2.87 ± 0.16 g/g). According to Caballero, Trugo, and Finglas (2003) an increase in the WHC of flour can be due to the hydrolytic depolymerisation of starch.

The water binding capacity (WBC) of PTF increased with plasma treatment (Fig. 3.b). The WBC of control sample was 1.56 ± 0.16 g/g, rising to 1.82 ± 0.03 g/g for PTF-30. However, no significant change was found for WBC of PTWGF-30, which was 1.59 ± 0.09 g/g. In contrast, an increase in the WHC has been reported for DBD-ACP setup

on myofibrillar protein isolated from Alaska Pollock for higher voltage (50 kV and 60 kV) and low pressure plasma treated rice flour treated at 30 and 40 W for 5 and 10 min (Miao et al., 2019; Sarangapani et al., 2016). According to Los et al. (2019), DBD-ACP treatment at 80 kV decreased the water contact angle with direct treatment, leading to enhanced water permeability and a grain with increased affinity towards water. In our study, the flour hydration properties of PTF were higher than PTWGF, which indicates that the increased sample surface area exposed to the initial plasma treatment influences the hydration properties of the flours, which can be considered in process design for functionality requirement.

3.2. Gel hydration properties

The gel hydration properties are governed by water absorption and water solubility. Fig. 4 illustrates the effect of plasma treatment on the gel hydration properties of wheat flour and grain. The Water Absorption Index (WAI) of control, PTF-30 and PTWGF-30 was 6.28 ± 0.13 g/g, 6.23 ± 0.11 g/g and 5.73 ± 0.27 g/g respectively. The Water Solubility Index (WSI) of control, PTF-30 and PTWGF-30 was 3.20 ± 0.21 g/100 g, 2.78 ± 0.24 g/100 g, 3.63 ± 0.11 g/100 g respectively. As it can be

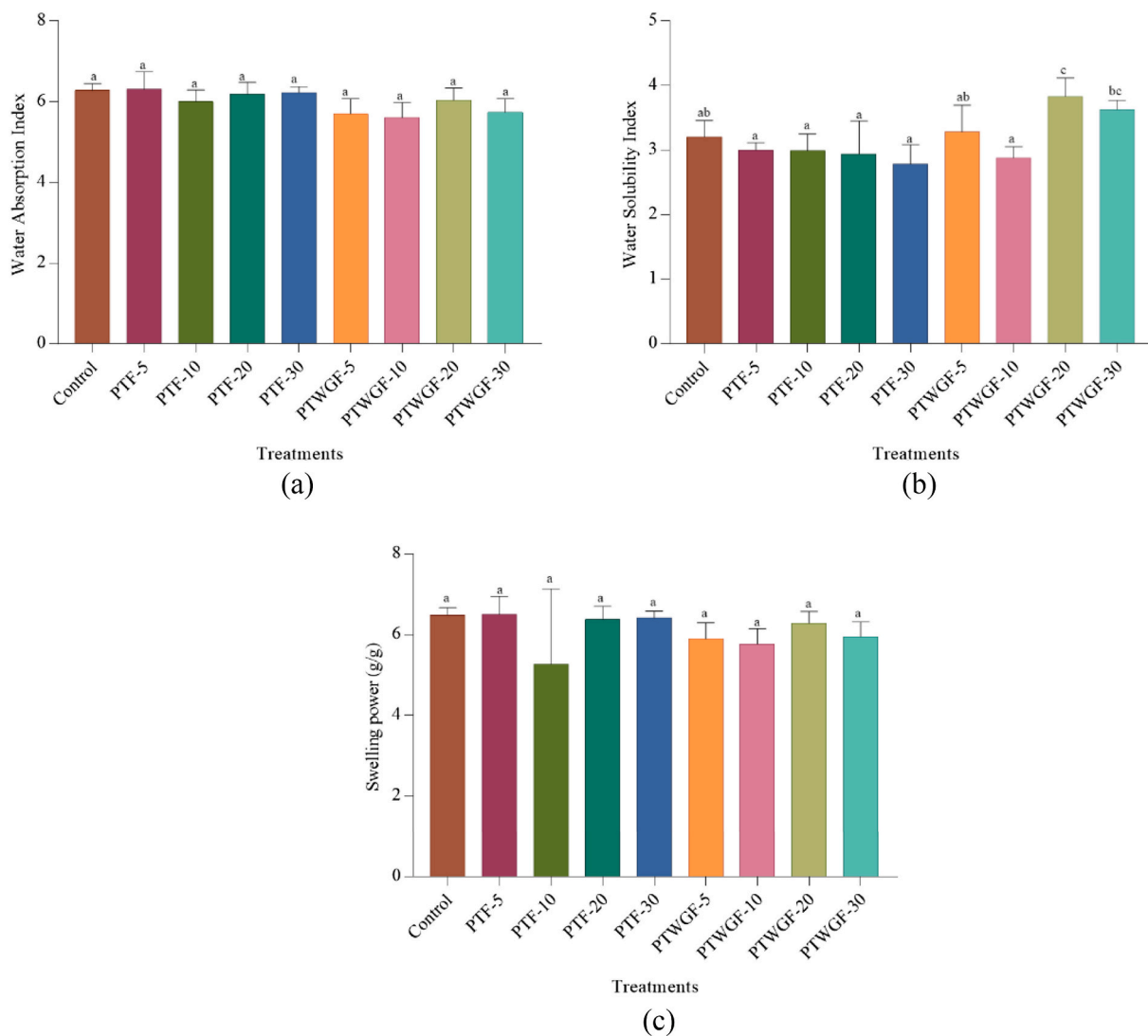


Fig. 4. Effect of plasma treatment on gel hydration properties of wheat flour and grain (a) Water absorption Index(g/g) (b) water solubility index(g/100 g) (c) swelling power(g/g) where, control is untreated flour; PTF-30, PTF-20, PTF-10, PTF-5 are flour treated for 30, 20, 10 and 5 min respectively; PTWGF-30, PTWGF-20, PTWGF-10, PTWGF-5 are grain treated for 30, 20, 10 and 5 min respectively. All column bars are expressed as mean and error bars stating \pm standard deviation. Means with different superscript letters differ significantly ($p < 0.05$).

observed that the WSI of flour decreased with increasing treatment time. The swelling power (SP) of control, PTF-30, PTWGF-30 was calculated as 6.49 ± 0.15 g/g, 6.41 ± 0.13 g/g and 5.95 ± 0.29 g/g respectively. There was no significant difference ($p < 0.05$) in gel hydration properties, whether samples were treated as flour or as grains when compared to control. Gel hydration properties indicates water intake during thermal treatment affecting starch gelatinization. Gelatinization is initiated when starch is heated up to 80°C with excess water. It causes the amylopectin to lose its crystalline structure and amylose to leach out of starch granules (Svihus, Uhlen, & Harstad, 2005). Previous studies showed an increase in gel hydration properties of rice flour when subjected to radiofrequency air plasma at two different power levels (40 and 60 W) (de Hera et al., 2013; Thirundas, Trimukhe, Deshmukh, & Annapure, 2017). However, wheat has a more complex structure of protein and carbohydrate containing different layers of outer pericarp, inner pericarp, aleurone layer and endosperm compared to one endosperm layer in white rice. The accessibility of starch can also have a major impact on the swelling power (Yadav et al., 2006). Previously, extracted starch had greater gel hydration properties due to the accessibility by comparison with native starch (Sandhu, Manthey, & Simsek, 2012). Food matrix components may play an important role, for example, wheat components such as protein and fat form a lipid-protein complex, which may inhibit plasma active species interactions with starch. Furthermore, protein decreases the availability of water for starch to form a gel and lipids can form amylose-lipid complexes which may inhibit changes in gel hydration properties. Variations in values for different treatment time (Fig. 4) could be due to presence of other non-starch components, which may have hindered the absorption of water. The gel hydration properties were not affected by ACP; however, flour hydration properties were significantly changed. These difference in behaviour between gel hydration and flour hydration properties was attributed to changes in the sample composition during treatment, as the plasma active species such as ozone and hydroxyl radicals may not diffuse through grains internal surface, but rather induce surface related chemical changes (Los et al., 2019). The microstructure characteristics of wheat grain (external, internal and extracted starch) were not damaged when exposed to ozone ($40\ \mu\text{mol/mol}$ and $60\ \mu\text{mol/mol}$) until 180 min (Savi, Piacentini, Bittencourt, & Scussel, 2014).

Table 1
L*, a*, b* and Whiteness Index (WI) for Control, flour and grain sample.

Samples	L*	a*	b*	Whiteness Index (WI)
Control	93.59 ± 0.01^a	1.70 ± 0.00^a	11.54 ± 0.00^a	86.70 ± 0.003^a
PTF5	93.72 ± 0.06^b	1.52 ± 0.07^b	10.75 ± 0.10^b	87.46 ± 0.075^b
PTF10	94.24 ± 0.02^c	1.54 ± 0.04^b	10.87 ± 0.06^b	87.60 ± 0.057^c
PTF20	94.26 ± 0.02^d	1.30 ± 0.03^c	10.09 ± 0.06^c	88.31 ± 0.045^d
PTF30	94.23 ± 0.04^e	1.23 ± 0.04^c	10.09 ± 0.04^c	88.31 ± 0.055^d
PTWGF5	83.60 ± 0.01^f	2.01 ± 0.00^d	10.75 ± 0.10^b	80.29 ± 0.047^e
PTWGF10	83.58 ± 0.05^g	1.97 ± 0.06^d	12.92 ± 0.010^d	79.02 ± 0.031^f
PTWGF20	83.50 ± 0.04^g	2.03 ± 0.04^d	12.56 ± 0.05^e	79.17 ± 0.008^f
PTWGF30	82.92 ± 0.05^h	1.21 ± 0.03^c	10.06 ± 0.02^c	80.14 ± 0.049^e

Table 1: Control is untreated flour; PTF-30, PTF-20, PTF-10, PTF-5 are wheat flour treated for 30, 20, 10 and 5 min respectively; PTWGF-30, PTWGF-20, PTWGF-10, PTWGF-5 are wheat grain treated for 30, 20, 10 and 5 min respectively. All the data are expressed as mean \pm standard deviations. Means with different superscript letters in a column differ significantly ($p < 0.05$).

3.3. Instrument color

The color values for grain and flour are shown in Table 1. The L* value of flour increased with treatment time. The L* of control and PTF-30 were 93.59 ± 0.01 and 94.23 ± 0.04 respectively while that of PTWGF-30 was 82.92 ± 0.05 . The higher L* value for flour than grain is attributed to the particle size variation between grain and flour. The a* value of flour decreased with increasing treatment time (control: 1.70 ± 0.00 , PTF-30: 1.23 ± 0.04 and PTWGF-30: 1.21 ± 0.03). Similarly, the b* value of flour and grain (control: 11.54 ± 0.00 , F30: 10.09 ± 0.04 and G30: 10.06 ± 0.02) decreased with treatment time. Overall, the L*, a* and b* value was changed significantly ($p < 0.05$) with increasing treatment time. The increase in L* value is possibly due to the degradation of conjugated double bonds of carotenoid pigments by ozone, as carotenoids are responsible for the pale yellow color detectable in flour (Mei, Liu, Huang, & Ding, 2016). PTF-30 showed the highest Whiteness Index (WI) value (88.31 ± 0.055). The WI increased with treatment time compared to control (86.70 ± 0.003) for all flour samples. Other authors have also reported a similar increase in WI for plasma treated grains including parboiled rice and black gram subjected to low pressure plasma treatment at treatment voltages of 30, 40 and 50 V (Saragapani, Devi, et al., 2017; Saragapani, Devi, Thirundas, Annapure, & Deshmukh, 2015).

3.4. Differential scanning calorimetry (DSC)

The thermal properties of control and plasma treated flours are shown in Table 2. The onset of gelatinization, peak and conclusion temperature were lowest for control samples. After plasma treatment, all gelatinization temperatures T_o , T_p and T_c increased. The peak gelatinization temperature increased with treatment time up to 30 min. Several authors have also observed an increase in peak gelatinization temperature with increasing plasma intensities (Kim & Min, 2017; Saragapani, Devi, et al., 2017). The increase in peak temperature is attributed to cross-linking on the surface and internal matrices of starch granules. The fluctuations in peak temperatures observed can be due to changes in plasma process parameters such as applied voltage, treatment time and

Table 2
Thermal properties of control, plasma treated wheat flour and wheat grains studied by DSC analysis

Samples	T_o ($^\circ\text{C}$)	T_p ($^\circ\text{C}$)	T_c ($^\circ\text{C}$)	Enthalpy (J/g of flour)
Control	58.88 ± 1.05^{abc}	62.85 ± 0.22^a	69.09 ± 0.35^{ab}	3.17 ± 0.01^a
PTF-5	59.23 ± 0.11^{17abc}	63.83 ± 0.12^b	69.95 ± 0.46^b	2.92 ± 0.04^b
PTF-10	58.58 ± 0.14^{ab}	63.81 ± 0.13^b	68.74 ± 0.13^{ac}	2.65 ± 0.00^c
PTF-20	59.56 ± 0.58^{bc}	63.73 ± 0.20^b	69.98 ± 0.37^b	2.56 ± 0.01^c
PTF-30	59.22 ± 0.61^{abc}	63.59 ± 0.42^{bc}	70.23 ± 0.13^b	2.33 ± 0.16^d
PTWGF-5	58.49 ± 0.45^{ab}	62.88 ± 0.17^a	67.10 ± 0.01^d	2.40 ± 0.04^d
PTWGF-10	59.54 ± 0.56^{abc}	63.20 ± 0.18^{ac}	68.25 ± 0.20^{ac}	2.68 ± 0.08^c
PTWGF-20	58.33 ± 1.07^a	62.30 ± 0.50^d	68.49 ± 1.07^{ac}	3.53 ± 0.08^e
PTWGF-30	59.88 ± 0.11^c	62.87 ± 0.08^b	68.31 ± 0.08^c	3.35 ± 0.01^f

Table 2: Onset gelatinization temperature (T_o), Peak gelatinization temperature (T_p), conclusion gelatinization temperature (T_c) and enthalpy for plasma treated samples where, control is untreated flour; PTF-30, PTF-20, PTF-10, PTF-5 are flour treated for 30, 20, 10 and 5 min respectively; PTWGF-30, PTWGF-20, PTWGF-10, PTWGF-5 are grain treated for 30, 20, 10 and 5 min respectively. All the data are expressed as mean \pm standard deviations. Means with different superscript letters in a column differ significantly ($p < 0.05$).

gas used. Previous results have showed that oxygen was the most effective gas for damaging starch (Zhang, Chen, Li, Li, & Zhang, 2015). Wongsagonsup et al. (2014) observed that an argon plasma jet initiated cross-linking at lower doses, while depolymerization was predominant at higher doses. In contrast to the present study, Zhang et al. (2015) observed a decrease in the gelatinization of potato starch when treated with nitrogen plasma (2000 Pa, 245 V and 1.1 A) for different treatment time (30, 45 and 60 min). DSC thermograms are provided in the supplementary copy.

The enthalpy of all samples decreased except for PTWG-20 and PTWG-30. There was a slight decrease in gelatinization enthalpy after treatment from 3.17 J/g to 1.69 J/g. The decrease in enthalpy values

post-treatment can be attributed to changes in crystallinity of starch in wheat flour. Wang et al. (2010), have reported that changes in enthalpy shows the loss of double helical structure of starch molecules. Furthermore, the loss in enthalpy might also depend on increase in T_o , T_p including the annealing time of starch gelatinization (Wu, Sun, & Chau, 2018). The decrease in enthalpy suggests that the cold plasma treated flours consume less energy for gelatinization. Thus, thermal analysis reveals that cold plasma treatment can be applied to elevate the gelatinization potential of starch.

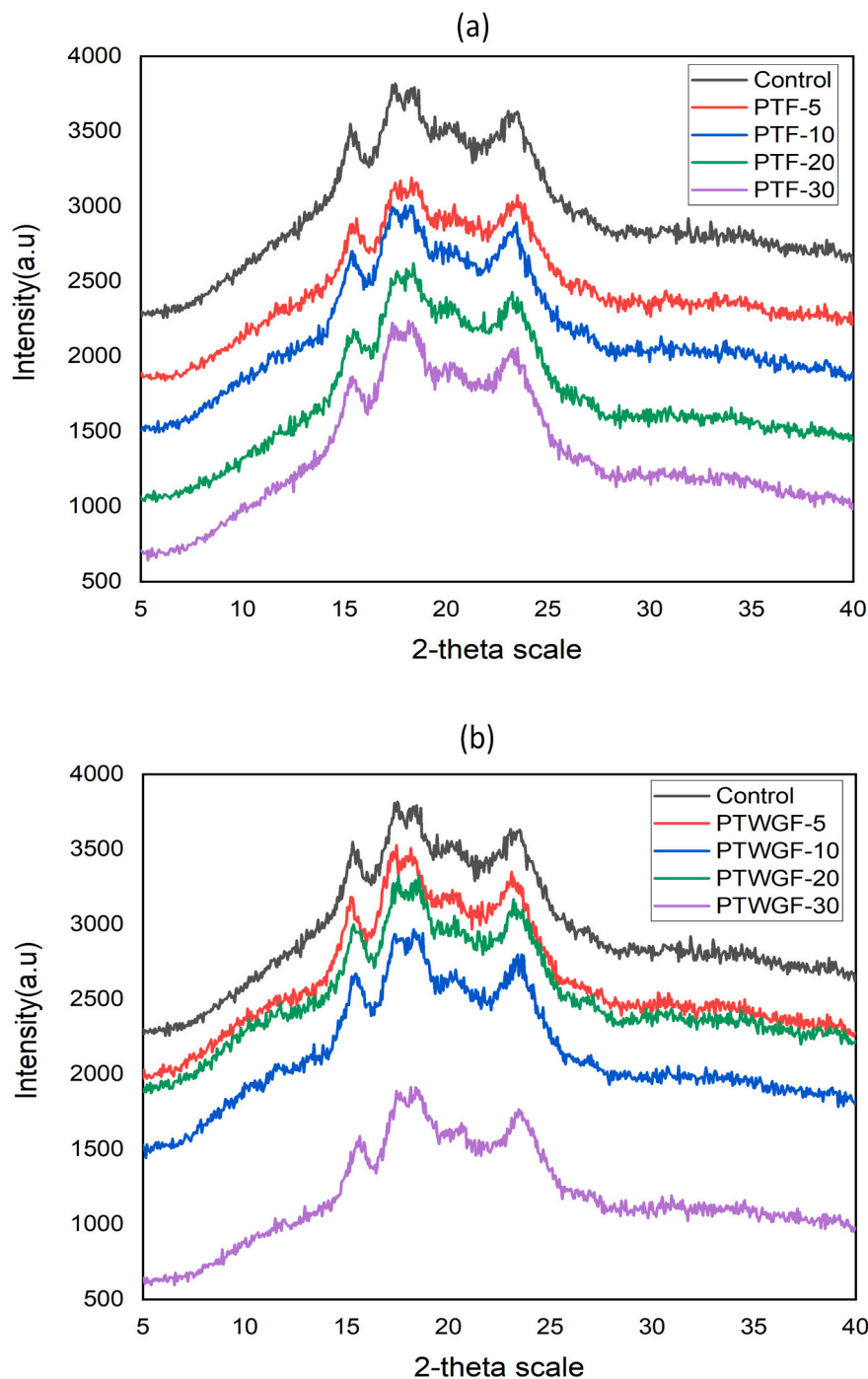


Fig. 5. X-ray Diffraction graph of (a) flour (b) grain where, control is untreated flour; PTF-30, PTF-20, PTF-10, PTF-5 are flour treated for 30, 20, 10 and 5 min respectively; PTWGF-30, PTWGF-20, PTWGF-10, PTWGF-5 are grain treated for 30, 20, 10 and 5 min respectively.

3.5. Fourier transform infrared analysis (FTIR)

FTIR spectra of PTF and PTWGF showed several characteristic peaks. The wide peak observed at 3436 cm^{-1} corresponds to the OH group of starch. As the treatment time increased, a slight increase in absorption peak of OH was observed. This might be due to depolymerization of starch glycosidic bonds by plasma reactive species. PTF and PTWGF proteins showed absorption peaks for amide I ($1600\text{--}1700\text{ cm}^{-1}$) and amide II ($1500\text{--}1600\text{ cm}^{-1}$). The stretching vibration of C=O bond is associated with amide I where bending vibrations of N—H bond associated with amide II. The absorption peak at 1167 cm^{-1} , 1079 cm^{-1} and 994 cm^{-1} corresponds to C—O—C, C—O and C—O—H stretching vibrations, respectively (Zhou, Yan, Shi, & Liu, 2019). In a study conducted by Misra et al. (2015) cold plasma treatment of wheat flour revealed an alteration of the secondary structure of gluten proteins in wheat dough, however in the current study, the FTIR spectra revealed no noticeable changes in peaks for both PTF or PTWGF samples. This suggests that the plasma exposure of flour at the stage when it is in the form of a hydrated dough may mediate structural changes to proteins, which were not observed with grain or flour.

3.6. X-ray diffraction analysis (XRD)

The XRD patterns of control, PTF and PTWGF are shown in Fig. 5. The four major peaks observed in XRD diffractograms at diffraction angles of 15.3° , 17.5° , 18.3° and 23.1° represent the crystalline portion, and the area below represents the amorphous portion. From Fig. 5, it can be observed that both PTF and PTWGF shows a typical A-type crystalline structure. These results are in support with the findings of Saiah, Sreekumar, Leblanc, Castandet, and Saiter (2007). In the present study, the XRD patterns did not exhibit significant shifts in the major peaks, however, changes in the overall intensities were observed in plasma treated samples. The change of intensities after plasma treatment is associated with the changes in crystallinity of wheat flour. As the treatment time increased, the crystallinity of wheat flour decreased from 13.88% to 11.88% for PTF samples, however no changes in crystallinity were observed in PTWGF samples. These results are in good agreement with Wu et al. (2018), who reported changes in peak intensities after corona discharge plasma treatment of banana starch at 30, 40 and 50 kV/cm for 3 min. The plasma generated reactive species such as hydroxyl radical and ozone reacted with wheat flour during jet atmospheric argon plasma treatment leading to cross-linking or depolymerization (Wongsagonsup et al., 2014). The decrease in crystallinity of the rice starch after the plasma treatment was previously reported by several authors (Saragapani, Thirumdas, et al., 2016; R. Thirumdas et al., 2017). In contrast, Wu et al. (2018) reported a decrease in the crystallinity of banana starch after, Zhang et al. (2015) reported no change in B-type crystalline pattern of potato starch after glow-plasma treatment for all treatment conditions. From these, results it can be inferred that cold plasma mediated effects on plant starches will depend on the system and process parameters applied, in this case,

Table 3

Pasting behaviour of the wheat flour and grain.

Treatments	Peak viscosity(cP)	Breakdown viscosity(cP)	Final viscosity(cP)	Set back (cP)	Peak time (min)	Pasting temperature ($^\circ\text{C}$)
Control	1348	671	1480	815	5.26	68.65
PTF-5	1372	693	1601	873	5.46	67.85
PTF-10	1707	834	1706	840	5.39	65.42
PTF-20	2013	999	1925	911	5.19	65.35
PTF-30	2167	1077	2096	1006	5.26	63.71
PTWGF-5	1241	625	1365	749	5.190	79.05
PTWGF-10	1487	643	1764	920	5.39	82.15
PTWGF-20	1523	646	1776	942	5.33	83
PTWGF-30	1509	640	1796	913	5.39	84.8

Table 3: Control is untreated flour; PTF-30, PTF-20, PTF-10, PTF-5 are flour treated for 30, 20, 10 and 5 min respectively; PTWGF-30, PTWGF-20, PTWGF-10, PTWGF-5 are grain treated for 30, 20, 10 and 5 min respectively.

treatment time, mode of treatment and type of starch. Whilst it is difficult to standardise and compare across different cold plasma systems and processes, an understanding of the impact of specific process parameters and the plasma reactive species profiles on the sample characteristics can be useful to promote critical appraisal of one process over another.

3.7. Pasting behaviour

The pasting properties of the PTF and PTWGF samples before and after treatment are presented in Table 3. Cold plasma treatment of PTF and PTWGF significantly increased the peak viscosities (PV) and final viscosities (FV) achieved as a function of increasing treatment time. Both PTWGF and PTF samples showed similar trends in pasting behaviours, but PTF samples showed higher peak and final viscosities compared to PTWGF grain samples. Peak viscosities (PV) after plasma treatment increased from 1372 cP (control) to 2044 cP (PTF-30) for flour samples, and to 1572 cP (PTWGF-30) was observed for grain samples. The increase in peak viscosities is attributed to cross-linking of starch induced by oxidation (Lee, Hong, Lee, Chung, & Lim, 2015). The action of plasma reactive species on starch may lead to destruction or loosening of starch granules, permitting greater swelling, leading to higher peak viscosities (Pal et al., 2016). The increase in breakdown viscosity from 644 cP to 1077 cP and final viscosity from 1601 cP to 2096 cP during the cooling process is due to the re-formation of bonds between molecules of chains. In addition, the disruption of hydrogen bonding promotes absorption of water molecules which results in higher viscosities. This reveals that plasma induces depolymerization of starch in wheat flour. The pasting temperature (PT) decreased as a function of increasing treatment time. These results are in support of the decrease in crystallinity of and peak gelatinization temperature of PTF by DSC. The increase in water binding capacity of PTF after treatment can be a possible explanation for such decrease in peak temperature. Several authors have reported cold plasma treatments etches the surface of polymeric films resulting in increase in hydrophilicity (S. K. Pankaj et al., 2014). Setback values were defined as the degree of re-association. The PTF had the highest setback values. Thus, cold plasma processes can be developed to alter the degree of retrogradation of starches in wheat flours.

4. Conclusion

These results demonstrate that DBD cold plasma processes may be tailored to regulate flour functionality and it is associated with partial disorganisation of the structure of starch in wheat flour. The plasma treatments applied using the prototype lab scale reactor induced changes in the flours and the flour hydration properties of flour. However, FTIR analysis indicated no change in the wheat proteins after the plasma treatments applied in this study. The decrease in peak gelatinization temperature and enthalpy of treated flour including changes in the crystallinity of the flour, indicate the plasma process induced modification of starch. The present study shows that the plasma process

parameters have an important role in modifying the properties of flour. Parameters such as applied voltage, feed gas, treatment duration and mode are factors that can influence functional properties using the lab scale prototype system, and may be important control parameters in scaling a plasma process that can be tuned to deliver a reactive species profile or intensity, that can regulate food ingredient functionality. However, there are limited reports on interaction of cold plasma species with starch/flour at a molecular level which requires further study. To conclude, the plasma functionalised flour or starch as an ingredient could be incorporated in the product formulation of various food products (e.g., frozen foods, sauces, baked goods). Cold plasma processing, using air as inducer gas with a contained reactor as employed here, may also be useful as a pre-treatment for starch ingredients that currently require a higher gelatinization temperature.

Credit author statement

PB, CS, SC, JJ conceived the work.
 SC, CS, JJ, AG, LC performed the experiments or analysed data.
 SC, CS, JJ, AG, BD, EC and PB wrote the manuscript.
 PB, SC, AG, JJ reviewed and edited the manuscript.

Industrial relevance

The requirements for clean labels and safe sustainability drives investigation of alternatives to current processing methods. This study examines the application of non-thermal Atmospheric Cold Plasma (ACP) as a potential alternative to current chemical treatments for improving functionality of wheat flour. The results showed improvements in flour hydration properties and crystallinity of wheat flour. Thus, plasma treatment can be used to enhance the functional properties of underutilized wheat. Plasma functionalised flour could be used as an ingredient in various wheat product formulations liked baked goods (leavened and unleavened products) and frozen products etc.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ifset.2020.102529>.

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