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Effects of incubation time of plasma activated water (PAW) combined with annealing for the modification of functional properties of potato starch

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ABSTRACT

The present study investigates the application of annealing and plasma-activated water (PAW) for starch modification, as alternative methods compared to the chemical one. Native potato starch was subjected to PAW, annealing with distilled water (DW-ANN), and the combination of the two (PAW-ANN) at different incubation times (1, 4, 8, and 12 h). The changes in rheological, pasting, and thermal properties were evaluated. The results showed that all treatments promoted significant modifications of the investigated parameters. In particular, while the pasting properties of the potato starch remained unchanged after the PAW treatment, G' (elastic) and G'' (viscous) of the PAW-treated starch were significantly ($p < 0.05$) higher than those of the native. DW-ANN significantly increased all rheological parameters and reduced peak viscosity, breakdown, and setback and significantly ($p < 0.05$) incremented pasting temperature, holding strength, and final viscosities of the potato starch may be due to cross-linking reaction. The combination of treatment (PAW-ANN) showed a synergistic effect leading to strong gel formation up to 4 h, while further treatment reduced the possibility of depolymerization. In conclusion, the combined treatment is a promising new green method to modify the properties of starch and improve its stability within a short treatment time.

1. Introduction

Starch is a biopolymer that is composed of amylose (linear with few branches, containing α-(1–4) linkages), amylopectin (branched, containing α-(1–4) and α-(1–6) linkages) chains (Fonseca, El Halal, Dias, & da Rosa Zavareze, 2021), and other minor components (Castanha, da Matta Junior, & Augusto, 2017). Starch is mainly a source of energy with multipurpose food ingredient employed as a confectionary, stabilizer, emulsifier, thickener, binding (encapsulating), and gelling agent in various food matrices (de Oliveira et al., 2018a). However, native starches are often characterized by drawbacks such as poor solubility of granules, tendency to syneresis, gel instability to heat, acidic environment and shear forces, weak structure and low shear resistance (Singh, Singh, Ezekiel, & Kaur, 2011). Consequently, to meet all industrial requirements, starch modification became important (Castanha, Santos, Cunha, & Augusto, 2019) to increase its application by providing products with higher thermal stability, mechanical (shear tolerance) and stable rheological and pasting properties (Falade & Ayetigbo, 2015). Conventional methods used for starch modification present many disadvantages, such as issues of environmental pollution, food safety concerns, chemical residues, waste, longer treatment time, and costs (Chaiwat et al., 2016).

Physical modification techniques, such as hydrothermal annealing and cold plasma technology (gas and plasma-activated water) are effective in changing the structure and functionality of starch; they do not produce hazardous chemical wastes, are cost-effective and environmentally sustainable (Chung, Liu, & Hoover, 2009; Zhu, 2017).

Annealing (ANN) consists of incubating starch for a certain period at a high moisture content (>40%) at a temperature slightly lower than gelatinization (Zheng et al., 2023). As a physical and green modification technique, ANN has received great attention in starch modification as only water and heat are used and the physicochemical properties of native starch can be changed to process and use in industrial food applications (Fonseca et al., 2021). According to literature results, ANN is able to reduce swelling power, solubility, amylose leaching, and to pasting parameters (breakdown, peak, final, and setback viscosities)

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(Shi et al., 2021). However, on the other side, it promotes increased crystallinity, pasting and gelatinization temperature (Hu, Wang, Li, Xu, & Zheng, 2020); finally, it requires a long time of incubation.

Plasma-activated water (PAW), generated by exposure of water to cold plasma, consists of an acidic environment with high redox potential, electrical conductivity and the presence of reactive oxygen and nitrogen species (Laurita, Gozzi, et al., 2021; Thirumdas et al., 2018). PAW has been applied for several applications such as disinfecting piping systems (Tan & Karwe, 2021), removing microorganisms (Han, He, Zhong, Wen, & Ni, 2022; Liu et al., 2020), enhancing seed growth (Fan, Liu, Ma, & Xiang, 2020), and modifying physicochemical and textural properties of various food matrices (Lotfy & Khalil, 2022; Muhammad et al., 2019; Patra, Prasath, Pandiselvam, Sutar, & Jeevarathinam, 2022). PAW can be exploited also to modify the structural and functional properties of food components; however, studies on the influence of PAW on starches are limited. Yan, Feng, Shi, Cui, and Liu (2020) exploited PAW-heat moisture treatment (treatment at 120 °C for 12 h in hot air) to modify normal and waxy maize starches. The authors observed an increase in resistant starch and solubility, improved short-range order, and a decrease in swelling power after treatment. Aaliya et al. (2022) investigated the effect of annealing (starch treated with water at a 1:2 ratio and for 24 h at 50 °C in a hot air oven) and heat moisture treatment (12 h at 100 °C in a hot air oven) with PAW on talipot starch. The authors reported increased crystallinity, enhanced thermal stability, and resistant starch content after treatment. Akua Y Okyere, Boakye, Bertoft, and Annor (2022) recently studied the effect of PAW (annealed by mixing starch (1.5 g) with PAW (100 mL) and incubated at 60 °C for 12 h) on waxy maize, waxy potato, and high amylose maize and potato starch. The authors reported increased gelatinization parameters (except enthalpy), water absorption capacity, and solubility while reducing swelling power. In addition, cross-linking was observed in waxy maize and high amylose potato. Yan et al. (2022) conducted a study of potato and pea starch with PAW and annealing with distilled water at 50 °C for 12 h, which showed no change in granular morphology, but a change in the long- and short-range order structure, rheological and thermal properties of the two starches.

However, in all the cited papers, a fixed incubation time (12 or 24 h) was investigated, while, considering that the stability, life cycle (life periods), and storability of PAW reactive species depend on time, it might be interesting to evaluate the effects of PAW (with and without annealing) incubation time on starch modification. Therefore, the present research aims to evaluate the effect of PAW in combination with annealing at different incubation times (1, 4, 8, and 12 h) on the modification of potato starch, investigating rheological, thermal, and pasting properties.

2. Materials methods

2.1. Materials

The potato starch used for the experiment was obtained from Ar. Pa S.r.l. (Ozzano dell'Emilia, Bologna, Italy).

2.2. Plasma activated water generation

The plasma-activated water (PAW) was obtained using a prototype developed by AlmaPlasma Srl, Bologna University, Italy, and generated by exposing distilled water to a corona discharge for 1 min using a voltage of 15 kV and a fixed frequency of 5 kHz as described by (Laurita, Contaldo, et al., 2021). Distilled water (500 mL) was grounded and placed in an Erlenmeyer flask on a stirrer (set at the speed of 700 rpm). The distance between the tip of the plasma source and the water was set at 5 mm, and the plasma discharge was generated for 1 min. The average discharge power (P), Hydrogen Peroxide (H2O2), pH, and NO_2^- concentrations of PAW generated was measured and reported in the our previous published paper (Gebremical et al., 2023).

2.3. Treatment of potato starch

Potato starch was subjected to three different treatments: the first was by immersion in the generated plasma activated water (PAW), the second by annealing in distilled water (DW-ANN), and the third by a combination of both, therefore annealing in the generated PAW (PAW-ANN). In detail, for the PAW treatment, the starch was mixed with plasma-activated water (starch:water ratio 1:2, w:w) at room temperature (20 ± 1 °C). Annealing was performed by mixing starch with distilled water (ratio starch: water 1:2, w:w) at 55 °C using a shaking water bath mod. ST30 (Nuve, Turkey). The combination (PAW-ANN) was obtained by mixing PAW with starch at the same ratio and incubating at 55 °C; the annealing temperature of 55 °C was chosen according to the literature (Castanha et al., 2017) and after preliminary DSC characterization of the gelatinization temperature on control samples. The untreated sample was considered as control (Native).

All samples were incubated for different times (1, 4, 8, and 12 h); after each time the paste was centrifuged at 2000 g (10 min), and the supernatant was carefully discarded. The paste was then washed with distilled water and centrifuged three times at 2000 g (10 min). After this, the remaining paste was dried at 40 °C (hot air drying), milled, sieved (100 mesh), packed and stored for further analysis for a maximum of 2 weeks.

2.4. Rheological analysis

2.4.1. Steady shear properties

A starch suspension (12%, w/w, dry basis) was prepared in the test tube and stirred for 2 min using a vortex mixer before heating at 90 °C for 30 min with occasional vortexing. The paste was then transferred onto the rheometer mod. MCR102 (Anton Paar, Graz, Austria) is equipped with a parallel plate (PP/50). It was equilibrated for 5 min at 25 °C before shearing from 0.1 to 100 s^{-1}. The power-law shear stress as a function of the shear rate was investigated using the following equation:

$$\tau = K\,\dot{\gamma}^{n} \tag{1}$$

Where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency index (Pa s^n), and n is the index flow behaviour (dimensionless).

2.4.2. Temperature sweep measurement

Dynamic oscillatory properties of potato were measured according to the method of Zhu and Cui (2020). Briefly, a starch suspension of 20% (w/w, dry basis) was mixed with a vortex mixer at room temperature (20 ± 1 °C) for 5 min before loading onto the bottom plate (PP/25) of the rheometer. The suspension was heated from 40 to 90 °C and cooled from 90 to 25 °C with a ramp rate of 2 °C/min. The sample edge was covered with a thin layer of sunflower oil to minimize water loss during the experiments. The shear strain was 1% and the frequency was 1 Hz. Dynamic rheological parameters, including storage modulus (G′), loss modulus (G″) and loss factor (tan δ = G″/G′), were recorded as a function of temperature.

2.4.3. Frequency sweep measurement

After the temperature sweep test, the resulting gel was held for 5 min at 25 °C to achieve a state of equilibrium before subjecting to frequency sweep at 25 °C from 0.1 to 100 Hz. The strain was kept at 1% (within the linear viscoelastic region). The G′ and G″ were plotted as a function of frequency.

2.5. Pasting properties

The potato starch pasting properties were measured according to the method of (Castanha et al., 2017) using a rheometer mod. MCR102 (Anton Paar, Graz, Austria), equipped with a starch cell (CC26ST aluminum) with continuous mixing (stirrer, ST24). Briefly, starch suspension (3 g starch in 25 mL) was rapidly mixed for 10 s, heated from 50 to 95 °C, held for 5 min at 95 °C before cooling from 95 to 50 °C, and finally held at 50 °C for 2 min. The rotation speed was held at 960 rpm for 10 s and the rest of the process was continued at 160 rpm. The obtained parameters were pasting temperature (PT), peak viscosity (PV), holding strength viscosity (HSV), and final viscosity (FV). Finally, breakdown (BDV = PV− HSV) and setback (SB=FV–HSV) viscosities were calculated.

2.6. Differential scanning calorimetry (DSC)

Starch gelatinization was measured using a DSC mod. Q20 (TA Instruments, USA). Starch (3 mg, dry basis) was mixed with water (7 μL) in aluminum crucibles (Tzero pans). An empty crucible was used as a reference. The sealed crucibles were heated from 25 to 120 °C at 10 °C/min. Onset (To), peak (Tp), conclusion (Tc) temperatures, and enthalpy change (ΔH) were obtained.

2.7. Data analysis

All obtained data (measurements were done in triplicate) were statistically analyzed by one-way ANOVA with SPSS software (Version 20.0, IBM, Armonk, New York, USA), and the Duncan Post Hoc Multiple Comparison tests were used to compare the statistically significant differences between means at $p < 0.05$.

3. Results and discussions

3.1. Steady shear rheological study

The efficiency of unit operations in the food industry is highly affected by the viscoelastic nature of food, and also the texture, stability and quality of starchy foods affect by viscosity (Kaavya et al., 2022). Increasing/decreasing viscosity/viscoelasticity is very important to form a paste that measures the thickening/thinning power of the starch for different applications (Thirumdas, Trimukhe, Deshmukh, & Annapure, 2017). Starch undergoes significant changes in its rheological properties due to exposure to high temperature, shear and acid during processing (Kaavya et al., 2023). Understanding these changes requires measuring rheology at functions of temperatures, shear rate and frequencies. Constant shear measurements were carried out and the results are shown in Fig. 1. The relationship between the shear stress and the shear rate was evaluated using the power-law model to describe the rheological flow behaviour. The detailed parameters of the regression power-law equations for different treatments are shown in Table 1. The correlation coefficients (R^2) ranged from 0.989 to 0.999, showing a strong power-law dependence of viscosity on shear rate. In addition, all values of the flow index (n) were much lower than 1, indicating that the potato starch treated at different incubation times behaved strongly like a non-Newtonian fluid and exhibited shear-thinning behaviour ($n < 1$).

The consistency coefficient (k) value was found to increase for all treatments with increased incubation time at the same shear rate, while the flow index (n) value decreased in starch annealed with PAW and DW, indicating that the potato starch becomes more resistant to flow as the treatment time increases.

As shown in Table 1, using PAW, the maximum k value was reached after 4 h, which may have been promoted by the reassociation of starch chains, following the reduction of hydroxyl groups in the starch structure due to PAW reactive species, that enhanced the starch interaction (Banura, Thirumdas, Kaur, Deshmukh, & Annapure, 2018; Chou, Tseng, Hsieh, & Ting, 2023), causing increase resistance to flow; similar results were also observed in our previous published work (Gebremical et al., 2023). However, afterward, values started to decrease (although they always remained higher compared to the native one), possibly as a

Table 1
Power law parameters of potato starches subjected to plasma activated water (PAW), annealing with distilled water (DW-ANN), and their combination (PAW-ANN) for different incubation times.

Sample	Incubation time (h)	K	n	R^2
Native	–	23.25 ± 0.31^j	0.47 ± 0.007^a	0.991
PAW	1	30.64 ± 0.22^g	0.47 ± 0.005^{ab}	0.989
	4	37.95 ± 0.26^i	0.47 ± 0.003^a	0.991
	8	35.25 ± 0.29^h	0.47 ± 0.005^{ab}	0.990
	12	34.83 ± 0.26^h	0.47 ± 0.004^{ab}	0.999
DW-ANN	1	46.28 ± 0.39^c	0.45 ± 0.001^{abc}	0.999
	4	50.74 ± 0.60^e	0.43 ± 0.01^{bcd}	0.999
	8	55.05 ± 1.32^e	0.44 ± 0.001^{abcd}	0.999
	12	55.25 ± 0.60^e	0.41 ± 0.003^d	0.999
PAW-ANN	1	60.88 ± 0.75^c	0.44 ± 0.004^{bcd}	0.990
	4	66.07 ± 0.53^b	0.42 ± 0.003^d	1.000
	8	70.96 ± 0.54^a	0.42 ± 0.003^{cd}	0.990
	12	63.85 ± 0.01^b	0.41 ± 0.001^d	0.999

Superscripts with different letters in the same column depict significant differences ($p < 0.05$).

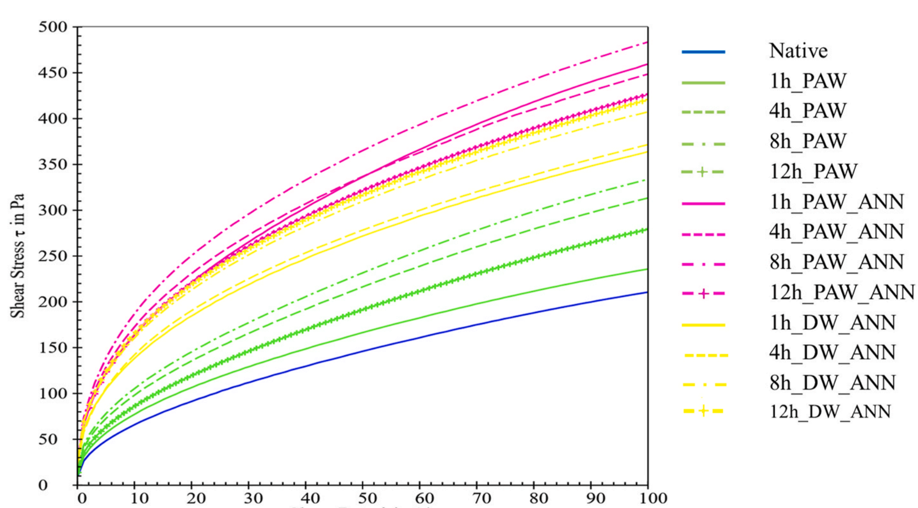

Fig. 1. Shear stress versus shear rate profile for potato starch samples subjected to plasma activated water (PAW), annealing with distilled water (DW-ANN), and their combination (PAW-ANN) for different incubation times.

consequence of depolymerization. Samples subjected to DW-ANN showed values that were doubled compared to the native one after only 1 h, with a further increase during the following incubation. It is known that annealing causes a more complete rearrangement of the potato starch molecules, resulting in tighter and more organized molecular structures with more rigid and cohesive starch that is resistant to flow. The combination of treatments (PAW-ANN) resulted in a remarkable increase in k value, which was around three times higher than the native one and significantly ($p < 0.05$) higher than starch subjected to both single treatments. After only 1 h of the PAW-ANN treatment, k values were higher compared to the maximum value reached for the longest ANN treatment (12 h).

The steady-state flow behaviour results indicate that in the PAW-ANN treatment, the presence of reactive species and the acidity of the PAW enhanced the cross-linking reaction occurring during annealing. Indeed, from our previous publication (Gebremical et al., 2023), a significant pH decrease (from 6.12 ± 0.05 to 3.48 ± 0.08) was observed during PAW generation. However, the degree of cross-linking was partially reduced after prolonged treatment time (12 h PAW-ANN). This reduction could be explained by a destabilization, breakdown, or damage to the starch granule due to the hydrolysis of amylose caused by the acidic pH of PAW and/or by a reduction of the efficiency of PAW reactive species which decreased the rigidity of the granules and the viscosity (Aaliya et al., 2022).

3.2. Dynamic shear rheological study

3.2.1. Temperature dependency frequency sweep

The dynamic viscoelastic properties of potato starch during the heating and cooling cycle are shown in Table 2 and Fig. 2. The elastic nature of the starch gel, indicated by the storage modulus (G′) as a function of temperature, increased progressively up to a maximum G' (G'_{mxa}, kPa) and decreased loss factor ($Tan\delta G'_{max}$) at maximum TG'_{max} (°C) (Table 2). The initial increase in G′ and decrease in $Tan\delta G'_{max}$ at a given temperature (TG'max, °C) is due to the development of a three-dimensional network, cross-linking, and swelling of the granules (Taslikh et al., 2022). The subsequent decrease observed with further heating (up to 90 °C) indicates that the gel structure weakens with increasing temperature; this might be due to the break-down of starch granules and intermolecular interactions, the melting of crystalline regions and deformation in the swollen granules, and the reduction in the degree of chain entanglement (Chen, Xie, Chen, & Zheng, 2019). As shown in Table 2, during cooling (90–25 °C), the G′ continuously increases for all samples.

Compared to the native starch, PAW-treated ones showed no differences in the $T_{G'max}$, but an increase in the G'_{max} that reached the highest value after 8 h and then decreased after 12 h. The annealing treatment promoted an increase of the two parameters during heating, significant only after 8 h for $T_{G'max}$ and after 12 h for G'_{max}. The combination of the treatments promoted in the PAW-ANN samples a faster increase in both parameters. In particular, the highest value was reached after 4 h (8.18 ± 0.24 kPa) and then decreased during the following incubation period. Also for this parameter, 1 h of PAW-ANN treatment led to higher values compared to 12 h of DW-ANN treatment, indicating a stronger gel formation in a remarkably shorter time.

During cooling, G'_{max} showed a similar behaviour and was increased in PAW, DW-ANN, and even more markedly by the combination of the two (PAW-ANN samples), while $Tan\delta G'_{max}$ decreased remarkably in DW-ANN and PAW-ANN samples. Also in this case, G'_{max} reached a maximum after 8 h of PAW-ANN, and then decreased in the 12 h treatment, still showing values similar to the ones obtained by the only DW-ANN treatment for the same incubation period.

Generally, all treatments showed an increase in molecular interactions in comparison to the native starch, with a synergistic effect in the PAW-ANN samples, that consequently resulted in a higher G' (Xu et al., 2022). Fig. 3 shows that G′ (elastic nature) and G″ (viscous nature)

Table 2
Rheological parameters during temperature sweep for potato starch subjected to plasma activated water (PAW), annealing with distilled water (DW-ANN), and their combination (PAW-ANN) for different incubation times.

Sample	Heating (40–90 °C)				Cooling (90–25 °C)	
	Incubation time(h)	$T_{G'max}$ (°C)	G'_{max} (kPa)	$Tan\delta G'_{max}$	G'_{max}	$Tan\delta G'_{max}$
Native	–	65.86 ± 0.34^{e}	4.03 ± 0.02^{f}	0.22 ± 0.01^{a}	2.47 ± 0.01^{g}	0.10 ± 0.01^{ab}
PAW	1	66.35 ± 0.35^{e}	3.40 ± 0.15^{g}	0.19 ± 0.01^{ab}	3.00 ± 0.09^{f}	0.09 ± 0.01^{b}
	4	65.10 ± 0.01^{e}	7.18 ± 0.04^{c}	0.23 ± 0.01^{a}	4.91 ± 0.6^{d}	0.11 ± 0.01^{a}
	8	65.10 ± 0.02^{e}	7.59 ± 0.04^{bc}	0.22 ± 0.02^{a}	4.97 ± 0.08^{d}	0.11 ± 0.01^{a}
	12	66.11 ± 0.72^{e}	4.84 ± 0.21^{e}	0.19 ± 0.01^{ab}	3.00 ± 0.02^{f}	0.10 ± 0.01^{ab}
DW-ANN	1	71.55 ± 0.62^{c}	4.83 ± 0.09^{e}	0.17 ± 0.02^{ab}	4.58 ± 0.06^{e}	0.07 ± 0.01^{cd}
	4	72.87 ± 0.35^{bc}	4.54 ± 0.32^{ef}	0.16 ± 0.01^{ab}	5.01 ± 0.02^{d}	0.06 ± 0.01^{de}
	8	75.65 ± 0.04^{a}	4.87 ± 0.06^{e}	0.17 ± 0.01^{ab}	7.51 ± 0.19^{b}	0.05 ± 0.01^{f}
	12	75.37 ± 0.35^{a}	6.37 ± 0.08^{d}	0.14 ± 0.01^{b}	7.62 ± 0.02^{b}	0.04 ± 0.01^{f}
PAW-ANN	1	69.62 ± 0.71^{d}	7.09 ± 0.07^{c}	0.17 ± 0.02^{ab}	6.34 ± 0.06^{c}	0.07 ± 0.01^{c}
	4	74.12 ± 0.35^{ab}	8.18 ± 0.24^{a}	0.15 ± 0.01^{ab}	9.46 ± 0.01^{a}	0.05 ± 0.01^{ef}
	8	74.12 ± 0.03^{ab}	7.85 ± 0.01^{ab}	0.14 ± 0.01^{b}	9.41 ± 0.08^{a}	0.05 ± 0.01^{f}
	12	75.37 ± 0.35^{a}	4.84 ± 0.21^{e}	0.14 ± 0.02^{b}	7.62 ± 0.05^{b}	0.04 ± 0.01^{f}

Superscripts with different letters in the same column show significant differences ($p < 0.05$) Where; TG'_{max} (°C), temperatures at which G′ reaches its maximum during temperature sweep; G'_{max}, maximum G′ during temperature sweep; $tan\delta G'_{max}$ at G'_{max}.

increased over the angular frequency range (0.1–100 rad/s), and the values of G′ were higher than those G″ which indicates the prevalence of the elastic over the viscous behaviour. This was also confirmed by the loss tangent (Tanδ at 100 Hz) values (Table 3) which were always <1. G′ values increased compared to the native starch (3.41 kPa) with PAW treatment reaching a maximum value after 4 h (7.35 kPa), and then decreasing after and with DW-ANN treatment proportionally to incubation time up to 9.8 kPa after 12 h.

When comparing the viscoelastic behaviour based on the treatments, the increase in the G′ suggests a starch characterized by more organized chains, higher paste stability, strength, and resistance to heat and shear (Aaliya et al., 2022). This effect was more pronounced with the combination of the treatments (PAW-ANN), resulting in a G' of 13.74 kPa after only 4 h. However, as it occurred for PAW, prolonging the incubation time up to 12 h, the values decreased significantly. This might be due to the granule corrosion and molecular degradation of starch exposure to PAW. In addition, the increase in the elastic moduli with an increase in frequency depends on the microstructural and conformational changes taking place in the potato starch granules, as a result of the cross-linking of the molecules and an augmentation in the gel

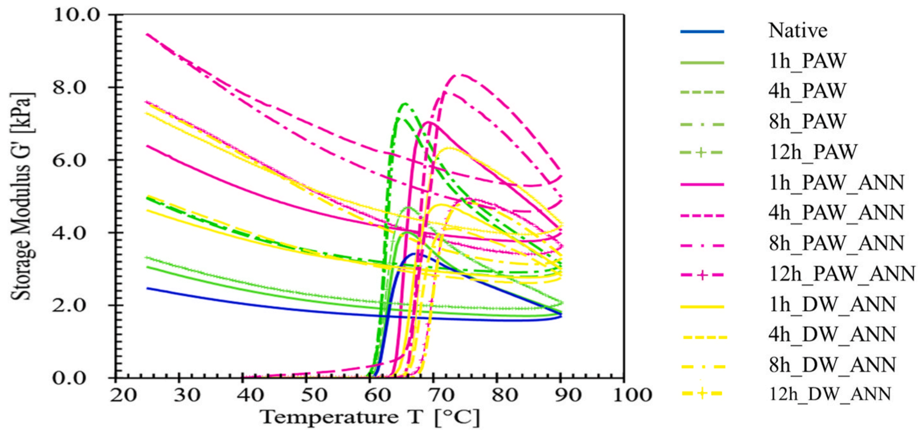

Fig. 2. Storage modulus (G′) as a function of temperature during dynamic oscillatory temperature sweep for potato starch subjected to plasma activated water (PAW), annealing with distilled water (DW-ANN), and their combination (PAW-ANN) for different incubation times.

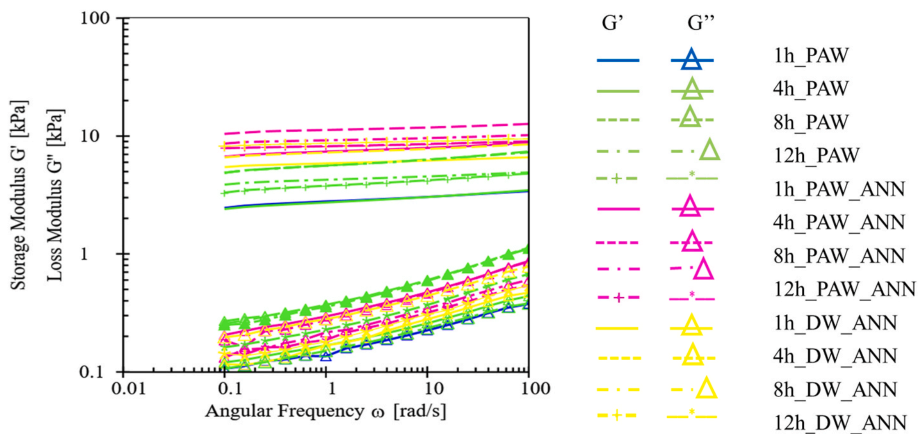

Fig. 3. The rheological curves of potato starch subjected to plasma activated water (PAW), annealing with distilled water (DW-ANN), and their combination (PAW-ANN) for different incubation times during the frequency sweep. G': storage modulus, G'': Loss modulus.

Table 3
Frequency sweep test at 100 Hz for potato starch subjected to plasma activated water (PAW), annealing with distilled water (DW-ANN), and their combination (PAW-ANN) for different incubation times.

Sample		Frequency sweep (0.1 → 100 Hz)	
	Incubation time (h)	G' (kPa)	Tanδ
Native	–	3.41 ± 0.3^{f}	0.15 ± 0.20^{a}
PAW	1	3.40 ± 0.08^{f}	0.14 ± 0.01^{a}
	4	7.37 ± 0.04^{cde}	0.12 ± 0.01^{ab}
	8	7.23 ± 0.04^{cde}	0.13 ± 0.02^{a}
	12	4.71 ± 0.37^{ef}	0.14 ± 0.02^{a}
DW-ANN	1	6.71 ± 0.05^{de}	0.08 ± 0.01^{bcd}
	4	7.93 ± 0.81^{cd}	0.07 ± 0.01^{cd}
	8	9.44 ± 0.05^{bcd}	0.06 ± 0.01^{cd}
	12	9.8 ± 2.27^{ab}	0.06 ± 0.01^{cd}
PAW-ANN	1	8.64 ± 0.18^{bcd}	0.06 ± 0.01^{bc}
	4	13.74 ± 1.52^{a}	0.05 ± 0.01^{cd}
	8	11.26 ± 1.59^{ab}	0.07 ± 0.01^{cd}
	12	9.91 ± 1.30^{bc}	0.06 ± 0.01^{cd}

Superscripts with different letters in the same column indicate significant differences ($p < 0.05$); where G': storage modulus, and Tanδ: loss factor.

strength during PAW-ANN treatment (Aaliya et al., 2022; da Rosa Zavareze & Dias, 2011).

3.3. Pasting properties

The pasting properties of native potato, treated with PAW, annealed with distilled water (DW-ANN) and with PAW (PAW-ANN), and incubated for different times are given in Table 4, and the related figure is in Fig. 4. Results show no differences in all the considered parameters for the PAW samples compared to the native starch. In a previous work (Gebremical et al., 2023), we observed an increase of most of the pasting parameters in potato starch after PAW treatment. This discrepancy might be due to the use of starches deriving from different manufacturers however, to clarify this, a deeper characterization of the raw material would be necessary. On the other side, significant differences ($p < 0.05$) were observed for DW-ANN and PAW-ANN. In particular, peak viscosity (PV), breakdown viscosity (BDV), and setback viscosity (SBV) were reduced while pasting temperature (PT), holding strength (HSV), and final viscosity (FV) of the potato starch were increased.

The increase in PT suggest the rising in resistance to swelling, agglomeration of starch granules, increased crystallinity, development of intermolecular cross-links, and a reduction in the space between molecules (Aaliya et al., 2022; Q. Wang, Li, & Zheng, 2021; Xia, Gou, Zhang, Li, & Jiang, 2018), which hinders the viscosity development during annealing, as shown in Fig. 4. The interaction between starch chains in the amorphous and crystalline regions makes the starch more

Table 4
Pasting properties of potato starch subjected to plasma activated water (PAW), annealing with distilled water (DW-ANN), and their combination (PAW-ANN) for different incubation times.

Sample	Incubation Time(h)	PT (°C)	PV(Pa.s)	BDV(Pa.s)	HSV(Pa.s)	SBV(Pa.s)	FV(Pa.s)
Native	–	64.50 ± 0.03^{e}	13.75 ± 0.13^{a}	10.89 ± 0.07^{a}	2.85 ± 0.06^{d}	1.63 ± 0.07^{ab}	4.49 ± 0.01^{e}
PAW	1	64.49 ± 0.01^{e}	13.15 ± 0.17^{ab}	10.33 ± 0.17^{a}	2.82 ± 0.03^{d}	1.56 ± 0.17^{bc}	4.38 ± 0.14^{e}
	4	64.63 ± 0.75^{e}	13.57 ± 0.11^{ab}	10.75 ± 0.13^{a}	2.82 ± 0.01^{d}	1.61 ± 0.05^{ab}	4.43 ± 0.04^{e}
	8	64.99 ± 0.69^{e}	12.83 ± 0.07^{ab}	10.05 ± 0.10^{a}	2.78 ± 0.02^{d}	1.60 ± 0.08^{abc}	4.38 ± 0.06^{e}
	12	64.71 ± 0.77^{e}	13.20 ± 0.17^{ab}	10.45 ± 0.16^{a}	2.75 ± 0.02^{d}	1.58 ± 0.01^{bc}	4.33 ± 0.03^{e}
DW-ANN	1	68.05 ± 0.74^{d}	12.56 ± 1.00^{ab}	8.74 ± 0.87^{b}	3.83 ± 0.13^{c}	1.43 ± 0.21^{c}	5.26 ± 0.08^{d}
	4	70.11 ± 0.76^{bcd}	9.16 ± 0.09^{cd}	4.08 ± 0.09^{c}	5.08 ± 0.01^{a}	1.55 ± 0.04^{bc}	6.63 ± 0.05^{c}
	8	71.19 ± 0.74^{abc}	9.77 ± 0.07^{c}	4.14 ± 0.11^{c}	5.64 ± 0.03^{b}	1.59 ± 0.29^{abc}	7.22 ± 0.25^{b}
	12	72.21 ± 0.72^{ab}	8.43 ± 0.41^{de}	2.41 ± 0.28^{d}	6.02 ± 0.12^{a}	1.92 ± 0.01^{a}	7.93 ± 0.13^{a}
PAW-ANN	1	68.78 ± 0.3^{cd}	12.16 ± 0.16^{b}	8.11 ± 0.31^{d}	4.23 ± 0.13^{d}	1.54 ± 0.12^{c}	5.70 ± 0.12^{d}
	4	72.30 ± 0.42^{ab}	7.83 ± 0.05^{e}	2.07 ± 0.05^{d}	5.66 ± 0.14^{a}	1.44 ± 0.03^{bc}	7.21 ± 0.03^{b}
	8	72.15 ± 0.27^{a}	7.76 ± 0.06^{e}	2.20 ± 0.06^{d}	5.77 ± 0.29^{a}	1.97 ± 0.24^{abc}	7.75 ± 0.05^{a}
	12	71.65 ± 0.76^{ab}	7.89 ± 0.30^{e}	2.31 ± 0.28^{d}	5.57 ± 0.02	1.62 ± 0.17^{ab}	7.57 ± 0.18^{ab}

Superscripts with different letters in the same column indicate significant differences ($p < 0.05$); where PT: pasting temperature, PV: peak viscosity, BDV: breakdown viscosity, HSV: holding strength viscosity, SBV: setback viscosity and FV: final viscosity.

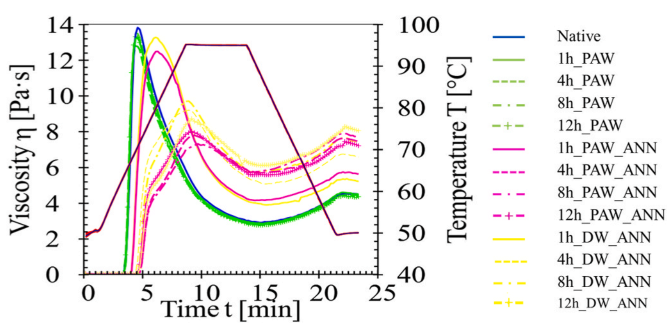

Fig. 4. Pasting curves of potato starches subjected to plasma activated water (PAW), annealing with distilled water (DW-ANN), and their combination (PAW-ANN) for different incubation times.

stable, so it probably needs a higher temperature for the rupture of the structure (Van Hung, Huong, Phi, & Tien, 2017).

Peak viscosity (PV) is a measure of the fragility and the maximum degree of swelling of starch granules before disintegration when subjected to heating (Xia et al., 2018). The observed decrease might indicate that the binding forces within the granules were strengthened, limiting starch swelling and structural disintegration. Studies have shown that annealing with PAW improves interactions between starch molecules and strengthens the structure of crystallites, making it more difficult for amylose to dissolve from the granules and resulting in a decrease in PV, similar results were observed in this study (Aaliya et al., 2022; Flores-Silva et al., 2023).

Samples with high breakdown viscosity (BDV) values (native and PAW) imply granule fragility at higher temperatures and shear rates. On the other hand, lower BDV values (DW-ANN and PAW-ANN) indicate higher thermal stability, higher resistance against shear exerted from high temperature and a lower deterioration tendency (Akua Yeboah Okyere, Bertoft, & Annor, 2019; Xia et al., 2018). In this study, lower BDV was promoted by annealing and to a higher extent by the combination with PAW. PAW-ANN after 4 h treatment time, showed values lower than those reached by DW-ANN after 12 h, this indicates that annealing with PAW treatment could improve the stability against mechanical shear and thermal stability within a short time.

The SBV and FV values can be used to understand the level of retrogradation in a starch paste and to measure the effects of different processing conditions on starch retrogradation (Kumar, Singh, Sharanagat, Patel, & Kumar, 2020). Low SBV values indicate the stability of starch against deterioration, staling, and the resistance to form an extensive network structure during the cooling stage (Xia et al., 2018), while an increase in FV refers to the stability of starch paste and a slower tendency to retrograde due to structural dissociations in amylose-amylopectin networks. Therefore, also for these parameters, while ANN favoured a better thermal resistance and weaker retrogradation tendency compared with those of the native starches, the combination with PAW allowed to reach the same values in a shorter time (4 h compared to 12 h).

3.4. Thermal properties

The gelatinization temperatures (T_o, T_p, and T_c), and gelatinization enthalpy (ΔH) values are presented in Table 5 and Fig. 5. The magnitude

Table 5
Thermal properties of potato starches subjected to plasma activated water (PAW), annealing with distilled water (DW-ANN), and their combination (PAW-ANN) for different incubation times.

Sample	Incubation Time (h)	T_O (°C)	T_p (°C)	T_c (°C)	△H (J/g)
Native	–	60.72 ± 0.24^{f}	65.31 ± 0.27^{f}	74.04 ± 1.23^{de}	10.45 ± 0.42^{bcd}
P AW	1	60.96 ± 0.26^{f}	65.37 ± 0.07^{f}	74.49 ± 0.99^{d}	11.87 ± 0.07^{abc}
	4	60.96 ± 0.43^{f}	65.01 ± 0.16^{fg}	74.78 ± 0.95^{d}	11.29 ± 0.46^{ab}
	8	60.79 ± 0.05^{f}	65.11 ± 0.08^{fg}	73.67 ± 0.60^{de}	11.69 ± 0.35^{abc}
	12	60.62 ± 0.68^{f}	64.27 ± 0.24^{fg}	72.23 ± 0.13^{e}	11.68 ± 0.21^{abc}
DW-ANN	1	65.21 ± 0.31^{e}	68.18 ± 0.32^{e}	78.32 ± 1.49^{abc}	11.92 ± 0.54^{abc}
	4	67.2 ± 0.15^{d}	70.21 ± 0.25^{d}	78.49 ± 1.03^{abc}	9.45 ± 0.78^{d}
	8	68.07 ± 0.04^{c}	71.07 ± 0.05^{cd}	78.21 ± 0.25^{abc}	10.95 ± 0.72^{abcd}
	12	69.62 ± 0.29^{a}	73.5 ± 0.91^{a}	80.19 ± 1.66^{a}	$10.68 \pm 0.65b^{cd}$
PAW-ANN	1	64.96 ± 0.11^{e}	67.88 ± 0.07^{e}	77.15 ± 1.48^{c}	12.54 ± 0.29^{a}
	4	67.15 ± 0.15^{d}	70.23 ± 0.03^{d}	77.61 ± 0.76^{bc}	12.20 ± 1.54^{ab}
	8	68.29 ± 0.03^{bc}	71.26 ± 0.04^{bc}	78.15 ± 1.07^{bc}	10.57 ± 0.72^{abc}
	12	68.94 ± 0.12^{ab}	72.05 ± 0.10^{b}	79.20 ± 0.69^{ab}	10.31 ± 0.67^{cd}

All data represent with mean ± standard deviation. Values followed by different superscripts in the same column indicate a significant difference ($p < 0.05$). To: onset temperature, T_P: peak temperature, Tc: conclusion temperature, and ΔH: enthalpy.

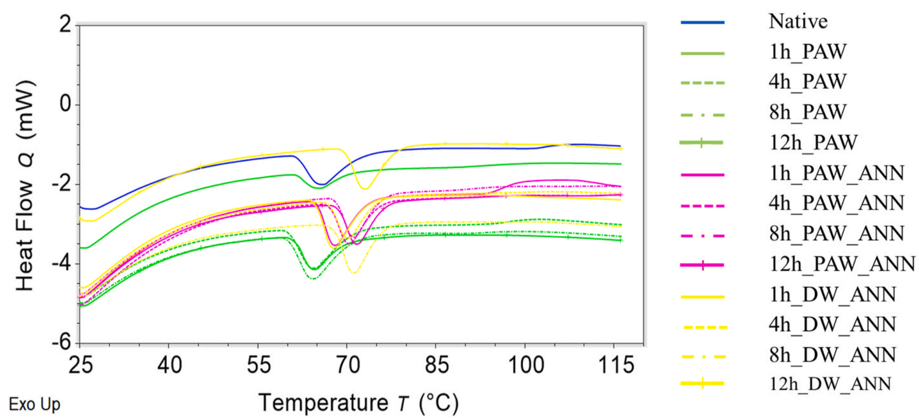

Fig. 5. Thermal profiles of potato starches subjected to plasma activated water (PAW), annealing with distilled water (DW-ANN), and their combination (PAW-ANN) for different incubation times.

of the gelatinization parameters is affected by the double helix order, amylose-amylopectin ratio, and by the molecular arrangement of the crystalline region (L. Wang, Wang, Zhou, Wu, & Ouyang, 2022). T_o, T_p and T_c represents the melting temperature of weak, general, and strong crystals of starch, respectively (Hu et al., 2020).

T_o, T_p, and T_c of native starch were on average 60.72 ± 0.24, 65.31 ± 0.27, and 74.04 ± 1.23 °C respectively, and ΔH was 10.45 ± 0.42 J/g. In PAW samples, although values of T_c after 8 and 12 h were slightly lower compared to the native ones, differences were not significant ($p > 0.05$). Instead, for the DW-ANN and PAW-ANN samples, all the gelatinization temperatures were increased significantly ($p < 0.05$), in line with the increase in the pasting temperature (PT) (Table 4) for the PAW-treated starch with increasing treatment time. This might have resulted from the increasing mobility of the non-crystalline (amorphous) regions and interactions between the amylose–amylose and amylose–amylopectin (Yan et al., 2020), which led to a reassociation of the starch molecules and an ordered arrangement of the crystalline structure (de Oliveira et al., 2018a; Hu et al., 2020). However, no differences were observed between DW-ANN and PAW-ANN at each incubation time, suggesting that the effect was mainly due to the annealing treatment.

Regarding, the values of ΔH as indicated in Table 5, PAW and DW-ANN however, did not modify the values, while a significant increase compared to the control sample was observed in the PAW-ANN sample, but only after 1 h of incubation. The value slightly decreased prolonging the treatment, but resulted significantly lower compared to the 1 h only after 12 h. This may be due to the presence of more heterogeneous crystals, resulting in the formation of amylose and amylopectin reaction chains by degradation of PAW reactive species (Aaliya et al., 2022; Alimi & Workneh, 2018). However, further treatment resulted in a decrease of enthalpy to 10.31 J/g (12 h), which could be due to the rupture of amylopectin, weakening its double helices and resulting in a transformation from the crystalline to the amorphous state (de Oliveira et al., 2018b).

4. Conclusions

In conclusion, the applied treatments, especially PAW-ANN and DW-ANN, caused a significant change in the rheological, thermal and pasting properties of potato starch at different incubation times (1, 4, 8 and 12 h). The combination of PAW with annealing showed a synergistic effect in thermal stability and shear resistance, resulting in lower breakdown viscosity, higher elasticity and gelatinization enthalpy up to 4 h, possibly due to crosslinking. This effect may be due to the acidic environment and the reactive species of PAW, which enhance the annealing effect. The techno-functional properties of the potato also changed significantly after DW-ANN treatment. PAW-ANN modified potato starch might be used as a sustainable raw material in food applications, such as a base for baked goods, thickening agent, candies, ice cream and in processed foods because of its ability to better withstand high thermal and mechanical processing conditions in the food industry. Therefore, the PAW-ANN combination represents a promising technology for the modification of starch, which is an environmentally friendly alternative to chemical modifications. However, it should be noted that further research is needed to improve the understanding of the mechanism of the modification induced by the combination of treatments and the modification of microstructure, amylose/amylopectin ratio after treatment, and as well as for the optimization of PAW parameters.

CRediT authorship contribution statement

Gebremedhin Gebremariam Gebremical: Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Silvia Tappi:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Romolo Laurita:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Filippo Capelli:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Federico Drudi:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Santina Romani:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Pietro Rocculi:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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