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Enhancing starch functionality through synergistic modification via sequential treatments with cold plasma and electron beam irradiation

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ABSTRACT

The impact of dual sequential modifications using radio-frequency (RF) plasma and electron beam irradiation (EBI) on starch properties was investigated and compared with single treatments within an irradiation dose range of 5–20 kGy. Regardless of sequence, dual treatments synergistically affected starch properties, increasing acidity, solubility, and paste clarity, while decreasing rheological features with increasing irradiation dose. The molecular weight distribution was also synergistically influenced. Amylopectin distribution broadened particularly below 10 kGy. Amylose narrowed its distribution across all irradiation doses. This was due to dominating EBI-induced degradation and molecular rearrangements from RF plasma. With the highest average radiation-chemical yield (G) and degradation rate constant (k) of $(2.12 \pm 0.14) \times 10^{-6}$ mol·J^{-1} and $(3.43 \pm 0.23) \times 10^{-4}$ kGy^{-1}, respectively, upon RF plasma pre-treatment, amylose underwent random chain scission. In comparison to single treatments, dual modification caused minor alterations in spectral characteristics and crystal short-range order structure, along with increased granule aggregation and surface irregularities. The synergistic effect was dose-dependent, significant up to 10 kGy, irrespective of treatment sequence. The highest synergistic ratio was observed when RF plasma preceded irradiation, demonstrating the superior efficiency of plasma pre-treatment in combination with EBI. This synergy has the potential to lower costs and extend starch's technological uses by enhancing radiation sensitivity and reducing the irradiation dose.

1. Introduction

Starch is among the most used raw ingredients by mankind, being considered a veritable naturally occurring biopolymer for the current economic revolution [1]. Its applications range from human food, cosmetics, and pharmaceuticals to textiles, adhesives, and even paints. Even if, at first sight, we can be impressed by its wide applicability, starch as a native form still has technological constraints related to its functionality. In this respect, native starch has limits in terms of its solubility, swelling capacity, viscosity, heat stability, freeze-thaw stability, film-forming properties, etc. [2]. Consequently, research groups across the globe are diligently exploring the methods, techniques, solutions, and tools that can support the functionalization of starch in an efficient, fast, and cheap way. There is a fundamental requirement for integrating different approaches that should be efficient, cost-effective, eco-friendly, and socially accepted [3]. Thus, current trends in modifying the starch functionality and structural architecture involve combining existing methods [4,5], especially to reduce input parameters, resulting in final products aligned with present environmental standards.

Dual processing of starch macromolecules, particularly through the combination of emerging and non-polluting physical methods, has gained remarkable interest worldwide lately. Various starches derived from sources such as corn, mung bean, yam, and lotus root [6–10] have

Abbreviations: RF, radio-frequency; RF/EBI, radio-frequency plasma treatment followed by electron beam irradiation; EBI, electron beam irradiation; EBI/RF, electron beam irradiation followed by radio-frequency plasma; HVD, half-value dose; SR, synergistic ratio.

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been investigated involving dual physical modification methods employing environmentally friendly techniques such as ionizing radiation and plasma discharges. The outcomes of these investigations suggest that the observed changes in starch functionality can be attributed to either degradation or cross-linking effects. These modifications appear to be influenced by various factors, including the processing parameters, the order in which the treatments are applied, and the inherent features of the starch under investigation.

Physical techniques, such as irradiation and plasma treatment, are typically regarded as progressive means of starch modification due to their rapid, inexpensive, and eco-friendly nature. These techniques offer advantages such as avoiding the utilization of pollutants, preventing the leakage of hazardous substances into treated items, eliminating the production of unwanted byproducts and the need for catalysts and laborious preparation of samples [11]. Electron beams, a form of ionizing radiation, have been widely studied for their ability to induce both functional and structural alterations in starch. Changes in the size and structure of starch components caused by EBI are accompanied by modifications in hydration and pasting properties, rheological and thermal parameters, and digestibility [12–17]. EBI can directly and/or indirectly impact starch molecules through interactions with electrons, leading to such alterations by degradation and/or cross-linking reactions, depending on the processing parameters [6,11]. Similarly, plasma processing has emerged as a promising non-thermal technology for starch functionalization. Plasma treatment involves interactions of starch surfaces with numerous active species, including electrons, ions, free radicals, excited state and unionized neutral molecules, resulting in cross-linking or depolymerization (degradation) of starch chains [18,19].

In addition, according to Maniglia et al. [20], combining technologies is an innovative way to potentialize the functionalization of starch that needs to be investigated, and synergy opportunities need to be evaluated. The initial alteration in starch structure may serve as preparation, enabling the subsequent modification to exert a stronger effect on the starch structure and functionality [21]. Thus, by choosing a suitable combination, it is possible to complete various mechanisms and minimize certain limits. At the same time, the possible applicability of materials based on modified starch with a focus on a specific application by selecting the appropriate technology requires deeper knowledge about the structure-properties-processing relationship.

In line with this research trajectory, we aim to explore and discuss the effects of RF plasma and EBI on starch properties from a synergistic perspective and differentiate our approach from the existing plasma-irradiation dual treatment approach. RF plasma and EBI were applied sequentially, and the combined effects were evaluated using a range of analytical techniques, including rheological analysis, gel permeation chromatography (GPC), Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). For this study, the sample geometry and processing parameters were selected following the insights provided by prior research [6,8,22,23].

This novel contribution addresses the less explored realm of dual starch modification using solely physical techniques based on ionizing radiation and plasma treatment. While previous research has explored the combination of cold atmospheric pressure plasma (CAP) and EBI for starch modification, this study focuses on employing low-pressure RF plasma as the treatment source, offering distinct advantages and mechanisms. There are obvious differences between RF plasma and CAP in terms of their operating conditions, temperature control, chemical

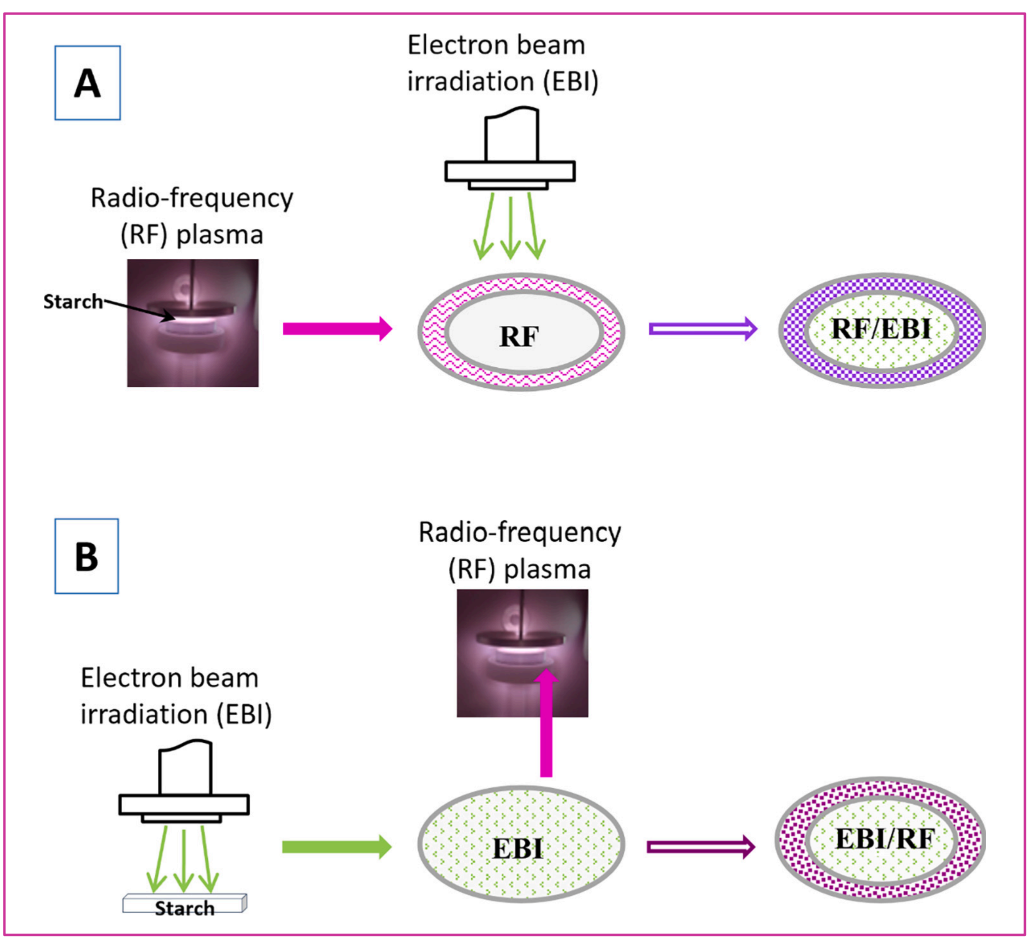

Fig. 1. Illustrative schematic of dual treatment approach for starch modification.

composition, penetration depth, and energy efficiency. RF plasma operates at lower pressure and higher frequency, enabling better temperature control. It generates a uniform plasma distribution [18], consisting of controlled reactive species such as ions and radicals. RF plasma typically affects surface layers due to its shallow penetration depth, making it suitable for surface modification [24]. Recent research has explored the alteration of physicochemical and structural properties in starch from various botanical sources through exposure to low-pressure radio-frequency (RF) plasma. These investigations suggest that the changes in RF plasma-treated starch are primarily influenced by degradation (depolymerization) and cross-linking processes [22,25–28].

This research intends to reveal freshness related to (1) the synergistic impact of low-pressure RF plasma and EBI on functional and structural properties and its level, (2) the importance of considering the treatment sequence when combining these methods for starch modification, which can offer guidance on optimizing the process for improved results, and (3) the indicators for assessing starch sensitivity to ionizing radiation, which can enable better control and predictability in processing. In addition, this study promotes the quantification of synergy in dual starch modification methods since existing literature often relies on qualitative assumptions to describe the synergistic consequences of dual methods on the properties of starch. This quantitative exploration of synergism addresses a notable gap in the contemporary research literature and contributes to a deeper understanding of starch modification for potential applications.

2. Materials and methods

2.1. Materials

Native regular corn starch (S4126; white powder; moisture content: 11.6 ± 0.5 %) was acquired from Sigma-Aldrich Company (St. Louis, MO, USA). The starch used in the processing experiments was in powder form. Additional analytical-grade chemicals were purchased from Sigma-Aldrich Company (St. Louis, MO, USA) and SC Chimreactiv SRL (Bucuresti, Romania).

2.2. Radio-frequency (RF) plasma exposure

Plasma exposures were carried out in an in-house experimental setup that was described in detail in a previous work [22]. Native starch (1 mg/mm^2) was exposed to low-pressure RF plasma at a power of 40 W in air for 10 min. Following exposure, the samples were kept in light-barrier plastic containers at room temperature (22 ± 1 °C), until further investigations were performed. A control sample of native starch was utilized.

2.3. Electron beam irradiation (EBI)

Samples of packed starch (4.5 cm × 4.5 cm) were irradiated in static configuration with an accelerated electron beam with a mean energy of 5.5 MeV, generated at a mean beam intensity of 4 μA by the linear accelerator ALID-7 (INFLPR, Magurele, Romania). In the current study, samples were irradiated at room temperature (22 ± 1 °C) and ambient pressure in an air environment. The irradiation doses (D) used ranged from 5 to 20 kGy, with a mean dose rate of 2.9 ± 0.2 kGy/min, checked by calorimetric dosimetry procedure. The samples were placed in the center of the irradiation field at 54 cm from the accelerator exit window, where the dose uniformity ratio ($U = D_{max}/D_{min}$) was 1.09. The irradiated samples were kept in the absence of light at room temperature (22 ± 1 °C) until subsequent measurements were carried out. A control sample of native starch was utilized.

2.4. RF plasma followed by electron beam irradiation (RF/EBI)

Starch samples were subjected to RF plasma as mentioned in Section 2.2. Afterward, within a 24-h timeframe, these samples underwent irradiation under similar conditions as presented in the preceding section (Fig. 1A). Following treatment procedures, the samples were stored in the absence of light at room temperature (22 ± 1 °C).

2.5. Electron beam irradiation followed by RF plasma (EBI/RF)

Starch samples underwent EBI in the same manner as described in Section 2.3. Subsequently, within a 24-h timeframe, they were subjected to RF plasma as explained in Section 2.2 (Fig. 1B). Afterward, the starches were kept in the absence of light at room temperature (22 ± 1 °C).

2.6. pH measurement

pH was determined for 1 % (w/v) aqueous starch samples as described in detail in [22], at room temperature (22 ± 1 °C).

2.7. Solubility and swelling power

Solubility and swelling power were evaluated according to the concept introduced by Leach et al. [29]. Starch was dispersed in water at room temperature (22 ± 1 °C) with magnetic stirring for 30 min. The mixture was subsequently centrifuged at 5000 ×g for 10 min, and the supernatant was collected. The supernatant was then evaporated at 120 °C until a constant weight was obtained, after which the residue was weighed. The solubility (s, %) was determined as the ratio of the weight of the dried supernatant (residue) to the initial starch weight. The wet sediment obtained after centrifugation was measured, and the weight of the swollen sediment divided by the initial starch weight was used to compute the swelling power (SP [g/g]).

2.8. Paste clarity

The paste clarity was assessed following the method suggested by Craig et al. [30]. The preparation of 1 % (w/v) starch samples involved suspending starch in distilled water within screwcap tubes, which were then immersed in a boiling water bath for 30 min, with intermittent shaking every 5 min to ensure uniform mixing. Subsequently, the samples were allowed to cool to room temperature. The paste clarity was quantified by measuring the light transmittance (T%) at 650 nm using a UV–Vis Carry 100 Bio spectrophotometer (Varian, Inc., USA), with distilled water as the reference, at room temperature (22 ± 1 °C).

2.9. Rheological measurements

The rheological investigation of starch samples was performed utilizing a HAAKE VT® 550 rotational viscosimeter (ThermoHaake, Germany) equipped with an NV coaxial cylinder in the steady shear regime as previously reported [6]. The shear stress, τ, was determined on 5 % (w/v) starch samples for various shear rates at room temperature (25 ± 1 °C). To fit the rheological behavior and to calculate the apparent viscosity, η_a, for shear rate $\dot{\gamma} = 200$ s^{-1} at 25 °C, the Ostwald de Waele rheological model (“power law” mathematical model) was applied:

$$\tau = k \cdot \dot{\gamma}^n \tag{1}$$

$$\eta_a = k \cdot \dot{\gamma}^{n-1} \tag{2}$$

where τ is the shear stress (Pa), k is the fluid consistency coefficient (Pa•s^n), n is the flow behavior index (dimensionless), $\dot{\gamma}$ is the shear rate (s^{-1}), and η_a is the apparent viscosity (Pa•s).

2.10. Gel permeation chromatography (GPC)

The analysis of samples by GPC was carried out according to the

Table 1
Investigated property values of native and modified starch samples.

Property	Irradiation dose (kGy)	Treatment type		
		EBI	RF/EBI	EBI/RF
pH	0	5.0 ± 0.1^{aA}	4.8 ± 0.1^{aB}	4.8 ± 0.1^{aB}
	5	4.3 ± 0.0^{bA}	3.7 ± 0.0^{bB}	3.7 ± 0.0^{bB}
	10	4.0 ± 0.0^{cA}	3.6 ± 0.0^{cB}	3.6 ± 0.0^{cdB}
	15	3.8 ± 0.0^{dA}	3.5 ± 0.0^{dC}	3.6 ± 0.0^{cdB}
	20	3.8 ± 0.0^{dA}	3.5 ± 0.1^{dB}	3.5 ± 0.1^{dB}
s (%)	0	1.34 ± 0.08^{aB}	0.82 ± 0.05^{aA}	0.82 ± 0.05^{aA}
	5	1.87 ± 0.05^{bA}	4.40 ± 0.33^{bB}	2.40 ± 0.32^{bA}
	10	1.82 ± 0.08^{bA}	4.86 ± 0.40^{bcC}	3.60 ± 0.29^{cB}
	15	1.93 ± 0.10^{bcA}	4.57 ± 0.35^{bcC}	3.82 ± 0.15^{cB}
	20	2.20 ± 0.28^{cA}	5.29 ± 0.66^{cC}	3.37 ± 0.37^{cB}
SP (g/g)	0	4.64 ± 0.45^{aA}	3.99 ± 0.38^{aA}	3.99 ± 0.38^{aA}
	5	4.08 ± 0.21^{abA}	3.56 ± 0.23^{abB}	3.87 ± 0.22^{aAB}
	10	3.97 ± 0.16^{bA}	3.54 ± 0.36^{abA}	3.56 ± 0.44^{abA}
	15	3.57 ± 0.27^{bcA}	3.33 ± 0.41^{bA}	3.47 ± 0.41^{abA}
	20	3.30 ± 0.43^{cA}	3.05 ± 0.10^{bA}	3.12 ± 0.35^{bA}
T (%)	0	4.9 ± 0.1^{aB}	2.7 ± 0.0^{aA}	2.7 ± 0.0^{aA}
	5	12.7 ± 0.3^{bC}	9.3 ± 0.1^{bB}	8.5 ± 0.1^{bA}
	10	19.5 ± 0.5^{cC}	11.9 ± 0.2^{cA}	13.6 ± 0.3^{cB}
	15	25.6 ± 0.1^{dC}	12.7 ± 0.2^{dA}	13.5 ± 0.2^{cB}
	20	25.9 ± 0.3^{dC}	15.1 ± 0.3^{eA}	16.1 ± 0.3^{dB}
CI	0	0.71 ± 0.01^{aA}	0.65 ± 0.02^{bB}	0.65 ± 0.02^{cB}
	5	0.70 ± 0.01^{aA}	0.71 ± 0.02^{aA}	0.68 ± 0.02^{bcA}
	10	0.71 ± 0.02^{aA}	0.66 ± 0.02^{bB}	0.73 ± 0.03^{abA}
	15	0.69 ± 0.01^{aA}	0.68 ± 0.01^{bA}	0.68 ± 0.03^{cA}
	20	0.68 ± 0.03^{aB}	0.68 ± 0.00^{abB}	0.76 ± 0.03^{aA}

The native starch form corresponds to the single EBI at 0 kGy, while the single RF plasma processing corresponds to a dual treatment (RF/EBI or EBI/RF) at 0 kGy irradiation dose.
Values within each column for each data set with different lower superscripts are significantly different ($p \leq 0.05$).
Values within each row for each data set with different upper superscripts are significantly different ($p \leq 0.05$).

method described by Han and Lim [31] and Nemtanu and Brasoveanu [12], with slight modifications. Starch (10 mg) was moistened with ethanol (20 μl) and dispersed in 1 M NaOH (500 μl). The resulting mixture was magnetically stirred in a water bath (200 rpm, 15 min, 85 °C) and then diluted to 50 mM NaOH with water and gently stirred again (200 rpm, 30 min, 25 °C). A Waters chromatographic system (USA) consisting of a quaternary pump and an e2695 autosampler, a differential refractive index detector model 2414, an online degasser AF, and a temperature module system was used. Calibration was performed using pullulan standards for two Ultrahydragel columns and a pre-column (Waters, USA). The mobile phase consisted of 50 mM NaOH with a flow rate of 0.5 ml/min. Column and detector temperatures were set at 70 °C and 35 °C, respectively.

The average value of radiation-chemical yield (G) and degradation rate constant (k) were calculated based on the methodology described by Nemţanu and Braşoveanu [32], which applies to all initial molecular weight distributions [33]. The radiochemical yield and rate constant of degradation were estimated using the following equations:

$$\frac{1}{M_n} - \frac{1}{M_{n0}} = G \bullet D \tag{3}$$

$$\frac{M_{n0}}{M_n} - 1 = k \bullet \frac{M_{n0}}{m} \bullet D \tag{4}$$

where M_n and M_{n0} are the number average molecular weights before and after irradiation in (g·mol^{-1}), G is the radiation-chemical yield of degradation (mol·J^{-1}), D is the irradiation dose (kGy), k is the degradation rate constant (kGy^{-1}), and m is the molecular weight of a monomer unit (=162 g·mol^{-1}).

2.11. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of the samples were collected using a Spectrum 100 instrument (Perkin Elmer, Waltham, MA, USA) equipped with a diamond crystal and operated at room temperature (23 ± 1 °C). The spectral data were acquired in ATR mode, averaging 30 scans per sample and covering the wavelength range from 4000 to 600 cm^{-1}, with a resolution of 4 cm^{-1}. Normalization was applied to the recorded spectra employing the Spectrum v. 6.3.2 software. The samples' short-range molecular order was evaluated by deconvoluting the spectra within the range of 950–1120 cm^{-1} and subsequently using the absorbance ratio at 1046/1022 cm^{-1} [34].

2.12. Scanning electron microscopy (SEM)

The examination of granule morphology was conducted using a Thermo Fisher Scientific Apreo S LoVac scanning electron microscope (SEM). Samples were prepared by drop-casting a saturated suspension of starch particles onto aluminum SEM stubs. The dried samples were then blown by compressed nitrogen for excess material removal and analyzed by SEM in Low vacuum mode at 50 Pa water vapor pressure.

2.13. Data mining

The measurements were conducted three times, the mean values with standard deviation are the reported data. Statistical analysis involved a completely randomized design (CRD) and the use of analysis of variance with Duncan's post-hoc test using IBM SPSS Statistics V22.0 software. For statistical significance, a probability value of $p \leq 0.05$ was used. The Principal Component Analysis (PCA) with Spearman's rank correlation coefficients was performed by XLSTAT statistical software V21.5 (XLSTAT, Paris, France) [35], and the Agglomerative Hierarchical Clustering (AHC) method based on Spearman's dissimilarity matrix was carried out by the software MATLAB 2022a (R2022a, MathWorks, USA). Ward's method was applied as an AHC procedure.

Table 2
Parameters of rheological curves of modified starches by investigated treatments.

Irradiation dose (kGy)	EBI		RF/EBI		EBI/RF	
	k (mPa•s^n)	n	k (mPa•s^n)	n	k (mPa•s^n)	n
0	2075 ± 67^{aB}	0.521 ± 0.016^{aB}	4304 ± 57^{aA}	0.415 ± 0.018^{aA}	4304 ± 57^{aA}	0.415 ± 0.018^{aA}
5	722 ± 14^{bA}	0.636 ± 0.012^{bA}	311 ± 7^{bC}	0.686 ± 0.010^{bC}	520 ± 8^{bB}	0.658 ± 0.010^{bB}
10	267 ± 11^{cA}	0.715 ± 0.010^{cA}	110 ± 4^{cC}	0.767 ± 0.007^{cC}	175 ± 4^{cB}	0.748 ± 0.008^{cB}
15	121 ± 9^{dA}	0.782 ± 0.010^{dA}	41 ± 2^{dC}	0.865 ± 0.008^{dC}	73 ± 5^{dB}	0.838 ± 0.010^{dB}
20	63 ± 2^{eA}	0.810 ± 0.009^{eA}	22 ± 2^{dC}	0.899 ± 0.007^{eC}	41 ± 2^{dB}	0.864 ± 0.010^{eB}

The native starch form corresponds to the single EBI at 0 kGy, while the single RF plasma processing corresponds to a dual treatment (RF/EBI or EBI/RF) at 0 kGy irradiation dose.
Values within each column with different lower superscripts are significantly different ($p \leq 0.05$).
Values within each row with different upper superscripts are significantly different ($p \leq 0.05$).

3. Results and discussion

3.1. pH evaluation

The native starch exhibited an acidic pH, slightly increasing ($p \leq 0.05$) after RF processing (Table 1). EBI significantly decreased the pH ($p \leq 0.05$) with increasing irradiation doses up to 15 kGy. Combined treatments intensified starch's acidic character equally, with a greater effect than single treatments (Table 1). For example, irrespective of the treatment order, the combination of 15 kGy and RF plasma led to an ~30 % drop in pH level.

The intensification of starch's acidic nature irradiation in the presence of oxygen may result from the formation of free radicals and various radiolysis products from water molecules within the starch [36]. The plateau phenomenon of pH reduction at doses higher than 15 kGy can also be due to this process and the relatively low moisture content of the starch. In the combined treatments, pre-treatment with RF plasma resulted in sensitization of the starch to irradiation. On the other hand, the RF plasma as a post-treatment of starch had a supportive role in the continuation of radiation-induced degradation. Hence, reactive species generated by RF plasma in the air may alter the starch structure, inducing molecular rearrangements that potentiate the irradiation degradation, regardless of the treatment sequence. Thus, this process might facilitate the development of controlled and tailored starch modifications to meet specific industrial needs.

These findings are consistent with prior data on electron beam irradiation of corn starch [14,23,37,38]. However, combining EBI with corona electrical discharges resulted in varying rates of pH reduction, depending on whether the plasma was applied as a pre-treatment or post-treatment [6]. Instead, no similar outcomes were yielded by combining EBI with RF plasma. This disparity could be explained primarily by differences in the processing parameters such as energy, frequency, and duration of the applied treatment.

3.2. Solubility and swelling power

The native starch had a low solubility (~1.3 %) at room temperature, while reduced ($p \leq 0.05$) by ~40 % after RF plasma processing (Table 1). In contrast, irradiated starch showed enhanced solubility with the increment of irradiation dose ($p \leq 0.05$). Dual treatments significantly enhanced solubility compared to EBI alone (Table 1), with plasma pre-treatment resulting in the highest increase with the irradiation dose, about four times at 20 kGy.

The solubility decrease induced by RF plasma might be attributed to starch molecular rearrangement. During plasma treatment of starch, the dehydration process and generation of free radicals may induce cross-linking of starch chains, fragmentation of molecules, or macro-radical recombination, leading to the rearrangement of starch molecules [8,22,39]. The removal of water molecules during both the vacuum step and RF plasma action potentially reduced the availability of functional groups for solvation causing a decrease in solubility. Conversely, EBI at irradiation doses higher than 5 kGy predominantly causes degrading changes such as chain scission and oxidation [14], affecting starch solubility. EBI-induced solubility increase may be due to the long-chain fragmentation into smaller fragments more soluble in water. Recent investigations on diverse starch sources [14,16,40,41] found increased solubility with increasing electron beam irradiation dose. The significant solubility increase with RF/EBI dual treatment can be due to the sensitization of the chains on the starch granule surface consequent upon the plasma pre-treatment. Following EBI, starch degradation may yield more soluble components. Within EBI/RF treatment, the irradiation produced a sequence of chain degradation and destabilization, generating new ruptures of small and soluble fragments when RF plasma was applied. Therefore, whereas single plasma treatment facilitated molecular rearrangements, reducing solubility, its application as a post-treatment of radiation-degraded starch induced further degradation, emphasizing the solubility increase compared to irradiated starch. As poor starch solubility limits its industrial utilization [3], enhancing its solubility is highly advantageous. This enhancement can facilitate more efficient processing, broaden application possibilities, stimulate the development of innovative products, improve end-product quality, and contribute to environmental sustainability.

The native starch's swelling power insignificantly decreased ($p > 0.05$) following the treatment with RF plasma (Table 1). EBI resulted in a significant decrease ($p \leq 0.05$) in the SP with increasing irradiation dose (Table 1). A treatment of 10 kGy lowered SP by ~14 %, whereas an irradiation dose of 20 kGy reduced it by ~30 %. Similar results were observed when starches from various sources, including rice [13], corn [14], potatoes [16], and broad beans [41], were subjected to an electron beam. Combined treatments also determined a decrease in swelling power, slightly more pronounced than EBI alone (Table 1). RF plasma and EBI both reduced SP, although by different mechanisms. Irradiation may break the main chain into shorter ones with low availability to hold water readily, thus reducing SP [42]. In addition, according to Wang et al. [17], EBI can impair the chain length distribution of branched chain starch (amylopectin), further inhibiting SP. On the other hand, plasma treatment can induce molecular rearrangements [8,22,39] with an enhancement of interactions among starch molecules [10], limiting water infiltration inside the granules and resulting in lower SP values.

3.3. Paste clarity

The native starch showed a relatively low paste clarity ($T < 5$ %). RF plasma increased ($p \leq 0.05$) the opacity, while EBI increased paste clarity with the irradiation dose (Table 1), even at the lowest irradiation dose of 5 kGy. When the treatments were applied together, the paste clarity was enhanced, although to a lesser extent compared with the single EBI (Table 1).

The utilization of RF plasma resulted in an augmentation of the opacity in the starch paste by molecular rearrangements able to increase light reflection. The molecular aggregates likely generated during plasma treatment may facilitate the development of hydrogen bonds

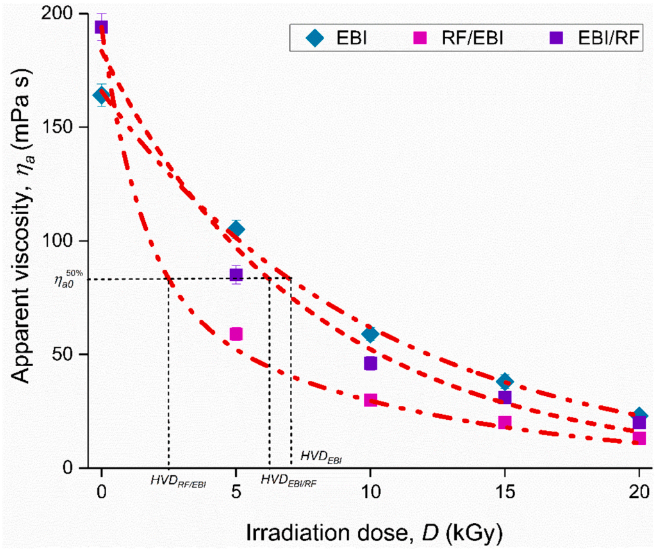

Fig. 2. Variation of apparent viscosity (25 °C, $\dot{\gamma} = 200\ s^{-1}$) for studied starches with the irradiation dose (the native starch form corresponds to the single EBI at 0 kGy, while the single RF plasma processing corresponds to a dual treatment (RF/EBI or EBI/RF) at 0 kGy irradiation dose).

between chains at the expense of bonds with water molecules, which results in light reflection by the granule [22]. Similar findings have been found in studies on different starches exposed to different plasma types [22,43,44]. Instead, the irradiation increased paste clarity due to associative interactions between starch molecules in granules that were weakened by the action of the ionizing radiation on the starch [6]. This evolution is consistent with previous reports on electron beam-irradiated starch from wheat [40], rice, and potatoes [38]. When the RF/EBI combination was applied to native starch, the irradiation led to the degradation of some molecules that had previously been affected by RF plasma pre-treatment. These degradations rather led to the development of soluble, but non-linear aggregates, such as amylopectin branches, and chain fragments linked together. However, when RF plasma was applied post-EBI, it reduced ($p \leq 0.05$) the capacity of the paste to transmit light. Residues of connected chains resulting from the radiation-induced degradation likely linked after plasma treatment, contributing to increased opacity in the irradiated samples.

3.4. Flow characteristics and apparent viscosity

The flow properties of starch samples were assessed using the Ostwald de Waele model. The flow behavior index, n, and consistency coefficient, k, are displayed in Table 2. The flow behavior index n was below 1 for all samples, showing the extent of shear-thinning nature as it deviates from 1, which is a value typical of a Newtonian fluid. The values of n increased with increasing irradiation dose for EBI-based treatments. These findings are consistent with those from corn and wheat starches subjected to an electron beam of 6 MeV [8,45]. Dual-modified starches had greater n values ($p \leq 0.05$) than their single EBI-modified counterparts, with RF/EBI treatment having the greatest effect on the flow behavior index. On the other hand, the consistency coefficient k decreased with increasing irradiation dose for both dual-modified and single-irradiated samples. This trend was opposite to n when considering the treatment methods investigated. Similar outcomes have been observed for corn starch treated by combining EBI and corona electrical discharges [8].

The native starch showed a non-Newtonian, shear-thinning (pseudoplastic) flow, meaning its shear stress increased nonlinearly with the shear rate. After single RF plasma processing, the modified starch retained its non-Newtonian nature, though it was impacted. The irradiation of native starch also influenced its flow, inducing a more Newtonian flow when the irradiation dose was increased. Dual processing of native starch resulted in even greater changes in its pseudoplastic behavior by increasing the irradiation dose relative to EBI alone. Because the consistency coefficient is strongly correlated with the viscosity [46,47], a similar decrease in overall viscosity was expected across the samples studied. These findings are likely due to changes in internal molecular variables, such as molecular weight and its distribution, and chain architecture, influenced by processing parameters. Moreover, these results are consistent with previous research in this field [6,8,48].

The apparent viscosity of starch changed in opposite ways through the two single treatments. When native starch was exposed to RF plasma, the viscosity (25 °C, $\dot{\gamma} = 200\ s^{-1}$) increased ($p \leq 0.05$) by almost 18 %. Conversely, the viscosity decreased exponentially ($p \leq 0.05$) when the irradiation dose increased following EBI ($R^2 = 0.9980$), as shown in Fig. 2. These observations are consistent with previous reports on both low-pressure plasma treatment [49] and e-beam irradiation [6,12,15] of different starches. Dual treatments caused a higher reduction in apparent viscosity compared to EBI alone (Fig. 2). For instance, at a dose of 5 kGy, RF/EBI led to a 68 % decrease in viscosity from its initial value, while EBI/RF caused a 48 % decrease, and single EBI treatment resulted in only a 35 % reduction. Based on these observations, a half-value dose (*HVD*) for apparent viscosity was determined, representing the irradiation dose at which the apparent viscosity decreases to 50 % of its initial value ($\eta_a^{50\%}$). The resulting *HVD* values were 7.2 kGy for EBI, 2.5 kGy for RF/EBI, and 6.3 kGy for EBI/RF. These findings reflect the starch sensitivity to radiation under the tested processing conditions. It is evident that the utilization of RF plasma, regardless of the application sequence in the combined treatment with EBI, increased the starch sensitivity to irradiation. This outcome implicitly led to a reduction in the irradiation dose required to have the same impact as a single EBI. Furthermore, pre-treating the starch with RF plasma before EBI sensitized it most effectively, reducing the irradiation dose necessary to produce the same effect as EBI by approximately threefold.

At the same time, we estimated the characteristic irradiation doses for starches modified under both single (EBI) and dual (RF/EBI and EBI/RF) modes, along with the proportion of the starch granule influenced by RF plasma. These doses reflect a nonlinear reduction in viscosity with increasing irradiation dose and were calculated using models of exponential-like viscosity decrease developed by Braşoveanu and Nemţanu [8]. Our results revealed characteristic irradiation doses of 10.2 ± 0.2 kGy ($R^2 = 0.9980$), 1.5 ± 0.5 kGy ($R^2 = 0.9972$), and 6.6 ± 1.1 kGy ($R^2 = 0.9634$) for EBI, RF/EBI, and EBI/RF, respectively. The proportion of the granule influenced by RF plasma had a value of 53 ± 3 %. While these values are similar in magnitude, they differ from those reported by Braşoveanu and Nemţanu [8] for treatments combining EBI and corona discharge. This difference is likely due to the type of plasma used in the present study, specifically RF plasma versus corona discharges, rather than differences in electron beam energy and irradiation dose rate.

The increase in viscosity induced by RF plasma processing can be assigned to a slight cross-linking-like event at the starch granule surface. The reactive species in plasma can facilitate the removal of a water molecule, resulting in the generation of a new bond between two starch chains [49,50]. Moreover, the resulting entanglement of the chains inhibits their motion and may potentially raise the molecular weight with a noticeable impact on viscosity [6,51]. Previous studies have reported such an increase in viscosity due to cross-linking induced by different plasma treatments, including those on starches from corn [22], sorghum [44], tapioca [49], and aria [50]. The viscosity of starch decreased after EBI in the air. This event is due to possibly oxidative degradation, resulting in smaller molecular fragments and, consequently, reduced viscosity [6,8].

The significant reduction in viscosity observed with dual RF/EBI

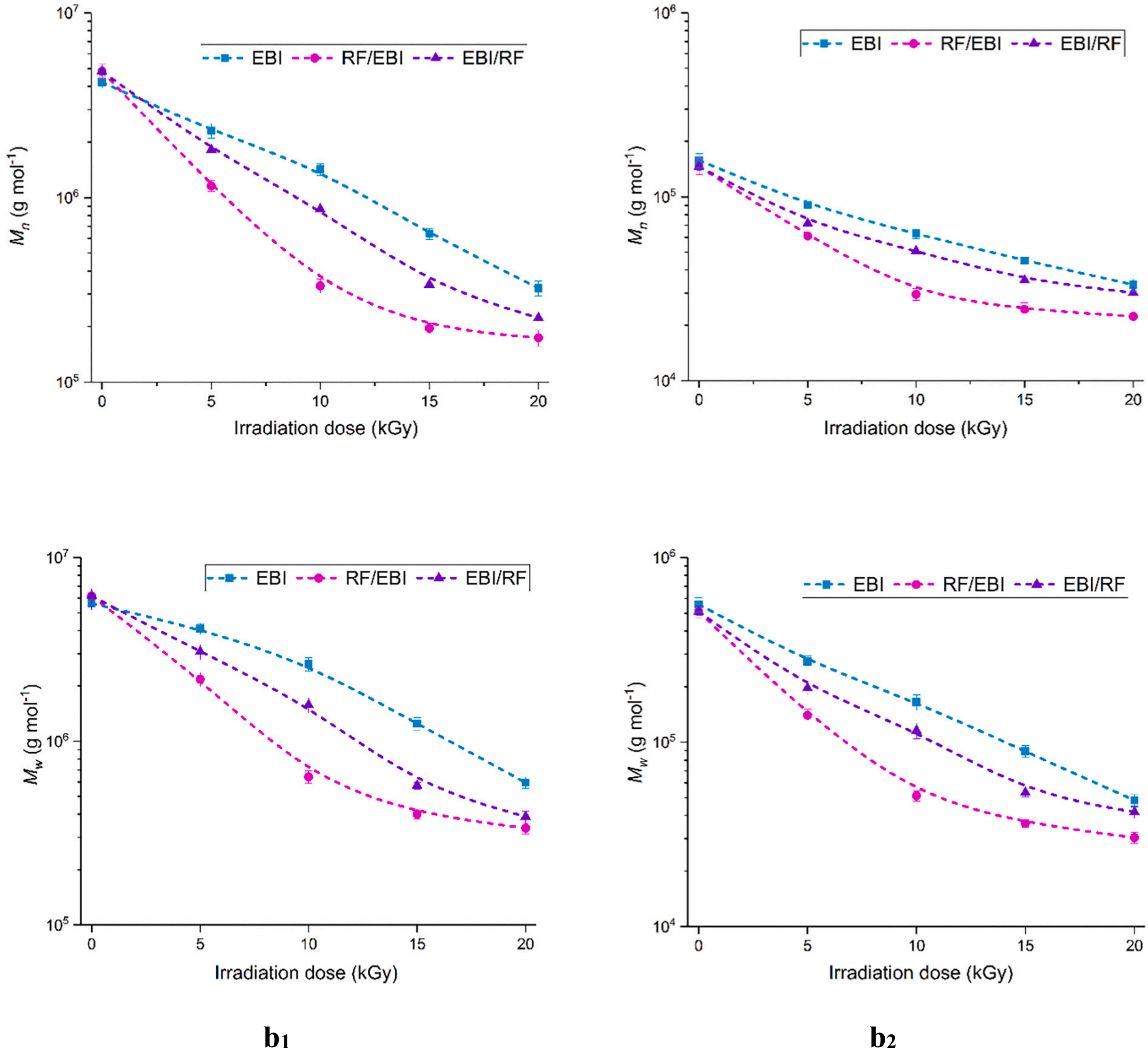

Fig. 3. Evolution of M_n (a_1–a_2) and M_w (b_1–b_2) for Peak 1 and Peak 2, respectively, with irradiation dose increase (the native starch form corresponds to the single EBI at 0 kGy, while the single RF plasma processing corresponds to a dual treatment (RF/EBI or EBI/RF) at 0 kGy irradiation dose).

modification was likely due to a combination of factors. When starch pre-treated with RF plasma underwent EBI, it caused expected degradation within the granule. Simultaneously, an attack occurred on the likely network formed on the granule's surface by RF plasma. This combined action resulted in a dual damage effect with a more pronounced overall impact than irradiation alone, as suggested earlier by Braşoveanu and Nemţanu [8]. All these findings indicate the synergistic impact of combining EBI with RF plasma on the rheological modifications in starch.

As the rheological features of starch are significantly influenced by the molecular weight distribution [6,12,38], it is therefore expected that the molecular weights of the dual-modified samples would also show decreasing dependence with the irradiation dose increase, more pronounced than in the case of EBI alone.

3.5. Molecular weight characterization

The chromatographic study of molecular weight evaluation revealed a bimodal distribution of molecular weight for all samples, due to the contribution of the two constituent starch molecules, amylopectin (Peak 1) and amylose (Peak 2). The molecular weight distribution (number average molecular weight, M_n, and weight average molecular weight, M_w) of native starch was slightly affected by RF plasma ($p > 0.05$), with an increase observed for Peak 1 and a decrease for Peak 2 (Fig. 3). Such a result can be explained by a molecular rearrangement probably similar to intermolecular cross-linking at the surface of the granule, induced by the combined action of vacuum and plasma-generated reactive entities. A comparable effect detected by GPC was found on aria starch using dielectric barrier plasma [50]. It was explained by the potential formation of intercrossed reactions under the plasma reactive species that likely contributed to the production of cross-linked starch. EBI diminished M_n and M_w for both peaks with increasing irradiation dose ($p \leq 0.05$) (Fig. 3). Dual treatments similarly declined the molecular weights ($p \leq 0.05$) as the irradiation dose increased, with greater reductions ($p \leq 0.05$) than EBI alone at the same irradiation doses. Moreover, between the dual treatments, the RF/EBI treatment exhibited the most pronounced effect. These changes in molecular weights indicated rearrangements in starch molecules, resulting in broadened molecular weight distribution for Peak 1, particularly evident at $D < 10$ kGy, and narrow distribution for Peak 2 over the entire range of irradiation doses. However, it can be observed in Fig. 3 that the curves tend to become parallel for $D > 10$ kGy, indicating that the EBI contribution is no longer

Table 3
Parameters of radiation-induced degradation of amylose.

Treatment	$G_{5-20kGy}$ ($\times 10^{-6}$ mol·J^{-1})	$k_{5-20kGy}$ ($\times 10^{-4}$ kGy^{-1})
EBI	1.11 ± 0.04^{a}	1.80 ± 0.07^{a}
RF/EBI	2.12 ± 0.14^{c}	3.43 ± 0.23^{c}
EBI/RF	1.36 ± 0.03^{b}	2.20 ± 0.05^{b}

Values within each column with different lower superscripts are significantly different ($p \leq 0.05$).

dependent on RF plasma, and a saturation effect of plasma contribution emerges. These findings are consistent with results obtained for rheological parameters and solubility.

The decrease in the molecular weights after EBI may be due to degradation processes, which led to fragments with lower molecular weight. This finding agrees with the viscosity decreasing noticed for starch following EBI. The radiation-induced degradation of polysaccharides in a solid state primarily involves dehydration and free radical depolymerization mechanisms, resulting in the production of smaller molecular weight fragments [32,52]. The significant alteration of the molecular weight distribution in dual treatments resulted from both the predominant effect of degradation induced by radiation and the molecular rearrangements caused by RF plasma, depending on the sequence of application. Thus, plasma pre-treatment of starch can induce structure alterations by rearranging amylopectin branched chains, increasing the starch susceptibility to irradiation and potentially more pronounced degradation.

Amylose, the predominantly linear component of starch, undergoes random main-chain scission when treated in the solid state by EBI with irradiation doses up to 30 kGy [32,53]. Considering the mathematical theory of random scission in polymer chains, applicable only to linear polymer molecules [54], we quantitatively characterized the degradation effect of amylose for both EBI alone and dual treatments. Consequently, we calculated the average value of radiation-chemical yield (G) and degradation rate constant (k) as described by Nemţanu and Braşoveanu [32], with $R^2 = 0.9821$–0.9981. The radiation-induced degradation yield refers to the quantity of chain break events occurring within the polymer upon absorption of radiation energy. The degradation rate constant quantifies the rate at which an average number of scissions take place in the initial amylose molecule under irradiation. Higher values for both G and k were noticed for combined treatments when compared to EBI alone (Table 3). RF plasma pre-treatment showed the most prominent degradation effect and resulted in the highest values for G and k among all treatments. The results observed for functional properties and molecular weight distribution aligned with the outcomes. The study found that the values obtained fall within a similar range as those reported previously for amylose and starch [6,12,32,53,55]. Another study has reported higher rate constant values for dual treatments with corona discharges and EBI [6]. However, various factors, including processing parameters, native material characteristics, and molecular weight determination methods, may have contributed to these differences.

Therefore, both amylose and amylopectin can be impacted to different degrees by dual alteration, involving multiple factors and complex reaction pathways. Further dedicated research should be performed for a better understanding of these complex reactions.

3.6. Spectral analysis

The spectral characteristics of the starch samples were evaluated using FTIR spectroscopy. Analyzing the FTIR spectrum of the native sample revealed the major bands' characteristics of starch: the O—H stretching band (3000–3700 cm^{-1}), the C—H stretching band (3000–2800 cm^{-1}), the O—H vibrations from bound water molecules (1550–1780 cm^{-1}), the fingerprint vibrational modes (1550–800 cm^{-1}), which include the C—O and C—C stretching bands and the glycosidic bond band, as well as the vibrational modes of the pyranose ring of the glycosidic unit (below 800 cm^{-1}). Fig. 4 shows both the complete (A) and deconvoluted (B) spectra of native starch and starches modified with single and dual treatments under the identical irradiation dose of 15 kGy.

All modified starches showed spectral patterns comparable to native starch. No new functional groups could be detected in the spectra of modified starches. Nevertheless, slight alterations in the intensity, width, or position of certain bands were noticed. For instance, plasma-modified starch had a shift in the band associated with O—H stretching vibrations to a higher wavenumber (from 3291 cm^{-1} for the control sample to 3307 cm^{-1}), along with a slight reduction in intensity and width. The band attributed to O—H deformation vibrations in the bound water [56,57] shifted from 1645 cm^{-1} for native starch to lower wavenumbers around 1639 cm^{-1} for plasma and dual modified starches, independent of the single treatment sequence. Dual-modified samples showed a significant decrease in the intensity and width of this band. The peak at 1150 cm^{-1} assigned to the C(1)—O—C(5) stretching band [58,59] maintained its position regardless of the applied treatment but broadened and increased for plasma-modified starch. In contrast, irradiated and dual-modified samples showed a narrowing of the band with decreased intensity. There was no consistent pattern of spectral changes as the irradiation dose increased. This finding aligns with previous observations on cereal starch modification using EBI [6,37,60].

Table 1 presents the results for the crystal short-range order structure (*CI*) using the ratio between bands in the crystalline region (1046 cm^{-1}) and amorphous region (1022 cm^{-1}). RF plasma modification of starch led to a decrease in this ratio ($p \leq 0.05$). A similar result was obtained when corn starch was exposed to RF plasma under a hexamethyldisiloxane atmosphere [56]. Increasing the irradiation dose caused a modest reduction in this ratio ($p > 0.05$). Dual-modified starches also revealed slight variations in this ratio compared to native starch. These observations are in agreement with a recent report on Chinese Yam starch modification through combined dielectric barrier discharge with EBI [9].

The FTIR analysis results showed distinct effects of single treatments on the starch molecular structure, detectable especially in the O—H and C(1)—O—C(5) stretch bands. RF plasma, under herein conditions, strengthened intermolecular bonds, indicating an apparent network formation and increased hydrogen bond robustness, potentially due to water molecule elimination through both vacuum step and plasma action. This finding is consistent with previous observations reported by Braşoveanu et al. [22]. On the contrary, irradiation degraded the starch molecular structure, weakening hydrogen bonds and breaking glycosidic bonds, potentially resulting in lower molecular weight structures by increasing the irradiation dose. This result concurs with previous research on corn starch exposed to the electron beam [6,37]. Dual treatments mainly resulted in starch structural breakdown comparable to single irradiation, without finding a distinct typology based on irradiation dose or single treatment sequence. This observation might indicate that random changes occurred in different molecule regions. Modifications to the overall treated starch structure can be complex and out of proportion to the irradiation dose or the single treatment applied, leading to such variability.

Although our recorded FTIR spectra revealed no new functional groups that could have been formed by plasma action or radiation-induced degradation, this absence may be explained by the formation of a negligible number of such groups. This assumption is supported by previous research on various starches modified with cold plasma [10,22,49], showing that even after treatments lasting up to 30 min, cross-linking reactions only altered the initial spectral characteristics of starch rather than producing new functional groups. Similarly, irradiating corn starch with electron beams up to 50 kGy at a dose rate of 2 kGy/min caused neither the appearance nor disappearance of functional groups [6,14]. Moreover, an insignificant number of oxidized groups in potato starch had no substantial impact on its chemical composition

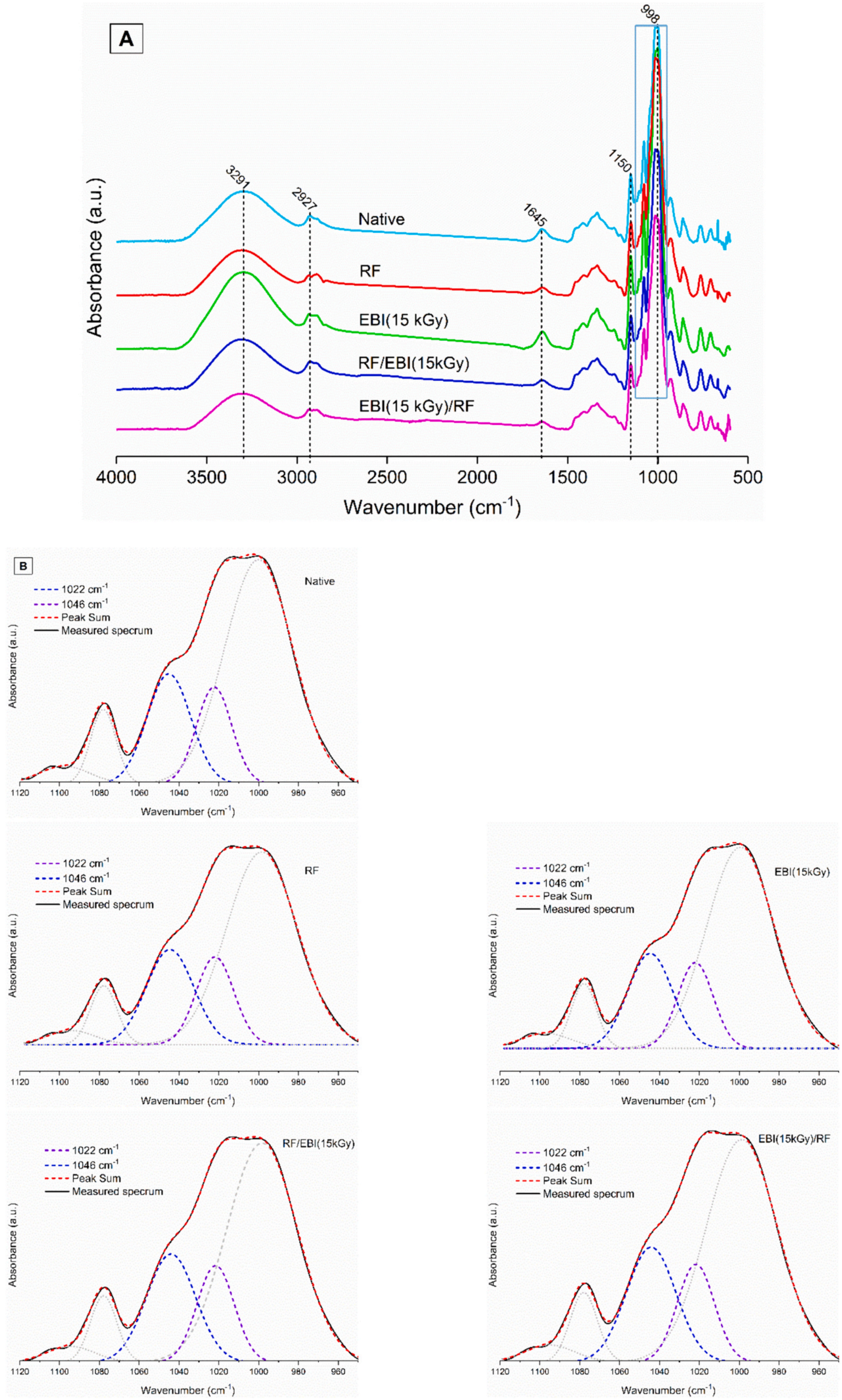

Fig. 4. (A) FTIR spectra of native and modified starches; (B) deconvoluted FTIR spectra within the range of 950–1120 cm^{-1} for native and modified starches.

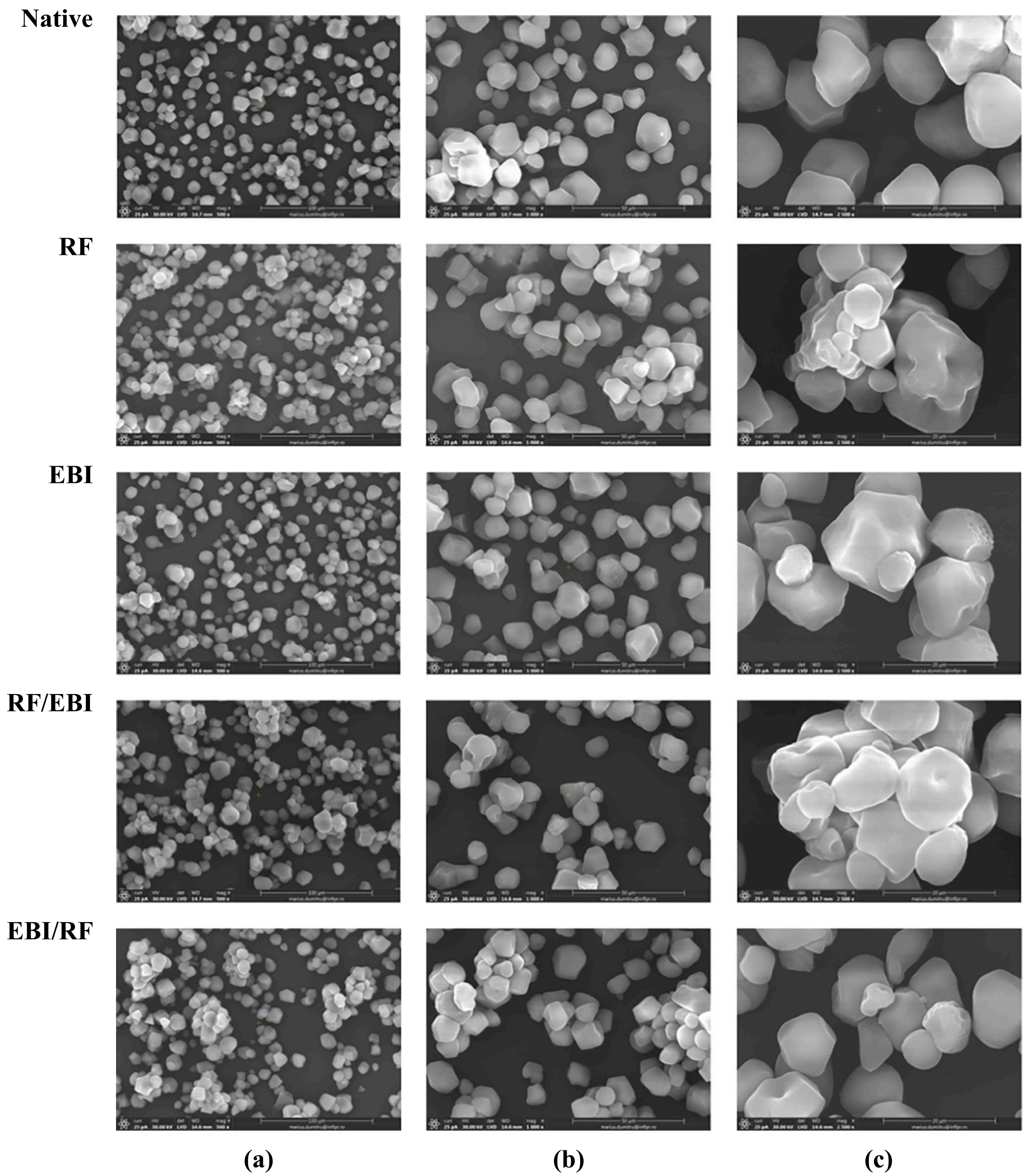

Fig. 5. SEM images for studied starches (irradiation dose of 20 kGy) corresponding to a magnification of (a) 500×, (b) 1000×, and (c) 2500×.

[61], as seen in similar IR spectra of native and EBI-modified starch in the 110–440 kGy range. According to Kizil et al. [62], irradiation of starches under non-aqueous conditions (granular form) leads to subtle structural changes that are challenging to detect using FTIR spectroscopy.

3.7. *Granule morphology*

SEM micrographs in Fig. 5 show the different magnifications of starch samples. Native corn starch granules appeared polyhedral with a slightly rough surface, sporadically concave, and tended to cluster together. Plasma-modified starch generally retained granule integrity, but with more aggregation than the control sample, as reported in previous studies [50,51,63]. Some small cavities or pores on the granule surfaces were also noted, consistent with earlier reports [9,26,63]. In contrast, the samples modified through EBI had fewer agglomerated granules than native starch. The overall granule shape remained unaffected, but smaller-sized granules and small pits appeared due to EBI. According to Luo et al. [64], the degradation of corn starch granules began at an irradiation dose of 10 kGy, with increasing doses resulting in further destruction. Yu et al. [15] found cavity formation and depressions on the surface of corn starch granules even at lower doses (<5 kGy). However, it is essential to note that factors like the amylose/amylopectin ratio, as suggested by Yu et al. [15], as well as processing parameters such as irradiation dose and dose rate, significantly influence the impact level of EBI on granule morphology.

Combined treatments reduced granule size and increased clustering, with holes or pores observed on granule surfaces, potentially influencing the modified starch properties. As suggested by Liang et al. [9], increased granule roughness and aggregation are mostly related to plasma treatment. Therefore, dual treatments with RF plasma and EBI induced more granule aggregation and surface irregularities than single

Table 4
Synergistic ratio for the investigated starch properties, at the same irradiation dose of 10 kGy.

Treatment	pH	*s* (%)	*SP* (g/g)	*T* (%)	*k* (mPa•s^n)	*n*	η_a (mPa•s)	*Mw* (Peak 1)	*Mw* (Peak 2)	*CI*
RF/EBI	1.2	15.9	0.9	1.2	1.3	5.1	1.4	1.8	1.2	0.8
EBI/RF	1.1	10.2	0.9	1.5	1.2	4.7	1.3	1.5	1.1	0.3

Table 5
Correlation matrix (Spearman = r_s) of the studied variables.

Variables	Irradiation dose (kGy)	Pre-RF time (min)	Post-RF time (min)	pH	*s* (%)	*SP* (g/g)	*T* (%)	*n*	η_a (mPa•s)	*Mw* (Peak 1)	*Mw* (Peak 2)	*CI*
Irradiation dose (kGy)	**1**	0.000	0.000	**−0.699**	0.511	**−0.876**	**0.806**	**0.941**	**−0.943**	**−0.927**	**−0.915**	0.021
pre-RF time (min)	0.000	**1**	−0.385	−0.442	**0.611**	−0.351	−0.364	0.221	−0.261	−0.260	−0.351	−0.454
post-RF time (min)	0.000	−0.385	**1**	−0.208	−0.013	−0.078	−0.130	−0.013	0.033	−0.026	−0.078	0.107
pH	**−0.699**	−0.442	−0.208	**1**	**−0.912**	**0.881**	−0.248	**−0.859**	**0.868**	**0.895**	**0.876**	0.070
s (%)	0.511	**0.611**	−0.013	**−0.912**	**1**	**−0.767**	0.095	**0.705**	**−0.733**	**−0.758**	**−0.770**	−0.156
SP (g/g)	**−0.876**	−0.351	−0.078	**0.881**	**−0.767**	**1**	−0.481	**−0.912**	**0.949**	**0.952**	**0.953**	0.111
T (%)	**0.806**	−0.364	−0.130	−0.248	0.095	−0.481	**1**	**0.618**	**−0.604**	**−0.569**	**−0.559**	0.291
n	**0.941**	0.221	−0.013	**−0.859**	**0.705**	**−0.912**	**0.618**	**1**	**−0.989**	**−0.987**	**−0.972**	−0.043
η_a (mPa•s)	**−0.943**	−0.261	0.033	**0.868**	**−0.733**	**0.949**	**−0.604**	**−0.989**	**1**	**0.993**	**0.992**	0.050
Mw (Peak 1)	**−0.927**	−0.260	0.026	**0.895**	**−0.758**	**0.952**	**−0.569**	**−0.987**	**0.993**	**1**	**0.981**	0.029
Mw (Peak 2)	**−0.915**	−0.351	0.078	**0.876**	**−0.770**	**0.953**	**−0.559**	**−0.972**	**0.992**	**0.981**	**1**	0.106
CI	0.021	−0.454	0.107	0.070	−0.156	0.111	0.291	−0.043	0.050	0.029	0.106	**1**

Values in bold are different from 0 with a significance level of alpha = 0.05.

treatments.

3.8. Remarks on the synergy effect

The data demonstrate that the combination of RF plasma and EBI dual treatments produced a more amplified effect compared to each treatment alone. To examine the treatment synergism, we calculated the synergistic ratio (SR), which compares the combined treatment effect to the sum of the effects of the two single treatments. Using the definitions of Kosman and Cohen [65] and Abbott's formula [66], Braşoveanu and Nemţanu [8] provided a detailed description for assessing the synergistic effect of combined treatments involving EBI and corona electrical discharges on apparent viscosity, which served as the basis for quantification of SR in the present study. SR values above one indicate synergy, below one suggest antagonism, and one imply additivity.

Both dual treatments, regardless of the treatment sequences, resulted in a synergistic impact ($p \leq 0.05$) on properties such as pH, solubility, clarity, rheological features, and molecular weights while they may have had a "protective" effect on properties like *SP*, and *CI* up to 10 kGy. Table 4 provides an example of SR for starches treated with both combined treatments at a 10 kGy irradiation dose. The utilization of RF plasma as a pre-treatment, rather than post-treatment, had a superior synergistic impact. This is consistent with earlier research on starch tailoring using EBI with corona electrical discharges. According to Braşoveanu & Nemţanu [8], this finding can be explained by dual damages within the granules caused by high-energy electrons and radicals from radiolysis during EBI, alongside disruption of the surface molecular rearrangements by plasma pre-treatment, while these molecular rearrangements during plasma post-treatment appear to be limited by the degradation induced by EBI.

This study contrasts the effects of combining 10 min of RF plasma (low-pressure plasma) with EBI at 3 kGy/min with the previous one [8] of combining 5 min of corona discharges (atmospheric plasma) with EBI at 2 kGy/min. It was noticed that the synergistic effect for apparent viscosity achieved with RF plasma and EBI is comparable to that obtained with corona discharges and EBI, though to a lesser extent, at the same dose of 10 kGy, irrespective of the sequence of treatments. This difference primarily arises from the processing parameters, including dose rate, type of plasma, and duration of plasma treatment. Therefore, this research reveals the importance of systematically investigating the combined treatments and refining the processing parameters for each treatment combination to enhance its efficiency and cost-effectiveness.

3.9. Data mining

To evaluate the variable interrelation in the dual processing of starch for a clear distinction of the dual modification methods depending on the order of single method application, the experimental data underwent dimensionality reduction through the utilization of Principal Component Analysis (PCA) and observation categorization using the Agglomerative Hierarchical Clustering (AHC) method.

In Table 5, the correlation matrix for the studied variables, mostly utilizing Spearman's rank r_s correlation coefficients, reveals significant relationships between processing parameters (applied treatments) and starch properties (output variables). Specifically, irradiation dose (*D*) showed strong negative correlations with viscosity (η_a) and molecular weights (M_w Peak 1 and M_w Peak 2), while pH and swelling power (*SP*) had good negative correlations, and paste clarity (*T*%) exhibited a good positive correlation. Solubility (*s*%) exhibited a moderate positive correlation, while crystallinity index (*CI*) had no significant correlation with irradiation. Correlations between starch properties and RF plasma were generally weak (r_s = 0.30–0.49) for plasma pre-treatment and negligible ($r_s < 0.29$) for plasma post-treatment. However, plasma pre-treatment notably impacted solubility more than plasma post-treatment and EBI. Furthermore, among the properties under investigation, the highest significant correlations were found between (η_a and M_w Peak 1 and M_w Peak 2), (η_a and *SP*), (η_a and pH), (pH and M_w Peak 1 and M_w Peak 2), (pH and *SP*), (*SP* and M_w Peak 1 and M_w Peak 2) and with correlation coefficients of 0.993, 0.992, 0.949, 0.868, 0.895, 0.876, 0.881, 0.9952, and 0.953, respectively.

In PCA, cumulative variability reached 80.544 % (~81 %) after the two principal components (**F2**), 90.622 % (~91 %) after the three principal components (**F3**), 96.113 % (~96 %) after the fourth principal components (**F4**). An optimal minimum cumulative variance, often used to determine the number of factors, is typically considered to be 80 % [67]. Thus, two factors were suitable for dimension reduction in this study. However, the highest squared cosines for each variable, up to the fourth factor, were as follows: **F1**: *D*, pH, *s*%, *SP*, *n*, η_a, and M_w; **F2**: pre-

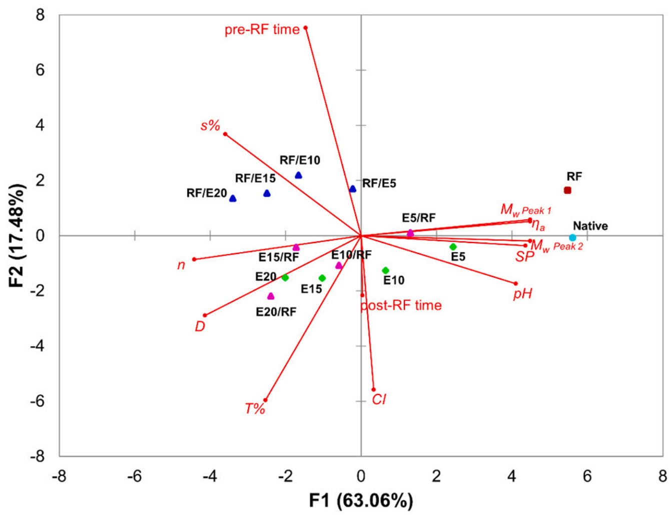

Fig. 6. Factor scores in PCA Biplot.

RF time and *T*%; **F3**: post-RF time; and **F4**: *CI*, reflecting the correlation of these variables with their corresponding principal component (or axis). Consequently, **F1** and **F2**, which collectively explain 80.54 % of the cumulative variance, exhibited major associations with EBI and functional properties, particularly evident in the case of **F1**. Conversely, **F2** had predominant correlations with plasma pre-treatment. Fig. 6 illustrates the samples depicted as points mostly characterized by factor scores, while the initial variables are represented as vectors within the plane defined by the main principal components (**F1** and **F2**). Dual-modified starches occupied distinct quadrants relative to native starch. In general, dual-modified samples shifted significantly to the left side of F1. The samples pre-treated with plasma moved to the left with $F1 < 0$ while retaining $F2 > 0$, mirroring the behavior of plasma-modified starch. Conversely, samples subjected to post-irradiation plasma treatment tended to be located in the same quadrants as the EBI-modified samples, when $F2 < 0$. This visual distinction shows the divergence of dual-modified samples from both native starch and single-modified starches. This analysis revealed the significant influence of the sequence of single treatments in determining the nature and extent of the modifications, as demonstrated by the observable physicochemical variations among the tested samples.

AHC clustering, based on Spearman's dissimilarity (Fig. 7), classified samples into three main clusters at a cut-off value of approximately 0.045. This classification demonstrated the distinct grouping of samples based on their treatment methods (Table 6), with each cluster representing a specific treatment category: non-irradiated samples, namely native and plasma-modified starches (Cluster 1), irradiated starches (Cluster 2), and dual-modified starches (Cluster 3).

4. Conclusions

The effects resulting from the sequential modification derived from the joint utilization of RF plasma and EBI on the physical, chemical, and structural attributes of starch were investigated, comparing them with the single methods for irradiation doses ranging from 5 to 20 kGy. Key findings include:

Table 6
AHC analysis results through class.

Class	1	2	3
Objects	2	5	7
Sum of weights	2	5	7
Within-class variance	0.003	0.001	0.001
Minimum distance to centroid	0.040	0.014	0.014
Average distance to centroid	0.040	0.026	0.028
	0.040	0.037	0.047
	Native	E5	RF/E5
	RF	E10	RF/E10
Maximum distance to centroid		E15	RF/E15
		E20	RF/E20
		E5/RF	E10/RF
			E15/RF
			E20/RF

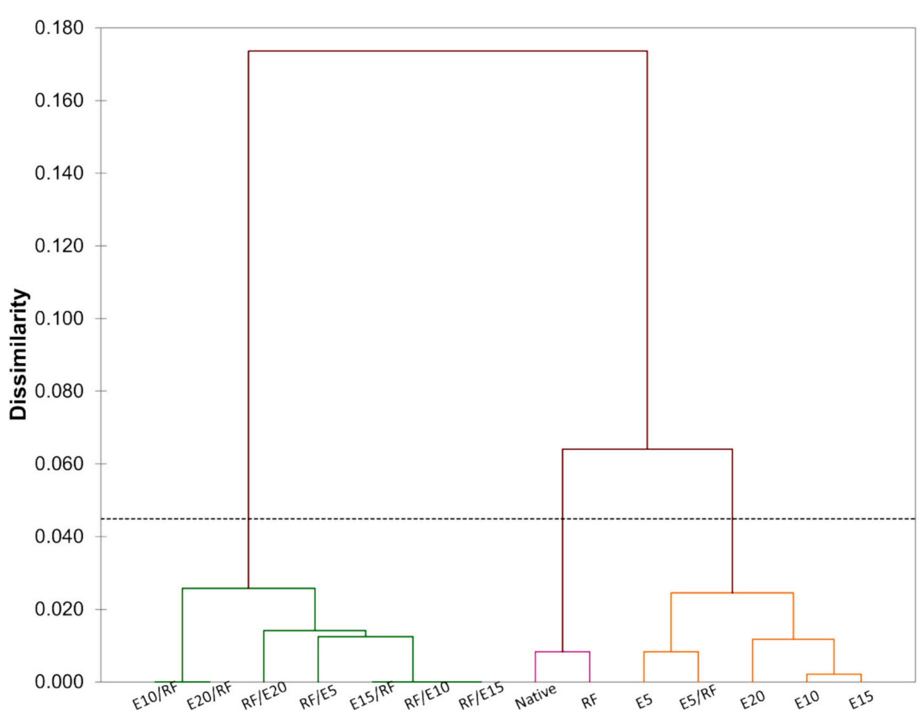

Fig. 7. Dendrogram obtained from AHC.

- Dual treatments, regardless of sequence, had a significant synergistic effect on starch properties up to 10 kGy. These treatments enhanced acidity, solubility, and paste clarity while reducing rheological characteristics and preserving non-Newtonian behavior.
- Dual modification led to enhanced granule aggregation and surface imperfections, with slight changes in spectral characteristics and crystal short-range order structure compared to single treatments.
- Sequential modification resulted in a broader molecular weight distribution of amylopectin below 10 kGy and a narrower distribution of amylose over the entire radiation dose range. This was due to the predominant degradation induced by EBI and molecular rearrangements at the granule surface caused by RF plasma, each exerting distinct effects on various molecular regions. Regardless of the treatment sequence, amylose experienced random chain scission.
- RF plasma pre-treatment significantly impacted starch, leading to the highest observed synergistic ratio, as well as the highest average radiation-chemical yield and degradation rate constant for amylose.
- Correlations between starch properties and processing parameters were primarily identified for irradiation dose, with RF plasma playing a less significant role.

This study shows that synergy between EBI and RF plasma can reduce the irradiation dose, making starch more sensitive to radiation, with the advantages of cost savings and technological applications broadening. For example, these findings can have a notable impact on the utilization of starch in food applications, where it is generally desirable to limit the maximum absorbed irradiation dose under 10 kGy. This research provides valuable insights for experimental designs and enhances understanding of plasma treatment and electron beam irradiation in starch-based material studies. Although the study has examined a well-defined range of processing parameters, further investigation is required to generalize its findings. Future research should focus on addressing these limitations by elucidating the specificity of the processes across various parameter domains and evaluating both technical-operational and economic feasibility.

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CRediT authorship contribution statement

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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.

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