

Starches Modified by Combination of Phosphorylation and High-Voltage Electrical Discharge (HVED) Treatment

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Starch is extensively used in the food industry as a texture modifier, a fat substitute, and in other applications. To optimise starch functional properties for specific use, it is subjected to various modifications. High-voltage electrical discharge (HVED) treatment, as a non-thermal and rapid process, was applied in this research as a single method and in combination with phosphorylation in order to explore its potential for improving starch physicochemical properties. Maize, wheat, potato, and tapioca starches were modified, and $\text{Na}_3\text{P}_3\text{O}_{10}$ and Na_2HPO_4 were used for phosphorylation. Starch gelatinisation parameters (by DSC); paste clarity; and contents of amylose, damaged starch, and resistant starch were determined; and FTIR-ATR spectra were recorded. All modifications reduced the enthalpy of gelatinisation and decreased contents of amylose, resistant starch, and damaged starch. The effect of the HVED treatment on starch properties depended on starch type and combinations with chemicals. HVED could act as an aid in the starch phosphorylation process since the properties analysed were more effectively improved when HVED was combined with phosphorylation than by phosphorylation alone.

ABBREVIATIONS

HVED – High-voltage electrical treatment, DSC – Differential scanning calorimetry, FTIR-ATR – Fourier-transform infrared spectroscopy-attenuated total reflectance, and % T – % transmittance.

INTRODUCTION

Starch has an already well established role in food, pharmaceutical, paper, and packaging industries; however, in its native form it often does not come up to desired functional properties. Therefore, different modifications have been applied to improve its properties, including mainly chemical processes. Increasing demands for reducing chemical usage, waste generation, and energy exploitation have led to novel technique applications in all aspects of food processing and production. High-voltage electrical discharge (HVED)

treatment has emerged as one of the non-thermal and rapid techniques applicable in decontamination of food, waste treatment, and extraction of bioactive compounds [Barišić *et al.*, 2020]. It is a process of releasing energy between two electrodes submerged in a liquid medium [Rosello-Soto *et al.*, 2015], which leads to the disruption of organic cells and enhanced extraction of different compounds. In addition, electrical discharge causes the formation of electrically-charged compounds (ions, free radicals), which induce chemical changes of the treated material. Most often, a pin-to-plate configuration is used, generating direct current glow or pulsed corona [Vanraes *et al.*, 2016]. If gas is available (air, N_2 , CO_2 , Ar, *etc.*) during the HVED treatment, plasma will be generated. There is a significant number of articles dealing with the influence of cold plasma on starch properties, with reported changes in starch structure, accompanied with cross-linking and/or formation of new functional groups [Bie *et al.*, 2016a,b; Khorram *et al.*, 2015; Thirumdas *et al.*, 2017]. However, to the best of authors' knowledge, there is no relevant data on the influence of other types of HVED or combination of HVED with chemical

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modification of starch. The aim of this research was, therefore, to explore if HVED treatment in a water suspension induces changes in the physicochemical properties of starch, how it reflects on starch properties, and if it may be used as an aid in chemical modification of starch to increase process efficiency, by activating starch molecules. Phosphorylation of starch with $\text{Na}_5\text{P}_3\text{O}_{10}$ or Na_2HPO_4 was chosen for the research because it has been reported that these modifications also result in a combined effect of cross-linking and substitution [Sechi & Marques, 2017]. The additional treatment of HVED could favour one of the reactions, enabling easier control of the process and obtaining a desired compound (cross-linked or substituted).

MATERIAL AND METHODS

Materials and chemicals

Tapioca, potato, and maize starches were kindly provided by Cargill (Minneapolis, MN, USA). Wheat starch was isolated at the Faculty of Food Technology Osijek, Josip Juraj Strossmayer University of Osijek (Croatia) as described in our previous paper [Ačkar *et al.*, 2010]. $\text{Na}_5\text{P}_3\text{O}_{10}$ or Na_2HPO_4 used for phosphorylation were obtained from Acros Organics (Geel, Belgium). Resistant starch assay kit, amylose/amylopectin assay kit, and starch damage assay kit were purchased in Megazyme (Bray, Ireland).

High-voltage electrical discharge (HVED) treatment of starch

Starch was suspended in demineralised water (1 g/mL) and treated with HVED (30 kV, 70 Hz, 30 min) with stirring at a magnetic stirrer. The HVED device was custom-made for the Faculty of Food Technology in Osijek by Inganire CPTS1. It consists of a chamber, containing a stainless steel needle (diameter 2.5 mm) and a plate electrode (diameter 45 mm), connected to a high-voltage pulse generator. The distance between the electrodes during all treatments was 2 cm. After treatment, the starch suspension was centrifuged at 3000 rpm/5 min (Centra MP4R, IEC, Needham Heights, MA, USA) to remove excess water, air-dried overnight, and then dried in the oven at 130°C until the moisture content of <85 g/100 g was achieved.

Phosphorylation of starch with $\text{Na}_5\text{P}_3\text{O}_{10}$

Starch was phosphorylated with $\text{Na}_5\text{P}_3\text{O}_{10}$ according to the method described by Lim & Seib [1993]. Briefly, 2.5 g of $\text{Na}_5\text{P}_3\text{O}_{10}$ and 2.5 g of Na_2SO_4 was dissolved in 200 mL of demineralised water. Next, 100 g of starch was suspended in the solution by stirring at a magnetic stirrer for 20 min. The pH value of the suspension was set to 10.0 by drop-wise adding of NaOH (0.45 M), and the suspension was stirred for another hour. Then, it was centrifuged at 3000 rpm for 5 min (IEC Centra MP4R), water was discarded, and starch was air-dried overnight and thermally-treated at 130°C/2 h. Starch was then suspended in 250 mL of water, centrifuged, and washed three times with water. Thus obtained starch was dried at ambient temperature until the moisture content of <85 g/100 g was achieved.

Phosphorylation of starch with Na_2HPO_4

Phosphorylation with Na_2HPO_4 was conducted according to the method described by Sung *et al.* [2005] and Prasanthi & Rama Rao [2010] with slight modifications. Briefly, starch (100 g) and Na_2HPO_4 were suspended in 200 mL of demineralised water and stirred at a magnetic stirrer for 30 min. The suspension was centrifuged, starch was thermally treated and washed as described above.

Combinations of HVED treatment and phosphorylations

When starch was first treated with HVED, a suspension was prepared as described in the “High-voltage electrical discharge (HVED) treatment of starch” section and then phosphorylated with $\text{Na}_5\text{P}_3\text{O}_{10}$ or Na_2HPO_4 . When starch was first phosphorylated, the chemical modification with $\text{Na}_5\text{P}_3\text{O}_{10}$ or Na_2HPO_4 was done prior to the HVED treatment.

Differential scanning calorimetry (DSC) analysis

A DSC 822E differential scanning calorimeter (Mettler Toledo, Giessen, Germany) was used to determine gelatinisation parameters of starches. Samples were analysed without pretreatment, as dry powders. Starch (on dry matter – d.m. – basis) and water were mixed in a ratio of 1:3 (w/w) in standard Al-crucibles (40 μL), hermetically sealed, and equilibrated at ambient conditions for 20 min. An empty aluminium pan was used as a reference. Starch samples were heated at the rate of 4°C/min from 25°C to 100°C and changes in enthalpy (ΔH in J/g d.m. of starch), onset temperature (t_o), peak temperature (t_p), and endset temperature (t_e) were obtained from the exotherm DSC curves. Analyses were done in triplicates.

Paste clarity determination

Paste clarity was determined (in triplicates) according to the method described by Raina *et al.* [2006]. To this end, 1% starch suspensions (on starch d.m. basis) were heated for 30 min in boiling shaking water bath (200 rpm). After 1-h holding at room temperature, % transmittance (%T) was measured at 650 nm against distilled water as a blank (Camspec M501 spectrophotometer, Leeds, UK).

Determination of contents of amylose, damaged starch, and resistant starch

Megazyme kits were used for analyses. Amylose content was determined according to the method described by Gibson *et al.* [1997]. Amylopectin was precipitated with concanavalin A and separated from amylose by centrifugation. Both amylose and amylopectin were enzymatically hydrolysed to glucose, which was measured spectrophotometrically (Camspec M501 spectrophotometer) after reaction with glucose oxidase with peroxidase and 4-aminoantipyrine reagent. Damaged starch content was determined according to the American Association of Cereal Chemists Method 76–31.01 [AACC, 2010] and resistant starch content according to the Association of Official Analytical Chemists Method 2002.02 [AOAC, 2005]. The contents of amylose (%), damaged starch (%), and resistant starch (g/100 g d.m.) were determined in triplicates.

Fourier-transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR) analysis

FTIR-ATR spectra were recorded using a Cary 630 spectrometer (Agilent, Santa Clara, CA, USA) in the range of 4000–650 cm^{-1} . In total, 32 spectra of each sample were recorded and averaged with a spectral resolution of 16 cm^{-1} . Samples were analysed without pretreatment, as dry powders.

Statistical analysis

All modifications were repeated three times, and for each modification one composite sample was made and taken for further analysis. All analyses were done in triplicates, and the results obtained were statistically analysed by the analysis of variance and Fischer LSD test ($p < 0.05$) in Statistica® 13 software (TIBCO Software Inc., Palo Alto, CA, USA). Results are expressed as mean value \pm standard deviation.

RESULTS AND DISCUSSION

In order to explore HVED influence on starch properties and the potential of combining HVED with chemical modifications, four starch types (maize, wheat, potato, and tapioca) were treated with HVED alone, phosphorylated ($\text{Na}_5\text{P}_3\text{O}_{10}$ or Na_2HPO_4) alone, and phosphorylated in combination with HVED prior or after the chemical modification. Gelatinisation parameters (t_0 , t_p , t_e , and ΔH), paste clarity, FTIR-ATR spectra, as well as contents of amylose, damaged starch, and resistant starch were determined to evaluate the effect of the treatments.

The gelatinisation parameters of native and modified starches are shown in Table 1. Generally, the HVED treatment resulted in a decrease in gelatinisation temperatures (with the exception of endset temperature of maize starch), but the change was not always statistically significant. Similarly, although not always statistically significant, the enthalpy of gelatinisation of the HVED-treated starches was lower compared to that of native ones (except for tapioca starch, where it was higher). This implies that the HVED treatment could cause changes in the morphology of starch granules. Other researchers reported the formation of fissures and cavities [Thirumdas *et al.*, 2017], enlargement of channels, and partial fractioning of starch granules [Bie *et al.*, 2016a]. All these changes facilitate water penetration into the granules and gelatinisation, reflected in decreased gelatinisation temperature and enthalpy. The difference observed for tapioca starch after the HVED treatment regarding gelatinisation enthalpy (Table 1) may be explained by low contents of fat, protein, and ash; a lower content of amylose; and higher molecular weights of amylose and amylopectin compared to all other starches [BeMiller & Whistler, 2009], which probably makes it less sensitive to granule damage.

Changes in the chemical structure have been reported as a result of plasma treatment as well. Depending on plasma type, treatment conditions, and starch type, depolymerisation, cross-linking, and formation of new functional groups have been reported [Bie *et al.*, 2016b; Khorram *et al.*, 2015]. The HVED treatment applied in this research is not as severe as the plasma treatment in the manner of energy input over time. However, the time was prolonged compared to

the plasma treatment (which typically lasts from few seconds to several minutes), and this may have induced a low degree of depolymerisation, as indicated by the reduction in amylose content (Table 2), which, in turn may have contributed to the observed changes in gelatinisation parameters.

Both chemical modifications applied in this research (with $\text{Na}_5\text{P}_3\text{O}_{10}$ and Na_2HPO_4) resulted in decreased enthalpy of gelatinisation of most starch types (Table 1). Phosphorylation with Na_2HPO_4 had a more pronounced effect, probably because the smaller molecule of this reagent than $\text{Na}_5\text{P}_3\text{O}_{10}$ penetrated more easily through the active sites of starch. The decrease in gelatinisation enthalpy is typical of substitution reactions, along with a reduced content of resistant starch observed for all analysed samples, and increased paste clarity observed for maize and wheat starch paste (Table 2). Xie & Shao [2009] also found that mainly starch monoesters were formed over diesters during corn starch phosphorylation.

For most samples, the enthalpy of gelatinisation of the HVED-treated starches before chemical modifications was lower than that of only phosphorylated starches (Table 1). This indicates that starch phosphorylation may have been enhanced by the prior activation of starch molecules through the reaction with free radicals generated in water by HVED [Thirumdas *et al.*, 2018]. However, temperatures of gelatinisation did not follow this trend (Table 1), *i.e.* the HVED-treated maize starch phosphorylated with Na_2HPO_4 had significantly higher gelatinisation temperatures than the one that was only phosphorylated with Na_2HPO_4 . Since reactions of phosphorylation involve electrically-charged compounds [Sechi & Marques, 2017], the formation of compounds by starch reaction with free radicals formed by HVED in water does not necessarily imply the activation of a starch granule towards the phosphorylation reaction (the phosphorylation reaction would be enhanced only if the cationic character of starch increases).

When phosphorylated starches were treated with HVED, the enthalpy of gelatinisation was lower compared to both HVED-treated and chemically-modified counterparts with a more pronounced decrease when Na_2HPO_4 was used (Table 1). It is apparent that the increase of the anionic character of starch makes it more prone to the reaction with active compounds formed in water by HVED.

Paste clarity is a physical property of starch that is also indicative of its water binding affinity. Substituted starches generally form more clear pastes than native counterparts due to more extensive hydration of granules, while cross-linked starches form more opaque pastes compared to native ones [Bhandari & Singhal, 2002]. Paste clarity of the investigated starches is shown in Table 2. It is evident that the HVED-treatment resulted in the increase of paste clarity of maize and potato starches, and its decrease for tapioca and wheat starches, although the statistical analysis does not show significance in all cases. The most significant change (decrease) was observed for tapioca starch, and this is consistent with the increase of gelatinisation enthalpy observed for native and HVED-treated tapioca starch (Table 1). Tapioca starch tends to form clearer pastes than cereal starches (which is observed in this research as well) and, unlike potato starch, it has a large number of thin layers of polymers

TABLE 1. Parameters of gelatinisation of starches modified by high-voltage electrical discharge (HVED), phosphorylation with $\text{Na}_5\text{P}_3\text{O}_{10}$, and Na_2HPO_4 , and combination of the processes obtained using differential scanning calorimetry (DSC).

	Modification	t_o (°C)	t_p (°C)	t_e (°C)	ΔH (J/g)
Maize	Native	64.90±0.15 ^c	70.24±0.18 ^c	75.06±0.22 ^{c,d}	12.10±0.40 ^a
	HVED	64.71±0.03 ^c	70.13±0.19 ^c	75.42±0.18 ^c	11.74±0.07 ^{a,b}
	$\text{Na}_5\text{P}_3\text{O}_{10}$	63.32±0.05 ^d	69.40±0.11 ^{c,d}	74.87±0.18 ^{d,e}	11.19±0.20 ^{b,c}
	HVED+ $\text{Na}_5\text{P}_3\text{O}_{10}$	63.53±0.28 ^d	69.19±0.01 ^d	74.44±0.06 ^c	12.31±0.03 ^a
	$\text{Na}_5\text{P}_3\text{O}_{10}$ +HVED	63.63±0.16 ^d	69.16±0.12 ^d	74.47±0.19 ^c	10.96±0.43 ^{c,d}
	Na_2HPO_4	67.72±0.13 ^a	72.52±0.24 ^b	77.65±0.02 ^b	10.92±0.15 ^{c,d}
	HVED+ Na_2HPO_4	69.12±0.11 ^b	74.39±0.02 ^a	79.19±0.04 ^a	10.24±0.08 ^d
	Na_2HPO_4 +HVED	69.12±0.21 ^b	74.38±0.29 ^a	79.09±0.49 ^a	10.25±0.01 ^d
Wheat	Native	55.91±0.13 ^C	60.33±0.10 ^C	64.92±0.27 ^C	11.09±0.17 ^A
	HVED	55.60±0.01 ^C	60.03±0.01 ^{C,D}	64.56±0.12 ^C	10.25±0.18 ^B
	$\text{Na}_5\text{P}_3\text{O}_{10}$	55.00±0.22 ^D	59.38±0.14 ^{D,E}	63.84±0.03 ^D	9.66±0.27 ^{B,C}
	HVED+ $\text{Na}_5\text{P}_3\text{O}_{10}$	54.42±0.14 ^E	59.04±0.33 ^E	63.20±0.03 ^E	9.05±0.51 ^{C,D}
	$\text{Na}_5\text{P}_3\text{O}_{10}$ +HVED	53.97±0.07 ^F	58.74±0.17 ^E	63.40±0.07 ^{D,E}	9.46±0.18 ^C
	Na_2HPO_4	58.74±0.04 ^B	63.33±0.01 ^B	67.82±0.23 ^B	9.14±0.66 ^{C,D}
	HVED+ Na_2HPO_4	60.14±0.07 ^A	65.16±0.70 ^A	69.08±0.09 ^A	7.90±0.16 ^E
	Na_2HPO_4 +HVED	59.85±0.10 ^A	64.39±0.04 ^A	68.87±0.09 ^A	8.60±0.16 ^{D,E}
Potato	Native	57.23±0.21 ⁱ	63.12±2.07 ⁱ	66.33±0.08 ⁱ	18.68±0.15 ⁱ
	HVED	56.96±0.14 ⁱ	61.35±0.31 ⁱⁱ	66.36±0.48 ⁱ	17.88±0.36 ⁱⁱ
	$\text{Na}_5\text{P}_3\text{O}_{10}$	52.71±0.05 ⁱⁱⁱ	57.17±0.09 ^v	62.15±0.06 ^{iv}	15.53±0.62 ⁱⁱⁱ
	HVED+ $\text{Na}_5\text{P}_3\text{O}_{10}$	52.66±0.23 ⁱⁱⁱ	57.09±0.01 ^v	62.15±0.37 ^{iv}	15.15±0.19 ⁱⁱⁱ
	$\text{Na}_5\text{P}_3\text{O}_{10}$ +HVED	51.96±0.08 ^{iv}	56.62±0.07 ^v	61.75±0.08 ^{iv}	15.51±0.67 ⁱⁱⁱ
	Na_2HPO_4	52.77±0.14 ⁱⁱⁱ	58.10±0.21 ^{iv}	63.51±0.14 ⁱⁱⁱ	14.04±0.51 ^{iv}
	HVED+ Na_2HPO_4	54.10±0.01 ⁱⁱ	59.42±0.01 ⁱⁱⁱ	64.57±0.04 ⁱⁱ	12.95±0.02 ^v
	Na_2HPO_4 +HVED	54.10±0.22 ⁱⁱ	59.43±0.05 ⁱⁱⁱ	64.79±0.24 ⁱⁱ	13.36±0.36 ^{iv,v}
Tapioca	Native	63.48±0.30 ⁱⁱⁱ	69.74±0.12 ⁱⁱ	77.82±0.04 ⁱⁱ	10.55±0.64 ^v
	HVED	61.56±0.18 ^{iv}	67.25±0.40 ⁱⁱⁱ	75.17±0.14 ^{iv}	15.19±0.29 ⁱ
	$\text{Na}_5\text{P}_3\text{O}_{10}$	59.85±0.06 ^v	66.04±0.05 ^{iv}	73.19±0.12 ^v	14.72±0.30 ^{i, ii}
	HVED+ $\text{Na}_5\text{P}_3\text{O}_{10}$	59.96±0.02 ^v	65.88±0.04 ^{iv}	73.03±0.20 ^v	14.15±0.47 ⁱⁱ
	$\text{Na}_5\text{P}_3\text{O}_{10}$ +HVED	59.73±0.01 ^v	65.83±0.11 ^{iv}	73.24±0.23 ^v	14.47±0.19 ^{iv}
	Na_2HPO_4	64.29±0.02 ⁱⁱ	70.20±0.02 ⁱⁱ	77.16±0.01 ⁱⁱⁱ	13.29±0.17 ⁱⁱⁱ
	HVED+ Na_2HPO_4	66.01±0.29 ⁱ	71.83±0.62 ⁱ	78.70±0.64 ⁱ	12.13±0.41 ^{iv}
	Na_2HPO_4 +HVED	65.77±0.21 ⁱ	71.76±0.07 ⁱ	78.77±0.14 ⁱ	12.03±0.47 ^{iv}

t_o , onset temperature; t_p , peak temperature; t_e , endset temperature; ΔH , enthalpy of gelatinisation. The results are expressed as mean±standard deviation (n=3). Values with different superscripts in the same column are different for the same starch type ($p<0.05$).

[Banura *et al.*, 2018]. This may be the reason for the unique effect of HVED on its clarity. However, Banura *et al.* [2018] reported that plasma treatment increased the swelling capacity of both maize and tapioca starches, which would imply that clarity should also increase. In turn, Bie *et al.* [2016a] reported that glow plasmas broke the hydrogen bonding

network at the molecular level of tapioca starch and change starch crystallites. Unlike these researches, results obtained in the present study (increased enthalpy of gelatinisation, reduced paste clarity) imply that HVED, unlike plasma treatment, causes better alignment of starch chains and more ordered structural organisation of tapioca starch polymers.

TABLE 2. Paste clarity, contents of amylose, damaged starch, and resistant starch of starches modified by high-voltage electrical discharge (HVED) treatment, phosphorylation with $\text{Na}_5\text{P}_3\text{O}_{10}$, and Na_2HPO_4 , and combination of the processes.

	Modification	Paste clarity (%T)	Amylose (%)	Damaged starch (%)	Resistant starch (g/100 g d.m.)
Maize	Native	8.31±0.28 ^d	15.80±0.05 ^a	2.41±0.08 ^a	2.40±0.54 ^a
	HVED	8.97±0.16 ^d	15.20±0.06 ^a	0.98±0.04 ^b	2.06±0.34 ^{ab}
	$\text{Na}_5\text{P}_3\text{O}_{10}$	18.90±0.42 ^a	14.19±0.11 ^b	0.82±0.01 ^d	1.04±0.89 ^{ab}
	HVED+ $\text{Na}_5\text{P}_3\text{O}_{10}$	13.15±0.21 ^c	13.88±0.14 ^{bc}	1.02±0.04 ^b	0.80±0.19 ^{ab}
	$\text{Na}_5\text{P}_3\text{O}_{10}$ +HVED	16.15±0.07 ^b	12.54±0.18 ^d	0.89±0.00 ^c	1.48±0.53 ^{ab}
	Na_2HPO_4	9.94±0.03 ^d	13.15±0.62 ^d	0.86±0.03 ^{cd}	0.59±0.06 ^b
	HVED+ Na_2HPO_4	8.73±0.06 ^d	13.50±0.57 ^{bc}	0.68±0.02 ^c	0.52±0.16 ^b
	Na_2HPO_4 +HVED	9.43±0.01 ^d	15.75±0.33 ^a	0.54±0.00 ^f	0.50±0.01 ^b
Wheat	Native	8.60±0.03 ^C	20.27±0.32 ^A	0.64±0.01 ^C	0.66±0.01 ^A
	HVED	7.66±0.19 ^C	17.03±0.93 ^D	0.67±0.06 ^C	0.22±0.01 ^A
	$\text{Na}_5\text{P}_3\text{O}_{10}$	17.50±0.42 ^A	18.12±0.02 ^{B,C}	0.83±0.05 ^A	0.29±0.02 ^A
	HVED+ $\text{Na}_5\text{P}_3\text{O}_{10}$	11.45±0.64 ^B	17.96±0.15 ^{C,D}	0.75±0.01 ^B	0.29±0.17 ^A
	$\text{Na}_5\text{P}_3\text{O}_{10}$ +HVED	11.90±0.28 ^B	20.73±0.91 ^A	0.75±0.04 ^B	0.18±0.01 ^A
	Na_2HPO_4	12.75±0.07 ^B	19.04±0.01 ^B	0.45±0.00 ^D	0.15±0.03 ^A
	HVED+ Na_2HPO_4	11.70±0.00 ^B	16.03±0.21 ^E	0.35±0.02 ^E	0.17±0.00 ^A
	Na_2HPO_4 +HVED	12.35±0.35 ^B	17.52±0.52 ^{C,D}	0.37±0.04 ^E	0.16±0.01 ^A
Potato	Native	87.10±0.57 ⁱⁱ	14.14±1.54 ⁱ	1.89±0.02 ⁱ	69.50±3.09 ⁱ
	HVED	91.15±0.35 ⁱ	11.87±0.16 ⁱⁱ	0.35±0.02 ⁱⁱⁱ	69.39±0.00 ⁱ
	$\text{Na}_5\text{P}_3\text{O}_{10}$	63.15±0.92 ⁱⁱ	11.77±0.10 ⁱⁱ	0.42±0.03 ⁱⁱ	34.32±0.99 ^v
	HVED+ $\text{Na}_5\text{P}_3\text{O}_{10}$	82.00±3.25 ⁱⁱⁱ	9.73±0.25 ⁱⁱⁱ	0.25±0.02 ^{iv,v}	46.52±0.39 ⁱⁱ
	$\text{Na}_5\text{P}_3\text{O}_{10}$ +HVED	77.90±0.99 ^{iv}	9.45±0.18 ⁱⁱⁱ	0.42±0.03 ⁱⁱ	38.24±1.29 ^w
	Na_2HPO_4	28.20±0.28 ^{iv}	11.63±0.71 ⁱⁱ	0.41±0.01 ⁱⁱ	47.44±1.77 ⁱⁱ
	HVED+ Na_2HPO_4	23.90±0.14 ^{vii}	11.66±0.02 ⁱⁱ	0.22±0.01 ^v	41.57±0.04 ⁱⁱⁱ
	Na_2HPO_4 +HVED	24.05±0.35 ^{vii}	12.05±0.20 ⁱⁱ	0.29±0.02 ^{iv}	4.48±0.08 ^{vi}
Tapioca	Native	48.85±0.35 ^I	12.85±0.36 ^I	0.02±0.00 ^V	15.15±0.10 ^I
	HVED	40.40±1.70 ^{III}	10.73±0.13 ^{II,III}	0.01±0.00 ^V	5.42±0.57 ^{IV}
	$\text{Na}_5\text{P}_3\text{O}_{10}$	38.20±0.57 ^{IV}	7.29±0.10 ^{IV}	0.18±0.00 ^{II}	3.16±2.26 ^V
	HVED+ $\text{Na}_5\text{P}_3\text{O}_{10}$	40.15±0.21 ^{III}	10.06±0.33 ^{III}	0.34±0.03 ^I	11.30±0.12 ^{II}
	$\text{Na}_5\text{P}_3\text{O}_{10}$ +HVED	44.00±0.99 ^{II}	10.99±0.00 ^{III}	0.29±0.01 ^I	2.81±0.59 ^V
	Na_2HPO_4	26.95±0.49 ^V	10.17±0.04 ^{II,III}	0.13±0.01 ^{II,III}	3.32±0.05 ^V
	HVED+ Na_2HPO_4	27.65±0.35 ^V	10.19±0.11 ^{II,III}	0.09±0.01 ^{II,III}	9.97±0.06 ^{II, III}
	Na_2HPO_4 +HVED	28.80±0.85 ^V	11.52±0.54 ^{II}	0.04±0.01 ^{IV,V}	9.54±0.00 ^{III}

%T, transmittance of 1% starch suspension measured at 650 nm against distilled water. The results are expressed as mean±standard deviation (n=3). Values with different superscripts in the same column are different for the same starch type (p<0.05).

Generally, phosphorylation induced an increase of paste clarity of the investigated cereal starches, and a decrease of this property for tuber starches, as shown in Table 2. Here, a more pronounced effect on the increase of paste clarity in tuber starches was observed for starches phosphorylated with $\text{Na}_5\text{P}_3\text{O}_{10}$, than with Na_2HPO_4 , probably due to larger mol-

ecules which keep starch chains more spaced and make light passing easier. Tuber starches, like potato and tapioca ones, are characterised by larger granules and a higher degree of polymerisation of amylose than maize and wheat starches [Banura *et al.*, 2018], which may be the reason for the observed opposite trend in paste clarity.

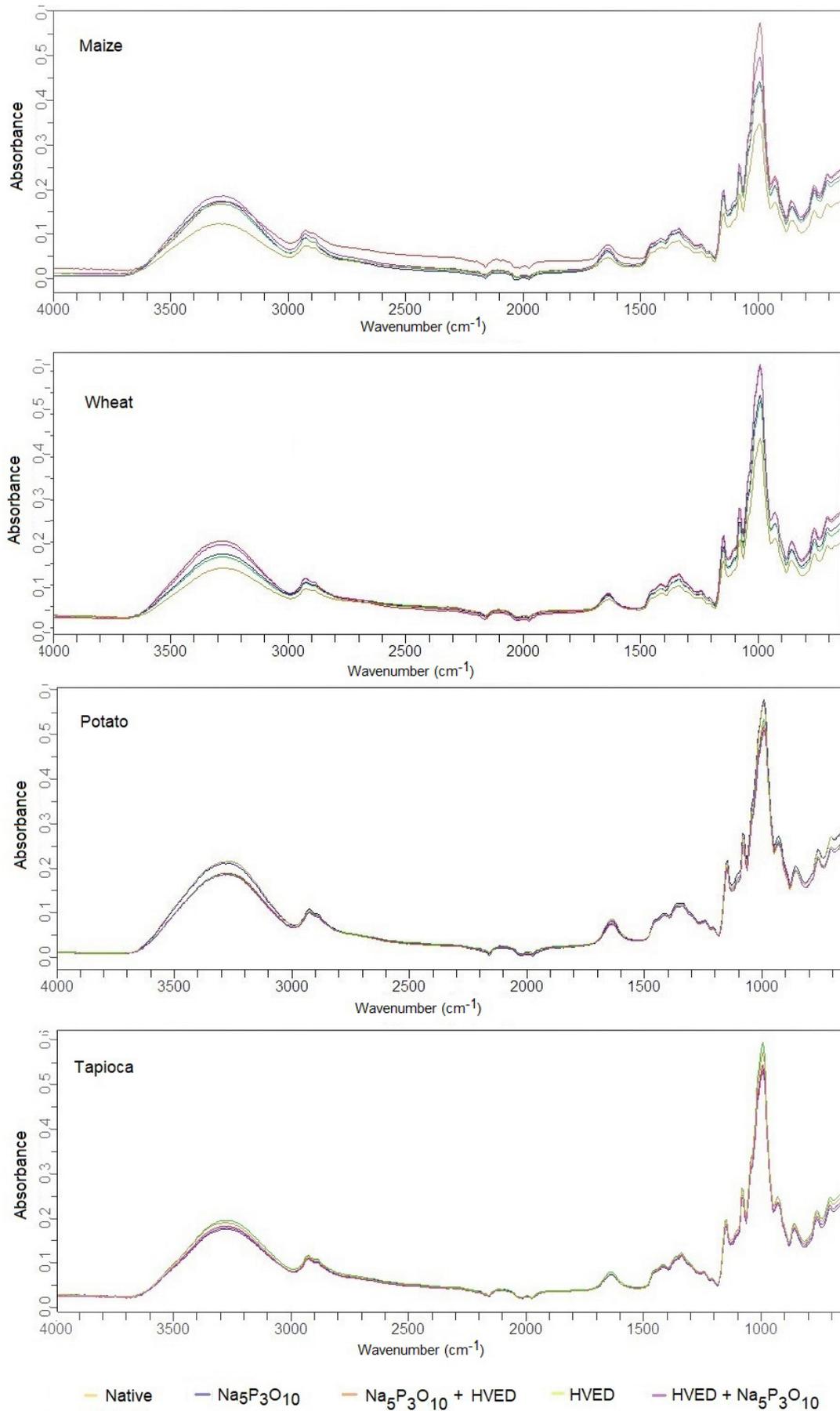


FIGURE 1. Fourier-transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR) spectra of starches modified with Na₅P₃O₁₀, with and without high-voltage electrical discharge (HVED) treatment.

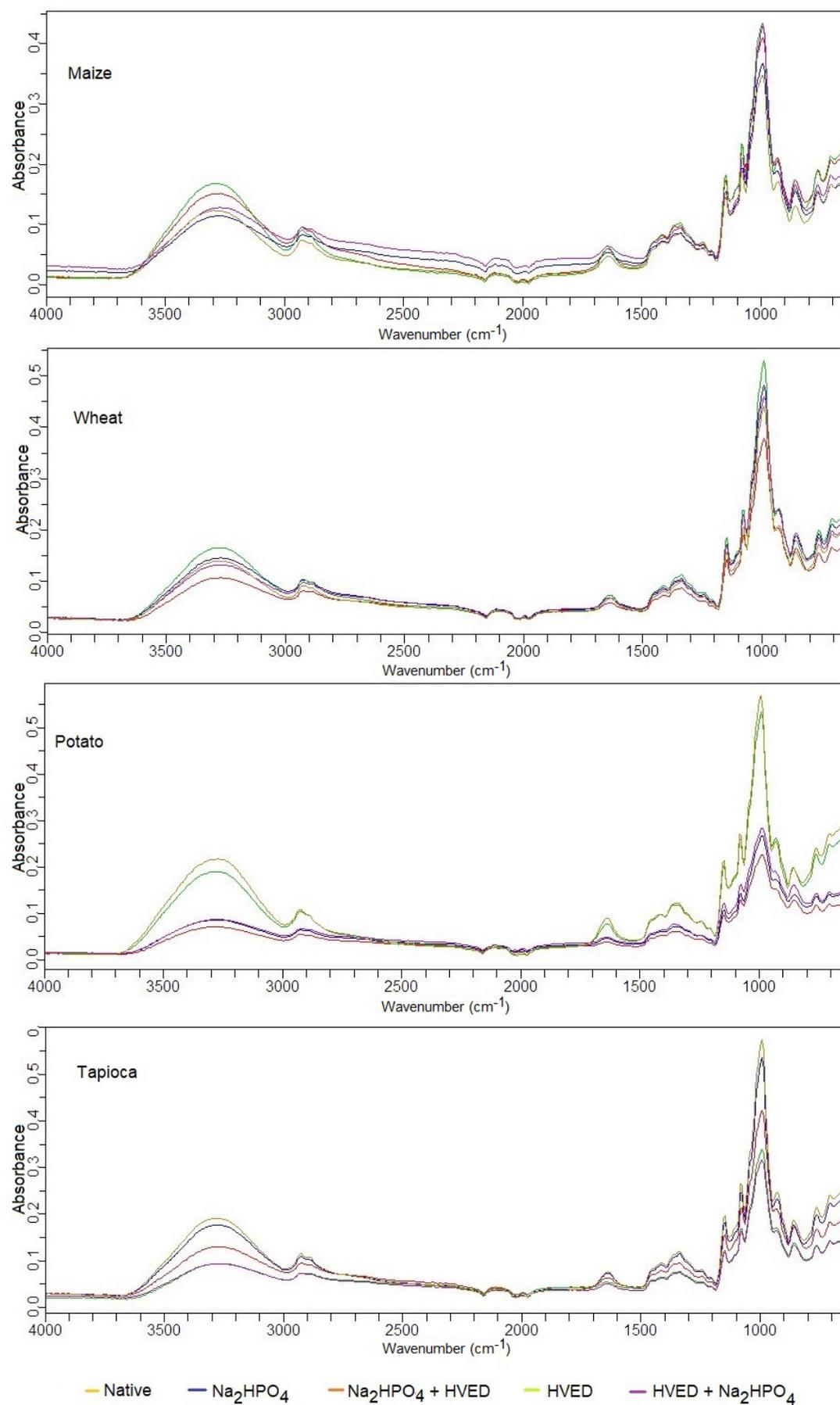


FIGURE 2. Fourier-transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR) spectra of starches modified with Na₂HPO₄, with and without high-voltage electrical discharge (HVED) treatment.

The HVED treatment before phosphorylation resulted in a reduced paste clarity compared to the phosphorylated counterpart of cereal starches. Although statistical difference was not observed for wheat starch, the values are indeed lower (11.45 %T compared to 12.75 %T) for this sample as well. Again, for tuber starches the trend was opposite, *i.e.*, only the HVED-treated potato starch modified with Na_2HPO_4 revealed lower pasted clarity than the phosphorylated counterpart (Table 2). Generally, for cereal starches, paste clarity followed the order: HVED-treated starch < HVED-treated + phosphorylated < phosphorylated starch; and for tuber starches it followed the order: phosphorylated < HVED-treated + phosphorylated < HVED-treated starch. When HVED was applied after the phosphorylation, the clarity of pastes made of cereal starches decreased in the following order: phosphorylated > phosphorylated + HVED treated > HVED treated starch, while the clarity of pastes made of tuber starches did not show any trend.

Chaiwat *et al.* [2016] stated that variations in paste clarity of starch after HVED treatment depended on the final outcome of two competing reactions: cross-linking and depolymerisation. Cross-linking produces a more ordered structure and prevents the dissociation of starch chains during pasting, while depolymerisation increases paste clarity.

Amylose content in all analysed starches decreased after HVED treatment (Table 2) (although, the differences were not always statistically significant), which is consistent with results reported for plasma-treated starch [Bie *et al.*, 2016b; Thirumdas *et al.*, 2018]. Thirumdas *et al.* [2018] ascribed this phenomenon to the depolymerisation of amylose, since physical modification predominantly affects amorphous regions of starch granules [Ascheri *et al.*, 2014]. In this research, a decrease in amylose content was observed after phosphorylation as well (Table 2). Ascheri *et al.* [2014] also reported decreased amylose content after phosphorylation of *S. lycocarpum* starch. In most samples treated by a combination of HVED and phosphorylation, the amylose contents were also lower compared to native starches (Table 2). However, it is difficult to reveal any trend regarding the coupled use of these methods. Starch damage was smaller after all modifications, except for tapioca starch, where virtually no damaged starch was present in native starch, and for wheat starch modified with $\text{Na}_3\text{P}_3\text{O}_{10}$ alone and in combination with HVED. Since damaged starch is a portion of starch that is physically broken or fragmented, and therefore has more affinity to water [Tian & Sun, 2020], it is possible that damaged starch was simply washed out during modification reactions.

Changes in the chemical nature of starches due to the HVED treatment and phosphorylation, both alone and in combination, are further supported by a reduction in resistant starch content determined in most samples (Table 2). Although the resistant starch content is typically linked to a higher amylose content (commonly high-amylose starches are used to produce resistant starch) [Liu *et al.*, 2020] and resistance to gelatinisation (temperatures and enthalpy of gelatinisation) [Apostolidis & Mandala, 2020], this research showed no obvious correlation between these parameters (results not shown). Furthermore, FTIR-ATR spectra (Figures 1 and 2) revealed that the number of –OH groups increased, which is visible from the increase in the heights of peaks

at 993 cm^{-1} (C-O-H bending vibrations) and 3440 cm^{-1} (O-H stretching vibrations) [Deeyai *et al.*, 2013; Guntzler & Gremlich, 2006]. This implies that starch depolymerisation was the dominant reaction during the HVED treatment. However, a band at around 1000 cm^{-1} is also used as an indicator of crystallinity [Deeyai *et al.*, 2013]. The higher peak at 1000 cm^{-1} for modified starches would show that the crystalline order of starch granules was increased after all modifications, especially in the case of maize and wheat starches (Figures 1 and 2). Capron *et al.* [2007] reported that most authors use bands at 1047 and 1022 cm^{-1} to evaluate crystalline order of starch and link the band at 1000 cm^{-1} to intramolecular hydrogen bonds of hydroxyl groups, which “could allow inter-double helices associations”, and concluded that the band at 1000 cm^{-1} was the result of hydrated crystalline regions. Since bands corresponding to phosphorus-containing groups of starches overlap with bands associated with C-O stretching vibrations of the glycosidic bond (1085 cm^{-1}), pyranose ring vibrations (929 cm^{-1}) [Deeyai *et al.*, 2013; Delval *et al.*, 2004], and the band showing the crystalline order of starch (around 1000 cm^{-1}) [Ispas-Szabo *et al.*, 1999], it is hard to confirm by FTIR-ATR that –OH groups were indeed substituted.

CONCLUSIONS

The HVED treatment of starch in an aqueous suspension may be used as an effective tool to modify starch properties both as a single method and in combination with its phosphorylation. As a result of the HVED treatment, generally, a reduction was observed in gelatinisation enthalpy, amylose content, and resistant starch content. The magnitude of these changes significantly depended on starch type. The HVED treatment prior to phosphorylation resulted in more pronounced changes of the investigated properties compared to phosphorylation alone, indicating that electrical discharges in water activate starch molecules, facilitate penetration of water to starch, and make them more prone to the reaction with phosphorylation agents. Additional examination of starch properties, such as swelling power, solubility, pasting properties, colour, morphology of starch granules, texture properties, *etc.* is, however, needed to get a better insight of the applicability of such modified starch in the food industry.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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