

Electrolytic oxidation of gelatinised tapioca starch: effect of sodium chloride content on structural and physicochemical properties

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Abstract

This is the first study on electrolytic oxidation of tapioca starch in the gelatinised state. Tapioca starch was first gelatinised and then oxidised in an electrolytic tank with NaCl as the electrolyte. The structural and physicochemical properties of the native and the oxidised starches were characterised by FTIR, XRD, molecular mass distribution, degree of oxidation, and thermal gravimetry. FTIR spectra and degree of oxidation analyses showed that 2 - 3% NaCl concentration in the electrolytic tank resulted in the highest content of carbonyl and carboxyl groups in the oxidised starch. The depolymerisation of starch increased with the concentration of NaCl. As a result of these structural changes, the thermal stability of starch samples decreased with the increase of NaCl concentration. The present work demonstrated a fast, simple, and benign process of oxidising starch that can be used in industrial production.

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Introduction

Starch is a natural polymer mixture of amylose and amylopectin molecules. Native starch granules exist in a semi-crystalline form with different shapes and sizes (Cui, 2005). Starch plays a major role in food applications and other industries. However, native starches do not have the properties that match specific requirements in industrial production. Because of these shortcomings, starch is normally modified to have the desired properties to meet specific needs. Oxidising starch with different oxidants is one of the strategies used to modify starch. Depending on the starch origin and the reaction conditions, the oxidation process can convert the hydroxyl groups in starch molecules into carbonyl and carboxyl groups, and even cause the depolymerisation of amylose and amylopectin molecules (Cui, 2005; Trinh and Dang, 2019). Some published studies have suggested that oxidation preferably takes place in the amorphous region of the semi-crystalline starch (Kuakpetoon and Wang, 2001; Zhou *et al.*, 2016).

Various oxidising agents have been studied such as hypochlorite, hydrogen peroxide, periodate, permanganate, dichromate, persulfate, and chlorite (Cui, 2005). However, these chemical agents raise concerns about starch contamination with the chemicals absorbed on and inside the starch granules. Electrolytic techniques are an appropriate approach to oxidise starch because they use food-grade chemicals

that are approved to be used in the food industry, namely NaCl, NaOH, and HCl. The amounts of these substances used in electrolytic techniques are low; so, they can be removed easily from the final products (Farley and Hixon, 1942; Trinh and Dang, 2019). Moreover, the use of small amounts of NaCl in the reaction has no significant effect on the environment. Usually, the oxidation of starch is conducted in the slurry state at low temperatures, so that the starch granules are kept intact for easier separation afterwards (Kuakpetoon and Wang, 2001; Zhou *et al.*, 2016; Trinh *et al.*, 2019). In the present work, to facilitate the oxidation reaction, tapioca starch was completely gelatinised to produce an amorphous form before the treatment with oxidising agents generated in an electrolytic tank. The treated starch was characterised for their structural and physico-chemical properties. To the best of our knowledge, this is the first study on electrolytic oxidation of starch in the gelatinised state.

Materials and methods

Electrolytic oxidation of starch

Tapioca starch (400 g) was suspended in 4.0 L of NaCl solutions (0 - 5.0%, w/v). This starch slurry was gelatinised sequentially by heating (95°C, 30 min) and autoclaving (121°C, 15 min). The obtained starch gel was cooled to room temperature (about 30°C), and then placed in a cylindrical electrolytic tank (radius of 12 cm). The electrolytic treatment followed a

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previously published method (Trinh and Dang, 2019). Two titanium electrodes (anode and cathode) were placed into the tank at a distance of 10 cm, and were supplied with a 3 A DC electric current and 10 V voltage. After 1 h of electrolysis, the electric current was switched off, and the pH was adjusted to 7 using 1 M HCl solution. The oxidised starch was precipitated by adding absolute ethanol to a final concentration of 70% (v/v) with continuous stirring. The obtained slurry was centrifuged (3,000 g, 15 min) to obtain the solid product. The procedure of adding ethanol and subsequent centrifugation was repeated two more times to wash the solid product. The sample was then dried in a convection dryer (40°C, 24 h) to reach a final moisture content of about 12%. The electrolytically oxidised starch samples treated in 0, 0.5, 1.0, 2.0, 3.0, and 5.0% NaCl solutions are represented as G0, G0.5, G1, G2, G3, and G5, respectively.

Total chlorine (TC), oxidation-reduction potential (ORP), and pH measurements

The total chlorine (TC) of the electrolytic tank during the electrolysis was determined based on a method of ISO 7393-3:1990 (ISO, 1990). The oxidation-reduction potential (ORP) was determined using a Portable Digital ORP Meter (ORP-2069, Walfront, USA). The pH was determined using a pH meter (SevenEasy S20, Mettler Toledo, USA).

Fourier-transform infrared (FTIR) spectroscopy measurement

FTIR spectra of the starch samples were recorded following the method of Kizil *et al.* (2002) at wavenumbers from 400 to 4500 cm⁻¹ (FTIR-8400S, Shimadzu, Japan) for 20 scans with a resolution of 2 cm⁻¹. Starch (2.0 mg) and KBr (200 mg) were thoroughly mixed and pressed (8.0 bar, 10 min) before the measurement.

X-ray diffraction (XRD) measurement

XRD spectra of the oxidised starch samples were recorded using a powder X-ray diffractometer (Model D5005, Bruker, Karlsruhe, Germany). The operating conditions were: 40 kV and 40 mA with Cu-K α radiation of 0.15406 nm (Nickel filter); step size: 0.01°; and step time: 4 s. Each scan was performed from 5 to 30° (2 θ) (Trinh *et al.*, 2012).

Molecular weight distribution and average molecular weight

Molecular mass distribution of the oxidised starch samples was determined by gel permeation chromatography (GPC). An HPLC 1100 (Agilent

Equipment, USA) with an RID refractive index probe was used for the measurement. The mobile phase was: 0.5 M KNO₃ aqueous solution; stationary phase: Ultrahydrogel 500 column (300 × 7.8 mm, ID); stationary phase temperature: 40°C; probe temperature: 35°C; flow rate: 1 mL/min; and sample volume: 20 μ L.

Carbonyl content

The carbonyl content of the oxidised starch samples was determined based on the method of Smith (1967), and was expressed as the number of carbonyl groups per 100 g of glucose unit (CO/100GU).

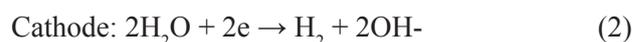
Thermogravimetric analysis (TGA/DTG)

The thermogravimetric measurements were performed on a TG apparatus (Shimadzu TGA-60, Japan). Each oxidised starch sample (10 mg) was heated from 20 to 500°C at a rate of 10°C/min, and a nitrogen flow rate of 100 mL/min in a nitrogen atmosphere (Wang *et al.*, 2008).

Results and discussion

Properties of the electrolyte solution

During the electrolysis of NaCl solutions, the following main reactions occur (Venkitanarayanan *et al.*, 1999; Hricova *et al.*, 2008):



The main oxidising agents in the electrolytic tank were ClO⁻ and dissolved Cl₂, due to reactions (1) and (3). Their overall concentrations are critical during the electrolytic process, and were monitored by TC and ORP. pH is another important parameter affecting any redox reaction. Their values during electrolysis are presented in Figures 1A - 1C.

In the range of NaCl concentration from 0.5 to 5.0% (w/v), the pH value of the electrolyte solutions increased rapidly and reached their equilibrium value (from 8.4 to 9.7) after 10 min of electrolysis due to reaction (2) (Figure 1A). When the NaCl concentration was higher, the extents of reactions (1) - (3) were higher, which is indicated by the higher value of equilibrium pH (Al-Haq *et al.*, 2005).

The ORP and TC values of the electrolyte solution increased with reaction time due to the formation of oxidising agents (ClO⁻ and Cl₂) (Figures

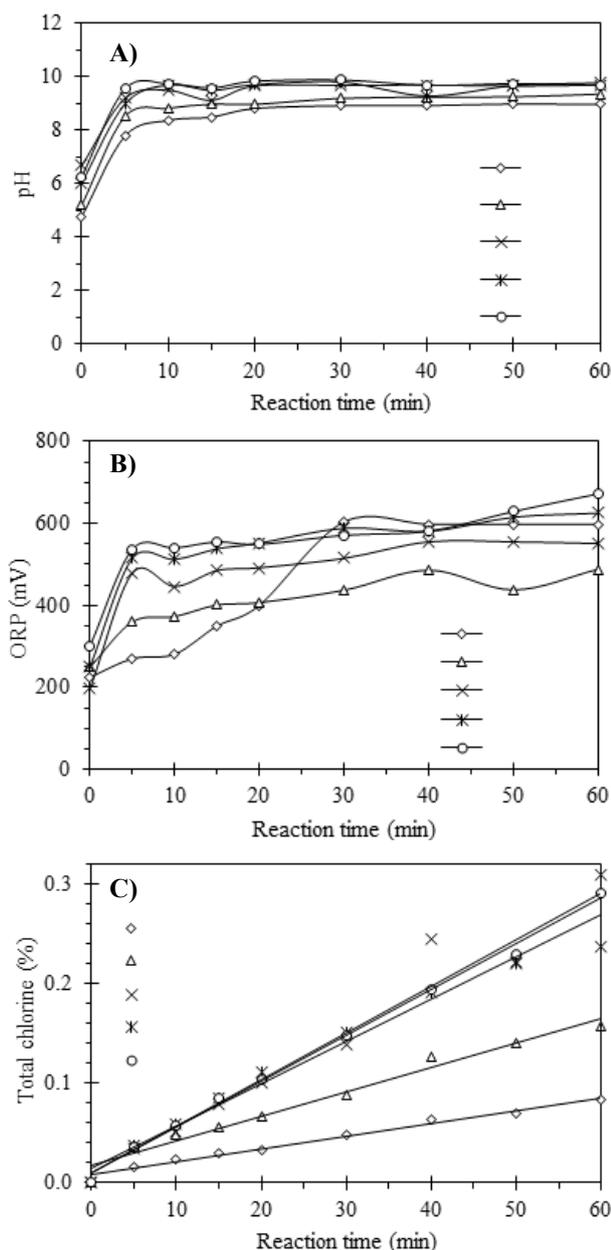


Figure 1. pH (A), oxidation-reduction potential (B), and total chlorine content (C) of starch samples following electrolytic treatment with different NaCl concentrations..

1B - 1C). The pH of the electrolytic solutions stabilised after only 10 min, while the ORP and TC values kept increasing even after 60 min. This is evidence of the oxidation reaction taking place between the oxidising agents and the starch molecules. The oxidising agents were formed at a constant rate by a constant electrolytic current, and simultaneously consumed by the reaction with starch. Initially, the consumption reaction was fast, which resulted in low TC and ORP values. Gradually, when more starch was oxidised, the consumption reaction became slower, and the TC content with ORP value increased over time.

FTIR spectra and carbonyl content

Infrared spectroscopy is an important physicochemical technique to characterise the chemical transformations occurred in the starch molecules after the electrolytic oxidation. The FTIR spectra of the samples are presented in Figure 2A. In general, the spectra were quite similar to those of previous studies (Fanning and Vannice, 1993; Kizil *et al.*, 2002; Trinh and Dang, 2019). According to Fanning and Vannice (1993), in the oxidised starch sample, the carboxyl group (-COOH) had strong absorption at three regions: 1120 - 1200, 1665 - 1760, and 2500 - 3300 cm^{-1} . The intensity of these regions can be a quantitative indicator of the number of carboxyl groups in the starch molecule (Figure 2B). Figure 2B shows that the carboxyl content in starch increased with the NaCl concentration to a maximum at about 2 - 3% NaCl, and then decreased at higher concentrations of NaCl.

The absorption peak of carbonyl C=O groups (1722 cm^{-1}) was not obvious in the FTIR spectra, possibly because of their low content. The titration results (Figure 2C) showed that the carbonyl content was actually low (0.05 - 0.4%), and had a maximum also in 2 - 3% NaCl solution. During the reaction, the hydroxyl groups at the C-2, C-3, and C-6 sites were first oxidised to the carbonyl groups, and then to the carboxyl groups (Cui, 2005). Therefore, high NaCl concentrations up to 2 - 3% increased both the carbonyl and carboxyl groups. The apparent decrease of both carbonyl and carboxyl groups at 5% NaCl was possibly due to the depolymerisation of starch by the high concentration of oxidising agents (Parovuori *et al.*, 1995; Cui, 2005). Starch fractions with small molecular weights have higher solubility and could be lost during the washing steps. An evidence of this loss was the observed recovery efficiency (% w/w) of starch samples after electrolysis which decreased with increasing NaCl concentrations. Samples G0, G0.5, G1, G2, G3, and G5 had recovery efficiencies of 96.82, 95.34, 93.25, 93.79, 93.37, and 92.61%, respectively.

To evaluate the effect of pregelatinising starch before electrolysis, we compared the maximum value of carbonyl contents in the present work (approximately 0.4%) with that (< 0.12%) of a previous work on electrolytic oxidation of native granular starch (Trinh and Dang, 2019). This higher oxidation degree when using gelatinised starch is due to the fact that in the granular state, the oxidising agents have to penetrate into the starch granules through capillary channels, thus limiting their oxidation ability (Sandhu, 2008; Kuakpetoon and

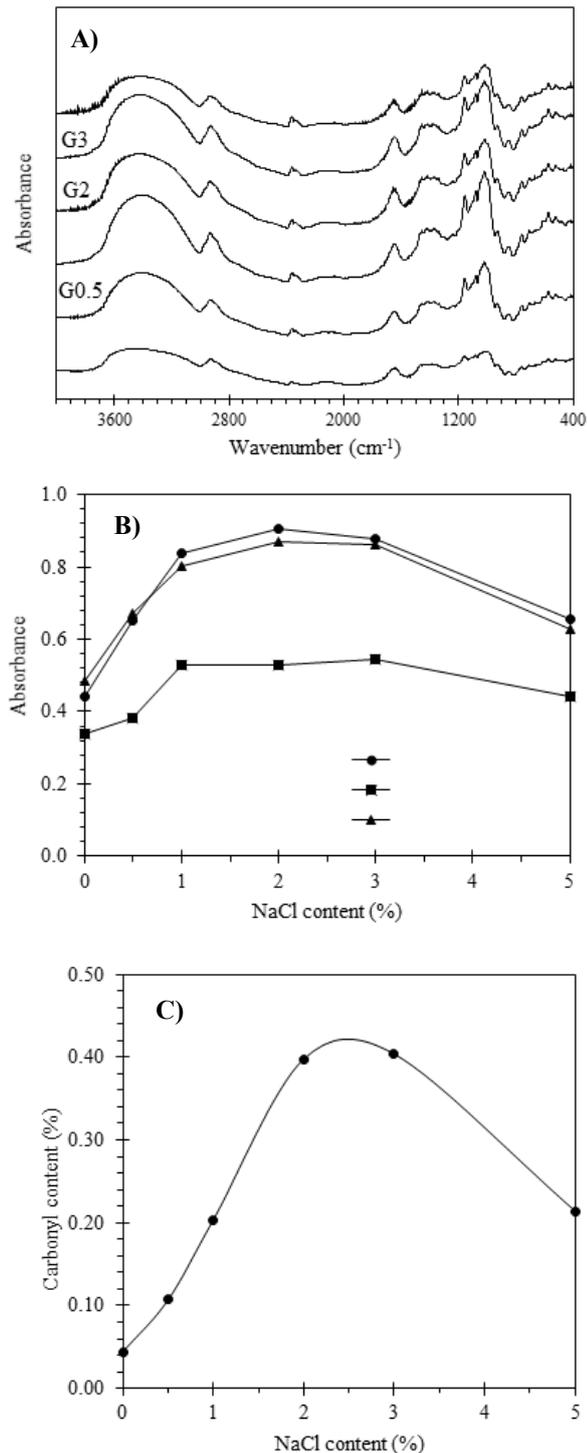


Figure 2. FTIR spectra (A), absorbance of carboxyl groups (B), and carbonyl contents (C) in the oxidised starch samples following electrolysis treatment with different NaCl concentrations.

Wang, 2001). In contrast, in the gelatinised (amorphous) state, the starch granules are unfolded, and may be easily attacked by the oxidising agents.

X-ray diffraction (XRD) patterns

Native starch granules are a semicrystalline

mixture of amylose and amylopectin. The crystallinity of starch affects its many functional properties, including gelatinisation temperature, pasting temperature, and thermal stability (Copeland *et al.*, 2009). Therefore, it is important to evaluate the crystallinity of the starch samples after electrolytic oxidation. Figure 3 shows the X-ray diffraction patterns of the starch samples. The G0 sample lost all characteristic peaks of the A-type crystals of tapioca granular starch and was in a complete amorphous state (Tester *et al.*, 2004). The samples of G0.5, G1, G2, G3, and G5 showed new peaks at 2θ values of 13 and 20°. These are the typical peaks for V-type starch crystal structures (Liu *et al.*, 2016). These structures were formed during the precipitation of the oxidised starch in 70% ethanol.

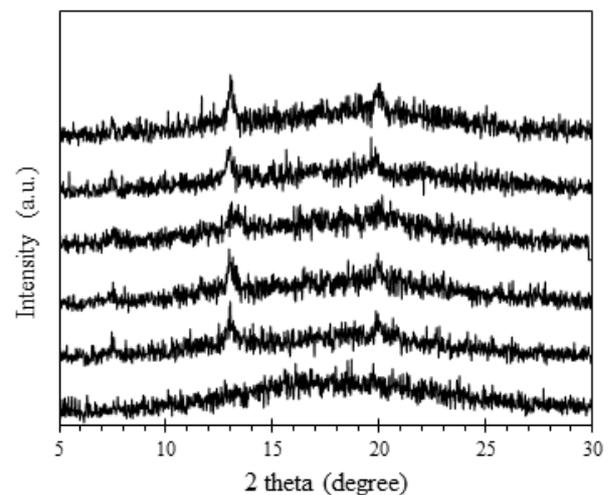


Figure 3. X-ray diffraction patterns of the oxidised starch samples following electrolytic treatment with different NaCl concentrations.

During electrolytic treatment, the gelatinised starch was partially depolymerised to shorter amylose chains. The presence of ethanol during the collection process induced a compact helical conformation of amylose, which resulted in a helix with a hydrophobic cavity surrounding the ethanol molecules (Liu *et al.*, 2016). The obtained inclusion complexes acquired a certain degree of order, resulting in the formation of crystals and the peaks in the XRD patterns (Putseys *et al.*, 2010).

It is well-known that amylopectin does not form inclusion complexes with alcohols (Polaczek *et al.*, 1999). Therefore, the native gelatinised starch with amylopectin as the major component (about 83%) remained in the amorphous form, and did not show peaks in the XRD patterns. At the same time, there were no significant differences between the peak heights of the oxidised starch samples when increasing the NaCl content from 0.5 to 5%. This result demonstrates that the electrolytic oxidation did

not selectively trim the initial amylopectin molecules into amylose chains, but mainly into smaller amylopectin molecules. Only almost constant amount of amylose was formed when increasing NaCl content from 0.5 to 5%, which resulted in similar XRD peaks.

Molecular weight distribution

To confirm the depolymerisation of the starch molecules, the molecular weight distribution chromatograms (GPC) of the oxidised starch samples were measured, and shown in Figure 4. The first peak (1) with higher molecular weight is of amylopectin, while the second peak (2) is of amylose molecules (Trinh, 2015). Increasing the NaCl concentration (from 0 to 5%) in the electrolytic tank (from G0 to G5) moved the first peak gradually towards smaller molecular weights with lower heights and areas. At the same time, the heights and areas of the second peak increased accordingly. This demonstrates that the electrolysis products of NaCl breaks down the amylopectin molecules into smaller fractions, thereby reducing the average molecular weight of starch (Kuakpetoon and Wang, 2001; Chan *et al.*, 2011). During the NaCl electrolysis, many strong oxidising agents, including hypochlorite ions (ClO^-), hydrogen peroxide (H_2O_2), and free radicals ($\text{HO}\cdot$ and $\text{HOO}\cdot$) can form and attack the glycoside C-O and C-C bonds in the starch molecules, thus resulting in their depolymerisation (Venkitanarayanan *et al.*, 1999; Wang and Wang, 2003; Stan *et al.*, 2005).

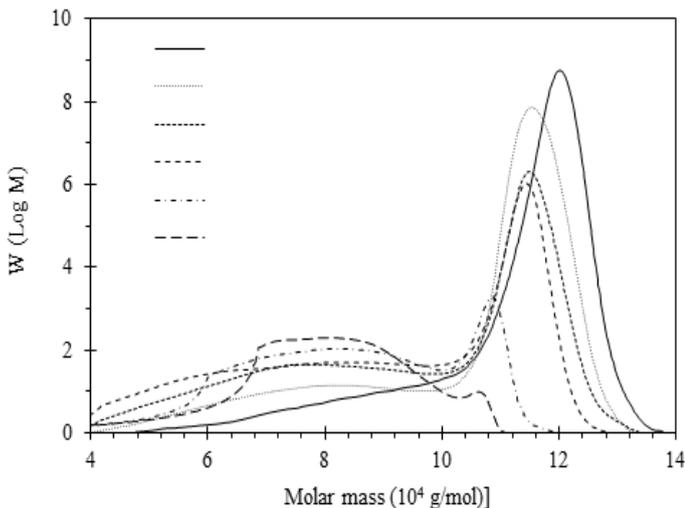


Figure 4. Molecular weight distribution of the oxidised starch samples following electrolytic treatment with different NaCl concentrations.

Thermal degradation

One of the practical concerns when processing oxidised starches at high temperatures is

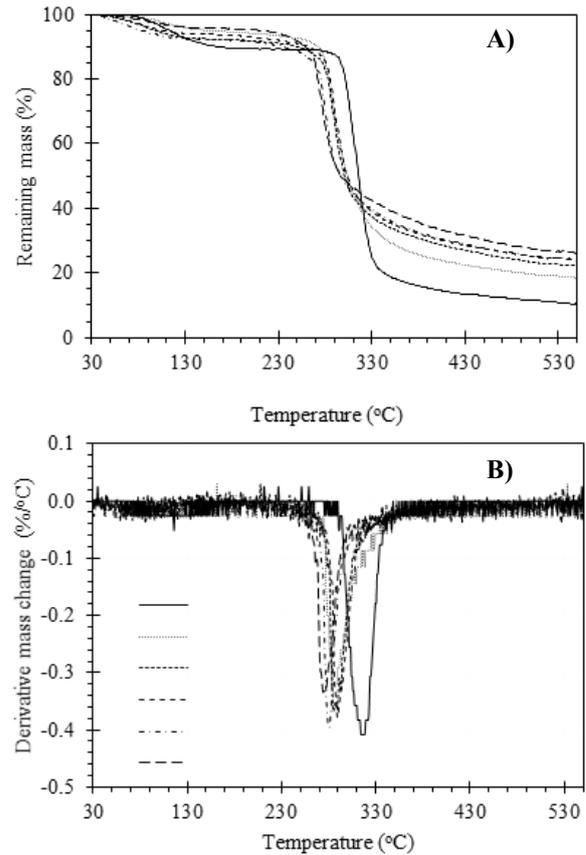


Figure 5. TGA (A) and DTG (B) spectra of the oxidised starch samples after electrolytic treatment with different NaCl concentrations.

their thermal stability. Figures 5A and 5B shows the TGA and DTG curves of the native and the oxidised starch samples, respectively. The mass losses of starch samples under heating are divided into three stages: (i) water evaporation (60 - 110°C), (ii) starch dehydration and subsequent depolymerisation (110 - 300°C); and (iii) carbonisation and ash formation (> 300°C) (Wang *et al.*, 2008). At stage 1, the native and oxidised starch samples had similar TGA/DTG spectra. At stage 2 of the DTG curve, the position of the peak (maximum mass loss) decreased gradually from 315 to 277°C (from G0 to G5), and their heights decreased from -4.08 to -3.26 (%/°C), thus indicating a decrease in thermal stability. This result is in accordance with other studies, where the thermal stability of oxidised starch decreases with an increase in the degree of oxidation (Zhang *et al.*, 2012; Moreno *et al.*, 2017). The reason for this is that the carbonyl and carboxyl groups in oxidised starch are more sensitive to thermal degradation to produce CO , CO_2 , and H_2O (Wei *et al.*, 2015). Although the onset temperatures of thermal degradation of the oxidised starch samples shifted to lower values, they were still higher than the normal processing temperatures of food (< 200°C).

Conclusion

For the first time, tapioca starch was electrolytically oxidised in the gelatinised state. The electrolysis of NaCl solution created an oxidising environment with mild alkaline pH. Increasing the concentration of NaCl in the electrolytic tank led to higher degrees of starch oxidation reaction, thus resulting in higher contents of carbonyl and carboxyl groups, lower average molecular weight, and lower thermal stability. The pre-gelatinised starch had an amorphous state, while ethanol precipitation gave the oxidised starch a V-type crystal structure. From a practical point of view, starch oxidation using NaCl electrolysis is more convenient because the oxidising agents are generated at point-of-use, thus avoiding the storage of corrosive and unstable chemicals like hypochlorites, peroxides, or permanganates. The high amount of carbonyl and carboxyl groups in the oxidised starch may increase the affinity for proteins and can be used for many interesting food applications.

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