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Dibasic Magnesium Hypochlorite as an Oxidant to Tune Pasting Properties of Potato Starch in One Step

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Abstract: Modified starches are used widely in the food industry but often have a low nutritional value, lacking minerals vital for the human body, such as magnesium. Magnesium addition to native starches has been shown to result in changes in pasting properties. However, little work has been done on the addition of magnesium and other divalent cations to highly oxidised starches. In this work, we used dibasic magnesium hypochlorite (DMH) to oxidise potato starch to an industrially relevant degree of oxidation while at the same time introducing magnesium into the starch structure. We found that magnesium incorporation changes the pasting properties of starch and increases the gelatinisation temperature significantly, possibly due to an ionic cross-linking effect. These properties resemble the properties found for heat-moisture-treated potato starches. This change in properties was found to be reversible by performing a straightforward exchange of metal cations, either from sodium to magnesium or from magnesium to sodium. We show in this work the potential of the addition of divalent cations to highly oxidised starches in modifying the rheological and pasting properties of these starches and at the same time adding possible health benefits to modified starches by introducing magnesium.

Keywords: oxidation; modified starch; sodium hypochlorite; divalent cations; food industry

1. Introduction

Starch is a major part of the modern human diet. Even though native starches have been an important part of the human diet for centuries, nowadays our diet contains more processed foods, including modified starches. Modification of native starch is performed, as for many applications, specific pasting properties (low retrogradation and viscosity) and high stability are required [1]. A drawback is that these processed foods contain little nutrients found in natural products, such as vegetables, fruits and seeds. Therefore, a deficiency in vital nutrients can become a factor in a society that consumes large amounts of processed foods [2]. Much research has focused on finding ways to improve the nutritional value of starches and limit their negative health effects. For example, recent studies have focused on the influence of modified starch on the gut microbiome and its associated health effects [3,4]. Another focus point is the addition of minerals to starch, especially ones whose deficiencies are identified in modern diets.

Several studies have focused on the addition of salts with various minerals to starches. A study by Zhou et al. compared the properties of native potato starch (NPS) with and without salt solutions [5–7]. The authors found that important properties, such as swelling power, solubility and pasting properties, follow the Hofmeister series closely. This study highlights that adding minerals to starch is not innocent and can have a large influence on the physical and chemical properties of starch. A recent study by Noda et al. focussed

on the addition of divalent cations Mg^{2+} and Ca^{2+} to starch as chloride salts [8]. Divalent cations have been hypothesised by several authors to alter the properties not just via the mentioned solubility changes but also by ionic cross-links of the native phosphate groups [8–10]. Especially, potato starch is naturally rich in phosphate groups [11]. Due to the divalent nature of Mg^{2+} and Ca^{2+}, they might bind two phosphate groups to form an ionic cross-link. Noda et al. found that salt addition of up to 400 ppm of magnesium and 600 ppm of calcium lowers the peak viscosity and breakdown of the starches significantly. Later, the same group found that calcium lowers the swelling power of starch as well [12].

Magnesium and calcium were not just chosen for their divalent nature. Calcium is essential for bone health and lowers the risk of fractures and bone disease, such as osteoporosis and immune function. Magnesium has been found to be crucial for many bodily functions, such as the activity of many enzymes and ATP and stress regulation, and magnesium deficiency increases the risk of many diseases [2,13–15]. A recent Special Issue of *Nutrients* focusing on the role of magnesium in human health and disease shows that the prevention and detection of magnesium deficiency, the latter of which is currently expensive and difficult [2], is a current challenge for academia and industry [16]. Thus, the addition of magnesium to (modified) starch, a major component of the current diet, is a possible way of preventing or lowering the risk of magnesium deficiency.

Many types of modified starches can be used in processed foods, but one of the main modifications is oxidation. Industrially, oxidation is performed with NaOCl, which is cheap and allows control over the final starch properties. The drawback of NaOCl is that it generates waste in the form of salts and chlorinated by-products [17–19]. During oxidation, alcohol groups are converted sequentially to aldehyde and acid groups. The acid groups provide chain repulsion, preventing retrogradation of the samples, which is ideal for (frozen) foods, which should not harden/crystallise once processed. Furthermore, oxidised starches are often used in the paper industry as binders or surface sizers [20–22]. The acid groups formed in the oxidation process have potential to bind minerals, such as magnesium, just like the phosphate groups in native starch. Potentially, with a high degree of oxidation (DS_{COOH}), a high mineral content can be achieved. Pietrzyk et al. added potassium, magnesium and iron salts to oxidised starch and analysed the cation content along with other starch properties, such as water-binding capacity, gelatinisation temperature and paramagnetism [23]. However, the degree of oxidation was not discussed and the content of cations was lower than for the native starches with added salts, as discussed by Noda et al. [8]. This, along with limited changes in the thermodynamic properties, indicates the degree of oxidation was likely not high enough for most industrial applications.

The displacement of sodium after NaOCl oxidation with other cations is a viable strategy. Still, hypochlorite salts with other cations can be used directly for oxidation as well. Potassium, calcium, barium and magnesium hypochlorite can be synthesised, for example [19]. The oxidation of starch and low-molecular-weight alcohols with calcium hypochlorite has been demonstrated [24–26]. The hypochlorite analogue with magnesium, dibasic magnesium hypochlorite ($Mg(OCl)_2 \cdot 2Mg(OH)_2$, DMH), has been hardly studied in the literature. The use of DMH in detergent compositions and for textile bleaching has been patented [27,28], but to the best of our knowledge, no detailed studies on the use of DMH for alcohol or starch oxidation are available. The use of DMH is interesting for starch oxidation as it can incorporate magnesium into starch while performing the oxidation in one step, eliminating the need for a second substitution step. Secondly, the oxidation of starch is performed at basic pH and requires the addition of a base during the reaction to keep the pH constant [29]. The two $Mg(OH)_2$ in the complex can act as an inherent buffer and increase the pH, lowering the amount of base that needs to be added during the reaction. Moreover, since the properties of highly oxidised starches with magnesium have not been studied in detail yet, we were interested in the influence of a high content on the starch properties.

In this work, we studied the use of DMH to reach an industrial standard degree of oxidation. This is challenging as DMH has low solubility in water, a hurdle that we aimed

to overcome by tuning the reaction conditions. Furthermore, we studied whether the incorporation of magnesium influences the pasting properties, which was expected due to possible ionic cross-linking between magnesium and the introduced carboxylic acid groups. In this way, the potential health benefits of incorporated magnesium might be combined with the desired property changes of oxidised starch.

2. Materials and Methods

2.1. General Considerations and Materials Used

Native, granular potato starch (NPS, moisture content approx. 15 wt%, approx. 20% amylose) was provided by Royal Avebe (The Netherlands), which was used as received. Dibasic magnesium hypochlorite (DMH) and magnesium hydroxide (52–54 wt% suspension in water) were kindly provided by Nedmag (the Netherlands) and used as received. NaOCl (10–15 wt% in water) was purchased from Acros Organics. NaOH was purchased from Merck. Phenolphthalein (reagent grade), $MgCl_2$ (anhydrous), NaCl, $Ca(OCl)_2$ (for synthesis) and concentrated H_2SO_4 were purchased from Sigma-Aldrich. Concentrated HCl (36–38 wt%) was purchased from Boomlab. All chemicals were used as received unless specified otherwise. The reproducibility of analytical techniques was confirmed by performing selected measurements in triplicate. The methods for analysis of the degree of oxidation, pasting properties, starch structure and making of the scanning electron microscope images were identical to those used in our previous work [30]. For clarity, they have been added to the Supplementary Materials.

2.2. Oxidation of Starch by DMH

This adapted procedure from our previous work on the oxidation of starch with NaOCl (see Supplementary Materials for details) and H_2O_2 [30] uses DMH and shows general reaction conditions. For details (amounts of oxidant/pH/temperature) and results, see the main text and Supplementary Materials (Tables S1–S4).

Demiwater (~55 mL) and native potato starch (39 g, 0.241 mol, dry basis) were mixed to obtain a 39 wt% suspension (dry basis) under vigorous stirring with an overhead stirrer. A Julabo 4 thermostat bath was used to heat the suspension to 35 °C and keep it at that temperature during the reaction (Figure S2). Next, dibasic magnesium hypochlorite was added (2.51 g, 10.2 mmol, 4.2 mol%, 6.4 wt% to dry starch), causing the pH to increase to 9.5–10.5. After addition and for the entire reaction time (from the addition of DMH to the depletion of DMH and base post-treatment), a WTW InoLab pH 7310 pH meter using a WTW SenTix 81 Precision pH electrode with a temperature sensor was used to monitor the pH and temperature. Using an Arduino Uno with Atlas Scientific EZO circuit, the pH was set and kept constant at 8.2 by dosing 0.5 M H_2SO_4 automatically with a Watson Marlow 101 dosing pump, which was controlled with an Atlas Scientific pH electrode. The verification of -OCl consumption was performed with a test strip, after which the pH was raised to 10.5 for 1 h. During this base post-treatment, several rearrangement and elimination reactions, such as beta-elimination, take place, modifying the pasting properties of the oxidised starches. As an example, two samples with and without post-treatment were analysed using RVA, showing a more stable starch with lower viscosity after treatment (Figure S4, Table S4, entries 6/9). After the base treatment, acidification to pH 5 (conc. H_2SO_4), vacuum filtration and washing with 1 L of demiwater gave the oxidised starch product. The starch product was air-dried overnight in a fume hood, and the product yield was determined gravimetrically. This was performed by correcting for the moisture content, which was determined with a DBS 60-3 Kern moisture analyser, to obtain the final starch loss and recovered starch yield (97%, dry basis).

2.3. Metal Cation Exchange of Oxidised Starch

2.3.1. NaOCl Oxidised—Mg Exchange

Briefly, 22.51 g (moisture content = 18.37%) of NaOCl-oxidised starch (previous procedure, 5.5 mol% to starch, Tables S1–S4, entry 3) was suspended in ~60 mL of 0.1 M

HCl for 30 min. Next, it was vacuum-filtered and washed with 1 L of deionized water. Subsequently, it was stirred in 50 mL of 5 wt% $MgCl_2$ (anhydrous) for 3 h, followed by vacuum filtration and washing with 1 L of deionized water. The magnesium-fortified starch was dried overnight, and 21.60 g of it was obtained (MC = 16.82%, yield = 98%).

2.3.2. DMH Oxidised—Na Exchange

Briefly, 21.24 g (moisture content = 13.24%) of DMH-oxidised starch (previous procedure, 4.2 mol% to starch, Tables S1–S4, entry 6) was suspended in ~60 mL of 0.1 M HCl for 30 min. Next, it was vacuum-filtered and washed with 1 L of deionized water. Subsequently, it was stirred in 50 mL of 5 wt% NaCl for 3 h, brought to pH 9 with dilute NaOH (0.1 M), followed by vacuum filtration and washing with 1 L of deionized water. The sodium-fortified starch was dried overnight, and 20.63 g of it was obtained (MC = 13.58%, yield = 97%).

2.4. DSC Analysis

Differential scanning calorimetry (DSC) was performed on a TA Instruments DSC 25 using Tzero Pans with Tzero Hermetic Lids. Sample preparation was performed by suspending 140 mg of a starch (wet weight) in 220 mg of H_2O (35 wt% suspension db). Next, 5–10 mg of this suspension was weighed into a DSC pan, which was subsequently closed. The analysis was run with an empty pan as a reference from 35 °C to 95 °C at a heating rate of 10 °C/min, and the relevant DSC parameters were obtained with TA Instruments software.

2.5. ICP-OES Analysis

Inductively coupled plasma–optical emission spectroscopy (ICP-OES) analysis was performed on an Agilent Technologies 5110 instrument with an SPS 4 Autosampler. Calibration curves for the amounts of Ca (317.933 nm), K (766.491 nm), Na (588.995 nm), Mg (383.829) and P (213.618 nm) were made using Sc (361.383 nm) as an internal standard to be able to accurately determine the mineral content in the starch samples. The following is the general procedure for sample preparation:

Ashing step (omitted for phosphorus determination): Briefly, ~5 g of starch (wet weight) was weighed in a porcelain cup and placed in an oven at 500 °C for 30 min. The temperature was increased to 600 °C for another 1 h.

Acid treatment: For phosphorus determination, ~1 g of starch was weighed in a digestion beaker (DigiTUBE). For metal cation determination, the prepared ashed sample was used, as described before. The samples were suspended in ~60–80 mL of ultrapure water, and 12 mL of 37 % HCl and 4 mL of 65 % nitric acid were added. The temperature was set to 130 °C, and the digestion beakers were placed in a Digi PREP jr from SCP Science. After ca. 30 min, the starch was dissolved and the mixture was heated for 45 to 60 min in total. After cooling, ultrapure water was added to yield 100 mL in total. If ash particles were present, the sample was filtered using LLG-syringev filters (SPHEROS, PES, 0.22 μm) to avoid contamination of the detector.

3. Results and Discussion

3.1. Properties of Starch after Oxidation with Dibasic Magnesium Hypochlorite

3.1.1. Initial Screening of the Oxidation Potential of Dibasic Magnesium Hypochlorite

We performed initial experiments with DMH to investigate the potential for the oxidation of starch and make a comparison to the industrial standard of the oxidation of starch with NaOCl. The reaction time to full depletion of hypochlorite (test strips were used to confirm depletion) was monitored along with the pH, and changes in the degree of oxidation (DS_{COOH}) and yield were determined. Our starting point was the industrial process of starch oxidation with NaOCl, which was performed at pH 8.2, at 35 °C and with 8.5 mol% -OCl (Figure 1). After full conversion of hypochlorite (~2 h), a base post-treatment was performed by increasing the pH to 10.5 for 1 h. Under these conditions, a DS_{COOH}

of 0.029 mol/mol was obtained at a starch recovery yield of 97% in 3.5 h (Figure 1c). ICP analysis showed a large increase in the Na content compared to native starch (Figure 2, Table 1, entry 2). The viscosity and paste stability improved, which will be discussed later (vide infra). Subsequently, oxidation reactions with DMH were performed under different conditions (Figure 1a), with the goal of achieving the oxidation of starch and benchmark the DS_{COOH} and yield with NaOCl-oxidised starches. Prior to the experiment, the DMH was ground in a mortar to improve the surface area. Davey and Buckley mentioned in a patent on detergents containing DMH that the solubility of DMH improves dramatically at a lower particle size [28]. We found the same effect in small-scale experiments and therefore ground the DMH before every experiment.
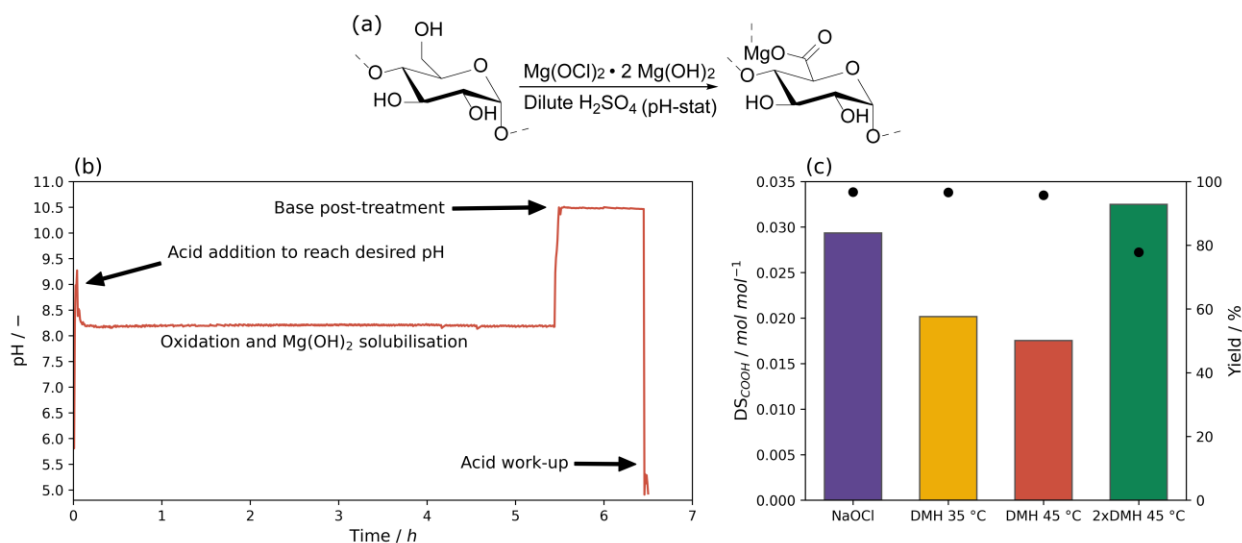

Figure 1. (**a**) Reaction scheme for the oxidation of starch using dibasic magnesium hypochlorite (DMH), acid addition needed to promote the solubility of DMH. The oxidation of the primary alcohol is taken as an example; see Scheme S1 for the full mechanism and oxidation on other positions. (**b**) pH in time of a reaction of starch with DMH (pH 8.2, 45 °C, 4.2 mol% DMH), showing the various stages of the reaction. (**c**) Degree of oxidation (DS_{COOH}, bars) and starch recovery yield (dots) of starch after oxidation with NaOCl (pH 8.2, 35 °C, 8.5 mol% -OCl) and DMH under various reaction conditions (pH 8.2, 8.5 mol% -OCl, except 2xDMH, which was with 17 mol% -OCl).
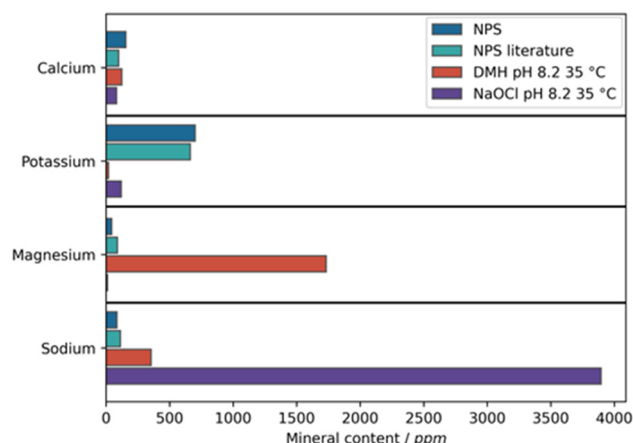

Figure 2. The mineral content of the native potato starch used in this paper (dark blue) compared to the NPS used by Noda et al., data from ref [8] (light blue), showing similar contents of all minerals. In red, the mineral content after oxidation by DMH at pH 8.2, at 45 °C and with 8.5 mol% -OCl is shown. In purple, the mineral content after oxidation by NaOCl at pH 8.2, at 35 °C and using 8.5 mol% -OCl is shown.

Table 1. Degree of oxidation (DS_{COOH}) and mineral content of NPS and starches oxidized with NaOCl and DMH.

Entry	Sample [a]	Temp. (°C) [b]	DS_{COOH} (mol/mol) [e]	Ca (ppm)	K (ppm)	Mg (ppm)	Na (ppm)	Mg/COOH (mol/mol)
1	Native potato starch [c]	–	0.004	155 ± 0.2	701 ± 1.3	43 ± 0.0	85 ± 0.8	0.07
2	NaOCl (8.5)	35	0.029	82 ± 0.0	119 ± 0.9	11 ± 0.1	3896 ± 16	0.003 [d]
3	NaOCl (5.5)	35	0.022	83 ± 0.2	111 ± 0.3	17 ± 0.3	3287 ± 10	0.005 [d]
4	Entry 3—Mg exchanged NaOCl	35	''	19 ± 0.3	12 ± 1.2	873 ± 0.2	69 ± 0.9	0.26
5	(5.5)—Mg(OH)$_2$ added (8.5)	45	0.022	69 ± 0.0	48 ± 0.4	862 ± 1.1	1352 ± 2.4	0.26
6	DMH (4.2)	35	0.020	124 ± 0.5	19 ± 0.5	1732 ± 4.4	354 ± 0.6	0.57
7	DMH (4.2)	45	0.018	113 ± 0.0	11 ± 0.5	1597 ± 2.3	308 ± 0.8	0.61
8	Entry 7—Na exchanged	45	''	23 ± 0.0	7 ± 0.7	65 ± 0.3	2804 ± 4.3	0.02 [d]
9	DMH (8.5)	45	0.033	82 ± 0.0	16 ± 0.7	2406 ± 0.2	681 ± 1.2	0.49

[a] Values within parentheses indicate the amount of oxidant in mol%; [b] all experiments performed at pH 8.2; [c] phosphorus content of 823 ppm, assumed to not change for oxidised starches; [d] Na/COOH of entries 2, 3 and 8: 0.9, 1.0 and 1.1, respectively; [e] average error determined to be <0.001 mol/mol.

We performed oxidation with DMH (Figure 1a) under the same conditions used for NaOCl oxidation, yielding a moderate DS_{COOH} of 0.020 mol/mol at a yield of 97%, although at a much longer total reaction time of 8 h (Figure 1c, Table S1, entries 2/5). ICP analysis showed 1732 ppm of Mg was introduced into the starch after oxidation (Figure 2, Table 1, entry 6). Since the active oxidant (-OCl) is the same as for NaOCl, we expect the oxidative mechanism to be the same as well. Therefore, oxidation likely takes place primarily at the C6-, C2-/C3-positions along with β-elimination, causing glycosidic bond cleavage (Scheme S1) [31,32]. An important factor is the addition of acid during the reaction. When performing the reaction with NaOCl, the pH drops quite rapidly after oxidant addition, setting the pH to 8.2. Additional NaOH is needed to keep the pH at the set value. This is not necessary when using DMH, as it is inherently basic due to the presence of Mg(OH)$_2$. Actually, acid is needed to prevent the pH from increasing to ~9.5, at which value the oxidant is unreactive due to poor solubility. A reaction performed at a pH of 9 showed that the oxidant was not consumed in 7 h (Table S1, entry 13). A DS_{COOH} of just 0.005 mol/mol was obtained, similar to the DS of native potato starch (NPS), which is 0.004 mol/mol (Table 1, entry 1). This shows that DMH needs to be dissolved to release the Mg(OCl)$_2$ in solutions as an effective oxidant. Thus, the addition of acid to start the reaction and maintain a certain pH (below a certain reactivity threshold of about ~8.5) is required to make DMH a potent oxidant (Figure 1b). Another experiment was performed at benchmark conditions but at an increased temperature of 45 °C to further promote DMH solubility. We found the reaction time decreased by 1.5 h to 6.5 h in total (Figure 1b,c, Table S1, entry 6), indicating that DMH solubilization improved. However, the DS_{COOH} (0.018 mol/mol) and yield (96%) were slightly lower compared to the reaction performed at 35 °C. Furthermore, the average M_w was much higher for the starch oxidised at 45 °C (337.6 vs. 174.7 kDa, Table S1, entries 5/6), indicating lower oxidative performance (less oxidative cleavage, see Scheme S1c) as well. Thus, the oxidant is more efficient in oxidising starch at 35 °C, while the reaction is faster at 45 °C.

We envisioned that since the formation of carboxylic acid groups during oxidation lowers the pH and the solubilisation of DMH increases the pH, a balance between these two effects could be achieved, making a pH-stat unnecessary. We performed an experiment in which we added large batches of acid to start the reaction, and let the system reach equilibrium (Table S1, entry 10). Again, 8.5 mol% -OCl was used at 45 °C. The higher temperature was chosen to improve the solubility of DMH. The reaction without pH-stat

allowed us to reach a pH of 8 with two batches of acid (3.09 g HCl, 31 mmol), after which no further acid was required to reach a stable pH, showing that a balance was achieved. At longer reaction times, the pH was not entirely constant, increasing to 8.4 (Figure S1). The lower pH allowed a shorter reaction of time of just 3 h (without base post-treatment). A DS_{COOH} of 0.019 mol/mol was reached at a yield of 96%, showing the potential of DMH as an oxidant with inherent buffering capacity (Table S1, entry 10). For control and comparison purposes, however, we used the pH-stat for all other experiments.

Comparing the obtained degree of oxidation to the NaOCl reference (0.029 mol/mol), for all reaction conditions, the DS_{COOH} of the oxidised starches was found to be lower at similar yields (Figure 1c). We hypothesise that because the hypochlorite of DMH is originally in solid suspension, it reacts more with the recalcitrant outside of the granule, leading to less efficient oxidation compared to fully homogenized NaOCl (which has been found to oxidise throughout the starch granule [31]). Scanning electron microscope images of starches oxidised by both NaOCl and DMH at similar DS_{COOH} were taken and compared to support this hypothesis (Figure 3). After oxidation, several rough areas could be observed on the surface of the granule, unlike the smooth granule of native potato starch (Figure S5). For NaOCl, the damage was limited (Figure 3a). The same was true for DMH-oxidised granules when 8.5 mol% -OCl was used, although the damage was more pronounced (Figure 3b). By doubling the amount of oxidant (pH 8.2, 45 °C, 17 mol% -OCl), we could study whether increasing the amount of oxidant would increase the damage as well. The effect was pronounced, with pits observed in the starch and parts of the surface being lost (Figure 3c). The latter was highlighted by the drop in yield to 78%, indicating that, indeed, large parts of the starch were solubilised and lost in the filtration step. The oxidation was successful in that a DS_{COOH} of 0.033 mol/mol could be reached, the highest of the DMH-oxidised starches (Figure 1c, Table S1, entry 8). Still, the low yield makes it unsuitable for large-scale application.
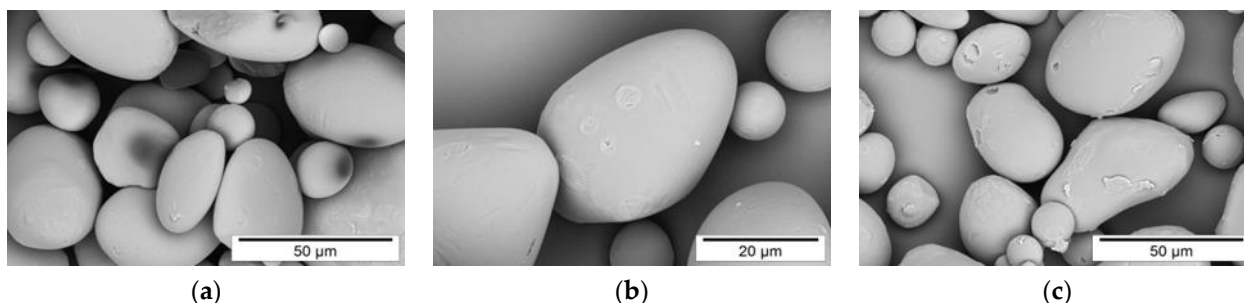

(a)   (b)   (c)

Figure 3. Scanning electron microscope (SEM) images of starches oxidised with (**a**) NaOCl (pH 8.2, 35 °C and 5.5 mol% -OCl), (**b**) DMH (pH 8.2, 35 °C and 8.5 mol% -OCl) and (**c**) DMH (pH 8.2, 45 °C and with 17 mol% -OCl).

3.1.2. Pasting Properties of Starches after Oxidation with DMH

We studied the oxidised starches further by looking at their pasting properties. These are vital for most food and paper applications as they provide insight into the way the starches behave in the products. For this, analyses using a Rapid Visco Analyser (RVA) and differential scanning calorimetry (DSC) were performed. RVA spectra of experiments performed at pH 8.2 showed that at 45 °C, a higher peak viscosity (7109 mPa·s, Figure 4, Table S4, entry 6) and setback viscosity (4747 mPa·s) were obtained compared to oxidation with NaOCl (5595 and 381 mPa·s, respectively, entry 2). The low setback for NaOCl-oxidised starches makes them useful for food applications that require high stability. A high setback indicates facile, undesired retrogradation, meaning that the starch can crystallise or harden in the product. NaOCl-oxidised starch is thus stable, while DMH-oxidised starch still retrogrades quite easily. This can be ascribed to the moderate DS_{COOH} of 0.018 mol/mol and the oxidation on the outside of the granule. The carboxylic acid groups introduced by oxidation provide chain repulsion, which prevents the chains from forming a double

helical structure and crystallise. When the chains on the inside are oxidised less, they have a higher tendency to retrograde and the lowering in setback is only moderate. The higher DS_{COOH} of 0.020 mol/mol for oxidation at 35 °C lowers the setback to 1966 mPa·s, with a peak viscosity of 4507 mPa·s (Figure 4, Table S4, entry 5). The lower peak viscosity is a sign of additional chain cleavage during oxidation, which is reflected by the average M_w, which is 175 kDa compared to 338 kDa for the higher reaction temperature (Table S1, entries 5–6). When doubling the amount of hypochlorite compared to the other experiments, a stable starch was obtained (setback = 178 mPa·s) at a low peak viscosity of 1210 mPa·s. With this amount of oxidant, additional oxidative cleavage (average M_w = 112 kDa) causes a low setback, which is reflected in the low yield of 78%. However, the average M_w is higher for the aforementioned starches compared to the NaOCl-oxidised starch (104 kDa, entry 2), another sign that the oxidation with NaOCl is more homogeneous throughout the granule. This strengthens our hypothesis that DMH oxidises the outside of the granules preferentially. This causes highly oxidised chains on the outside, which dissolve more readily to be lost, causing the yield to be lower (Figure 3).
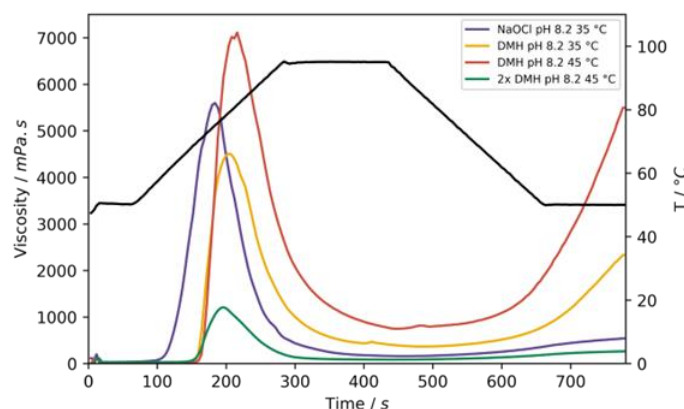

Figure 4. RVA pasting curves (30 wt% in water) of starches oxidized with NaOCl or DMH at pH 8.2 using 8.5 mol% (purple, red yellow) or 17 mol% -OCl (green).

The most striking difference when comparing DMH-oxidised starches to the NaOCl-oxidised one is the large shift in gelatinisation onset and peak viscosity temperatures (Figure 4, Table 2). The most facile explanation for this shift is the high content of magnesium in the DMH-oxidised starches (Figure 2). Indeed, the starches shown in Figure 4 have a magnesium content of 1597, 1732 and 2406 ppm (Table 1, entries 7, 6 and 9), respectively (cf. the highest content of 400 ppm for native potato starch by Noda et al. [8]). These values were related to the DS_{COOH} of these starches (0.018, 0.020 and 0.033 mol/mol, respectively). Mg/COOH ratios of 0.61, 0.57 and 0.49 mol/mol (Table 1) were obtained. This shows the starches bind magnesium closely and primarily on the acid groups formed during oxidation. The ratio of approximately 1:2 links to the divalent nature of the magnesium ions. We envision two likely hypotheses for the observed changes in properties. One is the formation of ionic cross-links between acid groups formed during oxidation. This ionic cross-linking of divalent minerals, as described by various authors in the literature (see Section 1), has an impact on the starch properties. Because the degree of oxidation is higher than for native starches (0.004 mol/mol for NPS), the change in the starch properties is higher as well. Due to the ionic cross-links formed, the swelling of the starches is inhibited. This causes a shift in the gelatinisation temperature of almost 6 °C (compare Table 2, entries 3/6) for starches close in the degree of oxidation (Table 1, 0.022 and 0.020, respectively). The second hypothesis is that the Mg primarily binds the C2-/C3-position dicarboxylic acid moieties (Scheme S1b). Due to the close proximity of the two groups, they bind the Mg strongly. The bound magnesium shields the charges of the carboxylic acid groups, thereby lowering the tendency for granular swelling and causing a shift in the gelatinisation temperature.

Table 2. Gelatinisation properties of NPS and starches oxidised with DMH and NaOCl as determined by DSC. T_O = gelatinisation onset temperature; T_P = gelatinisation peak temperature; T_E = gelatinisation end temperature; ΔH = gelatinisation enthalpy.

Entry	Sample [a]	Temp. (°C) [b]	T_O (°C)	T_P (°C)	T_E (°C)	ΔH (J/g)
1	Native potato starch	−	61.3	65.2	71.5	18.6
2	NaOCl (8.5)	35	55.8	60.4	66.7	15.8
3	NaOCl (5.5)	35	59.9	63.1	69.0	20.8
4	Entry 3—Mg exchanged	−	63.0	66.9	73.8	20.3
5	NaOCl (5.5)—Mg(OH)$_2$ added (8.5)	45	63.1	66.9	74.6	18.2
6	DMH (4.2)	35	62.5 ± 0.06	66.2 ± 0.1	72.6 ± 0.7	19.3 ± 2.3
7	DMH (4.2)	45	64.5	68.0	74.6	19.9
8	Entry 7—Na exchanged	−	60.6	65.0	73.4	20.4
9	DMH (8.5)	45	61.7	66.4	74.1	17.6

[a] Values within parentheses indicate the amount of oxidant used in mol%; [b] all experiments performed at pH 8.2.

A combination of the two is likely, considering that during the oxidation with hypochlorite, oxidation can take place at C6, C2/C3 or the glycosidic linkages. Regardless of the mechanism, the shift in gelatinisation temperature is a major difference that may be useful in a variety of applications. Such a shift due to starch inhibition was observed before for heat-moisture-treated starches [33]. The benefit of magnesium inclusion is that it is facile and the property changes are reversible by removing the magnesium or replacing it with a (monovalent) mineral (vide infra).

3.2. Exchange of Magnesium and Sodium of Oxidised Starch

We established the effect on the properties of magnesium-fortified oxidised starches. We were interested whether exchange after oxidation would show the same effect and whether the removal of magnesium would have an opposite effect. After oxidation with both NaOCl (5.5 mol%, Table 1, entry 3) and DMH (4.2 mol%, entry 7), half of the oxidised starch was subjected to exchange with MgCl$_2$ (entry 4) and NaCl (entry 8), respectively. First, the samples were stirred in 0.1 M HCl for 30 min to protonate the acid groups and remove the attached cations. Next, the samples were stirred in the chloride salt of the desired cation. In the case of Na exchange, NaOH was used to increase the pH and promote the exchange. ICP analysis showed that exchange was possible with this facile method. The DMH-oxidised starch with a Mg content of 1597 was fortified with Na to a content of 2804 ppm (Na/COOH = 1.1 mol/mol, Table 1, entries 7/8, Table S2, entries 6/7). The exchange with Mg was only partial, with the NaOCl-oxidised starch with a Na content of 3287 ppm fortified with Mg to a content of 873 ppm (Mg/COOH = 0.26 mol/mol, Table 1, entries 3/4, Table S2, entries 3/4). After this, RVA and DSC analysis were performed on the exchanged samples to see changes in the thermodynamic and pasting properties.

A large effect on the properties was observed for both samples after exchange. For the NaOCl-oxidised starch that was exchanged with MgCl$_2$, the peak viscosity increased from 7129 to 9771 mPa·s. The increase in peak viscosity was surprising, as most oxidised starches fortified with Mg show a decrease in peak viscosity (cf. Figures 4 and S3). We hypothesise that the lower Mg content of 873 ppm caused the increase. With this content, the ratio of Mg/COOH is 0.26, showing that not all COOH groups are involved in ionic cross-linking (Na content is low as well at 20 ppm). These free COOH groups may allow the starch to swell better and increase the viscosity, as was observed by Karim et al. for a higher phosphate content in potato starches [34]. A shift in the gelatinisation temperature indicative of a high magnesium content in oxidised starches was observed after exchange as well (3.1 °C increase, Figure 5b, Table 2, entries 3/4). This shows that exchange is also a potential method of tuning the properties of an oxidised starch. With this simple method, a starch with a relatively low DS$_{COOH}$ can be given a higher gelatinisation temperature, while also having the potential health benefits of a high magnesium content.
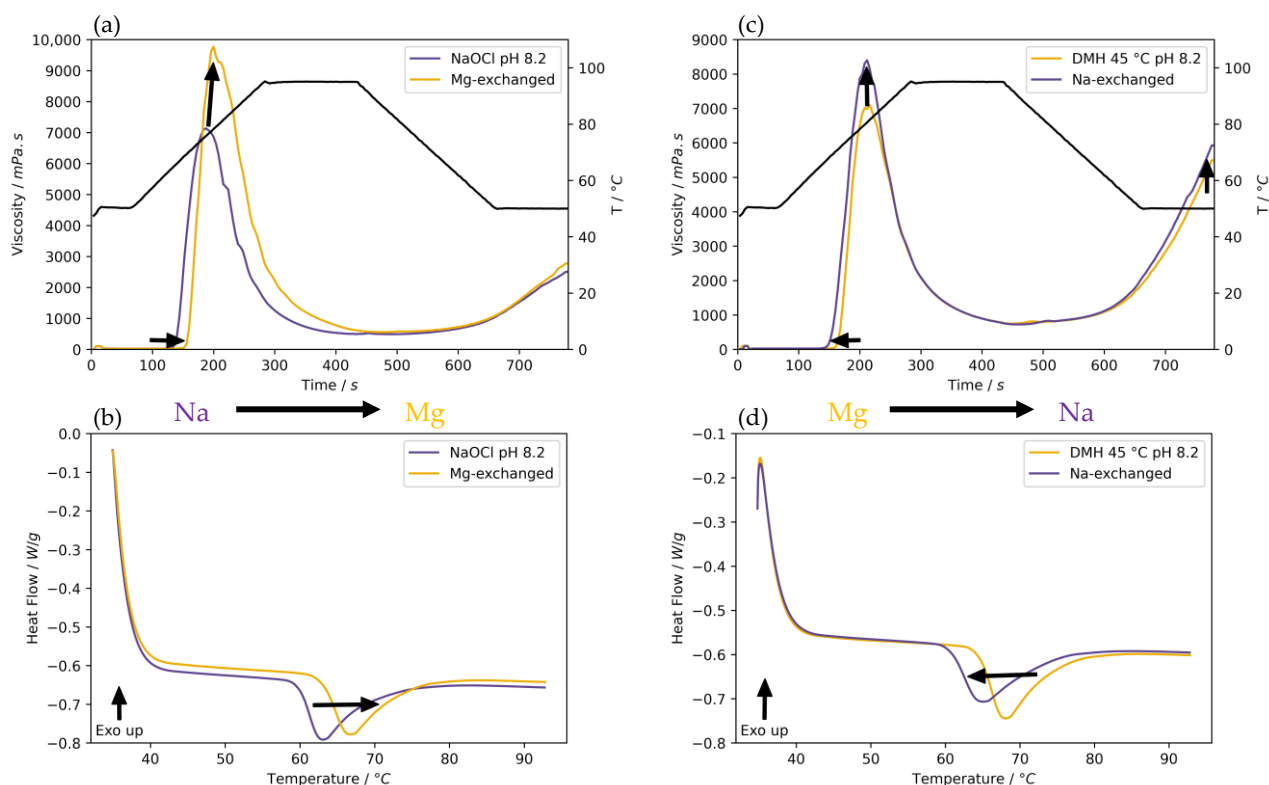
Figure 5. Result of the exchange of minerals in oxidised starch. On the left, NPS was oxidised with NaOCl at pH 8.2, at 35 °C using 5.5 mol% -OCl, followed by exchange with MgCl$_2$. The samples were analysed using (**a**) RVA and (**b**) DSC. On the right, NPS was oxidised with DMH at pH 8.2, ta 45 °C using 8.5 mol% -OCl, followed by exchange with NaCl and NaOH to increase the pH. The samples were analysed using (**c**) RVA and (**d**) DSC.

Reversibility was shown by exchange of a DMH-oxidised starch with sodium. The pasting properties of the Na-fortified starch changed, as expected, although to a lesser extent (Figure 5c), which we ascribe to the moderate DS$_{COOH}$ of 0.018 mol/mol (peak viscosity from 7109 to 8401 mPa·s, setback from 4747 to 5200 mPa·s, Table S4, entries 6/7) and because of the oxidation taking place mainly on the surface of the starch granule. A shift in the gelatinisation temperature of 4 °C (Figure 5d, Table 2, entries 7/8) was observed, showing again that magnesium has a large impact on the gelatinisation behaviour of highly oxidised starches. Overall, we can see that changing the properties of oxidised starches significantly by exchange with metal cations is possible in a simple and fast way.

We would like to highlight that divalent metal cations can change the properties of oxidised starch. Therefore, we also performed an experiment using Ca(OCl)$_2$ as an oxidant, and the resulting starch properties were analysed. The reaction was performed at pH 8.2 and 35 °C with 8.5 mol% -OCl. The resulting starch had a DS$_{COOH}$ of 0.027 mol/mol and an average M$_w$ of 288 kDa at a yield of 98 % (Table S1, entry 14). A calcium content of 3369 ppm was obtained, which is equivalent to a Ca/COOH ratio of 0.51 (Table S2, entry 14). So, most acid groups introduced by oxidation were bound by another divalent cation in a 1:2 ratio similarly to what we observed for magnesium. The peak viscosity was found to be lower than for the NaOCl-oxidised starch (4336 compared to 5595 mPa·s, Tables S3 and S4, entries 2/14). Moreover, the gelatinisation temperature shifted by 4 °C compared to NaOCl-oxidised starch to 61 °C. A setback of 699 mPa·s was obtained, indicating a stable starch. With this experiment, we showed that it is not just magnesium that can bring about such major changes to oxidised starches and that the modification of oxidised starches with divalent cations has a lot of potential in a more general sense.

4. Conclusions

We showed in this work that oxidation with dibasic magnesium hypochlorite (DMH) to industrial standard levels of oxidation can be performed successfully. In contrast to NaOCl oxidation of starches, the current industry standard, oxidation seems to mainly occur at the surface of the granule. Furthermore, the divalent magnesium cation is able to 'bridge' neighbouring carboxyl groups within the starch chains. The combined effects result in different properties of DMH-oxidised starches compared to NaOCl-oxidised starches. This is reflected in the different rheological behaviour and gelatinisation temperatures of DMH-oxidised granules. Exchange reactions with Na and Mg salts showed that these properties can be altered reversibly. In addition to the property changes, the nutritional value of these starches is enhanced for food applications due to fortification with magnesium. The use of these starches could help to lower the risk of magnesium deficiency. We showed two ways of achieving Mg fortification in oxidised starches. One is using ubiquitous NaOCl, followed by fortification via exchange with a Mg salt, a simple and fast method. The other is a one-step process allowing direct Mg fortification during oxidation, using DMH as a Mg-containing oxidant. Finally, we showed that oxidation with $Ca(OCl)_2$ is possible as well, and property changes in the oxidised starch similar to those due to Mg fortification were observed, indicating that this one-step oxidation process with divalent cations is applicable more broadly.

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