



# Impact of conjugation with maltodextrin on rheological properties of sodium caseinate

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## ABSTRACT

Aqueous dispersions of sodium caseinate-maltodextrin (NaCas-MD) conjugates were prepared by heating to study the impact of Maillard reaction on protein functionality. Conjugation with MD affected rheological properties, colour and microstructure of NaCas-MD dispersions. Gelation temperature of NaCas-MD dispersions increased by 7 °C after conjugation, whereas the gelation time was not significantly affected. Shear thickening of the dispersions occurred at high shear rates, and temperature-viscosity relationship was described by the Arrhenius equation. Gels formed at 25 °C had a lower gel strength after conjugation, associated with changes in microstructure. Strain hardening appeared at large amplitude oscillatory shear, which was more profound after conjugation. Moreover, a higher dextrose-equivalent-value of maltodextrin resulted in conjugates with higher degree of conjugation, darker colour, and larger particulates on a microscale. The study indicates that rheological properties of NaCas can be modified by conjugation with maltodextrin, which is in principle applicable for other protein-polysaccharide combinations.

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## 1. Introduction

Conjugation between proteins and polysaccharides via Maillard reaction is a spontaneous and catalyst free reaction, which can be greatly accelerated by heat (Kato, 2002). The Maillard reaction can be divided into three stages: (i) the early stage when reactions between the amino groups in proteins/peptides and the carbonyl groups of reducing carbohydrates form a Schiff base; (ii) the intermediate stage when the Amadori and/or Heyn's rearrangement products degrade; (iii) the advanced stages when advanced Maillard reaction products and melanoidins are produced (O'Mahony, Drapala, Mulcahy, & Mulvihill, 2017). The conjugation reaction between proteins and polysaccharides is primarily based on the formation of Amadori-type linkage (i.e., a covalent attachment) (Shepherd, Robertson, & Ofman, 2000). Modifying protein functionality via the Maillard reaction requires no extraneous chemicals, in contrast to acetylation, deamidation, succinylation and other chemical methods.

Hence, the Maillard reaction is a promising approach to improve protein functionality for food applications (Mulcahy, Park, Drake,

Mulvihill, & O'Mahony, 2016; Oliver, Melton, & Stanley, 2006; Zhang et al., 2020). Research has shown that conjugation with polysaccharides leads to significant improvements of the functional properties of proteins such as solubility, emulsifying properties, heat stability and antimicrobial activity (Al-Hakkak & Kavale, 2002; Fechner, Knoth, Scherze, & Muschiolik, 2007; O'Regan & Mulvihill, 2009, 2010a,b). Conjugation reactions may also modify rheological properties of proteins (e.g. gelation and viscoelastic properties), which has not been extensively studied and thus poorly-documented in literature (Spotti et al., 2019). However, knowledge about the rheological properties of proteins is essential for designing formulations and processing steps of protein-based foods such as protein gels.

Many food products, such as yoghurt, cheese, tofu, etc., are protein-based gels. Depending on the properties of proteins, gelation can be induced by heating, cooling, changing pH, adding salt, enzymatic crosslinking and/or applying high pressure. Sodium caseinate (NaCas) is a dairy protein ingredient that is widely applied in food industry due to its excellent emulsifying, thickening and foaming abilities (Moreira, Pereira, Vicente, & da Cunha, 2019). A suspension of sodium caseinate contains protein particles rather than monomeric casein (Huppertz et al., 2017), and the aqueous dispersion of sodium caseinate exhibits thermo-reversible gelation

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behaviour, i.e., the dispersion shows a liquid-like behaviour above its gelation temperature ( $T_{gel}$ ) and solidifies upon cooling at a temperature below its  $T_{gel}$  (Schutyser, Houlder, de Wit, Buijsse, & Altling, 2018).

As mentioned above, conjugation with polysaccharides may affect the rheological properties of proteins. Caseins have been successfully conjugated with maltodextrin (MD) (Morris, Sims, Robertson, & Furneaux, 2004). The acid gelation properties of casein were modified by conjugation with MD. The acid gelation time of sodium caseinate by adding glucono- $\delta$ -lactone decreased slightly, and the storage modulus ( $G'$ ) of acid gels was significantly lower after conjugation with maltodextrin. These were probably because MD polysaccharide moiety hindered the aggregation and rearrangements of the casein network (Zhang, Gong, Khanal, Lu, & Lucey, 2017). In another study, a 'synergistic' increase in the mechanical strength of gels formed from NaCas-MD composites (without conjugation) was observed, and the solvent partition between NaCas and MD phases was rationalised by the concept of kinetically trapped mixed gels (Manoj, Kasapis, & Chronakis, 1996). However, to date, the possible impact of conjugation with MD on the cold gelation of NaCas without acidification has not been systematically studied. Therefore, it is interesting to characterise NaCas-MD conjugates formed via Maillard reaction in terms of gelation temperature, gelation time, and other viscoelastic properties. The obtained conjugates may be used in food applications, for example as food inks for extrusion-based 3D printing of protein gels (Schutyser, Houlder, Wit, Buijsse, & Altling, 2018).

This research therefore investigated the impact of conjugation with maltodextrin on selected functional properties of sodium caseinate (i.e., gelation behaviour, viscoelastic properties, colour and microstructure). Maltodextrins with two different DE-values were chosen to investigate the influence of DE on the conjugation reaction and physicochemical properties of the conjugates. Specifically, NaCas-MD conjugates were made by heating the mixtures of NaCas (15%, w/w) and maltodextrin (DE5 or DE29, 15%, w/w) at 90 °C for 0, 2, 4, 6 h, respectively. Subsequently, the obtained conjugates were characterised in terms of degree of conjugation, rheological properties, colour and microstructure.

## 2. Materials and methods

### 2.1. Materials

Concentrated sodium caseinate dispersion (NaCas, 43%, w/w) was kindly supplied by FrieslandCampina (Wageningen, the Netherlands) and stored at -20 °C. Maltodextrin with a dextrose equivalent (DE) value of 5 (MD5) or 29 (MD29) was provided by Roquette (Lestrem, France). 2,4,6-Trinitrobenzenesulfonic acid solution (TNBS, 5%, w/v, in H<sub>2</sub>O; Sigma-Aldrich, Germany) was used to determine primary amines. Other reagents were of analytical grade, i.e., NaHCO<sub>3</sub>, NaOH, sodium dodecyl sulphate (SDS), lysine (all from Sigma-Aldrich) and HCl (VWR, the Netherlands). Milli-Q water (Merck KGaA, Germany) was used to prepare all the solutions and dispersions used in this study.

### 2.2. Preparation of conjugates

Frozen sodium caseinate dispersion was defrosted and stored at 4 °C before experimental use. Maltodextrin powder (MD5 or MD29) was dissolved in Milli-Q water at 15%, w/w, and NaCas was added as solid chunks at 15%, w/w. The total dry matter in the NaCas-MD dispersion was 30%, w/w. The sample in an airtight centrifuge tube (capacity: 15 mL) was heated in a water bath at 90 °C for approximately 2 min to melt the solid chunks of

NaCas. Subsequently, samples were thoroughly homogenised by high-speed vortexing. The pH of fresh NaCas-MD5 and NaCas-MD29 dispersions were 6.84 and 6.74, respectively, as measured by a pH meter (Hanna Instruments, Romania). The pH of all the samples was not adjusted. The conjugation reaction between NaCas and MD5 was induced by heating the samples in a water bath at 90 °C for 0, 2, 4, and 6 h, respectively. Samples prepared with MD29 were heated for 0 h and 6 h, respectively (Zhang et al., 2017). After conjugation reaction, samples were cooled down immediately in ice-water bath and were stored at 4 °C. Samples were melted and homogenised before further characterisation.

### 2.3. Degree of conjugation

The degree of conjugation between NaCas and MD was determined based on the change in the number of free amino groups. The concentration of available amino groups in NaCas-MD dispersions after conjugation reaction were determined using the trinitrobenzene sulfonate method (TNBS) which was adapted from O'Regan and Mulvihill (2009).

To obtain a lysine standard curve, 25 ~ 150  $\mu$ L of the lysine stock solution ( $2.7 \times 10^{-4}$  mol L<sup>-1</sup>, freshly prepared in Milli-Q water) was added into Eppendorf centrifuge tubes. A 0.1 mol L<sup>-1</sup> NaHCO<sub>3</sub> buffer solution (pH adjusted to 8.5 with 0.2 mol L<sup>-1</sup> NaOH) was added to reach a total volume of 500  $\mu$ L in the tubes, e.g., 450  $\mu$ L NaHCO<sub>3</sub> buffer was added into a centrifuge tube containing 50  $\mu$ L lysine stock solution. Subsequently, 250  $\mu$ L of trinitrobenzenesulfonic acid solution (TNBS, 0.01%, w/v) was added to each centrifuge tube. The tubes were vigorously vortexed and incubated at 40 °C for 2 h in a thermomixer (Eppendorf, Germany). After incubation, 250  $\mu$ L of 10%, w/v, sodium dodecyl sulphate (SDS) solution and 125  $\mu$ L of 1 mol L<sup>-1</sup> HCl were added into each sample to terminate the reaction. The concentration of  $\epsilon$ -triphenyl-lysine was determined by measuring the absorbance of the sample solutions at 335 nm ( $A_{335nm}$ ) using a spectrophotometer (Beckmann Coulter Inc, USA) while Milli-Q water was used as the blank. A lysine standard curve was obtained by plotting  $A_{335nm}$  against the known lysine concentrations (Supplementary material Fig. S1).

To obtain a calibration curve of protein content, NaCas was reconstituted in the NaHCO<sub>3</sub> buffer solution to reach a concentration of 1%, w/w. The NaCas solution was diluted using buffer solution to the desired concentrations (i.e., 0.01–0.2%, w/w). The diluted samples were transferred into quartz cuvettes and the absorbance at 280 nm ( $A_{280nm}$ ) was measured using a spectrophotometer (Beckmann Coulter Inc, USA). The buffer solution was used as the blank. The measured  $A_{280nm}$  were plotted against the known concentrations of NaCas (Supplementary material Fig. S2).

Similarly, NaCas-MD dispersions (before and after conjugation reaction) were reconstituted in NaHCO<sub>3</sub> buffer solution to a concentration of approximately 0.1%, w/w. The  $A_{280nm}$  of the sample was measured and the actual protein content in the diluted samples was determined by comparing the results to the aforementioned calibration curve. Subsequently, the concentration of available amino groups in the same diluted samples was determined using the same protocol for the lysine standard curve as described above. Specifically, the lysine stock solution (50  $\mu$ L) was replaced by 50  $\mu$ L of the diluted sample solution for the TNBS measurement, and the concentration of available amino groups was determined by comparing the results with the lysine standard curve.

Subsequently, the degree of conjugation reaction can be calculated as Eqn. (1):

$$d_c = \left(1 - \frac{C_{a,t}/C_{p,t}}{C_{a,0}/C_{p,0}}\right) \times 100 \quad (1)$$

where  $d_c$  was the degree of conjugation (%),  $C_{a,t}$  ( $\text{g L}^{-1}$ ) and  $C_{p,t}$  (% w/w) were the concentrations of residual amino groups and protein after conjugation reaction, respectively; whereas  $C_{a,0}$  ( $\text{g L}^{-1}$ ) and  $C_{p,0}$  (% w/w) were the concentrations of initial amino groups and protein in the untreated NaCas-MD mixture (i.e., 0 h sample).

## 2.4. Rheological properties

### 2.4.1. Gelation temperature

The gelation temperature of NaCas-MD dispersions was determined by a temperature ramp test using a rheometer equipped with a heating cap (Anton Paar Physica MCR502, Anton Paar GmbH, Graz, Austria) (Loveday, Rao, Creamer, & Singh, 2010). A 20 mm cone and plate geometry (CP20-2) was used for the measurement. Each sample was melted at 80 °C for 5 min prior to the test, and was transferred to the preheated plate (i.e., at 60 °C). The cone probe was brought down, and any excess of materials was carefully removed. The rim of the sample was coated with high viscosity paraffin oil to prevent water evaporation during measurement. The strain and frequency for the temperate ramp test were set to 3% and 1 Hz, respectively. The temperature of the system was gradually decreased from 60 °C to 10 °C at a rate of 2.5 °C  $\text{min}^{-1}$ , and then increased again from 10 °C to 60 °C at the same rate. The temperature, storage modulus ( $G'$ , Pa) and loss modulus ( $G''$ , Pa) of the system were recorded at a time interval of 1.5 s. The gelation temperature ( $T_{gel}$ , °C) was established at the temperature when the storage modulus  $G'$  exceeded that of the loss modulus  $G''$ .

### 2.4.2. Gelation time

After measuring the gelation temperature, the gelation time of the same sample was characterised by a time sweep test adapted from Liu, Bhandari, Prakash, Mantihal, and Zhang (2019). Specifically, the temperature of the system was decreased from 60 °C to 25 °C at a rate of 10 °C  $\text{min}^{-1}$  and was then kept at 25 °C for 3 min. The temperature, storage modulus ( $G'$ , Pa), loss modulus ( $G''$ , Pa) and complex modulus ( $G^*$ , Pa) of the system were recorded at a time interval of 1.5 s. The gelation time ( $t_{gel}$ , s) was defined as the time needed until a plateau of the complex modulus  $G^*$  was reached, i.e., when the value of  $|(G_{n+1}^* - G_n^*)/G_n^*|$  was  $\leq 0.001$  ( $n = 1, 2, 3, 4 \dots$ ).

### 2.4.3. Viscosity

The temperature-dependent viscosity of NaCas-MD dispersions was determined using a temperature ramp measurement. The temperature was increased from 35 °C to 90 °C at a rate of 5 °C  $\text{min}^{-1}$  while the shear rate was fixed at 50  $\text{s}^{-1}$ , and the viscosity was recorded. In addition, the shear viscosity of NaCas-MD dispersions was measured using a shear rate sweep test at 35 °C which was slightly above the measured  $T_{gel}$  (Liu et al., 2019). The shear rate was increased from 0.1 to 100  $\text{s}^{-1}$  in a timeframe of 250 s, and the corresponding viscosity was recorded.

### 2.4.4. Gel strength

The gel strength of NaCas-MD dispersions at 25 °C was determined using a frequency sweep test. The angular frequency was decreased from 100 to 0.1  $\text{rad s}^{-1}$ , and the corresponding complex modulus  $G^*$  (Pa) was recorded. The gel strength was calculated as Eqn. (2) (Díaz et al., 2019):

$$G^* = A\omega^n \quad (2)$$

where  $G^*$  was the complex modulus (Pa),  $\omega$  the angular frequency ( $\text{rad s}^{-1}$ ), parameter  $A$  represented the gel strength ( $\text{Pa s}^n$ ) and  $n$  was a power-law relaxation exponent (–). The reciprocal of  $n$  (i.e.,  $1/n$ ) was presented as  $z$  (–) which was a coordination number.

### 2.4.5. Oscillatory shear test

A dynamic oscillatory shear sweep test was employed to investigate the mechanical response of NaCas-MD dispersions to the change in shear strain/stress. During the test, the shear stress was increased from 0.01 to 1000 Pa at 25 °C and a fixed frequency of 1 Hz, the storage modulus ( $G'$ , Pa) and the loss modulus ( $G''$ , Pa) were recorded.

## 2.5. Colour measurement

The colour change in NaCas-MD dispersions due to conjugation reaction was determined by measuring the CIELab parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) using a colorimeter (Chroma Meters CR-400, Konica Minolta, USA). The colorimeter was calibrated with a standard white tile. Prior to the measurement, NaCas-MD dispersions were melted at 90 °C in a water bath and centrifuged at 4700× $g$  for 10 min to remove air bubbles. Subsequently, 5 g liquid sample was poured into a quartz cell and was left at room temperature until the sample solidified. The  $L^*$   $a^*$   $b^*$  values were determined while the standard white tile was placed on top of the quartz cell as a background. The colour difference ( $\Delta E$ ) between the untreated NaCas-MD dispersion and the conjugated ones was calculated as Eqn. (3):

$$\Delta E = \sqrt{(L^* - L_{ref}^*)^2 + (a^* - a_{ref}^*)^2 + (b^* - b_{ref}^*)^2} \quad (3)$$

where  $L_{ref}^*$ ,  $a_{ref}^*$  and  $b_{ref}^*$  were the  $L^*$ ,  $a^*$ , and  $b^*$  values, respectively, of the untreated NaCas-MD dispersion (i.e., 0 h sample).

## 2.6. Scanning electron microscopy

The particulate network microstructure of the samples was studied by scanning electron microscopy (SEM) analysis. The untreated and 6 h-conjugated NaCas-MD dispersions were freshly prepared. Samples were fixed in 2.5% glutaraldehyde at room temperature for 4 h. After rinsing, the samples were dehydrated in a graded ethanol solutions in water (10%–100%, 10 min per step). The samples were subsequently critical point dried (CPD) with carbon dioxide in a Leica EM CPD300 automated critical point dryer. Subsequently, the samples were mounted on a SEM stubs by carbon adhesive glue (EMS, Washington USA) and coated with 12 nm Tungsten (Leica MED 020). Samples were analysed at 2 kV, 13 pA, in a field emission scanning electron microscope (Magellan 400, FEI, Eindhoven, the Netherlands).

## 2.7. Statistical analysis

All the experiments were done independently in at least duplicate and all the data are presented as mean  $\pm$  standard deviation (SD). Student's  $t$ -test was performed using Microsoft Excel 2010, and a  $p$ -value smaller than 0.05 meant the difference between two means was statistically significant.

### 3. Results and discussion

#### 3.1. Preparation of conjugates

Heating can accelerate the Maillard reaction between maltodextrin and sodium caseinate (Zhang et al., 2017). Fig. 1 shows that the degree of conjugation between NaCas and maltodextrin (i.e., the loss of available amino groups) was increasing during the 6 h of reaction, to  $19.3 \pm 6.3\%$  and  $23.5 \pm 0.8\%$  for MD DE5 and DE29, respectively. However, longer reaction time (i.e., 8 h) did not further increase the degree of conjugation in NaCas-MD5 dispersion. Therefore, the 6 h-conjugated systems were selected for further investigation.

The degree of conjugation can be affected by the concentrations of NaCas and MD, as well as by the heating method (i.e., wet heating or dry heating) and time applied. Zhang et al. (2017) reported a degree of conjugation of 10.8% in a 5%, w/v, NaCas and 5%, w/v, MD (DE = 4–7) mixture after 10 h wet heating at  $90^\circ\text{C}$  in a water bath. Nevertheless, the degree of conjugation in the NaCas-MD5 dispersion was found to be 19.3% in this study, after 6 h wet heating at  $90^\circ\text{C}$ . The higher degree of conjugation found in the current study might be due to the higher concentrations of NaCas and MD (15%, w/w): the higher concentrations may have led to more effective interactions between protein and polysaccharide molecules due to macromolecular crowding effects, resulting in a higher degree of conjugation (Perusko, Al-Hanish, Velickovic, & Stanic-Vucinic, 2015). A research on conjugation between soy protein and dextran has shown that the degree of glycosylation (DG) under macromolecular crowding conditions in aqueous systems was four times higher than the DG achieved by dry heating (Zhuo et al., 2013).

In another study, a degree of conjugation of 35.6% was determined in a mixture of NaCas and MD (DE = 12) after dry heating, i.e., freeze-dried NaCas-MD12 mixture was dry heated at  $60^\circ\text{C}$  and 79% relative humidity for 96 h (O'Regan & Mulvihill, 2009). Although the final degree of conjugation (35.6%) is higher than the value found in the current study (20–25%), the average reaction rate ( $\sim 0.37\text{ h}^{-1}$ ) was actually much lower than during wet heating ( $\sim 3.33\text{ h}^{-1}$ ) due to the longer time used for dry heating (i.e., 96 h). This could be due to the easier interactions between proteins and polysaccharides molecules during wet heating as they are more

mobile in an aqueous dispersion than in a dry state (Sedaghat Doost, Nikbakht Nasrabadi, Wu, A'yun, & Van der Meeren, 2019).

Furthermore, the degree of conjugation in the 6 h-conjugated NaCas-MD29 dispersion was found to be  $23.5 \pm 0.8\%$ , which was slightly higher than in the NaCas-MD5 dispersion. This is in line with previously reported results (O'Regan & Mulvihill, 2009; Zhang et al., 2017), where maltodextrins with a higher dextrose equivalent (DE) results in a higher degree of conjugation when the same condition is applied. Maltodextrin with a higher DE is expected to have a wider molecular weight distribution, i.e., more shorter-chain polysaccharides. The molecular weight distributions of MD DE5 and DE29 used in this study are determined by size exclusion chromatography in another study by the same research group (Siemons, Politieka, Boom, van der Sman, & Schutyser, 2020). The present of more short-chain polysaccharides in MD DE29 was confirmed, which may facilitate the conjugation reaction (Castro, Durrieu, Raynaud, & Rouilly, 2016). For future research, the reaction kinetics of conjugation between NaCas and maltodextrin as influenced by the concentrations of reactants and conditions for heat treatment (i.e., heating method, temperature and time) and type of maltodextrin (i.e., varied DE numbers) could be studied (Wang, Wang, Guo, Ma, & Yu, 2013).

#### 3.2. Colour

Table 1 shows the colour of the untreated and conjugated NaCas-MD dispersions. A brown colour was developed during the heat treatment, indicating the occurrence of Maillard reaction. As discussed, a longer reaction time resulted in a higher degree of conjugation (see Fig. 1); however, the formation of brown/yellow colour was more pronounced at the meantime. This was probably due to the production of post-Amadori products at the advanced stages of the Maillard reaction (Corzo-Martínez, Moreno, Olano, & Villamiel, 2009). Moreover, 6 h-conjugated NaCas-MD29 had the darkest brown colour among all the samples (see Table 1). This could probably be explained by the presence of more low molecular weight oligosaccharides in MD29 (Castro et al., 2016). These low molecular weight oligosaccharides could have facilitated the formation of conjugates (Zhang et al., 2017) and the formation of advanced Maillard reaction products with darker colours. It was worth noting that the colour of 6 h-conjugated NaCas-MD29 was significantly darker than the 6 h-conjugated NaCas-MD5, although no significant difference was found in the corresponding degrees of conjugation (see Fig. 1), which is, however, difficult to explain at this stage. Nevertheless, an off-white appearance of ingredients might not be favourable for certain food applications. The colour formation could be reduced by modifying the reactant or process conditions if a darker colour is undesired. For example, maltodextrin could be dialysed to significantly remove low molecular weight sugars and dextrans. Thus, dialysed maltodextrin could be used to effectively reduce browning in NaCas conjugated with MD (Shepherd et al., 2000). In addition, a high-temperature-short-time process has been proved to effectively reduce the colour formation in whey protein isolate glycosylated with maltodextrin (Liu & Zhong, 2015), which might be more economically feasible than the dialysing method. This leaves space for further investigation.

#### 3.3. Microstructure

Microstructure of foods plays an important role in their oral processing and sensory perception (Stieger & Van de Velde, 2013). The microstructure of gels formed by untreated and conjugated NaCas-MD dispersions at room temperature was observed under scanning electron microscopy (SEM) (Fig. 2). These microscopic images illustrated a homogenous distribution of protein material in

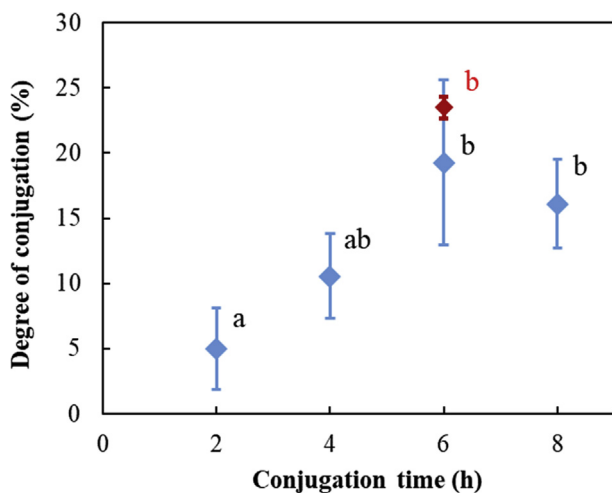








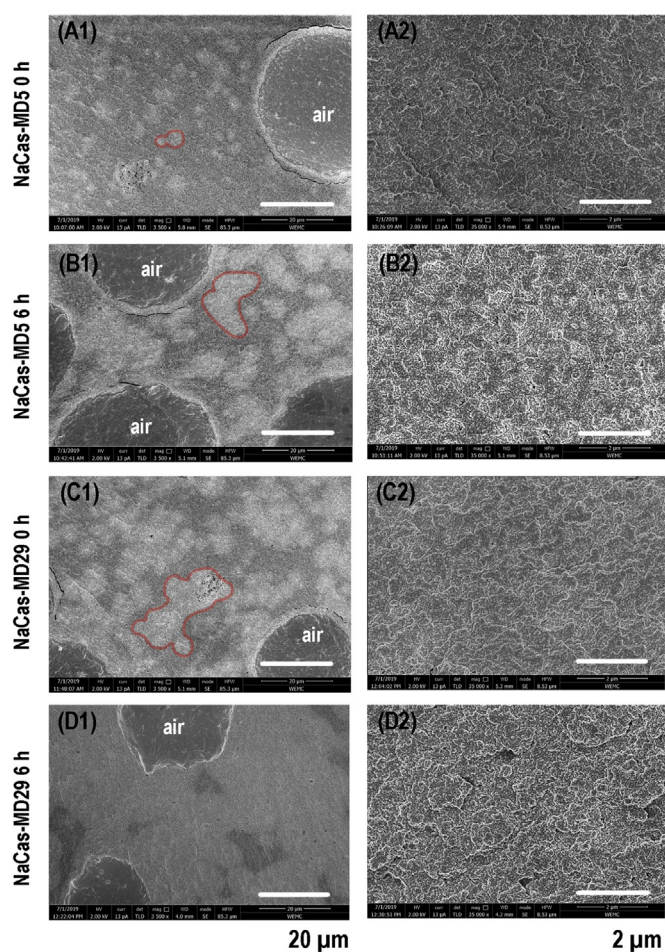
Fig. 1. Degree of conjugation between sodium caseinate (NaCas) and maltodextrin (MD) in the NaCas-MD (◆, DE5; ◆, DE29) dispersions during heating at  $90^\circ\text{C}$  (mean  $\pm$  SD,  $n = 3$ ): difference between data points marked with different letters was statistically significant ( $p < 0.05$ ).

**Table 1**

Colour analysis ( $L^*$ ,  $a^*$ ,  $b^*$  values) of NaCas-MD (DE5 or DE29) dispersions after conjugation reaction at 90 °C for 6 h and the colour difference ( $\Delta E$ ) between samples before and after the conjugation reaction.<sup>a</sup>

Sample	Time (h)	$L^*$	$a^*$	$b^*$	$\Delta E$	Colour <sup>2</sup>
DE 5	0	36.62 <sup>a</sup> ± 0.23	-1.21 <sup>a</sup> ± 0.16	-0.16 <sup>a</sup> ± 0.49	–	
	2	34.04 <sup>b</sup> ± 0.06	0.79 <sup>b</sup> ± 0.07	5.50 <sup>bc</sup> ± 2.12	6.55 <sup>a</sup> ± 1.55	
	4	32.61 <sup>c</sup> ± 0.30	2.34 <sup>c</sup> ± 0.20	6.40 <sup>b</sup> ± 1.37	8.59 <sup>ab</sup> ± 0.95	
	6	31.44 <sup>bc</sup> ± 1.19	3.60 <sup>c</sup> ± 0.77	9.36 <sup>c</sup> ± 0.78	11.90 <sup>c</sup> ± 1.10	
DE 29	0	42.38 <sup>a</sup> ± 0.55	-2.02 <sup>a</sup> ± 0.06	-0.19 <sup>a</sup> ± 0.37	–	
	6	28.84 <sup>b</sup> ± 0.16	5.82 <sup>b</sup> ± 0.09	8.65 <sup>b</sup> ± 0.26	18.00 <sup>a</sup> ± 0.67	

<sup>a</sup> Results (mean ± SD,  $n \geq 2$ ) with different superscript letters within the same column have significant differences ( $p < 0.05$ );  $L^*$ ,  $a^*$  and  $b^*$  values of the 0 h samples were used as references when calculating colour difference; colour charts were subtracted from digital photos of the samples.



**Fig. 2.** SEM images of gels formed with untreated (A, C) and conjugated (B, D) NaCas-MD [DE5 (A,B) and DE29 (C,D)] dispersions: scale bars [20 µm (1) and 2 µm (2)] and air bubbles are marked in the images; representative particulate microstructures in the gel are highlighted in red (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

the gel network, i.e., a ‘particulate’ microstructure (Van der Linden & Foegeding, 2009). The size of particles forming the gel structure seemed larger in the conjugated NaCas-MD dispersions than that in

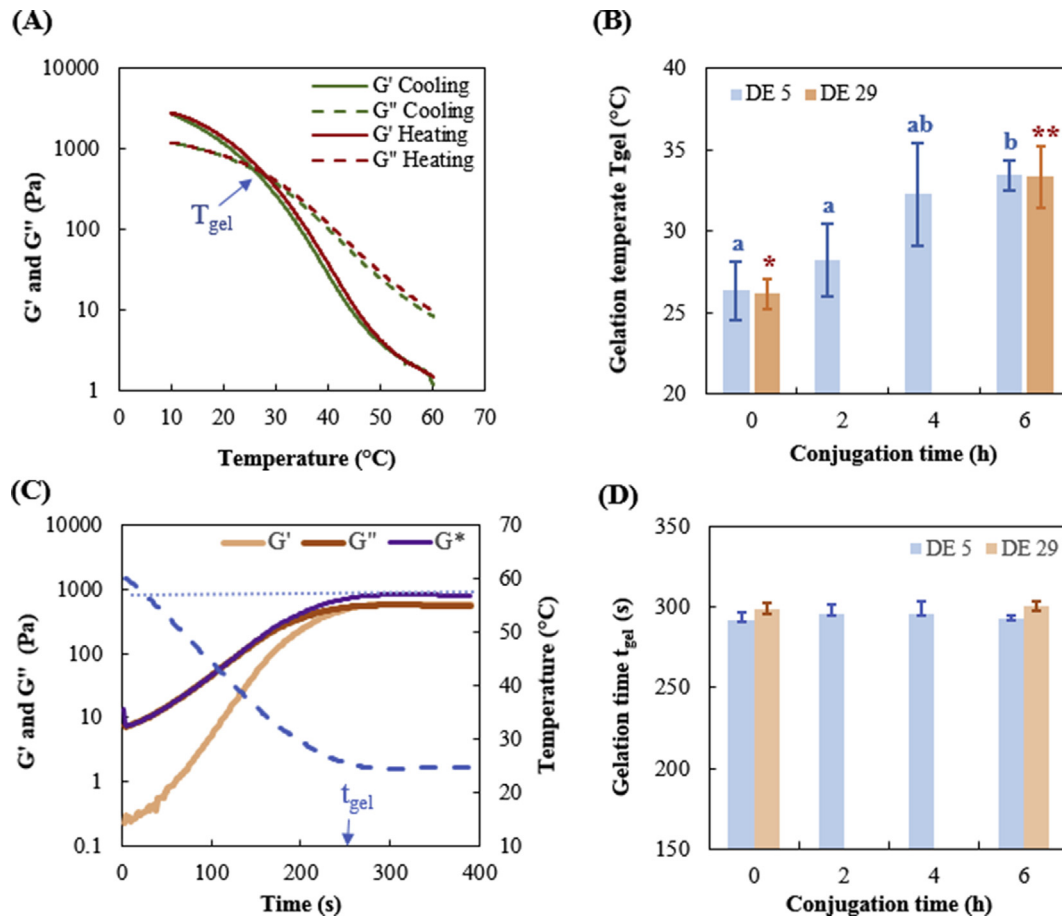
the untreated ones, i.e., the lighter particles shown in the SEM images (at a scale of 20 µm). The change in microstructure of the gels after conjugation could be linked to the change in the texture (e.g., reduced gel strength) (Morales, Martinez, & Pilosof, 2015) and the viscoelastic response (Manski, van der Goot, & Boom, 2007). Moreover, the size of clusters or particles appeared to be affected by the DE number of maltodextrin applied. The hygroscopicity of maltodextrin increases with increasing DE (Chronakis, 1998). Thus, formation of NaCas-MD gels using maltodextrin with a higher DE might result in different hydrophobic interactions between protein molecules which is then responsible for the formation of larger particulates in the microstructure (Van der Linden & Foegeding, 2009).

### 3.4. Rheological properties

The impact of conjugation with maltodextrin on rheological properties of sodium caseinate will be discussed in this section. The rheological properties studied include gelation temperature, gelation time, temperature-dependent viscosity, shear-dependent viscosity, gel strength and oscillatory shear behaviour, which are relevant for the application of the conjugates in foods.

#### 3.4.1. Gelation temperature

The gelation of NaCas containing systems can be induced by heating, cooling, acidification and high pressure (Dickinson, 2006). One convenient and widely adopted criterion for the sol-to-gel transition is when the value of the solid-like elastic modulus  $G'$  exceeds that of the liquid-like viscous modulus  $G''$  at a fixed frequency, and the corresponding temperature is determined as the gelation temperature ( $T_{gel}$ ) (Loveday et al., 2010) (Fig. 3A). In this study, the gelation temperature of the untreated and conjugated NaCas-MD5 and NaCas-MD29 dispersions was determined (Fig. 3B). The  $T_{gel}$  of NaCas-MD dispersions increased as the conjugation time increased when the total dry matter content of the dispersion was kept the same at 30%, w/w. More specifically, the  $T_{gel}$  of the 6 h-conjugated NaCas-MD5 (33.5 ± 0.9 °C) and NaCas-MD29 (33.4 ± 1.8 °C) was significantly higher ( $p < 0.05$ ) than the untreated ones (i.e., 0 h samples,  $T_{gel} \approx 26$  °C). Nevertheless, no significant difference between the  $T_{gel}$  of the 6 h-conjugated NaCas-MD5 and NaCas-MD29 was found ( $p > 0.05$ ), despite the difference in the microstructure of the gels formed. This might be attributed to the similar degree of conjugation in these two systems which may affect the sol-to-gel transition process.



**Fig. 3.** Panel A, representative results for the gelation temperature measurement: the  $T_{gel}$  of NaCas-MD dispersions was determined when the storage modulus ( $G'$ , solid lines) exceeded that of the loss modulus ( $G''$ , dashed lines) during the temperature sweep tests (green, cooling; red, heating); panel B, gelation temperature of the untreated and 2 h-, 4 h-, and 6 h -conjugated NaCas-MD (■, DE5; ■, DE29) dispersions; results (mean  $\pm$  SD,  $n = 3$ ) marked with different letters have significant differences ( $p < 0.05$ ); panel C, representative results for the gelation time measurement: the time when the value of  $|(G_{n+1}^* - C_n^*) / C_n^*| \leq 0.001$  ( $n = 1, 2, 3, 4 \dots$ ) (i.e.,  $G^*$  reached a plateau) was defined as the gelation time  $t_{gel}$ , and the dashed blue line represented the temperature profile of the materials during measurement (light orange line,  $G'$ ; dark orange line,  $G''$ ; dark blue line,  $G^*$ ); panel D, gelation time of the untreated and 2 h-, 4 h-, and 6 h-conjugated NaCas-MD (■, DE5; ■, DE29) dispersions (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

The sol-to-gel transition of NaCas upon cooling may be attributed to the increased relative contribution of attractive inter-chain interactions (especially hydrogen bonding) at lower temperatures (Dickinson & Casanova, 1999). The cold gelation of 14%, w/w NaCas with added salt was first reported by Carr and Munro (2004). Those authors hypothesised that the addition of salt to NaCas promotes attractive intermolecular protein interactions and the gelation at lower temperatures by removing water molecules from the protein backbone allowing easier protein-protein interaction. In this study, maltodextrin and NaCas-MD conjugates obtained after Maillard reaction may act as macromolecular crowding agents, which could hinder electrostatic repulsions and protein-water interactions and thus bring polypeptide chains of proteins closer (Carr & Munro, 2004). This phenomenon may have a similar effect on the sol-to-gel transition as increasing the protein concentration in the system, which also enhances intermolecular contacts. The gelation temperature of 33.5 °C of the conjugated samples (i.e., NaCas concentration 15%, w/w) was close to the gelation temperature of pure NaCas dispersion with a protein concentration of 40.5%, w/w (Loveday et al., 2010). Hence, the increase in  $T_{gel}$  of NaCas-MD dispersions observed in the current study might as well be due to the enhancement in protein-protein interaction via hydrogen bonds among NaCas particles after conjugation.

Moreover, maltodextrin with DE smaller than 10 are gelling dextrans with long, linear macromolecular chains can form double helices. These helices can aggregate with each other and form crystalline domains (Loret, Meunier, Frith, & Fryer, 2004). The crystalline domains serve as nucleation sites for the development of a continuous gel network (Manoj et al., 1996). Therefore, it is envisaged that the conjugation reaction between NaCas and MD resulted in an interconnected network with strong intermolecular interactions, which led to an increase in the  $T_{gel}$ .

#### 3.4.2. Gelation time

A fast gelation of caseinate could be preferable for certain food applications, e.g., extrusion-based 3D printing of protein gels (Schutyser et al., 2018). An enhanced gelation upon cooling could ensure the solidification of extruded filaments and thus the stability of 3D-printed structures (He, Zhang, & Fang, 2019). In this study, the gelation time ( $t_{gel}$ ) of NaCas-MD dispersion was determined as the time when the complex modulus  $G^*$  reached a plateau (slope  $\approx 0$ ) upon cooling (Ross-Murphy, 2007). The temperature of the system was first decreased from 60 °C to 25 °C in 210 s and then kept at 25 °C for 180 s (Fig. 3C). This temperature profile could represent the change in temperature during the extrusion-based 3D printing to some extent (Liu et al., 2019). We found that the  $G^*$  of NaCas-MD dispersions increased gradually during cooling and

quickly approached a plateau once the temperature of the system reached 25 °C (Fig. 3C). It was observed that the gelation time (i.e., gel point, ~300 s) of NaCas was not significantly affected by the conjugation with maltodextrin under tested conditions ( $p > 0.05$ ; Fig. 3D). Possibly since the cooling rate applied in this study was 10 °C min<sup>-1</sup>, which was relatively high. The cooling rate during gelation may influence the gelation kinetics as well as the strength of the gel formed (Zhong & Daubert, 2004), which might differ for different applications. Moreover, the gelation time of NaCas dispersion measured during rheological experiments depends also on the chosen frequency (Loveday et al., 2010). This leaves space for further investigation.

### 3.4.3. Viscosity

Fig. 4 shows the viscosity of the untreated and 6 h-conjugated NaCas-MD5 and NaCas-MD29 dispersions as a function of shear rate (Fig. 4A) and temperature (Fig. 4B). The dispersions showed initially shear-thinning behaviours at 35 °C ( $T > T_{gel}$ ) when the shear rate was increased from 0.01 s<sup>-1</sup> to 10 s<sup>-1</sup>. Later, shear-thickening occurs when higher shear rates were reached, and a “dilatancy peak” was observed (Fig. 4A) (Holland, Foster, & Tuck, 2019). This

might be because the NaCas particles increasingly made contact with one another as the shear rates increased at the condition used in this study (i.e., 35 °C), which resulted in an increase in flow resistance, i.e., a higher viscosity (Mezger, 2014). The occurrence of the “dilatancy peak” might also be a result of the addition of maltodextrin because dense suspensions of NaCas without maltodextrin were shear thinning (Pitkowski, Durand, & Nicolai, 2008; Schutyser et al., 2018). A similar “dilatancy peak” is observed when salt is added to NaCas containing systems which is explained by attractive interactions between caseinate particles (Thomar, Benyahia, Durand, & Nicolai, 2014). In addition, for both the NaCas-MD5 and NaCas-MD29 systems, the dilatancy peak seems to be shifted towards a lower shear rate after conjugates being formed. This could be because conjugation with maltodextrin eased the compression/deformation of NaCas particles under high shear.

On the other hand, the temperature-dependent viscosity of NaCas-MD dispersions can be described using the Arrhenius equation (see Eqn. A1) (Barreto et al., 2003):

$$\ln(\eta) = \ln(C) + \frac{E_a}{RT} \quad (4)$$

where  $\eta$  (mPa s) was the viscosity, C was a fitting parameter, R the ideal gas constant, 8.314 J mol<sup>-1</sup> K<sup>-1</sup>, and  $E_a$  (kJ mol<sup>-1</sup>) the activation energy of flow. The viscosity of NaCas-MD dispersions shows a strong temperature dependence (Fig. 4B). The viscosity drastically decreased from approximately 10,000 mPa s to 100 mPa s when the temperature was increased from 35 °C to 90 °C and the shear rate was kept constant (i.e., 50 s<sup>-1</sup>) (Fig. 4B). The decrease in viscosity could be caused by increased mobility of the molecules at elevated temperature (Mezger, 2014). In addition, similar observations were reported before by (Pitkowski et al., 2008), and the decrease in viscosity with increased temperature was speculated to be caused by a decrease in the effective volume fraction or an increase in hydrophobic interactions between the particles.

Moreover, the Arrhenius relationship was generally applicable for the viscosity of NaCas-MD dispersions measured over the temperature range applied in this study. However, conjugation with maltodextrin showed very little impact on the temperature-dependent viscosity profiles of sodium caseinate. The activation energy of flow  $E_a$  was found to be between 75.3 and 81.5 kJ mol<sup>-1</sup> (Supplementary material Table S1) which has the same order of magnitude as the data reported for 18.1% and 41.9% NaCas dispersions (Loveday et al., 2010).

### 3.4.4. Gel strength

The complex modulus of the untreated and 6 h-conjugated NaCas-MD5 and NaCas-MD29 dispersions was determined as a function of the angular frequency at room temperature (Supplementary material Fig. S3). The model (Eqn. (2)) was fitted to the data and the resulting parameters are shown in Table 2. The physical meaning of the fitting parameters (i.e., A, n and z) is explained in Section 2.4.4. Results show that the untreated NaCas-MD dispersion has a higher value of A than the conjugated samples,

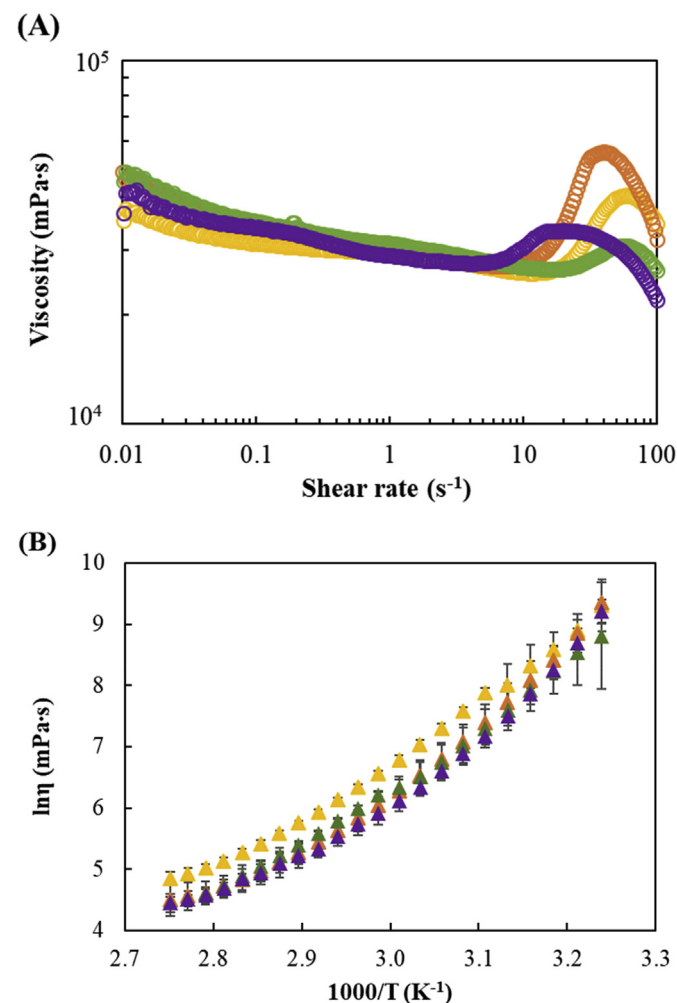


Fig. 4. Viscosity at 35 °C (A) as influenced by shear rate (0.1–100 s<sup>-1</sup>) and effect of temperature (35–90 °C: Arrhenius plots) on the viscosity (B) of untreated and 6 h-conjugated NaCas-MD5 and NaCas-MD29 dispersions. Untreated, light orange and green symbols; 6 h-conjugated, dark orange and blue symbols; NaCas-MD5, light and dark orange; NaCas-MD29, green and blue (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

Table 2

Coefficients A, n and z for the untreated and 6 h-conjugated NaCas-MD5 and NaCas-MD29 dispersions.<sup>a</sup>

Sample	Sample	A (Pa s <sup>n</sup> )	n (-)	z (-)	R <sup>2</sup>
NaCas-MD5	0 h	230	0.46	2.19	0.99
	6 h	25	0.59	1.69	0.99
NaCas-MD29	0 h	142	0.51	1.95	0.99
	6 h	93	0.47	2.13	0.99

<sup>a</sup> R<sup>2</sup> is coefficient of determination for the regression; experimental data can be found in the Supplementary material.

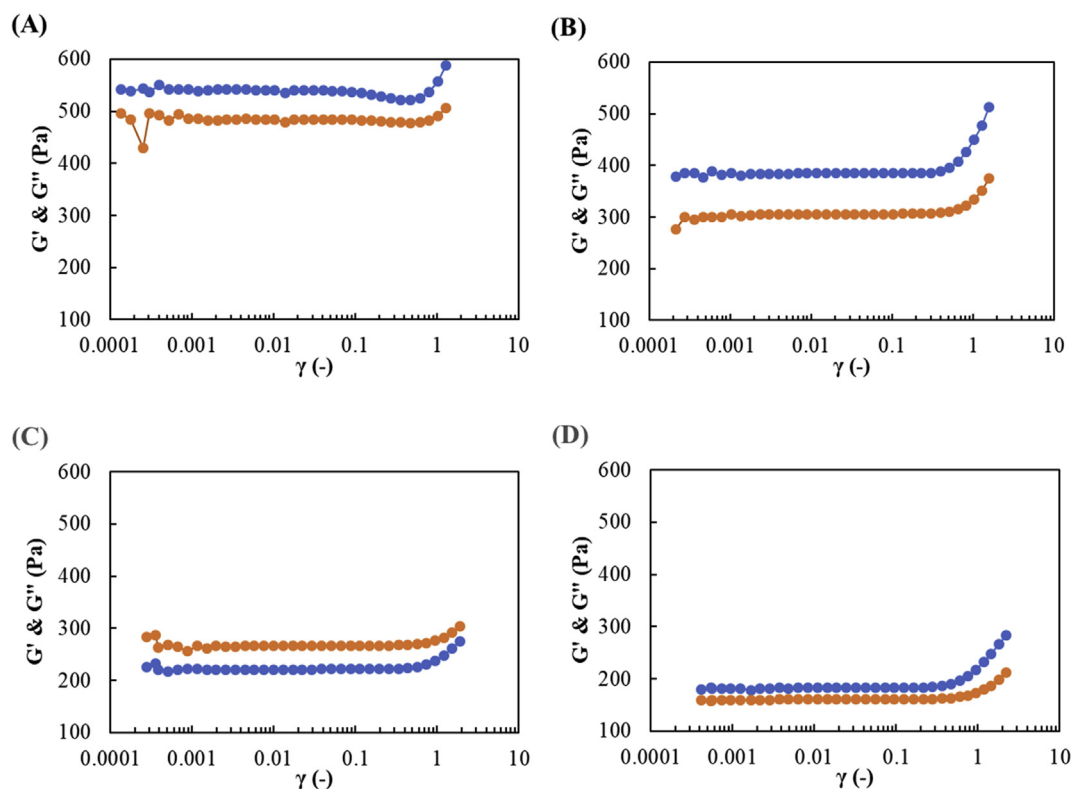
which is related to a higher gel strength. This means a firmer gel can be formed using this material at 25 °C. The observed decrease in gel strength of the conjugated sample is in line with other studies. For example, the gel formed from whey protein-dextran conjugates is found less firm and hard compared with the mixture of whey protein and dextran (Spotti et al., 2013; Zhang et al., 2017). The decrease in the strength of NaCas gel after conjugation with maltodextrin might be caused by the impairment of hydrophobic bonds and the formation of coarse protein networks (Luo et al., 2019). Nevertheless, the power-law relaxation exponent  $n$  and its reciprocal of the untreated and conjugated dispersions were not significantly different ( $p > 0.05$ ), which indicated the number of interacting rheological units within the 3D gel system did not differ after conjugation reaction (Gabriele, De Cindio, & D'Antona, 2001). Unfortunately, the influence of different DE values of maltodextrin on the gel strength of NaCas-MD dispersion cannot be derived from the results of this study.

#### 3.4.5. Oscillatory shear behaviour

The linear (small amplitude oscillatory shear, SAOS) and non-linear (large amplitude oscillatory shear, LAOS) viscoelastic response regimes can be determined by dynamic oscillatory shear test. Fig. 5 shows the viscoelastic response of NaCas-MD dispersions under shearing. The results indicate that the NaCas-MD dispersions showed a linear viscoelastic response when the strain amplitude is sufficiently small (from around 0.0001 to 0.5). In this linear response regime, both viscoelastic moduli ( $G'$  and  $G''$ ) are independent of strain amplitude and the oscillatory stress response is sinusoidal. The nonlinear regime occurred at larger strain amplitude (i.e.,  $>0.5$ ), indicating strain hardening behaviour. This strain hardening is believed to be

associated to strong interactions between molecules and the formation of complex microstructures or networks inside the fluid under shearing (Hyun et al., 2011). Similar strain hardening phenomena of cross-linked sodium caseinate or milk protein gels (i.e., Mozzarella cheeses) have been reported before (Manski et al., 2007; Sharma, Munro, Dessev, Wiles, & Foegeding, 2018). Moreover, the strain hardening behaviour of conjugated NaCas-MD dispersions (Fig. 5B,D) seems to be more profound than that of their untreated counterparts (Fig. 5A,C). This might as well be associated with the microstructures of the system (Fig. 2), e.g., clusters of casein polymers were formed after conjugation with maltodextrin which are assumed to be stiffer under shearing than individual casein molecules (Sharma et al., 2018).

In this section, we discussed the changes in some rheological properties of NaCas caused by conjugation with maltodextrin. These changes might influence certain applications of NaCas in foods. For instance, some preliminary results show that the increase in  $T_{gel}$  could enable extrusion-based 3D printing of protein structures at room temperature (Supplementary material). Potentially, the printing results (Supplementary material Fig. S4) could be significantly improved via adjusting printing conditions or via incorporation of additives, which leaves space for further investigation. Moreover, the viscosity of NaCas-MD dispersions could reach a lower level ( $<100$  mPa s) when the correct combinations of concentration, temperature and shear rate are applied (see Section 3.4.3). Therefore, it might be interesting to use this material to create nutritious food structures through 3D binder jetting, where a low viscosity of binder (e.g., 1 ~ 40 mPa s) is often required (Holland, Tuck, & Foster, 2018; Yang et al., 2013).



**Fig. 5.** Strain dependence of the storage modulus  $G'$  (●) and loss modulus  $G''$  (●) for NaCas-MD5 and NaCas-MD29 before (A and C, respectively) and after (B and D, respectively) 6 h conjugation; measurement was done at 1 Hz and 25 °C.



#### 4. Conclusions

Conjugation with maltodextrin affected the rheological properties of sodium caseinate dispersions. The gelation temperature of the NaCas-MD dispersions increased after conjugates being formed, whereas the gelation time was not affected by conjugation. The addition of maltodextrin affected the shear viscosity of the NaCas containing systems, i.e., a “dilatanity peak” occurred at high shear rates. In addition, shear thickening occurred at a relatively lower shear rate for conjugated samples in comparison with the untreated ones. The gels formed by conjugated NaCas-MD dispersions at 25 °C showed a weaker texture on a macroscale. On a microscale, larger gel-forming particulates were observed in NaCas-MD dispersion after conjugation, which was associated with strain-hardening occurred during dynamic oscillatory shear tests. Moreover, a higher DE of maltodextrin resulted in conjugated dispersions with higher degree of conjugation, darker colour and slightly different rheological properties and microstructure. For future research, kinetics of the conjugation reaction as influenced by the concentrations of reactants, conditions for heat treatment and type of polysaccharides could be of interest. To conclude, the results of this study showed that functional properties of protein can be modified by conjugation with polysaccharide.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.idairyj.2020.104660>.

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