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Physicochemical properties of a non-reducing maltoheptaose prepared by dual-enzyme cascade reaction from starch

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Abstract

The existence of a reducing end in the structure of maltodextrin can limit its applications as undesirable Maillard reaction would occur in some food processing steps. Consequently, a non-reducing maltoheptaose (N-G7) with a single degree of polymerisation was prepared through a cascade reaction of cyclodextrinase and maltooligosyltrehalose synthase, using β-cyclodextrin as substrate. The physicochemical properties of N-G7 were investigated. N-G7 exhibited low moisture absorption ability (8.91 and 18.02% at 43 and 81% relative humidity, respectively), excellent pH stability and thermostability (less than 10% N-G7 was hydrolysed between pH 4 and 10, even at 100°C), and a melting point higher than that of maltodextrin, as well as a typical gel-like behaviour. Most importantly, the results of Maillard reaction indicated that N-G7 was considered to be non-reducing, which suggested that it could be used in food processing where Maillard reaction should be avoided. Overall, the present work may provide important implications for the development and application of N-G7 in food products.

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Introduction

Maltodextrin is a mixture of saccharides with a broad molecular weight distribution between oligosaccharides and polysaccharides, and dextrose equivalent (DE) value lower than 20 (Chronakis, 1998). Low DE values of maltodextrin contribute to some of its functional properties including gelling, bulking, prevention of crystallisation, and promotion of dispersibility. Therefore, it plays an important role in the development of products low in calories and reduced-fat products (Blanchard and Katz, 1995). Additionally, maltodextrin is increasingly utilised in a variety of processed or commercial foods such as infant formulas, sports drinks, and energy supplements (Rezende and Hashizume, 2018). However, due to some disadvantages such as multiple degrees of polymerisation (DP), and high reducibility and instability, browning reaction can occur during processing, thus leading to undesirable colour and/or flavour (Zheng *et al*., 2021). There has, therefore, been a concerted effort to modify maltodextrin to improve its physicochemical properties.

Chemical modifications such as esterification, cross-linking, and etherification are the main approaches to generate branches and enhance the stability of maltodextrin. In an effort to improve the viscosity and solubility of maltodextrin, Zheng *et al*. (2007) found that cross-linking and esterification significantly decreased the hygroscopicity of maltodextrin, while esterification increased the intrinsic viscosity more than cross-linking. Hydroxypropyl maltodextrin was prepared from maltodextrin as a raw material by using epoxy propane as the etherification agent. The transparency of hydroxypropyl maltodextrin with low DE values was higher than that of native maltodextrin, and the transparency stability was also increased (Zheng *et al*., 2013). However, as the existence of chemical residues and introduced by-products can pose safety risks, enzymatic modification has been explored as an alternative approach. Maltodextrin was modified with

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starch-branching enzyme and β-amylase to introduce highly branched modifications and significantly enhance its slow digestion (Aga *et al*., 2010). Lee *et al*. (2013) reported that the addition of β-amylase increased the content of *α*-1,6-glycosidic bonds in maltodextrin, and further improved its slow digestion. An amount of α-amylase and glucoamylase were added to the starch after it was decomposed, and baked under acidic and heat conditions. Indigestible dextrin was obtained which improved the resistance to digestion (Su and Lin, 2014). Furthermore, Huang *et al.* (2021) prepared maltooligosyl trehalose from commercial maltodextrin using maltooligosyltrehalose synthase (MTSase) directly. The non-reducing oligosaccharides produced were shown to have unique emulsion-stabilising properties and thermal stability.

Information about the use of transglycosidase to prepare maltodextrin with a non-reducing end is currently very limited, and only a few studies to date have focused on the physicochemical properties of the functional non-reducing oligosaccharide. Therefore, the aim of the present work was to characterise the physicochemical properties of a non-reducing maltoheptaose with a single degree of polymerisation prepared by dual enzyme treatment and purified using Na^+ cation exchange resin chromatography. The preparation method of N-G7 stands for simple, cost-effective, and can readily be used for large-scale preparation. The results of the present work would add to more evidence to support its application in the food industry.

Materials and methods

Materials

Cyclodextrinase (CDase) and maltooligosyltrehalose synthase (MTSase) were prepared by the State Key Laboratory of Food Science and Technology, Jiangnan University, China. The CDase (GenBank: X62576) and MTSase (GenBank: D63343) enzymes were synthesised, and *E. coli* BL21 (DE3) was used as host for overexpression of the enzyme. DIAION UBK530Na ion-exchange resins were provided by Mitsubishi Chemical Co. Ltd. (Tokyo, Japan). Maltodextrin with DE 12, 15, and 19 (MD12, MD15, and MD19) were obtained from Roquette Frères (Lestrem, France). Lysine, sucrose, K_2CO_3, and $(NH_4)_2SO_4$ were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All other reagents were of reagent grade or higher purity, and purchased from Sangon Biotech Co. Ltd. (Shanghai, China).

Preparation and purification of N-G7

The synthesis of N-G7 from β-cyclodextrin (β-CD) by cascade reaction of CDase and MTSase was reported previously by our laboratory (PCTCN2020097622). In brief, β-CD was hydrolysed to linear maltoheptaose (G7) by CDase, and the α-1,4-glycosidic linkage at the reducing end of G7 was subsequently converted to an α-1,1-glycosidic bond by MTSase. The crude oligosaccharide solution (5.0 mL) containing 6.8% solids was loaded onto a column (1.6 × 100 cm) of UBK530Na ion-exchange resin at 60°C, and the column was then eluted with deionised water at a flow rate of 0.5 mL/min. The eluates were collected and analysed using a Waters e2695 HPLC, equipped with an Agilent Zorbax NH_2 column (4.6 × 250 mm; Palo Alto, CA, USA) with a refractive index detector (Waters 2414, Milford, MA, USA). Fractions containing the main elution peak with a purity higher than 98% were collected, lyophilised, placed in desiccators, and referred to as N-G7 (Nobre *et al*., 2014).

Evaluation of moisture absorption ability

The moisture absorption ability was determined following a previously reported method with slight modifications (Wang *et al*., 2013). Samples were ground into a fine powder with a mortar and pestle, and then oven-dried at 105°C for 4 h. For evaluating the moisture absorption, each 100 mg sample was placed in a desiccator with the bottom filled with K_2CO_3 (43% relative humidity, RH) or $(NH_4)_2SO_4$ (81% RH) at 25°C. The vials were weighed regularly during the storages until the water content levelled-off. The moisture absorption rate (R_a) was evaluated using Eq. 1:

$$R_a\ (\%) = (H_2 - H_1) / (H_1 - H_0) \times 100 \qquad \text{(Eq. 1)}$$

where, H_0 = net weight of the vial, H_1 = initial weight of vial, and H_2 = final weight of vial.

Effects of pH and temperature on the stability of N-G7

The stability of N-G7 at different pH values and temperatures was determined following established methods (Seo *et al*., 2007; Glibowski and Bukowska, 2011) with slight modifications. To

confirm the accuracy of pH regulation, the pH of 50 mL of crude oligosaccharide solutions (stock solution, 10-fold dilution) were subsequently adjusted to different values using 1 M HCl, 0.1 M HCl, 1 M NaOH, or 0.1 M NaOH (pH 2, 4, 6, 8, or 10, respectively). Next, 1 mL of samples of solution were then placed in test tubes, and incubated at different temperatures (60, 80, or 100°C) for 0, 4, 8, 12, or 24 h. The samples were then immediately neutralised after this heating step using 0.1 M or 1 M HCl, or NaOH solution, and afterwards cooled to ambient temperature under running tap water. The residual concentration of N-G7 was determined using the HPLC method as earlier described, and the stability was defined as the percentage share of residual N-G7 in the initial sample of N-G7.

Maillard reaction

To assess the extent of the Maillard reaction, equal amounts of samples (sucrose; N-G7, MD12, MD15, and MD19) and lysine were dissolved in water (15 mL; 10 mg/mL) and then adjusted to pH 9.0 with 2 M HCl. Aliquots (5 mL) of these solutions were then placed in 10 mL test tubes, and incubated at 90°C. After heated for various durations (2, 4, 6, 8, 10, 12, 24, and 36 h), the tubes were immediately cooled in an ice-water bath, and the absorbance at 420 nm was determined (Ajandouz *et al*., 2001; Li *et al*., 2011) using a UV-Visible spectrophotometer (MADAPA-P7, Shanghai, China).

Thermogravimetric analysis (TGA)

The thermal stability of N-G7 was determined following the method described by Luo *et al*. (2019) using a thermal gravimetric analyser (TGA2, Zurich, Switzerland). Approximately, 2 mg of each sample was weighed and placed in an alumina crucible, and then heated at a constant rate of 10°C/min over a temperature range of 25 to 450°C. The experiments were performed in a nitrogen atmosphere at a flow rate of 50 mL/min.

Differential scanning calorimetry (DSC)

The thermal properties of N-G7 were analysed following the method described by Miao *et al*. (2015) using DSC (TA-Q200, New Castle, DE, USA). Approximately, 3 mg of dried sample was placed in an aluminium pan before the pan was sealed and analysed. An empty pan was used as a reference. The onset temperature, peak temperature, and enthalpy change were recorded. The samples were heated in the range of 0 – 200°C at a rate of 10°C/min under a nitrogen atmosphere.

Rheological properties

The flow properties of N-G7 in deionised water were analysed at 25 ± 0.1°C using a DHR-3 rheometer (New Castle, DE, USA) equipped with a parallel plate measuring 40 mm in diameter (gap = 1,000 μm). For each measurement, 2.5 mL of samples at concentrations of 0.1, 1, and 10% were loaded onto the Peltier plate of rheometer. Flow curves were obtained with a shear rate ranging from 0.01 to 200 s^{-1}. To further investigate the storage modulus (G′) and the loss modulus (G”), a small amplitude oscillatory shear measurement was conducted within the region of linear viscoelasticity (LVR), under the condition of 1% strain and 1 Hz frequency. Dynamic frequency sweep testing was undertaken at a constant strain of 1% by an amplitude sweep measurement from 0.1 – 10 Hz at 25°C. The effects of temperature on modulus were recorded by a temperature ramp from 25 to 80°C, under a constant strain (1%) and frequency (1 Hz), with a heating rate of 5°C/min (Li *et al*., 2019).

Statistical analysis

All experiments were conducted in triplicate, and mean with standard deviation were obtained. Data were analysed through one-way analysis of variance (ANOVA) using SPSS software (Version 21.0). Significance level was determined at $p < 0.05$.

Results and discussion

Evaluation of moisture absorption ability

The chromatograms of crude and purified N-G7 were illustrated in Figure 1A. These indicate that the N-G7 had been successfully separated from the other products. As shown in Figures 1B and 1C, the weight of moisture absorption in all samples increased rapidly in the first 4 h, slowed after 4 h, and reached a steady state 24 h later at an RH of 43 and 81%. After being exposed to 43% RH for 48 h (Figure 1B), the R_a values of N-G7, MD12, MD15, and MD19 were 8.91, 9.21, 10.03, and 10.69%, respectively. To further characterise the moisture absorption properties, the samples were hydrated at 81% RH for 48 h. The overall trend of the water absorption ability of all of the samples remained unchanged, and the R_a values of N-G7, MD12, MD15, and MD19 were 18.02, 20.55, 28.37, and 30.99%, respectively, at the end of the experiment

(Figure 1C). Therefore, the relative moisture absorption abilities of the maltodextrins and N-G7 was as follows: MD19 > MD15 > MD12 > N-G7 (at both 43 and 81% RHs), which represented that N-G7 may have potential as a drying agent on powder. The results also indicated that the carbohydrate chain length and molecular weight may play key roles in the moisture absorption abilities. Maltodextrin with high DE value has more short chains which result in a high moisture carrying ability. The carbohydrate chains are interwoven with each other, and formed a network structure which is important for moisture absorption (Zhang *et al*., 2012).

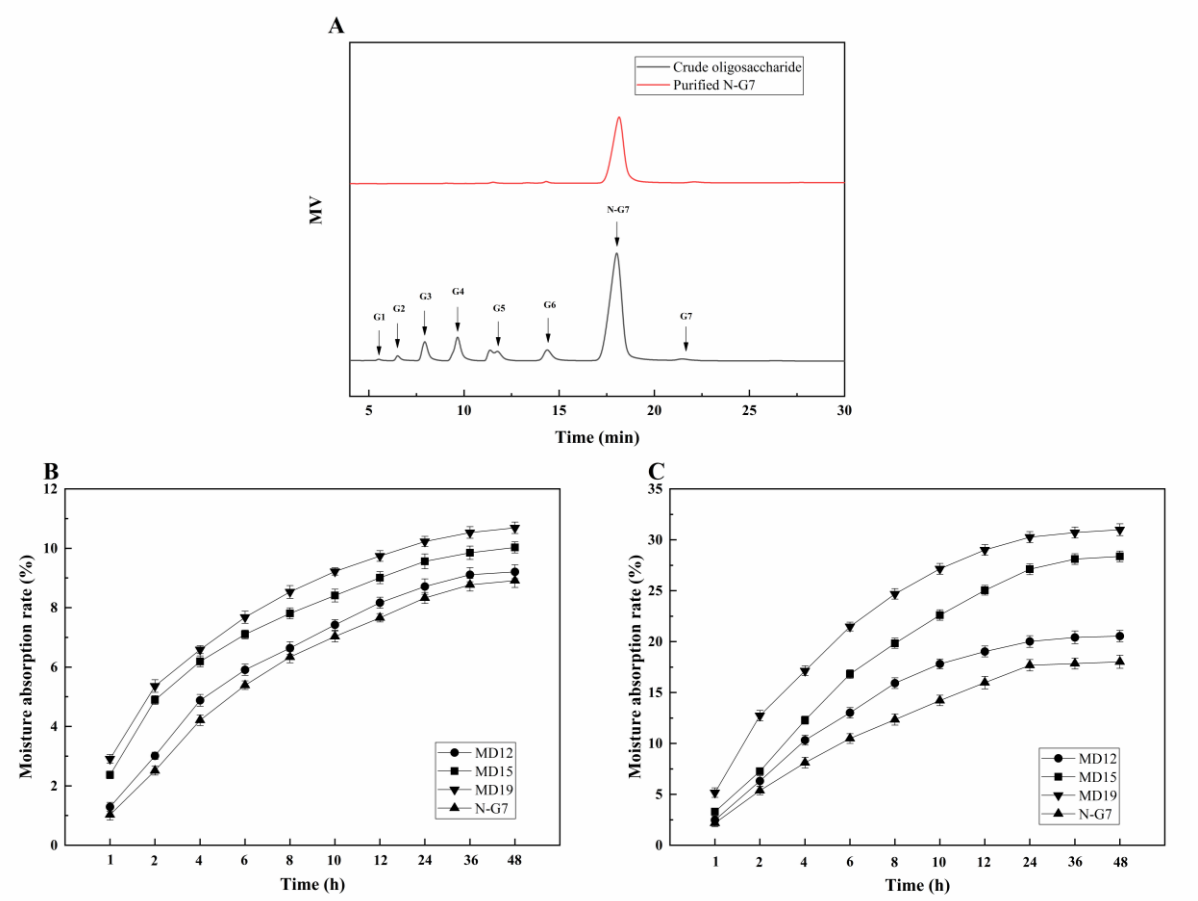

Figure 1. (A) comparative HPLC chromatogram of crude oligosaccharide and purified N-G7; **(B)** and **(C)** moisture absorption ability of the N-G7, MD12, MD15, and MD19. The samples were placed in a desiccator containing saturated K_2CO_3 [**(B)**; 43% RH] and saturated $(NH_4)_2SO_4$ [**(C)**; 81% RH], respectively. Moisture absorption ability was assessed by weight loss of the samples.

Effects of pH and temperature on the stability of N-G7

It is well known that malto-oligosaccharides are stable at acidic pH, and resistant to heat stress (Seo *et al*., 2007). Figure 2 depicts the stability of N-G7 at different pH levels and elevated temperatures. In a highly acidic environment (pH 2), all of the N-G7 solutions underwent a degree of decomposition at the three temperatures tested (60, 80, and 100°C), with 70.76% decomposition after 4 h of incubation at 100°C (Figure 2A). However, only 8.73% N-G7 breakdown occurred at the relatively low temperature (60°C) after 24 h (Figure 2C), thus indicating that N-G7 underwent reduced decomposition at low temperatures. The thermostability of N-G7 was higher than that of commercial fructo-oligosaccharides (Courtin *et al*., 2009). Previous studies have also reported that glycosidic bonds can be destroyed under highly acidic conditions (Courtin *et al*., 2009). As shown in Figures 2A - 2C, N-G7 resisted glycosidic linkage hydrolysis between pH 4 and 10, regardless of the duration and temperature of the thermal treatment (less than 10% N-G7 was hydrolysed). Generally, it can be concluded from the present work that N-G7 can be applied in food systems which contain considerable amount of fat (Glibowski and Bukowska, 2011).

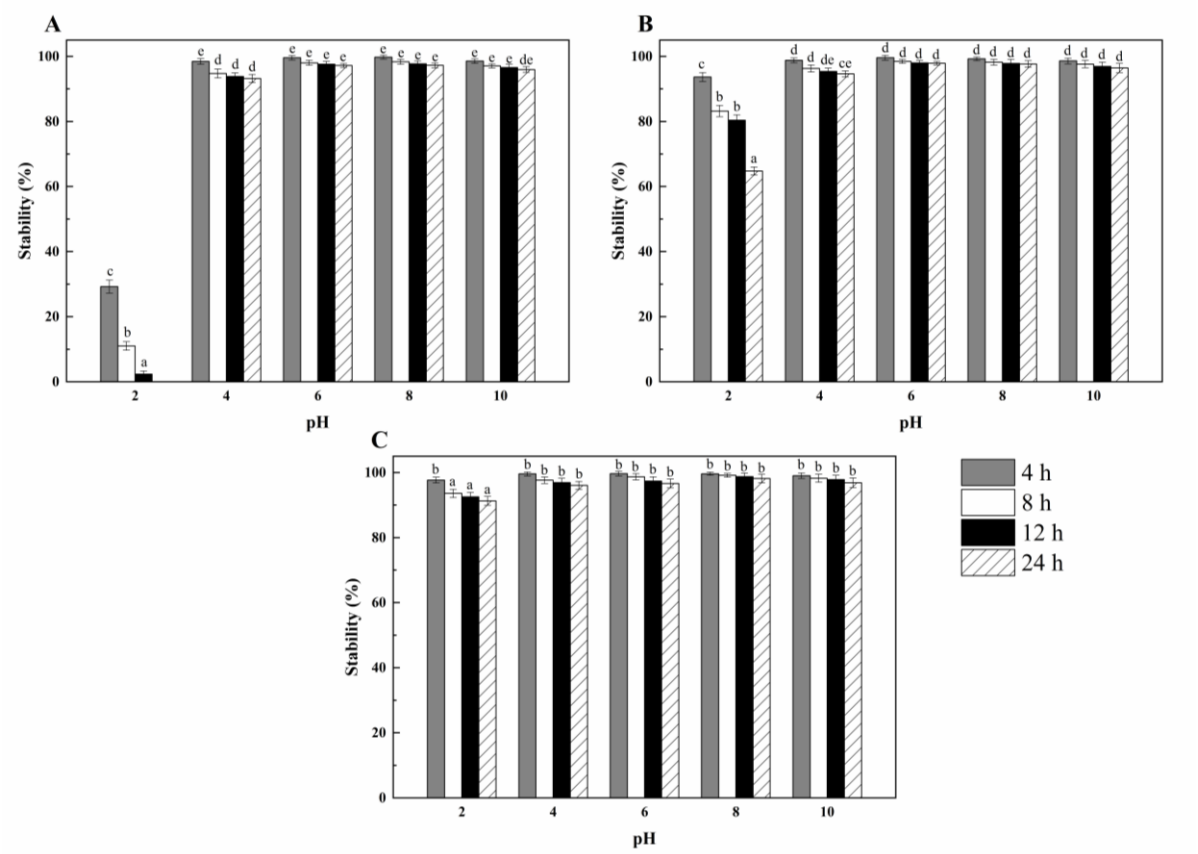

Figure 2. Effects of pH and temperature on the stability of N-G7. N-G7 solutions were placed under different pH levels (2, 4, 6, 8, and 10) at **(A)** 100°C, **(B)** 80°C, and **(C)** 60°C, respectively. The residual concentration of N-G7 were determined using the HPLC method at different heating durations (4, 8, 12, and 24 h). Different lowercase letters indicate significant difference ($p < 0.05$).

Determination of the extent of the Maillard reaction

Overall, the type of reducing sugar plays a major role in regulating the Maillard reaction rates and pathways. The data shown in Figure 3A indicate that the absorbance increased with the reaction time, which is an indication of the extent of the reaction. Moreover, sucrose did not react with amino acids. As expected, the low absorbance was obtained with N-G7. The difference in the development of absorbance and the browning intensity indicates different degrees of reactivity of the reducing sugar. This is because maltodextrins with higher DE values have a higher proportion of low molecular weight reducing oligosaccharides, which have less steric hindrance to lysine and are hence more likely to come into contact with the reactive sites of amino compounds (Liu *et al*., 2014). Moreover, the absorbance of the various types of malto-oligosaccharides reaction systems (MD12, MD15, and MD19) exhibited significant differences ($p < 0.05$) after a reaction time of 8 h. This could be interpreted by the fact that low molecular weight oligosaccharides reacted with lysine first, and then the large sugars reacted again after the consumption of the smaller sugars, thus the reaction rate diverged gradually.

TGA

The TGA was carried out dynamically (the weight loss of the sample as the temperature increased), and the experimental results are presented in Figure 3B. Based on these curves, the weight loss of maltodextrin (MD12, MD15, and MD19) occurred in three stages, while the decomposition profile of N-G7 was divided into four stages. In the first stage, when the temperature was increased to 156.5, 115.0, 138.5, and 142.5°C for MD12, MD15, MD19, and N-G7, respectively, 8% to 12% weights were lost, which were mainly represented by physically absorbed water. Above this temperature, the weight remained unchanged until a second degradation process of decomposing started at around 240°C, continuing for up to 350°C for maltodextrin with a pronounced

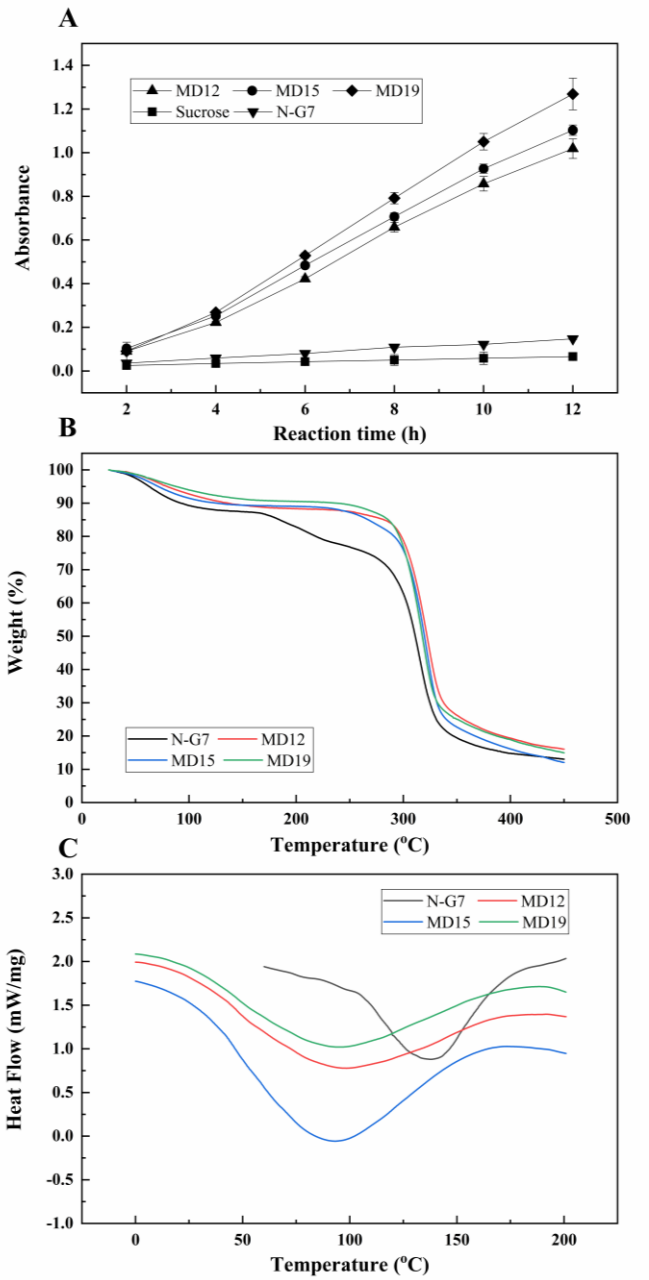

Figure 3. (A) effects of malto-oligosaccharide substrates on absorbance of Maillard reaction; **(B)** TGA curves of N-G7, MD12, MD15, and MD19 in the range of 25 to 450°C; and **(C)** DSC curves of N-G7, MD12, MD15, and MD19 in the range of 0 to 200°C.

weight loss of about 63%. In this stage, the weights of the samples decreased dramatically, which was mainly owing to the extensive thermal-degradation of the carbohydrate chain, and the disruption of C–O and C–C linkages on the pyran ring, thus leading to the formation of CO, CO_2, and H_2O (Zamora *et al.*, 2002). The second and third thermal degradation phases of N-G7 were obvious. The weight of N-G7 gradually decreased as the temperature increased from 167 to 274°C, and then dramatically decreased to 19.46%. These results were consistent with those of previous research (Branca *et al.*, 2001; Megarry *et al.*, 2011). In light of the results, the following considerations could be made. A gentle descending step occurred under the influence of α-1,1-glycosidic bonds. Some α-1,4-glycosidic bonds were rapidly decomposed prior to small amounts of α-1,1-glycosidic bonds being degraded. The last stage of depolymerisation from 350 - 450°C was also recorded, where around 12% weight remained with a low rate of weight loss. Finally, a progressive carbonised structure was formed as the temperature was increased further (Zamora *et al.*, 2002).

DSC

The DSC curves further elucidated the thermal transitions of N-G7 when compared with that of MD12, MD15, and MD19. As shown in Figure 3C, four major endothermal valleys were observed. The melting points of N-G7, MD12, MD15, and MD19 were 138.38, 93.96, 90.29, and 92.62°C, respectively, and the endothermic enthalpy changes (ΔH) required to melt 1 g samples were 91.4, 193.4, 215.6, and 214.6 J, respectively (Table 1). These results were similar to those observed by Raimi-Abraham *et al.* (2014) who identified the presence of two distinct thermal behaviours influenced by different pan types. The endothermic process suggests the loss of peripheral oligosaccharide chains and dehydroxylation reactions (Mohammed *et al.*, 2020). The melting point of N-G7 was higher than that of maltodextrin, which implied that N-G7 has a better thermal stability, and the water trapped in N-G7 requires more energy to escape than maltodextrin.

Rheological properties

The steady flow curves of N-G7 are shown in Figure 4A. It can be seen that the apparent viscosity of the samples increased as the concentration increased, which implied that the increased entanglement between molecular chains limited the individual movement and stretching of the chains of these oligomers, especially at higher concentrations (Bae *et al.*, 2008). However, the apparent viscosity of N-G7 at different concentrations decreased steadily as the shear rate increased, and ultimately the apparent viscosity values were almost identical when the shear rate was in the range of 10 - 200 s^{-1}. The N-G7 solution behaved as a typical pseudo-plastic fluid, thus indicating a more pronounced shear-thinning flow behaviour at higher concentrations. In particular,

Table 1. Thermal properties of N-G7, MD12, MD15, and MD19 as determined by DSC.

Sample	Onset temperature (°C)	Peak temperature (°C)	Enthalpy (J/g)
N-G7	106.33 ± 1.27[a]	138.38 ± 1.84[a]	91.4 ± 1.1[a]
MD12	28.24 ± 0.62[b]	93.96 ± 0.65[b]	193.4 ± 0.6[b]
MD15	28.76 ± 0.41[b]	90.29 ± 1.03[b]	215.6 ± 0.6[b]
MD19	24.51 ± 0.29[b]	92.62 ± 0.83[b]	214.6 ± 0.7[b]

Means within a column followed by different lowercase superscripts are significantly different ($p < 0.05$).

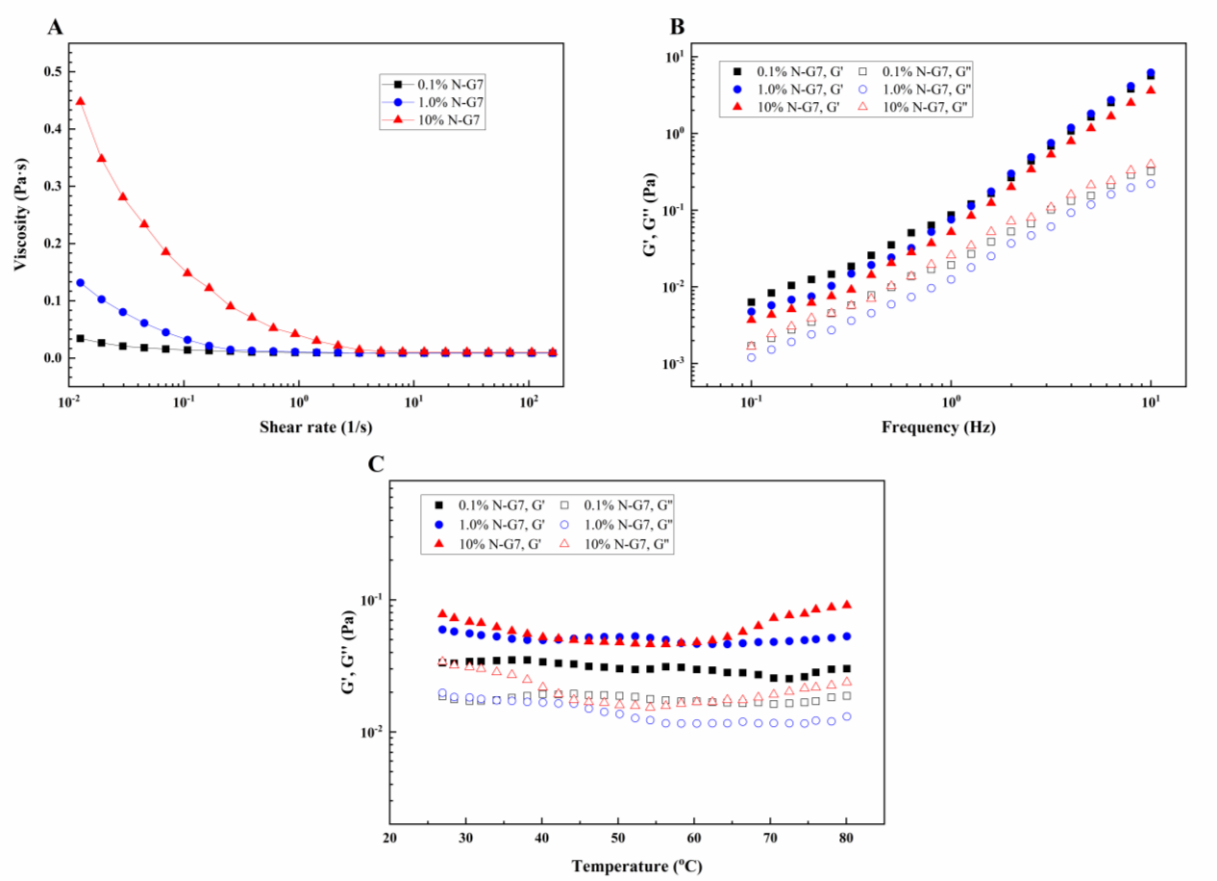

Figure 4. (A) effect of shear rate on the apparent viscosity of N-G7 solutions; **(B)** effect of frequency on storage modulus (G') and loss modulus (G") of aqueous N-G7 solutions at 25°C under 1% strain; and **(C)** effect of temperature on G' and G" of aqueous N-G7 solutions at constant 1% strain and 1 Hz.

the apparent viscosity of N-G7 at the low concentration exhibited little variation at different shear rates, which suggested that the N-G7 solution (0.1%, w/v) was close to a Newtonian fluid. This may be related to straightened linear molecular structure with increased shear rates, thus resulting in decreased flow resistance of N-G7 in solution. In the end, the entanglement between molecules was completely lost, and the apparent viscosity of the solution no longer decreased when the shear rate increased above a certain threshold value. In addition, the pseudo-plastic property of N-G7 makes it ideal for use in the preparation of liquid food that can readily be pumped, and also provides a pleasant mouthfeel.

The dynamic modulus is considered to be a good characterisation tool to study the viscous and elastic properties of samples. Frequency sweep experiments were performed at a constant strain of 1%, which was in the linear viscoelastic region (Figure 4B). The variations of the G' and G" values as a function of frequency are depicted. Figure 4B shows that both the G' and G" values at all N-G7 sample concentrations increased from 0.1 - 10 Hz, and the G' values were greater than the corresponding G" values,

which signified that the elasticity of N-G7 was always greater than the viscosity in the test frequency range, and that N-G7 exhibited a typical weak gel behaviour (Singh *et al*., 2017). At the various concentrations tested, the increased values of G' and G" in the frequency range could be attributed to the increase in the amounts and complexity of the connection points between the oligomer chains (Simastosin *et al*., 2010).

The N-G7 solutions were studied in terms of their dynamic rheological properties by employing a temperature range of 25 - 80°C (Figure 4C). Following heating, the storage modulus and loss modulus barely changed for N-G7 solutions of 0.1 and 1.0%, respectively, thus indicating that the G' and G" values were largely independent of the temperature during heating. As the temperature increased, the G' and G" values of N-G7 at a concentration of 10% decreased, and reached a steady value below 65°C later. This decrease in the modulus values revealed the existence of a weaker molecular structure at high temperatures, and a high dependency of the dynamic modulus on temperature change. Moreover, N-G7 did not exhibit a crossover point, and G" was always greater than G' in the tested temperature range. This demonstrated that N-G7 exhibited viscous deformation and weak gelling behaviour even at high temperatures.

Conclusion

In the present work, the physicochemical properties of N-G7 were studied. N-G7 was synthesised by the cascade reaction of CDase and MTSase, and then purified using cation exchange chromatography. Maltodextrin and N-G7 had different thermodynamic properties. N-G7 had a weak gel-like structure, and solutions of N-G7 exhibited shear-thinning behaviour. Furthermore, N-G7 did not undergo the Maillard reaction which suggested that it could be used as an alternative to maltodextrin in food processing, where the Maillard reaction should be avoided. All these results indicated that N-G7 has the potential as a novel food ingredient with a low moisture absorption ability, and high pH, thermal stability, and pseudo-plastic properties for use as a bulk ingredient in the food industry. In future studies, the influence of N-G7 on the rheological properties and functional performances of different food products, as well as its processing in the human body should be investigated to provide further support for its application in food products.

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