1 **Impact of glucose polymer chain length on heat and physical stability of milk**
2 **protein-carbohydrate nutritional beverages**

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Abstract

This study investigated the impact of glucose polymer chain length on heat and physical stability of milk protein isolate (MPI)-carbohydrate nutritional beverages containing 8.5% w/w total protein and 5% w/w carbohydrate. The maltodextrin and corn syrup solids glucose polymers used had dextrose equivalent (DE) values of 17 or 38, respectively. Increasing DE value of the glucose polymers resulted in a greater increase in brown colour development, ionic calcium, protein particle size, apparent viscosity and pseudoplastic rheological behaviour, and greater reduction in pH, hydration and heat stability on sterilisation at 120°C. Incorporation of glucose polymers with MPI retarded sedimentation of protein during accelerated physical stability testing, with maltodextrin DE17 causing a greater reduction in sedimentation velocity and compressibility of sediment formed than corn syrup solids DE38. The results demonstrate that chain length of the glucose polymer used strongly impacts heat and physical stability of MPI-carbohydrate nutritional beverages.

Key Words: protein beverage, glucose polymer, heat stability, viscosity, sedimentation

1. Introduction

Over the past 15 years, there has been considerable interest and research in nutritional beverages, which are normally based on protein and carbohydrate ingredients (Foster-Schubert *et al.*, 2008). The functions of such beverages are primarily to prevent dehydration, to supply energy, to provide protein and electrolytes to replace losses and to be palatable (Coombes & Hamilton, 2000). For these reasons, formulations typically consist of water to facilitate rehydration, carbohydrate to supply energy, protein to provide amino acids for muscle maintenance and energy replacement and minerals to replace electrolytes. According to the Euromonitor report (2010), the off-trade volume of sports nutritional beverages increased from 7563 to 9679 million litres globally between 2004 and 2009, which translates to an average annual increase of approximately 5% (Cochrane *et al.*, 2012).

Cockburn, Stevenson, Hayes, Robson-Ansley, & Howatson (2010) reported that milk protein-carbohydrate nutritional beverages had a positive impact on exercise-induced muscle damage and attenuated decrements in muscle performance. Milk protein concentrate (MPC) and milk protein isolate (MPI) ingredients are produced by partially removing the lactose and minerals from skim milk using ultrafiltration, followed by diafiltration, evaporation and spray drying to produce dry powder ingredients (Kelly, 2011) commonly used in the formulation of nutritional beverages. Arising from the formulation complexity associated with nutritional beverages, challenges, such as protein coagulation, sedimentation and possibly gelation, are often experienced on incorporation of MPC and MPI ingredients in nutritional beverages (Eshpari, Tong, & Corredig, 2014; Crowley, Megemont, Gazi, Kelly, Huppertz, & O'Mahony, 2014a).

With respect to the choice of carbohydrates, osmolality, nutritional contribution, sensory attributes and cost are the main determinants of the type and level of carbohydrate used in nutritional beverage formulations. Starch hydrolysis product-based carbohydrates consist of different chain length glucose polymers and provide many benefits in the formulation of protein-carbohydrates beverages; for example on an equal contribution of caloric density, high chain length glucose polymers (e.g., maltodextrins) contribute to lower osmolality, sweetness and cost, compared with low chain length glucose polymers (e.g., glucose) (Marchal, Beeftink, & Tramper, 1999).

It is often challenging to develop protein-carbohydrate nutritional beverages that are stable to heating (e.g., UHT and in-container sterilisation), have acceptable viscosity and remain physically stable during distribution and shelf life due to the many possible interactions between the ingredients (Anema, Pinder, Hunter & Hemar, 2006; Liang, Matia-Merino, Patel, Ye, Gillies, & Golding, 2014).

Heat stability analysis normally involves transferring solutions into glass tubes sealed with silicone caps and immersing them in an oil bath thermostatically controlled at either 140°C for unconcentrated milk or 120°C for concentrated milk, with constant shaking speed (8 rev/min) (O'Connell and Fox, 2003). The time that elapses between placing samples in the oil bath and the first visible onset of coagulation is recorded as the HCT. It has been reported that a number of compositional factors affect the heat stability of milk including initial heating pH, protein concentration, additives, polyphosphate and type of carbohydrate (O'Connell and Fox, 2003; Horne and Muir, 1990; Chen et al. 2015; Walstra et al. 2006; de Kort et al. 2012; Tsioulpas, 2010; Chen et al. 2012; Cruijsen, 1996; Tan-Kintia and Fox, 1996). The heat stability of MPC and MPI has been the subject of a small number of recent studies (Kaliappan & Lucey, 2011; Eshpari *et al.*, 2014). It has been shown that high protein content MPC's

and MPI have maximum heat stability in the pH range 6.7 to 6.8 and that high calcium ion concentration is the dominant factor influencing the heat stability of high protein content MPC's and MPI (Crowley *et al.*, 2014a). Meanwhile, it has been reported (Eshpari *et al.*, 2014) that the partial acidification of milk by glucono- δ -lactone to pH 6 before membrane filtration significantly decreased the heat stability of 5% MPC65 (65% protein) and MPC80 (80% protein) and that pH is a major factor affecting heat stability of MPC.

Incorporation of carbohydrate ingredients with milk proteins generally decreases heat stability and increases susceptibility to protein aggregation and sedimentation in nutritional beverage formulations (Holt, Muir & Sweetsur, 1978; Tan-Kintia & Fox, 1996; Cruijssen, 1996; Jain & Roy, 2009). Liang *et al.*, (2014) reported that the addition of maltodextrin, glucose, maltose, sucrose and trehalose decreased the heat coagulation time at 140°C of 10% MPC80 (82% protein) solutions in the pH range 7.0 to 7.2. Increasing concentration of maltodextrin from 10% to 30% shifted the pH of maximum heat stability to more alkaline values while the addition of glucose, maltose, sucrose and trehalose shifted the pH of maximum heat stability to more acidic values. It has also been shown that maltodextrin has less of an influence on heat stability of MPC80 than lactose, due to the slower heat-induced degradation of the former to acidic compounds (Crowley *et al.*, 2014a).

To the authors knowledge, there are no published studies systematically studying the influence of dextrose equivalent (DE) value of glucose polymer ingredients on heat stability of milk protein-carbohydrate nutritional beverages. Therefore, the objective of this study was to determine how a range of chain length of glucose polymers ingredients (maltodextrins DE 17 and corn syrup solids DE 38) influences the rheological properties, heat and physical stability of MPI-carbohydrate nutritional

beverages on sterilisation at 120°C. In addition, the dry sediment percentage (Chen, Grandison, & Lewis, 2012), as an objective heat stability measurement, will be determined in order to link the new understanding of heat stability to physical stability of MPI-carbohydrate beverage systems during accelerated storage.

2. Materials and methods

2.1. Materials

Milk protein isolate (MPI; 85.9% w/w protein, 4.23% w/w moisture, 7.59% w/w ash, 0.37% w/w lactose) was supplied by NIZO food research (Ede, the Netherlands). Maltodextrin (MD) with dextrose equivalent (DE) value of 17 (4.37% moisture and 0.10% ash) and corn syrup solids (CSS) with DE value of 38 (2.68% moisture and 0.13% ash) were supplied by Corcoran Chemicals Ltd. (Dublin, Ireland). Glucose (9.47% moisture and 0.01% ash) and all other chemicals used were of analytical grade and supplied by Sigma-Aldrich (Dublin, Ireland) unless otherwise specified.

2.2. Preparation of protein-carbohydrate solutions

MPI was reconstituted to 8.5% protein in simulated milk ultrafiltrate (SMUF; Jenness & Koops, 1962) at 22°C using a polytetrafluoroethylene magnetic stirring bar (50 × 8 mm). MD (5% w/w), CSS or glucose (DE value of 100) were then added and stirred magnetically at 500 rpm using a Stuart SB.162-3 hotplate stirrer (Bibby Scientific Limited, Stone, Staffordshire, UK) at 22°C for a minimum of 3 h. These solutions were adjusted to pH 6.8 using 1 M NaOH or 1 M HCl before storing at 4°C for 18 h to facilitate complete rehydration. MPI-C, MPI-MD17, MPI-CSS38 and MPI-G

solutions' were defined as 8.5% milk protein isolate (MPI) with no added carbohydrate, and 8.5% MPI contained 5% maltodextrin DE 17, 5% corn syrup solids DE 38 and glucose DE 100, respectively.

2.3. Determination of heat coagulation time

The heat coagulation time (HCT) of the protein-carbohydrate solutions was determined as a function of pH in the range 6.3 to 7.3 at 120°C in accordance with the method of Davies & White (1966) as described by Crowley, Desautel, Gazi, Kelly, Huppertz, & O'Mahony (2015a).

2.4. Sterilisation

Protein-carbohydrate solutions (400 mL) were placed in glass bottles (500 mL capacity) and sterilised using a Falcon 30 Laboratory autoclave (LTE Scientific Ltd, Greenfield, Oldham, UK) under static conditions at $121 \pm 1^\circ\text{C}$ for 15 min. The heat-up time was 40 min and samples were cooled naturally until the temperature reached 60°C (after approximately 50 min), after which, the samples were removed from the autoclave and further cooled by immersing in cold running water to reach 22°C. All in-container sterilised samples were stored at 4°C prior to analysis.

2.5. Measurement of pH, ionic calcium concentration and freezing point depression

The pH was measured using a Sentron 3001 pH meter (Meterlab, Radiometer Analytical, Villeurbanne, Lyon, France), which was calibrated with standard buffer

solutions of pH 4.0 and 7.0 at 22°C. Ionic calcium concentration (Ca^{2+}) was measured using a polymer membrane Ca-ion-selective electrode (Metrohm Ireland Ltd., Carlow, Ireland) at 22°C as described by Crowley, Kelly, & O'Mahony (2014b). Freezing point depression (FPD) was measured using an Advanced Milk Cryoscope 4L2 (Advanced Instruments Inc., Metuchen, NJ, USA) and results were expressed in millidegrees Celsius (m°C).

2.6. Colour analysis

A pre-calibrated Minolta chromameter CR-400 microcolour tristimulus colorimeter (Konica Minolta Sensing Inc., Milton Keynes, UK) was used for the determination of L^* , a^* and b^* colour parameters of the protein-carbohydrate solutions as described by Crowley *et al.* (2014b).

2.7. Measurement of rheological properties

The apparent viscosity of the protein-carbohydrate solutions before and after sterilisation was measured using a Haake RotoVisco viscometer (Thermo Electron, GmbH, Karlsruhe, Germany), equipped with a double gap concentric cylinder and DG43 cup (cup radius 21.77 mm) and inside and outside geometry gaps of 0.6 mm and 0.71 mm, respectively. Samples (12 mL) were loaded into the gap and allowed to equilibrate at 20°C for 2 min before analysis. Samples were pre-sheared at 500 s^{-1} for 1 min and then held at 0 s^{-1} for 1 min, after which the samples were subjected to a shear rate ramp from 10 to 1000 s^{-1} over 2 min, and held at 1000 s^{-1} for 2 min before returning shear rate from 1000 to 10 s^{-1} . The average apparent viscosity was taken at

the shear rate of 1000 s⁻¹. The power law (see Equation 1) was used to describe the rheological properties of these samples.

$$\tau = K\dot{\gamma}^n \quad (\text{Equation 1})$$

where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s⁻¹), K is the consistency coefficient (Pa.sⁿ) and n is the flow behaviour index (Anema, Lowe, Lee, & Klostermeyer. 2014). **Triplicate measurements were conducted for all MPI solutions.**

2.8. Physical stability testing

2.8.1. Dry sediment

The quantity of sediment in the samples was measured using a centrifugation method as described by Chen *et al.*, (2012).

2.8.2. Accelerated physical stability

Accelerated physical stability testing of protein-carbohydrate solutions was conducted using a LUMiSizer analytical centrifuge (Lum GMBH, Berlin, Germany), equipped with the software package SepView 4.1 (Lum GMBH). The operation principle of LUMiSizer has been described by Tobin, Fitzsimons, Kelly, & Fenelon (2011). Samples (0.4 mL) were filled into polycarbonate cells (PC 110-131XX; Lum GMBH) using a syringe with wide-bore needle and centrifuged at 2300 g for 3 h. The sedimentation velocity, sediment height and compressibility of the sediment were measured as described by Crowley *et al.*, 2015a).

2.9. Protein particle size distribution and hydration

The protein particle size distribution of the samples was measured using dynamic light scattering using a Zetasizer Nano series HT (Malvern Instruments Ltd., Malvern, UK) with Malvern Zetasizer v.2.2 software (Malvern Instruments Ltd.). Samples (0.125 mL) were diluted to 25 mL with ultra-pure water. For each sample, 10 measurements were performed and the output number was a mean average of the 10 measurements. Experimental parameters were as follows: temperature, 22 °C (120 s equilibration); material refractive index, 1.39; dispersant refractive index, 1.33; material absorption, 0.001; viscosity, 0.8872 mPa.s. The intensity-weighted mean particle diameter parameter was used for size measurements and distribution.

Protein hydration was measured using an ultracentrifugation method as outlined by Huppertz *et al.*, (2004).

2.10. Statistical data analysis

All samples were prepared in three independent trials and analysed in triplicate. Statistical analysis of all data was conducted using Statistical Package for the Social Sciences (SPSS 18; IBM Corporation, New York, United States) software version 18. Mean values, number of determinations, regression and univariate analysis was calculated using SPSS 18 one-way ANOVA and independent *t*-tests. The threshold level of significance of $p < 0.05$ was used in all analyses.

3. Results and discussion

3.1. Composition and colour

The pH of the control MPI solution (MPI-C) was 6.78 before heat treatment. Addition of MD17 or CSS38 had no significant effect on the pH of the MPI solutions which ranged from 6.77 to 6.78 (Table 1). The total solids of the MPI-C, MPI-MD17 and MPI-CSS38 solutions were 10.04%, 15.86% and 16.03%, respectively.

On sterilisation, the pH of the MPI-C, MPI-MD17, MPI-CSS38 solutions decreased by 0.19, 0.23 and 0.32 pH units, respectively. The magnitude of pH reduction on sterilisation was broadly in agreement with a previous study involving in-container sterilisation of milk (Chen, Grandison, & Lewis, 2015). Increasing DE value of the glucose polymers resulted in a greater reduction in pH on sterilisation which could probably be attributed to greater acid formation by the Maillard reaction with the higher proportion of reducing groups available in the high DE value samples (van Boekel, 1998) and also due to heat-induced precipitation of tertiary calcium phosphate (Visser, Minihan, Smits, Tjan, & Heertje, 1986). It has been reported (Semenova, Antipova, & Belyakova, 2002) that as glycation involved covalent interactions with basic amino acid side-chains, it caused a reduction in pH.

The ionic calcium (Ca^{2+}) concentration in the MPI-C solution was 2.17 mM before sterilisation (Table 1) and increased slightly on addition of glucose polymers ingredients to levels of 2.25 and 2.33 mM in the MPI-MD17 and MPI-CSS38 solutions, respectively. This increase in Ca^{2+} may be caused by a reduction in casein hydration (see Table 3) and the changes in protein surface activity (Semenova, Belyakova, Antipova & Jubanova, 1999) arising from the addition of glucose polymers. In keeping with this, Gao, van Leeuwen, Temminghoff, van Valenberg,

Eisner, & van Boekel (2010) reported that the addition of 10% sucrose, trehalose, lactose and maltose caused an increase in Ca^{2+} in milk due to a combination of volume exclusion and hydration effects.

The concentration of Ca^{2+} decreased significantly on sterilisation from 2.17 to 1.85 mM and from 2.25 to 2.13 mM for the MPI-C and MPI-MD17 solutions, respectively (Table 1). Similar decreases have been reported by Geerts, Bekhof, & Scherjon (1983) in a study of cow's milk. The decrease in Ca^{2+} may arise from calcium-mediated protein aggregation and precipitation of tertiary calcium phosphate during the heat treatment (O'Connell & Fox, 2001). Conversely, the Ca^{2+} concentration in the MPI-CSS38 solution increased from 2.33 to 2.61 mM on sterilisation. This sample displayed the greatest reduction in pH on sterilisation, which would be expected to increase Ca^{2+} (Chen *et al.* 2015). In addition, the ability of casein to bind Ca^{2+} has been shown to be impaired during high temperature heating as a consequence of dephosphorylation (Mohammad, 1985). It also should be noted that hydrophobic interactions were possibly not greater than steric repulsion for the MPI-MD17/CCS38 solutions before heat treatment, while heat denatured protein in MPI-MD17/CCS38 on in-container sterilisation would enhance hydrophobic interactions (Yüksel & Erdem, 2005).

The L^* , a^* and b^* values of the MPI-C, MPI-MD17 and MPI-CSS38 solutions before and after sterilisation are presented in Table 1. There were no significant differences in L^* , a^* and b^* values between the unheated solutions, with values for L^* , a^* and b^* ranging from 80.7 to 81.7, -2.59 to -2.73 and -0.81 to -1.02, respectively. The lightness (i.e., L^* value) increased significantly from 81.7 to 83.9 on sterilisation for the MPI-C solution, whereas the L^* value decreased significantly on sterilisation from 80.7 to 79.1 in the MPI-MD17 and from 80.8 to 74.2 in the MPI-CSS38 solution,

respectively (Table 1). Similar decreases have also been reported by Liu & Zhong (2015) on sterilisation of whey protein-carbohydrate solutions. The decrease in lightness/whiteness of the MPI-MD17 and MPI-CSS38 solutions may be attributed to brown melanoidin-based pigments produced in the final stages of the Maillard reaction (Boekel, 1998) on sterilisation.

The freezing point depression (FPD) is an important physico-chemical qualitative indicator of milk composition and quality (Chen, Lewis & Grandison, 2014). The compositional factors, such as lactose, chloride salts and other water-soluble constituents (such as calcium, potassium, and magnesium), contribute significantly to the FPD of milk by about 55%, 25% and 20%, respectively (Jenness & Patton, 1959). It has been reported (Chen et al., 2015) that the FPD of raw milk in different seasons was significantly positively correlated with the heat stability of UHT milk, although this correlation could probably be linked indirectly to the significant correlation between the protein content and heat stability.

In the present study, the freezing point depression of the unheated MPI-C solution was 225m°C and increased considerably on addition of glucose polymer ingredients to values of 329 and 447m°C in the MPI-MD17 and MPI-CSS38 solutions, respectively. The increase in value of FPD was due to the linear relationship between the FPD and the molality of the solute (Rong, Sillick, & Gregson, 2009).

On sterilisation, the FPD of the MPI-C, MPI-MD17, MPI-CSS38 solutions increased by 45, 72 and 90m°C, respectively (Table 1). It has been reported that heat treatment resulted in a reduction in the FPD of pasteurised milk (Janštová, Dračková, Navrátilová, Hadra & Vorlová, 2007; Kessler, 1984) due to the a change in the calcium phosphate complex and in the pressure of carbon dioxide (Rohm,

Pleschberger & Foissy, 1991). However, in the present study, the increase in FPD may be mainly caused by the formation of acids and other low molecular weight Maillard reaction products such as formic acid and melanoidins on sterilisation (Boekel, 1998).

3.2. Protein particle size distribution

The mean protein particle diameter of the MPI-C solution was 208 nm before heat treatment. Addition of MD17 or CSS38 had no significant effect on the distribution of protein particle size of the MPI solutions which ranged from 199 to 208 nm (Table 2). Similar results have been reported by Liang *et al.*, (2014) in a study of MPC-carbohydrate solutions. The mean protein particle size increased substantially on sterilisation (Table 2) for all 3 solutions and the magnitude of increase in mean particle size increased with increasing DE value of the glucose polymers (Table 2; Figure 1). This increase in protein particle size could probably be attributed to heat-denatured whey proteins, especially β -lactoglobulin, complexing with κ -casein on the surface of casein micelles, along with possible deposition of calcium phosphate onto the micelles (Singh and Fox, 1987) and aggregation of small casein micelles, or further association of whey-casein protein aggregates (Mohammad & Fox, 1987). It is known that in-container sterilisation at 120°C for 15 min caused complete denaturation of whey proteins (Lewis & Heppell, 2000).

3.3. Rheological properties

The apparent viscosity of the MPI-C solution was 7.07 mPa.s before heat treatment (Table 1) and increased significantly on addition of glucose polymers to levels of 9.79 and 9.22 mPa.s in the MPI-MD17 and MPI-CSS38 solutions, respectively. The increase in apparent viscosity is caused mainly by increases in protein hydrodynamic volume in the presence of carbohydrate (Gao *et al.*, 2010), the increased total solids and viscosity of addition of MD and CSS itself (Murray and Luft, 1973). It should be noted that, compared with the viscosity of MD, viscosity development of shorter polymers (CCS) benefits little from chain entanglement and hydrodynamic volume. For the 3 unheated solutions, the apparent viscosity decreased with increasing shear rate, which is typical of pseudoplastic or shear-thinning solutions (Figure 2 A, C). Using the power law (Equation 1), the rheological behaviour of a complex fluid can be described with two values: the “consistency coefficient” K, and the “flow behaviour index” n. There were no significant differences in K or n between the 3 unheated solutions, with values for K and n ranging from 23.3×10^{-3} to 35.2×10^{-3} Pa.sⁿ and from 810×10^{-3} to 847×10^{-3} , respectively.

The apparent viscosity increased significantly on sterilisation from 9.79 to 17.0 mPa.s and from 9.22 to 19.9 mPa.s in the MPI-MD17 and MPI-38 solutions, respectively (Table 1). The apparent viscosity of the heated MPI-C, MPI-MD17 and MPI-CSS38 solutions decreased considerably with increasing shear rate up to approximately 50 s^{-1} (Figure 2 D) and then decreased more slowly with increasing shear rate. It was also observed that, at all shear rates, higher shear stress and apparent viscosity was measured for the MPI-CSS38 solution than for the MPI-C or MPI-MD17 solutions (Figure 2 B, D). While there was no significant increase in apparent viscosity of the MPI-C solution, the MPI-MD17 and MPI-CSS38 did show considerable increases in viscosity on sterilisation. The sample with the greatest increase in apparent viscosity

(i.e., MPI-CSS38) had the greatest increase in protein particle size on sterilisation. Therefore, heat-induced (promoted further by the low pH of the heated WPI-CSS38 solution) protein denaturation and aggregation, leading to increases in protein particle size, had a strong influence on changes in apparent viscosity, while other factors, such as increases in hydrodynamic volume caused by water-binding by Maillard reaction products may also have had a contributory role (Hiller & Lorenzen, 2010).

3.4. Hydration

The hydration of the unheated MPI-C solution was 2.54 g H₂O/g and decreased significantly on addition of glucose polymers to levels of 2.19 and 2.11 g H₂O/g in the MPI-MD17 and MPI-CSS38 solutions, respectively. The reduction in hydration was in agreement with a previous study of cow's milk (Sood, Sidhu, & Dewan, 1976). On sterilisation, the hydration of the MPI-C, MPI-MD17, MPI-CSS38 solutions generally decreased by 0.14, 0.10 and 0.15 g H₂O/g, respectively, although it was not statistically significant. The magnitude of hydration reduction on sterilisation was broadly in agreement with a previous study of Rüegg, Blanc, & Lüscher (1979) who reported the hydration of casein micelles prepared from raw and heat-treated milk as measured by ultracentrifugation. The slight reduction in hydration of the 3 heated solutions may arise from the pH reduction on sterilisation. It has been reported (Creamer and Matheson, 1980) that when the pH value was readjusted to the original value after heat treatment at 100°C for 20 min, no significant effect on the casein micelle hydration was found.

3.5. Heat coagulation time

The pH-heat coagulation time (HCT) profiles of the MPI-C, MPI-MD17, MPI-CSS38 and MPI contained glucose (MPI-G) solutions were given in Figure 3. They all had similar shape but had maximum HCT (HCT_{max}) at pH 6.8. The HCT_{max} of MPI-C solution was 38 min and decreased substantially on addition of glucose polymers to HCT_{max} of 30, 26 and 18 min in the MPI-MD17, MPI-CSS38 and MPI-G solutions, respectively. The pH value of HCT_{max} in all 4 solutions remained unchanged of pH 6.8. This result was partially in agreement with Liang *et al.*, (2014), who reported that the HCT_{max} of 10% MPC (82% protein content) solutions shifted from pH 7.0 to pH 6.8 for 10% glucose but was unaffected for 10% MD with a DE value of 18. It was noted that the pH-HCT profiles of the MPI-MD17 solution did not exhibit a HCT_{min} . Instead, MPI-MD17 solution had the pH-HCT profile with very low HCT values at the acidic side of the spectrum. Above the pH 6.8, HCT of the MPI-MD17 decreased with increasing pH (Figure 3). The pH-HCT patterns (Figure 3) in the present study have not been reported in the literature previously for sterilisation of MPI-carbohydrate solutions, although most studies have dealt with non-reducing carbohydrates or starch rather than the carbohydrates contained the different chain length of glucose polymers.

When considering the heat stability of MPI-carbohydrates nutritional beverage, a number of factors related to the soluble phase are key contributors, most notably the influence of κ -casein dissociation (Singh, 2004), Ca^{2+} , hydration, protein particle size, Maillard reaction and heat-induced acidification.

In terms of HCT-pH profile, heat stability goes through a HCT_{max} and HCT_{min} for type A milk sample and it has been reported that this trend is probably mainly

attributed to heat induced dissociation of κ -casein (more dominate at $\text{pH} > 6.7$) and strong Ca^{2+} activity from the micelle (Singh, 2004). However, in the present study, based on the protein profile distribution results in the heated MPI suspensions from reversed phase HPLC, addition of MD17, CSS38 and glucose had no significant effect on the dissociation of κ -casein (data not shown).

So, when considering the various contributors outlined above in relation to the markedly reduced heat stability of MPI-carbohydrates nutritional beverages, increased Ca^{2+} concentration caused by the decreased hydration effect is identified readily as a primary factor to reduced heat stability. The negative effect of increased Ca^{2+} concentration on the heat stability of dairy products has been extensively reported (Boumpa, Tsioulpas, Grandison & Lewis, 2008; Chen *et al.* 2012; 2015; Singh, 2004).

Another factor, the increasing protein particle size arising from the addition of glucose polymers (Table 2), also had a significant effect on the decreasing heat stability of MPI-carbohydrates solutions. It has been reported (O'Connell & Fox, 2000) that heat stability of cow's milk is inversely proportional to the micelle size and the negative effect of increased protein particle size on the heat stability of dairy products has been extensively reported (Anema & Li, 2003; Chen *et al.*, 2012; O'Connell & Fox, 2000).

Bridging due to the Maillard reaction has often been shown when glucose polymers are present on sterilisation (Ashoor & Zent, 1984). Since the melanoidins caused by the final stages of Maillard reaction were mainly bound to milk protein (Boekel, 1998), it was suggested that the Maillard reaction in the MPI-C and MPI-MD17 solutions probably had less contribution to the formation of covalent bond than in the MPI-CSS38 solution which involved the aggregation of casein micelle .

The different rate of glucose polymers degradation on sterilisation was likely to be the final contributory role. Compared with the MPI-CSS38 solution, the MPI-MD17 solution will be probably slower degraded into organic acids through Maillard reaction on sterilisation (Crowley *et al.*, 2014a). This slower degradation reaction probably limited the influence of MD17 on the heat stability of MPI solution.

3.6. Physical stability testing

3.6.1. Dry sediment percentage

Sediment formation in MPI-carbohydrate nutritional beverage is one manifestation of poor heat stability and it is mainly formed by aggregation of casein micelle. In this respect, another heat stability method was developed which referred primarily to coagulation and sediment formation, arising either during processing or subsequent storage in the present study.

The sediment formation of the MPI-C and MPI-MD17 solutions on sterilisation was 1.45% and 1.63%, respectively, and promoted significantly on addition of CSS38 to levels of 5.88% (Table 2). Increasing DE value of the glucose polymers resulted in a greater increase in sediment formation on sterilisation which could probably be attributed to the increasing protein particle size and Ca^{2+} (Table 2). It has been reported that sediment formation arising from heat treatment of good quality cow's milk was below 0.5% (Chen *et al.*, 2015), but no data was given for milk MPI-carbohydrate beverage.

3.6.2. Accelerated physical stability testing

There was no significant difference in sedimentation profiles between the all 3 unheated solutions (data not shown). The sedimentation profiles of the all 3 unheated solutions post sterilisation are presented in Figure 4. Centrifugation of MPI-C, MPI-MD17 and MPI-CSS38 solutions created different sediment heights, as indicated by the formation of an optically dense (low transmission) region at the base of the centrifuge tube (see R2 region in Figure 4), with concomitant stable dispersion in the region (R1 in Figure 4) between the meniscus and the top of the sediment layer. The compressed sediment was formed (R3 region in Figure 4) in some solutions after 3 h centrifugation, with this effect being more pronounced in the MPI-C solution than in the MPI-MD17 and MPI-CSS38 solutions (Figure 4). The sediments velocity in the MPI-C solution was 7.57 mm/min on sterilisation and decreased considerably on addition of glucose polymers ingredients to 3.70 and 5.20 mm/min in the MPI-MD17 and MPI-CSS38 solutions, respectively. Based on Stokes' Law, sedimentation velocity is dependent on the density difference between the particle and the liquid phase, the particle size, and viscosity (van Vliet & Walstra, 1989; Mäkinen, Uniacke-Lowe, O'Mahony, & Arendt, 2015) as shown in equation (2).

$$V_g = d^2 (\rho_p - \rho_l) g / 18 \eta \quad (\text{Equation 2})$$

where V_g is sedimentation velocity, d is the particle diameter, ρ_p is the particle density, ρ_l is the liquid density, g is the gravitational acceleration and η is the viscosity. It was noted that the sedimentation velocity (V_g) was positively correlated with the particle size (d), but negatively with viscosity (η) which was two competing effects influencing the sedimentation velocity.

In the present study, increasing DE values of glucose polymers resulted in a greater increase in both mean protein particle size and apparent viscosity (Table 1 and 2) on sterilisation. It was calculated that the ratio of mean protein particle size to apparent

viscosity on sterilisation was 52.7, 26.4 and 32.8 for the MPI-C, MPI-MD17 and MPI-CSS38 solutions, respectively. So, it is clear that the greater ratio of mean protein particle size to apparent viscosity for the MPI-C solution probably resulted in the greater sedimentation velocity than for the MPI-MD17 and MPI-CSS38 solutions. Meanwhile, the sediment compressibility (R3 region, Figure 4) in the MPI-C and MPI-CSS38 solutions on sterilisation was 33% and 22%, respectively, whereas no compressed sediment was detected in the MPI-MD17 solution. The compressed sediment in the MPI-C and MPI-CSS38 solutions could probably be attributed to the casein entrapment within the surface of initial sediments (Crowley *et al.*, 2014b). Compared with the physical stability profiles of MPI-C and MPI-CSS38 solutions, there was no sharp, vertical, intersection characteristic of the phase boundary of aqueous/sediment in the profile of MPI-MD17 solution and, as centrifugation time extended, the gap distance (shown in Figure 4) between each profile after 3h in the profile of MPI-MD17 was constant (but gradually narrower gap distance was observed in the MPI-C and MPI-CSS38 profiles). These characteristics suggested that a ‘true’ sediment in the MPI-MD17 solution probably was not actually formed. So, it is suggested that the increasing ratio of mean protein particle size to apparent viscosity in MPI-carbohydrate solutions resulted in a greater increase in sedimentation velocity, sediment compressibility and a reduction in the final sediment height and *vice versa*.

Overall, when considering the heat stability of MPI-carbohydrate beverage on sterilisation, the MPI-C solution presented the highest heat stability due to the longest HCT_{max} and lowest sediment formation (Figure 3 and Table 2). However, when considering the physical stability of MPI-carbohydrate beverage in the subsequent storage, the greater ratio of mean protein particle size to apparent viscosity in MPI-C

solution may resulted in the sediment formation more rapidly and even highly compressed than the others. This unexpected sedimentation and compression was unacceptable for the MPI-carbohydrate products. For these reasons, it is suggested that incorporation of MD17 with MPI solution would be expected to impart both acceptable heat and stored stability to MPI-carbohydrate nutritional beverage due to its lowest ratio of mean protein particle size to apparent viscosity and acceptable sediment formation (Table 2).

4. Conclusion

This study demonstrated that the chain length of glucose polymer ingredients used in the formulation of protein-carbohydrate beverages impacted strongly on the heat and physical stability of the beverage formulations during sterilisation. The higher dextrose equivalent ingredient resulted in lower pH, with concomitantly higher levels of ionic calcium on sterilisation, which contributed to greater changes in viscosity and rheological properties of the beverages due to their combined roles in aggregating/destabilising milk proteins on heating. Incorporation of glucose polymers with milk protein isolates retarded sedimentation of protein during accelerated physical stability testing. The results demonstrate that the incorporation of maltodextrin DE17 resulted in the second highest heat stability and best physical stability and it would be expected to be the most suitable glucose polymer for inclusion in such MPI-carbohydrate nutritional beverages.

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References

Anema, S. G., & Li, Y. (2003). Association of denatured whey proteins with casein micelles in heated reconstituted skim milk and its effect on casein micelle size. *Journal of Dairy Research*, 70, 73-83.

Anema, S. G., Lowe, E. K., Lee, S. K., & Klostermeyer, H. (2014). Effect of the pH of skim milk at heating on milk concentrate viscosity. *International Dairy Journal*, 39, 336-343.

Anema, S. G., Pinder, D. N., Hunter, R. J., & Hemar, Y. (2006). Effects of storage temperature on the solubility of milk protein concentrate (MPC85). *Food Hydrocolloids*, 20, 386-393.

Ashoor, S. H., & Zent, J. B. (1984). Maillard browning of common amino acids and sugars. *Journal of Food Science*, 49, 1206-1207.

Boumpa, T., Tsioulpas, A., Grandison, A. S., & Lewis, M. J. (2008). Effects of phosphates and citrates on sediment formation in UHT goats' milk. *Journal of Dairy Research*, 75(02), 160-166.

565 Chen, B. Y., Grandison, A. S., & Lewis, M. J. (2012). Comparison of heat stability of
 566 goat milk subjected to ultra-high temperature and in-container sterilization. *Journal of*
 567 *Dairy Science*, 95, 1057-1063.

568 Chen, B. Y., Grandison, A. S., & Lewis, M. J. (2015). Effect of seasonal variation on
 569 some physical properties and heat stability of milk subjected to ultra-high temperature
 570 and in-container sterilisation. *Food Chemistry*, 181, 227-234.

571 Chen, B. Y., Lewis, M. J., & Grandison, A. S. (2014). Effect of seasonal variation on
 572 the composition and properties of raw milk destined for processing in the UK. *Food*
 573 *Chemistry*, 158, 216-223.

574 Cochrane, N. J., Yuan, Y., Walker, G. D., Shen, P., Chang, C. H., Reynolds, C., &
 575 Reynolds, E. C. (2012). Erosive potential of sports beverages. *Australian Dental*
 576 *Journal*, 57, 359-364.

577 Cockburn, E., Stevenson, E., Hayes, P. R., Robson-Ansley, P., & Howatson, G.
 578 (2010). Effect of milk-based carbohydrate-protein supplement timing on the
 579 attenuation of exercise-induced muscle damage. *Applied Physiology, Nutrition, and*
 580 *Metabolism*, 35, 270-277.

581 Coombes, J. S., & Hamilton, K. L. (2000). The effectiveness of commercially
 582 available sports drinks. *Sports Medicine*, 29, 181-209.

583 Creamer, L. K., & Matheson, A. R. (1980). Effect of heat treatment on the proteins of
 584 pasteurized skim milk. *New Zealand Journal of Dairy Science and Technology*, 15,
 585 37-49.

586 Crowley, S. V., Boudin, M., Chen, B. Y., Gazi, I., Huppertz, T., Kelly, A. L., &
587 O'Mahony, J. A. (2015b). Stability of milk protein concentrate suspensions to in-
588 container sterilisation heating conditions. *International Dairy Journal*, 50, 45-49.

589 Crowley, S. V., Desautel, B., Gazi, I., Kelly, A. L., Huppertz, T., & O'Mahony, J. A.
590 (2015a). Rehydration characteristics of milk protein concentrate powders. *Journal of*
591 *Food Engineering*, 149, 105-113.

592 Crowley, S. V., Kelly, A. L., & O'Mahony, J. A. (2014b). Fortification of
593 reconstituted skim milk powder with different calcium salts: Impact of
594 physicochemical changes on stability to processing. *International Journal of Dairy*
595 *Technology*, 67, 474-482.

596 Crowley, S. V., Megemont, M., Gazi, I., Kelly, A. L., Huppertz, T., & O'Mahony, J.
597 A. (2014a). Heat stability of reconstituted milk protein concentrate powders.
598 *International Dairy Journal*, 37, 104-110.

599 Cruijsen, J. M. M. (1996). Physical stability of caseinate-stabilized emulsions during
600 heating. Agricultural University of Wageningen.

601 Davies, D. T., & White, J. C. D. (1966). The stability of milk protein to heat. 1.
602 Subjective measurement of heat stability of milk. *Journal of Dairy Science*, 33, 67-81.

603 Eshpari, H., Tong, P. S., & Corredig, M. (2014). Changes in the physical properties,
604 solubility, and heat stability of milk protein concentrates prepared from partially
605 acidified milk. *Journal of Dairy Science*, 97, 7394-7401.

606 Euromonitor. *Sports drinks market sizes*. 2010. URL
607 <http://www.euromonitor.com/sports-drinks>. Accessed 11.09.15.

608 Foster-Schubert, K. E., Overduin, J., Prudom, C. E., Liu, J., Callahan, H. S., Gaylinn,
609 B. D., Thorner, M. O., & Cummings, D. E. (2008). Acyl and total ghrelin are
610 suppressed strongly by ingested proteins, weakly by lipids, and biphasically by
611 carbohydrates. *The Journal of Clinical Endocrinology & Metabolism*, 93, 1971-1979.

612 Gao, R., van Leeuwen, H. P., Temminghoff, E. J., van Valenberg, H. J., Eisner, M. D.,
613 & van Boekel, M. A. (2010). Effect of disaccharides on ion properties in milk-based
614 systems. *Journal of Agricultural and Food Chemistry*, 58, 6449- 6457.

615 Geerts, J. P., Bekhof, J. J., & Scherjon, J. W. (1983). Determination of calcium ion
616 activities in milk with an ion selective electrode. *Netherlands Milk and Dairy Journal*.
617 37, 197–211.

618 Hiller, B., & Lorenzen, P. C. (2010). Functional properties of milk proteins as
619 affected by Maillard reaction induced oligomerisation. *Food Research International*,
620 43, 1155-1166.

621 Holt, C., Muir, D. D., & Sweetsur, A. W. M. (1978). The heat stability of milk and
622 concentrated milk containing added aldehydes and sugars. *Journal of Dairy Research*,
623 45, 47-52.

624 Jain, N. K., & Roy, I. (2009). Effect of trehalose on protein structure. *Protein Science*,
625 18, 24-36.

626 Janštová, B., Dračková, M., Navrátilová, P., Hadra, L., & Vorlová, L. (2007).
627 Freezing point of raw and heat-treated goat milk. *Czech Journal of Animal*
628 *Science*, 52, 394-398.

629 Jenness, R., & Koops, J. (1962). Preparation and properties of a salt solution which
630 simulates milk ultrafiltrate. *The Netherlands Milk and Dairy Journal*, 16, 153-164.

631 Jenness, R., & Patton, S. (1959). Principles of dairy chemistry. New York, USA:
 632 Wiley, pp. 258–261.

633 Kaliappan, S., & Lucey, J. A. (2011). Influence of mixtures of calcium-chelating salts
 634 on the physicochemical properties of casein micelles. *Journal of dairy science*, 94,
 635 4255-4263.

636 Kelly P. (2011). Milk Protein Concentrate. In: Editor-in Chief: John W. Fuquay,
 637 editor. *Encyclopedia of Dairy Sciences* (Second Edition). 2nd ed. San Diego:
 638 Academic Press. pp 848-54.

639 Kessler, H.G. (1984): Effects of the technological processes on the freezing point of
 640 milk. *Milchwissenschaft*, 39, 339–341

641 Lewis, M. J., & Heppel, N. (2000). Continuous Thermal Processing of Foods. Aspen
 642 Publishers, Gaithersburg, MD.

643 Liang, Y., Matia-Merino, L., Patel, H., Ye, A., Gillies, G., & Golding, M. (2014).
 644 Effect of sugar type and concentration on the heat coagulation of oil-in-water
 645 emulsions stabilized by milk-protein-concentrate. *Food Hydrocolloids*, 41, 332-342.

646 Liu, G., & Zhong, Q. (2015). High temperature-short time glycation to improve heat
 647 stability of whey protein and reduce color formation. *Food Hydrocolloids*, 44, 453-
 648 460.

649 Mäkinen, O. E., Uniacke-Lowe, T., O'Mahony, J. A., & Arendt, E. K. (2015).
 650 Physicochemical and acid gelation properties of commercial UHT-treated plant-based
 651 milk substitutes and lactose free bovine milk. *Food Chemistry*, 168, 630-638.

652 Marchal, L. M., Beftink, H. H., & Tramper, J. (1999). Towards a rational design of
 653 commercial maltodextrins. *Trends In Food Science & Technology*, 10, 345-355.

654 Mohammad, K. S. (1985). *Heat-induced changes in micellar and soluble casein*. Ph.D.
 655 Thesis, National University of Ireland, Cork.

656 Mohammad, K. S., & Fox, P. F. (1987). Heat-induced association-dissociation of
 657 casein micelles preceding coagulation. *Journal of Dairy Research*, 54, 377-387.

658 Murray and Luft, 1973; courtesy of Grain processing Corp. Reprinted from Food
 659 Technolo. 27 (3), 33, 1973. Copyright by Institute of Food Technologists.

660 O'Connell, J. E., & Fox, P. F. (2000). The two-stage coagulation of milk proteins in
 661 the minimum of the heat coagulation time-pH profile of milk: effect of casein micelle
 662 size. *Journal of Dairy Science*, 83, 378-386.

663 O'Connell, J. E., & Fox, P. F. (2001). Effect of β -lactoglobulin and precipitation of
 664 calcium phosphate on the thermal coagulation of milk. *Journal of Dairy Research*. 68,
 665 81-94.

666 Rohm, H., Pleschberger, C., Foissy, H. (1991). Der Gefrierpunkt österreichischer
 667 Rohmilch. 1. Einflussfaktoren auf den originären Gefrierpunkt. *Ernährung*, 15, 333-
 668 337

669 Rong, Y., Sillick, M., & Gregson, C. M. (2009). Determination of dextrose equivalent
 670 value and number average molecular weight of maltodextrin by osmometry. *Journal*
 671 *of Food Science*, 74, 33-40.

672 Rüegg, M., Blanc, B., & Lüscher, M. (1979). Hydration of casein micelles: kinetics
673 and isotherms of water sorption of micellar casein isolated from fresh and heat-treated
674 milk. *Journal of Dairy Research*, 46, 325-328.

675 Semenova M, Belyakova L, Antipova A, Jubanova M. (1999). Influence of
676 maltodextrins with different dextrose equivalent on the thermodynamic properties of
677 legumin in a bulk and at the air–water interface. *Colloids and Surfaces B:
678 Biointerfaces*, 12, 287–297.

679 Semenova, M. G., Antipova, A. S., & Belyakova, L. E. (2002). Food protein
680 interactions in sugar solutions. *Current Opinion In Colloid & Interface Science*, 7,
681 438-444.

682 Singh, H. (2004). Heat stability of milk. *International Journal of Dairy Technology*,
683 57, 111-119.

684 Singh, H., & Fox, P. F. (1987). Heat stability of milk: role of β -lactoglobulin in the
685 pH-dependent dissociation of micellar κ -casein. *Journal of Dairy Research*, 54,
686 509-521.

687 Sood, S. M., Sidhu, K. S., & Dewan, R. K. (1976). Voluminosity of different-sized
688 casein micelles from the buffalo and the cow. *New Zealand Journal of Dairy Science
689 and Technology*, 11, 79-82.

690 Tan-Kintia, R. H., & Fox, P. F. (1996). Effect of enzymic hydrolysis of lactose on the
691 heat stability of milk or concentrated milk. *Netherlands Milk and Dairy Journal*, 50,
692 267-277.

693 Tobin, J. T., Fitzsimons, S. M., Kelly, A. L., & Fenelon, M. A. (2011). The effect of
 694 native and modified konjac on the physical attributes of pasteurized and UHT-treated
 695 skim milk. *International Dairy Journal*, 21, 790–797.

696 Van Boekel, M. A. J. S. (1998). Effect of heating on Maillard reactions in milk. *Food*
 697 *Chemistry*, 62, 403-414.

698 Van Vliet, T., & Walstra, P. (1989). Weak particle networks. In R. D. Bee, P.
 699 Richmond, & J. Mingings (Eds.), *Food Colloids* (pp. 206–217). London: Royal
 700 Society of Chemistry [Special Pu].

701 Visser, J., Minihan, A., Smits, P., Tjan, S. B., & Heertje, I. (1986). Effects of pH and
 702 temperature on the milk salt system. *Netherlands Milk and Dairy Journal*. 40, 351-
 703 368.

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1 **Table 1 pH, ionic calcium concentration, colour, freezing point depression and rheological properties of MPI-C, MPI-MD17 and MPI-CSS38 protein-**
2 **carbohydrate solutions before and after sterilisation**

		pH	Ca ²⁺ (mM)	Chromaticity co-ordinate			Freezing point depression (m°C)	Apparent viscosity (mPa. s)	Consistency coefficient (×10 ⁻³ Pa.s ⁿ)	Flow behaviour index (×10 ⁻³)
				L*	a*	b*				
Before sterilisation										
	MPI-C	6.78±0.01 ^{aA}	2.17±0.07 ^{aA}	81.7±0.08 ^{aA}	-2.59±0.06 ^{aA}	-0.86±0.04 ^{aA}	225±10.6 ^{aA}	7.07±0.09 ^{aA}	23.3±9.10 ^{aA}	847±70.8 ^{aA}
	MPI-MD17	6.77±0.02 ^{aA}	2.25±0.05 ^{aA}	80.7±0.06 ^{aA}	-2.73±0.09 ^{aA}	-1.02±0.07 ^{aA}	329±10.7 ^{bA}	9.79±0.25 ^{bA}	26.8±7.10 ^{aA}	854±34.4 ^{aA}
	MPI-CSS38	6.77±0.02 ^{aA}	2.33±0.06 ^{aA}	80.8±0.11 ^{aA}	-2.73±0.11 ^{aA}	-0.81±0.08 ^{aA}	447±14.0 ^{cA}	9.22±0.70 ^{bA}	35.2±6.90 ^{aA}	810±54.0 ^{aA}
After sterilisation										
	MPI-C	6.61±0.00 ^{aB}	1.85±0.02 ^{aB}	83.9±0.20 ^{aB}	-0.05±0.18 ^{aB}	4.10±0.19 ^{aB}	270±7.33 ^{aB}	6.91±0.79 ^{aA}	20.9±5.30 ^{aA}	836±52.2 ^{aA}
	MPI-MD17	6.57±0.00 ^{bB}	2.13±0.03 ^{bA}	79.1±0.47 ^{bA}	1.80±0.11 ^{bB}	11.9±0.29 ^{bB}	401±8.41 ^{bB}	17.0±6.31 ^{bA}	57.9±3.34 ^{bB}	823±42.5 ^{aA}
	MPI-CSS38	6.48±0.01 ^{cB}	2.61±0.04 ^{cB}	74.2±0.90 ^{cB}	3.52±0.25 ^{cB}	18.7±0.69 ^{cB}	537±11.3 ^{cB}	19.9±7.77 ^{bB}	97.9±5.87 ^{cB}	766±50.8 ^{bA}

3 ^{a-c}Means (±SD) within a column followed by the same letter are not significantly different (*P* > 0.05).

4 ^{A-B}Means (±SD) within a column and within each sample followed by the same letter are not significantly different arising from sterilisation treatment (*P* > 0.05).

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14 **Table 2 Physicochemical properties of MPI-C, MPI-MD17 and MPI-CSS38 protein-carbohydrate solutions before and after sterilisation**

	Mean protein particle size (d. nm)		Hydration (H ₂ O g/g dry weight)		Dry sediment (%)	Initial sediment height (mm)	Final sediment height (mm)	Compressibility (%)
	Before sterilisation	After sterilisation	Before sterilisation	After sterilisation	After sterilisation	After sterilisation	After sterilisation	After sterilisation
MPI-C	208±3.29 ^{aA}	364±14.6 ^{aB}	2.54±0.14 ^{aA}	2.40±0.26 ^{aA}	1.45±0.13 ^a	6.72±1.07 ^a	4.50±0.88 ^a	33.1±2.56 ^c
MPI-MD17	205±6.88 ^{aA}	448±13.4 ^{bB}	2.19±0.05 ^{bA}	2.09±0.08 ^{bA}	1.63±0.11 ^a	7.11±0.73 ^a	7.11±0.73 ^a	0 ^a
MPI-CSS38	199±6.89 ^{aA}	652±67.0 ^{cB}	2.11±0.07 ^{bA}	1.96±0.04 ^{bA}	5.88±2.26 ^b	7.69±1.23 ^a	6.14±1.51 ^a	22.4±4.88 ^b

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16 ^{a-c}Means (±SD) within each property followed by the same letter are not significantly different (*P* > 0.05).

17 ^{A-B}Means (±SD) within a column and within each sample followed by the same letter are not significantly different arising from sterilisation treatment (*P* > 0.05).

18 **Figure Captions**

19 **Figure 1** Particle size distribution data for milk protein isolate (MPI) with no added no added carbohydrate
20 (CHO) (□), and 8.5% MPI contained 5% maltodextrin DE 17 (Δ), 5% corn syrup solids DE 38 (○) and
21 glucose DE 100 (◇) before (A) and after (B) sterilisation. Values are presented as average data from three
22 independent trials

23 **Figure 2** Flow curve of the shear stress vs shear rate (A, B), and apparent viscosity vs shear rate (C, D) for
24 milk protein isolates (MPI) with no added carbohydrate (□), and 8.5% MPI contained 5% maltodextrin DE
25 17 (Δ) and 5% corn syrup solids DE 38 (○) before (A, C) and after (B, D) sterilisation. Values are presented
26 as average data from three independent trials

27 **Figure 3** pH-Heat coagulation time profiles for 8.5% (w/w) milk protein isolate (MPI) with no added
28 carbohydrate (□), and 8.5% MPI contained 5% maltodextrin DE 17 (Δ), 5% corn syrup solids DE 38 (○)
29 and glucose DE 100 (◇) at 120 °C. Values are presented as mean ± standard deviation of data from three
30 independent trials

31 **Figure 4** Representative sedimentation profiles for milk protein isolates (MPI) with no added carbohydrate
32 (A), 8.5% MPI contained 5% maltodextrin DE 17 (B) and 5% corn syrup solids DE 38 (C) after in-
33 sterilisation, showing transmission of NIR light through sample cells as a function of time and position in
34 the sample cell at 22 °C for 3 h. Profiles were recorded every 60 s during each run, with every 15th profile
35 (moving from first to last) presented for clarity. Regions of stable dispersion (R1), initial sediment (R2) and
36 compressed sediment (R3) during centrifugation are presented. Schematic representations in sedimentation
37 sample tube are shown for last (3 h) profiles of centrifugation. Phase separation profiles are from one trial
38 but are representative of data from three individual trials.

Figure 1

Figure 2

Figure 3

Figure 4