Alteration of physicochemical properties and heating stability of reconstituted acid whey powder by calcium chelating salts

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Abstract

Trisodium citrate (TSC) and ethylenediaminetetraacetic acid disodium salt (Na_2-EDTA) were applied in reconstituted acid whey powder (AWP) at 20% w/w, which mimicked acid whey concentration during industrial whey processing. Physicochemical properties and heat stability of the AWP suspensions with 0–50 mM TSC and Na_2-EDTA at pH 6.2 were investigated. TSC-containing suspensions prior to heating had decreasing Ca^{2+} activity, levels of sedimentation, and subtle reduction of aggregate size with increasing TSC concentrations (0–50 mM). Unheated Na_2-EDTA-containing suspensions had lower levels of sedimentation and smaller aggregate sizes than unheated TSC-containing suspensions; however, reduction of Ca^{2+} activity was only observed up to 20 mM Na_2-EDTA. Stronger effects of Na_2-EDTA than TSC on levels of sediment, viscosity, and aggregate size of AWP

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suspensions were observed after heating, except for 50 mM Na_2-EDTA. A remarkable difference between TSC and Na_2-EDTA addition was the nature of aggregates formed in heated suspensions. TSC-containing suspensions contained larger aggregates than corresponding Na_2-EDTA-containing suspensions, which exhibited increasing shear thinning behavior as a function of concentration. In contrast, the smaller aggregates in the corresponding Na_2-EDTA-containing suspension showed shear thickening. The inverse relationship between aggregate size and levels of sediment for TSC-containing suspensions post-heat treatment may indicate the formation of loose aggregates that resist sedimentation.
Keywords: acid whey; calcium chelating salts; physicochemical properties; heat stability

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List of abbreviations

ANS	:	8-Anilino-1-naphthalenesulfonic acid	Na_2HPO_4	:	Sodium phosphate dibasic
AWP	:	Acid whey powder	NaH_2PO_4	:	Sodium phosphate monobasic
AW	:	Acid whey	P	:	Phosphate
Ca	:	Calcium	PBS	:	Phosphate-buffered saline
Ca^{2+}	:	Calcium ion	PHE	:	Plate heat exchange
$CaCl_2$	:	calcium dichloride	RFI	:	Relative Fluorescence Intensity
CCS	:	Calcium chelating salts	S	:	Sulfur
CaP	:	Calcium phosphate	SDHP	:	Sodium dihydrogen orthophosphate
DSHP	:	disodium hydrogen orthophosphate	SHMP	:	Sodium hexametaphosphate
DTNB	:	5,5´-Dithi-bis(2-nitrobenzoic acid)	STPP	:	Sodium tripolyphosphate
HCl	:	Hydrochloric acid	TSC	:	Trisodium citrate
KCl	:	Potassium chloride	TPC	:	Tripotassium citrate
KOH	:	Potassium hydroxide	WPC	:	Whey protein concentrate
Na_2-EDTA (EDTA)	:	Ethylenediaminetetraacetic acid disodium salt	WPI	:	Whey protein isolate

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Introduction

Mineral acid whey (AW) is obtained by the precipitation of caseins and denatured whey from milk using hydrochloric acid (HCl), resulting in a stream consisting of whey proteins, minerals, and lactose. Nanofiltration is commonly applied to the AW to increase its total solids and also to reduce its level of mono- and divalent salts (Purwanti et al., 2022); however, the mineral content in AW remains high, e.g., the calcium (Ca) and phosphate (P) contents of the AW are higher than those in milk by 75 and 45%, respectively (Drapala et al., 2018). Previous research reported that the Ca level in AW, on average, was three times higher than in sweet whey (Donovan & Mulvihill, 1987; Nishanthi et al., 2017a, b; Wong et al., 1978). The presence of Ca leads to the formation of calcium phosphate which limits permeation during membrane processing, resulting in scaling, a barrier to filtration, and loss of throughput (Chandrapala et al., 2015). The presence of Ca has also frequently been reported to be responsible for whey fouling in heat exchangers (Belmar-Beiny & Fryer, 1993; Blanpain-Avet et al., 2020; Joyce et al., 2018; Khaldi et al., 2015; Xiong, 1992). Calcium-linked protein aggregates or calcium lactate formation can result in increased viscosity with subsequent negative implications for drying efficiency (Chandrapala et al., 2015; Mimouni et al., 2007).

The two major mechanisms of heat-induced fouling in dairy processes are a) protein denaturation/aggregation and b) precipitation of salts, in the form of calcium phosphate (CaP) complexes or CaP-protein/fat complexes) (Blanpain-Avet et al., 2020; Felfoul et al., 2016; Petit et al., 2016; Scudeller et al., 2021). The amount of deposit formed during heating of 1% whey protein concentrate (WPC) solution in a plate heat exchanger (PHE) has been found to increase with calcium concentration (Guérin et al., 2007; Khaldi et al., 2015).

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Different amounts of deposit were obtained in a fouling rig with WPC35 (35 %w/w protein) and WPC75 (75%w/w protein), at 1% protein concentration in the solution, which was attributed to the difference in their Ca contents (Belmar-Beiny & Fryer, 1993). Petit et al. (2016) demonstrated that increasing Ca in model whey systems enhanced β-lg aggregation, which highlighted the role of Ca in the formation of deposits in whey systems.

The first layer of deposit was dominated by protein, which was identified within 4 s of contact time between the protein solution and the heating surface. Ca and sulfur (S) were identified in the deposit after 150 s of contact time, without P present (Belmar-Beiny & Fryer, 1993). Recent research showed that a spongy fouling layer of 0.5% w/v WPI solution in a PHE consisted of unfolded protein strands bound by calcium ions (Ca^{2+}) at a P concentration ≤ 20 mg/L, whereas, a fouling layer formed of CaP clusters covered by proteins was observed at a P concentration > 20 mg/L (Scudeller et al., 2021). CaP clusters were also observed in the sediment of heated bovine milk (80 °C for 60 min) (Felfoul et al., 2016). The sediment contained 69% protein with a ratio of protein/mineral of 2.5 and the deposited minerals mainly comprised Ca and P, with a P:Ca ratio of 6:1. CaP formation only occurs under supersaturated conditions and consists of nucleation and crystal growth stages (Rosmaninho & Melo, 2006). The presence of CaP crystals that further grow into microsphere-type structures was observed in AW (Drapala et al., 2018) and may promote the formation of protein-CaP networks (Tercinier et al., 2013).

Increasing the heat stability of whey systems has been widely investigated, e.g., by pH shifting (Stănciuc et al., 2012), mechanical pre-treatments (Koh et al., 2014), or by reducing Ca^{2+} concentration using calcium chelating salts (CCS) added prior to heating (Lewis, 2011). The heating stability of a 5% WPC solution at 85°C for 30 min was better at pH 7.5 than

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6.6, as indicated by lower solution turbidity and degree of denaturation (Stănciuc et al., 2012). The aggregate size and dynamic viscosity of the sonicated and homogenized 5% WPC solution after heating were much smaller than those of the solution without sonication and homogenization, which indicated an improved heating stability (Koh et al., 2014). Fouling in dairy systems can be reduced by adding CCS, but the effectiveness of CCS is salt-dependent.

CCS applied for dairy systems can be classified as citrate-, phosphate-, oxalate-, and ethylenediamine-based CCS (Murphy, 1992; Odagiri & Nickerson, 1965). The CCS that are usually applied are TSC and tripotassium citrate (TPC), which are classified as citrate-based CCS (De Kort et al., 2011; Keowmaneechai & McClements, 2006; Rosmaninho & Melo, 2006); sodium hexametaphosphate (SHMP), disodium hydrogen orthophosphate (DSHP), sodium dihydrogen orthophosphate (SDHP), and sodium tripolyphosphate (STPP), which are classified as phosphate-based CCS (Boumpa et al., 2008; Choi & Zhong, 2020; De Kort et al., 2011); disodium oxalate, ammonium oxalate, and potassium oxalate, which are classified as oxalate-based CCS (Murphy, 1992; Odagiri & Nickerson, 1965; Spiegel & Huss, 2002); and ethylenediaminetetraacetic acid disodium salt (Na_2-EDTA) (De Kort et al., 2011; Sikand et al., 2016). The addition of CCS in the forms of citrates, phosphates, and EDTA to increase heat stability of dairy systems has been extensively studied in cow's milk (Choi & Zhong, 2020; McCarthy et al., 2017; Renhe et al., 2018; Sikand et al., 2016; Tsioulpas et al., 2010; Udabage et al., 2000, 2001), goat's milk (Boumpa et al., 2008; Chen et al., 2012; Montilla & Calvo, 1997; Prakash et al., 2007), and casein systems (De Kort et al., 2011; Kaliappan & Lucey, 2011; McIntyre et al., 2016; Mizuno & Lucey, 2005; Wu et al., 2020). Conversely, there are relatively few studies on the application of CCS to increase

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the heat stability of whey systems, most of which have focused on highly purified forms of whey, e.g., WPI (Blanpain-Avet et al., 2020; Hebishy et al., 2019; Keowmaneechai & McClements, 2006; Scudeller et al., 2021).

Hebishy et al. (2019) showed that 5 mM of TSC and TPC was sufficient to increase heat stability and reduce fouling of WPI dispersions in a heat exchanger; meanwhile, higher concentrations of DSHP were required to impart the same degree of heat stability. While the existing studies of CCS with WPI as the model system provide scientific insights on whey fouling, investigation of AW systems would be of great interest to processors due to the lack of previous studies pertaining to this calcium-rich dairy stream. Therefore, this study aimed to investigate the physicochemical properties and heat stability of reconstituted acid whey powder (AWP) upon addition of two types of CCS (TSC and Na_2-EDTA). TSC was selected because it is widely applied in dairy systems, whereas Na_2-EDTA has been reported to be more effective in preventing Ca-induced aggregation than TSC (Keowmaneechai & McClements, 2002). TSC has a high Ca^{2+} binding affinity when it is applied to a WPI solution with a pH close to the basic pKa value of TSC, e.g., 6.4 (Hebishy et al., 2019). Na_2-EDTA is often studied in milk systems, where it works by partially removing Ca^{2+} from casein micelles at low EDTA concentrations and then disaggregating the micellar structures at high concentrations of EDTA (Griffin et al., 1988). Na_2-EDTA was shown to be a stronger chelating agent than TSC in the milk system (Udabage et al., 2000); however, a study of TSC and Na_2-EDTA applied in the whey protein system is scarce. Based on the literatures, the mechanisms by which TSC and Na_2-EDTA affect physicochemical properties and heat stability depend on the types of dairy system, i.e., milk, whey, or casein systems. Therefore, detailed understanding relating to the impact of specific CCS on physicochemical properties

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of AWP may allow manufacturers and end-users to have better control over heat-induced changes, both during manufacture and in high-temperature applications.

Materials and Methods

Materials

Mineral acid whey powder (AWP) was provided by Arrabawn Co-operative Society Ltd., Nenagh, Co. Tipperary, Ireland. TSC, Na_2-EDTA, HCl, KOH, anhydrous $CaCl_2$, ANS, Na_2HPO_4 dihydrate, NaH_2PO_4 monohydrate, and DTNB were purchased from Merck (Wicklow, Ireland).

Compositional analysis of whey powder

The protein content of AWP was measured using a Leco Nitrogen Analyser (Leco FP-628, Leco Corporation, USA) using a nitrogen-to-protein conversion factor of 6.38. Fat content was analyzed using a gravimetric method (ISO 1211 IDF 1:2010), and ash and moisture content was also analyzed gravimetrically using a TGA701 thermogravimetric analyzer (Leco Corporation, USA). Lactose content was calculated by difference. Each analysis was performed at least in duplicate.

Reconstitution of whey powder

AWP was reconstituted at 20%w/w total solids in milli-Q water which corresponds to the average total solids of industrial AW after nanofiltration. Milli-Q water was heated in a microwave until its temperature reached 60–65°C and then AWP was slowly dispersed in the water at approximately 60°C while rigorously stirred on a magnetic stirring plate. The suspension was continuously stirred at room temperature overnight. TSC and Na_2-EDTA were added to the suspension to obtain final salt concentrations of 5, 10, 20, 30, 40, and 50

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mM. An AWP suspension without salt was prepared as a control sample. The initial pH of AWP suspensions was in the range of 6.1–6.3. The addition of TSC increased the pH; in contrast, the addition of Na_2-EDTA decreased the pH. Therefore, the pH of all suspensions was fixed to 6.2 using HCl or KOH before further analysis. Each salt concentration in AWP suspension was prepared in at least two batches for all analyses, unless otherwise stated.

Calcium Ion Activity

Calcium ion activity in freshly prepared AWP suspensions at pH 6.2 was measured at 21°C using a Ca^{2+}-ion selective electrode (MM374, SensION+, Hach, MM374, Colorado, USA) according to Crowley et al. (2014) with slight modification. As recommended by the manufacturer, 3M KCl was added (1% v/v) to 10 mL of AWP suspensions followed by 1 min of stirring after which the electrode probe was immersed in the suspension. Calcium ion activity was inferred from the electrode potential (mV). Calcium ion activity is commonly reported as mM Ca^{2+} and is generally achieved by calibrating known quantities of Ca^{2+} against electrode potential. However, such calibration curves are heavily influenced by ionic strength; therefore, achieving an accurate calibration may prove difficult in the high mineral environment of AW, especially when further increasing the mineral content through the addition of variable quantities of CCS. Accordingly, electrode potential is reported here as a qualitative measurement of the effect of CCS addition on Ca^{2+} ion activity.

The relationship between Ca^{2+}-ion activity and electrode potential is governed by the Nernst equation:

$$E = E_0 + 2.303\frac{RT}{nF}log_{10}[Ca^{2+}] \quad (1)$$

where E is measured electrode potential (mV), E_0 is a constant relating to any given electrode (mV), R is the universal gas constant (8.3145 J mol^{-1} K^{-1}), T is the temperature (K), F is

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Faraday's constant (96485.33 C mol^{-1}), n is the ionic charge (+2 for Ca^{2+}), and $[Ca^{2+}]$ is Ca^{2+} activity in millimolars. Resolving the equation using known factors and constants reveals that E should theoretically change by 29.2 mV at 21°C for each log change in activity.

Heat treatment of AWP suspension

Heating was applied to AWP suspensions using an AR 2000ex rheometer (TA Instruments, New Castle, USA). About 28 g of whey suspension was placed in a starch pasting cell geometry which was chosen for its rapid heating and cooling capabilities. The starch pasting cell had an internal cell diameter of 36 mm and a rotor diameter of 32 mm, and the gap between two elements at the base of the geometry was 0.55 mm. Viscosity changes of the suspension with temperature were recorded according to Murphy et al. (2014) under a constant shear rate of 16.78 s^{-1} (Kett et al., 2013). The temperature was increased from room temperature (~25°C) to 40°C over 3 min, from 40 to 95°C over 3 min, held at 95 °C for 5 min, and finally decreased to 40 °C over 5 min. The apparent viscosity of the suspension initially decreased and then subsequently increased during heating. The temperature at which the apparent viscosity started to increase indicates the point at which denaturation/aggregation events became physically evident (Murphy et al., 2014). Each batch of AWP suspension was heated in duplicate, and the heated suspensions were collected from the starch pasting cell after the rheological measurement were completed. Some analyses were performed immediately after heating, i.e., pH of the suspension, size distribution of aggregates, and weight of sediment. The unheated suspensions were used as the reference samples for those analyses.

Surface hydrophobicity

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A method adapted from Chandrapala et al. (2011) was applied to determine the surface hydrophobicity of unheated and heated AWP suspensions. ANS was used as a fluorescence probe because the fluorescence intensity of ANS increases upon binding to hydrophobic sites in proteins. A stock solution of 8 mM ANS was prepared in 0.1 M PBS pH 7.0. This stock solution was always prepared fresh on the day of measurement and was protected from exposure to light. AWP suspensions were diluted to protein concentrations of 0.005, 0.001, 0.015, 0.020, and 0.025% w/w in 0.1 M PBS pH 7.0. Each dilution of the AWP suspensions (4 mL) was placed in an amber vial to which 60 μL of ANS was added. This volume of ANS was the optimum volume of ANS needed to be added to the diluted AWP suspension, which was determined through separate experiments. This volume of ANS (60 μL) was found to be the volume at which the slope of relative fluorescence intensity (RFI) started to plateau. The mixtures of diluted AWP suspensions and ANS were vortexed and kept in the dark for 15 min. Three milliliters of each mixture was then placed in cuvettes with 4-sided optical pathways, and hydrophobicity measurements were performed using a Cary Eclipse Fluorescence Photometer (Agilent Technologies, USA) set at 25°C. The excitation and emission slits and wavelengths were set at 5/5 and 390/470 nm, respectively. Diluted AWP suspensions without the addition of ANS were used as blanks. RFI was defined with the following equation:

$$\mathrm{RFI} = (F - F_0)/F_0 \qquad (2)$$

where F is the fluorescence intensity of the protein conjugated with ANS and F_0 is the fluorescence intensity of the protein without ANS. The plot of RFI values against diluted AWP concentrations was computed using linear regression analysis from which the slope

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was derived and used to represent surface hydrophobicity. The assay was performed in duplicate for each batch and the intensity value was recorded in triplicate for each assay.

Shear rate sweep of heated AWP suspension

Heated AWP suspensions were subjected to shear rate sweeps using an AR 2000 rheometer (TA Instruments, New Castle, USA) fitted with a parallel plate geometry. The following shear rate profile was applied: pre-shear at 300 s^{-1} for 1 min; equilibrate at 0 s^{-1} for 1 min; increase from 5 to 300 s^{-1} over 2 min; hold at 300 s^{-1} for 1 min; and decrease from 300 to 5 s^{-1} over 2 min. The shear rate sweep was performed at 25°C. Two batches of each sample were analysed, with each batch was measured once. The results were analyzed in terms of the apparent viscosity at 300 s^{-1} and the flow behaviour index associated with the increase in shear from 5 to 300 s^{-1}.

Analysis of sediment and serum phase

Approximately 30 mL of unheated and heated AWP suspensions was poured into 50 mL centrifuge tubes and accurately weighed. The suspensions were centrifuged at 15,000 ×g (Heraeus, Thermo Scientific, Osterode am Harz, Germany) for 25 min at 25°C. The supernatant was carefully removed from the sediment and the sediment was weighed. The amount of sediment as a function of CCS concentration was expressed as the weight ratio between the sediment and initial weight of the suspension. For the heated suspensions, ash and crude protein contents of the sediment and the supernatant were analyzed using a thermogravimetric analyzer (TGA701, Leco Corporation, USA) (Perring & Tschopp, 2019) and a nitrogen analyzer (Leco FP-628, Leco Corporation, USA) (Renhe et al., 2018), respectively.

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Measurement of aggregate size

The size distribution of aggregates present in unheated and heated AWP suspensions was measured using a Mastersizer 3000 equipped with a Hydro MV dispersion unit (Malvern Instruments Ltd., UK). The refractive index (RI) of AWP aggregates was 1.54 with an absorption index of 0.001 (Walkenström et al., 1999). The dispersant was water with RI of 1.33, and the analysis applied Mie scattering model. The size was recorded as volume mean diameter (D[4,3]) (Drapala et al., 2018). The volume mean diameter was deemed appropriate as all peaks observed were monomodal and similar to a normal distribution curve, save for a slight tail at lower size ranges. Size measurements were performed using three batches of AWP suspensions, each of which was heated in duplicate. Three readings were recorded for each measurement.

Statistical analysis

All results of the analysis were presented as the mean ± standard deviation. A one-way ANOVA with a least squares difference post-hoc test was performed to assess the concentration effects of TSC or Na_2-EDTA on the various parameters tested. Differences were deemed statistically significant when $p < 0.05$.

Results and Discussion

The composition of mineral AWP, prior to reconstitution and other treatments, is listed in Table 1.

Ca^{2+}-ion activity

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Figure 1 shows the change in electrode potential of a Ca^{2+}-selective probe as a function of CCS concentration at pH 6.2, which reflected available Ca^{2+} in AWP suspensions prior to heating. Ca^{2+} concentration as a function of electrode potential is governed by the Nernst equation (Ross, 1967), which states that every 29.2 mV change in potential corresponds to a log change in [Ca^{2+}], as calculated using Eq. (1). According to the equation, the [Ca^{2+}] of AWP suspension without CCS addition is about 1.7 mM, which resembles the ionic calcium concentration in fortified bovine milk (Nguyen et al., 2014).

Figure 1 shows that the electrode potential of AWP suspensions significantly decreased ($p < 0.001$) with TSC concentration ≥ 20 mM. Using Eq. (1), theoretical [Ca^{2+}] in AWP suspension reached as low as ~ 0.43 mM with 50 mM TSC. In contrast to TSC, Na_2-EDTA significantly reduced ($p < 0.001$) the electrode potential of the AWP suspension with only 5 mM; however, it seems that effective chelation of Ca^{2+} by Na_2-EDTA was only observed up to 20 mM. At Na_2-EDTA concentrations > 20 mM, the electrode potential becomes erratic; nevertheless, the statistical significance suggests that the chelating effect of EDTA at a very high concentration, i.e., 50 mM, was the same as the effect at 20 mM. At this concentration, theoretical [Ca^{2+}] in AWP suspension was 0.71 mM with Na_2-EDTA and 0.78 mM with TSC. Figure 1 and the theoretical values of [Ca^{2+}] suggest that Na_2-EDTA has a stronger binding capacity toward Ca^{2+} than TSC in AWP suspension, but this only applies up to 20 mM. A reduction of [Ca^{2+}] in the AWP suspension was 21.7% and 28.9%, as compared to suspensions with no salt addition, by adding 20 mM TSC or Na_2-EDTA, respectively. The measurement is, however, strongly influenced by ionic strength, meaning that the above correlation can only be assumed to be valid in a system where total ionic strength is less than 1 mM, a condition that AWP suspension does not meet. Therefore, Fig.

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1 can be said to qualitatively show that TSC in particular reduced Ca^{2+} activity in the AWP suspensions whereas Na_2-EDTA also reduced the activity but only to a certain extent.

A study on TSC and EDTA in milk systems showed that adding 5 and 10 mM of EDTA/kg of milk reduced Ca^{2+} activity by 18% and 30%, respectively, at a fixed pH of 6.65, whereas, adding 5 and 10 mM of citrate/kg of milk reduced Ca^{2+} activity by 16% and 31% (Udabage et al., 2000). Another study also showed that the addition of 20 mM of TSC or EDTA to skim milk at pH 5.9 reduced Ca^{2+} by 54.9% and 57.7%, respectively, whereas, the soluble calcium remained constant with TSC and increased with EDTA (Ramchandran et al., 2017). However, elsewhere, TSC was reported to release calcium from casein micelles in reconstituted skim milk powder, as indicated by an increase of Ca^{2+} concentration in the serum phase by 77% by adding 5 mM TSC (Choi & Zhong, 2020). The same study also reported increasing soluble Ca concentrations with TSC, which was explained by depleting casein-bound Ca. Based on those studies, the mechanisms of EDTA in chelating calcium in the milk system consist of two steps: i.e., partial removal of Ca^{2+} and phosphate from casein micelles without disrupting micellar structures, and then major depletion of Ca^{2+} that leads to disaggregation of the micelles (Griffin et al., 1988). The same mechanisms were also shown for TSC, but to a lesser extent (Udabage et al., 2000). However, AWP suspension does not contain casein micelles; thus, the same mechanisms may not be valid. One study on the application of CCS in WPI solution (3% w/w, pH 6.4) reported a decrease of Ca^{2+} concentration by 60% with 1 mM TSC, 87.5% with 5 mM TSC, and by > 90% with 45 mM TSC (Hebishy et al., 2019), which showed stronger effects of TSC than those reported in milk systems or the AWP suspension. Another study reported that either TSC or EDTA bind Ca^{2+} in a 1:1 ratio but the calcium-binding capacity of EDTA is about $10^{3.5}$ times the capacity

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of TSC (Keowmaneechai & McClements, 2002). The same study also indicated that levelling-off effect of EDTA on the mean diameter of WPI-stabilized emulsion may indicate that all free Ca^{2+} had been sequestered by EDTA and the Ca-EDTA complex was incapable of ion binding effects. Based on those limited studies, the types and states of whey protein may dictate calcium-chelating mechanisms of TSC or Na_2-EDTA.

pH of AWP suspension

The pH of AWP suspensions increased as a function of TSC concentration (Fig. 2). Therefore, monitoring and controlling pH changes in AWP suspensions upon addition of CCS is important because Ca^{2+} activity plays a role in determining pH and vice versa (De Kort et al., 2009). Previous studies also reported an increase in pH upon the addition of TSC in WPI dispersion (Hebishy et al., 2019), milk protein concentrate (MPC) dispersion (McCarthy et al., 2017), WPI-stabilized emulsions (Keowmaneechai & McClements, 2006), and goat's milk (Boumpa et al., 2008; Chen et al., 2012). The breakup of colloidal tricalcium phosphate by the formation of calcium complex salt and liberation of phosphate ions, which further formed ionic equilibria through uptake of the proteins, was suggested to be the general reaction that produce a higher pH (Odagiri & Nickerson, 1965). In contrast to the effect of TSC, the pH of AWP suspensions decreased significantly with Na_2-EDTA concentrations (Fig. 2). In this case, binding of protons by phosphate ions upon Ca^{2+} chelation by Na_2-EDTA in the suspensions might also be overshadowed by proton release according to the equation of $Ca^{2+} + H_2Y^{=} \rightleftharpoons CaY^{=} + 2H^{+}$, which resulted in pH reduction (Odagiri & Nickerson, 1965). As a result of the large variations in pH, all dispersions were readjusted to 6.2 prior to subsequent heating experiments.

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In general, heating leads to a pH reduction in dairy systems, but the extent depends on calcium chelating salt and its concentrations (Choi & Zhong, 2020). The pH decreased to around 6.0 in TSC-containing suspensions which did not vary significantly ($p > 0.05$) between 0- and 50-mM salt concentrations. At low levels of addition ≤ 10 mM, the pH of Na_2-EDTA-containing suspensions was similar to that of suspensions with added TSC. However, at 20 mM < Na_2-EDTA ≤ 50 mM, the pH of suspensions after heating was significantly lower than the TSC-containing suspensions. Ca^{2+} concentration is known to increase with decreasing pH (Tsioulpas et al., 2007); however, this is counteracted by a decrease in CaP solubility and a decrease in soluble Ca^{2+} at high temperature (On-Nom et al., 2010). The results here are in keeping with the majority of the literature i.e. pH of dairy systems decreases during heating (Ma & Barbano, 2003; On-Nom et al., 2010; Walstra et al., 2006), which relates to the formation of CaP (Fox et al., 2015), as shown below.

$$3Ca^{2+} + 2HPO_4^{2-} \rightleftharpoons Ca_3(PO4)_2 + 2H^+$$

Viscosity changes during heating

Viscosity changes upon heating of AWP suspensions may indicate physical evidence of denaturation and aggregation. As the temperature increased to 95°C using a starch pasting cell, the viscosity of suspensions decreased first until a critical temperature, after which it increased due to protein changes (Hebishy et al., 2019; Murphy et al., 2014). This critical temperature is depicted in Fig. 3 as a function of CCS concentrations. The viscosity changes during heating and the determination of the critical temperature are described in the supplementary information (SI. 1). As the rate of change of temperature applied during heating from 40 to 95 °C was constant across all samples, the temperature at which

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denaturation/aggregation events became evident was taken to be a result of the stability of the proteins in the system, which in this case, was deemed to be a function of salt addition.

This temperature did not change significantly with the addition of TSC up to 10 mM. A significant decrease in critical temperature took place at 20 mM TSC which may indicate that protein changes are physically evident at lower temperatures. However, a significant increase in the critical temperature took place with a further increase in TSC concentrations. This observation contrasted with the results of dynamic scanning calorimetry (DSC) (Table 3, Appendix 1) which indicated no substantial changes in the thermal transition of AWP suspension with TSC. Meanwhile, the critical temperatures of AWP suspensions were relatively constant with EDTA, which was in line with DSC results. It is apparent that the critical temperature at which protein changes start to be physically evident is not linearly correlated with thermal transitions of the protein that take place at the molecular level as shown by DSC. All critical temperatures of AWP suspensions without or with CCS were above 80 °C; whereas, the first peak of thermal transition of the suspensions shown by DSC was between 73 and 76 °C. These are in the range of denaturation temperature reported for β-lg fraction (74 °C) and WPI solution (75.4 °C) (Toro-Sierra et al., 2013). It is therefore likely that the differences in the temperature at which thermal changes become apparent are related to aggregation events taking place after the initial denaturation event.

The average viscosities of AWP suspensions (measured using a starch pasting cell) before and after heating are shown in Fig. 4. Variations of viscosity between suspensions of different TSC/Na_2-EDTA concentrations were mostly significant before heating, but these changes were very small and did not follow any noticeable trend. Heating, regardless of the

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414 type and concentration of the CCS, increased the viscosities of the AWP suspensions.
415 Noticeably different effects between TSC and EDTA were observed after heating. There
416 were significant differences in the post-heating viscosity of TSC-containing suspensions as
417 the CCS concentration was increased from 0 to 50 mM. However, the differences between
418 TSC concentrations are small and do not follow any discernible trend. Therefore, TSC did
419 not seem to have a real effect on post-heating viscosity as a function of concentration. In
420 contrast, the viscosity of suspensions after heating steadily decreased with increasing EDTA
421 despite the observation that reduction of the apparent activity of Ca^{2+} occurred only up to 20
422 mM EDTA concentration (Fig. 1). This trend of lower post-heating viscosity was observed
423 up to 50 mM where viscosity drastically increased after heating. The results of this analysis
424 suggest that there is a narrow range of TSC concentration which may lead to a reduced post-
425 heating viscosity of AWP suspension, i.e., 20–30 mM. On the other hand, EDTA addition
426 seemed to impact post-heating viscosity in a concentration-dependent manner, with a turning
427 point at 50 mM. It is possible that viscosity changes of the AWP suspensions did not linearly
428 correlate with the reduction of Ca^{2+} activity (Fig. 1) because whey viscosity is a parameter
429 manifested by various factors, such as composition, pH, and heat treatment (Schmidt, 1984).
430 Hebishy et al. (2019) showed that a reduced post-heating viscosity of WPI solution was
431 observed with 1 and 5 mM TSC or TPC, but a much higher viscosity than the control solution
432 was observed with 45 mM TSC or TPC.

433 **Aggregates formed during heating**

434 Unheated AWP suspensions contained large aggregates (D[4,3]~ 41 μm), which was larger
435 than the aggregate size reported in native WPC solution (D[4,3]~25 μm) (Koh et al., 2014).
436 This supports another observation that unheated AWP suspensions contained significant

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sedimenting materials, which is described in a later section of this manuscript. The aggregate size in unheated suspensions remained unchanged with the addition of TSC up to 10 mM and started to decrease with TSC additions of 20 mM and beyond (Fig. 5A). In contrast, the addition of EDTA did not significantly decrease average D[4,3] until added at concentrations ≥ 40 mM (Fig. 5B) with a particularly noticeable reduction occurring at 50 mM.

Heating suspensions without the addition of CCS resulted in a reduction in aggregate size (D[4,3] ~34 μm). The effect of heating on the aggregate size was in contrast with Koh et al. (2014) who reported increased aggregate size; however the reported D[4,3] for holding 5% WPC solution at 80°C for 1 min is similar to this research (D[4,3] ~35 μm). The reduction of aggregate size in AWP suspensions was perhaps due an increase in hydration or solubilization of components (Benoit et al., 2013). However, the addition of TSC followed by heating had a large effect on the size of aggregates, which steadily increased as a function of TSC concentration and reached a maximum of 40 mM. This trend was in contrast with the starch-pasting cell results depicted in Fig. 4a. A previous study with heated reconstituted skim milk powder showed that the addition of TSC up to 7 mM reduced the aggregate size, but the size increased at 10–30 mM, albeit in a random manner, i.e. not as a function of TSC concentration (Choi & Zhong, 2020). In contrast to the effect of TSC on heated AWP suspension, Fig. 5b illustrates that the addition of Na_2-EDTA resulted in a reduction of aggregate size at concentrations ≤ 40 mM. This follows a similar trend to that observed for heat-induced viscosity development (Fig. 4b). With 50 mM Na_2-EDTA, the smallest aggregate size in unheated suspension led to the largest aggregate size after heating. These results suggest that an optimum concentration of CCS is required to reach the optimum stability of AWP suspension against aggregation during heating. An equimolar ratio of

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460 EDTA-to-Ca^{2+}, indicated by the number of calcium-binding sites per EDTA molecule is
461 almost one (N = 0.94 ± 0.01), leading to monomodal particle distribution with the smallest
462 mean particle diameter compared to other ratios, which might indicate sequestering of all
463 free Ca^{2+} by EDTA (Keowmaneechai & McClements, 2002). The same article also
464 demonstrated that the optimum TSC-to-Ca^{2+} ratio was rather arbitrary because TSC's ability
465 to bind Ca^{2+} is weaker than that of EDTA. This ability relies on the valency of the chelator,
466 the type of ionic species forming the CCS, pH, and temperature (Lucey et al., 2011).

467 The AWP suspensions without and with CCS had negative charges. The charges
468 increased after heating; however, the results showed no apparent correlation with aggregate
469 size, viscosity, etc. Hence, the charges of the suspension, presented as ζ-potential, are
470 presented in Appendix 2 (Fig. 11).

471 **Surface hydrophobicity**

472 Figure 6 shows that the hydrophobicity of TSC- and Na_2-EDTA-containing suspensions
473 increased but the increment was not concentration-dependent. The main contributory factor
474 for changes in surface hydrophobicity of whey proteins is conformational change
475 (Dissanayake et al., 2013). Figure 6a and b suggests that the addition of TSC or Na_2-EDTA
476 may lead to conformational changes in unheated AWP suspension, but the changes are not
477 concentration-dependent. Whether calcium sequestration by TSC or Na_2-EDTA is the
478 leading factor of conformational changes merits further study. The surface hydrophobicity
479 of the suspension increased substantially post-heating, which is a general occurrence in dairy
480 systems (Joyce et al., 2017, 2018).

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Levels of hydrophobicity in heated suspension have similar trends as viscosity (Fig. 4) and aggregate size (Fig. 5), especially in the suspensions with added Na_2-EDTA. A previous study showed acid whey has the highest level of surface hydrophobicity compared to native, sweet and salty whey, which is concomitant with the largest aggregate size (Nishanthi et al., 2017a, b). Upon heating and with calcium chelated by CCS, hydrophobic attractions would be responsible for aggregate formation rather than electrostatic interactions (Chandrapala et al., 2015; Keowmaneechai & McClements, 2006). Therefore, similar trends between aggregate size and surface hydrophobicity were observed. However, the mechanism(s) by which CCS affects surface hydrophobicity needs further study.

Shear rate sweep of heated AWP suspension

The poor correlation between aggregate size (Fig 5a) and viscosity (Fig 4a) of heated TSC-containing suspensions was surprising. The viscosity of protein suspensions generally correlates with the size of the constituent protein aggregates, i.e., larger protein aggregates lead to a more viscous suspension (Inthavong et al., 2019). Therefore, shear rate sweeps were performed on heated suspensions to better understand their flow behavior.

The apparent viscosity of heated AWP suspensions as a function of TSC concentration (Fig. 7a) follows a similar trend to the aggregate size of heated TSC-containing suspensions (Fig. 5a). The correlations between the apparent viscosity and the aggregate size are depicted in the supplementary information (SI. 2). The trend shows that the apparent viscosity of TSC-containing suspension increased with the aggregate size. This was also the case for Na_2-EDTA suspensions (Fig. 7b compared to Fig. 5b; SI. 2). However, the flow behavior index of TSC-containing suspensions approached unity as TSC $\leq$ 10 mM, indicating Newtonian

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behavior. The index steadily decreased as a function of TSC concentration at ≥20 mM, which indicated increasing shear thinning behaviour. Na_2-EDTA containing suspensions were also Newtonian up to 10 mM; however at 10 mM < Na_2-EDTA ≤ 40 mM, the flow index steadily increased as a function of concentration, indicating shear thickening behavior. However, the index dropped to below one when the Na_2-EDTA concentration was 50 mM. These results may explain the discrepancy between the size of aggregates and the viscosity of TSC-containing suspensions obtained using starch pasting cell. That is, the relatively high shear of the starch pasting cell in combination with the shear-thinning nature of the aggregated systems may have masked the real differences in viscosity, which were discernible using a more precise configuration (i.e., parallel plate geometry). This points to some limitations in the application of starch pasting systems for detailed rheological analysis.

The flow behavior of protein aggregates is related to the size and shape of the molecules (Vardhanabhuti & Foegeding, 1999). Many studies have been performed to elucidate the effects of heat treatment and Ca^{2+} content on the size of whey protein aggregates, but there are a limited number of studies relating to aggregate structure and flow behavior. The addition of up to 2.2 mM Ca^{2+} to an 8% w/w WPI solution promoted larger protein aggregates and viscosity increases (Joyce et al., 2018). A recent study showed that the size of whey-stabilized emulsions and their flocculation degree after sterilization increased rapidly when Ca^{2+} concentrations were above 1.5 mM (Du et al., 2021). Interestingly, the flow index turned from shear thickening to shear thinning when the Ca^{2+} was above 1.5 mM. However, while TSC-containing dispersions exhibited increasing shear-thinning behavior at higher CCS concentrations, this was not the case for Na_2-EDTA-containing dispersions, with the exception of 50 mM. This indicates that other factors apart

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526 from soluble Ca^{2+} play a role in determining the flow index of heated AWP suspensions. The
527 complexity of such systems is highlighted by the completely different behavior of TSC- and
528 Na_2-EDTA-containing suspensions. Increasing TSC content tended to produce bigger
529 aggregates with shear-thinning properties, whereas Na_2-EDTA produced small aggregates
530 with shear-thickening properties. Further work is required to determine the mechanism(s)
531 behind these observations.

532 **Analysis of sediment**

533 The adddition of either TSC or Na_2-EDTA has a strong liner correlation with the reduction
534 of sediment in unheated AWP suspensions (Fig. 8a, b), which is in agreement with Sikand
535 et al. (2016), who observed decreasing sedimentation with increasing concentrations of
536 EDTA, TSC, and sodium polyphosphate. Reduced sedimentation indicates that the overall
537 solubilization of AWP was higher upon the addition of either TSC or Na_2-EDTA. The
538 reduced amount of sediment may also reflect lower quantities of insoluble minerals as a
539 function of increasing CCS addition. This supports our previous hypothesis that CaP
540 constitutes a significant part of the sedimenting materials in unheated AWP suspensions.
541 Heating increased the amount of sediment in the suspension compared to unheated samples,
542 but sediment in heated samples gradually decreased as TSC or Na_2-EDTA concentration was
543 increased (with the exception of 50 mM EDTA) (Fig. 8a, b).

544 The ash content in the sediment of the heated suspension decreased with increasing
545 CCS concentrations; in contrast, the ash content in the supernatant increased with
546 concentration (Table 2). This further confirms the hypothesis that CCS improves the heat
547 stability of the AWP suspensions by reducing the formation of CaP. Silva et al. (2013)

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548 demonstrated that Ca-containing sediment was reduced by demineralization of the casein
549 system; whereas, Boumpa et al. (Boumpa et al., 2008) showed a linear correlation between
550 the amount of sediment in heat treated goat milk and apparent Ca^{2+} concentration, as well as
551 pH. In this study, Ca^{2+} was indicated by the electrode potential of a Ca^{2+}-ion selective probe.
552 Electrode potential had a strong linear correlation with levels of sediment when TSC was
553 added to the AWP suspensions. This correlation was applied for either unheated (Fig. 8c) or
554 heated (Fig. 8d) AWP suspensions. However, no such correlation was obtained with Na_2-
555 EDTA. This is likely attributed to the fact that electrode potential was not linearly correlated
556 with Na_2-EDTA concentration, as shown in Fig. 1.

557 Figure 9 shows the relationship between the level of sedimentation and aggregate size.
558 In unheated samples, lower levels of sediment contained aggregates with smaller D[4,3].
559 This somewhat remained valid in the heated Na_2-EDTA-containing suspensions; however,
560 the reverse was observed with TSC. Larger aggregates were correlated with lower levels of
561 sedimentation, which may indicate the formation of loose aggregate structures. This may be
562 somewhat related to the flow index results, which indicated that different aggregate
563 structures were formed upon the addition of either TSC (i.e., shear thinning behavior; Fig.
564 7a) or Na_2-EDTA (i.e., shear thickening behavior, Fig. 7b).

565 **Conclusions**

566 Heated Na_2-EDTA-containing suspensions had lower levels of sediment, lower viscosity and
567 smaller aggregate size when compared to equivalent TSC-containing suspensions, apart
568 from when very high concentrations were employed (50 mM). It may be argued that at low
569 addition rates $\leq$ 10 mM, both salts have similar effects on the level of sedimentation;

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however, there was a marked difference in the levels of aggregation observed during heating. This was immediately evident in the post-heating aggregate size which increased for TSC-containing suspensions and decreased for Na_2-EDTA-containing suspensions. The nature of aggregates was also different, with TSC-containing suspensions exhibiting increasing shear thinning behavior as a function of concentration; in contrast, the smaller aggregates in corresponding Na_2-EDTA-containing suspensions showed shear thickening. Most interestingly, this resulted in an unexpected inverse relationship between aggregate size and sediment levels for TSC-containing suspensions, indicating the formation of loose aggregates that resist sedimentation.

While CCS was applied to reduce the concentration of ionic calcium, it should be noted that when Na_2-EDTA was applied at > 20 mM, calcium ion activity measurements did not adequately describe the behavior observed. Activity was observed to increase in the region of 20 to 40 mM; however, this was not correlated with the increased extent of heat-induced changes, e.g., viscosity remained constant and aggregate size was smaller. Likewise, while activity in TSC-containing suspensions decreased as a function of CCS concentration, the observed heat stability of suspensions was observed to decreased, i.e., the formation of large aggregates was observed. Therefore, application of such measurements in the high-ionic environment of acid whey may be problematic and merits further study.

Overall, it may be concluded that Na_2-EDTA is superior to TSC in reducing the extent of heat-induced changes in reconstituted acid whey. Indeed, the presence of larger particle sizes and higher viscosities in TSC-containing suspensions suggests that they are unsuited for this application.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix

Appendix 1. Differential Scanning Calorimetry (DSC)

The effect of CCS on the thermal denaturation of AWP suspensions was measured by a differential scanning calorimetry (DSC) 8000 (Perkin Elmer Inc., Massachusetts, USA) equipped with Pyris™ software (Perkin Elmer Inc., Massachusetts, USA). About 30 µL of AW sample was placed into a stainless-steel pan (large volume capsule) and accurately weighed. The sample was scanned during a heating ramp from 20 to 120°C at 10°C/min. An empty reference pan and a sample pan containing 30 µL distilled water were used to establish a baseline. Typical thermograms obtained from DSC are depicted in Fig. 10.

The thermograms were analyzed to obtain the onset denaturation temperature (T_0), the peak of denaturation temperature (T_p) and the enthalpy of denaturation (ΔH) according

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to Ju et al. (1999). The T_0 was estimated by extrapolating the maximum deflection of the thermogram onto the baseline. The ΔH was calculated by integrating the area of the peak, which was then divided by the protein content of the sample. The results are tabulated in Table 3.

Appendix 2. Measurement of ζ-potential

Unheated and heated AWP suspensions were centrifuged at 20,000 ×g (Eppendorf, Hamburg, Germany) for 20 min at 4°C to remove all aggregated proteins. The supernatant was diluted 10-times using milli-Q water and then the pH of the dilution was brought back to pH 6.2 using 1 N HCl. The diluted sample was placed in a folded capillary cell DTS1070 (Malvern Panalytical, Worcestershire, UK). The electrophoretic mobility in the sample was measured using Malvern Zetasizer Nano ZS (Malvern Panalytical, Worcestershire, UK) with a backscattering detection at a constant 173° scattering angle, from which the ζ-potential value was analyzed by Zetasizer software (Version 7.11, Malvern Panalytical, Worcestershire, UK). The measurement was performed at 25°C with an automatic selection of measurement scans, attenuation and voltage. Each batch was measured in duplicate with five readings per replicate. The instrument performed 10-15 scans on average until a reading was displayed. A delay of 30 s was allowed between each reading to avoid over-heating due to the applied voltage. The results are depicted in Fig. 11.

Supplementary Information

Supplementary Information 1. Determination of critical temperature (Fig. 3)

The heating profile of AWP suspensions and the typical viscosity changes developed during heating are shown in SI. 1. The critical temperature reported in Fig. 3 was determined by

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analyzing data similar to that depicted in SI. 1. The temperature at which the measured viscosity increases for the first-time during heating was determined to be the temperature at which the effect of denaturation/aggregation became physically apparent within the system (shown as the circled area). The viscosities observed pre- and post-heating were also represented in Fig. 4. Therefore, in the context of this study the critical temperature of viscosity increase was determined to be representative of the heat stability of the system, whereas the difference between pre- and post-heating viscosity was deemed to be indicative of extent of heat-induced protein changes (under the shear conditions applied). This analysis was performed on the data of AWP suspensions at each concentration TSC/Na_2-EDTA and the control suspension.

Supplementary Information 2. Correlation of viscosity and aggregate size

See Figure S1. 2

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FIGURE CAPTIONS

Fig. 1 Electrode potential as a function of CCS concentrations. Statistically significant effects (p<0.05) of CCS concentration are indicated by uppercase letters (A–E) for TSC, whereas lowercase lettering (a–d) is used for Na2-EDTA. The order of the respective lettering indicates the difference in magnitude of the effect as compared to the highest value, e.g., A was assigned to the highest value, with C indicating a reduction of greater significance than B

Fig. 2 pH changes of the AWP suspension with increasing CCS concentrations, before heating without pH adjustment, and after heating

Fig. 3 Temperature at which denaturation events became physically evident during heating ranged from 40 °C to 95 °C. Statistically significant effects (p<0.05) of CCS concentration are indicated by uppercase letters (A–E) for TSC, whereas lowercase lettering (a and b) is used for Na_2-EDTA. The order of the respective lettering is indicative of the difference in magnitude of the effect as compared to the highest value, e.g., A was assigned to the highest value, with C indicating a reduction of greater significance than B. Note: Information on how the above temperature was determined can be found in the Supplementary Information section (SI. 1)

Fig. 4 The average viscosity of AWP suspension before and after heating, measured at 40 °C, as a function of TSC (a) and EDTA (b) concentrations. Statistically significant effects (p <0.05) of CCS concentration are indicated by uppercase letters for pre-heating values,

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Fig.5 The average aggregate diameter as a function of TSC (a) and Na_2-EDTA (b) concentrations in unheated and heated AWP suspensions. Statistically significant effects (p <0.05) of CCS concentration are indicated by uppercase letters for pre-heating values, whereas lowercase lettering is used for after-heating values. The order of the respective lettering is indicative of the difference in magnitude of the effect as compared to the highest value, e.g., A was assigned to the highest value, with C indicating a reduction of greater significance than B

Fig. 6 Surface hydrophobicity of unheated and heated AWP suspensions, presented as the slope of relative fluorescence intensity (RFI), as affected by TSC (a) and Na_2-EDTA (b) concentrations. Statistically significant effects (p <0.05) of CCS concentration are indicated by uppercase letters for pre-heating values, whereas lowercase lettering is used for after-heating values. The order of the respective lettering is indicative of the difference in magnitude of the effect as compared to the highest value, e.g., A was assigned to the highest value, with C indicating a reduction of greater significance than B

Fig. 7 Flow behavior index and apparent viscosity of heated AWP suspensions as a function of TSC (a) and Na2-EDTA (b) concentrations after cooling. Flow index was measured by shear rate sweep at 25 °C. The horizontal line indicates Newtonian flow. The apparent viscosity was recorded at 300 s^{-1}. Statistically significant effects (p <0.05) of CCS concentration are indicated by uppercase letters for viscosity values, whereas lowercase lettering is used for flow index values. The order of the respective lettering is indicative of the difference in magnitude of the effect as compared to the highest value, e.g., A was

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942 suspensions and CCS concentrations, as well as electrode potential

943 **Fig. 9** Relationship between aggregate size and level of sedimentation in unheated (a) and
944 heated (b) AWP suspensions

945 **Fig. 10** Thermograms of AWP suspensions without and with TSC addition obtained from
946 DSC

947 **Fig. 11** Average ζ-potential values of the supernatant from the unheated and heated AWP
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961 **TABLE CAPTIONS**

962 **Table 1** Composition of mineral acid whey powder

963 **Table 2** The ash content on dry matter basis (% d.m) in the sediment and supernatant of the
964 heated AWP suspensions

965 **Table 3** Thermal properties of AWP suspensions with added CCS obtained during heating
966 ramp in DSC. T_0 is the onset denaturation temperature, T_p is the peak of denaturation
967 temperature and ΔH is the enthalpy of denaturation. The following subscripts, i.e., $_1$ and $_2$,
968 refer to the first and second peak, respectively

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SUPPLEMENTARY INFORMATION CAPTIONS

SI. 1 Heating profile of AWP suspensions and the typical viscosity changes recorded during heating

SI. 2 Correlations between apparent viscosity at 300 s^{-1} (as depicted in Fig. 7) as function of aggregate size (as depicted in Fig. 5). Labels written in black represent concentrations of TSC and labels in grey represent concentrations of Na_2-EDTA

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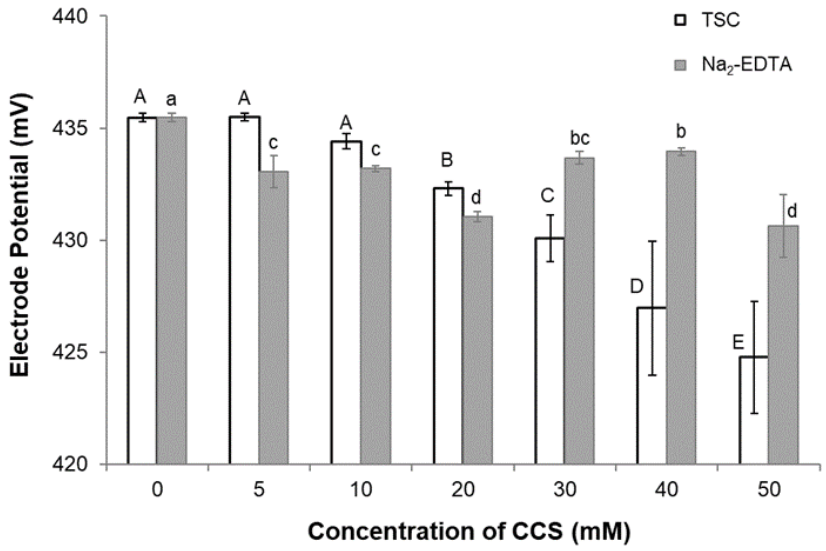

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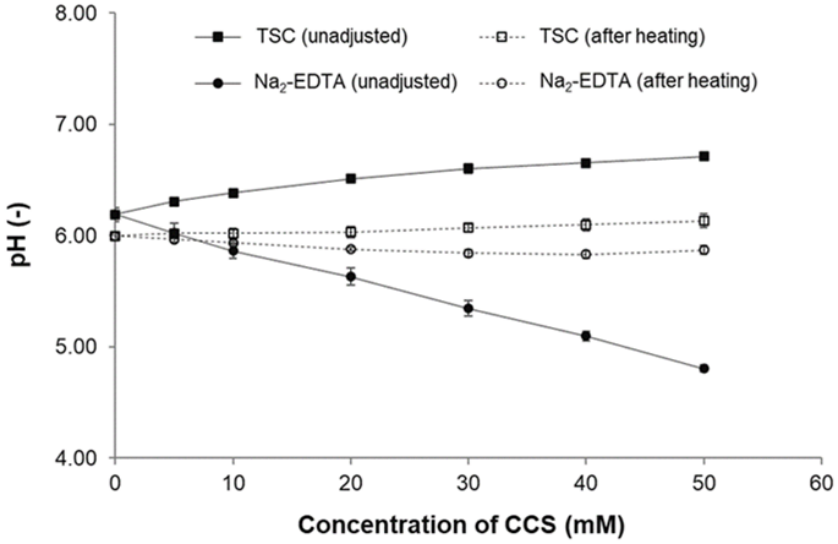

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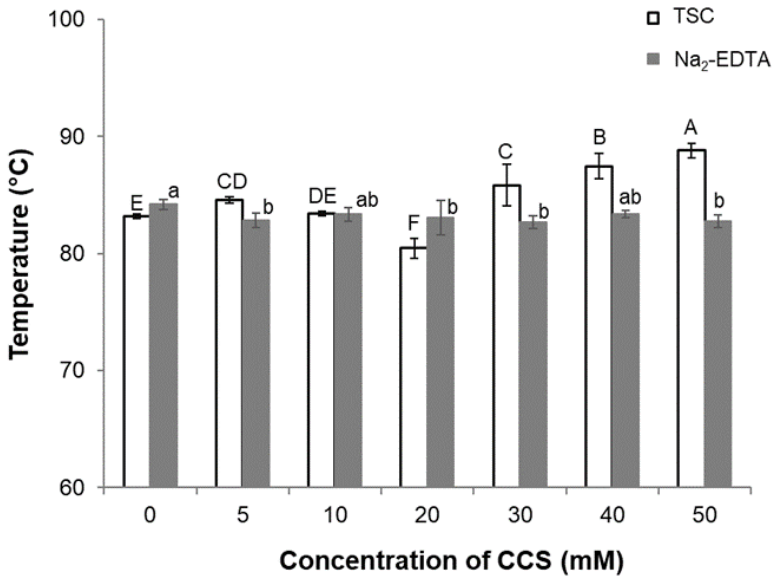

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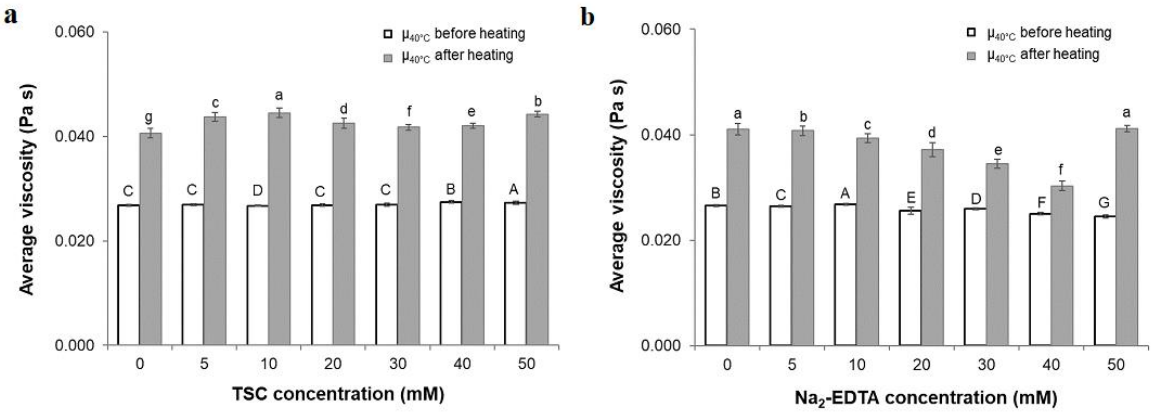

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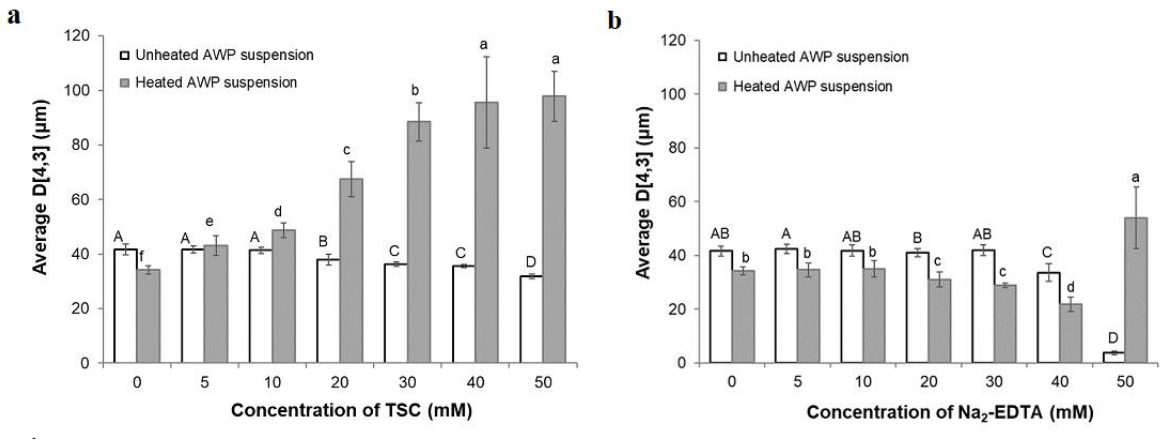

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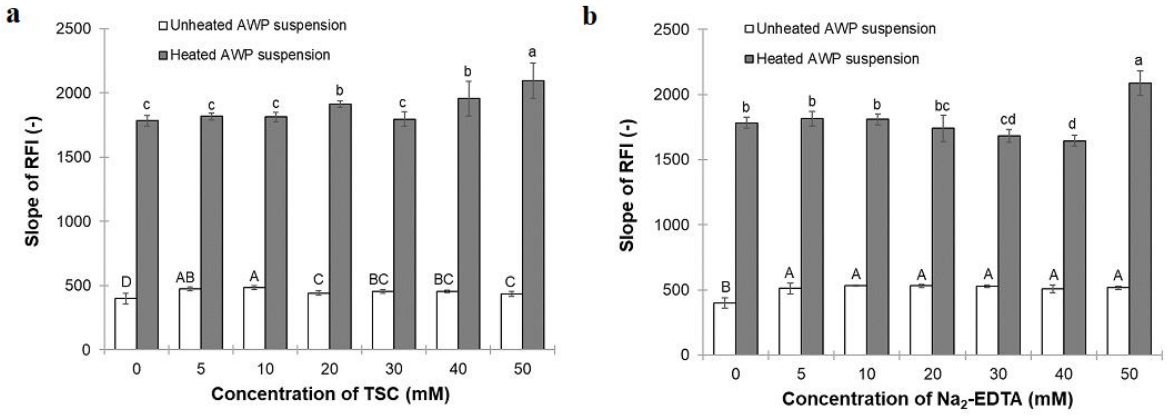

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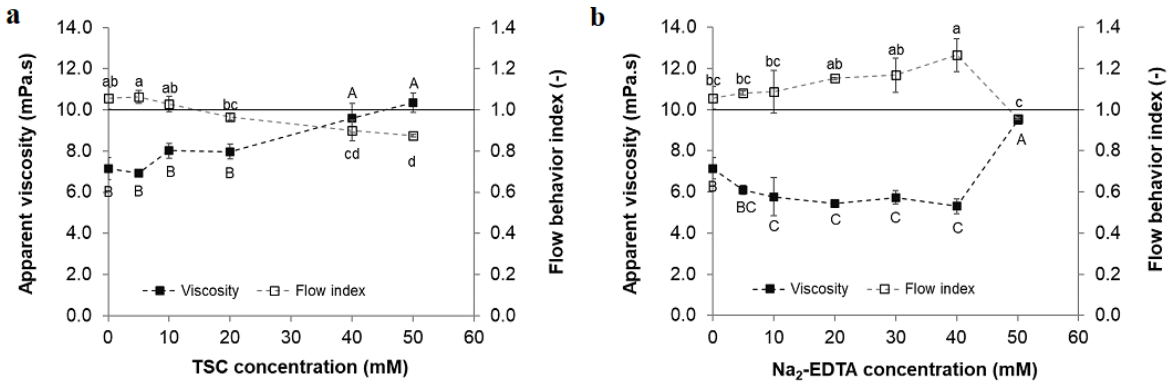

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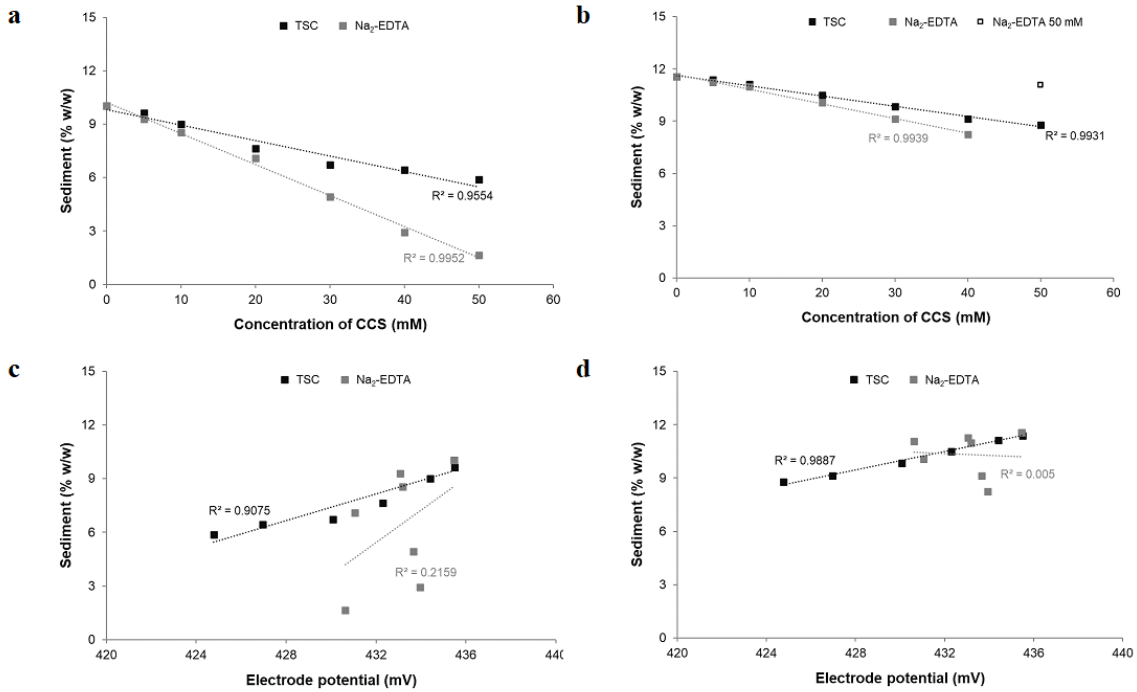

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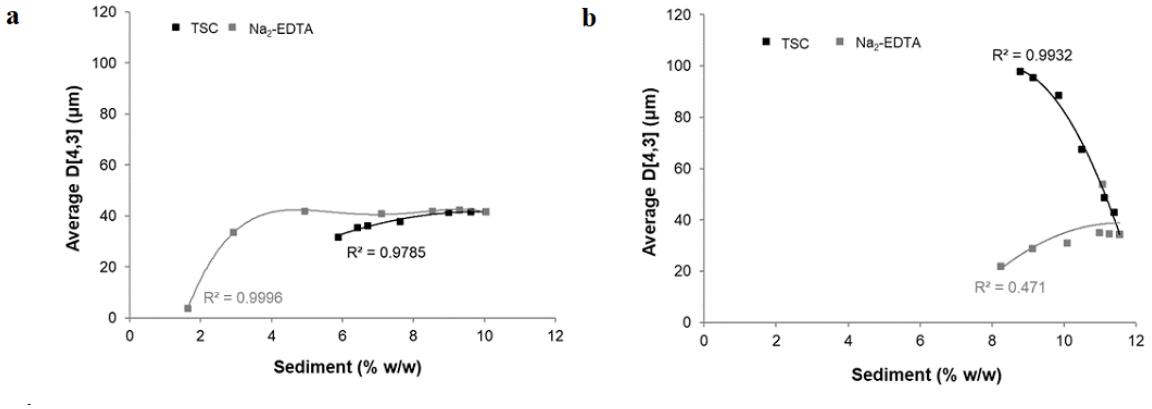

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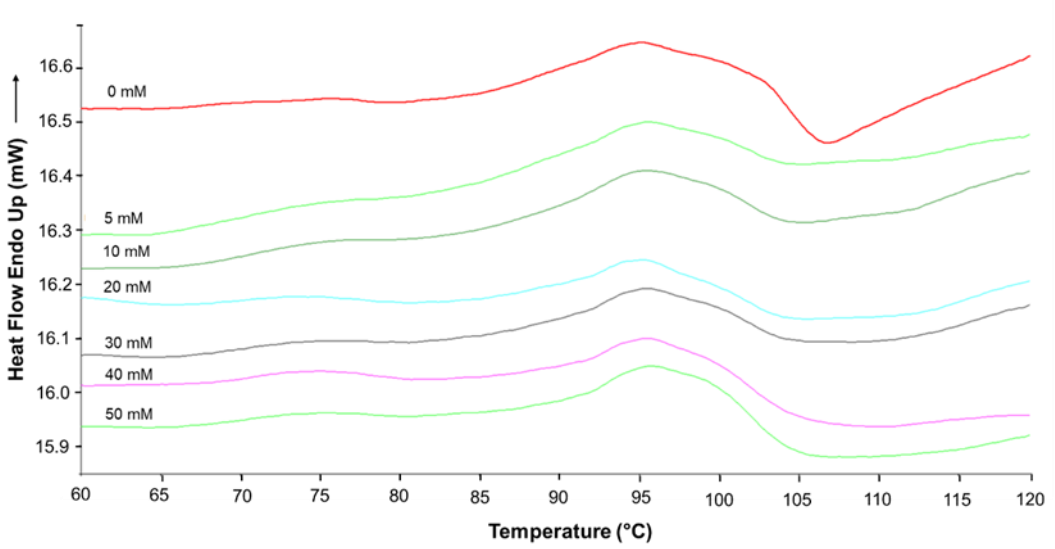

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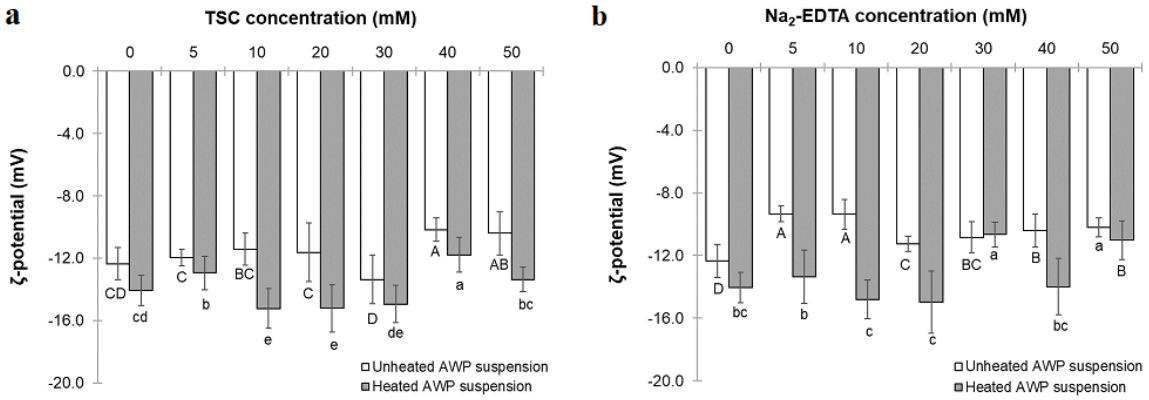

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Table 1 Composition of mineral acid whey powder

Composition	% w/w
Total solids	98.74 (±0.14)
Protein	9.20 (±0.02)
Fat	0.55 (±0.01)
Ash	8.39 (±0.02)
Lactose	80.59

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Table 2 The ash content on dry matter basis (% d.m) in the sediment and supernatant of the heated AWP suspensions

Concentration (mM)	Ash (% d.m) in sediment		Ash (% d.m) in supernatant	
	TSC	Na_2-EDTA	TSC	Na_2-EDTA
0	17.67^{A}	17.52^{A}	6.39^{G}	6.46^{G}
5	16.84^{AB}	17.06^{AB}	6.90^{F}	6.74^{F}
10	16.38^{B}	16.39^{B}	7.43^{E}	7.03^{E}
20	15.40^{C}	15.17^{C}	8.50^{D}	8.14^{D}
30	14.31^{D}	13.55^{D}	9.43^{C}	9.10^{C}
40	13.58^{DE}	11.76^{E}	10.22^{B}	10.06^{B}
50	13.35^{E}	9.80^{F}	10.83^{A}	11.23^{A}

For each column, the letters indicate statistical significance between concentrations.

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Table 3 Thermal properties of AWP suspensions with added CCS obtained during heating ramp in DSC

Concentration (mM)	DSC											
	TSC						EDTA					
	$T_{0,1}$ (°C)	$Tp_{,1}$ (°C)	ΔH_1 (J/g)	$T_{0,2}$ (°C)	$Tp_{,2}$ (°C)	ΔH_2 (J/g)	$T_{0,1}$ (°C)	$Tp_{,1}$ (°C)	ΔH_1 (J/g)	$T_{0,2}$ (°C)	$Tp_{,2}$ (°C)	ΔH_2 (J/g)
0	67.4	74.6	1.4	86.8	95.2	11.7	67.4	74.6	1.4	86.8	95.2	11.7
5	68.1	74.4	1.2	89.7	95.4	10.8	68.2	75.2	1.7	86.1	93.36	8.3
10	68.4	74.4	1.1	87.8	95.5	11.0	68.8	74.4	1.1	86.2	94.5	11.2
20	68.3	73.2	1.2	88.4	95.5	11.1	69.6	75.1	0.8	87.3	95.2	12.2
30	68.3	73.0	1.1	88.6	95.5	12.5	69.1	75.7	1.3	88.3	95.4	11.4
40	68.3	73.5	1.6	89.0	95.6	12.2	69.4	74.6	1.2	88.8	95.3	10.8
50	68.4	73.7	1.5	89.3	95.6	11.4	70.2	74.6	0.9	89.2	95.4	8.74

The following subscripts, i.e., $_1$ and $_2$, refer to the first and second peak, respectively.

T_0 is the onset denaturation temperature, T_p is the peak of denaturation temperature and ΔH is the enthalpy of denaturation.

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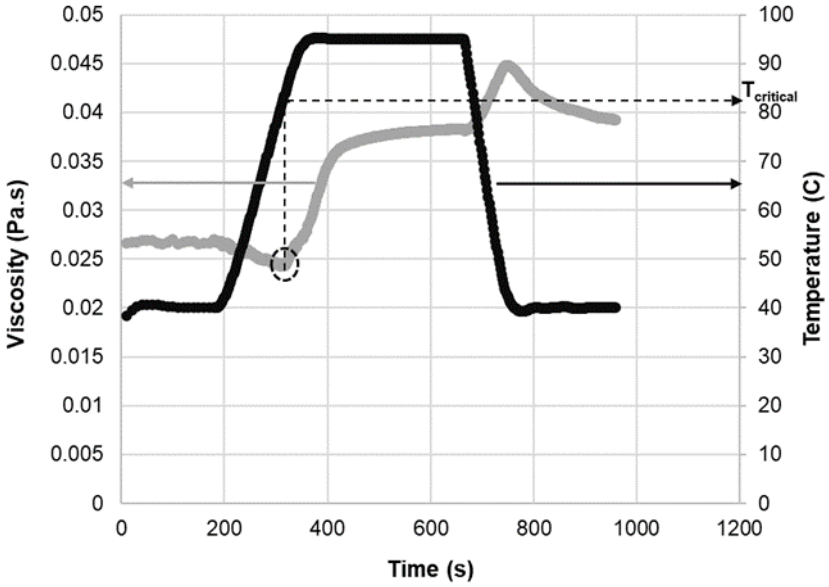

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SI. 1 Heating profile of AWP suspensions and the typical viscosity changes recorded during heating. By Purwanti, Mulcahy, and Murphy. 10.1007/s11947-023-03198-8 SI. 1

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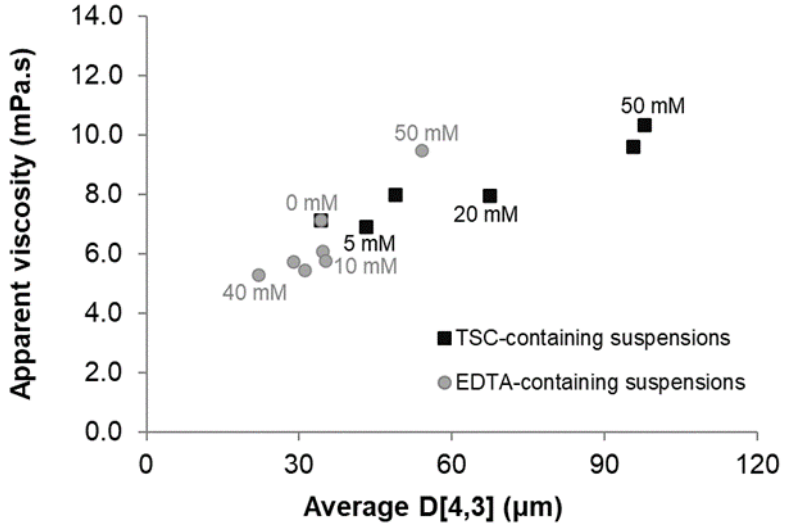

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SI. 2 Correlations between apparent viscosity at 300 s^{-1} (as depicted in Fig. 7) as function of aggregate size (as depicted in Fig. 5). Labels written in black represent concentrations of TSC and labels in grey represent concentrations of Na_2-EDTA. By Purwanti, Mulcahy, and Murphy. 10.1007/s11947-023-03198-8 SI. 2

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