

# THE EFFECT OF CELLULOSE AND STARCH ON THE VISCOELASTIC AND THERMAL PROPERTIES OF ACID- SWOLLEN COLLAGEN PASTE

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## ABSTRACT

Collagen pastes are processed materials obtained through the swelling of minced bovine hides using acids into a fibrous swollen structure. Depending on the application, there is a need to improve the performance of these pastes in terms of rheological properties and mechanical strength of the final product. In this work, the addition of cellulose fibres and starch granules as fillers in acid swollen collagen paste was investigated. The influence of cellulose fibre length and starch granules with different amylose and amylopectin content on the viscoelastic and thermal properties of acid swollen paste were studied as a function of mixing ratio and collagen paste concentration. Addition of cellulose and starch granules resulted in an increase in the elastic modulus of the collagen paste with the starch granules having the highest impact. Addition of cellulose and starch also affected the  $\tan \delta$  peak of collagen paste at different collagen concentrations as a function of temperature. The micro differential scanning calorimetry (microDSC) results indicated that the denaturation temperature value of collagen was not influenced by the presence of cellulose and starch. However, upon reheating the denaturation temperature of collagen pastes with starch granules shifted to lower temperatures.

Keywords: Corn starch, Denaturation, Rheology, Micro differential scanning calorimetry (DSC).

## 1 INTRODUCTION

Collagen is the major structural protein of connective tissues such as tendons, bones and skins. It consists approximately 30% of the total protein in human and animal bodies (Hashim et al., 2015). At least 27 forms of collagen have been identified in mammalian tissue of which collagen type 1 has been identified as the major structural component of the connective tissue (Pati et al., 2010; Zhang et al., 2010; Wang et al., 2017). Collagen's primary structure consists of repeating units of glycine (Gly)-proline (X)-hydroxyproline(Y). The collagen molecule is made of three polypeptide chains which are coiled in a left-handed helix, these chains are further twisted into a right-handed superhelix that are stabilised by hydrogen bonds (Komsa-Penkova et al., 1996; Schroepfer and Meyer, 2017). Due to its unique properties, such as weak antigenicity, biodegradability, biocompatibility, bioactivity and tensile strength, collagen has been extracted into different forms such as gels, pastes, films, sponges and fibres. It is widely used in the food, pharmaceutical, chemical and cosmetics industries and for scaffolds in tissue engineering (Friess, 1998; Ding et al., 2014). However, there are a number of challenges such as low thermal stability and poor water vapour barrier properties, which limits wider applications (Bigi et al., 2004; Mu et al., 2007). A possible means to circumvent the suboptimal properties of collagen and increase its application, is to incorporate other natural polymers such as polysaccharide fibres or fillers (Wang et al., 2018). These will act as a reinforcement by enhancing the strength, barrier properties and thermal stability of the resulting composite structures (Wolf et al., 2009) . In the food industry, collagen type 1 has been extracted into a paste that is used for sausage casings. Collagen casings have been applied successfully as an alternative to the relatively high cost of casings made from animal intestines (Barbut, 2010). There is an interest in modifying the mechanical properties of collagen casings through the addition of natural biopolymers such as cellulose fibres and starch granules. These natural biopolymers are of a particular interest as particulate fillers due to their natural abundance, biodegradability, and low cost (Ding et al., 2014; Basiak et al., 2017). They have also been shown to provide great reinforcement to polymer matrices due to their high mechanical

79 strength (Dufresne and Vignon, 1998;Bledzki and Gassan, 1999). Cellulose is the most  
80 abundant natural polymer on earth and is a major component of cell walls of higher plants and  
81 some bacteria, such as *Acetobacter Xylinum* strains. Cellulose possess a complex and highly  
82 ordered chemical structure akin to that of collagen giving high mechanical strength, albeit of  
83 polysaccharide rather than protein origin. It is a linear homopolymer composed of  $\beta$ -D-  
84 glucopyranose units linked with  $\beta$ -1, 4 glycosidic linkages. It is used in the food industry as a  
85 texturiser, non-caloric bulking agent, thickener, stabiliser and raising agent (Yoon and Lee,  
86 1990;Ang and Miller, 1991;Harris and Smith, 2006).

87 Starch is a well-known storage carbohydrate and is found in the form of insoluble granules  
88 within plant cells (Sullo and Foster, 2010). Semi-crystalline starch granules are made up of  
89 two polysaccharides. The first is amylose which contains the amorphous region of the granule  
90 and has long linear (1-4) linked  $\alpha$ -D-glucopyranose residues. The second is amylopectin,  
91 which is composed mainly in the crystalline region of the granule and is a highly branched  
92 molecule consisting of shorter chains of (1-4)  $\alpha$ -D-glucopyranose residues with (1-6) -  $\alpha$ -D-  
93 glycosidic branched linkages (Bul  on et al., 1998;Jane et al., 1999). Starch is obtained from  
94 various sources such as cereal, tuber and root crops and used in wide range of applications,  
95 such as food, pharmaceutical, paper and plastic. Starch has unique physicochemical and  
96 functional properties hence it is used as a thickener, colloidal stabiliser, gelling agent, adhesive  
97 and water retention agent in various industries (Singh et al., 2003;Copeland et al., 2009).

98 In recent years, there has been an increased interest in the blending of collagen with other  
99 natural biopolymers in order to develop products with new and modified functional properties  
100 as well as providing cost and processing advantages. Several studies demonstrated that the  
101 addition of polysaccharides into collagen solutions can modify the thermal and rheological  
102 properties of collagen solutions. (Ding et al., 2014) reports enhancement upon the  
103 Hydroxypropyl Methylcellulose (HPMC) improved the on the rheological and thermal stability  
104 of collagen solutions and was attributed to a hydrogen bond interactions as well as a  
105 compatibility between the collagen and HPMC molecules. Nicoleti and Telis (2009) reported  
106 that concentrations of about 0.1% xanthan gum increased the gel strength of collagen solution,

when heated. Increasing the concentration of xanthan to about 0.3% resulted in a weaker gel while the addition of Maltodextrin led to a more fluid-like structure and this was suggested to be due to the thermodynamic incompatibilities between the biopolymers. Similarly, Oechsle et al. (2015) studied the influence of incorporating co-gelling biopolymers of different molecular weights; low molecular weight (whey protein isolate and blood plasma protein), or high molecular weight (soy protein isolate and gluten) into a collagen matrix and they reported that they displayed effects when added to the collagen gels, affecting the collagen network and embedding within it.

Therefore, the aim of this study was to understand how the addition of cellulose with different fibre lengths and starch granule fillers affects the viscoelastic and thermal properties of acid swollen collagen pastes. The viscoelastic properties were investigated using small oscillation rheological measurements and the thermal changes were studied using a microDSC. Both rheological and thermal measurements were studied as a function of collagen paste concentration. The effect of biopolymer morphology and aspect ratios was also considered. The hypothesis underpinning this research is that the viscoelastic and thermal properties can be modified by the addition of non-charged polysaccharides. Also, it was hypothesised that similar effect will be observed when the phase volumes of the polysaccharide are matched.

## **2 Materials and Methods**

### **2.1 Materials**

Two types of cellulose with different aspect ratios were used in this study i.e. Solka-Floc 300 (SF3) and Solka-Floc 900 (SF9) with fibre lengths of 22 and 100  $\mu\text{m}$  respectively. Cellulose powders were supplied by the International Fibre Corporation (New York, USA). Two types of starches with different amylose and amylopectin contents were used, waxy maize starch (WS: 1-2% amylose) and high amylose starch (HAS: 70% amylose) were both supplied by Ingredion (Manchester, UK). A pH 2.0 acid (HCl) swollen paste was made from fibrous collagen donated by Devro plc (Scotland, UK).

## 2.2 Determination of Phase Volume ( $\Phi$ )

The phase volume of cellulose and starch dispersions were measured by centrifugation (Thermo Electron Corporation, Ohio, USA) using a centrifugation force of 2000g for 20 mins at 20 °C. Cellulose and starch dispersions at a total solid concentration ranging from 1% to 7% were transferred into 50 ml conical bottom centrifuge tubes. After centrifugation, the total height  $H_T$  of the sample and the height of the sediment  $H_S$  were measured and the phase volume of the cellulose and starch dispersions were calculated by the total height divided by the height of the sediment. The concentrations of cellulose and starch dispersions needed for a 15% phase volume were extrapolated from a graph of phase volume against concentration (not shown).

## 2.3 Preparation of Collagen-Cellulose and Collagen-Starch blends.

The concentrations of cellulose and starch (refer to **Error! Reference source not found.**) corresponding to a phase volume of 15% were prepared by dispersing the powders in deionised water. The dispersions were mixed with collagen paste at different collagen/cellulose (solka floc 300 and solka floc 900) and collagen/starch (waxy starch and high amylose starch) mixing ratios i.e., 80:20, 70:30 and 50:50. The final concentrations of the collagen pastes at the various mixing ratios were 4% (w/w), 3.5% (w/w) and 2.5% (w/w). The collagen paste with cellulose dispersions and starch dispersions were mixed by gently stirring at room temperature for 15 mins using an overhead stirrer (yellow line IKA OST 20 high torque Overhead Stirrer). The pH of the final blends was between 2 and 2.5. Samples were degassed using an Audionvac VMS 53 multivac vacuum packager (Audion Elektron, Netherlands) in order to remove the air bubbles incorporated during mixing, before further analyses were performed. The blends formulation and sample code is shown in Table 1.

Table 1. Formulation of collagen-cellulose and collagen-starch blends at various mixing ratios and different concentrations of collagen. SF3 = Solka floc 300, SF9 = Solka floc 900, WS = Waxy starch, HAS = High amylose starch,  $\Phi$  = phase volume

Collagen Paste: Suspension	Conc. of Collagen in the mixture (%wt)	Conc. (%wt) of cellulose and starch for $\Phi = 15\%$ in the mixture (%wt)
80:20 CollSF3	4	2.7
80:20 CollSF9		1.8
80:20 CollWS		7
80:20 CollHAS		6.6
70:30 CollSF3	3.5	2.7
70:30 CollSF9		1.8
70:30 CollWS		7
70:30 CollHAS		6.6
50:50 CollSF3	2.5	2.7
50:50 CollSF9		1.8
50:50 CollWS		7
50:50 CollHAS		6.6

## 2.4 Measurement of paste viscoelasticity

Small amplitude oscillatory rheology of the collagen pastes with and without cellulose/starch was performed using a controlled stress rheometer (Physica MCR 301, Anton Paar, Austria),

using a parallel plate (diameter 50 mm, gap 1.5 mm) geometry. To prevent the pastes from drying during the experiment, the edges of the measuring geometry was covered using a low viscosity mineral oil, which is known to have no effect on the rheological measurements. The samples were equilibrated for 5 mins before measurements were conducted, this was performed in order to prevent temperature variations within the samples. The dynamic viscoelastic measurements were performed by subjecting the samples to various profiles: (i) amplitude sweeps were conducted at a constant angular frequency (10 rad/s) to determine the maximum deformation attained by the sample in the linear viscoelastic range (ii) frequency sweeps were performed from 0.1-100 rad/s at 20 °C and a constant strain of 0.1% (selected from LVE region) (iii) dynamic temperature sweeps were conducted at a constant strain of 0.1% and frequency of 10 rad/s. Collagen pastes with/without cellulose and starch were heated from 5 to 80 °C at a rate of 5 °C/min. The storage modulus ( $G'$ ), loss modulus ( $G''$ ), and loss factor ( $\tan \delta = G''/G'$ ) were recorded as a function of frequency or temperature. The data reported represent the mean values from three replicates.

## **2.5 Differential Scanning Calorimeter (microDSC)**

The denaturation temperature ( $T_d$ ) of the pastes was determined using a micro DSC (MicroDSC III, SETARAM Instrumentation, Caluire, France). Approximately 0.8 g of the samples were weighed into Hastelloy cells and sealed with O rings and Hastelloy screw tops. Samples were loaded in the DSC instrument at 20°C and then cooled to 3°C at 1°C/min. Collagen pastes with/without cellulose and starch were heated up to 95 °C. Samples were then held at 95°C for 15 mins and cooled down to 3°C and reheated to 95 °C at 1°C/min. A Hastelloy cell filled with RO water was used as a reference. The onset ( $T_o$ ), offset, peak temperatures ( $T_p$ ) and enthalpy of transition ( $\Delta H$ ) were processed using Calisto Processing software v1.43 (AKTS, Switzerland). The peak temperature of the thermogram was taken as the melting or denaturation temperature of collagen. Runs were performed in duplicate and the average and standard deviation reported.



## 195    **2.6   Statistical analysis**

196    A one-way ANOVA was used to determine the difference between samples using Statistical  
197    Package for Social Science Software (SPSS Inc.). When there were any differences between  
198    samples, Tukey's test was used to determine the significance of the average ( $P < 0.05$ ).

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## 3 Results and Discussion

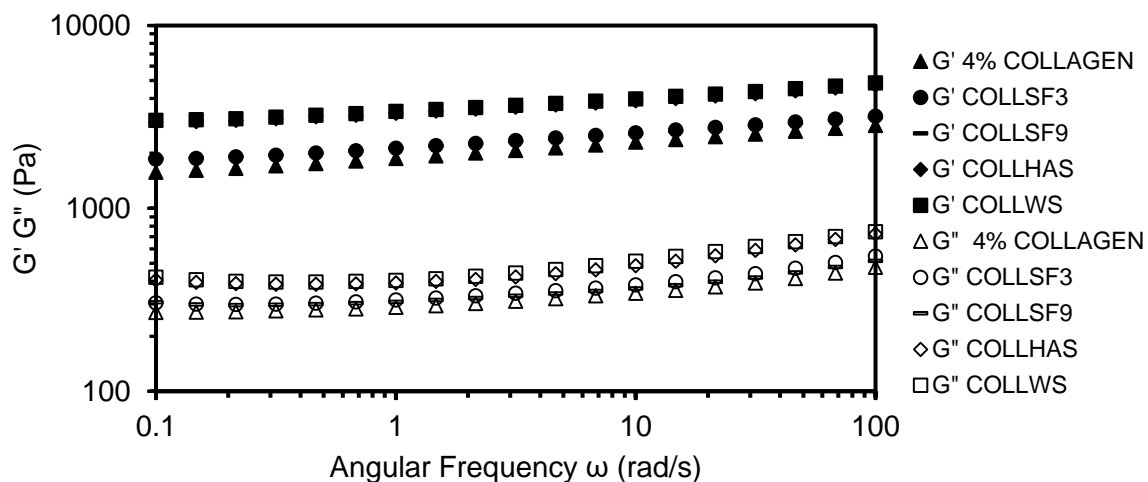
### 3.1 Dynamic Frequency Sweep

Figure 1 and Figure 2 shows the frequency dependency of the storage modulus ( $G'$ ), the loss modulus ( $G''$ ) and the loss tangent ( $\tan \delta$ ) for COLLSF9, COLLSF3, COLLWS and COLLHAS pastes at the different mixing ratios and collagen concentrations. The mechanical spectra of collagen pastes with the addition of starch granules and cellulose fibres at all mixing ratios showed that all the pastes had a solid like behaviour as the storage modulus was higher than the loss modulus throughout the frequency range investigated. This is the typical behaviour of biopolymer gel where  $G'$  (storage modulus) is usually greater than the  $G''$  (loss modulus) at the range of angular frequencies investigated (Ross-Murphy, 1995). Similar behaviour has also been reported by (Oechsle et al., 2015) who studied the influence of gluten, soy isolate and blood plasma proteins on the viscoelastic properties of collagen gels. The magnitude of the dynamic mechanical spectra of the collagen paste was modified after the addition of cellulose and starch, although the enhancement in  $G'$  was dependent on collagen concentration. COLLWS and COLLHAS pastes had higher values of storage modulus than that of collagen/cellulose pastes which suggests that the addition of starch promoted the formation of a stronger network structure (Figure 1A-C). However, at mixing ratio of 80:20 the effect of starch was more pronounced than cellulose and this was attributed to the poor dispersibility of the cellulose fibres in the collagen matrix. Ahmed and Jones (1990) reported that the viscoelastic properties of composite materials are dependent on factors such as size, shape, concentration and distribution of the reinforcing polymers. Thus, the higher storage modulus observed for COLLWS and COLLHAS pastes might be due to the higher concentration of starch granules needed to match the phase volume of cellulose, as well as differences in particle shape, sizes, and packing. Also, according to (Tatsumi et al., 2002), it was reported that fibre suspensions would give higher elasticity than spherical suspensions when matched at equal volume concentration. In contrast, results of this study show that when

starch granules and cellulose fibres were compared at the same phase volume, starch granules were more effective at reinforcing the elastic modulus of acid swollen collagen pastes. In addition, at all the mixing ratios studied, cellulose fibres with longer (COLLSF9) and shorter (COLLSF3) fibre lengths both had similar effects on the  $G'$  and  $G''$  of the pastes. This could be attributed to the fact that the volume fraction of the shorter fibre length (SF3) and longer fibre length (SF9) were matched in the final mixtures at all the mixing ratios studied. Hemar et al. (2011) indicated that the rheological behaviour of dispersions is related to the volume fraction that the particles occupy. Moreover, if the volume fraction of the cellulose fibers with different fibre lengths were not matched, differences in their packing densities and aspect ratios might have an effect on the viscoelastic properties of collagen. Philipse (1996) reported that the packing densities of high aspect ratio rod-like colloids are lower compared to rods with small aspect ratios. . The  $\tan \delta$  values of the blends at the various formulations were lower than one which indicates that the elastic component dominated over the viscous component (Figure 2A-C). The  $\tan \delta$  value was dependent on the collagen concentrations, higher  $\tan \delta$  values were observed for COLLSF3, COLLSF9, COLLWS and COLLHAS pastes at a ratio of 50:50 (2.5% collagen concentration). This indicates that the pastes were weaker in structure as compared to pastes at ratios 80:20 (4% collagen concentration) and 70:30 (3.5% collagen concentration). The most striking observation to emerge from the  $\tan \delta$  data is the difference in the  $\tan \delta$  shape at the various formulations. The  $\tan \delta$  values of collagen with cellulose and starch mixed at a ratio of 80:20 and 70:30 decreased when the oscillation frequency increased from 0.1 to 1 angular frequency with a further increase in  $\tan \delta$  values at higher frequencies. A similar frequency dependency of  $\tan \delta$  was found for chicken-wheat flour doughs (Mohammed et al., 2011). However, at a ratio of 50:50,  $\tan \delta$  values decreased when the frequency increased from 0.1 to 10 angular frequency and was almost constant at higher frequencies. This indicates that the pastes were solid-like when a slow change in stress is imposed but when subjected to fast motions the pastes behaves more like a liquid .

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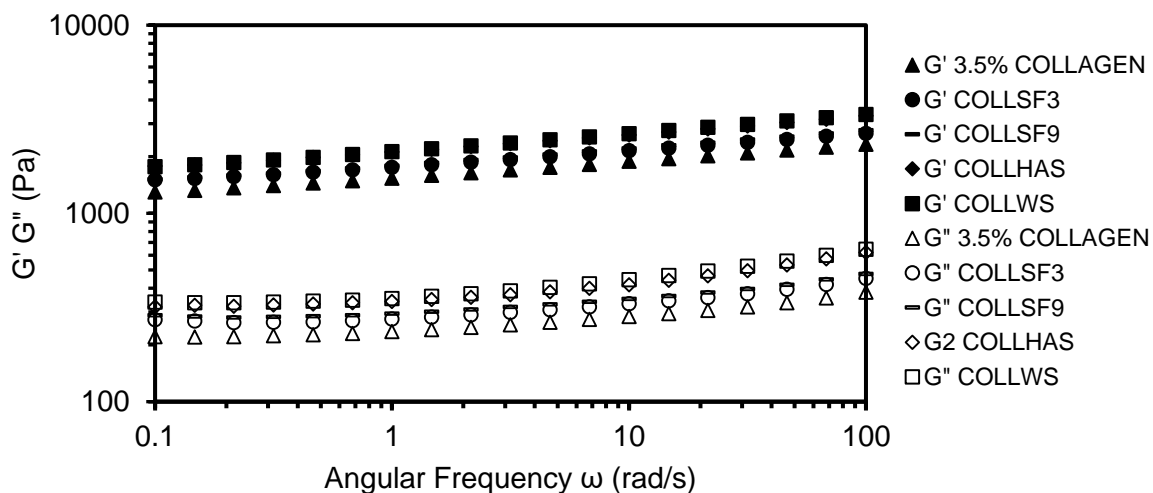
(A)



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(B)

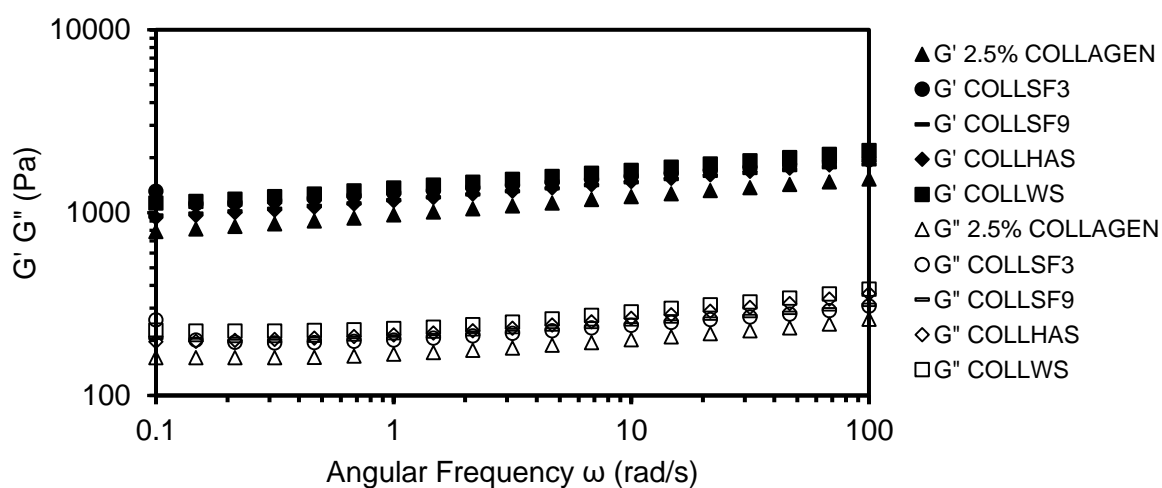


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(C)



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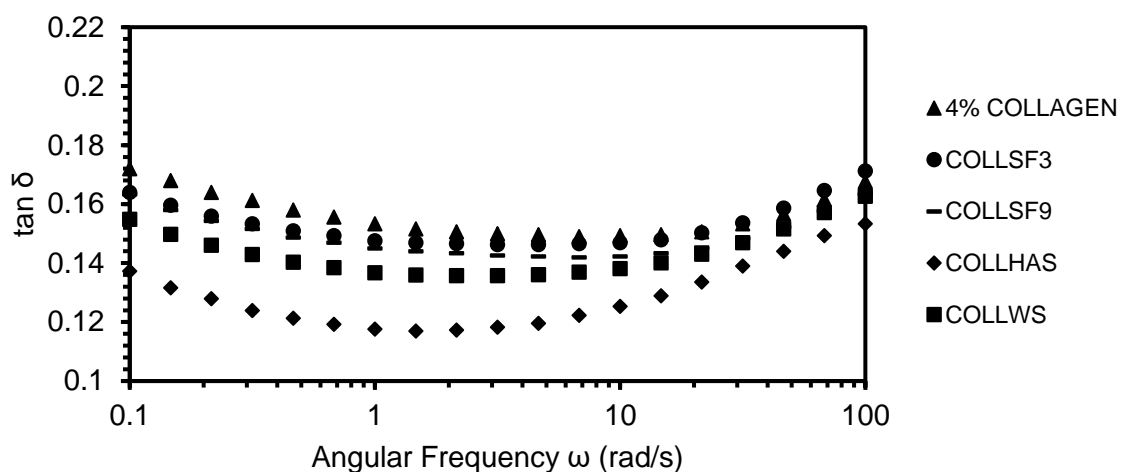
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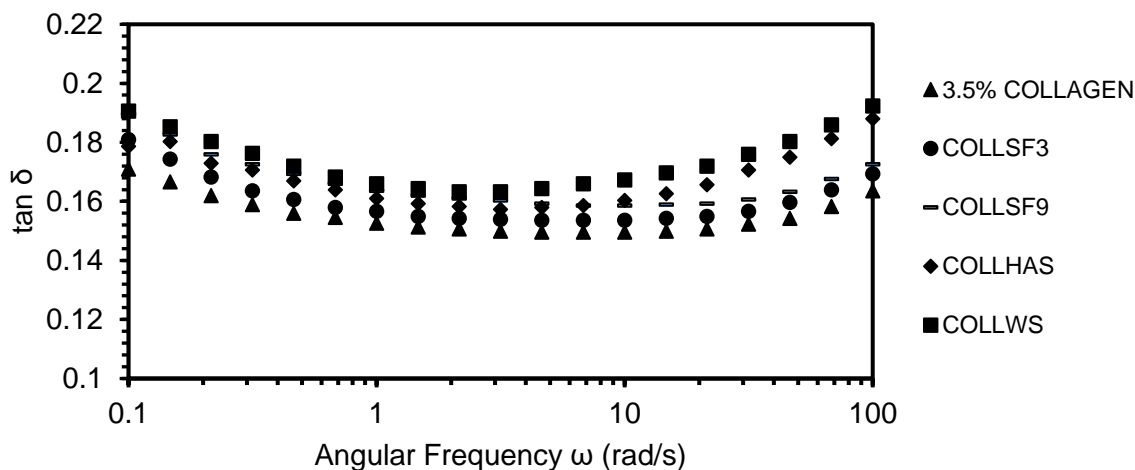
Figure 1. Storage modulus  $G'$  (closed symbols) and loss modulus  $G''$  (open symbols) as a function of angular frequency for COLLSF3 (●), COLLSF9 (—), COLLWS (■), and COLLHAS (◆) and COLLAGEN

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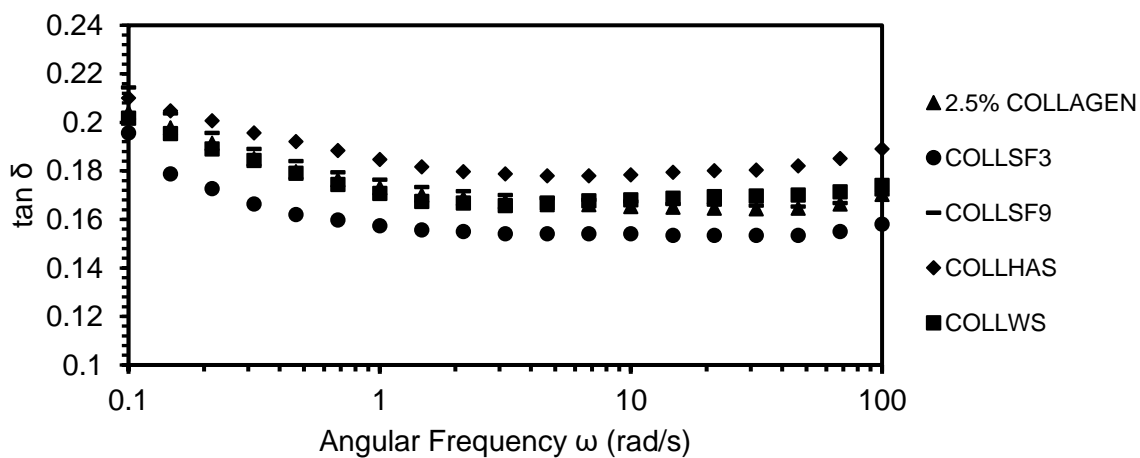
263 (A)



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266 (B)  
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268  
269 (C)  
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271  
272 Figure 2. Loss factor  $\tan \delta$  as a function of angular frequency for COLLSF3 (●), COLLSF9 (—),  
273 COLLWS (■), and COLLHAS (◆) and COLLAGEN (▲) pastes at different collagen concentrations (A)  
274 4% collagen (B) 3.5% collagen (C) 2.5% collagen.

## 3.2 Dynamic Temperature Sweep

**Error! Reference source not found.**Figure 3 and Figure 4 shows the changes in storage modulus ( $G'$ ), loss modulus ( $G''$ ) and loss factor ( $\tan \delta$ ) of COLLSF3, COLLSF9, COLLWS and COLLHAS pastes at various collagen concentrations as a function of temperature. All the samples at the various mixing ratios showed no changes in  $G'$  with stable  $\tan \delta$  values at temperatures from 10 °C to 33 °C, however, the  $G'$  rapidly decreased at approximately 34 °C (Figure 3) with a corresponding increase in  $\tan \delta$  (Figure 4). Similar behaviour was observed when a collagen solution was studied as a function of concentration and temperature (Lai et al., 2008). This behaviour was attributed to the loss of mechanical strength as a result of the structural transition of the collagen triple helix to a random coil conformation. The temperature where  $G'$  decreased steeply closely matched the onset of denaturation ( $T_o$ , about 34 °C) as measured by the micro differential scanning calorimeter, indicating helix to random coil transition (detailed later in section 3.3). Furthermore, as depicted in Figure 3A-C, the addition of waxy starch to different concentrations of collagen pastes resulted in an increase in  $G'$  between 68 °C and 76 °C and a maximum at 76 °C with a corresponding decrease in  $\tan \delta$  values at a temperature of 72 °C. This increase in  $G'$  and decrease in  $\tan \delta$  can be ascribed to the swelling of the starch granules as a result of the melting of the amylopectin region (Donovan, 1979; Tester and Morrison, 1990; Hsu et al., 2000). However, with further increases in temperature above 76 °C, a decrease in  $G'$  was observed for COLLWS pastes which was attributed to the loss of granule integrity and subsequent break down of the starch granules at higher temperature. Similar findings were reported by Sullo and Foster (2010) for waxy maize starch/hydrocolloid mixtures. It was shown in this work that the  $G'$  of the mixture increased at about 60 °C with a further decrease at higher temperatures (Sullo and Foster, 2010). On the contrary, the addition of high amylose starch to different concentrations of collagen paste did not result in an increase in  $G'$  at higher temperatures, which means that the granules of amylose starch did not swell under the range of temperatures the rheological measurements

were performed. This is expected, as it has been reported that amylose molecules swell in the temperature range of 104 °C and 125 °C (Kibar et al., 2010).

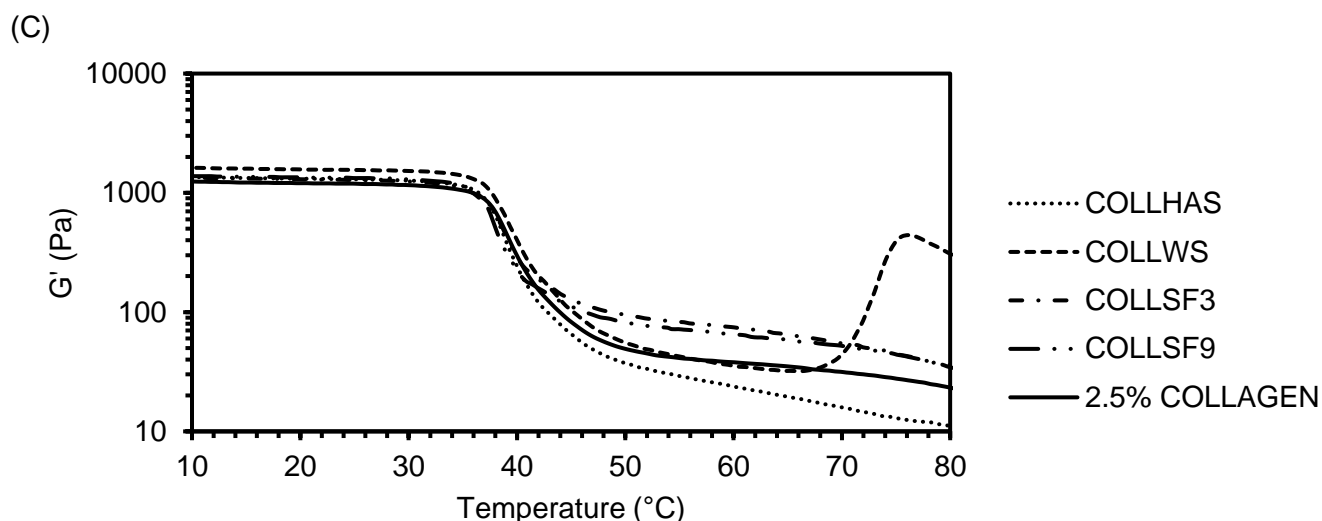
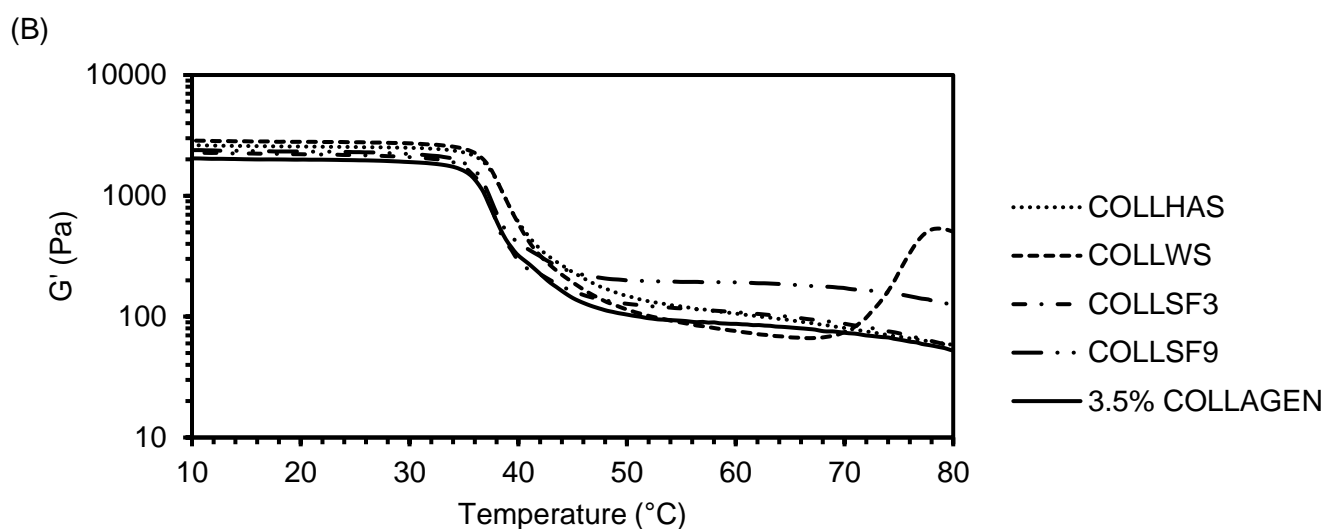
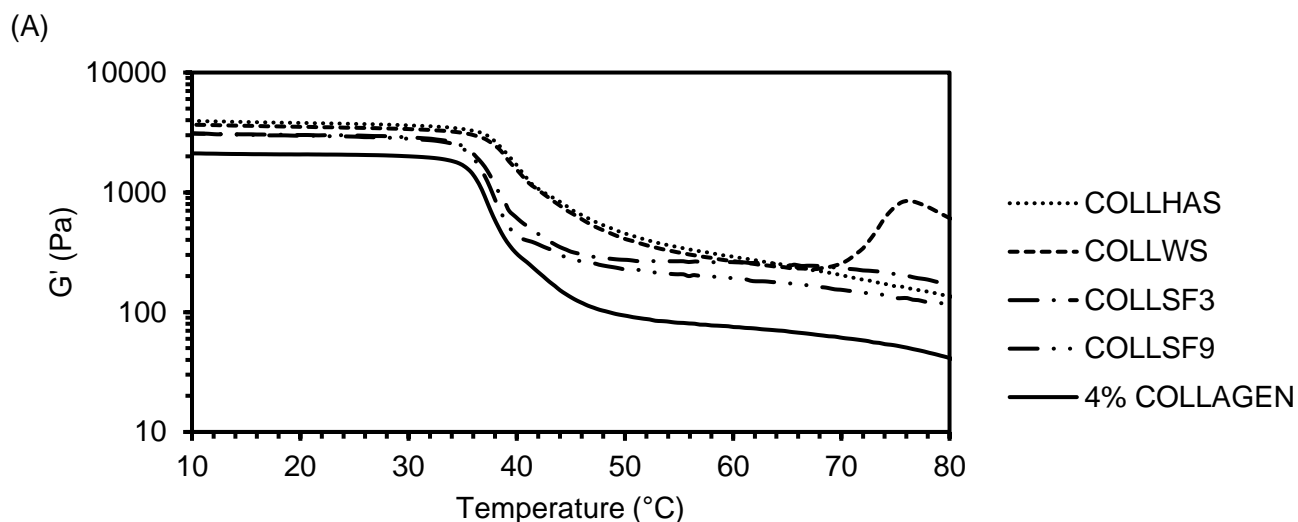
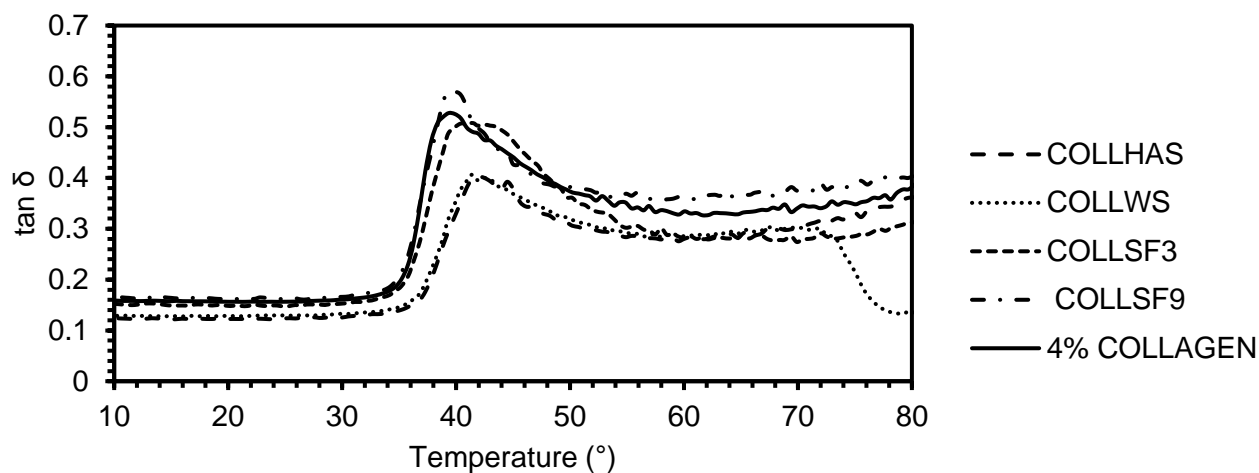


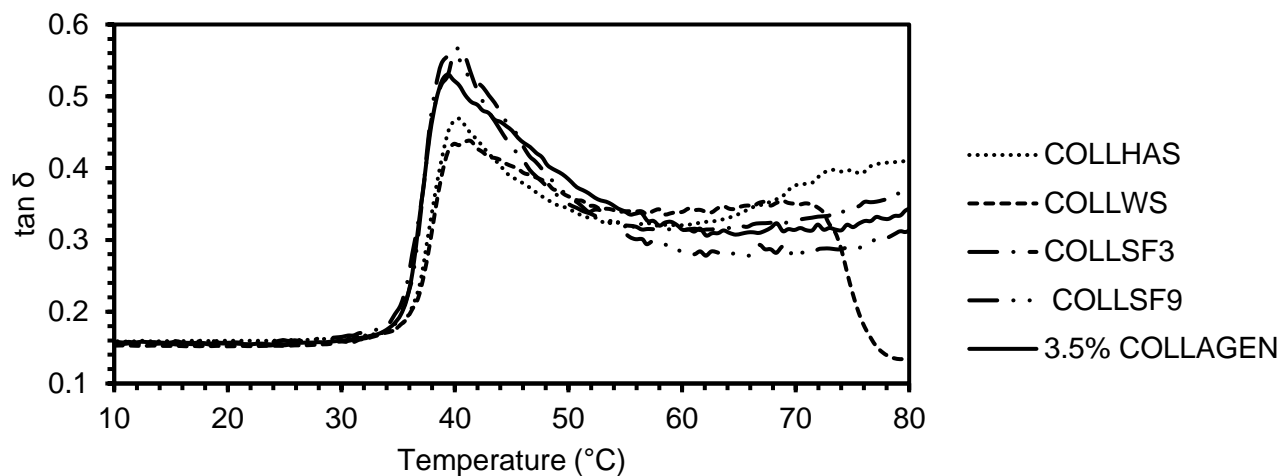
Figure 3. Storage modulus  $G'$  as a function of temperature for COLLHAS (.....), COLLWS (----), COLLSF3 (- · -), COLLSF9 (— · ) and COLLAGEN (—) pastes at different collagen concentrations (A) 4% collagen (B) 3.5% collagen and (C) 2.5% collagen.



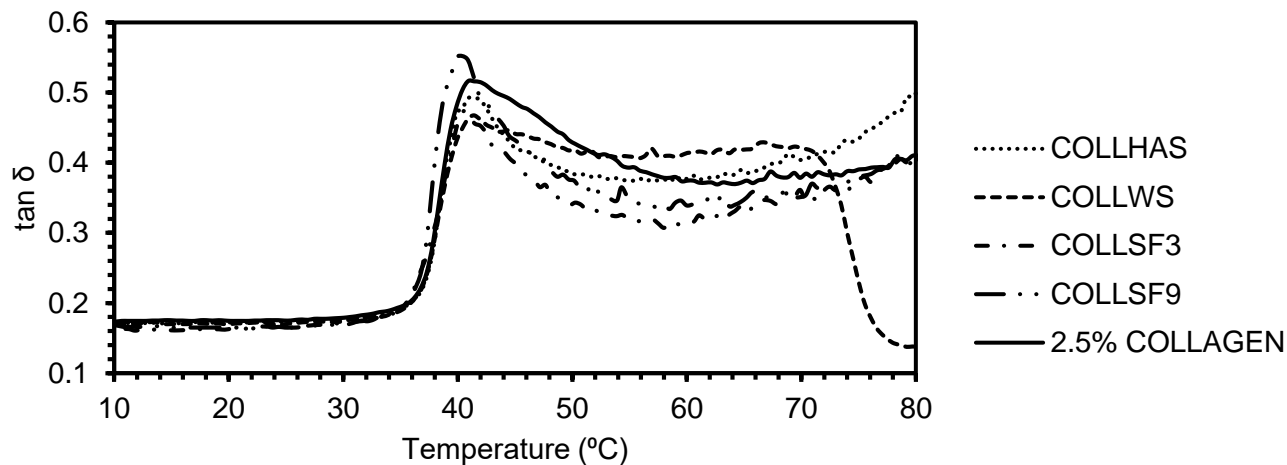
339 (A)



340 (B)



342 (C)



344 Figure 4. Storage modulus  $G'$  as a function of temperature for COLLHAS (·····), COLLWS (-----),  
 345 COLLWS (-----), COLLSF3 (- · -), COLLSF9 (— · —) and COLLAGEN (—) pastes at different collagen concentrations  
 346 (A) 4% collagen (B) 3.5% collagen and (C) 2.5% collagen.  
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349 The loss factor ( $\tan \delta$ ) values of collagen with and without cellulose/starch pastes at the various  
350 collagen concentrations were lower than 1 indicating that their overall behaviour was solid-  
351 like. Some studies have reported that during oscillatory rheological measurements, the  
352 temperature at which  $\tan \delta$  reached a peak value could be taken as the dynamic denaturation  
353 temperature of collagen ( $T_{dd}$ ) (Lai et al., 2008; Zhang et al., 2010; Ding et al., 2014). Hence the  
354 dynamic denaturation temperature of COLLSF3, COLLSF9, COLLWS and COLLHAS at the  
355 different collagen paste concentrations was recorded as 42 °C, which was slightly higher than  
356 that measured by micro DSC (37 °C). In addition, the different measurements report on the  
357 melting at different length scales. DSC report on the helix-coil transition directly, whereas the  
358 rheological measurement is dependent on the melting and softening of the helical aggregates  
359 as the helices melt. This result implies that the thermal denaturation of the collagen triple helix  
360 could be influenced by differences in the heating rate used by each instrument. Furthermore,  
361 a drastic drop in  $\tan \delta$  was observed for all the samples at about 42 °C. This has been  
362 attributed to the glass/rubbery transition of the denatured collagen (Pietrucha, 2005). It is also  
363 interesting to note that the  $\tan \delta$  peak value of COLLWS and COLLHAS pastes were lower  
364 than that of COLLSF3 and COLLSF9 pastes, indicating that the collagen pastes with starch  
365 granules were more rigid in comparison to collagen pastes with cellulose fibres. The lower  $\tan$   
366  $\delta$  values observed for COLLWS and COLLHAS pastes might be due to the presence of closer  
367 packing of the starch granules in the collagen matrix, which reduced the molecular mobility of  
368 the collagen triple helix molecules during heating. This is in comparison to cellulose fibres that  
369 were not closely attached to the collagen matrix, thus allowing the easy mobility of the collagen  
370 chains. Another explanation is that the higher concentrations of starch granules resulted in a  
371 stronger network structure which led to an increase in the restriction of the collagen chains  
372 and reduction in the damping values. Mohanty suggested that the magnitude of damping factor  
373 ( $\tan \delta$ ) peak of composite materials are affected by the incorporation of fillers, the extent of  
374 packing and the concentration of the fillers (Mohanty et al., 2006).

### 375    **3.3   Thermal properties**

376    The thermal properties of the collagen pastes with and without the addition of cellulose and  
377    starch at various mixing ratios are summarised in

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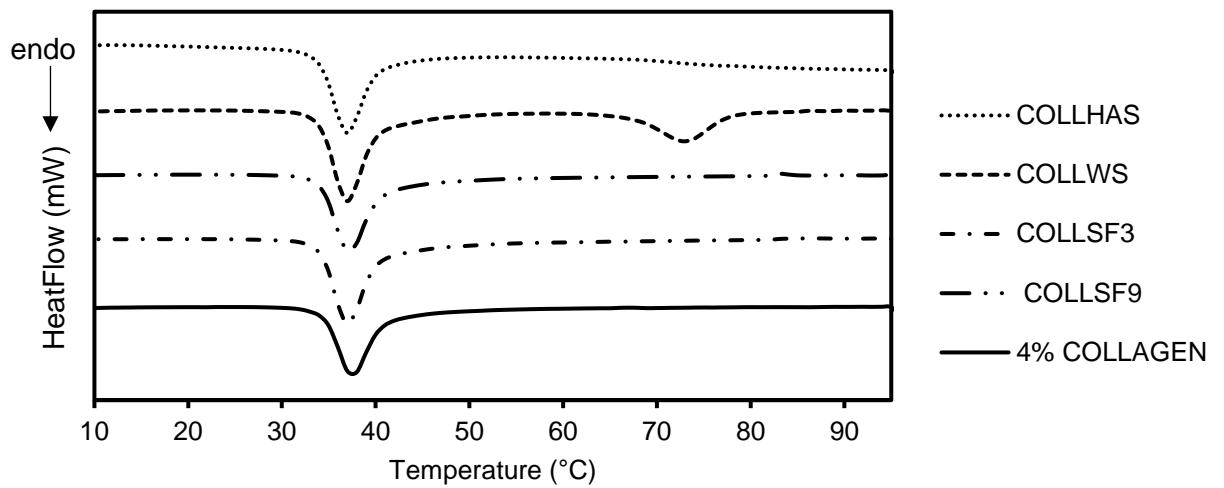
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397    (A)



(B)

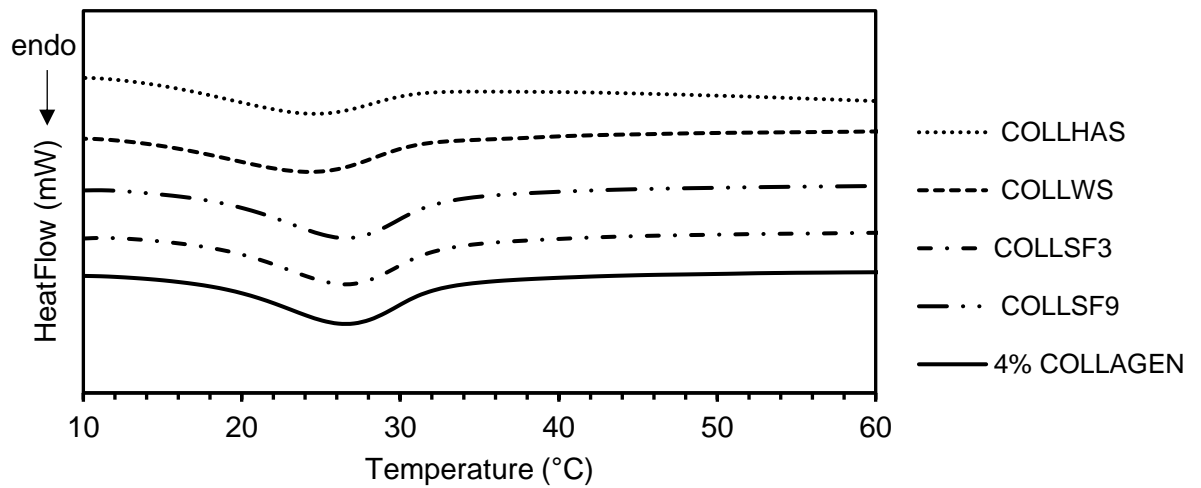


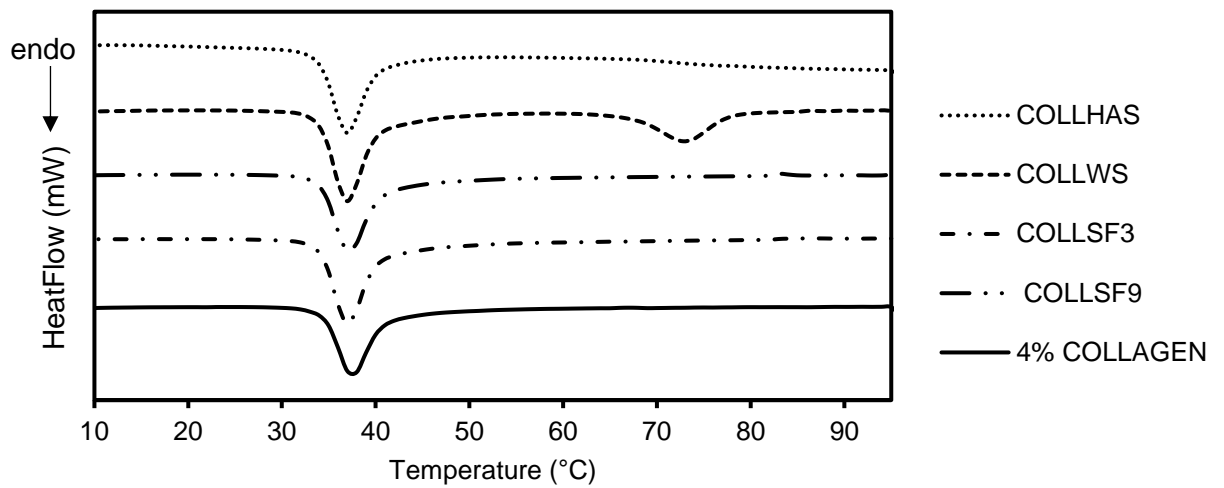
Figure 5. DSC thermograms of (A) first heating and (B) second heating of COLLHAS ( ), COLLWS ( ), COLLSF3 ( ), COLLSF9 ( ) and 4% COLLAGEN ( ) pastes.

Table 2. As depicted in (Figure 5A), pure collagen, COLLSF3 and COLLSF9 pastes (various formulations) exhibited a single endotherm at approximately 37°C. The transition temperature was associated with the transition of collagen molecules from the triple helix to a randomly coiled conformation which is induced by the thermal disruption of hydrogen bonds in the collagen molecule (Bigi et al., 2004). In contrast, COLLWS and COLLHAS exhibited two endothermic peaks, the first peak was related to the denaturation of collagen and the second peak corresponds gelatinisation of the starch granules (Figure 5A). Starch gelatinisation involves the irreversible swelling of starch granules when starch is heated in excess water (Morris, 1990;Tester and Morrison, 1990) It was observed that the COLLWS showed a pronounced gelatinisation peak at 73°C similar to previous reports (Liu et al., 2006). This endotherm can be attributed to the widely accepted gelatinisation of the amylopectin starch granules which is due to the disruption of the amylopectin crystallites (Donovan, 1979;Tester and Morrison, 1990;Koganti et al., 2011). However, the endotherms for COLLHAS pastes were not as sharp as those of the waxy maize starch which contains mainly amylopectin, instead the beginning of a broad endotherm can be observed from 68 °C. Such a broad peak was also observed previously for corn starch with high amylose content, within the temperature range of 65 to 115 °C (Liu et al., 2006), suggesting it resulted from a composite consisting of both gelatinisation and a phase transition of the amylose lipid complex. In measurements of pasting properties of high amylose starch up to 140 °C, we have observed gelatinisation peaks beginning at 100 °C and peaking at 120 °C (data not shown). Therefore, we would support the fact that the broad peak seen in the DSC endotherms would be consistent with starch-lipid complexes. These results were expected because waxy maize contains about 99% amylopectin which constitute the crystalline regions of the starch. On the other hand, amylose restricts the swelling of the starch due to the leaching out of amylose during the gelatinisation and formation of complexes with the lipid's presence in starch granules, which limits the rate at which water penetrates into the starch granules. In addition, it was observed that decreasing the collagen concentrations from 4% to 2.5% resulted in a slight increase of the peak value temperatures of the collagen/cellulose and collagen/starch

437 pastes. Overall, the transition temperatures of COLLSF3, COLLSF9, COLLWS and COLLHAS  
438 pastes were not significantly different ( $P>0.05$ ) from pure collagen pastes at the collagen  
439 concentrations studied. The endothermic peaks of the COLLSF3, COLLS9, COLLWS and  
440 COLLHAS pastes at a mixing ratio of 80:20 observed on reheating are shown Figure 5B and  
441 the thermal transition parameters of all formulations are detailed in Table 3. The endothermic  
442 peak appeared at lower temperatures between 24°C - 26°C (Figure 5B) for all the samples.  
443 During the thermal denaturation of collagen, the triple helix is disrupted to give a random coil  
444 confirmation (gelatin). However, on cooling, the random coils undergo a conformational  
445 disorder-order transition and partly renatures to a triple-helix structure which is similar to that  
446 of collagen but with a lower molecular weight (Machado et al., 2002; Bigi et al., 2004). Gilsenan  
447 and Ross-Murphy (2000) suggested that lower molecular weight gels melt at lower  
448 temperatures than gels of high molecular weight. Also, the lower transition temperature could  
449 indicate the formation of lower and less ordered helical bundles as well as reduced helix length  
450 (Michon et al., 1997; Aymard et al., 2001). Thus, this might explain the lower denaturation  
451 temperatures observed on the second DSC scans. Furthermore, as shown in Table 3, the  
452 thermal transitions temperatures of COLLWS and COLLHAS pastes were significantly lower  
453 ( $P<0.05$ ) than those of the COLLSF3, COLLSF9 and pure collagen pastes. The decrease in  
454 the denaturation temperature and enthalpy can be related to a decrease in the ordering and  
455 semi-crystalline nature of the reformed gelatin-like matrix, after collagen melting, upon cooling.  
456 Such a decrease might not be expected if the two materials do not affect one another. Lorén  
457 et al. (2001) have shown that phase separation and subsequent phase concentration does  
458 not impact on the amount of gelatin helices formed. Therefore we do not expect that  
459 confinement effects would reduce the ability of the collagen to reform triple helices. Therefore,  
460 an unexpected molecular interaction between collagen chains and starch may reduce the  
461 creation of gelatin-like network. Indeed, when mixtures highlighted in table 2 are dried, then  
462 mixtures containing starch also show a decrease in enthalpy. This is a phenomenon that is  
463 currently under further exploration.

Finally, the transition enthalpy for COLLWS pastes was slightly higher than COLLHAS pastes, however, this increase in enthalpy for COLLWS was not significantly different ( $P>0.05$ ) from enthalpy values of COLLHAS (Table 2). An explanation for this might be due to the high molecular weight and branched chains of amylopectin, indicating a larger amount of energy was needed to melt the regenerated triple helix of the COLLWS paste.

(A)



(B)

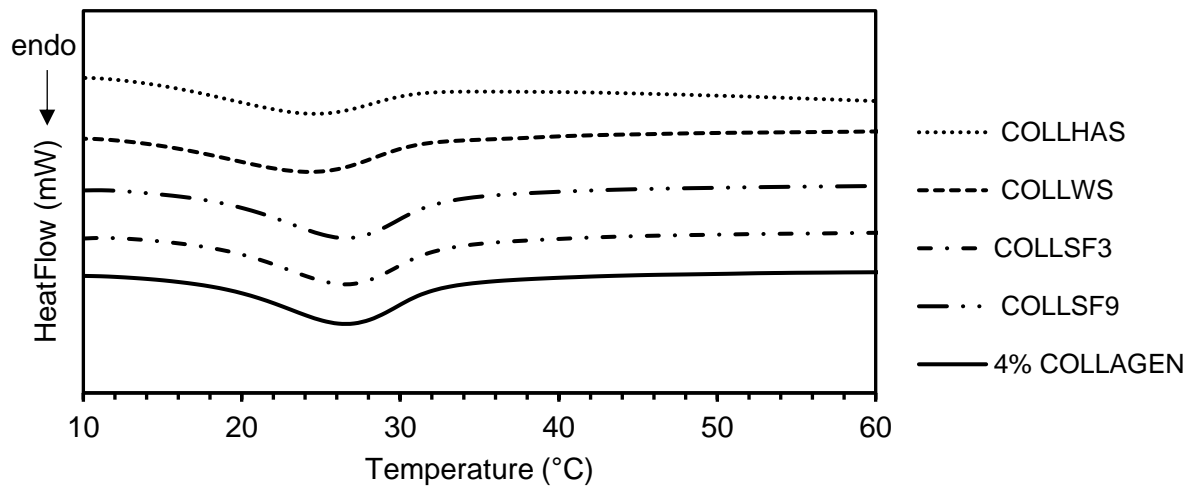


Figure 5. DSC thermograms of (A) first heating and (B) second heating of COLLHAS (.....), COLLWS (-----), COLLSF3 (- · - ·), COLLSF9 (- · · ·) and 4% COLLAGEN (——) pastes.



500 Table 2. DSC parameters after first heating of collagen/cellulose and collagen/starch pastes  
501 at different collagen paste concentrations and mixing ratios. T<sub>P</sub> = peak temperature; T<sub>O</sub>= Onset  
502 temperature; T<sub>e</sub>= end set temperature and ΔH= enthalpy

Samples	T <sub>onset</sub> (°C)	T <sub>Peak</sub> (°C)	ΔH (J/g of collagen)	T <sub>end</sub> (°C)
4% COLLAGEN	33.90 ± 0.01 <sup>a</sup>	36.80 ± 0.02 <sup>a</sup>	40.36 ± 0.02 <sup>a</sup>	0.47 ± 0.02 <sup>a</sup>
COLLSF3 80:20	34.00 ± 0.05 <sup>a</sup>	37.01 ± 0.07 <sup>a</sup>	40.91 ± 0.05 <sup>a</sup>	0.43 ± 0.01 <sup>ab</sup>
COLLSF9 80:20	33.99 ± 0.01 <sup>a</sup>	37.01 ± 0.00 <sup>a</sup>	40.60 ± 0.23 <sup>a</sup>	0.45 ± 0.45 <sup>ab</sup>
COLLWS 80:20	34.05 ± 0.01 <sup>a</sup>	36.97 ± 0.02 <sup>a</sup>	40.38 ± 0.00 <sup>a</sup>	0.46 ± 0.01 <sup>ab</sup>
COLLHAS 80:20	34.03 ± 0.03 <sup>a</sup>	36.97 ± 0.02 <sup>a</sup>	40.37 ± 0.06 <sup>a</sup>	0.41 ± 0.01 <sup>b</sup>
3.5% COLLAGEN	34.16 ± 0.07 <sup>a</sup>	37.23 ± 0.05 <sup>bc</sup>	40.91 ± 0.27 <sup>b</sup>	0.42 ± 0.06 <sup>a</sup>
COLLSF3 70:30	34.10 ± 0.02 <sup>a</sup>	37.21 ± 0.07 <sup>abc</sup>	40.96 ± 0.16 <sup>ab</sup>	0.46 ± 0.00 <sup>a</sup>
COLLSF9 70:30	34.17 ± 0.02 <sup>a</sup>	37.29 ± 0.07 <sup>c</sup>	40.99 ± 0.99 <sup>b</sup>	0.49 ± 0.01 <sup>a</sup>
COLLWS 70:30	34.12 ± 0.02 <sup>a</sup>	37.06 ± 0.02 <sup>ab</sup>	40.48 ± 0.12 <sup>ab</sup>	0.45 ± 0.01 <sup>a</sup>
COLLHAS 70:30	34.01 ± 0.11 <sup>b</sup>	37.06 ± 0.02 <sup>ab</sup>	40.30 ± 0.12 <sup>a</sup>	0.48 ± 0.02 <sup>a</sup>
2.5% COLLAGEN	34.63 ± 0.05 <sup>a</sup>	37.77 ± 0.03 <sup>a</sup>	41.63 ± 0.08 <sup>a</sup>	0.48 ± 0.00 <sup>b</sup>
COLLSF3 50:50	34.46 ± 0.07 <sup>a</sup>	37.66 ± 0.09 <sup>a</sup>	41.09 ± 0.03 <sup>a</sup>	0.45 ± 0.01 <sup>ab</sup>
COLLSF9 50:50	34.62 ± 0.04 <sup>a</sup>	37.77 ± 0.07 <sup>a</sup>	41.49 ± 0.03 <sup>a</sup>	0.44 ± 0.00 <sup>ab</sup>
COLLWS 50:50	34.35 ± 0.36 <sup>a</sup>	37.79 ± 0.15 <sup>a</sup>	41.02 ± 0.06 <sup>a</sup>	0.40 ± 0.01 <sup>a</sup>
COLLHAS 50:50	34.63 ± 0.01 <sup>a</sup>	37.67 ± 0.02 <sup>a</sup>	41.01 ± 0.13 <sup>a</sup>	0.42 ± 0.00 <sup>ab</sup>

503 <sup>a-c</sup> Mean ± standard deviation. Means in the same column with different superscript letters  
504 are significantly different (P<0.05).

Table 3. DSC parameters (second heating) of collagen/cellulose and collagen/starch at various collagen concentrations (4%, 3.5% and 2.5%). T<sub>P</sub> = peak temperature; T<sub>O</sub>= Onset temperature; T<sub>e</sub>= end set temperature and ΔH= enthalpy

Samples	T <sub>onset</sub> (°C)	T <sub>Peak</sub> (°C)	ΔH (J/g of denatured collagen)	T <sub>end</sub> (°C)
4% COLLAGEN	17.67 ± 0.11 <sup>b</sup>	26.66 ± 0.11 <sup>b</sup>	0.27 ± 0.01 <sup>c</sup>	33.29 ± 0.14 <sup>b</sup>
COLLSF3 80:20	17.68 ± 0.13 <sup>b</sup>	26.49 ± 0.05 <sup>b</sup>	0.27 ± 0.00 <sup>c</sup>	33.38 ± 0.05 <sup>b</sup>
COLLSF9 80:20	17.46 ± 0.10 <sup>b</sup>	26.55 ± 0.02 <sup>b</sup>	0.27 ± 0.01 <sup>c</sup>	33.41 ± 0.05 <sup>b</sup>
COLLWS 80:20	13.76 ± 0.20 <sup>a</sup>	24.37 ± 0.09 <sup>a</sup>	0.18 ± 0.00 <sup>b</sup>	31.67 ± 0.00 <sup>a</sup>
COLLHAS 80:20	14.33 ± 0.42 <sup>a</sup>	24.22 ± 0.00 <sup>a</sup>	0.14 ± 0.01 <sup>a</sup>	31.33 ± 0.03 <sup>a</sup>
3.5% COLLAGEN	18.13 ± 0.05 <sup>b</sup>	26.81 ± 0.04 <sup>b</sup>	0.26 ± 0.00 <sup>b</sup>	33.60 ± 0.04 <sup>b</sup>
COLLSF3 70:30	18.09 ± 0.13 <sup>b</sup>	26.79 ± 0.04 <sup>b</sup>	0.27 ± 0.00 <sup>b</sup>	33.61 ± 0.02 <sup>b</sup>
COLLSF9 70:30	19.07 ± 0.17 <sup>b</sup>	26.72 ± 0.02 <sup>b</sup>	0.27 ± 0.00 <sup>b</sup>	33.57 ± 0.00 <sup>b</sup>
COLLWS 70:30	14.06 ± 0.37 <sup>a</sup>	24.45 ± 0.02 <sup>a</sup>	0.18 ± 0.00 <sup>a</sup>	31.47 ± 0.02 <sup>a</sup>
COLLHAS 70:30	14.14 ± 0.37 <sup>a</sup>	24.22 ± 0.09 <sup>a</sup>	0.16 ± 0.01 <sup>a</sup>	31.41 ± 0.01 <sup>a</sup>
2.5% COLLAGEN	18.96 ± 0.04 <sup>b</sup>	27.35 ± 0.02 <sup>b</sup>	0.24 ± 0.02 <sup>b</sup>	33.98 ± 0.06 <sup>b</sup>
COLLSF3 50:50	19.21 ± 0.36 <sup>b</sup>	27.48 ± 0.09 <sup>b</sup>	0.23 ± 0.02 <sup>b</sup>	33.89 ± 0.16 <sup>b</sup>
COLLSF9 50:50	19.13 ± 0.02 <sup>b</sup>	27.38 ± 0.02 <sup>b</sup>	0.24 ± 0.00 <sup>b</sup>	34.02 ± 0.08 <sup>b</sup>
COLLWS 50:50	15.48 ± 0.07 <sup>a</sup>	25.08 ± 0.11 <sup>a</sup>	0.14 ± 0.01 <sup>a</sup>	31.80 ± 0.02 <sup>a</sup>
COLLHAS 50:50	15.98 ± 0.20 <sup>a</sup>	24.99 ± 0.06 <sup>a</sup>	0.12 ± 0.01 <sup>a</sup>	31.56 ± 0.13 <sup>a</sup>

<sup>a-c</sup> Mean ± standard deviation. Means in the same column with different superscript letters are significantly different (P<0.05).

## 4 Conclusions

The elastic properties of collagen pastes were modified by the addition of fillers i.e. cellulose fibres and starch granules at a range of different collagen concentrations. The results indicate that different materials, when matched for phase volumes, have very different effects on the elastic properties of collagen pastes. In addition, these materials did not affect the thermal stability of collagen. However, on reheating, the addition of starch granules had an impact on

the melting of denatured collagen. The findings in this study may serve as a platform to improve the rheological properties and processing performance of collagen pastes in order to improve their product or processing performance. Further research is still necessary to understand the mechanisms or interactions underlying the effect of starch granules on the melting of denatured collagen.

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