INVESTIGATION INTO INTERACTIONS BETWEEN EMUSIFIERS AND WHEAT STARCH/WHEAT FLOUR

by

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Abstract

One long-term goal of cereal science research is to increase the shelf life of baked products and one approach to that goal has been the use of emulsifiers. Emulsifiers are surface-active agents, some of which improve specific properties of baked products. Two emulsifiers, sodium stearoyl lactylate (SSL) and oleyl lactylic acid (OLA) are similar in structure, the difference being the acid moiety: stearic versus oleic acid. Widely researched, SSL has found many uses in baked products. However, how OLA interacts with starch/flour in baked products or how that interaction affects product quality is not well understood. The objective of this study was to understand the interactions between normal wheat starch and these emulsifiers, as well as the differences between SSL and OLA in those regards.

The effects of the two emulsifiers on the pasting properties of wheat starch and wheat flour were determined using a MicroViscoAmylograph. Heating at 6°C/min with increased emulsifier concentration (0-2%) resulted in increased hot paste viscosity and apparent interaction of both emulsifiers with wheat starch. Solid content affected both maximum hot and final viscosity. OLA produced increased maximum hot and final viscosities with increased starch concentration and emulsifier concentrations. For wheat flour, higher concentrations of SSL reduced both maximum hot paste and final viscosities. OLA behaves differently with wheat flour. At lower flour concentrations, higher concentrations of OLA reduced maximum hot viscosity. The effects of SSL on starch were affected by an increased heating and cooling rate (10°C/min). Heating stage microscopic analysis revealed that the presence of emulsifiers inhibited granular swelling beyond the normal gelatinization temperature (65°C) in both starch and flour systems. OLA's effect in this respect was greater than that of SSL. Complex formation between emulsifiers and wheat starch and wheat flour was determined using differential scanning calorimetry (DSC) and X-ray diffraction (XRD). For isolated wheat starch and hard wheat flour, DSC and XRD indicated that OLA complexes to a greater extent than does SSL. For flour, XRD indicated no difference exhibited in the interaction of the emulsifiers with the flours.

KEYWORDS: Emulsifiers, Starch, Wheat flour

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Dedication

This thesis is dedicated to all those people who were told that anything they wanted to do was impossible. As long as you have the motivation and the drive, you can do anything.

Also, I would like to dedicate this paper to my beautiful daughter. She has provided my soul with light and strength that I never knew I possessed. The joy in her eyes provided the motivation to keep pushing me to finish.

Chapter 1 - Interactions between normal wheat starch and emulsifiers

Introduction

The effects of a variety of emulsifiers on starch pasting properties, their complexation with starch, and the relationship of these properties to emulsifier function in baked products have been documented. Studying starch pasting properties helps in understanding starch and emulsifier functions in baked products (Carson and Sun, 2000; Ravi et al., 1999; Gomez et al., 2004; Chin et al., 2007; Singh et al., 1991; Serna-Salvidar et al., 1988; Indrani and Rao, 2003; Jacob and Leelavathi, 2007; Kaur et al., 2000; Conforti and Smith, 1998; Jyotsna et al., 2004; Tenney et al., 1972; Lakshminarayan et al., 2005; Kamel and Rasper, 1988). These characterizations of starches and emulsifiers by the analysis of pasting properties interactions have been extensive (Azizi and Rao, 2005; Eliasson, 1985; Krog, 1973; Lin et al., 1990; Numfor et al., 1996; Xu et al., 1992). The complexes formed between starches and emulsifiers have been studied extensively using differential scanning calorimetry (DSC) (Eliasson, 1994; Evans, 1986; Lai et al., 1998; Numfor et al., 1996) and X-ray diffraction (XRD) (Birnbaum, 1977; Ghiasi et al., 1982).

Wheat flour is a fundamental ingredient in baked products. Even so, there are fewer studies of interactions between wheat flours and emulsifiers compared to studies using isolated starches. Extensive literature exists regarding emulsifier interactions with wheat starches, but is lacking in regard to interactions with wheat flours. In most cases, studies using wheat flours are focused on emulsifier effects on final baked product quality (Gomez et al., 2004; Jyotsna et al., 2004; Sargent, 2008; Ravi et al., 1999; Chin, 2007). The interactions of emulsifiers with wheat flours are slightly more complicated to characterize because of the potential for interaction with the flour protein as well as with starch.

In general, researchers have used combinations of gums, dough conditioners, and dough strengtheners to achieve and maintain quality over an extended shelf life. Some emulsifiers such as polysorbate 60 and monostearate are known to improve specific properties of interest to baked food manufacturers including but not limited to dough strengthening, crumb structure, and shelf life (Kohajdova et al., 2009). Two emulsifiers of interest to this study are sodium stearoyl lactylate (SSL) and oleyl lactylic acid (OLA); both approved food grade molecules similar in structure, but differing in the acid moiety (stearic acid versus oleic acid).

SSL is one of the most commonly and effectively used emulsifiers as an ingredient in baked products. It provides a range of functional characteristics such as shelf life extension that appeals to the food industry. SSL has a saturated fatty acid and is a powder at room temperature, whereas OLA is a liquid, pumpable emulsifier with an unsaturated fatty acid. The differences in their structures may well affect the functional properties associated and the complexes they formed. Longer saturated fatty acids chains are known to complex better than those containing double bonds (Eliasson, 2006). Although OLA has potential benefits, the functional properties of OLA and its interactions with wheat starch or flour are not yet characterized.

In order to compare OLA and SSL, a thorough study on the effects of both on wheat starch needs to be completed. The goal of this study was to obtain a fundamental understanding of OLA's functions and interactions with wheat starch/flour, and to compare its behavior to that of SSL.

The specific objectives were:

1) to study the effects of SSL and OLA on pasting properties [Micro ViscoAmylograph (MVA)], swelling (hot stage microscopy), swelling power, and solubility

2) to quantify the complex formed between wheat starch and SSL and OLA (differential scanning calorimetry and X-ray diffraction)

In this study, the definition of interaction differs with the experiment performed. In MVA analyses, an interaction was considered greater or stronger if viscosity increased. In DSC analyses, an interaction was deemed greater or stronger if the peak or ΔH increased. In XRD analyses, the larger the relative intensity, the greater or stronger the interaction of the emulsifier with starch/flour.

Experimental

Materials

Wheat starch (Midsol 50) was obtained from MGP Ingredients, Inc. (Atchinson, KS). Its moisture was determined in duplicate according to the Approved Method 44-19 (AACC International, 1999). Hard wheat flour from the 2006 crop provided by Foundation Seed (Manhattan, KS) was milled to straight grain flour on the Buhler Mill in August 2008. Soft wheat flour, SSL, and OLA were donated by Caravan Ingredients (Lenexa, KS). Amylose content was determined using the method developed by Yun and Matheson (1990) using Megazyme Assay Kit K-AMYL 04/06 (Wicklow, Ireland).

Pasting properties

The pasting behavior of wheat starch/flour in the presence of SSL or OLA was analyzed via MVA (C.W. Brabender; Hackensack, NJ). The pasting onset temperature and maximum hot viscosity were measured during heating, while final viscosity was measured at the end of the MVA run. Starch or flour/water mixtures were created at 6, 7, and 8% solid content level at a total sample weight of 115 grams. SSL or OLA was tested at 0.5, 1.0, 1.5, and 2.0% (w/w of starch/flour). All analyses were carried out in duplicate using the following MVA temperature profiles of starting 30°C, ramping to 95°C at 6°C/min, holding for 5 minutes, and ramping to 50°C at 6°C/min to hold for 2 minutes. The effects of heating and cooling rates were assessed on duplicate samples by changing the heating rate from 6°C/min to 10°C/min and cooling rate from 6°C/min to 10°C/min.

Swelling and solubility

Starch swelling power and water solubility were measured and calculated using a modified method of Shi et al. (1991). Starch (0.1g) was weighed into 10 ml glass screw cap centrifuge tubes. Then, emulsifiers were added to create 1% and 2% (starch weight basis) mixtures. The tubes were placed on a vortex mixer for 10 seconds and then moved to a water bath starting at 65°C for 30 minutes; increasing the temperature 10°C every 30 minutes up to 95°C. At 10-minute intervals, the tubes were stirred with a vortex mixer for 10 seconds and returned to the water bath. After 30 minutes, the tubes were centrifuged at 3000g x 15min, the supernatant was decanted, and the uncapped tube placed in an oven at 85°C until completely dry. Once dry, the weight of the remaining dry matter was determined.

Visual analysis of starch and flour swelling and the effects of emulsifiers on granular swelling were carried out via an Olympus BX51 microscope with an Instec heater (Hitschel Instruments, Inc; Kansas City, MO). Five-gram samples of the starch/flour-emulsifier mixtures were created as 8% wheat starch or wheat flour in water and 2% emulsifier (starch/flour weight basis). This mixture was diluted 50% with water and a small drop of that solution was placed on a microscope slide, covered with a cover slip for microscopic examination. The hot stage temperature profile started at 25°C for 1 minute, ramped to 95°C at 5°C/min and held at 95°C for

1 minute. A photographic image of the field of view was taken every 20 seconds: during the 25°C hold and every 20 seconds during heating.

Amylose-lipid complex formation

The samples [7% starch/flour, 4% emulsifier (based on the weight of starch/flour)] were prepared in the MVA with the same temperature profile used to determine the effect of solid content on viscosity. The cooked samples were quick frozen in an acetone and dry ice bath and placed in a standard refrigerator freezer for one hour. Those frozen samples were freeze dried (Labconco Corp; St. Louis, MO) overnight or until completely dry. A portion of each freeze-dried sample was ground by a mortar and pestle and placed in a petri dish. The sample was then conditioned to 15% moisture before being placed in a sealed jar. The moisture conditioned sample (200 mg) was densified using a Manual Tablet Compaction Machine (MTCM-I; New Brunswick, NJ) at 100 psi and then reground by a mortar and pestle for the DSC and XRD.

Differential scanning calorimetry (DSC)

DSC was carried out using a TA DSC Q200 V24.4 (TA Instruments, Inc; New Castle, DE). A sample (≈ 10 mg) was weighed into a stainless steel high volume DSC pan. Then, water was added to the pan at a 1:3 starch/flour:water ratio. The pan was sealed and scanned using the following temperature profile: heating from 10°C to 140°C at 10°C/min, holding for 1 minute, cooling from 140°C to 10°C at 10°C/min and rescanning from 10°C to 140°C at 10°C/min. All samples were analyzed in duplicate DSC curves using the TA Instruments Universal Analysis 2000 software provided with the instrument to identify and quantify the onset temperature (°C), the peak temperature (°C), final temperature (°C), and the ΔH (J/g) for each thermogram.

X-ray diffraction (XRD)

The X-ray diffraction patterns were obtained by a Phillips APS 3520 X-ray Diffractometer and Phillips XRG 3100 X-ray generator with Cu-Ka radiation at 35KV and 20mA, a theta compensating slit and a diffracted beam monocromator. The scan was taken at Bragg angle 2θ from 2^o to 40^o at 0.05 step increments with 1.5 seconds/step.

Results and Discussion

The chemical composition (%) of hard wheat and soft wheat flour was shown in Table 1.1. The protein content of hard wheat flour (14.2%) was higher than that of soft wheat flour (8.6%), but values fall in the normal ranges for their hard wheat and soft wheat classification. Amylose content for the flours were similar.

Pasting Properties

Effect of solid content on viscosity

A previous study (Mira et al., 1986) used only 8% solid content samples when evaluating the interactions between starch and SSL. Varying the solid content may have an effect on the interaction between emulsifiers and wheat starch/wheat flour. A reduction in the solid contents should result in a lower maximum hot paste viscosity. This was true generally for the controls; 6% solids had the lowest maximum hot and final viscosities and viscosities increased with solids content (Fig. 1.1). The effects of SSL and OLA on starch were then studied at each solid level. In this case, “lower” emulsifier concentrations refers to 0.5% and 1.0% (starch/flour weight basis), while “higher” refers to 1.5% and 2.0%.

In general, as the solid content increased, the pasting temperature decreased (Fig. 1.1). This phenomenon was more prominent for SSL at 0.5, and 1.0%, and least at 2.0% where differences were the smallest. Further, as the concentration of emulsifier increased at a solid level, the pasting temperature increased (pasting was delayed) (Fig. 1.1) as reported previously by Deffenbaugh & Walker (1990). The pasting temperature was affected more by solid content than by the addition of emulsifiers. These results were similar to those of Krog (1973) who found that the addition of emulsifiers increased pasting temperature and to those of Lin et al. (1990) who reported that the higher the solids concentration, the lower the pasting temperature. Pasting onset temperatures increased with the addition of SSL (Fig. 1.1a) and increased further at higher SSL levels, which agrees with previous finding by Numfor et al. (1996) and Xu et al. (1992).

Maximum hot paste and final viscosity values as a function of solids content and emulsifier concentration (0-2%) are presented in Figure 1.2. SSL had minimal effect (Fig. 1.2a) on maximum hot viscosity at 6% starch; with only the 2.0% emulsifier values, being significantly increased (Fig. 1.2a). SSL had a more significant effect on the final viscosity at 6% solids (Fig. 1.2b), increasing viscosity at all emulsifier concentrations with maximum effect at 1.5% SSL. For 7% starch pastes, maximum hot viscosity at any emulsifier concentration was greater than the control values (Fig. 1.2c). However, maximum hot viscosities did not differ among the emulsifier levels tested. Krog (1973) reported that hot paste viscosity increased with the addition of emulsifiers, but not by large amounts due to the anionic nature of SSL. Our results agree in part with those findings. Final viscosity at this solid level (7%) was increased much greater than that of the control, but higher levels of SSL diminished the effect on viscosity (Fig. 12.d). At an 8% solid level, there was no consistent pattern of maximum hot viscosity response to SSL presence (Fig. 1.2e). In contrast, final viscosity increased with an increased amount of SSL to starch (Fig. 1.2f).

The addition of OLA to normal wheat starch produced somewhat different pasting results both for maximum hot and final viscosities at the lower solid content levels. The presence of

OLA increased hot paste viscosities at all inclusion levels above control (Fig. 1.2a), peaking at 1.5% OLA, and higher inclusion levels resulted in higher values. Final viscosity at 6% solids responded similarly (Fig. 1.2b). Similar to SSL, at 7% solids, the presence of OLA at any level resulted in increased maximum hot viscosity, but there was no clear pattern of response as a function of the amount of OLA present (Fig. 1.2c). OLA's affect on final viscosity was similar to that of SSL (Fig. 1.2d). Emulsifier presence increased final viscosity, regardless of the amount and the response, and increased at higher OLA levels (Fig. 1.2d). Maximum hot viscosity response to OLA was similar to that of SSL (Fig. 1.2e). No consistent pattern existed although it appears that OLA presence increased viscosity slightly over control values. The final viscosity at 8% solids increased with increased OLA concentration except at 1.0% OLA (Fig. 1.2f). There was no consistent pattern regarding the effects of maximum and final viscosity. The presence of OLA and SSL produced similar values, greater than the control values.

Compared SSL to OLA at the lowest solid content, the addition of OLA seemed to result in more interaction with normal wheat starch, especially at higher emulsifier concentrations (Fig. 1.2a). As solid content increased, the results of the maximum hot and final viscosities with the addition of SSL and OLA appeared to be equivalent.

Flour with the emulsifiers demonstrated similar pasting results equivalent to wheat starch systems, while the difference between the two flours was minimal. The two flours had similar amylose contents (Table 1.1) suggesting minimal difference in their affect on the pasting properties. As the solid content level increased, the pasting onset temperatures decreased, but the effect of emulsifiers became less monumental (Fig. 1.3). At low solid content levels, the pasting onset temperature (Fig. 1.3a) increased with increasing emulsifier concentration, similar to wheat starch. In contrast, flours, at higher solid content levels with the addition of either emulsifier showed no change in pasting onset temperature, especially in soft wheat flour (Fig. 1.3b).

Hot maximum and final viscosity values for the flour systems as a function of solids content and emulsifier concentration (0-2%) are presented in Figure 1.4 (hard wheat flour) and Figure 1.5 (soft wheat flour). As reported by Mira et al. (2005), an earlier onset of pasting temperature means a shorter time to reach viscosity, resulting in lower peak viscosities. Generally, this was true for the flours as the viscosities of hard wheat and soft wheat flour were lower than for normal wheat starch. Serna-Salvidar et al. (1988) reported that SSL has the ability to complex with both amylose and amylopectin, so when the protein denatures during heating, SSL strongly binds to the starch. Hard wheat and soft wheat resulted in interesting pasting results in that at certain solid levels depending on maximum hot and final viscosity, more interaction was observed in the presence of SSL, while at others more interaction was observed in flour with OLA.

At 6% solids, maximum hot viscosity indicated more interaction with hard wheat flour in the presence of OLA (Fig. 1.4a). While for soft wheat at this solid level (6%), both emulsifiers resulted in similar viscosities (Fig. 1.5a). Similarly, the final viscosity trends observed were different between hard wheat and soft wheat. For hard wheat, higher levels of both emulsifiers (Fig. 1.4b) decreased the viscosity, while for soft wheat both emulsifiers increased final viscosity (Fig. 1.5b). At 7% solids, emulsifiers reacted similarly for maximum hot viscosities of hard wheat and soft wheat flour. In contrast, at 7%, final viscosity trends were different between the two flours (Fig. 1.4c, 1.5c). Similar to 6% starch systems, hard wheat plus either emulsifier resulted in lower final viscosity at higher inclusion levels (Fig. 1.4d). In contrast, the final viscosity of soft wheat flour increased slightly compared to the control at 1.5% emulsifier and decreased at 2% emulsifier (Fig 1.5d).

At 8% solids, the presence of SSL or OLA had little effect on the maximum hot paste viscosities (Fig. 1.4e) of hard wheat flour. At this same solids level (8%), soft wheat flour had greater at maximum hot viscosity (Fig. 1.5e) when mixed with OLA. The maximum hot viscosities differed only slightly between emulsifiers and across the concentrations of emulsifier tested. Similar to results at the lower solid content levels, hard wheat flour final viscosity at 8% decreased at the higher inclusion levels and equivalent results were observed for both emulsifiers (Fig. 1.4f). In contrast, final viscosity of soft wheat was not decreased when mixed with 1.5% and 2.0% emulsifiers, and 2.0% OLA resulted in higher viscosity (Fig. 1.5f).

Effect of heating and cooling rate on viscosity

Earlier studies used the conventional RVA heating and cooling rate of 6^{o}C/min (Ryu & Walker, 1993). However, varying heating and cooling rate have been used (Seib, 1994; Ellis et al., 1989) to understand pasting properties as they affect the swelling of granules. In this study, heating and cooling rate did affect wheat starch behavior (Fig. 1.6). At 8% solids, both control (0% emulsifier) maximum hot and final viscosities were substantially lower at the increased heating and cooling rate (Fig. 1.6). Maximum hot viscosity of control starch was decreased by almost 300BU (Fig. 1.6a, 1.6b) and final viscosity by approximately 500BU (Fig. 1.6c, 1.6d). Seib (1994) reported that wheat starch increased hot paste and cold viscosity when heating rate was increased from 1.5^{o}C/min to 4^{o}C/min at 7.7% starch solids. In this study, at 8% starch solids, the normal wheat starch gave the same maximum hot viscosity at both heating rates when SSL or OLA was added (Fig. 1.6a, 1.6b) even at the lowest concentration tested.

The effect of increased cooling rate on final viscosity was slightly different from the effect on maximum hot viscosity. SSL presence increased final viscosity (Fig, 1.6c) to values greater than those observed at the lower cooling rate. Seib (1994) postulated that increased pasting with an increased heating rate from 1.5^{o}C/min to 4^{o}C/min may be due to increased swelling of the starch granules. Regardless, the amount of change in final viscosity was significantly greater with a faster cooling rate. The relative amount of increase was greater at 10^{o}C/min and the positive relationship with emulsifier concentration was more pronounced from 0.5-1.5%. The same is true for final viscosity response to OLA at increased cooling rates (Fig. 1.6d) in that they mimicked the effect of SSL; both in the change from control values and the effects of emulsifier concentration.

The effects of an increased heating and cooling rate on hard wheat and soft wheat flours at 8% solids in the presence of SSL or OLA are shown in Figures 1.7 and 1.8, respectively. The faster heating rate had no effect on the pasting onset temperature for either SSL or OLA (Fig. 1.3). The results obtained for pasting temperature at both heating rates were almost identical (Fig. 1.3). At 8% solids, the maximum hot viscosity of hard wheat flour was not affected by the heating rate (Fig. 1.7a). In contrast, a higher heating rate (10^{o}C/min) resulted in a higher maximum hot viscosity for soft wheat flour (Fig. 1.8a). SSL had no effect on hot maximum viscosity of hard (Fig. 1.7a) and soft wheat flour (Fig. 1.8a).

When heated at 10^{o}C/min (Fig. 1.7), the maximum hot viscosity of hard wheat flour plus OLA responded in the same manner as hard wheat plus SSL (Fig. 1.7b). There was no apparent effect of emulsifier concentration. In the case of soft wheat flour plus OLA heated at 10^{o}C/min (Fig. 1.8b), lower emulsifier concentrations decreased the maximum hot viscosity. The higher inclusion levels responded in the same manner as with SSL. At the increased cooling rate in the presence of SSL, hard wheat flour (Fig. 1.7c) and soft wheat flour (Fig. 1.8c) exhibited similar final viscosities. The same was true for final viscosity response of hard wheat flour and OLA at the increased cooling rate (Fig. 1.7d); both in the change from control values and the effects of emulsifier concentration similar to wheat starch. Similarly, the final viscosity response to OLA with soft wheat flour at increased cooling rates (Fig. 1.8d) mimicked its maximum hot paste viscosity response.

Visual characteristics of gels

The analysis of the visual differences between starch gels with either emulsifier is shown in Figure 1.9. At 6% solids, differences of the emulsifiers were clear (Fig, 1.9a). Starch plus OLA formed a gel and increased viscosity more than starch with SSL. As the solid level increased, the difference in gel formation between the two emulsifiers was less noticeable. This may be due to the increase of available starch with increased solid content level.

Swelling and solubility

Swelling power and solubility

The swelling power and solubility of normal wheat (control) starch and starch plus either emulsifier are shown in Table 1.2. As expected, as temperature increased, past gelatinization, swelling power and solubility increased. The greatest increase occurred from 85 to 95^{o}C. This was consistent with Ikegwu et al. (2010) who reported that the highest swelling power and solubility was across the temperature range of 80-90^{o}C. Previous results reported by Ghiasi et al. (1982) found that at temperatures below 85^{o}C, the presence of SSL reduced the swelling power. Eliasson (1985) suggested that depending on the polar group of the added emulsifier, the solubility of the complexes formed might differ. Moreover, it has been suggested that the swelling power of the granules reflect the extent of associative forces within the granule (Moorthy and Ramanujam, 1986).

For swelling power and solubility, all values followed the expected pattern with respect to temperature (Table 1.2a) and in agreement with Ikegwu et al. (2010) who reported that the solubility pattern had a correlation with swelling power. At temperatures below 85^{o}C, swelling power values paralleled those of the control starch (Ghiasi et al., 1982) except at 2.0% OLA that appeared most restricting. Above 85^{o}C, swelling power values were reduced with the addition of both emulsifiers (Table 1.2a). The solubility (Table 1.2b) results found were similar to Shi et al (1991) and were interesting in that the emulsifiers appear to act differently at lower temperatures. At 65^{o}C, the difference in solubility interaction showed starch samples containing OLA reducing it, while in the presence of SSL (Table 1.2b), starch samples had similar or an increased solubility like the control. With emulsifiers present, swelling power and solubility were dependent on both temperature and the amount of emulsifier.

Effects on granular swelling

Figure 1.10 compares the swelling of normal wheat starch (control), while Figure 1.11 compares the swelling of hard wheat flour (control) and samples containing emulsifiers (a-d), all in excess water. Row 1 presents the control starch/flour (no emulsifiers), Row 2, the control plus SSL, while Row 3 shows the control plus OLA.

The swelling of normal wheat starch granules during gelatinization has been shown to be delayed in the presence of a certain emulsifier (Eliasson, 1985). For temperatures between 30-50^{o}C, no major changes occurred in granular size or shape for the starch and flour granules with both emulsifiers. The swelling onset temperature for wheat starch/flour was established at or around 60^{o}C comparable to results reported by Maningat and Seib (2010). At 60^{o}C, the control starch and starch plus SSL demonstrated slight swelling (Fig. 1.10a), while the starch plus OLA granules showed no visible change in granule shape or size. By 70°C, control wheat starch granules had swollen extensively and started to deform (Fig. 1.10b). At 80°C, granular swelling in the presence of OLA was still quite limited (Fig. 1.10c). By 90°C, control starch and starch in the presence of SSL appeared similar, while starch in the presence of OLA granular swelling was still occurring (Fig, 1.10d). Granules with OLA were swollen less than control starch or starch plus SSL.

At 60°C, wheat flour plus SSL displayed more visible swelling than did flour plus OLA (Fig. 1.11a). By 70°C, granules in the presence of SSL continued to swell in flour (Fig. 1.11b), while granules in the presence of OLA had only started to swell. At 80°C, flour with SSL had granular swelling and deformation more extensive than control flour or flour plus OLA (Fig. 1.11c). By 90°C, wheat flour plus SSL exhibited more prominent granular swelling and deformation, while flour plus OLA continued to swell and was beginning to exhibit granular deformation (Fig. 1.11d).

Amylose-lipid complex formation

DSC

Table 1.3 presents the thermal properties of granular wheat starch (control) and pre-cooked starch plus SSL or OLA as measured by DSC. During the initial heating, two endothermic peaks were evident for the control samples (un-cooked). One peak was observed between 50-85°C (Table 1.3), while the second peak was found around 100°C (Table 1.3). Results found agree with Moorthy et al. (2006) that when a starch suspension was heated in the presence of a surfactant, two endotherms were present one corresponding to starch gelatinization and the second due to the melting of the amylose-lipid complex. Only one peak was visible during cooling and a subsequent 2nd heating (Table 1.3) showed the complex melting in agreement with Evans (1986) who concluded that the melting temperature of the amylose-lipid complex was a measure of the average stability of a lengthy stretch of amylose interaction with lipid (emulsifier).

The first peak is the irreversible gelatinization peak (Evans, 1986) and occurs only during the 1st heating. For control starch, a gelatinization temperature peak was observed at ≈ 65°C; while the peak for starch plus SSL was almost 10°C lower (Table 1.3) similar to results reported by Evans (1986). The gelatinization temperatures at peak and ΔH values of control starch (11.4 J/g) were in agreement with the results reported by Abdel- Aal et al. (2002). For the starch pre-cooked with SSL, a small peak at around 52°C noted and probably due to the melting of SSL (Table 1.3). No peak was identified for pre-cooked starch with OLA (Table 1.3). When viewed microscopically in excess water (Fig. 0.2), the granular structure of starch was still visible after cooking with SSL and freeze-drying (Fig. 0.2a). While, pre-cooked starch and OLA was completely gelatinized (Fig. 0.2b).

The initial heating scans of wheat starch with and without emulsifiers confirmed a second peak around 100°C (Table 1.3) close to the temperatures reported by Xu et al. (1992) and Eliasson (1985) as the thermal transition temperature at peak of the amylose-lipid complex. OLA reduced these temperatures, but the ΔH values of the amylose-lipid complex peaks were greater with either emulsifier, than were those of the control. Kugimiya and Donovan (1981) found that high temperature endotherms indeed correspond to the melting of an amylose-lipid complex during initial heating. Comparing SSL and OLA (Table 1.3), the amylose-lipid complex melting temperature and ΔH values were not significantly different from each other, but were different from those of wheat starch alone (Table 1.3).

Wheat starch showed only one exothermic peak during cooling (Table 1.3). The cooling peak temperatures of control and SSL containing samples were equivalent, while temperatures were lower for starch with OLA (Table 1.3). Statistically, the two emulsifiers were different from each other in this regard, as well as from normal wheat starch alone. Both emulsifiers increased the ΔH values of the cooling peak, with a very small difference between the effect of SSL and OLA. ΔH values were consistently greater for starch with OLA, than for starch with SSL. This contradicts the theory that heat stability of complexes between fatty acid anions and amylose increased with acid chain length and decreases with unsaturation (Blazek and Copeland, 2009).

A peak was also observed during the 2^{nd} heating (rescan) with and without the presence of emulsifiers. With control starch, the peak centered around a temperature of 104^{o}C and a ΔH value of 1.4J/g (Table 1.3.4). Eliasson (1994) reported that if a starch-lipid sample was heated, cooled, and then reheated, higher enthalpy values would occur during the second heating, as was found in the present study (Table 1.3). However, there were differences in the effects of the two emulsifiers. The ΔH of starch plus SSL rescan peak was lower than those equivalent peaks of starch plus OLA. Further, the onset and peak temperatures were greater for SSL containing samples (Table 1.3). Statistically, the effect of OLA presence was different from starch plus SSL for the rescan peak, while there was no difference, statistically, between normal wheat starch and starch in the presence of SSL.

The amylose-lipid complex peak was smaller for the control than for SSL or OLA containing samples for the initial heating, cooling, and rescanning (Table 1.3). Onset and final temperatures, as well as thermal energy (ΔH) were affected by the starch-emulsifier interaction. The amylose-lipid complex peaks resulting from an addition of SSL were similar to those of

OLA containing samples (Table 1.3). Consistently, the resulting ΔH values found were greater in the presence of OLA than of SSL suggesting that starch in the presence of OLA complexes more than starch with SSL.

Table 1.4 and Table 1.5 presents results of equivalent studies using hard wheat flour and soft wheat flour with and without SSL and OLA. In both flour systems, the control exhibited a gelatinization peak, while the pre-cooked samples containing emulsifiers showed no peak. This was expected because samples had gelatinized during MVA cooking. The ΔH values of flours containing SSL or OLA were not statistically significantly different from each other. Peak temperatures for both flours during the initial heating, cooling, and reheating (Table 1.4, 1.5) were similar to results found in this study for wheat starch as well as other previous studies (Evans, 1986; Moorthy et al., 2006). The ΔH values for the flour systems were close to those findings reported by Munzing (1991) (Table 1.4, 1.5). The effect on hard wheat flour did not differ between emulsifiers, but in both cases, their presence differed from control flour values (Table 1.4). In contrast, with soft wheat flour, emulsifier presence produced results significantly different from the control and each other, in all cases, except gelatinization (Table 1.5).

With hard wheat flour, the amylose-lipid complex peak during both initial heating and cooling was affected by emulsifier presence, but the effect was equivalent for both SSL and OLA (Table 1.4). During the 2^{nd} heating, hard wheat plus SSL's peak temperature were greater than with OLA, while the ΔH value of hard wheat plus OLA was greater than hard wheat with SSL (Table 1.4). Endotherm values indicate soft wheat complexes more with SSL (Table 1.5) and soft wheat plus SSL was found to be significantly different from both soft wheat plus OLA and soft wheat alone.

XRD

XRD was used to examine the type and extent of complexity resulting from cooling of the starch paste in the presence of SSL or OLA. Increased relative intensity would generally reflect a greater degree of complexity.

Without emulsifiers, control starch showed a typical A-type pattern (Fig. 1.12a) in agreement with previous reports (Blazek & Copeland, 2009). With the addition of either of the emulsifiers, wheat starch exhibited a typical V-type pattern after cooking and cooling (Fig. 1.12b, 1.12c), again similar to findings in previous reports (Birnbaum, 1977; Ghiasi et al., 1982).

When Tang and Copeland (2007) mixed stearic acid (Fig. 0.4a) and wheat starch the diffraction pattern had peaks at 2θ at 7.4^{o}, 12.7^{o}, and 19.8^{o}; the same V-type pattern was produced for starch with SSL in the present study. The same work (Tang and Copeland, 2007) studied oleic acid/starch XRD patterns. No specific data was published, but the authors stated the presence of oleic acid (Fig. 0.4b) reduced the relative intensity of the diffraction patterns of the starch. Results of the current study found that patterns of starch/OLA had slightly greater relative intensity than did the patterns of starch plus SSL. Copeland et al. (2009) postulated that some lipids tend to self-associate and Tang and Copeland (2007) reported that lipids with low water solubility have a tendency to self-associate rather than form complexes. This may well explain the decrease in relative intensity.

In the flour systems, XRD patterns in the presence of either emulsifier were identical in respect to the type of crystalline structure, crystallinity, and shift in peaks found with wheat starch. Like wheat starch, diffraction patterns of OLA plus either flour (Fig. 1.12b) had a slightly greater intensity than did the diffraction patterns of either flours plus SSL (Fig. 1.12c). Unlike wheat starch, diffraction peaks were not as prominent with either emulsifier. With the addition of emulsifiers, wheat starch and wheat flour produced a V-type diffraction pattern, while native wheat starch and flour had a typical A-type pattern similar to results reported by Abdel-Aal et al. (2002).

Conclusions

Increased solid content diminished the effect of the emulsifiers on the pasting onset temperature of starch and flour. Increased solid content level increased maximum hot and final viscosities. However, the maximum and final viscosity of both starch and flour systems appeared dependent on the presence of either emulsifier, especially at the lowest solid content level (6%). In addition, increased heating rate increased the maximum hot viscosity of wheat starch plus SSL and of flour plus OLA over flour plus SSL. An increased cooling rate increased the final viscosities of starch plus both emulsifiers, while neither emulsifier had more of an effect on final viscosity than did the other for flour. Yet, neither emulsifier provided any interaction with an effect on pasting onset temperature with an increased heating and cooling rate.

Swelling power and solubility values of control starch and all inclusions of emulsifiers increased with increased temperature. Lower temperatures determined the interaction of either emulsifier, but neither emulsifier provided more of an effect on swelling power or solubility at increased temperatures. In the presence of OLA, granular swelling of starch/flour was inhibited more than was in the presence of SSL. DSC determined that starch and hard wheat flour samples containing OLA complex more than in the presence of SSL, while soft wheat samples containing SSL seemed to complex more than soft wheat plus OLA. XRD showed no difference in crystalline pattern between the two emulsifiers (V-type) for starch or flour and it appeared that more interaction was found in the presence of OLA.

Figures and Tables

Table 1.1 Chemical composition of the wheat flours

	Moisture (%)	Protein (%)	Ash (%)	Amylose (%)
Hard wheat flour	13.0 ± 0.27	14.2 ± 0.01	0.53 ± 0.02	23.5 ± 0.9
Soft wheat flour	10.0 ± 0.02	8.6 ± 0.02	0.42 ± 0.00	26.8 ± 0.6

Table 1.2 Effect of temperature on a) swelling power and b) solubility of normal wheat starch with SSL or OLA

a)

Swelling Power (g/g)

Sample	**65°C**		**75°C**		**85°C**		**95°C**	
Control	10.2 ± 0.5	**a**	12.9 ± 0.8	**a,b**	15.2 ± 0.4	**a**	30.4 ± 0.1	**a**
1% SSL	10.0 ± 0.1	**a,b**	11.8 ± 0.3	**a**	13.5 ± 0.6	**b**	28.4 ± 1.1	**b,c**
2% SSL	9.3 ± 0.1	**b,c**	12.2 ± 0.6	**a,b**	13.7 ± 0.1	**b**	29.3 ± 0.0	**a,b**
1% OLA	9.3 ± 0.3	**c**	11.4 ± 0.6	**a**	13.6 ± 0.2	**b**	28.9 ± 0.4	**a,b,c**
2% OLA	7.5 ± 0.2	**d**	10.2 ± 0.6	**b**	13.9 ± 0.6	**b**	27.3 ± 1.0	**c**

Mean ± Standard deviation; n=2
Values in the same column with the same letters do not differ significantly ($p < 0.05$).

b)

Solubility (%)

Sample	**65°C**		**75°C**		**85°C**		**95°C**	
Control	1.4 ± 0.1	**a,b**	7.3 ± 0.4	**a**	5.5 ± 0.7	**a**	23.8 ± 0.3	**b**
1% SSL	2.5 ± 0.3	**a**	3.0 ± 1.0	**b**	6.5 ± 1.4	**a**	23.3 ± 0.0	**b**
2% SSL	1.4 ± 0.1	**a,b**	4.8 ± 0.1	**b**	5.9 ± 1.6	**a**	29.6 ± 2.5	**a**
1% OLA	0.8 ± 0.3	**b**	5.0 ± 1.3	**b**	6.4 ± 0.1	**a**	22.1 ± 0.3	**b**
2% OLA	1.2 ± 0.8	**b**	0.5 ± 0.3	**c**	9.5 ± 1.5	**a**	21.9 ± 0.7	**b**

Mean ± Standard deviation; n=2
Values in the same column with the same letters do not differ significantly ($p < 0.05$).

Table 1.3 Thermal properties of granular normal wheat starch and pre-cooked starch with emulsifiers determined by differential scanning calorimetry

	T_0 (^OC)		T_p (^OC)		T_c (^OC)		ΔH (J/g)	
1st scan - Gelatinization								
***Starch**	52.2 ± 0.0	**a**	65.6 ± 0.6	**a**	83.0 ± 1.0	**a**	11.4 ± 0.5	**a**
4% SSL	42.8 ± 0.8	**b**	51.6 ± 0.5	**b**	62.5 ± 0.3	**b**	1.7 ± 0.3	**b**
4% OLA	np	**c**	np	**c**	np	**c**	np	**c**
1st scan- Amylose Lipid Complex								
***Starch**	91.6 ± 0.1	**a,b**	101.0 ± 0.6	**b**	112.3 ± 0.1	**b**	0.9 ± 0.4	**b**
4% SSL	92.2 ± 1.5	**a**	105.8 ± 0.8	**a**	116.5 ± 0.5	**a**	4.6 ± 0.2	**a**
4% OLA	88.5 ± 1.0	**b**	99.7 ± 0.1	**b**	113.2 ± 1.4	**b**	5.6 ± 0.6	**a**
Cooling								
***Starch**	88.1 ± 0.7	**a**	81.2 ± 1.6	**a**	76.8 ± 1.7	**a**	-0.7 ± 0.2	**c**
4% SSL	90.1 ± 0.0	**a**	82.2 ± 0.5	**a**	73.6 ± 1.0	**a**	-2.7 ± 0.4	**b**
4% OLA	85.3 ± 0.8	**b**	75.4 ± 1.4	**b**	64.3 ± 2.5	**b**	-3.9 ± 0.0	**a**
2nd scan –Rescan								
***Starch**	90.6 ± 1.4	**b**	103.8 ± 0.8	**a**	114.3 ± 1.5	**a,b**	1.4 ± 0.1	**b**
4% SSL	93.9 ± 0.1	**a**	102.3 ± 0.4	**a,b**	110.8 ± 2.1	**b**	2.5 ± 0.5	**b**
4% OLA	86.4 ± 0.3	**c**	97.0 ± 0.0	**b**	115.8 ± 0.3	**a**	4.9 ± 0.7	**a**

Values in the same column (per group of 3) with the same letters do not differ significantly ($p < 0.05$)
*: un-cooked
np: no peak

Table 1.4 Thermal properties of granular hard wheat flour and pre-cooked flour with emulsifiers determined by differential scanning calorimetry

	T_o (^OC)		T_p (^OC)		T_c (^OC)		ΔH (J/g)	
1st scan - Gelatinization								
***HWF**	55.5 ± 1.3	**a**	67.5 ± 1.0	**a**	87.7 ± 1.0	**a**	7.8 ± 0.7	**a**
4% SSL	np	**b**	np	**b**	np	**b**	np	**b**
4% OLA	np	**b**	np	**b**	np	**b**	np	**b**
1st scan – Amylose Lipid Complex								
***HWF**	89.4 ± 0.2	**a**	98.6 ± 0.9	**b**	113.5 ± 0.6	**c**	1.4 ± 0.9	**b**
4% SSL	84.1 ± 0.2	**b**	101.5 ± 0.6	**a**	121.9 ± 0.1	**b**	6.4 ± 1.1	**a**
4% OLA	82.5 ± 0.6	**b**	98.8 ± 0.0	**b**	115.0 ± 0.2	**a**	7.2 ± 0.2	**a**
Cooling								
***HWF**	80.0 ± 0.1	**b**	71.3 ± 0.7	**a**	61.0 ± 0.1	**b**	-1.3 ± 0.7	**a**
4% SSL	90.6 ± 1.5	**a**	73.5 ± 1.7	**a**	62.1 ± 0.1	**a**	-2.5 ± 0.5	**a**
4% OLA	82.2 ± 1.9	**b**	70.9 ± 0.7	**a**	58.6 ± 0.5	**c**	-2.0 ± 0.0	**a**
2nd scan – Rescan								
***HWF**	88.9 ± 0.7	**a**	103.4 ± 3.0	**a**	114.2 ± 0.6	**b**	1.2 ± 0.6	**b**
4% SSL	83.3 ± 0.2	**b**	99.6 ± 0.5	**a,b**	120.1 ± 0.8	**a**	4.8 ± 0.5	**a**
4% OLA	79.6 ± 0.6	**c**	96.7 ± 0.2	**b**	116.3 ± 0.8	**b**	5.3 ± 1.4	**a**

Values in the same column (per group of 3) with the same letters do not differ significantly ($p < 0.05$)

*: un-cooked

np: no peak

Table 1.5 Thermal properties of granular soft wheat flour and pre-cooked flour with emulsifiers determined by differential scanning calorimetry

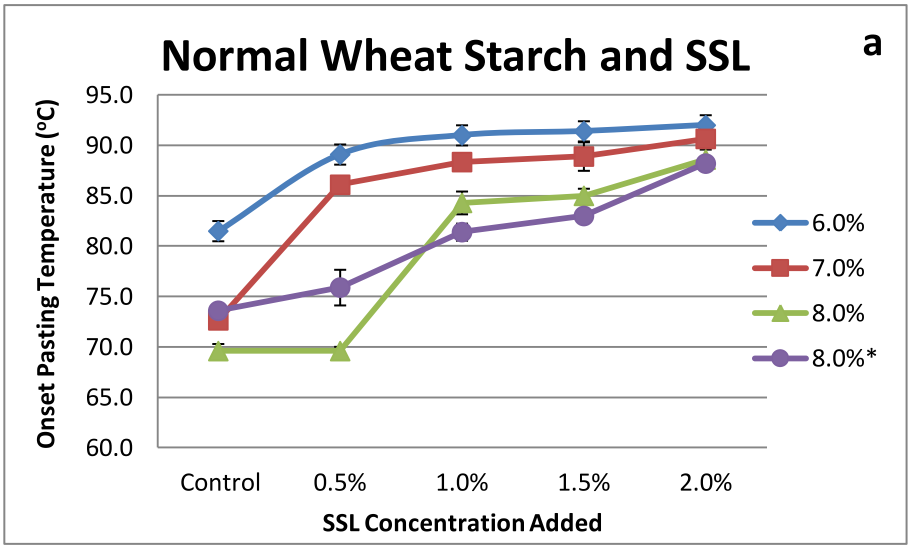
	T_o (^OC)	T_p (^OC)	T_c (^OC)	ΔH (J/g)
1st scan - Gelatinization				
***SWF**	54.5 ± 0.1 **a**	68.4 ± 0.0 **a**	88.0 ± 2.9 **a**	10.1 ± 1.4 **a**
4% SSL	np **b**	np **b**	np **b**	np **b**
4% OLA	np **b**	np **b**	np **b**	np **b**
1st scan – Amylose Lipid Complex				
***SWF**	87.2 ± 0.2 **a**	98.9 ± 0.5 **b**	108.6 ± 0.4 **c**	0.6 ± 0.0 **c**
4% SSL	83.1 ± 0.3 **b**	102.2 ± 0.5 **a**	123.2 ± 1.0 **b**	7.1 ± 0.3 **a**
4% OLA	81.3 ± 0.1 **c**	98.4 ± 0.1 **b**	116.4 ± 1.8 **a**	5.3 ± 0.3 **b**
Cooling				
***SWF**	79.4 ± 0.1 **b**	72.2 ± 0.7 **a**	55.7 ± 0.8 **b**	-1.1 ± 0.2 **c**
4% SSL	82.9 ± 0.4 **a**	73.7 ± 0.7 **a**	60.4 ± 0.0 **a**	-2.8 ± 0.1 **a**
4% OLA	79.9 ± 0.9 **b**	69.9 ± 0.9 **a**	53.0 ± 2.0 **b**	-2.4 ± 0.0 **b**
2nd scan - Rescan				
***SWF**	79.5 ± 0.0 **b**	100.5 ± 0.6 **a**	113.8 ± 0.0 **b**	1.3 ± 0.1 **c**
4% SSL	81.9 ± 0.6 **a**	100.1 ± 0.1 **a**	118.3 ± 0.6 **a**	4.9 ± 0.0 **a**
4% OLA	81.9 ± 0.5 **a**	97.3 ± 0.6 **b**	113.2 ± 0.2 **b**	3.6 ± 0.3 **b**

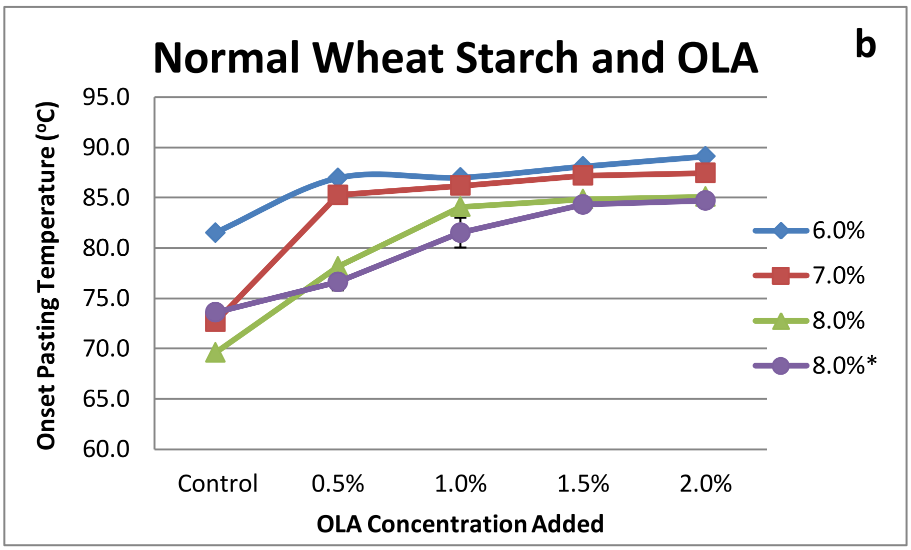
Values in the same column (per group of 3) with the same letters do not differ significantly ($p < 0.05$)

*: un-cooked

np: no peak

Figure 1.1 Pasting onset temperature of normal wheat starch a) with SSL and b) with OLA





*Increased heating rate of 10°C/min

Figure 1.2 Effects of emulsifier types on maximum and final viscosity of normal wheat starch all at heating rate of 6°C/min

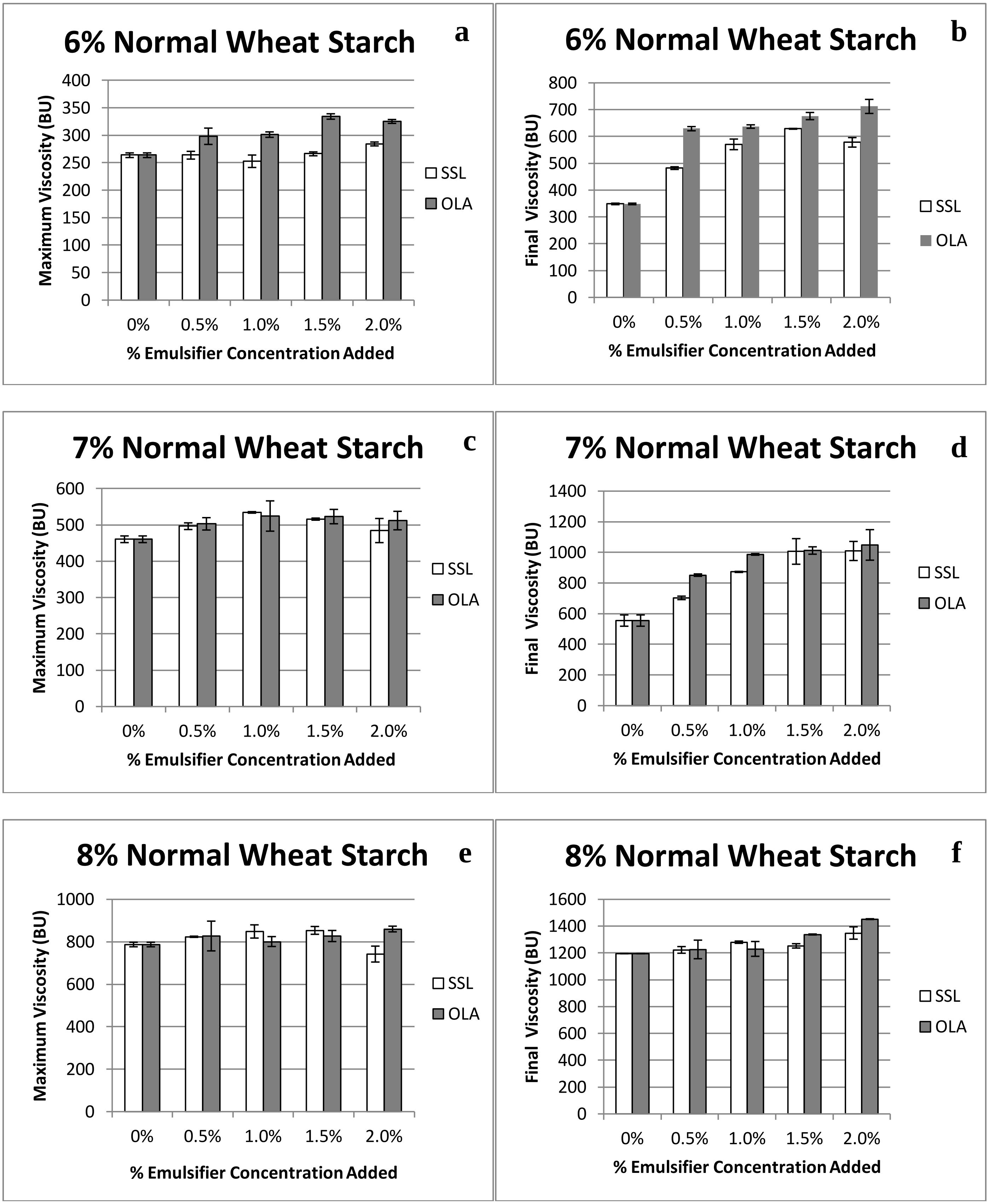
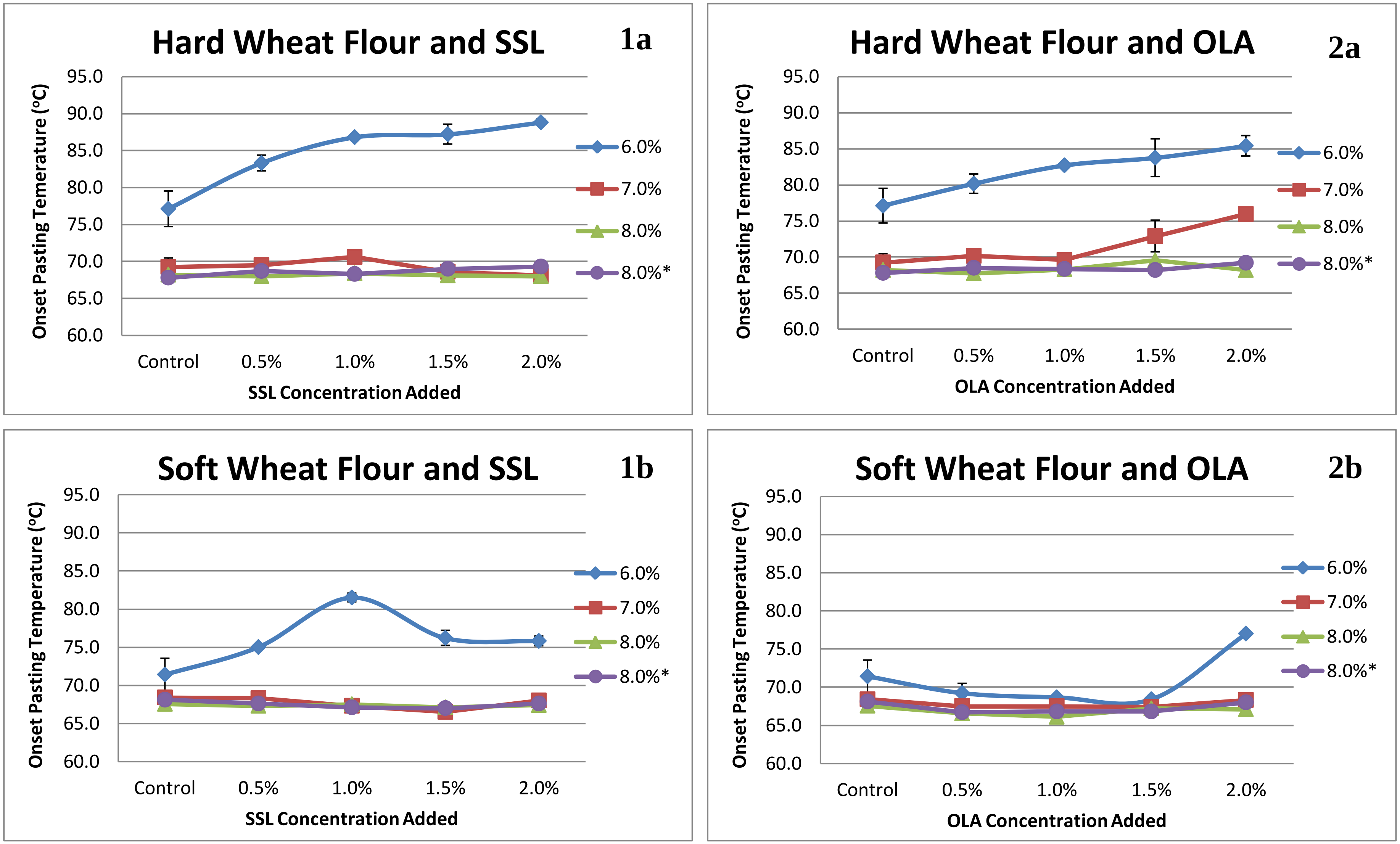


Figure 1.3 Pasting onset temperature of SSL and OLA a) with hard wheat and b) with soft wheat flour



*Increased heating rate of 10°C/min

Figure 1.4 Effects of emulsifier types on maximum and final viscosity of hard wheat flour all at heating rate of 6°C/min

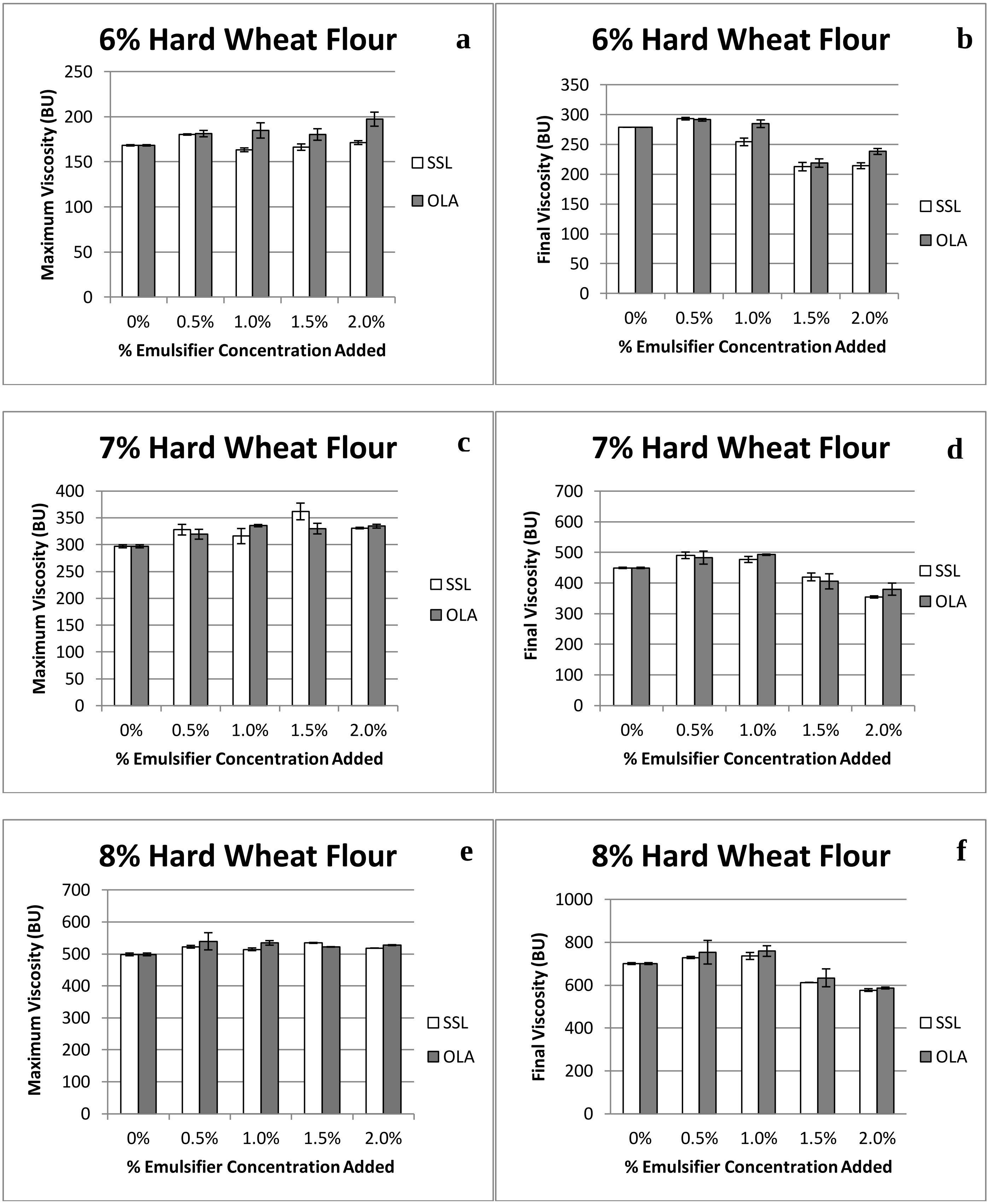


Figure 1.5 Effects of emulsifier types on maximum and final viscosity of soft wheat flour all at heating rate of 6°C/min

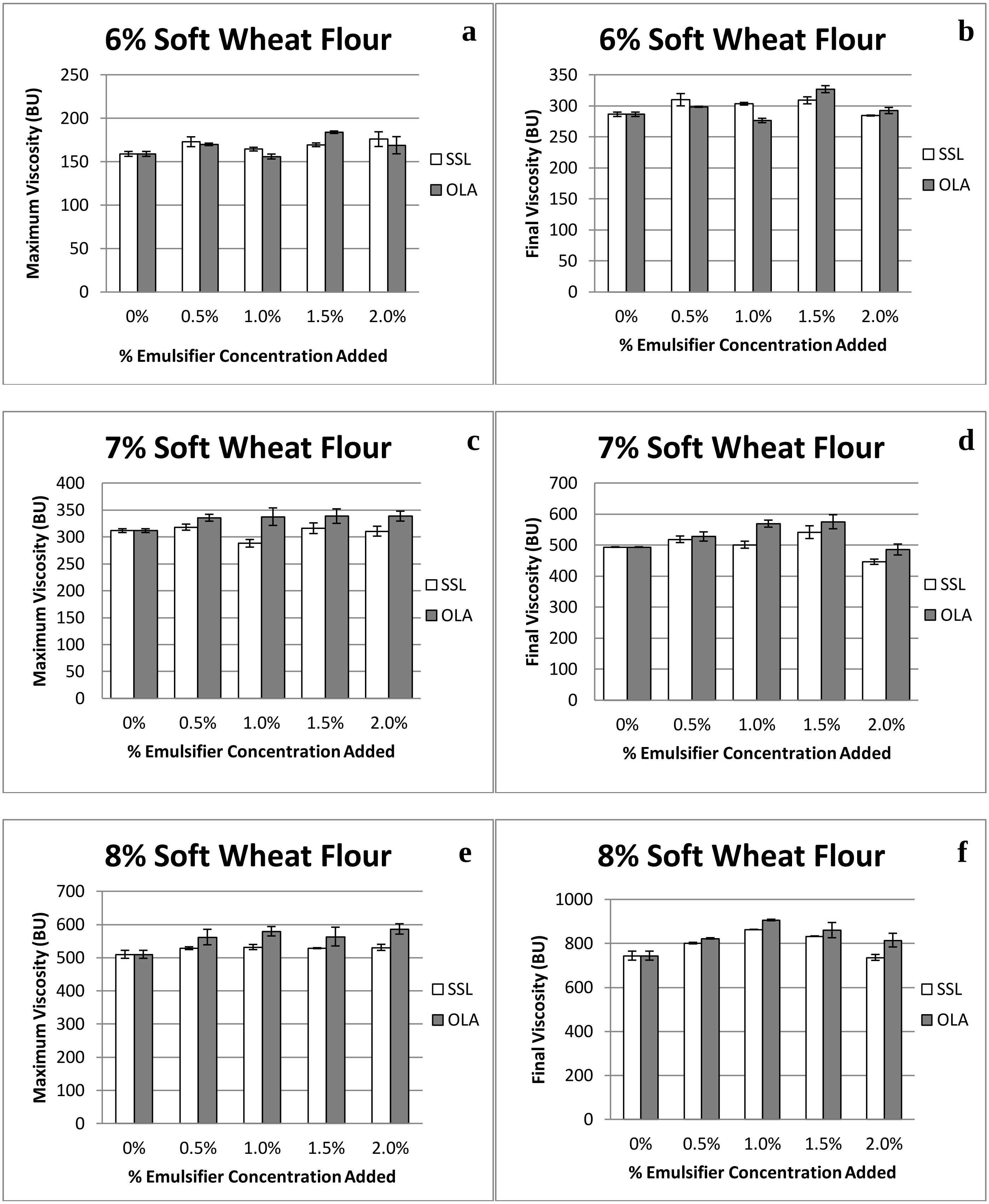


Figure 1.6 Effects of emulsifier types on maximum and final viscosity of normal wheat starch all at 8% solids level

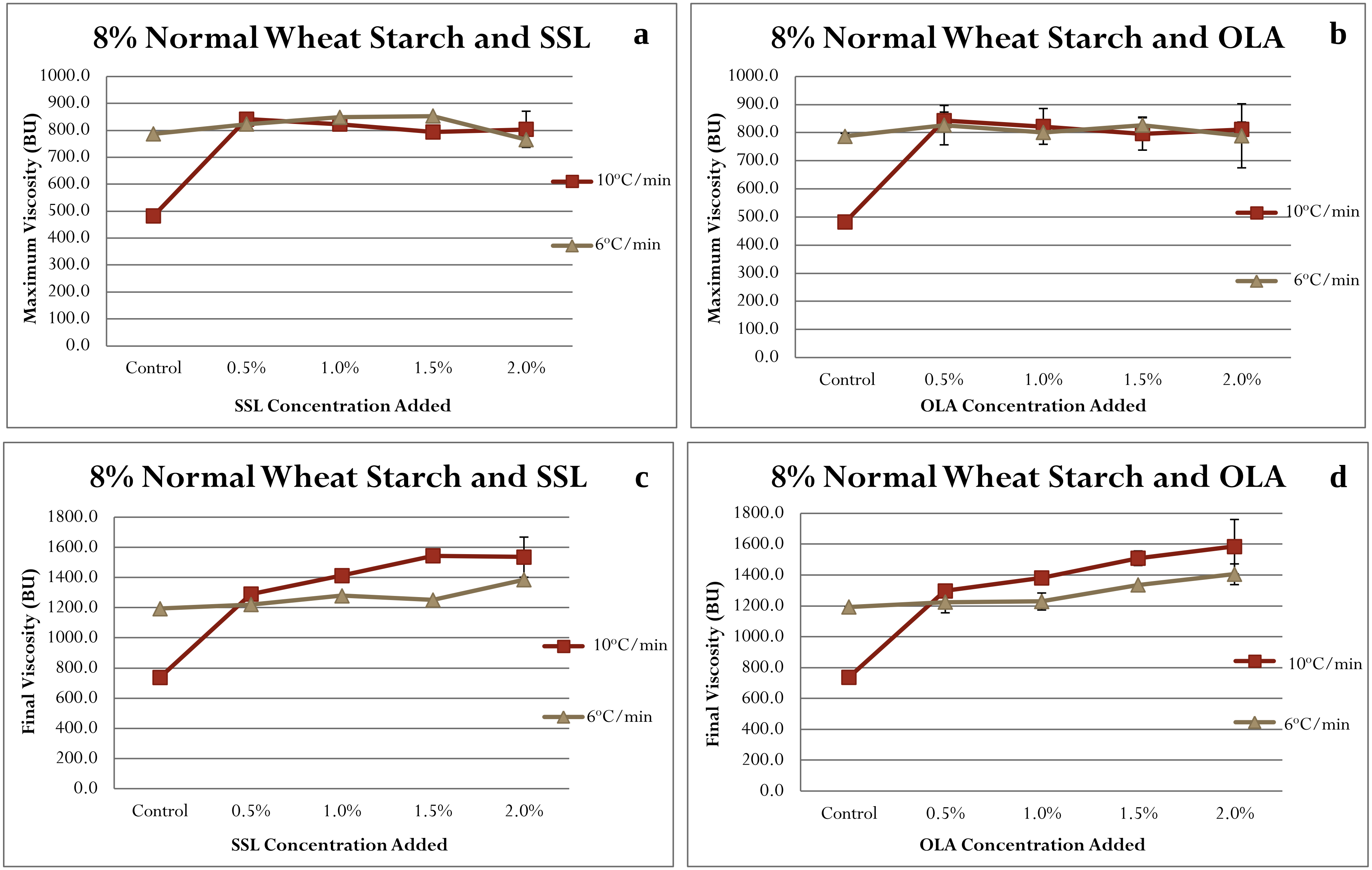


Figure 1.7 Effects of emulsifier types on maximum and final viscosity of hard wheat flour all at 8% solids level

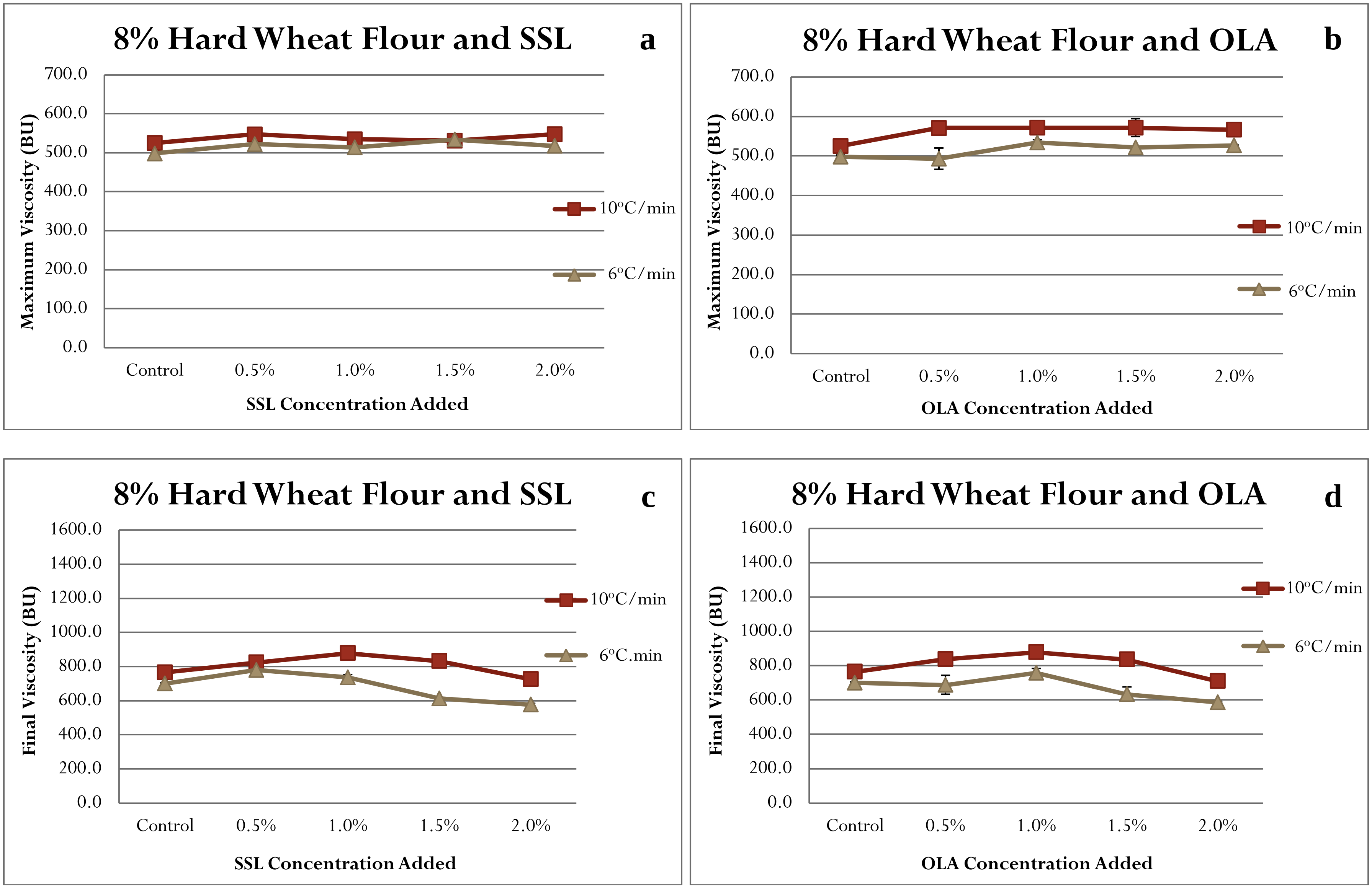


Figure 1.8 Effects of emulsifier types on maximum and final viscosity of soft wheat flour all at 8% solids level

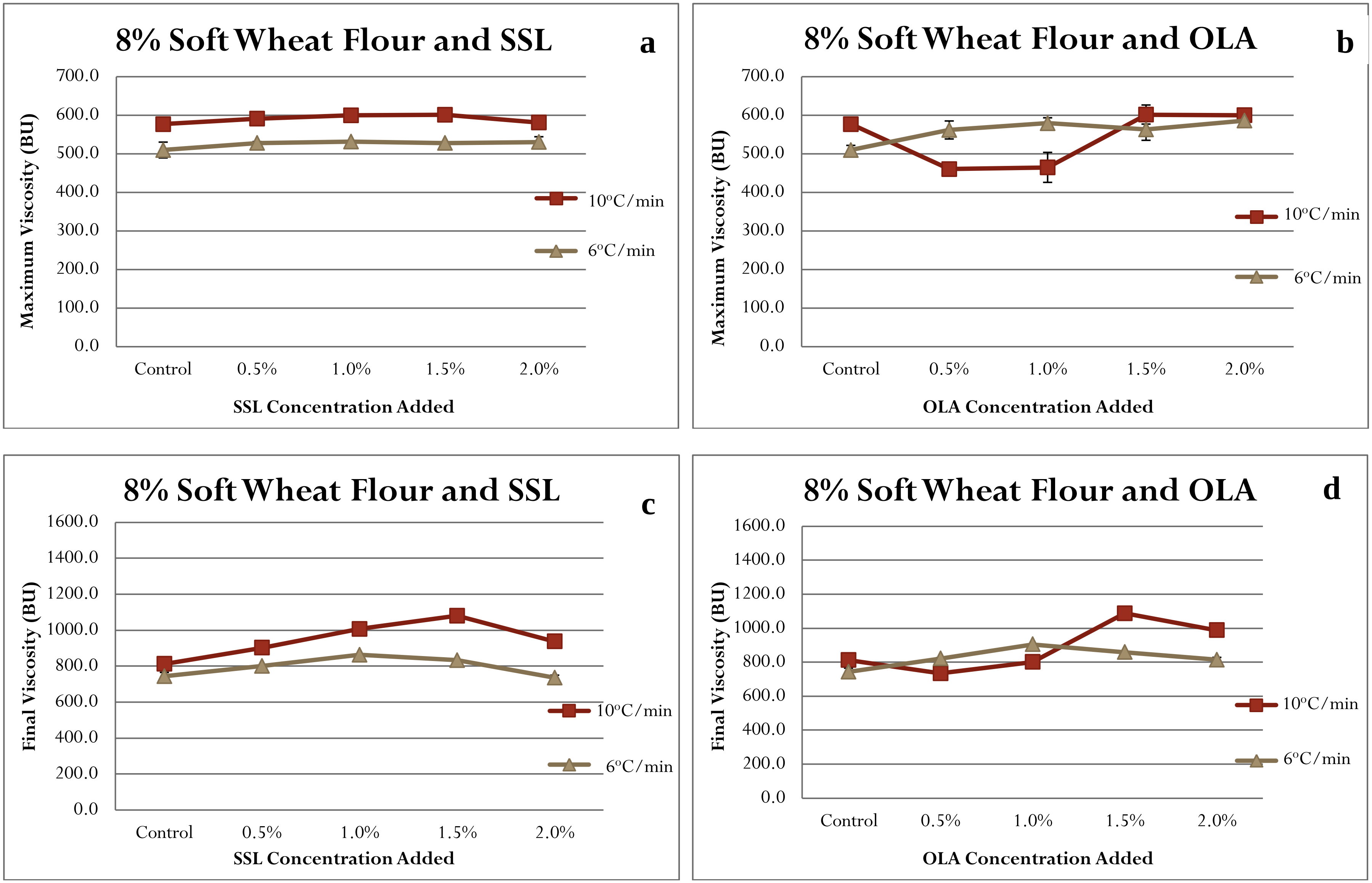


Figure 1.9 Images of normal wheat starch pastes after gelatinization and cooling at room temperature (25^{o}C) for 15 hours

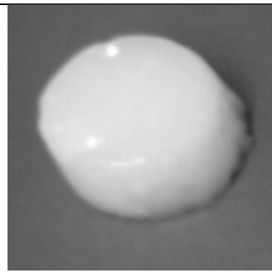
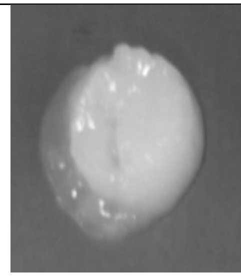
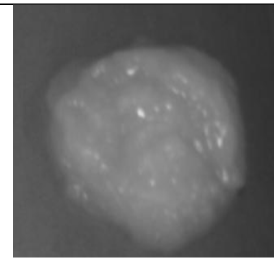
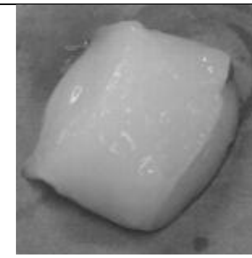
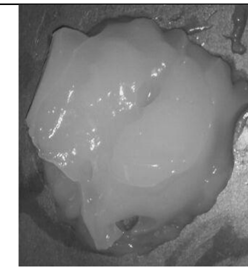
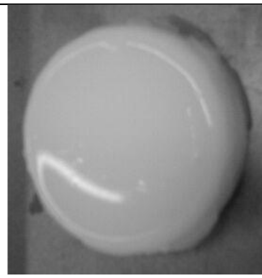
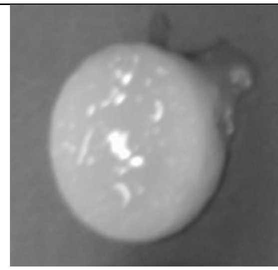
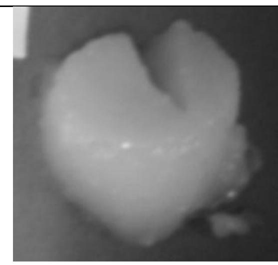
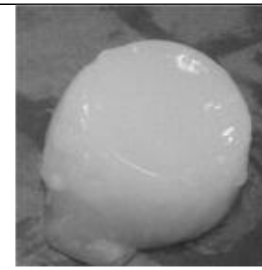
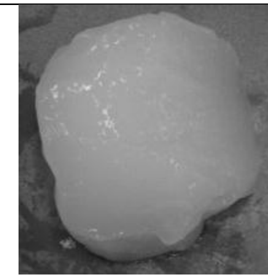
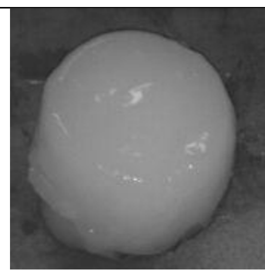
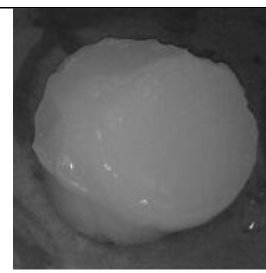
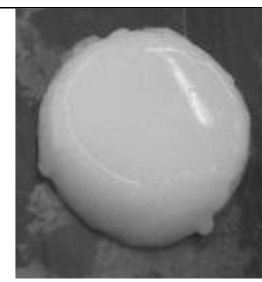
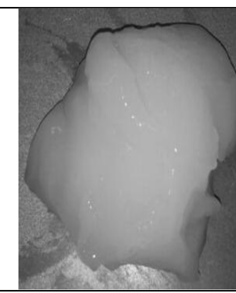
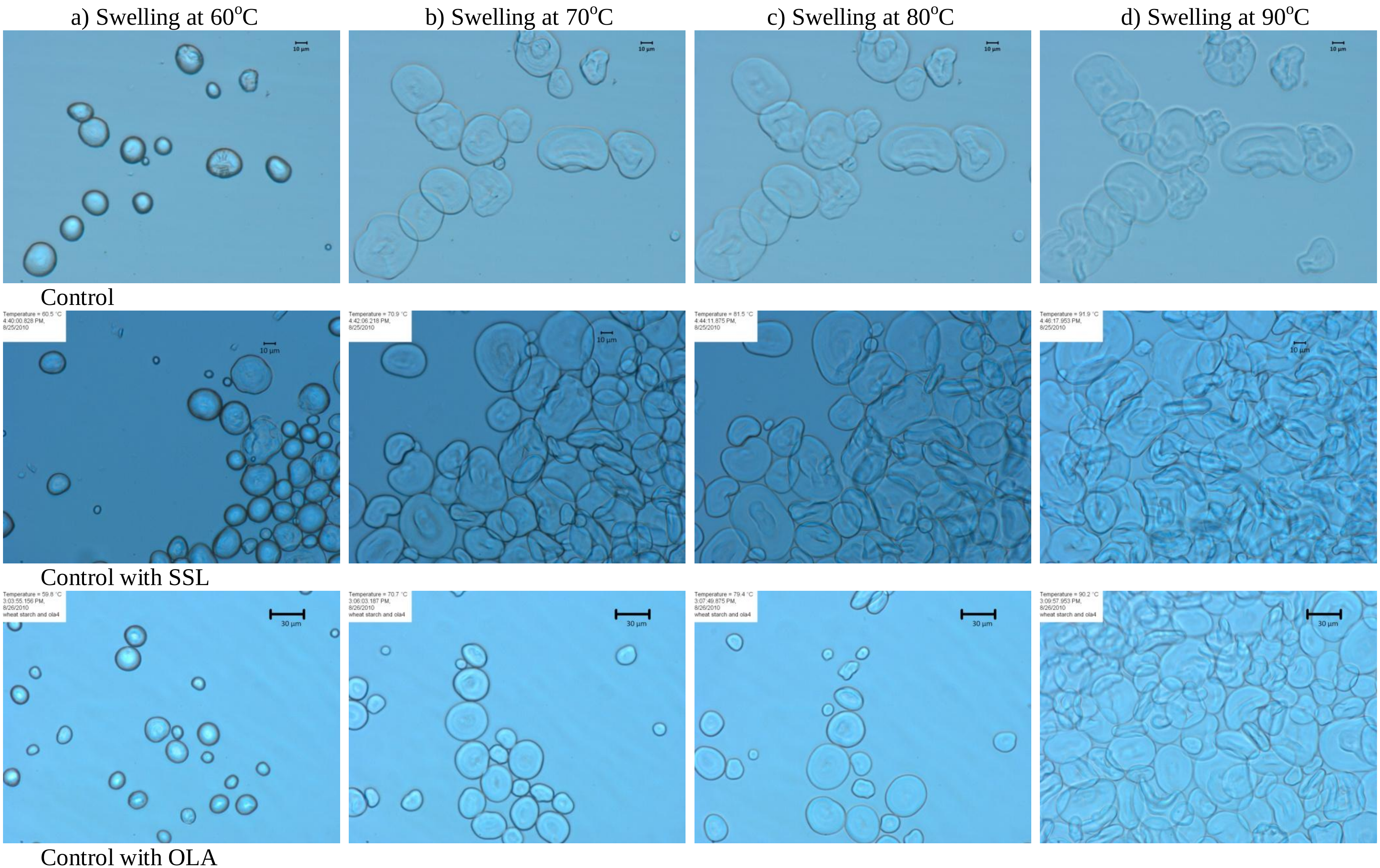
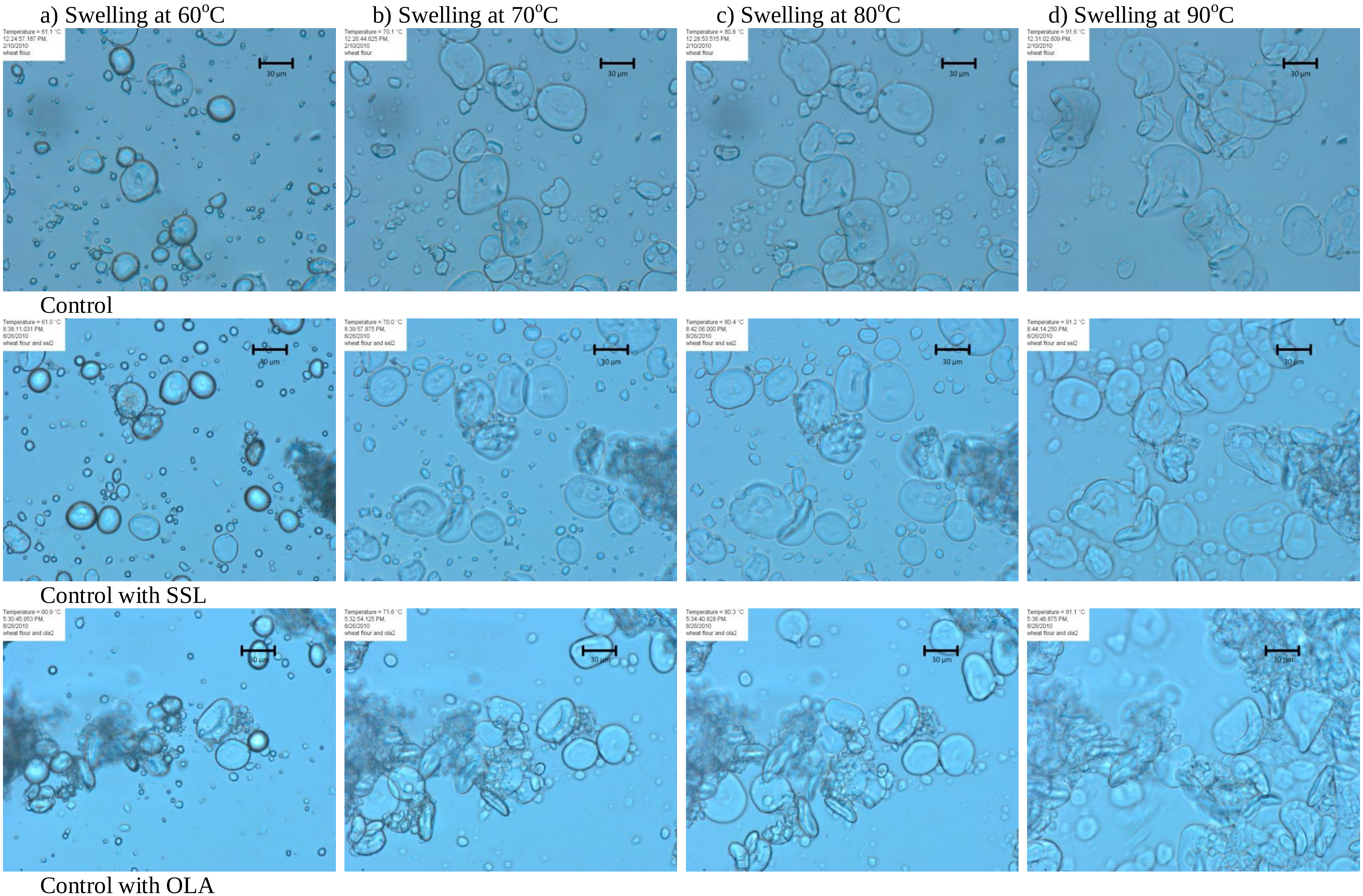
	CONTROL	1% SSL	2% SSL	1% OLA	2% OLA
6% SOLIDS (a)					
7% SOLIDS (b)					
8% SOLIDS (c)					

Figure 1.10 Microscopic images of the effect of temperature of granular swelling of normal wheat starch and emulsifiers



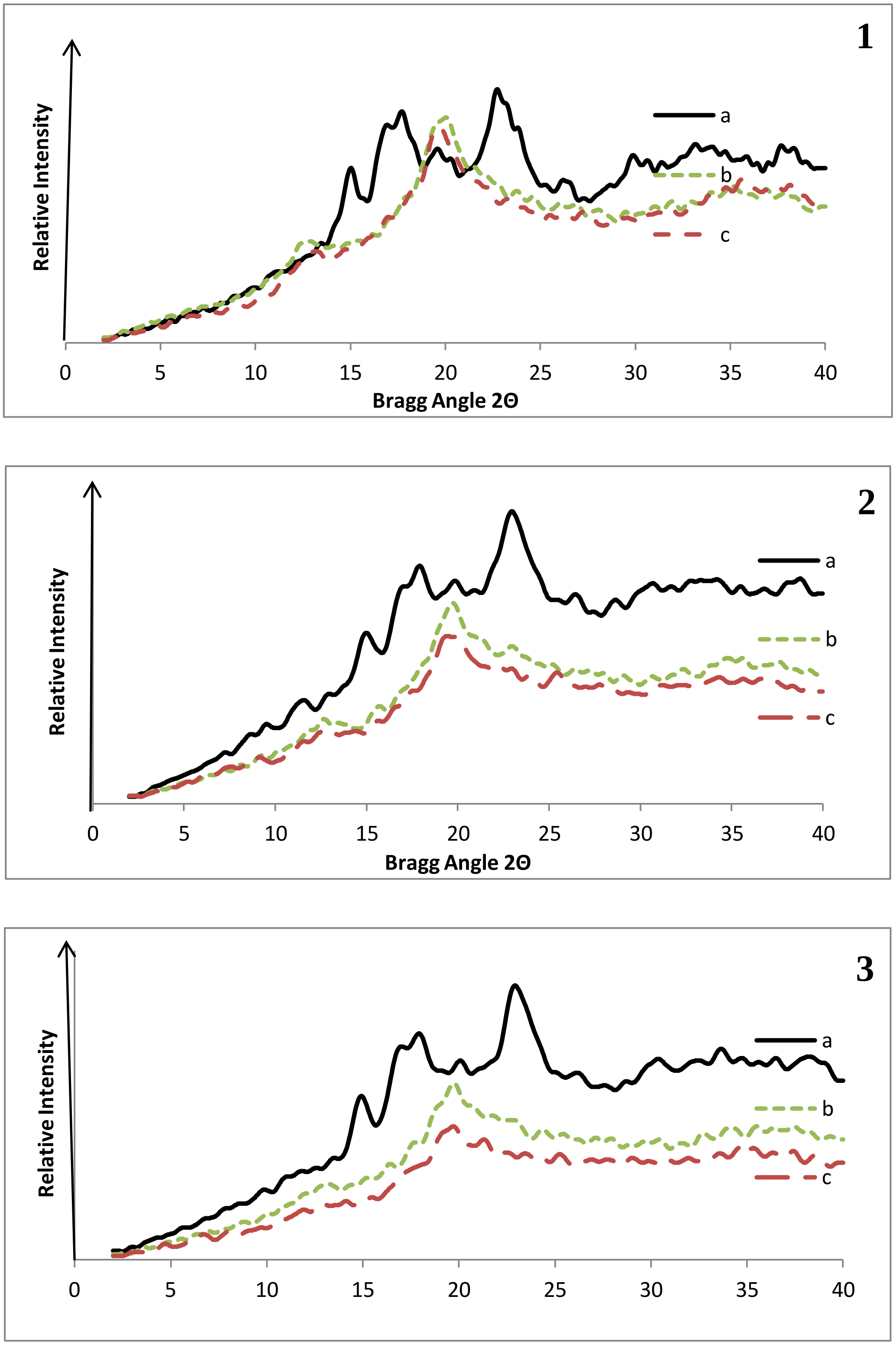
*Temperatures between 30-50°C showed no change; Not displayed

Figure 1.11 Microscopic images of the effect of temperature of granular swelling of hard wheat flour and emulsifiers



*Temperatures between 30-50°C showed no change; Not displayed

Figure 1.12 X-ray diffraction patterns of 1) normal wheat starch, 2) hard wheat flour and 3) soft wheat flour b) with OLA and c) with SSL



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Appendix

Figure 0.1 Normal Micro Visco Amylograph pasting curve of starch and emulsifier

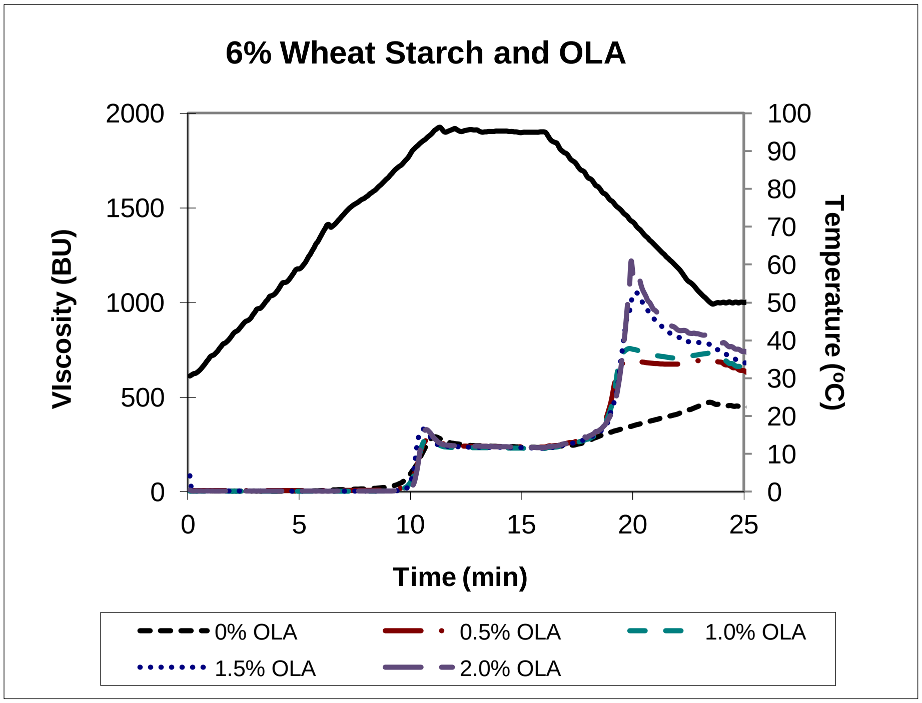
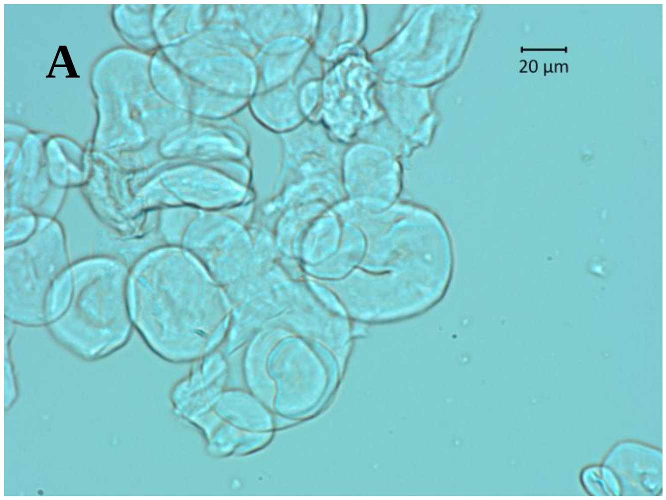


Figure 0.2 Microscopic images of cooked and freeze-dried normal wheat starch a) with 4% sodium stearoyl lactylate and b) with 4% oleyl lactylic acid



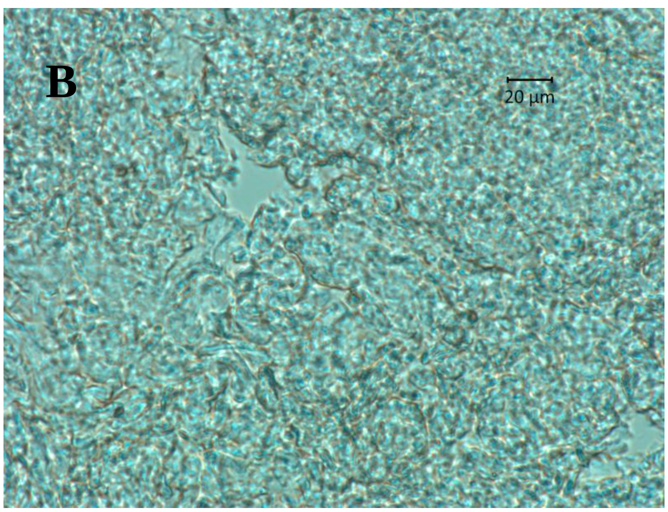
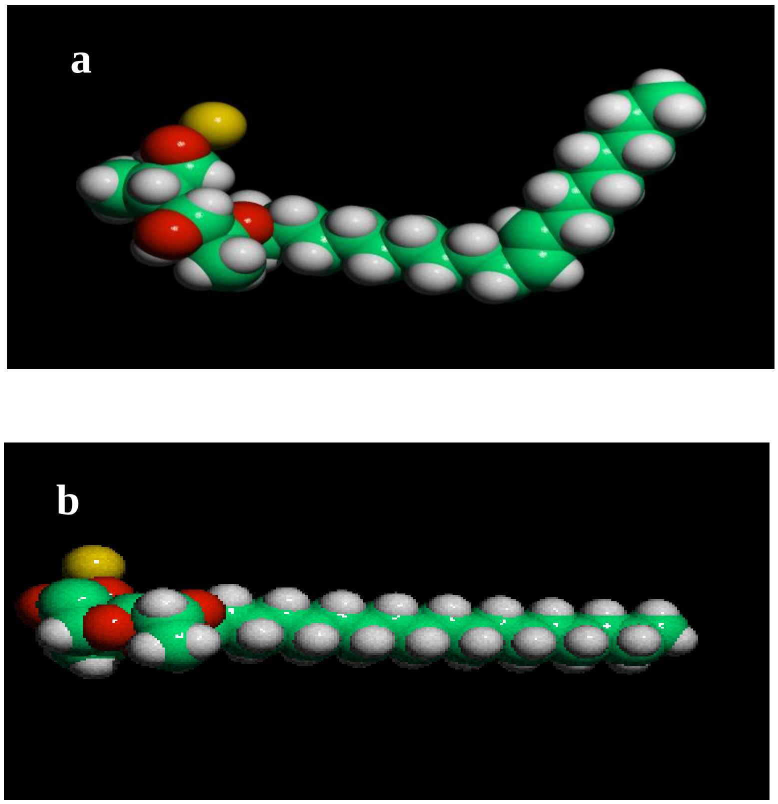
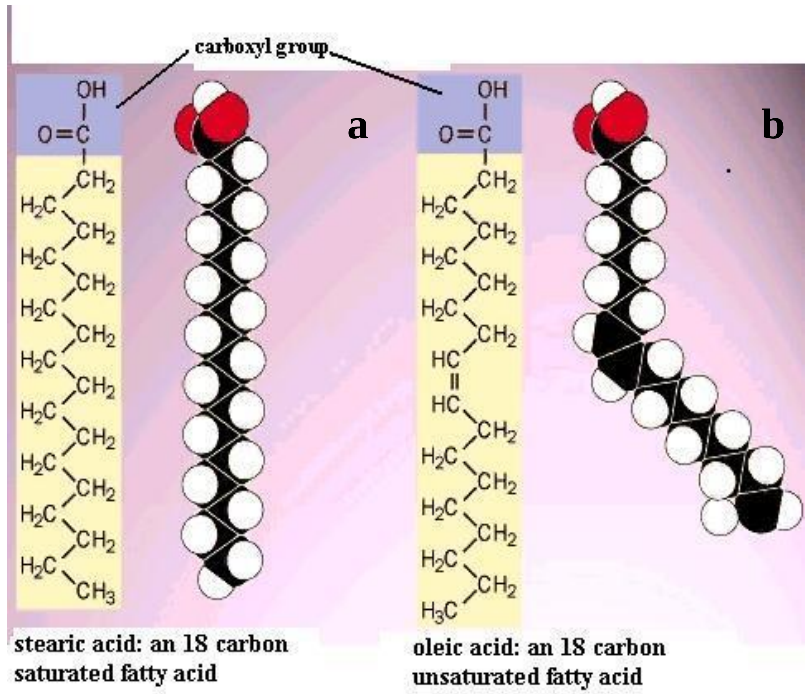


Figure 0.3 Structural comparison of a) sodium stearoyl lactylate (SSL) and b) oleyl lactylic acid (OLA)



Courtesy of Caravan Ingredients

Figure 0.4 Structural comparison of a) stearic acid and b) oleic acid



http://pirate.shu.edu/~rawncarr/molmodel/fattyacids.jpg