

“Curdling” of soymilk in coffee: A study of the phase behaviour of soymilk coffee mixtures



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ABSTRACT

Soymilk is often observed to “curdle” (become inhomogeneous) and then sediment in coffee, which makes it an undesirable product for consumers. This work investigates the phase behaviour (curdling) of an organic, unsweetened, long-life soymilk in instant coffee solutions for a range of coffee and soymilk concentrations, temperature and pH. The temperature vs soymilk concentration phase diagram exhibits a coexistence curve, separating a two-phase region at high temperature from a single-phase region at low temperature. The phase transformation is reversible and it is possible to recover a single phase mixture from the “curdled” mixture either by cooling the mixture or by increasing the soymilk concentration. Image analysis was used to investigate the time evolution of the phase separation and sedimentation processes. Fourier transformation of the images resulted in peaks corresponding to the growing length scale of the phase separation. The rate of growth of peak intensity over time shows a power law dependence on temperature characteristic of spinodal decomposition. However, the observed separation kinetics are not entirely straightforward to explain with common phase separation models.

1. Introduction

Switching from animal-to plant-based food sources would significantly alleviate local and global environmental problems to which livestock is a major contributor, including land degradation, climate change, water shortage and loss of biodiversity (Steinfeld, 2006). For example, in comparison to dairy milk, the production of an equivalent volume of soymilk is much less energy (Smedman, Lindmark-Mansson, Drewnowski, & Modin Edman, 2010) and water intensive (Ercin, Aldaya, & Hoekstra, 2011). However, one of the challenges of plant-based food alternatives is that they may not have the texture, taste or physicochemical behaviour of meat and dairy products. One example of this is the use of soymilk in coffee drinks, where the soymilk is often observed to “curdle” or phase separate into two phases with different soymilk concentrations (see Fig. 1) and the similarity in appearance to spoiled dairy milk makes the product unappealing to drink.

Anecdotal evidence suggests that the curdling behaviour is influenced by the concentration of soymilk, the acidity of the coffee, temperature and even the order of adding/mixing the coffee, water and soymilk. There are several brands of soymilk that do not readily exhibit the curdling effect in coffee, and these generally contain acidity regulators and stabilisers, such as monopotassium phosphate and gellan gum, respectively. This suggests that the occurrence of curdling of soymilk in coffee, which involves protein coagulation or aggregation, is

influenced by temperature and pH.

While there are numerous studies on isolated soy protein systems, which differ considerably from soymilk, soymilk and soymilk particles have received less attention. Soymilk is a hydrocolloid containing proteins, lipids, minerals and other components, and is much more complex than the soy protein isolate. Soymilk is extracted from whole soybeans and is prepared by soaking soybeans in water, wet grinding, heat treatment, and filtration (Giri & Mangaraj, 2012). The lipid, saccharide, and protein components of soybeans are retained in the soymilk (Guo, Ono, & Mikami, 1997). The composition of soymilk varies depending on processing conditions and bean variety but in general contains about 2%w/w lipid, 3–4%w/w protein, and 2%w/w carbohydrate (Cruz et al., 2007; Giri & Mangaraj, 2012; Iwuoha & Umunnakwe, 1997). The main proteins present in soybean are glycinin (11S) and β -conglycinin (7S), which constitutes about 70% of the total soy protein (Euston, Al-Bakkush, & Campbell, 2009). The complex behaviour of colloidal systems under various conditions (pH, ionic strength, buffer salts, temperature) is still not well understood and the conditions under which aggregation phenomena occur can vary significantly, depending on the system and the application targeted.

In this work we determine the conditions leading to curdling of soymilk in coffee, and provide insight into the separation and sedimentation mechanisms. A phase diagram for soymilk in coffee is developed for a range of soymilk-coffee-water concentration, temperature

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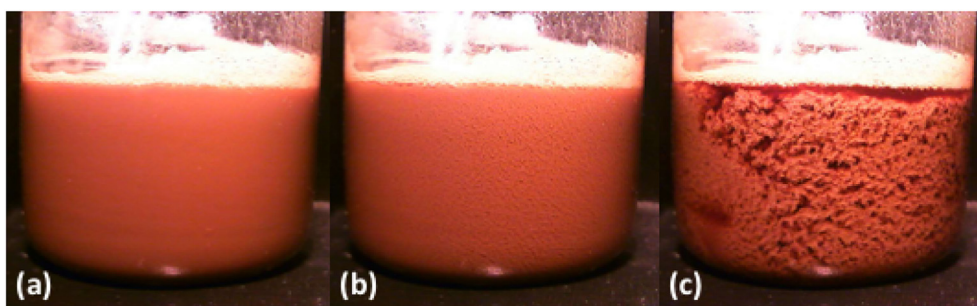


Fig. 1. Images of soymilk coffee mixtures in (a) single phase, (b) separating into two phases and (c) sedimentating.

and pH. Image analysis is used to quantify the phase separation kinetics during curdling, and thus obtain information on the mechanism of the separation process and its dependence on the location in the phase diagram.

2. Method

The experiments used Tesco Gold freeze-dried instant coffee granules, strength 3, and Tesco organic long-life unsweetened soymilk (both from Tesco PLC, UK). The soymilk ingredients were water, organic soy bean (6%) and natural flavouring. The composition of 100 ml of soymilk is 1.9 g of fat, 0.4 g of carbohydrate, 3.4 g of protein and 0.1 g of salt. The jar of coffee granules was stored in the lab at room temperature (approx 21 °C) and the soymilk was kept refrigerated once opened.

To prepare the solutions coffee granules were weighed to an accuracy of 0.1 mg and transferred to a 200 ml glass beaker. 100 ml (100 g) of deionised water was added to the coffee granules and the mixture was stirred until the coffee was dissolved. Soymilk was then added to the coffee solution. Experiments were carried out using coffee to water ratios of 0.0125, 0.025, 0.05 and 0.075 g coffee/g water, and soymilk concentrations from 2 to 25%w/w. The coffee to water ratios and soymilk concentrations were chosen based on realistic measures typical of those used in preparing coffee drinks.

The pH of the coffee/water/soymilk mixture was measured using a Jenway 3510 pH meter. After every use of the pH meter probe, it was rinsed with distilled water, wiped with a paper towel and placed back into the buffer storage solution. The pH of the pure soymilk was also recorded at the start of every experiment to check for changes over time after opening the carton. We observed that after opening the soymilk carton, the pH of the soymilk reduced from 7.25 to 7.10 over a period of 10 days, as can be seen in Supplementary Information. A given carton of soymilk was used for no longer than two weeks after opening.

A magnetic pellet was placed into the mixture and the beaker placed onto a Stuart temperature-controlled heat plate and magnetic stirrer. An additional thermocouple, connected to the pH meter, was placed into the beaker to check the accuracy of the thermocouple on the temperature-controlled heat plate and to provide a temperature correction for the pH readings. A webcam was used to record images of the mixture. The experimental set up is shown in Supplementary Information.

To obtain the temperature dependent phase behaviour, the mixture was stirred and heated from room temperature to 25 °C, then heated in 5 °C increments to 60 °C. At and above 40 °C the beaker was covered with Parafilm to minimise evaporation. Once a temperature increment was reached the webcam imaging was started and the stirrer was switched off. The webcam recorded pictures of the beaker every 5 s for 5 min. At the end of the 5 min the pH and phase were recorded (single phase, two phase, or “unclear” if separation could not be unambiguously detected by eye).

To obtain information about the phase separation and curdling rate, webcam images were analysed by computing the two-dimensional Fourier transform of the pixel intensity, after cropping an area at the

centre of the image and subtracting a reference background image to minimise the effect of fixed optical imperfections such as extraneous reflections. The reference background image was taken while the magnetic stirrer was still switched on. A peak in the radially-averaged Fourier transform magnitude $S(Q)$ at a given wave number magnitude Q essentially identifies significant image intensity variations around the length scale $L = 2\pi/Q$ (Poon & Haw, 1997). Hence, for example, separation and coarsening into phases of differing soymilk concentration at a growing length-scale L would be indicated by a peak in $S(Q)$ which grows in intensity and moves to smaller Q over time. The time-dependence of peak position and intensity can then be used to compare the soymilk separation process with common phase separation and flocculation models such as classical spinodal decomposition (Tanaka, 2000), cluster aggregation (Poon & Haw, 1997) and protein cluster formation (Stradner et al., 2004).

3. Results and discussion

3.1. Phase diagrams

Fig. 2 shows the temperature vs soymilk concentration phase diagrams for a 0.05 g coffee/g water solution. The phase diagrams for different coffee concentrations, namely 0.0125, 0.025 and 0.075 g coffee/g water, on heating show similar trends (see Supplementary Information). The phase diagram reveals a coexistence curve separating a two phase region at high temperature from a single phase region at

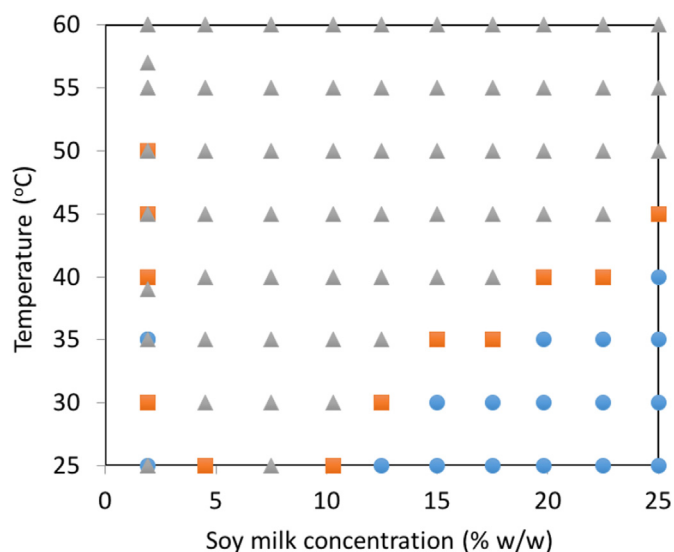


Fig. 2. Temperature and soymilk concentration phase diagram for a 0.05 g coffee/g water solution. Blue circles are single phase, grey triangles are two phase, and orange squares show where the phase was unclear. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

low temperature. For low soymilk concentrations, the phase coexistence boundary is high, and then sharply decreases as the concentration increases, reaching a minimum below 25 °C between 5 and 10%w/w soymilk concentration. As the soymilk concentration is further increased, the coexistence temperature increases more slowly. At low concentrations the coexistence curve is very sensitive to small fluctuations in mixture composition and temperature.

Interestingly, this phase separation behaviour is reversible, demonstrating that this is a true phase transformation and not an irreversible change due to e.g. protein denaturation. Experiments showed that it is possible to recover the homogeneous mixture in two ways: by cooling or by increasing the soymilk concentration. Mixtures in a two-phase state at high temperature were cooled (maintaining fixed composition) and at low enough temperature (and after stirring) became homogeneous. In addition, the effect of increasing soymilk concentration in mixtures at constant temperature with a coffee concentration of 0.0125 g coffee/g water was tested. In these experiments soymilk was gradually added to the mixture, held at either 25, 30, 40 or 50 °C, and the resulting phase after monitoring for 5 min at each concentration was recorded. It was observed that the mixture was homogeneous at low concentrations (around 2%), then phase separated at intermediate concentrations, and became homogeneous again at high soymilk concentrations, depending on temperature (see Fig. 2).

The relationship between the phase of the mixture and its pH was also investigated although there was no clear pH transition. The phases for pH vs soymilk concentration and for pH vs temperature are shown in Supplementary Information. In general, we see that two-phase mixtures have a lower pH than single phase. However, pH differences in these mixtures are small.

3.2. Separation and sedimentation rates

In this section we explore the time evolution of the curdling and sedimentation processes and the dependence on soymilk concentration and temperature. Fig. 1 shows example images of a mixture with 0.05 g coffee/g water and a soymilk concentration of 15.0%w/w at 60 °C that starts as a single phase, separates into a two phase mixture, and subsequently sediments. The images were taken at 5 s, 65 s, and 125 s after stirring was stopped. We note that throughout the phase separation process the flocs can be seen to sink slowly, and at some stage they undergo a rapid collapse, in a behaviour reminiscent of so-called delayed sedimentation (Bartlett, Teece, & Faers, 2012).

Fig. 3 shows radially averaged Fourier transforms, $S(Q)$, of a fixed 280×280 pixels section of images for the example mixture of Fig. 1. One pixel corresponds to approximately 0.08 mm. After 10 s a shoulder develops, then at 15 s, a peak becomes visible at around $Q \approx 0.6$ inverse pixels, which shifts to smaller Q (larger separation length scale) and grows in intensity (greater contrast between phases) as separation and coarsening proceeds. $S(Q)$ rises monotonically as $Q \rightarrow 0$ and after 115 s the peak disappears, corresponding to the final macroscopic collapse of the suspension. Similar qualitative behaviour is seen for all mixtures at temperatures that undergo separation.

To analyse the time-dependence of the peak position, Q_{\max} , and intensity, $S(Q_{\max})$, Q_{\max} and $S(Q_{\max})$ at each time were estimated by fitting polynomials to the $S(Q)$ curves in the region of the peak. The temporal behaviour of the peak position for a mixture with 0.05 g coffee/g water and soymilk concentration 15.0%w/w at different temperatures is shown in Fig. 4 (a). At low temperatures of 25 °C and 30 °C, there was no separation and hence no peak, as expected from the phase diagram in Fig. 2. At intermediate temperatures of 35 °C and 40 °C, the mixture partially separates as demonstrated by a clear peak in $S(Q)$, but within the time of the experiment does not undergo sudden collapse. The peak in $S(Q)$ is at an approximately constant position Q_{\max} over a long time period: separation of the phases appears to stabilise at an approximately constant temperature-dependent length scale, although peak intensity (see below) continues to increase implying an

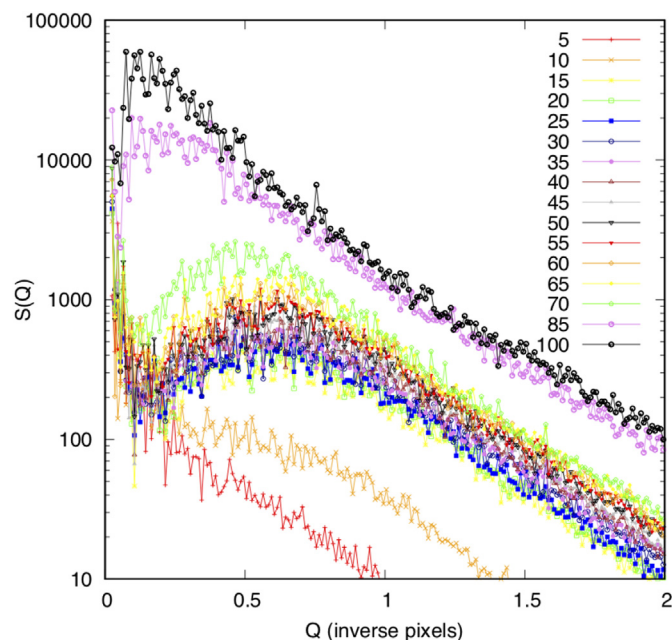


Fig. 3. Intensity $S(Q)$ vs Q (in inverse pixels) for Fourier transforms of series of images, taken every 5 s up to 100 s, for a mixture of 0.05 g coffee/g water and soymilk concentration of 15.0 %w/w, at a temperature of 60 °C.

increase in the density “contrast” between the phases. The temperature dependence of approximately constant Q_{\max} is consistent with the mixture becoming unstable at a smaller length scale as we go deeper into the two-phase region i.e. at higher temperature. At temperatures of 45 °C and higher, separation involves a shorter time with approximately constant Q_{\max} followed by rapid collapse, where we see a fast increase in length scale (decrease in Q_{\max}). The time at which rapid collapse occurs is generally shorter for higher temperatures, although measurement of collapse time is subject to uncertainties because collapse was sometimes observed to be position-dependent in the sample vial: images were obtained only from one position but on some occasions the onset of collapse could be observed elsewhere in the vial before becoming visible in the image region. For example, it can be seen from the $S(Q)$ data that the 50 °C mixture appears to collapse before the 55 °C mixture, possibly because the images in the 55 °C case did not capture the earliest instants of collapse. Further study is required to fully characterise the late-stage complete collapse. Comparison with detailed studies of delayed sedimentation in colloidal gels (Bartlett et al., 2012) would be interesting.

The temporal behaviour of the peak intensity $S(Q_{\max})$ for a mixture with 0.05 g coffee/g water and soymilk concentration 15.0%w/w at different temperatures is shown in Fig. 4(b). At any given temperature, the intensity increases with time as separation proceeds, with higher temperatures generally giving higher intensities and faster increase. For mixtures at 35 °C and 40 °C, even though a constant Q_{\max} persists for significant time, $S(Q_{\max})$ continues to increase. For higher temperatures, rapid collapse of the mixture coincides with rapid late-time increase of $S(Q_{\max})$. In fact, $S(Q_{\max})$ increases approximately exponentially over time (before rapid collapse due to sedimentation), with a characteristic time, τ , that decreases as temperature increases: the deeper the sample in the two-phase region, the faster the separation. Plotting characteristic time vs the quench depth, $T - T_c$, where we estimate the critical temperature, T_c , to be 24 °C from the experimental phase diagram, gives a power law with exponent -2.08 , which is consistent with spinodal models (see below) predicting an exponent of -2 (Fig. 5). Details about the fitting is given in Supplementary Information.

The effect of varying soymilk concentration on the $S(Q)$ peak during

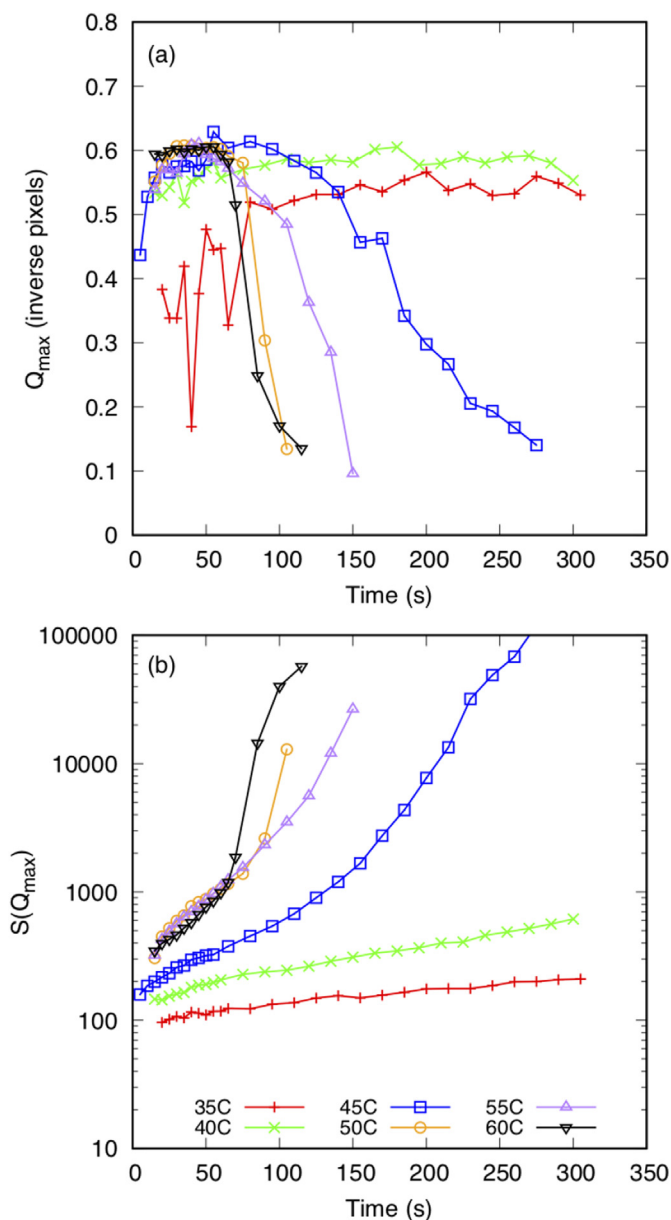


Fig. 4. Variation of peak position Q_{max} (a) and intensity $S(Q_{max})$ (b) with time for different temperatures at a soymilk concentration of 15%w/w.

separation and collapse at fixed temperature is shown for a temperature of 35 °C in Fig. 6. From the phase diagram in Fig. 2 we expect to see no separation at soymilk concentrations above 17.5%w/w. At concentrations of 4.5%w/w and 7.5%w/w, which are near the phase boundary (see Fig. 2) we observe peaks at smaller Q_{max} compared with higher concentration. Peaks at the higher concentrations of 12.5%w/w, 15.0% w/w and 17.5%w/w appear to stabilise at a particular Q_{max} and the samples do not undergo collapse. Intermediate concentrations of 7.5% w/w and 10.3%w/w show separation followed by collapse. The decrease of the typical scale of separation (the inverse of Q_{max}) from large scale near the low-concentration phase boundary, to a minimum near the middle of the two-phase region, and subsequent increase again as the high concentration boundary is approached, is consistent with the relative volumes of the two phases expected (as predicted through a tie-line construction): nearer the phase boundary, a small amount of one of the phases is expected (the dense phase, near the low concentration boundary; the dilute phase, near the high concentration boundary), so that regions of this phase will appear at lower number concentration as

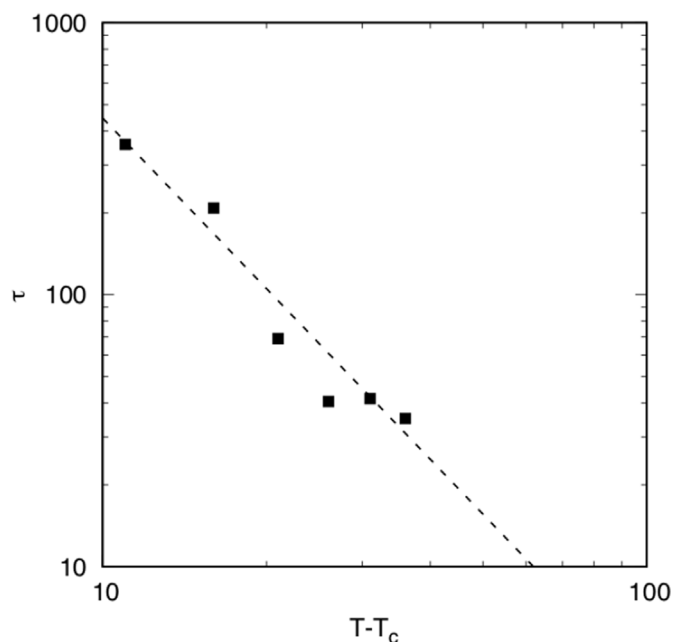


Fig. 5. Variation of the characteristic time τ with the quench depth $T - T_c$ showing a power law with exponent -2.08 .

separation begins, i.e. dispersed at greater distance from each other. Hence the concentration modulation giving rise to the $S(Q)$ peak is at larger characteristic scale/smaller Q_{max} near the phase boundaries. Conversely, at maximum distance from the boundaries, i.e. midway between the boundaries, one expects approximately 50% by volume of each phase and a maximum number concentration of separating phase regions, i.e. a minimum characteristic scale of density modulation or maximum peak Q_{max} .

The peak intensities $S(Q_{max})$ follow a similar temporal trend as before, increasing until the onset of collapse and rapid sedimentation. Compared to the fixed concentration case, shown in Fig. 4 at different concentrations, the rate of growth in intensity is less clearly exponential but qualitatively the variation with concentration is consistent with the samples' location in the phase diagram: the rate of intensity growth reaches a maximum around the middle of the two-phase region (7.5% and 10.3% w/w soymilk) i.e. for the deepest quench into the two-phase region.

Models of spinodal decomposition (Tanaka, 2000) predict, at the earliest stage of separation, a fastest growing concentration fluctuation at a constant lengthscale given by the characteristic spatial scale at which the mixture is maximally unstable. At first sight this constant length scale matches our results at temperatures/concentrations where Q_{max} is approximately constant during separation. The exponential growth of peak intensity over time, and the power law dependence of the characteristic growth time on the quench depth, with an exponent close to -2 , are also consistent with expectations from spinodal models. However, such early time spinodal separation is usually expected to rapidly transition to a growing characteristic scale as regions of the two phases coarsen to larger scale (e.g. by coalescence, surface-tension driven coarsening, or other mechanisms). Alternative explanations for separation displaying a constant characteristic scale include gelation, where growth is halted by formation of a system-spanning gel phase (Bartlett et al., 2012; Poon & Haw, 1997) which in many systems then suddenly collapses after a certain delay time due to thermal consolidation, and formation of a 'cluster phase' due to the combination of short-range attraction and longer-range weak repulsion between particles (Stradner et al., 2004/cross-ref >). However, the fact that the peak intensity continues to grow during the constant Q_{max} period is difficult to explain if growth were really frustrated by either of these

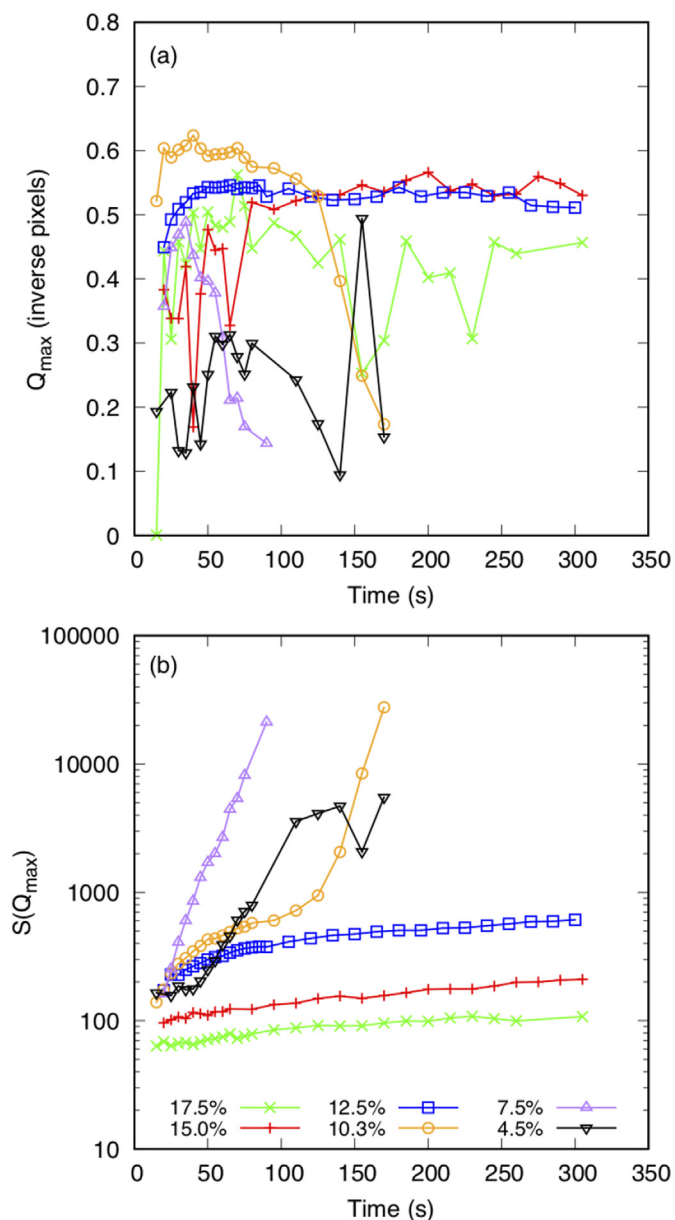


Fig. 6. Variation of peak position (a) and intensity (b) with time for different soymilk concentrations at a temperature of 35 °C.

mechanisms, unless consolidation and local densification could occur that increased the density contrast without changing the length scale. A study of the dynamics of the particles in this regime, for example by dynamic light scattering, could be used to confirm gelation, although the turbidity of the samples makes this challenging.

In the separating mixtures, especially near the phase boundaries, there is evidence of an increase in peak position at the earliest times (see Figs. 4(a) and 6(a)), implying a decreasing length scale during the initial stages of phase separation. Such a decrease in characteristic scale does not agree with expectations from aggregation and gelation models (Poon & Haw, 1997) or general models of diffusion-driven phase separation (Tanaka, 2000) and seems to effectively contradict coarsening-based models where regions of separating phases should be growing. A possible explanation, suggested by the fact that we observe this for the shallowest quenches into the two-phase region, i.e. near the phase boundaries, is that we are observing ongoing nucleation of droplets at a rate faster than growth of existing nuclei. At the earliest stages of separation as the first droplets of the separating phase nucleate, their

number concentration will be low giving a large droplet-to-droplet distance and hence large characteristic scale of concentration modulation i.e. a small Q_{\max} . With increasing time more droplets nucleate, increasing the number concentration, decreasing the characteristic distance between droplets and thus increasing Q_{\max} . Further experiments with better spatial and time resolution would be useful to explore this nucleation region in more detail: the image quality and time resolution of the experiments described here provides relatively low contrast in the early-time $S(Q)$ peaks, leading to difficulties with precise peak position estimation in some datasets.

4. Conclusions

The phase behaviour of soymilk coffee mixtures with temperature, pH, and soymilk and coffee concentrations were investigated. Mixtures of known composition were heated to a fixed temperatures and their phase behaviour over a period of 5 min was recorded. In general, the mixtures phase separated at high temperatures and the temperature vs soymilk concentration phase diagram exhibits a coexistence curve, separating a two-phase region at high temperature from a single-phase region at low temperature. The phase transition occurred at higher temperatures for high and low soymilk concentrations and a temperature minimum at intermediate concentrations.

Image analysis was used to explore how the kinetics of separation depended on position in the phase diagram. At temperatures and soymilk concentrations near the coexistence boundary, the characteristic separation length-scale was found to remain approximately constant for a significant time and rapid sedimentation collapse was not observed over the timescale of our measurements. The measured behaviour of the characteristic length scale during phase separation is partly consistent with spinodal models, although questions remain over this interpretation. Deeper within the two-phase region, the period of constant length scale was shorter and subsequent rapid sedimentation and collapse occurred. There was some evidence that at the earliest stages of separation, the characteristic length scale decreases, which may indicate a nucleation mechanism, however more detailed data are required to verify and explore this regime.

The chemistry of phase separation in soymilk systems is not well understood. Our measurements, while partly consistent with well-established separation models such as spinodal decomposition and nucleation, indicate significant complexities, which is not surprising given the interplay of the protein physics and chemistry involved in soymilk stability. We note that some types/brands of soy milk are less prone to curdling in coffee, which is likely due to the addition of stabilisers. Further work on the chemistry and physics of phase behaviour in soymilk, and other important dairy alternatives such as almond or oat milk, would be of significant predictive and product-development value in the food and drink industry. In particular, the reversibility of the soymilk phase separation could be a useful property to exploit in food product engineering.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodhyd.2019.04.032>.

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