1 Ultrasound characterisation of the rheology of crystallising anhydrous milk fat

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Abstract

Relationships between acoustical parameters and rheological ones permit the non-invasive determination of the elastic moduli in crystallising anhydrous milk fat during cooling between 43 °C and 17 °C. Whilst the real (elastic) moduli are dominated by the bulk modulus with a small contribution from the real shear modulus, the imaginary parts of the moduli (relating to viscosity) have significant contributions from both bulk and shear viscosities. Ultrasound techniques significantly expand the application of rheological techniques, they can be implemented in a non-invasive manner into a process, and therefore augment and enhance conventional rheology.

1 Introduction

Many important materials exhibit the characteristics of soft solids, with partially liquid-like and partially solid-like properties. One category of such materials is a crystalline medium forming from the melt, where solid crystals (or their nucleic precursors) are present in a liquid continuum, and where longer-range structuring to form a crystalline network may create further solid-like character.

Anhydrous milk fat (AMF) is a complex heterogeneous soft solid derived through the separation of milk fat from cow's milk by centrifugation; it is a valuable dairy fat with wide application in high quality confectionery, breads and baked goods. AMF is a well-studied (Arita-Merino et al., 2020) exemplar for the acoustical characterisation of a material in which a continuous deformable crystal network (20% - 40% for the β' phase cooling between 16 °C and 10 °C (Van Aken & Visser, 2000)) interacts with the majority fluid phase trapped within to produce soft solid rheological behaviour. Acoustic techniques have in-line capability for the characterisation of dairy fat rheology during bulk industrial crystallisation processes which would be invaluable in quality control, product and process development.

Both rheological and ultrasonic measurement techniques provide insight into the properties of such materials. Indeed, oscillatory rheometry and ultrasonic techniques are closely related, due to their connection to the complex elastic and shear moduli of the material. However, rheometry is often explored at lower frequencies than those accessible by ultrasound and classical rheometry is sensitive to the macroscopic material response to strain and stress, whereas ultrasound can also probe the material response at a microscopic length scale for example where solid microparticles exist in the liquid. In rheological measurements, it is normal to probe the shear viscosity, be it that experienced by a probing particle (micro-rheology (Cicuta & Donald, 2007)) or in the entire sample. In the case of acoustic/ultrasound attenuation and velocity spectroscopy of fluids and soft solids, the adiabatic bulk modulus, longitudinal and bulk viscosity are the determined parameters, setting it apart from more

usual rheological measurements of shear viscosity, complex rigidity, and stress/strain/strain rate plots.

In this work, the results of an experimental study using both longitudinal acoustic spectroscopy and a low frequency (1 Hz) oscillatory controlled strain rheometer are presented to demonstrate how acoustics can be an effective rheological probe. Our investigation explores the properties of the complex heterogeneous soft solid anhydrous milk fat (AMF) during cooling crystallisation. The quantitative relationships between the material properties are explored. The longitudinal viscosity η_{long} is contrasted (A. S. Dukhin & Goetz, 2010) to the shear viscosity (η_s) which can also be determined from the attenuation and reflectance coefficient of shear waves (Runrun et al., 2020); (Yamaguchi & Kikuzawa, 2019); (Rigolle et al., 2016). To the best of our knowledge, this is the first detailed comparison of rheological quantities determined by acoustical and rheological means on a complex heterogeneous material of industrial significance. Our intention is to demonstrate the value of using both acoustical and rheological methods for the characterisation of the dynamic flow properties of industrial materials with acoustics having the added benefit of convenient implementation in processes and process analytical technology (PAT). Consequently, an entirely new area of application for rheological measurements is opened by, for example, widely available Doppler flow meters (which are based on ultrasound waves), which could be adapted to provide rheological data without any modification to the instrument hardware, simply requiring a change in software. (Schroyen et al., 2020)) has recently published a comprehensive review of bulk rheometry at high frequencies, however the review does not present a consistent and systematic theoretical framework for data interpretation and the comparison of low and high frequency methods.

We will show that in homogeneous materials, longitudinal (compressional) wave acoustics gives the bulk modulus through the speed of sound and the bulk viscosity through the attenuation and shear viscosity; and that because longitudinal waves can propagate over long distances, they can inform on these properties in bulk materials. On the other hand, because shear waves/forces in soft materials

have a very limited range, only very low frequencies can probe reasonable distances. A number of interesting features are highlighted from the ultrasonic measurements during the crystallisation process that indicate the influence of solid-liquid interactions on the macroscopic measurements. Firstly, the relationship between rheology and ultrasonics is presented in order to set the context for the experimental investigation.

2 Rheological relations

Relationships and variables widely used in practical rheology are reprised here for later use. These relationships are presented in more detail in the appendix

The adiabatic compressibility is related to the adiabatic bulk elastic modulus K [Pa] and the isothermal bulk elastic modulus K_T [Pa] through the ratio of specific heats Υ.

$$K = \gamma K_T = \frac{1}{\kappa} \tag{1}$$

The relationship between fluid viscosity η_{long} [Pa s] and longitudinal sound attenuation α_{long} [Np m^{-1}] was first derived by Stokes. (Pierce, 1994) and (Morse & Ingard, 1968) present a more general expression that incorporates thermal effects.

$$\alpha_{long} = \frac{\eta_{long}\omega^2}{2\rho {c_{long}}^3}\left(\frac{4}{3} + \frac{\eta_b}{\eta_{long}} + \frac{(\gamma - 1)\kappa}{\eta_{long} C_p}\right) \tag{2}$$

here γ is the ratio of specific heats, κ [W m^{-1} .K^{-1}] is the thermal conductivity; C_p [J K^{-1}] is the specific heat at constant pressure; ω is radial frequency [rad s^{-1}]; longitudinal speed c_{long} [m s^{-1}] and η_{long} [Pa s] is longitudinal viscosity.

In most liquids, the ratio of specific heats is close to one (in water at 10 KPa and 25 °C it is 1.0106) and the thermal term is usually neglected, so that rearranging we get

$$\eta_{long} = \frac{3\alpha_{long}\rho {c_{long}}^3}{2\omega^2} \tag{3}$$

where the relationship

$$\eta_{long} = \eta_b + \frac{4\eta_s}{3} \quad (4)$$

(here η_b is bulk viscosity and η_s is shear viscosity) is analogous to the relationship between elastic moduli in solids

$$M = K + \frac{4}{3}G \quad (5)$$

where M [Pa] is the elastic modulus, G the shear modulus and K is the bulk modulus defined above. In rheology the moduli are each expressed as two numbers, a 'real' part, relating to strains that are in step with the applied stress and an 'imaginary' part where the strains are $\pi/2$ radians out of step,

$$M = M^{'} + iM^{''} = K^{'} + iK^{''} + \frac{4}{3}(G^{'} + iG^{''}) \quad (6)$$

where $i = \sqrt{-1}$.

For a harmonic oscillation of the form $e^{-i\omega t}$ the strain rate is equal to the strain multiplied by a factor $-i\omega$, and the relation between complex elastic moduli and viscosity enables us to re-express the acoustic properties. In solids, the equivalent of the Newton-Laplace equation is

$$c_{long} = \sqrt{\frac{M^{'}}{\rho}} = \sqrt{\frac{K^{'} + {}^{4}/_{3}G^{'}}{\rho}} \quad (7)$$

In fluids and soft solids $K' >> G'$, for example in a yoghurt G' ~ a few Pa whilst K' is ~ 10^{10} Pa so that

$$c_{long} = \sqrt{\frac{K^{'}}{\rho}}. \quad (8)$$

We can now rewrite Eq. 2 in terms of the complex elastic moduli (Eq.3, Eq. 4 and Eq. 6)

$$\alpha_{long} = \frac{2M^{''}}{3\rho c_{long}^{3}}\omega = 2\frac{K^{''} + {}^{4}/_{3}G^{''}}{3\rho c_{long}^{3}}\omega = \frac{2\omega^{2}\eta_{long}}{3\rho c_{long}^{3}} \quad (9)$$

where

$$M^{''} = K^{''} + {}^{4}/_{3}G^{''} = \eta_{long}\omega \quad (10)$$

For a complex shear modulus

$$G^{*} = G^{'} + iG^{''} \quad (11)$$

which can be expressed as (noting that G^{*} is formally the same as G)

$$G = |G^*|e^{i\delta} \quad (12)$$

with magnitude and phase given by $|G^*| = \sqrt{G'^2 + G''^2}$ (13)

$$tan\delta = \frac{G''}{G'} \quad (14)$$

where δ is the phase angle between stress and strain which varies between $\pi/2$ for fluids when $G' = 0$, to 0 in the case of elastic solids where $G'' = 0$. The stress-strain relations can be written

$$G = \frac{\tau}{\gamma} \quad (15)$$

or

$$\eta_s = \frac{\tau}{\dot{\gamma}} \quad (16)$$

where τ is shear stress [Pa], γ is shear strain and $\dot{\gamma}$ is shear strain rate [s^{-1}]. In general, and particularly in soft solid materials τ and γ are complex quantities as is G.

For a harmonic strain, for example in an oscillatory system where

$$\gamma = \gamma_0 e^{i(\omega t + \delta)} \quad (17)$$

then

$$\dot{\gamma} = i\omega\gamma_0 \quad (18)$$

So that from Eq. 16

$$\tau = i\omega\eta_s\gamma \quad (19)$$

Therefore

$$G = i\omega\eta_s \quad (20)$$

so in materials with no elastic component (such that $G' = 0$)

136 $$G'' = \omega\eta_s \quad (21)$$

137 Having explored the theoretical and physical relationships between acoustics and rheological material
138 properties we next present the results of the experimental investigation of anhydrous milk fat during
139 cooling crystallisation by using rheometry and ultrasonics.

140 3 Methods and Materials

141 Ethics approval was not required for this work

142 Absolute acoustic attenuation values over the frequency range 2 to 100 MHz were measured using a
143 Malvern Ultrasizer-MSV. The working principle of this ultrasound spectrometer is described in detail
144 elsewhere (Meyer et al., 2006); (Ghosh et al., 2017) . Measurements were carried out with the stirrer
145 set at an angular speed of 240 rpm (Heidolph RZR 2051) and a torque determined to within ± 1 N m.
146 Stirring the sample reduces flocculation and creaming effects and controls temperature with a
147 precision ± 0.2 °C throughout the sample volume. Temperature control by a Huber (MiniStat cc) is
148 accurate to ± 0.2 °C and the in-cell thermometer is similarly accurate. Attenuation is determined
149 absolutely, including appropriate diffraction corrections with a total error of ± 1 dB. Frequency
150 measurement is accurate to 1 Hz.

151 Anhydrous milk fat (AMF) was kindly provided by Arla Foods (Denmark) and without any further
152 purification. Samples were heated to 65 °C for 30 minutes to ensure dissolution of high-melting
153 crystalline lipids (Wang, 2016) at which temperature 500 ml of AMF was poured into the Ultrasizer
154 cell. The sample was then cooled whilst stirring at 240 rpm in steps (43, 40, 36, 26, 23 and 19-17 °C)
155 and then held isothermally at each temperature for 30 minutes to allow thermal equilibration.
156 Subsequently three measurements of attenuation spectra were taken, approximately 7 minutes apart
157 and obtaining the average of three attenuation spectra. The range 19-17 °C appears because it proved
158 impossible to hold the 500 ml sample at a constant temperature whilst it was crystallising extensively.

The measured stirrer torque varied between 1.9 N cm at the highest temperature to 5.2 N cm at the lowest temperature. Overall, temperature control achieved is accurate to within $\pm$ 0.5 °C.

Speed of sound was measured in a Resoscan RU(RZ21) (TF Instruments, Heidelberg) high precision ultrasound resonator system allowing simultaneous velocity and attenuation measurements (Svalova et al., 2017) The system includes a two-channel resonator unit with gold plated lithium niobate piezo crystals, two 250 µl sample cells and a Peltier thermostat. Temperature control and measurement are performed via two external units enabling a resolution of 0.001 °C and precision of ±0.005 °C. The instrument features a small sample requirement of 170–250 µl and a high temperature stability. At about 25 °C the sound velocity in an ultrapure deionized water standard (MilliQ, resistivity >18 MΩ cm) changes by around 5 ms^{-1} $°C^{-1}$. Therefore, the precision limit of temperature corresponds to a systematic error in velocity of around 0.025 m s^{-1}. The fundamental frequency of the instrument is 10 MHz, with range of 7–11.5 MHz; the precise frequency is automatically selected to maximise the quality factor of the acoustic resonant cell according to the sample properties.

Shear rheology measurements were carried out in an Anton Paar MCR 302 rheometer (Anton Paar GmbH, Austria) at a controlled strain of 0.001% , temperature controlled to within $\pm$ 0.05 °C, oscillatory (1 Hz, 6.28 rads s^{-1}) amplitude sweep measurements.

4 Results and Discussion

4.1 Microstructure of crystallised anhydrous milk fat

During cooling, AMF becomes a soft solid with inhomogeneity characterised by large crystal clusters (Figure 1). These clusters agglomerate and create an additional elastic modulus called the frame modulus (Narine & Marangoni, 1999) and have a significant impact on the rheology and the acoustics. To illustrate the microstructure, we sandwiched a small amount of sample preheated to 65°C in between 2 glass microscope slides separated by 5 mm. Cooling the sample was achieved by convective heat loss to the environment at an average rate of 0.2°C/min. Digital images of the sample at various

temperatures were acquired with a Canon DLSR camera at 40× magnification attached to a Leitz Dialux 22 polarized microscope (Leica, Germany). Similar images can be found in (Kaufmann et al., 2012a).

Figure 1

4.2 Complex shear modulus determined by conventional shear rheology

The authors of a detailed study of the rheology of pure AMF and its blends with rapeseed oil (Kaufmann et al., 2012b) point out that shearing of the sample changes the crystallisation behaviour during cooling. Hence, they subjected the sample to a period of no shear whilst cooling the sample from 65 °C down to 15 °C at 5 °C/min and then held isothermally with different shear rates of 0, 1, 50 and 500 s^{-1} . Low frequency oscillatory shear measurements of the real part of the shear modulus are also given in (Wang et al., 2019); (Shukla and Rizvi, 1995) . (Shukla and Rizvi, 1995).

Published data (Rønholt et al., 2014; Shukla & Rizvi, 1995a) for AMF indicates that $G^{'}$ is independent of frequency up to an angular frequency of 500 rad s^{-1} (~80 Hz), and it is therefore reasonable to conclude that our low frequency measurements (70 rad s^{-1}) of $G^{'}$ may be used at the high frequency of our ultrasound data. This assumption is supported by our observation of approximately Newtonian behaviour of the AMF melt at the lower end of the ultrasound frequency range studied here and reported below.

As the sample cools and crystals form, we observed the emergence of a storage modulus of order MPa, this is the elastic, solid-like part of the shear modulus. Our own data for AMF Figure 2 is consistent with the appearance of soft solid material $G^{'} \gg G^{''}$ when AMF is cooled to 15 °C as described above. Viscosity cannot be reliably obtained from our measurement of $G^{''}$, which varies by an order of magnitude, even at the lowest temperature and longest time (15 °C and 8000 s) due the torque being below the resolution of the rheometer. We note a significant disparity between the oscillatory shear data for AMF in (Kaufmann et al., 2012b) where $|G^*|$ at the end of cooling has a value of 0.3 MPa whilst in (Rønholt et al., 2014) *G*' has the corresponding value of 1.2 MPa. These disparities are

likely due to different stirring conditions (Tran & Rousseau, 2016) and are also influenced by the fact that $G^{'} \gg G^{''}$; for example, (16 indicates that $G^{'} > G^{*}$ is physically impossible. Other rheological measurements in AMF also show that $G^{'}$, $G^{''}$ and hence η_s are highly dependent on shear conditions during measurement (Kaufmann et al., 2012b).

Figure 2

The most consistent rheological data is available for the crystallisation of Palm Oil (Shukla & Rizvi, 1995b) which exhibits similar crystallisation behaviour to AMF. Hence we use what must be an approximate value 0.09 Pa s for the molten material (Shukla & Rizvi, 1995b), (rising to extremely variable values at 22 °C in the range 0.6-10 Pa s.) , because the sample has a low viscosity, also confirmed by (Shukla & Rizvi, 1995b).

4.3 Elastic modulus determined by acoustic velocity spectroscopy

In Figure 3 we plot the velocity of sound (Resoscan, ~ 10MHz) against temperature for AMF cooling down at a constant rate of 0.035 °C min^{-1} from 40 °C to 15 °C. The velocity of sound cannot be measured reliably in the Resoscan when crystalline material is in equilibrium with its melt due to the transfer of acoustic energy into the crystallising system (Akulichev & Bulanov, 1983). Like other animal fats(Pratama et al., 2021), AMF comprises a wide range of triacylglycerol materials and as a result does not have a sharp melting point and possesses a wide melting range. Data is further confused by the existence of a frame modulus associated with crystalline material and associated dissipation mechanisms which are poorly understood. Nevertheless, accurate speed of sound data was measured over a wide range of temperatures prior to the onset of crystallisation and whilst the behaviour of the measured speed of sound in the crystallising region is complicated, data is nevertheless still available. Crystallisation is also a kinetic process, so this is not a constitutive relationship but rather depends on the cooling rate and shearing conditions.

Figure 3

Equation 5 relates the speed of sound to *K'* (the real part of the bulk modulus) using the temperature dependent density for AMF (Jenness et al., 2010) and the results are shown in Table i.

4.4 Determination of longitudinal viscosity from acoustic attenuation measurements

We use measurements of the ultrasound attenuation spectrum between 2 and 100 MHz (Ultrasizer, Figure 4), and the velocity of sound measured in the Resoscan (Figure 3) to determine the longitudinal viscosity of AMF using Equation 2. The results are plotted in Figure 5 as a function of ultrasound wavelength.

Recent measurements of AMF bulk viscosity were included in previous works (A. Dukhin et al., 2020; A. S. Dukhin & Goetz, 2009; Ghosh et al., 2017; Holmes & Povey, 2011). The longitudinal viscosity was transformed to the corresponding imaginary part of the longitudinal modulus (Equation 11); the corresponding (imaginary) shear modulus at the ultrasonic frequency was obtained from the shear viscosity as described above, thus also determining the imaginary part of the bulk modulus (which directly relates to bulk viscosity). We have therefore determined both real and imaginary parts of the bulk modulus at ultrasonic frequencies from acoustic speed and attenuation measurements, together with an estimate of shear viscosity from low frequency rheological measurements. This analysis treats the material as homogeneous, with any dissipation mechanisms being attributed to the bulk viscosity and shear viscosity.

Figure 4

Figure 5.

Figure 6

At low ultrasound frequency (2 – 6 MHz), molten AMF samples step-cooled from 65 °C to temperatures above the crystallisation point (43 – 26 C) behave approximately as a Newtonian fluid (long wavelength fit in Figure 5), whose viscosity is almost independent of frequency (frequency

independence of viscosity is equivalent to shear rate independence). This is indicated by a power law exponent of approximately zero. For a Newtonian fluid (such as water), the attenuation has a frequency exponent of 2 and the viscosity is independent of frequency (Equation 1). Adopting a Newtonian shear viscosity from literature as described above, the bulk viscosity (Table i) is larger than the shear viscosity. Below 26 °C, the fitted power law exponent of the longitudinal viscosity departs significantly from zero indicating non-Newtonian behaviour and at 19 °C to 17 °C measurement errors become large as the material rheology becomes increasingly solid-like ($G' >> G''$). Additional contributions to attenuation may become important, which are not correctly described through the bulk viscosity, for example effects due to interaction of acoustic waves with crystals and crystal clusters, and the effects of the longer-range material structuring (frame modulus). At high ultrasound frequency (60 – 90 MHz), the viscosity consistently behaves in a non-Newtonian manner, probably because at short wavelengths (20 – 80 µm) and low temperature, shear interactions between the crystal clusters that form at 23 °C become significant. Even in the melt there is significant frequency dependence in this high frequency range, possibly arising from relaxation phenomena.

In Figure 6, (but not in Figure 4) data in the region 10 MHz to 40 MHz has been omitted because of an instrumental artefact arising from a coincidence between the size of the crystal clusters (Figure 1) in relation to wavelength (Figure 4 and Figure 5), the stirring speed alters the time spent by a cluster in the measurement zone. In the overlap region and the averaging time window, the instrument attempts to reconcile data from the high frequency (10 MHz to 120 MHz) and low frequency (2 MHz to 20 MHz) transducers. The effects of this are apparent in Figure 4, Figure 5 and Figure 6 at around 10 MHz and a wavelength of 100 µm which is of the same order as the size of the crystal clusters. These effects are absent in a homogeneous sample such as DI water.

Table 1:

5 Discussion

Table 1 shows that acoustical and rheological data can be used together to determine both low frequency and high frequency rheological measurements in a heterogeneous system such as AMF. From Table it is worth noting that although the real part of the longitudinal modulus is dominated by the bulk modulus (with negligible storage modulus, $G^{'} \ll K^{'}$) under the assumptions applied here, the imaginary part of the longitudinal modulus has a significant contribution from both bulk and shear components ($K^{''}$and G'' are of the same order at 10 MHz). Our analysis is limited by the reliance on a shear viscosity measured on liquid molten samples (Shukla & Rizvi, 1995b), which will not fully reflect the true viscosity of the system once crystals are formed and soft solid features emerge in the rheology. The longitudinal viscosity responds strongly to the emergence of solid crystalline material, probably because it relates to the acoustical loss mechanisms arising in part from viscous dissipation in the bulk liquid but also due to dissipation mechanisms at the crystals. So, the change in longitudinal viscosity could be used as a surrogate for bulk viscosity, due to a relatively small contribution from shear viscosity. The velocity also changes markedly as crystals form on cooling, reflecting the change in the real (elastic) part of the bulk modulus, or equivalently the compressibility.

Our rheological data agrees with that of the other groups to within an order of magnitude, the differences are unsurprising due to the sensitivity of AMF rheology to the conditions under which it is cooled and stirred. Nevertheless, the general trend of increasingly solid-like behaviour is evident in the complex elastic moduli.

5.1.1 Conclusions

Our rheological and ultrasonic measurements in different frequency regimes have been used to extract the complex elastic and shear moduli of AMF undergoing crystallisation by cooling. The results indicate the effect of the solid structure emerging from the liquid melt, imbuing an increasingly solid-like character to the soft-solid material, owing not only to the formation of microcrystals, but also to

their longer-range structuring corresponding to a dramatic increase in the real part of the shear modulus observed in oscillatory rheometry at 1Hz. Whilst the imaginary part of the shear modulus also increases dramatically as the AMF crystallises, it remains much smaller than the real part of the modulus.

The ultrasonics, at much higher frequencies, 2-100MHz, demonstrated that the longitudinal attenuation exhibits a change in frequency-dependence as crystallisation develops compared with the liquid melt, owing to the solid-liquid interactions of the crystals in the liquid phase and in the presence of the longitudinal acoustic oscillatory wave. This frequency-dependence of attenuation corresponds to a frequency-dependent (or equivalently non-Newtonian) longitudinal viscosity, a combination of both shear and bulk viscosities. In different parts of the spectrum, a different exponent for the frequency-dependence is observed; and these frequency regimes are determined by the crystal size. The ultrasonic measurements are therefore sensitive to the development of crystalline structure and of changes in viscosity. Whilst the real (elastic) moduli are dominated by the bulk modulus with negligible contribution from the real shear modulus, the imaginary parts of the moduli (relating to viscosity) have significant contributions from both bulk and shear viscosities. In addition, the resonant ultrasound measurement of speed was found to be indeterminate when crystal nucleation and growth were taking place, due to the interaction of the oscillatory acoustic energy with the crystallisation process.

The close relationship between rheology and ultrasonics due to their connection to the complex elastic and shear moduli is important for the extension of the study of soft solid materials to a wider frequency range, and to provide further complementary information from the two techniques. Acoustic techniques significantly expand the application of rheological techniques, they can be implemented in a non-invasive manner into a process, and therefore augment and enhance conventional rheology.

6 Data Availability

The data in this paper is available on request from the authors in the form of an Excel Spreadsheet.

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8 Figure Captions

Figure 1 Polarized optical microscopy pictures of AMF crystals and crystal clusters formed during cooling

Figure 2 Plot of G' (closed blue circles) and G'' (open red circles) against temperature and time for AMF sample provided by Arla foods, measured in an Anton Paar oscillatory rheometer at a controlled strain of 0.001% and a frequency of 1 Hz.

Figure 3 Velocity of sound in AMF, measured in the Resoscan and cooled from 40 °C to 15 °C over 12 hours. Fit to cooling data between 40°C and 26°C, y= -3.7629x + 1636.2, r^2 = 0.992; fit to cooling data between 20°C and 15°C, y= 7.5535x + 1332.7, r^2 = 0.5848.

Figure 4 Selected attenuation spectra for anhydrous milk fat. Filled blue circles, 43 °C; red open squares, 40 °C; black open circles, 23 °C. 43 °C fit, y= $0.56x^{1.885}$ r^2=0.9992. 23 °C fot, y=$1.464x^{1.7703}$, r^2 = 0.9976.

Figure 5 Longitudinal Viscosity plotted against wavelength at 40 °C, derived from data in Figure 4. Short wavelength – black crosses, power law fit y=$2.4562x^{0.2427}$, r^2= 0.996 Long wavelength – red open circles, power law fit, y= $0.4125x^{0.0349}$ r^2=0.938

Figure 6 Longitudinal viscosity plotted vs frequency step cooling to constant thermostated temperatures of 43, 40, 36, 26,23 and 19-17. Error bars represent standard error and are plotted for the smallest and the largest deviation. Stirring speed was 13 rad s^{-1}. Red +, 43 °C; orange diamond, 40 °C; green triangle, 36 °C; orange square, 26 °C; green diamond, 23 °C; black circle, 19-17 °C.

9 Table Caption

Table 1 Experimental and calculated acoustical and rheological quantities for crystallising AMF. Real part of the shear modulus G' determined from rheology and assumed frequency-independent, imaginary part of shear modulus G'' at low frequency determined from rheology (Fig 1). Real part of bulk modulus K' determined from longitudinal speed c_{long} (Eq. 8) and density ρ (Jenness et al., 2010). Longitudinal viscosity η_{long} determined from attenuation α_{long} (Fig 3), using longitudinal speed c_{long} (Fig 6) with Eq 9 and density ρ (Jenness et al., 2010) . Bulk viscosity η_b from Eq 2 using shear viscosity from (De Graef et al., 2009). Power-law fits of frequency-dependence of viscosity were made in two sections, <10MHz and >40MHz. Imaginary parts of the moduli (M'',K'',G'') at high frequency from attenuation Eq. 4, Eq. 6, Eq. 21 from corresponding viscosities.

10 Appendix

10.1 Rheology and acoustics and the complex moduli

Rheology and acoustics share a common basis in the physics and mathematics of elastic materials, and both begin from generalisations of force/stress and displacement/strain. The most general statements involve relations between a stress (force per unit area) tensor and a strain (or strain-rate) tensor, which need not be linear, isotropic or even time reversible (Povey, 1997). Examples of this approach in acoustics can be found in a number of works (Povey, 1997); (Epstein and Carhart, 1953) (Allegra & Hawley, 1972) but this is not the place to pursue these ideas. Instead, we will develop a relatively straightforward mathematical relationship between rheology and acoustics using simple rheological expressions.

It might be thought that compressional waves involve only normal stresses whilst rheology is often about shear stresses. However normal stresses contain a term depending on viscosity and as observed by Stokes (Stokes, 1845, 1848) the compressional wave is therefore linked with viscosity, mostly in the dissipation (attenuation terms).

The relationship between fluid viscosity η_{long} [Pa s] and longitudinal sound attenuation α_{long} [Np m^{-1}] was first derived by Stokes. Pierce (Pierce, 1994) and Morse & Ingard (Morse & Ingard, 1968) present a more general expression that incorporates thermal effects

$$\alpha_{long} = \frac{\eta_{long}\omega^2}{2\rho {c_{long}}^3}\left(\frac{4}{3} + \frac{\eta_b}{\eta_{long}} + \frac{(\gamma-1)\kappa}{\eta_{long}C_p}\right) \quad (22)$$

here γ is the ratio of specific heats, κ [W m^{-1} .K^{-1}] is the thermal conductivity; C_p [J K^{-1}] is the specific heat at constant pressure; ω is radial frequency [rad s^{-1}] and η_{long} [Pa s] is longitudinal viscosity.

This equation does not apply at very high frequency. When attenuation is plotted as a function of frequency, a normal distribution curve is obtained (Eq. 10-8.10c and Fig. 10-12 in Pierce (Pierce, 1994)) and the frequency at the peak of attenuation is the inverse of the viscous relaxation time τ. (This equation is the same as that in (Epstein & Carhart, 1953b) Eq 7.4, using their Eq 3.5 and knowing that their μ=(3/2)*η_b. This is also Eq 3.14 in the 2002 edition of Dukhin and Goetz (A. S. Dukhin & Goetz, 2002). The equation is low frequency because of the neglect of second order terms in the thermal and viscous contributions; the frequency range of validity is therefore below around 10^{14} Hz in water). It should be emphasised at this point that other phenomena (scattering, molecular relaxation, diffraction) may form a significant component of the measured attenuation, in addition to viscosity. It is therefore important, in the first place, to verify the frequency-squared dependence of the attenuation and account for those mechanisms not associated with viscosity.

In most liquids, the ratio of specific heats is close to one (in water at 10 KPa and 25 °C it is 1.0106) and the thermal term is usually neglected, so that rearranging This equation we get

$$\eta_{long} = \frac{3\alpha_{long}\rho c_{long}^3}{2\omega^2} \quad (23)$$

where the relationship

$$\eta_{long} = \eta_b + \frac{4\eta_s}{3} \quad (24)$$

(here η_b is bulk viscosity and η_s is shear viscosity) is analogous to the relationship between elastic moduli in solids

$$M = K + \frac{4}{3}G \tag{25}$$

where *M* [Pa] is the elastic modulus, *G* the shear modulus and *K* is the bulk modulus defined above. In rheology the moduli are each expressed as two numbers, a 'real' part, relating to strains that are in step with the applied stress and an 'imaginary' part where the strains are $\pi/2$ radians out of step,

$$M = M' + iM'' = K' + iK'' + \frac{4}{3}(G' + iG'') \tag{26}$$

where $i = \sqrt{-1}$.

For a harmonic oscillation of the form $e^{-i\omega t}$ the strain rate is equal to the strain multiplied by a factor $-i\omega$, and the relation between complex elastic moduli and viscosity enables us to re-express the acoustic properties. In solids, the equivalent of the Newton-Laplace equation is

$$c_{long} = \sqrt{\frac{M'}{\rho}} = \sqrt{\frac{K' + {}^{4}/_{3}G'}{\rho}} \tag{27}$$

In fluids and soft solids *K*' >> *G*', for example in a yoghurt *G*' ~ a few Pa whilst *K*' is ~ 10^{10} Pa so that

$$c_{long} = \sqrt{\frac{K'}{\rho}}. \tag{28}$$

We can now rewrite Eq. 4 in terms of the complex elastic moduli

$$\alpha_{long} = \frac{2M''}{3\rho c_{long}^3}\omega = 2\frac{K'' + {}^{4}/_{3}G''}{3\rho c_{long}^3}\omega = \frac{2\omega^2 \eta_{long}}{3\rho c_{long}^3} \tag{29}$$

where

$$M'' = K'' + {}^{4}/_{3}G'' = \eta_{long}\omega \tag{30}$$

Examination of the above equations shows that the real part of the shear modulus can contribute to the sound speed and the imaginary part of the shear modulus (which relates to the viscosity) contributes to the sound attenuation, clearly linking acoustics and rheology.

The relationship between length of the longitudinal wave, and the size and geometry of sample is important, due to additional restoring forces arising at boundaries when the longitudinal acoustic wavelength is of the order of or greater than any sample dimension. These additional forces result in acoustic parameters becoming a function of sample geometry, heterogeneity and dimensions. In the following we assume that the longitudinal wavelength is always smaller than any dimension of a sample holder.

10.2 Shear Acoustics

In the field of shear acoustics (Manfredi et al., 2019); (Schroyen et al., 2020) , a highly attenuated shear wave propagates over just a few wavelengths and much more slowly (A. Dukhin, 2021) than the longitudinal wave. Oscillatory shear rheology (Weitz et al., 2007) shares much in common with shear acoustics. The speed of an acoustic shear wave is given by

$$c_{shear}^{2} = \frac{G'}{\rho} \tag{31}$$

and its penetration depth δ_{shear} [m] (Povey, 1997)

$$\delta_{shear} = \sqrt{\frac{2\eta_{shear}}{\omega\rho}} \tag{32}$$

So in a typical yogurt, $c_{shear} \cong 0.03\ m\ s^{-1}$ whereas $c_{long} \cong 1450\ m\ s^{-1}$. At 1 MHz the equivalent wavelengths are $\lambda_{shear} \cong 0.03\ \mu m$ and $\lambda_{long} \cong 1.45\ mm$, at 100 Hz are $\lambda_{shear} \cong 0.3\ mm$ and $\lambda_{long} \cong 14.5\ m$. The shear penetration depth of a yogurt with shear viscosity 2 mPa s (van Marle et al., 1999) is 2.5 mm at 1 Hz, 0.25 mm at 100 Hz, 0.025 mm at 10 kHz and 2.5 µm at 1 MHz. Schroyen (Schroyen et al., 2020) comments that “The penetration depth is of particular importance at very high frequencies since it defines the maximal distance over which the sample can be probed … : “At large ultrasonic frequencies (>1 MHz), the penetration depth becomes so small that surface properties rather than bulk properties are probed”.