J Therm Anal Calorim (2009) 98:7–12
DOI 10.1007/s10973-009-0271-5

Thermo-mechanical method for the determination of the fractal dimension of fat crystal networks

Ricky Lam · Michael A. Rogers · Alejandro G. Marangoni

Special Chapter dedicated to the memory of dr. Michel Ollivon

Abstract The physical properties of a fat are determined by its thermal history, processing conditions, chemical composition, polymorphism and spatial distribution of mass. The mass fractal dimension of a fat crystal network can be determined from the slope of the log–log plot of the storage modulus (G') versus the solid fat content (SFC). Different SFCs were obtained by melting the fat, diluting it to different extents in liquid oil, and crystallizing the blends under specific conditions. One of the major drawbacks of this method is the inability of characterizing the native structure of an already crystallized fat. In the thermo-mechanical method developed in this study, SFC is changed via temperature variations instead. At each temperature, the G' and SFC were measured and the fractal dimension calculated as described above. The thermomechanical method proved to be a simpler and more reliable estimator of the fractal dimension of a fat crystal network than the dilution method.

Keywords Fat · Microstructure · Fractal dimension · Small deformation · Yield stress · Solid fat content

Introduction

Fats are the main structural constituents of many food products including margarine, chocolate, butter and spreads. The sensory textural characteristics (i.e., spreadability, hardness, snap) of fat structured foods are dependent on their macroscopic rheological properties, which are a consequence of the structure of their underlying fat crystal network [1, 2]. This network arises from the interactions between polycrystalline fat particles, and provides the elastic component, or the solid-like behavior, of a plastic fat [3]. The sensory properties of the fat-structured foods are dependent not only on the amount of solid fat crystals present and their polymorphism, but also their geometry and the spatial distribution of crystalline material [4–7].

Fractal theories have commonly been applied to colloidal gels in characterizing their microstructure. However, it was not until 1992 that Vreeker et al. first proposed that a network of tristearin crystals in paraffin oil was similar in nature to a colloidal gel, allowing for possible fractal analysis of the crystal network structure [8]. Extensive work from our group has focused on relating microstructural characteristics in fat crystal networks to mechanical properties using fractal scaling relationships [3], in particular the scaling behavior of the shear storage modulus (G') with the solid fat content (SFC). In this body of work, the fractal dimension was used as a means to characterize the spatial distribution of the mass and was determined using several techniques including light scattering [8], rheology [9–11] and microscopy techniques [12, 13]. Here we will re-examine the most widely used technique to determine the fractal dimension of a fat crystal network, where small deformation rheological methods are employed to measure G' at different volume fractions of solid network mass ($\Phi = \mathrm{SFC}/100$). The fractal dimension can then be determined using the following power–law relationship:

$$G' = \lambda \Phi^{\frac{1}{3-D}} \tag{1}$$

R. Lam · A. G. Marangoni (✉)
Department of Food Science, University of Guelph,
Guelph, ON N1G2W1, Canada
e-mail: amarango@uoguelph.ca

M. A. Rogers
Department of Food and Bioproduct Sciences,
University of Saskatchewan, Saskatoon, SK S7N5A8, Canada

where λ is related to the size of the primary crystals and the strength of their interactions, and D is the fractal dimension [14, 15].

Most commonly, the fractal dimension is determined by melting and diluting a fat to different extents in a solvent so as to change the SFC. Although this method yields reproducible results, there are also several drawback to this method. Firstly, it is a relatively time-consuming analysis which involves the preparation of several blends of the fat in oil and crystallization of the blend under specific conditions. Secondly, the melting and dilution of the fat results in a loss of native structure of the fat.

To overcome these limitations, a thermo-mechanical method for the determination of the mass fractal dimension was developed. It is also possible to modify the solids' volume fraction by progressively heating a fat while simultaneously measuring the G' and SFC. The fractal dimension can then be determined from the log–log plot of the G' versus Φ (=SFC/100) at the different temperatures. The main advantage of the thermo-mechanical method is the ability to determine the fractal dimension of a pre-formed fat crystal network without any loss in native structure. Here we also show that the variation in the yield stress as a function of temperature (and thus SFC), determined by cone penetrometry can also be used to estimate the value of the fractal dimension of a fat crystal network.

Experimental

Materials

Canola oil (Sunfresh limited, Toronto, ON, CAN), Cocoa Butter (CB) (Mars, Mississauga, ON, CAN), Anhydrous Milk Fat (AMF) (Parmalat, London, ON, CAN), fully hydrogenated canola oil (FHCO) (Bunge, Toronto, ON, CAN) and high oleic sunflower oil (HOSO) (Nealanders, Toronto, ON, CAN) were obtained and used as received.

Chemical interesterification (CIE)

A sample of 500 g of 30% (w/w) FHCO in was melted at 85 °C under nitrogen. Interesterification was initiated by the addition of 1.5 g 0.3% (w/w) sodium methoxide catalyst (Sigma-Aldrich, Ontario, Canada) and was maintained under a nitrogen environment by passing nitrogen gas through the sample for 60 min. Four percent acidic water (20% citric acid, w/v) was used to stop the reaction. Following the reaction, the product was washed with eight parts hot water (1:8) followed by the addition of sodium sulfate (5%). 1.5% (w/w) Bleaching clay was added and the sample was heated to 88 ± 2 °C for 20 min, under nitrogen. Finally, the blend was vacuumed filtered through a hot Buchner funnel using filter paper no. 4 (Whatman, no. 4, Florham Park, NJ, USA).

Dilution method

The samples were heated to 45 °C for AMF, 70 °C for CB and 90 °C for CIE, diluted with canola oil in 10% (w/w) increments from 10% canola oil to 40% canola oil, and held for 20 min to erase crystal memory. The blends were crystallized, quasi-isothermally, in a 5 °C incubator for 24 h in 20 mm diameter by 3000 μm cylinder aluminum moulds. All measurements were carried out using an AR 2000 rheometer (TA instruments, New Castle, DE) using a 20 mm flat stainless steel plate with 60-grit wood sandpaper glued to the spindle and peltier plate using an epoxy glue. The sample underwent a 10% compression to ensure sufficient contact between the spindle and peltier plate to prevent slippage. A stress sweep, at 1 Hz, from 0.1 to 400 Pa was used to ensure that measurements were carried out in the linear viscoelastic region. Each measurement was done with six replicates and the means and standard deviations are reported. The SFC of the samples prepared was measured using pulsed nuclear magnetic resonance spectroscopy using an mq20 series NMR Analyzer (Bruker, Milton, Ontario, Canada).

Thermo-mechanical method—small-deformation rheology

Samples were prepared and measured in a similar fashion as the dilution method. SFC was measured using pulsed nuclear magnetic resonance spectroscopy (mq20 Series NMR Analyzer, Bruker, Milton, Ontario, Canada) every 2.5 °C after equilibration for 1 h at each temperature. Each sample was analyzed in triplicate and the average SFC was reported at each temperature. Samples were stored at 5 °C for 24 h prior to analysis on an AR 2000 rheometer (TA instruments, New Castle, DE). Samples were then heated in 2.5 °C intervals and equilibrated for 1 h before the stress sweep was applied.

Thermo-mechanical method—large-deformation rheology

Fats were crystallized in 8 cm diameter by 4.5 cm deep cups at 5 °C for 24 h. The AOCS method Cc 16-60 for cone penetrometry was used to measure the yield stress of the samples. The cone was placed at the surface of the sample and released for 5 s and the penetration depth was recorded. Due to the large sample container the samples were equilibrated for 3 h. Ten penetrations were carried out for each temperature. The yield stress was calculated as described by Haighton [14]:

$$\text{Yield Stress} = \frac{K \cdot m}{P^n} \qquad (2)$$

where the constant K ($K = 19{,}000$) is dependent on the geometry of the cone, m corresponds to the mass (g) of the whole cone assembly, p corresponds to the cone penertration in 1/10 of mm, while the exponent n has a value of 1.6 for both shortenings and butter [16].

Polarized light microscopy

Samples were imaged on a Leica microscope using polarized light (Leica Microsystems: DM-RXA2/CTR-MIC, Richmond Hill, ON, Canada). The polarizer was set to full extinction (90° relative to the analyzer). The 40× objective lens had a numerical aperture of 0.85. The set of images were collected using a cooled CCD camera (Q-Imaging: Retiga 1300, Burnaby, BC, Canada). Focused images were acquired as uncompressed 8-bit (256 greys) grayscale TIFF files with a 1280 × 1024 spatial resolution. Four slides were made where four sets images were taken from each slide.

Data analysis

All data analysis including statistical analysis was carried out using GraphPad Prism 4.0 (GraphPad Software, La Jolla, CA). Unpaired t tests were done for statistics whereby a Welch's correction was applied when the variances were not equal.

Results and discussion

The dilution method and thermomechanical methods were used to determine the fractal dimension and λ parameter of three fat samples using Eq. 1 (Table 1). For the thermomechanical method, the SFC, G' and Yield Stress were measured in the temperature range where the SFC was most sensitive to temperature for AMF (Fig. 1a, b), CB (Fig. 2a, b) and the CIE fat (Fig. 3a, b).

The thermo-mechanical method using small deformation rheology and cone penetrometry produced similar trends

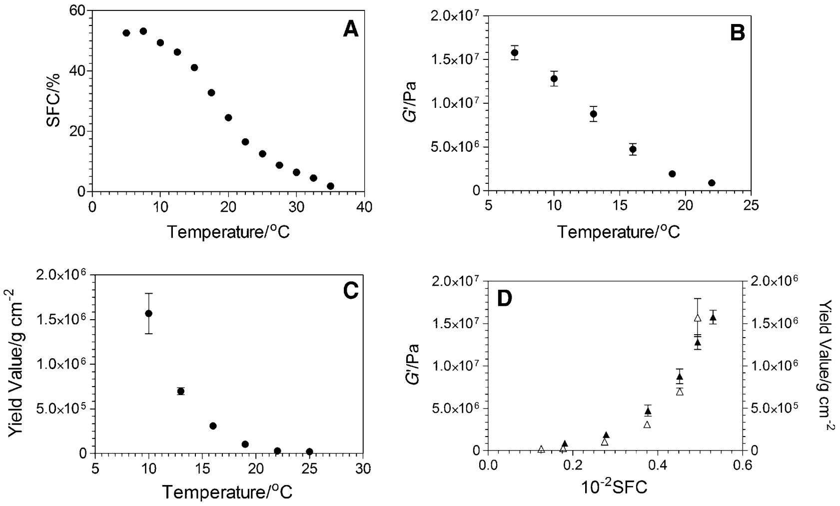


Fig. 1 Required analyses for the thermo-mechanical determination of the fractal dimension of a fat crystal network of the anhydrous milkfat (AMF) sample: **a** SFC-temperature profile **b** G' (Pa) versus temperature (°C) profile from small-deformation rheology **c** yield value (g/cm^2) versus temperature profile (°C) from large-deformation rheology and **d** overlay of the yield value (*open triangle*) and G' (*filled triangle*) versus the solids' mass fraction (SFC/100) profile

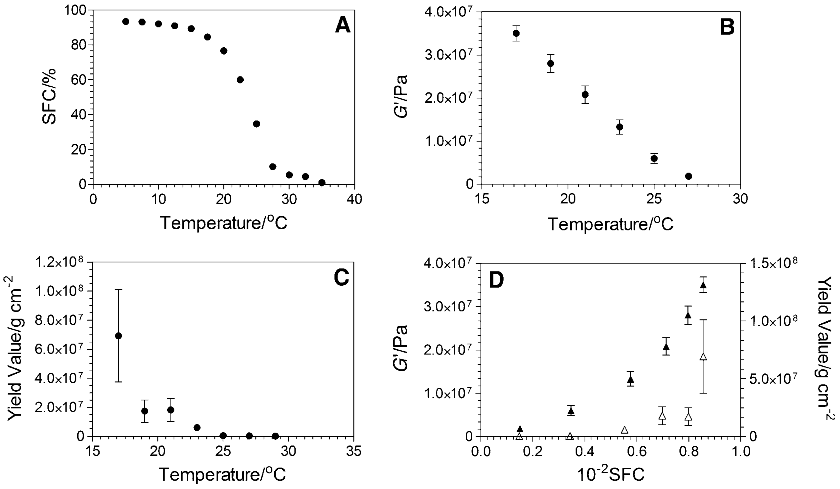


Fig. 2 Required analyses for the thermo-mechanical determination of the fractal dimension of a fat crystal network of the cocoa butter (CB) sample: **a** SFC-temperature profile **b** G' (Pa) versus temperature (°C) profile from small-deformation rheology **c** yield value (g/cm^2) versus temperature profile (°C) from large-deformation rheology and **d** overlay of the yield value (*open triangle*) and G' (*filled triangle*) versus the solids' mass fraction (SFC/100) profile

(Figs. 1, 2, 3d). These similar trends indicate that the structures being probed using small deformation rheology are similar to those being probed using large deformation rheology. deMan et al. suggested that cone penetrometry is sensitive to the inter-floc bonds that connect the crystal clusters and not the clusters themselves [17]. It is these inter-floc bonds which provide the elasticity that is stressed during small deformation rheology [18]. Thus it is not surprising that both large and small deformation rheology provide similar results. D and λ values were obtained by fitting Eqs. 1 and 2 to their respective data sets (Figs. 1, 2, 3d) and reported in Table 1. Statistical analysis revealed that the fractal dimension measured using the dilution method is significantly different than the fractal dimension determined using the thermo-mechanical method (AMF p-value $= 0.0001$, Welch's corrected $t(5) = 10.58$; CB p-value $= 0.0038$, Welch's corrected $t(5) = 5.082$; CIE p-value < 0.0001, $t(10) = 10.03$). Differences between the dilution and thermo-mechanical methods may have arisen because the mechanism of assembly may have been altered during initial crystallization at different dilutions. However,

Table 1 The fractal dimension (D) and λ parameter for the three samples used in this study, determined by three different methods

	D	λ (Pa)	r^2
AMF_d	2.83	1.254×10^9	0.7125
AMF_t	2.65	8.264×10^7	0.9185
AMF_c	2.68	8.121×10^6	0.9380
CB_d	2.72	1.631×10^8	0.4787
CB_t	2.51	4.358×10^7	0.8060
CB_c	2.62	2.186×10^7	0.6971
CIE_d	2.81	5.295×10^9	0.8622
CIE_t	2.71	1.283×10^8	0.8784
CIE_c	2.76	1.540×10^7	0.9876

The letters d, t, c in the subscript indicate dilution method using small deformation oscillatory rheology, indicates thermo-mechanical method using small deformation oscillatory rheology, thermo-mechanical method using cone penetrometry, respectively

The parameter λ is inversely proportional the size of the primary particles and directly proportional to the strength of their van der Waals' interactions

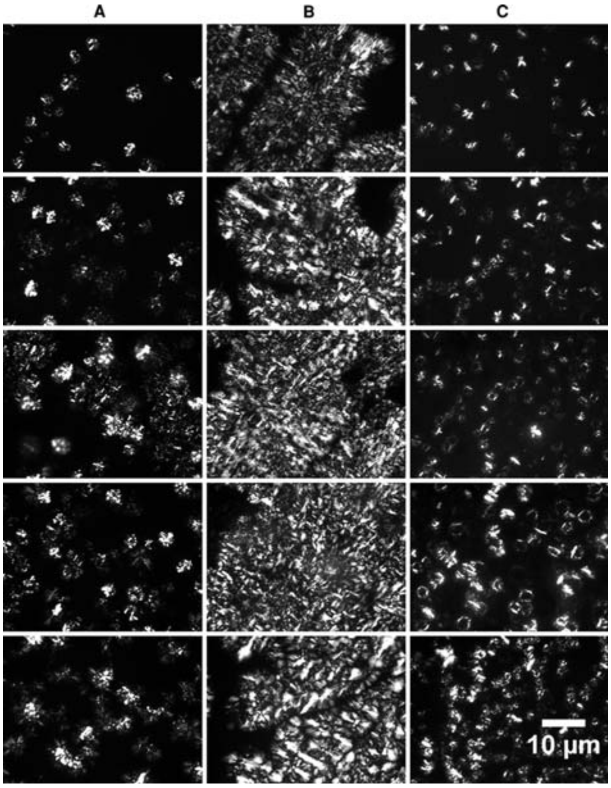
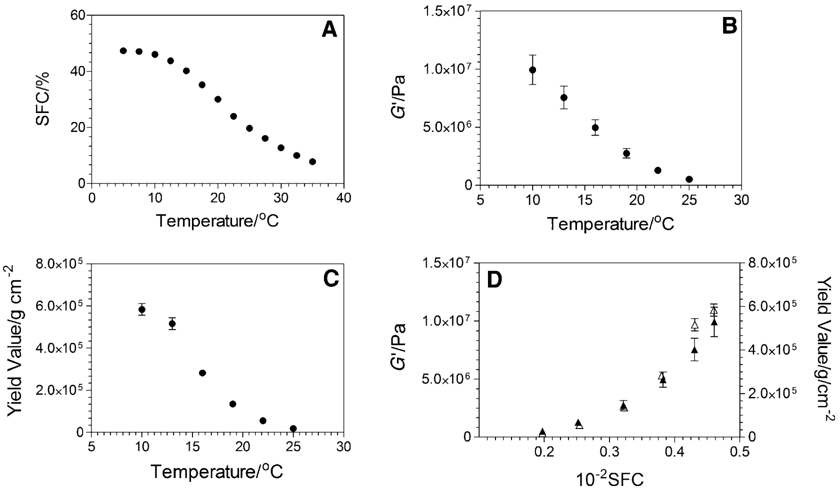


Fig. 4 Polarized light micrographs of samples prepared using the dilution method. Pictures from top to bottom have increasing ratios of fat:oil (w:w): **a** AMF:canola oil 30:70, 35:65, 40:60, 45:55, 50:50 (w/w); **b** CB:canola oil 25:75, 30:70, 35:65, 40:60, 45:55 (w/w); **c** CIE:canola oil 20:80, 25:75, 30:70, 35:65, 40:60 (w/w)

Fig. 3 Required analyses for the thermo-mechanical determination of the fractal dimension of a fat crystal network of the chemically interesterified (CIE) sample: **a** SFC-temperature profile **b** G' (Pa) versus temperature (°C) profile from small-deformation rheology **c** yield value (g/cm^2) versus temperature profile (°C) from large-deformation rheology and **d** overlay of the yield value (*open triangle*) and G' (*filled triangle*) versus the solids' mass fraction (SFC/100) profile



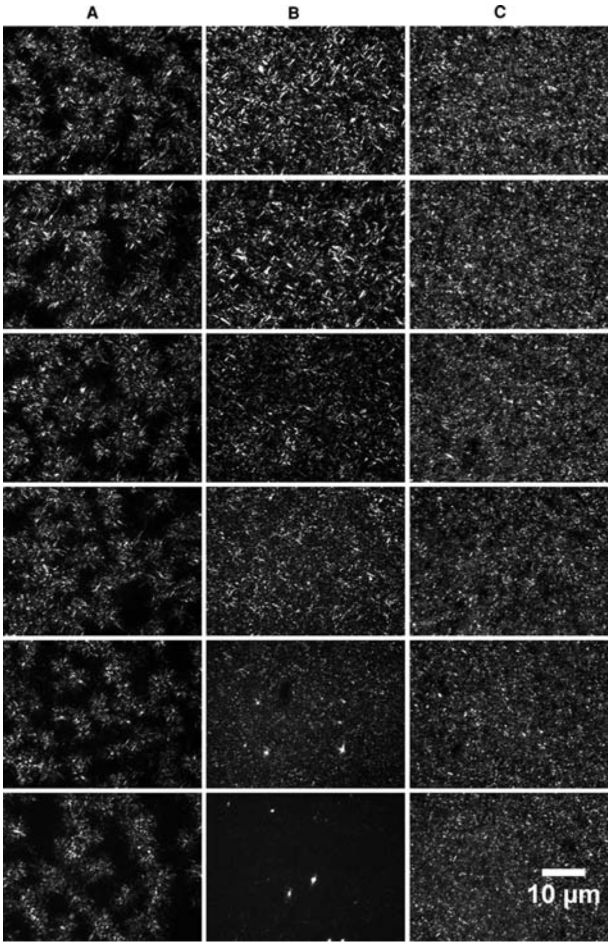


Fig. 5 Polarized light micrographs of samples prepared using the thermo-mechanical method. Pictures from top to bottom are at increasing temperatures: **a** AMF:canola oil 7, 10, 13, 16, 19 and 22 °C; **b** CB:canola oil 17, 19, 21, 23, 25 and 27 °C; **c** CIE:canola oil 10, 13, 16, 19, 22 and 25 °C

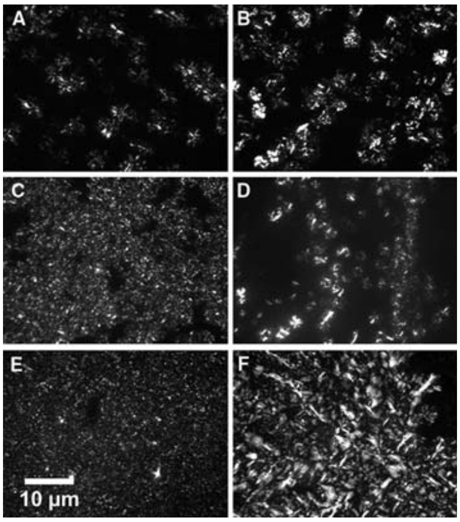


Fig. 6 Polarized light micrographs of samples prepared using the thermomechanical method compared to the dilution method at similar SFCs: **a** thermo-mechanical method of AMF at 22 °C and 17.7% SFC; **b** dilution method of AMF with 45:55 (w/w) fat:oil and 17.7% SFC; **c** thermo-mechanical method of CIE at 25 °C and 19.7% SFC; **d** dilution method of CIE with 40:60 (w/w) fat:oil and 14.2% SFC; **e** thermo-mechanical method of CB at 25 °C and 34.3% SFC; **f** dilution method of CB with 45:55 (w/w) fat:oil and 35.7% SFC

there was no statistical difference ($p > 0.05$) between the fractal dimensions obtained using the thermo-mechanical method with large deformation rheology (using cone penetrometry), and small deformation rheology (AMF 0.011%, CB 0.043% and CIE 0.018%). This indicates that the thermo-mechanical method can be carried out using large deformation mechanical testes, such as cone penetrometry, for the determination of a fractal dimension. The advantage to using cone penetrometry as opposed to small deformation oscillatory rheology is the ease of analysis and the low cost of a cone penetrometer. This would allow food manufactures to employ this technique as a quality control measure. In principle, this technique could be fully automated to yield a value of fractal dimension.

Statistical analysis shows that there is a difference between the λ parameters obtained using the dilution method and the thermo-mechanical method (AMF p-value $= 0.0053$, Welch's corrected $t(5) = 4.713$; CB p-value $= 0.0216$, Welch's corrected $t(5) = 3.296$; CIE p-value $= 0.0002$, Welch's corrected $t(5) = 9.308$). This would indicate that the dilution method will modify the native structure of the fat, which is not surprising, yielding structural features (crystals, crystal clusters) that have different sizes and/or interactions. Therefore polarized light microscopy (PLM) was employed to observe if there were obvious differences in their microstructure. The crystal morphology is different between the dilution method (Fig. 4) and the thermo-mechanical method (Fig. 5). The difference is particularly evident when comparing micrographs of the dilution method and the thermo-mechanical method at similar SFCs (Fig. 6).

Conclusions

The thermo-mechanical method proved to be a simple, accurate and convenient method for determining the fractal dimension of fat crystal networks, particularly when the native structure of a fat needs to be preserved. Moreover, simple large deformation mechanical tests such as cone penetrometry can be used instead of small deformation oscillatory rheology. This allows the mass fractal dimension to be used as a quality control tool in the food industry.

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