Analysis of Rheological Time Series Data

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ABSTRACT

Analysing rheological time series data for milk coagulation is challenging. The coagulation curves carry very important information for the dairy investigator. However, it is often difficult to identify the significant differences between curves, especially when the dataset is large, and the coagulation patterns appear very similar. The aim of this work was to develop a model that reduces the measured coagulation data into a number of quantitative parameters that can be analysed statistically. The Gompertz model for growth curves was used first to fit the experimental rheological curves, resulting in very high correlation coefficients, and followed by a non-linear regression curve fitting in MATLAB. It was then possible to extract values of maximum growth rate, value at infinite time, growth starting time, time of maximum growth rate, etc, from the fitted analytical functions. Each repetition of a coagulation curve yielded a complete set of extracted values that were possible to analyse statistically. ANOVA and Tukey test were then possible to run to identify significant differences between the variables. Application of the Gompertz equation on time sweep data from the gel formation of concentrated milk casein proved to work satisfactorily in the determination of the characteristic variables and to determine when differences were significant.

INTRODUCTION

Time is an important parameter for product processing in the dairy industry and in all other industries dealing with biological raw materials. Monitoring coagulation time for instance to cut the curd in cheese or the formation of gel in yogurt helps the dairy technologist deciding on the cutting time and/or control the gel firmness. Oscillatory shear is a transient dynamic test where a sample is subjected to either a stress, strain or frequency that varies harmonically with time, and is the most popular method widely used to characterize viscoelastic materials, such as dairy products[1, 2]. Time sweep test is a type of oscillatory test used to monitor the formation or breakdown of structure and is now widely used for measuring milk coagulation[3].

When measuring the coagulation/gelation profile of a milk system; the temperature, frequency and strain amplitude, within the LVR (e.g. $\leq$ 1% strain to not disrupt the structure development) are fixed. Thus, the measured values of and the storage modulus (G’) and loss modulus (G’’) are a function of time. A typical milk coagulation graph will start by G’ being below G’’ with a constant gap between them, indicating a more liquid state of the matter. Over time, G’ and G’’ will converge and cross, and then deviate again while keeping a constant gap between each other. The time when G’ starts increasing and G’’ starts decreasing marks the ‘onset of gelation’ until the point where G’ and G’’ cross and the phase angle is 45° indicating the 'gel point'[4].

The coagulation curves obtained by the time sweep test carries a large amount of information, however several challenges encounter the dairy investigator in extracting it. Some of these challenges could be the large number of datasets. Another challenge may be similarity for curve patterns, the absence of a clear 'gel point' and the measurable rate of gel structure build up. Some dairy systems, i.e. casein concentrates, are viscoelastic and tend to show a more elastic behaviour than a viscous one. When carrying out the time sweep test, their coagulation curves may already have a higher G' value than G'' at the start of measurement and before the gel has been formed. Which consequently causes a lack of a recorded crossover data point and a clear phase angle of 45°. It is therefore crucial to find a systematic method to identify data points and extract them for statistical testing.

Coagulation curves resemble to a very high extent the bacterial growth curves in their three phases; lag, exponential and stationary. Researchers have been able to describe biological meaning in growth curves using mathematical parameters by using Gompertz equation[5]. The Gompertz model became currently one of the most sigmoid models used for fitting growth data[6]. The model is possible to re-parametrise and apply to different fields and types of growth curves.

The objective of this work was to:

- Develop a model that reduces the measured coagulation curve data into a number of quantitative parameters that can be analysed statistically.
- Demonstrate the potential application of Gompertz equation.

MATERIAL AND METHODS

Milk casein concentrates production: The milk was separated (Westfalia Separator AG, MSD50-01-076, Oelde, Germany) at 55 °C into skimmed milk and cream. Pasteurization of skimmed milk was performed at 73 °C for 15 s using a plate heat exchanger (A3-HRB, Alfa Laval, Lund, Sweden) prior to MF. Pasteurized skimmed milk was fractioned into casein concentrate (CC) and MF-permeate (containing proteins and lactose and minerals) using a 0.14 µm ceramic membrane at uniform trans-membrane pressure (UTP) with average flux of 66±4.5 Lh^{-1} m^{-2} to a volume concentration factor of ~2.5 at 50 °C. When the protein concentration reached 8.0 ± 0.1%, aliquots of 10 L of CC were collected and cooled to 4 °C prior to experimental treatment.

Rheological analysis

Acid coagulation properties were measured using an MCR 301 Rheometer with a concentric- cylinder (C-CC27/Ti diameter: 26.657 mm, length: 40.003 mm) and cup (C-CC27/T200/Ti diameter: 28.926mm) (Anton Paar GmbH, Graz, Austria). Prior to measurement, the CC sample (30 ml) was kept in a water-bath at 32 °C for 30 min. Further 4.5 % of glucono delta lactone (GDL) (Sigma, Italy) was added to mimic the acidification occurring during fermentation and the sample was stirred for 20 seconds. Further 15 mL was transferred into the rheometer cup for immediate measurement. Small amplitude oscillatory measurements were performed at 32 °C with a very low constant strain of 0.001 within the viscoelastic range. During measuring the constant frequency of 1Hz were applied to avoid any influence on the gel formation properties. Storage modulus (G'), loss modulus (G''), phase angle (δ) and the complex viscosity (η') were recorded for 45 min. Each sample was analysed in duplicate as a minimum; that is, more replicates were run when the replicates did not follow the same coagulation pattern.

Gompertz Model fitting:

To obtain a direct estimation of the rheological parameters in the Gompertz equation; the mathematical parameters were substituted with rheological meaning, as explained by Zwietering *et al*[5] and Boiani *et al*[7] for growth curves re-parameterization.

The model was tested on the G' data output of the coagulation curves.

The employed Gompertz function and the derivatives used in this work are the following:

$$G' = A + Be^{Ce^{Dt}}, \quad (1)$$

$$\frac{dG'}{dt} = BCDe^{Dt}e^{Ce^{Dt}}, \quad (2)$$

$$\begin{aligned}\frac{d^2G'}{dt^2} &= BCD\left[De^{Dt}e^{Ce^{Dt}} + e^{Dt}\frac{d}{dt}\left(e^{Ce^{Dt}}\right)\right] \\ &= BCDe^{Dt}\left[De^{Ce^{Dt}} + e^{Ce^{Dt}}CDe^{Dt}\right] \\ &= BCD^2\, e^{Dt}e^{Ce^{Dt}}\left[1 + Ce^{Dt}\right], \quad (3)\end{aligned}$$

Where A, B, C and D are constants of the equation.

The maximum slope occurs at the time where the second derivative is zero, i.e.

$$\frac{d^2G'}{dt^2} = 0, \quad (4)$$

$$Ce^{Dt} = -1, \quad (5)$$

$$t = \frac{\ln(-1/C)}{D}. \quad (6)$$

Substituting this time into the expression for the derivative gives the value of the maximum growth rate.

A MATLAB program was used to fit the model and calculate the equations for different parameters. The non-linear equation was fitted to the coagulation data by nonlinear regression using the nonlinfit function. The goodness-of-fit of the model was assessed by R-squared (R^2). Means and standard deviation were calculated using their corresponding program functions. A MATLAB script was developed to calculate the following parameters: 1. Time at max coagulation rate, 2. Max rate, 3. End time, 4. G' at end time, 5. G' at max rate, 6. The intercept and 7. Start time of coagulation period: intersection with G'=A and maximum gradient line. Following the model fit and generation of new parameter dataset, ANOVA and multicomparison test were performed to identify significant differences.

RESULTS AND DISCUSSION

The experimental design resulted in at least 150 coagulation curves with 180-time data point recorded for every measured parameter, i.e. G' and G''.

Fig 1 shows some of the coagulation curves from rheological software obtained by the time sweep test. Visually, some curves appeared similar and some appeared different in pattern. The use of a very low strain in the measurement process resulted mainly in two types of coagulation curves: 1. A curve that started with high noise, Fig 1. This is possibly due to air bubbles in the sample and small-suspended particles. 2. A curve with an early dominant elastic behaviour before start of the gel building structure and formation, Fig 2. In both cases, the raw data failed to record the 'crossover'' time where δ = 45° indicating gelling point/time, and the visual differentiation between the curves is not accurate.

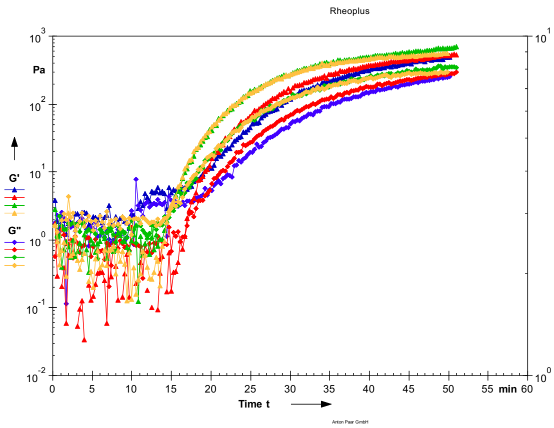

Figure 1. Storage and Loss Modulus (Pa) of acid gel formation as function of time (min).

The coefficient of the determination value indicated that 98.4% of the data had an average R^2= 0.99, while the remaining 1.6% had a minimum R^2= 0.97. Figure 3 shows some of the coagulation curves before and after the model fitting. Therefore, we can conclude that the Gompertz model present an excellent tool to fit the time series curves.

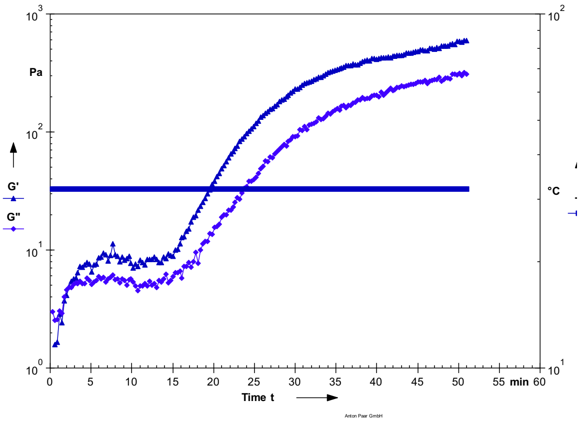

Figure 2. Storage and Loss Modulus (Pa) of acid gel formation as function of time (min). Complete absence of cross-over point.

Following the successful fitting of the model, it was possible to extract and calculate values like the start time of coagulation, i.e. crossover point, and the G' at end time. These are very important parameters for the dairy investigator regarding milk coagulation. With the presence of replicates per sample, it was moreover possible to use the generated numerical values in statistical analysis to compare significant differences within the studied factors, using ANOVA and pairwise comparison.

Significant differences in coagulation parameters between curves that appeared visually similar were now perceived.

CONCLUSION

The Gompertz modified model was successfully fitted into the oscillatory time sweep data of casein concentrates acid coagulation curves. The current finding provides an effective and systematic analytical method for the dairy industry to investigate (monitor) coagulation properties in milk.

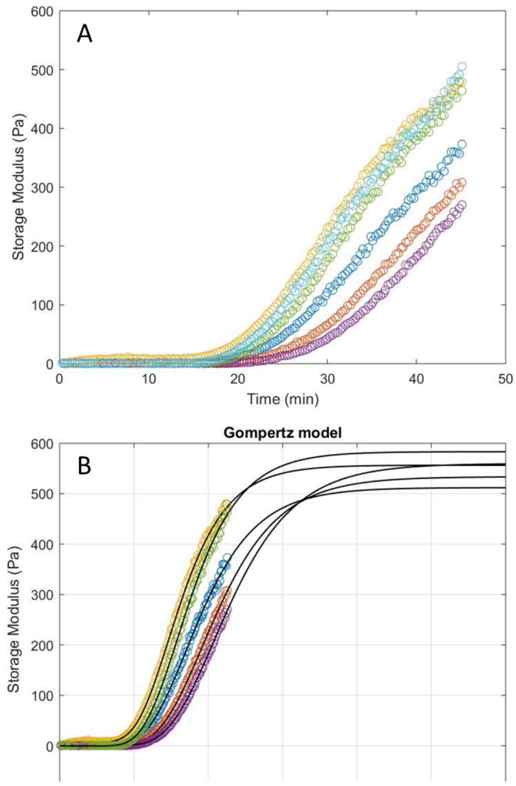

Figure 3. ln scale of storage modulus (G') data of some acid gels (A), Gompertz model fitted G' of the same acid gel data (B).

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