Activation energy measurements in rheological analysis of cheese

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A B S T R A C T

Activation energy of flow ($E_a$) between 30 and 44 °C was calculated from temperature sweeps of cheeses with contrasting characteristics to determine its usefulness in predicting rheological behavior upon heating. Cheddar, Colby, whole milk Mozzarella, low-moisture part-skim Mozzarella, Parmesan, soft goat, and Queso Fresco cheeses were heated from 22 to 70 °C, and $E_a$ was calculated from the resulting Arrhenius plots. Protein and moisture content were highly correlated with $E_a$. The $E_a$ values for goat cheese and Queso Fresco, which did not flow when heated, were between 30 and 60 kJ mol$^{-1}$. Cheddar, Colby, and the Mozzarella did flow upon heating, and their $E_a$ values were between 100 and 150 kJ mol$^{-1}$. Parmesan, the hardest cheese, flowed rapidly with heat and had an $E_a > 180$ kJ mol$^{-1}$. $E_a$ provides an objective means of quantitating the flow of cheese, and together with elastic modulus and viscous modulus provides a picture of the behavior of cheese as it is heated.

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1. Introduction

Cheese was one of the first foods to be examined by fundamental rheological methods. Being a classic viscoelastic material, cheese lends itself to small amplitude oscillatory shear analysis (SAOSA), where stress and strain are varied harmonically with time within the linear viscoelastic region. SAOSA is a nondestructive technique that provides information on the energy stored and released in the sample being tested. The data obtained during a test include the complex modulus G*, which is a measure of the total energy required to deform the specimen and is defined as:

$$|G^*|^2 = (G''^2 + (G')^2)$$

(1)

where $G'$ is the elastic modulus (or storage modulus), a measure of the energy stored in the material during an oscillation, and $G''$ is the viscous modulus (or loss modulus), a measure of energy dissipated as heat. The complex viscosity $\eta^*$ is the resistance to flow under oscillatory shear conditions and is the ratio of $G^*$ to frequency (Tunick & Nolan, 1992). A sample exhibits predominately viscous properties when its tan $\delta$ (equal to $G''/G'$) exceeds 1.0. When the tan $\delta$ is less than 1.0, a sample is predominately elastic.

When conducting a temperature sweep (increasing temperature while maintaining steady oscillatory strain and frequency), the activation energy of flow, $E_a$, may be calculated over a limited temperature range by substituting the complex viscosity, $\eta^*$, and absolute temperature, T, in the Arrhenius equation:

$$\eta^* = A \exp\left(-E_a/RT\right)$$

(2)

where $A$ is the pre-exponential factor and $R$ is the gas constant, 8.314 J K$^{-1}$ mol$^{-1}$. $E_a$ is proportional to the slope of the line in the Arrhenius plot of $\ln \eta^*$ versus 1/$T$. $E_a$ has long been applied to liquid foods (Rao, 1977) since it measures the energy required to overcome resistance to flow, but it may also be used to examine the flow of cheese during heating. Milk fat melts between approximately 40-44 °C (Tunick, 1994) and cheese starts to flow at the upper end of this range. A convenient section of the Arrhenius plot for determination of $E_a$ is therefore 30-44 °C, in which the fat completes its melting and the cheese structure is flowing. This range is small enough for the Arrhenius relationship to be valid and large enough for a representative portion of the flow to be observed.

A number of authors have published findings on SAOSA of cheese over the past 30 years, starting with Taneya, Izutsu, and Sone (1979), who performed temperature sweeps of Cheddar, Gouda, and processed cheese. Most of the work since then has centered on Cheddar, Feta, Gouda, Mozzarella, and processed cheeses, as reviewed by Gunasekaran and Ak (1993). Temperature sweeps of natural cheese (as opposed to processed or imitation cheese) have been reported in less than 20 papers since 1980, and few of these compared cheese varieties. Published studies on $E_a$ of natural cheese are even scarcer. Tunick et al. (1990) measured $\eta^*$ of Cheshire cheeses after 20 and 60 wk of aging, and calculated $E_a$ to...
be 102 and 85 kJ mol\(^{-1}\), respectively. Cheddar aged 60 wk exhibited a higher \(E_g\) of 137 kJ mol\(^{-1}\), which was attributed to its stronger protein network. Korolczuk and Mahaut (1989, 1990, 1992) compared apparent viscosity of fresh cheeses with nonfat versions and calculated \(E_g\) to be 13–40 kJ mol\(^{-1}\) for the nonfat cheeses, which was about one-third the \(E_g\) of fat-containing cheeses between 15 and 20 °C.

Whereas the measurement of \(G'\), \(G''\), and \(\eta^t\) yields information on the strength of the protein matrix in cheese, \(E_g\) quantitates how quickly the structure degrades with heating. Differential scanning calorimetry may be performed on cheese samples to obtain a thermal profile of the fat in cheese and the oil that leaks out with heat (Tunick, 1994). However, the thermal profile is similar for all bovine milk cheeses, the heat of fusion of the fat is always between 70 and 80 J g\(^{-1}\), and information on protein matrix degradation is not provided.

Several empirical techniques for measuring melting quality of cheese have been developed (Park, Rosenau, & Peleg, 1984), but SAOSA is a fundamental technique that uses specific specimen geometries and instruments, allowing systematic and mathematical interpretation of the results (Tunick & Van Hekken, 2002). Correlations between empirical cheese melting tests and SAOSA data have been found for Cheddar (Ustunol, Kawachi, & Steffe, 1994) and imitation cheese (Mounsey & O’Riordan, 1999). The determination of \(E_g\) along with \(G'\), \(G''\), and \(\eta^t\), should provide more extensive knowledge on the behavior of cheese during heating. The purpose of this research was to evaluate the usefulness of \(E_g\) data in cheese analysis by examining a range of cheeses with a variety of characteristics.

2. Materials and methods

2.1. Cheese samples

Queso Fresco (QF) was prepared in the laboratory using 205 kg of milk from a local dairy. The milk, which contained 3.5% fat, was pasteurized at 73 °C for 15 s and homogenized at 10.3 MPa. It was then warmed to 32 °C and CaCl\(_2\) was added to a concentration of 0.1%. Chymoferment-produced chymosin (14 ml; Chr. Hansen’s Laboratory, Milwaukee, WI, USA) was diluted in 500 mL of water and added to the milk, and coagulation occurred in 30–35 min. The curd was cut with 9.5 mm knives, and after 5 min of resting the curd and whey were heated to 39 °C over a period of 35 min. The whey was drained after 30 min of cooking and the curd was trenched and drained for 15 min. The curd was finely milled and divided into equal portions that were dry salted to levels of 0.7 and 2.2% NaCl. The curd was tightly packed into molds, held over-night at 4 °C, vacuum packed, and stored at 4 °C. Samples were taken for analysis after 4 wk.

Other cheeses were purchased at local stores: mild Cheddar and Colby aged approximately 4 wk (Great Value, Bentonville, AR, USA), Parmesan aged at least 10 mo (Giant, Landover, MD, USA), whole milk (WM) Mozzarella and low-moisture part-skim (LMPs) aged approximately 4 wk (Lucerne, Pleasanton, CA, USA), and chèvre, or soft goat cheese aged approximately 2 wk (Betin, Inc., Belmont, WI, USA).

2.2. Compositional analyses

Moisture was determined by the forced draft oven method 948.12 (AOAC International, 1997) and fat was determined by the modified Babcock method (Kosikowski & Mistry, 1997). Moisture in nonfat substance (MNFS) was calculated as percent moisture divided by (100 – percent fat). Fat in dry matter (FDM) was calculated as percent fat divided by (100 – percent moisture). Protein was determined by a 1112 series nitrogen analyzer (CE Elantech Inc., Lakewood, NJ, USA). NaCl was determined by Quantab chloride test strip (Hach Co., Loveland, CO, USA) and pH by model 611 pH meter (Orion Research Inc., Cambridge, MA, USA) equipped with a spear-tip electrode (Sensorex Corp., Garden Grove, CA, USA).

2.3. Rheological analyses

Cheese samples were allowed to come to room temperature and were cut into disks measuring 25.4 mm diameter and 4–5 mm thick using piano wire and a cork borer. Specimens were then placed in an AR-2000 rheometer (TA Instruments, New Castle, DE, USA) at 22 °C between parallel aluminum plates. To prevent slippage, the specimens were glued to the plates with cyanoacrylate adhesive. Strain sweeps were performed to determine the linear viscoelastic range, which was between 0.05 and 1.3–1.5% for each cheese. A strain of 1.0% and a frequency of 1.00 rad s\(^{-1}\) were selected for each specimen. Using the instrument’s sealed environmental chamber, samples were heated from 24 to 70 °C over a period of 70–85 min, corresponding to a heating rate of 0.54–0.66 °C min\(^{-1}\). \(G'\), \(G''\), tan \(\delta\), and \(\eta^t\) were recorded every 2 °C. Three different cheeses of each type were analyzed, and three specimens of each sample were run once, producing nine replicates. Calculation of \(E_g\) was made by determining the slope of the plot of \(\ln \eta^t\) versus 1/\(T\) between 30 and 44 °C.

2.4. Statistical analyses

Linear regressions, slope, and \(R^2\) values for the Arrhenius plots were calculated using Excel software (version 2002 SP-1, Microsoft Corp., Redmond, WA, USA). Statistical analyses were performed by analysis of variance with mean separation using the Bonferroni technique (SAS, 2004). Differences were identified as significant only when \(P < 0.05\). Averages of all nine replicates of each cheese variety are reported.

3. Results and discussion

3.1. Composition and rheology

Table 1 shows the composition of the cheeses. The Cheddar and Colby had nearly the same composition, and contained the most fat and least NaCl of the cheeses tested. The QF types were also compositionally similar to each other, except for NaCl content. The Parmesan and goat cheeses were at the extremes for moisture, MNFS, and protein, with Mozzarella in between them.

The rheological results are shown in Tables 2 and 3. Parmesan exhibited the highest \(E_g\) and highest initial values (at low temperature) for \(G'\), \(G''\), and \(\eta^t\), which were due to the strong nature of the.

<table>
<thead>
<tr>
<th>Cheese</th>
<th>pH</th>
<th>Component (%)</th>
<th>Moisture</th>
<th>Fat</th>
<th>Protein</th>
<th>NaCl</th>
<th>MNFS</th>
<th>FDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheddar</td>
<td>5.40c</td>
<td>37.8d</td>
<td>34.1a</td>
<td>22.3c</td>
<td>0.6e</td>
<td>57.4d</td>
<td>54.8a</td>
<td></td>
</tr>
<tr>
<td>Colby</td>
<td>5.40c</td>
<td>39.3d</td>
<td>33.3a</td>
<td>21.1c</td>
<td>0.6e</td>
<td>58.9d</td>
<td>54.9a</td>
<td></td>
</tr>
<tr>
<td>Whole milk Mozzarella</td>
<td>5.66b</td>
<td>49.6c</td>
<td>22.7b</td>
<td>22.9c</td>
<td>1.5c</td>
<td>64.2c</td>
<td>45.0b</td>
<td></td>
</tr>
<tr>
<td>Mozzarella</td>
<td>5.41c</td>
<td>46.6c</td>
<td>19.5c</td>
<td>27.6b</td>
<td>1.9b</td>
<td>57.9d</td>
<td>36.5d</td>
<td></td>
</tr>
<tr>
<td>Parmesan</td>
<td>5.30d</td>
<td>37.6d</td>
<td>24.4b</td>
<td>29.4a</td>
<td>2.4a</td>
<td>49.7e</td>
<td>39.1c</td>
<td></td>
</tr>
<tr>
<td>Goat</td>
<td>4.63e</td>
<td>60.5a</td>
<td>21.0b</td>
<td>17.1d</td>
<td>1.2d</td>
<td>76.6a</td>
<td>53.2a</td>
<td></td>
</tr>
<tr>
<td>2.2% NaCl QF</td>
<td>6.25a</td>
<td>54.8b</td>
<td>23.5b</td>
<td>17.4d</td>
<td>2.2a</td>
<td>71.8b</td>
<td>52.0a</td>
<td></td>
</tr>
<tr>
<td>0.7% NaCl QF</td>
<td>6.30a</td>
<td>54.2b</td>
<td>25.0b</td>
<td>17.5d</td>
<td>0.7e</td>
<td>72.3b</td>
<td>54.6a</td>
<td></td>
</tr>
</tbody>
</table>

* Abbreviations are: MNFS, moisture in nonfat substance; FDM, fat in dry matter. Values in the same column followed by different letters are significantly different \((P < 0.05)\).
Colby softened and disrupted and the casein begins to aggregate. Both Cheddar and fat melting has completed as protein liquefaction of fat, fat coalescence, and protein aggregation. Solid observed by Guinee, Auty, and Mullins (1999) and attributed to its own weight. Cheddar had the higher protein matrix was degraded to the extent that it could not support of 20\(^0\)C required more energy for the fat to melt and Cheddar, perhaps because of the different whey removal tech-

Table 2

<table>
<thead>
<tr>
<th>Cheese</th>
<th>Elastic modulus (G') (kPa)</th>
<th>Viscous modulus (G'') (kPa)</th>
<th>Complex viscosity (\eta') (kPa s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheddar</td>
<td>29.1b</td>
<td>0.38d</td>
<td>9.9b</td>
</tr>
<tr>
<td>Colby</td>
<td>27.6b</td>
<td>0.32d</td>
<td>10.3b</td>
</tr>
<tr>
<td>Whole milk Mozzarella</td>
<td>34.3b</td>
<td>1.04c</td>
<td>12.3b</td>
</tr>
<tr>
<td>Low-moisture part-skim Mozzarella</td>
<td>30.1b</td>
<td>1.71b</td>
<td>11.2b</td>
</tr>
<tr>
<td>Parmesan</td>
<td>69.3a</td>
<td>2.45a</td>
<td>67.3a</td>
</tr>
<tr>
<td>Goat</td>
<td>9.3d</td>
<td>1.14c</td>
<td>3.3c</td>
</tr>
<tr>
<td>2.2% NaCl Queso Fresco</td>
<td>13.6c</td>
<td>4.51a</td>
<td>3.9c</td>
</tr>
<tr>
<td>0.7% NaCl Queso Fresco</td>
<td>10.5d</td>
<td>4.50a</td>
<td>3.0c</td>
</tr>
</tbody>
</table>

\(^a\) Values in the same column followed by different letters are significantly different \((P<0.05)\).

Table 3

<table>
<thead>
<tr>
<th>Cheese</th>
<th>Activation energy (kJ mol(^{-1}))</th>
<th>A (Pa s)</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheddar</td>
<td>141.1 ± 9.5</td>
<td>46.2</td>
<td>0.998</td>
</tr>
<tr>
<td>Colby</td>
<td>131.3 ± 8.3</td>
<td>42.5</td>
<td>0.997</td>
</tr>
<tr>
<td>Whole milk Mozzarella</td>
<td>113.3 ± 2.0</td>
<td>34.1</td>
<td>0.990</td>
</tr>
<tr>
<td>Low-moisture part-skim Mozzarella</td>
<td>104.4±</td>
<td>31.0</td>
<td>0.996</td>
</tr>
<tr>
<td>Parmesan</td>
<td>182.5 ± 16.8</td>
<td>61.2</td>
<td>0.993</td>
</tr>
<tr>
<td>Goat</td>
<td>69.0 ± 0.4</td>
<td>15.5</td>
<td>0.985</td>
</tr>
<tr>
<td>2.2% NaCl Queso Fresco</td>
<td>34.7 ± 4.4</td>
<td>1.6</td>
<td>0.935</td>
</tr>
<tr>
<td>0.7% NaCl Queso Fresco</td>
<td>30.4 ± 4.5</td>
<td>1.7</td>
<td>0.977</td>
</tr>
</tbody>
</table>

\(^a\) Abbreviations are: MNFS, moisture in nonfat substance; FDM, fat in dry matter.

\(E_a\) values for WM and LMPs Mozzarella were similar (Table 3). The \(\eta'\) values for LMPs Mozzarella dropped slightly below those for WM Mozzarella between 58 and 62\(^0\)C and then the value began to increase (Fig. 2). The increase was due to heat aggregation of caseins, which starts to occur around that temperature range and reorganizes the structure of the matrix (Repatet & Noël, 2003).

At 22\(^0\)C, WM and LMPs Mozzarella cheeses yielded \(G'\), \(G''\), and \(\eta'\) values similar to those of Cheddar and Colby, but at 50\(^0\)C the values for the Mozzarellas were much higher (Table 2 and Fig. 2). Mozzarella is a pasta filata cheese that is heated and stretched

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instead of milled, causing the casein fibers to be elongated and aligned, unlike cheeses that are not stretched. The tan δ for the WM and LMPS Mozzarella cheeses began to exceed 1.0 starting at 46–48 °C, much the same as the Cheddar and Colby samples. Mozzarella flows when heated but retains some structural integrity because of the alignment of the casein fibers, which traps melted fat in columns (Guinee et al., 1999). As a result, the $G'_i$, $G''$, and $\eta'$ values at elevated temperatures were higher than those of Cheddar and Colby. The LMPS Mozzarella exhibited greater rheological values than the WM Mozzarella at 50 °C because of the differences in protein content.

### 3.4. Parmesan

A typical Arrhenius plot and temperature sweep for Parmesan is shown in Fig. 3A. The $E_A$ (182.5 kJ mol$^{-1}$) was clearly higher than those of the other cheeses (Table 3). Parmesan has a low MNFS because it is cooked at 50 °C instead of the 30–39 °C used for Cheddar, Colby, Mozzarella, QF, and most other cheese varieties. Moisture acts as a lubricant in the casein matrix and hydrates the protein, so low-moisture levels in cheese result in a more rigid structure that takes longer to soften with heat and thus a very high $E_A$. Once the Parmesan did begin to soften, after 30 °C, its structure weakened quickly, resulting in a steep drop in $\eta'$.

Despite the hardness of the cheese at room temperature, the $G''$ and $\eta'$ values at 50 °C were the lowest in this study, and the $G'$ at 50 °C was lower than all but the Cheddar and Colby (Table 2). The $G'_i$, $G''$, and $\eta'$ values decreased by two orders of magnitude from 22 to 50 °C, the largest drop of the cheeses tested. However, unlike Cheddar, Colby, and Mozzarella, the tan δ for Parmesan was less than 0.82 throughout the temperature sweep, which showed that the cheese retained its predominately elastic behavior.

### 3.5. Goat cheese

The low values for the viscoelastic properties of the goat cheese at 22 °C (Table 2) demonstrate the lack of strength of the bonds holding the cheese together at room temperature (Van Hekken, Tunick, & Park, 2005). Its $E_A$ was less than half of the values for Cheddar, Colby, Mozzarella, and Parmesan (Table 3). Ordinarily, a soft cheese would be expected to melt easily, especially one with such a high MNFS level (76.6%). The goat cheese did not flow or release oil when heated, however, because of its unusually low pH of 4.63, which is at the isoelectric point of casein. There is therefore no electrostatic repulsion between casein molecules in goat cheese, but there are hydrophobic attractions that increase with temperature and inhibit melting (Lucey et al., 2003). Curd cohesion and ability to flow when heated occurs between pH 4.8 and 5.8 (Lawrence, Creamer, & Gilles, 1987).

Fig. 3B shows that $G'_i$ was greater than $G''$ at all temperatures, $G'_i$, $G''$, and $\eta'$ decreased by less than an order of magnitude from 22 to 50 °C. Though the goat cheese was the softest of the samples tested, its tan δ was 0.43 at 50 °C, meaning that it was more solid-like when heated than the Cheddar, Colby, and Mozzarella (tan δ > 1.1).
Fig. 3B also shows that the η∗ values for goat cheese decreased steadily, in contrast to Parmesan.

3.6. Queso Fresco

QF is a crumbly rennet-set cheese made from homogenized milk and finely milled curd, as opposed to acid-set Queso Blanco, which is made from non-homogenized milk and is not finely milled (Van Hekken & Farkye, 2003). The pH of QF was above 6.0, outside the upper limit for coagulation and flow of heated cheese (Lawrence et al., 1987). As a result, all of the QF samples were predominately elastic-like (tan δ < 0.75) throughout the temperature sweeps (Fig. 4). The G′, G″, and η∗ values were in a narrow range compared with the other cheeses, and were far higher than the other cheeses at 50 °C (Table 2). The Arrhenius plots reveal that the steady decreases in η∗ with heating stopped earlier than the other cheeses. The Ea values for the QF samples were the lowest in this study. The smaller and more uniform fat droplets resulting from homogenization complete their melting at a lower temperature than normal droplets (Tunick, 1994). This effect along with fine milling will alter the properties of cheese.

All measured rheological values for 0.7% NaCl QF increased after 54 °C (Fig. 4B), apparently because of casein aggregation. The pH of QF was above 6.0, outside the temperature range of (Pastorino, Hansen, & McMahon, 2003). QF maintained its ability to dissipate applied energy, as reflected by the relatively steady G″ values, throughout the heating range. At the same time, QF lost its ability to store energy, as reflected by the drop in G′, from 22 to 54 °C after 4 wk storage.

3.7. Correlations

At 22 °C, G′, G″, and η∗ were positively correlated with protein and negatively correlated with MNFS, moisture, and FDM (Table 4). Ea, which is related to η∗ and therefore to G′ and G″, also showed the same sign for the correlations. MNFS is essentially a ratio of moisture to protein; the correlations for moisture were lower than those for MNFS, which showed that the relationship between the rheological parameters and both moisture and protein in cheese is more important than with moisture alone. Similarly, FDM is basically a ratio of fat to protein, and its correlations with the rheological results were higher than those for fat, which were insignificant (R2 < 0.2). At 50 °C, the correlations between the compositional parameters and the rheological results were all insignificant. Once fat melts, the structure of cheese is degraded and the rheological values no longer depend upon the composition.

3.8. Impact on research

The determination of Ea allows a number to be assigned to the extent at which a cheese sample melts with heat. A cheese with an Ea under 60 kJ mol⁻¹ does not flow. Ea values over 100 kJ mol⁻¹ indicate that the cheese will flow, and values over 150 kJ mol⁻¹ point toward a rapid collapse of the protein matrix once the energy barrier is overcome by heating. Coupled with the simultaneous determination of G′, G″, and η∗, the melting behavior of cheese during heating may be quantitated objectively, as opposed to a subjective comparison of thermal curves.

The differing values among cheeses for G′, G″, and the temperature (if any) at which tan δ exceeded 1.0 led Reparet and Noël (2003) to suggest that cheese varieties have their own “rheological signatures.” It is possible that the G′, G″, η*, and Ea results will allow unique rheological profiles for cheeses to be determined. It is also possible that the melt and flow characteristics of other fat-containing foods that are often heated, such as chocolate and dough, may be found by conducting temperature sweeps.

4. Conclusions

Cheddar, Colby, and Mozzarella flow when heated (tan δ > 1 at 50 °C) and have Ea values in the 100–150 kJ mol⁻¹ range, but Mozzarella has a stronger structure than Cheddar and Colby after the fat has melted (higher G′, G″, and η* at 50 °C). Parmesan does not become viscous-like when heated, although its structure weakens considerably (large decreases in G′, G″, and η* with heating) and its fat eventually melted quickly (very high Ea). Goat cheese and QF, which contain the most moisture, have relatively weak protein bonds (low G′, G″, and η* at room temperature), but these bonds do not weaken much further with heat (Ea between 30 and 60 kJ mol⁻¹). Goat cheese and QF do not flow when heated because of their extreme pH levels (4.6 and >6.0, respectively).

It is difficult to find two more dissimilar cheese varieties than chèvre, a soft goat milk cheese, and Parmesan, a hard cow milk cheese. Yet the results demonstrate that the rheological properties of these and other cheeses at room temperature and up to 50 °C can be determined by performing temperature sweeps and calculating Ea.

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References


