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Interpretive Summary

The stretchability of Mozzarella cheese evaluated by a temperature-controlled 3-prong hook test. By Ma et al. A novel and objective method for evaluating the stretchability of Mozzarella cheese with a tensile tester using a 3-prong hook is described. The stretching takes place in a temperature-controlled oil bath to ensure a constant temperature profile and to prevent moisture loss. In addition, a parameter from studies of polymer materials is used to evaluate stretchability, and is found to apply very well to studies of cheese.

STRETCHABILITY TEST FOR MOZZARELLA CHEESE

The stretchability of Mozzarella cheese evaluated by a temperature-controlled 3-prong hook test

X. X. Ma,* B. James,* L. Zhang,† and E. A. C. Emanuelsson-Patterson‡†
*Department of Chemical and Materials Engineering, University of Auckland, Auckland 1142, New Zealand
†Fonterra Research Centre, Fonterra Co-operative Group Limited, Palmerston North 4442, New Zealand
‡Department of Chemical Engineering, University of Bath, Claverton Down, Bath BA2 7AY, United Kingdom
††Corresponding author: Emma Anna Carolina Emanuelsson-Patterson, Tel.: 0044 1225 385312, Fax: 0044 1225 385713, Email: eaep20@bath.ac.uk
ABSTRACT

Stretchability is one of the most important functional properties of Mozzarella cheese, but there is lack of an objective and widely accepted technique for evaluation; most of the cheese stretchability tests are influenced by the ambient conditions. This paper demonstrates a technique, which is novel and relatively simple, to evaluate the stretchability of Mozzarella cheese objectively. In an oil bath, melted cheese is stretched by a hook probe, controlled by an Instron tensile tester; cheese strands are lifted from the melted cheese reservoir to a stretch length of 300 mm, when the load and extension data are recorded by Instron. This test overcomes other tests’ drawbacks, such as subjective stretching speeds and variations in ambient temperature and humidity. Through the test comparison on standard Mozzarella, the modified stretching test in an oil bath has greater repeatability than the original test without oil bath.

From the load and extension curve, the yield load is measured to evaluate the stretchability. In the meantime, the inversion point extension of necking is also measured based on polymer study, producing more repeatable results than the yield load. The modified 3-prong hook test was applied to Mozzarella cheese samples with different processing conditions, including the screw conditions (screw time, speed, and temperature), draining pH, calcium content, and fat content, and significant differences were found between these samples and the control one.

Key words: Mozzarella, stretchability, yield load, inversion point extension
INTRODUCTION

Mozzarella is one of the most widely sold cheeses. Its main use is as a pizza topping because of its stretchability, meltability, and shredability. Stretchability is defined as the ease and extent to which cheese strands are formed (Gunasekaran and Ak, 2003). During manufacture, Mozzarella cheese is stretched in hot water using a mechanical mixer with screws, which results in high stretchability because the proteins are aligned into fibers with the fat and the serum incorporated between the fibers (McMahon et al., 1993).

The stretching of polymers is analogous to the stretching of cheese. When some polymers are stretched uniformly for a few percent, they form a neck rather than break. Either the neck becomes steadily thinner until it breaks, or it stabilizes at some point and then propagates along the specimen (Vincent, 1960), which is often called “cold drawing”. In the stretching of cheese, neck thinning is combined with neck propagation as observed. Factors that are related to load and deformation, before, during, and after the formation of a stable neck, can be applied to distinguish the stretch profiles of different cheeses.

Researchers have developed a range of techniques to evaluate the stretchability of Mozzarella cheese. At a specific time after a pizza had been baked, the “fork test” measured the stretch length of cheese lifted by a fork until it broke (USDA, 1980). This method was fast and easily implemented, but the results were dependent on the individuals performing the test (specifically the uncontrolled lifting speed, load, etc.), which decreased the reproducibility. An improved method is the tensile stretch test; this is a more objective method, in which a circular piece of cheese is lifted vertically out of a baked cheese pizza at a constant speed (Apostolopoulos, 1994). The stretchability was defined as the point at which all cheese strands break off the pizza. Guinee and O’Callaghan (1997) cut a pizza base into two equal halves, before covering it with shredded cheese and baking. The cooked pizza was then placed on the platform of a stretching apparatus. The sides of the pizza were clamped
and stretched apart until the extended strings of cheese broke completely (Guinee and O'Callaghan, 1997).

To prevent the problems associated with temperature, humidity, and moisture loss and to increase the repeatability, the cheese was stretched in mineral oil using a uniaxial horizontal extension test (Ak et al., 1993). A tensile tester stretched the dumbbell shaped cheese sample by moving the clamp attached to the sample, at a constant speed. The limitation with this study was that the cheese samples could be evaluated only between 10 and 40°C, because the cheese became soft and difficult to clamp at higher temperatures.

Instead of using clamps, Fife et al. (2002) used a 3-prong hook method to provide a simple and objective way of measuring the stretchability of melted Mozzarella cheese using a tensile tester. A steel cup containing Mozzarella cheese was heated in a water bath and fixed on the platform of a tensile tester; once the temperature was uniform, the hook was inserted into the sample and the cheese was pulled vertically to obtain a load–distance curve. The main drawback with this technique was that the results were dependent on the environmental conditions such as temperature and humidity, resulting in poor repeatability. To overcome the drawbacks, the present study improved on this method by performing the tensile test in a temperature-controlled oil bath. Oil has been used previously as a medium for the evaluation of cheese stretchability, to maintain a uniform temperature and to prevent the cheese from drying out (Ak et al., 1993, Hicsasmaz et al., 2004, Joshi et al., 2004), and has been shown not to affect the stretchability (Ak and Gunasekaran, 1995).

The aim of this study is, firstly, to verify whether stretching the cheese in oil overcomes the issue of poor reproducibility; secondly, to investigate the stretchabilities of Mozzarella cheeses of different compositions and manufactured using different processing conditions. In addition, based on this technique, parameters to evaluate the stretchability of Mozzarella cheese are to be defined.
MATERIALS AND METHODS

3-Prong Hook Test
A double-walled glass cylinder (Figure 1), which allowed hot water to circulate between the glass walls to control the temperature, was built. The height is 350 mm and the internal diameter is 50 mm. Plugs (21 ± 0.3 g) cut from Mozzarella cheese blocks were placed in separate beakers (with a diameter of 36 mm and a height of 50 mm), covered with aluminum foil to prevent water evaporation, and heated in a water bath for 30 min at 70 or 90°C. Individually, beakers containing melted cheese were put into the bottom of the glass cylinder, which contained the oil already heated to the appropriate temperature. A 3-prong hook connected to a tensile tester (Instron 5543, Norwood, MA) was inserted into the melted cheese until it was 5 mm from the bottom of the beaker. The hook lifted cheese strands at a speed of 1000 mm/min to a distance of 300 mm (Fife et al., 2002).

To test for repeatability, defined as the variation in measurements taken by the same person or instrument on the same item and under the same conditions, 3-prong hook tests, with and without an oil bath (at 70 or 90°C), were performed on a Mozzarella cheese (Fonterra Brands Limited) bought from a local supermarket at each set of conditions with 9 replications. Mozzarella cheeses with different compositions and manufactured using different processing conditions were produced at the Fonterra Research Centre (Palmerston North, New Zealand). Samples of these cheeses were tested using the 3-prong hook test, with the oil bath at 70°C and with 3 replications for each of them.

Stretch Profile
A load–extension curve, i.e., the “stretch profile”, for a sample of Mozzarella cheese is shown in Figure 2; it can be divided into 2 regions. In region R1, the load increases until the hook has travelled around 10 mm and then decreases rapidly during an unstable deformation period as the hook leaves the cheese reservoir. The cheese finally forms a stable neck and the...
rate of the change in load decreases. Region R2 starts at the inversion point, when the stable neck has formed, and the load increases gradually until the end of the test.

The maximum load of the cheese stretch profile is defined as the “yield load”. The slope of the dramatic decrease in load is defined, in this study, as the unstable deformation gradient (UDG) and relates to the rapid formation of the stable neck that propagates in R2. This is analogous to polymer stretching, when a stable neck forms during a tensile test after a region of rapid load or stress drop (Nazarenko et al., 1994). The load and extension values at the inversion point provide further parameters for quantifying stretchability.

Microstructure

Confocal laser scanning microscopy (CLSM) was used to examine the microstructure of the cheese samples. Mozzarella cheese samples were sectioned into slices 50 μm thick using a cryotome (Leica CM1850, Leica Microsystems, Buffalo Grove, IL), soaked in a 0.2% (wt/wt) Nile blue fluorescent probe (Sigma–Aldrich, St Louis, MO), diluted in Citifluor to prevent photobleaching, and placed between a slide and a cover slip overnight. Images were taken using a confocal microscope with 40× objective (Leica TCS SP2, Leica Microsystems, Buffalo Grove, IL). A CLSM laser wavelength of 488 and 633 nm was used to excite the Nile blue used for fat and protein staining, with an emission wavelength of 514 and 645 nm individually for fat and protein. Samples from interrupted stretching tests were taken at extensions of 100, 150, 200, and 300 mm.

Manufacturing and Analysis of Different Mozzarella Cheese Samples

Mozzarella cheese samples were manufactured at the Fonterra Research Centre (Fonterra Co-operative Group Limited, Palmerston North, New Zealand) with different processing conditions. The control sample was produced using standard processing conditions (CT); samples were also manufactured with longer screw time (ST), higher screw speed (SPD), higher screw temperature (TM), both higher screw speed and higher screw temperature
(TMP), lower draining pH (LPH), higher draining pH (HPH), higher calcium content (HCA), higher fat content (HFT), and lower fat content (LFT); the detailed processing conditions are shown in Table 1.

Fresh milk was standardized to a protein-fat ratio of 1.31 (except 0.71 for HFT and 3.49 for LFT). 19.8 g/kg CaCl2 was added to the milk for HCA. The milk was pasteurized, cultured (with Mesophilic starter culture), stirred, and let stand for 15 min. The curd was cut, and then whey was drained when pH decreased to 5.9 (except 5.8 for LPH and 6.1 for HPH). Then the curd was milled (at pH of 5.3), salted, and dry stirred. Twin screw was used to stretch the curd with a speed of 17.6 rpm (except 20.8 rpm for SPD) in hot water at 58 °C (except 68 °C for TMP and ST) for 2 min (the total transit time from the point when the curd entered the mixer to when it exited, except 10 min for TM). Samples were vacuum-packed and stored at 4 °C, and their stretchabilities were analyzed after 12 weeks of aging.

The compositions of the cheeses were confirmed using a number of techniques. Fat content and moisture content were determined using a FoodScan dairy analyzer (FOSS, Hillerød, Denmark). Salt content was measured using an autotitrator (Metrohm Ltd., Herisau, Switzerland) and calcium content was measured using inductively coupled plasma optical emission spectrometry (Varian Ltd., Palo Alto, CA). To measure Non-casein nitrogen (NCN) content, the cheese was dissolved using 1 mL of 0.1 M NaOH, and then adding 2 mL of Acetic acid solution. Once the filtrates were prepared the nitrogen content is determined using the Kjeldahl method (BOCHI Kjeldahl, BUCHI Labortechnik AG, Flawil, Switzerland). All chemical analyses were carried out by the Analytical Services Group at the Fonterra Research Centre.

Statistical Analysis
One-way ANOVA was performed to investigate the significant difference between cheese samples with different compositions and manufacturing conditions by OriginPro 8 (OriginLab Corporation, Northampton, MA).

RESULTS

Stretching Test with and without an Oil Bath

Table 2 shows that the 3 parameters of stretchability under the conditions with oil bath had obviously lower standard deviation percentages than those without oil bath at the same temperature, except that the standard deviation percentages of yield load at 70°C with and without oil bath were similar. In addition, for the tests with oil bath, the standard deviation percentages at 90°C were less than those at 70°C. However, the higher temperature oil bath significantly decreased the yield load, which inevitably lowered the differences in yield load between different cheese samples. Therefore, for the Mozzarella cheeses of different compositions and manufactured using different processing conditions, the stretching test with the oil bath at 70°C was applied.

The microstructures of standard Mozzarella cheese samples at various extensions are shown in Figure 3. It is clear that the anisotropy of the protein strands was maintained during this extension and that there was no substantial breakdown of the structure.

Stretch Profiles of Mozzarella Cheese Samples

The stretch profiles of the different Mozzarella cheese samples in the 70°C oil bath are shown in Figure 4, in which substantial differences among the yield loads and the inversion points of the different samples can be found.

The parameters: yield load, UDG, and inversion point are shown in Figures 5, 6, and 7, respectively. Figure 5 shows that the largest difference in yield load was between LFT and HFT with values of 328 and 49 g, respectively. HPH and HCA had similar yield load values.
(P>0.05), and both higher than that of CT, (P<0.05), whereas LPH had a similar yield load value to CT (P>0.05). Among the samples manufactured using different screw conditions, only SPD, with higher screw speed, had a lower yield load than CT (P<0.05); TMP, TM, and ST, with higher screw temperature, longer screw time, and both higher screw speed and higher screw temperature, respectively, had increased yield loads (P<0.05).

Figure 6 shows the UDG of SPD and HFT were lower, and TMP, ST, HPH, and LFT were higher than that of CT (P<0.05). TM, LPH, HCA did not differ from that of CT (P>0.05).

Figure 7 shows that the inversion point extension values of all samples were higher than CT (P<0.05) except SPD (lower than CT, P<0.05) and HFT (not significantly different from CT, P>0.05).

**DISCUSSION**

**Modified Stretching Test**

The high variability of the load–extension curves that were produced during the stretching test without the oil bath was due to the rapid decrease in temperature and the dramatic moisture loss of the cheese strands when stretched in air. The modified stretching test overcomes these problems with the temperature-controlled oil bath, which provides a uniform environment with a constant temperature, preventing temperature decrease and moisture loss.

**Stretchability of Mozzarella Cheese Samples**

Both the yield load and the inversion point can be used to quantify stretchability. The yield load reflected the cheese viscosity and its capacity to resist deformation (Fife et al., 2002). The inversion point indicates the extension at which a stable neck is formed, after which the material stretches uniformly with apparent strain hardening.

Calcium acts as a cementing agent that cross links, and thus strengthens, the casein network. A cheese with a higher calcium content has been found to require higher loads when using a uniaxial horizontal extension test ((Joshi et al., 2004), which was corroborated by our
results. Calcium content has been found to control the stretchability of nonfat Mozzarella cheese (McMahon et al., 2005); in the current study, both HPH and HCA, with higher calcium content (see Table 1), had higher yield load than CT.

The high yield load of LFT was probably due to its lower fat content and higher protein content, in addition to its higher calcium content, compared with CT. This is in agreement with previous literature; low fat cheese has fewer and smaller fat globules embedded in the protein matrix than full fat cheese, and the dominating protein matrix results in the firm texture of low fat cheese (Mistry and Anderson, 1993). Even though LFT had low fat, and no butter oil was added on the surface during heating in the water bath, surface cooling and desiccation were minimized by its natural free oil formation, due to the aging effect (Kindstedt and Kiely, 1992).

SPD was compositionally similar to CT in terms of fat, moisture, and protein contents; this was in contrast to other research (Renda et al., 1997), in which differences in moisture content and protein content were related to the screw speed (this was probably because the screw speed for SPD in the current study was only slightly higher than that for CT). However, it should be noted that SPD had a significantly higher NCN content, which is an index of proteolysis, indicating that SPD had higher protein degradation than CT. As a result, SPD had a less dense casein structure than CT, which explains its lower yield load. Similarly, TM, TMP, and ST, with higher NCN, had higher yield loads than CT.

The UDG and inversion point extension results were similar to the yield load results. In addition to the stretchability of the cheese, the UDG is also affected by how much the cheese is lifted by the hook, as indicated by the higher UDG value for HPH than for HCA. This difference in the amount of cheese lifted by the hook results in relatively high standard deviations of UDG, which is a limit to this parameter. The inversion point extension results had smaller standard deviations than the yield load results, indicating that, compared with
yield load, the inversion point extension is a more repeatable parameter for the evaluation of stretchability and can distinguish smaller differences between the stretchabilities of Mozzarella cheese samples.

Consumers may have different preferences on the stretchability of Mozzarella cheese. The higher values of yield load and inversion point extension respectively indicate that it is more difficult to stretch the cheese, and it takes longer for the cheese strings to be stable. As a result, Mozzarella samples with lower yield load and inversion point extension values have greater stretchability.

**CONCLUSIONS**

The modified 3-prong hook stretching tests with oil bath had better repeatability than the same tests without oil bath. Yield load was selected for the evaluation of cheese stretchability. As a similar necking phenomenon to that observed for some polymers occurred in the stretching test, the inversion point extension was also measured. Both increasing the calcium content and increasing the draining pH increased the yield load. Compared with a control sample, a high fat Mozzarella had around 70% the yield load, and a low fat Mozzarella had a yield load 3 times higher. Mozzarella cheeses manufactured using a longer screw time or a higher screw temperature had a higher yield load, and those manufactured using a higher screw speed had lower yield load. As the inversion point extension and the yield load results behaved similarly, both parameters can be used to quantify the stretchability of cheese using this temperature-controlled 3-prong hook test.

**ACKNOWLEDGEMENTS**

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glass workshop at the University of Auckland, and Andrew Huxford, Bruce Allen, Elizabeth Nickless, and Errol Conaghan at Fonterra Research Centre.


Table 1. Chemical compositions of Mozzarella cheese samples manufactured using different processing conditions

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CT</th>
<th>TM</th>
<th>SPD</th>
<th>TMP</th>
<th>ST</th>
<th>LPH</th>
<th>HPH</th>
<th>HCA</th>
<th>HFT</th>
<th>LFT</th>
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<tr>
<td>Fat (%)</td>
<td>21.1</td>
<td>20.1</td>
<td>21.1</td>
<td>21.1</td>
<td>21.5</td>
<td>21.0</td>
<td>21.6</td>
<td>20.8</td>
<td>29.4</td>
<td>11.8</td>
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<tr>
<td>Moisture (%)</td>
<td>47.4</td>
<td>48.3</td>
<td>47.2</td>
<td>47.3</td>
<td>46.5</td>
<td>49.4</td>
<td>46.1</td>
<td>48.0</td>
<td>44.9</td>
<td>51.8</td>
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<td>Protein (%)</td>
<td>27.0</td>
<td>27.3</td>
<td>27.3</td>
<td>27.1</td>
<td>27.7</td>
<td>25.5</td>
<td>27.7</td>
<td>26.8</td>
<td>22.0</td>
<td>31.9</td>
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<td>Salt (%)</td>
<td>1.58</td>
<td>1.51</td>
<td>1.70</td>
<td>1.77</td>
<td>1.76</td>
<td>1.63</td>
<td>1.52</td>
<td>1.41</td>
<td>1.57</td>
<td>1.84</td>
</tr>
<tr>
<td>Calcium (mg/g)</td>
<td>6.37</td>
<td>6.37</td>
<td>6.49</td>
<td>6.26</td>
<td>6.46</td>
<td>5.98</td>
<td>7.76</td>
<td>7.44</td>
<td>5.34</td>
<td>8.11</td>
</tr>
<tr>
<td>pH</td>
<td>5.50</td>
<td>5.54</td>
<td>5.49</td>
<td>5.48</td>
<td>5.46</td>
<td>5.55</td>
<td>5.56</td>
<td>5.64</td>
<td>5.52</td>
<td>5.51</td>
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<tr>
<td>FDM&lt;sup&gt;2&lt;/sup&gt; (%)</td>
<td>40.11</td>
<td>38.88</td>
<td>39.96</td>
<td>40.04</td>
<td>40.19</td>
<td>41.50</td>
<td>40.07</td>
<td>40.00</td>
<td>53.36</td>
<td>24.48</td>
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<tr>
<td>M:P&lt;sup&gt;3&lt;/sup&gt; (%)</td>
<td>1.756</td>
<td>1.769</td>
<td>1.729</td>
<td>1.745</td>
<td>1.679</td>
<td>1.937</td>
<td>1.664</td>
<td>1.791</td>
<td>2.041</td>
<td>1.624</td>
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<td>S in M&lt;sup&gt;4&lt;/sup&gt; (%)</td>
<td>3.33</td>
<td>3.13</td>
<td>3.60</td>
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<td>3.78</td>
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<td>Ca:Pr&lt;sup&gt;5&lt;/sup&gt; (%)</td>
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<td>23.33</td>
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<td>23.45</td>
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<td>27.76</td>
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<tr>
<td>NCN&lt;sup&gt;6&lt;/sup&gt; (%)</td>
<td>0.929</td>
<td>0.754</td>
<td>1.030</td>
<td>0.820</td>
<td>0.640</td>
<td>0.718</td>
<td>0.885</td>
<td>0.895</td>
<td>0.730</td>
<td>0.911</td>
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</table>

<sup>1</sup>CT = control processing conditions; TM = longer screw time; SPD = higher screw speed; TMP = higher screw temperature; ST = higher screw speed and higher screw temperature; LPH = lower draining pH; HPH = higher draining pH; HCA = higher calcium content; HFT = high fat content; LFT = low fat content.

<sup>2</sup>Fat in DM.

<sup>3</sup>Ratio between moisture content and protein content.

<sup>4</sup>S in M = salt in moisture.

<sup>5</sup>Ratio between calcium content and protein content.

<sup>6</sup>Non-casein nitrogen.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test conditions</th>
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<tbody>
<tr>
<td></td>
<td>Without oil bath</td>
<td>With oil bath</td>
<td>Without oil bath</td>
<td>With oil bath</td>
<td></td>
</tr>
<tr>
<td></td>
<td>70 °C</td>
<td>90 °C</td>
<td>70 °C</td>
<td>90 °C</td>
<td></td>
</tr>
<tr>
<td>Yield load (g)</td>
<td>298.94</td>
<td>82.71</td>
<td>146.09</td>
<td>68.39</td>
<td></td>
</tr>
<tr>
<td>SD (%)</td>
<td>16.1</td>
<td>20.4</td>
<td>16.3</td>
<td>17.0</td>
<td></td>
</tr>
<tr>
<td>Unstable deformation gradient (g/mm)</td>
<td>4.90</td>
<td>0.92</td>
<td>4.41</td>
<td>1.92</td>
<td></td>
</tr>
<tr>
<td>SD (%)</td>
<td>62.9</td>
<td>78.3</td>
<td>24.0</td>
<td>14.6</td>
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<tr>
<td>Inversion point extension (mm)</td>
<td>61.00</td>
<td>53.00</td>
<td>50.78</td>
<td>47.67</td>
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<td>SD (%)</td>
<td>19.0</td>
<td>14.1</td>
<td>17.7</td>
<td>9.1</td>
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FIGURE LEGENDS

Figure 1. Modified 3-prong hook stretching test.

Figure 2. Parameters to evaluate the stretch profile of cheese.

Figure 3. CLSM microstructures of standard Mozzarella cheese during a stretching test at extensions of (a) 100 mm, (b) 150 mm, (c) 200 mm, and (d) 300 mm.

Figure 4. Stretch profiles of Mozzarella cheese samples manufactured using different processing conditions: CT, control processing conditions; TM, longer screw time; SPD, higher screw speed; TMP, higher screw temperature; ST, higher screw speed and higher screw temperature; LPH, lower draining pH; HPH, higher draining pH; HCA, higher calcium content; HFT, high fat content; LFT, low fat content.

Figure 5. Yield load of Mozzarella cheese samples manufactured using different processing conditions (The error bars represent SD, and the values with different letters are significantly different, P<0.05.)

Figure 6. Unstable deformation gradient of Mozzarella cheese samples manufactured using different processing conditions (The error bars represent SD, and the values with different letters are significantly different, P<0.05.)

Figure 7. Inversion point extension of Mozzarella cheese samples manufactured using different processing conditions (The error bars represent SD, and the values with different letters are significantly different, P<0.05.)