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1 ***Interpretive Summary***

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

8

9 **STRETCHABILITY TEST FOR MOZZARELLA CHEESE**

10

11 **The stretchability of Mozzarella cheese evaluated by a temperature-controlled**
12 **3-prong hook test**

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22 **ABSTRACT**

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

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

40 **Key words:** Mozzarella, stretchability, yield load, inversion point extension

INTRODUCTION

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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

66 and stretched apart until the extended strings of cheese broke completely (Guinee and
67 O'Callaghan, 1997).

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

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

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

MATERIALS AND METHODS

91

92 **3-Prong Hook Test**

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

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

111 **Stretch Profile**

112 A load–extension curve, i.e., the “stretch profile”, for a sample of Mozzarella cheese is
113 shown in Figure 2; it can be divided into 2 regions. In region R1, the load increases until the
114 hook has travelled around 10 mm and then decreases rapidly during an unstable deformation
115 period as the hook leaves the cheese reservoir. The cheese finally forms a stable neck and the

116 rate of the change in load decreases. Region R2 starts at the inversion point, when the stable
117 neck has formed, and the load increases gradually until the end of the test.

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

124 ***Microstructure***

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

135 ***Manufacturing and Analysis of Different Mozzarella Cheese Samples***

136 Mozzarella cheese samples were manufactured at the Fonterra Research Centre (Fonterra
137 Co-operative Group Limited, Palmerston North, New Zealand) with different processing
138 conditions. The control sample was produced using standard processing conditions (**CT**);
139 samples were also manufactured with longer screw time (**ST**), higher screw speed (**SPD**),
140 higher screw temperature (**TM**), both higher screw speed and higher screw temperature

141 (TMP), lower draining pH (LPH), higher draining pH (HPH), higher calcium content
142 (HCA), higher fat content (HFT), and lower fat content (LFT); the detailed processing
143 conditions are shown in Table 1.

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

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

163 ***Statistical Analysis***

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

167

168

RESULTS

169 ***Stretching Test with and without an Oil Bath***

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

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

182 ***Stretch Profiles of Mozzarella Cheese Samples***

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

186 The parameters: yield load, UDG, and inversion point are shown in Figures 5, 6, and 7,
187 respectively. Figure 5 shows that the largest difference in yield load was between LFT and
188 HFT with values of 328 and 49 g, respectively. HPH and HCA had similar yield load values

189 ($P>0.05$), and both higher than that of CT, ($P<0.05$), whereas LPH had a similar yield load
190 value to CT ($P>0.05$). Among the samples manufactured using different screw conditions,
191 only SPD, with higher screw speed, had a lower yield load than CT ($P<0.05$); TMP, TM, and
192 ST, with higher screw temperature, longer screw time, and both higher screw speed and
193 higher screw temperature, respectively, had increased yield loads ($P<0.05$).

194 Figure 6 shows the UDG of SPD and HFT were lower, and TMP, ST, HPH, and LFT were
195 higher than that of CT ($P<0.05$). TM, LPH, HCA did not differ from that of CT ($P>0.05$).
196 Figure 7 shows that the inversion point extension values of all samples were higher than CT
197 ($P<0.05$) except SPD (lower than CT, $P<0.05$) and HFT (not significantly different from CT,
198 $P>0.05$).

199 DISCUSSION

200 ***Modified Stretching Test***

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

206 ***Stretchability of Mozzarella Cheese Samples***

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

211 Calcium acts as a cementing agent that cross links, and thus strengthens, the casein
212 network. A cheese with a higher calcium content has been found to require higher loads when
213 using a uniaxial horizontal extension test ((Joshi et al., 2004), which was corroborated by our

214 results. Calcium content has been found to control the stretchability of nonfat Mozzarella
215 cheese (McMahon et al., 2005); in the current study, both HPH and HCA, with higher
216 calcium content (see Table 1), had higher yield load than CT.

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

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

233 The UDG and inversion point extension results were similar to the yield load results. In
234 addition to the stretchability of the cheese, the UDG is also affected by how much the cheese
235 is lifted by the hook, as indicated by the higher UDG value for HPH than for HCA. This
236 difference in the amount of cheese lifted by the hook results in relatively high standard
237 deviations of UDG, which is a limit to this parameter. The inversion point extension results
238 had smaller standard deviations than the yield load results, indicating that, compared with

239 yield load, the inversion point extension is a more repeatable parameter for the evaluation of
240 stretchability and can distinguish smaller differences between the stretchabilities of
241 Mozzarella cheese samples.

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

247 **CONCLUSIONS**

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

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- 296

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

Parameters	Mozzarella cheese samples ¹									
	CT	TM	SPD	TMP	ST	LPH	HPH	HCA	HFT	LFT
Fat (%)	21.1	20.1	21.1	21.1	21.5	21.0	21.6	20.8	29.4	11.8
Moisture (%)	47.4	48.3	47.2	47.3	46.5	49.4	46.1	48.0	44.9	51.8
Protein (%)	27.0	27.3	27.3	27.1	27.7	25.5	27.7	26.8	22.0	31.9
Salt (%)	1.58	1.51	1.70	1.77	1.76	1.63	1.52	1.41	1.57	1.84
Calcium (mg/g)	6.37	6.37	6.49	6.26	6.46	5.98	7.76	7.44	5.34	8.11
pH	5.50	5.54	5.49	5.48	5.46	5.55	5.56	5.64	5.52	5.51
FDM ² (%)	40.11	38.88	39.96	40.04	40.19	41.50	40.07	40.00	53.36	24.48
M:P ³	1.756	1.769	1.729	1.745	1.679	1.937	1.664	1.791	2.041	1.624
S in M ⁴ (%)	3.33	3.13	3.60	3.74	3.78	3.30	3.30	2.94	3.50	3.55
Ca:Pr ⁵ (%)	23.59	23.33	23.77	23.10	23.32	23.45	28.01	27.76	24.27	25.42
NCN ⁶ (%)	0.929	0.754	1.030	0.820	0.640	0.718	0.885	0.895	0.730	0.911

¹CT = 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.

²Fat in DM.

³Ratio between moisture content and protein content.

⁴S in M = salt in moisture.

⁵Ratio between calcium content and protein content.

⁶Non-casein nitrogen.

Table 2. Repeatability of stretchability test for Mozzarella under different test conditions

Parameter	Test conditions			
	Without oil bath		With oil bath	
	70 °C	90 °C	70 °C	90 °C
Yield load (g)	298.94	82.71	146.09	68.39
SD (%)	16.1	20.4	16.3	17.0
Unstable deformation gradient (g/mm)	4.90	0.92	4.41	1.92
SD (%)	62.9	78.3	24.0	14.6
Inversion point extension (mm)	61.00	53.00	50.78	47.67
SD (%)	19.0	14.1	17.7	9.1

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, $P < 0.05$.)