Effect of Transglutaminase on Yield, Compositional and Functional Properties of Low-fat Cheddar Cheese

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The effect of transglutaminase (TG) on the yield, composition, proteolysis and functional properties of low-fat Cheddar cheese were investigated. By adding TG, the protein, fat recoveries and the yield of low-fat cheese were improved significantly. In addition, owing to the increase in moisture content, the degree of proteolysis of the TG-treated low-fat cheese was higher than that of untreated cheese during the first 15 days, and the hardness was also reduced significantly during early ripening. On the other hand, the additional covalent cross-linking catalyzed by TG had a slight adverse effect on the proteolysis during later ripening and resulted in a harder texture and lower meltability than the low-fat cheese without TG. In conclusion, TG is promising for increasing the yield and improving the texture properties of low-fat cheese, which makes it very beneficial for the cheese making.

Keywords: yield, functional properties, transglutaminase, Cheddar cheese, low fat

Introduction

Cheddar cheese is one of the most widely consumed traditional cheeses in the world. The demand for low-fat Cheddar cheese has steadily increased because of the trend towards healthier eating (Haque et al., 2007). However, reducing the fat has a negative effect on the sensory and functional qualities of cheese, resulting in a rubbery texture, lack of flavor and poor meltability (Rogers et al., 2009). Moreover, there are also negative economic consequences to consider, such as lower cheese yield (Rodrı́guez, 1998). Because lower fat cheeses have higher protein-to-fat ratios than full-fat cheeses, they have a denser protein network and firmer texture (Rogers et al., 2009). Several strategies have been proposed to compensate for the increased protein concentration: making-process modifications; starter culture selection and use of adjunct cultures; and the use of additives such as stabilizers and fat replacers (Fenelon and Guinee, 2000). However, reducing fat and simultaneously maintaining the texture of a comparable full-fat cheese remains a challenge.

In recent years, an attempt to improve the qualities of low or reduced fat dairy products by using transglutaminase (TG; E.C. 2.3.2.13) has been made (Ozer et al., 2007, Yokoyama et al., 2004). TG is a transferase naturally present in most animal tissues and body fluids, and can form both inter- and intra-molecular iso-peptide bonds ($\varepsilon$-(γ-glutamyl)lysine) between many proteins by cross-linking of the amino acid residues of protein-bound glutamine and lysine (Yokoyama et al., 2004). Many food proteins are good substrates of TG, especially casein, which is the principle protein in milk. The introduction of additional covalent cross-linking by TG represents a promising tool to improve the functional properties for casein-based dairy products (Özrenk, 2006). The effect of TG-catalyzed cross-linking on the rheology and structure of acidified milk gels have been largely discussed and the results showed that TG-treated gels had higher serum binding capacity and lower permeability than the control sample (Anema et al., 2005, Færgemand and Qvist, 1997, Schorsch et al., 2000). Bönisch et al. (2008) investigated the influence of TG on the rennet coagulation of casein. The results showed that the simultaneous reaction of TG and rennet markedly increased the yield after centrifugation and

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decreased gel firmness due to the enhanced serum binding of the gel network stabilized by additional covalent bonds.

From an industrial standpoint, compared with the other method, using TG has the following advantages: it is simpler, a lower dose is required and the reactions have higher specificity (Özrenk, 2006). Many patents indicate the high economic potential of TG application for enhancing cheese yield and improving cheese functionality (Miwa et al., 2002). Comparatively, there are few related scientific studies. Desá and Bordignon-Luiz (2010) investigated the influence of TG on rennet coagulation properties and the properties of processed cheese. TG improves the physical properties (decreased syneresis index and increased consistency index) of processed cheese, possibly by the occurrence of enzymatic cross-linking. Pierro et al. (2010) used TG to obtain a novel “cross-linked cheese”. After adding TG, water content and cheese yield were higher and the proteolysis of cross-linking cheese after 35 days of ripening markedly reduced. However, there are still no reports on the effect of TG on long-aging cheese such as Cheddar cheese. Whether TG would be effective for low-fat cheese needs to be investigated.

In our study, the effect of TG on the yield, composition, proteolysis and changes in textural properties during the ripening of low-fat Cheddar cheese were investigated. Full-fat cheeses are also examined as a control. The aim was to determine whether TG can be applied to improve the quality of low-fat Cheddar cheese.

**Materials and Methods**

**Materials**  
Ca²⁺ independent microbial transglutaminase (ACTIVA-WM) derived from Streptovercilium was supplied by Ajinomoto Co. Inc. (Tokyo, Japan). The mean enzymatic activity was 100 U/g (data supplied by the manufacturer). The other reagents used were all of analytical grade and from China.

Raw and skim milk were obtained from the China Agriculture University (CAU) dairy farm. Milk was standardized to 0.12% and 3.9% fat (w/w) for making low-fat and full-fat cheeses, respectively.

**Cheddar cheese manufacture**  
Four different cheeses (in duplicate) were made in a randomized order in the experiment. Two TG-treated cheeses (full-fat and low-fat) and two untreated cheeses (full-fat and low-fat) were coded as FFT, LFT, FFC and LFC, respectively.

The standardized milk was sterilized at 63°C for 30 min. After being cooled to 32°C, the milk (pH 6.6) was inoculated at 32°C for 30 min with 0.004% lactic bacteria starter (R704, CHR Hansen, Hørsholm, Denmark) which shifted the milk pH value to 6.5. Then, 0.005% calf rennet (120000 U/g, Stamiix 1150, CHR Hansen, Hørsholm, Denmark,) and 0.02% calcium chloride were added. Curd formed after about 30 min. The curd was subsequently cut and left to heal for 15 min and then heated to 38°C at a rate of 1°C/5 min. During the cooking step, curd was stirred gently to avoid crushing the curd particles. The whey was drained off when the pH value decreased to 6.10. The cheddaring lasted until the pH value drop to 5.45. After milling, the curd was salted (3.0 g/L milk). Thereafter, the resulting curd was filled in the hoop and pressed at 2.46 kPa overnight. The cheese blocks were packed under vacuum and ripened at 4°C for 90 days. TG was added at a concentration of 2 U/g protein 7 min after adding the rennet (Desá and Bordignon-Luiz, 2010). The physicochemical analyses and functional properties were carried out at 1, 15, 30, 60 and 90 days of ripening.

**Analysis of chemical composition of cheese**  
Cheeses (1, 15, 30, 60 and 90 days old) were analyzed for moisture content by oven drying (at 100°C for 24 h) of a 2 g sample until a constant weight was obtained, fat content by the Babcock method (Richardson, 1985) and total protein (TN) by the Kjeldahl method (Anonymous, 1990). Each analysis was performed in triplicate.

**Analyses of fat, protein recovery and cheese yield**  
The fat recovery (%, w/w) is the fat retained in the cheese product × 100/the total fat of cheese-milk. Similarly, the total protein recovery (% w/w) is the protein retained in the cheese product × 100/the total protein of cheese-milk. The yield of cheese (% w/w) was determined by weighing the cheese obtained in each vat after removal from the press, and it was expressed as cheese weight × 100/cheese-milk weight.

**Analysis of proteolysis**

**Determination of nitrogen fractions**  
The level of proteolysis was assessed at 1, 15, 30, 60 and 90 days after ripening. The soluble fractions of the total N at pH 4.6 of cheeses (pH 4.6 SN) were determined according to the method of Kuchroo and Fox (1989) and expressed as a percentage of total cheese N (pH 4.6 SN/TN). The protein levels of those fractions were determined by the Kjeldahl method (Anonymous, 1990). Each analysis was performed in triplicate.

**Gel electrophoresis analysis**  
Urea-polyacrylamide gel electrophoreograms (Urea-PAGE) was conducted on cheese samples at 1, 15, 30, 60 and 90 days of ripening using the Mini-Protean Tetra Electrophoresis system (BioRad Laboratories, Hercules, CA, USA) as described by Andrews (1983), with staining using Coomassie Brilliant Blue G250 as described by Blakesley and Boezi (1977).

**Analyses of functional properties**

**Textural analysis**  
Texture profile analysis (TPA) including the measurement of hardness, springiness and cohesiveness was performed on cheese samples (1, 15, 30, 60 and 90 days old) using a TMS-Pro Texture Analyzer (Food Technol-
Effect of Transglutaminase on Low-fat Cheddar Cheese

Compositional analysis  The components of cheeses are summarized in Table 1. LFC had higher moisture, protein contents and lower fat content on a percentage fat on dry basis (FDB) \( (P < 0.05) \) than FFC due to the lower fat content. Because the moisture did not replace the fat on an equal basis, there was a significant decrease in the moisture in the nonfat substance (MNFS) and in the moisture-to-protein ratio (W/P) in the cheese, which is in agreement with results reported by Fenelon and Guinee (2000). Compared with LFC, LFT showed significant increases \( (P < 0.05) \) in moisture, MNFS and W/P content. Besides, the MNFS and W/P of LFT were higher than FFC. According to Gauche et al. (2009) and Özrenk (2006), TG-catalyzed cross-linking in casein micelles show a better water-holding capacity, meaning that more free water can be entrapped in the rennet gel network, and therefore the moisture content increases. Additionally, this effect was more obvious for low-fat cheese than full-fat cheese. Similar results were observed for yogurt with low fat content (Lorenzen et al., 2002). And, there were no significant differences in the moisture, fat and protein contents for all cheese samples during the 90 days of ripening \( (P > 0.05) \) (data not shown). In this study, all cheese samples had higher moisture levels than what would be expected for commercial Cheddar cheese. This may be because the pressure used during cheese making in our laboratory conditions was lower than that used in the commercial process. Ong et al. (2007) also used a lower pressure in their study.

Component recovery and cheese yield  As shown in Table 2, the protein and fat recoveries for LFC were significantly, markedly lower than FFC. While for the low-fat cheeses, TG significantly reduced fat loss \( (P < 0.05) \). And the nitrogen recovery was also improved \( (P > 0.05) \). As shown in Table 2, LFT had higher nitrogen and fat recoveries (71.7%, 83.4%) than LFC.

Table 1. Major compositions of cheeses at day 1

<table>
<thead>
<tr>
<th>Item</th>
<th>FFC</th>
<th>FFT</th>
<th>LFC</th>
<th>LFT</th>
</tr>
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<tbody>
<tr>
<td>Protein (g/100 g)</td>
<td>19.3 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.4 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.6 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.4 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat (g/100 g)</td>
<td>26.6 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.0 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.3 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.2 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Moisture (g/100 g)</td>
<td>46.2 ± 0.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>52.6 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.1 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65.7 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>FDB (g/100 g)</td>
<td>49.5 ± 4.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.7 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MNFS (g/100 g)</td>
<td>63.0 ± 2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>69.2 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.9 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66.4 ± 1.5&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>W/P</td>
<td>2.39 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.02 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.78 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.49 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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</table>

<sup>a,b</sup> Means within the same column with different superscripts are significantly different (Tukey test, \( P < 0.05 \)).

<sup>1</sup> The results are expressed as mean ± standard deviation.

<sup>2</sup> FFC: full-fat cheese; FFT: full-fat cheese treated with TG; LFC: low-fat cheese; LFT: low-fat cheese treated with TG.

<sup>3</sup> FDB: Fat content on a dry weight basis; MNFS: moisture in the nonfat substance; W/P: Ratio of water to protein.
shown in Table 1, the addition of TG made up for the defects significantly decreased due to the reduction of the fat content. As by the reduction in fat (Table 1). Therefore, the yields significantly decreased due to the reduction of the fat content. As shown in Table 1, the addition of TG made up for the defects by increasing the cheese moisture significantly. By adding TG, the yield increased by 2.2% for low-fat cheese (Table 2).

Cheese yield is very important for a cheese manufacturer. The results showed that the yield of LFC was significantly less than FFC. Milk fat, one of the major components in milk, is trapped in the protein matrix during cheese making. The reduced fat in cheese can be replaced by moisture, but the increased moisture was not enough to fill the volume left by the reduction in fat (Table 1). Therefore, the yields significantly decreased due to the reduction of the fat content. As shown in Table 1, the addition of TG made up for the defects by increasing the cheese moisture significantly. By adding TG, the yield increased by 2.2% for low-fat cheese (Table 2).

Table 2. Protein, fat recoveries and yields of cheeses.

<table>
<thead>
<tr>
<th>Item</th>
<th>FFC</th>
<th>FFT</th>
<th>LFC</th>
<th>LFT</th>
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<tr>
<td>Protein recovery (%)</td>
<td>74.4 ± 2.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.2 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.2 ± 2.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.7 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Fat recovery (%)</td>
<td>83.0 ± 2.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.2 ± 2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.2 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.4 ± 2.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>12.2 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.5 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.5 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.7 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

<sup>a</sup> Means within the same column with different superscripts are significantly different (Tukey test, P < 0.05).
<sup>1</sup> The results are expressed as mean ± standard deviation.
<sup>2</sup> FFC: full-fat cheese, FFT: full-fat cheese treated with TG, LFC: low-fat cheese, LFT: low-fat cheese treated with TG.

Table 3. SN/TN of cheeses ripened for 1, 15, 30, 60 and 90 days.

<table>
<thead>
<tr>
<th>Cheese age(days)</th>
<th>FFC</th>
<th>FFT</th>
<th>LFC</th>
<th>LFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.9 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.8 ± 0.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.5 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.1 ± 0.6&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>15</td>
<td>13.0 ± 0.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>15.1 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.1 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.2 ± 0.0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>16.4 ± 0.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>17.5 ± 0.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.1 ± 0.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>10.1 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>18.8 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.8 ± 0.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>13.1 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.9 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>90</td>
<td>22.7 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.4 ± 0.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16.2 ± 0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.4 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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</table>

<sup>a</sup> Means within the same column with different superscripts are significantly different (Duncan test, P < 0.05).
<sup>1</sup> The results are expressed as mean ± standard deviation.
<sup>2</sup> FFC: full-fat cheese, FFT: full-fat cheese treated with TG, LFC: low-fat cheese, LFT: low-fat cheese treated with TG.

* pH 4.6 SN/TN = pH 4.6 acetate buffer-soluble nitrogen as a percentage of total nitrogen.

(64.2%, 71.2%). As the fat content of the cheese decreased (i.e., ratio of casein to fat increased), the rennet curd matrix at cutting, during cooking and during milling became more fragile (Rudan et al., 1999). In our experiment, there were more curd chippings and fines during cheese making for LFC and this may cause a higher percentage of protein and fat loss, while the above-mentioned phenomenon did not happen to LFT. Many investigators indicated that TG can increase the gel strength of dairy products by catalyzing the covalent bond of γ-glutamyl)lysine (Bönisch et al., 2008, Lorenzen et al., 2002). Thus, during cheese making, a stronger rennet curd matrix was lower than that of full-fat cheeses, which may be partly due to the decrease of both W/P and MNFS. The decrease in W/P and MNFS, which means a decrease in the freedom of moisture, decreased the activity of enzymes and microorganisms and therefore the degree of proteolysis (Lane et al., 1997, Rudan et al., 1999). The result also indicated that in the TG-treated samples, the degree of proteolysis was higher during the first 15 days and lower at later ripening times than the corresponding untreated samples. Compared with LFC and FFC, cheeses obtained by adding TG showed higher W/P and MNFS, which led to more proteolysis initial-
Effect of Transglutaminase on Low-fat Cheddar Cheese

as discussed above. At later ripening days, TG catalyzed additional cross-linking of proteins and TG interference with the action of the coagulant enzyme may lead to the slower degradation of the TG-treated samples (Pierro et al., 2010). And, the inhibitory effect on the proteolysis of low-fat cheese was more obvious than that of full-fat cheese. The bands created by TG-treated samples were not exact but broad bands scattered significantly downwards on the gel (Fig. 1(b), (d)); the same phenomenon was observed by Ercili-Cura et al. (2012).

**Functional properties**

**Texture**  At the first day of cheese ripening, the hardness, springiness and cohesiveness of LFC were higher than those of FFC (Fig. 2(a) – (c)). In accordance with our results, an increase in hardness due to a reduction in the fat content of cheese was reported for low-moisture, part-skim Mozzarella (Rudan et al., 1999) and Cheddar cheeses (Bryant et al., 2009). Consistent with the predictions of the filled gel composite theory and the work of Rudan et al. (1999), the increase of hardness was due to the decrease of the fat content and total filler volume and the increase of the amount of matrix (protein). Although the moisture content increased, it did not completely offset the decrease in fat as indicated by the steady increase in protein content with the reduction of fat content (Table 1). Rudan et al. (1999) found that the springiness was higher for low-fat Mozzarella cheese than for full-fat Mozzarella cheese. They indicated that the absence of fat resulted in a more flexible protein network. From the comparison of LFT with LFC, the TG treatment cheese had softer texture and exhibited increased springiness and cohesiveness. Compared with LFC and FFC, the filler volume increased by approximately 9% and 4% for LFT and FFT, respectively. All the TG-treated cheeses possess more filler volume than untreated cheeses. And, this trend of low-fat cheese was more obvious than full-fat cheese. Therefore, the addition of TG significantly decreased the hardness of low-fat cheese. These higher values of springiness and cohesiveness may be due to the additional cross-linking effect by TG,

![Urea-PAGE of FFC (a), FFT (b), LFC (c) and LFT (d) ripened for 1, 15, 30, 60 and 90 days ((1) – (5), respectively); milk (S).](image)
which led to a compact protein network and increased the association between casein micelles (Gauche et al., 2009).

As LFC and FFC matured, the values for hardness, springiness and cohesiveness decreased (Fig. 2(a) – (c)). During ripening, proteolysis by enzymes led to a reduction in the levels of intact casein in the cheese and therefore may contribute to the overall weakening of this structure (Hort and Grys, 2001, Lane et al., 1997). For FFT, the hardness decreased during the first 15 days and increased at later ripening days. And for LFT, the hardness exhibited an increasing trend during whole 90 ripening days. As a result, the hardness of TG-treated cheeses surpassed that of untreated cheese until about 60 days of ripening. During ripening, the cohesiveness value of the TG-treated cheeses decreased at a very slow rate, and the springiness value increased slowly and fluctuated. It could be due to the formation of protein intermolecular cross-linking (Kwan and Easa, 2003). The reduction of hardness of TG-treated cheeses during early ripening was probably because of proteolysis (Pierro et al., 2010). At later aging stages, the higher hardness and springiness values may be due to the new covalent bonds created by TG. The intensity of the bands in $\beta$-CN and $\alpha_s$-CN was reduced considerably with the addition of TG as shown in Fig. 1. This was probably due to the formation of high molecular weight polymers catalyzed by TG. It has been reported that excessive protein polymerization leads to a structure with higher stress values for fracture (Pierro et al., 2010). In addition, the lower degree of proteolysis in TG-treated cheeses (Table 3, Fig. 1(b), (d)) may account for the higher values in both hardness and cohesiveness.

**Meltability** As shown in Fig. 3, fat percentage significantly affected the meltability of the cheese. LFC had less melting area than FFC. In accordance with our results, impairment in meltability due to a reduction in fat content were reported for reduced-fat Cheddar (Kim et al., 2011) and Mozzarella (Rudan et al., 1999, Tunick et al., 1993) cheeses. As suggested by the latter authors, the poor meltability could be attributable to a stronger protein network and a lower ability of fat and protein phases to move in relation to each other (Tunick et al., 1993). For this reason, both TG-treated cheeses with stronger and more compact protein network showed poor meltability compared with untreated cheeses.

Aging also had a large impact on the meltability of cheese (Fig. 3). During matured storage, the meltability of the LFC and FFC significantly increased, which was consistent with the observations reported by other researchers.

![Fig. 2. The hardness (a), springiness (b) and cohesiveness (c) of FFC, FFT, LFC and LFT ripened for 1, 15, 30, 60 and 90 days.](image)

![Fig. 3. The melting area of FFC, FFT, LFC and LFT ripened for 1, 15, 30, 60 and 90 days.](image)
Effect of Transglutaminase on Low-fat Cheddar Cheese

(Rudan et al., 1999, Tunick et al., 1993). As the protein matrix was degraded by proteolysis, the ability of the cheese to maintain its original structure during heating decreased. Thus, for a given amount of heat, the melting diameter of the cheese discs increased in relation to the increase in the amount of proteolysis during storage. For the 90-day storage, LFT had stable and poor meltability (Fig. 3). The meltability of FFT decreased after 30 days of aging and remained stable, like that of LFT at later ripening days. This was because during ripening, TG catalyzed additional covalent cross-linking, which resulted in a denser protein network with higher heat stability, as reported by Schorsch et al. (2000).

**Rheological properties** Storage modulus ($G'$) of a given material is a measurement of energy stored and recovered per cycle, such as heating during a test cycle (Rogers et al., 2010). The changes in $G'$ of cheeses with maturing are shown in Fig. 4(a) – (e). As the heating temperature increased, the $G'$ value of FFC decreased slowly. With respect to LFC, two steps were visible. The $G'$ value of LFC was always higher than others in the test process. On the first day of ripening (Fig. 4(a)), the initial $G'$ was maintained at a very high level, then decreased noticeably to a low value and increased slightly with temperature during the later course. After ripening for 15 days, the initial $G'$ decreased to a lower level than that at the first day. From 20 to about 35°C, $G'$ decreased slightly with temperature. Above 35 to 45°C, $G'$ increased obviously.

![Fig. 4](image-url). The storage modulus of FFC, FFT, LFC and LFT ripened for 1 (a), 15 (b), 30 (c), 60 (d) and 90 (e) days, with heating from 20 to 80°C at a rate of 3°C/min.
up to a high value and then remained almost constant. The $G'$ showed a similar trend during the later maturing course. And during this course, LFC had higher $G'$ value than FFC, as has previously been observed by other researchers (Hennelly et al., 2006, Wang et al., 2011). During heating, the protein – protein bonds within the casein network were weakened, and the fat globules liquefied and deformed (<40°C), which may have plasticized the protein matrix and allowed it to flow (Hennelly et al., 2006, Rogers et al., 2010). For low-fat cheese, this temperature behavior of $G'$ can be mainly attributed to the alterations in the protein-gel network (Rogers et al., 2010). The decrease of rheological properties with cheese ripening has been observed in other studies, and the reduction of intact caseins as a result of proteolysis accounted for the decrease of the storage modulus and the consequent softening effect (Rogers et al., 2010, Yang et al., 2011). Therefore, cheeses ripened for a shorter time had higher $G'$ compared with the mature cheeses. The result for FFT showed that the $G'$ values of the five cheeses of different maturity decreased first and increased subsequently with increasing temperature. A similar result was observed in imitation cheese containing inulin (Hennelly et al., 2006). For LFT, a similar trend of the changes in $G'$ was observed during the first 60 days of ripening. After 90 days of aging (Fig. 4(e)), like the $G'$ of LFC on the first day, $G'$ was maintained at a very high level within 20–30°C, and then decreased noticeably to a lower value from 30–40°C. The later trend was similar to that for FFT. The increase of $G'$ may indicate that additional bonds are formed between proteins in the presence of TG and result in a strengthening of the cheese matrix (Hennelly et al., 2006, Schorsch et al., 2000).

**Conclusions**

In this study, TG improved the yield of low-fat cheese by enhancing the serum holding capacity as a result of additional covalent bonds. The protein and fat recoveries of low-fat cheese were also increased by adding TG. In addition, TG catalyzed cross-linking which influenced both the proteolytic pattern and the texture properties of cheese. During the early ripening stage, the hardness of low-fat cheese was significantly reduced. However, this cross-linking inhibited protein proteolysis at later ripening days, which resulted in a harder texture and lower meltability than untreated cheese. TG is a promising agent which could be used to increase the yield and improve the texture properties of fresh and short-aging low-fat cheeses.

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**References**


of fat-replacing ingredients on process and age induced soluble nitrogen content and ultrastructure of low-fat Cheddar cheese.


