Original Research Article

Effect of Transglutaminase Treatment on Functional Properties of Paneer

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ABSTRACT

Paneer is a traditional Indian milk product popular among vegetarians. It is valued for its nutritional and health benefits. Application of microbial transglutaminase (protein-glutamine γ-glutamyl transferase) (MTGase) in improving the physical and textural properties of paneer was investigated. Paneer was prepared from milk treated with different concentrations of MTGase using citric acid as coagulant. Milk samples were treated with MTGase at 4°C for 18 h or 50°C for 1 h. Parameters such as yield, cooking loss, water content and textural parameters were analyzed. The batch treated with MTGase showed increase in yield at both the temperatures. MTGase at a concentration of 1 U/g of protein was found to be optimum in improving textural properties and water holding capacity while minimizing the cooking losses in paneer. The studies suggested that paneer with improved functional qualities can be produced using enzymatic cross linking technique with MTGase.

Keywords

Microbial transglutaminase, Milk, Paneer, Cooking loss, Texture

Introduction

Paneer is a traditional Indian milk product popular among vegetarians. It is valued for its nutritional and health benefits. It mainly consists of milk solids and is prepared by coagulating casein with the help of citric, lactic or tartaric acid. Moisture is then removed from the product by applying pressure. In India, paneer production has been largely confined to the non-organized sector of dairy industry. An estimated one per cent of the country’s total milk production is converted into paneer. Its annual production is estimated at 1, 50,000 tones. It is widely used as a base material for preparation of a variety of culinary dishes, stuffing material for various vegetable dishes, snacks and sweet meats (Mathur, 1991; Agnihotri and Prasad, 1993).

The enzyme transglutaminase (TGase) catalyzes the acyl transfer reaction between γ-carboxamide groups of peptide bound glutamine (acyl donor) and the primary amino groups in a variety of amine compounds (acyl acceptor), including peptide bound ε-amino groups of lysine residues. As a result of this inter and intra-molecular protein cross linking, high molecular weight bio-polymers are formed (Kashiwagi et al., 2002). Transglutaminases
have been found in animals and are involved in the cross linking of fibrin molecules during blood clotting. Mammalian TGase is $\text{Ca}^{2+}$ dependent and its isolation is tedious and costly. Furthermore, its substrate specificity and different reaction rates for catalyzing different proteins restrict its use in industrial applications. The production of TGase from the bacterial strain *Streptoverticillium* opened up the possibility of a large scale production (Ando *et al.*, 1989). Advantages of microbial transglutaminase (MTGase) include calcium independence, low production cost, easy separation and amenability to genetic manipulation, which is of interest to the food industry as this enzyme can be used for the modification of foods containing proteins. Moreover, the enzyme treatment is more accepted by consumers as it is used in minute quantities for catalytic purposes and is considered to be more ‘natural’ than the chemical treatments. Industrially, MTGase is mainly used to improve the texture, stability, water binding, and other functional properties of food products (Gauche *et al.*, 2010; Kuraishi *et al.*, 2001). Utility of MTGase in reducing retort induced water loss and produce a harder tofu using either glucono delta lactone (GDL) or calcium sulfate as the coagulant has been demonstrated (Kwan and Easa, 2003; Yasir *et al.*, 2007). MTGase has also been reported to induce the coagulation of soymilk and was used to prepare tofu in the absence of other coagulants (Tang, 2007).

*Paneer* is commercially prepared from milk by coagulating milk proteins using acidulant like citric acid. The major milk protein, casein, is a good substrate for the enzyme MTGase (Lorenzen *et al.*, 1998). Due to its high reactivity with milk proteins, application of MTGase in dairy products has been widely investigated (Bönisch *et al.*, 2007; Myllärinen *et al.*, 2007; Rodriguez-Nogales, 2006; Ozrenk, 2006). The aim of the present study was to investigate technological potential of MTGase in the preparation of *paneer* and to correlate the variations in textural properties of *paneer* and other parameters like yield, cooking loss and water content with the concentration of MTGase added for cross-linking.

### Materials and Methods

#### Milk

Full cream pasteurized milk (Mother Dairy, Mumbai) was procured from a local market. The composition of the milk as given by the manufacturer was as follows.

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>Fat</td>
<td>6.2%</td>
</tr>
<tr>
<td>Protein</td>
<td>3.3%</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>5.1%</td>
</tr>
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</table>

#### Enzyme

The $\text{Ca}^{2+}$ independent enzyme MTGase “Activa” was gifted by Shanghai Kinry Foods Ingredients (P) Ltd, China. The enzyme, supplied by the manufacturer having an activity of 100 U/g was used as such without further modification or purification. The activity was determined according to Folk (1970). The method is based on cross linking of hydroxyl amine with benzoyloxycarbonyl-L-glutaminyl glycine. The resulting hydroxamate further reacted with $\text{FeCl}_3$, forming a ferric complex, which was detected using spectrophotometer at 525 nm. The assay of the activity was carried out at 37°C. All chemicals used for analysis are HiMedia/Sigma make AR grade chemicals.

#### Paneer preparation

Milk (1.5 L) in a stainless steel vessel was heated till it boiled. It was then allowed to cool to room temperature (~ 25°C) in a glass beaker. The enzyme was dispersed in 5 ml...
distilled water and added slowly with constant stirring. The beaker was covered to prevent loss of water due to evaporation and then refrigerated at 4°C for 18 h.

Another set was prepared by following the same procedure and kept at 50°C for 1 h in a constant temperature water bath. Three beakers with enzyme concentrations of 150 mg/L (0.5 U/g of protein), 300 mg/L (1.0 U/g of protein), and 600 mg/L (2.0 U/g of protein) of milk were prepared. Control sample contained no enzyme. After incubation, the beakers were allowed to attain room temperature.

Coagulation of milk is the most important step in the preparation of paneer. In the present study citric acid was employed as coagulant because it is a component of natural lime juice.

Milk samples (control as well as enzyme treated) were boiled (this resulted in inactivation of MTGase enzyme) and 35 ml of 10% freshly prepared citric acid solution was added slowly with constant stirring till clear whey (yellowish-greenish color) separated out. The curd was left to settle down for 10 min without agitation. The curd was then filtered through a muslin cloth. After filtration, the curd was tied in the muslin cloth and squeezed to remove excess water.

The curd was then cast to form paneer by placing on a flat surface and ~ 2 kg weight was placed on the curd for 1 h. The pressed blocks of curd were removed and immersed in chilled water (4°C) till further used. The paneer formed was cut into pieces of desired size using a sharp knife. Paneer pieces (size of 1.8 cm dia X 1.5 cm height) were used for TPA analyses. Before analyses the samples were placed on a wooden plank for 10–15 minutes to allow loose water to drip down.

**Yield of paneer**

Weight of paneer (in g) obtained from 1 L of milk sample was measured to determine yield of paneer.

**Total water content**

Initially weighed paneer (W₁) was kept in laboratory oven at 100 ± 2°C for 16 h. The samples were weighed again (W₂) after attaining room temperature and the difference in weight was expressed as water content in percentage.

\[
\text{Water content (\%)} = \frac{W_1 - W_2}{W_1} \times 100
\]

**Cooking loss**

Cooking loss of paneer was estimated according to the methods of Nonaka *et al.* (1996a, b) with some modifications. Paneer samples of approx 40 g weight cut into rectangular blocks of 3 x 1.5 x 1.5 cm (initial weight W₁) were put into a beaker containing water and kept in boiling condition for 15 m. After 10 m the paneer blocks were filtered through a mesh and allowed to cool to room temperature and the final weight W₂ was taken. The cooking loss of paneer was calculated as

\[
\text{Cooking loss (\%)} = \frac{W_1 - W_2}{W_1} \times 100
\]

**Texture analyses**

Texture characteristics of paneer were determined using Texture Analyzer TA-HD plus (Stable Micro Systems, Surrey, UK) in compression mode. Texture Profile Analysis (TPA) was carried out on cylindrical paneer samples having dimensions 1.8 cm dia. and 1.5 cm height. The samples were compressed twice by a cylindrical aluminium probe of 36 mm dia. (P/36R) to 33.3% of their original height at a constant
cross head speed of 1 mm/sec in ambient laboratory condition (25±2°C). From the TPA curve, hardness, toughness, springiness, cohesiveness and gumminess were estimated. Hardness was calculated as peak force (N) during the compression cycle. Toughness was calculated as the area under first curve. Springiness was calculated as ratio of second compression distance divided by the first compression. Cohesiveness was calculated as the ratio of area under the second curve to the area under the first curve (dimensionless). Gumminess was calculated by multiplying cohesiveness and hardness. The textural characteristic values were means of 5 measurements performed on 3 batch replicates.

**SDS-polyacrylamide gel electrophoresis (SDS-PAGE)**

SDS-PAGE was performed using 4% stacking gel and 10% running gel according to the method of Laemmli (1970) with a vertical gel electrophoresis unit (Mini-kin, Techno Source, Mumbai, India). Protein (25 μg) was applied to the gel. The electrophoresis was carried out at 20 mA. Medium range molecular weight markers (RPMWM) (Bangalore Genei Pvt. Ltd. India) were used for calibration. After separation, protein bands were stained using Coomassie Brilliant Blue R-250 (0.2%) in 25% methanol and 10% acetic acid. Destaining was performed using 40% methanol and 10% acetic acid.

**Sensory evaluation**

*Paneer* samples prepared from milk treated with different concentration of MTGase were analyzed for their sensory attributes. A panel of twelve scientists carried out the sensory analysis. The sensory attributes evaluated were appearance, color, odor, texture, taste and overall acceptability of the product using a 9-point numerical scale, where 9 corresponded to a most liked sample and 0 corresponding to a least liked sample. A score of 5 was taken as the lower limit of acceptability.

**Result and Discussion**

**Yield**

*Paneer* is prepared by the addition of coagulants to denature the proteins present in the milk. In the process of *paneer* making, some solids are lost in the whey. Effect of MTGase treatment on yield of *paneer* is shown in figure 1. It was seen that addition of MTGase enzyme resulted in concentration dependent increase in the yield of *paneer*. In the samples prepared from milk incubated at 4°C for 18 h, control sample yielded 155.7 ± 2.5 g of paneer/L of milk, whereas, in samples treated with 0.5, 1 and 2 U of enzyme the yield increased by 6.4, 9.6 and 10.7 % respectively. Similarly in samples prepared from milk incubated at 50°C for 1 h, control sample yielded 163.7 ± 3.8 g/L of *paneer* whereas, in samples treated with 0.5, 1 and 2 U of enzyme yield increased by 4.2, 7.6 and 8.7 % respectively. These results are in agreement with Pierro et al. (2010), Sayadi et al. (2013) and Özer et al., (2013) who reported improved yield of cheese due to addition of MTGase. Enzymatic modification of milk proteins provides protein in network to distribute more evenly and homogeneously which increases the gel stability in turn and affects the techno-functional properties of milk products (Faergamand et al., 1999).

**Moisture content of *paneer***

Water content is an important parameter that contributes to the palatability of *paneer*. Figure 2 shows that TGase treatment increased the water content of *paneer*. In case of control samples moisture content
was 42 ± 1 % and was not affected by incubation conditions. In case of enzyme treated samples moisture content was affected by both enzyme concentration as well as incubation conditions (Fig. 2). In samples prepared from milk incubated at 4°C for 18 h, the moisture content in samples treated with 0.5, 1 and 2 U of enzyme was 45, 47.8 and 45.6 %, respectively, whereas, in samples prepared from milk incubated at 50°C for 1 h, the values were 44, 46.9 and 44.6%, respectively. It can be seen that application of MTGase resulted in higher levels of water in the experimental paneer than that in control samples. This can be attributed to the increased water retention capacity of citric acid induced gels formed with cross-linking of bonds ε-(γ-glutamyl) lysine by MTGase (Farnsworth et al., 2006; Lorenzen et al., 2002). Higher moisture content in cheese prepared from MTGase treated milk has been reported earlier (Pierro et al., 2010; Özer et al. (2013). Previous studies have also shown that addition of MTGase in the production of yoghurts increased the gel strength and viscosity while decreased the syneresis due to the increased water binding capacity (Faergamand et al., 1999; Motoki and Seguro, (1998).

Cooking Loss

_Paneer_ is cooked during consumption which leads to moisture loss and has adverse effect on textural attributes. The _paneer_ samples without the transglutaminase treatment shrunk and lost about 14% of its weight after cooking (Fig. 3). The loss consisted of water and some water soluble compounds. This result suggested that the structure of _paneer_ is changed because of heat treatment and that the water entrapped in the microstructure of the _paneer_ is released. The enzyme-treated _paneer_ also shrunk and decreased in weight, but the weight loss was improved in a concentration dependent manner up to 1 U/g of protein. Further increase in enzyme resulted in increased cooking loss (Fig. 3). These findings suggested that the protein network formed by MTGase of 1 U/g protein was more cohesive keeping water within the network during heat treatment. Incorporation of additional MTGase resulted in the formation of excessive cross-links that prevents the formation of a uniform protein network upon heating. In earlier studies on tofu, addition of transglutaminase is reported to reduce cooking loss in tofu due to the formation of intermolecular cross-links during incubation with enzyme (Kwan and Easa, (2003); Nonaka et al., (1996a, b). Concentration dependent improvement in gelation of protein gel and adverse effect of higher MTGase concentration on protein gels has been reported (Sakamoto et al., 1994; Imm et al., 2000).

Textural analysis

The results obtained from the Textural Profile Analysis on hardness, springiness, cohesiveness and gumminess of _paneer_ samples are presented in figure 4a-d, respectively. It can be seen that the _paneer_ samples prepared from MTGase treated milk were harder than those prepared from untreated milk. Inter and intramolecular cross linking of protein by the addition of enzyme resulted in improvement of textural attributes. It can be seen that in _paneer_ samples prepared from milk pretreated with MTGase, both hardness as well as toughness peaked at 1 U/g protein and further increase in the MTGase concentration had adverse effect on these textural attributes. Similar trend has been reported by Sakamoto et al., (1994), who hypothesized that excessive incorporation of ε-(γ-Glu)-Lys cross-links by MTGase, may prevent the formation of a uniform protein network upon heating.
Modification of milk proteins in the presence of active TGase results in improvement of consistency of set-type yoghurts have been reported by Lorenzen et al. (2002) and Sanli et al., (2011).

Toughness of the paneer also showed trend similar to hardness (data not shown). It increased up to the enzyme concentration of 1.0 U/g of protein and started declining at higher concentration.

The results of springiness and cohesiveness of paneer samples are shown in figure 4 b, c. Springiness which is often called as elasticity of the sample is a measure of ability of food to return to its original form after being compressed. Cohesiveness indicates how well the product withstands a second deformation relative to how it behaved under the first deformation. It can be seen that MTGase treatment did not produce any significant change in the springiness and cohesiveness characteristics of paneer (Fig 4 b,c). A similar trend has been reported in other studies where there was no significant change in these properties of whole soybean curd (Joo et al., 2011), procine plasma gel (Saguer et al., 2007), surimi (Kaewudom et al. 2013) and meat (Carballo et al., 2006).

There are contrasting reports in literature on the effect of MTGase treatment on springiness and cohesiveness for food proteins. Some studies have reported increase in springiness and cohesiveness of products such as beef gels (Pietrasik and Li-Chan, 2002; Pietrasik and Jarmoluk, 2003) restructured fish product (Ramirez et al., 2002), fish patties (Min and Green, 2008), bread (Caballero et al., 2007). Adverse effect of MTGase treatment on cohesiveness value of tofu gels has also been reported (Chang et al., 2011).

Effect of MTGase treatment on gumminess of paneer is shown in Figure 4d. It can be seen that gumminess which is product of hardness and cohesiveness followed the same trend as hardness and toughness. In samples treated at 4°C, it increased with enzyme concentration up to 1.0 U/g of protein and then started showing a declining trend. The value went up from 22.0 (± 0.9) in control to 28.4 (±2.3). In the samples treated at 50°C, gumminess increased in the sample treated with MTGase but no significant effect of enzyme concentration was observed. Studies on tofu have shown positive correlation between gumminess and enzyme concentrations (Chang et al., 2011). Improvement in gumminess of surmi, glycinin gels and soybean yoghurt by MTGase treatment has been reported (Kaewudom, 2013; Zhu et al., 2011; Gauche et al., 2009).

Electrophoretic pattern of paneer proteins

The inter-molecular cross-linking of the milk proteins through the action of MTGase was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). This is considered an acceptable method to monitor the cross-linking of proteins by MTG, because it shows inter-molecular cross-linking through the formation of new high-molecular-weight bands (Pinterits and Arntfield, 2008). The electrophoretic pattern of paneer prepared from milk treated with different concentrations of MTGase is shown in fig. 5. The rate of cross linking is quite clear in treated samples in comparison with untreated samples. Incubation of milk with MTGase resulted in a noticeable reduction of low molecular weight bands with concomitant increase in high molecular bands (Fig. 5).
Fig. 1 Effect of MTGase Treatment on yield of paneer

![Graph showing the effect of MTGase treatment on yield of paneer.](image)

Fig. 2 Effect of MTGase treatment on moisture content of paneer

![Graph showing the effect of MTGase treatment on moisture content of paneer.](image)

Fig. 3 Effect of MTGase treatment on cooking loss of paneer

![Graph showing the effect of MTGase treatment on cooking loss of paneer.](image)
**Fig. 4** Effect of MTGase treatment on textural properties of paneer

![Graph showing textural properties](image)

**Fig. 5** SDS-PAGE protein pattern of paneer samples. Lane 1 & 10 Molecular weight markers, Lanes 2-5 Paneer samples prepared from milk treated with MTGase (0, 0.5, 1 and 2 U/g of protein for 16 h at 4°C respectively); Lanes 6-9 Paneer samples prepared from milk treated with MTGase (2, 1, 0.5 and 0 U/g of protein for 1 h at 50°C respectively)
Reduction of low molecular weight bands and increase in high molecular bands was proportional to MTGase concentration. Addition of MTGase resulted in partial transformation of proteins into high molecular polymers (Wróblewska et al., 2009). Nonaka et al. (1997) and Sharma et al. (2001) reported that milk caseins, α-lactalbumin and β-lactoglobulin as well as many other albumins have been shown to be excellent substrates for MTGase-induced cross-linking.

Sensory analyses

The objective of this study was to investigate effect of MTGase treatment of milk on techno-functional properties of paneer. It was concluded from above results that 1U/ g of protein was optimum in terms of improving yield and textural properties and reducing cooking loss of paneer, hence sensory quality of paneer prepared from milk treated with MTGase (1 U/g of protein) was evaluated with the aim of predicting the applicability of MTGase in terms of consumer acceptance. The results of the sensory evaluation of samples are given in figure 6. It can be seen that appearance and odour of the paneer were not affected by MTGase treatment. These findings suggested that MTGase treatment did not lead to any objectionable change in odor and appearance of the products, which could lead to rejection of products by the consumer. However, samples treated with MTGase at 50ºC for 1 h had lower textural score resulting in lower acceptability of the product compared to that of samples incubated at 4ºC for 16 h. These results are sustaining the above observations that the samples treated with MTGase at 50ºC for 1 h had higher cooking loss resulting in rigid paneer. Effect of MTGase treatment on sensory attributes of various food products has been investigated by other researchers. Results of present study are agreement with Kaewudom et al. (2013) who studied the effect of MTGase treatment on fish protein gel and observed that addition of MTGase resulted in reduced score of texture and overall acceptability due to formation of rigid gel. In another study addition of different levels of MTGase to chicken meat did that have any significant effect on texture and overall acceptability of chicken meat balls (Tseng et al., 2000). Previous studies on yoghurt have also suggested that addition of MTGase treatment had no effect on ethanol and diacetyl formation which are
responsible for aroma of yoghurt samples (Özer et al., 2007, Sanli et al., 2011), whereas, Wróblewska et al. (2009) have reported improvement in sensory attributes of kefir upon MTGase treatment.

The results show that important parameters of paneer production and its palatability can favorably be altered by MTGase treatment (1.0 U/g of protein and incubation at 4°C for 16 h). The yield of paneer increased from 149.7 g/L to 161.1 g/L. Other important parameters like total water content and cooking loss were favorably altered by the addition of MTGase. The sensory attributes of panner were not adversely affected by MTGase treatment. The treatment of milk proteins with MTGase addition was found to be an effective method to increase techno-functional properties of paneer.

References


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