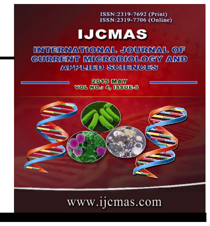
Int.J.Curr.Microbiol.App.Sci **(2015) 4(5): 227-238**



International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 5 (2015) pp. 227-238**
http://www.ijcmas.com

Original Research Article

Effect of Transglutaminase Treatment on Functional Properties of Paneer

V. Prakasan, S. P. Chawla* and Arun Sharma

Food Technology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India

**Corresponding author*

A B S T R A C T

Paneer is a traditional Indian milk product popular among vegetarians for its nutritional and textural properties. 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 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.

Fat	: 6.2%
Protein	: 3.3%
Carbohydrate	: 5.1%

Enzyme

The 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 benzyloxycarbonyl-L- glutaminyl glycine. The resulting hydroxamate further reacted with $FeCl_3$, 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* (W_1) was kept in laboratory oven at 100 ± 2 °C for 16 h. The samples were weighed again (W_2) after attaining room temperature and the difference in weight was expressed as water content in percentage.

$$\text{Water content (\%)} = (W_1 - W_2) / W_1 \times 100$$

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 W_1) 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 W_2 was taken. The cooking loss of *paneer* was calculated as

$$\text{Cooking loss (\%)} = (W_1 - W_2) / W_1 \times 100$$

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 shrank 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 shrank 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 *panner* 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

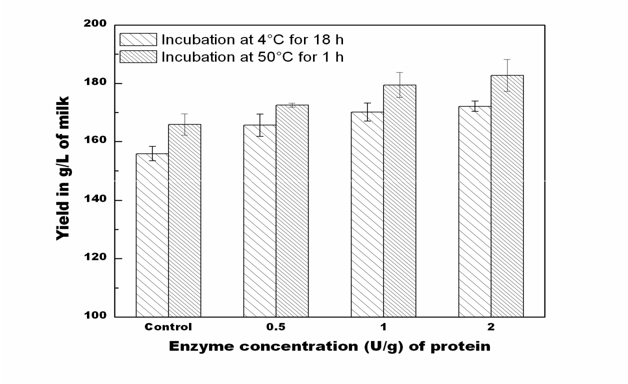


Fig.2 Effect of MTGase treatment on moisture content of paneer

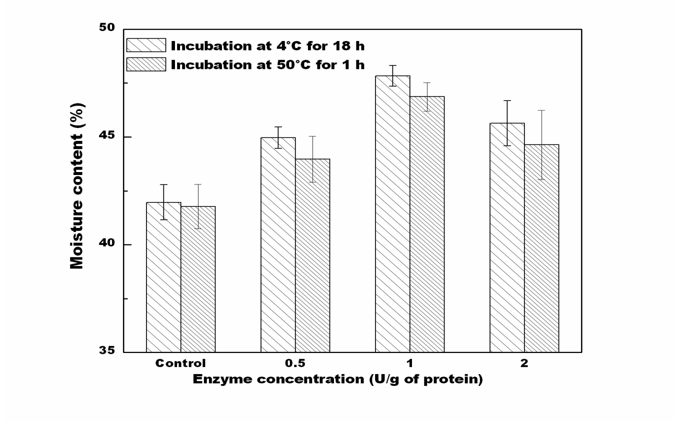


Fig.3 Effect of MTGase treatment on cooking loss of paneer

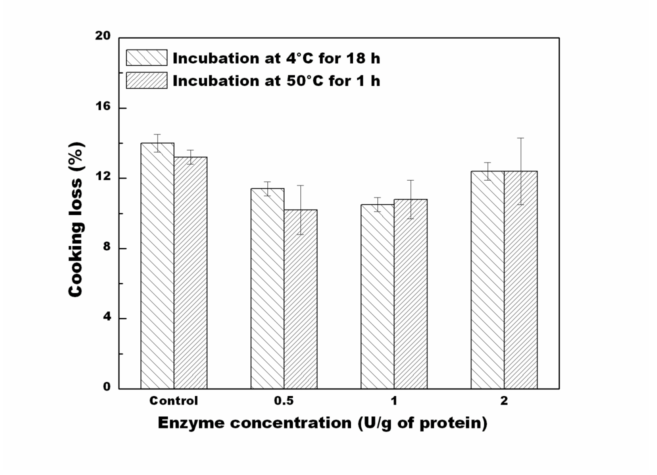


Fig.4 Effect of MTGase treatment on textural properties of paneer

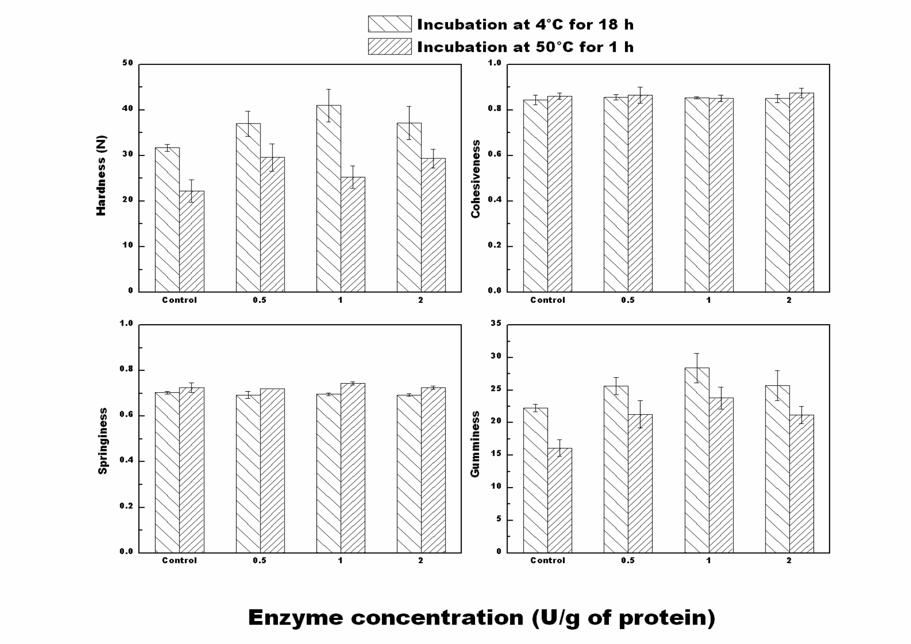


Fig.5 SDS-PAGE protein pattern of paneer samples. Lane 1 & 10 Molecular weight markers, Lanes 2-5 Paneer samples prepared from milk treated with MTGase (0, 0.5, 1 and 2 U/g of protein for 16 h at 4ºC respectively); Lanes 6-9 Paneer samples prepared from milk treated with MTGase (2, 1, 0.5 and 0 U/g of protein for 1 h at 50ºC respectively)

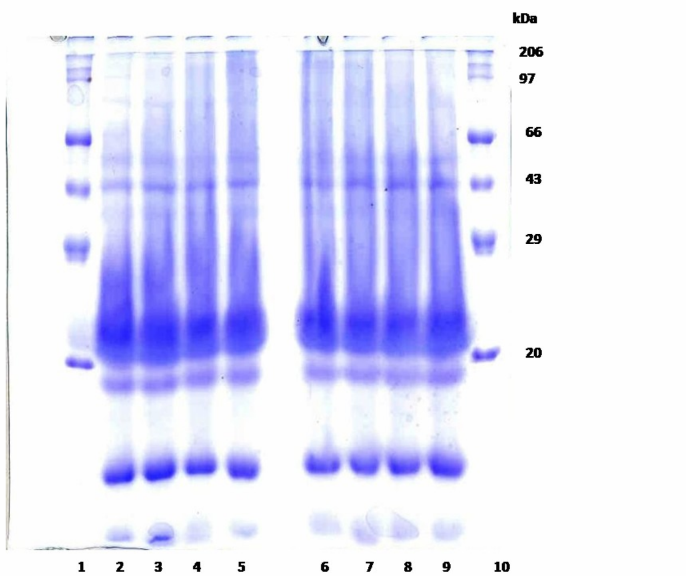
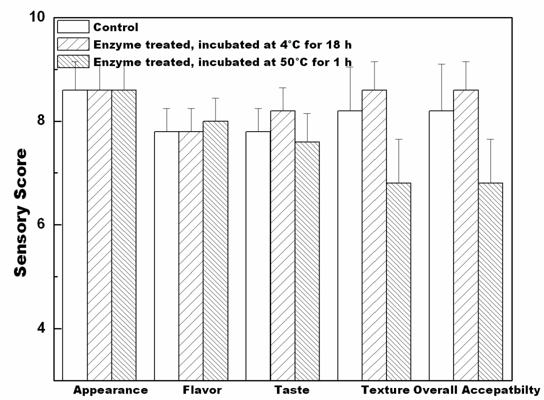


Fig.6 Effect of MTGase treatment sensory scores of paneer



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 over all 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 4ºC 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

Aagnihotri, M.K., Prasad, V.S.S. 1993. Biochemistry and processing of goat milk and milk products. *Small Ruminant Res.*, 12: 151–170.

Ando, H., Adachi, M., Umeda, K., Matsuura, A., Nonaka, M., Uchio, R., Tanaka, H., Motoki M. 1989. Purification and characteristics of a novel transglutaminase derived from microorganisms. *Agr. Biol. Chem. Tokyo,* 53: 2613–2617.

Bönisch, M.P., Huss, M., Weitl, K., Kulozik, U. 2007. Transglutaminase cross-linking of milk proteins and impact on yoghurt gel properties. *Int. Dairy J.*, 17: 1360–1371.

Caballero, P.A., Go´mez, M, Rosell, C.M. 2007. Improvement of dough rheology, bread quality and bread shelf-life by enzymes combination. *J. Food Eng.,* 81: 42–53.

Carballo, J., Ayo J., Jimēnez, F.C. 2006. Microbial transglutaminase and caseinate as cold set binders: Influence of meat species and chilling storage. *Food Sci. Technol-Leb.*, 39: 692–699.

Chang, Y.H., Shiau, S.Y., Chen, F.B., Lin, F.R. 2011. Effect of microbial transglutaminase on the rheological and textural characteristics of black soybean packed tofu coagulating with agar. *Food Sci. Technol-Leb.*, 44: 1107–1112.

Faergamand, M., Murray, B.S., Dickinson, E., Qvist, K.B. 1999. Cross-linking of absorbed casein films with transglutaminase. *Int. Dairy J.*, 9: 343–346.

Farnsworth, J.P., Li, J., Hendricks, G.M., Guo, M.R. 2006. Effects of transglutaminase treatment on functional properties and probiotic culture survivability of goat milk yogurt. *Small Ruminant Res.*, 65: 113–121.

Folk, J.E. 1970. Transglutaminase In *Method in Enzymology* Ed: Tabor H and Tabor CW, Academic Press, New York. Pp. 889–894.

Gauche, C., Barreto, P.L.M., Bordignon-Luiz, M.T. 2010. Effect of thermal treatment on whey protein polymerization by transglutaminase: Implications for functionality in processed dairy foods. *Food Sci. Technol-Leb.*, 43: 214–219.

Gauche, C., Tomazi, T., Barreto, P.L.M., Bordignon-Luiz, M.T. 2009. Physical properties of yoghurt manufactured with milk whey and transglutaminase, *Food Sci. Technol-Leb.*, 42; 239–243.

Imm, J.Y., Lian, P., Lee, C.M. 2000. Gelation and water binding properties of transglutaminase –treated skim milk powder. *J. Food Sci.*, 65: 200–205.

Joo, S.I., Kim, J.E., Lee, S.P. 2011. Physicochemical properties of whole soybean curd prepared by microbial transglutaminase. *Food Sci. Biotechnol.,* 20: 437–444.

Kaewudom, P., Benjakul, N.S., Kijroongrojana, K 2013, Properties of surimi gel as influenced by fish gelatin and microbial transglutaminase. *Food Biosci.,* 1: 39-47.

Kashiwagi, T., Yokoyama, K. I., Ishikawa, K., Ono, K., Ejima, D., Matsui, I Suzuki, E. 2002. Crystal structure of microbial transglutaminase from *Streptoverticillium mobaraense. J Biol. Chem.,* 277: 44252–44260.

Kuraishi, C., Yamazaki K., Susa, Y. 2001. Transglutaminase: its utilization in the food industry. *Food Rev. Int.,* 17: 221–246.

Kwan, S.W., Easa, A.M. 2003. Comparing physical properties of retort-resistant glucono-δ-lactone tofu treated with commercial transglutaminase enzyme or low levels of glucose. *Food Sci. Technol-Leb.,* 36*:* 643–646.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* 227: 680–685.

Lorenzen, P.C., Neve, H., Mautner, A., Schlimme, E. 2002. Effect of enzymatic cross linking of milk proteins on functional properties of set-style yoghurt. *Int. Dairy J.,* 55: 152–157.

Lorenzen, P.C., Schlimme, E., Roos, N. 1998. Crosslinking of sodium caseinate by a microbial transglutaminase, *Nahrung,* 42: 151–154.

Mathur, N. 1991. Indigenous milk products of India. The related research and technological requirements. *Indian Dairyman,* 42: 61–74.

Min, B., Green, B.W. 2008. Use of microbial transglutaminase and non meat proteins to improve functional properties of low NaCl, phosphate-free patties made from channel catfish (Ictalurus punctatus) belly flap meat. *J. Food Sci.,* 73: 218–226

Motoki, M., Seguro, K. 1998. Transglutaminase and its use for food processing. *Trends Food Sci. Tech.,* 9: 204–210.

Myllärinen, P., Buchert, J., Autio, K. 2007. Effect of transglutaminase on rheological properties and microstructure of chemically acidified sodium caseinate gels. *Int. Dairy J.,* 17: 800–807.

Nonaka, M., Matsuura, Y., Motoki, M. 1996a Incorporation of lysine- and lysine dipeptides into αs1-casein by Ca2+ -independent microbial transglutaminase. *Biosci. Biotech. Biochem.,* 60: 131–133.

Nonaka, M., Matsuura, Y., Nakano, K., Motoki, M. 1997. Improvement of the pH-solubility profile of sodium caseinate by using Ca^{2+}-independent microbial transglutaminase with gelatin. *Food Hydrocolloid,* 11: 347–349.

Nonaka, M., Sakamoto, H., Toiguchi, S., Yamagiwa, K., Soeda, T., Motoki, M 1996b. Retort-resistant tofu prepared by incubation with microbial transglutaminase. *Food Hydrocolloid.,* 10: 41–44.

Özer, B., Hayaloglu, A.A, Yaman, H., Gürsoy, A., Şener, L. 2013. Simultaneous use of transglutaminase and rennet in white-brined cheese production. *Int. Dairy J.,* 33: 129–134.

Özer, B., Kirmac, H. A, Oztekin, S., Hayaloglu, A., Atamer, M. 2007. Incorporation of microbial transglutaminase into non-fat yogurt production. *Int. Dairy J.,* 17: 199–207.

Ozrenk, E. 2006. The use of transglutaminase in dairy products. *Int. J. Dairy Technol.,* 59: 1–7.

Pierro, P.D., Mariniello, L., Sorrentino, A., Valeria, C., Giosafatto, L., Chianese, L., Porta, R. 2010. Transglutaminase induced chemical and rheological properties of cheese. *Food Biotechnol.,* 24: 107–120.

Pietrasik, Z., Jarmoluk, A. 2003. Effect of sodium caseinate and j-carrageenan on binding and textural properties of pork muscle gels enhanced by microbial transglutaminase addition. *Food Res. Int.,* 36: 285–294.

Pietrasik, Z., Li-Chang, Y.C.E. 2002. Binding and textural properties of beef gels as affected by protein, j-carrageenan and microbial transglutaminase addition. *Food Res. Int.*, 35: 91–98.

Pinterits, A. Arntfield S.D. 2008. Improvement of canola protein gelation properties through enzymatic modification with transglutaminase. *Food Sci. Technol-Leb.,* 41: 128–138.

Ramirez, J., Uresti, R., Tellez, S., Vazquez, M. 2002. Using salt and microbial transglutaminase as binding agents in restructured fish products resembling hams. *J. Food Sci.,* 67: 1778–1784

Rodriguez-Nogales, J. M. 2006. Enhancement of transglutaminase-induced protein cross-linking by preheat treatment of cows' milk: a statistical approach. *Int. Dairy J.,* 16: 26–32.

Saguer, E., Fort, N., Pare´S, D., Toldra, M., Carretero, C. 2007. Improvement of gelling properties of porcine blood plasma using microbial transglutaminase. *Food Chem.,* 101: 49–56

Sakamoto, H., Kumazawa, Y., Motoki, M. 1994. Strength of protein gels prepared with microbial transglutaminase as related to reaction conditions. *J. Food Sci.,* 59: 866–871.

Sanli, T., Sezgin, E., Deveci, O., Senel, E., Benli, M. 2011. Effect of using transglutaminase on physical, chemical and sensory properties of set-type yoghurt. *Food Hydrocolloid.,* 25: 1477–1481

Sayadi, A., Madadlou, A., Khosrowshahi, A. 2013. Enzymatic cross-linking of whey proteins in low fat Iranian white cheese. *Int. Dairy J.,* 29: 88–92.

Sharma, R., Peter, C., Lorenzen, K., Qvist, B. 2001. Influence of transglutaminase treatment of skim milk on the formation of ε(γ-glutamyl) lysine and the susceptibility of individual proteins towards cross linking. *Int. Dairy J.,* 11: 785–793

Tang, C.H. 2007. Effect of thermal pretreatment of raw soymilk on the gel strength and microstructure of tofu induced by microbial transglutaminase. *Food Sci. Technol-Leb.*, 40: 1403–1409.

Tseng, T.F., Liu, D.C., Chen, M.T. 2000. Evaluation of transglutaminase on the quality of low salt chicken meat balls. *Meat Sci.,* 55: 427–431.

Wróblewska, B., Kołakowski, P., Pawlikowska, K., troszyńskaa A, Kkaliszewskaa, A. 2009. Influence of the addition of transglutaminase on the immunoreactivity of milk proteins and sensory quality of kefir. *Food Hydrocolloid.,* 23: 2434–2445.

Yasir, S.B.M., Sutton, K.H., Newberry, M. P., Andrews, N.R., Gerrard J.A. 2007. The impact of transglutaminase on soy proteins and tofu texture. *Food Chem.,* 104: 1491–1500.

Zhu, J.H., Yang, X.Q., Ahmad. I., Zhong, R.M., Qi, J.R. Zhum D.H. 2011. Physical properties of yoghurt manufactured with milk whey and transglutaminase. *Int. J. Food Sci. Tech.,* 46: 963–971.