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Minimizing textural defects in heat and acid coagulated dairy gel- (Paneer) during sub-zero temperature storage using protein cross-linking enzyme (Microbial transglutaminase)

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Abstract
The current study was taken up to investigate the effect of Microbial Transglutaminase (MTGase) on textural and viscoelastic properties of Paneer stored at -15±1 ºC for 60 days. The prolonged storage of control samples at sub-zero temperature exhibited textural defects post thawing, such as fluffy body, crumbly and weak texture with reduced textual integrity as compared to fresh samples. The application of protein cross linking enzyme was hypothesized to reduce such defects by increasing the textural compaction due to enhanced covalent cross linking of proteins. Application of MTGase had significant impact on the textural attributes of Paneer viz. hardness, cohesiveness, chewiness and springiness. Modulus of elasticity (MPa), coefficient of viscosity (MPa.s) and creep compliance ($M.Pa^{-1}$) was calculated using stress relaxation and creep retardation viscoelastic tests. Periodic study of textural and viscoelastic properties of thawed samples (at 30 ºC/2hr) revealed positive impact of MTGase incorporation on Paneer samples. Results were supported by obtained scanning electron micrographs, percentage porosity value and sensory scores.

Keywords: Textural defects, heat and acid, coagulated dairy gel, Paneer, microbial transglutaminase

1. Introduction
Paneer is a famous heat and acid coagulated, non-fermented, non-renneted, non-melting and fresh, soft cheese type of dairy product popular in the south Asian region. It holds a strong demand between both vegetarian and non-vegetarian class of consumers. It contributes as a major protein source for vegetarian class of consumers due to its superior digestibility and high biological value (80 to 86) [28]. Raw Paneer is characterized by marble white to creamy white colour, firm and chewy body, close-knit texture and sweetish acidic nutty flavour [13, 21].
The water activity (a_w) of the product is 0.99, which limits its shelf life to up to 1 day at ambient temperature and up to 6 days at 10 °C but suffers loss of freshness after 2-3 days [3]. Several attempts have been made to increase the shelf life of the Paneer which includes in-can thermal sterilization [25], ultrafiltration followed by in-package texturization [20], dehydration up to 15-18% moisture content [33], dipping in brine solutions of various concentrations [30], use of sorbic or benzoic acids [29], application of bacteriocins viz. nisin, pediocin and their combinations along with NaCl and EDTA [19], use of fungicide (delvocid) and germicide (H_2O_2) [26], application of antioxidants (BHA and TBHQ) [14], Lysozyme and Lactoperoxidase treatments [1]. Apart from these, intermediate moisture food (IMF) technique, individual quick freezing (IQF), blast freezing, modified atmospheric packaging (MAP), UV treatment, smoking and a combination of hurdles have also been tried to extend the shelf life of Paneer [23]. However, only partially relevant successes have been achieved. IQF and blast freezing have achieved significant success in extending the shelf life but that requires large capital investments which restricts its use to large scale manufacturers. It was concluded that Paneer can be stored for up to 120 days at -13 and -32 ºC without significant decline in flavour and colour but the texture becomes fluffy and crumbly due to formation of large ice crystals which after thawing causes voids formation in Paneer matrix [3]. This has been a major hurdle in application of deep freezing as a means to extend the shelf life, otherwise freezing is the most effective method of extending shelf life without subjecting original product to further modifications. As availability of domestic refrigerators with dedicated freezing cabinets is common among retailers and consumers, any intervention which can protect or minimize the

Textural damage during frozen storage would be very helpful in utilizing the existing cold chain for extended storage of Paneer. Hence this research has been taken up to study the effect of incorporation of MTGase, a protein cross-linking enzyme on textural, viscoelastic and sensory acceptability of Paneer during frozen storage.

The enzyme transglutaminase is known for altering the techno-functional properties of protein containing foods [22]. Microbial transglutaminase (protein-glutamine γ -glutamyl-transferase, EC 2.3.2.13) in particular is an extracellular, low molecular weight enzyme of class transferase obtained from a variant of *Streptomyces mobaraensis* [34, 2, 35]. It is a calcium independent enzyme capable of forming ε-(γ-glutamyl) lysine bond by acyl transfer from donor γ-carboxyamide group of a peptide bound glutaminyl residue to several acyl receptors. MTGase efficiently catalyses polymerisation or cross linking between most of the food proteins like caseins, soy proteins, muscle proteins, egg and cereal derived proteins etc., by inducing inter or intra acyl transfer reaction between glutamine- the acyl donor and lysine- the acyl receptor [10, 15, 17, 35]. A spectrum of techno-functional changes viz. textural, rheological, water holding, gelling and emulsifying ability in food systems have been reported due to MTGase induced covalent bonding in proteins. These have been attained without affecting the native sensory and compositional aspects of food, albeit, an increase in nutritional profile due to possible binding of essential amino acids has been predicted [2, 8, 15]. It was reported that an increase in firmness, gelation temperature and reduced cooking loss in soybean tofu prepared using MTGase and agar [6]. An increase in gel strength and reduced syneresis in set yoghurt using transglutaminase was also reported [17]. Improved textural property of Paneer prepared using 1U/g of MTGase have been reported [22]. However, there are not much reported study on effect of MTGase incorporation on textural and viscoelastic property of heat and acid coagulated dairy gels like Paneer during frozen storage.

2. Materials and Methods

2.1 Materials

Fresh cow milk was procured from Livestock Research Centre (LRC) of ICAR- National Dairy Research Institute, Bengaluru which was filtered and standardized to approximately 3.5% fat and 8.5% SNF by mixing required quantity of skim milk and cream, followed by batch pasteurisation at 62±1 ºC for 30 min. MTGase (100U/g activity) was provided by Amano Enzyme Inc., Japan through Anthem Cellutions, Bangalore (India) Ltd. No modifications were done in the enzyme. All chemicals used were analytical grade procured from M/s Hindustan Dehydrated Media (Hi-Media) Laboratories Ltd., Mumbai (India).

2.2 Preparation of Paneer

For every trial, two batches each of 5 litres standardized and pasteurized milk was taken for making MTGase treated Paneer and control Paneer. The level of MTGase addition was fixed to 2U/g protein in milk based on conducted preliminary trials. Protein estimation done by Kjeldahl method using Gerhardt Kjeldahl estimation unit (Gerhardt GmbH and Co. Germany). Calculated amount of MTGase was dispersed in 20ml of distilled water at 40 ºC and added slowly with light stirring in milk previously maintained at 50±1 ºC in thermostatically controlled water bath (M/s KEMI, Bengaluru, Model no. 210A). After incubation of 1h, milk was heated up to 85 ºC without holding then cooled to 80 ºC followed by coagulation using citric acid solution 2% (w/w) maintained at 80 ºC. After clear separation of whey (greenish-yellow in colour), the curd was left undisturbed for 8-10 min. settled curd was filled in hoops (14*14*9 cm^3) and pressed at 2 bar pressure for 10 min in microprocessor based pneumatic Paneer press (Designed and developed at ICAR-NDRI, Bangalore). Pressed curd was soaked in chilled water (4-6 ºC) for 1 h. Chilled-pressed curd was kept on a flat surface to allow drainage of excess water for 5 min then were cut into pieces of three sets of dimensions for different intended tests viz. 2×2×2 cm^3 size for texture profile analysis, viscoelastic measurements and sensory evaluation, 1×1×1mm^3 for SEM analysis and 1.5×1.5×1.5 cm^3 for porosity measurements. All cut samples were packed in polypropylene containers with HDPE lids. Packed samples were kept at -15±1 ºC in vertical chest freezer (Middleby Celfrost Innovations Pvt. Ltd., Bengaluru). Same procedures were followed for preparing and storing control Paneer samples except addition of MTGase. Textural, viscoelastic and sensory evaluation were done at every 10 days, porosity measurement every 20 days and SEM analysis done at the end of 60 days storage period. Frozen samples of Paneer were thawed at 30±1 ºC for 2h prior to every analysis.

2.3 Texture analysis of Paneer

Textural profile analysis for three measured attributes viz. hardness, cohesiveness, springiness and one calculated attribute i.e. chewiness, was done according to Bourne, (1978). For TPA measurement TA-XT plus texture analyser (Stable Micro Systems, Surrey, UK) was used with test settings as given in Table 1. Load cell of capacity 50kg with platen probe (P/75 of 75mm diameter) was used for the tests. The mean of 9 measurements from three trials have been considered for analysis. Hardness (N) is the maximum peak force (F) during the first compression cycle (first bite). Cohesiveness indicates as to how well the product withstands a second deformation relative to how it behaved under the first deformation. It is measured as the area of work during the second compression divided by the area of work during the first compression. Springiness is the ratio of time difference between the time of first bite and the time of second bite. Chewiness (N) was calculated from the product of Hardness x Cohesiveness x Springiness.

2.4 Measurement of viscoelastic properties of Paneer

Paneer samples were subjected to stress relaxation tests and Creep retardation test using TA-XT plus texture analyser (Stable Micro Systems, Surrey, UK). The tests gave the measurements of Modulus of elasticity (MPa) which indicates the degree of solid character in the sample. Coefficient of viscosity (MPa.s) was calculated as the product of stress relaxation time (τ) and modulus of elasticity (ε_o). Creep compliance which is inverse of Modulus of elasticity indicates about the liquid character of the product. Tests were done using platen probe (P/75) with 75mm diameter and with test settings as given in Table 1. The values are reported after taking mean of nine readings from three trials conducted.

2.5 Scanning electron microscopy of Paneer

Samples intended for SEM imaging were cut to the size of 1 mm× 1mm×1mm and kept at similar storage condition. SEM was done at the end of 60 days storage period. The primary fixation of Paneer was done by fully immersing the cube of Paneer sample of size 1 mm× 1mm×1mm in 3% concentration solution of glutaraldehyde buffered with 0.1 M

phosphate buffer at room temperature or 0-4 °C and left for incubation for 2 h. Sample was rinsed with 0.1 M phosphate buffer solution (pH=7.2) three times, each for ten min. Post-fixation, sample was immersed in 1-2% osmium tetra-oxide in 0.1 M phosphate buffer pH=7.2 (2 – 4 h) at room temperature. Washing was carried out in 0.1 M phosphate buffer (pH=7.2) three times, each for ten min, followed by dehydration in graded ethanol and critical point drying (CPD). After that samples were mounted, metal coated and then SEM images were acquired using Zeiss EVO LS15 scanning electron microscope (Zeiss GmbH., Germany) at accelerating voltage of 15kV.

2.6 Sensory evaluation
Sensory evaluation of frozen Paneer samples were conducted at every 10 days intervals after thawing at 30±1 °C for 2 hr. Samples were evaluated for its sensory attributes like colour and appearance, body and texture, flavour and overall acceptability by expert sensory panel comprising of 6 members using a 9 - point hedonic scale [16]. The sensory panel included scientists and research scholars of the Institute.

2.7 Porosity of Paneer
Porosity in percentage was evaluated using the following method: Initial weight (M_0) of thawed Paneer cubes of size 1.5 × 1.5 × 1.5 cm^3 (V_0= 3.375 cm^3) were taken and noted down, then the weighed sample cubes were soaked in distilled water (1:20 ratio by volume) for about 12 hours in the refrigerator (6-8 °C). Samples were taken out from distilled water and after gentle wiping the surface to remove loose water, the weight (M_1) was recorded. The % porosity was evaluated as,

$$\text{Porosity}(\%)=\frac{M_1-M_0}{V_0.\rho}\times 100$$

Where, ρ is the density of water = 1 g/cm^3 at 4 °C
The standard equation for evaluating the bulk density in g/cm^3 is,
Bulk density = (M_0 / V_0) g/ cm^3.

2.8 Moisture determination
Moisture content in Paneer was determined using gravimetric method according to BIS (1983) procedure specified for Paneer under IS: 10484.

2.9 Statistical analysis
Data obtained during the present project work were subjected to two-way analysis of variance (ANOVA) using SPSS Software (Version 16.0, SPSS Inc. Chicago, USA). The results are expressed using mean and standard deviation of 9 observations taken during 3 trials. The comparison between treatment means was done for statistical significance by Duncan's test.

3. Results and Discussion
3.1 Effect of MTGase incorporation on textural attributes of sub-zero temperature stored Paneer
The effect of MTGase treatment on textural attributes of Paneer stored under sub-zero temperature is given in Table 2. The hardness value at day zero of MTGase incorporated Paneer samples were significantly higher than control samples. This may be attributed to the fact that formation of protein cross links (3-(g-glutamyl)-lysine crosslinks) led to increased water holding and greater hydration of protein matrix in product. With increased inter or intra protein interaction and hydration, the gel strength of proteinaceous foods has been found to be increasing [6, 17]. It concluded that use of MTGase improved the hardness of Paneer samples [22]. Study of effect of MTGase treatment on set type yoghurt reveals that gel strength and water holding improved by incorporation of protein crosslinking enzyme [17]. At the end of 60 days of frozen storage the hardness value of control samples reduced significantly. For the first 20 days of frozen storage the hardness value increased for both the samples. This phenomenon is in support of findings that hardness of frozen (-15 °C) and refrigerated (6 °C) Paneer samples increased from 1869.05g to 2450.54g for frozen samples and up to 3126.60g for refrigerated samples during first 2 weeks of storage and concluded that during low temperature storage of Paneer the protein fibres swells by hydration which causes increased hardness [9]. It is in support of this finding as when fresh Paneer samples were kept in deep freezer, the time required for attaining the deep freezer temperature might have given time for hydration of proteins. However, after 20 days the hardness value started reducing for control samples. This may be due to progressive loss of moisture (given in Table 5) by exudation due to melting of frozen water while thawing. Although % moisture reduction was higher in MTGase incorporated samples but due to high initial moisture content the net moisture content was still significantly higher than control samples. This may have resulted in higher hardness value of MTGase Paneer than control throughout the storage study. A time and temperature dependent loss in moisture content of Paneer samples stored at -13 °C and -32 °C for 120 days was reported [3]. The cohesiveness and springiness values did not vary significantly for control and MTGase incorporated samples at day zero. This was in agreement with the findings of Prakasan *et al*. (2015) [22]. However, over the period of storage, both the values reduced significantly as compared to day zero. It was concluded that the cohesiveness and springiness values of Paneer stored at -15 °C for 15 weeks shown decreasing trend throughout the storage period [9]. Similar findings were reported for frozen storage of mozzarella cheese [32]. At the end of 60 days storage period the cohesiveness and springiness values were significantly higher in MTGase samples than control Paneer samples. The formation of large ice-crystals causes void formation in product matrix after thawing this might cause complete and permanent collapse of voids during compression by probe and may result in reduced springing back of product after removal of compression [3, 32, 22].
The brittleness resulted from dehydration may be attributed to reduction in cohesiveness value of samples during frozen storage. The chewiness value observed the trend followed by hardness change. Significantly higher chewiness value was found in MTGase Paneer samples at day zero. Similar observations have been reported for Paneer and for soybean curd [22, 11]. Although the value reduced throughout the frozen storage period in agreement with the reported literature but remained higher for MTGase incorporated samples [9].

3.2 Effect of MTGase incorporation on viscoelastic properties of sub-zero temperature stored Paneer
Effect of MTGase incorporation on viscoelastic properties of Paneer during frozen storage is given in Table 3. Modulus of elasticity (ME) indicates the solid character of a specimen [21]. MTGase incorporated Paneer shown higher ME value than control. Increased cross-linking of protein increases the

firmness and elastic properties of protein gels [18]. Over the period of storage, the ME value first increased up to 20 days and afterwards it reduced significantly. Similar observation has been reported where the ME value of Paneer stored at -15 °C and 6 °C increased up to first 2 weeks of storage then after the solid character progressively reduced due to moisture loss [9]. Coefficient of viscosity decreases as the liquid character increases [9]. However, an increasing trend in coefficient of viscosity was observed for both control and MTGase samples but control samples at day zero shown less viscosity than MTGase samples and the trend followed throughout the 60 days of storage with significant difference. The liquid character is also indicated by compliance which is inverse of ME [21]. It was found that the compliance value remained higher for control samples than MTGase samples throughout the storage period, in agreement with the reported study [9]. MTGase and control samples shown compliance value in the range of 0.0666 to 0.0786 $M.Pa^{-1}$ and 0.0716 to 0.1056 $M.Pa^{-1}$ respectively. Higher compliance value of control samples suggests that the solid character in control Paneer progressively reduced during frozen storage and vice versa is true for MTGase Paneer samples. The results were supported by obtained instrumental hardness value which indicated that MTGase samples were significantly firmer than control Paneer samples. Improved viscoelastic properties of tofu by using MTGase has been reported [6]. Increased storage modulus (G') of soy protein isolate gels by treatment with MTGase has been reported [31].

3.3 Effect of MTGase incorporation on sensory attributes of sub-zero temperature stored Paneer

Various researchers have found that the sensory score [Colour and appearance (CA), flavour] of Paneer remains unaffected by the sub-zero temperature storage up to 120 days, however the body and texture (BT) attribute adversely affected [3, 9, 12]. It was found that mozzarella cheese when stored at -10 °C and -15 °C became crumbly and fluffy [32]. Minimal change in sensory perception score associated with CA and flavour was found for both control and MTGase samples. The sensory score data is given in Table 4. The BT score and overall acceptability (OA) score reduced over the period of frozen storage for both samples however the MTGase incorporated Paneer scored significantly higher than control samples. This can be correlated well with instrumental textural analysis values.

3.4 Porosity of Paneer

Porosity is an important parameter which affects the textural and rheological attributes of Paneer [7]. The porosity of control samples at day zero was found significantly higher than MTGase samples. Increased moisture holding by MTGase Paneer samples may have resulted in reduced empty space in the matrix thus lesser water uptake during porosity test. Over the period of frozen storage, progressive loss of moisture from both of the samples resulted in increased porosity % throughout the 60-day storage. However due to higher initial moisture content in MTGase samples the % porosity remained significantly less than control samples which indicates more compact Paneer matrix would have formed by enzymatic cross linking. Reduction in porosity in protein network and increased homogenous distribution of protein in product treated with MTGase was also reported [18].

3.5 Microstructure of Paneer with MTGase

Scanning electron micrographs were obtained at the end of 60 days storage at -15 °C. The micrographs are given in Fig. 1A & 1B, Fig. 2A & 2B and Fig. 3A & 3B. At all the three magnifications (100X, 500X and 750X), the control samples shown more roughness with cracks like and open type structure. MTGase incorporated samples were more compact and shown relatively smoother surface morphology. This suggests that increased cross-linking due to MTGase treatment caused more regular and compact network formation in Paneer. Similar findings were reported for use of MTGase in yoghurt [18, 27]. The increased viscosity and elasticity of MTGase treated samples may have possessed some protective effect against damage due to large ice-crystal formation as concluded in a study [24].

Table 1: Instrument settings used for texture profile analysis, stress relaxation test and creep test.

Test Mode	Texture profile analysis	Stress relaxation test	Creep retardation test
Pre-test speed	1 mm/sec	1 mm/sec	1 mm/sec
Test Speed	5 mm/sec	5 mm/sec	2 mm/sec
Post-test speed	5 mm/sec	10 mm/sec	10 mm/sec
Target Mode	Distance	Distance	Force
Distance	10mm	5mm	
Force	-	-	1000 g
Hold time	-	180 sec	180 sec
Trigger type	Auto (Force)	Auto (Force)	Auto (Force)
Trigger force	2g	0.5 g	5 g
Advance options	Off	Off	Off

Table 2: Texture profile analysis of Paneer samples stored at -15 °C for 60 days. Mean ± SD from three determinations.

Days	Hardness (N)		Cohesiveness		Springiness		Chewiness (N)	
	Control	TG	Control	TG	Control	TG	Control	TG
0	19.83 ± 0.379^{aE}	22.30 ± 0.457^{bCD}	0.59 ± 0.006^{aD}	0.60 ± 0.017^{aB}	0.85 ± 0.006^{aD}	0.86 ± 0.012^{aC}	9.46 ± 0.228^{aD}	11.21 ± 0.145^{bB}
10	21.83 ± 0.351^{aF}	23.43 ± 0.651^{bB}	0.55 ± 0.015^{aD}	0.60 ± 0.030^{aAB}	0.83 ± 0.010^{aCD}	0.84 ± 0.010^{aBC}	10.03 ± 0.526^{aD}	11.41 ± 0.389^{bAB}
20	22.87 ± 0.321^{aG}	23.60 ± 1.054^{aA}	0.53 ± 0.006^{aC}	0.58 ± 0.021^{bAB}	0.82 ± 0.013^{aC}	0.83 ± 0.006^{aB}	9.85 ± 0.231^{aD}	11.29 ± 0.966^{bA}
30	18.04 ± 0.252^{aD}	21.79 ± 1.431^{bC}	0.51 ± 0.006^{aBC}	0.56 ± 0.017^{aAB}	0.81 ± 0.010^{aBC}	0.81 ± 0.017^{aA}	7.50 ± 0.102^{aC}	9.95 ± 0.953^{bAB}
40	16.92 ± 0.208^{aC}	22.23 ± 0.569^{bD}	0.50 ± 0.010^{aAB}	0.55 ± 0.047^{bAB}	0.79 ± 0.012^{aAB}	0.81 ± 0.014^{aA}	6.71 ± 0.219^{aB}	9.96 ± 1.145^{bAB}
50	16.62 ± 0.153^{aB}	23.60 ± 0.265^{bE}	0.49 ± 0.015^{aA}	0.55 ± 0.036^{bAB}	0.79 ± 0.012^{aA}	0.81 ± 0.010^{bA}	6.46 ± 0.436^{aB}	10.33 ± 0.840^{bAB}
60	15.32 ± 0.200^{aA}	24.47 ± 0.351^{bE}	0.48 ± 0.006^{aA}	0.54 ± 0.015^{bA}	0.78 ± 0.015^{aA}	0.81 ± 0.006^{bA}	5.80 ± 0.243^{aA}	10.48 ± 0.375^{bAB}

Superscript in small letters: Different superscripts in the same row indicate significantly different means at ($P < 0.05$). Superscript in capital letters: Different superscripts in the same column indicate significantly different means at ($P < 0.05$)

Table 3: Viscoelastic property of Paneer stored at -15 °C for 60 days. Mean ± SD from three determinations.

Days	Modulus of Elasticity (MPa)		Coefficient of Viscosity (MPa.s)		Creep Compliance ($M.Pa^{-1}$)	
	Control	TG	Control	TG	Control	TG
0	0.0356 ± 0.0025^{aB}	0.0419 ± 0.0017^{bAB}	0.653 ± 0.0261^{aA}	0.739 ± 0.0064^{bA}	0.0870 ± 0.0016^{bC}	0.0786 ± 0.0015^{aC}
10	0.0426 ± 0.0037^{aC}	0.0480 ± 0.0012^{bC}	0.710 ± 0.0106^{aB}	0.830 ± 0.010^{bB}	0.0734 ± 0.0021^{bAB}	0.0715 ± 0.0042^{aB}
20	0.0439 ± 0.0021^{aC}	0.0460 ± 0.0018^{bBC}	0.903 ± 0.0705^{aC}	0.868 ± 0.055^{aB}	0.0716 ± 0.0034^{bA}	0.0666 ± 0.0023^{aA}
30	0.0401 ± 0.0017^{aC}	0.0447 ± 0.0024^{bBC}	1.051 ± 0.0172^{aD}	1.159 ± 0.0360^{bC}	0.0773 ± 0.0012^{bB}	0.0686 ± 0.0021^{aA}
40	0.0335 ± 0.0023^{aB}	0.0387 ± 0.0012^{bA}	1.105 ± 0.0245^{aE}	1.212 ± 0.0074^{bD}	0.0892 ± 0.0047^{bC}	0.0760 ± 0.0018^{aBC}
50	0.0289 ± 0.0058^{aAB}	0.0408 ± 0.0034^{bAB}	1.149 ± 0.0648^{aE}	1.274 ± 0.0125^{bE}	0.0958 ± 0.0006^{bD}	0.0739 ± 0.0034^{aBC}
60	0.0275 ± 0.0013^{aA}	0.0419 ± 0.0028^{bAB}	1.201 ± 0.0086^{aF}	1.305 ± 0.0155^{bF}	0.1056 ± 0.0063^{bE}	0.0723 ± 0.0015^{bB}

Superscript in small letters: Different superscripts in the same row indicate significantly different means at ($P < 0.05$). Superscript in capital letters: Different superscripts in the same column indicate significantly different means at ($P < 0.05$)

Table 4: Sensory score of Paneer samples stored at -15 °C for 60 days. Mean ± SD from three determinations.

Days	Colour and appearance		Body and Texture		Flavour		Overall appearance	
	Control	TG	Control	TG	Control	TG	Control	TG
0	8.23 ± 0.06^{aB}	8.53 ± 0.06^{bB}	8.30 ± 0.10^{aF}	8.43 ± 0.06^{bD}	8.25 ± 0.06^{aB}	8.23 ± 0.06^{aA}	8.33 ± 0.06^{aC}	8.43 ± 0.06^{aC}
10	8.23 ± 0.06^{aB}	8.50 ± 0.10^{bB}	8.20 ± 0.10^{aF}	8.40 ± 0.10^{bD}	8.27 ± 0.12^{aAB}	8.17 ± 0.06^{aA}	8.43 ± 0.12^{aC}	8.37 ± 0.12^{aC}
20	8.23 ± 0.06^{aB}	8.47 ± 0.06^{bAB}	7.87 ± 0.12^{aE}	8.27 ± 0.12^{bCD}	8.23 ± 0.06^{aA}	8.27 ± 0.15^{aA}	8.27 ± 0.06^{aC}	8.37 ± 0.06^{aC}
30	8.13 ± 0.06^{aAB}	8.47 ± 0.06^{bAB}	7.50 ± 0.10^{aD}	8.23 ± 0.06^{bBC}	8.27 ± 0.06^{aAB}	8.27 ± 0.12^{aA}	7.40 ± 0.17^{aB}	8.33 ± 0.06^{bC}
40	8.07 ± 0.06^{aA}	8.43 ± 0.12^{bAB}	7.17 ± 0.06^{aC}	8.10 ± 0.10^{bBC}	8.23 ± 0.15^{aA}	8.23 ± 0.06^{aA}	7.27 ± 0.31^{aB}	8.07 ± 0.12^{bB}
50	8.03 ± 0.06^{aA}	8.43 ± 0.06^{bAB}	6.90 ± 0.10^{aB}	8.03 ± 0.21^{bB}	8.17 ± 0.12^{aA}	8.27 ± 0.06^{aA}	6.73 ± 0.40^{aB}	7.93 ± 0.12^{bAB}
60	8.03 ± 0.06^{aA}	8.33 ± 0.12^{bA}	6.50 ± 0.10^{aA}	7.83 ± 0.06^{bA}	8.22 ± 0.08^{aA}	8.20 ± 0.17^{aA}	6.47 ± 0.06^{aA}	7.87 ± 0.12^{cA}

Superscript in small letters: Different superscripts in the same row indicate significantly different means at ($P < 0.05$). Superscript in capital letters: Different superscripts in the same column indicate significantly different means at ($P < 0.05$)

Table 5: Variation in % porosity and moisture content of Paneer samples stored at-15 °C for 60 days. Mean ± SD from three determinations.

Days	% Porosity in Paneer		% Moisture in Paneer	
	Control	TG	Control	TG
0	13.17 ± 0.57^{bA}	8.36 ± 0.96^{aA}	51.97 ± 0.35^{aC}	58.53 ± 0.16^{bD}
20	15.35 ± 0.06^{bB}	10.89 ± 0.64^{aB}	50.67 ± 0.47^{aB}	55.17 ± 0.21^{bC}
40	16.25 ± 0.90^{bC}	12.40 ± 0.26^{aC}	49.22 ± 0.58^{aB}	53.57 ± 0.32^{bB}
60	17.24 ± 0.41^{bC}	13.30 ± 0.15^{aD}	47.35 ± 0.53^{aA}	52.60 ± 0.60^{bA}

Superscript in small letters: Different superscripts in the same row indicate significantly different means at ($P < 0.05$). Superscript in capital letters: Different superscripts in the same column indicate significantly different means at ($P < 0.05$)

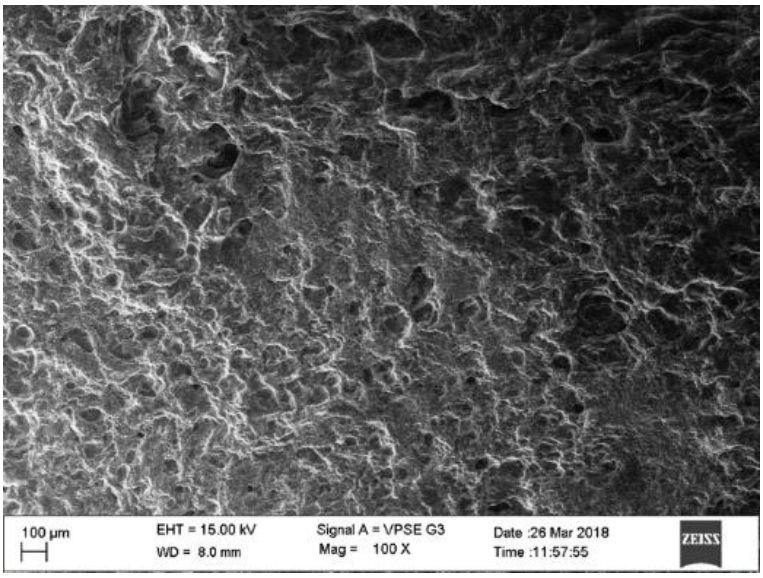

Fig 1 A: Scanning electron micrographs of Paneer samples stored at -15 °C for 60 days (Control sample at 100X magnification.)

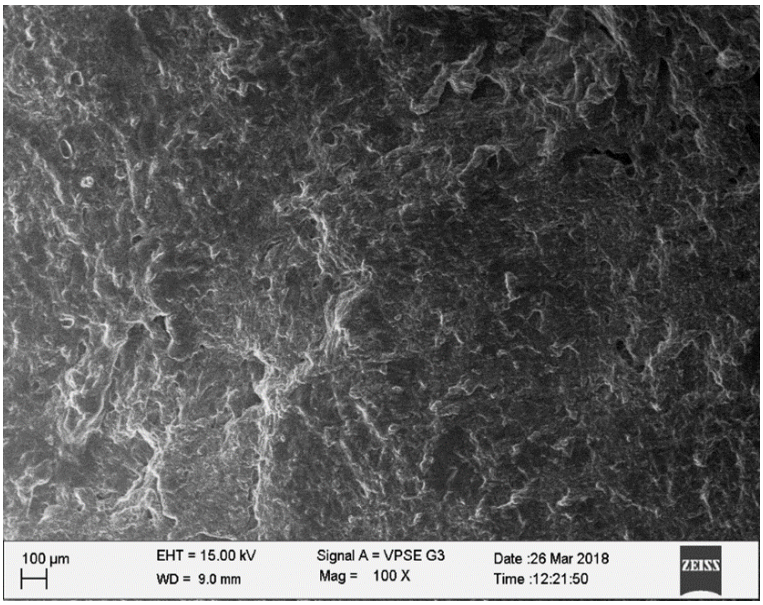

Fig 1 B: Scanning electron micrographs of Paneer samples stored at -15 °C for 60 days (MTGase incorporated sample at 100X magnification.)

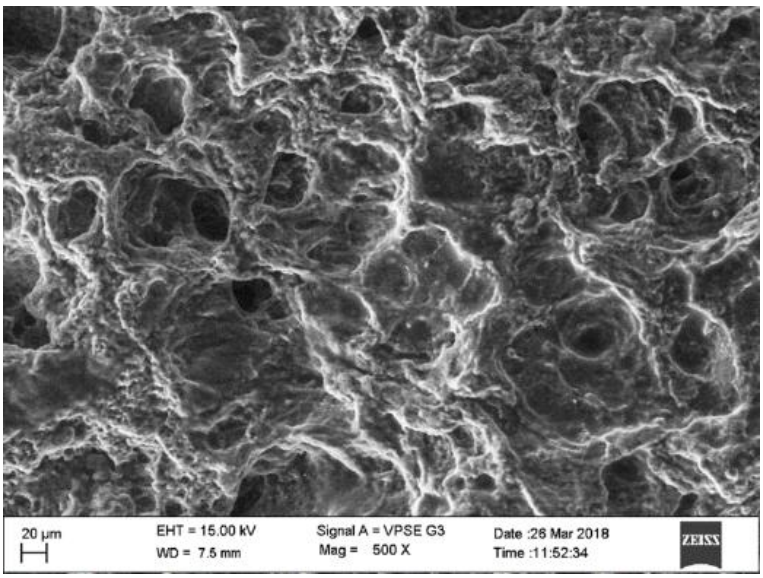

Fig 2 A: Scanning electron micrographs of Paneer samples stored at -15 °C for 60 days. (Control sample at 500X magnification.)

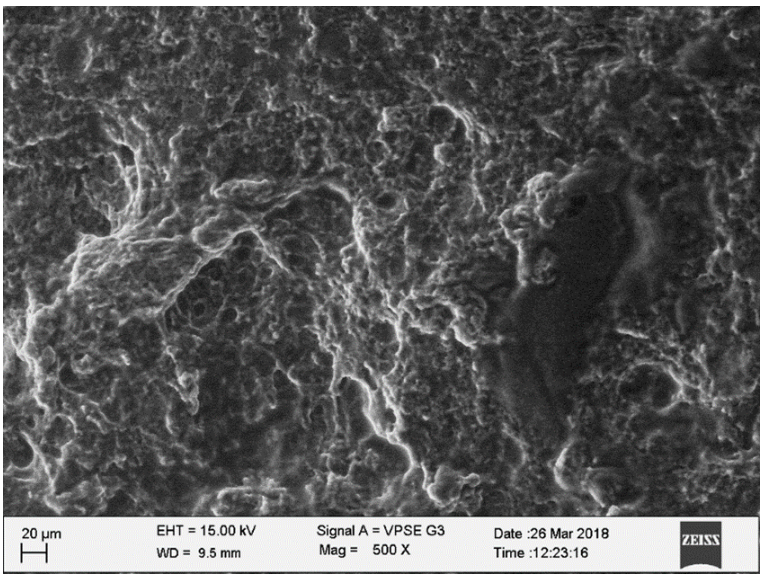

Fig 2 B: Scanning electron micrographs of Paneer samples stored at -15 °C for 60 days. (MTGase incorporated sample at 500X magnification.)

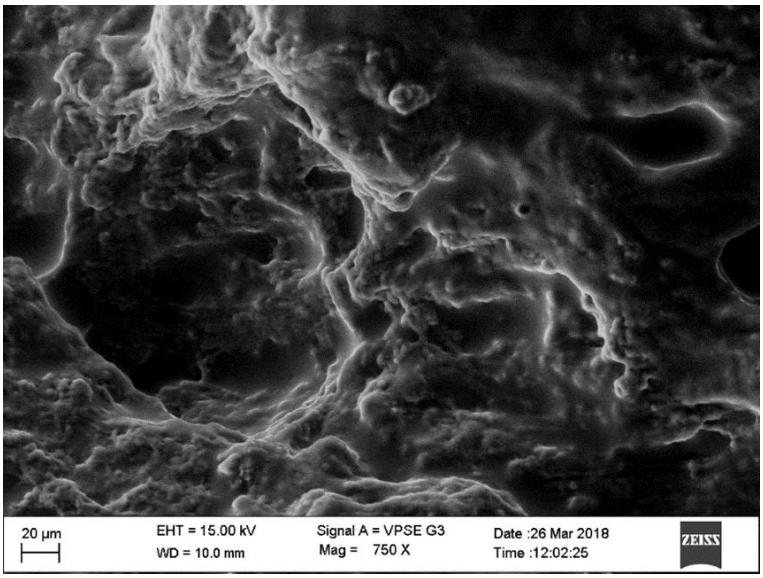

Fig 3 A: Scanning electron micrographs of Paneer samples stored at -15 °C for 60 days (Control sample at 750X magnification)

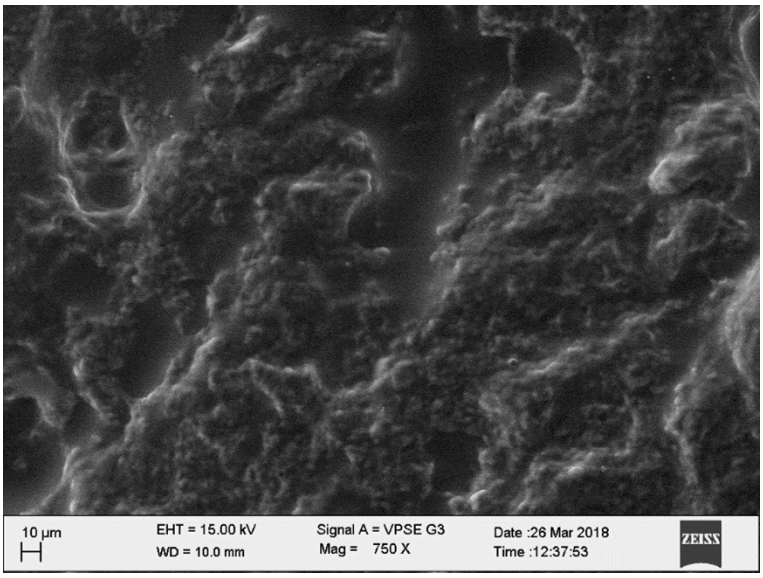

Fig 3 B: Scanning electron micrographs of Paneer samples stored at -15 °C for 60 days (MTGase incorporated sample at 750X magnification.)

4. Conclusion

During sub-zero temperature storage, addition of MTGase had positive impact on its textural and viscoelastic properties. Although, both control and MTGase samples suffered loss of textural, sensorial and associated viscoelastic attributes, the loss in MTGase treated samples were significantly less than control samples. As samples without MTGase became softer with reduced textural integrity during progressive duration of frozen storage, the MTGase incorporated samples were rated higher by sensory panel. The instrumental textural and imaging analysis supports that MTGase has definite favourable impact on texture of Paneer during sub-zero temperature storage. Hence, its use can be recommended as potential intervention in proteinaceous foods such as Paneer for texture protection during frozen storage.

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