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Research Paper / Araştırma Makalesi

Effect of Transglutaminase on Textural and Microstructural Properties of Probiotic Yoghurt Produced With Mixture of Cows' Milk and Soy Drink

Hasan Temiz, Elif Büşra Ersöz

Department of Food Engineering, Engineering Faculty, Ondokuz Mayis University, 55139, Samsun, Turkey

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Corresponding author (Yazışmalardan Sorumlu Yazar): hasant@omu.edu.tr (H. Temiz)
+90 362 312 19 19 +90 362 457 60 35

ABSTRACT

This study surveyed the effect of the addition of microbial transglutaminase (m-TGs) on textural, microstructural, FTIR spectra and SDS-PAGE electrophotogram parameters of probiotic yoghurts made with mixture of cows' milk and soy drink during refrigerated storage for 28 days. Mixture was treated with different rates of m-TGs (0, 0.5, 1, 1.5 U/g protein) and incubated with *Lactobacillus acidophilus* La-5, *Bifidobacterium lactis* Bb-12, *Streptococcus thermophilus* probiotic starter cultures. Yoghurts prepared with m-TGs had higher textural parameters than samples without m-TGs. SDS-PAGE patterns, SEM imagines and FTIR spectra demonstrated that milk caseins were well cross-linked by transglutaminase. Scanning electron microscopic studies showed that the microstructure of m-TGs added probiotic yoghurt samples appeared denser than that of control. Results of this study indicated that the textural and microstructural properties of probiotic yoghurt prepared with soy drink could be improved by incorporating m-TGs up to a level of 1.5 U/g protein.

Keywords: Transglutaminase enzyme (m-TGs), Probiotic yoghurt, Reology, Microstructure, Soy drink

İnek Sütü ve Soya İçeceği Karışımından Üretilen Probiyotik Yoğurtların Tekstürel ve Mikroyapısal Özellikleri Üzerine Transglutaminaz İlavesinin Etkisi

ÖZ

Bu çalışmada, inek sütü ve soya içeceği karışımı ile yapılan probiyotik yoğurtların 28 günlük depolama süresince tekstürel, mikroyapısal, FTIR spektrumları ve SDS-PAGE elektrofotogram parametreleri üzerine, mikrobiyal transglutaminazın (m-TG) etkisi araştırılmıştır. Karışıma, farklı m-TG oranları (0, 0.5, 1, 1.5 U/g protein) ilave edilmiş ve *Lactobacillus acidophilus* La-5, *Bifidobacterium lactis* Bb-12, *Streptococcus thermophilus* probiotic starter kültürleri ile inkübe edilmiştir. m-TG ile hazırlanan yoğurtların, m-TG ilave edilmemiş olanlarla kıyaslandığında daha yüksek tekstürel özelliklere sahip olduğu görülmüştür. SDS-PAGE desenleri, SEM görüntüleri ve FTIR spektrumları, süt kazeinlerinin transglutaminaz ile iyi bir şekilde birbirine bağlandığını göstermiştir. Taramalı elektron mikroskobik çalışmalar, m-TG ilave edilmiş probiyotik yoğurt örneklerinin mikro yapılarının kontrolden daha yoğun göründüğünü göstermiştir. Bu çalışmanın sonuçları soya içeceği ile hazırlanan probiyotik yoğurtların dokusal ve mikroyapısal özelliklerinin 1.5 U/g protein seviyesine kadar m-TG ilave edilerek geliştirilebileceğini göstermektedir.

Anahtar Kelimeler: Transglutaminaz enzimi (m-TGs), Probiotik yoğurt, Reoloji, Mikroyapı, Soya içeceği

INTRODUCTION

Several fermented products are produced with the use of probiotic bacteria, such as *Streptococcus* spp., *Lactobacillus* spp., and *Bifidobacterium* spp. Probiotic bacteria are the natural inhabitant of the gut of warm-blood animals. Those organisms have an important role in human health with regular consumption because of reduction of serum cholesterol, inhibition of the growth of potential pathogens and improvement of intestinal bacterial composition [1].

Soy drink is produced by extracting soy with water and is a cheap source of protein and calories in the human diet. Similarly, it doesn't contain cholesterol or lactose. It has important nutritional constituents such as calcium, high quality protein, polyunsaturated fatty acids, isoflavones, phytosterols, lignans, saponins coumestans and phytates [2, 3]. Soybean can help to decrease the harmful low-density lipoprotein cholesterol level, prevent heart diseases and the colon, prostate and breast cancers and also osteoporosis. It may help to decrease women's menopausal symptoms by reduction of estrogen production by the ovaries [4]. The direct consumption of soybeans is limited by the raw bean flavor it possesses and the presence of α-D-galactosyl oligosaccharides such as stachyose and raffinose. Due to the unpleasant beany flavour, insufficient acidity, rigid and brittle gel structure soy yoghurt has not been generally accepted among consumer. To overcome these limitations, fermentation procedures with various microorganisms have been experienced. For example, *Bifidobacterium* strains have been used for soymilk [5, 6, 7]. Soybeans are a great raw material for the development of probiotics and can promote the growth of many probiotic strains such as *Lactobacillus acidophilus*, *Lactobacillus casei* and *Streptococcus thermophilus* [8].

The most significant physical feature of yoghurt is its rheological quality which is affected by milk composition, processing, heating, bacterial culture selection, incubation temperature, packaging and storage processes [9]. The most typical defects of yoghurt are low viscosity and reduced firmness. Because, the protein gels is stabilized by weak non-covalent interactions in yoghurt. Different materials have been used in the production of yoghurt such as fibers [10, 11], starches [12] and transglutaminase [13, 14] to increase water holding capacity or to improve the emulsifying behavior.

m-TGs (EC 2.3.2.13) has been used to improve stability and textural properties in dairy and many other food products. [15-17]. m-TGs catalyzes the covalent cross-linking of proteins by γ-carboxyamide groups of peptides bound glutamine residues and the ε-amino groups of lysine residues and cross-linked protein polymers are formed [18, 19].These polymers change functional, rheological, immunoreactivity of the casein fraction and sensorial properties of the products [20, 21]. m-TGs cross-linking may be used to improve the mechanical properties of protein films and the structure of stirred yoghurt with reduced or no addition of protein ingredients such as whey protein concentrate, sodium caseinate and skim milk powder.

The objective of this research was to determine the effects of the cross-linking reaction of transglutaminase (m-TGs) on the textural and microstructural properties of probiotic yoghurt produced with mixture of cows' milk and soy drink with m-TGs addition of different rates.

MATERIALS AND METHODS

Preparation of Soy Drink

Soybeans, Asgrow 3935 were obtained from Black Sea Agricultural Research Institute, Samsun, Turkey. Soy drink production was carried out as described in our previous article Temiz and Çakmak [22]. Soybeans were kept in water for 18 hours at 4°C. After crust separation, soybeans were ground at high speed for 2 minutes in a Waring blender (Waring Commercial Blender, Waring, Torrington, CT, USA) with water at 95°C. Then, dilution, boiling and filtering processes were done respectively. The nutrient concentrations of the soy drink produced; 7.0% total solid, 3.26% protein, 2% fat, pH 6.7 and 0.09% titratable acidity.

Preparation of Yoghurts

Yoghurt production was carried out as described in our previous article using freeze-dried probiotic yoghurt starter culture (10^8 cfu/g), type ABT-2 1000 I (*Lactobacillus acidophilus* La-5, *Bifidobacterium lactis* Bb-12, *Streptococcus thermophylus*, Danisco Biolacta sp.z o.o. ul. Tuwima I A: 10-747 Olsztyn-Poland) and m-TGs (ACTIVA WM, Ajinomoto Foods Europe S.A.S, France) [22]. Bovine milk and soy drink were mixed as 75% bovine milk/25% soy drink. Yoghurt samples were coded according to the ratio of used: KY (0 U m-TGs /g protein), Y1 (0.5 U m-TGs /g protein), Y2 (1 U m-TGs /g protein) and Y3 (1.5 U m-TGs /g protein). Yoghurt samples were stored at 4°C until analysis. Instrumental texture analysis was carried out at 1st, 7th, 14th, 21st and 28th days of storage while SEM images, FTIR spectra and SDS–PAGE were obtained only at the 7th day of storage.

Instrumental Texture Analysis

Yoghurt samples were stored in original containers at 4°C for 24 h for texture analysis. Texture analysis was performed using a Texture Analyser (TA-XT Plus, Stable Micro Systems Co, Ltd. Surrey, England). For each sample four measurements were carried out using a cylindrical probe (SMS P/50, 50 mm diameter) attached to 30 kg load cell. The speed of measurement was set at 1.0 mm/s. Force versus time plots were used for the calculations of texture profile analysis (TPA) values, such as hardness, cohesiveness, gumminess, adhesiveness and springiness. All experiments were repeated three times.

Scanning Electron Microscopy (SEM)

SEM images of samples were analyzed at 7th day of storage. Samples were freeze-dried in a lyophilizer after storage at 4°C. After then, the samples were coated with a 15 nm gold-palladium (Model SC7620; Quorum Technologies, Laughton, UK) layer performed by a cathodic coater as described in by Espirito-Santo et al. [9] with some modifications. Eight areas of the samples were imaged on a scanning electron microscope (SEM; Model JSM-7001F; JEOL, Tokyo, Japan) operating at a voltage of 10 kV. Photomicrographs were recorded under 100 to 5,000 x magnified images and structural differences were analyzed in 5,000 x times magnified images [23].

Sodium Dodecylsulphate Polyacrylamide Gel Electrophoresis

In electrophoresis studies, SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) technique was applied according to the method described in detail by Laemmli [24] with BIO-RAD Protean II xi Cell gel electrophoresis device powered by PowerPac Universal. The sample loading volume, concentrations of separating and stacking gels were 25 µL, 18.5% and 6.0%, respectively

Fourier-Transform Infrared Spectroscopy (FTIR)

The Fourier-transform infrared (FTIR, spectrometer (Model Spectrum Two; Perkin Elmer, Akron, OH, USA) spectras of the samples were measured using reported method by Tural and Turhan [25].

Statistical Analysis

Statistical analysis was conducted using SPSS Statistical Software (2000) (SPSS Inc., Chicago, IL) and results were given as mean ± standard deviation significance differences ($P<0.05$) between the samples and the effect of storage time were analyzed by ANOVA, followed by Duncan multiple range tests. All measurements were repeated twice.

RESULTS AND DISCUSSION

Rheological Properties

The textural parameters of probiotic yoghurts were tested during storage at 4°C and are shown Table 1. Various textural parameters like adhesiveness, cohesiveness, gumminess and hardness showed significant change with addition of m-TGs in probiotic yoghurts.

Hardness values varied between 528.01-737.50 g and showed significant differences ($P<0.05$) during storage. The effect of m-TGs on the hardness values was only found to be significant at 21st days of storage time. Samples made from 1.5 U m-TGs/per g protein exhibited an increase in hardness compared to samples made from the other treatments and the higher value was determined at Y3 sample at 21st days of storage. The hardness values increased during storage time, however after 7th days of storage there were no differences in the KY sample with any m-TGs. Samples made with m-TGs showed the highest increase in hardness throughout the storage time.

Table 1. Rheological properties of probiotic yoghurt samples during storage time

		Storage time (days)				
Attributes	Treatments	1	7	14	21	28
Hardness	KY	561.56±4.62^{B}	622.29±17.67^{A}	630.00±28.28^{A}	632.60±20.71^{bA}	615.40±23.20^{A}
	Y1	528.01±8.18^{B}	622.71±31.67^{A}	596.42±41.23^{AB}	676.51±4.45^{bA}	620.36±9.46^{A}
	Y2	534.59±6.36^{C}	627.69±25.51^{B}	660.48±7.53^{AB}	649.67±27.81^{bAB}	680.66±13.37^{A}
	Y3	573.78±5.38^{C}	601.72±27.66^{C}	650.56±7.07^{B}	737.50±17.67^{aA}	654.18±21.35^{B}
Adhesiveness	KY	-462.29±40.75^{bcAB}	-385.10±0.40^{cBC}	-357.90±17.92^{cC}	-516.62±8,25^{bA}	-468.41±71.00^{bAB}
	Y1	-431.70±7.36^{cAB}	--370.68±17.78^{cB}	-212.22±58.17^{cC}	-536.01±87.79^{bA}	-483.62±0.00^{bAB}
	Y2	-515.87±30.21^{bB}	-693.20±11.27^{bA}	-574.55±2.415^{bB}	-446.34±28.05^{bC}	-436.62±29.73^{bC}
	Y3	-705.52±18.64^{aBC}	-938.45±21.21^{aA}	-791.74±58.40^{aB}	-671.38±6.747^{aBC}	-621.95±4.53^{aC}
Cohesiveness	KY	0.31±0.02^{b}	0.32±0.00^{b}	0.32±0.02	0.29±0.01	0.30±0.07
	Y1	0.28±0.00^{b}	0.27±0.01^{c}	0.31±0.01	0.29±0.04	0.25±0.04
	Y2	0.32±0.02^{ab}	0.27±0.00^{c}	0.31±0.03	0.33±0.00	0.29±0.00
	Y3	0.36±0.00^{a}	0.35±0.01^{a}	0.31±0.04	0.38±0.02	0.33±0.02
Gumminess	KY	160.21±2.92^{bB}	170.19±0.04^{cB}	184.03±2.00^{C}	201.77±8.03^{bA}	191.86±9.54^{bA}
	Y1	172.37±2.87^{b}	199.32±16.60^{ab}	177.23±23.06	196.71±7.13^{b}	187.11±4.94^{b}
	Y2	165.69±13.13^{b}	178.52±9.33^{ab}	199.29±17.30	193.00±22.19^{b}	188.41±18.66^{b}
	Y3	210.26±0.36^{aBC}	212.13±15.88^{aBC}	206.65±12.36^{A}	243.52±6.25^{aAB}	259.11±4.95^{aA}

Values are means ± standard deviation of three replicates. KY; 75% cow milk + 25% soy drink without m-TGs. Y1;75% cow milk + 25% soy drink + 0.5 U m-TGs/per g protein. Y2; 75% cow milk + 25% soy drink + 1 U m-TGs/per g protein and Y3; 75% cow milk + 25% soy drink 1.5 U m-TGs/per g protein. Small letters (a, b, c) indicate significant differences between means within a column. Capital letters (A, B, C) indicate significant differences ($P<0.05$) between means within a row. There is no statistically difference between non-letter columns

Our results indicated that m-TGs can help to provide the desired hardness in dairy products. Similar results were reported by Yüksel and Erdem [26], Tsevdou et al. [14], and Garcia-Comez et al. [27]. The higher numbers of glutamyl residues in cows' and soy drink, which acts as acyl donor, and the high numbers of ε-amine groups in lysine residue, which works as acyl acceptor, help to increase the cross-linking induced by transglutaminase [16].

Adhesiveness values, varied between -212.22, -938.45, were significantly affected by enzyme application and storage time. m-TGs added samples had higher adhesiveness values compared to those made with no m-TGs (Table 1). These findings could be seen as the

result of the different effects of m-TGs on the gel properties of the final product. The highest value was determined in Y3 sample at 7th day of storage while the lower values determined in KY and Y1 samples at 14th day of storage. Similar results have been reported by Domagala et al. [13], Tsevdou et al. [14].

The cohesiveness values measured during storage are shown in Table 1. In terms of cohesiveness, no significant differences (P<0.05) were observed during the storage time but cohesiveness values significantly increased with increase of m-TGs at 1st and 7th days of storage, and the higher values were observed in Y3 samples. Similar behavior was observed by Tsevdou et al. [14].

The changes of gumminess values in yoghurt samples are shown in Table 1. KY and Y3 samples increased during storage statistically and highest values were observed at 28th day of storage. In terms of m-TGs treatments, gumminess values were determined to be significant except 14th day of storage (P<0.05). Gumminess values of yoghurt samples increased with increasing m-TGs concentration. Similar results were reported by Yüksel and Erdem [26].

Microstructure of Yoghurt

SEM micrographs (x5000) of yoghurt gels prepared with and without m-TGs are illustrated in Figure 1. SEM revealed that the protein matrices of the m-TGs added samples appeared to be relatively more compact than the control (Figure 1). The differences were mainly associated with the compactness of the protein matrix. The microstructures of the yoghurts made with m-TGs treated milk changed with increasing amounts of the enzyme added to the milk. The samples made with the m-TGs showed lower pore sizes with a well-defined porous web like structure. Similar results were reported by Şanlı et al. [28].

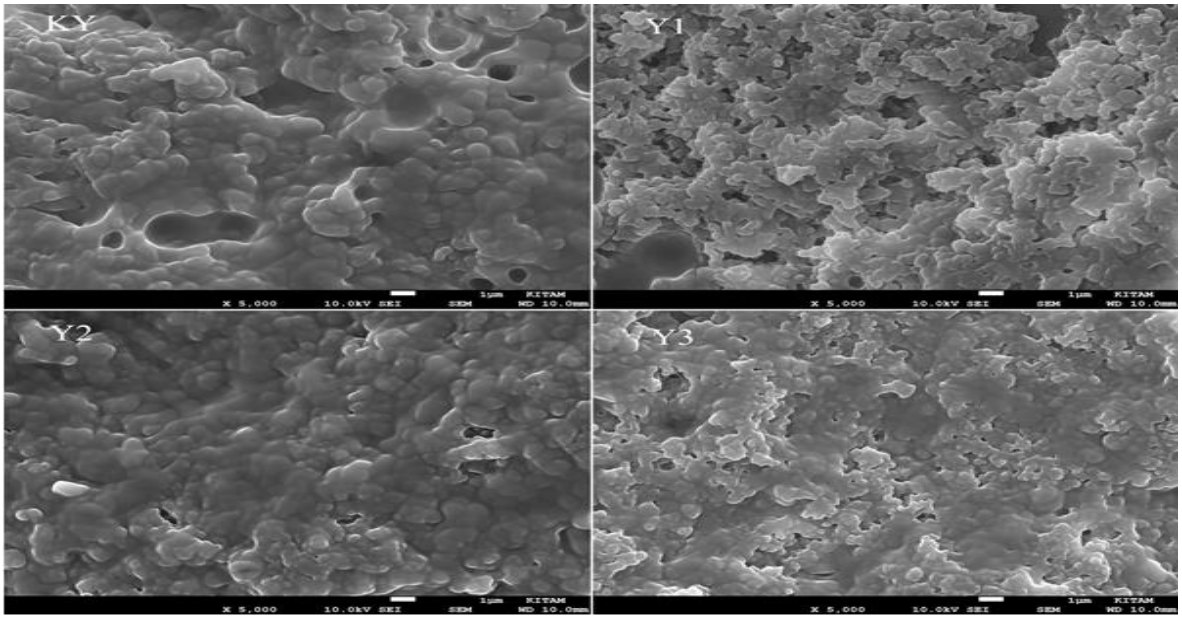

Figure 1. Scanning electron microscopy (SEM) of probiotic yoghurts. note: KY: (control) 0 U m-TGs/g protein Y1: 0.5 U m-TGs/g protein, Y2: 1 U m-TGs /g protein Y3: 1.5 U m-TGs/g protein.

These results suggested that m-TGs had some interaction with the milk proteins. These indications can be explained with the existence of m-TGs, which may have interacted with proteins to form a more compact structure with ability to catch the whey phase. Similar observations were reported by Domagala et al. [13] that the use of m-TGs improved the gel strength of the product.

Electrophoretic Studies

In this study, SDS-PAGE was used to elucidate the cross-linking of protein in m-TG treated samples. The SDS-PAGE electrophotograms of samples are shown in Figure 2. As it can be seen in fist line 9 whey proteins as well as caseins are presented (bands of 25-30 kDa and 14-19 kDa), respectively. In samples line, whey proteins have been denaturalized due to thermal treatment and thus, their bands are not present. Increase of enzyme concentration leads to the formation of new protein bands. These new bands were higher-molecular-weight bands, resulting from cross-linked proteins and a concurrent increase in polymers, which did not enter the stacking gel. The m-TG has a molecular weight of 37 kDa and thus, it is not visible in the m-TG-treated samples. Wroblewska et al. [15] reported that the addition of m-TG caused partial transformation of proteins into high molecular polymers. Similar results were reported by Tsevdou et al. [14], Al- Saadi et al. [16], Chen et al. [17].

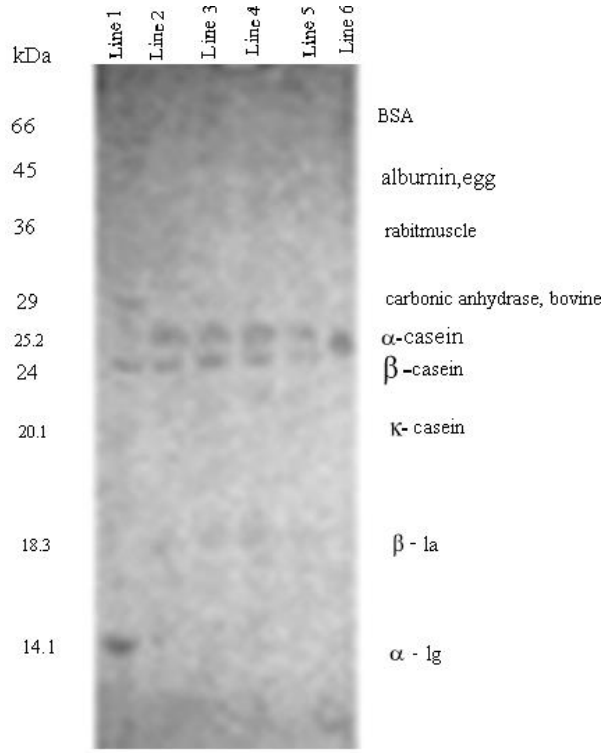

Figure 2. SDS-PAGE electrophotogram of yoghurt. line 1; molecular weight markers. line 2; KY (control) 0 U m-TGs/g protein, line 3; Y1 0.5 U m-TGs/g protein, line 4; Y2 1 U m-TGs /g protein, line 5; Y3 1.5 U m-TGs/g protein, line 6; α-casein.

Fourier-Transform Infrared Spectroscopy

The effect of m-TG was investigated with FTIR spectroscopy in yoghurt samples. The FTIR spectra of control yoghurt and yoghurt containing 0.5, 1.0 and 1.5 U m-TG/g protein are shown in Figure 3. The spectra were recorded within the range of 650-4000 cm^{-1}. The shifts of particular bands, as well as the differential spectra, clearly indicated that the processes led to the complex formation. For all yoghurt samples, an amide A peak (N-H stretch, coupled with hydrogen bonding) was found at v=3281-3275 cm^{-1} and an amide B peak (representing CH stretching and NH_3^+) was found at v=2921-2922 cm^{-1}. All samples had major peaks at v=1633-1645 cm^{-1} (amide I, representing C=O stretching/hydrogen bonding coupled with COO), 1538-1543 cm^{-1} (amide II, arising from bending vibration of NH groups and stretching vibrations of CN groups) and 1238 cm^{-1} (amide III, illustrating the in-plane bending vibrations of CN and NH groups of bound amide or vibrations of CH_2 groups). The peak situated around 1034-1037 cm^{-1} was observed in all samples, corresponding to the OH group. The main bands of samples were: 1100 and 1000 cm^{-1} which give information on sugar molecules (polysaccharide ring vibrations); 1633-1645 cm^{-1} (amide i) and 1538-1543 cm^{-1} (amide ii) are used to obtain information on protein structure and 2921-2922 cm^{-1} (amide b) was associated with the vibration from fatty acid [4].

As shown in Figure 3, both wavelengths of Amide A and amide B shifted to the higher wavenumber and the intensity of the two peaks also increased as the m-TG increased. In addition, both the intensity of amide I and amide II were slightly enhanced and shifted to the higher wavenumber with the addition of m-TG into the samples. The results showed that the cross-linking of m-TG were facilitated the increase of amplitudes in the amide I bands. The high number of bands is correlated with the complexity of the food composition which contains proteins, carbohydrates, lipids etc.

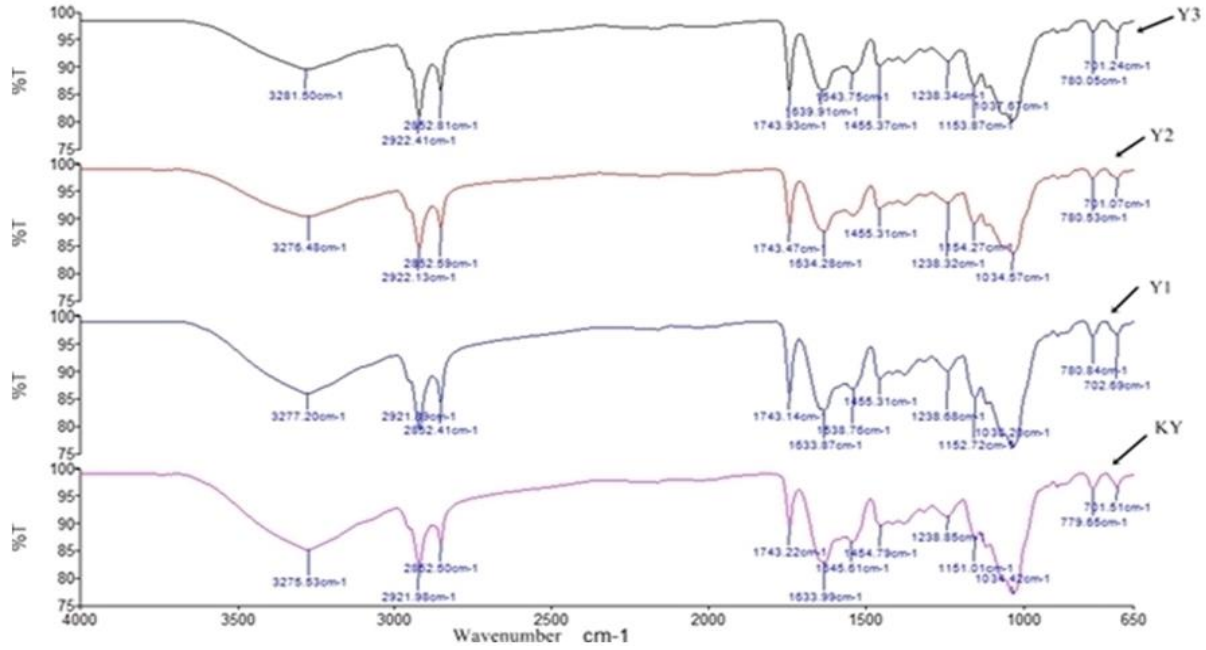

Figure 3. FTIR spectra of probiotic yoghurts, KY: (control) 0 U m-TGs/g protein Y1: 0.5 U m-TGs/g protein, Y2: 1 U m-TGs/g protein Y3: 1.5 U m-TGs/g protein.

Interestingly, the peak corresponding to Amide B (3281 cm^{-1}) was highly intense at 1.5 U m-TGs/g protein added samples, indicating that the functional groups from m-TG has cross-linked with some milk components and helped to development of gel matrix. Similar results were also found by Cheng et al. [29].

CONCLUSION

The textural properties of the probiotic yoghurts were significantly improved by addition of m-TGs and also storage period. SEM images demonstrated that addition of m-TGs could be used to improve the microstructure of the probiotic yoghurt gel. The results indicate that enzymatic cross-linking of milk proteins by m-TGs improved the properties of probiotic yoghurts. Crosslinking of milk proteins by means of m-TGs appears to be an acceptable alternative way to addition of extra stabilizers in probiotic yoghurt. According to data obtained in this study, m-TGs concentration was seen practical up to 1.5 U/g proteins when textural and microstructural properties of the probiotic yoghurts examined.

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