International Dairy Journal 99 (2019) 104544

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

# Production of skim milk powder by spray-drying from transglutaminase treated milk concentrates: Effects on physicochemical, powder flow, thermal and microstructural characteristics

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ARTICLE INFO

*Article history:*
Received 2 April 2019
Received in revised form
29 July 2019
Accepted 29 July 2019
Available online 9 August 2019

ABSTRACT

The effects of transglutaminase (TGase) treatment of skim milk concentrates on physicochemical, powder flow, thermal, microstructural characteristics and protein profiles of skim milk powders (SMP) were investigated. Spray-dried SMP samples produced from milk concentrates were TGase-treated at enzyme concentrations of 0 (control), 0.020% (TG-20), 0.030% (TG-30) and 0.035% (TG-35). Lower particle size values were observed in TGase-treated samples. TGase treatment slightly decreased zeta potential and increased the a* value (redness) of SMPs. Up to a concentration of 0.030%, TGase treatment decreased caking characteristics and increased glass transition temperatures of samples. The TGase treatment above 0.035% significantly decreased cohesion index and compaction coefficient at different speeds. TG-35 showed free flowing flow behaviour depending on the cohesion index, whereas control, TG-20, and TG-30 showed easy flowing behaviour. SDS-PAGE analysis revealed that high molecular mass bands indicated cross-linking in TGase-treated milk powders.



## 1. Introduction

Milk powder is usually manufactured by spray drying from evaporated milk to extend shelf life. Skim milk powder can be stored for about 18–24 months without considerable deterioration at ambient temperatures. In addition, milk powders are generally preferred due to convenience in shipping, handling, processing, and formulations of food products. Milk powders are used for reconstituted or recombined dairy products as well as bakery, confectionery and meat products. Therefore, improved milk powders with high functionality and quality are crucial in the dairy industry.

Transglutaminase (TGase) is an enzyme that induces intramolecular or intermolecular covalent cross-linking by catalysing reactions of acyl-transfer between residues of lysine (acyl acceptor) and glutamine (acyl donor) of proteins (Lauber, Henle, & Klostermeyer, 2000). Apart from the cross-linking reaction, deamidation and amine incorporation reactions are catalysed by TGase (Özrenk, 2006). Incorporating amines, intra- and intermolecular cross-links or deamidation by TGase causing profound changes in the molecular structure of proteins results in modifying proteins (Gaspar & de Góes-Favoni, 2015). When lysine residues, free lysine or primary amines are absent from the reaction system, water reacts as the acyl acceptor and hydrolytic deamidation of the glutamine residues occurs, transforming them into glutamic acid. This deamidation reaction changes protein charge and protein solubility (Sharma, Lorenzen, & Qvist, 2001; Yokoyama, Nio, & Kikuchi, 2004). TGase is safe for human consumption; it has GRAS (generally recognised as safe) statute.

As one of the milk proteins, casein is a suitable substrate for the TGase reaction, whereas TGase-induced cross-linking is not very useful for whey proteins (Guyot & Kulozik, 2011). Thus, treatment of TGase-induced cross-linking is mainly performed to dairy products that consist of mainly casein (Ikura, Yoshikawa, Sasaki, & Chiba, 1981). Milk proteins affect the structure of various dairy products. Therefore, TGase-induced cross-linking in caseins causes

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https://doi.org/10.1016/j.idairyj.2019.104544
0958-6946/

specific changes in many dairy products. Modification of proteins caused by TGase induced crosslinking varies depending on pH, temperature, concentration, substrate availability and specificity of the enzyme (Romeih & Walker, 2017).

The utilisation of TGase in the dairy industry has increased to improve competitive functional modifications in dairy products (Romeih & Walker, 2017). Previous studies have focused on the utilisation of TGase on milk proteins (Hinz, Huppertz, & Kelly, 2012), cheese types (Aaltonen, Huumonen, & Myllärinen, 2014; Özer, Hayaloglu, Yaman, Gürsoy, & Şener, 2013), fermented milks (Chen et al., 2018; Imm, Lian, & Lee, 2000; Jaros, Heidig, & Rohm, 2007; Schorsch, Carrie, & Norton, 2000), caseinate (Flanagan, Gunning, & FitzGerald, 2003) and other dairy products (Rossa, Burin, & Bordignon-Luiz, 2012). TGase treatment in dairy products has changed techno-functional characteristics of milk proteins such as emulsification, foam stability, gelation, heat stability, viscosity and water holding capacity (WHC).

To the best of our knowledge, only limited research has been reported on the TGase-treated milk powders. Imm et al. (2000) investigated the water binding and gelation characteristics of freeze-dried TGase-treated SMP, whereas Guyot and Kulozik (2011) produced yoghurt from TGase-treated SMP (spray dried) and reported improved viscosity, gel strength and WHC. However, there is no research reported on spray-dried skim milk powder produced from TGase-treated milk concentrate. Modification of dairy proteins by an enzymatic method is an alternative treatment for improved new functional properties. The aim of this study was to determine the effects of transglutaminase (TGase) treatment to skim milk concentrates on physicochemical, powder flow, thermal and microstructural characteristics of skim milk powders.

## 2. Material and methods

### 2.1. Materials

Production of skim milk concentrate (SMC), transglutaminase treatment of SMCs and spray drying of milk powders were carried out in Enka Dairy Company (Konya, Turkey). Total dry matter, protein and fat content of SMC were 45%, 16%, 0.2%, respectively.

Activa MP (Ajinomoto Co., Ltd., Japan) was used as a microbial TGase (E.C. 2.3.2.13) enzyme in this study. It was provided by Ajinomoto Foods Europe SAS (Hamburg, Germany) with a specific enzyme activity of 100 U $g^{-1}$ and consisted of 1% enzyme, lactose, and maltodextrin. TGase has optimum enzyme activity at 50 °C between pH 5 and 8 (Motoki & Seguro, 1998).

### 2.2. Skim milk powder production

SMP production was done as described in the flow chart in Fig. 1. Following the production of milk concentrate, TGase was included into skim milk concentrates at concentrations of 0% (control), 0.020% (TG-20; 12 U 100 $g^{-1}$ milk concentrate), 0.030% (TG-30; 18 U 100 $g^{-1}$ milk concentrate) and 0.035% (TG-35; 21 U 100 $g^{-1}$ milk concentrate). After enzyme addition, milk concentrates were incubated at 50 °C for 2 h (Ajinomoto, 2008). Then, concentrates were homogenised (20 MPa) and they were heated to 63 °C by a tubular heat exchanger. SMCs were held at 63 °C for 15 min in the holding tank to inactivate TGase. Then, SMC was spray-dried. Followed by spray-drying, SMP samples were packed. Rotary atomiser with a speed of 12, 000 rpm was used in spray-drying of samples (GEA Niro Atomizer, GEA Process Engineering A/S, Soeborg, Denmark). Spray-drying conditions were: feed temperature 63 °C, inlet temperature 180 °C, outlet temperature 70 °C, air velocity 30,000 kg $h^{-1}$, drying time of 40–50 s.

### 2.3. Determining texture, particle size, colour and pH parameters of TGase-treated milk concentrates

A texture analyser (TA.XTPlus, Stable Micro Systems, Godalming, Surrey, UK) equipped with back extrusion rig was used to determine textural properties (firmness, consistency, cohesiveness, and index of viscosity) of milk concentrates. Particle size measurements of milk concentrates were carried out according to Mercan, Sert, and Akın (2018b). For particle size measurements, a Malvern

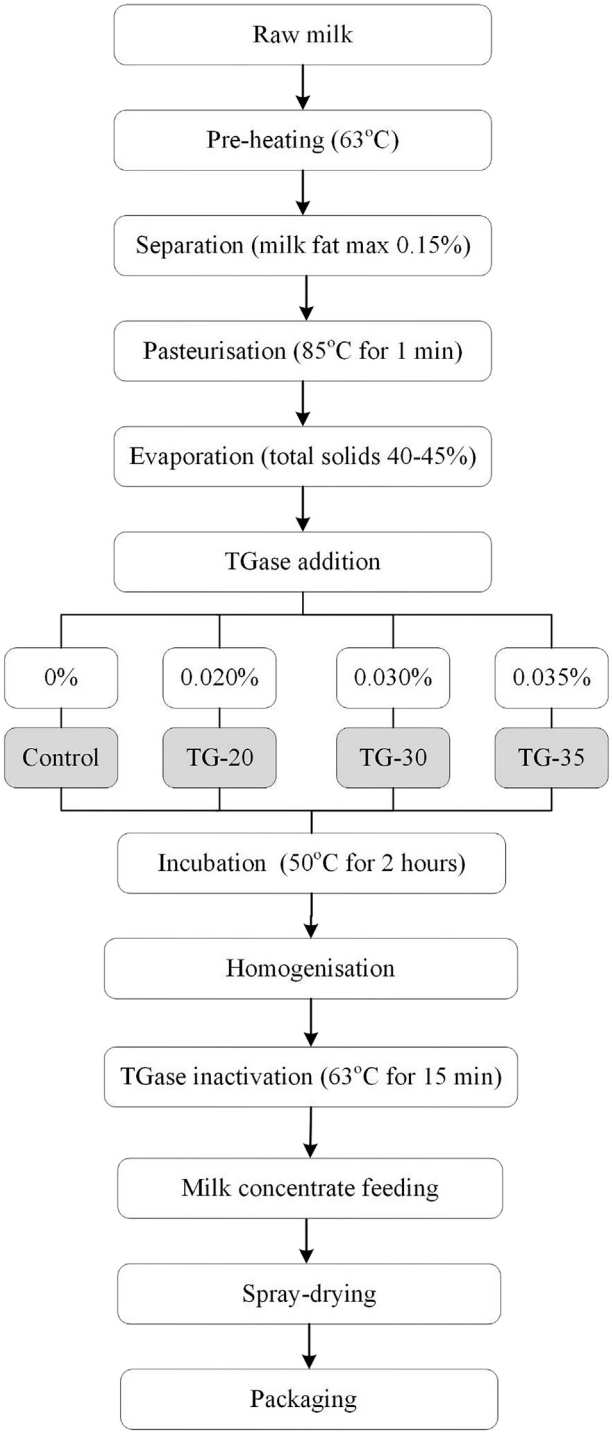


**Fig. 1.** Production flow chart for control skim milk powder and those (TG-20, TG-30, TG-35) prepared by spray-drying transglutaminase treated milk concentrates.

Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, UK) was used. Particle size parameters depending on Mie theory were given as follows: span, which normalises the width of the distribution relative to the median value; D[4.3], volume mean diameter; D[3.2], surface area mean diameter; and d(0.1), d(0.5) and d(0.9) [i.e., 10%, 50% and 90% of the sample particles are below this diameter, respectively (Sert, Mercan, Aydemir, & Civelek, 2016)]. A Minolta Chroma Meter CR-400 (Osaka, Japan) was used to determine colour parameters of milk concentrates. The pH of milk concentrates was measured by a 315i/SET pH-meter (WTW, Germany).

### 2.4. Analysis of milk powders

#### 2.4.1. Proximate analyses

For moisture determination, approximately 3 g of the SMP sample was dried in the drying oven at a temperature of 102 °C for 3 h (GEA, 2006b). GEA (2005) method was used for determination of fat content by Gerber centrifuge. The Kjeldahl method described by Sert et al. (2016) was used to determine the protein content of SMP. In the calculation of percent protein, the conversion factor of 6.38 was used to multiply the percent nitrogen value. For determination of ash content, 1–5 g of milk powder sample was weighed into a pre-ignited crucible and the sample was heated in a heating furnace at a temperature of 525 °C until carbon-free (GEA, 2004).

#### 2.4.2. Titratable acidity

To determine titratable acidity, reconstituted milk was titrated with 0.1 N NaOH solution. The titratable acidity of samples was calculated as % lactic acid (%LA) (GEA, 2006a).

#### 2.4.3. Particle size, colour and pH

Particle size, colour and pH analyses of milk powders were determined as stated in Section 2.3. In particle size measurement of powder samples, a Scirocco 2000 (Malvern Instruments Ltd) sampler for powders was used.

#### 2.4.4. Zeta potential

Zeta potentials of samples were carried out using a NanoPlus-3 (Micromeritics Instrument Corporation, USA) at 25 °C. Purified water was used as a diluent.

#### 2.4.5. Powder flow

A powder flow analyser (PFA) connected to TA.XTPlus texture analyser (Stable Micro Systems) was used for determination of flow characteristics, which comprised: a load cell (49.0 N capacity), a specified cylindrical glass vessel (220 mL total capacity; height: 120 mm and internal diameter: 50 mm) and a specified rotating helical blade (Rotor no. R48/50/10/2/A). Before analyses, force (using 19.6 N force) and height calibration (using height and target calibration disc) was carried out. Caking, cohesion, cohesion at 4 speeds and powder speed flow dependence (PFSD) analyses of SMP were carried out using the instrument's software (Exponent, Version 6, Stable Micro Systems).

*2.4.5.1. Caking.* Before the caking analysis, 2 conditioning cycles were performed to remove differences of user loading and to reorganise the column of milk powder. To level off the peak point of column of powder and to quantify the height of the column, the rotating blade moved to a force of 49 mN with a speed of 20 mm $s^{-1}$ and angle of 2°. Then the blade moved down through the column (20 mm $s^{-1}$ and 20°) and compacted the powder to a predetermined 7.35 N force to form a cake. When the rotating blade achieved the target force (7.35 N), it was determined the formed cake height. Then, the blade sliced up the powder with a tip speed of 10 mm $s^{-1}$ and a path angle of 45°. Totally five compaction cycles were carried out. In the fifth cycle, the blade sliced through the occurred cake of samples with a speed of 20 mm $s^{-1}$ and an angle of 170° and quantified the required force to do it. This force was expressed as cake strength (mN m). Also, mean cake strength (N) was determined. It is described as the average force for cutting the formed cake in grams. To calculate the cake height ratio for each cycle, cake height divided by the first column height (Bansal, Premi, Sharma, & Nanda, 2017).

*2.4.5.2. Cohesion.* Cohesion analysis was performed according to Mercan, Sert, and Akın (2018a). Before the cohesion analysis, 2 conditioning cycles were performed. Cohesion analysis consisted of downward (50 mm $s^{-1}$ and angle of 170°) and upward (50 mm $s^{-1}$ and angle of 170°) movements. In the upward movement of the cycle, the force on the vessel was expressed as cohesion coefficient (mN m). To calculate the cohesion index, cohesion coefficient of the sample was divided to the weight of sample (g). Flow characteristics of milk powders depending on cohesion index values are given in Table 1 (Benković, Srečec, Špoljarić, Mršić, & Bauman, 2013b).

*2.4.5.3. Cohesion.* Cohesion at four speeds analysis was determined as described in Mercan et al. (2018a). It measures cohesion index values from the upward movement of the cycle at four different speeds as a controlled flow. Firstly, a conditioning cycle was performed. Then, 1 cycle at 10 mm $s^{-1}$; at 20 mm $s^{-1}$; at 50 mm $s^{-1}$; at 100 mm $s^{-1}$ and finally at 10 mm $s^{-1}$ in downward and upward directions were carried out. The downward movements compacted the milk powder as a compacting action at a path angle of 5°, whereas the upward movements lifted the powders as a lifting action (178°). Then, cohesion index at 10, 20, 50 and 100 mm $s^{-1}$ were calculated by dividing the cohesion coefficient (upward movement) of the sample for each speed to powder sample weight (g).

*2.4.5.4. Powder speed flow dependence.* PFSD analysis carried out according to Mercan et al. (2018a). It consisted of 5 sets of 2 cycles. The first 4 sets of two cycles were carried out at 10, 20, 50, 100 mm $s^{-1}$, respectively; the final, 2 cycles were done at 10 mm $s^{-1}$. These speeds indicated downward action that compacted powder. In upward actions of all cycles, a speed of 50 mm $s^{-1}$ was used as a lifting action. The positive area of the compaction curves was averaged over the two cycles at each speed and gave the compaction coefficient (mN m) at each of the speeds tested. The negative area of curve of the first 10 mm $s^{-1}$ speed was calculated as cohesion coefficient at 50 mm $s^{-1}$. When dividing the compaction coefficient of the last 10 mm $s^{-1}$ cycles by the compaction coefficient of the first 10 mm $s^{-1}$, flow stability value was obtained (Bansal et al., 2017).

#### 2.4.6. Thermo-gravimetric analysis

Thermo-gravimetric analyses (TGA) of SMPs were performed using a TGA analyser (Setaram LabSys Evo, Caluire, France). Samples were scanned from 25 to 800 °C at a rate of 10 °C $min^{-1}$, under nitrogen gas at a flow rate of 20 mL $min^{-1}$.

**Table 1**
Classification of flow characteristic of milk powders based on cohesion index.

| Cohesion index | Flow characteristic |
|---|---|
| >19 | Hardened/extremely cohesive |
| 19–16 | Very cohesive |
| 16–14 | Cohesive |
| 14–11 | Easy flowing |
| <11 | Free flowing |

#### 2.4.7. Differential scanning calorimetry

Glass transition temperature and other thermal reactions of SMPs were examined by differential scanning calorimetry (DSC; DSC 25, TA Instruments, New Castle, DE, USA). SMPs were scanned from −20 to 250 °C at a rate of 10 °C $min^{-1}$, under nitrogen gas at a flow rate of 50 mL $min^{-1}$.

#### 2.4.8. Scanning electron microscopy

To determine the microstructure of SMPs, scanning electron microscopy (SEM) (Hitachi SU 1510, Hitachi Ltd., Tokyo, Japan) was used. Before SEM imaging, samples were coated with a thin layer of gold in an ion sputter coater. SEM instrument was operated at 20 kV during imaging. SEM images were taken at three different magnifications (100×, 500× and 1000×).

#### 2.4.9. SDS-PAGE

The changes of casein fractions and serum proteins of each milk powder samples were determined by SDS-PAGE according to Laemmli (1970). The acrylamide content of the stacking and separating gels was 4.0 and 12.5%, respectively. The samples were dissolved in sample buffer (10 mg $mL^{-1}$) and 8 μL aliquots were loaded onto the gel and run through the stacking and separating gels at 200 mV. Before staining, gels were kept in fixing solution (100% trichloroacetic acid, methanol, deionised water, 1:3.3:5.7 by vol) for 60 min. Gel electrophoresis was performed using a Protean II XL vertical electrophoresis unit (Bio-Rad Laboratories Ltd., Watford, UK) (Bulat & Topcu, 2019). The gels were scanned using an Agfa Arcus 1200 image scanner (Agfa-Gevaert NV, Mortsel, Belgium) controlled with Agfa Fotolook v3.5 (Agfa-Gevaert NV).

#### 2.4.10. Statistical analysis

At each level of treatment, powders were produced from TGase-treated milk concentrate as triplicate. ANOVA test at a 0.05 level ($P \leq 0.05$) was performed to evaluate significant differences between the results. In statistical analysis, Minitab 18 (Minitab LLC., State College, PA, USA) software was used. Results are presented as mean ± standard deviation.

## 3. Results and discussion

### 3.1. Texture, particle size, colour and pH parameters of milk concentrates

Table 2 shows the texture, particle size, colour and pH values of milk concentrates. Firmness, cohesiveness, and index of viscosity values of samples were not affected by TGase treatment ($P > 0.05$). However, TGase treatment of milk concentrates increased consistency ($P < 0.01$). Consistency value of TG-30 and TG-35 were significantly higher than the control. TGase treatment significantly affected particle size parameters of milk concentrates ($P < 0.01$). Particle size values [D[4.3], D[3.2], d(0.1), d(0.5), d(0.9)] of milk powders increased with increasing concentration of TGase treatment. It was thought that changes in texture and particle size parameters of milk concentrates were affected by the cross-linking of milk proteins by TGase treatment. TGase treatment caused a slight decrease in L* values of concentrates. However, a* and b* values were not affected by TGase treatment ($P > 0.05$).

### 3.2. Physicochemical characteristics of milk powders

Physicochemical characteristics of skim milk powders are presented in Table 3. The moisture content of milk powders varied from 1.53 to 2.35%. The fat content of milk powder samples was found to be 0.5% and there were no changes between samples ($P > 0.05$). TGase treatment did not significantly affect titratable acidity (TA) value of milk powders ($P > 0.05$) and TA was between 0.11 and 0.13% (% lactic acid). The protein content of milk powders was not considerably affected by the TGase treatment of milk concentrates. Moisture, fat, protein and titratable acidity of TGase-treated milk powders fulfilled to standards specified by American Dairy Products Institute (ADPI). TGase treatment of evaporated skim milk in milk powder production significantly affected the ash content of samples ($P < 0.01$). The ash content of samples increased related to treatment with higher TGase concentration. It was related to scorched particle content which was increased depending on TGase treatment. Civelek (2005) reported that there was a positive correlation between ash and scorched particle contents. The pH value of samples was found to vary from 6.64 to 6.68.

TGase treatment decreased zeta potential of SMP samples. This was related to deamidation and reticulation reactions by TGase. The findings of the current study were consistent with those of Broyard and Gaucheron (2015) who reported that release of ammonia from glutamine transformed in glutamic residues via deamidation and cross-link between lysine and glutamine residues via reticulation cause more negative in electrical charge of caseins.

TGase treatment significantly affected the particle size values of milk powder samples. TGase treatment decreased the span value of milk powders and so TGase-treated milk powders showed narrow particle size distribution. Powders with narrower distribution show

**Table 2**
Texture, particle size, colour and pH values of TGase-treated of milk concentrates.[a]

| Parameter | Control | TG-20 | TG-30 | TG-35 | *P* |
|---|---|---|---|---|---|
| Texture | | | | | |
| Firmness (N) | 6.915 ± 0.121 | 6.577 ± 0.119 | 6.639 ± 0.537 | 7.081 ± 0.459 | ns |
| Consistency (N s) | 79.98 ± 1.91 | 82.06 ± 7.53 | 92.50 ± 6.27 | 92.18 ± 1.80 | ** |
| Cohesiveness (N) | −1.510 ± 0.013 | −1.500 ± 0.079 | −1.560 ± 0.125 | −1.600 ± 0.003 | ns |
| Index of viscosity (N s) | −0.046 ± 0.001 | −0.046 ± 0.007 | −0.050 ± 0.002 | −0.047 ± 0.003 | ns |
| Particle size | | | | | |
| Span | 3.07 ± 0.37 | 3.16 ± 0.28 | 3.07 ± 0.44 | 3.11 ± 0.26 | ns |
| D[4.3] (μm) | 47.70 ± 2.45 | 69.60 ± 3.95 | 80.90 ± 4.48 | 81.60 ± 4.97 | ** |
| D[3.2] (μm) | 22.30 ± 0.94 | 28.10 ± 1.45 | 30.70 ± 3.25 | 31.90 ± 2.35 | ** |
| d(0.1) (μm) | 10.30 ± 1.36 | 11.90 ± 2.47 | 12.60 ± 1.85 | 13.10 ± 2.38 | ** |
| d(0.5) (μm) | 30.50 ± 2.75 | 45.20 ± 3.96 | 53.40 ± 5.56 | 56.10 ± 4.74 | ** |
| d(0.9) (μm) | 104.00 ± 8.96 | 154.00 ± 12.44 | 176.00 ± 20.40 | 187.00 ± 25.55 | ** |
| Colour | | | | | |
| L | 78.47 ± 0.34 | 75.37 ± 0.19 | 75.67 ± 0.23 | 75.34 ± 0.19 | * |
| a | −8.45 ± 0.13 | −8.20 ± 0.08 | −8.17 ± 0.08 | −8.18 ± 0.12 | ns |
| b | 13.04 ± 0.37 | 13.61 ± 0.22 | 13.64 ± 0.16 | 13.27 ± 0.36 | ns |
| pH | 6.24 ± 0.02 | 6.26 ± 0.01 | 6.27 ± 0.01 | 6.27 ± 0.01 | ns |

[a] Values are the mean ± standard deviation (n = 3); asterisks indicate significant difference: *$P < 0.05$; **$P < 0.01$; ns, not significant.

**Table 3**
Physicochemical characteristics of milk powders.[a]

| Parameter | Control | TG-20 | TG-30 | TG-35 | *P* |
|---|---|---|---|---|---|
| Moisture (w/w) | 2.35 ± 0.07 | 1.53 ± 0.11 | 1.79 ± 0.04 | 2.23 ± 0.12 | ** |
| Fat (w/w) | 0.5 ± 0.0 | 0.5 ± 0.0 | 0.5 ± 0.0 | 0.5 ± 0.0 | ns |
| Protein (w/w; N × 6.38) | 34.80 ± 0.13 | 34.99 ± 0.18 | 34.98 ± 0.10 | 35.02 ± 0.16 | ns |
| Ash (w/w) | 7.32 ± 0.07 | 8.62 ± 0.09 | 8.89 ± 0.10 | 8.85 ± 0.12 | ** |
| Titratable acidity (% LA) | 0.11 ± 0.01 | 0.13 ± 0.00 | 0.12 ± 0.01 | 0.11 ± 0.01 | ns |
| pH | 6.67 ± 0.02 | 6.65 ± 0.04 | 6.64 ± 0.02 | 6.68 ± 0.02 | ns |
| Zeta potential (mV) | −14.69 ± 1.44 | −16.13 ± 0.53 | −15.36 ± 0.19 | −18.89 ± 0.35 | ** |
| Particle size | | | | | |
| Span | 1.441 ± 0.012 | 1.053 ± 0.002 | 1.218 ± 0.039 | 1.149 ± 0.011 | * |
| D[4.3] (μm) | 50.43 ± 0.50 | 40.22 ± 0.08 | 44.30 ± 3.16 | 46.34 ± 0.34 | ** |
| D[3.2] (μm) | 37.80 ± 0.06 | 34.04 ± 0.05 | 33.84 ± 0.32 | 37.57 ± 0.16 | ** |
| d(0.1) (μm) | 22.03 ± 0.01 | 21.17 ± 0.03 | 20.51 ± 0.06 | 23.20 ± 0.07 | ** |
| d(0.5) (μm) | 43.33 ± 0.06 | 38.51 ± 0.06 | 38.00 ± 0.22 | 42.06 ± 0.17 | ** |
| d(0.9) (μm) | 84.44 ± 0.61 | 61.70 ± 0.18 | 66.78 ± 1.81 | 71.54 ± 0.75 | ** |
| Colour | | | | | |
| L | 97.73 ± 0.02 | 97.62 ± 0.04 | 97.41 ± 0.00 | 97.53 ± 0.22 | ns |
| a | −4.46 ± 0.03 | −4.40 ± 0.06 | −4.31 ± 0.16 | −4.07 ± 0.18 | * |
| b | 14.17 ± 0.06 | 14.23 ± 0.18 | 14.55 ± 0.49 | 13.70 ± 0.59 | * |

[a] Values are the mean ± standard deviation (n = 3); asterisks indicate significant difference: $^{*}P < 0.05$; $^{**}P < 0.01$; ns, not significant.

good flow characteristics (Sharma, Jana, & Chavan, 2012). Similar span values for skim milk powders were reported by Nikolova et al. (2014). D[4.3] values of TGase-treated skim milk powders ranged from 40.22 to 46.34 μm and these values were smaller than that of the control sample (50.43 μm). However, the increase in TGase concentration resulted in higher D[4.3] value ($P < 0.01$). This could relate to cross-linking in TGase-treated powders. A higher concentration of TGase slightly increased high molecular mass bands (Fig. 4) indicating increased cross-linking between casein fractions. TGase treatment of milk concentrates relatively decreased D[3.2] values of milk powders. Pugliese et al. (2017) reported that D[4.3] and D[3.2] values of the spray-dried skim milk powder changed from 72.60 to 122.75, and from 25.86 to 60.79 μm, respectively. The d(0.1) value of samples was between 20.51 and 23.20 μm; d(0.5) values of milk powders varied from 38.00 to 43.33 μm and d(0.9) value of milk powder samples was between 61.70 and 84.44 μm. It was found that particle size values of TGase-treated milk powders were generally smaller than those of control. This may relate to the increased consistency of milk concentrates depending on TGase treatment. The consistency (viscosity) of milk concentrates is one of

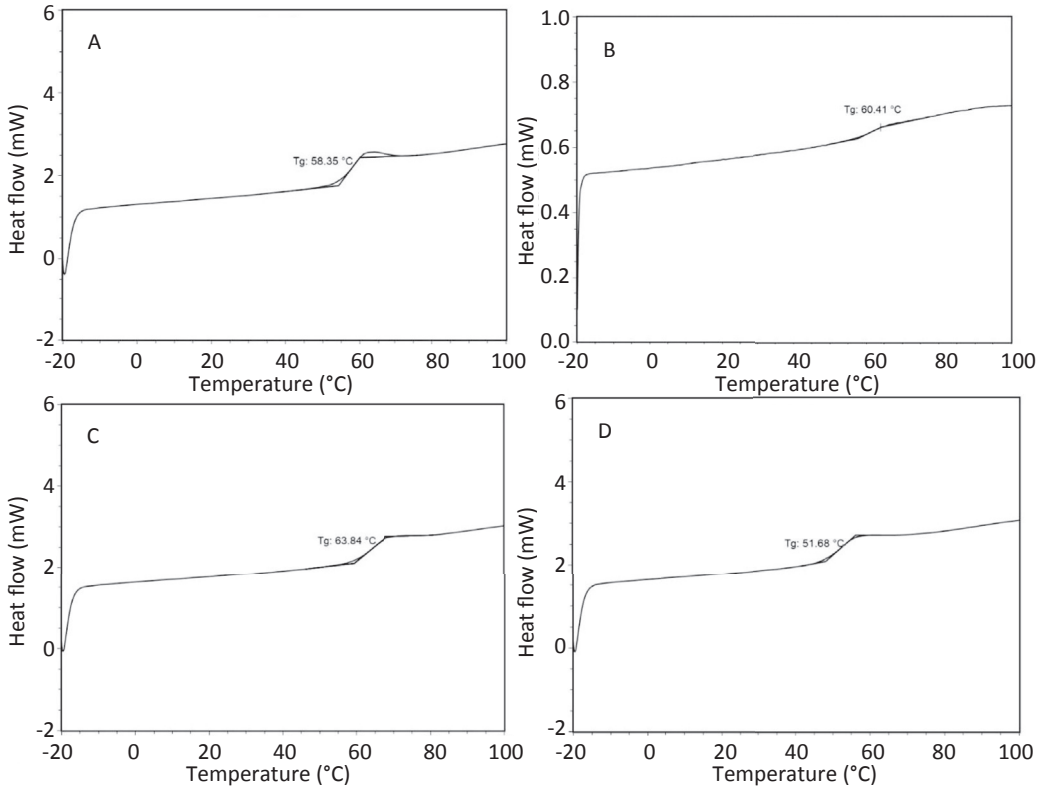


**Fig. 2.** DSC thermograms of (A) control and skim milk powders (B, TG-20; C, TG-30; D, TG-35) prepared by spray-drying transglutaminase treated milk concentrates.

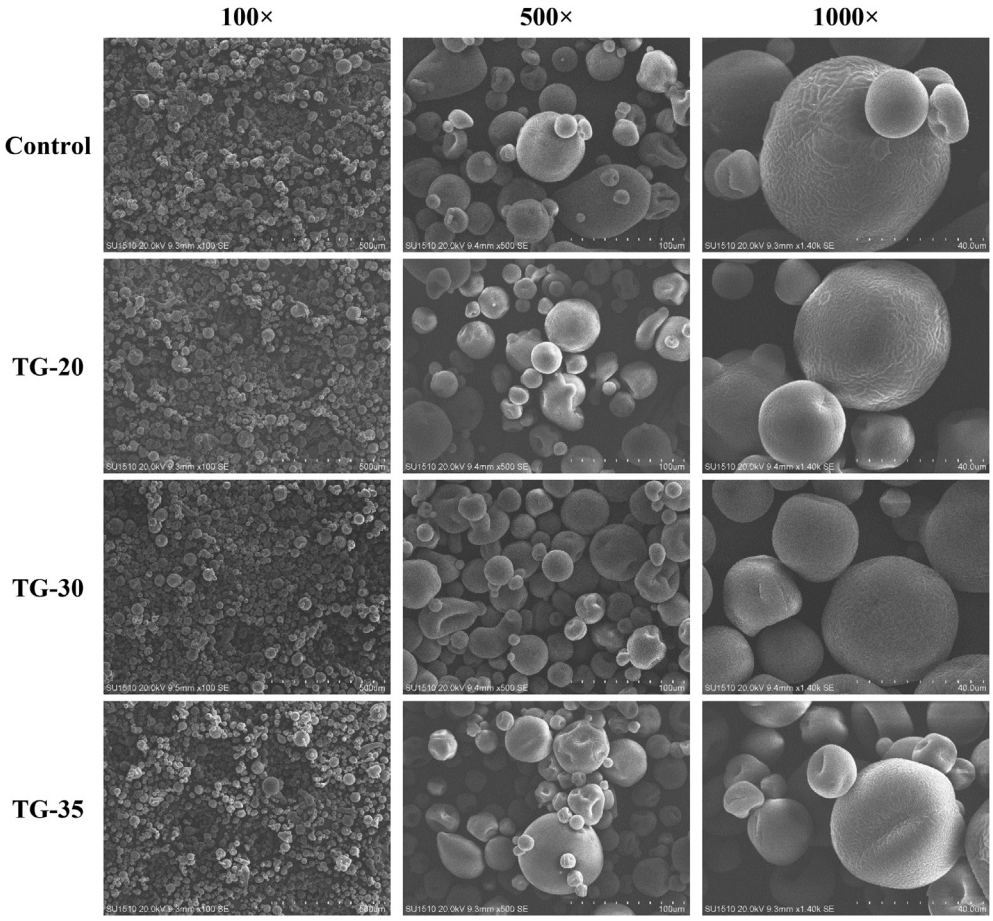


**Fig. 3.** SEM images of control and skim milk powders (TG-20, TG-30, TG-35) prepared by spray-drying transglutaminase treated milk concentrates.

the crucial parameters to affect the particle size of milk powder (Pisecký, 1997).

TGase treatment did not significantly affect the L* value of milk powders ($P > 0.05$). The lowest and highest a* values were found in the control and TG-35, respectively; i.e., TGase treatment increased the redness of samples related to TGase concentration. There was a slight increase in b* value of samples up to TGase concentration of 0.030%. However, a significant increase was observed in a* value of TG-35.

### 3.3. Powder flow characteristics of milk powders

Cake height ratios, cake strength and mean cake strength of TGase-treated skim milk powders are presented in Table 4. Cake height ratios for each cycle give data on the level of cake which is formed at the caking analysis. Cake height ratio 1 of samples ranged from 0.125 to 0.190, whereas cake height ratio 5 changed from 0.273 to 0.987. At the end of the analysis (at 5 cycle) cake height ratios of samples increased about 1-fold when compared with 1 cycle except TG-20. There was a slight increase in the cake height ratio of all milk powders related to the number of cycles. This situation indicated that all samples were slightly prone to caking. These results were similar to the previous study who reported that there was a slight increase in cake height ratios of skim milk powders (Bansal et al., 2017). However, sharp increases in cake height ratios indicate that the powder sample is considerably prone to cake formation (Benković, Belščak-Cvitanović, Bauman, Komes, & Srečec, 2017).

TGase treatment significantly affected cake strength and mean cake strength of skim milk powders ($P < 0.01$). TG-20 and TG-30 have considerably lower cake strength as compared with the control. However, TGase treatment at a concentration of 0.035% significantly increased cake strength of the sample. Increase in the cake strength indicated that more work required to cut the formed cake in the cylinder. Mean cake strength of milk powder samples ranged from 0.07 to 0.24 N and similar trend to cake strength was observed. According to cake strength and mean cake strength, TGase treatment at concentration of 0.020% and 0.030% significantly decreased caking characteristics of skim milk powders. TGase treatment at a concentration of 0.020−0.030% can therefore be used for controlling of cake formation in skim milk powders.

Table 5 shows cohesion coefficient, cohesion index and cohesion index at four speeds of skim milk powders. Cohesion coefficient is the required force to lift the milk powder through the column by the rotating blade. In cohesion coefficient, decreased cohesion coefficient shows increased cohesiveness. Cohesion coefficients of skim milk powders ranged from −6.21 to −8.52 (mN m). Cohesion coefficients of TG-20 and TG-30 were similar to the Control sample. There is a reverse relationship between cohesion coefficient and cohesion index. Cohesion index is practical analysis for determining flow properties of milk powders due to the fact that it removes influence of sample weight on the end result (Mercan et al., 2018a). Flow characteristics of milk powders are classified depending on cohesion index values (Table 1). Cohesion index values of samples ranged from 8.66 to 11.47. Control, TG-20, and TG-30 showed easy flowing behaviour whereas TG-35 had free-flowing behaviour;

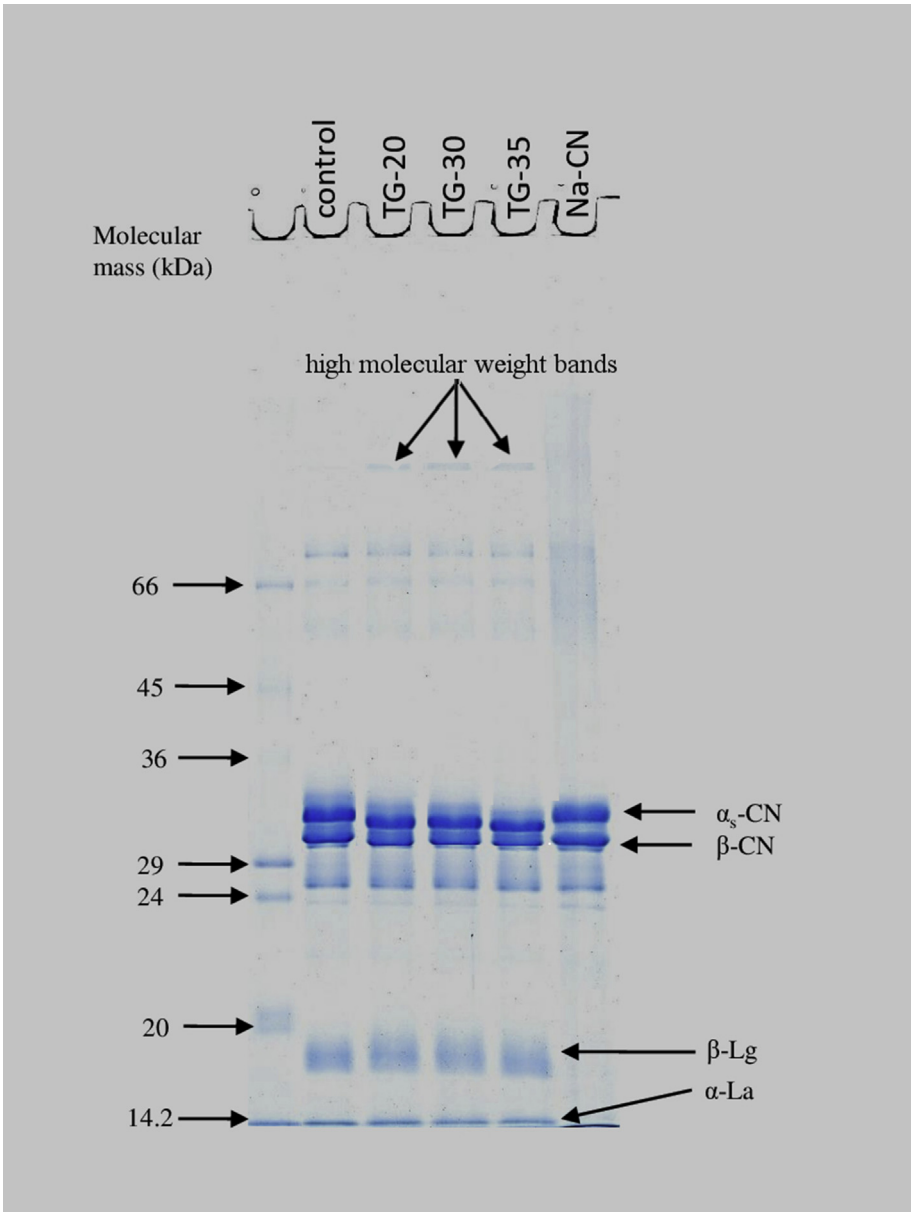


**Fig. 4.** SDS-PAGE profiles of control and skim milk powders (TG-20, TG-30, TG-35) prepared by spray-drying transglutaminase treated milk concentrates; Sodium caseinate (NaCN) was used as a milk protein standard.

therefore TGase treatment at a concentration of 0.035% improved flowing behaviour of skim milk powders when compared with the control sample. Cohesion characteristics of TGase-treated skim milk powders can be related to deamidation and cross-link reactions by TGase. Deamidation and polymerisation (cross-linking) reactions significantly change the hydrophobic/hydrophilic ratio of the protein surface and protein solubility (Damodaran & Agyare, 2013). Alteration in the hydrophobic/hydrophilic ratio on the protein surface can affect cohesive characteristics of final products (Gaspar & de Góes-Favoni, 2015). Apart from this situation, cohesion characteristic of milk powder was influenced by various factors such as hygroscopicity, electrostatic activity, porosity, particle size, and shape, etc. (Thomas, Scher, Desobry-Banon, & Desobry, 2004).

Cohesion at four speeds analysis determines the flow behaviour of milk powders related to flow rate. It measures the resistance of powder samples to controlled flow, which consists of four different flow rates. Flow speeds of 10, 20, 50, and 100 mm $s^{-1}$ were used in cohesion index of skim milk powders due to the fact that these speeds simulate some conditions of milk powder during shipping and processing in the dairy industry (Mercan et al., 2018a). TGase treatment considerably affected the values of cohesion index at four different speeds ($P < 0.01$). The results of cohesion at four speeds analysis possessed that milk powder samples showed easy flowing and free-flowing behaviours depending on cohesion index classification (8.24–13.04). While cohesion index values of control sample relatively decreased related to increasing flow rate, cohesion indexes of TG-20 and TG-30 increased depending on increasing flow rate. However, TG-30 showed almost stabile flow characteristic depending on the flow rate. In addition, TGase-treated milk powders had more flowable behaviour at lower flow rates when compared with the control. Also, TG-30 was more flowable than other samples at all speeds. Flowable characteristic of milk powders is crucial for the dairy industry (Mercan et al., 2018a).

**Table 4**
Caking characteristics of milk powders.[a]

| Sample | Cake height | | | | | Cake strength (mN m) | Mean cake strength (N) |
|---|---|---|---|---|---|---|---|
| | Ratio 1 | Ratio 2 | Ratio 3 | Ratio 4 | Ratio 5 | | |
| Control | 0.172 ± 0.003 | 0.310 ± 0.012 | 0.354 ± 0.009 | 0.383 ± 0.011 | 0.351 ± 0.010 | 1.26 ± 0.04 | 0.13 ± 0.01 |
| TG-20 | 0.190 ± 0.005 | 0.274 ± 0.008 | 0.347 ± 0.007 | 0.366 ± 0.009 | 0.987 ± 0.015 | 0.33 ± 0.01 | 0.07 ± 0.00 |
| TG-30 | 0.125 ± 0.002 | 0.199 ± 0.005 | 0.243 ± 0.005 | 0.273 ± 0.007 | 0.273 ± 0.005 | 0.58 ± 0.02 | 0.10 ± 0.01 |
| TG-35 | 0.173 ± 0.003 | 0.260 ± 0.007 | 0.311 ± 0.006 | 0.334 ± 0.010 | 0.343 ± 0.011 | 2.39 ± 0.08 | 0.24 ± 0.02 |

[a] Values are the mean ± standard deviation (n = 3).

**Table 5**
Cohesion characteristics of milk powders.[a]

| Sample | Cohesion coefficient (mN m) | Cohesion index | Cohesion index at four speeds | | | |
|---|---|---|---|---|---|---|
| | | | 10 mm $s^{-1}$ | 20 mm $s^{-1}$ | 50 mm $s^{-1}$ | 100 mm $s^{-1}$ |
| Control | −8.52 ± 0.18 | 11.40 ± 0.22 | 13.04 ± 0.24 | 12.76 ± 0.29 | 12.02 ± 0.25 | 12.13 ± 0.33 |
| TG-20 | −7.95 ± 0.11 | 11.06 ± 0.20 | 11.02 ± 0.18 | 11.07 ± 0.22 | 11.40 ± 0.24 | 11.41 ± 0.29 |
| TG-30 | −8.35 ± 0.19 | 11.47 ± 0.23 | 10.61 ± 0.27 | 11.23 ± 0.25 | 12.19 ± 0.30 | 12.59 ± 0.35 |
| TG-35 | −6.21 ± 0.17 | 8.66 ± 0.14 | 8.33 ± 0.17 | 8.24 ± 0.20 | 8.24 ± 0.19 | 8.36 ± 0.24 |

[a] Values are the mean ± standard deviation (n = 3); TG-35 values significantly different at $P < 0.01$.

In general, TGase treatment can improve the flow behaviour of skim milk powders except for some situations.

PFSD analysis measures the flow characteristics of powders depending on flow speed. These data are crucial in processing and transporting. Table 6 shows compaction coefficients at 10, 20, 50 and 100 mm $s^{-1}$ flow speeds, flow stability and cohesion coefficient at 50 mm $s^{-1}$. Compaction coefficients of milk samples ranged from 45.63 to 84.95 mN m at all speeds. Except for TG-35, other samples had similar compaction coefficients. However, TG-35 had lower compaction coefficients as compared with other samples ($P < 0.01$). The results revealed that compaction coefficients of all samples decreased related to increasing flow speeds; i.e., samples had more free-flowing behaviour depending on increasing flow speeds. A similar trend was reported by previous studies (Bansal et al., 2017; Mercan et al., 2018a). Free-flowing behaviour of milk powders is advantageous in the dairy industry due to the fact that conveying this powder requires less work.

Besides ensuring essential data on the flow resistance of milk powder samples, flow stability is also known as an indicator of the susceptibility of the product to attrition (breakdown). Flow stability of skim milk powders changed from 1.00 to 1.05. TGase treatment did not affect the flow stability of samples ($P > 0.05$). When flow stability is close to 1.00, the powder sample is not considerably affected during the analysis (Benković, Belščak-Cvitanović, Komes, & Bauman, 2013a). Cohesion coefficients at 50 mm $s^{-1}$ determined by PFSD test of skim milk powder ranged from −5.76 to −9.10 mN m. These results were in accordance with the results of cohesion analysis as given in Table 4.

### 3.4. Thermal characteristics of milk powders

TGA analysis determines physical changes and force reactions, leading to the weight loss or gain due to decomposition, oxidation, dehydration, or desorption of a sample with time and temperature. Decomposition temperatures, rate of degradation, life time of product, thermal stability and moisture content can be measured by TGA. TGA is crucial for food analysis due to the fact that it can simulate different types of thermal food processes and storage conditions such as drying, cooking, and moisture uptake (Lin & Lin, 2015). TGA data of skim milk powders are given in Table 7. It was

**Table 6**
Powder flow speed dependency (PFSD) characteristics of milk powders.[a]

| Sample | Compaction coefficient (mN m) | | | | Flow stability | Cohesion coefficient 50 mm $s^{-1}$ (mN m) |
|---|---|---|---|---|---|---|
| | 10 mm $s^{-1}$ | 20 mm $s^{-1}$ | 50 mm $s^{-1}$ | 100 mm $s^{-1}$ | | |
| Control | 84.94 ± 0.28 | 84.95 ± 0.31 | 68.47 ± 0.33 | 47.42 ± 0.44 | 1.04 ± 0.02 | −8.57 ± 0.24 |
| TG-20 | 84.61 ± 0.30 | 83.53 ± 0.36 | 65.65 ± 0.36 | 45.63 ± 0.40 | 1.02 ± 0.02 | −8.05 ± 0.33 |
| TG-30 | 84.83 ± 0.42 | 84.23 ± 0.29 | 69.37 ± 0.30 | 50.83 ± 0.59 | 1.05 ± 0.03 | −9.10 ± 0.43 |
| TG-35 | 76.33 ± 0.25 | 75.50 ± 0.30 | 62.10 ± 0.19 | 46.35 ± 0.50 | 1.00 ± 0.01 | −5.76 ± 0.32 |

[a] Values are the mean ± standard deviation (n = 3); TG-35 compaction coefficient (except at 100 mm $s^{-1}$) and cohesion coefficient significantly different at $P < 0.01$; flow stability not significantly different.

**Table 7**
Summary of TGA thermograms of milk powders.

| Sample | Step 1 | | | Step 2 | | | Step 3 | | | Step 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Temp. range (°C) | Max. peak (°C) | Total weight loss (%) | Temp. range (°C) | Max. peak (°C) | Total weight loss (%) | Temp. range (°C) | Max. peak (°C) | Total weight loss (%) | Temp. range (°C) | Max. peak (°C) | Total weight loss (%) |
| Control | 32–245 | 116 | 28 | 257–300 | 272 | 32 | 300–430 | ~340 | 60 | 500–900 | – | 69 |
| TG-20 | 36–245 | 112 | 26 | 246–300 | 272 | 36 | 300–410 | ~340 | 57 | 500–900 | – | 67 |
| TG-30 | 31–245 | 113 | 28 | 258–300 | 272 | 37 | 300–450 | ~340 | 61 | 500–900 | – | 68 |
| TG-35 | 30–245 | 127 | 28 | 258–300 | 272 | 37 | 300–450 | ~340 | 62 | 500–900 | – | 70 |

found that TGase treatment did not significantly affect the thermal stability of samples. TGA data of samples was examined in 4 steps using thermograms. The first step was between 30 and 245 °C and the weight loss was about 26–28% in all samples. The weight loss at this stage, the evaporation of water and the loss of volatile compounds, as well as the thermo-degradation of casein and whey proteins, can be considered.

The second step was approximately 246–300 °C. The total weight loss was 32% in the control sample, whereas it was 36–37% in the TGase-treated milk powders. In this step, the decomposition of the polymer structure could occur. As evaluated this temperature range, it can be thought that the thermal stability of milk powders partially decreased depending on TGase treatment.

In the third step, which took place in the 300–450 °C range, the total weight loss increased to 57–62%. At this stage, it was thought that degradation of glycosidic bonds, loss of hydroxyl groups, and $CO_2$ release occurred.

The last step was between 500 and 900 °C. The total weight loss was 67–70% in this step. This may relate to the decomposition of the residues may result in carbon and ash formation.

DSC thermograms of samples are given in Fig. 2. As shown in graphs, glass transition temperatures ($T_g$) of control, TG-20, TG-30, and TG-35 were 58.40, 60.41, 63.80 and 51.68 °C, respectively. These results were similar to those reported by Schuck, Jeantet, and Dolivet (2012). However, they were lower than results of other researches (Jouppila & Roos, 1994; Ozmen & Langrish, 2002). These differences may relate to composition of powders, which affects $T_g$ value (Ishwarya & Anandharamakrishnan, 2017). In addition, it would be difficult to ascribe the thermal characteristics of milk powders to particular components as complex individual as well as synergistic effects need to be considered due to the fact that milk is a multi-component mixture (Lapčík et al., 2015). $T_g$ of TG-20 and TG-30 was higher than that of the control, whereas that of TG-35 was significantly lower than the control sample. TGase treatment of up to 0.030% increased $T_g$. This was probably due to the cross-linking of casein which is a major component of milk and may dominate the $T_g$ of SMP. Mizuno, Mitsuiki, and Motoki (1999) reported that the polymerisation of α-casein by TGase induced cross-linking resulted in increased the Tg of α-casein.

According to DSC data, the first exothermic peak that occurred at about 162–165 °C could be attributed to lactose crystallisation and the second major peak reaching maximum rate at about 183–187 °C, which could be attributed to the Maillard reaction causing non-enzymatic browning between proteins and lactose (Rahman, Al-Hakmani, Al-Alawi, & Al-Marhubi, 2012; Vuataz, Meunier, & Andrieux, 2010). Considering the differences in moisture, exothermic peak values are consistent with those reported by Lapčík et al. (2015).

### 3.5. Microstructural characteristics of milk powders

Quality parameters of milk powders are significantly affected by the milk content, processing methods, thermal treatment throughout the process and especially the drying method. SEM is used to get data about the effects of these factors on the microstructure of milk powders. Fig. 3 shows scanning electron micrographs of TGase-treated skim milk powders. TGase treatment did not significantly affect the microstructure of skim milk powders. This may be related to the stability of the casein micelles when TGase is added to the milk. It was found that the shape of powder particles was whole and globular. In addition, wrinkles and some pores were determined on the surface of skim milk powders. These results were similar to reported by the previous studies (Buma & Henstra, 1971; Caric & Kalab, 1987). They reported that milk powders produced by spray drying have generally regular globular shapes. The surfaces of the milk powders are generally smooth, but varying levels of wrinkles (curved structures) can also be seen on the surface. Gaps in both smooth and wrinkled particles may sometimes involve small milk powder particles.

### 3.6. SDS-PAGE profile of proteins in milk powders

SDS-PAGE was performed to determine changes in protein profiles of milk powders. Fig. 4 shows the SDS-PAGE electrophoretograms of SMPs produced from TGase-treated milk concentrates. Some visual changes were observed in electrophoretograms depending on the samples. High molecular mass bands were determined in TGase-treated samples. In addition, casein bands in TGase-treated samples were smaller than control. This indicated that TGase treatment resulted in cross-link or deamidation reactions in proteins of milk concentrates.

## 4. Conclusions

Physicochemical, powder flow, thermal and microstructural characteristics of skim milk powders (SMP) produced from TGase-treated milk concentrates were evaluated. It was concluded that TGase treatment decreased particle size and zeta potential of SMP samples. TGase treatment increased the redness of samples related to TGase concentration. Control, TG-20, and TG-30 showed easy flowing behaviour, whereas TG-35 had free-flowing behaviour. TG-35 had lower compaction coefficients as compared with other samples. Glass transition temperatures of samples ranged from 51.68 to 63.80 °C. TGase treatment did not significantly affect the microstructure of skim milk powders. It is possible to modify the structure of acid-induced milk gels by pre-treating the milk with TGase. TGase-treated milk powder could possibly be utilised in the production of yoghurt from reconstituted milk due to the fact that cross-linking by TGase improve texture, gel formation dynamics, water holding capacity and microstructure, but further studies are required to determine applications in the dairy industry.

## Acknowledgements

We would like to thank Mustafa Civelek, Director of Research & Development Department of Enka Dairy Company, for his contributions during the production process of milk powder samples.

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