



How do different cooling temperatures affect the characteristics of set-type yoghurt gel?

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ABSTRACT

The impact of cooling temperatures on gel characteristics of set-type yoghurts was investigated through 28 days of storage. Yoghurt samples were cooled at four different temperatures ($-10\text{ }^{\circ}\text{C}$, $-5\text{ }^{\circ}\text{C}$, $0\text{ }^{\circ}\text{C}$, $4\text{ }^{\circ}\text{C}$) after incubation until the central temperature of yoghurt cups reached $4\text{ }^{\circ}\text{C}$. Cooling temperature and storage period were determined as significant factors affecting the yoghurt gel characteristics ($p < 0.05$). Yoghurts cooled at $0\text{ }^{\circ}\text{C}$ clearly differed from the other yoghurts in terms of gel firmness and consistency. Confocal images of yoghurts cooled at $0\text{ }^{\circ}\text{C}$ showed more homogeneous protein matrix than the other samples. Microbial counts and the acidity of the yoghurt samples increased with storage period. Textural parameters, water holding capacity and susceptibility to syneresis increased until 14th day of storage and then decreased. Sensory analysis of yoghurt samples confirmed the results obtained by microbiological and textural measurements.

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1. Introduction

Yoghurt is a semisolid fermented product made from heat-treated standardised milk with the activity of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* (Chandan, 2007; Shah, 2003; Vedamuthu, 1991). Two types of yoghurts are available on the market. One of these has a firm gel-like structure (set type) and the other has a thick consistency (stirred type). In set type yoghurt, milk inoculated with the yoghurt starter is packaged and then incubated while stirred type yoghurt gels are disrupted by stirring after fermentation (Tamime, 2007; Tamime & Robinson, 2000). A major aim of manufacturers is to optimise the texture of gels in the production of the yoghurt, because the overall sensory perception and functionality of fermented milks are affected by the properties of the milk gel (Tamime, 2007). The processing stages used in yoghurt production directly influence gel properties; these are standardisation, homogenisation and heat treatment conditions, starter culture, incubation temperature, time to start cooling, cooling rate and handling of the yoghurts after manufacture (Jaros & Rohm, 2003; Lucey, 2004; Lucey & Singh, 2003; Walstra, 1998).

The role of cooling is particularly important because it directly affects gel structure. The main purpose of cooling is to restrict the

activity of the starter culture and its enzymes as quickly as possible and to provide the desired pH, texture and structure (Akın, 2006). The cooling process should not occur too fast; cooling too rapidly may cause undesirable changes in the structure of the gel contributing to whey separation in the yoghurt (Chandan, 2007). Dairy manufacturers take different approaches to the cooling stage. Despite the fact that yoghurts supplied to the market are produced by similar methods, many differences in taste-aroma and structure are observed. If the cooling cannot be successfully performed, this stage leads to adverse effects such as weak body, whey separation and post acidification, which are considered significant quality defects (Özer, 2009). Therefore, the application of an optimum cooling process is very important to achieve the desired physical and sensory qualities in the final product and to be able to produce quality yoghurt to protect consumer health and to comply with regulatory requirements. Furthermore, these properties in yoghurt are important parameters influencing consumer preference (Park, 2007).

There are comprehensive studies in the literature regarding optimisation of quality parameters and manufacturing steps of yoghurts such as heat treatment, inoculation, incubation and storage, but there are no studies on the cooling step. In the present research, set yoghurt samples that were cooled at four different temperatures ($-10\text{ }^{\circ}\text{C}$, $-5\text{ }^{\circ}\text{C}$, $0\text{ }^{\circ}\text{C}$ and $4\text{ }^{\circ}\text{C}$ as the control) after incubation were analysed in terms of physicochemical, microbiological, textural and sensory characteristics.

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2. Materials and methods

2.1. Materials

Raw milk was obtained from Selçuk University Dairy Farm, Konya, Turkey; skim milk powder (0.50% fat, 34.65% protein, 3.90% moisture, 7.98% ash, 52.97% lactose, 99.91% solubility) was provided by ENKA Dairy Products (Konya, Turkey). Lyophilised DVS starter culture (*Lb. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*) was provided from Chr. Hansen-Peyma (Istanbul, Turkey). The yoghurt packaging material used in the research was purchased from Şekeroğlu Chemical-Plastic Industry and Trade Inc. (Konya, Turkey); round polypropylene containers were used for packaging the yoghurt. The outer diameter of the package used in yoghurt production was 112.65 mm; height and volume were 71 mm and 500 mL, respectively. The packaging has a plastic handle system, tamper evident system, easy to open snap-on lid, and is resistant to freezing, microwaves and dishwashers.

2.2. Production of set-style yoghurt

Raw milk (12.32% total solids, 3.95% total fat, 3.75% total protein) samples were standardised with skim milk powder to produce 16% total solids in the final products and heated at 90 °C for 5 min, then cooled to 45 °C. After cooling to incubation temperature, milk samples inoculated with yoghurt starter (2%, v/v) and were transferred to sterile plastic cups. Then the cups were incubated at 42 °C for 3 h until the pH of samples reached 4.60. Yoghurt samples for which fermentation was completed were cooled into a cold room at –10 °C, –5 °C, 0 °C and 4 °C. For monitoring of the decrease of temperature to 4 °C, thermocouples were located at the centre of the yoghurt cups and a data logger recorded the temperature of samples. When the yoghurt temperature reached 4 °C, cooling was terminated and cups were stored at 4 °C for 28 days.

2.3. Physicochemical analyses

The pH values of yoghurts were determined with WTW 315i Set brand pH meter. For the titratable acidity measurement, 10 g yoghurt sample was mixed with 10 mL distilled water then the mix was titrated with 0.1 N NaOH solution using phenolphthalein as indicator. The water holding capacity of samples were measured by the method reported by Senaka Ranadheera, Evans, Adams, and Baines (2012). Five grams of yoghurt sample were centrifuged at 4500 rpm for 30 min at 10 °C. After centrifugation, the weight of whey was calculated. The susceptibility to syneresis was determined as recommended by Sahan, Yasar, and Hayaloglu (2008) with slight modifications. Twenty-five grams of yoghurt sample on a filter paper (Whatman no: 1) were put on top of a funnel; the volume of the whey collected in a tube was measured after 2 h of filtration.

2.4. Microbiological analyses

Ten grams of yoghurt sample was diluted with 90 mL of sterile ¼-ringer solution and serial dilutions were prepared. The spread plate technique was used for determination of the counts of starter bacteria. MRS agar (Merck KGaA, Darmstadt, Germany) was used for the enumeration of *Lb. delbrueckii* ssp. *bulgaricus* under anaerobic conditions at 37 °C for 72 h (Cruz et al., 2012). For *S. thermophilus*, plates were incubated aerobically at 37 °C for 48 h (Dave & Shah, 1996). After incubation at certain temperatures, plates were counted and the data was expressed as a log cfu mL⁻¹. Microbiological analyses of yoghurt samples were carried out weekly intervals throughout the storage period.

2.5. Texture profile analysis

The texture profile analysis (viscosity index, consistency, firmness, cohesiveness) was performed using a texture analyser TA-XT2 (Stable Micro Systems, Goldaming, Worcestershire, UK) with a 5 kg load cell and back extrusion unit as recommended by Najgebauer-Lejko, Tabaszewska, and Grega (2015) with slight modifications. Texture studies were performed on the undisturbed yoghurt samples in polypropylene cups (112.65 mm external diameter and 71 mm height) removed from the cool store (4 °C) immediately before measurement. The cylinder probe with a diameter of 35 mm was thrust through the sample to a depth of 30 mm at a speed of 1 mm s⁻¹. The parameters that give information about the textural properties of yoghurt samples from the obtained force–time graphs were calculated with the software of Texture Analyzer TA Plus. The test was carried in three replicates for each sample at 1, 7, 14, 21 and 28 days of storage.

2.6. Gel microstructure

The microstructure of yoghurt samples was visualised using a method described in Pang, Deeth, Sharma, and Bansal (2015). The fluorescent protein dye Rhodamine B (0.1%, w/w, Fluka, Sigma–Aldrich, St. Louis, MO, USA) was added to milk samples prior to acidification (20 µL per sample). After stirring gently, a few drops of the milk were transferred to microscope slide covered with a coverslip. The slides were incubated at 45 °C for fermentation then cooled by transferring them into a refrigerator at –10 °C, –5 °C, 0 °C and 4 °C. After one day of storage at 4 °C gel microstructure was observed with a Confocal laser scanning microscope (A1, Nikon, Tokyo, Japan) using an Argon laser with a 40× oil immersion objective at the emission and excitation wavelength of 595 and 561.7 nm, respectively. The resolution of images was 1024 × 1024 pixels and 10 images were taken for each sample.

2.7. Sensory characteristics

Yoghurt samples stored at +4 °C for sensory analysis were taken from this temperature and presented to the panellists at 12–15 °C. The tasting panel consisted of academical personal of Food Engineering Department of Selçuk University, Turkey. Four samples were presented to the panellists and all samples were coded and arranged in random order. Sensory properties of yoghurt samples were determined by seven experienced panellists using a sensory rating scale of 1–5 (1: unacceptable; 5: excellent) for appearance, texture, mouth feel, odour and taste (Tamime, Barrantes, & Sword, 1996) on the 7th and 21st day of storage.

2.8. Statistical analysis

The data obtained for results of pH, titratable acidity, water holding capacity, syneresis, microbiological, textural and sensory analyses were analysed using SPSS programme and reported as the mean ± standard deviation of the three replicates. The significant differences compared by Tukey test at $p < 0.05$.

3. Results and discussion

3.1. Monitoring of the cooling process of yoghurt samples

Cooling of yoghurt can be carried out in single or double stage. In single-stage cooling, the temperature of the fermented milk is reduced directly from 43 °C to 10 °C. This model is more suitable for plain, set type yoghurts (Tamime & Robinson, 2000). For this reason, single stage cooling was preferred in this research. After

incubation, yoghurts were immediately transferred to the cold room set at $-10\text{ }^{\circ}\text{C}$, $-5\text{ }^{\circ}\text{C}$, $0\text{ }^{\circ}\text{C}$ and $4\text{ }^{\circ}\text{C}$. The temperature measurement values recorded with thermocouples placed in the packaging centre during cooling of the yoghurt samples at $-10\text{ }^{\circ}\text{C}$, $-5\text{ }^{\circ}\text{C}$, $0\text{ }^{\circ}\text{C}$ and $4\text{ }^{\circ}\text{C}$ are shown in Fig. 1. The time required for cooling to $4\text{ }^{\circ}\text{C}$ (cold storage temperature) was measured as 135, 195, 270, 565 min for cooling at $-10\text{ }^{\circ}\text{C}$, $-5\text{ }^{\circ}\text{C}$, $0\text{ }^{\circ}\text{C}$ and $4\text{ }^{\circ}\text{C}$, respectively. As shown in Fig. 1, the cooling time has increased as the cooling temperature increases. During cooling at $-5\text{ }^{\circ}\text{C}$ and $-10\text{ }^{\circ}\text{C}$, yoghurt samples did not freeze. When the central temperatures of yoghurts decreased from $41\text{--}43\text{ }^{\circ}\text{C}$ to $4\text{ }^{\circ}\text{C}$, the cooling process was terminated and the samples were stored at $4\text{ }^{\circ}\text{C}$ for further analysis.

3.2. pH and titratable acidity of yoghurt samples during storage

The pH of milk samples 6.36 ± 0.01 was reduced to 4.72 ± 0.01 throughout the fermentation in approximately 3 h. pH values for all yoghurt samples ranged from 4.66 ± 0.02 to 4.33 ± 0.01 during the 28 days of storage (Fig. 2). The initial average pH values of yoghurts cooling at $-10\text{ }^{\circ}\text{C}$, $-5\text{ }^{\circ}\text{C}$, $0\text{ }^{\circ}\text{C}$ and $4\text{ }^{\circ}\text{C}$ (control) were 4.66 ± 0.02 , 4.63 ± 0.01 , 4.53 ± 0.00 and 4.52 ± 0.01 , respectively, and at the end of the storage pH values were reduced to 4.38 ± 0.01 , 4.37 ± 0.01 , 4.35 ± 0.01 and 4.33 ± 0.01 , respectively. Yoghurts cooling at $-10\text{ }^{\circ}\text{C}$ had the highest values of pH among yoghurt samples ($p < 0.05$) during cold storage. In agreement with other studies (Bonczar, Wszotek, & Siuta, 2002; Vianna et al., 2017; Zare, Boye, Orsat, Champagne, & Simpson, 2011) pH values of all yoghurt samples decreased during the 28 days of storage. A continuous decrease of the pH values could be attributed to the residual activity of starter microorganisms especially *Lb. delbrueckii* ssp. *bulgaricus* under refrigerated temperatures (Lourens-Hattingh & Viljoen, 2001).

When the titration acidity data were analysed in terms of the storage period, the titration acidity was shown to increase as the storage period increased. The initial average titratable acidity of yoghurt samples was 1.01 ± 0.00 and increased to 1.26 ± 0.02 at the end of the storage period (Fig. 2). Similar to pH changes, titratable acidity values of yoghurts increased during storage due to the lactic acid. This increase in acidity is thought to be due to lactic acid, which is the result of lactic acid bacteria continuing to grow fermentation of lactose to lactic acid during the storage period (Lucey, 2004). The results for increased acidity during storage is in agreement with Mani-López, Palou, and López-Malo (2014), Serra, Trujillo, Guamis, and Ferragut (2009) and Shah, Lankaputhra,

Britz, and Kyle (1995). During the storage period average titratable acidity values of yoghurts cooling at $-10\text{ }^{\circ}\text{C}$, $-5\text{ }^{\circ}\text{C}$, $0\text{ }^{\circ}\text{C}$ and $4\text{ }^{\circ}\text{C}$ were 1.28 ± 0.14 , 1.29 ± 0.14 , 1.31 ± 0.14 and 1.33 ± 0.14 respectively. The effect of cooling temperature on titratable acidity was found statistically significant ($p < 0.05$) and the highest titratable acidity value was obtained at $4\text{ }^{\circ}\text{C}$. During the cooling process, temperatures of yoghurt samples reached the refrigerated temperature in 135, 195, 270, 565 min at $-10\text{ }^{\circ}\text{C}$, $-5\text{ }^{\circ}\text{C}$, $0\text{ }^{\circ}\text{C}$ and $4\text{ }^{\circ}\text{C}$ (control) respectively. Considering the whole period of cooling, the cooling period was prolonged as the cooling temperature increased. This may be explained that the titratable acidity tends to increase because of the yoghurt bacteria continue to work as the cooling process becomes longer.

3.3. Physicochemical characteristics

The physicochemical characteristics of samples were analysed during the 28 days of storage period and the results were displayed in Table 1. The differences in water holding capacity between cooling temperatures were statistically significant ($p < 0.05$). The results showed that cooling at $0\text{ }^{\circ}\text{C}$ and $-5\text{ }^{\circ}\text{C}$ increased water holding capacity (WHC) from that of $52.26 \pm 0.62\%$ for the control to $54.73 \pm 0.33\%$ and $53.46 \pm 0.64\%$, respectively. Cooling at $-10\text{ }^{\circ}\text{C}$ adversely affected the water holding capacity ($49.98 \pm 0.94\%$). The WHC can be defined as the retention of water by the proteins (Hongyu, Hulbert, & Mount, 2000). For this reason, this could be explained by the fact that the coagulum is constricted because of rapid cooling at $-10\text{ }^{\circ}\text{C}$ and therefore the serum is more easily separated from the curd. Lucey (2008) reported that the cooling of the milk gel caused an increase in the storage modulus, probably because of the rising in the area between the casein particles and swelling of the particles. Chandan (2007) also reported that rapid cooling caused undesirable changes in the structure contributing to whey separation especially due to very rapid interactions of the protein filaments. The effect of storage period on water holding capacity was found to be significant at $p < 0.05$. An increase in WHCs of all samples was determined throughout the 14 days and decreased slightly towards the end of the storage. These findings are in agreement with Küçükçetin, Demir, Aşci, and Çomak (2011) who observed a similar decrease throughout the storage period for yoghurt samples made from goat and cow milk. Similarly, Sert, Akin, and Dertli (2011) in a study that examined the effect of honey addition on the physicochemical, microbiological and sensory

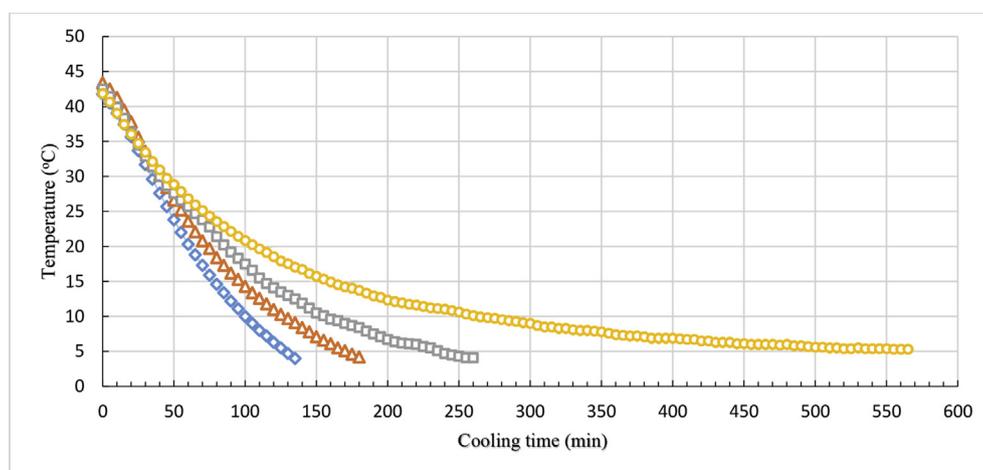


Fig. 1. Temperature curves of yoghurt samples cooling at $-10\text{ }^{\circ}\text{C}$ (\diamond), $-5\text{ }^{\circ}\text{C}$ (\triangle), $0\text{ }^{\circ}\text{C}$ (\square) and $4\text{ }^{\circ}\text{C}$ (\circ) after incubation.

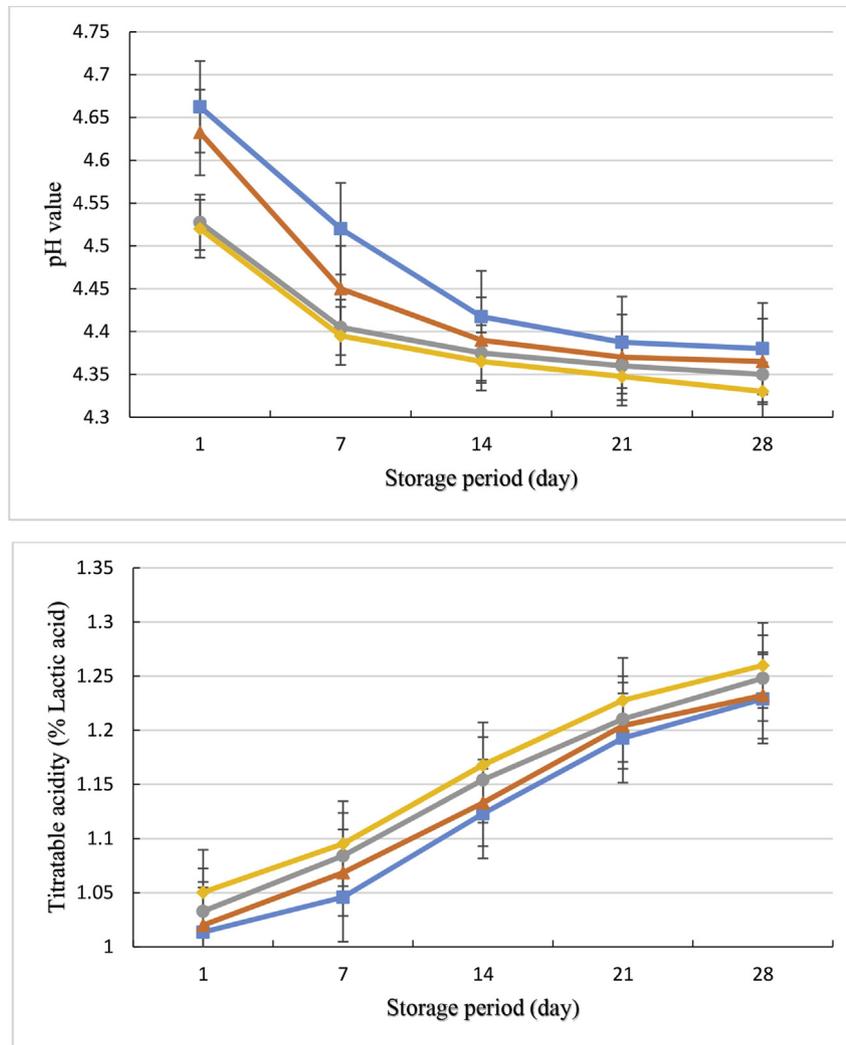


Fig. 2. pH and titratable acidity values of yoghurt samples cooling at $-10\text{ }^{\circ}\text{C}$ (■), $-5\text{ }^{\circ}\text{C}$ (▲), $0\text{ }^{\circ}\text{C}$ (●) and $4\text{ }^{\circ}\text{C}$ (◆) during 28 days of storage.

Table 1

Water holding capacity and syneresis of set type yogurt samples during cold storage.^a

Cooling temperature ($^{\circ}\text{C}$)	Period of storage (days)				
	1	7	14	21	28
Water holding capacity (%)					
-10	$42.37 \pm 0.53^{\text{c,C}}$	$52.17 \pm 0.74^{\text{b,AB}}$	$54.20 \pm 0.28^{\text{c,A}}$	$51.62 \pm 1.16^{\text{b,AB}}$	$49.53 \pm 2.02^{\text{b,B}}$
-5	$47.72 \pm 0.88^{\text{a,AB}}$	$54.37 \pm 0.46^{\text{ab,A}}$	$56.55 \pm 0.63^{\text{ab,A}}$	$54.62 \pm 1.23^{\text{ab,A}}$	$54.55 \pm 0.00^{\text{a,A}}$
0	$49.02 \pm 0.25^{\text{a,C}}$	$54.60 \pm 0.70^{\text{a,B}}$	$57.87 \pm 0.10^{\text{a,A}}$	$56.07 \pm 0.53^{\text{a,B}}$	$56.10 \pm 0.07^{\text{a,B}}$
4	$45.22 \pm 0.46^{\text{b,C}}$	$53.32 \pm 0.39^{\text{ab,AB}}$	$55.48 \pm 0.67^{\text{bc,A}}$	$54.55 \pm 0.92^{\text{ab,AB}}$	$52.72 \pm 0.67^{\text{ab,B}}$
Syneresis (%)					
-10	$36.16 \pm 0.19^{\text{a,A}}$	$34.93 \pm 0.62^{\text{a,AB}}$	$32.87 \pm 0.02^{\text{a,B}}$	$33.12 \pm 0.32^{\text{a,AB}}$	$33.38 \pm 0.25^{\text{a,AB}}$
-5	$34.84 \pm 0.32^{\text{bc,A}}$	$32.36 \pm 0.20^{\text{b,A}}$	$31.95 \pm 0.01^{\text{b,A}}$	$32.30 \pm 0.30^{\text{ab,A}}$	$32.51 \pm 0.04^{\text{ab,A}}$
0	$33.97 \pm 0.16^{\text{c,A}}$	$31.63 \pm 0.52^{\text{b,AB}}$	$30.02 \pm 0.23^{\text{c,B}}$	$31.34 \pm 0.16^{\text{b,AB}}$	$32.39 \pm 0.31^{\text{b,AB}}$
4	$35.97 \pm 0.49^{\text{ab,A}}$	$33.08 \pm 0.87^{\text{ab,A}}$	$32.03 \pm 0.18^{\text{b,A}}$	$32.56 \pm 0.09^{\text{a,A}}$	$33.24 \pm 0.24^{\text{ab,A}}$

^a Values are expressed as the means \pm standard deviation ($n = 3$). Superscript lowercase letters show differences between the yogurt samples and the superscript uppercase letters present differences between the storage time. Different letters in the same column and row are statistically different ($p < 0.05$).

characteristics of yoghurt, determined that the water holding capacity decreased due to cold storage.

It is desired that set yoghurts have semi-solid consistency with no cracks, holes and wheying-off (Lucey & Singh, 1997). Whey separation (syneresis) is defined as the liquid on the surface of a gel and it is one of the most common problem in fermented milks (McCann, Fabre, & Day, 2011). The effect of cooling temperatures on

whey separation (syneresis) was therefore measured and the values are shown in Table 1. The yoghurts cooled at $0\text{ }^{\circ}\text{C}$ after incubation had lower syneresis values compared with other yoghurt samples. Cooling at $-10\text{ }^{\circ}\text{C}$ and $4\text{ }^{\circ}\text{C}$ significantly increased syneresis. When the results are examined, it can be stated that the cooling of yoghurt samples below $-5\text{ }^{\circ}\text{C}$ and above $0\text{ }^{\circ}\text{C}$ has a negative effect on syneresis in similar proportions. Whey loss of all

samples decreased until 14th day of cold storage and then increased. It can be said that the increase in syneresis after 21st and 28th day of storage is related to increasing acidity, since it is known that higher acidity stimulates whey loss in yoghurts (Lucey, 2001; Lucey, Munro, & Singh, 1998; Tamime & Robinson, 2007).

3.4. Microbiological analyses

Microbiological counts of yoghurt samples were determined once a week in the 28-day storage period; this was to investigate the effect of cooling temperatures on yoghurt starter culture throughout the storage. Generally, the decrease in temperature prolongs the exponential phase and generation times of microorganisms and therefore microbial growth slows down. The activity of natural and microbial enzymes decreases as the temperature decreases (Ünlütürk & Turantaş, 2003). As a result of the microbial analysis, *Lb. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* counts were found to decrease due to a decrease in cooling temperature (Fig. 3). There were lower counts for *S. thermophilus* compared with *Lb. delbrueckii* ssp. *bulgaricus* in all samples and the difference between the cooling temperatures was statistically significant ($p < 0.05$). Moreover, after 28 days of storage microbial counts declined from $8.17 \log \text{ cfu mL}^{-1}$ to $7.46 \log \text{ cfu mL}^{-1}$ for *Lb. delbrueckii* ssp. *bulgaricus* and $7.58 \log \text{ cfu mL}^{-1}$ to $7.14 \log \text{ cfu mL}^{-1}$ for *S. thermophilus*. Several studies have reported a significant decrease throughout the storage period for *Lb. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* counts in yoghurts fortified with vegetables (Najgebauer-Lejko et al., 2015), strawberry (Oliveira et al., 2015), passion fruit peel powder (do Espírito Santo, Perego, Converti, & Oliveira, 2012), lentil flour (Zare et al., 2011) and yoghurts made from buffalo and bovine milk (Nguyen, Ong, Lefevre, Kentish, & Gras, 2014). In addition to these, in the present study, yeast-mould counts were found to be $< 1 \text{ cfu mL}^{-1}$ on the 28th day of the storage and there were no coliform group bacteria in any of the yoghurt samples.

3.5. Textural properties

The firmness of yoghurt is defined as the force required to compress the sample between the teeth or tongue and palate (Guggisberg, Cuthbert-Steven, Piccinali, Bütikofer, & Eberhard,

2009). In this study, the force required to push the cylinder probe into yoghurt samples was measured at different cooling temperatures and storage period and all textural parameters are shown in Fig. 4. The average firmness values of samples ranged between $268.24 \pm 6.42 \text{ g}$ and $572.30 \pm 17.70 \text{ g}$. The highest firmness values were obtained from yoghurts cooled at 0°C . Firmness increased until the 14th day of storage and slightly decreased on the 21st and 28th days of storage. However, there was no statistically significant difference between 0°C and -5°C throughout storage. Several researchers (Akalin, Unal, Dinkci, & Hayaloglu, 2012; do Espírito Santo et al., 2012; Mani-López et al., 2014) noted that firmness increased during the all storage period while Bonczar et al. (2002) stated that firmness reached the highest value on the 7th day of storage and then started to decrease. This situation can be caused by increasing acidity towards the last weeks of cold storage. Modler and Kalab (1983) stated in their study that there is a correlation between the firmness of yoghurt and its tendency to syneresis. Water holding capacity and syneresis data obtained from the present study also support these findings (Table 1).

The effect of cooling temperatures on the consistency was found to be significant ($p < 0.05$). The highest consistency values were in yoghurts cooled at 0°C whilst cooling at -10°C was lowest. Consistency values significantly increased throughout the storage, being the highest increase observed at 14th day. Similarly, do Espírito Santo et al. (2012) showed that the consistency of yoghurts increased during the storage period. It was determined that the effect of the cooling temperature and cold storage on cohesiveness and viscosity index was found to be statistically significant ($p < 0.05$). The highest value for these parameters is recorded at 0°C and the lowest is at -10°C . The researchers attributed the high cohesiveness value to a firmer, stronger, harder gel structure (Gastaldi, Lagaude, Marchesseau, & Fuente, 1997); the firmness data obtained support this result (Fig. 4). A reduction in cohesiveness and viscosity index values of all yoghurts was recorded after 14 days of storage. Aportela-Palacios, Sosa-Morales, and Vélez-Ruiz (2005) stated that this might be due to the stability loss of protein. Assem (2013) reported that cohesiveness increased in the last weeks of the storage compared with the first day but fluctuations were observed between storage weeks. Sahan et al. (2008) noticed that the highest viscosity values were recorded on the 15th day of storage during the storage period. The increase in viscosity during

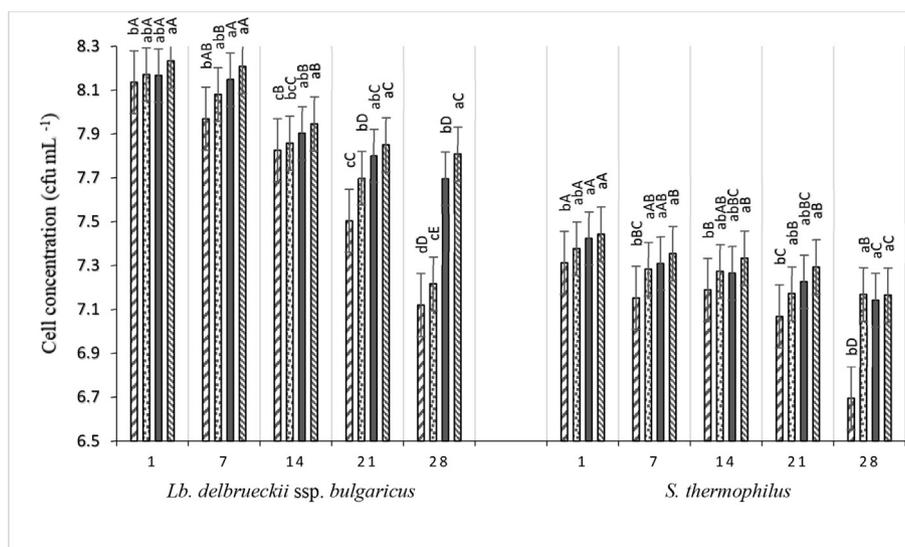


Fig. 3. Effect of cooling temperatures (-10°C , -5°C , 0°C , 4°C , unlabeled) on viability of yoghurt culture (*Lb. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*) during cold storage. Upper-case letters show the differences between the storage period and the lower-case letters present differences between the samples in the same storage period.

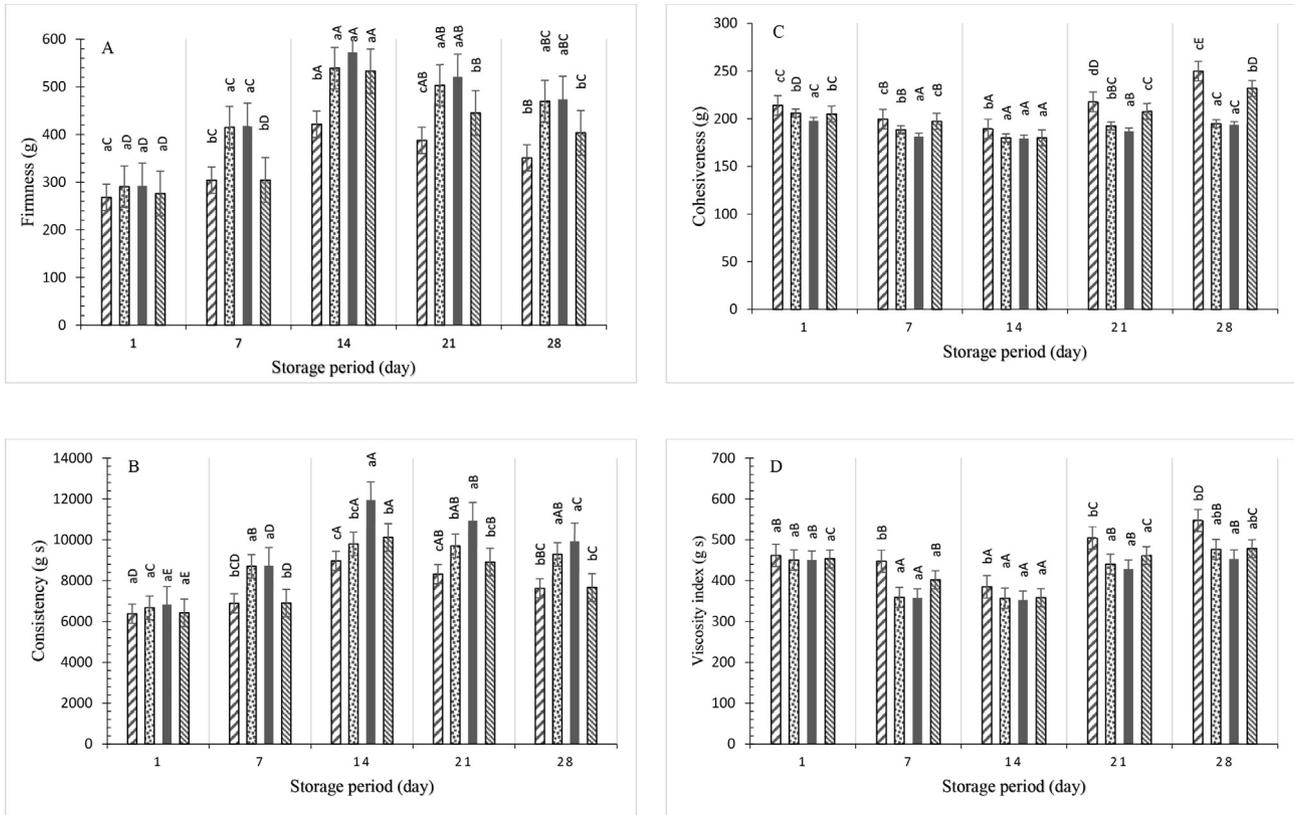


Fig. 4. Textural profile of set yoghurt samples cooling at -10°C (▨), -5°C (▩), 0°C (▣), and 4°C (▤) during storage: A, firmness; B, consistency; C, cohesiveness; D, viscosity index. Upper-case letters show the differences between the storage period and the lower-case letters present differences between the samples in the same storage period.

storage was also determined in a study by [Isleten and Karagul-Yuceer \(2006\)](#) and reported that this increase may be due to protein–protein interaction and protein rearrangement.

3.6. Gel microstructure

Confocal laser scanning microscope images were taken on the 1st day of the storage period. Confocal analysis images of samples are presented in [Fig. 5](#). The red areas in the images show Rhodamine B stained protein matrix and black areas are whey phase. To avoid texture defects in yoghurt, the whey phase must be homogeneously distributed and the whey area should be at a minimum level within the protein matrix. The presence of high serum area is the most important indicator that yoghurt has a greater tendency to syneresis.

The images showed that the whey phase is more than the other samples in yoghurt with cooling at -10°C . The casein network shows a heterogeneous structure with thicker chains and larger clusters. This irregularity in the microstructure of yoghurts is most likely a consequence of very rapid cooling that destroys the homogeneous network of gels. The yoghurt samples cooled at 0°C had more dense and homogeneous and less porous structure compared with the other samples. Some researchers have reported a correlation between the microstructure of the yoghurt, its firmness and its tendency to whey separation ([Modler & Kalab, 1983](#)). Also, [Lee and Lucey \(2004\)](#) noticed that high serum phase caused weak gel structure. These images support the present results of whey separation, water holding capacity and textural measurements at time 0. Numerous studies on the microstructure of

yoghurt have been done by researchers ([Ciron, Gee, Kelly, & Auty, 2010](#); [Kristo, Miao, & Corredig, 2011](#); [Krzeminski, Großhable, & Hinrichs, 2011](#); [Lucey, Munro, & Singh, 1999](#); [Yang et al., 2014](#)), but the effect of cooling temperature after incubation on yoghurt microstructure is not included in the literature.

3.7. Sensory evaluation

The average score for sensory attributes of set yoghurt samples is shown in [Table 2](#). Cooling temperatures and storage period significantly ($p < 0.05$) affected all sensory parameters. As a result of the sensory analyses, the highest score in terms of appearance, texture, mouth-feel, odour and taste was taken from the yoghurt sample that was cooled to 0°C . The lowest score for these parameters is recorded at -10°C . The high syneresis values of the yoghurts cooled at -10°C ([Table 1](#)) may be indicative of the negative impact of this parameter on consumer preference. No statistical differences were detected between 0°C and 4°C (control yoghurts) with regards to all parameter scores while these scores were lower in -5°C and -10°C . Decreasing of cooling temperatures below 0°C negatively influenced the scores for taste due to the low acid development and lactobacilli counts. Especially *Lb. delbrueckii* ssp. *bulgaricus* can influence the acetaldehyde content with yoghurt flavour ([Ekinci & Gurel, 2008](#); [Güler-Akin & Akin, 2007](#)). The pH, titration acidity and microbiological values of yoghurt samples supported this reduction in taste scores ([Figs. 2 and 3](#)). The appearance and taste were positively affected while the odour, texture and mouth-feel were adversely affected by the increasing storage time.

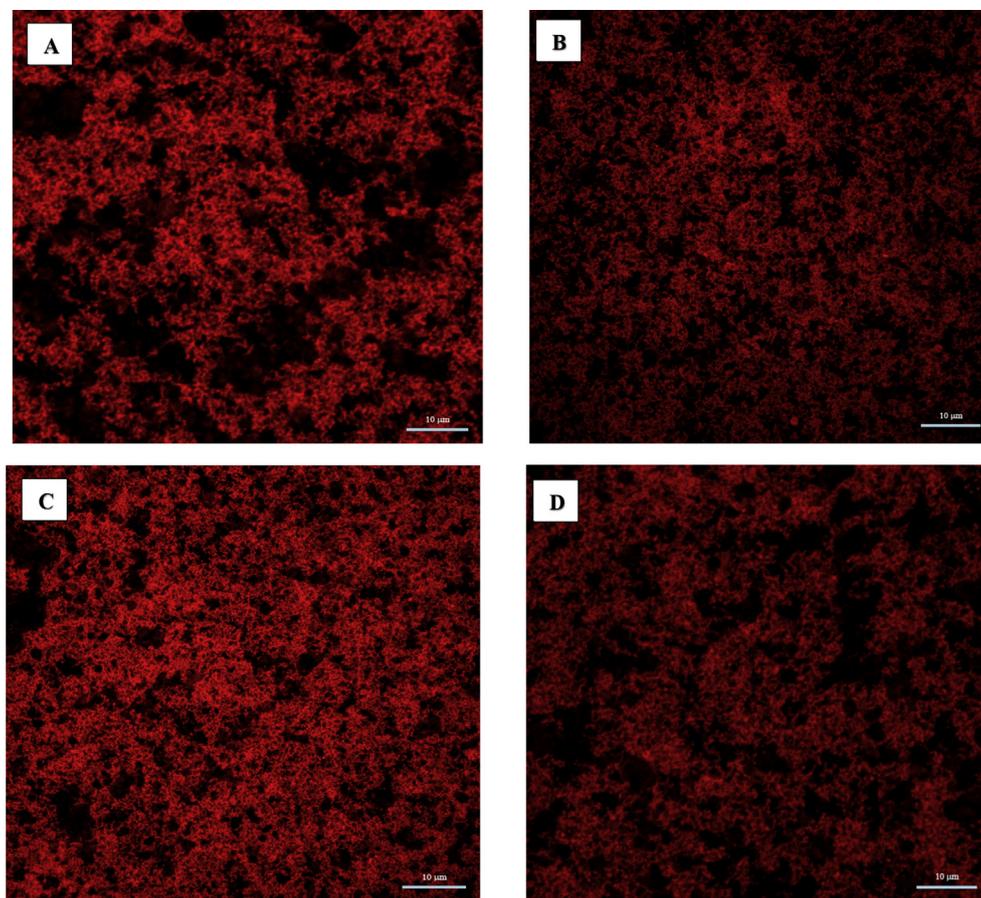


Fig. 5. Confocal laser scanning micrographs of yoghurt samples cooling at $-10\text{ }^{\circ}\text{C}$ (A), $-5\text{ }^{\circ}\text{C}$ (B), $0\text{ }^{\circ}\text{C}$ (C) and $4\text{ }^{\circ}\text{C}$ (D) on the 1st day of the storage period.

Table 2
Sensory attributes of set yoghurt samples.^a

Parameter	Appearance	Texture	Mouth feel	Odour	Taste
Cooling temperature ($^{\circ}\text{C}$)					
-10	3.65 ± 0.23^b	3.86 ± 0.24^b	3.89 ± 0.15^b	4.12 ± 0.2^b	4.05 ± 0.07^c
-5	3.71 ± 0.19^b	4.08 ± 0.07^{ab}	4.03 ± 0.17^{ab}	4.16 ± 0.13^b	4.25 ± 0.05^{bc}
0	4.41 ± 0.42^a	4.21 ± 0.15^a	4.31 ± 0.15^a	4.36 ± 0.22^a	4.49 ± 0.12^a
4	4.4 ± 0.44^a	4.32 ± 0.25^a	4.24 ± 0.2^a	4.35 ± 0.13^a	4.44 ± 0.19^{ab}
Storage period (days)					
7	3.96 ± 0.15^b	4.24 ± 0.25^a	4.25 ± 0.19^a	4.39 ± 0.12^a	3.96 ± 0.15^b
21	4.12 ± 0.68^a	4.0 ± 0.2^b	3.99 ± 0.19^b	4.1 ± 0.13^b	4.12 ± 0.68^a

^a Values are means \pm standard deviation ($n = 3$); different superscript letters within each column indicate statistically significant differences ($p < 0.05$).

4. Conclusion

In the study, it was investigated whether there was a physico-chemical, microbiological, textural and sensory difference between yoghurt samples that were cooled at 4 different temperatures after incubation. It was observed that the cooling temperature affects the properties of the yoghurt gel throughout the storage period. The effect of cooling temperatures on acidity, water holding capacity, syneresis, textural and microbiological properties and microstructure were found to be statistically significant. As the cooling temperature decreased, the cooling time was shortened. Cooling below $-5\text{ }^{\circ}\text{C}$ and above $0\text{ }^{\circ}\text{C}$ had a negative effect on the syneresis, water holding capacity and textural properties. Gel characteristics of yoghurt samples cooled to $0\text{ }^{\circ}\text{C}$ were better than the other groups. Cooling at $0\text{ }^{\circ}\text{C}$ has improved both gel properties and shortened the cooling time, saving time and energy. Achieving the

desired physical and sensory qualities will also increase consumer satisfaction. This work can be made more comprehensive with on-site applications and recommendations can be made on behalf of enterprises to obtain optimum gel properties.

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