



## Introduction

Yogurt making dates back to many centuries, although there is no accurate record of the date when it was first made. According to the legend, yogurt was first made by the ancient Turkish people in Asia (Tarakçı, 2010). For thousands of years, yogurt has been popular fermented milk in the Middle East and, for the most part, the product was made in individual households or on a limited communal scale (Robinson, 2002b). Both historically and commercially, yogurt is the most popular product made with thermophilic cultures, and a typical commercial sample will contain millions of viable cells of *Streptococcus salivarius subsp. thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* (Robinson, 2002a). The gel structure of yogurt results primarily from acid effect, which was created by these bacteria, on the integrity of the casein micelle (Rawson and Marshall, 1997).

At present, the retail markets of many countries are dominated by two types of yogurt. One type has a firm, gel-like structure together with a clean, mildly acidic and slightly aromatic flavour – ‘natural set yogurt’, while the other has the consistency of ‘double cream’ and the taste and aroma of yogurt is usually modified by the addition of fruit/favours and sugar – ‘stirred yogurt’ (Robinson, 2002a). Set type yogurt is more popular in Turkish market. People in Turkey still continue to make yogurt in their own homes. For this purpose, they can make yogurt using the same yogurt repeatedly. Also, when people do not have yogurts, they may request yogurt to use as inoculum from their neighbours. People who cannot find homemade yogurt as inoculum can also use commercial yogurts or cultures for this purpose. Recently, some consumers have complained that the quality of yogurts produced from commercial yogurts is not at the desired level. The problems are watery/weak texture and ropiness in structure. The aim of this study is to investigate the suitability of commercial yogurts as inoculum for yogurt production. Four different yogurt companies with the highest market share in Turkey was selected as the material. The yogurt was produced by the use of these commercial yogurts as the first inoculum. pH, titratable acidity, serum separation, viscosity values and *Lb. delbrueckii subsp. bulgaricus* and *S. salivarius subsp. thermophilus* counts in yogurts obtained by fermenting four times in succession and in yogurts stored at 4°C for 21 days were determined.

## Materials and Methods

Four commercial yogurt samples (A, B, C and D) were supplied from the local market in Çankırı (Turkey) for this study in April–2016. UHT milk which contains 3.1% fat, 2.8% protein, 4.7% lactose (Dost, Ak Gıda, Turkey) was used for yogurt production.

## Production of Yogurt

330 mL glass jars with metal lid were wrapped in aluminum foil and sterilized for 150 min at 170 °C. 200 mL of UHT milks, which were kept in 45 °C water bath for 1 hour in a packaged state, were transferred into sterile jars under aseptic conditions. 4 g (2%) of the commercial yogurts as inoculum source were added to the milk and mixed. They were incubated at 43 ±0.5 °C until pH was below 4.6 and then taken to the refrigerator at 4 °C. The next yogurt production was carried out using a 24-hour yogurt sample. Thus, 4 generation of yogurt productions were consecutively performed. In addition, first generation yogurts were stored in the refrigerator for 21 days and analyzed for the same parameters on the 1st, 7th, 14th and 21st days of storage. Two replicates of yogurt production were performed.

## Analytical Methods

The pH was measured through a pH meter (Ohaus, ST3100, Switzerland) on yogurt directly. The titratable acidity was determined as lactic acid percentage by titrating with 0.1 N NaOH, using phenolphthalein as an indicator. Viscosity measurement on stirred yogurt samples was performed under room temperature (23 ± 2 °C) using a Brookfield DV2T Viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA), equipped with a No. 3 spindle running at 15 rpm. Viscosity readings were carried out at the point of the 30th second. Syneresis of the yogurt samples were measured by the centrifugation. Yogurt (40 g) was weighed in centrifuge tubes and centrifuged at 2500 rpm for 10 min at 4 °C. The supernatant was separated, weighed and syneresis was calculated according to the following equation (Farnsworth et al., 2006):

$$\text{Syneresis (\%)} = \frac{\text{weight of supernatant (g)}}{\text{weight of yogurt sample (g)}} \times 100$$

## Microbiological Analyses

Ten gram yogurt sample was mixed with 90 mL of ¼ Ringer solution (Merck, Germany) and homogenized uniformly with a stomacher (BagMixer 400, Interscience, France). Subsequent serial dilutions were prepared and microbial numbers determined using pour plate technique. *S. salivarius subsp. thermophilus* counts were enumerated on ST agar (HiMedia, India) aerobically at 37°C for 72 h. MRS agar (Merck, Germany) was used for the enumeration of *Lb. delbrueckii subsp. bulgaricus* at 43°C for 5 days (Dave and Shah, 1997).

## Results and Discussion

### Analytical Characteristics

The pH profile of yogurt samples during the incubation period in 4 generations is shown in Figure 1. Except for the D sample, the slowest decrease in the pH between the generations was obtained in the first generation yogurt. This period lasted 210 minutes in samples A and B, 240 minutes in sample C and 180 minutes in sample D. In all samples, fermentations of the next 2nd, 3rd and 4th generation yogurts were completed in 180 minutes. Only the long incubation time of the first generation yogurt may be due to the adaptation of the culture to the new milk and waiting time of the commercial yogurts in the groceries' shelves. There was no difference between the incubation times of the 2nd, 3rd and 4th generation yogurts ( $P > 0.05$ ). The difference among the samples was significant ( $P < 0.05$ ). The highest pH value was found in the

sample C and the lowest was observed in the sample A ( $P < 0.05$ ). Robinson et al. (2006) stated that decreasing to the isoelectric point of caseins (pH 4.6) and increasing to the level of 1.0–1.2% (w/v) titratable acidity took in 3–4 hours. Mohammadi et al. (2011) and De Brabandere and De Baerdemaeker (1999) determined that incubation time was 190 min and 3–3.5 hours, respectively.

Figure 2 shows the change in the pH of the 1st generation yogurt samples during the 21-day storage period. There was a rapid decrease in pH until the 7th day and the pH tended to remain constant after the 7th day. Considering the mean values during storage, the highest pH was observed in yogurt C, while the lowest pH was determined in yogurt A ( $P < 0.05$ ). B and D yogurts showed similar pH values ( $P > 0.05$ ). Güler-Akın (2005), Tarakci et al. (2010) and Dabrowska et al. (2017) found similar results for pH in different yogurt samples.

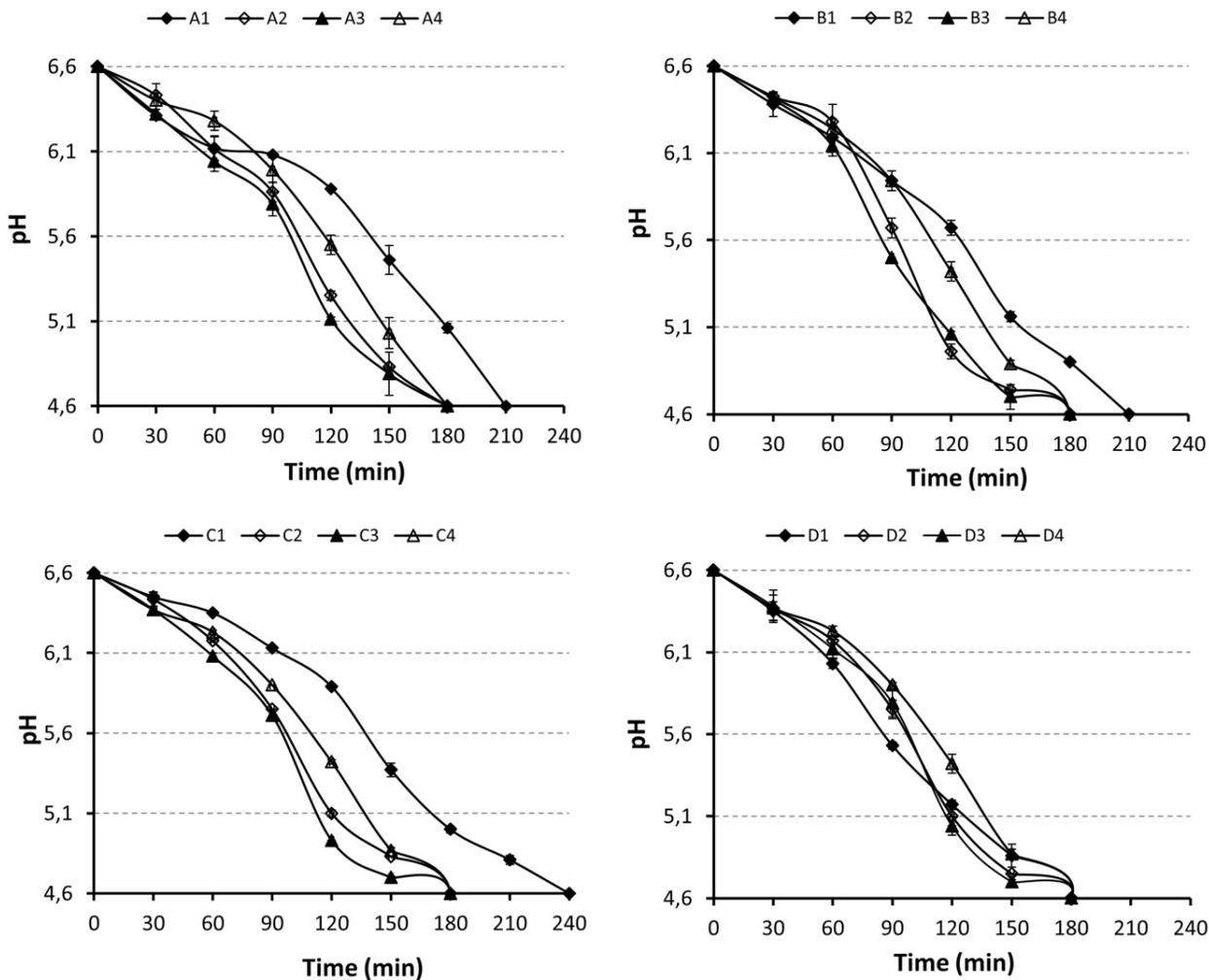
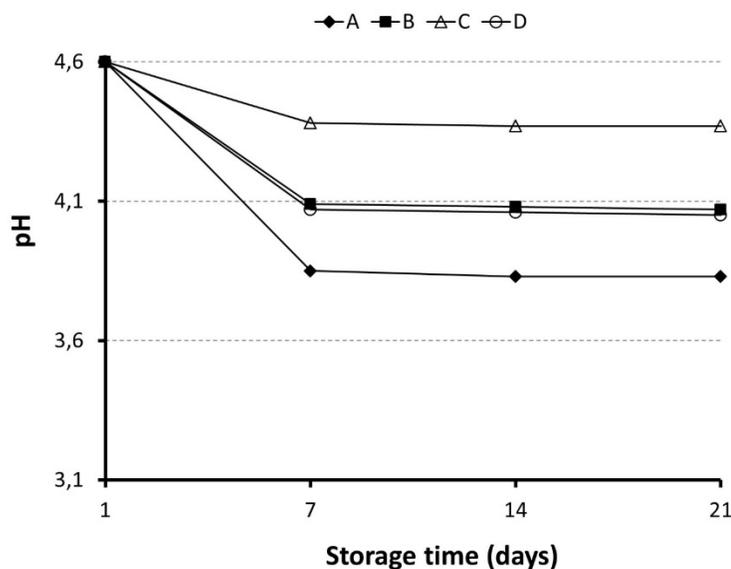


Figure 1. pH profil of generations in yogurt samples



**Figure 2.** pH in yogurts during cold storage

Titrateable acidity values in the 4 generations of samples are presented in Figure 3. While the differences among the generations were not significant ( $P>0.05$ ), there was a significant difference among the mean titrateable acidity values of the samples ( $P<0.05$ ). Titrateable acidity values ranged from 0.69 to 0.92% among the generations. The highest mean titrateable acidity values were observed in the sample A, the lowest titrateable acidity was determined in the sample C ( $P<0.05$ ). It was observed that these titrateable acidity values were compatible with pH values. Similar titrateable acidity values were obtained by Güler–Akın (2005) and Tarakci et al. (2010).

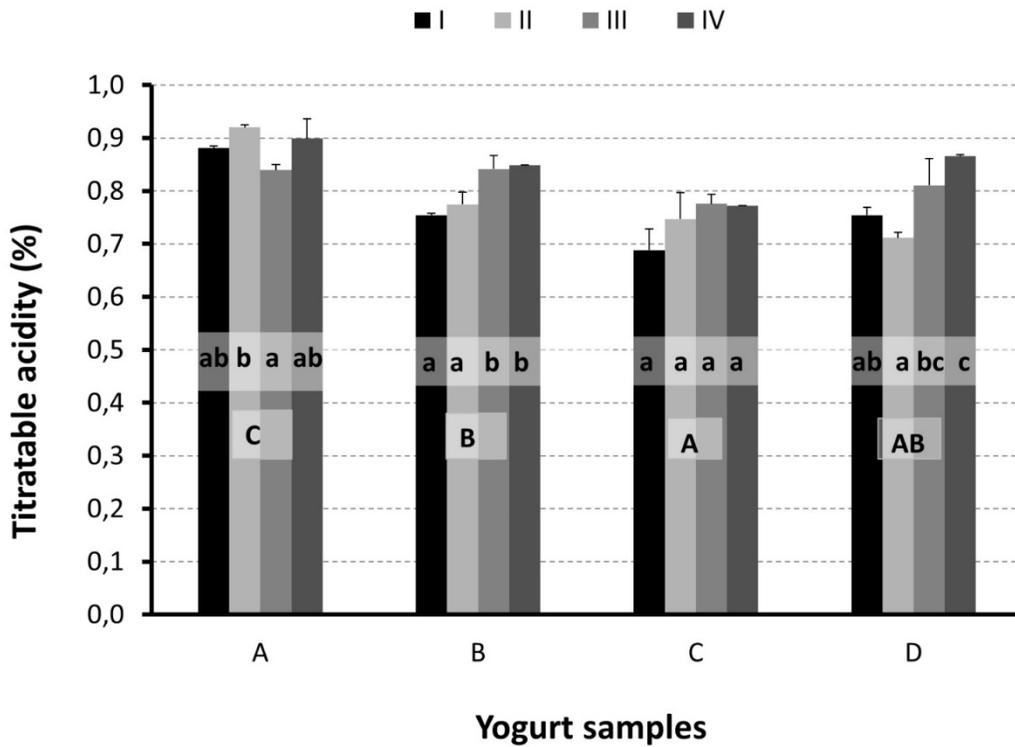
The titrateable acidity change of the 1st generation yogurt samples during 21 days of storage is shown in Figure 4. As the storage period progressed, the titrateable acidity values of the yogurts increased slightly. Considering the mean values during storage, the highest titrateable acidity was found in yogurt A, while the lowest acidity values were detected in C. B and D yogurts presented similar acidity values. For titrateable acidity, Güler–Akın (2005), Mudawi et al. (2014), Ramchandran and Shah (2010) Joung et al. (2016) found similar results during storage.

The viscosity values of yogurt samples are given in Figure 5. While the differences among the generations were significant ( $P<0.05$ ), the difference among the mean viscosity values of the samples was found to be not significant ( $P>0.05$ ). Viscosity values in samples ranged from 2374 (sample C) to 3153 (sample B) cP among the generations. The highest mean viscosity values were observed in the sample B, the lowest viscosity was determined in the sample A. Bacterial culture

compositions or specific strains, especially those releasing exopolysaccharides or containing probiotics, take an active role in the forming of yogurt texture (Espírito–Santo et al., 2013). While the first generation production gave the lowest viscosity ( $P<0.05$ ), no significant difference was observed among the other 2nd, 3rd and 4th generations. This may be related to the process of adaptation of starter bacteria to the new milk environment in the first generation production. Tarakci et al. (2010) found similar results for viscosity in different yogurt samples.

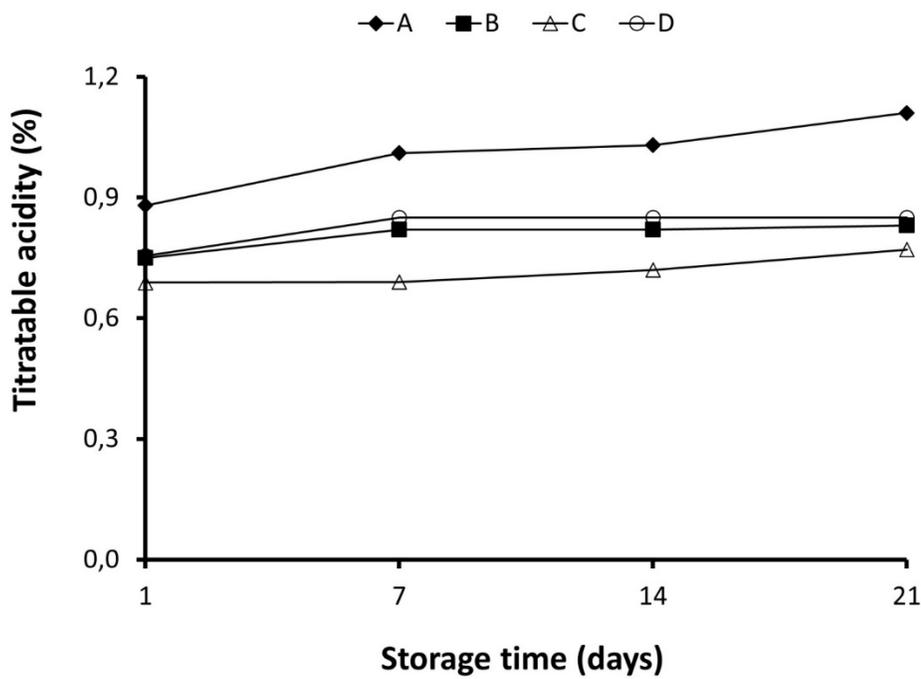
The viscosity values of the 1st generation yogurt samples during the storage are shown in Figure 6. The viscosity values of the yogurts increased till the 7th day. After the 7th day, viscosity decreased slightly. However, the viscosity values in the 1th day were similar to the values in the end of storage. Considering the mean values during storage, yogurts B and C showed higher viscosity than yogurts A and D ( $P<0.05$ ). Krisnaningsih et al. (2019) found similar results for viscosity in yogurt samples during storage.

Syneresis values in the 4 generations of samples are presented in Figure 7. While the differences among the generations were significant ( $P<0.05$ ), there was no significant difference among the mean syneresis values of the samples ( $P>0.05$ ). Syneresis values ranged from 18.36 (Sample B) to 27.78% (sample D) among the generations. The syneresis value of the 4th generation yogurts was significantly lower ( $P<0.05$ ). While Güler–Akın, (2005), Farnsworth et al. (2006) and Abbasi et al. (2009) determined lower syneresis than those of the present study depending on the total solids contents, heat treatment conditions, and presence of additives.

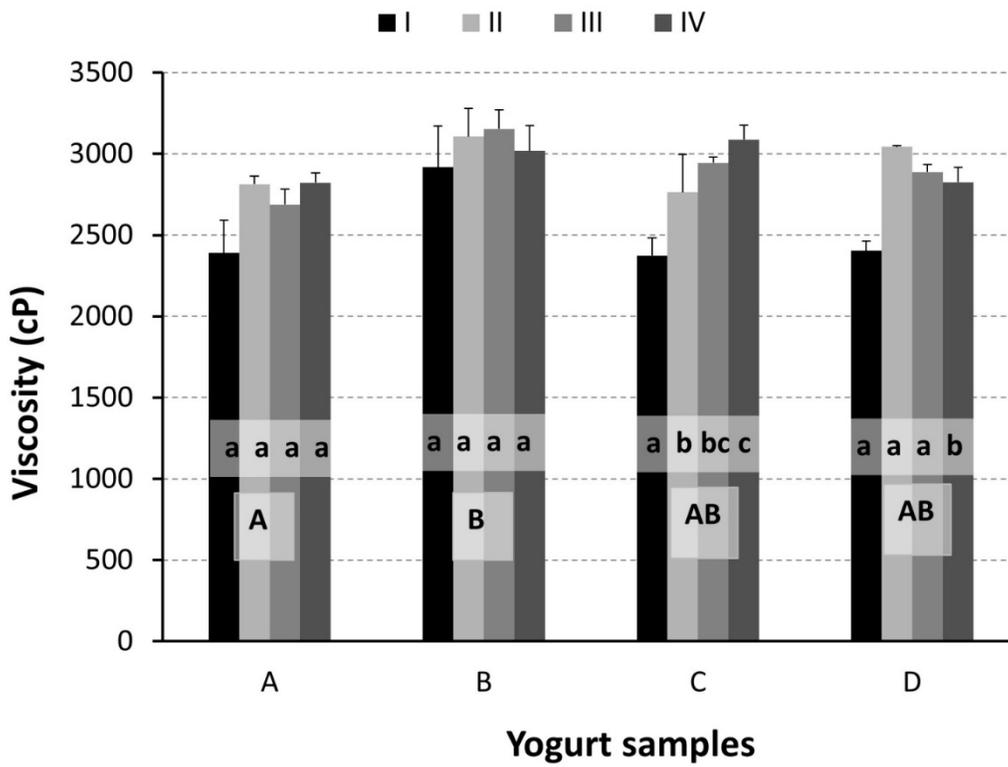


A-C: Means with same letters in a row within the category for samples are not significant at  $P > 0.05$   
 a-c: Means with same letters in a row within the sample for generations are not significant at  $P > 0.05$

**Figure 3.** Titratable acidity changes in yogurt samples.

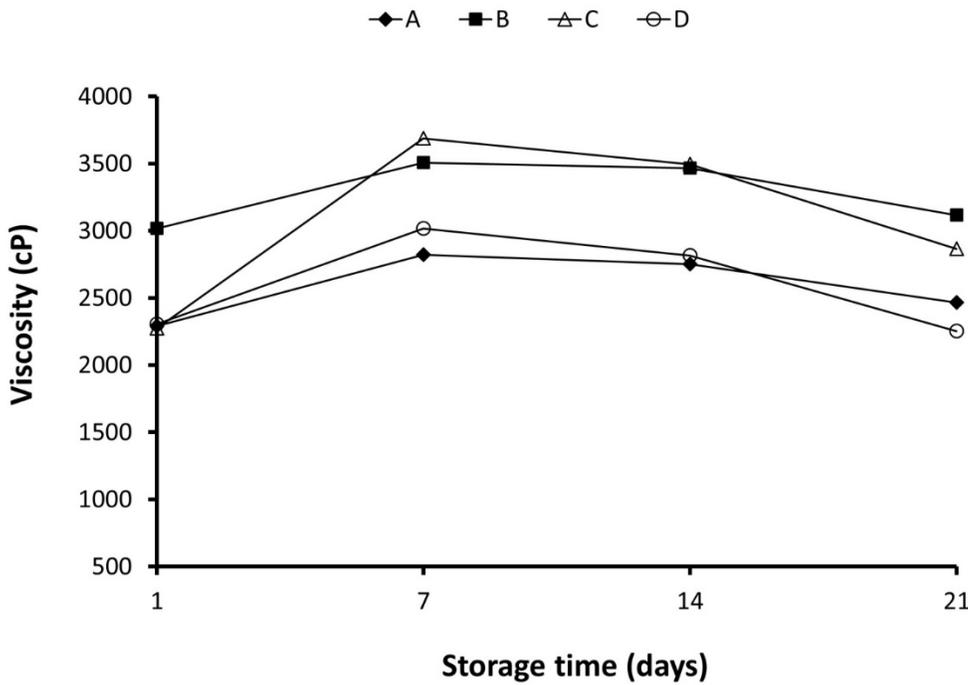


**Figure 4.** Titratable acidity in yogurts during cold storage

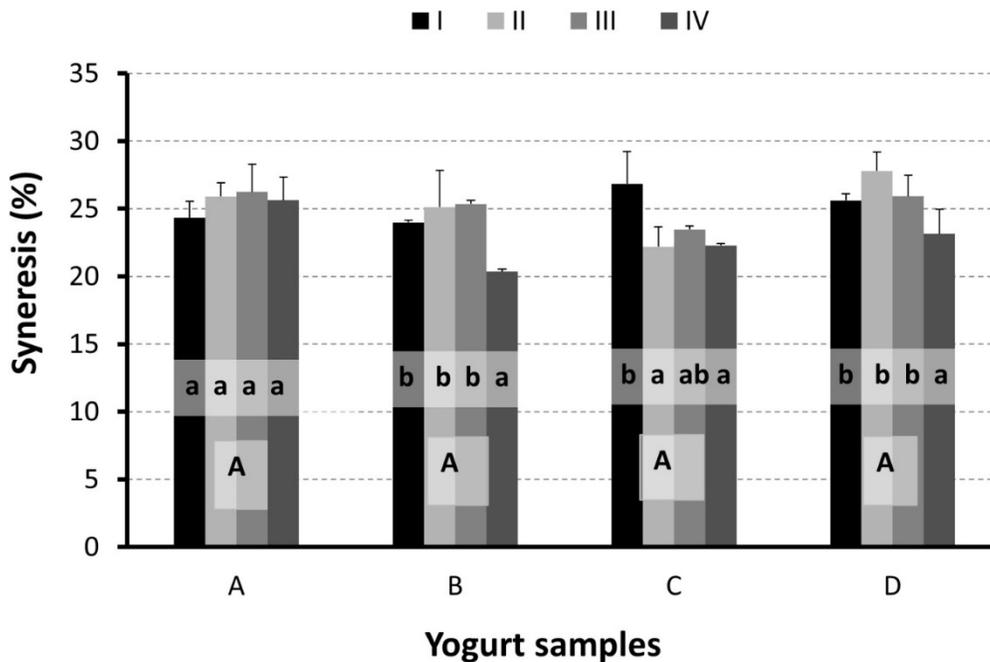


A-C: Means with same letters in a row within the category for samples are not significant at  $P > 0.05$   
 a-c: Means with same letters in a row within the sample for generations are not significant at  $P > 0.05$

**Figure 5.** Viscosity values in yogurt samples



**Figure 6.** Viscosity in yogurts during cold storage



<sup>A</sup>: Means with same letters in a row within the category for samples are not significant at  $P>0.05$

<sup>a-b</sup>: Means with same letters in a row within the sample for generations are not significant at  $P>0.05$

**Figure 7.** Syneresis values in yogurt samples

The profile of syneresis values of 1st generation yogurt samples during storage is shown in Figure 8. In general, syneresis values tended to remain constant during storage. Considering the mean values during storage, there was no significant difference among the yogurt sample ( $P>0.05$ ). While Güler-Akın (2005) and Tarakci et al. (2010) found a decrease in syneresis during storage, Mudawi et al. (2014) observed an increase in whey separation.

It is normal for yogurts produced by using commercial yogurts as inoculum in home conditions to be different from commercial yogurts in terms of structure and texture. Because milk is standardized, homogenized and evaporated in the production of commercial yogurts. The consistency of the yogurt produced at home using commercial yogurt will not be the same with original commercial yogurt. Yogurt produced at home will be relatively weaker.

#### Evaluation of Microbiological Counts

*Lb. delbrueckii* subsp. *bulgaricus* counts in the 4 generations of samples are shown in Figure 9. While the differences among the generations were not significant ( $P>0.05$ ), there was a significant difference among the mean *Lb. delbrueckii* subsp. *bulgaricus* counts of the samples ( $P<0.05$ ). *Lb. delbrueckii* subsp. *bulgaricus* counts ranged from 4.25 (sample

C) to 9.28 (sample A) log cfu  $g^{-1}$  among the generations. The highest mean *Lb. delbrueckii* subsp. *bulgaricus* counts were observed in the sample A, the lowest counts were determined in the sample C ( $P<0.05$ ). It was observed that these *Lb. delbrueckii* subsp. *bulgaricus* counts were compatible with titratable acidity pH values. Some authors reported similar *Lb. delbrueckii* subsp. *bulgaricus* results with yogurts A, B and D (Miller et al., 2002; Güler-Akın, 2005; Asensio-Vegas, et al., 2018). As in the C sample, there are also a few studies reporting a low *Lb. delbrueckii* subsp. *bulgaricus* counts (Dave and Shah, 1997; Lopes et al., 2019).

The *Lb. delbrueckii* subsp. *bulgaricus* counts of 1st generation yogurt samples during the storage are shown in Figure 10. The *Lb. delbrueckii* subsp. *bulgaricus* counts of the yogurts tended to decrease during storage. With the exception of the C sample showing the lowest *Lb. delbrueckii* subsp. *bulgaricus* counts ( $P<0.05$ ), the bacteria counts in the samples during storage slightly decreased. Considering the mean values during storage, yogurts A, B and D showed similar counts ( $P>0.05$ ). Güler-Akın (2005) and Asensio-Vegas, et al. (2018) found that *Lb. delbrueckii* subsp. *bulgaricus* counts decreased slightly during storage.

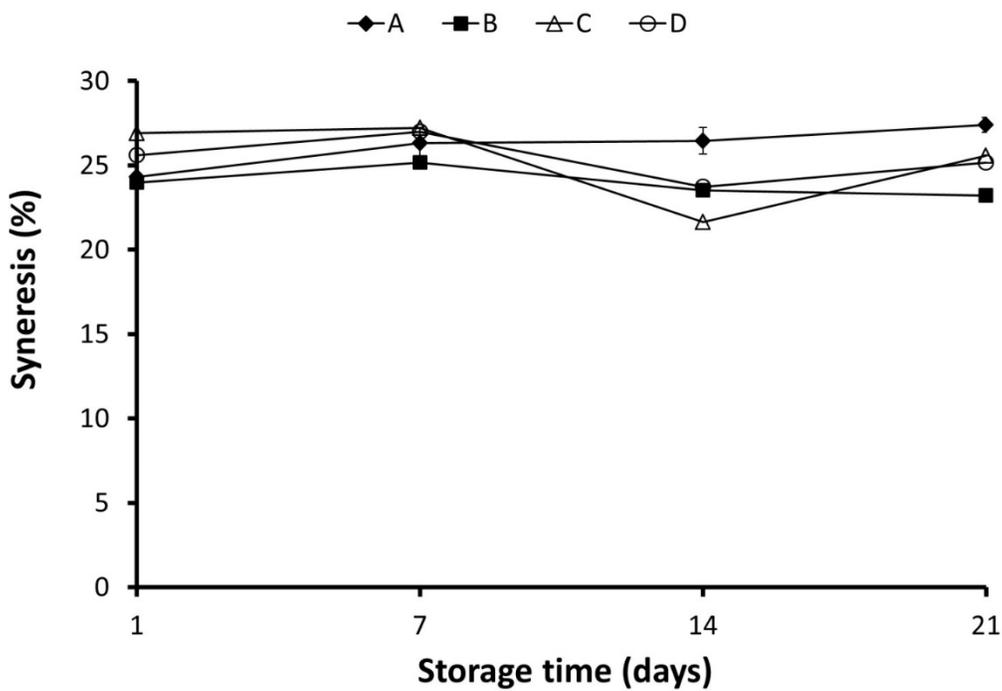
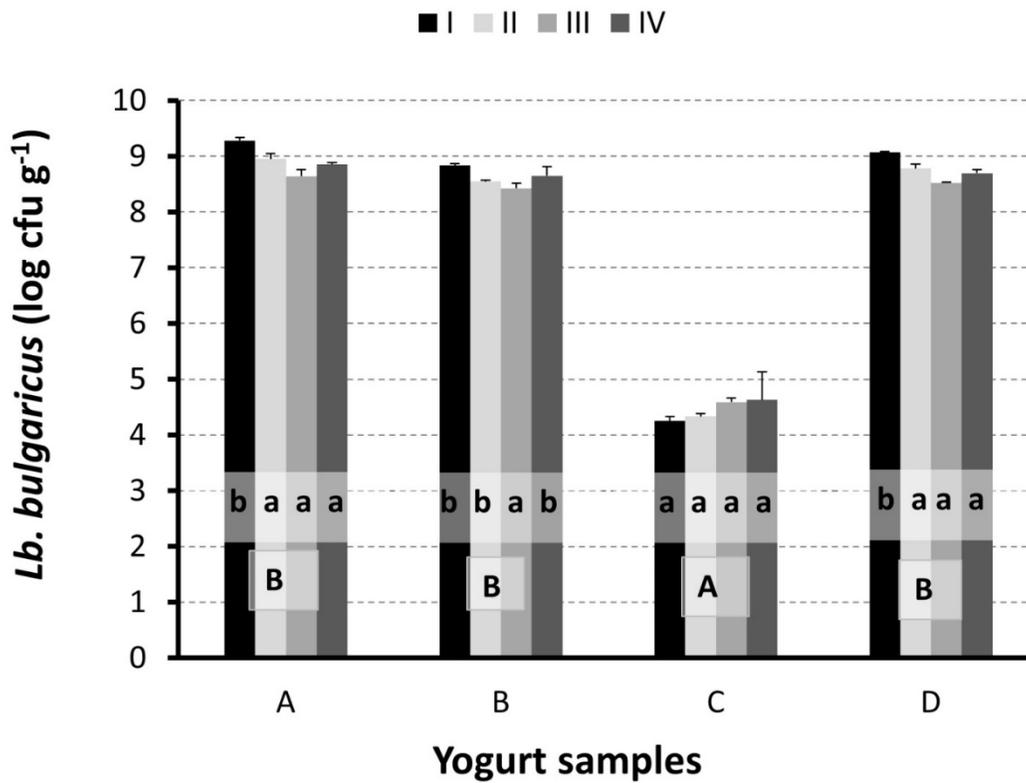


Figure 8. Syneresis in yogurts during cold storage



A-B: Means with same letters in a row within the category for samples are not significant at P>0.05  
 a-b : Means with same letters in a row within the sample for generations are not significant at P>0.05

Figure 9. *Lb. delbrueckii* subsp. *bulgaricus* counts in yogurt samples

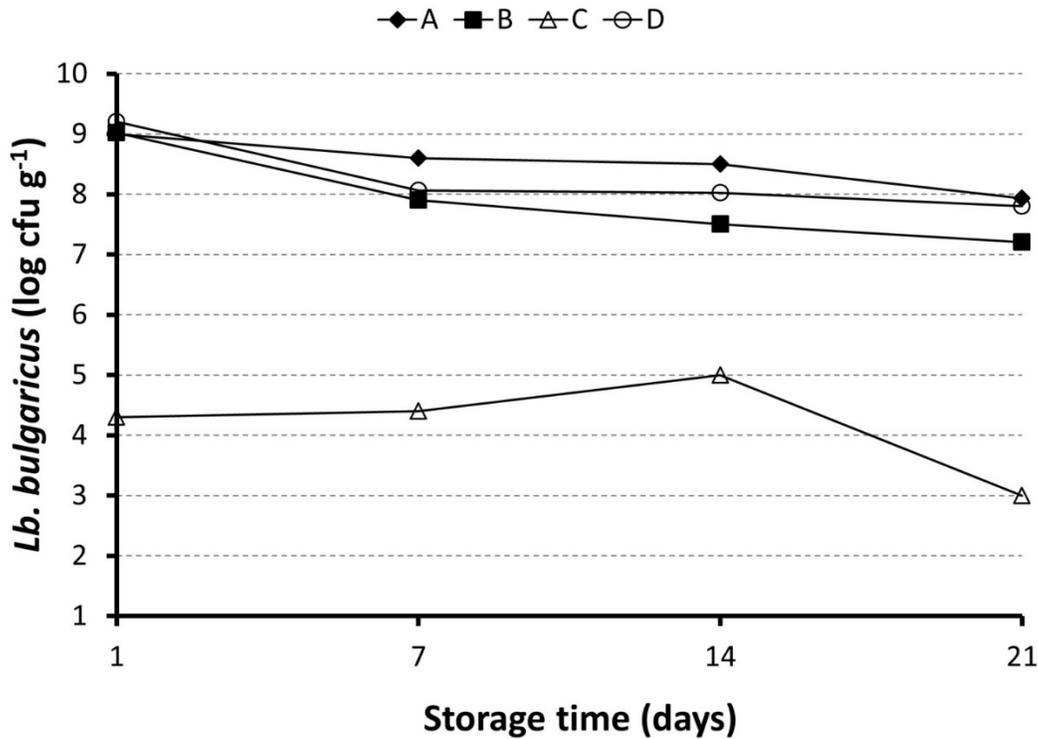


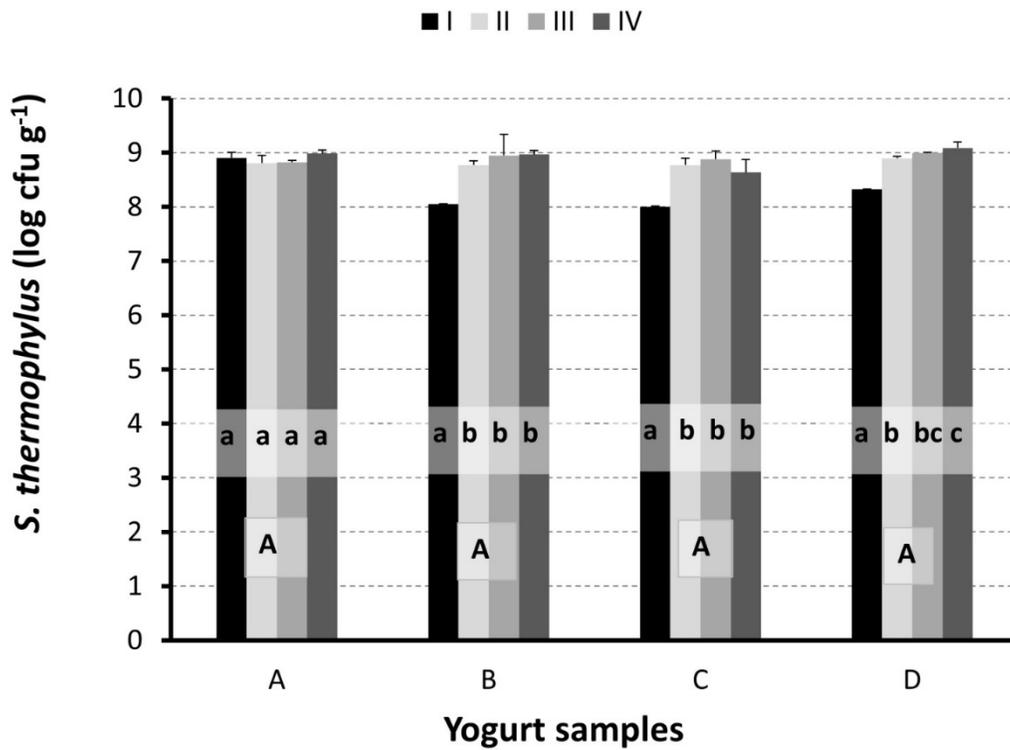
Figure 10. *Lb. delbrueckii* subsp. *bulgaricus* in yogurts during cold storage

*S. salivarius* subsp. *thermophilus* counts in the 4 generations of samples are presented in Figure 11. While the differences among the samples were not significant ( $P > 0.05$ ), there was a significant difference among the *S. salivarius* subsp. *thermophilus* counts of the generations ( $P < 0.05$ ). *S. salivarius* subsp. *thermophilus* counts ranged from 8.01 (sample C) to 9.09 (sample D) log cfu g<sup>-1</sup> among the generations. The highest mean *S. salivarius* subsp. *thermophilus* counts were observed in the sample A, the lowest counts were determined in the sample C. Miller et al., 2002 and Lopes et al., (2019) found similar results for *S. salivarius* subsp. *thermophilus* in yogurts.

*S. salivarius* subsp. *thermophilus* counts in 1st generation yogurt samples during the storage are presented in Figure 12. *S. salivarius* subsp. *thermophilus* counts of the yogurts increased till the 7th day. After 7th day, bacteria counts remained constant except sample A showed a slight decrease.

Considering the mean values during storage, there was no significant difference among the yogurt sample ( $P > 0.05$ ). Some authors reported similar *S. salivarius* subsp. *thermophilus* results and trend (Dave and Shah, 1997; Güler-Akın, 2005; Asensio-Vegas, et al., 2018).

Another reason why the yogurts produced by using commercial yogurts as inoculum in home conditions are different from the commercial yogurts in terms of structure and texture is that the required constant incubation temperature and the necessary hygienic conditions are not achieved in the home conditions. In addition, the hygienic condition (microflora) of the yogurt used as inoculum and the survival rates of the starter culture in this yogurt are of great importance. Of course, the characteristics of the strains in starter culture are very important in yogurt making.



<sup>A</sup>: Means with same letters in a row within the category for samples are not significant at P>0.05  
<sup>a-c</sup> : Means with same letters in a row within the sample for generations are not significant at P>0.05

Figure 11. *S. salivarius* subsp. *thermophilus* counts in yogurt samples

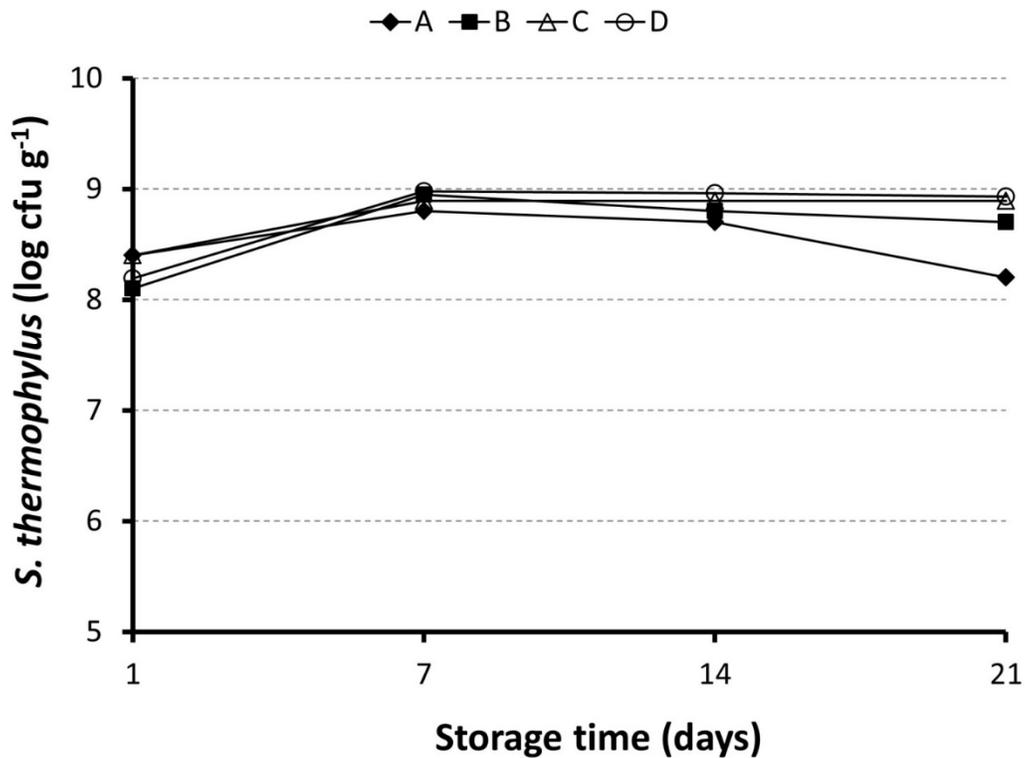


Figure 12. *S. salivarius* subsp. *thermophilus* in yogurts during cold storage

## Conclusions

In this study, it was investigated whether it is possible to make yogurt by using commercial yogurts as starter culture and as a result, it is observed that this is possible if the necessary hygienic conditions and incubation temperature are paid attention. Since these two conditions cannot be followed very well in the home environment and also the shelf life and hygienic quality of the yogurt that will be used as the source of inoculation cannot be standard, home yogurts cannot be expected to be of standard quality. In order to better illuminate yogurt inoculation and fermentation, studies on yogurt making processes where yogurt stored during different times can be used as the source of inoculation may be beneficial.

## Compliance with Ethical Standard

**Conflict of interests:** The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

**Ethics committee approval:** Author declare that this study does not include any experiments with human or animal subjects.

**Funding disclosure:** -

**Acknowledgments:** The author would like to thank Filiz Ezer, Fatma Uysal, Emine Ademi and Seda Özkan for their help. The author is also grateful to Çankırı Karatekin University Faculty of Engineering.

**Disclosure:** A part of this research was presented as oral and published in abstract form at the 2nd International Eurasian Conference on Biological and Chemical Sciences (EurasianBioChem 2019), 28–29 June 2019, Ankara, Turkey.

## References

- Abbasi, H., Mousavi, M.E., Ehsani, M.R., D-Jomea, Z.E., Vaziri, M. Rahimi J., Aziznia, S. (2009). Influence of starter culture type and incubation temperatures on rheology and microstructure of low fat set yoghurt. *International Journal of Dairy Technology*, 62(4), 550-555. <https://doi.org/10.1111/j.1471-0307.2009.00513.x>
- Asensio-Vegas, C., Tiwari, B., Gredilla, A.E., Bueno, F., Delgado, D., Martin-Diana, A.B. (2018). Development of yoghurt from ovine milk with enhanced texture and flavour properties. *International Journal of Dairy Technology*, 71(1), 112-121. <https://doi.org/10.1111/1471-0307.12341>
- Dabrowska, A., Babij, K., Szoltysik, M., Chrzanowska J. (2017). Viability and growth promotion of starter and probiotic bacteria in yogurt supplemented with whey protein hydrolysate during refrigerated storage. *Advances in Hygiene & Experimental Medicine*, 71, 952-959. <https://doi.org/0.5604/01.3001.0010.5866>
- Dave, R.I., Shah, N.P. (1997). Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. *International Dairy Journal*, 7, 31-41. [https://doi.org/10.1016/S0958-6946\(96\)00046-5](https://doi.org/10.1016/S0958-6946(96)00046-5)
- De Brabandere, A.G., De Baerdemaeker, J.G. (1999). Effects of process conditions on the pH development during yogurt fermentation. *Journal of Food Engineering*, 41, 221-227. [https://doi.org/10.1016/S0260-8774\(99\)00096-5](https://doi.org/10.1016/S0260-8774(99)00096-5)
- Espirito-Santo, A.P., Lagazzo, A., Sousa, A.L.O.P., Perigo, P., Converti, A., Oliveria, M.N. (2013). Rheology, spontaneous whey separation, microstructure and sensorial characteristics of probiotic yoghurts enriched with passion fruit fiber. *Food Research International*, 50, 224-231. <https://doi.org/10.1016/j.foodres.2012.09.012>
- Farnsworth, J.P., Li, J., Hendricks, G.M., Guo, M.R. (2006). Effects of transglutaminase treatment on functional properties and probiotic culture survivability of goat milk yogurt. *Small Ruminant Research*, 65, 113-121. <https://doi.org/10.1016/j.smallrumres.2005.05.036>
- Güler-Akın, M.B. (2005). The effects of different incubation temperatures on the acetaldehyde content and viable bacteria counts of bio-yogurt made from ewe's milk. *International Journal of Dairy Technology*, 58(3), 174-179. <https://doi.org/10.1111/j.1471-0307.2005.00209.x>
- Joung, J.Y., Lee, J.Y., Ha, Y.S., Shin Y.K., Kim, Y., Oh, N.S., (2016). Enhanced Microbial, Functional and Sensory Properties of Herbal Yogurt Fermented with Korean Traditional Plant Extracts. *Korean Journal of Food Science and Animal Resource*, 36(1), 90-99. <https://doi.org/10.5851/kosfa.2016.36.1.90>
- Krisnaningsih, A., Radiati, I., Evanuarini, H., Rosyidi D. (2019). The effect of incubation time to the physicochemical and microbial properties of yoghurt with local taro (*Colocasia esculenta* (L.) Schott) starch as stabilizer. *Current Research in Nutrition and Food Science*, 7(2), 547-554. <https://doi.org/10.12944/CRNFSJ.7.2.23>

- Lopes, R.P., Mota, M.J., Pinto, C.A., Sousa, S., Lopes da Silva, J.A., Gomes, A.M., Delgadillo, I., Saraiva, J.A. (2019).** Physicochemical and microbial changes in yogurts produced under different pressure and temperature conditions. *LWT - Food Science and Technology*, 99, 423-430. <https://doi.org/10.1016/j.lwt.2018.09.074>
- Miller, D.M., Dudley, E.G., Roberts, R.F. (2002).** Development of a quantitative PCR method for monitoring strain dynamics during yogurt manufacture. *Journal of Dairy Science*, 95, 4868-4872. <https://doi.org/10.3168/jds.2012-5445>
- Mohammadi, R., Rouhi, M., Mortazavian, A.M. (2011).** Effects of music waves on fermentation characteristics and viability of starter cultures in probiotic yogurt. *Milchwissenschaft*, 66(2), 193-196.
- Mudawi, H.A., Abdelrahim, S.M.K and Mustafa, A.M.I. (2014).** Effect of incubation and storage temperatures on quality of set yoghurt. *Egyptian Academic Journal of Biological Sciences*, 6(2), 131-137. <https://doi.org/10.21608/eajbsc.2014.16040>
- Ramchandran, L., Shah, N.P. (2010).** Characterization of functional, biochemical and textural properties of symbiotic low-fat yogurts during refrigerated storage. *LWT - Food Science and Technology*, 43, 819-827. <https://doi.org/10.1016/j.lwt.2010.01.012>
- Rawson, H.L., Marshall, V.M. (1997).** Effect of 'ropy' strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* on rheology of stirred yogurt. *International Journal of Food Science and Technology*, 32(3), 213-220. <https://doi.org/10.1046/j.1365-2621.1997.00395.x>
- Robinson, R.K. (2002a).** Fermented Milks/Yoghurt Types and Manufacture. In *Encyclopedia of Dairy Sciences*. H. Roginski (ed.) pp. 1055–1058, London, UK, Academic Press. ISBN 978-0-12-227235-6 <https://doi.org/10.1016/B0-12-227235-8/00171-1>
- Robinson, R.K. (2002b).** Fermented Milks/ Yoghurt, Role of Starter Cultures. In *Encyclopedia of Dairy Sciences*. H. Roginski (ed.) pp. 1059–1063, London, UK, Academic Press. ISBN 978-0-12-227235-6 <https://doi.org/10.1016/B0-12-227235-8/00172-3>
- Robinson, R.K., Lucey, J.A., Tamime, A.Y. (2006).** Manufacture of Yoghurt. In *Fermented Milks*. A. Y. Tamime (ed.), pp. 53-75, Oxford, UK, Blackwell Science Ltd. ISBN 0-632-06458-7 <https://doi.org/10.1002/9780470995501.ch3>
- Tarakçı, Z. (2010).** Influence of kiwi marmalade on the rheology characteristics, color values and sensorial acceptability of fruit yogurt. *Kafkas Universitesi Veteriner Fakültesi Dergisi*, 16(2), 173-178.