

The Effect of Microwave Heating on the Some Quality Properties and Shelf Life of Yoghurt

Tamer TURGUT ¹ 

¹ Atatürk Üniversitesi, Erzurum Meslek Yüksekokulu, TR-25240 Erzurum - TÜRKİYE

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Abstract

This study was carried out to determine the properties of set type yoghurts made using microwave heating after yoghurt fermentation during storage period of 28 d at 4±1°C. In this way, it will be possible to increase the yoghurt shelf-life, by preventing post-acidification. Yoghurts samples were subjected to three different microwave heating (10, 20 and 30 sec) at 720 Watt power level. Viability of yoghurt bacteria (*Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*) was assessed during 28 d of storage. Samples were subjected also physicochemical analysis (pH and titratable acidity). The results showed that microwave applications significantly affected viable counts of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* (P<0.05). The counts of *L. bulgaricus* and *S. thermophilus* increased in heated yoghurts but a delay was observed during of storage period. Also the results indicated that the microwave heating had significant effect on the titratable acidity values (P<0.05). The titratable acidity and pH showed similar patterns of increase or decrease during storage period. During refrigerated storage, decline in pH was greater in control group yoghurts than heated yoghurts. The titratable acidity increased in all yoghurt samples. However, the rate of increase was affected by the microwave heating time. The results of the study showed that the microwave heating had significant effect on the acceptability of yoghurt during shelf-life.

Keywords: Yoghurt, Microwave heating, Shelf-Life, *L. Lactobacillus delbrueckii* ssp. *bulgaricus*, *Streptococcus thermophilus*

Mikrodalga Isıtma Uygulamasının Yoğurdun Raf Ömrü ve Bazı Kalite Özellikleri Üzerine Etkisi

Özet

Bu çalışma yoğurt fermantasyonu sonrası mikrodalga ısıtma kullanılarak üretilen ve 4±1°C'de 28 gün depolanan set tipi yoğurtların bazı özellikleri ve mikrodalga ısıtmanın yoğurt bakterilerinin canlılıkları üzerindeki etkisini belirlemek amacıyla yapılmıştır. Bu sayede depolama sırasında asitlik artışı geciktirilerek yoğurdun raf ömrünün uzatılması mümkün olabilecektir. Yoğurt örnekleri fermantasyondan sonra 720 Watt mikrodalga güç seviyesinde üç farklı süreyle (10, 20 ve 30 sn.) mikrodalga ısıtmaya tabi tutulmuştur. Yoğurt bakterilerinin (*Lactobacillus delbrueckii* ssp. *bulgaricus* ve *S. thermophilus*) depolama boyunca sayıları ve yoğurt örneklerinin bazı fiziko-kimyasal özellikleri (pH ve titre edilebilir asitlik) belirlenmiştir. Bulunan sonuçlar mikrodalga uygulamasının, canlı *L. bulgaricus* ve *S. thermophilus* sayıları üzerinde etkili olduğunu göstermiştir (P<0.05). Sonuçlar aynı zamanda mikrodalga ısıtmanın titre edilebilir asitlik değerleri üzerinde de önemli etkiye (P<0.05) sahip olduğunu göstermiştir. Çalışma, mikrodalga ısıtma uygulamasının raf ömrü süresince yoğurdun kabul edilebilirliği üzerinde önemli bir etkisi olduğunu göstermiştir.

Anahtar sözcükler: Yoğurt, Mikrodalga ısıtma, Raf ömrü, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus*

INTRODUCTION

Fermented dairy products have been considered to have health-promoting properties on consumers' health for a long time. The consumption of fermented milk products is reported to have many benefits to human health around the world [1-6]. Yoghurt is the most known fermented dairy product with high consumption worldwide mainly because of the many health-promoting effects [7-9].

The shelf life of a product is described as the time in which the food product will remain safe, be certain to retain desired sensory, chemical, physical and microbiological characteristics, and will comply with any label declarations of nutritional data when stored under the recommended conditions [10]. The possibility to offer a higher shelf life than its competitors constitutes a primary competitive advantage for fresh food producers. As all fresh products, yoghurt has a relatively short shelf life. The shelf life of yoghurt produced under normal conditions is about



İletişim (Correspondence)



+90 442 2312705



tturgut@atauni.edu.tr

8-10 d at $<10^{\circ}\text{C}$. Furthermore, consumers tend to buy the product with the longest possible shelf-life ^[11].

Microwaves are basically high frequency electromagnetic energy in the frequency range of 300-3000 MHz generated by a magnetron. Electromagnetic energy at 915 and 2450 MHz can be absorbed by water containing materials and converted to heat ^[12]. Microwave energy contributes to the heat transfer by electromagnetic radiation and subsequent volumetric heating ^[13]. The purpose of thermal processing was to extend the shelf life of food products without compromising food safety ^[14]. The application of microwave heating in food processing is a partly recent development. Although microwave ovens are widely used as a means of food preparation, insufficient information is available on the consequences for the composition and nutritional quality of food ^[13].

The storage life of yoghurt depends on the degree of sanitation during processing and packaging. Several methods are used to increase the shelf life of the product such as different packaging ^[15], addition of antibiotics ^[16-18], addition of gas ^[19], application of preservatives, heat treatment after incubation ^[18] and carbonation process ^[20].

The aim of this study is to increase the yoghurt shelf-life, by preventing post-acidification, the effects of microwave heating on pH, titratable acidity and viable *S. thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* population, important in view of existing legal regulations for yoghurts, of yoghurt produced from cow's milk. The other aim of this study is to investigate the possibility of using microwave heating in the production of yoghurt and to make a contribution to the novel food manufacture techniques.

MATERIAL and METHODS

Raw Milk, Bacterial Cultures and Propagation

Raw cow's milk used in this study was obtained from the Atatürk University, Pilot Dairy Factory of Food Engineering, Erzurum, Turkey. The some physicochemical properties of the cow's milk were as follows: pH 6.48, titratable acidity 0.15%, dry matter 8.76% (Solid Not- Fat, SNF) and fat 3.4%.

Yoghurt starter cultures (*L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*) were obtained from the Food Engineering Department at Atatürk University. The yogurt strains were isolated from our previous one and then identified and stored. Strains were stored frozen (-80°C) as stock cultures in with 20% (v/v) glycerol (Merck, Darmstadt, Germany). Frozen cultures were prepared in the following way. The cells were routinely propagated on three successive days in MRS Agar (Merck) and M17 Agar (Merck) and incubated at 37°C anaerobically for *L. bulgaricus* (Anaerocult C system; Merck), and aerobically for *S. thermophilus* for 24-48 h respectively. Finally, the strains were inoculated in reconstituted (10%, w/w) skimmed milk and incubated at

$43\pm 1^{\circ}\text{C}$. Milk for the production of yoghurt was inoculated with this culture at a ratio of 2.5%. The identification of these strains was confirmed API 50 CH (bioMerieux, France) kits as previously described by Turgut et al.^[21].

In this study, 230 V- 50 Hz, 23 L. 1400 Watt programmable microwave oven (Vestel MD GD23, Manisa, Turkey) were used. Microwave oven's dimensions are $340 \times 220 \times 320$ mm and there is the round glass drying tray in the bottom. This study was carried out at 2450 MHz and 720 Watt microwave power level.

Experimental Design

The study designed completely randomized design in a factorial arrangement. There were four treatment groups with seven storage periods of 1, 5, 7, 10, 15, 20 and 28 d. The control yoghurt group was prepared without microwave treatment. Yoghurt samples were prepared by heating with microwave oven for 10, 20 and 30 sec at 2450 MHz. Every group consisted of 8 cups. One batch was taken as a control group (control) and not microwave heated. Other batches were transferred in microwave oven respectively. Other batches (H1, H2, and H3) heated at 720 W power level for 10, 20 and 30 sec respectively. Two replicates were accomplished for a total of 56 yoghurt samples. All these procedures were conducted twice and all analysis was carried out in parallel order.

Manufacture of Yoghurts

Yogurt samples were manufactured from cow's milk. The dry matter of homogenized milk was increased to 14% (SNF) by evaporation under vacuum pressure (60°C 450 mm Hg). Then evaporated milk heated for 10 min at 90°C and cooled to $43\pm 1^{\circ}\text{C}$ for incubation. The yoghurt culture was added to milk and the mixture was divided in to 150 mL plastic cups. The cups incubated at $42\pm 1^{\circ}\text{C}$ until the pH decreased to 4.7. After incubation, H1, H2 and H3 yoghurts were subjected to the microwave heating but control groups were not. All yogurt samples stored at $+4^{\circ}\text{C}$ for 28 d.

Enumeration of Yoghurt Bacteria

The viable *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* counts were determined 1, 5, 7, 10, 15, 20 and 28 d of storage periods at $+4^{\circ}\text{C}$. Samples of yoghurt (10 g) were serially diluted (w/v) with $\frac{1}{4}$ Ringer's solution and up to 10^{-7} , then spread-plated (0.1 mL) in duplicate onto plates of MRS agar for the enumeration of *L. delbrueckii* ssp. *bulgaricus* and M17 Agar for *S. thermophilus*. Inoculated plates were incubated anaerobically (Anaerocult C; Merck) at 43°C for 48-72 h for *L. bulgaricus* and aerobically for *S. thermophilus* at $35-37^{\circ}\text{C}$ for 48 h. The counts of *L. bulgaricus* were enumerated according to the method of Dave and Shah ^[22]. The counts of *S. thermophilus* were enumerated according to the method of Harrigan ^[23]. Plates containing 25 to 250 colonies were enumerated and the colony forming units per gram (CFU/g) of the product was calculated.

Yoghurt Analysis

All the analyses were carried out during storage on 1, 5, 7, 10, 15, 20 and 28th d. The pH values of the yoghurt samples were determined by direct measurement with a digital microprocessor pH meter (Hanna Instruments 211, Romania) after calibration with pH 4.0 and 7.0 standard buffer. Titratable acidity (lactic acid, %) was determined by the titrating with 0.1 N NaOH using phenolphthalein indicator previously described by Kurt et al.^[24]. All analyses were conducted twice.

Statistical Analysis

A factorial arrangement was set up to study the influence of microwave heating time (4) and storage periods (7) using 2 replicates. A total of 56 samples were investigated for microbiological, acidity and pH analyses on days 1, 5, 7, 10, 15, 20 and 28. All analyses were conducted twice. Data obtained from analysis of the samples were evaluated by variance analysis and the differences among means were detected by Duncan's multiple range tests (IBM SPSS ver 20).

RESULTS

Changes in pH and Titratable Acidity

The results of mean pH and acidity values are presented in Table 1. The pH value of the yoghurts was significantly affected by heat treatment after incubation. The initial mean pH values of the control sample and H1, H2 and H3 yoghurts were 4.22, 4.08, 4.20 and 4.41 respectively. At the end of 28 d, the values were 4.04, 3.99, 4.16 and 4.21. The pH values of the yoghurt samples during refrigerated

storage period are shown (Fig. 1). The initial pH values of the different yoghurt types were ranged from 4.08 to 4.41 and decreased slowly during storage. The declining trend in the pH was comparatively similar for all the yoghurt samples throughout storage. The yoghurt samples have significant difference ($P<0.05$) in pH measurements. The heated H2 and H3 yoghurt samples had higher pH values than the control and H1 yoghurt samples. Eventually, pH decreased for all samples and the decrease in pH values continued for up to 28 d. There were statistically differences between pH values and microwave heating time ($P<0.05$). The pH values of the H3 yoghurt samples were significantly different from each other ($P<0.05$) (Table 1).

Microwave treatment also significantly affected the acidity of the yoghurts. During the storage period, titratable acidity of the yoghurts samples increased and differences between the values were found significant ($P<0.05$). The effect of microwave treatment after incubation on titratable acidity values of yoghurts during storage are shown (Fig. 2). In the control and H1 yoghurts, a rapid increase continued for up to 28 d of storage, while the acidity of the H2 and H3 yoghurt increased less the during storage period. Acidity values also showed insignificant changes which supports the results obtained for pH.

There were statistically differences between titratable acidity values and microwave heating time ($P<0.05$). The titratable acidity of the H3 yoghurt samples was significantly different from each other ($P<0.05$). The titratable acidity of the H3 and H2 yoghurt samples had less than the control and H1 yoghurt. Though not close to the titratable acidity values of the H1 and H2 yoghurt, the differences were not found statistically significant ($P>0.05$).

Table 1. Viable counts of *L. bulgaricus* and *S. thermophilus* of yoghurt samples

Tablo 1. Yoghurt örneklerindeki *L. bulgaricus* ve *S. thermophilus* sayıları

Yoghurt samples	pH (X±Sx)		Titratable Acidity (%) (X±Sx)		<i>L. delbrueckii</i> ssp. <i>bulgaricus</i> (log cfu/g) (X±Sx)		<i>Streptococcus thermophilus</i> (log cfu/g) (X±Sx)	
Storage time (days)	**		**		**		**	
1	4.23	0.04 ^a	0.84	0.02 ^a	7.844	0.09 ^a	8.400	0.08 ^{ab}
5	4.21	0.04 ^{ab}	0.89	0.02 ^{ab}	7.937	0.09 ^a	8.367	0.08 ^{ab}
7	4.14	0.04 ^{ab}	0.91	0.02 ^{bc}	7.854	0.09 ^a	8.261	0.08 ^a
10	4.12	0.04 ^b	0.95	0.02 ^{cd}	7.884	0.09 ^a	8.497	0.08 ^{abc}
15	4.10	0.04 ^b	0.99	0.02 ^{de}	8.091	0.09 ^{ab}	8.618	0.08 ^{bc}
20	4.10	0.04 ^b	1.03	0.02 ^{ef}	8.246	0.09 ^b	8.710	0.08 ^c
28	4.10	0.04 ^b	1.07	0.02 ^f	8.285	0.09 ^b	8.707	0.08 ^c
Heating time	*		**		**		**	
Control	4.07	0.03 ^a	1.04	0.01 ^a	8.588	0.07 ^a	8.744	0.06 ^a
H1	4.03	0.03 ^a	1.04	0.01 ^a	8.378	0.07 ^b	8.597	0.06 ^{ab}
H2	4.19	0.03 ^b	0.92	0.01 ^b	8.099	0.07 ^c	8.474	0.06 ^b
H3	4.29	0.03 ^c	0.82	0.01 ^c	7.015	0.07 ^d	8.219	0.06 ^c

Means followed with the different superscript alphabet within each column are significantly different; *, ** are significant at 0.05 and 0.01 probability levels respectively

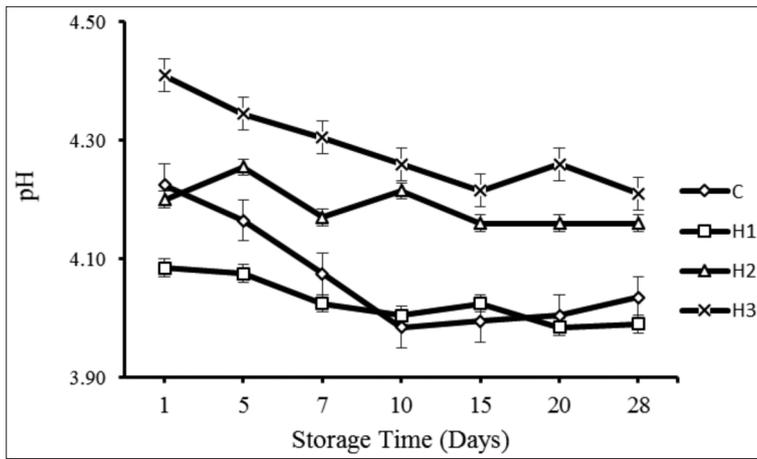


Fig 1. pH values of the yoghurt samples during refrigerated storage period

Şekil 1. Yoğurt örneklerinin depolama sırasındaki pH değerleri

Fig 2. Titratable acidity values of the yoghurt samples during refrigerated storage period
Şekil 2. Yoğurt örneklerinin depolama sırasındaki titre edilebilir asitlik değerleri

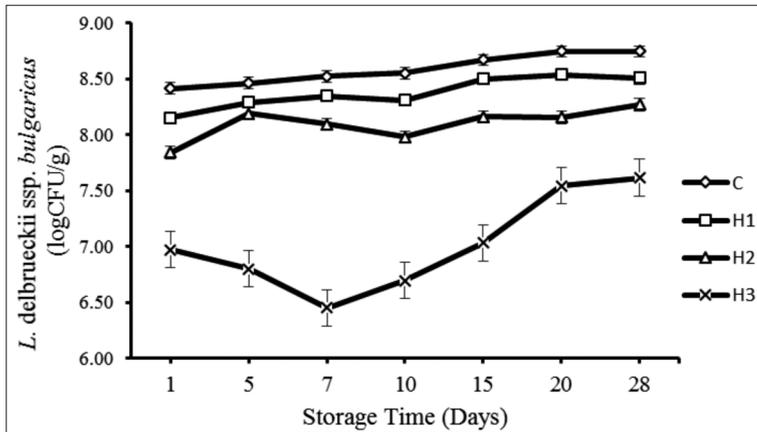
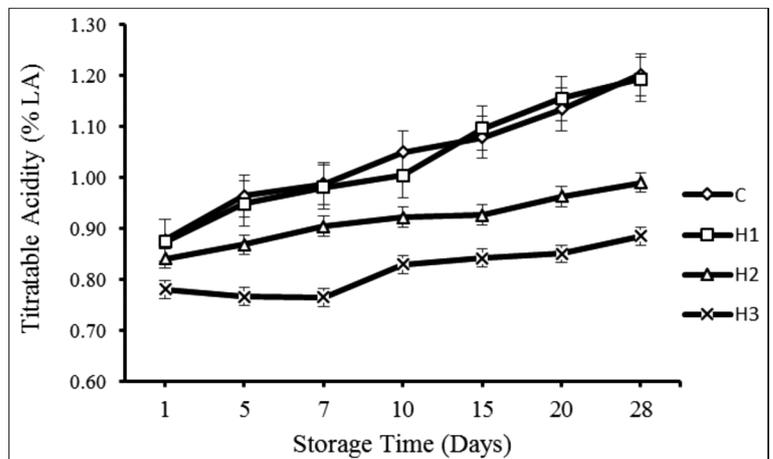


Fig 3. Viable counts of *L. delbrueckii ssp. bulgaricus* in the yoghurt samples during refrigerated storage period

Şekil 3. Depolama sırasında yoğurt örneklerindeki canlı *L. delbrueckii ssp. bulgaricus* sayıları

Changes in the Viable Counts

Microwave application significantly affected viable counts of *Lactobacillus delbrueckii ssp. bulgaricus* and *S. thermophilus*. Changes in the counts of yoghurt bacteria in during refrigerated storage are presented in Table 1. *L. bulgaricus* showed a more marked increase than *S. thermophilus* during the storage period. The counts of *L. bulgaricus* increased in each type of yoghurt samples during the storage time. The effects of microwave heating on viable counts of *L. bulgaricus* in yoghurts are shown

(Fig. 3). The effect of storage period on viable counts of *L. bulgaricus* was found statistically significant ($P < 0.05$). There were statistically differences between the counts of *L. bulgaricus* and microwave heating time. The values for the counts of *L. bulgaricus* in H3 yoghurt samples were significantly different from each other ($P < 0.05$). In H3 yoghurt, *L. bulgaricus* counts were lower by at least 1 log order than those for H1 and control yoghurts. Similarly, the values for the counts of *L. bulgaricus* in H1, H2 and control type yoghurt were not statistically significantly different for one another ($P > 0.05$).

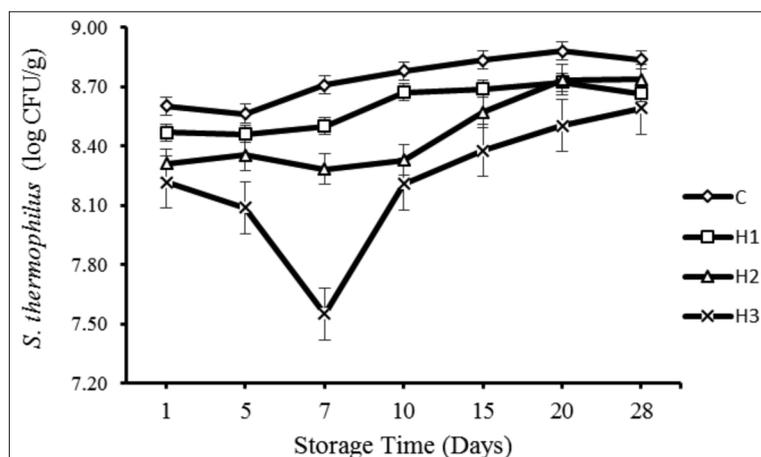


Fig 4. Average viable counts of *S. thermophilus* in the yoghurt samples during refrigerated storage period

Şekil 4. Depolama sırasında yoğurt örneklerindeki canlı *S. thermophilus* sayıları

The effects of microwave heating on viable counts of *S. thermophilus* in yoghurts during storage are shown (Fig. 4). There were small differences between the yoghurt samples and the number of bacteria increased slightly during the storage in terms of the counts of *S. thermophilus*. After 7 days of storage, the counts of *S. thermophilus* showed a decline but then the counts of *S. thermophilus* increased. At the end of storage, the number of bacteria increased almost 0.4 log unit in the samples and these increases were not found statistically significant from initial counts ($P > 0.05$). There were statistically differences between the counts of *S. thermophilus* and microwave heating treatment. The viable counts of *S. thermophilus* in the H3 yoghurt samples were significantly different from control group ($P < 0.05$). Similarly, the values for the counts of *S. thermophilus* in H1 and H2 yoghurt were not statistically significantly different from each other ($P > 0.05$).

DISCUSSION

Microwave heating application significantly affected the pH and acidity of yoghurt samples. The heated H2 and H3 yoghurt samples had higher pH values than the control and H1 yoghurt samples. There were significant differences in titratable acidity values between the groups of yoghurt samples. This difference in pH values are likely caused by exposure microwave heating time. The temperature of the yoghurt samples was rising a bit during the microwave heating. The results of the study showed that microwave heated yoghurt samples had lower acidity than no heated yoghurt. Titratable acidity of the all yoghurts samples tended to increase; however, the rate of increase was affected by the microwave heating time. There were statistically significant differences in titratable acidity values between the yoghurt samples. Whereas there was no statistical difference between pH values ($P > 0.05$). *L. bulgaricus* and *S. thermophilus* are responsible for the post acidification of yoghurt during cold storage. This study has proved that microwave heating can serve as an excellent method for long storage of yoghurt without adding any

preservative agents. It is possible to keep the acidity low through microwave heating for 30 sec. The microwave heating also did not change flavour or compositional characteristics of yoghurt.

Microwave application significantly affected viable counts of yoghurt bacteria also. Heat treatment at first resulted in a decline in counts of *S. thermophilus* and *L. bulgaricus*. Thus it will be possible to maintain yoghurt longer time without any additives. Karagül-Yüceer et al.^[20] studied on the protective effect of carbon dioxide in fruit-flavored yoghurt and reported that carbon dioxide did not affect the growth of yoghurt bacteria but carbonated yoghurt samples had lower acidity than noncarbonated yoghurt. In our study, H3 yoghurt samples had lowest titratable acidity values and lowest *S. thermophilus* and *L. bulgaricus* numbers. According for these results of microwave heating treatment it can be said to be more beneficial than CO₂ application. An increase in the viable bacterial numbers is most likely to be due to the long storage of the yoghurts, resulting in the growth of yoghurt bacteria. Contrary to our results, Akalın et al.^[25] concluded that viable counts of *L. bulgaricus* decreased in yoghurt containing prebiotic and probiotic culture during the storage. Hamann and Marth^[26] reported that the count of *L. bulgaricus* decreased during refrigerated storage of plain yoghurts.

The highest counts of *S. thermophilus* were found in control yoghurt and lowest numbers were found in the H3 yoghurt. Our results in terms of the viable number of *S. thermophilus* are compatible with those of Kim et al.^[27] and Akalın et al.^[25]. Karagül-Yüceer et al.^[20] stated that counts of *L. bulgaricus* and *S. thermophilus* decreased in yoghurt during 90 d of storage period. Dave and Shah^[28] found similar viable counts of *S. thermophilus* in yoghurts containing whey powder and cystein. Çakmakçı et al.^[29] also found similar results in probiotic yoghurt with banana marmalade. This study has proved that microwave heating can serve as an excellent method for long storage of yoghurt without adding any preservative agents.

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