Research Article **Open Access**

Functional Foods in Health and Disease

Effect of inoculation of *Lactobacillus acidophilus* on the level of nitrite and nitrate in yogurt containing spinach during refrigeration in 21 days

Mitra Keshavarz[1], Reza Barati-Boldaji[2, 1], Seyed Mohammad Mazloomi[3, 1*], Mohammad Hashem Yousefi [4]

[1]Nutrition Research Center, Shiraz University of Medical Sciences, Shiraz, Iran;[2]Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran;[3]Department of Food Hygiene and Quality Control, School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Shiraz, Iran;[4]Department of Food Hygiene and Public Health, Faculty of Veterinary Medicine, Shiraz University, Shiraz, Iran.

***Corresponding author:** Seyed Mohammad Mazloomi, Department of Food Hygiene and Quality Control, School of Nutrition and Food Sciences, Nutrition and Food Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

Submission Date: February 21st, 2022; **Acceptance Date:** April 11th, 2022; **Publication Date:** April 19th, 2022

Please cite this article as: Keshavarz M., Barati-Boldaji R., Mazloomi S. M., Yousefi M. H. Effect of inoculation of *Lactobacillus* acidophilus on the level of nitrite and nitrate in yogurt containing spinach during refrigeration in 21 days. Functional Foods in Health and Disease 2022; 12(4): 188-197 DOI: https://www.doi.org/10.31989/ffhd.v12i4.907

ABSTRACT

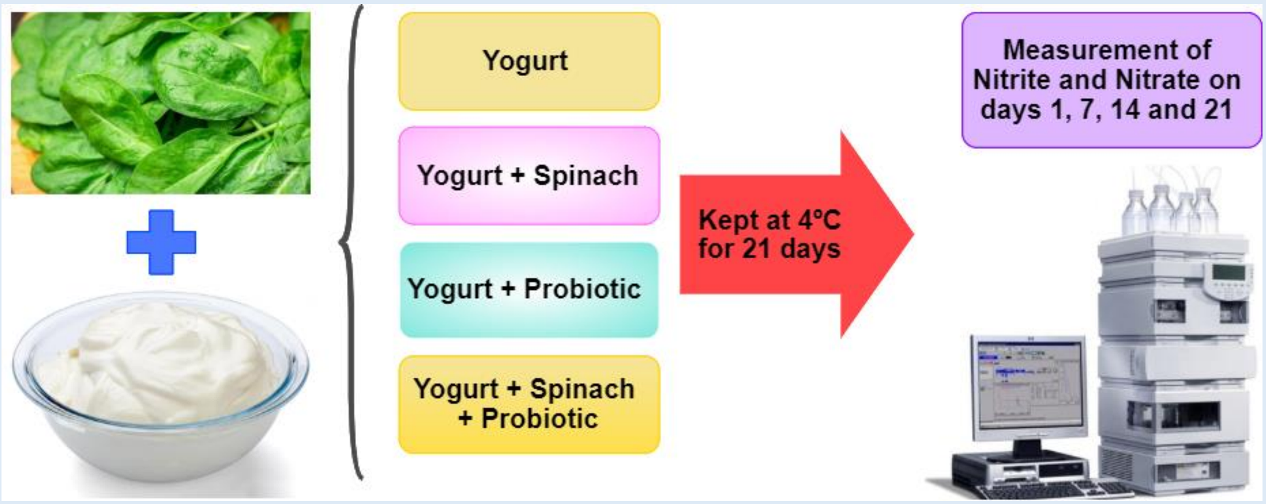
Introduction: Yogurt is mixed with spinach in some countries. Spinach can be a probable dietary source of Nitrate and Nitrite, and further N-nitrosamine.

Methods: The present study aimed to investigate the effect of inoculation of Lactobacillus acidophilus on the level of nitrite and nitrate in "yogurt containing spinach" (YS) during refrigeration in for 21 days. 4 types of yogurts including yogurt (Y), probiotic yogurt (PY), YS, and probiotic YS (PYS) were produced and kept at 4 °C for 21 days. Their nitrite and nitrate levels were measured by HPLC on days 1, 7, 14, and 21.

Results: There was no significant difference between the mean concentrations of nitrate and nitrite of Y and PY and between YS and PYS. Although the effect of refrigeration at 4 °C for 21 days was not significant on the nitrate amount of Y and PY, it was close to a significant level in YS and PYS. It also caused a significant increase in the nitrite content of all yogurts. This study showed that Lactobacillus acidophilus bacteria cannot affect the amount of nitrate and nitrite in yogurt or YS at 4°C.

Conclusion: Increasing the nitrite content of samples may enhance the possibility of nitrosamine formation. It is suggested that those who are at risk for all malformations caused by nitrites should not keep YS for a long time.

Keywords: nitrate; nitrite; Lactobacillus acidophilus; probiotic yogurt containing spinach; HPLC



INTRODUCTION

The compounds nitrates and nitrites are present naturally in the environment and can be consumed by humans through meats, vegetables and drinking water. Nitrate can convert into nitrite by bacterial activity [1]. Because of their carcinogenic effect [1-2], they may lead to some diseases including methemoglobinemia in infants [3-4] and congenital malformations in children [5], they have been considered as unwanted compounds in the diet. Furthermore, an increase in the nitrate level leads to block iodide uptake of the sodium iodide symporter [6]; also, it can lead to nutrient deficiency and raise the risk of kidney stones [7]. Many studies have indicated that nitrites can react with secondary amines and form carcinogenic compounds named N-nitrosamines in the stomach [1-2, 8-9]. However, some studies have revealed that nitrite accumulation in the tissues and blood is a biological source of nitric oxide (NO) which can enhance gastroprotection and reduce blood pressure. It possesses good effects on cardiac function and participate in mitochondrial respiration [10-11]. Nitrates are found in large quantities in vegetables, so that 70-90% of dietary nitrate intake comes from vegetables [12-16]. Green leafy vegetables contain higher levels of nitrate and spinach is one of them [14 - 15, 17].

One of the forms of spinach consumption in Iran is a combination of cooked spinach with yogurt, and it is not clear what effect has the combination of spinach with yogurt on the amount of nitrite and nitrate; furthermore, the prepared mix may be refrigerated for several days. However, it should be noted that nitrate and nitrite are compounds that have an Acceptable Daily Intake (ADI). The ADI for nitrate is 3.7 mg kg-1 day-1 [3, 15, 18] and for nitrite it is 0.07 mg kg-1 day-1 [19]. It means that the nitrate intake for a 60 kg adult should not be higher than 222 mg nitrate per day [3, 14-15, 18-19]. Therefore, nitrate and nitrite intake should be minimized, especially for those who consume lots of vegetables in their diet [18].

Today, a part of yogurt production in the world is in the form of probiotics. Probiotics are live microbial food supplements that have benefits for health [20]. Some beneficial effects of probiotic consumption include improvement of digestive system health, reducing the risk of certain cancers, contributing to relief from lactose intolerance symptoms [21-22], enhancement of the immune system, synthesis and enhancement of the bioavailability of nutrients, decreasing prevalence of allergies in susceptible individuals [22], anti-diarrhea effects [21, 23] and reducing serum cholesterol [21, 23-24]. In addition, the consumption of probiotic yogurt can also reduce the inflammatory, infectious, and metabolic effects of pregnancy [25]. Among the probiotic bacteria, lactic acid bacteria have been identified as the most important one [26].

Studies have shown that lactic acid bacteria are involved in the depletion of nitrate in many foods [4]. Recent findings indicate that lactobacillus can use nitrate and nitrite and produce NO in the digestive tract, which plays a role in physiologic events as told [27-28].

Yogurt is mixed with vegetables such as spinach in some countries, and there is not any study on the effect of yogurt starter and probiotic bacteria activity on the amount of nitrite and nitrate in these yogurts during the storage period in the refrigerator. Also, each kilogram of fresh spinach can contain up to 5 grams of nitrate [5, 29]; and still, there are questions about the usefulness or harmfulness of nitrate. Considering the above points, we aimed to investigate the effect of inoculation of *Lactobacillus acidophilus* on the level of nitrite and nitrate in yogurt containing spinach during refrigeration for 21 days.

MATERIALS AND METHODS

Instruments: According to Keshavarz et al. [14], the samples were analyzed using Waters HPLC (High-Performance Liquid Chromatography) system (made in waters company, US), which was equipped with a C18 Waters Spherisorb ® 5 µm ODs2 (250 × 4.6) column, UV Waters 2487 Dual λ Absorbance Detector and Waters 1525 Binary HPLC Pump. For degassing the solutions, YL9100 HPLC System Vacuum Degasser was utilized. PH measurements were determined using a Metrohm 827 pH meter (Switzerland (. Sigma Laboratory Centrifuges 3k30 (Germany) was utilized to centrifuge the samples before injection, and Elmasonic S60H ultrasonic system (Germany) was used for the preparation of samples.

Chemicals: All reagents were prepared using Merck Company (Germany). The YC-X11 yogurt starter (including *Streptococcus thermophilus* and *Lactobacillus bulgaricus*) and *Lactobacillus acidophilus* LA-5 produced by Hansen Company (Denmark) are used to produce a simple yogurt and probiotic yogurt.

Preparation of samples

1. ***Preparation of spinach*:** According to Keshavarz et al. [14], spinach was bought from green groceries and after removing their visible soil and non-edible parts, they were cut into 4-7 cm pieces. Then, they were washed with deionized water and drained. They were heated in a closed container for about 40 min without adding water.
2. ***Preparation of 4 types of yogurts*:** To prepare

yogurt, milk (3.2% fat) was heated at 90 ° C for 10 minutes. The heated milk was then divided into two parts. In one part, for one liter of milk, 0.05 gr of *lyophilized* powder of *Lactobacillus acidophilus* with 0.05 gr of lyophilized starter powder of yogurt, and in the other part, only starter powder of yogurt (0.1 g per liter of milk) was inoculated as the control. The milk was stored in 250 g disposable containers at 40°C incubator until the pH reached 4.6 and then transferred to the refrigerator. On the next day, some cooked spinach (in the amount of 15% by weight) was added to half of the simple and probiotic obtained yogurts. Then, all the samples including simple yogurt (Y), probiotic yogurt (PY), yogurt containing 15% cooked spinach (YS) and probiotic yogurt containing 15% cooked spinach (PYS) were kept in the 4ºC refrigerator for 21 days.

Sample preparation for analysis: After the initial preparation, the samples were prepared for HPLC injection based on the National Food Safety Standard of the People's Republic of China [30]. All glassware was based-washed by NaOH solution and then washed with deionized water several times.

Preparation of mobile phase and standard solutions: Various aqueous methanol concentrations (40, 30, 20, 15 and 10% v/v) and different pH values (4.5 - 7.5) were tested. PH adjustment was done by adding orthophosphoric acid. Different amounts of Octylamine (0.005, 0.0075, 0.010, 0.0125, 0.015, 0.020, 0.025 and 0.030 M) were added to the solutions. Finally, the solutions were passed from a 0.22µm filter. Eventually, the optimal condition of the mobile phase (10% methanol, pH 6.5 with 0.0125 M Octylamine) was used in the experiment. Standard solutions which were prepared on the day of the experiment and injected into the HPLC system contained 3.12, 6.25, 12.5, 25 and 50 µgmL of nitrite and nitrate.

HPLC analysis: After testing different flow rates (0.5, 0.8, 1 ml min^{-1}), the optimum flow rate was obtained 0.8 ml min^{-1}. The injection volume and the UV wavelength were 5 µL and 213 nm, respectively. HPLC column was washed and refreshed by passing a mixture solution of water and methanol (50:50 v/v) at the end of the analysis.

Statistical analysis: The obtained data were analyzed using SPSS, version 18. Descriptive statistics were used to present the mean and standard deviation. ANOVA with repeated measurements was used to compare the changes in nitrate and nitrite of Y, PY, YS and PYS overtime during days 1, 7, 14 and 21. One-way ANOVA test was used to compare the changes in nitrate and nitrite of groups on each measurement day. The significance level was $P<0.05$.

RESULTS

After examining various conditions to set up the HPLC system, the optimum mobile phase for detection and separation of two peaks of nitrate and nitrite was 10% (v/v) aqueous methanol with the addition of 0.0125 M Octylamine which was adjusted in 6.5pH value. The flow rate was 0.8 ml min^{-1}.

Table 1 shows nitrate changes in Y, PY, YS and PYS during the refrigeration storage period. The changes were not significant in any of the groups over time although in the PYS group it was close to a significant level (P=0.058). The trend of nitrate changes over time in the two groups of Y and PY (P= 0.50) and also in the two groups of YS and PYS (P=0.986) was similar. The amount of nitrate in the two groups of YS and PYS increased in the first and second weeks and decreased in the third week, and in the two groups of Y and PY this increase was seen only in the first week, and after that, the amount of nitrate was reduced. However, none of these changes was significant.

Table 1. Changes in nitrate level of yogurt, probiotic yogurt, yogurt containing spinach and probiotic yogurt containing spinach during refrigeration storage at 4 ºC in 21 days

groups	n	level of NO3 during refrigeration storage				p value
		1st day	7th day	14th day	21th day	
		Mean ± SD (ppm)	Mean ± SD (ppm)	Mean ± SD (ppm)	Mean ± SD (ppm)	
Y	6	11.74 ± 6.75 ᵃ	18.46 ± 6.87 ᵃ	14.36 ± 2.92 ᵃ	11.91 ± 6.57 ᵃ	**0.092**
PY	6	13.32 ± 5.26 ᵃ	13.60 ± 8.30 ᵃ	10.93 ± 5.73 ᵃ	9.63 ± 2.74 ª	**0.609**
YS	6	102.18 ± 29.56 ᵇ	133.55 ± 14.38 ᵇ	139.14 ± 8.91 ᵇ	136.01 ± 8.12 ᵇ	**0.071**
PYS	6	104.38±17.51 ᵇ	133.89 ± 9.73 ᵇ	139.47 ± 28.83 ᵇ	137.91 ± 9.06 ᵇ	**0.058**

Y: yogurt; PY: Probiotic yogurt; YS: yogurt containing spinach; PYS: Probiotic yogurt containing spinach

Differences between values in columns signed with the same letters are non-significant

Table 2 shows nitrite changes in Y, PY, YS and PYS during the refrigeration storage period. The trend of nitrite changes in each of the groups was significant during the storage period in the 4°C refrigerator. However, there was no significant difference in the trend of nitrite changes during the time between the groups (P = 0.252).

Table 2. Changes in nitrite level of yogurt, probiotic yogurt, yogurt containing spinach and probiotic yogurt containing spinach during refrigeration storage at 4ºC in 21 days

groups	n	level of NO2 during refrigeration storage				p value
		1st day	7th day	14th day	21th day	
		Mean ± SD (ppm)	Mean ± SD (ppm)	Mean ± SD (ppm)	Mean ± SD (ppm)	
Y	6	39.25 ± 8.64 ᵃ	50.56 ± 8.80 ᵃ	56.71 ± 5.85 ᵇ	45.08 ± 7.53 ᵇᵃ	**0.004**
P.Y	6	38.28 ± 8.03 ᵃ	46.87 ± 7.00 ᵃ	48.59 ± 7.15 ᵇᵃ	41.01 ± 2.25 ᵃ	**0.040**
YS	6	35.44 ± 12.50 ᵃ	47.35 ± 6.71 ᵃ	50.75 ± 4.87 ᵇᵃ	46.17 ± 5.42 ᵇᵃ	**0.034**
P.YS	6	34.72 ± 10.05 ᵃ	48.88 ± 2.72 ᵃ	44.90 ± 4.26 ᵃ	49.98 ± 3.13 ᵇ	**0.001**

Y: yogurt; P.Y: Probiotic yogurt; YS: yogurt containing spinach; P.YS: Probiotic yogurt containing spinach

Differences between values in columns signed with the same letters are non-significant

DISCUSSION

The aim of this study was to evaluate the effect of probiotic bacteria, *Lactobacillus acidophilus*, on the amount of nitrate and nitrite in yogurt containing spinach. The mean nitrate concentration of yogurt on the second day after the production was 11.74 ± 6.75 mg kg^{-1}. It is similar to the results of Kucukcetin et al.'s study, which measured the nitrate of strained yogurt at 16.6 mg kg^{-1} [31]. However, it was higher than the nitrate content reported by Martins et al. in yogurt. They reported the nitrate of yogurt was 3.07-4.77 mg kg^{-1} by spectrophotometry and 0.49-3.69 by flow injection analysis (FIA) [32]. Also, Luf et al. determined the nitrite concentration of yogurt, about 0.72 mg kg^{-1} [33]. Therefore, the nitrate concentration of yogurt in this study was similar to the results of some studies [31] although was high compared to the other two studies [32, 33]. The mean nitrite concentration of yogurt in this study on the second day was obtained at 38.28 ± 8.03 mg kg^{-1}, which is high in comparison to the nitrite content of yogurt reported in other studies. Kucukcetin et al. reported the mean nitrite concentration of strained yogurt as about 2.1 mg kg^{-1} [31] and it was reported 0.43-0.93 mg kg^{-1} and 1.06-1.86 mg kg^{-1} in yogurt by Martins et al. [32] and 0.002 mg kg^{-1} by Luf et al. [33]. A higher amount of nitrate in dairy products can be due to the water or gas used in the processing or because of the use of nitric acid as a cleaning agent in the factories [33, 34]. Also, milk contamination with nitrate can occur during or after secretion. Since the nitrate of forage is carried to milk in a low amount, most nitrate contamination has a post-secretory route [33]. As Kammerer et al. showed, contamination of the drinking water of cows with nitrates could not significantly change the nitrate levels of their milk [35]. However, Baranová et al. found that after feeding potassium nitrate (KNO3) to dairy cows, a considerable increase in nitrate of milk was observed, depending on the amount of KNO3 [36]. According to Bouchard's study, increased concentration of nitrate and nitrite in milk can also be due to endotoxin-induced mastitis [37]. Therefore, although the amount of nitrate and nitrite in milk and dairy products is very low in normal conditions, for other reasons, this can be higher than usual. To find the main reason for the higher nitrate and nitrite levels in the yogurts tested in this study, further evaluation should be done.

In this study, the mean nitrate and nitrite levels between samples of Y and PY groups were not significantly different on any days of measurement, as were not between samples of YS and PYS groups. This indicates that the *Lactobacillus acidophilus* bacteria could not affect the amount of nitrate and nitrite in yogurt or yogurt containing spinach at 4 °C. The effect of *Lactobacillus acidophilus* on the nitrate and nitrite content of Y and YS has not been studied yet, but there are some studies that investigated the effect of other lactic acid bacteria on nitrate and nitrite content of other foods; the results are different depending on the type of bacteria and foods. Gierschner and Hammes showed that the two bacteria, *Lactobacillus plantarum* and *Lactobacillus pentosus,* could not reduce the amount of nitrate in the vegetable extract. Andersson also showed that inoculation of *Lactobacillus plantarum* had no effect on the nitrate content of carrot, but the gram- negative flora of carrot reduced it [38]. However, Hybenova et al. observed that 92H and 90H strains of *Lactobacillus plantarum* and 37 H strain of *Lactobacillus delbrucki* bacteria effectively reduced the nitrate level in carrot and cabbage extract; the highest nitrate reduction occurred by strain 92H of *Lactobacillus plantarum* [39].

In another study, Yan et al. examined the effect of inoculating lactic acid bacteria starter cultures on the concentration of nitrite in fermenting Chinese Paocai and observed that in addition to their ability to deplete nitrite, by preventing the growth of nitrate-reducing bacteria such as Enterobacteria, lactic acid bacteria can reduce the formation of nitrite during fermentation of paocai (at 30 °C for 10 days) [4]. Oh et al. investigated the depletion of sodium nitrite in Lactobacilli MRS broth by lactic acid bacteria isolated from kimchi at different temperatures; they found that these bacteria are very effective in depleting sodium nitrite at high temperatures, so that more than 90% of sodium nitrite was depleted in the initial inoculation period (1-2 days) at 30 °C and 36 °C. However, as the temperature dropped, sodium nitrite depletion was reduced by all species so that at 5 °C, bacteria deplete less than 20% of sodium nitrite after 10 days [40]. In addition to the type of food and the type of bacteria inoculated, the difference in the results of this study and some other studies can be explained by the storage conditions of food, including temperature and storage time. The storage temperature of all types of yogurts in this study was 4 °C, which reduces the ability of bacteria to deplete nitrite according to Oh et al. [40]. Another probable cause is the reduction of the survival of probiotics in yogurt [41] affected by factors such as pH reduction and organic acid accumulation during growth in the storage period [42]; there are some studies that show that the pH of samples of probiotic yogurt decreased during storage at 4°C, and their acidity increased [43-45]. Another factor can be the inhibitory effect of nitrate and nitrite on bacterial growth; it has been shown in a study that nitrite can inhibit the growth of bacteria in certain concentrations and pH [46].

Although the effect of refrigeration at 4 °C for 21 days was not significant on the nitrate amount of Y and PY, it was close to a significant level in YS and PYS ($p = 0.071$ and $p = 0.058$ respectively). It caused a significant increase in nitrite content of all yogurts up to the third week ($p < 0.05$). The probable cause of increased nitrate (although wasn't significant) and nitrite during the refrigeration storage period can be evaporation of water and, consequently, the concentration of nitrate and nitrite in the samples. Spinach tissue decomposition by starter and probiotic bacterial enzymes and therefore the release of nitrate and nitrite from its tissue can also be one of the reasons for the increase of these two compounds. Anyway, this issue may increase the possibility of nitrosamine formation in yogurt, due to the high amount of protein and some nitrosatable amines in yogurt [47].

The mean nitrate concentrations of two groups of Y and YS, and two groups of PY and PYS were significantly different on all measuring days; this suggests that the addition of spinach influences the nitrite levels of yogurt, which seems to be natural due to the high amount of nitrate in spinach [14]. However, the mean nitrite concentrations were almost the same in all groups and did not have a significant difference. Since the amount of spinach added to yogurts in this study was 15 percent, and according to the amount of nitrite in the cooked spinach [14], this result is explainable.

CONCLUSION

This study showed that *Lactobacillus acidophilus* bacteria cannot affect the amount of nitrate and nitrite in yogurt or yogurt containing spinach at temperatures of 4°C. However, considering the effects of other probiotic bacteria on nitrate and nitrite in other studies, it is possible that other probiotic bacteria can affect the amount of nitrate and nitrite of Y and YS.

Although refrigeration at 4 °C for 21 days does not significantly affect the amount of nitrate, it causes an increment in the level of nitrite in samples up to the third week, and this issue may increase the possibility of nitrosamine formation. Therefore, it is advisable that those who are at risk for cancer and other malformations caused by nitrites should not keep yogurt for a long time.

List of Abbreviations: Yogurt: Y; Yogurt containing spinach: YS; probiotic yogurt: PY; probiotic yogurt containing spinach: PYS; nitric oxide: NO; acceptable daily intake: ADI; High Performance Liquid Chromatography: HPLC; potassium nitrate: KNO_3

Competing Interests: The authors declare that there is no conflict of interest.

Author Contributions: SMM: conceptualization, methodology and funding acquisition. MK, RBB & MHY: validation and writing-review and editing. MK: formal analysis, investigation, data curation and writing-original draft preparation.

Acknowledgements: The authors would like to thank Shiraz University of Medical Sciences for financial support and and Center for Development of Clinical Research of Nemazee Hospital and Dr. Nasrin Shokrpour for editorial assistance and also Ms Rousta for performing the statistical analysis.

Funding: This research was extracted from the MS thesis written by Mitra Keshavarz which was funded by Shiraz University of Medical Sciences (Project No. 6849-92).

REFERENCES

1. World Health Organization, *Guidelines for drinking-water quality, third edition, incorporating first and second addenda 2008.*http://www.who.int/water_sanitation_health/dwq/gdwq3rev/en
2. Machha A and Schechter AN, Dietary nitrite and nitrate: a review of potential mechanisms of cardiovascular benefits. Eur J Nutr 2011; 50(5): 293–303. https://doi.org/10.1007/s00394-011-0192-5
3. Katan MB, Nitrate in foods: harmful or healthy?. Am J Clin Nutr 2009; 90(1): 11-12. https://doi.org/10.3945/ajcn.2009.28014
4. Yan PM, Xue WT, Tan SS, Zhang H and Chang XH, Effect of inoculating lactic acid bacteria starter cultures on the nitrite concentration of fermenting Chinese paocai. Food Control 2008; 19(1): 50-55. https://doi.org/10.1016/j.foodcont.2007.02.008
5. Sobhan Ardakani S, Shayesteh K, Afyouni M and Mahbobi Soufiani N, Nitrate Concentration in Some Plants in Isfahan. J Environ Stud 2005; 31(37): 69-76. https://www.sid.ir/en/journal/ViewPaper.aspx?id=38073
6. Qiu W, Wang Z, Huang C, Chen B and Yang R, Nitrate accumulation in leafy vegetables and its relationship with water. J Soil Sci Plant Nutr. 2014; 14(4): 761-168. https://doi.org/10.4067/S0718-95162014005000061
7. Najafi N and Parsazadeh M, Effect of Nitrogen Form and pH of Nutrient Solution on the Shoot Concentration of Phosphorus, Nitrate, and Nitrogen of Spinach in Hydroponic Culture. ejgcst 2010; 1(1): 41-56.
8. Beard JC, Swager TM. An organic chemist's guide to N-nitrosamines: their structure, reactivity, and role as contaminants. J Org Chem 2021;86:2037-57. https://doi.org/10.1021/acs.joc.0c02774
9. Ma L, Hu L, Feng X, Wang S. Nitrate and Nitrite in Health and Disease. Aging Dis. 2018;9:938-45. https://doi.org/10.14336/AD.2017.1207
10. World Health Organization, Nitrate and nitrite in drinking-water ,Background document for development of WHO Guidelines for Drinking-water Quality 2011. http://www.who.int/water_sanitation_health/dwq/chemicals/nitratenitrite2ndadd.pdf
11. Sobko T, Marcus C, Govoni M and Kamiya S, Dietary nitrate in Japanese traditional foods lowers diastolic blood pressure in healthy volunteers. Nitric Oxide 2010; 22(2): 136-140. https://doi.org/10.1016/j.niox.2009.10.007
12. Hord NG, Tang Y and Bryan NS, Food sources of nitrates and nitrites: the physiologic context for potential health benefits. Am J Clin Nutr. 2009; 90(1): 1-10. https://doi.org/10.3945/ajcn.2008.27131
13. Karwowska M, Kononiuk A. Nitrates/Nitrites in Food—Risk for Nitrosative Stress and Benefits. Antioxidants. 2020;9. https://doi.org/10.3390/antiox9030241
14. Keshavarz M, Mazloomi SM and Babajafari S, The effect of home cooking method and refrigeration processes on the level of nitrate and nitrite In spinach. Health Sci Surveillance Sys 2015; 3(3): 88-93.
15. Ministry of Agriculture, Fisheries and Food, 2004 UK monitoring programme for nitrate and nitrite in lettuce and spinach 2000–2002.
16. Wolff IA and Wasserman AE, Nitrates, nitrites and nitrosamines. Science 1972; 177: 15-19. https://doi.org/10.1126/science.177.4043.15
17. Huckle A. Annual Report for: Food Standards Agency, Nitrate Surveillance Monitoring Programme May 2020 – March 2021. 2021. https://www.food.gov.uk/sites/default/files/media/document/Nitrate%20Data%20May%202020%20to%20March%202021.pdf
18. Pirsaheb M, Rahimian S and Pasdar Y, Nitrite and Nitrate content of fruits and vegetables in Kermanshah (2010). J Kerman Univ Medical Sci 2012; 16(1): 76-83. https://www.sid.ir/en/journal/ViewPaper.aspx?id=278888.
19. European Food Safety Authority. EFSA confirms safe levels for nitrites and nitrates added to food. 2017. https://www.efsa.europa.eu/en/press/news/170615#:~:text=The%20current%20acceptable%20daily%20intake,mg%2

Fkg%20bw%2Fday.

20. Roberfroi MB, Prebiotics and probiotics: are they functional foods?. Am J Clin Nutr 2000; 71: 1682–1687. https://doi.org/10.1093/ajcn/71.6.1682S
21. Mazloomi SM, Shekarforoush SS, Ebrahimnejad H and Sajedianfard J, Effect of adding inulin on microbial and physicochemical properties of low fat probiotic yogurt. Iran J Vet Res. 2011; 12(2): 93-98. https://www.sid.ir/en/journal/ViewPaper.aspx?id=278252.
22. Kopp-Hoolihan L, Prophylactic and Therapeutic Uses of Probiotics: A review. J Am Diet Assoc 2001; 101(2): 229–241. [2-7]. https://doi.org/10.1016/s0002-8223(01)00060-8
23. Baroutkoub A, Roushan Zamir M, Razmik B, Julayi H, Sohrabi Z, Mazloomi SM and Eskandari MH, Effects of probiotic yoghurt consumption on the serum cholesterol levels in hypercholestromic cases in Shiraz, Southern Iran. Sci Res Essays. 2010; 5(16): 2206-2209. https://doi.org/10.3168/jds.2014-8500
24. Lee DK, Jang S, Baek EH, Kim MJ, Lee KS, Shin HS, Chung MJ, Kim JE, Lee KO and Ha NJ, Lactic acid bacteria affect serum cholesterol levels, harmful fecal enzyme activity, and fecal water content. Lipids Health Dis 2009; 8: 21. https://doi.org/10.1186/1476-511X-8-21
25. He A, Chin J, Lomiguen CM. Benefits of Probiotic Yogurt Consumption on Maternal Health and Pregnancy Outcomes: A Systematic Review. Cureus. 2020;12:e9408. https://doi.org/10.7759/cureus.9408
26. Tofangsazan F, Shahidi F, Mortazavi S A, Milani E and Eshaghi Z, Investigation of antibacterial activity of Lactic Acid Bacteria isolated from traditional kordish cheese in comparison with commercial strains. Iran J Med Microbiol 2013; 7(3): 34-41. http://ijmm.ir/article-1-253-en.html
27. Sobko T, Huang L, Midtvedt T, Norin E, Gustafsson LE, Norman M, Jansson EA and Lundberg JO, Generation of NO by probiotic bacteria in the gastrointestinal tract. Free Radic Biol Med 2006; 41(6): 985–991. https://doi.org/10.1016/j.freeradbiomed.2006.06.020
28. Sobko T, Reinders CI, Jansson E, Norin E, Midtvedt T and Lundberg JO, Gastrointestinal bacteria generate nitric oxide from nitrate and nitrite. Nitric Oxide 2005; 13: 272–278. https://doi.org/10.1016/j.niox.2005.08.002
29. Tamme T, Reinik M, Roasto M, Juhkam K, Tenno T and Kiis A, Nitrates and nitrites in vegetables and vegetable-based products and their intakes by Estonian population. Food Addit Contam 2006; 23(4): 355-361. https://doi.org/10.1080/02652030500482363
30. China. Ministry of Health of the People's Republic of China. National Food Safety Standard of the People's Republic of China, National Food Safety Standard Determination of Nitrite and Nitrate in Foods; 2016. GB 5009.33-2016 https://www.chinesestandard.net/PDF.aspx/GB5009.33-2016
31. Kucukcetin A, Sik B and Demir M, Determination of Sodium Benzoate, Potassium Sorbate, Nitrate and Nitrite in Some Commercial Dairy Products. GIDA 2008; 33(4): 159-164. https://dergipark.org.tr/en/pub/gida/issue/6830/91671
32. Martins I, Silva T, Lima P, Figueiredo E and Maia P, Determination of nitrate and nitrite in milk products commercialized in the southern region of Minas Gerais State, Brazil: Comparison between the flow injection analysis and the spectrophotometric conventional method. Toxicology Letters 2010; 196: 292. https://doi.org/10.1016/j.toxlet.2010.03.923
33. Gilbert J, Senyuva H, editors. Bioactive compounds in Foods 2008. https://books.google.com/books?id=2myBYRAGogC&printsec=frontcover#v=onepage&q&f=false
34. Yeh TS, Liao SF, Kuo CY, and Hwang, W, Investigation of the Nitrate and Nitrite Contents in Milk and Milk Powder in Taiwan. J Food Drug Anal. 2013; 21(1): 73-79. https://doi.org/10.6227/jfda.2013210109
35. Kammerer M, Pinault L and Pouliquen H, Content of nitrate in milk. Relationship with its concentration in the water supply for livestock. Ann Rech Vet 1992; 23(2): 131-138.
36. Baranová M, Mal'a P and Burdová O, Transport of nitrates and nitrites into the milk of dairy cows through the digestive system. Vet Med (Praha) 1993; 38(10): 581-8.
37. Bouchard L, Blais S, Desrosiers C, Zhao X and Lacasse P, Nitric oxide production during endotoxin-induced mastitis in the cow. J Dairy Sci 1999; 82(12): 2574-81. https://doi.org/10.3168/jds.S0022-0302(99)75512-8
38. Salminen S, Wright AV and Ouwehand A, editors. Lactic Acid Bacteria. 3th ed. https://www.crcpress.com/Lactic-Acid-Bacteria-Microbiological-and-Functional-Aspects-Third-Edition/Salminen-von-Wright/p/book/9780824753320 [23 July 2004].
39. Hybenovfi E, Drdak M, Guoth R, and Jfin Gracak, Utilisation of nitrates - a decisive criterion in the selection of Lactobacilli for bioconservation of vegetables. Z Lebensm Unters Forsch 1995; 200: 213-216. https://doi.org/10.1007/BF01190497
40. Oh CK, Oh MC and Kim SH, The depletion of sodium nitrite by lactic acid bacteria isolated from kimchi. Med Food 2004; 7(1): 38-44. https://doi.org/10.1089/109662004322984680
41. Capela P, Hay TKC and Shah NP, Effect of cryoprotectants, prebiotics and microencapsulation on survival of probiotic organisms in yoghurt and freeze-dried yoghurt. Food Res Int. 2006; 39(2): 203-211.
42. Nikbakhe H, Fadaei Noghani V and Khosravi-Darani K, Investigation on the viability of selected probiotics in low fat set yogurt by homogenized milk at various temperatures. Innovative Food Technologies 2014; 1(4): 3-11. https://doi.org/10.22104/JIFT.2014.48
43. Dave RI and Shah ML, Ingredient supplementation effects on viability of probiotic bacteria in yogurt. J Dairy Sci. 1998; 81(11): 2804-2816. https://doi.org/10.3168/jds.S0022-0302(98)75839-4
44. Masoud R, Fadaei Noghani V and Khosravi-Darani K, Investigating effect of homogenization pressure and stages on some physicochemical properties of probiotic low fat yoghurt. Innovative Food Technologies 2014; 2(1): 39-48. https://doi.org/10.22104/JIFT.2014.67
45. Mortazavian AM, Ehsani MR, Mousavi SM, Rezaei K,

Sohrabvandi S and Reinheimer JA, Effect of refrigerated storage temperature on the viability of probiotic micro-organisms in yogurt. Int J Dairy Technol. 2007; 60(2): 123-127.

46. Xia DS, Liu Y, Zhang CM, Yang SH and Wang SL, Antimicrobial effect of acidified nitrate and nitrite on six common oral pathogens in vitro. Chin Med J 2006; 119(22): 1904-1909. https://doi.org/10.5555/cmj.0366-6999.119.22.p1904.01

47. El-Aaser AA, El-Merzabani MM and Zakhary NI, A study on the aetiological factors of bilharzial bladder cancer in Egypt-2. Nitrosamines and their precursors in Egyptian dairy products. Eur J Cancer 1979; 15(3): 293-298. https://doi.org/10.1016/0014-2964(79)90040-9