

## EFFECTS OF POWDERED *SPIRULINA PLATENSIS* BIOMASS ON PH AND TITRATABLE ACIDITY OF PROBIOTIC DOOGH CONTAINING POWDERED MINT DURING COLD STORAGE

Vajiheh Fadaei<sup>1</sup>, Atefeh Eslami-Moshkenani<sup>1\*</sup>, and Kianoush Khosravi-Darani<sup>2</sup>

<sup>1</sup>Department of Food Science & Technology, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran

<sup>2</sup>Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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### ABSTRACT

*Spirulina* has been used for many years as human food because of its high protein content and nutritional value. The plenty of biologically important compounds in the algae, provide new opportunity for producing functional dairy products. The main purpose of this study was to determine the effect of the powdered *Spirulina platensis* on the titratable acidity and pH of probiotic Doogh samples containing *Mentha piperita* during the refrigerated storage. In this research, Doogh samples were enriched with different concentrations of *Mentha piperita* (0.5 and 1%) and *Spirulina platensis* (0, 0.3, 0.5 and 0.8%). The treatments were stored at 4 °C for 21 days. pH and acidity of samples were measured at regular (7-day) intervals. The results showed that an increase in the concentration of *Spirulina platensis* induced a significant increase in the titratable acidity of probiotic Doogh during cold storage. However, the pH decreased slowly with the increasing alga content. There was no significant difference in pH and titratable acidity between samples with various concentrations of powdered *Mentha piperita* during storage time.

**Keywords:** Doogh, probiotics, *Spirulina platensis*, *Lactobacillus acidophilus* (La5), *Mentha piperita*.

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### INTRODUCTION

Doogh is a traditional Iranian drink based on fermented milk. Doogh, can be produced by adding water and salt into yoghurt. Beside local consumption, Doogh is exported to Afghanistan, Armenia, Azerbaijan, the Balkans, Iraq and few part of the Middle East and central Asia. Doogh, is a National Drink due to its organoleptic and hygienic characteristics (Anonymous, 2010).

Consumers would need to ingest considerably less medicine and artificially produced vitamin and mineral supplements if fermented milks were enriched with vitamins, proteins, essential fatty acids and trace elements of natural origin. A simple way of attaining this goal is the use of cyanobacteria in the manufacture of cultured dairy foods. (Varga *et al.*, 2012). Cyanobacteria or blue- green algae are photoautotrophic micro-organisms widely distributed in nature. They have been used as human food for centuries. *Spirulina platensis* is the best known genus because of its nutritional value (Parda *et al.*, 1998; Molnar *et al.*, 2005; Akalin *et al.*, 2009). It has been proved that consumption of *Spirulina* is beneficial for health due to its chemical composition including compounds like essential amino acids, vitamins, natural pigments, and fatty acids, especially  $\omega$ -6 representatives such as gamma-linolenic (GLNA) acid, a precursor of the prostaglandin hormones in the body. In addition to high quality proteins, it contains high amounts of calcium, vitamin B12, Vitamin A, B2, B6, E, K and H, many essential minerals and enzymes. *Spirulina* is also very rich in terms of iron content (Henrikson, 1994; de Caire *et al.*, 2000; Varga *et al.*, 2002; Akalin *et al.*, 2009; Radulović *et al.*, 2010).

*Mentha* spices are used for their flavoring and medicinal properties widely throughout different countries of the world. *Mentha piperita* is currently used to treat irritable bowel syndrome, Crohn's disease, ulcerative colitis, biliary tract disorders and liver complaints. It is cultivated in India, China, Europe, America, Australia, South Africa and some other countries. (Shah and Mello, 2004). Peppermint is on the FDA<sup>1</sup>'s GRAS (generally recognized as safe) list and whole herb peppermint has few side effects (Gardiner, 2000).

*Lactobacillus* strains containing products like yoghurt has gained popularity all over the world. The probiotic activity of some lactobacillus strains stabilize the intestinal microflora, (Parda *et al.*, 1998).

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\*Corresponding author: Atefeh Eslami-Moskenani, M.Sc. student of Food Science and Technology, Shahr-e-Qods branch, Islamic Azad University, Tehran, Iran; E-mail: atefeh.eslami.m@gmail.com

The objective of this research was to study the effects of various concentrations of natural additives, *Spirulina platensis* biomass and powdered *mint*, on the pH and titratable acidity of probiotic Doogh during cold storage (21 days at 4 °C).

## MATERIALS AND METHODS

### Cultures

Thermophilic yoghurt culture containing *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* (YF-3331) was supplied by Chr. Hansens (Horsholm, Denmark). Also, *L. acidophilus* (La-5) was used (from Chr. Hansens dairy cultures, Denmark) to produce probiotic dairy- beverage product. The cultures were used in a freeze dried direct vat set (DVS) and maintained according to the manufacturer's instruction at -18 °C until used.

### Additives

*Spirulina platensis* was used in powdered form obtained from Gheshm Sina Microalgae company, Tehran, Iran. *Menta piperita* was used in a powdered form obtained from Goar, Markazi, Iran.

### Study design and Doogh samples preparation

Cow's milk with 1.2% fat, 2.9% protein, 9.6 % total solid, 8.21% solids non fat, pH 6.63 and titratable acidity 14.5 (°D) was pasteurized at 85°C for 30 min. After cooling milk up to fermentation temperature (40 °C), it was inoculated with yoghurt culture, 0.1% (w/v) according to the manufacturer's instruction, and incubated. During fermentation, pH drop and titratable acidity increase were monitored every 30 min until pH reached 4.5±0.02 (Figs.1 and 2). After fermentation, yoghurt prepared was held in refrigerator (4°C). For producing Doogh, water (50% v/v) and salt (0.7% w/v) were added to yoghurt and mixed. Doogh prepared was heated to a high temperature (85 °C for 15 min), for deactivating yoghurt culture, tempered to 36 °C, and inoculated with 0.2 % (w/v) *L. acidophilus* (Culture was used according to the manufacturer's instruction). According to concentration of powdered *mint*, 0.5 and 1% (w/v), Doogh was divided into two groups and enriched with *S. platensis* at concentrations of 0.3, 0.5 and 0.8% (w/v); two samples with no added *S. platensis* were used as controls. Then Doogh samples were filled into 250-ml PET bottles and stored at 4 °C for 21 days. Doogh samples were taken for titratable acidity and pH measurements on 1, 7, 14 and 21 days of storage.

### Measurement of titratable acidity and pH

Samples (10 mL) were titrated with 0.1 N NaOH (Merck), using 0.5 % phenolphthalein as an indicator (Anonymous, 2005).

The pH of the samples were measured at room temperature using a pH meter (Metrohm, Germany) and combined glass electrode standardized with pH 4.00 and 7.00 standard buffer solutions (Anonymous, 2005).

### Statistical analysis

Each experiment was independently replicated three times in a completely randomized design. Analysis of variance (ANOVA) was applied using SAS 9.1 software. Lsmean's test was used to compare the difference among means values at the significant level of  $p < 0.05$ .

## RESULTS AND DISCUSSION

The pH of the probiotic Doogh samples during the storage are shown in Figs. 3 and 4. The pH of cyanobacterial samples was significantly higher than that of control at the beginning of storage time ( $p < 0.05$ ). This is in accordance with findings of Varga *et al.* (2012), who observed that the addition of *S. platensis* increased the pH in fermented milk, and Guldaz and Irikh (2010), who found the same result in yoghurt and acidophilus milk. According to results of Varga *et al.* (2012), an aqueous solution containing 3 g/dm<sup>3</sup> *Spirulina* has a pH of 9.9. The pH value decreased slowly during storage time. The pH of controls was lower than that of cyanobacterial samples at the end of storage time and there were no significant differences between treatments with 0.5 and 0.8% *S. platensis*. Slow decrease of pH in algal samples is presumably due to alkaline character of cyanobacterial biomass because it contains compounds, such as proteins, peptides and amino acids, with considerable buffering capacity (Varga *et al.*, 2012). Hence, greater buffering capacity led to slower pH drop and stimulate acidification rate by *L. acidophilus* (Varga *et al.*, 2012; Beheshti pour *et al.*, 2012). However, this is in contrast to other studies, which have shown that the pH values were lower in algal yogurt samples containing dried *S. platensis* biomass (Akalin *et al.*, 2009) and the *S. platensis*-supplemented fermented ABT milk (Varga *et al.*, 2012) than those of the control. As shown in Figs. 4 and

5, there was no significant difference in pH between samples with various concentrations of powdered *mint* during cold storage. The acidity values in Doogh inoculated with *L. acidophilus* and containing different concentrations of *S. platensis* increased more rapidly than those in the control samples during storage time (Figs. 5 and 6). The results revealed a significant relationship between *S. platensis* addition and titratable acidity; so that the acidity increased with the increasing *S. platensis* content. This is due to addition of alga caused to stimulate the growth of *L. acidophilus*. This effect can be attributed to the presence of free amino acids, peptone, adenine and hypoxanthine in the algal biomass. These nitrogenous substances are capable of significantly stimulating the growth and acid production of *L. acidophilus*. (Parada *et al.*, 1998; Akalin *et al.*, 2009; Guldass and Irikh, 2010). Also, the increments in acidity appear to be related to the fact that the growth of probiotic bacteria populations in the fermented functional foods are much lower in the presence of the starters than when the probiotics are grown alone (Guldass and Irikh, 2010); as yoghurt starters have been inactivated in Doogh and there is no any competitive microorganism with *L. acidophilus*. There was no significant difference in acidity between samples with various concentrations of powdered *mint* during storage.

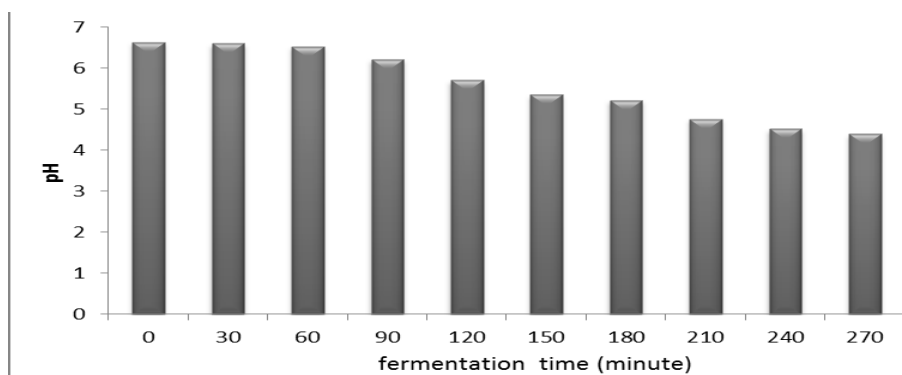


Fig.1. Changes of pH in yogurt during fermentation.

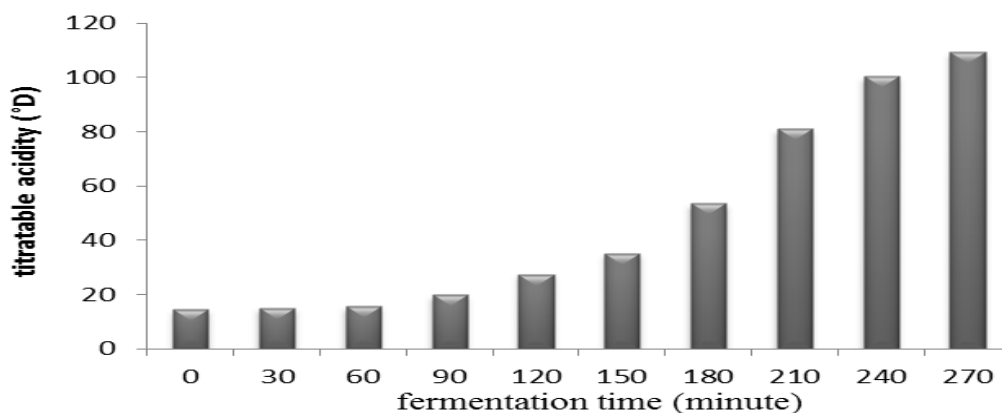


Fig.2. Changes of titratable acidity in yogurt during fermentation.

## Conclusion

The results obtained in the present study indicate that the addition of *S. platensis* decreased pH slowly and significantly enhanced the titratable acidity in probiotic Doogh samples during storage. It was noted that there was no significant difference in pH and titratable acidity between samples with various concentrations of powdered *mint* during cold storage.

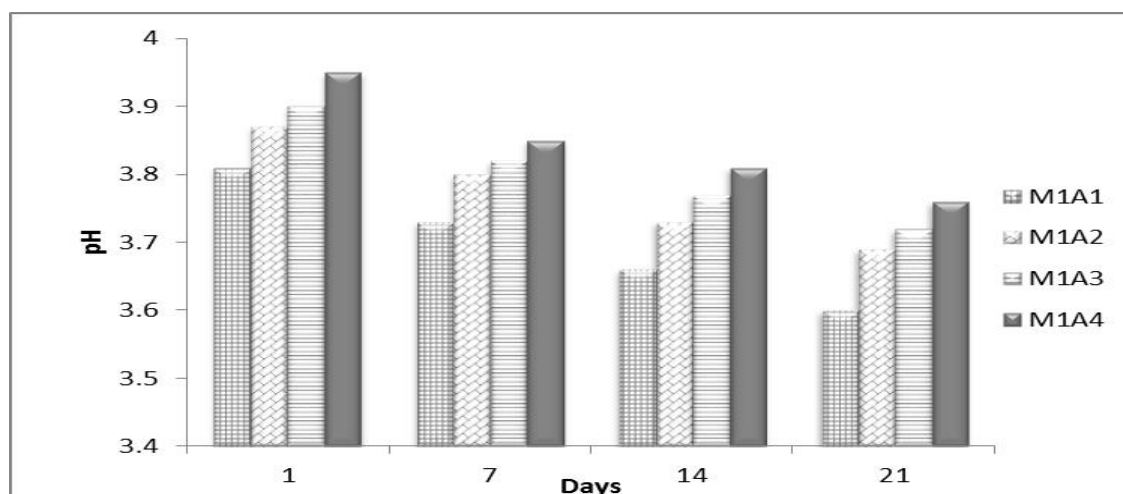


Fig. 3. Changes of pH in probiotic Doogh samples containing different concentrations of *Spirulina platensis* and 0.5% mint during storage ( $M_{2A1}$ :0%,  $M_{2A2}$ :0.3%,  $M_{2A3}$ :0.5%,  $M_{2A4}$ :0.8%).

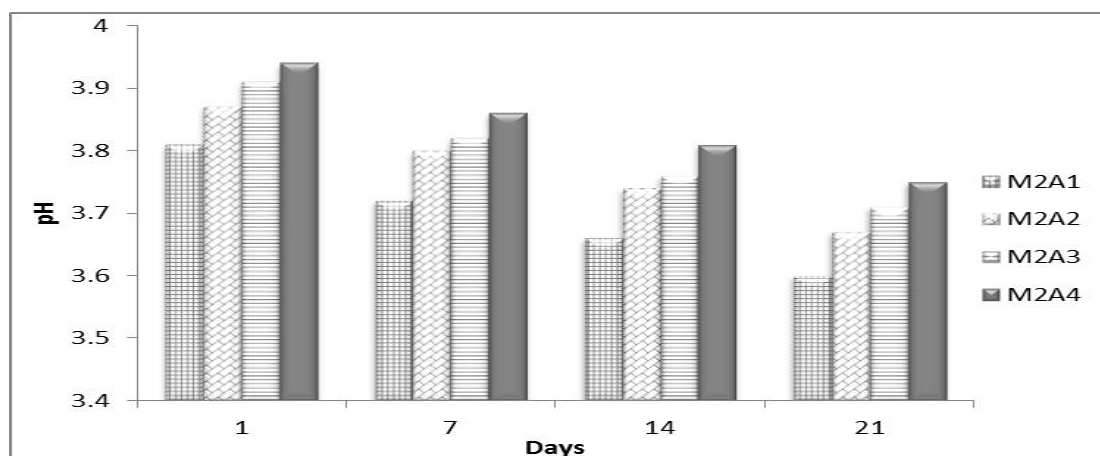


Fig. 4. Changes of pH in probiotic Doogh samples containing different concentrations of *Spirulina platensis* and 1% mint during storage ( $M_{2A1}$ :0% ,  $M_{2A2}$ :0.3%,  $M_{2A3}$ :0.5%,  $M_{2A4}$ :0.8%).

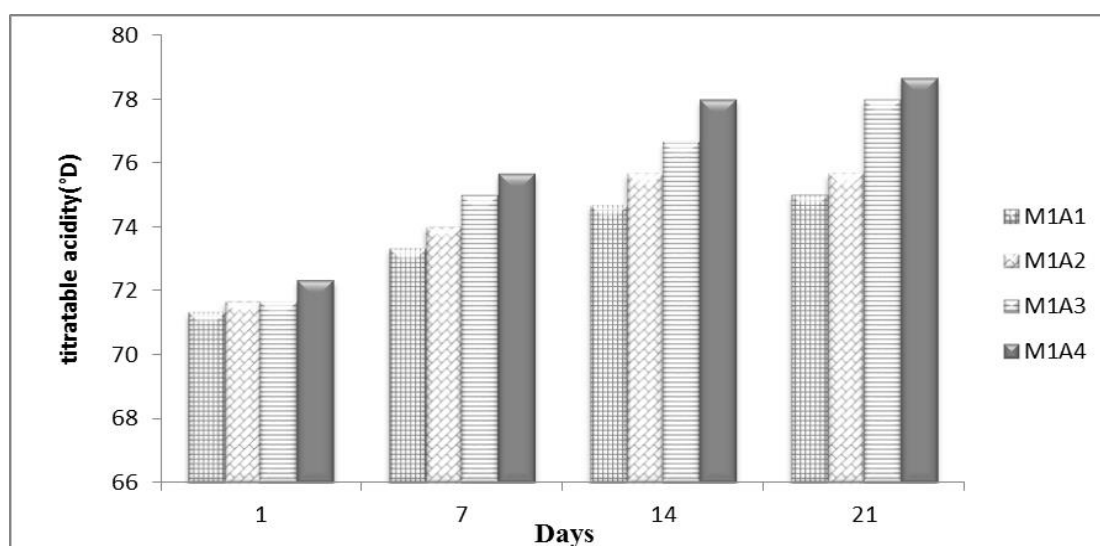


Fig. 5. Changes of titratable acidity (°D) in probiotic Doogh samples containing different concentrations of *Spirulina platensis* and 0.5% mint during storage ( $M_{2A1}$ :0% ,  $M_{2A2}$ :0.3%,  $M_{2A3}$ :0.5%,  $M_{2A4}$ :0.8%).

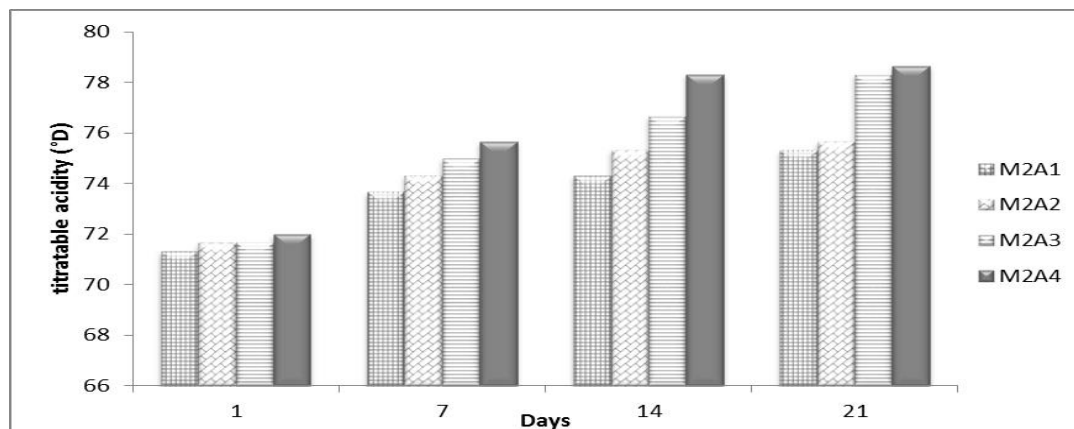


Fig. 6. Changes of titratable acidity (°D) in probiotic Doogh samples containing different concentrations of *Spirulina platensis* and 1% mint during storage (M<sub>2</sub>A<sub>1</sub>:0% , M<sub>2</sub>A<sub>2</sub>:0.3%, M<sub>2</sub>A<sub>3</sub>:0.5%, M<sub>2</sub>A<sub>4</sub> :0.8%).

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