

## Physicochemical properties and survivability of probiotics in bio-dough containing wild thyme essence and xanthan gum

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### Abstract

In this study, the effect of kakuti or wild thyme (*Ziziphora clinopodioids*) essence and Xanthan gum on survivability of *Bifidobacterium lactis* in Iranian yogurt drink (dough) was evaluated. The kakuti essence was added at concentrations of 50 and 75 µl. The levels of added Xanthan gum were 0, 0.075 and 0.15%. Samples were analyzed after 1, 15, 30 and 45 days of storage. The results of statistical analysis showed that by increasing the amounts of Xanthan gum to 0.075%, *B. lactis* count increased 1.2 log CFU/ml significantly ( $P < 0.05$ ), but further increasing of the gum, had no significant effect on *B. lactis* count ( $p > 0.05$ ). Moisture content was decreased as the amounts of Xanthan gum increased. Stability, viscosity and texture score were increased significantly ( $P < 0.05$ ). Samples containing 0.15% of xanthan gum were completely stable during storage period. The acidity increased in all samples during storage and the highest acidity was observed for samples containing xanthan gum. The results of sensory evaluation showed that the flavor scores were decreased significantly ( $p < 0.05$ ) as the amount of essence was increased from 50 to 70 µl. According to the obtained results, at least 0.15% of xanthan gum is necessary to produce a completely stable dough, although increasing the gum concentration to more than 0.075% did not have any significant effect on the viability of *B. lactis*. The favorable amount of kakuti essence was observed to be 50 µl. Thus, these additives could be used at the said amounts successfully without having detrimental effects on bio-dough.

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### Keywords

Bio-dough

Herbal flavorings

Probiotics

Wild thyme

Xanthan gum

Yogurt drink

### Introduction

Today, different kind of probiotic dairy products such as yoghurt drinks, kefir, cheeses and ice creams are produced in the world. The technological issues relating to the development of these products containing probiotic bacteria in sufficient numbers during storage need to be overcome (Stanton *et al.*, 2003). Dough is a kind of Iranian fermented acidic dairy drink which is prepared by mixing water, yoghurt, salt and herbal flavorings such as pennyroyal and spearmint. This product provides one fourth of daily requirement of calcium. It contains vitamin B and is useful for the health of teeth. Separation in to two phases due to its high acidity ( $pH < 4$ ) and aggregation of dough proteins is one of the technological issues of dough (Azarikia and Abbasi, 2010). To overcome this problem, many researchers have studied the effect of adding different hydrocolloids such as gelatin, pectin, guar, locust bean and tragacanth on the stability of this drink (Kiani *et al.*, 2010; Azarikia and Abbasi, 2010). Shafei *et al.* (2012) evaluated the antimicrobial activity of the essential oil of *Ziziphora clinopodioids*

on *Kluyveromyces marxianus*. They reported that the minimum inhibitory concentration (MIC) of the essential oil of *Ziziphora clinopodioides* was 0.25% for *Kluyveromyces marxianus*. They related this effect to phenolic compounds present in the oil. Vossough *et al.* (2009) showed that mint extract depending on its concentration could affect the survival of the bacteria *Lactobacillus acidophilus* and *Bifidobacterium lactis*, inoculated into yoghurt drinks containing 1% or 2% mint extract. The authors showed that after storing at 4°C, viable *B. lactis* reduced by 2 log CFU/ml, while *L. acidophilus* reduced to zero after eight weeks at 4°C. There was no significant difference ( $P > 0.05$ ) between pH and acidity of bio-dough during storage period. A few studies have been performed on the interaction between flavoring agents of Lamiaceae family and yoghurt starter culture activity. The number of starter culture in set yoghurt containing different concentrations of *Ziziphora clinopodioides* decreased during storage at 4°C. The extract of *Ziziphora clinopodioides* at high concentration (4000 µg/L) significantly ( $P < 0.01$ ) decreased the viability of starter culture

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of yoghurt after 19<sup>th</sup> day (Mehrabansangatash *et al.*, 2007). In another study, it was reported that among mint, clove, cinnamon and cardamom essential oils, the cinnamon essential oil at the concentration of 0.005-0.5% could inhibit the growth of the yoghurt starter bacteria completely (Bayoumi, 1992). The antibacterial effects of *Ziziphora clinopodioides* on different bacterial species have been studied by many researchers (Chitsaz *et al.*, 2007; Mehrabansangatash *et al.*, 2007; Jamalifar *et al.*, 2009). Azarikia *et al.* (2009) studied the effects of high-methoxyl pectin, tragacanth, locust bean, tragacanthin, and soybean soluble polysaccharides on the stability of sour Doogh. They showed that tragacanthin, tragacanth and locust bean gum could inhibit serum separation for 30 days at a concentration of 0.1, 0.2 and 0.3%, respectively, while soybean soluble polysaccharides caused the stabilization at a concentration of 0.6% only for 6 days (Azarikia *et al.*, 2009). In this study, we aimed to evaluate the effect of the kakuti essence addition on the survivability of *Bifidobacterium lactis* and the effect of xanthan gum concentration on the stability of bio doogh.

## Materials and Methods

### Materials

Milk was purchased from a local milk producer in Semnan (Iran). The milk contained 12.5% dry matter, 3% fat and 3.17% protein. Its acidity was 0.14% as lactic acid and its pH and density were 6.73 and 1.030, respectively. The commercial yoghurt starter was a mixed culture of streptococcus thermophiles and *Lactobacillus delbrueckii* subsp. *bulgaricus* obtained from Christian Hanson Inc., Denmark. *Bifidobacterium lactis* was purchased from DSM Company in Australia.

### Preparation of probiotic doogh

Milk was pasteurized for 15 minutes at 85°C and cooled up to 44°C. Then the starter culture was added to the milk according to the producer recommendations and incubated at 44°C to reach the pH of 4.4 and then cooled. Xanthan gum at three levels of 0 (as control), 0.075 and 0.15% was added to the water used for dilution of yoghurt. Then 0.8% of salt was added to the solution and it was pasteurized at 90°C for 15 minutes. Yoghurt was diluted at ratio of 50:50 with water containing xanthan gum and salt. After dilution, the kakuti essence at levels of 50 and 75 µl and *Bifidobacterium lactis* ( $6.4 \times 10^8$ ) were added. The samples were packaged in PET bottles and stored for 45 days at 5°C.

### Probiotic enumeration

Five milliliters of doogh samples were added to 45 ml of sterile 0.1% peptone solution to prepare 0.1 dilution. Other dilutions were prepared by adding 1 ml of the last dilution to 9 ml of sterile peptone solution. *Bifidobacterium lactis* was cultured on RCA (Reinforced Clostridia medium Agar) at 37°C for 72 hours under anaerobic conditions using gas pack (Krasaekoopt *et al.*, 2004).

### Chemical and physical measurements

Chemical and physical analysis including moisture content, pH, acidity, stability (serum separation) and apparent viscosity were conducted at days 1, 15, 30 and 45.

The stability of doogh was measured according to Amiriaghdaei and Alami method (2011) by use of the following equation:

Apparent viscosity was determined at 20°C by a rheometer equipped with concentric cylinder geometry. The shear rate was increased from 0.1S-1 to 100 at 10-minute intervals to measure the shear stress and viscosity of samples as a function of shear rate.

### Sensory evaluation

Sensory attributes of bio-doogh samples including taste and consistency were evaluated using a 5-point hedonic test by 15 trained panelists. The samples were prepared in coded containers presented to panelists one day after preparation at 5°C. Panelists used mineral drinking water after each test to rinse their mouth.

### Statistical analysis

This study was conducted using a completely randomized design based on factorial test with three replications. Analysis of variance (ANOVA) was performed on all experimental data and means were compared using Duncan's multiple range test (DMRT) at the statistical level of 95% ( $p < 0.05$ ) with Minitab 16 software. The treatments were xanthan gum at three levels (0, 0.075 and 0.15%) and kakuti essence at two levels (25 and 75 µl).

## Results and discussion

### Survival of *Bifidobacterium lactis*

It is shown in Figure 1 that the effects of storage time, the concentration of xanthan gum and their mutual effects on survival of *Bifidobacterium lactis* are significant ( $p < 0.05$ ). The number of *Bifidobacterium*

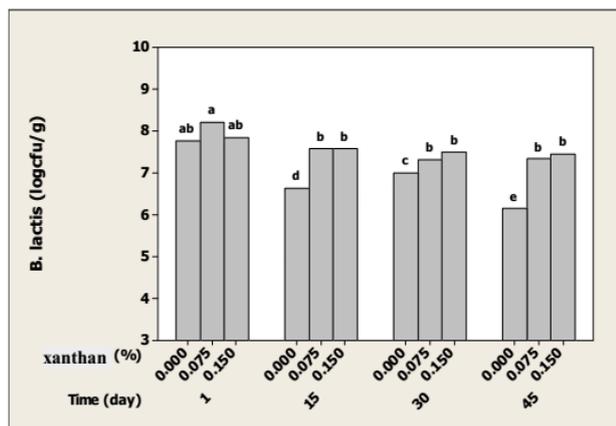


Figure 1. The effect of xanthan gum concentration on survival of *Bifidobacterium lactis*

colonies were significantly increased by addition of 0.075% of xanthan gum, but increasing the level of xanthan gum to more than this amount had no significant effect. The number of *B. lactis* bacteria decreased significantly during 45 days of storage and the lowest number of bacteria was observed for control samples (without gum). After 45 days, the log number of *B. lactis* in the samples containing 0, 0.075 and 0.15% xanthan gum were 6.15, 7.33 and 7.44 respectively. This showed that xanthan gum had increased the survivability of *B. lactis* to about 1.2 log. Xanthan gum, as a prebiotic has an activating effect on the growth of probiotics. Mohebbi and Ghoddousi (2008) reported that the mixture of lactulose–inulin could stimulate the growth of *Lactobacillus casei* and *L. acidophilus* (Mohebbi and Ghoddousi, 2010). Akalin and Erisir (2008) also showed that prebiotics such as oligofructose and inulin improved survivability of probiotics in ice cream. Bifidobacteria prefer fructo-oligosaccharides as carbon and energy sources (Akalin and Erisir, 2008). Inulin increases the survival of *L. acidophilus* and *B. lactis* in ice cream (Akin et al., 2007).

#### pH and acidity

Statistical analysis showed that the effect of storage time, xanthan gum and kacuti essence on the pH (Figure 2-A) and acidity (Figure 2-B) of bio-dough were significant ( $p < 0.05$ ). The acidity of control samples decreased until the day 30 and then it increased significantly. The acidity of samples containing 0.075 and 0.15% xanthan gum increased significantly during storage. The pH of the samples containing 0.075% of xanthan gum decreased during 30 days of storage and then increased at the day 45. However, the samples with 0.15% of gum showed no significant changes during storage. This is probably due to the buffer effect of xanthan gum hydrocolloid.

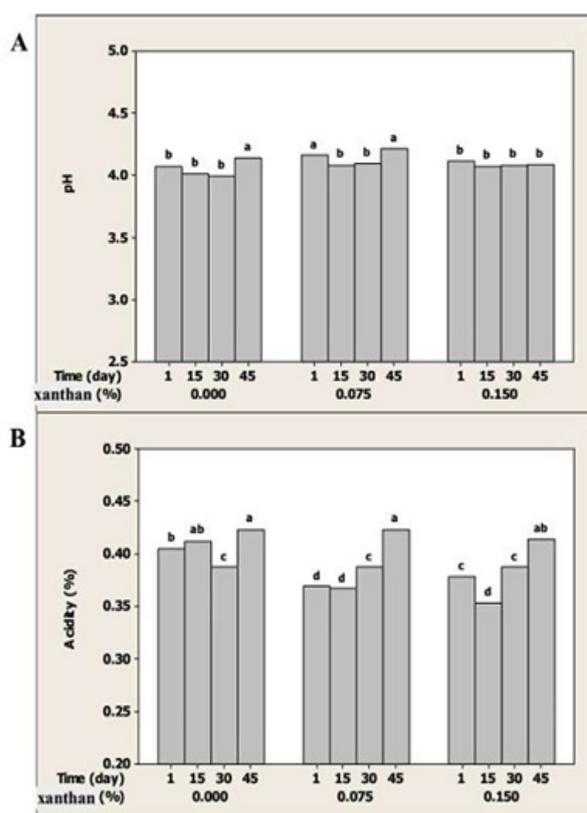


Figure 2. The mutual effect of storage time and xanthan gum concentration on the pH (A) and acidity (B) of bio-dough

#### Moisture

The effects of storage time and concentrations of xanthan gum on the moisture content of samples were significant ( $p < 0.05$ ). The moisture of samples decreased as the concentration of xanthan gum increased (Figure 3-B). Xanthan gum is a hygroscopic gum and entraps water.

#### Stability

The stability of bio-dough was increased as the concentration of xanthan gum increased and by extending the storage time from 15 days to 45 days, the stability decreased significantly (Figure 3-A). The stability of bio-dough increased with the concentration of xanthan gum. Samples containing 0.15% of xanthan gum were completely stable during storage period. The stability of samples with 0.075% xanthan gum decreased significantly during storage time. The formation of complexes between proteins and gum in the presence of free radicals is one of the reasons of stability loss during storage (Langendorff et al., 1997; Abbasi and Dickinson, 2004). Xanthan gum especially at high concentrations forms some hydrocolloid network throughout dough which entraps caseins and water and so prevents from syneresis (Syrbe et al., 1998). Foroughinia et al.

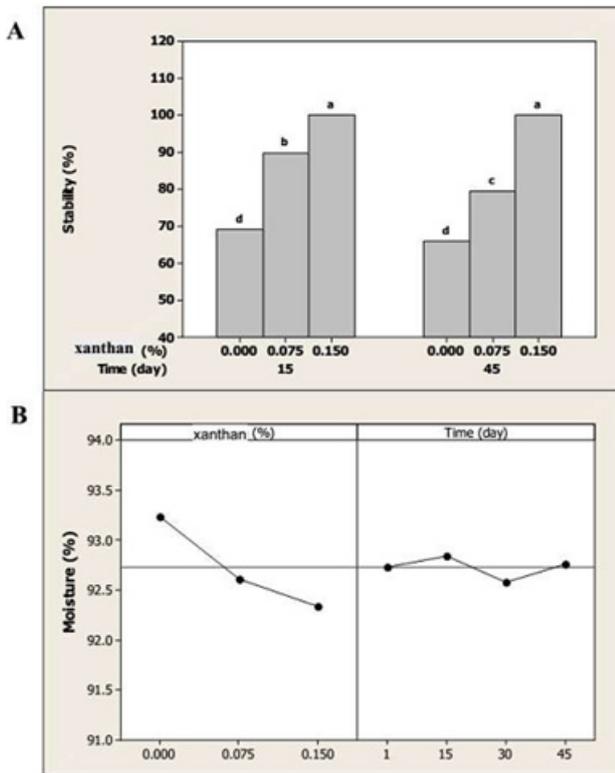


Figure 3. Effect of xanthan gum concentration and storage time on the stability (A) and moisture content (B) of bio-dough

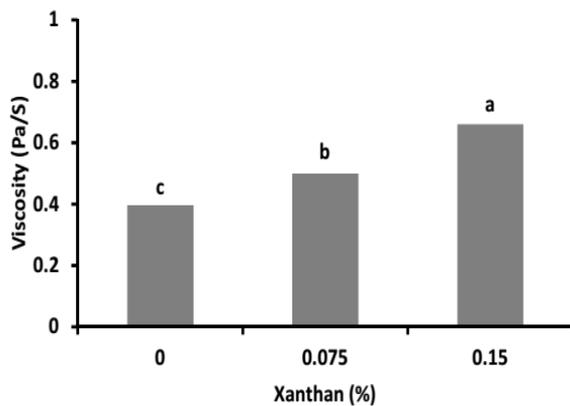


Figure 4. The effect of xanthan gum concentration on the viscosity of bio-dough

(2007) reported that guar gum at the level of 0.3% decreased syneresis significantly and stabilized the product completely at 0.5% level (Foroughinia *et al.*, 2007). Azarkia and Abbasi (2010) reported that soluble tragacanthin adsorbs onto casein and prevents Serum separation in Doogh via electrostatic and steric repulsions (Azarkia and Abbasi, 2010). Parker *et al.* (1995) suggested that the addition of xanthan gum to salad dressing leads to the formation of a particle network and the time-dependent yield stress of this network stabilizes the dressing (Parker *et al.*, 1995).

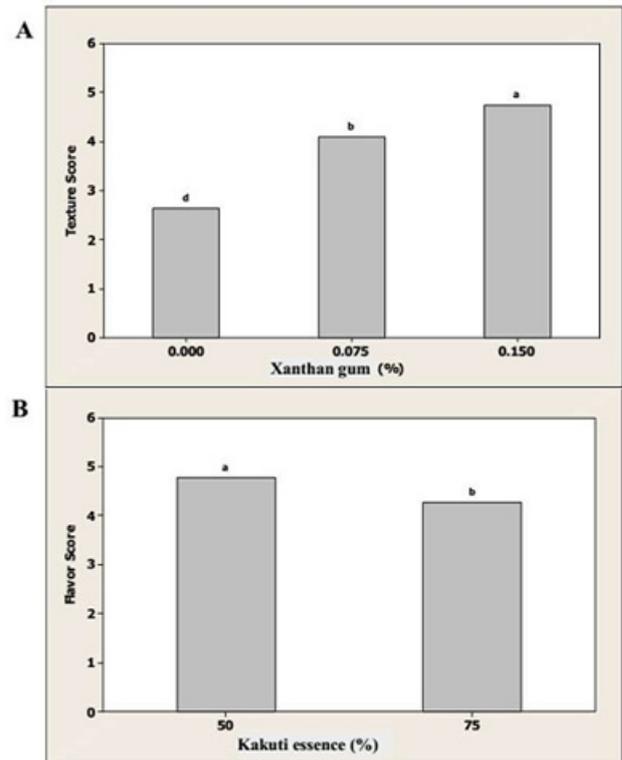


Figure 5. The effect of xanthan gum concentration on the texture score (A) and kakuti concentration on flavor score (B) of bio-dough

#### Viscosity

As it is shown in Figure 4, the viscosity of probiotic doogh was increased as the concentration of xanthan gum increased from 0 to 0.15%. The results of different studies showed that the presence of hydrocolloids has significantly changed the viscosity of yoghurt drinks (Paraskevopoulou *et al.*, 2003; Koksoy and Kilic, 2004; Foroughinia *et al.*, 2007; Janhoj *et al.*, 2008). Janhoj *et al.* (2008) showed that carboxymethyl cellulose increased the viscosity of drinking yoghurt (Janhoj *et al.*, 2008). When xanthan gum is used, cluster formation due to depletion flocculation is less extensive and the formation of weak gel by the polysaccharide molecules in the continuous phase will stabilize the casein micelles and fat globules against strong aggregation, cluster formation and precipitation (Paraskevopoulou, 2003).

#### Sensory evaluation

In Iran the traditional drinks flavored with herbs are very popular. Traditionally, yoghurt and doogh are flavored with herbs such as mint, dill or kacuti. The sensory evaluation of samples showed that the concentration of xanthan gum and kacuti had significant effects on the consistency (Figure 5-A) and flavor (Figure 5-B) of bio-dough, respectively. The consistency scores of samples containing higher concentrations of xanthan gum were higher and this

was in accordance with viscosity measurements. The panelists preferred bio-dough containing 15µl of kacuti. Higher levels of this herb caused a bitter flavor. Azarkia and Abbasi (2010) showed that the presence of local herbs extracts in doogh stabilized with tragacanthin could enhance its taste score. They also reported that gum tragacanth and tragacanthin did not significantly change the consistency of doogh (Azarikia and Abbasi, 2010).

## Conclusion

According to the results, adding xanthan gum at the level of 0.075% increased the number of *B. lactis* significantly. However, higher levels had no significant effect on the number of *B. lactis*. The extract of *Ziziphora clinopodioides* (kacuti) had no significant effect on the number of Bifidobacteria. Thus, we can use this flavoring herb in bio-dough at the level of 50 µl without any effect on the reduction of probiotics. Concentrations of kacuti more than 50 µl, made the flavor of bio-dough unfavorable. The bio-dough stability was the highest at the level of 0.15% of xanthan gum. Also, the viscosity of the product increased with the concentration of xanthan gum. The *Ziziphora clinopodioides* had no effect on these characteristics. It is recommended that the effect of other flavoring herbs and gums be investigated by other authors.

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