

## PHYSICO-CHEMICAL AND RHEOLOGICAL PROPERTIES OF DOOGH UNDER SPECIFIC FERMENTATION

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### ABSTRACT

Fermented dairy products such as doogh are more nutritious than the natural milk from which they are made. In this study enhancing the viscosity of *Doogh* (Iranian yogurt drink) fermented by *Leuconostoc mesenteroides* under the optimum condition of exopolysaccharide (EPS) production including temperature of 25°C, agitation of 120 RPM and different sucrose contents from 0 to 2%, w/w, was investigated. Addition of sucrose was not influenced the rheological and sensory characteristics of *Doogh* samples before the fermentation significantly according to sensory and rheological analysis. The results of chemical, microbiological, viscosity, serum separation and sensory analysis showed that viscosity of *Doogh* increased corresponding to sucrose contents during the fermentation up to 10.01 cp with no change in the taste of the *Doogh* samples. Also, sensory analysis of consistency confirmed the viscosity results. Serum separation values of samples also showed enhancement in stability after the fermentation and finally, this special fermentation process contributed to enhancement in viscosity of *Doogh* as an edible suspension, with reasonable economic targets compared with other stabilizing methods.

**Keywords:** Doogh, fermentation, *Leuconostoc mesenteroides*, physicochemical quality, rheological properties

### INTRODUCTION

Dairy drinks are a group of dairy products that have been known as acidified dairy drink (ADD) and produced variously, e.g. yogurt drink, buttermilk, whey drink, kefir, etc. These dairy products can be described as an acidified protein liquid system with lactic acid fermentation by lactic acid bacteria (LAB) with stability occasionally similar to natural milk (Laurent and Boulenguer, 2003). Yogurt, by its presence in the market and high consumption is the most important fermented dairy product (Ramírez-Sucre and Vélez-Ruiz, 2013). Also, the number of consumers of yogurt drinks and associated products increased in recent years (Gonzalez et al., 2011). Yogurt drinks are produced and consumed in various ways in the world. *Doogh* is a traditional Iranian yogurt drink usually is manufactured by mixing of water, salt, set or stirred yogurt and extract of some aromatic herbs (Azarikia and Abbasi, 2010). Ayran also is another traditional yogurt drink with high fat content that is produced and consumed in Turkey (Koksoy and Kilic, 2004). It has been reported that the fermented dairy products are more nutritious than the natural milk from which they are made. Importance of nutritional value in these products attributes to availability of certain nutrients (O'Connell and Fox, 2001) and hydrolysis of milk constituents with fermentation by LAB making them more digestible (Park, 1994). Yogurt and yogurt drink through being more digestible caused by lactic acid fermentation (Gonzalez et al., 2011), are enriched with bioactive peptides that are produced by proteolytic activity (Kunda et al., 2012) and the presence of LAB having functionality properties known as probiotic organisms (Ruas-Madiedo et al., 2002).

Yogurt drinks are produced from natural milk by lactic acid fermentation. Casein in milk at its natural pH of approximately 6.6 is in a stable form, micelle. When the pH of milk is decreased by lactic acid fermentation or other acidification process to 5.5 – 5.8, zeta potential decreases conducting the micelles to form clusters. During producing of lactic acid and lowering the pH to 5 micelles in clusters are in multi-strands structure. This position leads clusters to aggregation by more decreasing of pH to below the pH 5. These steps occur to construct suspension form of casein from its colloidal system in yogurt and yogurt drink under condition of decline in pH by lactic acid (Laurent and Boulenguer, 2003). Biochemical pathways in yogurt fermentation include carbohydrate, protein, fat and vitamins metabolic activities that their final products are lactic acid; peptides and amino acids; free fatty acids and vitamins of group B,

respectively. Moreover, metabolism of carbohydrate (lactose) and produced lactic acid is the most important biochemical reaction (O'Connell and Fox, 2001). Lactic acid fermentation can cause biochemical changes in the milk. It reduces the pH of the milk and leads to a progressive being soluble of micellar calcium phosphate, causing the demineralization of casein micelles and their destabilization, which generates the complete precipitation of casein in a pH range of 4.6-4.7. Also, produced lactic acid by LAB provides the yogurt or yogurt drink with its shape, acid taste and contributes to the flavor (Santos et al., 2000). Lactic acid is the most important product of LAB while the fermentation time increases in any substrate that this kind of fermentation has been done with it.

Despite lactic acid producing, LAB constructs some other fermentation products that significantly are produced by them. One group of these secondary metabolites is hetero polysaccharides, such as hydrocolloid materials, produced by some varieties of LAB; for example *leuconostoc* and some of the *lactobacillus* strains. The hetero polysaccharides that are secreted by LAB have been known as EPS (Johanningsmeier et al., 2007). EPS develops the texture, modifies the rheological properties and hence the stability of the products by increase in viscosity (Huang et al., 2013). EPS incorporate with casein when it is continuous to influence, increase the viscosity and decline the serum separation of suspension system (Kiani et al., 2010). The natural properties of milk protein in acidified condition are extensive casein micelle aggregation, sedimentation and subsequent macroscopic whey separation. It is well known that the stabilization of acidified milk drinks is dependent upon many factors including pH range, protein content, hetero polysaccharides etc. The presence of hetero polysaccharides improves the viscosity, serum separation and stability of the suspension system of acidified milk drinks significantly more than other factors. Consequently, addition of natural and microbial hetero polysaccharides is used in manufacturing acidified milk drinks such as yogurt drinks as a stabilization method (Thi and Ipsen, 2009). *Doogh*, the Iranian yogurt drink also is an edible suspension that its stability is developed by this method (Kiani et al., 2010).

Despite adding stabilizers, for increasing viscosity and declining serum separation to enhance the stability of suspension system, other methods specially are used to achieve viscosity development such as fermentation with specific microorganisms under characterized conditions (Edema, 2010). Increased the viscosity of formulated microbial culture by fermentation with *Streptococcus thermophilus* under developed condition of EPS producing was reported (Shene

et al. 2008). Also enhance the viscosity of sour maize meal was made by fermentation with *Leuconostoc mesenteroides* (Edema, 2010). EPS producer bacteria are used to increase the viscosity and stability of fermented materials. *Lactobacilluslactis*, *raffinolactis* and *graviæae*; *Leuconostoc pseudoplantarum* and *mesenteroides* are the lactic acid bacteria that can secrete -EPS or some other associated hydrocolloid materials and also develop the viscosity serum separation and stability of the suspensions during and after the fermentation (Adebayo-tayo and Onilude, 2008). In addition to these technological benefits, EPS have beneficial physiological effects on the consumer. It is assumed that, enhancement of viscosity in EPS containing foods may increase the residence time of ingested fermented milk in the gastrointestinal tract and therefore be beneficial to a transient colonization by probiotic bacteria as a functional product (Hemme and Foucaud-Scheunemann, 2004).

*Leuconostoc* represent a small percentage of the mesophilic micro-flora belonging to the group of LAB including a wide range of varieties that are well known in the dairy industry and functional foods (Kekkonen et al., 2008). *L. mesenteroides* between varieties of *Leuconostoc* has the highest efficiency in EPS producing. Strains of these bacteria that efficiently produce EPS include B-1355, B1498, B-1299 and B-1501 (Naessens et al., 2005). EPS produced by *L. mesenteroides*, has been secreted in a higher efficiency under best temperature and agitation conditions that are about 25°C and 120 RPM, respectively (Johanningsmeier et al., 2007). These conditions lead EPS better to be contributed in fluidal media that bacterium is growing in it. In this research enhancement in viscosity of Iranian yogurt drink (*Doogh*) by fermentation with *L. mesenteroides* strain of B-1501 was investigated. Manufactured *Doogh* has fermented by *L. mesenteroides* as mentioned above under the best cultural conditions of EPS producing that are temperature 25°C, agitation 120 RPM and the presence of different contents of sucrose (Edema, 2010). Viscosity, serum separation, consistency and stability of *Doogh* samples were studied during the fermentation time.

## MATERIALS

Pasteurized *Doogh* (pH: 4.3, Fat: 1.2%) and commercial sugar that were used for this study were purchased from the local market in Karaj, Iran. Packages were stored at 4°C until the re-fermentation and experiments; finally was divided into 200 ml containers for incubation by *L. mesenteroides* B-1501 and sampling for chemical, microbiological, rheological and sensory analysis, consequently.

## METHODS

### Fermentation of *Doogh*

#### Preparation of *L. mesenteroides* stocks for re-fermentation of *Doogh* samples

The activation of *L. mesenteroides* has been done by the procedure described by Santos et al. (2000). The *L. mesenteroides* B-1501 (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH., Germany) was supplied for this study obtained from microbial culture stock of Department of Food Science and Engineering (University of Tehran). Bacterial culture was stored frozen at -20°C in MRS medium (Merck., Germany) containing 20% glycerol. The strain has activated by incubation at 30°C for 24 hours in ten milliliters of MRS broth (dextrose 20.0 g/L; meat peptone 10.0 g/L; beef extract 10.0 g/L; yeast extract 5.0 g/L; sodium acetate 5.0 g/L; disodium phosphate 2.0 g/L; ammonium citrate 2.0 g/L; tween 80 1.0 g/L; magnesium sulfate 0.1 g/L, manganese sulfate 0.05 g/L). These stocks were grown for being appropriate for fermentation and EPS producing in *Doogh* substrate.

#### Fermentation process of *Doogh* samples

Fermentation experiments were conducted in test container, each containing 200 mL of *Doogh* yogurt drink. Sugar was added into *Doogh* containers in 0, 0.5, 1, 1.5 and 2% W/W. Adding these contents of sugar have not made any significant different in initial *Doogh* samples (similar viscosity compared with the blank sample before fermentation). All containers were inoculated with 24-h activated culture in 5% V/V and incubated at 25°C and agitation of 120 RPM, which are the best temperature and agitation conditions to EPS producing by *L. mesenteroides*, for 72 hours fermentation (Naessens et al., 2005). All samples were taken from the containers at the times 0, 24, 48, and 72 has the fermentation time increases.

#### Chemical properties

Titration acidity (TA, g lactic acid in 100 g of sample), was measured for all samples, using 0.1 mol L<sup>-1</sup>-NaOH (sodium hydroxide) and 10 g L<sup>-1</sup> phenolphthalein (Scharlau Co, Spain) solution in 950 mL L<sup>-1</sup> ethanol as an endpoint indicator. In short, 1 mL of sample was diluted with 9 mL distilled water and titrated against NaOH (Gonzalez et al., 2011). The pH of the samples was also determined by using a pH-meter (Methrohm 744, Netherland).

## Microbiological analysis

Microbiological analysis of this investigation includes measurement of cell growth in sampling times. Cell growth was measured by plating on MRS agar medium (Merck, Germany) and incubating anaerobically at 30°C for 48 h (Edema, 2010). The Initial lactic acid microbial population of *Doogh* samples before fermentation with activated *L. mesenteroides* was 2.17 log CFU g<sup>-1</sup>.

## Viscosity of *Doogh* samples

According to the method of Azarikia and Abbasi (2010) the dynamic viscosity of each sample was measured by using a rotational viscometer (DV-III digital viscometer; Brookfield RVT, USA). All measurements were done at room temperature (24 ± 1 °C) using coaxial cylinder geometry on a small sample adapter and an SC4-29 spindle set at 24 rpm (Calibrated with distilled water at the same temperature of experiment in the viscosity of 1 cp). Viscosity was recorded in cp. The viscosity of initial *Doogh* without any fermentation and adding sugar was 2.49 cp; without any fermentation and with adding 2% W/W sugar was measured 2.57 cp, whose difference is insignificant.

## Serum separation of *Doogh*

*Doogh* samples were placed in 50 ml graduated cylinders (1.2 cm radius, 12.5 cm height, and 1 ml = 0.25 cm) and stored at 4°C. The volume of separated serum at the top was read after storage for 15 days. Serum separation measured according to the method of Azarikia and Abbasi (2010).

## Sensory analysis of *Doogh* samples

*Doogh* samples were analyzed for taste, consistency and overall acceptability after the 72-hour fermentation. Sensory analysis was performed by using 20 panelists familiar with the product. A 7-point hedonic scale including both the number and verbal scores was provided to the panelists (Koksoy and Kilic, 2003). The scores were; Like extremely (7), Like very much (6), Like moderately (5), Neither like nor dislike (4), Dislike moderately (3), Dislike very much (2), Dislike extremely (1). A section for the panelist's comment was presented in the evaluation sheet. Consistency is associated to the viscosity perceived by the panelists. Mean scores for each attribute were calculated for comparison of the samples.

## Statistical analysis

All determinations were made in triplicate trials and the data generated in sensory analysis and were subjected to the one-way analysis of variance (ANOVA) at 5% level of significance using Minitab statistical software (V16, Minitab Inc., Pennsylvania, USA). Also, means were separated by Duncan's multiple range tests.

## RESULTS AND DISCUSSION

In this study we applied the fermentation with *L. mesenteroides* in *Doogh* samples to change the rheological and physicochemical property of drinks. Via measurement of viscosity and serum separation, changes in stability can be investigated during and after the fermentation. Sensory analyses such as taste and consistency evaluation of fermented samples were associated with pH, acidity and viscosity variations as the fermentation time increased (Koksoy and Kilic, 2004).

### pH and titrable acidity changes in *Doogh* samples

Chemical characteristics of the substrate during fermentation period significantly changed, because the during fermentation process the bacteria is active because of the activity of organisms, existed in the fermentation process. These changes indicate the growth and activity of the organism during the fermentation. Organic acids such as lactic, citric, propionic and acetic acid are produced by fermentative micro-organisms. These products considering their ionization constant are the most important factors that influence the final pH of fermentation products. pH and titrable acidity have shown the reverse correlation; increase in organic acid content and decline in pH value (Sefa-dede et al., 2003). Fermentation of *Doogh* with *L. mesenteroides* contributed to the reduction of the pH in all samples with fermentation time that has been shown in Fig 1. The pH of the samples after the fermentation varied from 3.81 to 4.05 and the initial pH of *Doogh* without fermentation was measured 4.45. Fermentation by this organism leads to decrease in pH significantly in dairy and nondairy substrates because of production of lactic acid predominantly and some other organic acids inconsiderably that also produced ordinarily by other LAB as the fermentation time increases (Sefa-dede et al., 2003). Production of lactic acid is contributed to enhancement of titrable acidity as it has shown in Fig 2. The initial acidity of *Doogh* sample without any fermentative treatment was measured 0.61% w/w lactic acid that increased after the fermentation; moreover titrable acidity of the

samples varied from 0.72 to 0.83% w/w lactic acid. Lactic acid in addition to have anti-pathogenic characteristics, influence the taste of the dairy and nondairy fermented drinks (Kiani et al., 2010). Range of changes in titrable acidity is the critical fact that determines the significant difference in taste of the fermented drinks (Balaswamy et al., 2011). The relationship between titrable acidity and taste of the fermented samples has discussed in sensory analysis section of this study. It should be noted that the importance of pH and titrable acidity in this investigation is for demonstration of microbial activity and growth during the fermentation process under the condition of sugar existence that leads to some special effects. Gonzalez et al (2011) have shown drop in pH and enhancement of titrable acidity or production of organic acids show the activity and growth of fermentative organism in substrate. For this reason, it should be resulted that *L. mesenteroides* have fermented the substrate (*Doogh*) after 72 h and it was confirmed by investigation of cell growth.

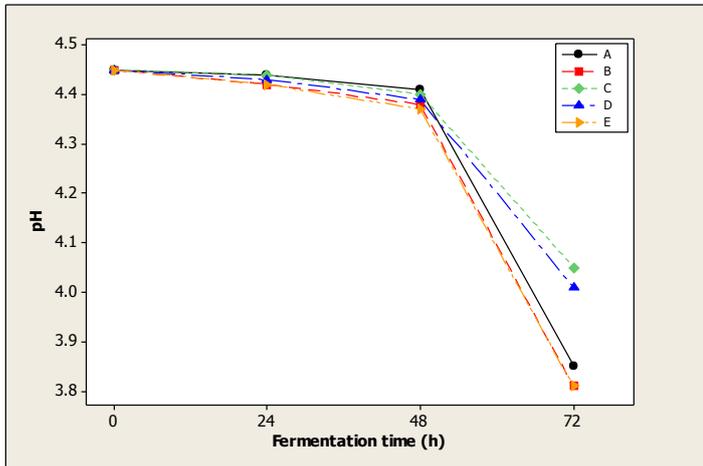


Figure 1 pH changes during fermentation of *Doogh* samples with *L. mesenteroides* under the optimum conditions of EPS producing, containing sugar contents of A = 0%, B = 0.5%, C = 1%, D = 1.5% and E = 2% (W/W)

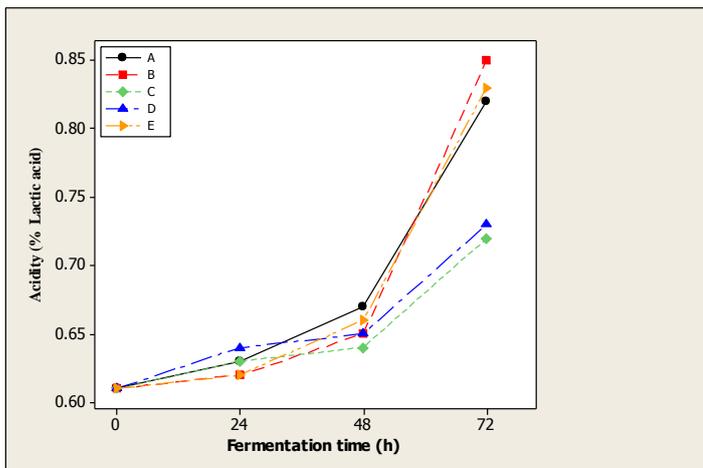


Figure 2 Titrable acidity variations during fermentation of *Doogh* samples with *L. mesenteroides* under the optimum conditions of EPS producing, containing sugar contents of A = 0%, B = 0.5%, C = 1%, D = 1.5% and E = 2% (W/W)

### Microbiological analysis of *Doogh* samples

In this study fermentation of *Doogh* with *Leuconostochas* progressed under the optimum conditions of EPS producing that are contributed to viscosity development after the fermentation. These constant conditions include temperature of 25°C and agitation of 120 RPM. Analysis of variables in this experimental investigation demonstrates that the fermentation process is running well so it has considered as an indication of progression in this bioprocess (Shene et al., 2008). Fig 3 shows the microbial count of *L. mesenteroides* during the fermentation of *Doogh* samples. The initial microbial count of *Doogh* (after the inclusion with *L. mesenteroides*) was measured 4.50 log CFU ml<sup>-1</sup> that after 72 h fermentation has increased to 6 log CFU ml<sup>-1</sup>. This enhancement causes the changes of pH and acidity that mentioned before. Acidity and pH variations are relevant to microbial count, but at all no significant difference are there between the different sugar contents of samples that shows existence of sugar have not influenced the growth of *Leuconostoc* (Santos et al., 2000).

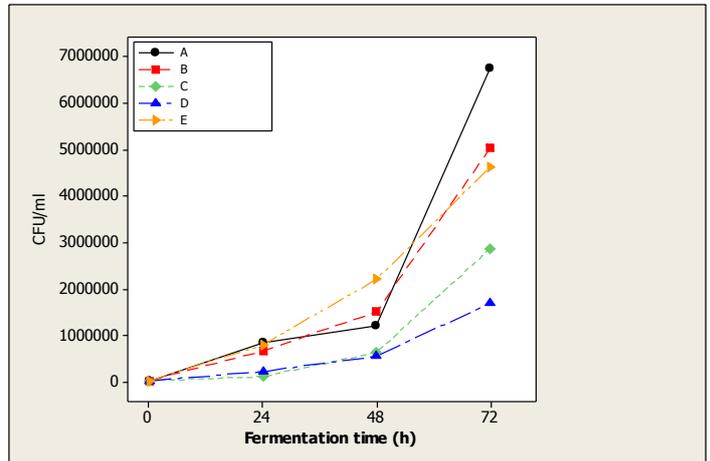


Figure 3 Microbial count of *L. mesenteroides* during fermentation of *Doogh* samples under the optimum conditions of EPS producing, containing sugar contents of A = 0%, B = 0.5%, C = 1%, D = 1.5% and E = 2% (W/W)

### Viscosity of *Doogh* samples

Indeed enhancement in viscosity of *Doogh* samples is the final goal of this investigation so it has shown in Fig 4, the viscosity of samples has increased during the progress of fermentation. As it is obvious in Fig 4 the sample without sugar did not show any significant difference in measured viscosity during and after the fermentation however in samples containing sugar, related to content of sugar in samples, the viscosity is considerably enhanced during 72-h fermentation and it has reached to 10.01 cp. *L. mesenteroides* produces EPS under the optimum conditions that are implemented in this fermentation; also this polysaccharide enhances the viscosity of *Doogh* by standing between yogurt drink particles are known the suspension casein and adsorbing water. Agitation also contributes to distribution of EPS through the substrate particles (Santos et al., 2000). By the progress of fermentation during 72 hours, EPS producing and viscosity of samples was intensified gradually. Ordinarily, the premier source of EPS that is produced by *L. mesenteroides* is sugar that metabolize to homopolysaccharides (Santos et al., 2000); therefore it is reasonable that viscosity of samples should be increased according to their sugar contents after and while the bioprocess time increases.

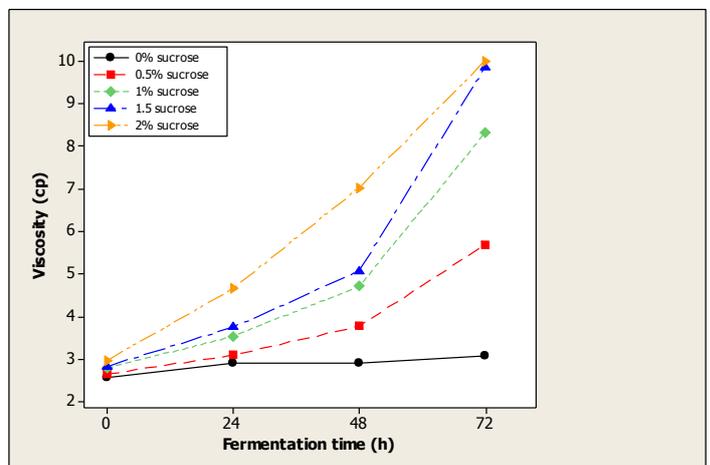
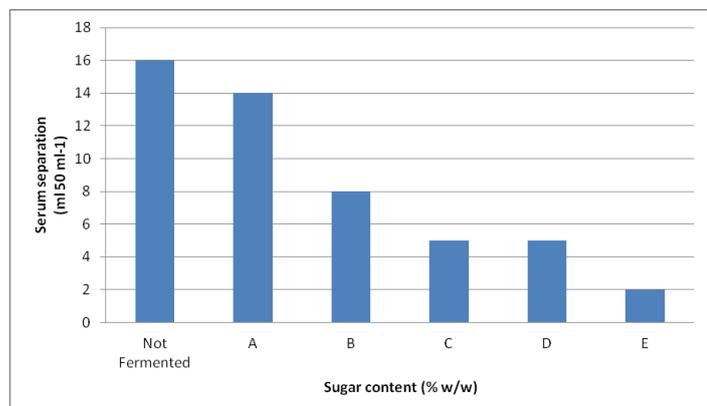


Figure 4 Viscosity changes (cp) of *Doogh* samples during fermentation with *L. mesenteroides* under the optimum conditions of EPS producing, containing sugar contents of A = 0%, B = 0.5%, C = 1%, D = 1.5% and E = 2% (W/W)

### Serum separation of *Doogh* samples

Serum separation occurs in fermented milk products due to the aggregation and sedimentation of casein particles during the storage. The use of stabilizers was found necessary to prevent serum separation in fermented milk beverages such as in yogurt drinks (Koksoy and Kilic, 2004). *Doogh* also is a unstable suspension that needs to be stabilized by stabilizers and additives so in this study when the fermentation by *L. mesenteroides* under condition of sugar existence occurred in *Doogh* samples, serum separation was reduced compared to that in *Doogh* without any fermentation process as it has shown in Fig 5. Samples without any fermentation are considered as blank and other samples have investigated in comparison to it. As it has shown in Fig 5 the fermented samples without sugar did not have any significant difference in volume of serum separation however other fermented samples according to enhancement of sugar contents in results, taken from volumes of serum separation, has shown decline appropriately. In

contrast, the samples containing 1 and 1.5% sugar have shown insignificantly similar serum separation volumes. **Koksoy and Kilic (2004)** found reverse correlation between viscosity and serum separation in Ayran samples with adding of stabilizers. Also in this study, we found that fermentation condition including sugar content that have contributed to higher viscosity leads to more prevention of serum separation in *Doogh* samples. When serum separation volume decreased, the stability of suspension intensified consequently (**Thi and Ipsen, 2009**). Serum separation volume of fermented sample containing 2% w/w sugar is declined down to 2 ml 50ml<sup>-1</sup>.



**Figure 5** Serum separation of *Doogh* samples fermented with *L. mesenteroides* under the optimum conditions of EPS producing, containing different sugar contents including A = 0%, B = 0.5%, C = 1%, D = 1.5% and E = 2% (W/W) and sample without any fermentation as a sample with original properties, after 15 days storage at 4°C

**Sensory analysis of *Doogh* samples**

Sensory analysis of samples has shown in table 1. They statistically analyzed by using Duncan’s multiple comparison test at 5% confidence level. Panelists did not find any differences in taste of all samples (fermented and unfermented), so it should be resulted that the fermentation process has no effect on taste and odor of the samples and this process can be implemented without any changes in flavor with mentioned conditions, however most of the fermentative processes influence the organoleptic characteristics of substrate (**Olanipekun et al., 2009**). Produced organic acids affect the taste of the fermented substrate but the range of acidity changes should be considerable (**Shene et al., 2008**). In *Doogh* samples, range of changes in titrable acidity of samples was not significant and the results of sensory analysis also have confirmed it. As it has shown in table 1, consistency and acceptability are significantly different in all groups between samples. Moreover, panelists found less consistency in samples without fermentation and fermented without adding sugar. In the other hand, panelists found other fermented samples more consistence so these results have proved the enhancement of viscosity of fermented samples containing sugar after the 72-h fermentation. Finally, panelists assessed the overall acceptability of samples that the results has shown satisfaction from the fermented *Doogh* samples in comparison with samples without fermentation and fermented samples without adding sugar. Fermentation with *L. mesenteroides* under optimum conditions of EPS producing, despite less changes in original taste of *Doogh* have caused to higher satisfactory of panelists in this study that demonstrates acceptability of this method of stabilization to enhance the viscosity of *Doogh* yogurt drink as an edible suspension.

**Table 1** Sensory evaluation of *Doogh* samples containing different sugar contents after 72-h fermentation with *L. mesenteroides* under the optimum conditions of EPS producing and sample without any fermentation as a sample with original properties

Fermentation sucrose content (%w/w) condition	Taste <sup>NS</sup>	Consistency	Overall acceptability
Not fermented	6.4	2.6 <sup>d</sup>	3.9 <sup>d</sup>
0	5.7	3.1 <sup>b</sup>	3.2 <sup>b</sup>
0.5	5.8	5.6 <sup>c</sup>	5.2 <sup>c</sup>
1	6.0	5.7 <sup>d</sup>	4.9 <sup>d</sup>
1.5	5.9	5.4 <sup>e</sup>	5.0 <sup>e</sup>
2	6.2	6.1 <sup>f</sup>	5.9 <sup>f</sup>

**Legends:** Means at the same column with different superscripts are different (P<0.05) by Duncan’s multiple comparison test. <sup>NS</sup> means Not Significant

**CONCLUSION**

Fermentation of *Doogh* by *L. mesenteroides* under optimum conditions of EPS producing including 25°C temperature, 120 RPM agitation and sugar existence

enhance the viscosity of *Doogh* after 72-h fermentation without any significant changes in the taste and flavor of *Doogh*. With increase in sugar content up to 2% w/w, the viscosity of *Doogh* enhanced appropriately. Enhancement of viscosity in *Doogh* is confirmed by the results of sensory evaluation, also serum separation volume of fermented *Doogh* samples have shown decline because of enhancement in viscosity. The viscosity of fermented *Doogh* sample contained 2% w/w sugar was increased up to 10.01 cp and consequently serum separation volume was decreased down to 2 ml 50ml<sup>-1</sup> that have shown increase of stability in fermented *Doogh* samples. Also assessment of consistency in samples by panelists confirmed the viscosity measurements. Finally, this process is known acceptable in experimental and sensory evaluation to intensify the viscosity of *Doogh* yogurt drink as an edible suspension in addition to its economic benefits in comparison with other stabilization methods based on additives.

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