An Investigation into the Characteristics of Super-Healthy Doogh: Application of Microbial Transglutaminase and Lipase Enzymes

Jokar, SH.[1]; Yazdanpanah, S.[1*]

[1] Department of Food Science and Technology, Kazerun Branch, Islamic Azad University, Kazerun, Iran.

*** Corresponding author:** S. Yazdanpanah, Department of Food Science and Technology, Kazerun Branch, Islamic Azad University, Kazerun, Iran, Email: Yazdanpanah2004@gmail.com

(Received: November 4, 2017; Accepted: January 11, 2018)

Abstract

Iran has a long history in the production of a wide variety of dairy products. Doogh is one of the traditional Iranian drinks which is made by mixing yogurt and water and a little salt. This beverage has a special place in the Iranian markets. During its storage, the main problem with doogh is the two phases that results from low pH and caseins accumulation. Doogh preparation, which has not been serum separation, is considered by researchers and industries. The transglutaminase enzyme has positive effect on the serum storage capacity and the strength of the food in the gel by creating cross linking at the proteins. Therefore, in the present study, the effect of using the transglutaminase and lipase enzymes was studied in order to improve the rheological properties and durability of doogh. The transglutaminase enzyme in doses of 10, 15 and 20 ppm and lipase enzyme in doses of 30, 45 and 60 ppm were added to doogh with fat 1.5% and 3%. Then, physicochemical, microbial, rheology properties, stability and microstructure were investigated. The results showed that treatment with these enzymes improved the stability. The treated samples showed a particulate accumulation, and casein clusters were more regular than the control sample. Added enzymes changed the flow behavior of all treatments and their behavior led to a behavior non-Newtonian shear-thinning. It also increased viscosity and decreased serum separation amount in two phases. This study showed that the combination of transglutaminase and lipase enzymes had a good potential for use in doogh as a stabilizing and viscosity improvement factor.

Keywords: *Doogh, Transglutaminase, Lipase Enzyme, Rheology*

Introduction

For the first time, pragmatic or super healthy foods were used in Japan from the early 1980's. In addition to the nutritional characteristics, this kind of foods are said to have health features for the consumer. In other words, beyond its nutritional value, it has medicinal importance as well. Meanwhile, foods are pragmatic as they have been modified by one or more of their combinations (Yasini and Bolandi, 2014). Dairy as a main food has a base in many food

diets and a rich source of essential nutrients to the human body (Blasi *et al*., 2008). Doogh is a dairy, fermented, acidic and native beverage of Iranians that is made by thinning yoghurt with adding water and salt or by direct milk fermentation (Kiani *et al*., 2008). Common micro-structure is used as a starter in the doogh production of streptococcus thermophilus and lactobacillus delbrueckii subsp.bulgaricus (Codex Alimentarius, 2013). One of the common problems of this fermented beverage is the two phases during its storage. This is due to the effect of low pH and acidic conditions on casein proteins that, at the isoelectric point, causes sediment leading doogh to experience two phases, creating an undesirable appearance in the product. One of the ways to prevent two phases in doogh production is to increase the viscosity of the continuous phase (watery phase) using various hydrocolloids (Tamine, 2006; Tamine and Robinson, 1985). However, the unpleasant taste would be the result of adding it as a limiting factor. Therefore, selecting the appropriate level of hydrocolloids is one of the important factors in the production of fermented milk products. Today, in proteins modification, enzymatic methods are more preferred than chemical methods, since in enzymatic methods and in the production of toxic substances the resulting of chemical methods are minimized (Sanli *et al*., 2011). The use of the transglutaminase enzyme can be used as a substitute for this method. The transglutaminase enzyme is a transferase enzyme that can interconnect the amino acid glutamine from a protein and lysine from another protein. If this enzyme does not have any undesirable effect on lysine bioavailability, the nutritional value of the resulting protein also does not change (Trachoo and Mistry, 1998). In fact, with the tangles of milk proteins to modify the functional properties of proteins and the formation of products with sensory and rheological properties this enzyme will be better (Bonisch *et al*., 2007). Research has shown that in 100 ml of cow's milk, there is 3.5-5 g of fat to form emulsified blood cells, the diameter of these blood cells varies from 0/1-1micrometers (Jensen *et al*., 1991; Mcpherson and Kitchen, 1983). The main fats in milk are triacylglycerides that forms 98% of total fat (Jensen *et al*., 1991; Gunstone *et al*., 1994). Lipases are enzymes that catalyze hydrolysis of triacylglycerols, this kind of hydrolysis is called lipolysis. Products derived from this reaction are free or with no ester fatty acids, mono and di-glycerides, and some glycerol (Jaeger *et al*., 1994). The importance of lipolysis in milk is due to two reasons: creating flavor in products and changing their properties. Free fatty acids, especially short and medium chain free fatty acids, have a very strong flavor and in many cases are

[DOR: 20.1001.1.2717378.2018.2.4.2.1]

considered undesirable (Fox *et al*., 2000). Raw milk is a source of 60 types of enzymes, and during the pasteurization process the lipase enzyme in the milk is inactive. The enzyme is added to pasteurized milk to maintain enzymatic activity in the doogh production, and after doing its activity and creating the desired aroma, it is inactive in the second pasteurization to prevent excessive activity and off-taste and bitterness. The contribution of lipase in dairy products is that lipolysis depends on the temperature and time of the milk heating. In effect pasteurization (72 $^{\circ C}$, 15 seconds), the lipase activity is reduced to the amount 83% and 100% will be inactive after heating for 15 seconds at 78 $^{\circ C}$ (Rao and Renner, 1989). In their studies, Aston and Creamer, (1986) demonstrated that concerning cheddar cheese the major flavor compounds are created through free fatty acids released from lipolysis which directly affects on the cheese taste. Akin *et al*. (2003) showed that by adding 4 g of lipase per 100 liters of cheese milk, salty water white cheese obtained very good sensory properties after 20 days. De-Pierro *et al*. (2010) observed that transglutaminase could enhance cheese, making efficiency by maintaining moisture in the curd. The purpose of this study, therefore, is to increase the stability of doogh produced through low viscosity, using new cross-linkages in milk proteins by the enzymes and also dough produced with desireable taste and stability. After the production of doogh with the microbial transglutaminase and lipase enzymes, the effect of adding these two enzymes on non-gas heated doogh and physicochemical properties, rheology, microbial, microstructure, free fatty acids, and sensory properties were examined.

Materials and Methods

Cow's fresh milk were prepared from valid cow breeding farm and under veterinary supervision of Fars province, Iran (Spring 2017), yogurt commercial starter contained streptococcus thermophilus and lactobacillus delbrueckii subsp.bulgaricus (Mediterranea Biotecnologie Company, Italy), transglutaminase enzyme with an enzyme power of 100 (C & P company, Germany) and calf lipase enzyme with enzyme power of 10 (Clerici, Italy).

To prepare the control doogh (no enzyme), milk was first standardized after weighing. The required milk sample was prepared with a maximum acidity of 15 durnick degree, solid non-fat at least 8, protein between 3 and 3/3 and fat with two degrees 1.5% and 3%. Then in order to pasteurize that, it was heated at 90 $^{\circ C}$ for 15 minutes. After reducing the temperature to about 43

°C, starter was added in order to make doogh yogurt. Enzymes were also added to the treatments in certain doses with starter. Doogh yogurt samples were incubated at 42 °C during production to reach to the optimal pH (from 4 to 4.2 °C). The yogurt was mixed with drinking water 50:50 to ratio and 0.5% w / w salt with a purity of 99.5%. Then, a second pasteurization in benmari was performed in order to prevent the direct heat to doogh. The treatment characteristics are presented in Table 1. Finally, the cooling and packaging steps were performed (Standard 10528, 2007).

Table 1: Treatments Specifications

Code	Fat (%)	The amount of transglutaminase enzyme	The amount of lipase enzyme
A (Control sample)	-	-	-
B	1.5	10 ppm	30 ppm
C	1.5	15 ppm	45 ppm
D	1.5	20 ppm	60 ppm
E	3	10 ppm	30 ppm
F	3	15 ppm	45 ppm
G	3	20 ppm	60 ppm

Counting mold and yeast were carried out according to the Institute of Standard and Industrial Research of Iran, No. 8923-5 and 2406. Coliforms counts were performed according to the Institute of Standard and Industrial Research of Iran, No. 9263and 11166.

Physico-chemical tests including acidity and pH, salt, fat, non-fat milk solids and density were conducted in accordance with the national standard methods of Iran, No. 2852, 694, 384, 11328 and 638, respectively.

To determine the stability of the treatments, 50 ml of sample was poured out inside sterilized graded falcon and covered. At ambient temperature, they were kept for 30 days. Finally, their stability was calculated in percent using Formula (1) (Amiri aghdayi and Alami, 2011).

1) Stability (%) $= \frac{V_1 - V_2}{V_1} \times 100$

V_1: Doogh initial volume

V_2: Serum volume

The rheological properties of the specimens were evaluated and measured using the rheometeric mechanical spectrometer (MCR 301), manufactured by Antonpaar (Austria),

equipped with concentric cylindrical geometry (CC27, Concentric cylinder geometry). Also, rheoplus/32, version V3.40 software was used to determine the test data and calculate rheological parameters including shear rate, shear stress, viscosity, speed and torque at 25 $^{\circ C}$. In addition, the fitting level of the data obtained with the Herschel-Bulkley model was examined according to the Formula (2):

2) $\tau = \tau_o + K\dot{\gamma}^n$

τ_0: Yield stress according to Pa

K: Consistency coefficient according to Pa.S^n

n: Flow behavior index

In order to study the microstructure of the doogh, an electron microscope ZEISS xio Scope.A1 (Germany) was used. To observe microscopy, 10 to 20 landaus from the sample were spread on a microscope slide and were covered by lamel and placed under a light microscope with a magnification of 1000.

The test design of this study was based on random block design, which was used to analyze the data using SPSS software. ANOVA was used for analysis of variance and Duncan test was used to obtain the difference between treatments (P≤0/05). All tests were compared with control treatment, which was pure and without enzyme doogh.

Results

Investigating the Microbial Agents

In order to ensure the health of the prepared specimens, all samples were evaluated for microbial agents in terms of the presence of mold, yeast, coliform and all were in the standard range which indicated the health of the product in assessing the sense by the panelists and also the absence of microbial agents in the results, specially micro structural evaluation.

Investigating the Physico-chemical Characteristics

With respect to the importance of the amount of compounds such as dry matter, fat, salt, density, acidity and pH on rheological and stability characteristics of doogh, it should be noted that the addition of transglutaminase and lipase enzyme did not significantly change the amount of salt, pH, and density of the samples. The samples prepared in this study were based on the amount of compounds containing 0.50 to 1.50% fat, 3.95 to 6.64% dry matter, 0.28 to 0.42 %

[DOR: 20.1001.1.2717378.2018.2.4.2.1]

salt, 1.0172 to 1.0256 g/cm^3 density, 49.30 to 52.90 acidity degrees durnick and 4.20 to 4.30 pH. The physicochemical properties of doogh samples corresponded to the Iran standards. According to the statistical results, there was a significant difference between the control and treated samples for titratable acidity ($p \leq 0.05$). The highest acidity level was observed in control samples and the lowest level in G treatment.

Investigating the Sustainability of Doogh Samples

The effect of added enzymes on the stability of doogh in different doses is shown in Diagram 1. The amount of water gush of the samples was decreased with increasing enzyme amount and subsequently it increased their stability. This increase in stability was statistically only between the control and D treatment (1/5 % fat, 20 ppm enzyme transglutaminase, 60 ppm lipase enzyme) ($p \leq 0.05$).

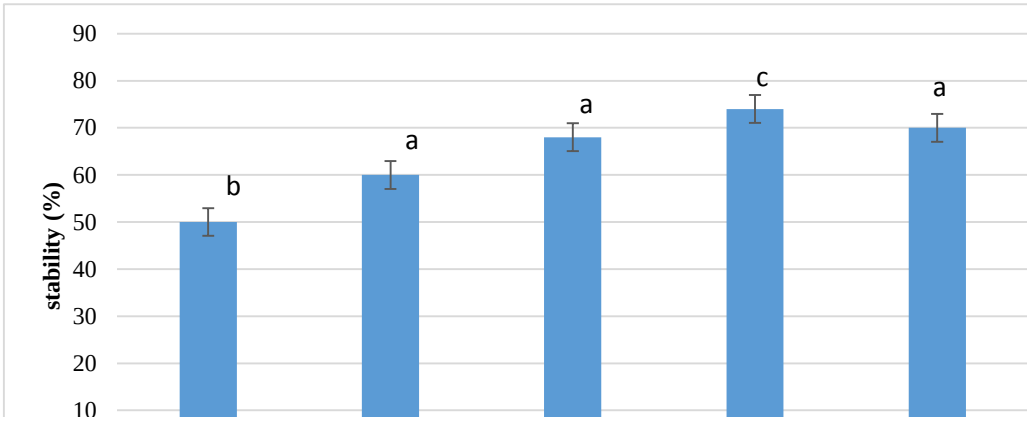


Diagram 1: The amount of doogh stability the affected various doses of transglutaminase and lipase enzyme in different fats

The same letters indicate no significant difference (p>0/05). The letters stand for: A (Control), B (1.5% fat, 10 ppm transglutaminase enzyme, 30 ppm lipase enzyme), C (1.5% fat, 15 ppm transglutaminase enzyme, 45 ppm lipase enzyme), D (1.5% fat, 20 ppm transglutaminase enzyme, 60 ppm lipase enzyme), E (3% fat, 10 ppm transglutaminase enzyme, 30 ppm lipase enzyme), F (3% fat, 15 ppm transglutaminase enzyme, 45 ppm lipase enzyme), and G (3% fat, 20 ppm transglutaminase enzyme, 60 ppm lipase enzyme).

[DOR: 20.1001.1.2717378.2018.2.4.2.1]

Investigating the Rheological Properties

According to the results, there was an increasing trend in all factors (shear rate, shear stress, speed and torque) except for viscosity during the measured time. Increasing fat in treatments also increased all variables indicators. Among the samples, the control sample had the lowest shear stress (0.276 Pa), viscosity (0.0126 pa.s) and torque (14.7 μnm). D treatment had the highest shear stress (2.07 pa) and torque (110 μnm) and F treatment had the highest viscosity (0.336 Pa). Also average shear rate and speed were the same in all samples.

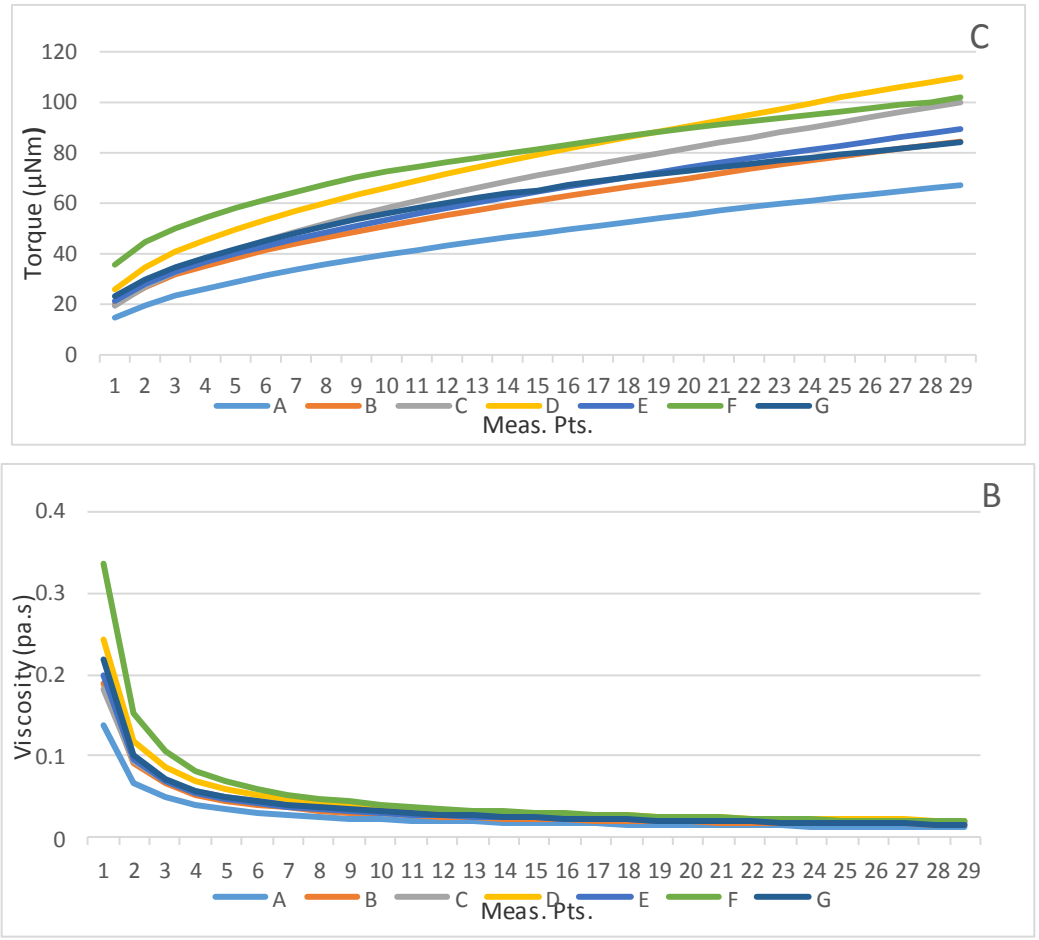


Figure1:Effect of transglutaminase and lipase enzyme on A: shear stress, B: viscosity, C: torque The letters

stand for: A (Control), B (1.5% fat, 10 ppm transglutaminase enzyme, 30 ppm lipase enzyme), C (1.5% fat, 15 ppm transglutaminase enzyme, 45 ppm lipase enzyme), D (1.5% fat, 20 ppm transglutaminase enzyme, 60 ppm lipase enzyme), E (3% fat, 10 ppm transglutaminase enzyme, 30 ppm lipase enzyme), F (3% fat, 15 ppm transglutaminase enzyme, 45 ppm lipase enzyme), and G (3% fat, 20 ppm transglutaminase enzyme, 60 ppm lipase enzyme)

The relationships concerning the shear rate and shear stress were fitted with Newtonian models, power law, Bingham and Herschel-Bulkley. Considering the amount of fitting of rheological data, from the four mathematical models evaluated to predict the rheological properties of doogh specimens, the most suitable model for samples, the Herschel-Bulkley model is shown in Table 2. As can be seen, the added enzymes altered the flow behavior of the treatments and their behavior led to behavior non-Newtonian shear-thinning. At lower shear rates, appear viscosity was higher and with increasing shear rates, viscosity decreased strongly.

Table 2: Calculated parameters related to Herschel-Bulkley model for treatments

Treatment	yield stress(pa) (τ_0)	Consistency coefficient (pa.s^n) (K)	Flow behavior index (n)	Correlation coefficient (R2)
A	0.153	0.082	0.562	0.99977
B	0.204	0.126	0.510	0.99982
C	0.173	0.129	0.559	0.99997
D	0.276	0.154	0.523	0.99992
E	0.256	0.105	0.560	0.99986
F	0.372	0.230	0.413	0.99922
G	0.248	0.127	0.522	0.99974

The letters stand for: A (Control), B (1.5% fat, 10 ppm transglutaminase enzyme, 30 ppm lipase enzyme), C (1.5% fat, 15 ppm transglutaminase enzyme, 45 ppm lipase enzyme), D (1.5% fat, 20 ppm transglutaminase enzyme, 60 ppm lipase enzyme), E (3% fat, 10 ppm transglutaminase enzyme, 30 ppm lipase enzyme), F (3% fat, 15 ppm transglutaminase enzyme, 45 ppm lipase enzyme), and G (3% fat, 20 ppm transglutaminase enzyme, 60 ppm lipase enzyme)

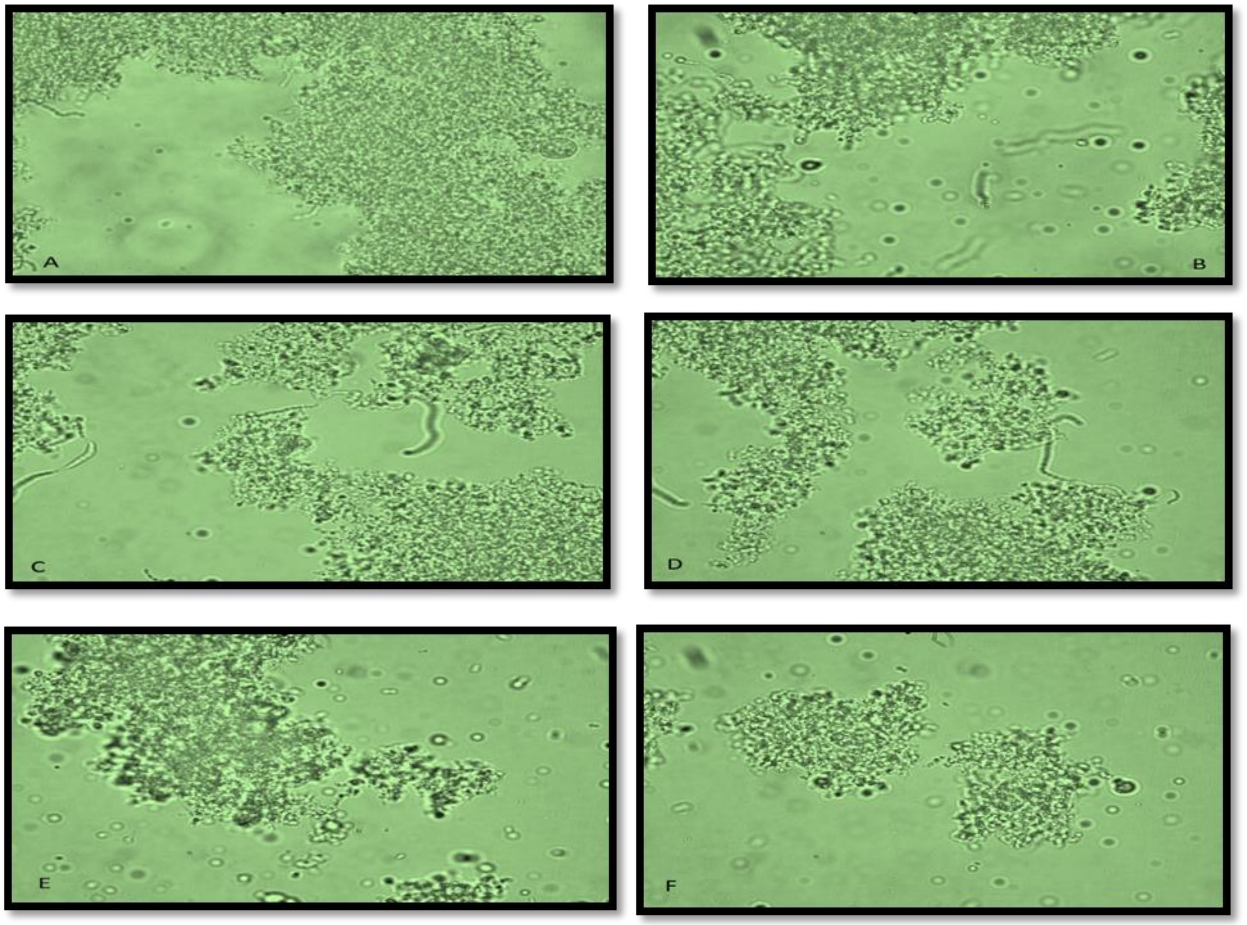
As shown in Table 2, the flow behavior index in all samples was less than 1 which represents the behavior non-Newtonian shear–thinning (pseudoplastic) of the samples (Newtonian fluid n=1, pseudoplastic fluid n<1, dilatant fluid n>1). Comparing the samples, the control sample showed the lowest yield stress (0.153 pa) and the consistency coefficient (0.802

[DOR: 20.1001.1.2717378.2018.2.4.2.1]

pa.s^n) and the highest yield stress was (0.372 pa) and the consistency coefficient (0.230 pa.s^n) was observed in sample F.

Investigating the Microstructure

The following figures demonstrate the microscopic images taken from the treatments. As shown in Figure 2, in the control sample, the particles distribution was very scattered and with increasing dosages of the enzymes consumed in the subsequent shapes, the accumulation of particles and casein clusters became more regular.



[DOR: 20.1001.1.2717378.2018.2.4.2.1]

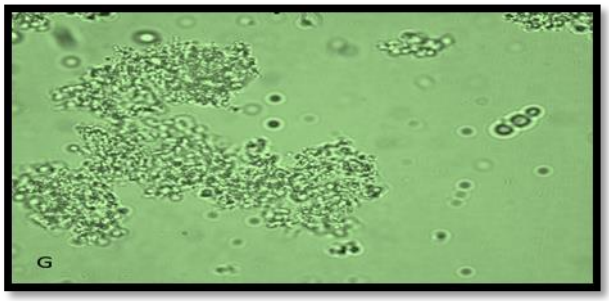


Figure 2. A (Control), B (1.5% fat, 10 ppm transglutaminase enzyme, 30 ppm lipase enzyme), C (1.5% fat, 15 ppm transglutaminase enzyme, 45 ppm lipase enzyme), D (1.5% fat, 20 ppm transglutaminase enzyme, 60 ppm lipase enzyme), E (3% fat, 10 ppm transglutaminase enzyme, 30 ppm lipase enzyme), F (3% fat, 15 ppm transglutaminase enzyme, 45 ppm lipase enzyme), and G (3% fat, 20 ppm transglutaminase enzyme, 60 ppm lipase enzyme)

In a study by Soleimanpuri *et al*. (2014), the creation of cross-links between milk and soy protein in non-fat yoghurt by the transglutaminase enzyme was examined. The microstructure of yogurt samples was kept at 4 ^{o}C for 21 days by electron microscopy. It was reported that the presence of isolated soy protein created a special quasi -sponge structure in yogurt, the connection between a particle of milk proteins with soy protein isolates led to the formation of tall and narrow chains. Moreover, Remeuf (2003) reported that the chains resulting from the accumulation of soy and milk proteins by heat increased the water holding capacity of enriched samples with soy protein isolated. In their studies on lactobacillus acidophilus and bifidobacterium lactise on microorganisms of doogh, Ebrahimzadegan and Zomorodi (2014) showed that in samples containing probiotics, clusters and large aggregations of protein particles would be seen which were separated by large pores containing water. Also, the distribution of particles in a sample containing lactobacillus acidophilus was more regular than that of bifidobacterium lactise. Therefore, it could be concluded that lactobacillus acidophilus caused a more regular accumulation of casein micelles.

Discussion

In the present study, the findings showed that samples under treatments demonstrated a higher level of fat with more positive effects of lipase enzyme than the control sample which was resulted in a significant difference between Acidity of treatments. Yüksel and Erdem (2010) reported a significant reduction in acidity for non-fat and with full fat yogurt treated with the transglutaminase enzyme compared to untreated samples. Farnsworth *et al*. (2006) did not

observe any significant difference between the pH of the sample treated with the enzyme and the control sample. His finding was consistent with the results obtained in this research. In a similar study on yogurt, Sanli *et al*. (2011) reported that the presence of transglutaminase enzyme in mold yogurt had no significant effect on acidity and pH.

The degree of water gush of the samples was decreased with increasing enzyme degree and increased their stability. Generally, ε- (γ-Gla) Lye permanent cross-linking which was created between milk proteins, the enzyme would reduce the permeability of the gel (Lauber *et al*, 2000; Faergemand *et al*, 1999). Also, the transglutaminase enzyme reduced pore size in the yogurt gel (Lorenzen and Schlimme, 1998).

In the sample containing the transglutaminase and lipase enzymes, viscosity was increased, while serum separation and two phase amount decreased. These enzymes caused yield stress in the drink and prevented the sedimentation of suspended particles in static condition.

Creating yield stress was reversible due to the presence of three-dimensional network. Since this network was easily removed by applying shear stress, it was possible to use this property in beverages. In general, adding a microbial transglutaminase enzyme to milk caused an increase in the viscosity of yogurt which was used in doogh production. This order increased the viscosity and improved the rheology properties of the doogh treated with this enzyme. Since the main function of this enzyme was the cross-linking of milk proteins in covalent form and consequently forming a stronger gel in the sample, which was different in structure, the results were expected to some extent (Schorsch *et al*., 2000).

In fact, the treatment with the microbial transglutaminase enzyme could improve the properties of the gel formation in casein by intermolecular cross-linking (Farnsworth *et al*, 2006). Moreover, similar results were reported from gel strength of yogurt samples obtained from milk by the microbial transglutaminase enzyme (Faergemand *et al*, 1999).

The particle distribution was more regular, the pores containing water and casein clusters were more regular and less water leak out of these networks (Schorsch *et al*, 2000). This result was also confirmed by stability study.

Conclusion

In this study, transglutaminase and lipase enzymes were used at different doses to produce stable doogh containing 1.5 and 3% fat. Based on the results of this study, the transglutaminase enzyme increased the viscosity of the samples by forming molecular weight polymers higher than protein monomers. By decreasing the gel permeability, as a result of creation the permanent cross-links between milk proteins, redounding to the water retained in the yogurt gel network was used to produce doogh. The lipase enzyme which broke down the fats and made a linkage with existing caseins, caused linkage stronger, and as a result, reduced the water gush, resulted in a more suitable flavor and aroma. The results obtained in this study are very effective in terms of economic and customer satisfaction. Of course, higher levels of enzymes can have a more desirable effect, however due to the economic issues and comparing the treatments with the control sample, it is not necessary to add higher enzymes.

References

Akin N., Aydemir S., Kocak C. and Yildiz MA. Changes of free fatty acid contents and sensory properties of white picked cheese during ripening. Food Chemistry, 2003; 80, 77-83.

Amiri Aghdayi S. and alami M. Effect of Basil Grain Mucilage on Doogh Rheology and stability characteristics. Journal of Food Science and Technology, 2011; 3 (3): 18-24 (In Persian)

Aston JW. and Creamer LK. Contribution of the components of the water soluble fraction to the flavor of Cheddar cheese. Journal of Dairy Science and Technology, 1986; 21: 229-248.

Blasi F., Montesano D., Deangelis M., Maurizi A., Ventura F., Cossignani L., *et al*. Results of stereo specific analysis of triacylglycerol fraction from donkey, cow, ewe, goat and buffalo milk. Journal of Food Composition and Analysis, 2008; 21: 1-7.

Bonisch MP., Huss M., Weitl K. and Kulozik U. Transglutaminase cross-linking of milk proteins and impact on yoghurt gel properties. International Dairy Journal, 2007; 17, 1360–1371.

Codex Alimentarius Commission. Joint FAO/WHO food standard programme, proposed draft codex regional standard for doogh. Beirut, Lebanon, 2013; 21-25 January.

De-Pierro P., Mariniello L., Sorrentino A., Gosafatto CVL., Chianese L. and Porta R. Transglutaminase-induced chemical and rheological properties of cheese. Food Biotechnology, 2010; 24: 107-120.

Ebrahimzadegan S. And Zomorodi SH. Survival of Lactobacillus acidophilus (LAFTI-L10) and Bifidobacterium lactis (LAFTI-B94) and their effect on quality and microstructure of doogh. Journal of Food Industry Research, 2014; 24 (4): 531-541 (In Persian)

Faergemand M., Sorensen MV., Jorgensen U., Budolfsen G. and Qvist KB. Transglutaminase: Effect on instrumental and sensory texture of set style yoghurt. Milchwissenschaft, 1999; 54(10): 563–566.

Farnsworth JP., Li J., Hendricks GM. and Guo MR. Effects oftransglutaminase treatment on functional properties and probiotic culture survivability of goat milk yogurt. Small Ruminant Research, 2006; 65: 113–121.

Fox PF., Guinee TP., Cogan TM. and McSweeney PLH. Fundamentals of cheese science. Gaithersburg, MD: Aspen Publishers Inc, 2000.

Gunstone FD., Harwood JL. and Padley FB. Milk lipids. In: The lipid handbook (2nd ed). London: Chapman & Hall, 1994; pp. 195-197.

Institute of standard and industrial research of Iran. Animal and vegetable fats and oils-Gas chromatography of fatty acid methyl esters- Part 1: Guidelines on modern gaschromatography of fatty acid methylesters, 2016. No. 13126-1.

Institute of standard and industrial research of Iran. Animal and vegetable fats and oils-Gas chromatography of fatty acid methyl esters- Part 2: Preparation of fatty acid methyl esters, 2015. No. 13126-2.

Institute of standard and industrial research of Iran. Butter - Determination of salt content-Test method, 2007. No. 694.

Institute of standard and industrial research of Iran. Determine the specific gravity of the milk (lacto-density meter method), 1991. No. 638.

Institute of standard and industrial research of Iran. Doogh- Code of practice and production, 2007. No. 10528.

Institute of standard and industrial research of Iran. Doogh – Specifications ans test method, 2008. No. 2453.

Institute of standard and industrial research of Iran. Microbiology of food and animal feeding stuffs – Horizontal method for the detectionand enumeration of coliforms –Most probable number technique, 2008. No. 11166.

Institute of standard and industrial research of Iran. Microbiology of food and animal feeding stuffs – Horizontal method for theenumeration of coliforms –Colony-count technique, 2007. No. 9263.

Institute of standard and industrial research of Iran. Microbiology of food and animal feedingstuffs - preparation of test samples, initialsuspension and decimal dilutions for microbiological examination - Part 5: specific rules for the preparation of milk and milk products, 2011. No. 8923-5.

Institute of standard and industrial research of Iran. Microbiology of milk and milk products-Specifications, 2008. No. 2406.

Institute of standard and industrial research of Iran. Milk and milk products - Determination of titrable acidity and value pH-test method, 2006. No. 2852.

Institute of standard and industrial research of Iran. Milk, cream and evaporated milk — Determination of total solids content (Reference method), 2014. No. 11328.

Institute of standard and industrial research of Iran. Milk. Determination of fat content, 2010. No. 384.

Jaeger KE., Ransac S., Dijkstra BW., Colson C., Van Heuvel M. and Misset O. Bacterial lipases. FEMS Microbiology Reviews, 1994; 15: 29-63.

Jensen RG., Ferris AM. and Lammi-Keefe CJ. The composition of milk fat. Journal of Dairy Science, 1991; 74: 3228-3243.

Kiani H., Ebrahimzadeh-Mousavi MA., Emam-Djomeh Z. and Yarmand MS. Effect of gellangum on the stability and physical properties of acidified milk protein solutions. Australian Journal of Dairy Technology, 2008; 63(3): 87-92.

Lauber S., Klostermeyer H. and Henle T. Influence of irreversible casein crosslinking on the gel strength of yoghurt. Czech Journal of Food Sciences, 2000; 18: 69–71.

Lorenzen PC. and Schlimme E. Properties and potential fields of application of transglutaminase preparations in dairying. Int Dairy Federation, 1998; 332: 47-53.

Mcpherson AV. and Kitchen BJ. Reviews of the progress of dairy science: The bovine milk-fat globule membrane-its formation, composition, structure and behavior in milk and dairy products, Journal of Dairy Research, 1983; 50: 107-133.

Rao DV. and Renner E. Studies on application of ultrafitration for the manufacture of Cheddar cheese. 3. Ripening characteristics. Milchwissenschaft, 1989; 44: 351-354.

Remeuf F., Mohammed S., Sodini I. and Tissier PJ. Preliminary observations on the effects of milk fortification and heating on microstructure and physical properties of stirred yogurt, International Dairy Journal, 2003; 13: 773–782.

Sanli T., Sezgin E., Deveci O., Senel E. and Benli M. Effect of using transglutaminase on physical, chemical and sensory properties of set-type yoghurt. Food Hydrocolloids, 2011; 25: 1477-1481.

Schorsch C., Carrie H. and Norton IT. Cross-linking casein micelles by a microbial transglutaminase: influence of cross-links in acid-induced gelation. International Dairy Journal, 2000; 10: 529-539.

Tamine AY. and Robinson RK. Yogurt science and technology. Oxford: Pergamon Press, 1985; 365–373.

Tamime AY. Fermented Milks. Blackwell Science Ltd, Oxford, England, 2006.

Trachoo N. and Mistry VV. Application of ultrafiltered sweet buttermilk and sweet buttermilk powder in the manufacture of nonfat and low fat yogurts. Journal of Dairy Science, 1998; 81: 3163-3171.

Yasini FS. and Bolandi M. Functional Food. National Congress on Science and New technologies in Food Industry, 2014; 1-8 (In Persian)

Yüksel Z. and Erdem YK. The influenc of transglutaminase treatment on functional proteins of set yogurt .International Journal of Dairy Technology, 2010; 63: 86-97.

[DOR: 20.1001.1.2717378.2018.2.4.2.1]