Heliyon

Received:
3 November 2018
Revised:
20 January 2019
Accepted:
30 January 2019

Cite as: Maryam Tavakoli, Mohammad B. Habibi Najafi, Mohebbat Mohebbi. Effect of the milk fat content and starter culture selection on proteolysis and antioxidant activity of probiotic yogurt. Heliyon 5 (2019) e01204. doi: 10.1016/j.heliyon.2019.e01204

Effect of the milk fat content and starter culture selection on proteolysis and antioxidant activity of probiotic yogurt

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Abstract

In this study, the effects of milk fat content (0%, 2% and 3.5%) and starter culture (autochthonous or commercial) on physicochemical properties, degree of proteolysis, antioxidant activity and viability of *Lactobacillus acidophilus*, within 21 days storage of probiotic yogurt at 5 ± 1 °C were investigated. Statistical analysis showed that the type of starter culture had a significant effect ($P < 0.05$) on proteolysis and antioxidant activity, in such a way that both of them were increased until the 14^{th} day of storage but they decreased after this period. Similarly, the pH value of all samples decreased during storage time. It ranged from 3.84-4.34 and 4.18−4.43 for yogurt samples made by autochthonous and commercial starter culture, respectively. According to the results, the survival of *Lactobacillus acidophilus* decreased during storage time ($P < 0.05$), although it stood at recommended levels for health effects (at least 10^6 cfu/ml in traditional yogurt). Milk fat content did not have significant effect on the survival of probiotic organisms ($P < 0/05$).

Keywords: Food science, Microbiology

https://doi.org/10.1016/j.heliyon.2019.e01204

1. Introduction

Yogurt is one of the most commonly used fermented milk products that has received special attention due to its proven health features [1].

Because of recent concerns about the safety of synthetic antioxidants, an increasing attention has been paid to natural antioxidants especially derived from natural sources such as protein hydrolysates. Protein hydrolysates and peptides showing health properties like antioxidant and ACE-inhibitory activities have been described to be generated from different sources of plant and animal proteins [1].

Thus, antioxidant enzymes combined with molecules, act against oxidant molecules and food content antioxidants causes a biological antioxidant barrier. Sometimes, defense system stop insulation of the body against oxidative stress. As a result, further investigation on possibility of reinforced antioxidant defense to improve physical health and overcome illnesses is necessary [2]. The emergence of probiotics with antioxidant capacity to decrease bodily oxidative stress is a new way. Protein content of milk is considered as replete with energy and the required amino-acids which are essential for the growth and proper functioning of physiological systems. Particular protein fragments with specific amino acid sequences are known as bioactive peptides which are active in a sequence of a parent protein. Enzymatic hydrolysis releases such peptides from milk proteins. Sometimes they released through the fragmentation of milk protein by proteolytic starter cultures and extracellular enzyme action. After releasing within the gastrointestinal digestion or treating food by proteolytic enzymes, they might leave different effects on metabolism [3]. Antioxidant activity is one of the key functions of the peptides taken from milk proteins. Such activities are mostly due to tiny peptides from casein as well as whey proteins [3, 4, 5] Chelating of transition metals as well as scavenging free radicals are among antioxidant functions of such peptides [6]. The probiotic effects of lactic acid bacteria and fermented dairy products do not only result from both microorganisms and their cell wall constituents, but they can also originate from metabolites such as peptides and extracellular polysaccharides produced in fermentation [7]. Many authors wrote about current awareness on producing and practicing bioactive peptides and lactic fermentation [8, 9]. Some of the key features of *Lactobacillus acidophilus* are: anti-oxidative activity [10], antimicrobial activity against different bacteria including *Escherichia coli* [11], *Staphylococcus aureus* [10, 12], *Shigella sonnei*, *Shigella flexneri* [13], *Campylobacter jejuni* [14] as well as *Salmonella typhimurium* [15], survival while stored within fermented milk products such as yogurt [16], making progress in immune system through cytokine production [17] and preventing the spread of bladder cancer [18]. Since, Bioactive compounds in fermented dairy products affect health thoroughly, more investigations are required to elucidate the other entire dimensions of such products, specially, on the type and quantity of the compounds in various circumstances.

https://doi.org/10.1016/j.heliyon.2019.e01204

Since proteolysis products in potential healthy functional foods are considered as natural alternatives to synthetic antioxidants, and in view of the special significance of dairy products, especially yogurt as an appropriate food for delivering probiotic bacteria, the aim of the present study was to delve into the effect of fat concentration and starter culture on proteolysis and antioxidant activity of the bioactive peptides freed in probiotic yogurt.

2. Materials and methods

2.1. Strains and ingredients

The commercial yogurt starter culture was made up of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* (1:1) and the probiotic was *Lactobacillus acidophilus* LAFTI® L10. The culture and probiotic bacteria were in a freeze-dried direct vat set form and were provided by DSM Food Specialties (Sydney, NSW, Australia). The autochthonous starter culture previously isolated from Iranian traditional yogurt [19] was provided by the microbial collection of Ferdowsi University of Mashhad (FUM). Skim milk powder (SMP) (0.05% fat, 34.03% protein, pH: 6.52) and whole milk powder (35% fat, 34.03% protein, pH: 6.59) were purchased from Golshad Dairy Product Company in Mashhad, Iran. Table 1 shows the composition of milk powders.

2.2. Chemicals and equipment

In the present research, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), O-phthalaldehyde (OPA) reagent, Methanol (HPLC grade) and β- Mercaptoethanol were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). MRS broth and M17 broth were obtained from Merck Co. Germany. All other reagents and solvents used were of

Table 1. Composition of milk powders and reconstituted milk used for yogurt production.

Chemical characteristics	Milk		Reconstituted milk		
	Skimmed milk powder	Whole milk powder	Fat free milk	Semi fat milk	Full fat milk
Fat (%)	0.05 ± 0.002	35 ± 0.65	<0.5	2.1 ± 0.31	3.6 ± 0.34
Protein (%)	34.03 ± 0.5	34.03 ± 0.24	3.4 ± 0.05	3.5 ± 0.07	3.4 ± 0.03
Total solids (%)	95.25 ± 1.23	95.12 ± 1.4	12 ± 0.38	11.5 ± 0.25	12 ± 0.18
Solid nonfat (%)	——	——	12 ± 0.50	9.4 ± 0.82	8.4 ± 0.38
pH	6.52 ± 0.07	6.59 ± 0.1	6.61 ± 0.15	6.63 ± 0.13	6.65 ± 0.11
Lactic acid	1.44 ± 0.09	1.31 ± 0.008	1.52 ± 0.1	1.45 ± 0.06	1.43 ± 0.08
Total plate count	5000	5000			
Coliform	<10	<10	<10	<10	<10

https://doi.org/10.1016/j.heliyon.2019.e01204

analytical grade. An 8453 UV/Vis Spectrophotometer (Agilent Technologies; USA) was used to get all spectrophotometric data.

2.3. Milk preparation and yogurt manufacture

Reconstituted milk was used to make set type yogurt. Low heat skim milk (SMP) or whole milk powder (WMP) with 34% protein content was used to prepare milk with 12% (w/w) total solids using deionized water. Briefly, deionized water was heated to 30−40 °C before adding SMP or WMP. The mixture was then heated to 50 °C while being continuously stirred for half an hour to dissolve completely all the solid materials. To produce yogurt, milk was heat treated at 85 °C for half an hour [4]. Table 1 represents the composition of milk powders and reconstituted milks, which were analyzed by reference method. The pH, acidity, total solid and fat contents of the milk powders and reconstituted milks were measured according to standards ISIRI 2852 [20]. Total coliforms and total count of samples were determined according to ISO standards 5541 [21] and 4833-1 [22] respectively.

According to the manufacturer's guidelines, the yogurt starter culture was added to 1 liter of sterilized milk at 40 °C and was thoroughly mixed. Afterwards, aliquot of 4 milliliters was added to the heat treated milk. Autochthonous starter culture was activated using M17 or MRS broth for *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*, respectively. Inoculated tube was incubated at 30 or 37 °C for 24−72 h under anaerobic conditions. The activated strain was then centrifuged (10000×g, 15 min, 4 °C) and the pellet was used in yogurt preparation [19].

The preparation mix was transferred to 100-mL plastic containers and incubated at 37 °C until the pH reached 4.65. At the end of the fermentation process, all containers were immediately cooled down and stored in a refrigerator at 4 °C for analysis. generally, 4 treatments were considered for each starter culture wherein treatments C refer to Control sample (yogurt without probiotic bacteria), treatments SY pertain Probiotic fat free yogurt (yogurt with probiotic bacteria and maximum 0.5% fat), treatments SWY relate to Probiotic semi fat yogurt (yogurt with probiotic bacteria and 2.1% fat and treatments WY connect to Probiotic full fat yogurt (yogurt with probiotic bacteria and 3.6% fat.

2.4. Chemical and microbial methods

ISIRI 2582 standard [23] was used as the criteria to measure pH and ISIRI 695 standard [24] was used to test the acidity of samples. To measure the syneresis, a process taken from [4], with some modifications, was followed. Nevertheless, the syneresis was reported as g of the whey isolated from the entire weight (=100 g) of yogurt. The 25-g yogurt sample of each batch was weighed with a centrifuge tube (Sigma, USA). It was then centrifuged at 3500× g at 4 °C for 10 minutes. The supernatant

https://doi.org/10.1016/j.heliyon.2019.e01204

was disposed of the whey extracted from the sample and the resultant yogurt in the centrifuge tube was then weighed again. The rate of the drained yogurt weight to the entire weight (100 g) of yogurt prior to the centrifuge was defined as syneresis.

2.5. Determination of antioxidant activity

2.5.1. DPPH$^{\bullet}$ radical scavenging activity

The DPPH radical scavenging activity was measured by the method McCue and Shetty (2005) with slight modifications [25]. Briefly, 2 ml of sample and 8 mL of ethanolic DPPH$^{\bullet}$ solution (.1 mmol.L^{-1} DPPH$^{\bullet}$ radical solution provided in 95% ethanol) were mixed and allowed to react for 30 min. The scavenged DPPH was then monitored by measuring the decrease in absorbance at 517 nm. The scavenging ability was estimated as:

$$\text{DPPH}^{*}\ \text{scavenging activity}\ \% = [(\text{control absorbance} - \text{extract absorbance})/(\text{control absorbance})] \times 100$$

2.5.2. Enumeration of L. acidophilus in yogurt

In order to quantify viable *L. acidophilus* cells, standard plate count (SPC) was employed which is a typical method to estimate cell count. 90 milliliters of sterile phosphate buffer saline (PBS), pH 7.2 was used to dilute 10 g of yogurt sample. PBS was used for 10-fold serial dilutions. Then, 1 milliliter of the diluted sample was spread evenly on MRS-maltose agar which is a selective medium for *Lactobacilli* [26, 27]. Once the anaerobic incubation was done at 37 °C (taking 48−72 hours), the colonies were counted.

2.5.3. Proteolysis (determination of degree of proteolysis)

The proteolysis in the probiotic yogurt were estimated according to the method described by Donkor et al. (2007) using OPA [4]. 2.5 ml of yogurt was added to 5 ml of 0.75 % TCA in a test tube. The mixture was vigorously vortexed and filtered through a whatman filter paper. Two hundred μL of filtrate was then added to 3 ml of OPA reagent and incubated at room temperature for 2 min. Finally, the absorbance of the mixture was read at 340 nm using a UV-visible spectrophotometer. For preparing OPA reagent 2.5 ml sodium dodecyl sulfate (SDS) (20 % w/w) was transferred into a glass flask containing 25 ml 100 mM sodium tetra borate. Then, 40 mg OPA reagent (previously dissolved in 1 ml pure methanol) was added to the flask. Finally, 150 μL of β-Mercaptoethanol was added to the flask and the mixture was reached to the final volume of 50 ml with distilled water.

https://doi.org/10.1016/j.heliyon.2019.e01204

2.6. Statistical analysis

A total of three separate experiments were carried out and assays were performed in triplicate. Data were expressed as mean ± standard deviation. One-way ANOVA was used to analyze the data with a general linear model implemented in SPSS 14.0 (SPSS Inc., Chicago, IL, USA). Significance level was set at $p < 0.05$ to make comparisons between the means using Duncan test.

3. Results and discussion

3.1. pH and acidity

Table 2 indicates the values of the pH of yogurt samples (different fat content as well as different type of starter culture) resulted from multiple formulations during the refrigeration storage. The pH values ranged from 3.84−4.34 and 4.18−4.43 for the yogurt made by autochthonous and commercial starter culture, respectively. An overall decrease of pH values of yogurt samples observed during storage in refrigeration conditions. In general, it is obvious that the decline in pH is due to bacterial activity and acid production which is in agreement with previous findings. The obtained results in this study are in agreement with our previous finding and those reported by Adriana Dabija-2018 et al., who concluded that pH values of yogurt samples decreased during storage time [1, 3].

3.2. Syneresis

The obtained results for syneresis of different yogurt samples containing different percentages of fat content and autochthonous/commercial starter culture are reported in Table 3. As considered by many researchers syneresis is one of the most important parameters indicating the quality of yogurt during storage and consumer satisfaction [6] which has an inverse correlation with water holding capacity and whey drainage [28]. Therefore, syneresis was analyzed in this study since it represents the physiochemical properties of the product. As expected, there was a significant decrease in syneresis of the yogurt samples with a rise in the percentage of fat content ($P < 0.05$). Also, using of different starter culture had a significant effect on syneresis ($P < 0.05$) such that syneresis of the samples containing commercial starter culture was lower than that of autochthonous containing samples. Then, lower syneresis were promoted in samples with 3.5% fat inoculated with the commercial starter culture on the 14th day (Table 3). Similar results in literature review were observed for the yogurt whey draining during storage time. By Barkallah et al. and other researchers have been reported that the ability of proteins to retain water and milk fat cells in the structure of yogurt is the main factor influencing syneresis and WHC in yogurt [3, 4, 29].

https://doi.org/10.1016/j.heliyon.2019.e01204

Table 2. pH values of yogurt made by autochthonous and commercial starter culture.[a]

Treatment	pH							
	Autochthonous Starter Culture				Commercial Starter Culture			
	Day 1	Day 7	Day 14	Day 21	Day 1	Day 7	Day 14	Day 21
C[b]	4.21 ± 0.03^{Aa}	4.18 ± 0.02^{Aa}	4.08 ± 0.02^{Ab}	3.94 ± 0.02^{Ac}	4.38 ± 0.05^{Ad}	4.35 ± 0.02^{Ad}	4.21 ± 0.03^{Aae}	4.18 ± 0.02^{Aae}
SY	4.3 ± 0.05^{Ba}	4.25 ± 0.06^{Ba}	4.11 ± 0.01^{Ab}	3.87 ± 0.03^{Bc}	4.35 ± 0.05^{Ad}	4.25 ± 0.06^{Be}	4.3 ± 0.03^{Bde}	4.27 ± 0.02^{Bde}
SWY	4.22 ± 0.02^{Aa}	4.09 ± 0.10^{Cb}	4.03 ± 0.02^{Bc}	3.84 ± 0.05^{Bd}	4.41 ± 0.07^{Ae}	4.36 ± 0.05^{Ae}	4.22 ± 0.08^{Af}	4.09 ± 0.02^{Cg}
WY	4.34 ± 0.02^{Ba}	4.27 ± 0.01^{Db}	4.15 ± 0.02^{Bc}	4.1 ± 0.06^{Cc}	4.43 ± 0.02^{Ad}	4.35 ± 0.06^{Ae}	4.34 ± 0.05^{Bef}	4.21 ± 0.05^{Ag}

[abcd] Means in the same row with different small letter superscripts are significantly different.
[ABC] Means in the same column with different capital letter superscripts are significantly different.

[a] Analyses were performed in triplicate. Values are means ± SD.

[b] C: control sample, SY: Probiotic fat free yogurt, SWY: Probiotic semi fat yogurt, WY: Probiotic full fat yogurt.

https://doi.org/10.1016/j.heliyon.2019.e01204

Table 3. Syneresis value of yogurt made by autochthonous and commercial starter culture.[a]

Treatment	Syneresis							
	Autochthonous Starter Culture				Commercial Starter Culture			
	Day 1	Day 7	Day 14	Day 21	Day 1	Day 7	Day 14	Day 21
C[b]	18.45 ± 0.11^{Aa}	18.32 ± 0.14^{Aa}	18.21 ± 0.21^{Aa}	18.4 ± 0.18^{Aa}	17.59 ± 0.15^{Ab}	17.23 ± 0.22^{Ab}	16.77 ± 0.19^{Ac}	16.33 ± 0.11^{Ac}
SY	18.92 ± 0.09^{Aa}	18.74 ± 0.18^{Aa}	18.56 ± 0.15^{Aa}	18.83 ± 0.22^{Aa}	17.92 ± 0.18^{Ab}	17.64 ± 0.18^{Ab}	17.45 ± 0.15^{Bb}	17.71 ± 0.22^{Bb}
SWY	17.85 ± 0.18^{Bb}	17.73 ± 0.19^{Bb}	17.6 ± 0.16^{Bb}	17.78 ± 0.11^{Bb}	16.93 ± 0.15^{Bb}	16.74 ± 0.13^{Bb}	16.62 ± 0.14^{Ab}	16.86 ± 0.15^{Ab}
WY	17.62 ± 0.2^{Bb}	17.42 ± 0.11^{Bb}	17.21 ± 0.19^{Bb}	17.58 ± 0.17^{Bb}	16.55 ± 0.18^{Bb}	16.43 ± 0.22^{Bb}	16.27 ± 0.13^{Ab}	16.6 ± 0.14^{Ab}

[abcd] Means in the same row with different small letter superscripts are significantly different.
[ABC] Means in the same column with different capital letter superscripts are significantly different.

[a] Analyses were performed in triplicate. Values are means ± SD.

[b] C: control sample, SY: Probiotic fat free yogurt, SWY: Probiotic semi fat yogurt, WY: Probiotic full fat yogurt.

https://doi.org/10.1016/j.heliyon.2019.e01204

3.3. Proteolysis

Following the OPA approach, during the fermentation process, extracellular proteinases of lactic acid bacteria hydrolyze milk proteins and release free amino groups. Fig. 1 is presented the proteolytic activity of yogurt samples over storage time. As the results shown, proteolysis levels increased for all samples during the storage period, representing proteolytic activity of lactic acid bacteria during the storage period [30, 31]. Also these results were in agreement with our previous study, where proteolysis increased over storage time until day 14, and decreased afterward until the 21st day ($P > 0.05$) [3]. The results were consistent with our previous findings that revealed probiotic organisms along with *L. delbrueckii ssp. bulgaricus* and *S. thermophilus* were elaborate proteolytic activity in yogurt and that the extent of proteolysis depended significantly ($p < 0.05$) on the storage time. Therefore, the degree of proteolysis was different among strains and seemed to be a function of time. Assessing the degree of proteolysis and release of bioactive peptides through commercial or autochthonous starter culture with/without probiotic microorganisms during yogurt production revealed that all yogurt samples enjoyed a desirable level of proteolytic activity. The experimental sample contained probiotic organisms showed higher proteolysis activity than the control sample contained only *L. delbrueckii ssp. bulgaricus* and *S. thermophilus* ($P < 0.05$). Perna et al. (2014) and Shakerian et al. (2015) reported an increase in the amounts of free amino groups during fermentation time [6, 32]. Donkor et al. (2006) and Yuksel and Erdem (2010) also achieved similar results about the dependence of proteolysis levels on the nutrients available to proteolytic microorganisms [4, 33]. The elevated proteolysis may induce improved survival of probiotic microorganisms [4]. The degree of proteolysis was significantly different during storage time ($P < 0.05$) for all treatments regardless of fat content. No significant differences ($P > 0.05$) was observed in proteolytic

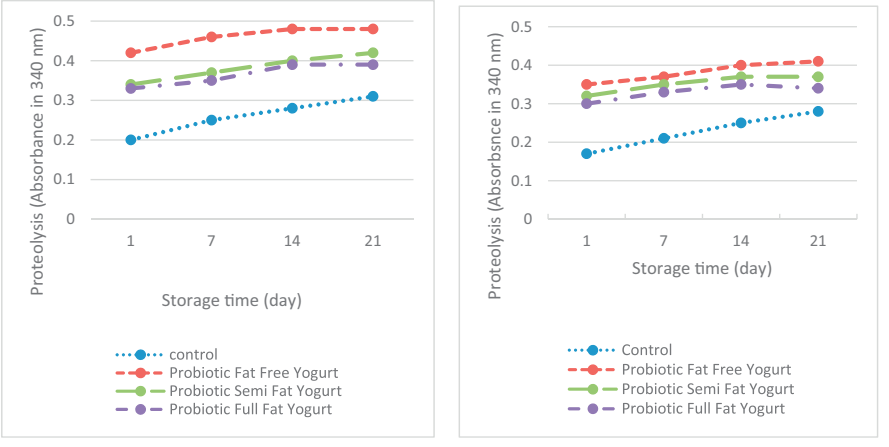

Fig. 1. Proteolysis in yogurt made by commercial starter culture (A) and autochthonous starter culture (B).

https://doi.org/10.1016/j.heliyon.2019.e01204

activities of treatments with different fat content with the exception of probiotic fat free yogurt made by commercial starter culture which in turn indicated a significant difference ($P < 0.05$). It can be concluded that the concentration of fat was ineffective on proteolysis. Both control yogurt and probiotic yogurt were significantly different ($P < 0.05$) in terms of proteolytic activity, which in turn might be due to the proteolytic activity of probiotic organisms. Similar findings have been reported by other researchers. The proteolytic system of LAB, *L. helveticus*, *Lc. lactis ssp. cremoris* and *L. delbrueckii ssp. bulgaricus* consisted of proteinases and peptidases is well characterized [34].

3.4. Survival of *Lactobacillus acidophilus*

Multiple factors can influence *Lactobacillus* sp. survival in yogurt including strains of probiotic bacteria, pH, and presence of hydrogen peroxide as well as dissolved oxygen, concentration of metabolites including lactic acid and acetic acids, the buffering capacity of the media along with the temperature of storage [35]. Fig. 2 shows the number of probiotic microorganisms during storage time. The entire yogurt treatments indicated a decline in the number of *Lactobacillus acidophilus L10* regardless of the concentration of fat. Count of *L. acidophilus L10* initially varied between 6−7.25 and 6.74−7.85 Log cfu/g for yogurt made by autochthonous and commercial starter culture, respectively, following a non-significant difference ($P > 0.05$) between the batches on the 1st day of fermentation. The same trend went on until the 21st day when the counts began to fall down, between the 14th and 21st day of storage. As the result indicate, the counts of *L. acidophilus* has decreased during storage, and this decline is noticeable from day 14−21, probably due to increased yogurt acidity. Similar results were reported by Akın and Guler-Akın (2005) and

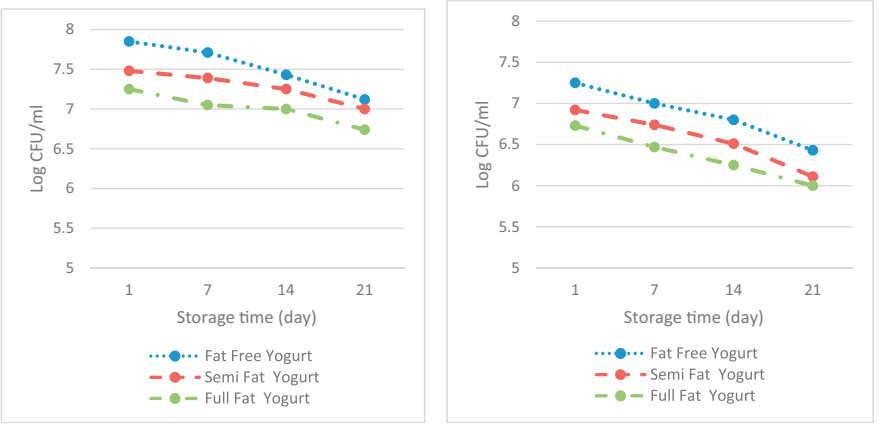

Fig. 2. Survival of *Lactobacillus acidophilus* in yogurt made by commercial starter culture (A) and autochthonous starter culture (B).

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Turgut & Cakmakcı (2017) [36, 37]. The consistent decrease in pH during the storage influenced the survival of *L. acidophilus L10*. However, probiotic organisms persisted to be viable above the therapeutic level of 6.00 log cfu/g which is suggested for health effects. As some researcher mentioned, yogurt starter cultures and probiotic bacteria produce extra- and intracellular enzymes that are able to generate biologically active peptides [38] and hydrolyze bradykinin [39]. Probably the survival of probiotics can be affected by proteolysis. Apparently, proteolysis provides the essential growth factors as peptides and amino acids for improved growth and survival of probiotic bacteria in products [3]. The growth of probiotic bacteria was observed highest in samples with a higher proteolytic activity. Nielsen et al (2009) also demonstrated that proteases are active during refrigerated storage that is in agreement with our finding [40]. No impact on the activity of probiotic organisms in the yogurt samples was observed in different levels of fat. Nevertheless, the survival of probiotic organisms in yogurt is strain dependent [4]. The use of commercial starter culture significantly affected the survival of probiotic organisms. A change in pH might have been the key factor in the survival of *L. acidophilus L10* since the decrease from the 14th to the 21st day was statistically significant ($P < 0.05$). Viability among all treatments followed a rather similar trend and no significant difference was noticed ($P > 0.05$). Moreover, viability is a function of the availability of nutrients, growth promoters/inhibitors, concentration of solutes (osmotic pressure), inoculation level, incubation temperature, fermentation duration and the temperature of storage [1, 41].

3.5. Antioxidant activity

The Fermentation makes some molecular changes in the milk which results in resulting different compounds such as peptides, free amino and fatty acids which possess antioxidant capacity. Investigation of DPPH radical scavenging as a result of antioxidant activity of protein hydrolysates resulted from bacterial activity showed in Table 4 in yogurt made by autochthonous and commercial starter culture, respectively. As shown in Table 4, the highest and the lowest value of antioxidant activity was 55.39% for yogurt made by commercial starter culture on day 14 (probiotic fat free yogurt made by commercial starter culture) and 41.18% for yogurt made by autochthonous starter culture on day 1 (control sample made by autochthonous starter culture), respectively. The results revealed that probiotic yogurt made by autochthonous or commercial starter culture has a good antioxidant activity for inhibiting lipid peroxidation. Similar studies describe that the antioxidant activity of yogurts was enhanced by the presence of probiotics [1, 4, 18, 41]. McCue and Shetty (2005) also investigated the DPPH$^{\bullet}$ scavenging activity of soy yogurt produced by Kefir cultures and reported the activity as 92.3% after 48 h of production [25]. We have presented the results in our previous research about antioxidant activity of probiotic bacteria. Based on that result, we supposed that the oxidative stability of

https://doi.org/10.1016/j.heliyon.2019.e01204

Table 4. Percentage of DPPH inhibition (Antioxidant activity) in yogurt made by autochthonous starter culture and commercial starter culture.

Treatment	Percentage of DPPH inhibition (Antioxidant activity)							
	Autochthonous Starter Culture				Commercial Starter Culture			
	Day 1	Day 7	Day 14	Day 21	Day 1	Day 7	Day 14	Day 21
C[b]	41.18 ± 1.05^{Aa}	43.52 ± 0.35^{Ab}	45.35 ± 0.54^{Ac}	44.08 ± 1.22^{Ad}	42.11 ± 0.72^{Ae}	44.18 ± 1.23^{Af}	46.14 ± 0.34^{Ag}	45.09 ± 0.26^{Ah}
SY	49.52 ± 1.24^{Ba}	51.23 ± 1.20^{Bb}	54.82 ± 0.67^{Bc}	52.31 ± 2.12^{Bd}	50.23 ± 0.46^{Be}	52.35 ± 0.56^{Bf}	55.39 ± 0.86^{Bg}	52.41 ± 1.27^{Bf}
SWY	49.38 ± 0.37^{Ba}	52.12 ± 0.46^{Cb}	55.28 ± 0.47^{Cc}	53.11 ± 1.23^{Cd}	49.52 ± 0.55^{Ce}	50.31 ± 1.27^{Cf}	53.18 ± 0.45^{Cg}	52.38 ± 1.15^{Ch}
WY	44.93 ± 0.54^{Ca}	46.25 ± 0.35^{Db}	48.01 ± 0.38^{Dc}	47.14 ± 0.54^{Dd}	48.51 ± 1.65^{De}	51.28 ± 0.84^{Df}	52.19 ± 0.78^{Dg}	50.31 ± 1.56^{Dh}

[a] Analyses were performed in triplicate. Values are means ± SD.
[b] C: control sample, SY: Probiotic fat free yogurt, SWY: Probiotic semi fat yogurt, WY: Probiotic full fat yogurt.
[abcd] Means in the same row with different small letter superscripts are significantly different.
[ABC] Means in the same column with different capital letter superscripts are significantly different.

https://doi.org/10.1016/j.heliyon.2019.e01204

yogurt might be due to antioxidant peptides released during the fermentation of milk by lactic acid bacteria [3].

4. Conclusion

In this study, the effect of milk fat content and starter culture on proteolysis and antioxidant activity of probiotic yogurt was investigated. It is obvious that fermentation increases the health benefits of milk. According to the results, the antioxidant activity and proteolysis between yogurt made by autochthonous and commercial starter culture were significantly different ($P < 0.05$). Increasing the storage time up to 14 days changed proteolysis and antioxidant activity. The antioxidant activity in fat free yogurt was higher than semi and full fat yogurt ($P < 0.05$). Furthermore, survival of *Lactobacillus acidophilus* decreased during storage period but was within the recommended level for health effect. The increase in proteolysis and antioxidant activity by starter culture type showed the importance of taking further investigation into account on bacteria isolated from traditional products.

Declarations

Author contribution statement

Maryam Tavakoli: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Mohammad B. Habibi Najafi: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mohebbat Mohebbi: Conceived and designed the experiments.

Funding statement

This work was supported by Ferdowsi University of Mashhad (Research affairs).

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

https://doi.org/10.1016/j.heliyon.2019.e01204

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