International Journal of Agriculture and Crop Sciences.
Available online at www.ijagcs.com
IJACS/2015/8-1/61-67
ISSN 2227-670X ©2015 IJACS Journal

IJACS

Study the possibility of producing symbiotic yogurt containing lactobacillus casei and wild thyme extract

Sana Nikjooy[1,*], Seyede Hoda Hashemi[2]

1. Department of Food Science & Technology, Damghan Branch, Islamic Azad University, Damghan, Iran
2. Department of Food Science & Technology, Khazar Institute of Higher Education, Iran

corresponding author email: sun313ni@yahoo.com

ABSTRACT: Symbiotic products consumption (presence of probiotic and prebiotic together) has some beneficial effects on consumer's health. In the present study, symbiotic yogurt was produced by adding extracts obtained from wild thyme at three levels (1.5, 1.3, 0.5),respectively, and from probiotic lactobacillus casei strains and then the sensory evaluation, physicochemical and antioxidant characteristics of this yogurt were examined during a 21 day period. Results showed that in the case of adding wild thyme extract to yogurt in the samples and the storage time, there wasn't a significant difference in syneresis, and the amount of thyme extract hadn't have any significant effect on this index. The effect of adding this extract to yogurt was quite significant on the pH and acidity, but the storage period had no effect on these indexes. Also, considerable antioxidant activities were seen in samples containing wild thyme extracts. The most antioxidant activity based on DPPH, was reported about 69 percent in the sample 1which contained the highest amount of extract, And also the increasing of the overall counts of probiotic bacteria were seen in the samples with wild thyme extracts. According to the remarkable antioxidant activities in samples containing wild thyme extracts, we can consider this yogurt as a functional food. Although there wasn't a significant difference in respect to majority of sample's characteristics such as color, flavor and appearance among samples and the number of storage days, but the acceptance level of produced product among assessors was quite high.
Keywords: Probiotic, yogurt, wild thyme extract, lactobacillus casei, prebiotic, symbiotic.

INTRODUCTION

Yogurt is a dairy product which is made by lactic acid fermentation in two types of starter bacteria of yogurt, lactobacillus delbrueckii and streptococcus thermophilus (Crittenden, 2006). Probiotics are live organisms available in foods which are believed that by making a microbial balance in intestine, have useful and healthy effects on their host. Improving lactose intolerance, rotavirus-induced diarrhea, and antibiotic associated diarrhea are some examples among all scientifically proved health benefits of these probiotics (Homayouni et al., 2008). Lactobacillus casei is a gram-positive, mesophyll, microaerophilic and non-spore forming bacteria. In order to achieve desirable nutritional, organoleptic and technological features, this bacterium is added to the yogurt individually or in combination with other probiotics (Larrauri et al.,1997). prebiotics are nutrients that are used up by certain bacteria as a rich source of carbon, and thus they can be added to the environment to increase the growth and survival of the bacteria. Herbal extracts are prebiotics that elevate and enhance the antioxidant content of products and cause the health of the product. Wild thyme extract is a rich source of flavonoids polymerized polyphenols, carotenes, tocopherol, betaine and choline (Cummings et al., 2001).

As far as yogurt is a product with a variety of nutritional values (Gad et al., 2010), therefore it is a good option to produce functional products and many studies have been carried out in this area (Ghiassi et al.,2011). In fact, dairy products and milk products, especially yogurt, are bioactive compounds which promote health. Today, yogurt as a dairy food especially low fat ones, is suggested to be consumed along with a group of herbal foods to treat obesity and to appropriately manage weight. Moreover, some observations demonstrate that yogurt as the most popular dairy food, and also phytoestrogens in spices and herbs are useful components used to treat obesity and manage weight, and besides they reduce chemical drug needs which results in much more limited side effects (Shahraki et al., 2013).

Accordingly, Gad and et al. (2010) after reviewing different characteristics of yogurt containing palm extract and vivid changes during the storage period, realized that the intended sample contains phenolic components, and in contrast to ordinary yogurt, antioxidant activities are more significant and so they called this yogurt a functional product (Ferna'ndez-Garci'a and McGregor, 1997). Eliana Pereira and et al. (2013) did some research on yogurt and natural food additives, and demonstrated an increase in antioxidant activities in yogurt contained additives compared with ordinary yogurt, which can protect the consumer against oxidative stress-induced diseases (Periera et al., 2013).

Zainoldin & baba (2009) investigated anti oxidative activities in yogurts containing 10 percent pytaya extract and found that compared to ordinary yogurt, this yogurt has more anti oxidative activities and suggested that adding extract is a factor that improves phenolic characteristics of an ordinary yogurt (Zainoldin and Baba, 2009).

Brinkso and et al. (2011) reported survival rate of Lactobacillus plantarum in yogurt and investigating under simulated digestive system condition and storing in a refrigerator. They found that survival of probiotics are seriously influenced by gastrointestinal condition, and during a 38 storage period in fridge, the number of survived organisms was declined to 0.55 logarithmic cycle (Lucey et al., 1997).

Sultana and et al, (2000) used alginate calcium to overlap lactobacillus acidophilus and they realized that the population of overlapped bacteria kept in yogurt during the 8 week storage period, was declined about 5 logarithmic cycle and declined to 1 logarithmic cycle when they are free (Sultana et al., 2000).

The purpose of this study was to examine the effects of adding wild thyme extract and probiotic lactobacillus casei strain to yogurt and produce a symbiotic yogurt.

MATERIALS AND METHODS

Materials used in this study

Microbial strains including yogurt starter bacteria with YC-X11 profile containing lactobacillus delbrueckii and streptococcus thermophilus and one-strain probiotic lactobacillus casei LC-01, which both were provided in a dried, frozen and DVS- form Christansen Honson Company in Tehran. Chemical compounds used in experiments includes MRS agar culture medium, MRS broth and peptone water culture. The rest of the chemical materials were bought from Merk Company.

CELLULAR SUSPENSION PREPARATION METHOD

First we inoculate a few pure, lyophilized LC-01 granules to 50 ml of Sterile MRS broth medium and then home heated in 37° C under anaerobic condition for 24 hours. Thus, the culture medium was supplemented with live cells that are cultured in MRS broth and agar dilution which suggests that every ml of the medium contains living cell number of 10^{10}-10^{9}. To obtain biomass, the whole area of the inside of centrifuge tubes were divided and centrifuged for 5 min at 4°C with 4600g round. After removing the additional medium, the excess biomass by using a solution of sterile peptone water 0.1% w/w, double the above conditions have been washed and the final cell mass was collected and was stored in a 0.1 percent peptone water solution in the refrigerator temperature before it is used.

Wild thyme extraction

to extract from wild Thyme, dried leaves were removed about 200 grams. Afterward the weighted leaves were mixed with 500 ml water, and then the final mixture was kept in a water bath machine in 70° C. After that extracting was done by soaking and Condensing with rotary device at 50 °C until it reaches the 35 BRICS, and after filtration under vacuum through a Buchner funnel using Whatman filter with a pore diameter of 1 micrometer, the extracts were filtered.

YOGURT PREPARATION METHOD

After standarding the fat (3.5 percent), acidity (0.16%) and nonfat dry milk (8.5 percent), the process of preparing yogurt was done based on the Tamim and Robinson (1999) proposed method. Pasteurization process was done at 90-85°C for 5 min. After cooling the milk to a temperature of 43°C, commercial yogurt starter in accordance with the manufacturer's instructions and cell mass that had been previously collected, were added to each treatment. After adding specific amount of thymus mountain to the milk in order to produce the yogurt, packaging samples in sterile containers were done in one liter containers and incubated at 42 ° C until the pH of the samples reaching to 0.6% according to lactic acid The next step was putting the samples in the cold storage at 4°C.Then, within 21 days, quality changes were examined in the flavored yogurt samples and the control sample

(Tamime and Robinson,1999). It is notable that in the control sample, the yogurt starters and probiotic bacteria were also inoculated, but the control sample was free of extract.

Factors to be examined

Microbial testing: to count probiotic lactobacillus casei, one gram of yogurt samples which contains this cell is diluted during the storage period with 9 ml sterile peptone water solution and after culturing in a vancomycine agar - MRS with pour plate method, plates were transferred to incubation with 37℃. Colony counts were performed after 72 hours of incubation. The number of viable cells after incubation were determined over time as the number of live (cfu) per gram by the colony counting machine (Homayouni et al ., 2008).

pH measurement: the pH was measured by using pH meter model (Metrohm 69, Swiss) which already had been calibrated (AOAC, 1995).

Acidity measurement: samples were measured based on AOAC method, 1995.

Water level measurement: 50 grams of yogurt were weighted in the filter paper in the funnel and after two hours putting in the refrigerator temperature, the water amount was weighted and water percentage was calculated (Zainoldin and Baba, 2009).

Antioxidative activities: measuring ant oxidation level among probiotic yogurts produced by DDPH solution (Alorich D913-2, Germany), was done by molecular weight of 394/32 and methanol, and the absorption level of each sample was in 517 nm (Zainoldin and Baba, 2009).

Sensory testing: sensory evaluation of samples were done after one whole night storing in 4°C by 10 witness reviewer. All yogurt samples in respect to organoleptic properties (taste, tissue, color and overall acceptance) were evaluated (Herald et al., 2008).

Statistical analysis: Wild thyme extract at 3 level and probiotic lactobacillus casei were added to each sample. All samples were produced in three times. In the present study, results and data were analyzed in a form of factorial design and random base by using statistical analysis software (SAS). Mean comparison done using Duncan Multiple range test at the confidence interval of 95 percent and drawing the curves was done by Microsoft Excel 2007 software

RESULTS AND DISCUSSION

Evaluating the physico-chemical characteristics of yogurts containing wild thyme extract

Evaluating Synersis in samples containing wild thyme extract

Analysis of variance table showed that in term of the value of synersis, there wasn't a statistically significant difference between sample no 2 (1.3% extract) and sample 3 (0.5% solvent), but sample 1 (1.5% solvent) with maximum synersis, has significant difference at the one percent level with the two other samples Figure 1 shows that the highest rate with 29 percent belong to the sample 1. The most synersis of the producing yogurt say was observed 26 percent, and then on days 7, 14 and 21 no significant differences were seen between yogurt samples. Days of storage in all samples had quite significant differences according to the synersis. The most synersis was seen on day zero and in sample 1. Blecker et al (2001) demonstrated that using inulin may result in a decrease in synersis value in yogurt and other kinds of Acidophilus milks (Blecker et al., 2001). Same results were reported in fruity yogurts by other researchers. Staffolo et al (2004) found that yogurts containing apple fiber and crop have less synersis compared to samples with no further additives (Staffolo et al., 2004).

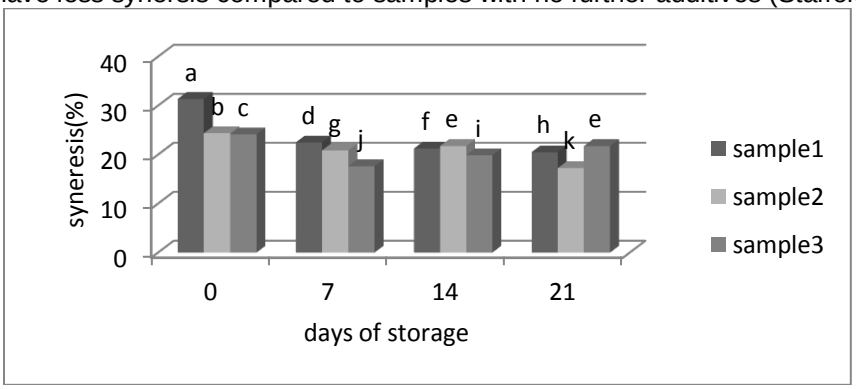

Figure 1. The effect of storage period and amounts of wild thyme extract on the synersis value in symbiotic yogurt (sample 1: 1.5 percent of extract, sample 2: 1.3 percent of extract and sample 3: 0.5 percent of extract)

pH changes evaluation in symbiotic yogurts

There was no significant difference between the two samples in respect to pH changes, however, sample 2 accounted for the highest pH (Figure 2). The amount of extract added to the yogurt was not sufficient to cause a change in pH and we can conclude that the addition of wild thyme extracts doesn't have a significant effect on increasing or decreasing the pH. Figure 2 shows that according to pH, there was no significant difference on the preserved days, but the pH value increased from the first day until the fourteenth day and then decreased until the twenty-first day. The highest and lowest pH was in day 14 and in sample 3, and the lowest pH was observed in day zero and in sample 3.

These results correspond with the findings obtained from Vahedi et al (1998) works. Ozer et al (1998) found that the pH value in Concentrated yogurts (16 percent of solid content) at the end of 240 minutes reaches to 4.3, while the decline in the value of pH in yogurts with more solid contents was more slowly, which is resulted from higher Buffering capacity of Concentrated milks (O'Connell and Fox, 2001).

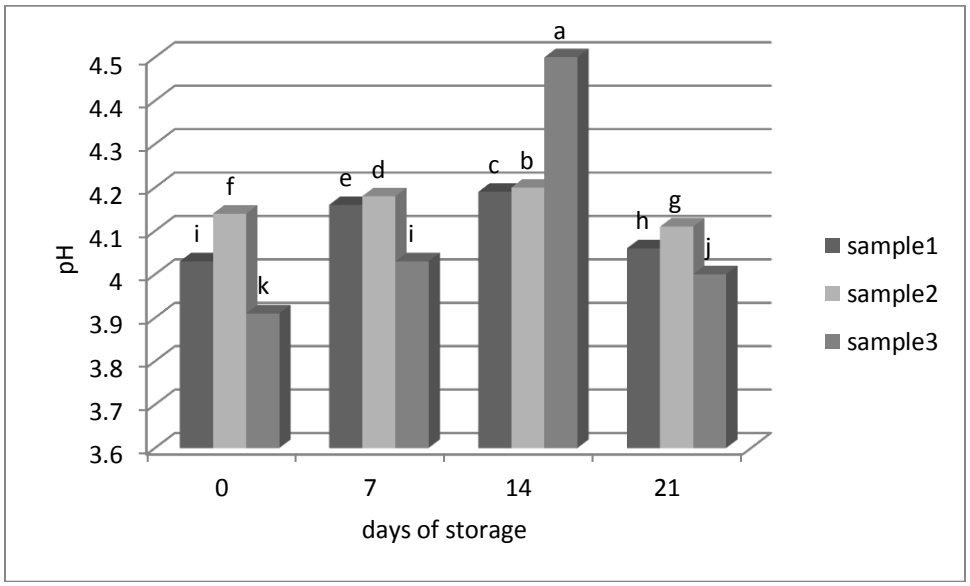

Figure 2. The effect of storage period and amounts of wild thyme extract on the pH value in symbiotic yogurt (sample 1: 1.5 percent of extract, sample 2: 1.3 percent of extract and sample 3: 0.5 percent of extract)

Acidity changes in samples containing wild thyme extract

According to the yogurt acidity, a significant difference was seen between the samples in the 1 percent level. The most acidity was in the sample 3 and the lowest acidity was in the sample 1 (figure 3). So we conclude that adding the wild thyme extract will have a significant effect in acidity level. According to the results from other researchers, this effect is because of the changes in the proportions of Bacilli and Cocci in the yogurt. So increasing the number of lactobacillies cause the more accidity flavor.

Figure 3, represents the non-significant acidity decrease by increasing the storage time to 7 days and then the Non significantly increases till day 14. According to Tehrani et al (2004), and Mahdian et al. (2006) findings, it can be proved that the amount of acidity increased at day 7 which is the result of lactic bacteria activities inside the yogurt in the storage time (Mazaheri Tehrani et al., 2004, Mahdavian et al., 2006).

Mortazavi et al (2002) suggested that during the 21day storage of the samples in refrigerator, the value of decreased pH and acidity had been increased, and the storing has a significant effect on acidity and pH, and the reason of decrease in pH or increase in acidity is resulted from helpful and harmful activities of microorganisms that by sugar consumption and organic acids production can be followed the decrease of pH or increase in acidity (Mortazavian and Sohrabvandi, 2006).

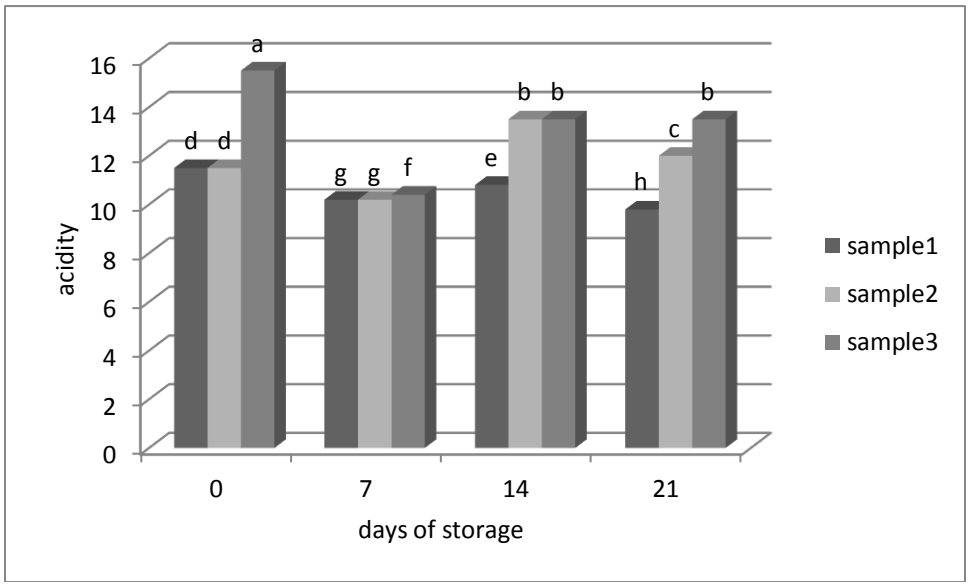

Figure 3. The effect of storage period and amounts of wild thyme extract on the acidity value in symbiotic yogurt (sample 1: 1.5 percent of extract, sample 2: 1.3 percent of extract and sample 3: 0.5 percent of extract)

Evaluation of antioxidant activity in yogurt samples containing extracts of wild thyme

There wasn't a statistically significant difference between samples 1, 2 and 3 in respect to the level of antioxidant activities. Figure 4 shows that sample 1 with 69 percent showed the highest and sample 3 with 64 percent showed the lowest antioxidant activity level among all. By increasing the amount of wild thyme extracts in yogurt formulations, antioxidant activity has been increased. In fact, the phenolic wild thyme extract compounds show the antioxidant activity which cause this process (Chen et al., 2003). The most magnificent antioxidant activity was about 69 % in day zero and in sample 1 which was not significantly different from sample 1 in day 7 and the lowest antioxidant activity level, was about 60 % in sample 3 and in day 14.

By adding Rosemary and Green Tea extract to the ordinary yogurt, Gad and et al. (2010) found that antioxidant characteristics haven't decreased during the storage and it can be noted that these components result in an increase in antioxidant features of yogurt (Gad et al., 2010).

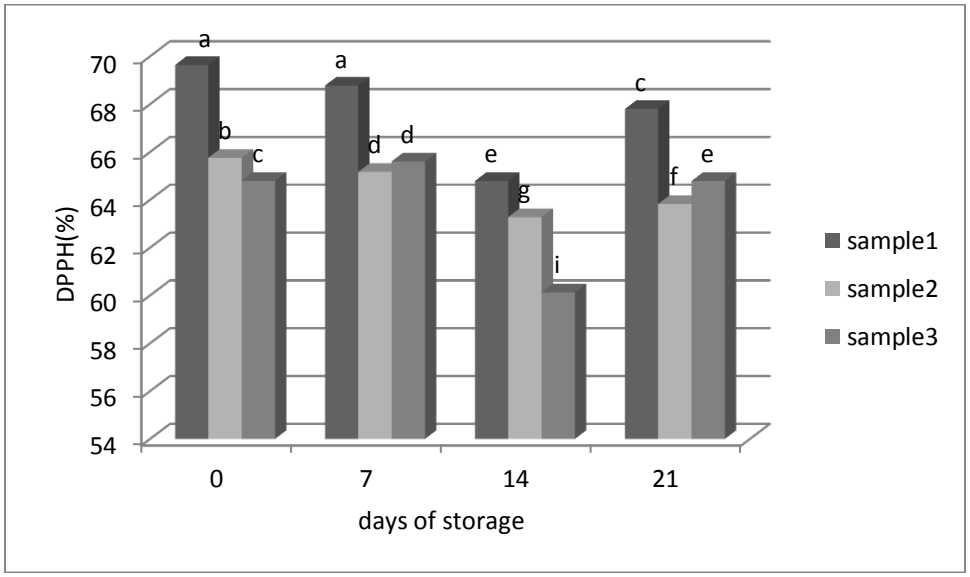

Figure 4. The effect of storage time and wild thyme extract on symbiotic yogurt antioxidant activity (sample 1: 1.5 percent of extract, sample 2: 1.3 percent of extract and sample 3: 0.5 percent of extract)

Sensory characteristics evaluation

During this study, the sensory features of produced yogurt samples including color utility, taste, perfume, and appearance were evaluated and the results were presented as follow:

Variation analysis table 1, in respect to yogurt color, showed that there was not a significant difference between all three samples, although the best score was given to sample 3 in which the amount of wild thyme extract was the lowest. Color desirability in the storage period decreased to day 21 and there was not a significant difference between the samples in accordance to color scores.

According to table 1, it's obvious that in respect to Perfume and aroma index, there wasn't a significant difference between three samples with different wild thyme extract compounds. Sample 3 received the lowest score and sample 2 and 1 were the same. Consuming wild thyme extract was caused the increase in the yogurt perfume, and therefore the samples containing more wild thyme extract received higher scores.

Best score was seen in day 7 in all samples, and we can conclude that the number of days and amount of extract added, did not have a significant effect on perfume character.

According to the variation analysis table, in respect to taste desirability of the yogurt, there was not a significant difference between samples and we can conclude that wild thyme extract addition didn't significantly influence on evaluators judgment. The best acceptance level of the taste with the score of 4.22 was referred to day 7 and the lowest one with the score of 3.33 belongs to day zero.

The variation analysis table showed no significant difference between samples according to the desirability of the yogurt appearance and the Sample 1 had the lowest and the sample 2 had the highest desirability of the appearance has been decreased by the time. Also, the effect of storage time and the amount of wild thyme extracts on the amount of the desirability of appearance in symbiotic yogurt was not significant.

Table 1. variation analysis of the examined characteristics changes in testing the related panel of the yogurt sample containing the Wild Thyme extract

appearance	taste	perfume	color	relief degree	changing source
0.141 ^{ns}	0.084 ^{ns}	0.057 ^{ns}	0.184 ^{ns}	2	repeating levels
0.333 ^{ns}	0.229 ^{ns}	0.634 ^{ns}	0.111 ^{ns}	3	Treatment levels
0.567	0.548	0.316	0.424	30	error
22.094	21.04	15.78	18.45		CV

Microbial Enumeration

According to results presented in table 2, extracts used in this study, at the end of day 21, had the least number of probiotic bacteria (6 log CFU/g) according to FIL-IDF - Food Standards for prebiotic products.

Table 2. Effect of wild thyme extract on lactobacillus casei counts (log cfu/g)

Time of storage(day)	1	7	14	21
Lactobacillus casei	9.48	9.16	8.48	7.32

Wiljanen et al. demonstrated that adding fruit extract as a major functional component can help the distribution of increased biomass from different bacteria species as well as increasing the lifetime of probiotic bacteria living Gastrointestinal Tract and therefore plays an important role in health promotion (Gad et al., 2010).

Sophorn hob (2012), suggested that strawberry and Blueberry extracts had a significant stimulating effect on Lactobacillus isolates and Bifidobacterium From yogurt (Hap, 2010).

CONCLUSION

In this study, the effect of wild thyme extract on sensory, physicochemical and antioxidant characteristics of symbiotic yogurt was examined. Results showed that when adding wild thyme extracts to yogurt, there wasn't a significant change among all samples and the storage time, and the amount of wild thyme extracts had not significant effect on this index. Also the most significant antioxidant activity was seen in the samples containing wild thyme extracts which the highest antioxidant activity was about 69 percent in sample 1 and in the storage day of 1 in the sample containing the extract and reported based on DPPH. Results showed that yogurt containing the extract had a significant antioxidant activity compared to ordinary yogurt thus we can introduce this yogurt as a functional food. Although there wasn't a significant difference in respect to majority of sample's characteristics such

as color, Perfume, taste and appearance among samples and the number of storage days, but the acceptance level among assessors was quite high.

REFERENCES

Anderson JJB, Garner SC.1998. Phytoestrogens and bone Baillieres Clin Endocrinol Metab,12:543-57.

AOAC. 1995. Official Methods of Analysis (14th Ed). Washington Dc: Association of Offocial Analytical Chemists.

Blecker C, Chevalier JP, Van Herck JC, Fougnies C, Deroane C, Paquot M. 2001. Inulin: Its physiochemical properties and technological functionality. Recent Research Development in Agric and Food Chem, 5: 125–131.

Chen J, Lindmark-Mansson H, Gorton L, Akesson B. 2003. Antioxidant capacity of bovine milk as assayed by spectrophotometric and amperometric methods.International Dairy Journal, 13: 927–935.

Crittenden R. 2006. Emerging prebiotic carbohydrates. In: Prebiotics: Development and Application. Gibson, G. R. & Rastall, R. A. (Eds.). John Wiley 7 Sons, Ltd. England, pp: 111-133.

Cummings JH, Macfarlane GR, Englyst HN. 2001. Prebiotic digestion and fermentation American Journal of Clinical Nutrition, 73: 415-420.

Ferna'ndez-Garci'a E, McGregor JU. 1997. Fortification of sweetened plain yogurt with insoluble dietary fiber.EuropeanFood Research and Technology, 204: 433-437.

Gad AS, Ebtisam I, Ghita Halam F, El-Din Sanaa MA, Badran TM, Messery El. 2010. Production Health benefit yoghurt supplemented with Rosemary and Green tea extract as Natural Anti oxidant.

Gad AS, Kholif AM, Sayad AF. 2010. Evaluation of the Nutritional Value of Functional Yogurt Resulting from Combination of Date Palm Syrup and Skin Milk.Aminrican Journal of Food Technology, 5: 250-259.

Ghiassi. 2011. Moulded fruit-flavored yogurt formulation using natural pigments and evaluating its features, Gorgan university of Agricultural Sciences and Natural Resources.

Hap S.2010. Functional Properties of Aqueous Fruit Extracts of Toward Probiotic and Pathogenic Bacteria. 50-53.

Herald TJ, Aramouni FM, Abu- Ghoush MH. 2008. Comparison study of egg yolks and egg alternatives in French Vanilla ice cream, Journal of Texture Studies, 39: 284-295.

Homayouni A, Ehsani MR, Azizi A, Razavi SH, Yarmand MS. 2008. Growth and survival of some probiotic strains in simulated ice-cream conditions. Journal of Applied Sciences, 8: 379–382.

Larrauri JA, Borroto B, Crespo AR. 1997. Water recycling in processing orange peel to a high dietary fiber powder. International Journal of Food Science and technology, 32:73-75.

Lucey JA.1997. Properties of acid casein gels made by acidification with glucono-δ-lactone. 2. Syneresis, permeability and microstructural properties. International Dairy Journal, **7**(6–7): p. 397-389.

Mahdavian A, Mazaheri Tehrani M, Karazhian R. 2006. The effect of solid substance of the whole milk on the growth of starter bacteria and quality of the concentrated yogurt. Journal of Agricultural and Industrial Chemistry, 4 (3): 59-67.

Mazaheri Tehrani M, Mahdavian A, Vakarazhian . 2004. The effect of fat amount of milk on the growth and activity of the starter bacteria and quality of the yogurt. Journal of Agricultural and Natural Resource Sciences, Vol XIII, Eighth No: Bahman-esfand 85.

Mortazavian AM, Sohrabvandi S. 2006. Probiotic and probiotic foods, Ata Publication, Tehran, 264 p.

O'Connell JE, Fox PF. 2001. Significance and applications of phenolic compounds in the production and quality of milk and dairy products: a review. *International Dairy Journal*, 11: 103–120.

Periera E, Barros L, Ferreira I. 2013 . Relevance of the mention of Antioxidant Properties in yoghurt labels: Invitro Evaluation and Chromatographic Analysis, 62-76.

Sayyadaki Shahraki M, Mohammadi Sani A, Hojatol Eslami M. 2013. Evaluation of the phenolic compounds and antioxidant activity of flavored yogurt enriched with red betalain beet and evaluation of its physic-chemical properties in the storage time, second national conference on food science and technology, Ghoochan, Islamic Azad university, Ghoochan Branch

Staffolo MD, Bertola N, Martino M, Bevilacqua A. 2004. Influence of dietary fiber addition on sensory and rheological properties of yogurt. International Dairy Journal, 14: 263–268.

Sultana K. 2000. Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. Journal of Food Microbiology, 62: p. 47-55.

Tamime AY, Robinson RK.1999. Yogurt science and technology 2nd 365-373.

Vahedi N, Mazaheri M.2009. Optimizing the fruit ordinary yogurt formulation and evaluating its quality in the storage time, Science and Technology of Agriculture and Natural Resources , Vol XIII, No.48

Viljanen M, Savilahti E, Haahtela T, Juntunen-Backman K, Korpela R, Poussa T. 2005. Probiptics in the treatment of atopic eczema/ dermatitis syndrome in infants: a double-blind placebo-controlled trial. *Allergy (Copenhagen),* 60(4), 494-500.

Yapo BM. 2009. Pineapple and banana pectins comprise fewer homogalacturonan building blocks with a smaller degree of polymerization as compared with yellow passion fruit and lemon pectins: implication for gelling properties. Biomacromolecules, 10:717-721.

Zainoldin KH, Baba AS. 2009. The effect of *Hylocereuspolyrhizus*and *Hylocereusundatus*on physicochemical,proteolysis, and antioxidant activity in yogurt. *World Academy of Science, Engineering and Technology* ,76:361- 366.