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ORIGINAL ARTICLE

Effect of Garlic and Dill Extract on Yoghurt Probiotic Bacteria (*Bifidobacterium bifidum* and *Lactobacillus acidophilus*) and their role in rat's Triglycerides and Cholesterol

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ABSTRACT
The purpose of this study was too compare dill and garlic extract on the viability of probiotic bacteria in yogurt. Also by giving the products is to rats, the effect of these two plants on cholesterol and Triglyceride levels in rats were investigated. It should be noted in this article probiotic yoghurt was been produced in presence of Lactobacillus acidophilus & Bifidobacterium bifidum. The probiotic yoghurt was produced by Tamime, standard methods. The samples were then examined in terms of pH, acidity and microbial count during the incubation period, and permanence. In 7 day, the products were sensory evaluated. The results of the filled questionnaires in statistical descriptive test were analyzed using SPSS version 17. In the samples containing Lactobacillus acidophilus and Bifidobacterium bifidum, it was observed that increased concentrations of dill and garlic extract create a favorable taste in yoghurt. Results showed that the yoghurt containing 0.6% dill extract had the best for taste, color, and insolubility. Also,no significant difference was observed in the dill and garlic yoghurt in terms of color, thickness, taste and scent. The control sample and yoghurt containing 0.2% dill extract and 0.1% garlic had greater viscosity than the other samples investigated. The bio ability of probiotic bacteria was measured by direct counting method. The shelf lives of products were determined to be 21 days during which the bacterial count decreased but not less than 10^9. The results showed that milk with Bifidobacterium bifidum was more effective in reducing cholesterol than the milk with Lactobacillus acidophilus. However, the probiotic Lactobacillus milk with dill extract was more effective in reducing serum Triglyceride in the rats. Although a positive correlation was observed between the increased concentration of garlic in the milk with Lactobacillus acidophilus, the milk with Bifidobacterium bifidum and reduced cholesterol and triglyceride, respectively.
Key words: Garlic, dill, Probiotic, Lactobacillus acidophilus, Bifidobacterium bifidum, Triglycerides, Cholesterol.

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INTRODUCTION

Consumers across the world are becoming more interested in foods with health promoting features as they gain more awareness of the links between food and health. Among the functional foods, products containing probiotics are showing promising trends worldwide. Probiotics such as *Lactobacillus* and *Bifidobacterium spp,*are bacterial members of the normal human intestinal flora (1). That exert several beneficial effects on human health and well-being through production of short chain fatty acids and improve the intestinal microbial balance, resulting in the inhibition of bacterial pathogens, reduction of colon cancer, improving the immune system and lowering serum cholesterol levels (2). Probiotics are recognized for their applications in dairy products, particularly yoghurts and the market for these products is still rising. To achieve the claimed health benefits, one of the most important requirements for manufacturing and marketing of probiotic yoghurt is to maintain a high number of probiotic organisms P6 log CFU/g at the point of consumption (3). However, in commercial products various probiotic lactobacilli and bifido bacteria show a decline in their viability during product's shelf life (4 and5).Recently, the food biotechnology industry has developed a number of commercial products containing a single probiotic strain or bacterial associations of various complexities. Yoghurt has been known for its nutraceutical, therapeutic, and probiotic effects (6).Several factors are responsible for the viability of probiotic organisms e.g. the strains used, culture conditions, antagonism among cultures present, storage time and temperature, initial counts, hydrogen peroxide and oxygen contents in the medium, and the amount of

organic acids in the product [5]. Recently, the design and production of plant-based probiotic products have received much attention chiefly due to their natural health benefits (protein, fiber, vitamin and salts) and also because of the variety in their production. Therefore, it seems that the issue of producing probiotic foods with appropriate qualities will be a major research topic for prospective researchers [7]. Development of new products with fragrance and flavor will increase sales and consumer satisfaction. It can be used in order to prepare materials such as soya yogurt, vegetables, sweet potatoes, pumpkin (8) to enhance the flavor and quality of feed used (9).Dill as aromatic herbs in the food industry, in the form of fresh vegetables, tea and cooked our food. Also in perfumes, sanitary and pharmaceutical industries as essential oils are used. Garlic, as a medicinal plant, possesses a lot of therapeutic properties of which we could mention antihypertensive, diuretics, anticancer and lipid reducing agent (10).Significant amounts of antioxidants and antimicrobial activity were observed in both plants (11). Also the anticancer properties of both plants are (12).The purpose of this study was to compare dill and garlic extract on the viability of probiotic bacteria in yogurt. Also by giving the products is to rats, the effect of these two plants on cholesterol and *Triglyceride* levels in rats were investigated. It should be noted in this article probiotic yogurt was been produced in presence of *Lactobacillus acidophilus* & *Bifido bacterium bifidum*.

MATERIALS AND METHODS

Materials

Garlic plant extract, Low-fat yoghurt from supermarket , Lyophilized *Lactobacillus acidophilus* (CHR Hansen company, Denmark), Lyophilized *Bifidobacterium bifidum* (CHR Hansen Company, Denmark), High fat feeding of the rats, Serum cholesterol measuring kit (Sinateb, Iran), Serum triglyceride measuring kit (Sinateb, Iran), MRS Agar culture medium (Merck, Germany).

Preparation of starters *(Bifidobacterium Bifidum & Lactobacillus acidophilus)*

For preparation starters, 1liter of sterile skim milk (1.5% fat) was considered. Then0.33 gram of the each lyophilized probiotic bacteria (*Bifidobacterium Bifidum*or *Lactobacillus acidophilus*) was separately added to one liter of low fat sterilized milk. Finally, sample, to reach42 °C in an incubator at 38 °C acidity were placed.

Preparation of probiotic yoghurt containing dill extract

To produce yoghurt in this stage, after providing 4 containers, 1 liter of the low fat sterilized probiotic milk (1.5 % fat) at first passage (*Lactobacillus acidophilus or Bifidobacterium bifidum*) and the (1.5%) starter of low-fat yoghurt (1.5%) were added to each container. Different concentrations of dill extract (0, 0.2, 0.4, and 0.6%) were added respectively to the containers and mixed properly so that dill was uniformly dissolved. Afterwards, all the containers were placed in the incubator at 38˚ C. Approximately every 2 hours, the acidity and pH tests were done until acidity reached 90˚ D. Then, the samples were taken out of the incubator and transferred to a refrigerator and stored at 2˚C.

Preparation of probiotic yoghurt containing garlic concentrations

All the same procedures were followed as mentioned above with the difference of using different concentrations of gralic (%0, %0.1, %0.2, and % 0.3) instead of concentrations of dill. Having produced the above-mentioned products, we stored each product in a refrigerator for 21 days. During this period, each sample was tested in days 1, 7, 14, and 21 for acidity, pH, and sensory properties.Also probiotic yoghurt was evaluated every 7 days by counting the microbes using direct counting method and after 10 days the yoghurt was evaluated for sensory properties, using questionnaires filled by 50 participants. The respondents were asked to rate the factors of scent, taste and permanence on a scale ranging from very good, good, medium, to weak.

Effect of probiotic yoghurt containing garlic extract on the cholesterol and *Triglyceride* level of the rats

In doing so, we initially prepared the rats with the same physiological, genetic and environmental features.Having bred the rats within a 45 day period, we came to mature rats of the same age (45 days), same sex (female), same weight (200 gram) and same environmental and nutritional features including temperature, humidity, light, living place, water and food. Then, we divided them into six groups of six rats as follows:

Group 1: first control group with balanced diet, Group 2: second control group with high fat diet , Group 3: the experimental group fed with *Lactobacillus acidophilus*yoghurt without garlic extract, Group 4: the experimental group fed with probiotic *Lactobacillus acidophilus* yoghurt with 0.1% garlic extract, Group 5: the experimental group fed with probiotic *Lactobacillus acidophilus*yoghurt with 0.2% garlic extract, Group 6: the experimental group fed with probiotic *Lactobacillus acidophilus*yoghurt with 0.3% garlic extract.At the beginning, blood samples were collected from the group 1 and transferred to the lab. In doing so, the rats were made unconscious in a cylinder with ether and blood was taken directly from their hearts through incision made on the chest. The remaining five groups were fed with high fat diet prepared

from plate and animal fat with 3 to 1 ratio, for 14 days. Afterwards, blood sampling was performed on group 2 and transferred to the lab, and the group was excluded.
The other four group received their feeding for 7 days as follows: group 3 received balanced diet, group 4 received probiotic *Lactobacillus acidophilus*yoghurt with 0.1% garlic, group 5 received probiotic *Lactobacillus acidophilus*yoghurt with o.2% garlic and finally group 6 received the same yoghurt but with 0.3% garlic. During this period, 30 ml/day of yoghurt was given to each rat and blood sampling was performed on the four remaining groups and transferred to the lab.All the same procedures were followed as mentioned above with the difference of using *Bifidobacterium bifidum* instead of *Lactobacillus acidophilus*.

Effect of probiotic yoghurt containing dill extract on the cholesterol and *Triglyceride* level of the rats

All the same procedures were followed as mentioned above with the difference of using dill instead of garlic.

Measuring serum cholesterol and serum *Triglyceride* levels

To prepare serum samples from the transferred blood samples, the samples were centrifuged once or twice with 3500 RPM for five minutes and serum samples were isolated. To prepare reagents for cholesterol and *Triglyceride* kits, Reagent A and Reagent B were mixed respectively with 2 to 1 ratio. The experiment parameters included: Temperate: 37^{0}C, Wave length: 450 Nanometer, Sample volume: 10 Micro liters, Cuveet: 1 cm, Reagent volume: 1 ml

Statistical analysis

All the above experiments were repeated three times. SPSS17 was used for one-way analysis of variance for all data, and significant differences ($p < 0.05$) among means were determined by the least significant difference test.

RESULTS AND DISCUSSION

pH and Acidity examination

Table 1 shows the acidity degrees of dill and garlic yoghurt *Lactobacillus acidophilus*, during storage time in the refrigerator. The *Lactobacillus acidophilus* yoghurt with 0.3% garlic reached the acidity of 90˚ D eeriest, followed by the samples with 0.2%, 0.1% and the control. And the *Lactobacillus acidophilus* yoghurt with 0.6% dill reached the acidity of 90˚D faster, andthenthesampleswere0.4, 0.2%andcontrol sample. All of which were then transferred to a refrigerator at 2˚C.The storage time in the refrigerator was found to be 21 days during which the acidity level of the yoghurt with *Lactobacillus acidophilus* containing 0.1% garlic was lower than that for other samples.

Table 1: Comparison of essential dill and garlic to the acidity of yogurt containing Lactobacillus *acidophilus* in for 21 days in the refrigerator.

Acidity level in Dornic degree									
Garlic yoghurt	1 day	7 day	14 day	21 Day	dill yoghurt	1 day	7 day	14 day	21 day
0%	100	95	114	92	0%	91	90	103	94
0.1%	91	91	114	80	0.2%	92	91	104	91
0.2%	90	93	107	85	0.4%	96	93	110	96
0.3%	99	92	108	95	0.6%	94	94	115	91

Table 2 shows the acidity degrees of dill and garlic yoghurt *Bifidobacterium bifidum*, during storage time in the refrigerator. The *Bifidobacterium bifidum* yoghurt with 0.2 and 0.6% dill extract reached 90˚Dornic acidity earliest, followed by the yoghurt sample with 0.4% and the control. The *Bifidobacterium bifidum* yoghurt with 0.1% and 0.3% garlic reached 90˚D acidity earliest, followed by the yoghurt sample with 0.2% and the control.

Table 2: Comparison of essential dill and garlic to the acidity of yogurt containing *Bifidobacterium bifidum* in for 21 days in the refrigerator.

Acidity level in Dornic degree									
Garlic yoghurt	1 day	7 day	14 day	21 Day	dill yoghurt	1 day	7 day	14 day	21 day
0%	94	130	116	85	0%	110	119	121	132
0.1%	91	130	118	75	0.2%	109	127	125	138
0.2%	97	140	117	90	0.4%	92	118	123	129
0.3%	98	140	114	90	0.6%	90	114	122	132

Sensory properties

Although the basic feature of the probiotic products consumption is their medicinal effects (bio value), their associated sensory properties are also important. In other words, sensory properties rather than medicinal effects play the most important role in their daily consumptions. Among the probiotic products, fermented ones especially the probiotic yoghurt is popular worldwide for its unique sensory properties (13).The sensory evaluation was performed by 50 participants for the probiotic *Lactobacillus acidophilus* yoghurt with varying concentrations of dill and garlic extract, after seven days. Check the results, no significant difference was found between samples containing dill with a sample containing garlic. Also there were significant differences between the samples ($p<0.05$) and it was shown that the increase of dill or garlic extract gives rise to favorable taste, color, scent and thickness. Also,no significant difference was observed in the *Bifidobacterium bifidum* yoghurt with dill and garlic extract in terms of color, thickness, taste and scent.The investigation showed that the yoghurt containing 0.6% dill extract had the best for taste, color, and insolubility.

Probiotics growing and their living

The minimum required level of probiotic bacteria to be useful for the consumer's body is 10^7CFU.ml^{-1} of living bacteria and the level in the present study was found to be 10^{10}, thus, it could be beneficial for the consumers (13).

Table 3 and 4, Represents growth of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in the presence of garlic and dill extract is. Upon evaluation of the samples on MRS Agar, the *Lactobacillus acidophilus* with dill extract had the counts equal to logarithmic 10^{10} in day 15, and the sample product with 0.2% dill extract possessed the highest count of bacteria. As revealed in direct microbial counting, the count in day 15 was lower, compared to day 1, for all dill concentrations, but possessed logarithmic coefficient 10^{10}. The bactericidal and inhibitory effect of low pH was stronger for *Bifidobacterium bifidum* than *Lactobacillus acidophilus* and it seems that during the storage time and enhanced fermentations process, decreased pH caused decreased growth of *Bifidobacterium bifidum*.While upon evaluation of the samples on MRS Agar, the *Lactobacillus acidophilus* with garlic extract had the counts equal to logarithmic 10^{10} in day 15, and the sample product with 0.3% garlic possessed the highest count of bacteria. In the *Bifidobacterium bifidum* yoghurt the sample with 0.1% garlic extract and the control were with highest bacterial counts, as revealed in the evaluation of the samples on MRS Agar medium.The *Bifidobacterium bifidum*yoghurt with 0.6% dill extract was with the highest bacterial counts, as revealed in the evaluation of the samples on MRS Agar medium.

Table 3: *Lactobacillus acidophilus* bacteria grown in the presence of the essential of garlic and dill

Garlic yoghurt	1 day	15Day	dill yoghurt	1 day	15 day
0%	7.6×10^{10}	4.6×10^{10}	0%	7×10^{10}	8.75×10^{10}
0.1%	4.2×10^{10}	6.2×10^{10}	0.2%	12.5×10^{10}	12.75×10^{10}
0.2%	8.6×10^{10}	7.2×10^{10}	0.4%	6.5×10^{10}	7.75×10^{10}
0.3%	6×10^{10}	8.6×10^{10}	0.6%	6.75×10^{10}	10×10^{10}

Table 4: *Bifidobacterium bifidum* bacteria grown in the presence of the essential ofgarlic and dill.

Garlic yoghurt	1 day	15 Day	Dill yoghurt	1 day	15 day
0%	3.8×10^{10}	3.4×10^{10}	0%	14.4×10^{10}	9×10^{10}
0.1%	11.2×10^{10}	5.4×10^{10}	0.2%	21.75×10^{10}	10.5×10^{10}
0.2%	5.8×10^{10}	2.8×10^{10}	0.4%	9.5×10^{10}	6.75×10^{10}
0.3%	9.8×10^{10}	3.2×10^{10}	0.6%	10.75×10^{10}	9.5×10^{10}

Effect of dill and garlic extract on the cholesterol and triglyceride level of the rats

The results showed that the increased dill concentration in *Lactobacillus acidophilus* yoghurt was positively solidarity with reduced serum *Triglycerides* in the rats (0.6%>0.4%>0.2%) and in comparison with the control, the different concentrations were more effective in reducing serum *Triglycerides*. The increased dill concentration in probiotic *Bifidobacterium bifidum* yoghurt was negatively correlated with reduced serum *Triglycerides* in the rats (0.6%< 0.4%< 0.2%), and the 0.2% concentration had the greatest effect, even more than the control sample. In sum, the *Bifidobacterium bifidum* yoghurt with dill extract was found to be more effective in reducing serum cholesterol in rats than the *Lactobacillus acidophilus* yoghurt. However, the probiotic *Lactobacillus acidophilus* yoghurt with dill extract was more effective in reducing serum *Triglyceride* in the rats. While the increase of garlic concentration in *Lactobacillus acidophilus* yoghurt was positively correlated with reduced serum cholesterol of the rats: (0.1<0.2<0.3%). However, the two concentrations 0.1 and o.3% were more effective in reducing serum cholesterol, compared to the controls. The increased garlic concentration in *Lactobacillus acidophilus* yoghurt was positively correlated with reduced serum *Triglyceride* in the rats (0.1<0.2< 0.3%) and in comparison with the control, the different concentrations were more effective in reducing serum *Triglycerides*. The increased garlic concentration in *Bifidobacterium bifidum* yoghurt was positively correlated with reduced serum cholesterol (0.2< 0.1<0.3%).As shown, the probiotic *Bifidobacterium bifidum* yoghurt 0.3% garlic extract was the most effective in reducing cholesterol and this reduction was even lower than that in the first control group. The increased garlic concentration in probiotic *Bifidobacterium bifidum* yoghurt was positively correlated with reduced serum *Triglyceride* in the rats (0.1< 0.2< 0.3%), and the 0.3% concentration had the greatest effect, even more than that in the first control group. In sum, the *Bifidobacterium bifidum* yoghurt with garlic extract was found to be more effective in reducing serum cholesterol in rats than the *Lactobacillus acidophilus* yoghurt. However, the probiotic *Lactobacillus acidophilus* yoghurt with garlic extract was more effective in reducing serum *Triglyceride* in the rats.The results of the studies addressing the probiotic bacteria have demonstrated the following: The increased concentration of malt and soya caused increase in the microorganism growth and rising acidity level which in turn resulted in shorter incubation time for the desired acidity. In a study on the effects of soya powder on the growth of the bacteria, *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, in probiotic products, it was demonstrated that the shelf life for the acidity reaching the desired level during incubation decreased for the milk with both bacteria and combined soya and malt, compared to the milk with only soya. As for the yoghurt with both bacteria, the same results were yielded and incubation time for the yoghurt with malt and soya was decreased (14 and15). In another study addressing the effect of avishan on the bacterial growth, it was demonstrated that the increased avishan concentration promoted the growth of the bacteria in probiotic milk and yoghurt (16).The effect of honey on the growth of the above-mentioned bacteria introduced simultaneously into dairy products and drinks was investigated, and the results indicated that yoghurt with only *Lactobacillus acidophilus* tasted sourer than the yoghurt with both bacteria. The products containing *Bifidobacterium bifidum*, compared to those with *Lactobacillus acidophilus*, were with slower growth rate and also tasted less sour and were of longer permanence. They were not of favorable taste when honey concentration increased and the control was of the best taste among all the samples (17).In another study addressing the effect of vanillin on the bacterial growth, it was demonstrated that the increased vanillin promoted the growth of the bacteria in probiotic milk and yoghurt (18).Taking into account the results of the above-mentioned studies investigating the effects of malt, soya, honey , thyme and mint on the growth of *L. acidophilus* and *B. bifidum*, we can conclude that they all enhance the bacterial growth in dairy products, either when the bacteria are introduced separately or in combination into the products.

CONCLUSION

The results of the experiments in this work showed that dill and garlic extract (*Anethumgraveolens L*) was a suitable support for these intestinal bacteria that were kept viable up to the end of fermentation (21days). All tested *Bifidobacterium bifidum* and *Lactobacillus acidophilus* were capable of growing well on dill and garlic yoghurt without nutrient supplementation. *Lactobacillus acidophilus* had the highest viability in all of the products investigated .The survival of probiotic bacteria in refrigerated conditions for at least 21 days were in number of greater than 10^9cfu.mL^{-1} which is essential if a product should have probiotic properties. It is important to emphasize that all the products possessed excellent stability during 21 days of storage. The sensory scores of the products were high and acceptance. From the foregoing results it can be concluded that dill and garlic extract can be successfully used in formulation of dairy products.

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