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Influence of addition of lavender (*Lavandula officinalis* L.) on the growth, acidification profile and viable counts of different probiotics in fermented milk and yoghurt during fermentation and refrigerated storage

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ABSTRACT

The growth and metabolism of four probiotic strains with documented health effects were studied in treated milk and yoghurt supplemented with 0%, 1%, 2% and 3%(w/v) lavender (*Lavandula officinalis L.)*. The probiotic strains were *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, *Lactobacillus Casei* and *Lactobacillus paracasei*. The addition of lavender appeared to support the viability of probiotic bacteria, with higher microorganism numbers observed in lavender milk and yoghurts than in plain throughout the shelf life. Addition of lavender resulted in a significant decrease in pH and increase acidity, syneresis, viscosity and water holding capacity of milk and yoghurt base ($p < 0.05$), and also enhanced their sensory acceptability. Among three treatments of lavender and control sample, all of sensory parameters at level of pd” 0.05 no significant difference was seen. However 3% lavender milk was the most unfavorable because of the unpleasant appearance. The results of this experiment demonstrated the positive relation between increased bacterial growth and increased lavender concentration. In conclusion, all these experiments provide convincing evidence that lavender have beneficial effects on the survival of these probiotic cultures in dairy products. As a result, such stored dairy products containing both probiotics and prebiotics have synergistic actions in the promotion of health.

KEYWORDS

Lavender;
Probiotic bacteria;
Dairy products.

INTRODUCTION

Nowadays, consumers are demanding for foods with increasingly properties, such as pleasant flavor, low-calorie value or low fat content, and benefic health effects[2].

Significant part of the world population suffers gastrointestinal diseases caused by pathogenic bacteria that

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invade the human intestine. A few days after the birth, the human intestine is colonized mainly by bifidobacteria which play a very important role in the maintenance of a good health[33]. So, in order to solve this health problem, food industry and in particular dairy technology has developed dairy functional products enriched with probiotics like lactobacilli (*Lactobacillus acidophilus*, *Lactobacillus casei*, etc.) and bifidobacteria[7].

Probiotics have been defined as selective viable microorganisms that, following consumption with food, have potential for improving the health and nutrition of the consumer[11]. Bifidobacteria and lactobacilli, which can be considered the most common probiotics[10,21], are associated with maintaining optimum microbial balance in the digestive tract with a number of well documented health benefits, including enhancement of the immune system[21], reduction of lactose intolerance[16] reduction of serum cholesterol level and possible anti carcinogenic properties[34]. Thus, these organisms have been extensively incorporated into dairy foods over the last decade and yogurts containing *Lactobacillus acidophilus* and/or *Bifidobacterium* species are widely marketed[11,42].

Kurman and Rasic[17] recommended that the minimum dose able to assure therapeutic effect should range between 8 and 9 log CFU/mL. Although this level is not well established, it should vary according to species and strains used.

Fermented milks containing probiotic microorganisms are generally considered as functional foods. Their consumption has the potential to improve lactose digestion[19] as well as to modulate immune function[8] and enteric flora[31]. This leads to the prevention of some gastro- intestinal disorders in humans such as antibiotic- associated diarrhoea[4], rotavirus gastroenteritis[15], traveller's diarrhoea[32] and radiation-induced diarrhoea[43]. In order to provide health benefits, fermented milks should contain a minimum level of living probiotic bacteria at the use- by date. Assuming a daily consumption of fermented milk equal to 100 g, a minimum level for probiotic bacteria of 10^6cfug^{-1}can be suggested in these products according to the daily efficient doses (10^8cfu) reported by[45]. This level is now recommended by Iran standard.

Development of dairy products with new products and flavors has potential health benefits thereby increasing sales and consumers satisfaction. Traditional preparation of yoghurt may be beneficial by including other ingredients such as soya protein, vegetables, sweet potato, pumpkin and plum[14] to enhance the flavor as well as the nutritional quality.

However, traditional medicinal plants such as lavender (*Lavandula officinalis L.*) has been proved to provide important therapeutic values. *L. officinalis* is a native of Southern Europe and the Mediterranean region and is commercially cultivated in France, Spain, Portugal, Hungary, the UK, Bulgaria, Australia, China and the USA. In Iranian flora, lavender is mainly distributed in the northern parts of the country[47]. (Figure 1)

Pharmaceutically, this plant and its preparations have long been used for carminative, antispasmodic,

Figure 1 : Lavandula officinalis.L flowers from Northwest Iran.

antidepressant, expectorant, anti-rheumatic, relaxant, sedative, anti-inflammatory and tonic properties. Moreover, its preparations are prescribed against flatulent dyspepsia, colic and depressive headache. It was also found to be active against some bacterial and fungal species. Traditionally, lavender has been used as an antiseptic agent in swabbing of wounds (wound healing), for burns and insect bites, and in veterinary medicine to kill lice and other animal parasites[9,20,46].

The pleasant aroma of this plant is mainly due to the occurrence of low molecular weight terpenoids synthesized and accumulated in aerial parts, especially in inflorescences. The distilled volatile oil of this plant has gained great importance in aromatherapy and in perfume, cosmetic and flavoring industries since antiquity. Furthermore, the volatile constituents of lavender are of special significance in pharmaceutical and food industries[6,9].

Although scientific evidence supports lavender consumption, many people are still unaware of the benefits provided by this product and its possible applications in the production of foodstuffs.

On the basis of this background, the present work aims at contributing to the knowledge of the: a) investigation the selected probiotic survival and chemical composition of lavender milk and yoghurt (pH, lactic acid and protein contents); c) kinetics of acidification of milk and yoghurt supplemented with 1%, 2% & 3% (w/w) of lavender during fermentation and refrigerated storage in order to produce a new functional food.

MATERIALS AND METHODS

Materials

Sample preparation

The aerial part of *L. officinalis*. Was obtained in Mars 2013 and was air-dried. The flowers of *L. officinalis* were dried under shade at room temperature for 2 months. Low-fat sterilized milk and yoghurt (1.5%) Were locally purchased (Kazerun, Iran). MRS Agar culture medium was used for carrying out the microbial test (Merck, Germany).

Probiotic cultures

Three commercial probiotic cultures (Cl, C2,C3) were used in this study. Cultures Cl contained *L. acidophilus* and *bifidobacteria* as constitutive microflora. In this commercial culture combination studied, the strains of probiotic organisms (*L. acidophilus* and *bifidobacteria*) were kept the same, Culture C3 and C4 contained a strain of *L.casei* and *L.paracasei* that produce polysaccharides during fermentation and was used for comparison. Lyophilized combination Probiotic strains, *Lactobacillus acidophilus* (La-5) and *Bifidobacterium bifidum* (Bb-12) were obtained from CHR Hansen Pty Ltd (CHR-Hansen, Denmark) and directly used as starter culture. *L. casei* and *L.paracasei* were obtained from Iranian Biological Resource Centre. (Tehran,Iran). The probiotic cultures were in freeze dried DVS (Direct Vat System) form. After procurement, the starter cultures were stored at -18°C in absence of atmospheric air.

Preparation of probiotic *Lactobacillus acidophilus* and *Bifidobacterium Bifidum* (C1) milk containing lavender (*Lavandula officinalis L.*) at first passage

In order to produce milk containing the probiotic bacterium C1, four containers each containing 1 liter of low-fat sterilized milk (1.5% fat) was considered as our four groups.

As recommended by the starter culture supplier, the rates of probiotic culture addition were 0.33gram. The starter *C1* was added directly to all the containers, followed by adding lavender powder of 0 (Control sample), 1%, 2%, and 3% (10, 20 and 30gram) to all the containers, respectively and finally they were placed in the incubator at 38°C. The acidity test was performed approximately every 2 hours until reaching 42° Dornic. The samples were then taken out of incubator and transferred to a refrigerator and stored at 2°C. The produced probiotic milk was evaluated once every 7 days by counting the microbes using direct counting method.

Preparation of probiotic *Lactobacillus acidophilus* & *Bifidobacterium Bifidum*(C1) yoghurt containing lavender (*Lavandula officinalis L.*) at the second passage

To produce probiotic yoghurt in this stage, after providing 4 containers, 1 liter of the low - fat sterilized probiotic milk (1.5 % fat) from the control group at first passage and the (1.5%) starter of low-fat yoghurt

(1.5%) were added to each container. Different concentrations of lavender powder 1%, 2%, and 3% (10, 20 and 30gram) were added respectively to the containers and mixed properly so that lavender (*Lavandula officinalis L.*) 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° Dornic. Then, the samples were taken out of the incubator and transferred to a refrigerator and stored at 2°C. The produced probiotic lavender 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 15 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. The results were analyzed in a statistical descriptive test by SPSS version 17software.

Preparation of probiotic *Lactobacillus Casei* milk containing lavender (*Lavandula officinalis L.*) at first passage

All the same procedures were followed as mentioned above with the difference of using *Lactobacillus Casei* instead of *Lactobacillus acidophilus and Bifidobacterium Bifidum.*

Preparation of probiotic *Lactobacillus paracasei* milk containing lavender (*Lavandula officinalis L.*) at first passage

All the same procedures were followed as mentioned above with the difference of using *Lactobacillus paracasei* instead of *Lactobacillus acidophilus and Bifidobacterium Bifidum.*

Having produced the above-mentioned products, we stored 1000 gram of each product in a disposable container placed 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.

Determination of titratable acidity (TA)

Titratable acidity (as % lactic acid) of milk and yoghurt was determined in triplicate according the AOAC titration method 947.05 using 0.1 M NaOH[3] and phenolphthalein as indicator

Determination of pH

All pH measurements were made during fermentation and storage using a Radiometer (model Labtron) pH meter with a combined glass electrode and temperature probe (Radiometer, Labtron, Denmark). The pH meter was calibrated using standard buffer solutions (Merck) at pH 4.0 and 7.0. All analyses were carried out in duplicate at 20 °C.

Chemical analysis

Protein content of heat-treated yoghurt mix was analysed by the Kjeldahl method using a Kjeltec digestion system and distillation unit (Tecator ab, Hoganas, Sweden). Total solids were determined by drying the samples at 110°C for 2 h.

Microbiological analyses

Probiotic cells were enumerated before fermentation, after fermentation, and each week during a 21days storage period at 2°C, to determine their growth during fermentation and their viability during storage. Appropriate dilutions of samples were prepared in 0.1% (w/v) Normal saline and subsequently plated in selective media. Populations of *Lactobacillus acidophilus*& *Bifidobacterium Bifidum*, *Lactobacillus casei* and *Lactobacillus paracasei* were enumerated on MRS agar(Merck, Germany) plates at pH 5.4 incubated at 42°C for 72 h in an anaerobic jar containing GasPak™.

Sensory evaluation

Sensory evaluation of plain and lavender milk and yoghurts (stored at 2°C) was conducted by 15 volunteer aged 19–27 years, one week after production. The tasting panel consisted of students from the Faculty of Veterinary of Kazerun, Iran. Each panelist received 4 samples of milk and yoghurt to taste, evaluate and comment on sensory characteristics at each serving. The panelists were asked to evaluate the color and appearance, aroma, body and texture, taste and overall acceptability, based on a 9 point hedonic scale; like extremely = 9, like very much = 8, like moderately = 7, like slightly = 6, neither like nor dislike = 5, dislike slightly = 4, dislike moderately = 3, dislike very much = 2, dislike extremely = 1.

Statistical analysis

All the above experiments were repeated three times with each test carried out in triplicate. 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

In the present study, the effects of lavender (*Lavandula officinalis L.*) on the growth and viability of the bacteria *Bifidobacterium bifidum* & *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus paracasei* in probiotic milk and yoghurt were investigated. The acidity, pH and survival of the bacteria in lavender probiotic milk and yoghurt were evaluated at 2 hours intervals till reaching 42°Dornic acidity degrees for milk and 90°Dornic degree for yoghurt in the incubator at 38°C. At the first hours of production, the *Lactobacillus acidophilus* & *Bifidobacterium bifidum* milk containing 3% and 2% lavender reached the acidity of 42°Dornic earliest, followed by 1%, and 0% milk. Once they reached this acidity level, they were transferred to a refrigerator at 2°C. The storage time in the refrigerator was determined to be 21 days.

In direct microbial counting in first inspection, the highest counts were sequentially in the samples with 1%, 2% & 3% and the controls, indicating the positive correlation between increased bacterial growth and increased lavender concentration.

Changes in Acidity level in Dornic degree during three-week storage of milk samples at 2°C are shown in Figure 2, 3.

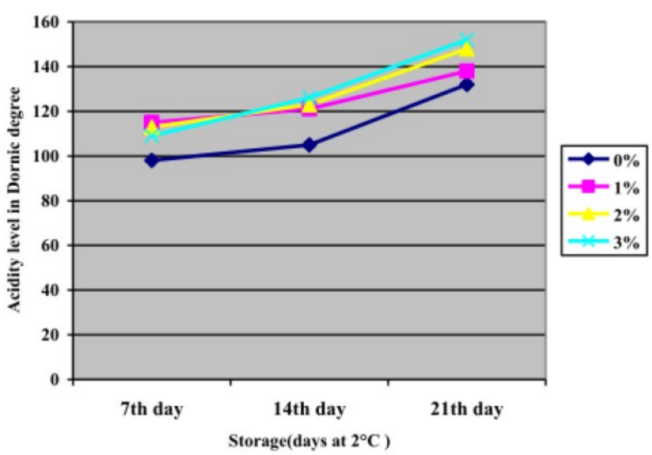

Figure 2 : Changes in acidity level based on Dornic degree in milk containing *Lactobacillus Casei* & *Lactobacillus paracasei* with lavender (Lavandula officinalis L.) within 21days storage in the refrigerator.

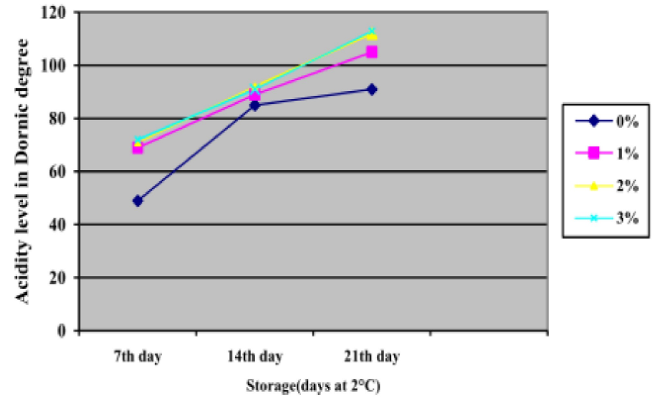

Figure 3 : Changes in acidity level based on Dornic degree in milk containing *Lactobacillus acidophilus* & *Bifidobacterium Bifidum* with lavender (Lavandula officinalis L.) within 21days storage in the refrigerator.

All the samples showed a decrease in pH (0.10-0.52). Some products like 2% and 3% samples in this study showed a smaller drop in pH than the other samples. The small drop in pH might have been due to the increased buffering action of milk proteins, as the protein content of these samples was higher than in the other samples(Figure 5). The chemical composition of treated samples showed that 3%lavender has higher protein content than the other investigated

The final pH of yoghurt can affect the growth and viability of *Lactobacillus acidophilus*& *Bifidobacterium Bifidum* most notably that of Bifidobacteria[12,18]. It has been found that acid production ability by lactic acid bacteria, especially post-incubation (post-acidification), affected cell viability of L. Acidophilus and *Bifidobacterium Bifidum*[13]. (Fig-

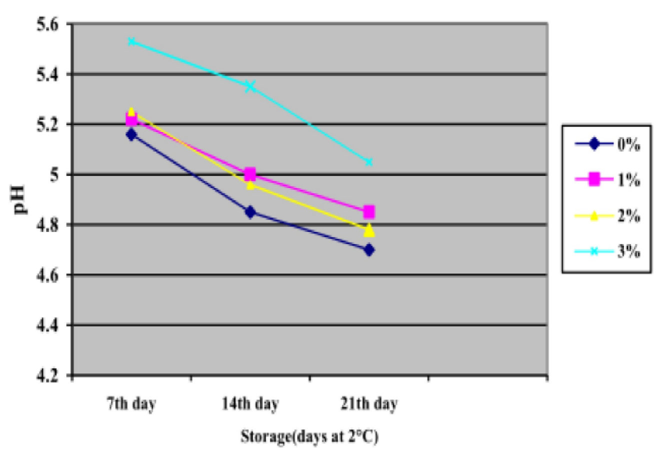

Figure 4 : Changes in pH of five yoghurt samples containing *Lactobacillus acidophilus*& *Bifidobacterium Bifidum* with lavender (*Lavandula officinalis L.*) within 21days storage in the refrigerator.

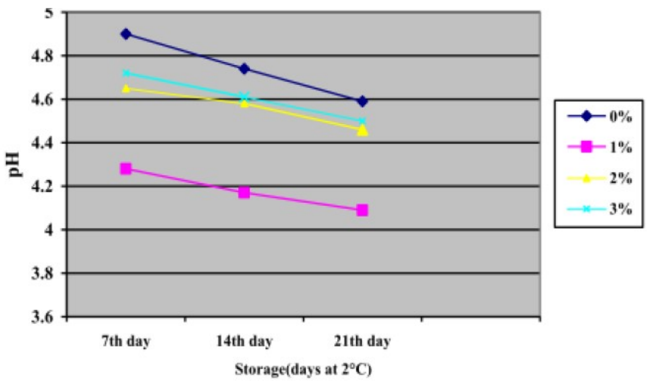

Figure 5 : Changes in pH of five milk samples containing *Lactobacillus acidophilus*& *Bifidobacterium Bifidum* with lavender (*Lavandula officinalis L.*) within 21days storage in the refrigerator.

ure 4, 7)

There is a major trend for consumers to purchase foods which provide excellent nutrition and health benefits, especially those that can prevent disease and/or maintain health. All using probiotic bacteria's in this study *Lactobacillus acidophilus* & *Bifidobacterium Bifidum*, *Lactobacillus Casei* and *Lactobacillus paracasei* have been proven to colonize the intestine and vagina following ingestion in skim milk, and to help prevent infections[5,35-39]. The presence of sufficient number of viable bacterial cells (10^9 cfu) appears to be necessary to provide therapeutic benefits[37]. Therefore, in order to call a product 'probiotic', such as with a new yogurt, the viability of probiotic bacteria must be maintained. Several studies have shown that when appropriate number of La-5 and Bb-12 has been consumed, these bacteria were capable of restoring a normal urogenital flora and reduce the risk of urinary tract infections, and also they were able to pass through the stomach and bile and colonize the intestine for several weeks[5,35-37].

Changes in the viable counts of *L. acidophilus* & *B.Bifidum* and *L. Casei* & *L.paracasei* in four samples of milk and yoghurt are given in Figure 6, 7. Products l-3 contained lo^7 to log 10^9 viable cells of *L. acidophilus* & *B.Bifidum* during storage time. In these products, the number of viable cells of *L. acidophifus* & *B.Bifidum* remained high until 21 days from the date of production and then declined. The control sample contained 7 log viable cells of L. Acidophilus when fresh and their number declined rapidly. It has been suggested that to have therapeutic effects, the minimum number of these organisms in a product should be $>10^5$ g^{-1} and that one should aim to consume100 million (10^8) live cells of *L. Acidophilus* and *B.Bifidum* per day.[1]. A 100 g serving of yoghurt containing lo^6 cells g^{-1} would supply this amount. Some products examined in this study would be suitable for this purpose. However, other samples had low levels of these organisms initially and contained a few at the end of storage, as the bacteria had died off in the product during the refrigerated storage, most likely due to post-incubation (post-acidification) by milk and yoghurt culture organisms. Several other factors may be responsible for the reduced viability of these organisms, such as hydrogen peroxide produced by yoghurt culture organisms, oxygen level in the product or oxygen permeation through the package.

Viable counts during storage showed that treated samples withstood storage better than control samples, irrespective of the time of inoculation or its level. (Figure 7, 8).

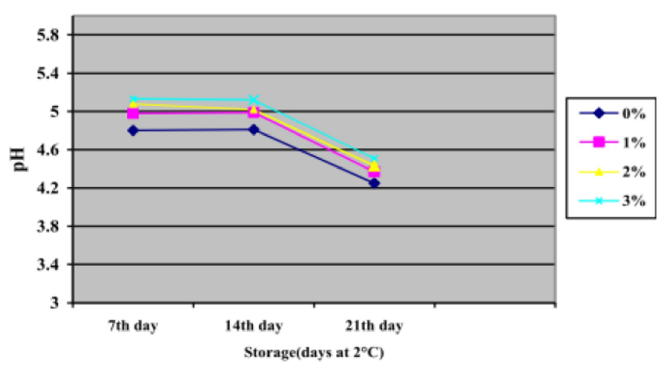

Figure 6 : Changes in pH of five milk samples containing *Lactobacillus Casei* & *Lactobacillus paracasei* with lavender (*Lavandula officinalis L.*) within 21days storage in the refrigerator.

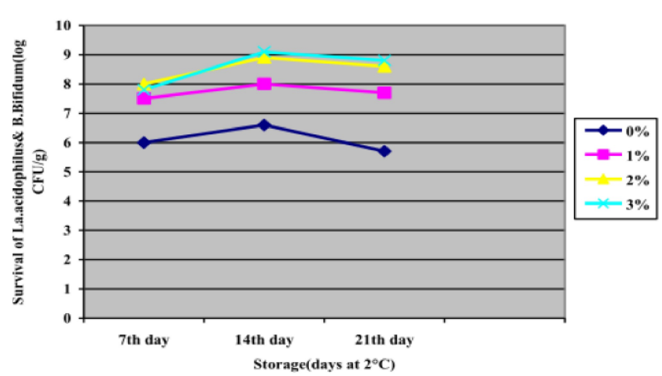

Figure 7 : Changes in viable count of *Lactobacillus acidophilus*& *Bifidobacterium Bifidum* in four milk samples over three weeks storage at 2°C.

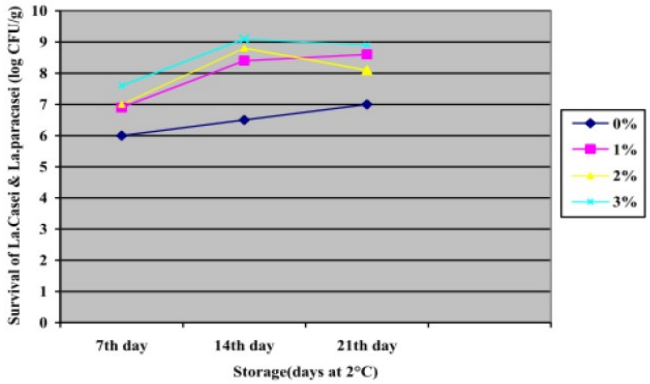

Figure 8 : Changes in viable count of *Lactobacillus Casei* & *Lactobacillus paracasei* in four milk samples over three weeks storage at 2°C.

After 21 days, the enumeration of *L. acidophilus* & *B. bifidum* was higher ($P < 0.05$) in milk and yoghurt. Between days 14 and 21, there were significant reductions ($P < 0.05$) in the *L. acidophilus* & *B. bifidum* counts in milks, with or without lavender (Figure 7).

The last enumeration at day 21 pointed to a lack of influence ($P > 0.05$) of lavender. This observation indicates a probable change of synergistic behavior previously observed at 14 days of storage.

In direct microbial counting in first day, the highest counts were sequentially in the samples with 3%, 2%, & 1% and the controls, indicating the positive correlation between increased bacterial growth and increased lavender concentration. Upon evaluation of the cultured samples on MRS agar media, the same correlation was revealed. The storage time in the refrigerator was found to be 21days.

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[30].

Descriptive sensory evaluation showed attributes identified in the color and appearance, aroma, body and texture, taste samples. Exception of 3% lavender milk treatment, all samples of milk and yoghurt were generally liked and consumer sensory evaluation indicated that there was no clear preference of one sample over the others, despite of differences observed through descriptive sensory analysis. Among three treatments of lavender and control sample, all of sensory parameters at level of pd" 0.05 no significant difference was seen. However 3% lavender milk was the most unfavorable because of the unpleasant appearance. General comments by the panelists regarding sensory attributes were also evaluated. The most common criticisms were related to the higher acidity and the semi-liquid texture of the products and non-typical milk and yoghurt taste. This treatment at level of pd" 0.05 had significant difference with others.

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[23].

Upon evaluation of the samples on MRS Agar, the *Lactobacillus acidophilus* & *Bifidobacterium bifidum* with lavender had the counts equal to logarithmic 10^9 in day 14, and the sample product with 3% lavender possessed the highest count of bacteria.

The milk containing *Lactobacillus Casei* & *Lactobacillus paracasei* with 3 and 2 % lavender reached 42°Dornic acidity earliest than others, followed by the milk with 1% and finally the control. Once reached 42°Dornic, the samples were transferred to a refrigerator at 2°C. The permanence of the product in the refrigerator was determined to be 21 days during which the acidity of control sample was lower than other samples.

The release of lactic acid is an indication of the activity of the probiotic bacteria[44] and the pH of all the cultures was monitored to provide an indication of The milk and yoghurt fermented with *Lactobacillus acidophilus* & *Bifidobacterium bifidum* and *Lactobacillus Casei* & *Lactobacillus paracasei* in the presence of lavender had a significant lower pH than the control, suggesting an appreciable amount of lactic acid had been produced. These results of lower pH values in the treated milk and yoghurt with lavender could be attributed to the phenolic compounds, which are known to serve as an oxygen scavenger and to reduce the redox potential of the growth media, as probiotic bacteria grow better in the absence of oxygen.

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[23,24].

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[28].

In another study addressing the effect of cinnamon on the bacterial growth, it was demonstrated that the increased cinnamon concentration promoted the growth of the bacteria in probiotic milk and yoghurt[48].

In another study addressing that investigated the effect of spearmint on the bacterial growth, it was demonstrated that increased spearmint concentration promoted the growth of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in probiotic milk and yoghurt[24].

In another study addressing the effect of juice on the bacterial growth, it was demonstrated that the increased juice product promoted the growth of the bacteria in probiotic orange and apple[22].

In a study that investigated the effect of garlic on bacterial growth and survival, it was observed that increased garlic concentration promoted the growth and viability of probiotic bacteria in milk and yoghurt during refrigerated storage[22].

In another investigation addressing the effect of dill extract on growth and survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, it was represented that dill extract has positive effect on growth and viability of probiotic bacteria in milk and yoghurt during permanence period and finally leaded to produce new fermented dairy product[22].

In experiment that researchers investigated the effect of permeate on the growth and survival of the above mentioned bacteria (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) was indicated that the permeate was suitable support for intestinal bacteria that had kept viable up during 21 days of refrigerated storage and final evaluation of products showed that permeate can be successfully used in the preparation of nutritive probiotic beverages[22].

CONCLUSION AND SUGGESTION

This study is the first to report the successful application of the newly identified potential lavender (*Lavandula officinalis L.*) in probiotic milk and yoghurt. The results indicated that the viability of this probiotic strain can be improved in milk and yoghurt by addition of lavender to the product. All tested strains were proved a good growth capacity in lavender milk and yoghurt without nutrients added, this being a guarantee on the one hand for the normal evolution of the fermentation and on the other hand for the stability of the final product. Thus it seems that the nutrients are available in acceptable forms and in optimal concentrations in the tested lavender milk and yoghurt. Addition of lavender resulted in a significant decrease in pH and increase acidity, syneresis, viscosity and water holding capacity of milk and yoghurt base. Addition of lavender also appeared to improve the sensory characteristics of probiotic milk and yoghurt. The results showed that fortification of the milk base with lavender affected the acidification profile, counts of viable bacteria and rheological properties of milk and yoghurt. These observations point to the applicability of lavender in the development of new high nutritional value-added probiotic milk and yoghurt. Finally, fortification of milk and yoghurt with lavender powder simultaneously enhanced the rheological properties of probiotic milk and yoghurt taking into account the kinetics of acidification and enumeration of viable bacteria; however, sensory profiling studies in the future are required to confirm these results including industrial trials.

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