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Effects of Chamomile Essence on the Growth of Probiotic Bacteria, *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in Milk and Yoghurt

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Abstract: Using combinations of natural antimicrobials including medicinal plants to conserve foodstuffs has considerably grown. Not only do such combinations act as antimicrobials, but also they have medicinal and antioxidant activities and can change the tastes. On the other hand, probiotic products as purposeful food have an important role in the production of healthful foods. This study seeks to evaluate the effects of chamomile plant essence on the probiotic bacteria, *Bifidobacterium bifidum* and *Lactobacillus acidophilus* acting as starter bacteria in probiotic yoghurt and fermented milk. At first stage, probiotic (milk) and at second stage (yoghurt) were produced to determine the effects of chamomile different doses (%0, 0. 2%, 0.4%, 0. 6%) on the growth of the two probiotic bacteria, i.e., *Bifidobacterium bifidum* and *Lactobacillus acidophilus*. The products were then examined in terms of pH, acidity and microbe counting in the shelf life period and permanence. In day ten, the quantities of the products were evaluated by a sensory method. The results of the questionnaire in statistical-descriptive test were analyzed using SPSS software version 16 system. Findings show that the samples with % 0.6 concentration chamomile were ranked the best for taste, color, insolubility. Although low fat milk was used to produce yoghurt, it was tasted full fat. Existence of probiotic bacteria were evaluated by direct count and cultivation on MRS environment. During the 20 day period of observation, the microbial counts were persistent and there was a significant difference between the controls. It was also revealed that the 0.6% concentration of chamomile increased the thickness of yoghurt, enhanced the fermentation process and the growth of bacteria, as observed in all the samples except the milk with *Bifidobacterium bifidum*. Also, when the combination of the two bacteria was used, the bacterial growth and number of colonies resulted from their cultivation were increased. The produced colonies were round and flat in the milk and broad and hurtful in the yoghurt. Addition of sugar enhanced the bacterial growth, like increased essence did.

Key words: Probiotic · *Bifidobacterium bifidum* · *Lactobacillus acidophilus* · Chamomile · MRS Cultivation Environment

INTRODUCTION

According to definition of FAO, probiotic microbes are living organisms that are used by eating and condition application in repellent number and caused creation of one or many healthy effects on host body. These include characteristic anticancer effects [1-4], regulating and enhancing safety system, anti microbial effect and decrease of serum cholesterol [5-8], increase of nutritional value [3] and improvement of lactose intolerance [7, 8].

Bifidus are the most common species in use among microbes of probiotic *Lactobacillus acidophilus* and *Bifidobacterium bifidum* [9-11].

Recently design and production of a probiotic with basic plant (protein, fiber, vitamin, solute) are considered for both rule of health nature and variation creation in product and consume. It seems that these probiotic products are of relevant quality property and will take considerable part of studied searches in the future.

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There is a board square of searches fields for researches to update subject of probiotic and related respects to them and their complexity and extent. The objective of this study was to create a technical method to produce and preserve a kind of fermented probiotic dairy with acceptable aroma, while containing reasonable counts of probiotic bacteria.

MATERIALS AND METHODS

Materials:

- Bacteria (lyophilized) including *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (CHR Hansen Company, Denmark).
- Low-fat sterilized milk (1.5% fat) and.
- Low-fat yoghurt from supermarket.
- Chamomile essence

The Effect of Chamomile on the Production of Probiotic *Bifidobacterium bifidum* Milk at First Passage: In order to produce milk containing the probiotic bacterium *Bifidobacterium bifidum*, four containers; each containing 1 liter of low-fat sterilized milk (1.5% fat) were considered as four groups. The starter (*Bifidobacterium bifidum*) was added directly to all the containers, followed by adding dried chamomile essence (0% to the control), 0.2, 0.4 and 0.6% to the other three containers, respectively and finally they were placed in hot -house at 38°C. The acidity test was performed approximately every 2 hours until reaching 42^0 Dornic [12]. The samples were then taken out of hot -house, transferred to a refrigerator and stored at 2°C [13-16]. The produced probiotic milk was evaluated once every 5 days by counting the microbes using direct counting method.

The Effect of Chamomile on the Production of Probiotic *Bifidobacterium bifidum* Yoghurt at the Second Passage: To produce *Bifidobacterium bifidum* yoghurt in this stage, after providing 4 containers, 1 liter of 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 chamomile (0, 0.2, 0.4 and 0.6%) were added respectively to the containers and mixed properly so that chamomile was uniformly dissolved. Afterwards, all the containers were placed in the hot - house at 38°C. Approximately every 2 hours, the acidity and pH tests were measured until acidity reached 90^0 Dornic. Then, the samples were taken out of the hot-house, transferred to a refrigerator and stored at 2°C. The produced probiotic chamomile yoghurt was evaluated every 5 days by counting the microbes using direct counting method and after 10 days the yoghurt was evaluated for sensory properties [17], using questionnaires filled by 40 people. 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 16 software.

The Effect of Chamomile on the Production of Probiotic *Lactobacillus acidophilus* Milk at First Passage: The procedure was done as mentioned above.

The Effect of Chamomile on the Production of Probiotic *Lactobacillus acidophilus* Yoghurt at Second Passage: The procedure was done as mentioned above.

The Effect of Chamomile on the Production of Probiotic *Lactobacillus acidophilus* and *Bifobacterium bifidum* Milk at First Passage: As previously described, four containers containing 1 liter of sterilized low-fat milk (1.5% fats) were considered as our four groups. The Lyophilized bacteria*Bifidobacterium bifidum* and *Lactobacillus acidophilus* were simultaneously added to all the containers. The first container was considered as the control group and dried chamomile essence 0.2, 0.4 and 0.6% were added respectively to the other containers. The procedures were followed as we did in the first stage.

The Effect of Chamomile on the Production of Probiotic *Lactobacillus acidophilus* and *Bifidobacterium bifidum* Yoghurt at Second Passage: Four containers, each containing 1 liter of sterilized low-fat milk were considered as four groups. Then, the starter of yoghurt and the probiotic milk from the control group in the previous stage were added to all the containers. Except for the first container which was considered as the control, chamomile 0.2, 0.4 and 0.6% were added to the other containers. Procedures of yoghurt production were the same as those for the yoghurt with the above - mentioned bacteria.

Determining the Product Shelf Life Duration: Having produced the above-mentioned products, we stored 1000 gm of each product in a disposable container placed in a refrigerator for 20 days. During this period, each sample was tested in days 1, 5, 10, 15 and 20 for acidity, pH and sensory properties.

RESULTS

Table 1 shows the acidity in Dornic degree in *Lactobacillus acidophilus* Chamomile yoghurt and milk at refrigerator during 20 days. Table 2 shows microbial growth by direct count of *Lactobacillus acidophilus* Chamomile milk and yoghurt at refrigerator during 20 days. Table 3 shows the growth on MRS-A cultivation environment of *Lactobacillus acidophilus* Chamomile milk and yogurt at refrigerator during 20 day insolubility.

Table 4 shows the acidity in Dornic degree in *Bifidubacterium bofidum* Chamomile yoghurt and milk at refrigerator during 20 days. Table 5 shows microbial growth by direct count of *Bifidobacterium bifidum* Chamomile milk and yoghurt at refrigerator during 20 days. Table 6 shows the growth on MRS-A cultivation environment of *Bifidobacterium bifidum* at refrigerator during 20 days.

Table 7 shows the acidity in Dornic degree in *Lactobacillus acidophilus* and *Bifidobacterum bifidum* Chamomile yoghurt and milk at refrigerator during 20 days.

Table 8 shows the microbial growth by direct count of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* Chamomile yoghurt and milk at refrigerator during 20 days.

Table 1: The acidity level based on Dornic degree in the chamomile *Lactobacillus acidophilus* milk and yoghurt within 20-day storage in the refrigerator

	Acidity level in Dornic degree										
Chamomile milk	1 day	5 day	10 day	15 day	20 day	Chamomile yoghurt	1 day	5 day	10 day	15 day	20 day
0%	50^{0D}	53^{0D}	50^{0D}	47^{0D}	49^{0D}	0%	98^{0D}	119^{0D}	99^{0D}	112^{0D}	100^{0D}
0.2%	43^{0D}	46^{0D}	42^{0D}	40^{0D}	45^{0D}	0.2%	113^{0D}	120^{0D}	95^{0D}	95^{0D}	97^{0D}
0.4%	50^{0D}	59^{0D}	52^{0D}	47^{0D}	51^{0D}	0.4%	97^{0D}	111^{0D}	93^{0D}	100^{0D}	113^{0D}
0.6%	53^{0D}	56^{0D}	53^{0D}	43^{0D}	52^{0D}	0.6%	105^{0D}	117^{0D}	102^{0D}	114^{0D}	116^{0D}

Table 2: Growth of microbes in the chamomile *Lactobacillus acidophilus* milk and yoghurt

Chamomile milk	1 day	5 day	10 day	15 day	20 day	Chamomile yoghurt	1 day	5 day	10 day	15 day	20 day
0%	$10^{10}\times14$	$10^{10}\times23$	$10^{10}\times34$	$10^{10}\times44$	$10^{10}\times74$	0%	$10^{10}\times19$	$10^{10}\times10$	$10^{10}\times26$	$10^{10}\times21$	$10^{10}\times30$
0.2%	$10^{10}\times9$	$10^{10}\times18$	$10^{10}\times51$	$10^{10}\times53$	$10^{10}\times18$	0.2%	$10^{10}\times35$	$10^{10}\times12$	$10^{10}\times18$	$10^{10}\times11$	$10^{10}\times28$
0.4%	$10^{10}\times10$	$10^{10}\times14$	$10^{10}\times51$	$10^{10}\times56$	$10^{10}\times66$	0.4%	$10^{10}\times21$	$10^{10}\times19$	$10^{10}\times26$	$10^{10}\times19$	$10^{10}\times41$
0.6%	$10^{10}\times12$	$10^{10}\times26$	$10^{10}\times57$	$10^{10}\times82$	$10^{10}\times46$	0.6%	$10^{10}\times35$	$10^{10}\times18$	$10^{10}\times40$	$10^{10}\times17$	$10^{10}\times44$

Table 3: Growth of microbes on MRS-A cultivation environment in *Lactobacillus acidophilus* Chamomile milk and yoghurt at refrigerator during 20 days insolubility

Chamomile milk	1 day	5 day	10 day	15 day	20 day	Chamomile yoghurt	1 day	5 day	10 day	15 day	20 day
0%	$10^{7}\times40$	$10^{7}\times43$	$10^{7}\times44$	$10^{7}\times34$	$10^{7}\times10$	0%	$10^{7}\times16$	$10^{7}\times160$	$10^{7}\times20$	$10^{7}\times24$	ÜÜ
0.2%	$10^{7}\times47$	$10^{7}\times40$	$10^{7}\times88$	$10^{7}\times55$	$10^{7}\times80$	0.2%	$10^{7}\times26$	$10^{7}\times16$	$10^{7}\times20$	$10^{7}\times24$	$10^{7}\times3$
0.4%	$10^{7}\times60$	$10^{7}\times92$	$10^{7}\times30$	$10^{7}\times30$	$10^{7}\times54$	0.4%	$10^{7}\times17$	$10^{7}\times26$	$10^{7}\times72$	$10^{7}\times20$	$10^{7}\times2$
0.6%	$10^{7}\times12$	$10^{7}\times80$	$10^{7}\times30$	$10^{7}\times55$	$10^{7}\times38$	0.6%	$10^{7}\times39$	$10^{7}\times36$	$10^{7}\times72$	$10^{7}\times10$	$10^{7}\times20$

Table 4: The acidity level based on Dornic degree in the chamomile *Bifidobacterium bifidum* milk and yoghurt within 20-day storage in the refrigerator

	Acidity level in Dornic degree										
Chamomile milk	1 day	5 day	10 day	15 day	20 day	Chamomile yoghurt	1 day	5 day	10 day	15 day	20 day
0%	52^{0D}	48^{0D}	43^{0D}	45^{0D}	52^{0D}	0%	102^{0D}	111^{0D}	95^{0D}	90^{0D}	94^{0D}
0.2%	60^{0D}	56^{0D}	53^{0D}	51^{0D}	62^{0D}	0.2%	90^{0D}	93^{0D}	85^{0D}	87^{0D}	98^{0D}
0.4%	57^{0D}	52^{0D}	50^{0D}	53^{0D}	64^{0D}	0.4%	112^{0D}	117^{0D}	98^{0D}	99^{0D}	116^{0D}
0.6%	64^{0D}	58^{0D}	51^{0D}	53^{0D}	66^{0D}	0.6%	114^{0D}	112^{0D}	99^{0D}	98^{0D}	108^{0D}

Table 5: Growth of microbes in the chamomile *Bifidobacterium bifidum* milk and yoghurt

Chamomile milk	1 day	5 day	10 day	15 day	20 day	Chamomile yoghurt	1 day	5 day	10 day	15 day	20 day
0%	$10^{10}\times34$	$10^{10}\times9$	$10^{10}\times14$	$10^{10}\times31$	$10^{10}\times34$	0%	$10^{10}\times37$	$10^{10}\times12$	$10^{10}\times24$	$10^{10}\times14$	$10^{10}\times73$
0.2%	$10^{10}\times21$	$10^{10}\times18$	$10^{10}\times42$	$10^{10}\times26$	$10^{10}\times39$	0.2%	$10^{10}\times34$	$10^{10}\times14$	$10^{10}\times19$	$10^{10}\times18$	$10^{10}\times36$
0.4%	$10^{10}\times24$	$10^{10}\times9$	$10^{10}\times22$	$10^{10}\times45$	$10^{10}\times22$	0.4%	$10^{10}\times46$	$10^{10}\times17$	$10^{10}\times19$	$10^{10}\times16$	$10^{10}\times22$
0.6%	$10^{10}\times41$	$10^{10}\times22$	$10^{10}\times26$	$10^{10}\times29$	$10^{10}\times46$	0.6%	$10^{10}\times44$	$10^{10}\times20$	$10^{10}\times27$	$10^{10}\times31$	$10^{10}\times28$

Table 6: Growth of microbes on MRS-A cultivation environment in *Bifidobacterium bifidum* at refrigerator during 20 days

Chamomile milk	1 day	5 day	10 day	15 day	20 day	Chamomile yoghurt	1 day	5 day	10 day	15 day	20 day
0%	$10^7\times19$	$10^7\times18$	$10^7\times25$	$10^7\times44$	$10^7\times74$	0%	$10^7\times23$	$10^7\times66$	$10^7\times40$	$10^7\times60$	$10^7\times21$
0.2%	-	-	$10^7\times38$	$10^7\times29$	$10^7\times34$	0.2%	$10^7\times25$	$10^7\times66$	$10^7\times10$	$10^7\times66$	$10^7\times46$
0.4%	-	-	$10^7\times60$	$10^7\times2$	$10^7\times30$	0.4%	$10^7\times88$	$10^7\times60$	$10^7\times66$	$10^7\times16$	$10^7\times13$
0.6%	-	-	$10^7\times8$	$10^7\times1$	$10^7\times13$	0.6%	$10^7\times76$	$10^7\times36$	$10^7\times45$	$10^7\times20$	$10^7\times31$

Table7: The acidity level based on Dornic degree in the chamomile *lactobacillus acidophilus* and *Bifidobacterium bifidum* milk and yoghurt within 20-days storage in the refrigerator

					Acidity level in Dornic degree					
Chamomile milk	1 day	5 day	10 day	15 day	20 day	Chamomile yoghurt	1 day	5 day	10 day	15 day	20 day
0%	52^{0D}	48^{0D}	43^{0D}	45^{0D}	52^{0D}	0%	100^{0D}	100^{0D}	102^{0D}	98^{0D}	112^{0D}
0.2%	60^{0D}	56^{0D}	53^{0D}	51^{0D}	62^{0D}	0.2%	120^{0D}	95^{0D}	102^{0D}	113^{0D}	114^{0D}
0.4%	57^{0D}	52^{0D}	50^{0D}	53^{0D}	64^{0D}	0.4%	112^{0D}	98^{0D}	100^{0D}	95^{0D}	98^{0D}
0.6%	64^{0D}	58^{0D}	51^{0D}	53^{0D}	66^{0D}	0.6%	111^{0D}	95^{0D}	98^{0D}	98^{0D}	104^{0D}

Table 8: Growth of microbes in the chamomile *Lactobacillus acidophilus* and *Bifidobacterium bifidum* milk and yoghurt

Chamomile milk	1 day	5 day	10 day	15 day	20 day	Chamomile yoghurt	1 day	5 day	10 day	15 day	20 day
0%	$10^{10}\times33$	$10^{10}\times18$	$10^{10}\times25$	$10^{10}\times32$	$10^{10}\times44$	0%	$10^{10}\times33$	$10^{10}\times34$	$10^{10}\times14$	$10^{10}\times26$	$10^{10}\times23$
0.2%	$10^{10}\times59$	$10^{10}\times11$	$10^{10}\times19$	$10^{10}\times37$	$10^{10}\times46$	0.2%	$10^{10}\times30$	$10^{10}\times30$	$10^{10}\times18$	$10^{10}\times28$	$10^{10}\times21$
0.4%	$10^{10}\times47$	$10^{10}\times20$	$10^{10}\times12$	$10^{10}\times36$	$10^{10}\times52$	0.4%	$10^{10}\times21$	$10^{10}\times34$	$10^{10}\times12$	$10^{10}\times55$	$10^{10}\times16$
0.6%	$10^{10}\times45$	$10^{10}\times4$	$10^{10}\times19$	$10^{10}\times36$	$10^{10}\times36$	0.6%	$10^{10}\times33$	$10^{10}\times29$	$10^{10}\times23$	$10^{10}\times10$	$10^{10}\times11$

Table 9: Growth of microbes on MRS-A cultivation environment in *Bifidobacterium bifidum* and *Lactobacillus acidophilus* Chamomile yogurt and milk at refrigerator during 20 days

Chamomile milk	1 day	5 day	10 day	15 day	20 day	Chamomile yoghurt	1 day	5 day	10 day	15 day	20 day
0%	$10^7\times70$	$10^7\times62$	$10^7\times30$	$10^7\times31$	$10^7\times17$	0%	$10^7\times60$	$10^7\times27$	$10^7\times13$	$10^7\times24$	$10^7\times33$
0.2%	$10^7\times56$	$10^7\times35$	$10^7\times14$	$10^7\times35$	$10^7\times79$	0.2%	$10^7\times20$	$10^7\times32$	$10^7\times14$	$10^7\times64$	$10^7\times25$
0.4%	$10^7\times36$	$10^7\times90$	$10^7\times60$	$10^7\times52$	$10^7\times35$	0.4%	$10^7\times87$	$10^7\times50$	$10^7\times15$	$10^7\times30$	$10^7\times20$
0.6%	$10^7\times60$	$10^7\times21$	$10^7\times56$	$10^7\times46$	$10^7\times31$	0.6%	$10^7\times14$	$10^7\times23$	$10^7\times52$	$10^7\times52$	$10^7\times32$

Table 9 shows the growth on MRS-A cultivation environment of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* Chamomile yoghurt and milk at refrigerator during 20 days.

DISCUSSION

Different products containing probiotic microorganisms have been produced, which can transfer the respective medicinal effects to the consumers. Of such products, milk and yoghurt play an important role [18]. Milk and its fermented products with their nutritional and medicinal properties, as the carrier of the probiotic bacteria, have been a major research topic in recent decades. In addition to being an appropriate environment for the growth and survival of bacteria, milk can serve the consumers as a rich product with nutritional values.

Following some initial experimentations, we came to the conclusion that the use of high concentration (0.6%) of chamomile extract and essence could enhance the growth of the bacterium *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. Similarly, the addition of sugar to the products promoted the bacterial growth. As revealed, when both the essence and the extract were introduced simultaneously to the products, the result was much more remarkable.

More specifically, the acidity levels in the control sample and those with o.2, o.4 and o.6% chamomile were decreasing from day1 to day 10.

The decrease was significant from day 10 to day 15, however, the acidity level increased from day 15 till day 20.

The acidity degree reached 42^0 Dornic earliest in the yoghurt sample 0.2% with both bacteria, followed by the yoghurt sample (0.6%) and the control sample and finally the sample with 0.4% chamomile concentration. As revealed, the yoghurt samples with *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were with decreasing acidity level in day 5 till day 20.

The enhanced bacterial growth and consequently shorter production time were observed in the milk and yoghurt containing both bacteria, compared with the samples with either of the bacteria. In addition, the colony counts increased in the cultures of the samples with both bacteria, indicating the co-existence of the two bacteria.

As for the milk with *Bifidobacterium bifidum*, the control sample reached the acidity of 42^0 Dornic degree earliest, followed by the samples with 0.2%, 0.4% and finally 0.6% chamomile concentration. Therefore, it can be suggested that in the above- mentioned milk, the increased essence leads to decreased bacterial growth. On the contrary, the increased essence resulted in the enhanced bacterial in the milk samples with *Lactobacillus acidophilus* and the sample with both bacteria.

Regarding the acidity level in the *Lactobacillus acidophilus* milk, we observed increasing trend from day 1 to 5, decreasing from day 5 to 10 and increasing from day 10 t0 day 20.

As for the yoghurt with *Lactobacillus acidophilus*, the sample with 0.6% concentration reached the favorite acidity degree 42^0 Dornic earliest, followed by the samples with 0.4%, 0.2% and finally the control. This shows the role of increasing concentration in the enhancement of bacterial growth. The respective acidity levels were increasing for the first five Days, decreasing for the next five days and increasing for the remaining 10 days.

It is worth mentoring that the acidity degrees in all the samples were constant for the first two hours of incubation, indicating lack of acidity within this two hour interval.

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 [18].

The statistical analysis of the date obtained from the sensory properties based questionnaires, using SPSS showed significant differences between the products in terms of color, taste and thickness ($P<0/05$). The samples with 0.6% concentration possessed highest thickness. The *Bifidobacterium bifidum* yoghurt tasted less sour than the yoghurt with *Lactobacillus acidophilus*.

Taking in to account the present findings and other reported ones, it can be suggested that the high acidity and pH may damage the survival of the probiotics in the fermented products.

The survival is at least 10 times growth than that in rate of the bacteria in non-sour milk and the fermented counterparts. *Bifidobacerium bifidum* enjoys a larger survival in the ice cream, compared with that in the yoghurt.

One way to prevent the decrease of probiotics, caused by increase in acidity and decrease in pH, is to terminate the fermentation stage in the products during incubation when the pH is about 4/5 [18].

The pH of the products with probiotic milk containing either of the bacteria during the incubation and shelf life was not lower than 4.5 and only in the product with combined bacteria it reached 4.26. As for the probiotic yoghurt, the respective pH reached 4/30 during production and it came to 3/79 during shelf life in the yoghurt with *Bifidobacterium bifidum*, 3.60 in the Lactobacillus acidophilus product and 4 in the combined product. The difference is due to higher acidity of the yoghurt.

The incubation time for the Lactobacillus acidophilus milk to reach the favorable acidity was shorter than that for *Bifidobacterium bifidum* milk and it was the shortest for the milk containing both the bacteria. The reason might be lower proteocafti activity and insufficient organic nitrogen in the milk which make it unsuitable for the growth of *Bifidobacterium bifidum* species.

In the samples containing either Lactobacillus acidophilus or their combination, the increased concentration of chamomile resulted in the favorable taste of yoghurt, but did not change the thickness and the yoghurt with 0.6% chamomile possessed greater thickness than others. Regarding the color, aroma and smell, the same sample of yoghurt was the best among the other samples.

The shelflife for the probiotic yoghurts was found to be 20 days and even after this period the products were still intact and the shelflife was expected to be longer.

Bifidobacterium bifidum, as a single species in the milk, is of very low growth due to insufficient proteocafti activity. Coexistence with Lactobacillus acidophilus can give rise to its enhanced growth. As revealed, the increased chamomile concentration did not lead to the growth of the colonies, but it was not the case with the controls and growth was detected. As for the yoghurt, the result was remarkably different. The *Bifidobacterium bifidum* growth was observed in the respective yoghurt when chamomile concentration increased, but on the contrary, the growth was inhibited once the concentration increased in the milk. The produced colonies were round and flat in the milk and broad and hurtful in the yoghurt.

The increased chamomile concentration in *Lactobacillus acidoph*ilus milk and yoghurt and *Bifidobacterium bifidum* yoghurt led to the bacterial growth and faster favourable acidity, but it was the reverse for the *Bifidobacterium bifidum* milk and increased 0.6% chamomile concentration slowed the bacterial growth. No regular pattern was observed for the combined bacteria and the result for the respective milk was sequentially 0.4%, 0.6%, 0.2% and finally the controls. The sequence for the yoghurt was 0.2%, 0.6%, control and 0.4%. The increased chamomile concentration led to increased thickness of the yoghurt and the products with bacteria in the three samples appeared full fat although they were made with low fat milk. The yoghurt with Lactobacillus acidophilus tasted more sour than other products.

Compared with the reported studies on thyme and malt, chamomile and malt extract can cause the increased concentration and acidity while thyme has no effect on the thickness of the yoghurt. As for malt extract, the increased malt concentration enhanced acidity and shorter shelf life, the same as chamomile. For instance, the Lactobacillus acidophilus yoghurt needed shorter time to reach 90 degree Dornic acidity and shorter storage in the refrigerator, when 0.6% chamomile was added. In day 20, the acidity was 116 for the yoghurt with 0.6% chamomile and 100 for the control. The same result appeared for the malt extract, i.e., increased malt extract like increased chamomile resulted in thicker yoghurt. The yoghurt with 0% chamomile contained more water than the one with 0.6% chamomile and the *Lactobacillus acidophilus* yoghurt with chamomile tasted more sour than *Bifidobacterium bifidum* counterpart [14, 16].

As shown, the probiotic milk with honey behaved differently than the milk with chamomile, i.e, the acidity in the former was hampered while in the latter enhanced. The honey probiotic milk tasted full fat while it was made with low fat milk, the same as we experienced with chamomile and malt yoghurts. The *Lactobacillus acidophilus* yoghurt was sourer than the *Bifidobacterium bifidum* one, indicating the effects of probiotics on the tastes of such products [15].

Growth of mold was observed in the honey milks after 15 days, but it was not the case with products with chamomile because they were in sterilized condition and no spoiling agents, so that they remained intact within the 20 day period.

As for the products with soya, the increased concentration led to enhanced acidity, as we observed in chamomile products [18].

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