The Effect of Tarragon on the Growth of *Lactobacillus casei* and *Lactobacillus paracasei* in Probiotic Milk

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Abstract

Probiotics are live microbial food supplements which benefit the health of consumer by maintaining or improving their intestinal microbial balance. This study aimed at investigating the effect of different doses of tarragon on the growth of *Lactobacillus casei* and *Lactobacillus paracasei* in probiotic milk produced at one step. The product was then examined in terms of pH, acidity and microbe counting during incubator setting period. In both *Lactobacillus casei* and *Lactobacillus paracasei* milk samples, the sample containing 3% tarragon reached acidity level more quickly. In the milk samples with either *Lactobacillus casei* or *Lactobacillus paracasei*; it was observed that during refrigeration, the control sample had the most duration. The bioability of probiotic bacteria was measured by direct counting method. Duration of the product permanence was determined within 21 days. Upon examination of the results, it was revealed that the increased concentration of tarragon had a positive effect on the growth of the probiotic bacteria, *Lactobacillus casei* and *Lactobacillus paracasei* in probiotic milk.

Key words: *Lactobacillus casei, Lactobacillus paracasei, Milk, Probiotic, Tarragon*

Introduction

Probiotics are "Living micro-organisms, which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition" (Guarner and Schaafsma, 1998). Foods containing such bacteria fall within the "functional Foods" category and these are described as "foods claimed to have a positive effect on health" (Lee and Salminen, 1995). Functional foods should contain at least 10^7 cfu g^{-1} probiotic bacteria and should be consumed at levels higher than 100 g day^{-1} to have positive effects on health (Heller, 2001; Ishibashi and Shimamura, 1993). Due to their health attributes, probiotic-containing products have become primary choices for the consumer. Therefore, the market for such products has rapidly grown (Heller, 2001; Lourens-Hattingh and Viljoen, 2001; Ross et al, 2002). Many different strains and species of lactobacilli and bifidobacteria have been used commercially as probiotcs. It is reported that the probiotic bacteria have health-promoting effects and antagonistic activity against food-borne disease agents (Gilliand and Speck, 1997).

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 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 (Guarner and Schaafsma, 1998; Jahanara and Haerizade, 2001).

Though the synthetic chemical drugs that make use of separation mechanisms have been much in demand in the past decades, their corresponding side effects have been gradually observed so much so that their irregular and improper consumption has turned out to be a critical issue. On the other hand, the benefits of medicinal plants and their little or zero side effects have made them a proper substitute, and highly appreciated by physicians and patients. Iran possesses a rich source of such plants and herbs in terms of variety and amount. The essence plants play a critical role in human life, and have been used for long by Iranians (Jahanara and Haerizade, 2001).

Tarragon is widely used around the world as a spice in foods. It is a perennial member of the Asteraceae family, related to the herb wormwood. The herb is widely used as food seasoning and as a primary flavor for some brands of carbonated beverages. The plant contains compounds that help alleviate pain associated with dental conditions, promote bile production and detoxification by the liver, thus aiding digestion, and may act as a mild sedative as well (Nesslany et al, 2010).

Even though the effects of several herbs have been investigated on dairy probiotic products, there still has been a renewed interest in tarragon. Several scientific studies investigated the isolation and identification of active constituents of tarragon, scientific verification of its constituents, and verification of the basis of the use of tarragon in some of several diseases and conditions (Kalantari, 2013).

The present study, therefore, was an attempt to evaluate the effect of tarragon on the growth of *Lactobacillus casei* and *Lactobacillus paracasei* in probiotic milk in order to develop a new probiotic product with reinforcement probiotic effect.

Materials and methods

In this study, materials included dried tarragon powder, Low fat sterilized milk from supermarket (1.5% fat), lyophilize *Lactobacillus casei* (isolated from Iranian dairy products, Takjen company, Iran), lyophilize *Lactobacillus paracasei* (isolated from Iranian dairy products, Takjen company, Iran) and MRS Agar (Merk Company, Germany).

The effect of tarragon on the production of probiotic *Lactobacillus casei* milk

In order to produce milk with the probiotic bacterium *Lactobacillus casei*, four containers each containing 250 cc of low-fat sterilized milk (1.5% fat) were considered as four groups. 0.1 gram starter (*Lactobacillus casei*) was added directly to all the containers, followed by adding tarragon powder 0 (the control sample), 1, 2 and 3% to all the containers, respectively and finally they were placed in an incubator at 38 ^{o}C. The acidity test was performed approximately every 2 hours until reaching 84-87 ^{o}Dornic.

The effect of tarragon on the production of probiotic *Lactobacillus paracasei* milk

To produce *Lactobacillus paracasei* milk, four containers each containing 1 liter of low-fat sterilized milk (1.5% fat) were considered as four groups. 0.2 gram starter (*Lactobacillus paracasei*) was added directly to all the containers, followed by adding tarragon powder 0% (the control sample), 1, 2 and 3% to all the containers, respectively and finally they were placed in an incubator at 38 ^{o}C. The acidity test was performed approximately every 2 hours until reaching 89 ^{o}Dornic.

After *Lactobacillus casei* and *Lactobacillus paracasei* milk samples reached 84-87 ^{o}Dornic and 89 ^{o}Dornic, respectively, they were taken out of the incubator and mixed together and then transferred to a refrigerator and stored at 2 ^{o}C. The produced probiotic milk was evaluated once every 7 days by counting the microbes using direct counting method.

Results

To give a vivid picture of the findings, the results are tabulated. Table 1 shows the pH level in the tarragon *L. casei* milk during incubation, and Table 2 presents the acidity level in the tarragon *L. casei* milk during the same time. Table 3 depicts the pH level in the tarragon *L. paracasei* milk during incubation, and Table 4 shows the acidity degree in the tarragon *L. paracasei* milk during incubation. Table 5 demonstrates the PH level in the tarragon *L. casei* and *L. paracasei* milk within 21-day storage in the refrigerator, and Table 6 shows the acidity level base on Dornic degree in the tarragon *L. casei* and *L. paracasei* milk within 21-day storage in the refrigerator. And finally, Table 7 illustrats the growth rate of microbes in the tarragon *L. casei* and *L. paracasei* milk.

Table 1. The pH level in the tarragon *L. casei* milk during incubation

Tarragon Milk (%)	00:00 (h)	02:00 (h)
0	↓	5.24
1	↓	4.97
2	↓	4.91
3	↓	4.87

Table 2. The acidity level in the tarragon *L. casei* milk during incubation

Tarragon Milk (%)	00:00(h)	02:00(h)
0	↓	90
1	↓	150
2	↓	151
3	↓	184

Table 3. The pH level in the tarragon *L. paracasei* milk during incubation

Tarragon Milk (%)	00:00(h)	02:00(h)
0	↓	5.28
1	↓	5.03
2	↓	4.89
3	↓	4.87

Table 4. The acidity level in the tarragon *L. paracasei* milk during incubation

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Tarragon Milk (%)	00:00(h)	02:00(h)
0	↓	83
1	↓	112
2	↓	115
3	↓	168

Table 5. The pH level in both tarragon *L. casei* and *L.paracasei* milk within 21-day storage in the refrigerator

	pH Level		
Tarragon Milk (%)	7 day	14 day	21 day
0	5.27	5.17	5.14
1	5.02	5	4.98
2	4.96	4.93	4.88
3	4.91	4.90	4.86

Table 6. The acidity level base on Dornic degree in both tarragon *L. casei* and *L.paracasei* milk within 21-day storage in the refrigerator

	Acidity Level in Dornic degree		
Tarragon Milk (%)	7 day	14 day	21 day
0	80	96	85
1	114	145	124
2	127	134	130
3	156	177	165

Table 7. Growth of microbes in both tarragon *L. casei* and *L.paracasei* milk

Tarragon Milk (%)	10^{-5} cfu/gr
0	8.75×10^{11}
1	15×10^{11}
2	17.5×10^{11}
3	16.25×10^{11}

Disscussion

Before a probiotic can benefit human health, it must fulfill several criteria. It must have good technological properties so that it can be manufactured and incorporated into food products without losing viability and functionality or creating unpleasant flavors or textures. Moreover, it must survive passage through the upper gastrointestinal (GI) tract and arrive alive at its site of action, and must be able to function in the gut environment. It is a particular concern, given that high levels at least 10^7 per gram or mg of live micro-organisms are recommended for probiotic products (Guarner and Schaafsma, 1998; Heller, 2001; Lee and Salminen, 1995).

Essence medicinal plants and herbs play a significant role in the human life and have been very popular for long among the Iranian. Tarragon health benefits are due to its liquid oil with a strong odor and flavor. Tarragon original oil has seventy percent estragole and twenty percent terpene. Tarragon is extensively used as food seasoning. It contains compounds that facilitate reducing pain associated with dental conditions. In addition, it promotes bile production and detoxification by the liver, leading to aid digestion, and it may operate as a mild sedative (Jahanara and Haerizade, 2001; Kalantari, 2013; Nesslany, 2010).

The aim of the present study was to investigate the effects of tarragon on the growth of the bacteria *Lactobacillus casei* and *Lactobacillus paracasei* (together) in probiotic milk. For this purpose, The acidity, pH and survival of the bacteria in the tarragon probiotic milk were evaluated at 2 h intervals till reaching 42 ^{o}Dornic acidity degrees for milk in the incubator at 38 ^{o}C and also within 21 day period of storage in the refrigerator.

The 3% tarragon milk in the sample containing *Lactobacillus casei* reached 84-87 ^{o}Dornic acidity much earlier than other samples, which was transferred to a refrigerator and stored at 2 ^{o}C. So, this sample revealed the most effect on the growth of bacteria during incubation. The sample with %2 tarragon and subsequently the sample with 1% and 0% (the control sample) tarragon and finally the sample containing both bacteria, Lactobacillus casei and *Lactobacillus paracasei*, reached 84-87 ^{o}Dornic. Thus, the *Lactobacillus casei* tarragon milk 0% demonstrated a minimal effect on the growth of bacteria during incubation.

The 3% tarragon milk in the sample containing *Lactobacillus paracasei* reached 89 ^{o}Dornic acidity earlier than others, which was transferred to a refrigerator and stored at 2 ^{o}C. Consequently, this sample showed the most effect on the growth of bacteria during incubation. The sample with 2% tarragon and subsequently the sample with %1 tarragon and the control sample and finally the sample containing both bacteria, *Lactobacillus casei* and *Lactobacillus paracasei* reached 89 ^{o}Dornic. Therefore, the *Lactobacillus paracasei* milk sample 0% presented a minimal effect on the growth of bacteria during incubation.

During the 21 days storage of milk samples containing bacteria as *Lactobacillus casei* and *Lactobacillus paracasei* in the refrigerator, the acidity levels in the sample with 3% tarragon was higher than others, and subsequently the samples with 2%, 1% and 0% (the control sample) were higher, respectively. So during refrigeration, the control sample revealed the most persistence, while the sample milk containing 3% tarragon demonstrated a minimal persistence.

In the direct counting method of bacteria, maximum number of microbes (the mixture of *Lactobacillus casei* and *Lactobacillus paracasei*) were observed in the milk sample with 2% and 3% tarragon, and subsequently the sample with 1% tarragon and finally the control sample were more, respectively in the first week. Maximum colony growth was observed in the sample with 2% tarragon, and subsequently the sample with 1%, 3% and %0 tarragon were more, respectively.

In a study on the effects of soya powder on the growth of the bacteria as *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in probiotic products, it was demonstrated that the increased concentration of soya caused an increase in the microorganism growth and acidity level which in turn resulted in shorter incubation time for the desired acidity (Marhamatizadeh, 2009).

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In a study, investigating the effect of garlic on the bacterial growth, it was shown that the increased garlic concentration promoted the growth of the bacteria in probiotic milk and yoghourt (Marhamatizadeh, 2012).

In another study, addressing the effect of spearmint on the bacterial growth, it was reported that the increased spearmint concentration promoted the growth of the bacteria in probitic milk and yoghurt (Marhamatizadeh, 2011).

Examining 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 milk (Marhamatizadeh, 2012).

Conclusion

Some other studies have been carried out on soy, honey, ginger, cinnamon, spearmint, garlic, dill, and chicory. Upon comparing the results, it was revealed that the increased concentration of tarragon had a positive effect on the growth of the probiotic bacteria, *Lactobacillus casei* and *Lactobacillus paracasei* in probiotic milk. The probiotic effect of *Lactobacillus casei* and *Lactobacillus paracasei* and some other properties in tarragon may be highlighted for medical benefits.

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