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The Effects of Origanum vulgare on the growth of Lactobacillus casei and Lactobacillus paracasei in probiotic milk

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Abstract

The objective of this study ware to investigate the effects of different doses of Origanum vulgare on the growth of Lactobacillus casei and Lactobacillus paracasei (separated from Iranian dairy products) in milk produced at one step. The product was then examined in terms of pH, acidity and microbe counting during incubator setting period. In the milk samples with Lactobacillus casei, the control sample reached acidity level more quickly and in the milk samples with Lactobacillus paracasei, the sample containing %1 Origanum vulgare reached acidity level earlier than other samples. 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 Origanum vulgare had a positive effect on the growth of the probiotic bacteria, Lactobacillus casei and Lactobacillus paracasei in probiotic milk.

Key words: Probiotic, Lactobacillus casei, Lactobacillus paracasei, Origanum vulgare, milk.

INTRODUCTION

Probiotics are “Living micro-organisms, which Upon ingestion in certion numbers exert health benefits beyond inherent basic nutrition”(Guarner & 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 &. Salminen, 1995). Functional foods should contain at least 10^7 cfug^{-1} probiotic bacteria and should be consumed at levels higher than 100g day^{-1} to have positives effects on health (Ishibashi & Shimamura, 1993). Probiotic-containing products have become primary choise for the consumer because of their health attributes. Therefor, the market for such products has rapidly grown (Ross et al, 2002). Many different strains and species of lactobacilli and bifidobacteria have been used commercially as probiotcs. It is well known that the probiotic bacteria have health-promoting effects and antagonistic activity against food-borne disease agents (Gilliand & Speck, 1977). 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

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prospective researchers (Mortazavian & Sohrabvandi, 2006). Although, in the past decades, the synthetic chemical drugs that make use of separation mechanisms have been much in demand, their corresponding side effects are being gradually observed so much so that their irregular and improper consumption has turned out to be a critical issue. On the contrary, the benefits of medicinal plants and their little or zero side effects have made them a proper substitute, highly appreciated by physicians and patients. Iran possesses a very rich source of such plants and herbs in the world in terms of variety and amount. Origanum Vulgare is from labiatae family and plantae kingdom (Christman, 2010). This herb is seen in vast areas of Europe, especially in southern Europe, the north of Africa and vast areas of Asia. In Iran it is distributed in the north and northwest regions but has not been observed in the hot regions of the south (El-Ashmawy et al, 2007). Origanum Vulgare in traditional medicine is utilized as disinfectant, antispasmodic, anti flatulence, anti-worm and is also used for liver and gallbladder discomforts (Bremness, 1994; Faleiro et al, 2005 ; Yazdanparast & Shahriyary, 2008 ; El-Ashmawy, 2005). Origanum Vulgare 1% essence consists of phenols, monoterpene main proportion of which consists of phenols, monoterpene hydrocarbons and alcohol. Generally Origanum Vulgare essence contains 25 compounds, such as 26.9% thymol, carvacrol 40.7% and 7.3% gamma Trypnyn (Novak et al, 2003; Fabio et al 2003; Hazzit et al, 2006). The aims of the present study ware to evaluate the effects of Origanum vulgare on the growth of Lactobacillus casei and Lactobacillus paracasei in probiotic milk.

MATERIALS AND METHODS

materials:
(i) Low fat sterilized milk (%1.5 fat)
(ii) Dried Origanum vulgare powder
(iii) Bacteria lyophilize Lactobacillus cacei (isolated from iraniandairy products)
(iv) Bacteria lyophilize Lactobacillus paracasei (isolated from iraniandairy products)
(v) MRS Agar (Merk company, Germany)

Effect of Origanum vulgare on the Production of Probiotic Lactobacillus casei milk as first passage:
In order to produce the milk containing the probiotic bacterium Lactobacillus casei, Four containers each containing 250 cc of low-fat sterilized milk (%1.5 fat) were considered as our four groups. 0.1 gram starter (Lactobacillus casei) was adde directly to all the containers, followed by adding Origanum vulgare powder 0 (the control sample), 1, 2 and %3 to all the containers, respectively and finally they were placed in an incubactor at $38^{\circ}C$. The acidity test was performed opproximately every 2 hours until reaching $84-87^{\circ}$ Dornic .

Effect of Origanum vulgare on the Production of Probiotic Lactobacillus paracasei milk at the second passage:

To produce Lactobacillus paracasei milk, four containers each containing 1 liter of low-fat sterilized milk (%1.5 fat) were considered as our four groups. 0.2 gram starter (Lactobacillus paracasei) was added directly to all the containers, followed by sdding Origanum vulgare powder 0 (the control sample), 1, 2 and %3 to all the containers, respectively and finally they were placed in an incubator at $38^{\circ}C$. The acidity test was performed opproximately every 2 hours until reaching 89° Dornic. After reaching Lactobacillus casei and Lactobacillus paracasei milk samples to $84-87^{\circ}$ Dornic and 89° Dornic,

respectively, they were taken out of the incubator and mixed together and then transferred to a refrigerator and stored at $2^{\circ}C$ produced probiotic milk was evaluated once every 7 days by counting the microbes using direct counting method. Some components including aroma, smell, taste and consistency in four levels of very good, good, fair and poor were considered in each questionnaire. The questionnaire results were analyzed by SPSS17 software based on descriptive statistics.

RESULTS

Table 1 shows the acidity degrees of Origanum vulgare milk Lactobacillus casei and Lactobacillus paracasei, during storage time in the refrigerator and Table 2 shows the PH level in the Origanum vulgare L. casei and L. paracasei milk with in 21-day storage in the refrigerator during the same cooling period.

DISCUSSION

Probiotic cultures are described as live microbial feed supplements that improve intestinal microbial balance and are intended for maintenance of health or prevention, rather than curing of disease. The demand for probiotic foods is increasing in Europe, Japan and the U.S. reflecting the heightened awareness among the public of the relationship between diet and health. Traditionally, the most popular food delivery systems for these cultures have been freshly fermented dairy foods, such as yoghurts and fermented milks, as well as unfermented milks with cultures added. However, in the development of functional foods, the technological suitability of probiotic strains poses a serious challenge since their survival and viability may be adversely effected by processing conditions. This is a particular concern, given that high levels cat least 10^{7} per gram or mg of live micro-organisms are recommended for probiotic products (Guarner & Schaafsma, 1998; Lee &. 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(Jahanara & Haerizade, 2001). Origanum Vulgare in traditional medicine is utilized as disinfectant, antispasmodic, anti flatulence, anti-worm and is also used for liver and gallbladder discomforts (Bremness, 1994; Faleiro et al, 2005 ; Yazdanparast & Shahriyary, 2008 ; El-Ashmawy, 2005). The aim of the present study, the effects of Origanum vulgare on the gtowth of the bacteria Lactobacillus casei and Lactobacillus paracasei (together) in probiotic milk sere investigated. The acidity, pH and survival of the bacteria in the Origanum vulgare probiotic milk were evaluated at 2 h intervals till reaching 42° Dornic acidity degrees for milk in the incubator at $38^{\circ}C$ and also within 21 day period of storage in the refrigerator. The probiotic Origanum vulgare milk %0 (the control sample) in the sample containing Lactobacillus casei reached $84-87^{\circ}$ Dornic acidity much earlier than other samples, which was transferred to a refrigerator and stored at $2^{\circ}C$. So, this sample had the most effect on the growth of bacteria during incubation. The sample with %2 Origanum vulgare and subsequently the samples with %1 and %3 Origanum vulgare and finally the sample containing both bacteria, Lactobacillus casei and Lactobacillus paracasei, reached $84-87^{\circ}$ Dornic. So, the Lactobacillus casei Origanum vulgare milk %3 had a minimal effect on the growth of bacteria during incubation. The probiotic Origanum vulgare milk %1 in the sample containing Lactobacillus paracasei reached 89° Dornic acidity earlier than others, which was transferred to a refrigerator and stored at $2^{\circ}C$. So, this sample had the most effect on the growth of bacteria during incubation. The sample with %3 Origanum vulgare and subsequently the control sample and the sample with %2 Origanum vulgare and finally the sample containing both bacteria, Lactobacillus casei and Lactobacillus paracasei reached 89° Dornic. So, the Lactobacillus paracasei milk sample %2 had a minimal effect on the growth of bacteria during incubation.

During the 21 days storage of milk samples containing both bacteria, Lactobacillus casei and Lactobacillus paracasei, in the refrigerator, the acidity levels in the sample with %2 Origanum vulgare was higher than others, and subsequently the samples with %3, %1 and %0 (the control sample) were higher, respectively. So, during refrigeration the control sample had the most persistence. The sample milk containing %2 Origanum vulgare had 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 %1 and %3 Origanum vulgare and subsequently the sample with %2 Origanum vulgare and finally the control sample were more, respectively. In a study on the effects of soya powder on the growth of the bacteria, Lactobacillus acidophilus and bifidobacterium bifidom, 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 yielded and incubation time for the yoghurt with malt and soya decreased (Marhamati Zade et al, 2009). In another study addressing the effect of spearmint on the bacterial growth, it was demonstrated that the increased spearmint concentration promoted the growth of the bacteria in probitic milk and yoghurt (Marhamati Zade et al, 2011). In other 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 milk (Marhamati Zade et al, 2012). The sensory scores of the products were high and acceptance. From the foregoing results it can be concluded that Origanum vulgare extract can be successfully used in formulation of dairy products.

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Table 1. The acidity level base on Dornic degree in the Origanum vulgare L. casei and L. paracasei milk with in 21-day storage in the refrigerator.

Acidity Level in Dornic degree				
Origanum vulgare Milk(%)	1 day	7 day	14 day	21 day
0	105	93	107	125
1	117	91	105	128
2	131	87	102	132
3	112	90	106	131

Table 2. The PH level in the Origanum vulgare L. casei and L. paracasei milk with in 21-day storage in the refrigerator.

PH Level in Dornic degree				
Origanum vulgare Milk(%)	1 day	7 day	14 day	21 day
0	4/82	4/80	4/81	4/25
1	4/91	4/89	4/99	4/37
2	5/03	5/08	5/02	4/43
3	5/18	5/13	5/12	4/51