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Research article

Viability of probiotic bacteria and some chemical and sensory characteristics in cornelian cherry juice during cold storage

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ABSTRACT

Background: Increased popularity of vegetarianism, lactose intolerance, and the high cholesterol content in dairy products, are all factors that have recently increased the demand for nondairy probiotic products. The objective of this study is to evaluate the effect of refrigeration on the viability of probiotics and asses some of the chemical and sensory characteristics in cornelian cherry juice.
Results: The Iranian native probiotic strain (*L. casei* T4) showed greater viability compared to industrial types (viable count of 8.67 log cfu/mL versus <6.0 log cfu/mL at d 28). However, this most tolerant Iranian strain, could not withstand the conditions of 'Natural juice' at pH 2.6 for more than 7 d. Following a pH adjusted treatment (to pH ~3.5), the viability of the strain was improved to 28 d with some evidence of increased growth of the probiotic. However, the level of antioxidant activity, anthocyanin and phenolic compounds, revealed a slight decrease during cold storage. The changes in the chemical profile of the sample containing *L. casei* T4 indicated fermentation activity during cold storage. Sensory evaluation results showed significant differences between samples containing *L. casei* TD4 and other samples in taste, odor and overall acceptance in a complimentary way.
Conclusion: The results showed that low pH and presence of inhibitor phenolic compounds of cornelian cherry juice have negative effect on viability of probiotics, especially industrial strains during refrigerated storage.

1. Introduction

An increased trend in consumer health consciousness and food selection has pushed for research and development of foods that promote health [1]. Foods that supply significant nutrition, have health advantages, inhibit disease and/or assist health have become more readily accepted by the industry and can be used as successful marketing/branding tools. This has caused the emergence of functional foods that comprise a wide range of components such as probiotics, prebiotics, vitamins, minerals and dietary fiber [2,3].

Probiotics are functional components that are defined as live and active bacteria food supplements, which upon consumption in adequate amounts, show health advantages beyond regular health benefits of foods without probiotics. Nowadays, probiotics are used broadly in dairy as well as non-dairy products, while in past decades they were principally applied in dairy products, especially yoghurt [4,5,6].

Recently, there has been an interest in the development of fruit and vegetable juices as functional beverages with probiotics. Probiotics are consumed universally by a wide range of people and the reason lies behind probiotics' inherently healthy characteristic and being free from incompatible components such as lactose and casein [1,7].

Fruits of cornelian cherry are currently used in medicine to modify kidney and liver functions for their diuretic and anti-diabetic characteristics. These fruits are a good source of anthocyanin, phenolic compounds and ascorbic acid [8]. It contains more than twice as much ascorbic acid as oranges. Cornelian cherry fruits have been used in Chinese medicine and known for their tonic and analgesic functions. Furthermore, this fruit is known as a good nutritional ingredient for the production of functional beverages [9].

Therefore, the juice of this fruit has the potential to be used for the production of functional food beverages. The main objective of this study was to incorporate some native Iranian probiotic strains, as well

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as, industrial (commercial) probiotics into both natural and pH-adjusted cornelian cherry juice (pHs 2.6 or 3.5) and monitor their viability during 28 d of refrigerated storage at 4°C. Meanwhile, chemical characteristics of the products (pH, antioxidant activity, titrable acidity, anthocyanin and phenolic content) were the subject of assessment.

2. Materials and methods

2.1. Study design and sample preparation

Cornelian cherry (*Cornus mas* L.) concentrate (Takdaneh, Tabriz, Iran) was diluted with sterilized distilled water to 8° brix. In a group of samples the pH was adjusted to 3.5 (using industrial sodium bicarbonate), while in the other group, the pH was kept intact (~2.6). The samples were pasteurized at 95°C for 15 min and cooled to 4°C followed by inoculation (8.0 log cfu/mL) of three industrial probiotics (*Lactobacillus rhamnosus*, *Lactobacillus plantarum* and *Lactobacillus casei*) as well as two Iranian native probiotics (*L. casei* TD4 and T4). The viability of probiotics, along with the changes in chemical properties including pH, titratable acidity, phenolic content, anthocyanin content and antioxidant activity, were measured during refrigerated storage (28 d, 4°C), within 7-d intervals.

2.2. Probiotic strains

Industrial probiotic lactobacilli (*L. plantarum* ATCC20174, *L. casei* ATCC 393 and *L. rhamnosus* ATCC 7469) were obtained from DSM Co., and the inoculum was prepared by incubating the culture at 30°C for 24 h in MRS broth. Two Iranian native lactobacilli (*L. casei* T4 and TD4) were supplied by Tak Gene Zist Co., (Tehran, Iran) in lyophilized type. The probiotics were kept according to manufacturers' instructions until used.

2.3. Chemical analysis

Total titrable acidity expressed as percent of malic acid (as the predominant acid in cornelian cherry) was measured by titrating the samples with 0.1 N NaOH [9]. Changes in antioxidant activity, phenolic and anthocyanin content of samples were determined by DPPH radical assay, Folin-ciocaltue and differential pH assay, respectively [9,10,11].

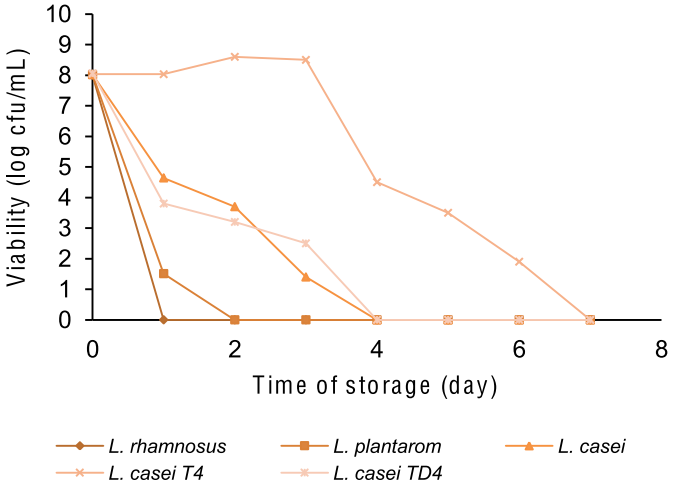

Fig. 1. Viability of probiotic bacteria in cornelian cherry juice with natural pH (2.6) during cold storage (4°C).

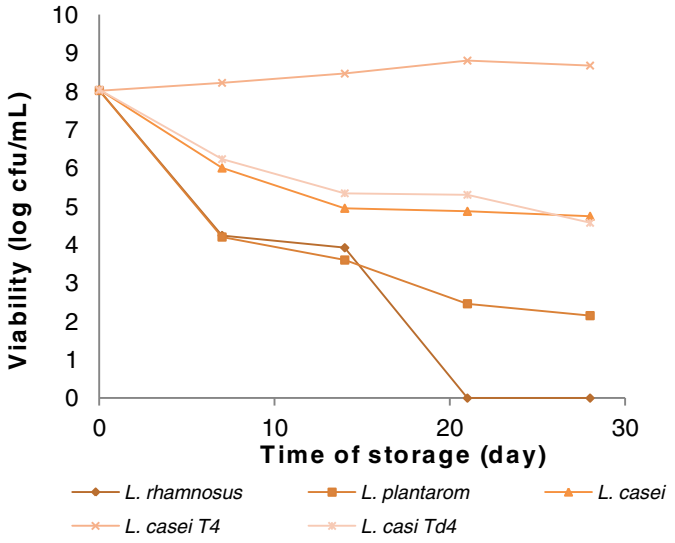

Fig. 2. Viability of probiotic bacteria in cornelian cherry juice with adjusted pH (3.5) during cold storage (4°C).

2.4. Microbiological analysis

Viable cell counts of probiotics (cfu/mL) were determined by plate count methodology (MRS agar medium, 72 h incubation at 30°C, aerobically) according to Mortazavian et al. [12].

2.5. Sensory evaluation

Sensory properties such as taste, odor and overall acceptance of different samples containing 5 probiotics were carried out by 9 trained test panelists with serial paired comparison test (DUO test). In this method, pH (3.5) adjusted sample devoid of probiotic was considered as control.

2.6. Statistical analysis

All experiments were performed in triplicate. Two-way Analysis of Variance (ANOVA) was performed to evaluate statistical difference between the treatments (samples containing *L. rhamnosus*, *L. casei*, *L. plantarum*, *L. casei* T4, *L. casei* TD4 and control) for chemical and sensory properties as well as the viability of probiotics over 7-d intervals of storage time. Statistical comparison of the effect of time on chemical and sensory properties of samples was done by repeated measure test with SPSS software (version 16) at significance level of 0.05.

Table 1
pH in cornelian cherry juice treatments with adjusted pH (3.5) during cold storage (4°C)*.

Treatments	Time of storage (day)				
	0	7	14	21	28
Control**	3.49^{aA**}	3.49^{aA}	3.49^{aA}	3.49^{aA}	3.49^{aA}
L. rhamnosus	3.50^{aA}	3.50^{aA}	3.50^{aA}	3.51^{abAB}	3.52^{bB}
L. plantarum	3.51^{aA}	3.50^{aA}	3.50^{aA}	3.51^{abA}	3.51^{bA}
L. casei	3.50^{aA}	3.49^{aA}	3.50^{aA}	3.51^{abA}	3.51^{bA}
L. casei T4	3.49^{aA}	3.50^{aA}	3.51^{aAB}	3.53^{bBC}	3.55^{cC}
L. casei TD4	3.50^{aA}	3.50^{aA}	3.50^{aA}	3.52^{bAB}	3.53^{bcB}

* Means shown with different small and large letters represent significant differences ($P < 0.05$) in each column and row, respectively.
** Control: sample without probiotic.