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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 assess 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.

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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].

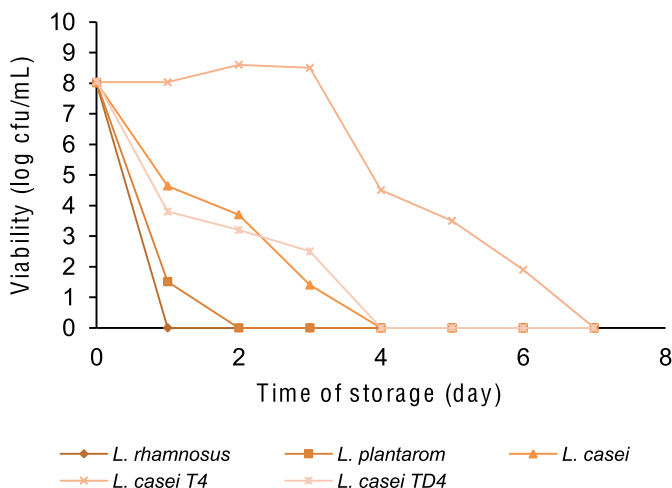


Fig. 1. Viability of probiotic bacteria in cornelian cherry juice with natural pH (2.6) during cold storage (4°C).

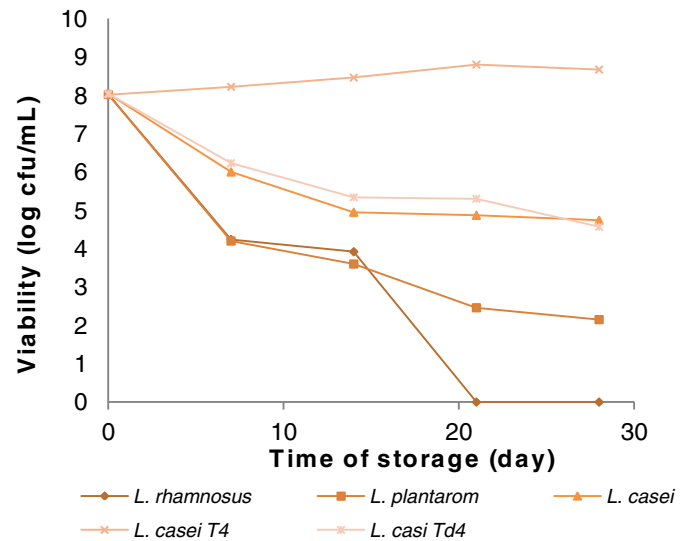


Fig. 2. Viability of probiotic bacteria in cornelian cherry juice with adjusted pH (3.5) during cold storage (4°C).

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}
<i>L. rhamnosus</i>	3.50 ^{aA}	3.50 ^{aA}	3.50 ^{aA}	3.51 ^{abAB}	3.52 ^{bB}
<i>L. plantarum</i>	3.51 ^{aA}	3.50 ^{aA}	3.50 ^{aA}	3.51 ^{abA}	3.51 ^{bA}
<i>L. casei</i>	3.50 ^{aA}	3.49 ^{aA}	3.50 ^{aA}	3.51 ^{abA}	3.51 ^{bA}
<i>L. casei</i> T4	3.49 ^{aA}	3.50 ^{aA}	3.51 ^{aAB}	3.53 ^{bBC}	3.55 ^{cC}
<i>L. casei</i> 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.

Table 2

Titration acidity (% malic acid) 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**	1.00 ^{aA**}	1.00 ^{aA}	1.01 ^{aA}	1.01 ^{aA}	1.01 ^{aA}
<i>L. rhamnosus</i>	1.00 ^{aA}	1.00 ^{aA}	1.01 ^{aA}	1.00 ^{aA}	1.00 ^{aA}
<i>L. plantarum</i>	1.00 ^{aA}	1.00 ^{aA}	1.00 ^{aA}	1.00 ^{aA}	1.00 ^{aA}
<i>L. casei</i>	0.99 ^{aA}	1.00 ^{aA}	1.00 ^{aA}	1.00 ^{aA}	1.00 ^{aA}
<i>L. casei</i> T4	1.00 ^{aAB}	1.02 ^{aA}	1.01 ^{aA}	0.98 ^{bBC}	0.97 ^{bC}
<i>L. casei</i> TD4	1.01 ^{aA}	1.02 ^{aA}	1.02 ^{aA}	1.01 ^{aA}	1.00 ^{aA}

* Means shown with different small and large letters represent significant differences ($P < .05$) in each column and row, respectively.

** Control: sample without probiotic.

3. Results

3.1. Viability of probiotic bacteria during cold storage

Changes in viable counts (log cfu/mL) of industrial and Iranian native strains in cornelian cherry juice with natural pH (2.6) and adjusted pH (3.5) during cold storage (4°C) over 7-d intervals are presented in Fig. 1 and Fig. 2, respectively. All strains could not tolerate detrimental conditions of product matrix especially a very low pH (2.6). They completely lost their viability during the early days of cold storage in such a way that even the most resistant strain (*L. casei* T4) reached to an all-dead population state at d 7.

In pH-adjusted treatment (Fig. 2), the viability of industrial strains *L. rhamnosus* and *L. plantarum* decreased from the initial number of 8.00 log cfu/mL to 4.24 and 4.20 log cfu/mL respectively, after 7 d. During the same time period, the viability Iranian native strain *L. casei* TD4 remained about 2 log cycles higher than industrial strains *L. rhamnosus* and *L. plantarum* (6.00 and 6.23 log cfu/mL, respectively). Of the mentioned strains, the native strain *L. casei* T4 not only fully retained its viability, but also showed significant increase ($P < 0.05$) in viability. On d 14 and 21 of cold storage all strains except *L. casei* T4 continued their decline trend in viability. For example *L. rhamnosus* lost its viability completely after 21 d. In contrast, *L. casei* T4 showed continual increasing trend until the end of storage period (d 28) and the viable count was more than 8 log cfu/mL.

3.2. pH and titration acidity in pH-Adjusted samples during cold storage

Table 1 and Table 2 indicate pH and titration acidity changes in treatments during 28 d of cold storage. Evidently, during this period, samples containing *L. casei* T4 exhibited significant increase in pH and significant decrease in titration acidity.

Table 3

Antioxidant activity (% scavenging of DPPH radical) 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**	90.40 ^{aA**}	90.20 ^{adB}	90.15 ^{aC}	89.20 ^{aD}	89.00 ^{aE}
<i>L. rhamnosus</i>	90.42 ^{aA}	90.19 ^{aB}	89.81 ^{bC}	88.24 ^{bD}	87.86 ^{bE}
<i>L. plantarum</i>	90.39 ^{aA}	89.60 ^{bB}	89.10 ^{cC}	88.71 ^{cD}	87.40 ^{cE}
<i>L. casei</i>	90.41 ^{aA}	90.33 ^{adB}	89.85 ^{bC}	89.29 ^{aD}	87.97 ^{bdE}
<i>L. casei</i> T4	90.41 ^{aA}	88.87 ^{cB}	88.67 ^{dC}	87.60 ^{dD}	86.95 ^{eE}
<i>L. casei</i> TD4	90.40 ^{aA}	90.37 ^{dB}	90.33 ^{cC}	89.14 ^{aD}	88.08 ^{dE}

* Means shown with different small and large letters represent significant differences ($P < 0.05$) in each column and row, respectively.

** Control: sample without probiotic.

Table 4

Anthocyanin (mg cyanidin 3-glycoside/100 mL) content 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**	88.45 ^{aA**}	88.33 ^{aB}	87.92 ^{aC}	87.07 ^{aD}	86.16 ^{aE}
<i>L. rhamnosus</i>	88.39 ^{aA}	87.75 ^{bB}	84.24 ^{bC}	83.49 ^{bD}	83.07 ^{bE}
<i>L. plantarum</i>	88.41 ^{aA}	87.83 ^{bB}	84.83 ^{cC}	84.07 ^{cD}	82.24 ^{cE}
<i>L. casei</i>	88.45 ^{aA}	86.16 ^{cB}	85.58 ^{dC}	81.57 ^{dD}	81.23 ^{dE}
<i>L. casei</i> T4	88.40 ^{aA}	85.58 ^{dB}	83.49 ^{eC}	80.07 ^{eD}	77.56 ^{eE}
<i>L. casei</i> TD4	88.46 ^{aA}	88.17 ^{aB}	87.91 ^{aC}	84.78 ^{dD}	80.48 ^{fE}

* Means shown with different small and large letters represent significant differences ($P < 0.05$) in each column and row, respectively.

** Control: sample without probiotic.

3.3. Antioxidant activity, phenolic content and anthocyanin content in pH-adjusted samples during cold storage

Table 3, Table 4, and Table 5 represent antioxidant activity, phenolic and anthocyanin content of treatments during 28 d of refrigerated storage. Mentioned parameters in all samples decreased significantly, but this decrease was less evident during the storage period ($P < 0.05$). The highest changes for all three parameters belonged to samples with native strains and the lowest change was observed in control (without added probiotic).

3.4. Sensory evaluation in pH-adjusted samples during cold storage

As seen in Table 6 there is no significant difference ($P > 0.05$) between control samples and samples containing probiotic strains except Iranian native strain *L. casei* TD4 during 28 d of cold storage. Sensory evaluation showed that this sample had pungent odor and astringent taste. The unusual taste detected in this sample was not off-flavor; on the contrary, it was special and even relatively pleasant.

4. Discussion

All strains could not tolerate detrimental conditions of product matrix especially a very low pH (2.6) and completely lost their viability at early days of cold storage. The impact of this condition was reflected on the most resistant strain (*L. casei* T4), which reached to all-dead population state at d 7.

Sheehan et al. [13] studied the suitability of probiotic cultures in orange juice, pineapple juice and cranberry juice during 12 weeks of refrigerated storage. The live cell counts of probiotics in cranberry juice (pH = 2.5) dropped by 3 log cycles after 2 d with no viable cells observed by d 9 [13]. Their results are somehow similar to our observed results.

In pH-adjusted treatment, the native strain *L. casei* T4 not only fully retained its viability, but also showed significant increase ($P < 0.05$) in

Table 5

Phenolic content (mg Gallic acid/100 mL) 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**	215.90 ^{aA**}	210.90 ^{aB}	206.89 ^{aC}	200.46 ^{aD}	199.03 ^{aE}
<i>L. rhamnosus</i>	216.20 ^{bA}	198.32 ^{bB}	196.32 ^{bC}	194.10 ^{bD}	191.75 ^{bE}
<i>L. plantarum</i>	215.00 ^{aA}	208.10 ^{cB}	200.39 ^{cC}	198.39 ^{cD}	191.03 ^{cE}
<i>L. casei</i>	215.80 ^{aA}	200.46 ^{dB}	194.17 ^{dC}	190.82 ^{dD}	186.03 ^{dE}
<i>L. casei</i> T4	216.10 ^{bA}	196.60 ^{eB}	185.30 ^{eC}	180.17 ^{eD}	172.50 ^{eE}
<i>L. casei</i> TD4	215.90 ^{aA}	200.67 ^{fB}	191.67 ^{fC}	190.32 ^{dD}	189.10 ^{fE}

* Means shown with different small and large letters represent significant differences ($P < 0.05$) in each column and row, respectively.

** Control: sample without probiotic.

Table 6
Sensory evaluation in cornelian cherry juice treatments with adjusted pH (3.5) during cold storage (4°C) using serial DUO test.

Treatments	Time of storage (day)														
	0			7			14			21			28		
	OA*	T	O	OA	T	O	OA	T	O	OA	T	O	OA	T	O
Control** and <i>L. rhamnosus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Control and <i>L. plantarum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Control and <i>L. casei</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Control and <i>L. casei</i> T4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Control and <i>L. casei</i> TD4	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05***

* O = odor; T = taste; OA = overall acceptance.

** Control: sample without probiotic.

*** Significant difference ($P < 0.05$) and non-significant difference (-).

viability. This gain has not been previously reported and showed the strain as a leading probiotic. According to Shahabbaspour et al. [14], the viability of *L. casei* increased during 14 d of refrigerated storage and then slightly reduced until d 21 in fermented drink flavored with 3 types of fruit [14].

Sheehan et al. [13] reported that in cranberry juice with adjusted pH (3.5), viability of strains was below the critical amount of 6 log cfu/mL at day four and by day five, the viability reached 5 log cfu/mL and remained at this level for the remaining four days of analysis. Also, they announced that cranberry juice matrix (even when adjusted to a similar pH of pineapple juice) was more detrimental than pineapple juice to *Lactobacillus paracasei* due to some ingredients such as benzoic acid [13].

Sadaghdar et al. [15] showed that viability of *Lactobacillus acidophilus* decreased about 2 and 3 log cycles in fermented milk flavored by peach strawberry, respectively; whereas the viability of *L. casei* strains decreased less than a log cycle during 21 d of refrigerated storage (4°C). Their results revealed that viability of probiotic bacteria could be significantly influenced by pH, inhibitory compounds as well as the type of probiotic strain [15].

Similar results were shown for several juices by other researchers. The viability of *L. paracasei* and *L. acidophilus* in pomegranate juice reduced approximately 3 log cycles during the first week of cold storage and lost their viability after 14 d at 4°C [16]. Also, Yoon et al. [17] represented that viability of *L. plantarum* and *Lactobacillus delbrueckii* was nearly 7 and 5 log cfu/mL, respectively, after 4 weeks of refrigerated storage (4°C); while *L. casei* could not tolerate the low pH and high acidity of cabbage juice and lost the viability completely after 2 weeks of storage [17].

It is well known that pH is one of the most important harsh environmental factors that can adversely affect the viability of probiotics [18]. Therefore, adjusting pH of juice in this research from 2.6 to 3.5 resulted in considerable viability retain of probiotics, as can be seen in Fig. 1 and Fig. 2. Increase in pH may be due to the conversion of malic and citric acids to lactic acid metabolized by the probiotic bacteria [16] or due to autolysis of bacterial body and release of peptides into juice [6]. Daneshi et al. [7] reported that pH of all probiotic carrot flavored milk treatments except *L. rhamnosus*-containing sample increase insignificantly ($P \leq 0.05$) in d 5 and 10 of refrigerated storage (4°C) [7].

The decrease in antioxidant activity, phenolic and anthocyanin content may be due to the slight activity of probiotic bacteria in refrigerated temperature situation as well as the presence of dissolved oxygen in samples, which resulted in oxidation of phenolic compounds. This oxidation is highest for samples containing *L. casei* T4 due to the growth of bacteria during cold storage. It has been reported that in the absence of light and oxygen, the amount of phenolic compounds did not considerably change during the refrigerated storage period [19]. Prasawang et al. [20] reported that antioxidant activity of health beverage from fermented purple rice supplemented with *L. acidophilus*

was not significantly reduced during 35 d of refrigerated storage (7°C) [20], which is in conformity with the results of present study.

Most of the research showed that probiotics lead to considerable off-flavor in products fortified with them that often cause consumer dissatisfaction [21]. Luckow and Delahunty [22] found that consumers can recognize a sensory difference between juices containing probiotic and conventional types and preferred the taste of natural fruit juice without probiotic. However it was found that the acceptance of these two types of juice depended on different factors such as age, sex and etc [22]. In the present study, unlike noted research, there is no off-flavor in cornelian cherry juice containing probiotic strains comparing with control sample (without probiotic) that can be due to differences between type of applied probiotic strains and type of fruit juice.

5. Conclusion

This research demonstrated that natural cornelian cherry juice with pH 2.6 was very detrimental to both industrial and native probiotic strains and even the most resistant probiotic strains will be completely diminished after about 7 d of refrigerated storage. By adjusting pH to 3.5 with no significant sensory change by consumer's point of view, the viability of all applied strains, particularly the native strain *L. casei* T4, dramatically increased in such a way that after 28 d its population was even more than the initial population (log 8.00 cfu/mL). Therefore, the aforementioned strain was realized as a golden resistant strain for industrialization in food matrices with very harsh conditions, particularly, the beverages such as fruit juices and beer.

Conflicts of interest

The authors declare that there is no conflict of interest in this work.

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