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Full Length Article

Production of Probiotic mixture of Barberry and Black cherry juice by lactic acid bacteria

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ABSTRACT
In this study, producing of fermentative functional drinks based on mixture of Barberry and Black cherry juice by lactic acid bacteria, including Lactobacillus casei and Lactobacillus acidophilus with 0.2% whey powder was investigated. In this paper, microbial growth, changes in pH, lactic acid and reducing sugars were analysed after fermentation, and also viability of probiotic bacteria, changes in pH and lactic acid were determined during 28 days storage at 4^{0}C. For producing probiotic mixture of Barberry and Black cherry juice microbial suspension with an initial concentration of 10^{7}cfu/ml was prepared. Various ratios of bacteria including(65% Lactobacillus casei + 35% Lactobacillus acidophilus), (50% Lactobacillus casei + 50% Lactobacillus acidophilus) and (35% Lactobacillus casei + 65% Lactobacillus acidophilus) were inoculumed to 5% and 10% concentrations of mixture of Barberry and Black cherry juice with0.2% whey powder. Fermentation process was carried out for 72 h at 30^{0}C. According to the obtained results, treatment A_1B_1 (35% Lactobacillus casei + 65% Lactobacillus acidophilus) with 10% concentration, was detected as the best treatment, because it had the maximum bacterial growth and cell viability, the minimum pH and the maximum amount of lactic acid. Reduction sugar dropped during fermentation($p<0.05$). Generally, the results of this study showed that, mixture of Barberry and Black cherry juice with 0.2% whey powder could be considered as a suitable matrix for growth of probiotic bacteria and functional beverage production.
Keywords: Barberry and Black cherry juice, probiotic, Lactobacillus casei, Lactobacillus acidophilus

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INTRODUCTION

In recent years many researchers have been carried out about production of functional foods for promotion of consumer healthy. Food industry with development of functional foodshas been able to reduce some of diseases related to life style (Mousavi et al ., 2011).so recently some foods with special propose are attractive for many of food science researchers. This group of foods that called functional food (Nosrati et al., 2014), are defined as foods containing health-promoting components that extend beyond traditional nutrients(Luckow&Delahunty,2004).These foods have been enriched with active components such as prebiotics, probiotics and symbiotic (Mousavi et al., 2011).FAO/WHO ,2001defined probiotic as living microorganisms which upon ingestion in certain numbers, exert health benefits beyond inherent general nutrition (Mokarram et al., 2009 ; Prado et al., 2008). In order to exert their health benefits the minimum concentration of live probiotic bacteria at the expiry date of the product should be around 10^{7}cfu/ml (Nualkaekul&Charalampopoulos, 2011 ;Kun et al., 2008).Most of probiotics belong to Bifido bacterium and Lactobacillus genus (Vitali et al., 2012). These micro-organisms have numerous health promoting effects such as treatment and prevention of antibiotic diarrhea, prevention of diabetes (Roble *et al*., 2010)، production of B vitamins and folic acid, Reduction of intestinal pH (Prado *et al.,* 2008)anti-carcinogenic and anti-mutagenic effects(Nosrati et al., 2014), improvement in lactose metabolism and absorption of calcium, zinc, copper, iron, manganeseand phosphor(Rivera et al., 2010), positive effects on bladder cancer, reduced in testinal ulcer (Marteau et al., 2002). Most of probiotic foods are based on dairy and cereals(Von Mollendorff, 2008),butincreasing of vegetarian consumers in over the world has rise require for vegetable probiotic products such as fruit and vegetable juices.Because they don't have dairy allergens, lactose and cholesterol that might prevent consumption by certain segments of

the population (Pereira et al., 2011;Roble*et al*., 2010;Hellandet al., 2004).Due to they contain potassium salt, vitamins, dietary fibers, antioxidants, bioflavones, positive effects on liver and gallbladder (Moraru et al., 2007)and according to liquids remove stomach faster than solids fruit and vegetable juices can be suitable carrier. So mixture of Barberry and Black cherry juice is a proper media for probiotic transmission.

Blackcherry (Prunuscerasus L.) is a deciduous tree. Its origin are around the Caspian Sea ,the Black Sea and Mediterranean regions. Black cherry contains C, A vitamins, iron, antioxidants, terpenoids and other phito chemical compounds. This fruit is effective on prevention of cancer and heart diseases; Black cherry is beneficial for liver failure, disposal of bladder stones and irritation of the urinary tract (Rad and Ghaseminezhad, 2007). Barberry (Berberis vulgaris L.) belongs to moderate and subtropical regions of Europe, Asia, Africa, North and South of America (JavadZadeh and Mokhtari, 2010). It is a good source of alkaloids(Berberine), calcium, phosphor, potassium, iron and C, B vitamins. Barberry is effective on bile, heart, liver and blood refining (Magsodi, 2010).

In present paper use of mixture of Barberry and Black cherry juice with 0.2% whey powder as a probiotic culture matrix. The aim of this study was investigation of desirability of mixture of Barberry and Black cherry juice with 0.2% whey powder as a substrate for probiotic bacterial growth, determination of optimal storage time and survival of these cultures in the product.

MATERIALS AND METHODS

Raw material

Dried Barberry was purchased from local store and washed. Then was brought to boil for obtaining its extract. After cooling mixture of barberries and water were filtered by cloth filter (Magsodi, 2010). This extract was frozen at at -20°C prior to use (Pereira et al., 2011).Black cherry concentrate was purchased from Sasan Shahd Co. and was kept at 4 °C prior to use (Yoon et al., 2004).

Preparation of microorganisms and stock culture

Probiotic lactic acid bacteria(Lactobacillus casei PTCC1608 Iranian Research Organization for Science and Technology , Lactobacillus acidophilus DSM Co.Netherlands) were activated by MRS broth (Merck, Germany) and were incubated at 37 °C for 24 h under aerobic conditions.In order to produce sub-cultures from the bacteria, about 10 cc of the 24-hour culture were centrifuged at 3000 g for 5 minutes at 25 °C (Mokarram et al., 2009).Sub-cultures were prepared by adding sterile glycerol (50%v/v) to the activated cultures. Sub-cultures were kept in sterile micro-tubes containing 8 cc of 24-h culture suspension at -20 °C (Pereira et al., 2011). The strains were reactivated by means of double passage on MRS broth when needed(48-hour culture) (Mokarram et al., 2009; Mousavi et al., 2011).

Pasteurization

Whey powder was pasteurized by water bath at 80 °C for 20 minutes[1]. Pasteurization of Barberry extract was carried outat 80 °C for 15 minutes (Kun et al., 2008). Also Black cherry concentrate was pasteurized for 5 min at 80°C (Mousavi et al., 2011).

Inoculum preparation

To determine the number of bacteria per ml was used of the 0.5 MacFarland standard suspension that its turbidity is equal 1.5×10^8cfu/ml[4] .In order to achieve 10^7cfu/ml of each strain, 10 cc of 48-hour culture was centrifuged at 3000 g for 5 minutes at 25°C,Then was washed twice by sterile peptone buffer 0.1% (Merck, Germany) (Mokarram et al., 2009).The cultures were diluted with sterile distilled water. Then was measured optical density of bacterial suspension by spectrophotometer at 625 nm. The optical density of bacterial suspension was corresponded with the optical density of 0.5 MacFarland [4]. Serial dilution was takenfor obtaining10^7cfu/ml of probiotic bacteria.

Fermentation of mixture of Barberry and Black cherry juice

Black cherry extract(with no preservatives) and pasteurized Barberry juice were mixed equally(50%-50%). 0.2% of pasteurized whey powder was added to the each sample, then were prepared 5% and 10% concentrations by adding sterile distilled water. L.casei and *L.acidophilus* with initial density of 10^7cfu/ml and the ratios of 35%, 50% and 65% were inoculumed in to the pasteurized mixture of Barberry and Black cherry juice with 0.2% whey powder. Two samples of Barberry and Black cherry juice with 0.2% whey powder with 5% and 10% concentrations were prepared as control.The samples were incubated at 30°C for 72 h. The samples were kept at 4°Cfor 4 weeks, for investigating considered factors (Yoon et al., 2005; Mousavi et al., 2011).

Microbiological analysis

Viable cell counts were determined by serial dilution and standard plate method after fermentation. Dilutions of 10^{-7} and 10^{-8}cfu/ml were prepared of the fermented samples and plated in double plate. Then, sterilized MRS agar (Merck, Germany) medium was poured on them(standard plate count method). The plates were incubated at 30°C for 48 h. Plates containing 20–350 colonies were measured and

recorded as colony forming units (CFU) per mL of solution (Vinderola and Reinheimer, 2000). Also the viability of lactic acid cultures was determined during cold storage period at weekly intervals by using the mentioned method and expressed ascfu/ml. The number of lactic acid cultures was calculated by equation (1).

Eq.1 $$N = \frac{\Sigma C}{V(n1+0/1n2)d}$$

ΣC: total colonies in plates of two successive dilutions, V: volume of inoculated dilution in each plate as ml, n_1: number of counted plate in the first dilution (thicker), n_2: number of counted plate in the second dilution(thinner),d: dilution index based on the first of counted dilution (thicker) (Iran NationalStandardNo. 5272).

Chemical analysis

Samples were taken at weekly intervals for chemical analysis.pH was measured with pH meter (SAT-2002, Iran) (Iran National StandardNo. 2685). Total acidity was measured by potentiometric withtitrazol0.1N NaOH(Merck, Germany) to pH 8.2 and expressed as lactic acid(gr/100cc) (Iran National StandardNo. 2685).Determination of reduction sugares(gr/100gr) were carried out by Fehling method, before and after fermentation of mixture of Barberry and Black cherry juice(Iran National StandardNo. 2685).

Eq.2 $$n = \frac{F \times 100 \times 100}{V \times 25}$$

F: Fehling factor, V: Consummated volume of solution, n: reduction sugars (before hydrolysis)(gr/100gr)

Statistical analysis

All experiments were carried out in triplicate. The results are expressed as mean ± S.D. (standard deviation). Mean analysis was carried outusing Duncan's multiple range tests at 95% level. The SPSS(version 20) statistical computer package was used to the experimental dataand the graphs were drawn by Excel 2007 software.

The used codes in this study were as following: A_1(65% Lactobacillus acidophilus + 35% Lactobacillus casei), A_2(50% Lactobacillus acidophilus + 50% Lactobacillus casei),A_3(35% Lactobacillus acidophilus + 65% Lactobacillus casei).B_1 and B_2at the end of the cods refer to the concentrations of10% and 5% of mixture of Barberry and Black cherry juicerespectively.C_1 and C_2 represented control samples with concentrations of 10% and 5% of mixture of Barberry and Black cherry juicerespectively.

RESULTS

3.1. Changes inpH and lactic acid after fermentation and during cold storage

Table 1.The mean of pH and lactic acid in fermented mixture of Barberry and Black cherry juice with 5% and 10% concentrations and various ratios of L.casei and L.acidophilus during 72 h fermentation at 30 ^{0}C and 4 weeks storage at 4^{0}C± standard deviation.

treatments	72 h		First week		Second week		Third week		Forth week	
	pH	Lactic acid g/100cc	pH	Lactic acid g/100cc	pH	Lactic acid g/100cc	pH	Lactic acid g/100cc	pH	Lactic acid g/100cc
C_1	3.490±.000^{g}	1.148±.000^{a}	3.490±.000^{f}	1.148±.000^{a}	3.490±.000^{e}	1.148±.000^{b}	3.490±.000^{c}	1.148±.000^{d}	3.490±.000^{d}	1.148±.000^{d}
C_2	3.560±.000de	.405±.000^{b}	3.560±.000^{c}	.405±.000^{c}	3.560±.000^{b}	.405±.000^{c}	3.560±.000^{a}	.405±.000^{f}	3.560±.000^{a}	.405±.000^{f}
A_1 B_1	3.553±.005^{ef}	1.136±.000^{a}	3.553±.005^{e}	1.151±.006^{a}	3.510±.010^{d}	1.196±.006^{a}	3.453±.005^{d}	1.237±.011^{ab}	3.410±.010^{f}	1.298±.011^{a}

A_3B_2	A_2B_2	A_1B_2	A_3B_1	A_2B_1
3.660±.010^{c}	3.656±.011^{c}	3.660±.010^{c}	3.553±.005^{ef}	3.546±.005^{f}
.390±.006^{b}	.390±.006^{b}	.386±.006^{b}	1.132±.006^{a}	1.132±.006^{a}
3.596±.005^{b}	3.596±.011^{b}	3.606±.005^{b}	3.553±.005^{e}	3.526±.005^{e}
.495±.011^{b}	.502±.028^{b}	.502±.011^{b}	1.144±.006^{a}	1.144±.006^{a}
3.573±.005^{b}	3.566±.005^{b}	3.573±.015^{b}	3.513±.005^{cd}	3.510±.010^{d}
.532±.016^{d}	.540±.011^{cd}	.531±.011^{d}	1.200±.006^{a}	1.192±.011^{a}
3.533±.015^{b}	3.531±.010^{b}	3.530±.010^{b}	3.460±.010^{d}	3.456±.005^{d}
.566±.017^{e}	.570±.006^{e}	.574±.011^{e}	1.241±.013^{a}	1.230±.017^{abc}
3.496±.011^{cd}	3.490±.010^{d}	3.486±.005^{d}	3.413±.005^{f}	3.413±.005^{f}
.581±.012^{e}	.577±.016^{e}	.592±.017^{e}	1.290±.006^{ab}	1.297±.006^{a}

Values in a same column and with the same letter do not have significant difference ($p > 0.05$)

According to the (Table1) initial pH of mixture of Barberry and Black cherry juice with 5% and 10% concentrations were 3.56 and 3.49 respectively. The pH of all samples increased and their lactic acid dropped during 72 h fermentation at 30 ^{0}C, but these factors didn't have significant difference between various ratios of bacteria($p>0.05$). The amount of pH in all samples decreased and their lactic acid increased during 4 weeks storage at 4^{0}C, therefore effect of time was significant on pH and lactic acid ($p<0.01$). The minimum amount of pH and maximum amount of lactic acid were related to A_1B_1in 10% concentration of mixture of Barberry and Black cherry juice during 4 weeks storage at 4^{0}C which had no significant difference with other ratios of lactic acid bacteria in this concentration($p>0.05$), but had significant difference with control sample($p<0.05$).The maximum of lactic acid and minimum of pH between 5% concentration juices were related to A_2B_2 and A_1B_2 after 14 and 28 days storage at 4^{0}C respectively which had nosignificant difference with other samples($p>0.05$). The lactic acid and pH of A_1B_2 had significant difference with control treatment during third and fourth weeks($p<0.05$). The results showed that, various ratios of probiotic bacteria had no main effect on pH and lactic acid($p>0.05$), but 5% and 10% concentrations of juices caused significant difference in pH reduction and increase of lactic acid($p<0.01$). Fig.1 and 2 show , A_1B_1(35% L.casei +65%L.acidophilus) with 10% concentration had maximum of lactic acid and minimum of pH in 28th day of storage at 4^{0}C.
(The mean of day1 in all graphs is the measurement after 72 h fermentation)

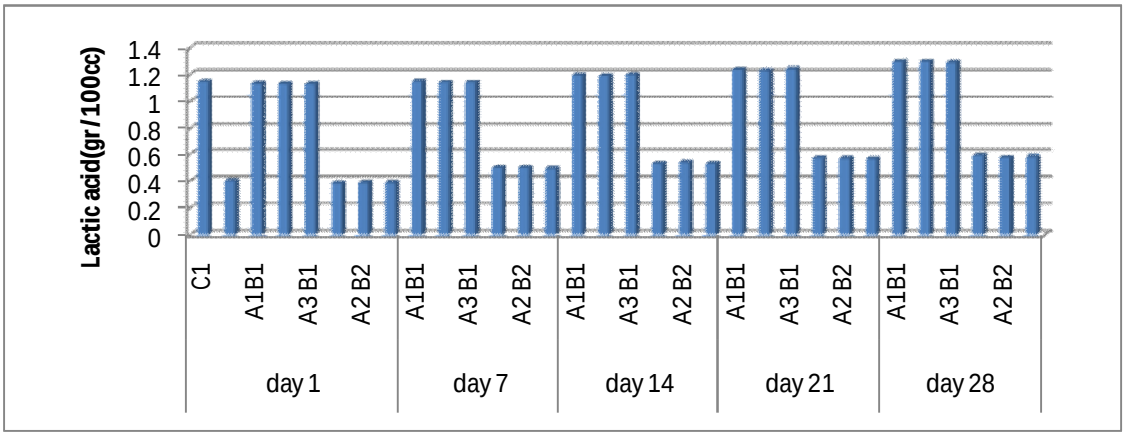

Figure 1.Effect of mixture of Barberry and Black cherry juice concentration and various ratios of bacteria in different days of cold storage on lactic acid

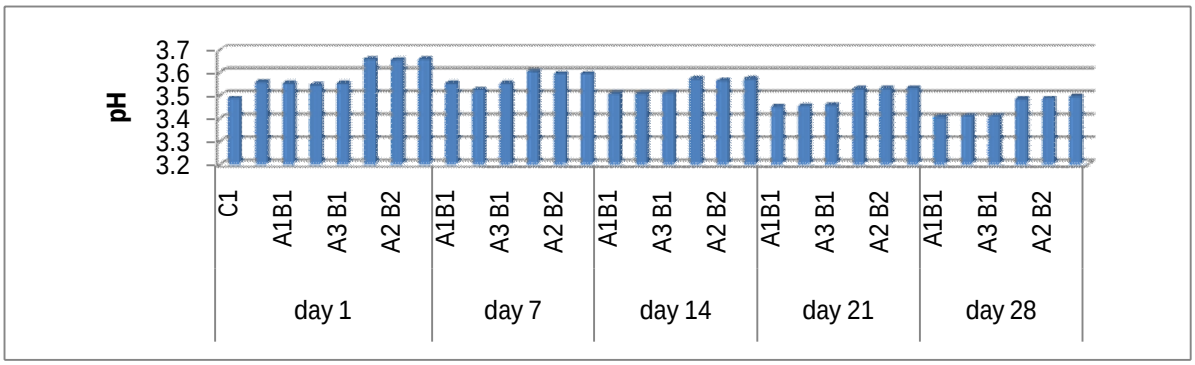

Figure 2.Effect of mixture of Barberry and Black cherry juice concentration and various ratios of bacteria in different days of cold storage on pH

Changes in bacterial growth after fermentation and during cold storage

Table 2. The mean of the bacteria survival as cfu/ml in fermented mixture of Barberry and Black cherry juice with 5% and 10% concentrations and various ratios of L.casei and L.acidophilus during 72 h fermentation at 30^0C and 4 weeks storage at 4^0C ± standard deviation.

treatment	survival (cfu/ml) 72 h	First week	Second week	Third week	Fourth week
$A_1 B_1$	$2.546\times10^{9}\pm.150\times10^{9a}$	$2.826\times10^{9}\pm.155\times10^{9a}$	$3.563\times10^{9}\pm1.173\times10^{9a}$	$1.466\times10^{9}\pm1.155\times10^{9a}$	$5.320\times10^{8}\pm1.655\times10^{8a}$
A_2B_1	$1.883\times10^{9}\pm.791\times10^{9b}$	$1.990\times10^{9}\pm.684\times10^{9b}$	$3.233\times10^{9}\pm.208\times10^{9a}$	$8.413\times10^{8}\pm2.014\times10^{8b}$	$3.400\times10^{8}\pm.888\times10^{8b}$
A_3B_1	$2.290\times10^{9}\pm.173\times10^{9ab}$	$2.346\times10^{9}\pm.144\times10^{9ab}$	$3.050\times10^{9}\pm.320\times10^{9a}$	$7.126\times10^{8}\pm.593\times10^{8bc}$	$1.856\times10^{8}\pm1.186\times10^{8c}$
$A_1 B_2$	$8.800\times10^{8}\pm1.216\times10^{8c}$	$1.026\times10^{9}\pm.220\times10^{9c}$	$1.160\times10^{9}\pm.211\times10^{9bc}$	$3.646\times10^{8}\pm1.462\times10^{8bc}$	$6.333\times10^{7}\pm2.589\times10^{7d}$
$A_2 B_2$	$8.000\times10^{8}\pm6.244\times10^{8c}$	$1.090\times10^{9}\pm.669\times10^{9c}$	$1.503\times10^{9}\pm1.470\times10^{9b}$	$2.606\times10^{8}\pm1.690\times10^{8bc}$	$3.220\times10^{7}\pm2.122\times10^{7d}$
A_3B_2	$3.000\times10^{8}\pm1.473\times10^{8de}$	$3.800\times10^{8}\pm1.833\times10^{8de}$	$1.083\times10^{9}\pm.793\times10^{9\ bc}$	$1.106\times10^{8}\pm.463\times10^{8c}$	$4.833\times10^{7}\pm1.887\times10^{7d}$

Values in a same column and with the same letter do not have significant difference (p> 0.05)

The results in table 2 presents that number of bacteria increased in all samples from an initial number of 10^7cfu/ml after 72 h fermentation at 30^0C and first week of storage at 4^0C. The maximum of growth was related to A_1B_1, that had significant difference with other samples, But A_3B_1 ($p<0.05$). Also population of lactic acid bacteria increased in all juices after 14 days storage and the highest rate of bacterial growth in 10% and 5% concentrations of mixture of Barberry and Black cherry juice were observed in A_1B_1(3.563×10^9cfu/ml) and A_2B_2(1.503×10^9cfu/ml) respectively which had no significant difference with other ratios of bacteria in these concentrations($p>0.05$). Viable cell counts of probiotic bacteria decreased in all treatments after third and forth weeks of storage at 4^0C. The maximum of viability was related to A_1B_1 by 5.320×10^8cfu/ml after 28 days of storage which had significant difference with other samples ($p<0.05$). The result showed that, time and mixture of Barberry and Black cherry juice concentration had a significant main effect on population of lactic acid cultures($p<0.01$). According to the fig3 the maximum of bacterial growth was observed in A_1B_1 after 14 days storage at 4^0C.

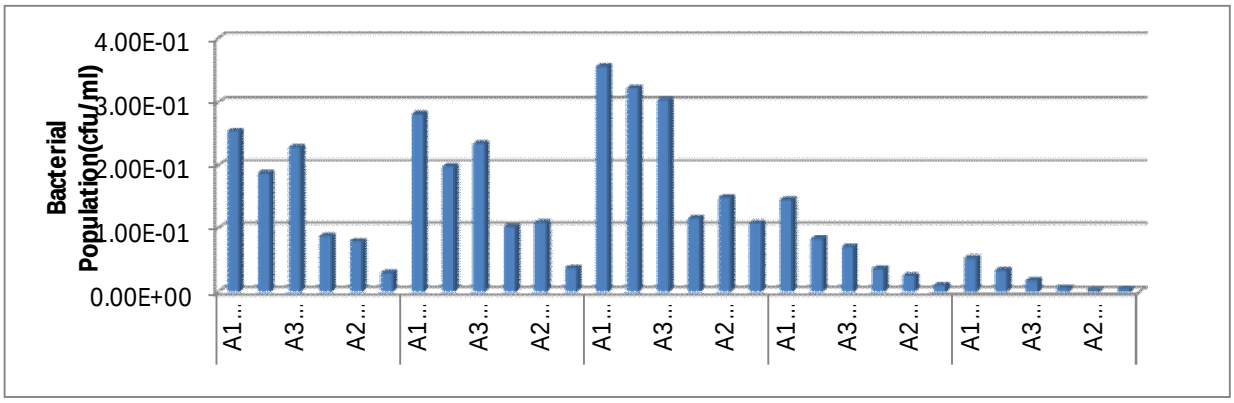

Figure 3. Effect of mixture of Barberry and Black cherry juice concentration and various ratios of bacteria in different days of cold storage on the bacteria population

Changes in reducing sugars content after fermentation

Table 3. Mean of reducing sugars (gr/100cc) in fermented mixture of Barberry and Black cherry juice with 5% and 10% concentrations and various ratios of L.casei and L.acidophilus during 72 h fermentation at 30 ^{0}C ± standard deviation

Treatment	Reducing sugar g/100cc
C_1	5.930±.000^{a}
C_2	3.690±.000^{d}
$A_1 B_1$	4.823±.015^{c}
A_2B_1	4.826±.020^{c}
A_3B_1	4.826±.011^{c}
$A_1 B_2$	3.100±.010^{g}
$A_2 B_2$	3.093±.037^{g}
A_3B_2	3.123±.015^{g}

Values in a same column and with the same letter do not have significant difference (p> 0.05)

The amount of reducing sugars in 5% and 10% concentrations of mixture of Barberry and Black cherry juice were measured before and after fermentation by Fehling method. The initial values of reducing sugars in 5% and 10% concentrations were 3.69 and 5.93 gr/100cc respectively.The amount of reducing sugars had no change in control samples after fermentation ,but this factor decreased in other samples(Fig4). Reducing sugars were dropped in all juices with 5% and 10% concentrations but was not observed significant difference in remained sugars of variation ratios of bacteria in these concentrations($p>0.05$). The maximum level of reducing sugars dropping were related to A_1B_1. The result showed that, mixture of Barberry and Black cherry juice concentration had a significant main effect on reducing sugars consumption($p<0.01$).

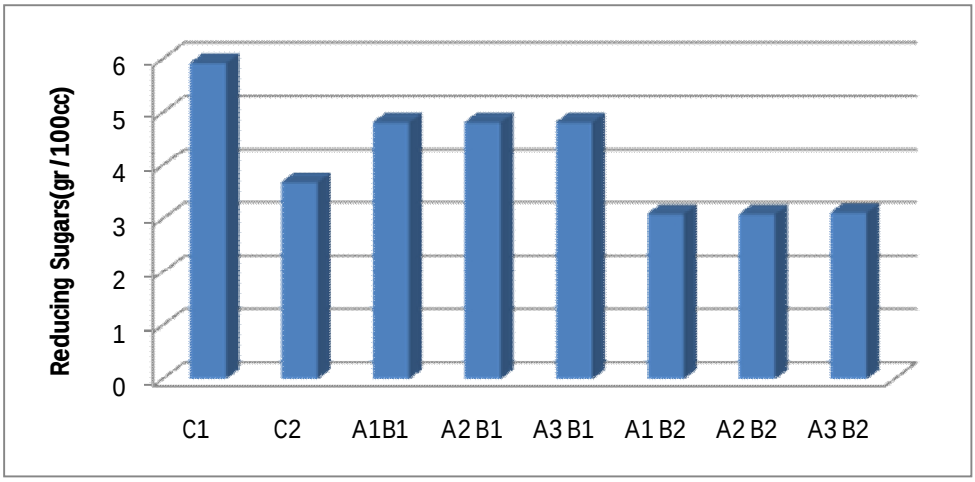

Figure 4. Effect of mixture of Barberry and Black cherry juice concentrations and various ratios of bacteria on reducing sugars during fermentation

DISCUSSION

Changes in pH and lactic acid during fermentation and cold storage

The results of this study showed that bacterial growth in the mixture of Barberry and Black cherry juice was led to increase of pH and lactic acid dropping after 72h fermentation. This may be attributed to very low sugar content in the mixture of Barberry and Black cherry juice and in contrast, its considerable amounts of organic acid i.e., lactic acid. Therefore, L.acidophilus and L.casei metabolized lactic acid as the major carbon source available in this juice during fermentation. This results are in agreement with those presented by Mousavi et al.,(2011) who studied fermentative pomegranate juice by L.acidophilus, L.paracasei, L.plantarum and L.delbrueckii. They reported the amount of citric acid from an initial value of 60 g/l reached 13-17 g/l after 48 h fermentation and selected probiotic bacteria were capable of metabolizing citric acid soon after fermentation starts, while sugar consumption by all the strains was much lower at this stage. During 4 weeks of cold storage at 4°C pH and lactic acid changes were decreasing and increasing respectively. One may explain this phenomenon due to sugars consumption and production of organic acids by lactic acid cultures(Kun et al., 2008). Some of researchers reported, pH of respberries juice after adjusting, an initial value of 4, 4.5, 5, 5.5 and 6 reached 3.9, 4.2, 4.4, 4.5 and 4.7 after 48 h fermentation at 25, 30 and 37°C(Pereira et al., 2011).

Sample of A_1B_1 (35% Lactobacillus casei + 65% Lactobacillus acidophilus) with 10% brix had minimum pH and maximum lactic acid during 4 weeks of cold storage. This results are in agreement with those presented by Yoon et al., (2005) who studied fermentative beet juice by L.casei, L.plantarum, L.acidophilus and L.delbrueckii(30°C for 72 h). They reported that, L.acidophilus decreased pH more than L.casei whereas, reduced the pH from an initial value of 6.3 to 3.7 after 72 h fermentation while, L.casei decreased pH from 6.3 to 5. Also the pH of A_1B_1 from an initial value of 3.49 reached 3.41 after 28 days cold storage. Priera et al., (2011) in an investigation showed that, inoculating L.casei to Cashew apple juice, dropped pH from an initial value of 4.28 to 3.79 at the end of 42 days of cold storage at 4^0C.Faria et al., (2006) reported, the pH decreased from 5.02 to 4 in the fermented buffalo milk containing L.casei after 30 days of refrigerated storage. Because there were much more minerals and sugars, the highest bacterial growth and therefore maximum pH reduction and increasing of organic acids were observed in 10% concentration of juices.

Changes in bacterial population during fermentation and cold storage

The data revealed, A_1B_1 had the maximum bacterial population during fermentation and the highest viable cell counts during 4 weeks of storage at 4^0C. Bacterial population of all samples increased after 2 weeks of cold storage and it dropped during the third and fourth weeks of refrigerated storage. The reason could be addressed to the lack of cultures ability to survive in the stressful condition of low pH and high acidity of the mixture of Barberry and Black cherry juice (Mousavi et al., 2011). Rivera et al., (2010) reported, in fermented milk increasing of fermentation time and pH reduction were due to accumulation of lactic acid, deacetil and acetaldehyde in the medium. These metabolites had an important effect on reducing of viable cell counts.Periera et al., (2011) found that, L.casei grew during cold storage, and viable cell counts from an initial value of 7.48 log cfu/ml reached more than 8 log cfu/ml during 42 days of storage at 4^0C. Also in present study, bacterial populationincreased during two weeks of refrigerated storage. Probably the more bacterial population in A_1B_1 could be attributed to the higher rate of L. acidophilus and faster growth of this strain than L.casei.Yoon et al., (2004) studied, fermented tomato juice by four lactic acid cultures (L.casei, L.plantarum, L.acidophilus and L.delbrueckii). They reported bacterial populationfrom initial concentration > 10^5cfu/ml reached more than 10^8cfu/ml after 48 h incubation at 30 ^{0}C. For example viable cell counts of L.acidophilus and L.casei were 1.7×10^9cfu/ml and 9×10^8cfu/ml respectively that indicated faster growth of L. acidophilus than L.casei.The viable cell counts of probiotic bacteria is important for performance of bacteria were higher than 10^7cfu/ml after 4 weeks of cold storage at 4^0C. Consequently, fermented mixture of Barberry and Black cherry juicewith 0.2% whey powder could be considered as a functional beverage.

Reducing sugars variations during fermentation

The results of sugars consumption showed, the amount of reducing sugars of all samples dropped after 72 h fermentation. The sample of A_1B_1 had the maximum reduction of reducing sugars. Sugar dropping is because of bacterial growth and organic acids production. (Tsen et al., 2003) investigated lactic acid production in medium based on mashed banana by L. acidophilus. They used of kapakaragynan gum for more efficiency of fermentation. The selected strain metabolized low molecular weight sugars i.e., fructose and glucose as a carbon source for acid production. They reported the amount of carbon sources with high molecular weight i.e., Fructo-oligo-Saccharide didn't change during fermentation, which are in agreement with the result of this study.Muraro et al., (2007) studied the production of fermented celery juice and beet juice with and without pulp by Bifidobacterium BB12.They revealed, fermentative sugar content in celery juice with pulp decreased 57.11% and viable cell counts increased 18.65% and reached 1.2×10^8cfu/ml after 48 h fermentation. In cloudy beet extract, fermentative sugar content reduced with less slope than cloudy celery extract but, increase of the bacteria number was the same as in the celery

extract. They also indicated, dropping of fermentative sugar content, increased treatable acidity. Also Buruleanuet al.,(2009) evaluated effect of inulin prebiotic on quality of produced lactic acid in carrot and beet extracts during fermentation by Bifidobacterium BB12. They reported, the amount of glucose reduced by selected strain inoculation after 48 h fermentation at 37^0C, and in contrast the amounts of lactic acid and acetic acid increased, which are in agreement with the data of present paper.

CONCLUSION

According to the results, L. casei and L. acidophilus were capable to growing and surviving in the fermented mixture of Barberry and Black cherry juicewith 0.2% whey powder during 4 weeks storage at 4^0C. This fermented beverage had adequate number of probiotic bacteria for exerting of maximum health benefits. Also results showed that, sample A1B1 (35% L.casei + 65% L.acidophilus) with 10% brix had the maximum growth during the fermentation and maximum viability during the refrigerated storage. It was due to faster growth of L. acidophilus than L. casei during the fermentation, the higher rate of this strain and much more concentration of extracts in this sample. Also, the minimum pH and maximum acidity was observed in this sample. pH reduction was due to the production of organic acids as a result of carbohydrates consumption by probiotic cells in the juice. Glucose sugar dropped during fermentation which was because of the bacterial growth and production organic acids.

REFERENCES

1. Aguirre-Ezkauriatza, E.J., J.M. Aguilar-Yanez., A. Ramirez-Medrano., M.M. Alvarez. (2010). Production of probiotic biomass (Lactobacillus casei) in goat milk whey: Comparison of batch, continuous and fed-batch cultures.Journal of Bioresource Technology. 101: 2837–2844
2. Anonymous.(2007). Fruit juices - Methods of test.Iran National Standard No. 2685.Institute of Standards and Industrial Research of Iran.
3. Anonymous.(2007). Microbiology of food and animal feed- Comprehensive approach for total counting of microorganisms at 30 ° C. Iran National standard No. 5272. Institute of Standards and Industrial Research of Iran.
4. Ashrafi, F. (2002). Practical microbiology.First published compilation, Ahsan Press.
5. Bomba, A., R. Nemcova., D. Mudronava and P. Guba. (2002). The possibilities of potentiating the efficacy of probiotics.Trends in Food Science and Technology. 13: 121-126
6. Buruleanu, L., I. Manea., M.G. Bratu., D. Avram. And C.L. Nicolescu. (2009). Effects of prebiotics on the quality of lactic acid fermented vegetable juices. Ovidius University Annals of Chemistry. 20(1):102-107.
7. Faria, C. P., H. D. Benedet and J. R. Guerrove. (2006). Parâmetros de produção de leite de búfalafermentadoporLactobacillus casei.PesquisaAgropécuáriaBrasileira. 41(3): 511–516
8. Helland, M. H., T. Wicklund and J. A. Narvhus. (2004). Growth andmetabolism of selected strains of probiotic bacteria, in maize porridge withadded malted barley. Journal of Food Microbiology. 91(3): 305– 313.
9. Kun, S., J.M. Rezessy_Szabo. Q.D. Nguyen and A. Hoschke.(2008). Changes of microbial population and some components in carrot juice during fermentation with selected Bifidobacterium strains.Process Biochemistry. 43: 816-821.
10. Uckow,T and C. Delahunty.(2004). Consumer acceptance of orange juice containing functional ingredients.Food Research International.37: 805- 814.
11. Marteau, PH., PH. Seksik and R. Jian. (2002). Probiotics and health: new facts and ideas. Food Biotechnology. 13(5):486-489
12. MazaheriTehrani, V., Yegane Azad, S.M., Moeinfard, S and Vahedi, S. (2010). Performance and use of various healthy additives in food industry. First published compilation, Mashhad: Ferdowsi University of Mashhad.
13. Mokarram, R.R., S.A. Mortazavi. M.B. HabibiNajafi and F. Shahidi.(2009). The influence of multi stage alginate coating on survivability of potential probiotic bacteria in simulated gastric and intestinal juice. Food Research International.42:1040- 1045
14. Moraru, D., I. Bleoanca and R.Segal. (2007). Probiotic vegetable juices.Food Technology.4: 87- 91.
15. Mortazavian, S. and Sohrabvandi, S. (2006). Probiotics and probiotic food products.First published compilation, Eta Press.
16. Moussavi, Z. E., S.M. Mousavi., S.H. Razavi., Z. Emam_DjomehandH. Kiani.(2011). Fermontation of pomegranate juice by probiotic lactic acid bacteria.world Microbiology &Biotechnol. 27:123-128.
17. Nosrati, R., M. Hashemiravan and M. Talebi. (2014).Fermentation of vegetable juice by probiotic bacteria .International Journal of Biosciences. 4(3): 171-180.
18. Nualkaekul, S and D. Charalampopoulos.(2011). Survival of Lactobacillus Plantarum in modle solution and fruit juices.International Journal of Food Microbiology.146: 111-117.
19. Pereira, A. L.F., T.C. Maciel and S. Rodrigues.(2011). Probiotic beverage from cashew apple juice fermented withLactobacilluscasei.Food Research International. 44: 1276-1283
20. Prado, F.C., J.L. Parada. A.Pandey and C.R. Soccol.(2008). Trends in non_dairy probiotic beverages.Food ResearchInternational. 41: 111-123
21. Rivera Espinoza,Y and Y. Gallardo Navarro. (2010). Non_dairy Probiotic products. Food Microbiology.27: 1-11.
22. Roble, C., M.A.E.Auty. N. Brunton. R.T. Gormley and F.Butler.(2010). Evaluation of fresh- cut appleslices enriched with probiotic bacteria. Innovative Food and Emerging Technologies.11: 203-209.

23. Tsen, J.H., Y. P. Lin and V.A.E. King. (2003). Banana purees fermentation by Lactobacillus acidophilus immobilized in Ca-alginate. Journal of General Application and Microbiology. 49(6):357–361.
24. Vitali, B., G.Minervini. C.G. Rizzello. E.Spisni. S. Maccaferri. P. Brigidi. M. Gobbetti and R.DiCagno. (2012). Novel probiotic candidates for humans isolated from raw fruits and vegetables.Food Microbiology.30:1- 10.
25. Vinderola, C. G., & Reinheimer, J. A. (2000). Enumeration of Lactobacillus casei in the presence ofL. acidophilus. Bifidobacteria and lactic starter bacteria in fermented dairy products. International Dairy Journal, 10(4), 271-275.
26. Von Mollendorff, J. W. (2008). Characterization of bacteriocins produced by lactic acid bacteria from fermented beverages and optimization of starter cultures.Thesis presented in partial fulfillment of the requirements for the degree of master of science at the university of Stellenbosch.
27. Yoon, K.Y., E.E. Woodams and Y.D. Hang.(2004). Probiotication of tomato juice by lactic acid bacteria. The Journal of Microbiology. 42:315-318.
28. Yoon, K.Y., E.E. Woodams and Y.D. Hang.(2005). Fermentation of beet juice by beneficial lactic acid bacteria.Lebensm._Wiss. U._Technol. 38: 73- 75