

Development of lactic acid fermented, probiotic sour cherry juice

JUDIT PERJÉSSY* , FERENC HEGYI, MAGDOLNA NAGY-GASZTONYI, RITA TÖMÖSKÖZI-FARKAS and ZSOLT ZALÁN

National Agricultural Research and Innovation Centre, Food Science Research Institute, Budapest, Hungary

CONFERENCE FULL PAPER

Received: January 31, 2020 • Accepted: September 8, 2020

Published online: October 13, 2020



© 2020 The Author(s)

ABSTRACT

Nowadays, demand for products which beyond the overall nutritional value have a feature that protects the consumer health, have increased. Several studies have proved that fruit juices can become a suitable carrier or medium for probiotic organisms. Therefore, the aim of our study was to investigate the possibility of the probiotication of sour cherry juice (SCJ) by fermentation with probiotic starter culture. During the fermentation 9 *Lactobacillus* strains were used and Újfehértói fűrtös sour cherry species as raw material. To reach the recommended probiotic cell count we investigated the pH adjustment, supplementation of nutrients, the effect of dilution, and strain adaptation to SCJ. In our study the properties of the strains – such as reproduction and metabolism – and its effect on the raw material were investigated. A significant difference was observed between the number of viable cells of certain *Lactobacillus* strains, that is important in point of view of the development of probiotic-containing products. Furthermore, the lactic acid fermented SCJ can enhance the polyphenol content and antioxidant activity to promote the health of consumers.

KEYWORDS

functional food, lactofermented, plant-based, probiotic, sour cherry juice

INTRODUCTION

Traditional fermented foods and beverages form an integral part of cultural heritage and these foods are widely consumed since ancient civilization and still form part of the human diet.

* Corresponding author. E-mail: perjessy.judit@eki.naik.hu

Fermented foods are less perishable than the original raw materials, their nutritional value may be enhanced, and the safety of these foods may be improved by the low pH and the presence of organic acids and antimicrobial compounds (Narvhus et al., 2003).

Lactic acid bacteria (LAB) are predominant microflora present in most of the traditional fermented foods and their role in fermentation is known since ages (Anandharaj and Sivasankari, 2013). *Lactobacillus* strains are important members of LAB and play essential role in lactic acid fermentation. Many of members of the *Lactobacillus* genus have been proven to have a health benefit effect on consumers and are considered as probiotics, live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2001).

Lactic acid fermentation of vegetables and the role of *Lactobacillus* strains in this process have been investigated for a long time and it is well-described in the case of, e.g., olive (Leal-Sánchez et al., 2003; Randazzo et al., 2004), kimchi (Rhee et al., 2011) and sauerkraut (Halász et al., 1999; Leroy and De Vuyst, 2004). Traditionally, many of the fermented foods are produced under spontaneous fermentation, however, by the application of *Lactobacillus* strains – which have good fermentation properties – as selected starter culture, microbiological safety and constant quality would be assured.

During the past few decades significant success has been achieved in the development of dairy products containing probiotics, such as fermented milks, ice cream (Akalin et al., 2018) and cheese (Blaiotta et al., 2017). But nondairy probiotic products have a large worldwide importance due to the ongoing trend of veganism and to a high prevalence of lactose intolerance in many populations around the world. Therefore, a product that combines the benefits of lacto-fermented plant-based products and probiotic microorganisms would be important. Development of fruit-based probiotic products could provide a solution. It would be an ideal substrate for a fermented product, as they play an important role in human nutrition and contain many beneficial ingredients (minerals, vitamins, and dietary fibers) and at the same time are free from milk's allergens.

Sour cherry is one of the oldest cultivated fruit species and its production is continuously increasing in the world. Hungary is one of the leading countries in sour cherry production (FAOSTAT, 2017), it always has been a popular fruit and its cultivation is very important in Hungary. Sour cherries offer a healthy dose of the antioxidant vitamin C (15 mg/100 g) (Sredojevic, 2014), and due to the high content of polyphenols – because the fruit juices, mainly prepared from red fruits, are the most available sources of anthocyanins and polyphenols (Chiara et al., 2011) – it has an important role maintaining human health (Repajić et al., 2015). It is suitable raw material in the food processing industry used in production juices, compotes, confectionery, liquor, and marmalade.

The aim of this study was to develop a fermented product that combines the beneficial effects of lacto-fermented sour cherry juices (SCJs) and probiotics. By investigating the fermentation properties of different *Lactobacillus* strains we wanted to find the most suitable probiotic strain(s) to produce a high added value fermented SCJ.

MATERIALS AND METHODS

Microorganisms

Six well-known strains, *Lactobacillus* (*L.*) *rhamnosus* GG, *Lactobacillus acidophilus* 150, *L. acidophilus* La-5, *Lactobacillus casei* Shirota, *L. casei* LC-01, *Lactobacillus reuteri* DSM 17938, and



three nonstarter dairy, nonprobiotic strains, *Lactobacillus plantarum* 2142, *L. acidophilus* N2, *Lactobacillus fermentum* DT41, were used in the experiments of fermentation.

Raw material

The Újfehértói fűrtös sour cherry species was provided by National Agricultural Research and Innovation Centre, Fruitculture Research Institute. Fruit juice was made with fruit centrifuge (Biovita-25 Automata Soy Milk Meaker) and the extracted juice filtered twice through two strainers with 1.0 and 0.8 mm pore diameter to make a clear juice. Following the processing of the fruit, due to the presence of high number of total autochthonous cell number on the raw sour cherries, pasteurization of the juice was necessary.

Fermentation experiments

Fermentation experiments were conducted in test tubes, each containing 10 mL SCJ media. All samples were inoculated with a 24 h old culture (1% (v/v), initial cell concentration in SCJ was $7 \log \text{cfu mL}^{-1}$) and were incubated aerobically at 30 °C for 24 h. At the 0th and 24th h of the fermentation sample was taken.

The SCJ was investigated in native form (without supplementation and pH adjustment, as control) and in supplemented form, with pH adjustment, dilution, and strain adaptation to SCJ, separately and in combination. In strain selection the SCJ was supplemented with 3 g L⁻¹ yeast extract (Lab M Ltd., United Kingdom) to promote the propagation of the starter probiotic strains, diluted with sterile water (60% SCJ, 40% water) and the pH was adjusted to 5.6 with sterile 2 M NaOH solution (according to the optimization, see in Effect of pH and strain adaptation to SCJ and Effect of dilution and supplementation).

Determination of viable cell number of *Lactobacillus*, pH, titratable acidity and degrees Brix

The Miles and Misra Method (Miles et al., 1938) was used to determine the exact number of colony forming units with de Man, Rogosa and Sharpe (MRS) agar (Merck KGaA, Germany) as a *Lactobacillus* selective medium. Samples from the juices were diluted (in 9 mL physiological salt solution (0.85%) complemented with peptone (0.1%)) by 10-fold and 20 µL of the aliquots were dropped onto Petri dishes. The plates were incubated at 30 °C for 48 h and the colonies were counted.

The pH was measured using digital pH meter (Mettler-Toledo GmbH, Switzerland). The titratable acidity in SCJ was determined by titration of 1 mL of juice with addition of 9 mL of distilled water, with 0.1 M NaOH solution and addition of phenolphthalein solution (1% (m/V) in ethanol) as indicator, until the pink phenolphthalein end-point. Titratable acidity was expressed as % (m/V) of lactic acid equivalents in SCJ. The total soluble solids content of SCJ was determined using a digital refractometer (Schmidt+Haensch GmbH & Co., Germany), and the results were expressed in °Bx.

Determination of bioactive compounds

The SCJs total phenolic content was estimated spectrophotometrically using Folin Ciocalteu method (Singleton et al.,1999) and antioxidant capacity was measured by ferric reducing



Table 1. Elution program for the separation of anthocyanins and phenolic components

Time (min)	A (%)	B (%)
0.01	98.5	1.5
5	85	15
12	85	15
20	75	25
28	50	50
30	50	50
33	98.5	1.5
35	98.5	1.5

antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods. The ferric reducing-antioxidant power test was performed according to [Benzie and Strain \(1996\)](#), while the methanolic extracts (80% (v/v)) were also used to assess the antioxidant capacity by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging method ([Brand-Williams et al., 1995](#)).

For the anthocyanins and flavonols content measurement we used high pressure liquid chromatography (HPLC) method. One gram of homogenized cherries was weighed into 2 mL Eppendorf tubes, centrifuged for 5 min (10,000 rpm). The supernatant was set aside, and the centrifugation residue was dissolved in 1 mL of eluent (acetonitrile : 1% formic acid in distilled water = 1.5 : 98.5) and vortexed. It was placed in an ultrasonic water bath for 10 min and then centrifuged again. The combined supernatants were filtered through 0.45 μm syringe filter and the filtrate was injected.

Waters Alliance (USA) chromatography system consisting of a Model 2695 separation module, a Model 2996 photodiode-array (DAD) and a Model 2475 fluorescence (FLD) detectors was used for the chromatographic analysis of the anthocyanin and flavonol components. Separation was performed using a MN Sphinx 5 μm 250 \times 4.6 mm column. The system was operated by Empower software. Gradient elution was used according to [Table 1](#), where the eluents were (A) 1% formic acid in distilled water and (B) acetonitrile. The flow rate was 0.7 mL/min and the injected volume was 10 μL . Anthocyanins were determined at 520 nm while phenolic compounds were measured at 320, and 355 nm. Standards were used to identify components. Calibration was performed with a rutin standard at different wavelengths, and the amount of components was given in rutin equivalents.

All the samples were analyzed in triplicates. The values from all determinations ($n = 3$) of each sample were averaged and represented as means with standard deviations.

Central Composite Design and statistical evaluation of data

The Response Surface Methodology (RSM) based on Central Composite Design (CCD) was used to minimize the heat treatment and to evaluate and optimize the combined effect of fermentation's parameters (such as pH, extent of strains' preliminary adaptation to the raw material, added yeast extract as well as dilution). The design has 4 factorial points, 4 star points and 2 central points (total runs = 10). For the distance between center points and star-points the axial distance $\alpha = 1.414$ was used. The experiment is designed to allow us to estimate interaction and even quadratic effects, and therefore give us an idea of the (local) shape of the response surface. For the minimalization of the heat treatment the independent variables of the



pasteurization were the time and the temperature, and the response (dependent variable) was the total mezophilic aerob living cell number. The range of the values of the variables were 0–35.8 min for time and 31.7–88.3 °C for temperature. To reach the recommended probiotic cell count the independent variables of the optimalization were the pH and the amount of the SCJ for the adaptation and the response was the viable cell count of *L. rhamnosus* GG. The range of the values of the variables was 2.62–6.58 for pH and 1.38–5.60 mL SCJ in 10 mL MRS–SCJ for adaptation. During the second CCD for optimalization the independent variables of the optimalization were the yeast extract concentration and the amount of the SCJ (for the dilution) and the dependent variable was the viable cell count of *L. rhamnosus* GG. The range of the values of the variables were 0.17–5.82 g L⁻¹ for yeast extract and 1.75–10 mL SCJ in 10 mL water–SCJ for dilution.

For statistical evaluation Minitab Statistical Software and Statistica (StatSoft Inc.) software were used. The one-way analysis of variance (one-way ANOVA) is a technique that can be used to compare means of two or more samples (using the *F* distribution).

RESULTS

Preliminary experiments

Because of the high number of total cell count in the raw sour cherries, after the processing of the fruit, pasteurization of the juices was necessary. To minimize the heat treatment, the process parameters for the pasteurization was optimized with the combined effect of temperature (range is between 31.7 and 88.3 °C) and time (range is between 0 and 35.8 min) by CCD and for the evaluation RSM was used (Fig. 1). Heating in a water bath at 60 °C for 15 min (and after cooled down back) proved to be the most effective pasteurization of the raw material,

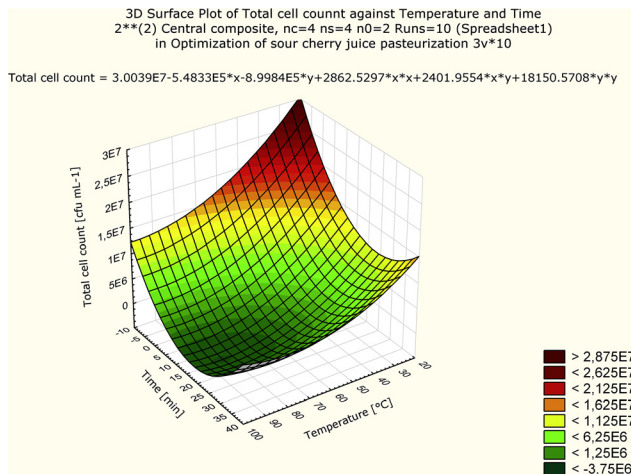


Fig. 1. Response surface plot for minimum total cell number of cherry juice as a function of temperature and treatment time

where the indigenous flora was totally eliminated, while the pasteurization effect was minimal. This pasteurization time and temperature fall within the range of mild temperature-long time (MTLT) pasteurization (Ağçam et al., 2018) that is applied for improving minimally processed food products with longer shelf life.

In preliminary experiments the juice was inoculated with *L. rhamnosus* GG in natural form and incubated at 30 °C for 24 and 48 h. It was observed that SCJ in its natural form does not provide an adequate environment for the growth of *Lactobacillus*, already after 24 h the fermentation showed a decrease of the viable cell number of *Lactobacillus* in SCJ (data not shown).

Effect of pH and strain adaptation to SCJ

Because of the optimum pH range of *Lactobacillus* is between 5.5 and 6.2 (Olivares et al., 2019), the pH adjustment of the original acidic pH to a neutral value with sterile NaOH resulted in a one-tenth increase in order of magnitude (data not shown).

Despite that phenolic compounds can also inhibit cell growth, this negative effect can be eliminated by adapting *Lactobacillus* to polyphenol (Perricone et al., 2014). To increase the viability by inducing a moderate resistance to phenols a preliminary adaptation was carried out. The SCJ was diluted with MRS broth in different ratio that inoculated with a 24 h old *L. rhamnosus* GG culture and incubated at 30 °C for 24 h. Afterwards, these cultures were used as an inoculum for SCJ fermentation. Therefore, the combined effect of pH (range is between 2.62 and 6.58) and adaptation (range is between 1.38 and 5.60 mL SCJ in 10 mL MRS–SCJ) on growth of *Lactobacillus* strain in SCJ was studied through a CCD. The growth of *Lactobacillus* is affected by only the linear variable of pH of the independent variables, the adaptation had no significant effect. At the same time, we could determine the initial optimal pH for the cell growing based on the response surface (Fig. 2), which has given for this parameter the value of 5.8.

Effect of dilution and supplementation

In order to reach the recommended probiotic cell count, we investigated the supplementation of nutrients, such as bacteriological peptone (10 g L⁻¹, Lab M Ltd., United Kingdom), dextrose (20 g L⁻¹, Lab M Ltd., United Kingdom) and yeast extract (4 g L⁻¹, Lab M Ltd., United Kingdom). To prevent the inhibitory effect of the polyphenols, the dilution of SCJ with water (ratio of water to SCJ was 1:1 and pH was adjusted to 5.8) was investigated, because the inhibitory effect of polyphenols on bacteria depends on the composition and the concentration of the polyphenols (Tabasco et al., 2011).

SCJ diluted with water as well as the addition of yeast extract have a positive effect on the *Lactobacillus* growth. Therefore, the ideal values of yeast extract concentration (range is between 0.17 and 5.82 g L⁻¹) and ratio of dilution (range is between 1.75 and 10 mL SCJ in 10 mL water–SCJ) were also optimized by CCD. As shown on the response surface (Fig. 3) to aid fermentation 3 g L⁻¹ added yeast extract will be sufficient if water is added to SCJ in ratio 4:6 (v/v) (60% SCJ, 40% water). The positive effect of yeast extract on the number of LAB was also confirmed in fruit juice, which favorable effect was already detected in vegetable juice (Rakin et al., 2007).

Strain selection based on *Lactobacillus* growth

For the strain selection 9, among them 6 probiotic *Lactobacillus* strains were used and one species of sour cherry (Újfehértói fürtös) as raw material. Because the added 3 g L⁻¹ yeast extract



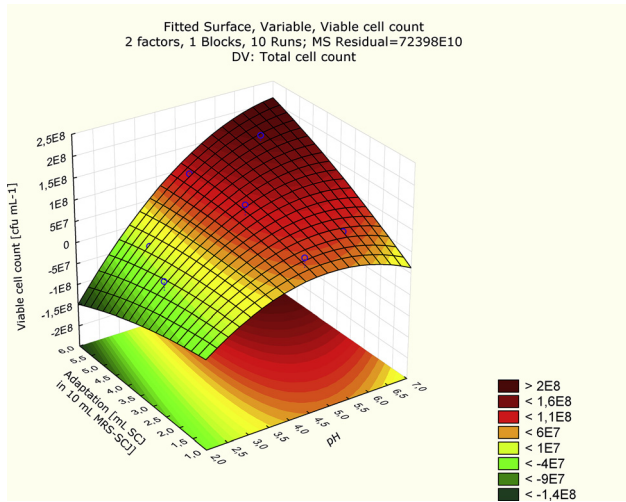


Fig. 2. Combined effect of pH and adaptation on the number of viable cells in sour cherry juice

as well as the pH adjusting to 5.8, and the dilution (ratio of SCJ to water was 6:4) resulted in the highest ($9.91 \log \text{cfu mL}^{-1}$) cell number and the pH decreasing to the sufficiently low value (4.28) after 24 h, these initial parameters were adjusted to the given values before the inoculation.

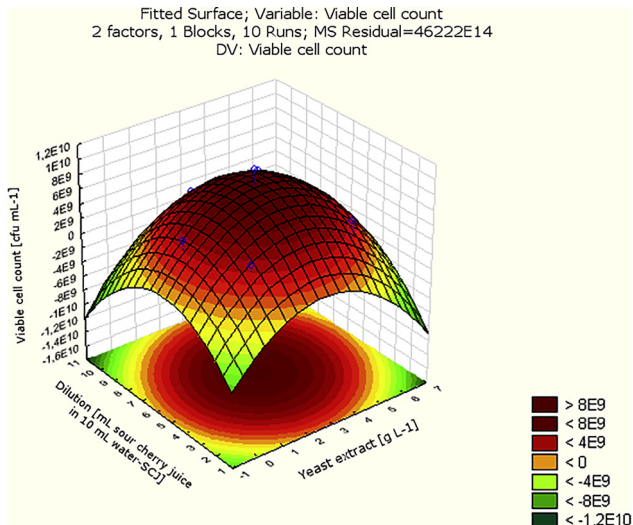


Fig 3. Response surface plot for maximum *Lactobacillus* cell number of fermented cherry juice as a function of dilution ratio and yeast extract concentration



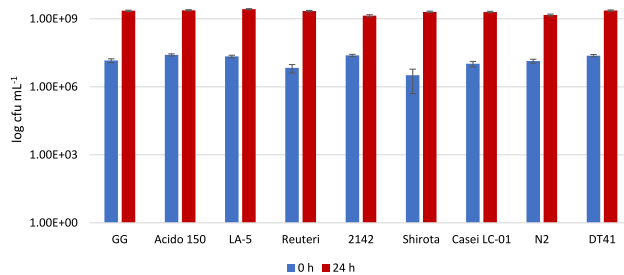


Fig. 4. Viable cell number of *Lactobacillus* strains in log cfu mL⁻¹ in sour cherry juice

Despite the fact that all investigated strains reached the desired 9 log cfu mL⁻¹ cell density (Fig. 4) after 24 h, a significant difference was observed between the number of viable cells of certain *Lactobacillus* strains. In Újfehértói fűrtös sour cherry the cell number of *L. acidophilus* La-5 strain resulted in the highest probiotic cell number (9.43 log cfu mL⁻¹) and it was statistically significant ($P < 0.05$) compared to the other strains. These cell concentrations are similar to the reported cell density reached in other high polyphenol containing fruit juices, such as in elderberry (7.05–9.50 log cfu mL⁻¹, Ricci et al., 2018, Cirlini et al., 2019) and sweet cherry (9.5 log cfu mL⁻¹, Di Cagno et al., 2011).

The effect of fermentation on pH, titratable acidity, and total soluble solids

Before the inoculation, the SCJs initial pH was adjusted to 5.8 following the supplementation and dilution. The pH decreased to the value 4.21–4.56 (Table 2) after 24 h fermentation, which pH value is still at and under the value of 4.5, which is low enough to prevent the growth of the most microorganisms (Lund et al., 2000). There was statistically significant difference between certain *Lactobacillus* strains, but from the point of view of fermentation and product development, that minimal deviation cannot be considered significant – similarly in terms of titratable acidity (0.4444–0.9548% (m/V) lactic acid) and total soluble solids (10.1–10.6 °Bx) (Table 2).

Bioactive compounds

Total polyphenol. At the end of the fermentation large difference was measured in polyphenol content (Table 2) between *Lactobacillus* strains, but statistical evaluation did not show significant differences, except between *L. acidophilus* N2 and *L. acidophilus* 150, and between *L. acidophilus* N2 and *L. fermentum* DT41 used as a starter culture. But in the case of some strains (e.g., *L. acidophilus* 150) the total polyphenol content was remarkably higher at the end of the fermentation than in the initial, adjusted juice. This can be explained by the lactic acid fermentation being capable of increasing the phenolic compounds by the release of the bioactive compounds from the fruit cells (Cirlini et al., 2019; Muhialdini et al., 2020), and by the lower pH formed by the acidification of LAB being favorable for the stability of polyphenols (Panda et al., 2016).

Antioxidant capacity (FRAP and DPPH). The LAB with the β -glucosidase activity (including *L. acidophilus*, *L. casei*, *L. plantarum*, *L. fermentum*) can increase the aglycone during the



Table 2. pH, titratable acidity (TA) in % (gram/100 mL), total soluble solids (TSS) in °Bx, polyphenol content in mg GAE kg⁻¹, antioxidant capacity (FRAP) in mM Fe(II) kg⁻¹ and free radical scavenging capacity (DPPH) in mMTr kg⁻¹ in fermented sour cherry juice

<i>Lactobacillus</i> strain	pH	TA (%)	TSS (Bx°)	Polyphenol (mg GAE kg ⁻¹)	FRAP (mM Fe(II) kg ⁻¹)	DPPH (mMTr kg ⁻¹)
∅ (0 h control)	5.82	0.1743	11.6	552	6.29	2.61
<i>L. rhamnosus</i> GG	4.21	0.8528	10.1	558	6.29	2.78
<i>L. acidophilus</i> 150	4.41	0.5585	10.3	706	6.44	3.69
<i>L. acidophilus</i> La-5	4.37	0.5044	10.3	584	6.46	3.27
<i>L. casei</i> Shirota	4.28	0.6426	10.2	573	6.57	3.36
<i>L. casei</i> LC-01	4.41	0.9548	10.5	505	6.22	3.29
<i>L. reuteri</i>	4.32	0.7807	10.1	569	6.21	3.66
<i>L. plantarum</i> 2142	4.56	0.4444	10.2	558	6.24	3.50
<i>L. acidophilus</i> N2	4.54	0.4444	10.6	449	6.28	3.02
<i>L. fermentum</i> DT41	4.37	0.6125	10.2	667	6.36	3.61

fermentation. The aglycone acts as an antioxidant (Marazza et al. 2009). Our results (Fig. 5) showed that *L. rhamnosus* GG can produce the fermented Újfehértói fűrtös SCJ by retaining the antioxidant activity. Although in the case of certain *Lactobacillus* strains in the fermented juice the antioxidant capacity decreased, while in the case of *L. acidophilus* 150, *L. acidophilus* La-5, *L. casei* Shirota and *L. fermentum* DT41 fermented SCJ has higher antioxidant activity compared to the initial juice, but there is no significant difference. The juice has showed higher free radical scavenging capacity after 24 h fermentation in the case of all *Lactobacillus* strains used as a starter culture (Fig. 6), that could mean up to 40% increase compared to the 0 h sample (in the case of *L. acidophilus* 150) and the statistical test showed significant differences between certain strains (e.g., *L. rhamnosus* GG and *L. acidophilus* 150).

Anthocyanins and flavonols. Anthocyanin is the most unstable compound among natural products that is degraded by many factors, such as temperature, oxygen, light, pH, and enzymes.

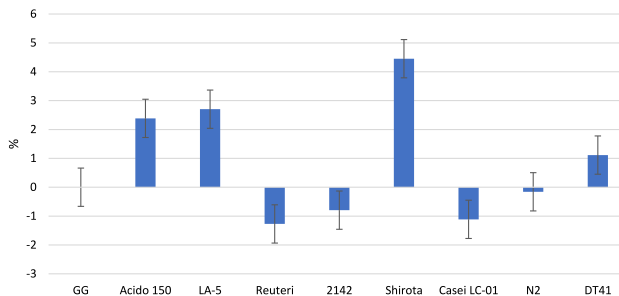


Fig. 5. Effect of lactic acid fermentation on antioxidant capacity in % in sour cherry juice compared to the 0 h control



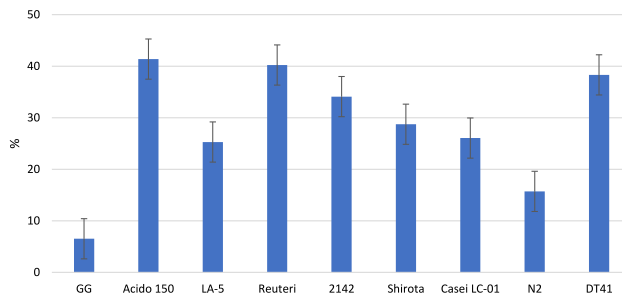


Fig. 6. Effect of lactic acid fermentation on free radical scavenging capacity in % in sour cherry juice compared to the 0 h control

It has different stabilities and color because its chemical structures depend on pH (Jung et al., 2020). Because of the acid that was produced by the LAB due to the fermentation the pH decreased, which caused the increasing of the most specific anthocyanin's, the cyanidine derivatives' content (Table 3). In 0 h control, compared to the raw material, the anthocyanin content decreased with dilution and pH adjustment, but the main anthocyanin constituents of sour cherry samples, the cyanidine derivatives increased remarkably in fermented juices (Fig. 7).

Dietary polyphenols, such as phenolic acids, are considered to be powerful antioxidants, but that is only one of the many mechanisms through which polyphenols can exert their actions (Mattila et al., 2006). From phenolic compounds, flavonols were measured at 355 nm, in fermented SCJ rutin and quercetin could be detected (Table 4). Rutin content increased in every sample compared to the 0 h control, but quercetin decreased with the fermentation (except *L. plantarum* 2142). In the case of the anthocyanins and flavonols content, a significant difference was not observed between the fermented SCJs. Jakobek and Seruga (2012) reported in the case of SCJ that the dominant hydroxycinnamic acids were caffeic acid derivatives, including neochlorogenic acid, chlorogenic acid, and caffeic acid. We detected five phenolic acid derivatives at 320 nm in the fermented SCJ (Table 5). The phenolic acid content – except coumaric acid – was higher at the end of the fermentation than in the initial,

Table 3. Anthocyanin content in fermented sour cherry juice (integrated areas were measured in a concentration of 1 g mL^{-1}), $\lambda = 520 \text{ nm}$

<i>Lactobacillus</i> strain	1. Cyanidine derivative (integrated areas)	2. Cyanidine derivative (integrated areas)
Ø (0 h control)	117,268 ± 9,986	13,239 ± 1,051
<i>L. rhamnosus</i> GG	637,722 ± 40,245	54,321 ± 332
<i>L. acidophilus</i> 150	650,213 ± 5,173	55,493 ± 2,044
<i>L. acidophilus</i> La-5	612,537 ± 12,256	54,643 ± 4,820
<i>L. reuteri</i> DSM 17938	633,482 ± 5,681	55,051 ± 972
<i>L. plantarum</i> 2142	652,538 ± 1,767	60,801 ± 241
<i>L. casei</i> Shirota	661,657 ± 27,189	62,386 ± 3,281
<i>L. casei</i> LC-01	633,266 ± 1,428	59,355 ± 2,017
<i>L. acidophilus</i> N2	594,577 ± 2,544	53,256 ± 343
<i>L. fermentum</i> DT41	650,631 ± 10,159	56,256 ± 1,850



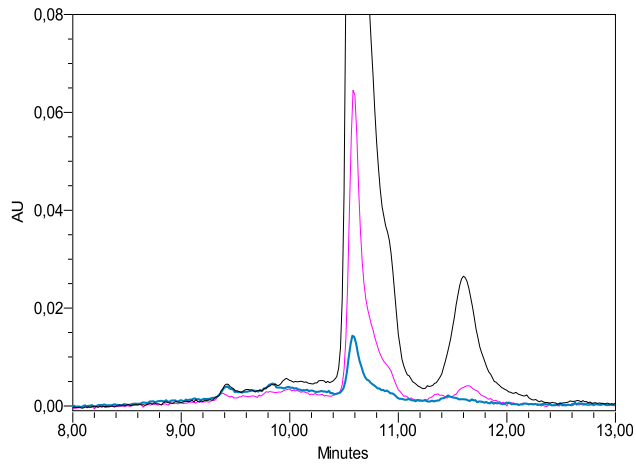


Fig. 7. Cyanidine content, chromatogram ($\lambda = 520$ nm) of sour cherry juice samples (blue: 0 h control, magenta: fermented juice (*L. casei* LC-01), black: raw sour cherry juice)

adjusted juice. This can be explained by the spontaneous or enzymatic degradation of the complex polyphenol components into smaller parts, for example phenolic acid. But *L. plantarum* has already been reported as having the ability to degrade some hydroxycinnamic acids (e.g., coumaric and caffeic acid) and some hydroxybenzoic acids involving decarboxylation and reduction reactions of phenolic acid (Rodríguez et al., 2008).

In the case of the anthocyanins and flavonols content, a significant difference was not observed between the fermented SCJs.

DISCUSSION

Our results showed the importance of ensuring an adequate environment for growth of *Lactobacillus* in SCJ. Adjusting pH to 5.8, added 3 g L⁻¹ yeast extract and dilution of SCJ (60%

Table 4. Polyphenol content in fermented sour cherry juice (rutin equivalent, mg L⁻¹), $\lambda = 355$ nm

<i>Lactobacillus</i> strain	Rutin (mg L ⁻¹)	Quercetin derivative (mg L ⁻¹)
Ø (0 h control)	0.81 ± 0.06	0.59 ± 0.04
<i>L. rhamnosus</i> GG	0.93 ± 0.01	0.43 ± 0.01
<i>L. acidophilus</i> 150	0.94 ± 0.02	0.48 ± 0.03
<i>L. acidophilus</i> La-5	1.03 ± 0.07	0.44 ± 0.01
<i>L. reuteri</i> DSM 17938	0.92 ± 0.04	0.48 ± 0.04
<i>L. plantarum</i> 2142	1.04 ± 0.04	0.63 ± 0.05
<i>L. casei</i> Shirota	0.87 ± 0.00	0.44 ± 0.02
<i>L. casei</i> LC-01	0.93 ± 0.02	0.45 ± 0.02
<i>L. acidophilus</i> N2	0.95 ± 0.06	0.38 ± 0.01
<i>L. fermentum</i> DT41	0.98 ± 0.10	0.43 ± 0.01



Table 5. Polyphenol content in fermented sour cherry juice (rutin equivalent, mg L⁻¹), $\lambda = 320$ nm

<i>Lactobacillus</i> strain	unknown (mg L ⁻¹)	Neochlorogenic acid (mg L ⁻¹)	Coumaric acid derivative (mg L ⁻¹)	Chlorogenic acid (mg L ⁻¹)	Cinnamic acid derivative (mg L ⁻¹)
Ø (0 h control)	2.15 ± 0.22	104.20 ± 11.05	18.93 ± 0.05	14.22 ± 0.62	7.44 ± 0.82
<i>L. rhamnosus</i> GG	2.37 ± 0.07	146.38 ± 4.51	17.50 ± 0.19	19.88 ± 0.46	18.99 ± 0.35
<i>L. acidophilus</i> 150	2.44 ± 0.08	139.35 ± 1.54	16.78 ± 0.23	19.66 ± 0.74	20.30 ± 0.26
<i>L. acidophilus</i> La-5	2.28 ± 0.05	141.79 ± 0.06	17.80 ± 0.12	19.40 ± 0.22	19.00 ± 0.30
<i>L. reuteri</i> DSM 17938	2.08 ± 0.10	141.18 ± 0.26	17.22 ± 0.38	19.27 ± 0.12	20.34 ± 0.35
<i>L. plantarum</i> 2142	2.50 ± 0.06	147.15 ± 1.23	14.74 ± 0.07	12.61 ± 0.04	9.39 ± 0.11
<i>L. casei</i> Shirota	2.43 ± 0.17	139.46 ± 5.23	16.59 ± 0.64	19.79 ± 0.73	20.55 ± 0.87
<i>L. casei</i> LC-01	2.40 ± 0.04	137.85 ± 1.22	16.43 ± 0.05	19.57 ± 0.22	24.17 ± 0.23
<i>L. acidophilus</i> N2	2.26 ± 0.03	134.78 ± 2.28	16.59 ± 0.89	19.18 ± 0.04	23.43 ± 0.79
<i>L. fermentum</i> DT41	2.62 ± 0.08	141.88 ± 0.19	16.87 ± 0.21	19.64 ± 0.24	20.45 ± 0.27

SCJ, 40% water) resulted in the highest (9 log cfu mL⁻¹) cell number and the pH decreasing to the sufficiently low value (4.28) after 24 h. During the fermentation all investigated *Lactobacillus* strains reached the desired 9 log cfu mL⁻¹ cell density, that is important in point of view of the development of probiotic-containing products, according to the recommendation by the International Dairy Federation (IDF) the minimum number of probiotic cells in the product at the moment of consumption should be ≥ 7 log cfu g⁻¹ (Beena Divya et al., 2012). But a significant difference was observed between the number of viable cells of some *Lactobacillus* strains. These cell concentrations are similar to the reported cell density reached in other high polyphenol containing fruit juices, such as in elderberry (Ricci et al., 2018, Cirlini et al., 2019) and sweet cherry (Di Cagno et al., 2011). Furthermore, it could be concluded from our results that the lactic acid fermentation could increase the concentration of the bioactive compounds. Like conversion of the phenolic acids and flavonoids in goji juice was influenced by fermentation, and the antioxidant capacity improved significantly (Liu et al., 2019).

CONCLUSIONS

The results suggest that fermented SCJ is a promising source for use as functional, nondairy probiotic food with a characteristic of preserving the health of the consumer. However, the lacto-fermentation of the SCJ in its natural form is not satisfying, especially from the point of view of the living cell number. We have determined and optimized the parameters, which promote the lacto-fermentation of sour cherry and minimize the supplementation and the level of treatments to develop a near-natural, probiotic juice. The preservative-free fermented SCJ, in



addition to their natural acidity resulting from the fermentation, preserves its organoleptic properties and natural components, moreover, the quantity of bioactive components increase.

REFERENCES

- Ağçam, E., Akyıldız, A., and Dündar, B. (2018). Thermal pasteurization and microbial inactivation of fruit juices. *Fruit Juices*: 309–339.
- Akalın, A.S., Kesenkas, H., Dinkci, N., Unal, G., Ozer, E., and Kınık, O. (2018). Enrichment of probiotic ice cream with different dietary fibers: Structural characteristics and culture viability. *Journal of Dairy Science*, 101(1): 37–46.
- Anandharaj, M. and Sivasankari, B. (2013). Isolation of potential probiotic *Lactobacillus* strains from human milk. *International Journal of Research and Development in Pharmacy & Life Sciences*, 1: 26–29.
- Beena Divya, J., Kulangara Varsha, K., Madhavan Nampoothiri, K., Ismail, B., and Pandey, A. (2012). Probiotic fermented foods for health benefits. *Engineering in Life Sciences*, 12(4): 377–390.
- Benzie, I.F.F. and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Analytical Biochemistry*, 239(1): 70–76.
- Bliotta, G., Murru, N., Di Cerbo, A., Succi, M., Coppola, R., and Aponte, M. (2017). Commercially standardized process for probiotic “Italice” cheese production. *LWT – Food Science and Technology*, 79: 601–608.
- Brand-Williams, W., Cuvelier, M.E., and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology*, 28: 25–30.
- Chiara, F., Dugo, L., D’Orazio, G., Lirangi, M., Dachà, M., Dugo, P., and Mondello, L. (2011). Analysis of anthocyanins in commercial fruit juices by using nano-liquid chromatography electrospray-mass spectrometry and high performance liquid chromatography with UV-vis detector. *Journal of Separation Science*, 34: 150–159.
- Cirlini, M., Ricci, A., Galaverna, G., and Lazzi, C. (2019). Application of lactic acid fermentation to elderberry juice: Changes in acidic and glucidic fractions. *LWT – Food Science and Technology*, 108779.
- Di Cagno, R., Surico, R.F., Minervini, G., Rizzello, C.G., Lovino, R., Servili, M., Taticchi, A., Urbani, S., and Gobetti, M. (2011). Exploitation of sweet cherry (*Prunus avium* L.) puree added of stem infusion through fermentation by selected autochthonous lactic acid bacteria. *Food Microbiology*, 28: 900–909.
- FAO (2001). *Report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria*. Cordoba, Argentina.
- FAOSTAT (2017). www.fao.org/faostat.
- Halász, A., Baráth, Á., and Holzapfel, W.H. (1999). The influence of starter culture selection on sauerkraut fermentation. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung. A*, 208: 434–438.
- Jakobek, L. and Seruga, M. (2012). Influence of anthocyanins, flavonols and phenolic acids on the anti-radical activity of berries and small fruits. *International Journal of Food Properties*, 15(1): 122–133.
- Jung, Y.-K., Joo, K.-S., Rho, S.-J., and Kim, Y.-R. (2020). pH-dependent antioxidant stability of black rice anthocyanin complexed with cycloamylose. *LWT – Food Science and Technology*, 109474.
- Leal-Sánchez, M.V., Ruiz-Barba, J.L., Sánchez, A.H., Rejano, L., Jiménez-Díaz, R., and Garrido, A. (2003). Fermentation profile and optimization of green olive fermentation using *Lactobacillus plantarum* LPCO10 as a starter culture. *Food Microbiology*, 20: 421–430.



- Leroy, F. and De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science & Technology*, 15: 67–78.
- Liu, Y., Cheng, H., Liu, H., Ma, R., Ma, J., and Fang, H. (2019). Fermentation by multiple bacterial strains improves the production of bioactive compounds and antioxidant activity of goji juice. *Molecules*, 24(19): 3519.
- Lund, B.M., Baird-Parker, T.C., and Gould, G.W. (2000). *The microbiological safety and quality of food*, Vol I, Aspen Publishers Inc., Gaithersburg, p. 2024.
- Marazza, J.A., Garro, M.S., and de Giori, G.S. (2009). Aglycone production by *Lactobacillus rhamnosus* CRL981 during soymilk fermentation. *Food Microbiology*, 26: 333–339.
- Mattila, P., Hellström, J., and Törrönen, R. (2006). Phenolic acids in berries, fruits, and beverages. *Journal of Agricultural and Food Chemistry*, 54(19): 7193–7199.
- Miles, A.A., Misra, S.S., and Irwin, J.O. (1938). The estimation of the bactericidal power of the blood. *The Journal of hygiene*, 38(6): 732–749.
- Muhialdin, B.J., Kadum, H., Zarei, M., and Hussin, A.S.M. (2020). Effects of metabolite changes during lacto-fermentation on the biological activity and consumer acceptability for dragon fruit juice. *LWT – Food Science and Technology*, 121: 108992.
- Narvhus, J.A. and Axelsson, L. (2003). *Encyclopedia of food sciences and nutrition*, 2nd ed., Chapter: Lactic acid bacteria, pp. 3465–3472.
- Olivares, A., Soto, C., Caballero, E., and Altamirano, C. (2019). Survival of microencapsulated *Lactobacillus casei* (prepared by vibration technology) in fruit juice during cold storage. *Electronic Journal of Biotechnology*, 42.
- Panda, S.K., Behera, S.K., Witness Qaku, X., Sekar, S., Ndinteh, D.T., Nanjundaswamy, H.M., Ray, R.C., and Kayitesi, E. (2016). Quality enhancement of prickly pears (*Opuntia* sp.) juice through probiotic fermentation using *Lactobacillus fermentum* – ATCC 9338. *LWT – Food Science and Technology*, 75: 453–459.
- Perricone, M., Corbo, M.R., Sinigaglia, M., Speranza, B., and Bevilacqua, A. (2014). Viability of *Lactobacillus reuteri* in fruit juices. *Journal of Functional Foods*, 10: 421–426.
- Rakin, M., Vukasinovic, M., Siler-Marinkovic, S., and Maksimovic, M. (2007). Contribution of lactic acid fermentation to improved nutritive quality vegetable juices enriched with brewer's yeast autolysate. *Food Chemistry*, 100: 599–602.
- Randazzo, C.L., Restuccia, C., Romano, A.D., and Caggia, C. (2004). *Lactobacillus casei*, dominant species in naturally fermented Sicilian green olives. *International Journal of Food Microbiology*, 90: 9–14.
- Repajić, M., Bursać Kovačević, D., Putnik, P., Dragović-Uzelac, V., Kust, J., and Čosić, Z. (2015). Influence of cultivar and industrial processing on polyphenols in concentrated sour cherry (*Prunus cerasus* L.) juice. *Food Technology and Biotechnology*, 53(2): 215–222.
- Rhee, S. J., Lee, J.-E., and Lee, C.-H. (2011). Importance of lactic acid bacteria in Asian fermented foods. *Microbial Cell Factories*, 10(Suppl 1): S5.
- Ricci, A., Cirlini, M., Levante, A., Dall'Asta, C., Galaverna, G., and Lazzi, C. (2018). Volatile profile of elderberry juice: Effect of lactic acid fermentation using *L. plantarum*, *L. rhamnosus* and *L. casei* strains. *Food Research International*, 105: 412–422.
- Rodríguez, H., Landete, J.M., Rivas, B.d.l., and Muñoz, R. (2008). Metabolism of food phenolic acids by *Lactobacillus plantarum* CECT 748T. *Food Chemistry*, 107(4): 1393–1398.
- Singleton, V.L., Orthofer, R., and Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299: 152–178.



- Sredojevic, Z. (2014). Value chain analysis of region specific organic production in Serbia. FAO Government Cooperative Programme. Assistance to the Development of Capacity and Support Services for Organic Agriculture in Serbia. GCP/SRB/001/HUN.
- Tabasco, R., Sánchez-Patán, F., Monagas, M., Bartolomé, B., Moreno-Arribas, M.V., Peláez, C., and Requena, T. (2011). Effect of grape polyphenols on lactic acid bacteria and bifidobacteria growth: Resistance and metabolism. *Food Microbiology*, 28(7): 1345–1352.

Open Access. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited, a link to the CC License is provided, and changes - if any - are indicated. (SID_1)

