Development of lactic acid fermented, probiotic sour cherry juice

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Abstract

Nowadays, demand for products which beyond the overall nutritional value have a feature that protects the consumers health, have increased. Several studies have proved fruit juices can become 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 by fermentation with probiotic starter culture. In the fermentation 9 *Lactobacillus* strains were used and two types of sour cherry as raw material. The Újfehértói fürtös and Petri species were provided by NARIC – Fruitculture Research Institute. To reach the recommended probiotic cell count we investigated the pH adjustment, supplementation of nutrients, the effect of dilution and strain adaptation to sour cherry juice. In our study the properties of the strains – such as reproduction and metabolism – and its effect on the raw material were investigated. As a result a significant difference was observed between the number of viable cells of certain *Lactobacillus* strains. Our results showed that the type of sour cherry also affects the fermentation, so it is important to select the starter culture for the given raw material.

Keywords: functional food, lactofermented, probiotic, sour cherry juice

1. Introduction

Traditional fermented foods and beverages form an integral part of cultural heritage, are widely consumed since ancient civilization and still part of the human diet. 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 present as predominant microflora in most of the traditional fermented foods and their role in fermentation is known since ages (Anandharaj et al., 2013). *Lactobacillus* strains are important members of LAB and play essential roles in lactic acid fermentation. Many members of the *Lactobacillus* genus proved to have a beneficial health

benefit for the consumer. Probiotics are defined as live microorganisms when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2001).

Lactic acid fermentation of vegetables and role of *Lactobacillus* strains in this process are investigated long ago and it is well-described in case eg. olive (Leal-Sánchez et al., 2003; Randazzo et al., 2004), kimchi (Rhee et al., 2011) and sauerkraut (Halász et al., 1999; Leroy & 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 probiotic, such as fermented milks, ice cream (Akalın et al., 2018) and cheese (Blaiotta et al., 2017). But non-dairy probiotic products have a large worldwide importance due to the ongoing trend of vegetarianism and to a high prevalence of lactose intolerance in many populations around the world. Therefore, would be important a product that combines the benefits of lacto-fermented plant-based products and probiotic microorganisms. Development of fruit-based probiotic products could provide a solution. It would be ideal substrate for a fermented product, as they play an important role in human nutrition and contain many beneficial ingredients (minerals, vitamins, dietary fibers) and at the same time are free from milk's allergens.

Some technical challenges have suggested that fruit juice could serve as a good medium for functional ingredients like probiotics (Nazzaro et al., 2008). The fruit juices, mainly prepared from red fruits, are the most available sources of anthocyanins and polyphenols (Chiara et al., 2011). Sour cherry is one of the oldest fruit species and its production is continuously increasing in the world. Hungary is one of the leading countries in sour cherry production (FAOSTAT, 2016), it always has been a popular fruit and its cultivation is very important. Sour cherries offer a healthy dose of the antioxidant vitamin C (15 mg) (Sredojevic, 2014), and due to the high content of polyphenols it has an important role in 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 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 sour cherry juice that helps maintain the gut flora.

2. Materials and methods

2.1. Microorganisms

Lactobacillus (L.) rhamnosus GG, *L. fermentum* D13 and *L. acidophilus* 150 – *Lactobacillus* strains with three different types of metabolism – were investigated as a starter culture in the preliminary experiments. In strain selection for the fermentation 9 – among them 6 probiotic – *Lactobacillus* strains (*L. rhamnosus* GG, *L. acidophilus* 150, *L. acidophilus* LA-5, *L. casei* Shirota, *L. casei* LC-01, *L. reuteri*, *L. plantarum* 2142, *L. acidophilus* N2, *L. fermentum* DT41) were used.

2.2. Raw material

Two types of sour cherry were used, the Újfehértói fürtös and Petri species were provided by National Agricultural Research and Innovation Centre – Fruitculture Research Institute. Fruit juice was made with centrifugal juicer and the extracted juice filtered twice through a 0.5 mm filter to make a clear juice. Following the processing of the fruit, because of the indigenous flora of the raw sour cherries, pasteurisation of the juices is necessary.

2.3. Fermentation experiments

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

2.4. Measured parameters

The Miles and Misra Method (Miles and Misra, 1938) was used to determine the exact number of colony forming units with de Man, Rogosa and Sharpe (MRS) agar as a selective medium. Samples from the fermented 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. 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 as indicator. Titratable acidity was expressed as g L^{-1} of lactic acid equivalents in fermented juice. The total soluble solids content of sour cherry juice was determined using a digital refractometer, and the results were expressed in ° Brix. The sour cherry juices' antioxidant capacity was measured by FRAP and DPPH methods.

2.5. Central Composite Design and statistical evaluation of data

To optimize the factors for all of the processes the combined effect of some parameters Box-Wilson central composite design (CCD) in response surface methodology (RSM) 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 we are investigating. 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).

3. Results

3.1. Preliminary experiments

In preliminary experiments the juices were inoculated, separately, with three *Lactobacillus* strains with different metabolic types (*L. rhamnosus* GG – facultative heterofermentative, *L. acidophilus* 150 – obligate homofermentative, *L. fermentum* D13 – obligate heterofermentative) in natural form and incubated at 30 °C for 24 and 48 hours. It was observed that sour cherry juice in its natural form does not provide an adequate environment for the growth of *Lactobacillus* and despite the careful preparation, a significant number of its indigenous microflora proliferated during the incubation.

Therefore after the processing of the fruit, because of the high number of total cell count present in the raw sour cherries, pasteurization of the juices is necessary. For the reason to minimalize the heat treatment the process parameters for the pasteurization was optimalized with the

combined effect of temperature and time by central composite design (CCD) and for the evaluation response surface methodology (RSM) was used *(Figure 1)*. Heating at 60 °C for 15 minutes proved to be the most effective pasteurization of the raw material while least modify the beneficial components in the sour cherry.

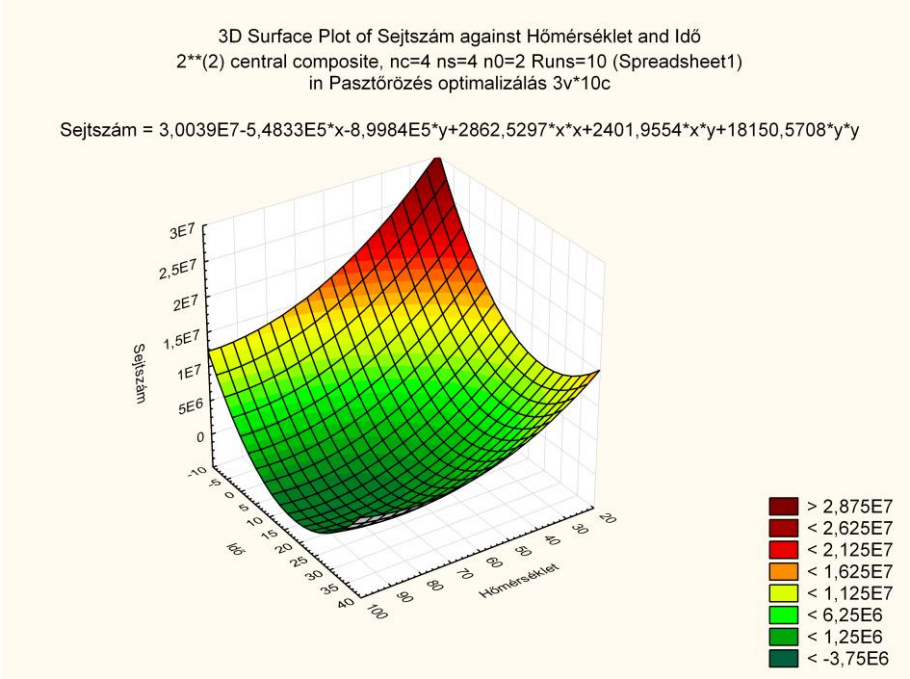

Figure 1. Response surface plot for minimum total cell number of cherry juice as a function of temperature and treatment time.

3.2. Effect of pH and strain adaptation to sour cherry juice

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 (10^8 cfu mL^{-1}) *(Table 1)*. To maximize the viable cell count, we investigated the strain's preliminary adaptation to the raw material since phenolic compounds can inhibit cell growth, but it can be eliminated by adapting *Lactobacillus* to polyphenol (Perricone et al., 2014). The SCJ was diluted with MRS broth in different proportion (SCJ to MRS is 10:0, 9:1, 8:2, 7:3, 6:4 and 5:5) and these mixtures were inoculated with a 24 h old *L. rhamnosus* GG culture and incubated overnight at 30 °C for 24 hours.

Table 1. Viable cell number of *Lactobacillus* strains in cfu mL^{-1} in sour cherry juices with adjusted pH

Type of sour cherry	*Lactobacillus* strain	Fermentation time	
		0 h	24 h
Újfehértói fürtös	*L. rhamnosus* GG	3,82E+07	8,03E+07
	L. fermentum D13	4,15E+07	1,02E+08
	L. acidophilus 150	2,92E+07	9,24E+07
Petri	*L. rhamnosus* GG	3,82E+07	1,89E+08
	L. fermentum D13	4,15E+07	1,42E+08
	L. acidophilus 150	2,92E+07	1,77E+08

To investigate the combined effect of pH and preliminary adaptation a CCD was used. As shown in Pareto chart *(Figure 2)* the growth of *Lactobacillus* is affected by only the linear variable of pH of the independent variables, the adaptation has no significance. At the same time we could determine the initial optimal pH for the cell growing based on the response surface *(Figure 2)*, which has given for this parameter the value 5.8.

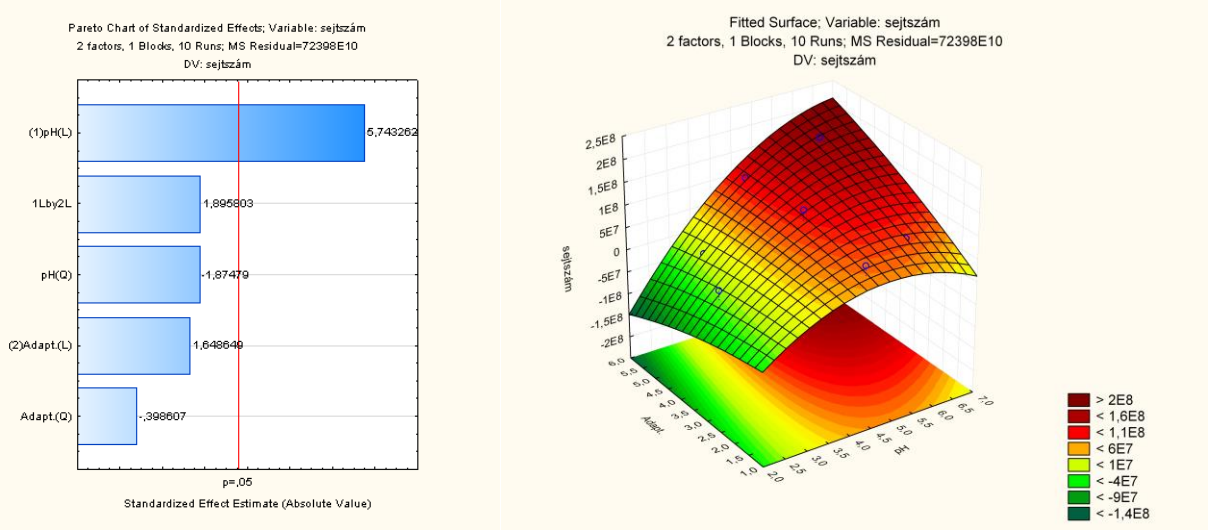

Figure 2. (Left) Pareto chart of standardized effects (pH and adaptation). (Right) Combined effect of pH and adaptation on the number of viable cells in sour cherry juice

3.3. Effect of dilution and supplementation

In order to reach the recommended probiotic cell count, we henceforth investigated the supplementation of nutrients, such as peptone, dextrose, yeast extract, and dilution of SCJ (with adjusted pH) with sterile water.

Sour cherry juice diluted with sterile water as well as the addition of yeast extract have a positive effect on the *Lactobacillus* growth. Therefore, the ideal values of these variables were also optimized by central composite design. As shown on the response surface *(Figure 3)* 3 g L^{-1} added yeast extract will be sufficient if the SCJ is diluted with water and the ratio of sour cherry juice to water is 6:4.

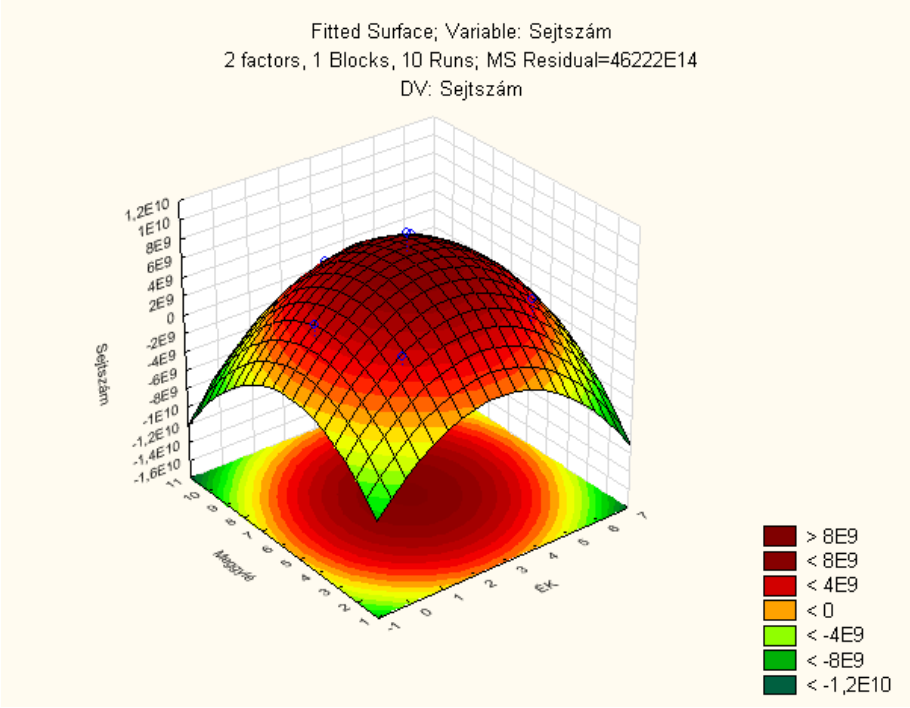

Figure 3. Response surface plot for maximum *Lactobacillus* cell number of fermented cherry juice as a function of dilution ratio and yeast extract concentration.

3.4. Strain selection

For the strain selection 9 – among them 6 probiotic – *Lactobacillus* strains were used and two types of sour cherry as raw material. Since the added 3 g L^{-1} yeast extract as well as the pH adjusting to 5.8, and the ratio of sour cherry juice to water is 6:4 resulted in the highest (10^9 cfu mL^{-1}) cell number and the pH decreasing to the optimal value after 24 hours, these parameters were adjusted to the given values before the inoculation. In this study the properties of the

strains – such as reproduction (viable cell count) – and its effect on the raw material (pH, titratable acidity, total soluble solids, polyphenol and antioxidant capacity) were also measured.

Despite the fact that all investigated strains reached the desired 10^9 cfu mL^{-1} cell density *(Figure 4)* after 24 hours, a significant difference was observed between the number of viable cells of certain *Lactobacillus* strains *(Figure 5)*.

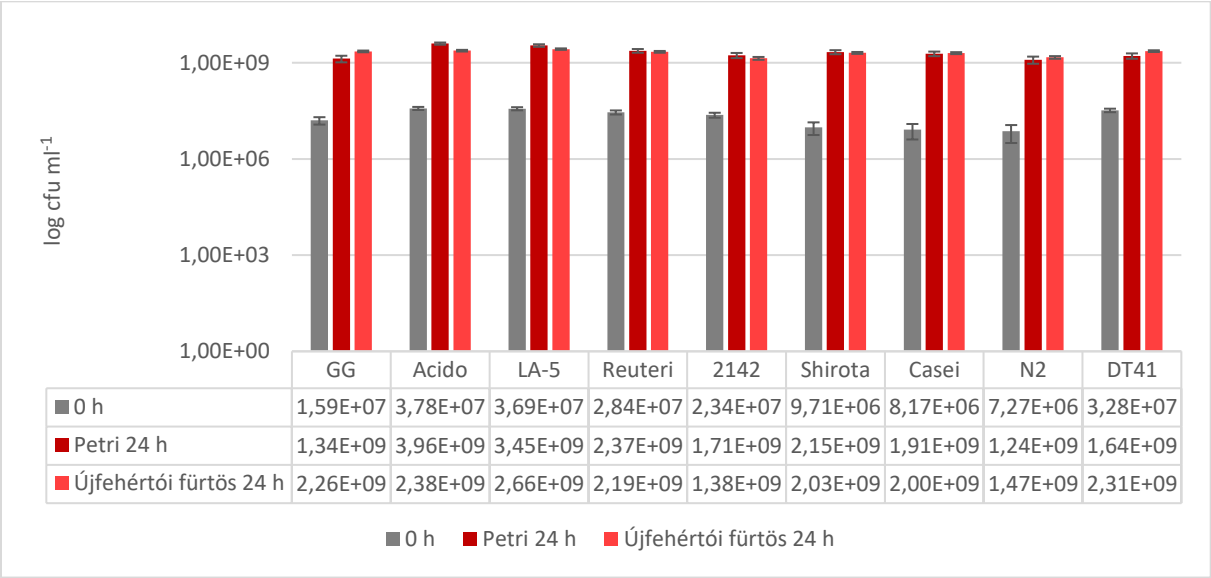

Figure 4. Viable cell number of *Lactobacillus* strains in log cfu mL^{-1} in sour cherry juices

In Petri sour cherry species the cell number of *Lactobacillus acidophilus* 150 strain was statistically significant in a positive direction compared to all other strains – except *L. acidophilus* LA-5. Furthermore, it can be concluded from our results that there is no significant difference between the sour cherry species for the general *Lactobacillus* cell growth, but the type of sour cherry influences the fermentation, so it is important to select the starter culture for the given raw material. Namely in the Újfehértói fürtös sour cherry *L. acidophilus* LA-5, while in the Petri species *L. acidophilus* 150 resulted in the highest probiotic cell number, that is important in point of view of the development of probiotic-containing products.

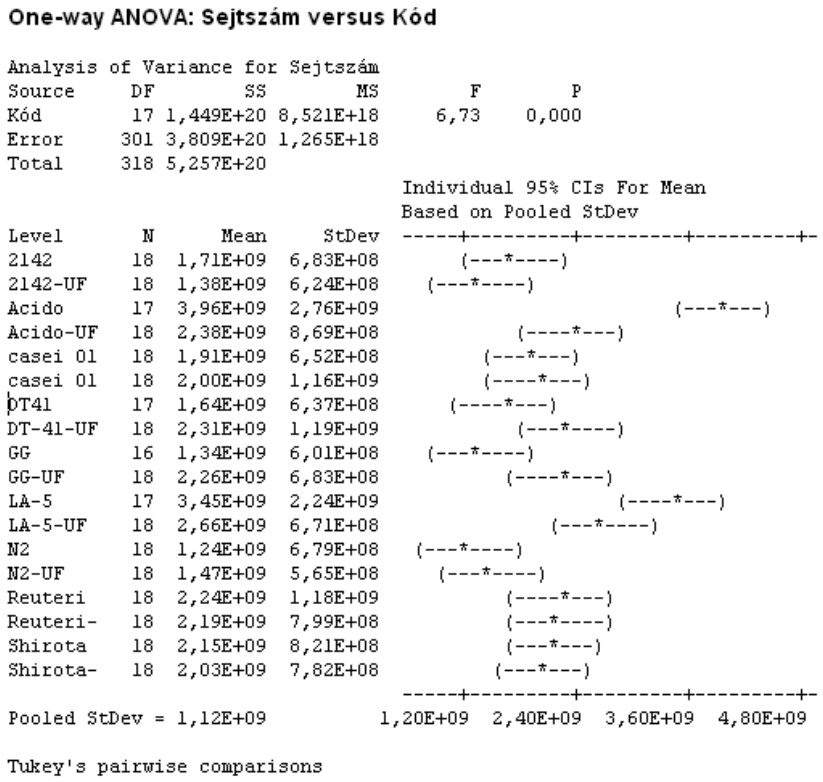

Figure 5. Comparison the means of *Lactobacillus* viable cell count in sour cherry juices with one-way ANOVA

The lactic acid bacteria with the β-glucosidase activity (including *Lactobacillus acidophilus*, *L. casei*, *L. plantarum*, *L. fermentum*) can increase the aglycone during the fermentation. The aglycone acts as an antioxidant (Marazza et al. 2009). Our results *(Figure 6)* showed that *L. rhamnosus* GG can produce the fermented Újfehértói fürtös sour cherry juice by retaining the antioxidant activity. Although in the same type of sour cherry in case of certain *Lactobacillus* strains (*L. acidophilus* 150, *L. acidophilus* LA-5, *L. casei* Shirota and *L. fermentum* DT41) in the fermented juice the antioxidant capacity has increased, while in case of *L. reuteri*, *L. plantarum* 2142, *L. casei* LC-01 and *L. acidophilus* N2 fermented SCJ has lower antioxidant activity compared to the initial juice. Nevertheless, in Petri SCJ the antioxidant activity increased during the fermentation (approximately between 5% and 25%) by all of the *Lactobacillus* strains used in the strain selection. According to the antioxidant capacity of the fermented juices the Petri species seems more applicable for a helathy, functional sour cherry juice production.

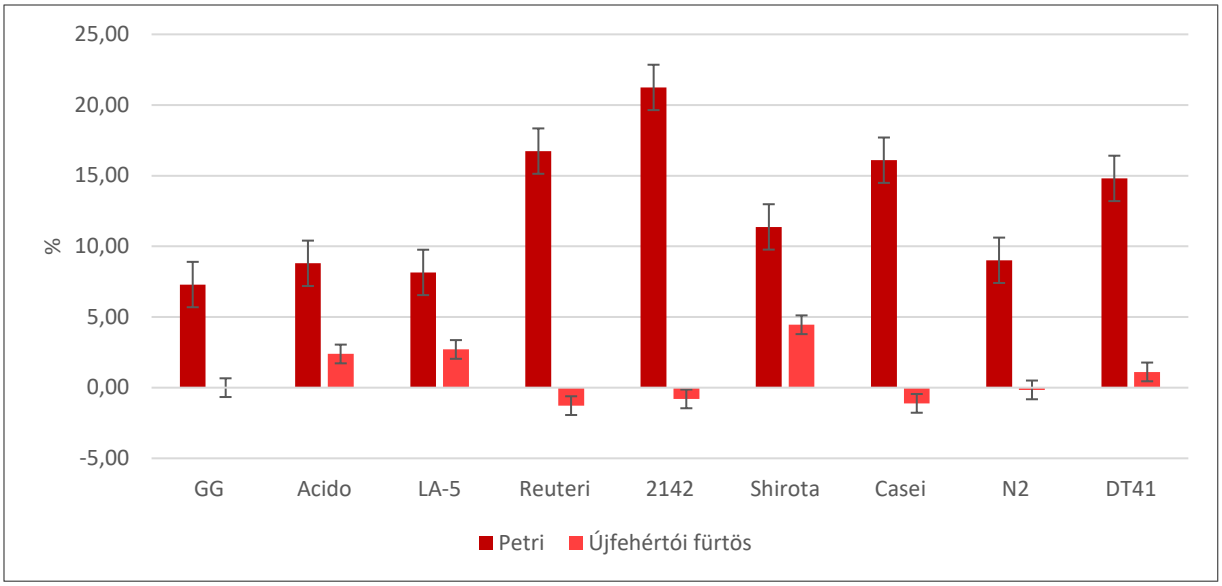

Figure 6. Effect of lactic acid fermentation on antioxidant capacity in % in sour cherry juices compared to the 0 h control

4. Conclusions

Our results showed the importance of ensuring an adequate environment for growth of *Lactobacillus* in sour cherry juices. Adjusting pH, added yeast extract and dilution of SCJ resulted in the highest (10^9 cfu mL^{-1}) cell number and the pH decreasing to the optimal value after 24 hours. The ideal values of variables were optimized by central composite design with response surface methodology. Initial optimal pH is 5.8 and 3 g L^{-1} added yeast extract would be sufficient if the ratio of sour cherry juice to water is 6:4. Despite during the fermentation all investigated *Lactobacillus* strains reached the desired 10^9 cfu mL^{-1} cell density, a significant difference was observed between the number of viable cells of some *Lactobacillus* strains. It can be concluded from our results that there is no significant difference between the sour cherry species for all strains, but the type of sour cherry influences the fermentation, so it is important to select the starter culture for the given raw material. Nevertheless, in point of antioxidant capacity worthy select the Petri species could be suggested for fermentation, because in Petri sour cherry juice the antioxidant activity increased in the fermentation by all of the *Lactobacillus* strains used in strain selection.

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