Malolactic fermentation of sea buckthorn (*Hippophaë rhamnoides* L.) berry juice with *Lactobacillus plantarum*: impact on sugars, sugars alcohols, and organic acids

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Abstract

Potential to decrease sourness, and thus increase sensory value of sea buckthorn berries, by using malolactic fermentation with *Lactobacillus plantarum* was investigated. Sea buckthorn juice samples were fermented with four different *L. plantarum* strains, and chemical changes related to fermentation were determined by analysing sugars, sugar alcohols and organic acids as trimethylsilyl-derivates from fermented sea buckthorn juices with GC-FID. There was a clear difference in fermentation efficiency between studied strains, strain 10492 being the most effective, resulting in total conversion of malic acid into lactic acid. Additionally, levels of total sugars maintained comparable to the non-treated juice with all strains, and thus sweetness was maintained. Therefore, *L. plantarum* with selected strains is potential candidate for malolactic fermentation of sea buckthorn.

Introduction

Sea buckthorn (*Hippophaë rhamnoides* L.) berries contain a versatile combination of chemical compounds having health promoting features such water-soluble vitamins (C, B1, and B2), fat-soluble vitamins (A, K, and E), fatty acids, flavonoids, and plant sterols [1]. However, the sour, bitter and astringent taste characteristics limit its regular consumption. The main chemical factors related to the sourness of sea buckthorn are the high concentrations of malic and quinic acids. Additionally, strong sourness intensifies the perception of astringency [2].

One potential treatment to increase the value of sea buckthorn would be to use malolactic fermentation, a method currently used for decreasing acidity of sour wines. In this process, certain lactic acid bacteria convert malic acid into lactic acid. In the wine industry, *Oenococcus oeni* is the most commonly used lactic acid bacteria [3]. However, while being effective in wines, *O. oeni* has specific nutrient requirements and a relatively slow growth rate [4], and thus other candidates for malolactic fermentation of atypical materials (such as sea buckthorn) are worth investigating. One potential candidate is *Lactobacillus plantarum*, a bacterial species commonly found in and responsible of the fermentation of plant-based lactic acid fermented foods such as sauerkraut and table olives. Potential benefit of *L. plantarum* is in its robustness: it has a relatively fast growth rate, tolerance of low pH, and low nutrient requirements [5]. However, studies related using *L. plantarum* as malolactic organism are currently limited. Here we evaluate the potential to use *Lactobacillus plantarum* to decrease acidity of sea buckthorn juice, and thus increase its sensory value.

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Experimental

Materials

Frozen sea buckthorn berries (*Hippophaë rhamnoides subp. mongolica*) were obtained from Asterpajutooted OÜ (Tõrva, Estonia). Berries were originated from South Estonia, collected from multiple producers by the distributor. Berries were stored at –20 °C until use.

Four strains of *Lactobacillus plantarum* (DSM 16365, DSM 20174, DSM 10492, DSM 100813) were obtained from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Leibniz, Germany) as freeze-dried cultures. Cultures were revived as instructed by the manufacturer, and stored in 10 % (v/v) glycerol in food-grade medium (FGM) [6] at –20 °C until use.

Sample preparation and fermentation set-up

In order to prepare the juice, frozen berries were thawed in a microwave at 650 W for 5 minutes with intermittent mixing. Berries were made into a mash with an immersion blender. Juice was extracted from the mash with mechanical pressing. Prior to pasteurization, the juice was diluted 1:1 with active-carbon filtered water. Juices were pasteurized in an autoclave (Systec D-150, Linden, Germany) at 85 °C for 5 minutes. After pasteurization, juice samples were cooled down on an ice bath and stored at +4 °C for 24 hours before inoculation.

To produce the starter cultures, each strains was inoculated into 250 ml of FGM by a scrape from the glycerol stock with a sterile inoculation loop. Cells were grown at +30 °C for 24 hours. From each culture, cells were collected with centrifugation at 3410 × *g* for 10 minutes from 200 ml of o/n growth. Cells were washed twice with sterile saline solution and concentrated into a volume of 4,5 ml. Each juice sample of 100 ml was inoculated with 1 ml of respective cell concentrate. Fermentation was performed for +30 °C for 72 h in iCinac equipment (Unity Scientific, Milford, USA) equipped with TW8 water bath (Julabo, Seelbach, Germany). Each fermentation was prepared in duplicates. After fermentation, samples were collected in sterile 2 ml tubes and stored at –80 °C until analysis.

Analysis of sugars, sugars alcohols, and organic acids

First, the juice samples were diluted with reverse-osmosis water to achieve an appropriate concentration for the analyses. Aliquots of the diluted samples were dried under nitrogen flow, followed by derivatization of the sugars, sugars alcohol and organic acids with chlorotrimethylsilane reagent with pyridine and hexamethylsilazane (Tri-Sil HTP, Thermo Scientific, Bellefonte, PA, USA). Each sample was prepared in triplicate.

TMS-derivated samples were analysed by using a Shimadzu 2010Plus gas chromatograph (Kyoto, Japan) equipped with flame ionization detector and Shimadzu AOC-20i autosampler. Analyses were performed on SPB-1 column (30 m x 0,25 mm ID, liquid film 0,25 μm, Supelco, Bellefonte, PA, USA). Internal standards were used for quantification, xylitol for sugars and sugar alcohols, and tartaric acid for organic acids. External standards were used for the calculation of correction factors and for the identification of the analytes.

Results and discussion

The purpose of this work was to investigate the potential to utilize malolactic fermentation with *Lactobacillus plantarum* to decrease the acidity in sea buckthorn juice. The content of malic acid in the control juice was 11,5 ± 0,08 mg/ml (Figure 1). The level of malolactic fermentation varied greatly among the studied *L. plantarum* strains. Strain DSM 10492 was the most effective, with all malic acid converted into lactic acid. Strains DSM 20174 and 100813 had moderate conversions, and malic acid contents were reduced to 8,73 ± 0,19 and 8,34 ± 0.14 mg/ml, respectively. No conversion was detected with the strain 16365.

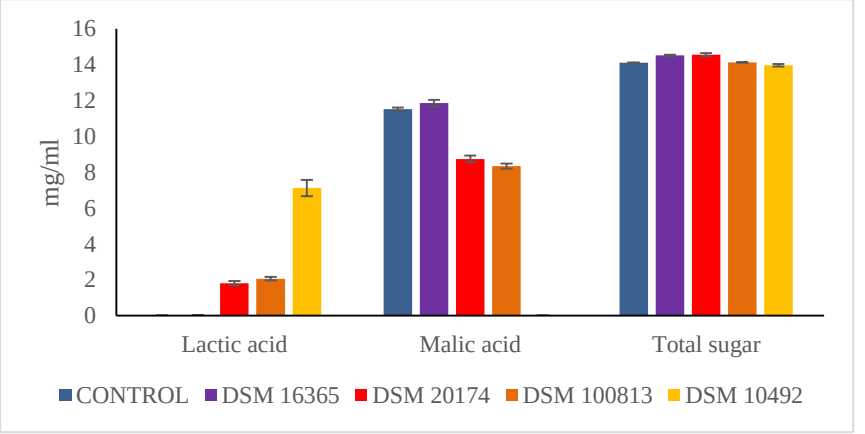

Figure 1: Lactic acid, malic acid and total sugars (sum of fructose, glucose, myo-inositol and methyl-myo-inositol) in control sea buckthorn juice and juices fermented with *L. plantarum*. Error bars present standard deviation (N = 6).

As malic acid is converted into lactic acid, acidity is reduced due to decarboxylation, as was observed in the increase in pH of the fermented juices (Figure 2). Although lactic acid bacteria can use a variety of carbon sources, including monocarbohydrates such as glucose and fructose, the content of total sugars remained similar in the fermented juices compared to the control. A similar phenomenon was observed when sea buckthorn juice was fermented with other malolactic bacteria, *Oenococcus oeni* [7]. This is most likely due to the high acidity of the material. At low pH, *L. plantarum* seems to prefer malic acid as an energy source over sugars, possibly due to passive diffusion, as the acids are predominately present in the protonated form [3].

Other identified and quantified compounds were the organic acids citric acid, quinic acid, and ascorbic acid, and the sugars ethyl-glucose, myo-inositol, and methyl-myo-inositol. Additionally, the sugar alcohol L-quebrachitol was identified. Compared to the control, samples fermented with strain 10492 had a small but significant ($P<0,05$) decrease in levels of citric acid, fructose, and quinic acid. Comparing the same samples, significantly higher levels ($P<0,05$) of ascorbic acid and ethyl-glucose were measured in the fermented samples compared to the control.

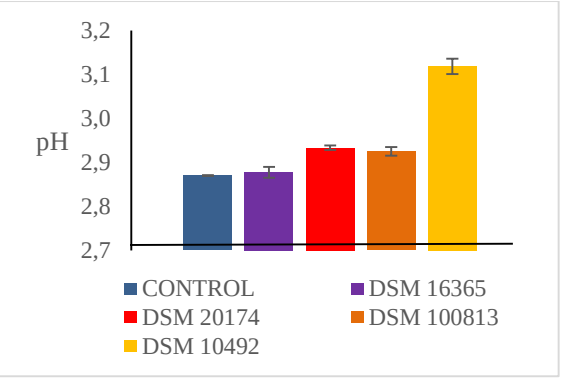

Figure 2: Measured pH in sea buckthorn control juice and in juices fermented with *L. plantarum*. Error bars present standard deviation (N = 4).

All in all, our results indicate that *L. plantarum* could be used for malolactic fermentation of sea buckthorn juice without additional nutrients or by increasing the pH. Additionally, other acids, sugars and sugar alcohols remained mostly unfermented. Therefore, this method also maintains the sweetness of the berry juice. However, the effectiveness of the fermentation is highly affected by the strain. Thus, prior investigation of the suitable strains is important. On the other hand, prolonged malolactic fermentation can also produce unwanted off-flavours on strain-dependent basis [7], possibly due to the production of alcohols [8]. On the other hand, malolactic fermentation with *O. oeni* was shown to increase fruity notes in sea buckthorn juice by releasing more ethyl esters or acetate esters of fatty acids [8]. Our work should therefore in the future be combined with aroma analysis and sensory evaluations to confirm how the sensory value and the flavour of sea buckthorn are affected by lactic acid fermentation with *L. plantarum*.

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