Fermented birch sap

Birch sap fermentation

Birch sap is traditionally used as a refreshing beverage in the springtime in northern Europe. Without any additional processing the storage time of fresh birch sap is relatively short, therefore fermentation of birch sap is common. Before the use of pasteurization and other possibilities for increasing storage time, the main means of preserving birch sap was through fermentation. The most common fermentation for birch sap was wild fermentation. This means that birch sap is fermented by the bacteria and yeast that are naturally present in the birch sap. Therefore, the microflora of fermented birch sap is specific to collecting environment and can variate also from batch to batch.

Wild fermentation is something that people tend to think of as more natural than fermentation with selected microorganism. In fact, that is not the case. Wild fermentation means that fermentation is conducted by microorganisms that are already in the product. This means that without specific analysis, it is unknown what kind of microorganisms are in our product and who will conduct the fermentation. Therefore, it is hard to predict what kind of quality the end product will have. Wild fermentation can give a product of high quality as well as a product of low quality. Therefore, wild fermentation is common only in craft beverage industries that have small production volumes and who can afford “losing” small batches. Producers who aim for high and continuous quality use starter cultures: specific microorganisms selected from nature. Before the availability of specific bacteria and yeast as starter cultures, different additives were used as a starter culture source. For example raisins, fruit juice, grains, bark etc.

Center of Food and Fermentation Technologies conducted research to measure the bacteria naturally occurring in birch juice and some traditional fermented birch sap drinks to find the best starters for fermented birch sap. The results from metagenomics analysis showed a clear difference between wild fermentation and fermentation with traditional starters. Results obtained from this study helped to find the best lactic acid bacteria for the fermentation of birch sap. These findings were used in the further production of fermented birch sap beverages to have the fermented birch sap with highest possible sensory quality.

Microbiota of fermented birch sap and health claims

Metagenomics analysis of birch saps have showed that birch sap contains different microorganisms and many of them have a probiotic effect according to the literature. The fermentation of birch sap increases the quantity of different microorganisms and probiotics: therefore, fermented birch sap can be classified as a probiotic drink that contains different lactic acid bacteria. The popularity of probiotic/functional products is wide and although there are many products available, most of them are dairy-based products: this means they are not suitable for individuals who are lactose intolerant or have a milk protein allergy or are on cholesterol-restricted diets. In addition, vegetarianism and veganism are becoming more and more widespread. Thus, the development of non-dairy-based functional/probiotic products has been in the spotlight during recent years. Fermented birch sap drinks are one of the best options to produce the non-dairy-based probiotic beverage.

Although fermentation could enrich birch sap with probiotic bacteria, the use of health claims, in this case, is not allowed without human clinical studies. In Europe it is very complex to use claim "probiotic" on food products because European Food Safety Authority (EFSA) has not approved mentioned health claim. Therefore, only specific bacteria can be named on the product, e.g. "contains lactic acid bacteria *Lactobacillus rhamnosus GG*". As wild fermentation varies a lot, it might be difficult to use specific bacteria claim on the product. Nevertheless, the total amount of bacteria and/or amount of lactic acid bacteria could be referred if approved with acceptable documentation.

Chemical composition

The chemical composition of fermented birch sap is rather similar to the chemical composition of starter material with one important difference: sugars are converted to organic acids, mainly to lactic acid, and other metabolites. If the lactic acid bacteria are dominant in fermentation process, then lactic acid is main product and beverages with dominating sour taste profile is obtained. In addition to mentioned lactic acid the fermented birch sap can contain also other metabolites obtained form fermentation process, e.g. acetic acid, gluconic acid, different aroma compounds, vitamins, enzymes etc. Their occurrence and quantity depend on fermentation processes parameters: fermentation temperature, microflora, fermentation time etc.

The fermented birch sap has no sugars if the fermentation is complete. This means that the beverage has almost no calories: only organic acids give a few calories to the drink. The claim "sugar-free" is also suitable for the product if fermentation completion is confirmed with residual sugar analysis.

Example of metagenomic analysis of fermented birch sap

Sequence Data Analysis Report

Date: 15.02.2021

Contact Person: Valjo Liivamägi

E-mail: valjo.liivamagi@gmail.com

Samples for analyses:

Sample #	*Description*	*Sequence protocol*	*DNA extraction (if applicable)*	*Notes*
1	***Fermented birch sap***	*16S (v4)*	*yes*	

Total - ***1*** samples to sequence.

Timeline:

Activity	**Date**
Samples arrived	01.02.21
DNA extraction	03.02.21
16S Library preparation	4-5.02.21
16S Sequencing	10-11.02.21
Report	15.02.21

Fermented birch sap was in two plastic bottles (500 ml) with a screw cap. The liquid was opalescent. 500 ml of birch sap was taken as a sample for analysis and genomic DNA was extracted using Quick DNA Fungal/Bacterial Miniprep Kit (Zymo Research, Lot: ZRC205481).

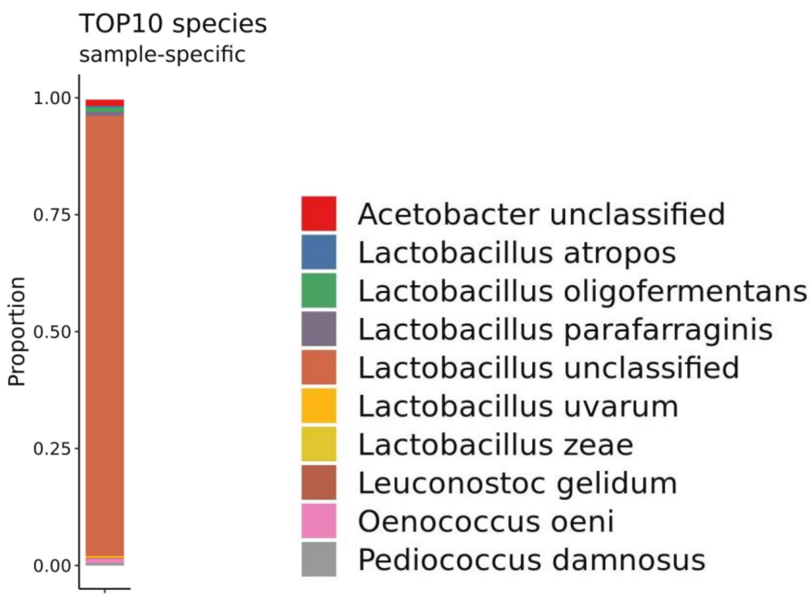
For estimation of bacterial cell number, quantification by 16S qPCR method was applied.

Data Analysis

After application of 0,001 threshold, 10 most abundant bacteria were identified, they belonged to 5 taxonomic genera (*Acetobacter, Lactobacillus, Leuconostoc, Oenococcus, Pediococcus*), but the main represented genus with 97% of bacterial presence in birch sap was referred to *Lactobacillus*.

The bacteria concentration in 1 ml of fermented birch sap was 2 ng/µL, which corresponds to about 3x10 ^ 5 cells in 1 ml of fermented birch sap.

Top10 the most represented bacteria:



Lactobacillus is a common group of bacteria that is characterised by their ability to produce lactic acid as a by-product of glucose metabolism. They are widely distributed in various fermented products, animal feeds, silage, and milk foods. Various species of *Lactobacillus* are used commercially during the production of sour milks, cheeses, and yogurt, and they have an important role in the manufacture of fermented vegetables (pickles and sauerkraut), beverages (wine and juices), sourdough breads, some sausages etc. Presence of *Lactobacillus* in the fermented birch sap shows on the fermented beverage with definite healthy benefits.

The other represented bacteria (1,3% of all species) belongs to *Acetobacter* genus. Acetic Acid Bacteria (AAB) oxidise sugars and alcohol into acetic acid. All AAB are specialised for rapid oxidation and are strict aerobes therefore oxygen availability is very important. Their growth and metabolism are markedly enhanced with the addition of oxygen to their environment. *A. pasteurianus* prefers to grow at pH ranging from 5.5-6.3 and at temperatures of 25-30 °C. Preferred carbon sources are glucose, mannitol, and ethanol. In nature, the endophytic AABs exist on sugar-rich substrates such as fruits, flowers, and vegetables.

Oenococcus oeni (known as *Leuconostoc oeni* until 1995), is the primary bacterium involved in completing the malolactic fermentation, process in which tart-tasting malic acid is converted to softer-tasting lactic acid.

Taxonomic Level	Taxonomic Unit	Normalized Fraction
g	*d__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__casei_41% / paracasei_34% / buchneri_7% / diolivorans_5% / rhamnosus_3% / farraginis_1% / kisonensis_1% / parakefiri_1% / amylovorus_1%*	**0.940637846**
g	*d__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodospirillales; f__Acetobacteraceae; g__Acetobacter; s__pasteurianus_62% / fabarum_15% / ghanensis_15% / cibinongensis_3% / orientalis_1%*	0.013067094
s	*d__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Leuconostocaceae; g__Oenococcus; s__oeni*	0.009186293
s	*d__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__parafarraginis*	0.009156612
s	*d__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__oligofermentans*	0.008934004
s	*d__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Pediococcus; s__damnosus*	0.005372275
s	*d__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__atropos*	0.003673033
s	*d__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__uvarum*	0.002715819
s	*d__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Leuconostocaceae; g__Leuconostoc; s__gelidum*	0.001491474
s	*d__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__zeae*	0.0010982

References

1. Svanberg I., Sõukand R., Łuczaj Ł., Kalle R., Zyryanova O., Dénes A., et al. Uses of tree saps in northern and eastern parts of Europe, Acta Soc. Bot. Pol., 2012, 81(4), 343–357
2. Semjonovs, P., Denina, I., Fomina, A., Patetko, A., Auzina, L., Upite, D., Upitis, A. & Danilevics, A. Development of birch (Betula pendula Roth.) sap based probiotic fermented beverage, International Food Research Journal, 2014, 21(5), 1763-1767.
3. European Food Safety Authority: https://www.efsa.europa.eu/

Rain Kuldjärv,

Project leader, Center of Food and Fermentation Technologies

Ph.D. student, Tallinn University of Technology