Enzymatic, yeast and lactic acid fermentation of Cloudberry (*Rubus chamaemorus* L.) leaves

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Introduction

Cloudberry (Rubus chamaemorus L.) grows on peatland and occurs most abundantly in Northern Scandinavia. Berries and berry extractives of cloudberry are utilized in food and cosmetics. Fermentation process is mostly utilized in food applications but it is also interesting pre-treatment process for cosmetic ingredients. Through the process of fermentation, organic compounds are decomposed to the smaller size and new/different organic compounds are produced. Plant phenolics possess a significant potential to inhibit or even reverse the signs of aging, such as wrinkles or hyperpigmentation marks (Działo et al. 2016). Fermentation is known to raise levels of active components (Mossou et al. 2005), which are also interesting ingredients for cosmetic applications.
To the best of our knowledge there is no publications available considering fermentation of cloudberry leaves. In this study, fermentation of cloudberry leaves were performed by enzymatic, yeast and lactic acid fermentation methods and the profiles of the fermented products were analyzed by LC-MS/MS system combined with high-resolution accurate-mass detection (HRAM).

Materials and methods

Cloudberry leaves were collected from Kajaani, Finland and the leaves were stored in the freezer before processing. Thawed leaves were cut and fermentations were performed by four replicates using B. Braun Biotech International Biostat Q Bioreactor System, which contains four chambers (1L) with stirrers and sensors for pH, oxygen and temperature. This instrument is specifically designed to accommodate the requirements of process optimization.

Alcohol fermentation was performed by *Saccharomyces bayanus* and lactic acid fermentation by *Lactobacillus reuteri*, whereas enzymatic fermentation was performed by activating the plant own enzymes with optimal conditions (temperature, pH and particle size). In addition, a reference sample was prepared by deionized water extraction.

After fermentation, samples were raw filtered and the filtrates were stored in a freezer. Thawed subsamples were diluted with mixture of methanol and water (1/1 v/v), centrifuged and further diluted with deionized water. Profile analyses (qualitative) of samples were performed by LC-MS/MS system combined with high-resolution accurate-mass (HRAM) Orbitrap detection (Q Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer, Thermo Scientific™) performed with both negative and positive ion modes. Compounds were identified using library search (Xcalibur and Metlin).

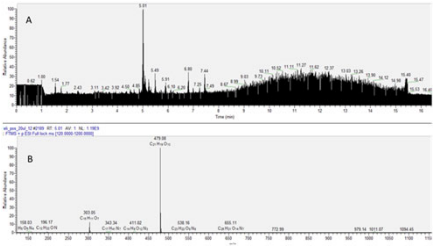
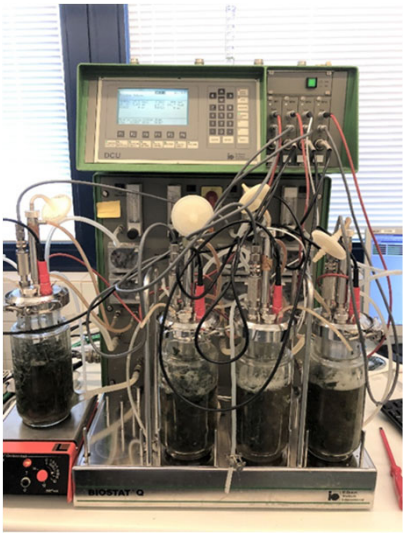


Figure 1.A) LC-MS/MS chromatogram of the enzymatic fermentation product of cloudberry leaves and B) mass spectrum (positive ionization) of the highest response (5.01 min) detected in the chromatogram A.



Results and conclusions

The products of alcoholic, lactic acid and enzymatic fermentations and water extract of cloudberry leaves analyzed by LC-MS/MS system showed very similar chromatogram profiles. The chromatogram of enzymatic fermentation product is shown in figure 1A as an example. In all studied samples the highest response was detected for compound eluting at a retention time of 5.01 minutes (Fig 1A), which was, based on the library search of the spectrum (Fig. 2B), obviously quercetin glycoside (m/z=479.08). Also other phenolic compounds were identified by library search. More accurate identification and quantitation of the compounds will be performed using authentic standards. Based on those results more detailed information of the samples and their appropriate utilization possibilities will be obtained.

References

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