The probiotic nature of novel kombucha drinks

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Introduction

Kombucha is non-alcoholic refreshing beverage with mild acidic taste originated from East. It's popular also on the European market and was claimed to have several health beneficial effects, including anti-diabetic, blood cholesterol reducing, and anti-cancer effects. Traditionally kombucha is produced by microbial fermentation of sweetened black or green tea. Nonetheless herbal teas can be utilized for fermentation as well opening new possibilities for Finnish local producers. The probiotic potential of the microbial consortium of kombucha drinks prepared from nettle or black currant leaves tea were investigated in the LuoPro project run by MITY of the University of Oulu.

Materials and Methods

Microbial plate counts (MPC) for total bacteria, yeast, molds, and fungi were determined using standard live plating techniques on different selective cultivation media. Representative yeast isolates were identified by sequencing D1/D2 region of the 26S rRNA gene using universal primers NL-1 and NL-4 (*Kurtzman and Robnett 1997*). For identification of the bacterial isolates, 16S rRNA gene amplification followed by sequencing were carried out using the universal primers BSF8 and BSR1541 (*Wilmotte, 1993*). Anaerobic growth of kombucha microbes was verified using Anaerocult® A mini (Millipore).

Resistance of the kombucha microbial consortium to simulated gastric acid environment (pH 2, pepsin 3g/L, mucin 3g/l) and bile acids (0,6% w/v) resistance was performed at 37ºC for 3h, followed by plating on several selective media.

Bacterial community profiles of Kombucha samples were analyzed by modified Terminal restriction fragment-length polymorphism (T-RFLP) method (*De Vrieze, 2018*) using Phusion Bacterial Profiling Kit (Thermo Scientific). PCR product of the 16S RNA gene was amplified with tagged universal bacterial primers, digested and fragments analyzed against the database.

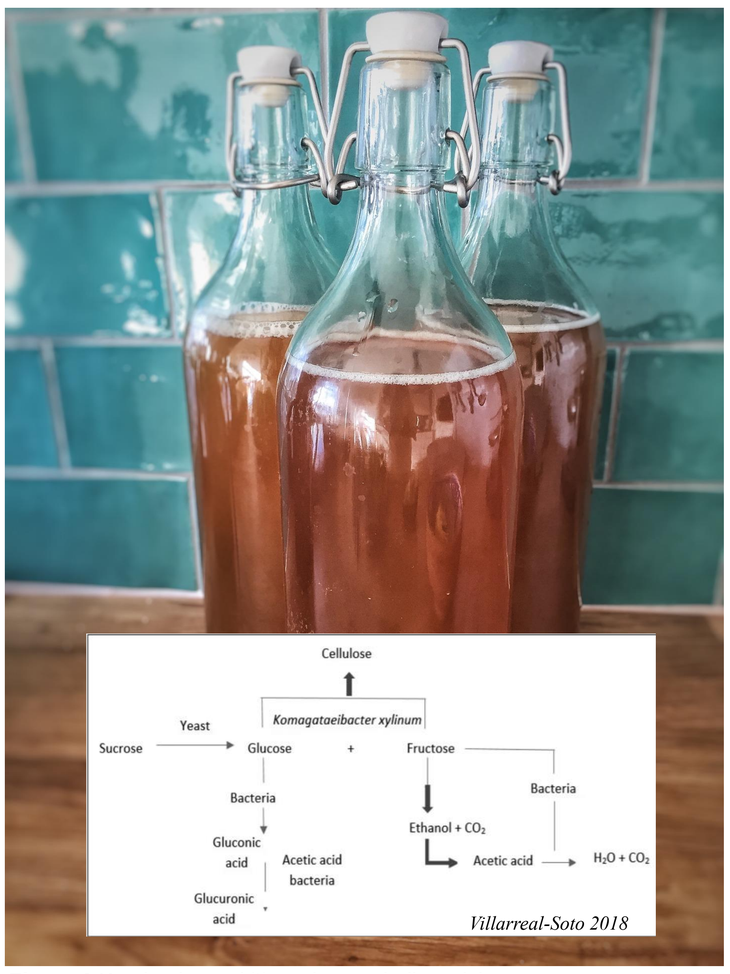

Figure 1 *Kombucha and it's main metabolic activity*

Organic acids and sulfate content were measured with capillary electrophoresis.

Oxygen radical absorbance capacity (ORAC) was performed by modified method of *Huang et al. 2002.*

Results

All tested kombucha drinks contained live microorganisms that were able to be cultured both aerobically and anaerobically. Among the identified yeasts species were: *Pichia occidentalis, Pichia membranifaciens, Zygosaccharomyces parabailii, Kluyveromyces marxianu, Brettanomyces anomalus, Brettanomyces bruxellensis,* and *Saccharomyces cerevisiae.* The most abundant bacterial species were acetic acid bacteria of the genus: *Gluconobacter, Gluconacetobacter, and Acetobacter.*

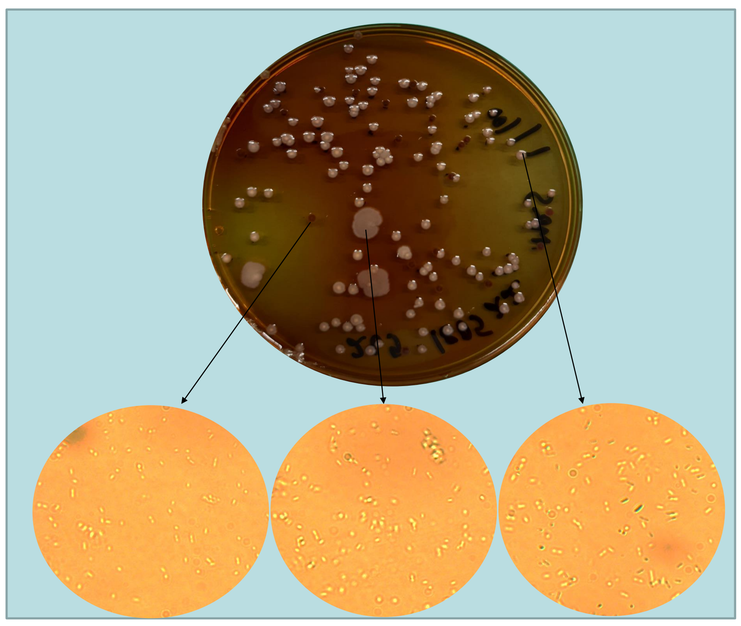

Figure 2 *Microbial characterisation and identification*

Colonies of different morphology cultivated on various selective media were further inspected under the microscope and subjected to molecular identification by sequencing.

The major organic acids measured were: acetic, malic, lactic and citric acids. Although all four samples were prepared from the same pre-culture, slight variation of the acid content was detected. This variation seemes to be due the natural flavoring extracts used at the final stage of the kombucha fermentation.

Concentration (g/L):	Sulfate	Acetic acid	Lactic acid	Malic acid	Citric acid
Sample 1	0,042 ± 0,001	1,654 ± 0,052	0,311 ± 0,012	0,018 ± 0,001	0,010 ± 0,001
Sample 2	0,026 ± 0,002	2,589 ± 0,089	0,084 ± 0,001	0,026 ± 0,010	0,014 ± 0,003
Sample 3	0,023 ± 0,002	1,042 ± 0,025	0,302 ± 0,012	0,255 ± 0,080	0,319 ± 0,024
Sample 4	0,044 ± 0,005	2,396 ± 0,042	1,289 ± 0,020	0,054 ± 0,009	0,492 ± 0,046

Table 1–General chemical composition of Kombucha. (Source: doi: 10.1111/1750-3841.14068)

	Compound	Average composition	Initial sucrose	Fermentation time (days)	References
Organic acids	Acetic acid	5.6 g/L	70 g/L	15 d	Blanc (1996)
	Acetic acid	8.36 g/L	100 g/L	18 d	Jayabalan et al. (2007)
	Acetic acid	11 g/L	100 g/L	30 d	Chen and Liu (2000)
	Gluconic acid	39 g/L	100 g/L	60 d	Chen and Liu (2000)
	Glucuronic acid	0.0160 g/L	70 g/L	21 d	Lončar et al. (2006)
	Lactic acid	0.18 g/L	100 g/L	18 d	Jayabalan et al. (2007)

Table 1 *Organic acids and sulfate content of the nettle Kombucha drinks. Organic acids concentrations of traditional Kombucha drinks are taken for comparison from: doi: 10.1111/1750-3841.14068)*

Antioxidant properties of the nettle Kombucha starter cultures varied from 66-127 µmol TE/100mL (µmol of Trolox equivalent per 100ml) and was slightly higher than that of the nettle tea (69-103 µmol TE / 100mL). Antioxidant properties of the ready nettle Kombucha drinks varied from 101 to 1267 µmol TE / 100mL. Antioxidant activity of the currant leaf kombucha was approx. 800 µmol TE /100mL and was in the range of the antioxidant properties of some juices.

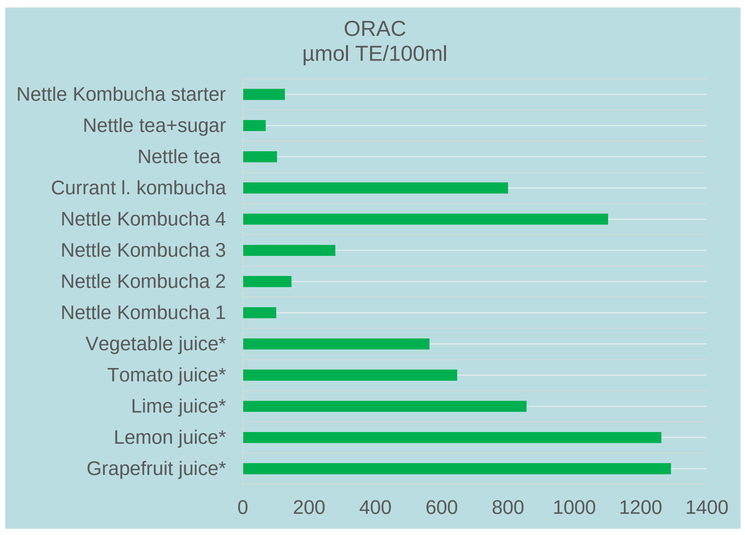

Figure 3 *Oxygen radical absorbance capacity (ORAC) of Kombucha samples in comparison to some juices.*

**ORAC measurements from juices were taken for comparison from* https://pubs.acs.org/doi/10.1021/jf049696w

Conclusions

All the tested kombucha drinks contained several live yeasts, acetic and lactic acid bacteria species able to survival in human gastrointestinal tract, namely can survive in the absence of O2, presence of gastric acid juice (pH 2) and/or bile acids (0,6%).

The pH of the Kombucha drinks was acidic and varied from 3,25-3,4. This acidity can assist natural digestion process taking place in the stomach when consumed with meal.

Among the organic acids tested, the acetic acid prevailed, followed in different samples either by lactic, citric or malic acids. Organic acids can act as mild antimicrobials, reduce in digesta pH and increase pancreatic secretion, thus improving food digestibilities. In addition, they have positive trophic effects on the gastrointestinal mucosa (*Dibner, J. J, 2002*).

Although the antioxidant properties of the nettle kombucha products were only slightly influenced by the fermentation process itself, addition of the natural wild berries flavoring extracts at the final stage of the fermentation process strongly increased the antioxidative capacity of the final product. The antioxidant properties of the black currant leaf kombucha without additives was in the range of the antioxidant properties of some juices.

Kombucha drinks prepared by microbial fermentation of nettle or black currant leaf tea represent a worthy alternative for non-alcoholic beverages.

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