Utilization of Stir Bar Sorptive Extraction – Gas Chromatography – Mass Spectroscopy Technique to Study the Aroma Active Compound Production during Kombucha Fermentation

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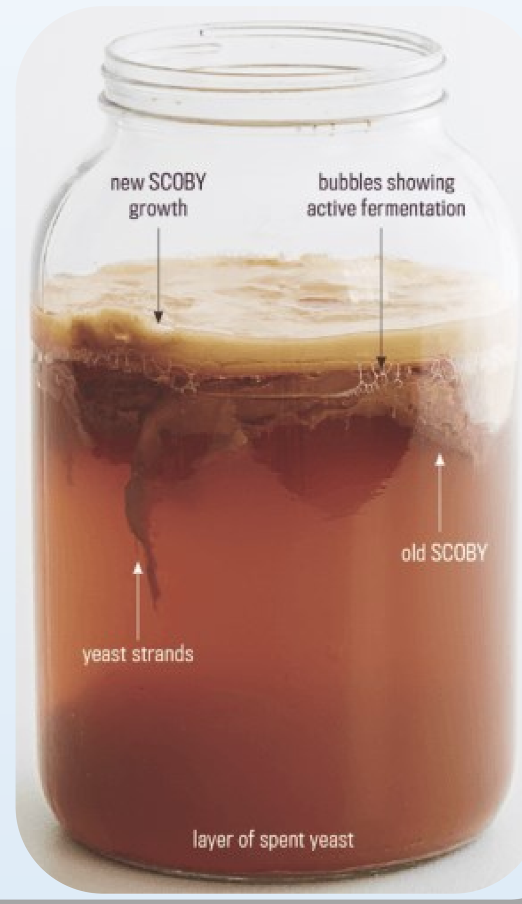
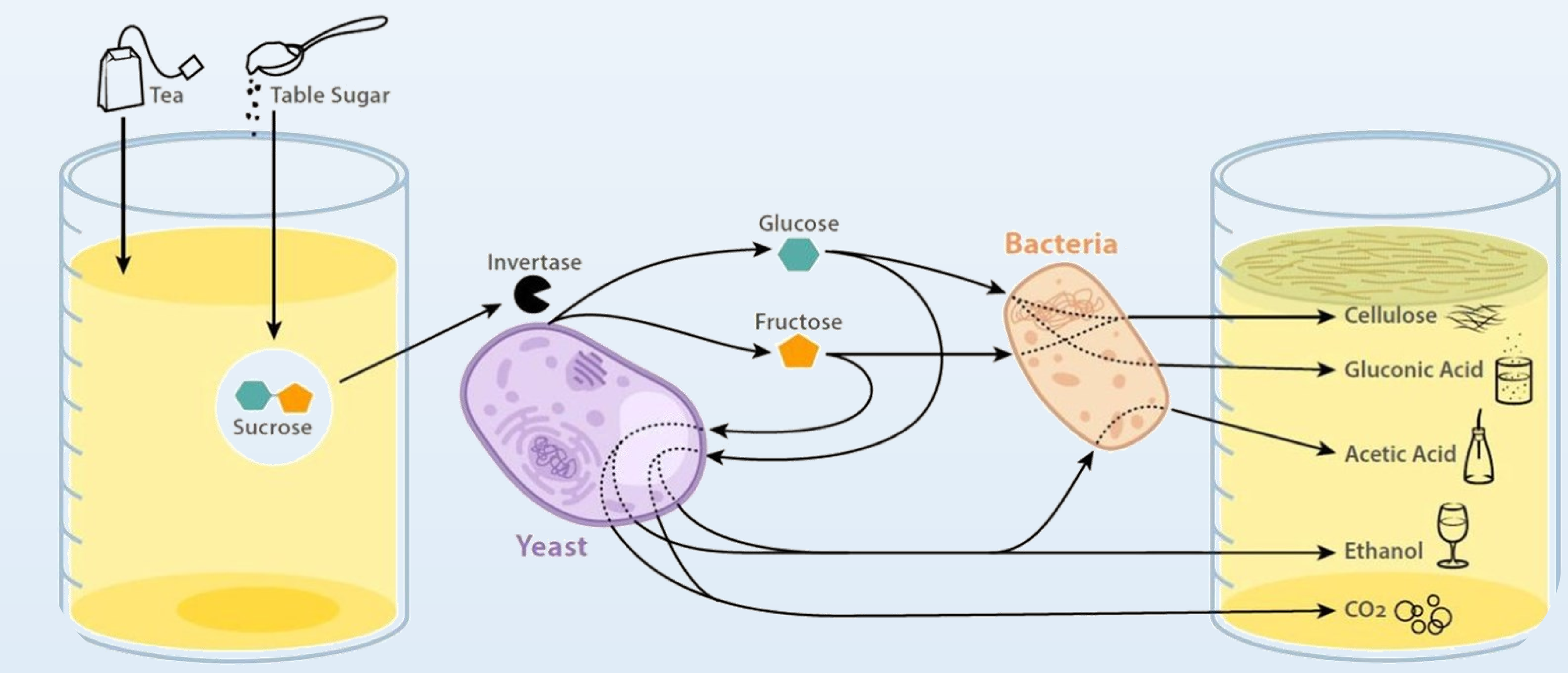
Introduction

Kombucha (from the Japanese for "tea algae") is a drink with the characteristics of a functional food: it satisfies hunger and provides nutrients, but also helps to prevent chronic diseases and improve physical and mental health. Originating in China, Korea and Japan (220 B.C.), it is popular for its detoxifying, antioxidant and energizing properties, as well as its ability to combat digestive problems. The drink is made by fermenting sweet tea, using bacteria and yeast. This combination is a symbiotic culture known as SCOBY (Symbiotic Culture of Bacteria and Yeast), which more commonly refers to the gelatinous substance that develops on the surface of the kombucha during fermentation. Today, kombucha is one of the most popular fermented beverages among many traditional fermented foods.

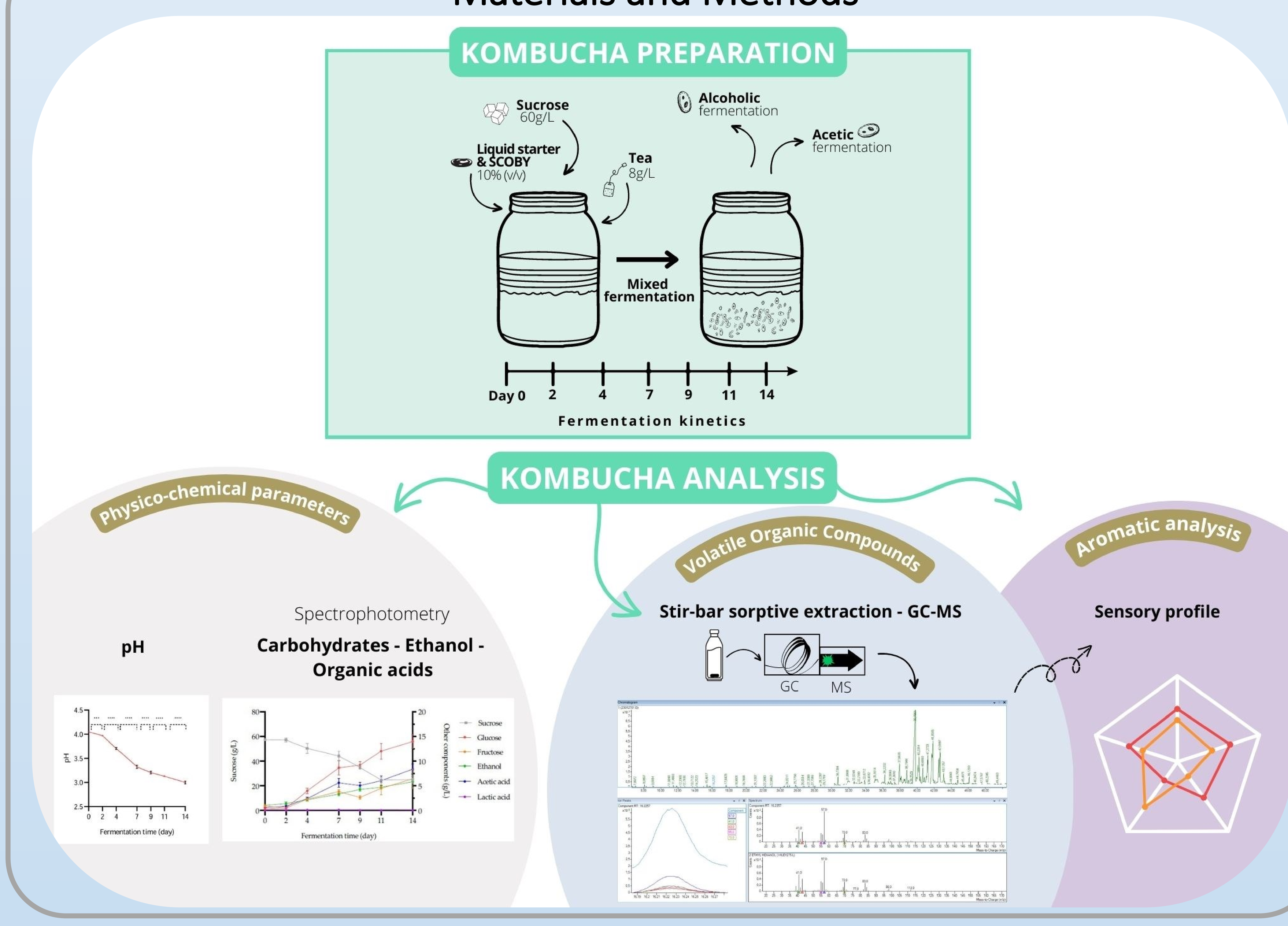
Since the sensorial profile is the cornerstone for the development of kombucha as a beverage with mass market appeal, advanced analytical tools are needed to gain a better understanding of the kinetics of aromatic compounds during the fermentation process to control the sensory profiles of the drink.



Mixed Fermentation Process of Kombucha

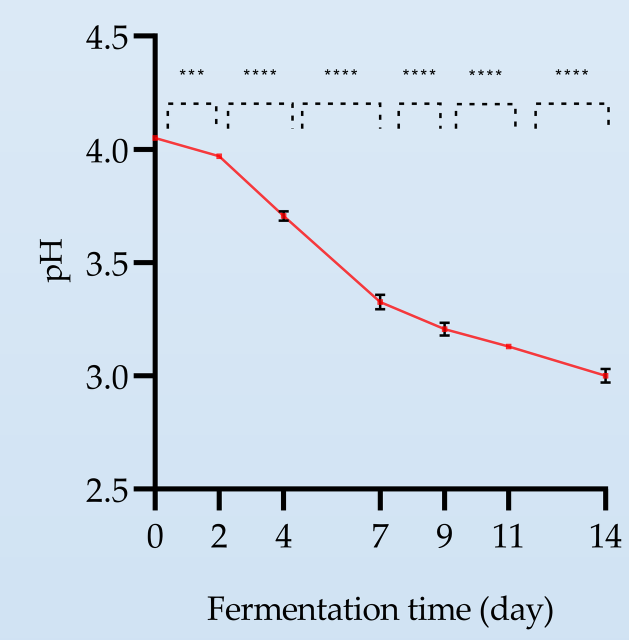


Materials and Methods

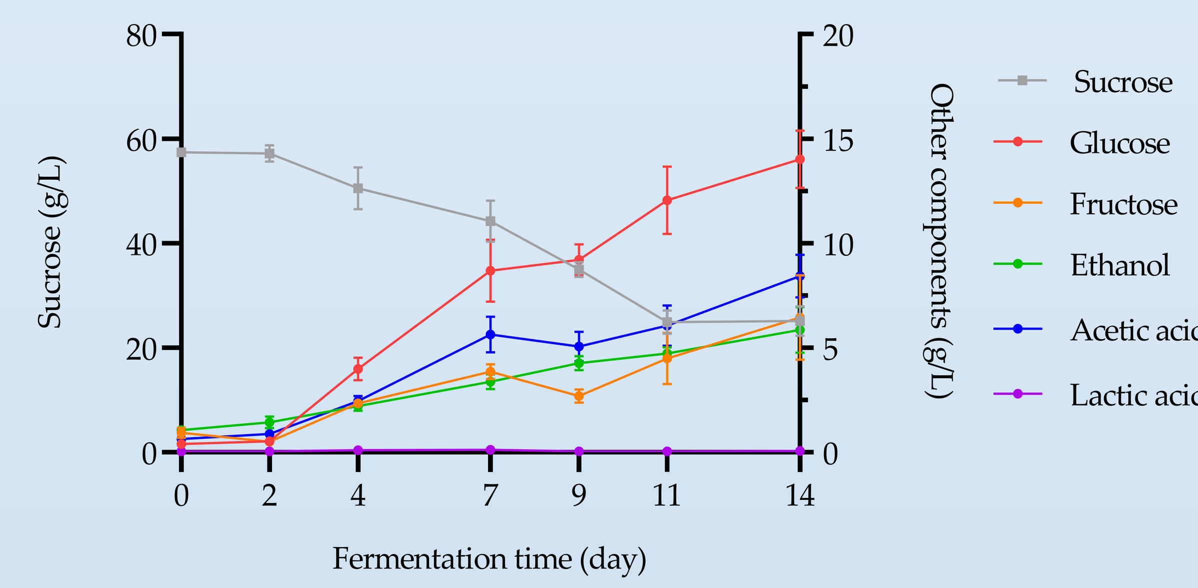


Results and Discussions

Physico-chemical characterization of kombucha during fermentation



pH evolution of kombucha samples in function of fermentation time (day) at 30°C (mean ± SD; n=3; Student's t-test; ****, p-v < 0.0001; ***, p-v < 0.001)

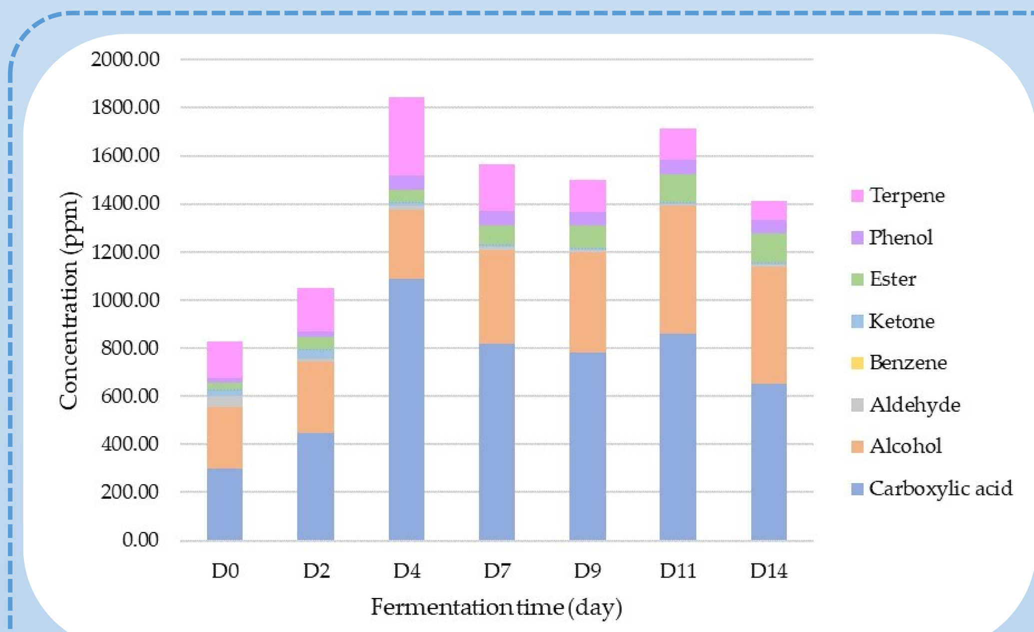


Evolution of carbohydrates, alcohol, and acids concentration (g/L) in function of fermentation time (day) at 30°C(mean ± SD; n=3)

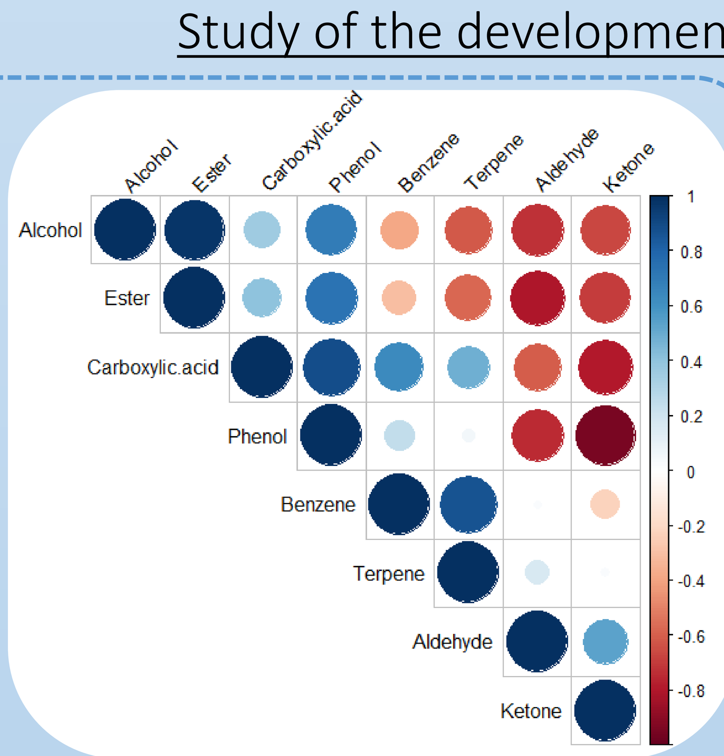
Concerning physico-chemical characterization of kombucha, a pH decrease reflects an acidification in the fermentation medium and illustrates organic acids synthesis by the fermentation microbiome. In these conditions, low pH (below 4.5) ensures food safety by inhibiting spoilage and pathogens development.

Through the major compounds kinetic, main microbial consortium pathways are highlighted, such as glycolysis pathway and AAB pathways.

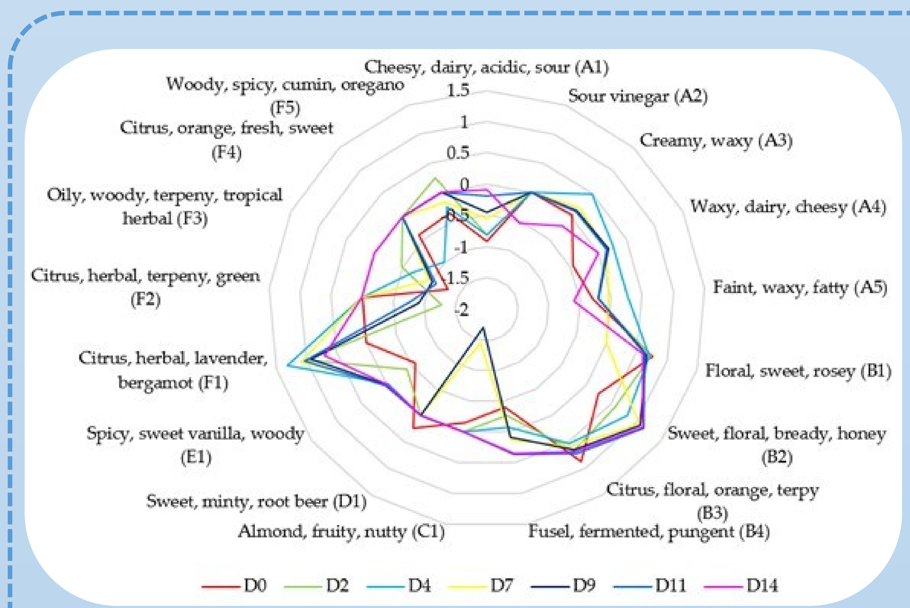
Study of the development kinetics of volatile organic compounds



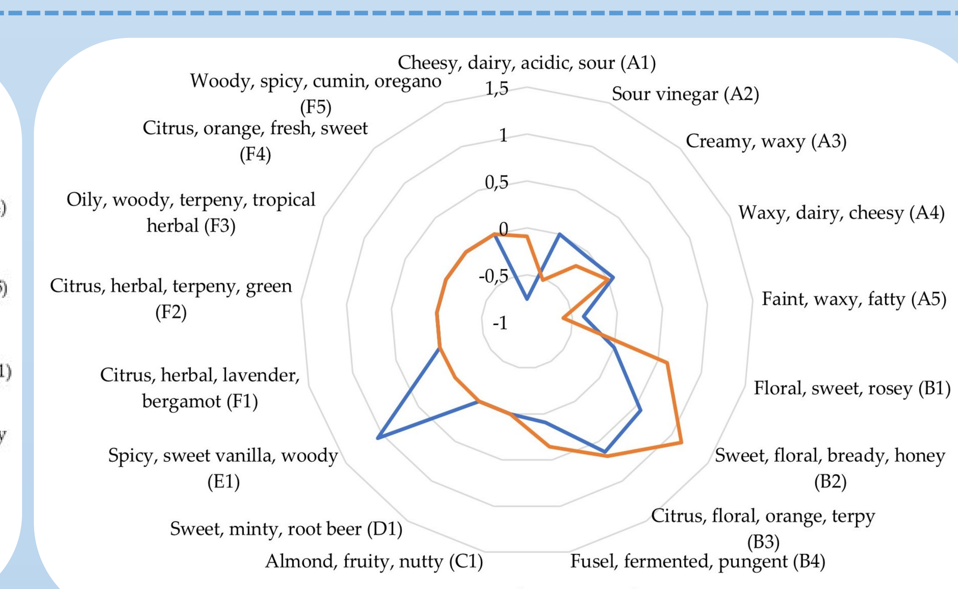
Changes in average contents (ppm) of different classes of VOCs during kombucha fermentation time (day) at 30°C



Correlation between different classes of VOCs during kombucha fermentation stages (a color gradient denotes the Spearman's correlation coefficients)

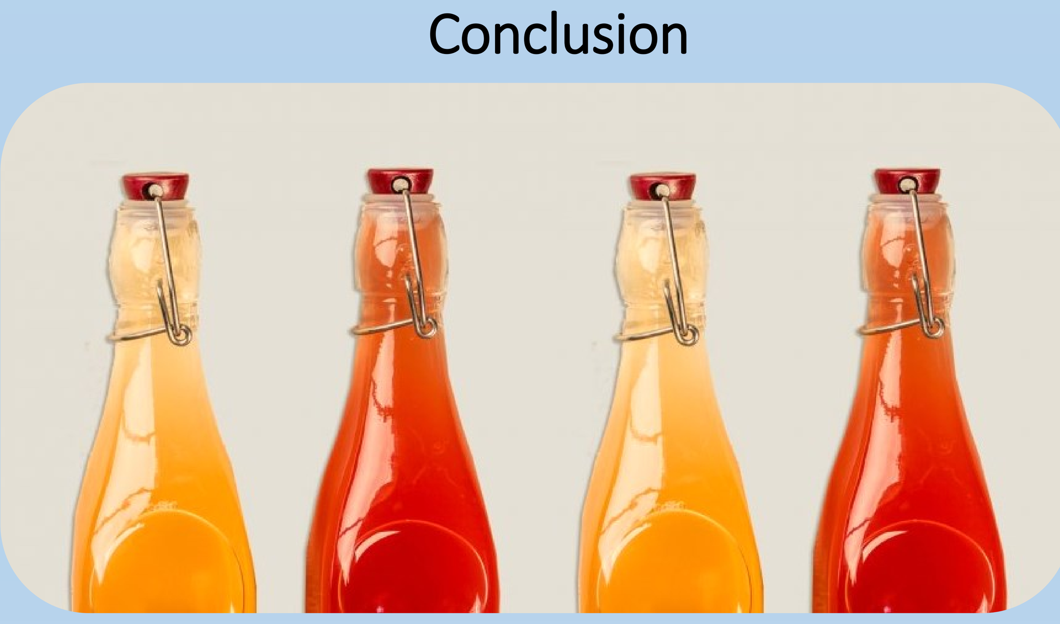


Estimated sensory profile is expressed as the log of the OAV for most representative volatile organic compounds (%area > 1%) with kombucha fermentation time (day) at 30°C (OAV = concentration/ perception threshold)



Variation of the estimated sensory profile (expressed as the log of OAV; OAV = concentration/ perception threshold) of most representative volatile compounds (%area > 1%) with temperature (°C) on kombucha fermentation final stage (D14)

Conclusion



Dynamic changes in VOCs were investigated using SBSE-GC-MS during kombucha fermentation. A total of 87 VOCs were detected in kombucha during the fermentation stages, including 28 carboxylic acids, 23 alcohols, 10 ketones, 9 terpenes, 7 esters, 7 aldehydes, 2 phenols and 1 benzene. The fermentation process changes the VOC tea profile dynamically into a different mixture of compounds. The synthesis of mainly phenethyl alcohol and isoamyl alcohol probably by *Saccharomyces* genus leads to ester formation (ethyl acetate main structure). Moreover, the terpene synthesis occurring at the beginning of fermentation (Δ-3-carene, α-phellandrene, γ-terpinene, and m- and p-cymene) could be related to yeast activity as well.

The aromatic analysis, based on odor-active values, accounts for 17 aroma-active compounds. These changes in the evolution of compound concentrations led to flavor variations at the different fermentation stages: from citrus-floral-sweet and orange notes at the initial stage (geraniol and linalool domination), with stages D2-D4 fermentation bringing intense citrus-herbal-lavender-bergamot notes (synthesis of α-farnesene). From stage D7, sweet-floral-bready-honey notes dominates the kombucha flavor (2-phenylethanol synthesis). However, although the final flavor of kombucha keeps characteristic aroma compounds from its original substrate, several common compounds (such as 2-phenyl ethanol, nonanoic, and octanoic acids) may also contribute to the signature aroma of kombucha.

Despite the need of sensory analysis to evaluate consumer acceptance and preferences on the beverage, the fermentation time control seems to give an overview of potentially developed aromas and perceptions. Moreover, as temperature may have an impact on final kombucha flavor, effect of crossed fermentation parameters (temperature, time) on the aroma-active compounds development should be further studied, towards the control of fermentation and the production of quality flavored beverages.

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Acknowledgements

The research leading to these results has been founded by the Public Service of Wallonia (Economy, Employment and Research), under the FoodWal agreement n°2210182 from the Win4Excellence project of the Wallonia Recovery Plan