Sweeteners and gut flora: harmless flavor enhancers or hidden stress factors?

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Introduction

High consumption of added sugars has long been associated with various health problems and as a result, sugar substitutes were introduced to reduce caloric intake and limit health risks. Today, sweeteners are widely used in 'zero' and 'light' products, leading to frequent exposure through the daily diet. Although these sweeteners were intentionally considered a healthy alternative, growing evidence suggests that they may have negative effects on human health. This study investigates the effects of the sweeteners sorbitol, mannitol and saccharin on representative gut bacteria such as *Escherichia coli* and *Enterococcus faecalis*, with a focus on bacterial growth, survival and osmotic stress-related gene expression.

Materials and methods

CFU-counting

Colony-forming unit (CFU) analysis was performed by plating tenfold serial dilutions of *E. coli* and *E. faecalis* onto LB and TSA agar, respectively, containing sorbitol, mannitol and saccharin at varying concentrations.

OD_{600}-measurement

E. coli and *E. faecalis* were grown in LB or TSB broth, respectively, containing sorbitol, mannitol and saccharin at varying concentrations. Bacterial growth was then monitored every hour by measuring optical density at 600 nm using a spectrophotometer.

qPCR

The liquid cultures were used to quantify gene expression by qPCR. Expression of osmotic stress-related genes, such as osmY and proX, was monitored in E. coli, while rpoD served as a housekeeping control.

Results and discussion

CFU-counting

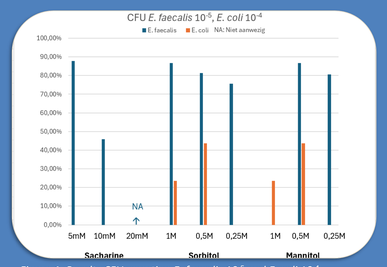

Figure 1: Results CFU-counting E. faecalis 10^{-5} and E. coli 10^{-4}

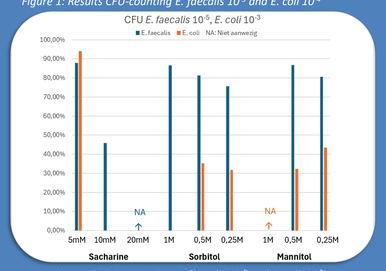

Figure 2: Results CFU-counting E. faecalis 10^{-5} and E. coli 10^{-3}

qPCR

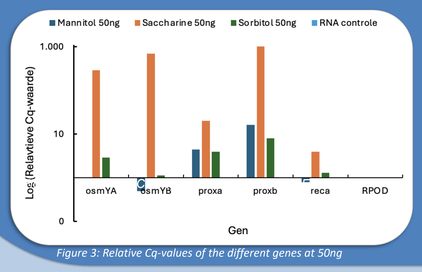

Figure 3: Relative Cq-values of the different genes at 50ng

OD-measurement

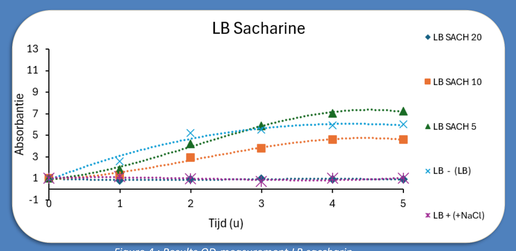

Figure 4 : Results OD-measurement LB saccharin

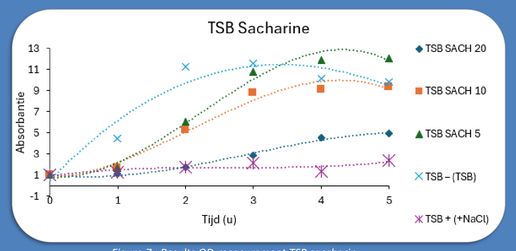

Figure 7 : Results OD-measurement TSB saccharin

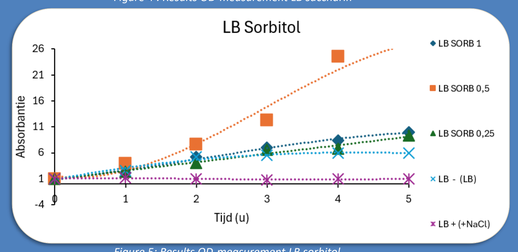

Figure 5: Results OD-measurement LB sorbitol

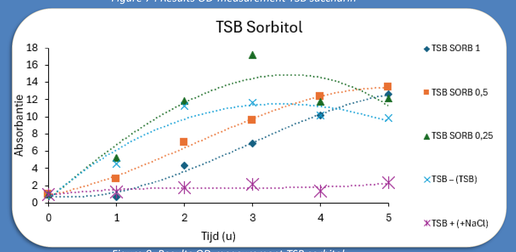

Figure 8: Results OD-measurement TSB sorbitol

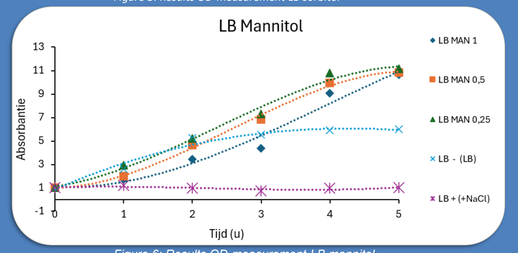

Figure 6: Results OD-measurement LB mannitol

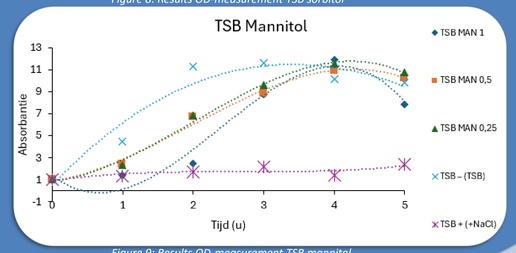

Figure 9: Results OD-measurement TSB mannitol

Conclusion

The results indicate that the sweeteners sorbitol, mannitol and saccharin affect the growth and osmotic stress response in gut bacteria, while *E. faecalis* is generally more tolerant than *E. coli*. Saccharin caused the strongest stress response across assays, while sorbitol and mannitol produced milder effects consistent with their polyol nature. Higher sweetener concentrations consistently reduced growth and altered physiological outputs like pH changes and OD_{600} - readouts. In addition, RT-qPCR confirmed that all sweeteners triggered transcriptional stress responses and decreased bacterial growth.