In situ tracking of CO_2 bubble dynamics in kefir
"The Champagne of Dairy"

O. Brosnan[1], C. Schmidmeier[2], S.V. Crowley[2]

[1]School of Biochemistry and Cell Biology, University College Cork

[2]School of Food and Nutritional Sciences, University College Cork

University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Introduction

Kefir is a CO_2 containing and slightly alcoholic beverage made through fermentation, typically of milk or water, with cultures of bacteria and yeasts. Traditionally, cultures come as grains – bacteria and yeasts embedded in a strong, carbohydrate and protein containing matrix – but more recently, also freeze dried cultures are available for large scale kefir production. During fermentation, bacteria produce acids and break down disaccharides into sugar monomers which are then metabolised by the yeasts, resulting in acidification of the beverage (and thickening in the case of milk) and the release of CO_2 bubbles, giving kefir its effervescent character. This project explores CO_2 bubble dynamics in both milk and water kefir.

The traditional milk-based beverage, with roots in Europe and Asia, has recently been gaining popularity as a health product due to its pre- and probiotic nature, in addition to its effervescence which gives it a refreshing quality. However, the scientific community is only starting to investigate biological, nutritional and physical properties of the highly complex beverage.

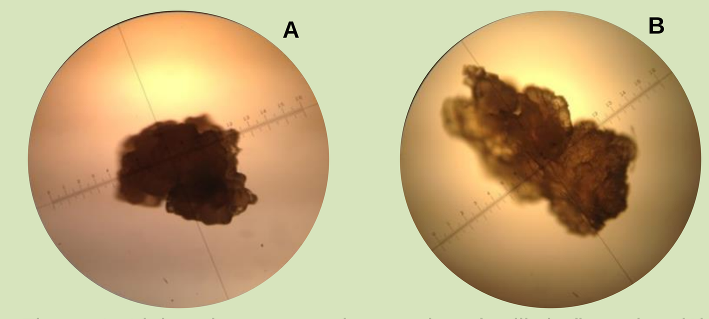

Figure 1: Light microscope micrographs of milk kefir grains (A) before culturing and (B) after one day culturing in whole milk. 10x magnification. No scale bar.

Materials & Methods

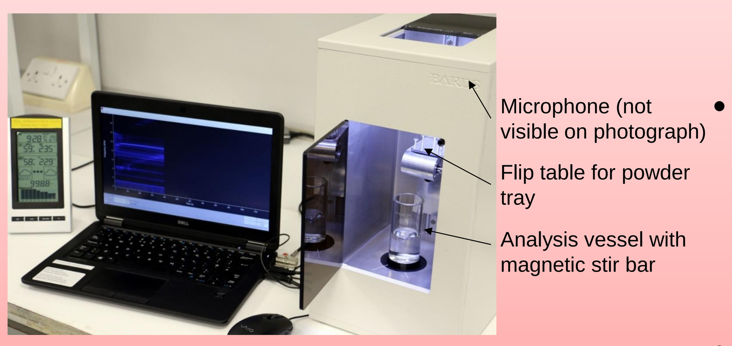

Figure 2: BARDS instrument during sample analysis.

- **Kefir grains** were cultured in about 200ml whole milk and water accordingly (water supplemented with sucrose and raisins) and changed after 24 hrs of incubation. Filtrate was kept for second fermentation at 4°C up to 14 days.
  - BARDS was used to monitor release of CO_2 bubbles from second ferments
- **Freeze dried cultures (milk Kefir 1 & Kefir 2)** were inoculated at 0.01 U in 100ml reconstituted skim milk (12% total solids) and analysed as follows
  - **Conductivity** (Metrohm conductivity probe) and **pH-value** (HACH benchtop pH-meter) were monitored as indicators of milk kefir fermentation
  - **Viscosity** was measured with a Haake RotoVisco to compare different cultures as well as commercial kefir drinks
  - **Protein profiles** were generated using reducing SDS-PAGE to monitor protein break down during kefir fermentation
  - **CO_2 bubble development** was followed using BARDS
- A **4.8% lactose solution** was used as a model system to investigate
  - Lactase activity, indicated by **freezing point depression**, as a preliminary experiment for fermentation trials with yeast (LAF-5) only

Results: BARDS

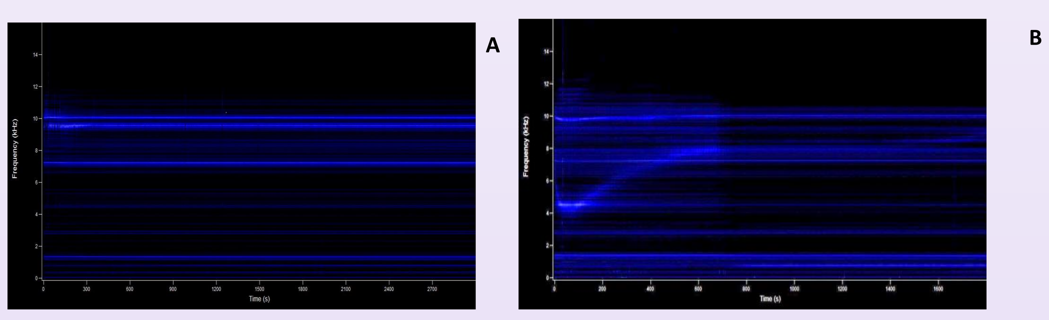

Figure 3: Kefir 1 at t= 4hrs (A) and t=16hrs (B)

- After 16 hours the milk culture began to thicken causing interference and merging of background frequencies with the fundamental curve (curve of interest), making monitoring bubble formation difficult
- In Figure 3(B), outgassing may be observed in the first 10 minutes of testing (increase in the fundamental curve) at which point the frequency appears to reach a plateau of 8kHz (equilibrium of gas release and introduction), however this was the only successful outgassing event visible throughout the experiment

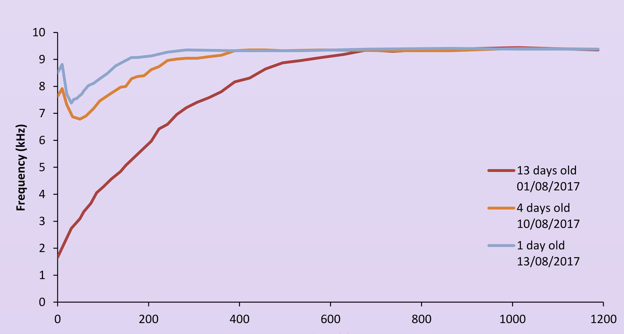

Figure 4: BARDS analysis of water Kefir secondary ferments

- As can be seen in Figure 4 the longer the Kefir second ferments have to continue fermenting, the more CO_2 gas they produce
- In Figure 4, the 13 day old Kefir seems to have a longer outgassing phase to reach plateau than the 4 day old and 1 day old Kefirs, suggesting it has a higher CO_2 content and therefore a higher effervescence

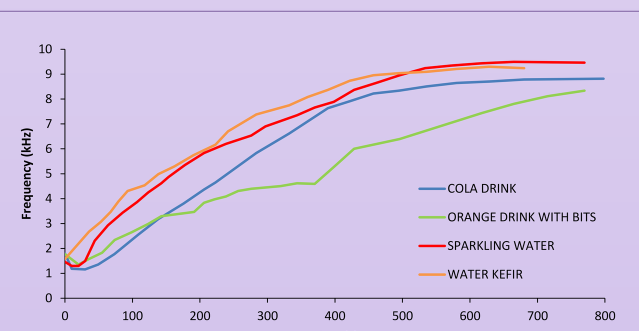

Figure 5: BARDS analysis of soft drinks and water kefir to compare degassing after opening

- It appears the water kefir loses its fizziness the fastest followed by sparkling water, orange drink with bits and cola drink respectively (Figure 5)
- The data suggest that the orange drink with bits is slowest to release the CO_2 bubbles which could be due to its chemical composition but also may be connected to the real fruit bits which give it texture, compared to the other drinks which have a lower viscosity
- Plots for water kefir, sparkling water and orange drink appear as uneven curves, compared to a smoother, more controlled release of bubbles from the cola drink

Results: Freezing Point Depression

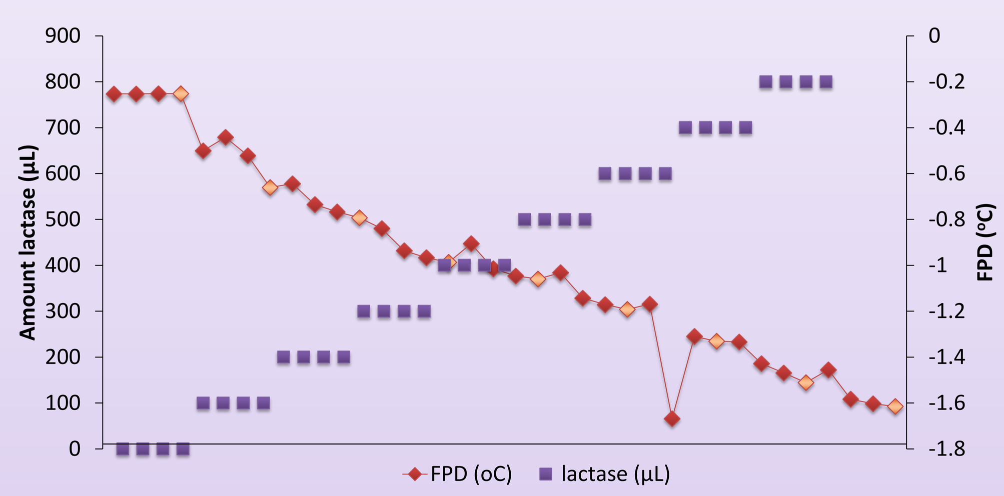

Figure 6: Graph of freezing point depression (FPD) of 4.8% lactose with increasing amounts of Colief lactase enzyme

- The higher the concentration of molecules in the solution, the lower the freezing point that will be detected by the Cryoscope
- Therefore the lower freezing point here indicates more lactose hydrolysis by the lactase enzyme, into glucose and galactose
- It appears that at 800uL lactase the graph begins to plateau out, suggesting that hydrolysis of lactose is close to completion

Results: Viscosity

- Viscosity, thickness due to the internal friction of a liquid, was compared between Kefir cultures and commercial Kefirs (Lowicz and Milko)
- It appears that Lowicz had the highest viscosity, followed by Milko, Kefir 1 and Kefir 2
- It was also described, during a taste test, that Lowicz was more sparkling than Milko, which could be caused by the high viscosity trapping the CO_2 bubbles
- The viscosity therefore may contribute to the effervescence of Kefir

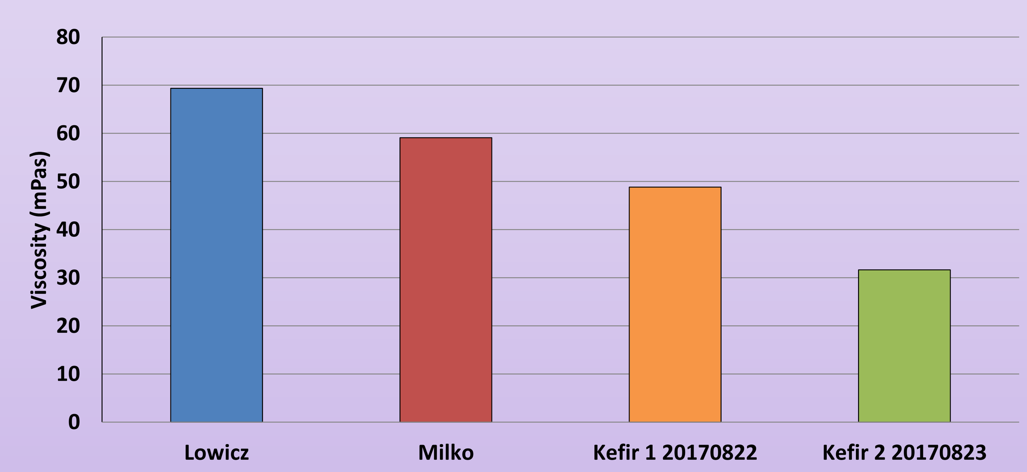

Figure 8: Bar chart of viscosity of commercial and lab grown Kefir beverages

Results: SDS PAGE

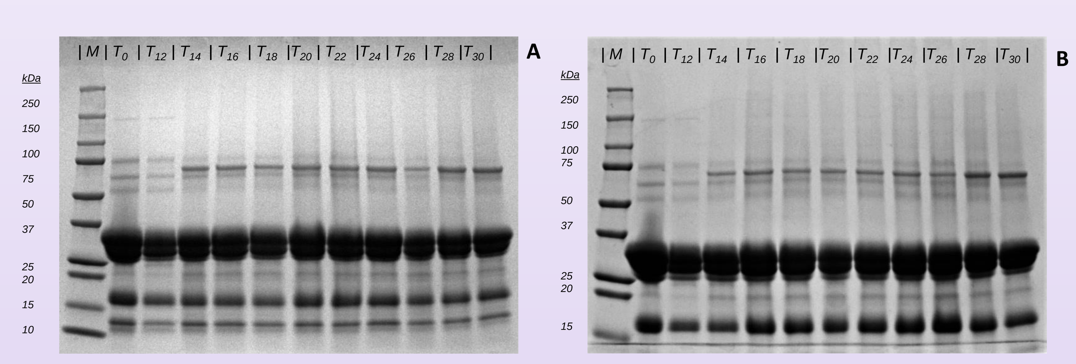

Figure 7: SDS PAGE analysis of Kefir 1 (A) and Kefir 2 (B) culture time-course

- Analysis of kefir 1 (A) suggests that during the course of the fermentation there is proteolysis mainly of high molecular weight proteins
- At 150 kDa (xanthine-oxidoreductase, XOR), 75 kDa (lactoferrin, LF) and 50 kDa (IgG heavy chain), decrease in band intensity after 12 hours of fermentation suggests proteolysis. (Proteins suggested according to literature values.)
- In addition to proteolysis there also seems to be an increase in band intensity at ~70kD after 12 hours which may be larger protein fragments but also could be indicative of the proteins produced by Kefir bacteria to create their protective biofilm-like matrix
- As literature suggests both α-LA and β-LG are unaffected by degradation
- Qualitatively both gels for Kefir 1 and 2 were similar

Results: Conductivity & pH

- As expected, pH drops due to acidification and conductivity increases due to microbial secretions
- Different duration of lag-phases, rates of acidification and pH end-levels suggest that Kefir 1 and Kefir 2 cultures contain different bacterial and/or yeast strains
- Similarly for the conductivity measurement, the two cultures appear to vary slightly with Kefir 2 reaching a plateau/ equilibrium state more quickly than Kefir 1. This may be due to different yeast strain metabolisms contributing ethanol and CO_2 bubbles.

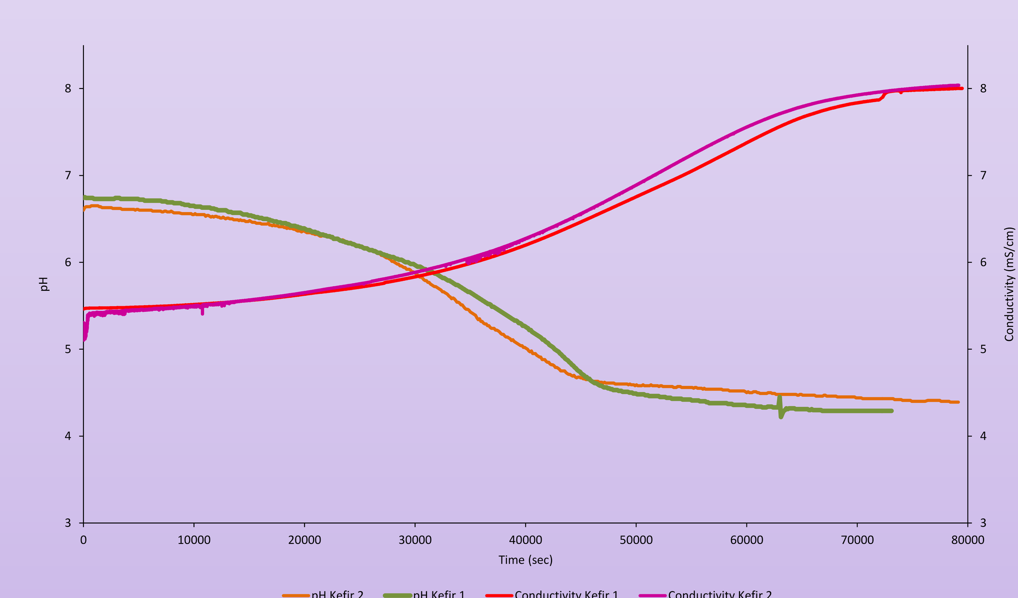

Figure 9: Conductivity and pH time-course of Kefir 1 and 2

Discussion & Future work

- BARDS is not well suited for application on milk kefir under normal fermentation conditions due to viscosity increase. However there is some evidence of CO2 release after 16 hours as seen in Figure 3(B). To exploit this, maintaining a constant initial pH in the milk system to prevent thickening may allow monitoring of the complete fermentation process in BARDS
- Results of the water kefir second ferments may be used to monitor primary fermentation, e.g. to test inter-batch and intra-batch variability
- Kefir 1 and 2 seem to have different conductivity and pH profiles which could be due to different microbial compositions
- Preliminary experiments with LAF-5 yeast and lactose suggested that CO_2 bubbles (popping sound upon opening flask) are produced so this could be continued and tested in the BARDS to monitor the CO_2 bubble release
- From the SDS PAGE gels it was not possible to qualitatively detect new populations of bands of digested peptides as expected at ~10kD which should be explored as it is known the larger proteins are digested

Acknowledgements

- UCC School of Science, Engineering & Food Science (SEFS Summer Bursary)
- Dr Seamus O'Mahony, UCC (Laboratory & equipment use)
- Chr. Hansen (provision of cultures used in project)