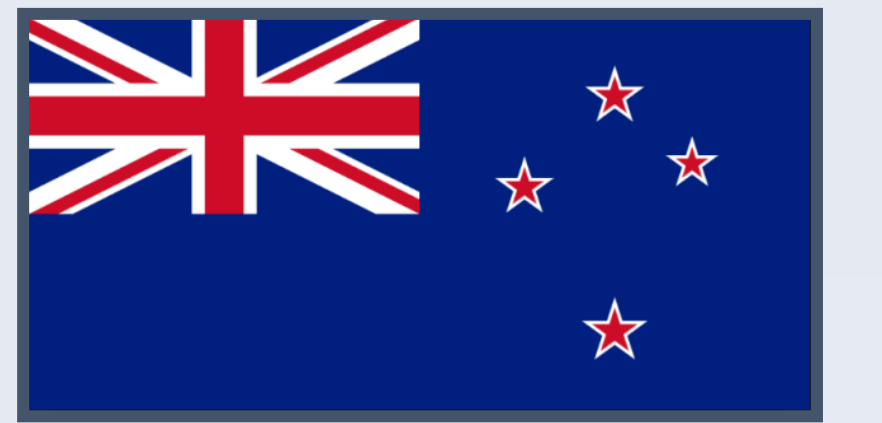




Chemometric Analysis of Alternative Plant-based Milks

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Why Plant-Based Milks?

In recent years, there has been a notable **rise in the popularity and consumption of plant based ‘milks’/mylks**. This trend is the result of a range of factors related to increasing levels of concern amongst consumers with the **ethical, environmental, nutritional and health implications** related to the consumption of animal milks, particularly bovine milk.¹ Plant based milks can be produced from **any number of ingredients that fall within the families of legumes, grains and nuts/seeds** and while “traditional” plant-based milks such as soy, almond, rice and oat milks are popular commercial products, there is surprisingly little scientific literature concerning the chemical profiles of these substances, beyond their percentage nutritional composition. Additionally, **many “traditional” plant-based milks have unfavourable nutritional attributes and have questionable long-term sustainability**.²⁻⁴

Despite the fact that this is an extremely exciting area, the scientific literature exploring the composition of different plant-based milks is scarce. **More needs to be done to understand the chemical signature of these new milks and also explore how their chemical profiles change with altering conditions**. Not only does this allow understanding of the benefits and potential of these new milks, but allows for comparison of their attributes and resulting viability and suitability for future use.

Aims

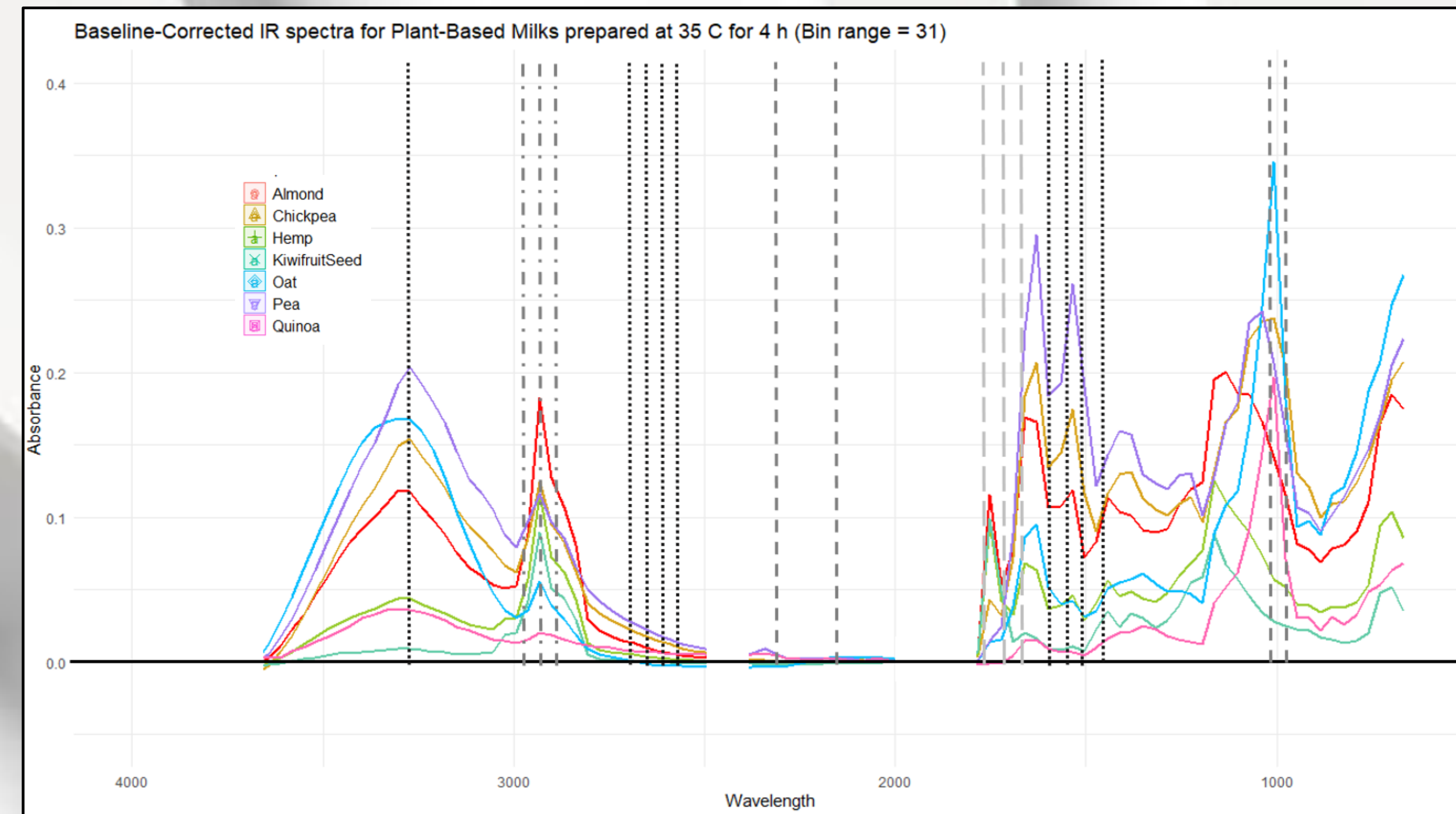
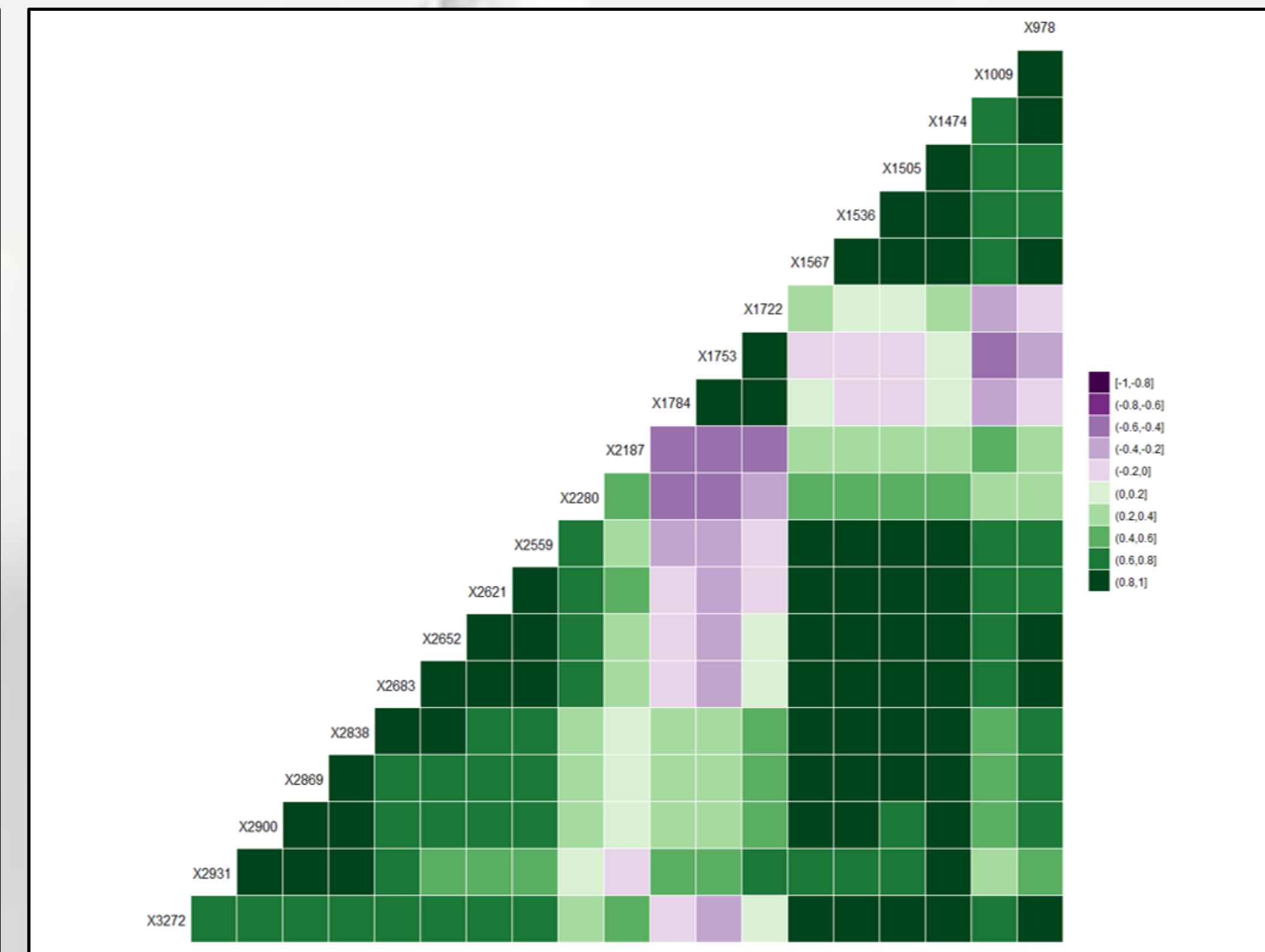
- The overall aims of this work was to;
- i) **produce a range of plant-based milks using different experimental conditions, and**
 - ii) **analyse their chemical signatures through ATR-FTIR and untargeted analysis of the resulting spectra**, allowing an understanding of the overall profile of the milks, as well as the effect of different experimental conditions.

Methodology

To gain representation of both traditional and alternative plant-based milks in this study, seven types of milks representing each of the three main ingredient types; legume (chickpeas and yellow split peas), grain (oats and quinoa) and nut/seed (almonds, hemp seeds and kiwifruit seeds) were investigated.

Making the milks: The raw ingredients were soaked in Type I water (3:1 w/w) under one of three different experimental conditions (20 °C for 2 hours, 35 °C for 4 hours or 50 °C for 8 hours, before undergoing blending (60 s using an immersion blender) and filtration, producing the plant based milk. Each milk (type and condition) was made in triplicate.

Spectroscopic analysis: A drop of the homogenised milk sample was placed onto the FT-IR crystal and then dried using a stream of air for ~ 2 mins until the sample was dry by visual inspection. The absorption spectra of the samples were recorded and processed as described.

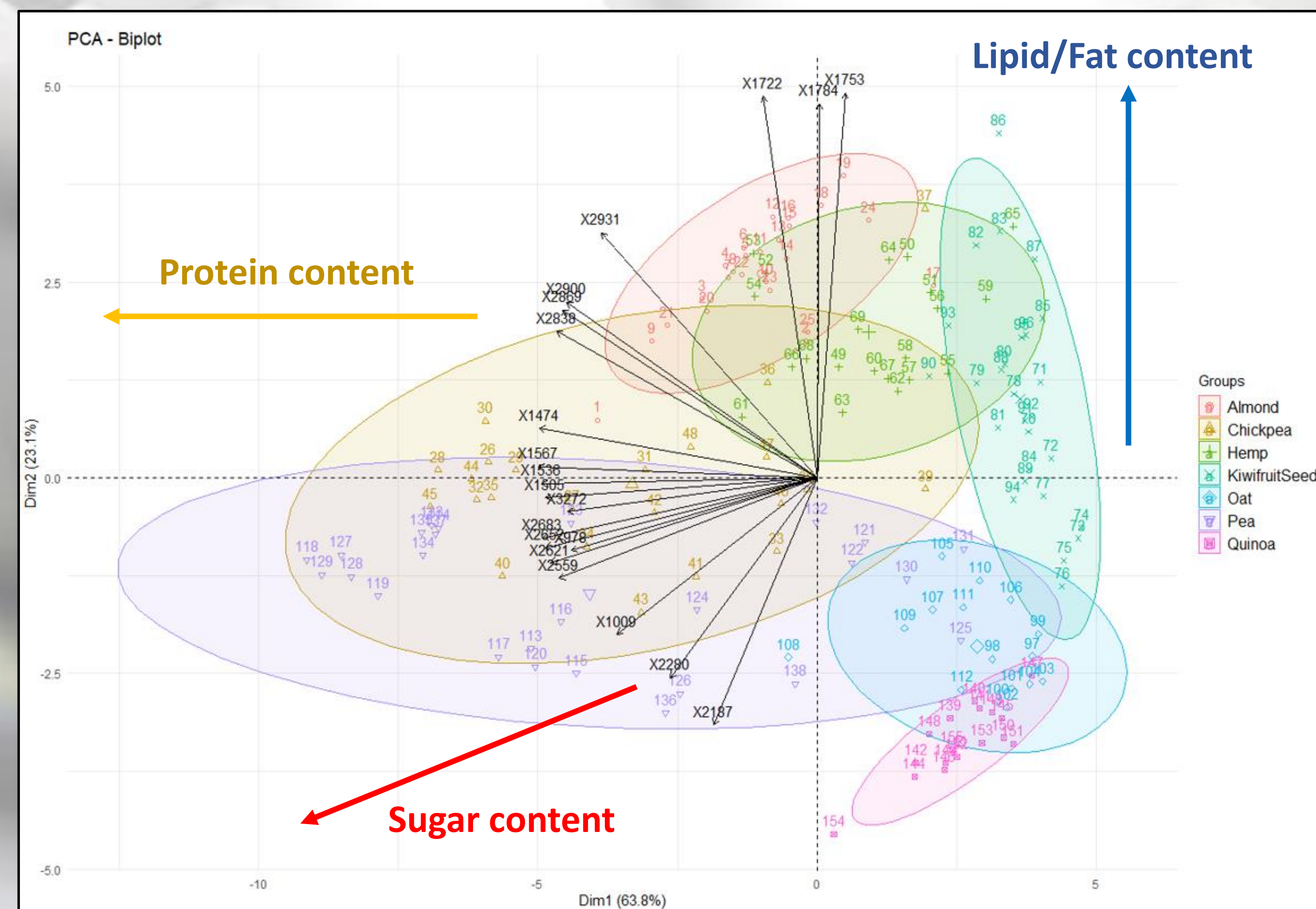
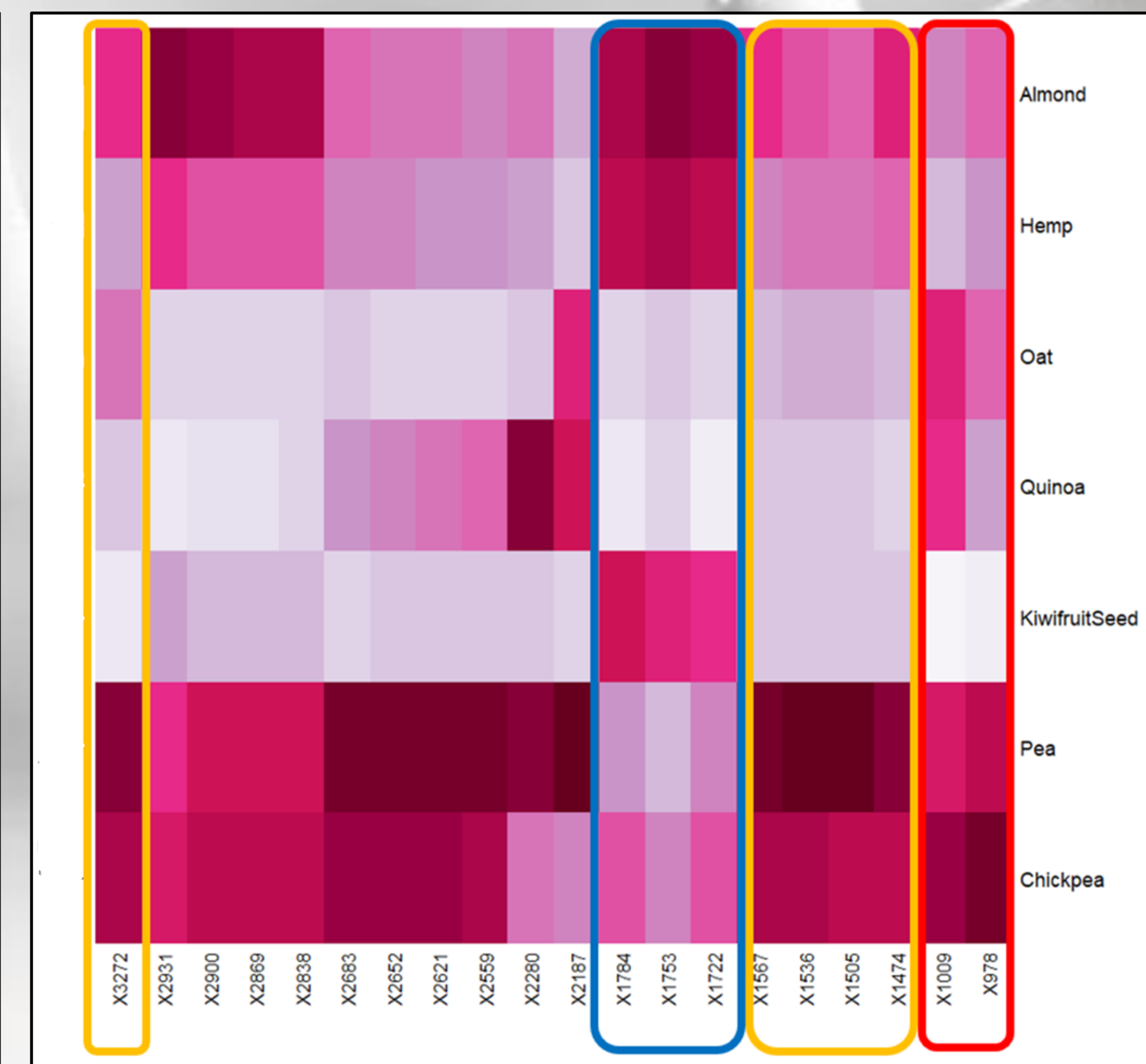


Data pre-processing: The FT-IR data first underwent a set of processing steps; to reduce the dimensionality, a range of binning ranges were trailed, where it was ultimately decided to employ a binning range of 31. In addition to binning the data, the spectra were all baseline corrected and regions of little to no variability (>3800 cm⁻¹, 2550-2400 cm⁻¹ and 1800-2000 cm⁻¹) were also removed prior to further analysis (**above, left**)

Further variable reduction: Classification random forest models were fit to the data determine which of the ~100 remaining features were most important for milk-type determination. The features with a mean decrease in Gini > 2, averaged across ten different random forest models, indicating that they are important for classification of the milks, were chosen for the reduced-variable analysis (**above left, grey lines**)

Feature analysis: The interrelationships between the 20 selected wavelengths for all the spectra were analysed through the use of correlation analysis (**above, right**).

Milk type analysis: The chemical signatures of milk types were statistically different (PERMANOVA), and analysed through Principal Component Analysis (**PCA, below, left**) and profiled (**below, right**).



Results

Initial analysis: Overlaid, pre-processed (see *Methods*) and averaged spectra for each of the milk types made under the medium of the three conditions (35 °C for 4 hours) are displayed in the **top left figure**. As can be seen, **while there are common bands present in all/almost all milk types, there is a high degree of variability in the FTIR spectra, particularly in the intensity of the peaks seen**.

Feature analysis: Following variable selection (see *Methods*) 20 features were found to be the most important in differentiating the milk types. The 20 selected features covered a broad range of positions in the FTIR spectra (**grey dashed lines in the top left figure**) and most correspond to wavelengths where there are observable peaks present for some or all milk types. Furthermore, **selected features were able to be linked with characteristic signals corresponding to the three main nutritional components present in food matrices:**

- Highly-correlated absorbances at **1722, 1753 and 1784 cm⁻¹** align with the triglyceride ester carbonyl found in **lipids**.
- Strongly positively-correlated absorbances at **978, 1009 cm⁻¹** relate to C-C/O stretching-related signals in **sugar**.
- Amide II (C-N) signals at **1474, 1505, 1536 and 1567 cm⁻¹** and NH₂ at **3272 cm⁻¹** represent the **protein** present.

While most of the features appeared to have positive correlations, sugar content was either uncorrelated or low-moderately negatively correlated to protein and fat (lipid) content (**top right figure**).

Milk type analysis: PCA and heatmaps were used to analyse the data to investigate differences in the milk types (**bottom figures**). It was found that, relative to the other milks:

- The grain-derived milks (quinoa, oat) are low in all of protein (yellow), sugar (red) and lipids (blue) content.**
- Legumes (pea, chickpea) have moderate-low fat content (chickpea generally higher than pea), but high protein and sugar contents.**
- Almond milk has the highest fat content, along with the other nuts/seeds, kiwifruit seed and hemp. Of the nuts/seeds, almond and hemp milk have higher protein and sugar content than kiwifruit seed, which was the milk type that had one of the lowest protein levels.**

Got Mylk? Summary

This study is the first of its kind, analysing the chemical profile of a range of plant-based milks using FT-IR. In this work:

- Several alternative plant-based milks were made**, many for the first time
- It was shown that **the chemical signature of plant based milks can be appropriately analysed used FT-IR**
- It was found that the studied **plant-based milks have very different chemical profiles** and the effect of changing experimental conditions was different for each milk (results not shown).
- Potential options for new plant-based milks were found, to be explored further.**

References

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