# Improved salt predictions with FT-NIR understanding signal-to-noise sources

Anders Larsen[1]*, Lone Vejgaard[1] and Anne Grete Johansen[2]

[1]Q-Interline A/S, Stengaardsvej 7, DK-4340 Tølløse, Denmark
[2]Arlafoods, DK-7500 Holstebro Denmark
*Corresponding author: al@q-interline.com

## Introduction

The food and dairy industry is faced with upcoming demands to control and lower NaCl content in food products. Salt consumption above 5-6 g.day$^{-1}$ is considered unhealthy[1] and a cause for high blood pressure and higher incidences of cardiovascular disorders. Salt is as an analytical component moving from "nice to know" in a production to "need to know".

Historically near infrared (NIR) calibrations for salt concentration have had very mixed results, most of which were not successful over time. Since the industry needs to monitor salt levels and NIR is extensively used within the food industry this work examined factors which affect the outcome of NIR calibrations. This study showed that FT-NIR technology can improve accuracy and robustness of salt predictions in butter. This becomes especially interesting as salt does not have a vibrational feature of its own, and therefore these calibrations were dependent on the water response to salt concentration. It was further shown that understanding sources of noise yields better and more robust predictions.

The water spectrum is known by many as a couple of big hills on the spectral landscape (Figure 1), but these dominant NIR macro features of water can be used for more than predictions of water/solids concentrations if the effect of temperature and electrolytes (salts) on the spectrum is understood.

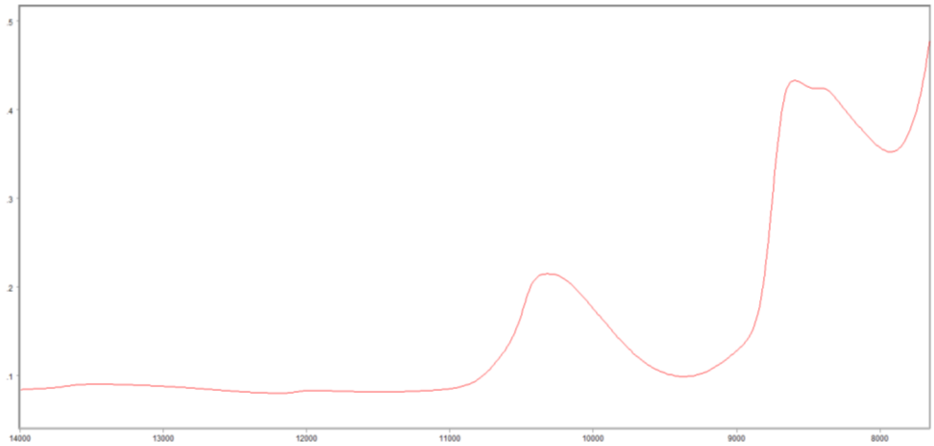

**Figure 1.** Typical water spectrum.

Water molecules in the liquid phase are mutually interlinked by means of hydrogen bonds, and in the presence of minerals may also create hydrogen bonds to mineral surfaces, forming mono layers and similar. In liquid form a water molecule can donate and receive 2 hydrogen bonds (Figure 2), and will form structures in the shape of tetrads. Studies have shown that at room temperature 43% of all water molecules have 4 hydrogen bonds,[2-3] as temperature increases the number of hydrogen bonds decreases. The water structure with 4 hydrogen bonds is more stable, i.e. lower potential energy, than water structures with less hydrogen bonds. It is therefore expected that the NIR spectrum of water will change shape with changing temperature.

In the same studies as referenced above[2-3] it was also shown, by means of Raman spectroscopy, that adding salt (NaCl) in the range 0–5 mol.kg$^{-1}$ increases the number of water molecules holding the tetrad bond pattern; we therefore see a shift towards lower energy in the NIR spectrum (Figure 3). The peak at 8620 cm$^{-1}$ attributed to free water lowers in intensity and 8000–8333 cm$^{-1}$ increases in intensity as free water becomes hydrogen bonded to 2, 3, or 4 other water molecules. This effect must very carefully be modeled together with structural changes caused by temperature since temperature and salt concentration have similar but opposite effects. It has been shown by experiment that a net analytical change is created at around 8500 cm$^{-1}$

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Edited by M. Manley, C.M. McGoverin, D.B. Thomas and G. Downey, Cape Town, South Africa, pp. 382-385.

of 0.0025 abs per °C and 0.002 abs per 1% w/w salt added, hence the design of experiments (DOE) must be very precise.

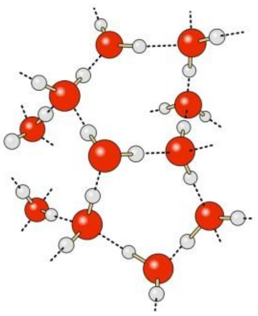

**Figure 2.** Water tetrad structure.

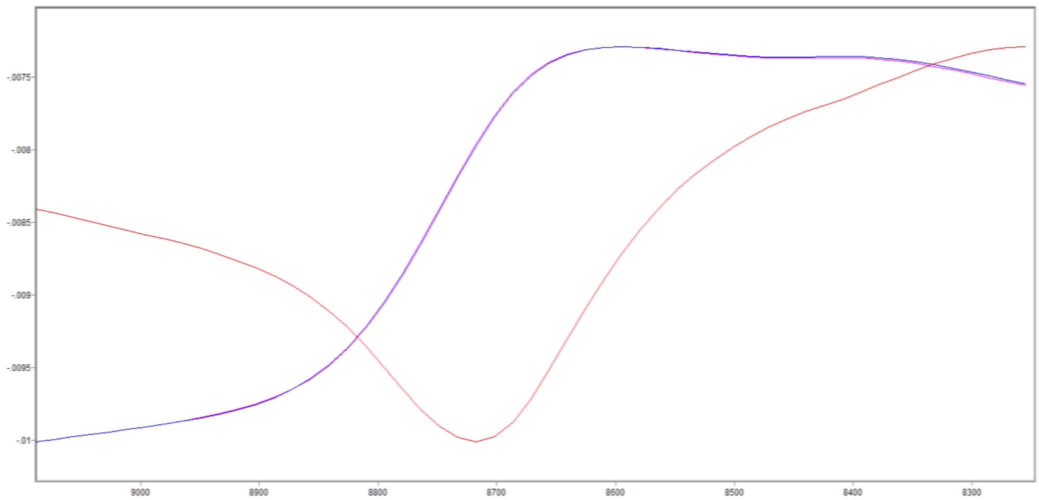


**Figure 3.** Salted water (blue) and water (purple) (44°C) subtracted (red).

The water band also shifts with increasing salt concentration (Figure 4). From pure water to a 1% salt solution a band shift from 8663.1 to 8662.3 $cm^{-1}$ or 1154.32 to 1154.42 nm is observed. Not all NIR technologies will produce adequate x-axis stability to predict salt over time, as many NIR technologies have reproducibility in the 0.1 nm range, making it impossible to see if salt or the instrument change over time. Knowing this, FT-NIR was tested for salt applications; FT technology has very high x-axis reproducibility, typical better than 0.1 $cm^{-1}$. This work has tested FT acquisition using spectral resolutions from 4 to 64 $cm^{-1}$ and argues that for butter the best signal to noise vs spectral quality is obtained at 32 $cm^{-1}$ resolution, traditionally considered low for FT. However, spectral features are not to be resolved by eye but rather by chemometrics, hence priority should be given to low noise (Figure 5).

## Materials and Methods

All butter samples (n = 245) were scanned with FT-NIR technology (DairyQuant-B, spinning CupSampler Q-Interline Tølløse, Denmark). The samples were all standard production samples from Arlafoods site in Holstebro, Denmark. The FT-NIR scanning used diffuse reflection mode. All FT spectral data were collected with 32 $cm^{-1}$ resolution and 30 s observation time to create very low noise data. High resolution FT data can be fairly noisy compared to a more conservative use of FT technology, but the real value of the FT lies in the stability and x-axis reproducibility, not in its ability to resolve spectral features.

Product temperature was varied within the range of later use during calibration steps in a controlled pattern. The samples were spun at 0.33 $turns.s^{-1}$ to ensure a representative spectrum, as it cannot be expected that salt added after the churn is homogeneously distributed. Dissolved salt was injected into the butter samples. A van't Hoff factor of 2 should be ideally assumed, but this may not always be the case, especially not for NIR samples containing dry matter as well as water.

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PLS-1 calibrations for salt concentration were developed using PLSIQ/Grams software. Selected segments from the overall spectral range 4000–10 000 cm$^{-1}$ were used containing both CH and OH bands, primarily in the 7344 to 4597 cm$^{-1}$ range various preprocessing steps was tested, but first derivate (SG) 5 points were found to give the best model overall.

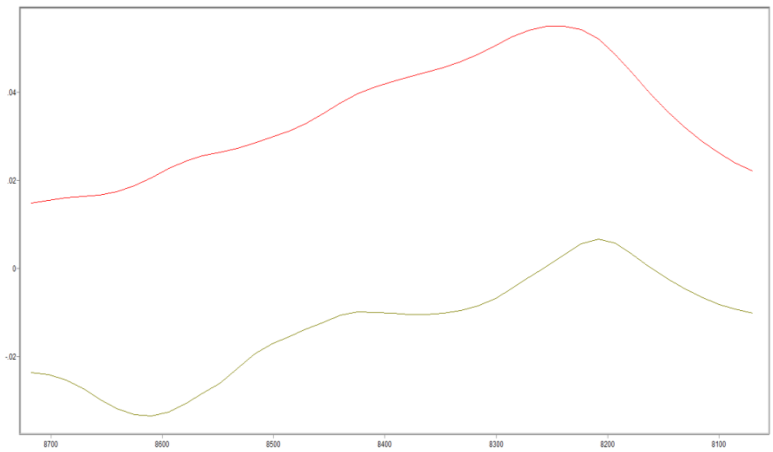


**Figure 4.** Loadings of PLS factors 1 and 2 from butter model.

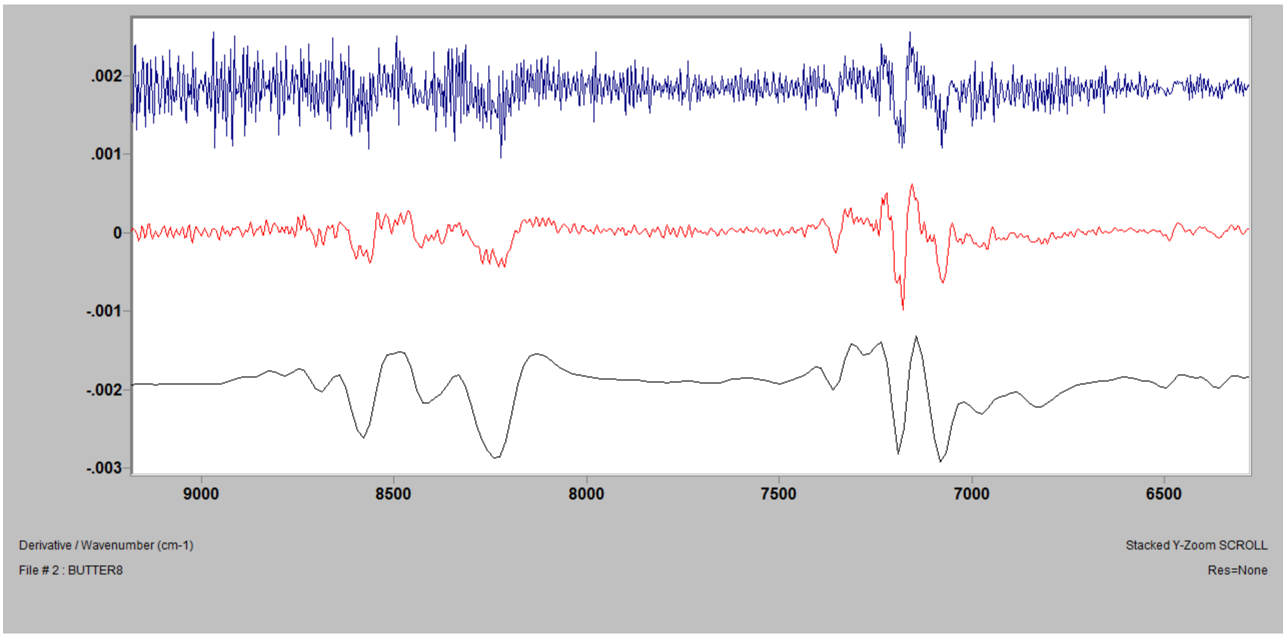


**Figure 5.** Second derivative (Savitzky-Golay smoothing, 5 point window) butter spectra of recorded using spectral resolutions of 4, 8 and 32 cm$^{-1}$.

## Results and Discussion

The agreement between the lab and the NIR results were compared and validated over a one month period and showed a standard deviation (1 sigma) of 0.035% w/w, compared to a 0.062% w/w Standard error of cross validation. The improved performance from calibration to validation can be related to less outliers and more experience from operators after 2 month calibration. The short term stability (repeatability) was on average 0.01% w/w and thus not a critical limiting factor for the performance. The longer term stability was tested over a period of 3 month and no significant effects were observed, all bias values were within the variance of agreement.

From loading plots (Figure 4) the effects consolidating the ability to predict salt is seen. Temperature effects as well as the shift and secondary effects on the water band are picked up by the model and creates loadings with shapes that can be assigned to these effects. Further work is planned to address other water/fat ratios, added taste and spicing as well as a wider temperature span. The concept has been applied to cheese

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as well and offers stable salt readings, however more work is required to fully examine the mentioned effects in samples not containing high levels of liquid water.

## Conclusion

Analysis of parameters, such as water and salt, in products like butter is to a large degree a study of the water spectrum. The water spectrum can be influenced by many factors which will cause the method to perform less than adequately. By carefully understanding the water spectrum and the underlying physical chemistry, i.e. effects from water structure, temperature, density and van't Hoff factors, one can apply NIR technology in a better way, by choosing amongst various basic NIR technologies and setting up a proper design of experiment for the calibration work. It is believed that the solid performance of salt measured with FT-NIR comes from the core technology having a superior x-axis performance over other technologies, combined with a solid understanding of other sources of "spectral noise" and creating a calibration set with sufficient variation between the interlinked water effects.

## Acknowledgements

To the lab and process persons at Arla foods Holstebro DK for supplying the high amount of representative samples with very good reference values and to Ole F. Nielsen for good hints and inspiration to add the section on physical chemistry.

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