Maximize Efficiency and Quality in Cultivated Meat Production

Real-time Glucose and Lactate Monitoring with Process Raman Analyzer and Accurate Transferable Models

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In collaboration with

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Introduction

Cultivated meat, also known as cell-based or lab-grown meat, represents an innovative and emerging field of food technology. In this process, meat products are grown from animal cells in a controlled laboratory environment without the need for traditional animal farming. This emerging field has gained significant interest due to its potential to address various environmental, ethical, and sustainability challenges associated with conventional meat production.[1,2]
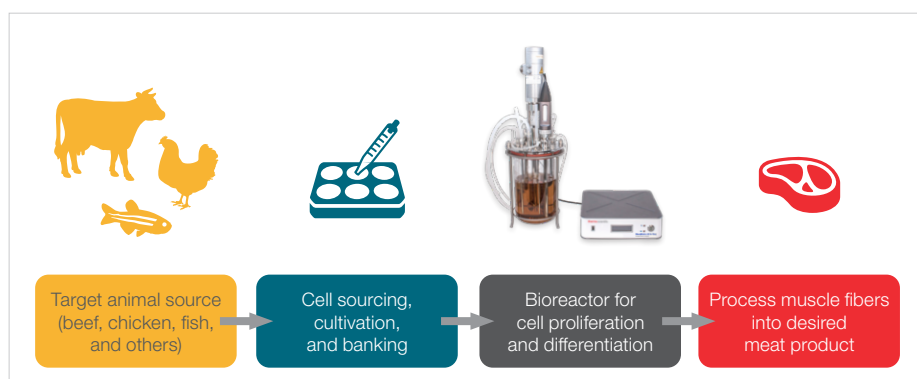

Figure 1. Schematic of process for cultivated meat production. In this study, a Raman process analyzer was used to monitor a bioreactor run.

A typical bioprocess of cultivated meat includes cell sourcing, cell culture, cell differentiation, and tissue engineering, resulting in meat products (Figure 1) that closely resemble traditional animal-based meat in terms of taste, texture, and nutritional composition.[3] Bioreactors are essential components in the overall process, as they provide a controlled environment for cell growth and tissue development. Several process parameters, including nutrients, oxygen, pH, temperature, antiapoptotic and differentiation factors, waste removal, and scaling up, must be carefully controlled within bioreactors to optimize the production of high-quality cultivated meat.

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Raman spectroscopy is increasingly being used as a process analytical technology (PAT) for simultaneous monitoring and control of multiple parameters of bioreactors, especially in biopharmaceutical development. Established PAT applications for Raman include evaluating the components of the cell culture, glucose and lactate concentrations, amino acid consumption, and titer production.[4] Of particular interest in this study are glucose and lactate.

Proper monitoring of nutrient concentration and measurement of secondary metabolites are important. Glucose is the primary source of energy and carbon in most of the bioreactor runs. However, the aldehydic functional group of glucose is reactive toward primary amines of biomolecules, leading to undesirable product glycation. Thus, the glucose level in the bioreactor is a critical process parameter that needs to be monitored and maintained at an optimum to ensure high quality and quantity of products.[5] Recently, we demonstrated the feasibility of accurate monitoring, control, and automated glucose feeding for the entire CHO cell bioreactor run using process Raman as a real-time sensor.[6] A byproduct of anoxygenic cell metabolism, lactate, is often used to indicate cell health in response to oxygen availability. Excessive lactate hinders cell viability and product yield.

In this study, we present a proof of concept (PoC) study for monitoring glucose and lactate in cultivated meat production using a Thermo Scientific™ MarqMetrix™ All-In-One Process Raman Analyzer, in collaboration with Ivy Farm Technologies within one of their bench-scale processes. Also, we demonstrate the transferability of chemometric models for glucose and lactate across bioprocesses and different instruments.

Experimental
Cultivated meat bioreactor runs
The sourced bovine cells were seeded at a low density of 1×10^5 cells/mL to culture for one week in a batch bioreactor at approximately 3 g/L glucose. No additional glucose was added to the bioreactor. The dissolved oxygen, pH, temperature, and agitation speed were controlled by respective sensors integrated into the bioreactor runs. A MarqMetrix All-In-One Process Raman Analyzer with the immersible probe was also integrated into the bioreactor to monitor the progress of the process.

Each Raman spectrum was acquired using the acquisition (ACQ) parameters of power 450 mW, integration time 3000 ms, and 20 averages. All data acquisition was performed using the Thermo Scientific™ Lykos™ PAT Software with built-in cosmic ray removal. The software uses Open Platform Communication Unified Architecture (OPC™ UA) that allows users to do the following:

- Connect multiple instruments within the workflow (machine-to-machine communication).
- Pass chemometric predictions in real-time to an OPC client.
- Streamline results into another software system.
- Use results in an automated feedback loop.

The samples were pooled during the runs, and the reference values were measured offline. The prediction from the model and the reference values were used to calculate the root mean square error of prediction (RMSEP).

Chemometric models
The chemometric partial least square (PLS) models for glucose and lactate deployed in this study were previously built using the Raman data acquired from various bioreactor runs, including monoclonal antibody production, viral vector production, and others. The ACQ parameters for the training data were the same as mentioned above. After data acquisition, five spectra with timestamps close to the reference values were further averaged to improve the signal-to-noise ratio (SNR) before building the chemometric models. The training dataset was preprocessed using normalization to water band, Sav-Gol 1st derivative filter (order=2, smoothing window=13), and mean centering. The partial least square (PLS) models were built with appropriate region selection specific for glucose and lactate. The number of latent variables for the PLS models was selected using a leave-out-one-run cross-validation strategy. Each run was excluded once during the cross-validation for these models. The five latent variables of the PLS model were selected for glucose and lactate based on the objective function of minimizing root mean square error of cross-validation. No data from cultivated meat was included in the training set. The chemometric models were applied to the Raman data collected on the cultivated meat bioreactor after preprocessing in the same manner as carried out on the training data. Different instruments were used to collect the training data and monitor the cultivated meat bioreactor; however, no transfer function was applied to mitigate inter-instrument variance.

Results and Discussion

Initial Data Assessment

Figure 2 shows the representative Raman spectra collected from the bioreactor run of the cultivated meat. Using the appropriate ACQ, the intensity of all peaks in the entire spectrum was kept below 45,000 counts (intensity in Y axis) throughout the run to avoid signal saturation. All spectra were free of cosmic ray interference as the Lykos PAT software was operated by enabling the inbuilt cosmic ray removal algorithm. The cell density increases with the progression of the bioreactor run, causing baseline shift due to background scattering and fluorescence effect. In this study, Sav-Gol filters and water band normalization were applied to all spectra to remove the baseline and correct for the path length differences before building the chemometric models or performing prediction on the test samples.

Figure 3 shows the Sav-Gol 2nd derivative (order = 2; window width=13) plot of Raman spectra from the bioreactor run for cultivated meat production, emphasizing characteristic Raman peaks corresponding to glucose and lactate. All traces are color-coded based on the reference analyte concentration, shown as the gradient bar in the measured range, with yellow being high and blue being low. The inverted peak at 1,125 cm^{-1} is mainly ascribed to the stretching of C-O and C-C and asymmetric out-of-plane vibration of the C-O-C bond in the glucose molecule.[7] The peak at 855 cm^{-1} is attributed to the C-C symmetric stretching of the C-COO^{-} bond (deprotonated form) in the lactate molecule.[8] The appearance of these characteristic peaks assures the presence of glucose and lactate in the cultivated meat bioreactor.

Variable importance in projection (VIP) Analysis of PLS calibration models

The calibration PLS models for glucose and lactate were built using Raman data collected primarily on the CHO cell bioreactors for monoclonal antibody production. Before applying the model to the cultivated meat process, variable importance in projection (VIP) analysis was performed to ensure the specificity of the model for the target analytes; this provides a measure of transferability across bioprocesses. The VIP score analysis is the statistical approach to estimate the importance of each predictor variable (also known as the independent variable) in regards to the response variable. In this PLS model, the Raman shift is the independent variable and the concentration of analytes is the response variable.[9] Not all Raman shifts are of equal importance; variables with high VIP scores are vital to the model. For instance, the VIP plot for the glucose-specific model will have high VIP scores for the Raman shift that corresponds to the vibrational modes of glucose molecules. In this aspect, the VIP scores plot is also indicative of model specificity, which is essential for its transferability across processes.
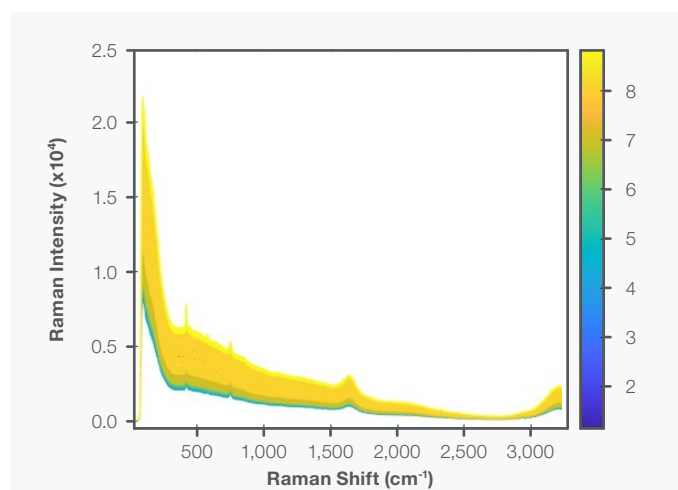

Figure 2. Raman spectra of cultivated meat bioreactor run; color-coded by days. The baseline increased with the days' progress primarily due to cell density increase and background fluorescence.
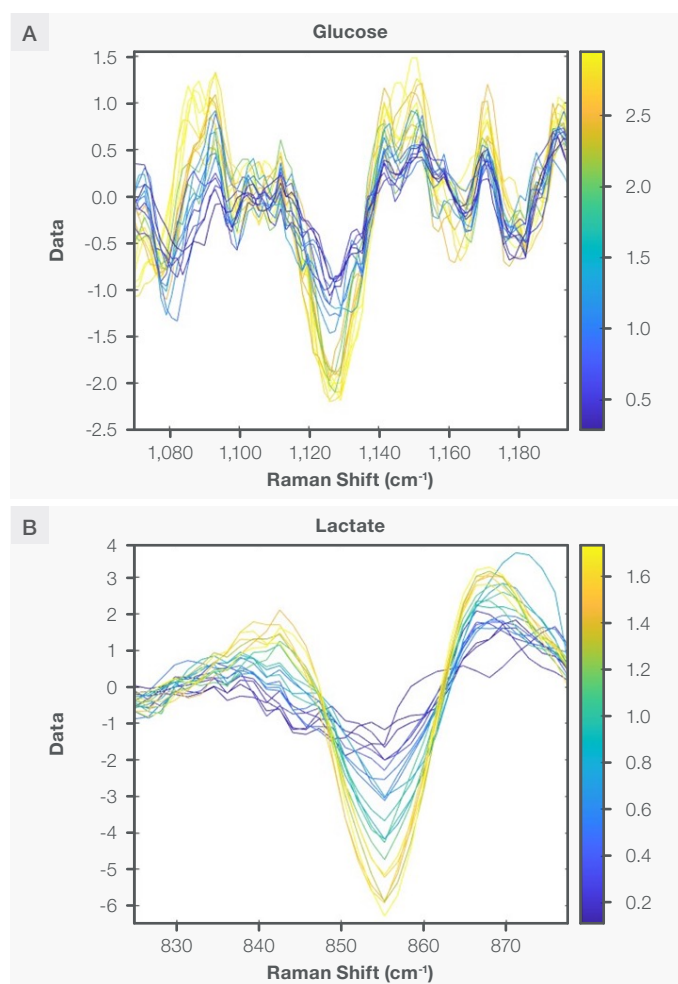

Figure 3. The Sav-Gol filter 2nd derivative plot of the Raman spectra of the bioreactor run for cultivated meat production, highlighting the characteristic Raman peaks for (A) glucose and (B) lactate as explained in the text.

Figure 4 shows the VIP score plot for the glucose and lactate PLS models deployed in this study. It is noted that although the Raman spectra of the bioreactor are highly complex due to the presence of multiple analytes, in the PLS models, the spectral regions of 1,100–150 cm⁻¹ for glucose and 830–870 cm⁻¹ for lactate have high VIP scores, indicating that these regions are "important" for the models. As described above, these regions contain Raman features specific to glucose and lactate. Thus, despite the complex intricacies of bioreactors with multiple overlapping Raman signals, the PLS models are highly specific to the analytes of interest, making them independent of other variables in the processes. In other words, the models are potentially transferable to an independent future dataset.

Predictive Performance of PLS Models

Figure 4 shows the performance of the PLS glucose and lactate on the bioreactor run for the cultivated meat. Key performance statistics are also summarized in Table 1.

The correlation plot of predicted value vs. reference value is shown in Figure 5. The root mean square error of predictions (RMSEPs) are 0.24 g/L for the glucose model and 0.21 g/L for the lactate model, respectively, demonstrating excellent correlation between the predicted and the reference values. The prediction R^2 is 96% for glucose and 87% for lactate, indicating that the models account for 96% of any variances observed for glucose and 87% of those for lactate. *The low RMSEP and high prediction R2 demonstrate excellent model transferability across different processes and instruments.*

	Glucose	Lactate
RMSEC	0.337 g/L	0.181 g/L
RMSECV	0.413 g/L	0.250 g/L
RMSEP	0.239 g/L	0.212 g/L
R^2 (Pred.)	96.1%	87.4%

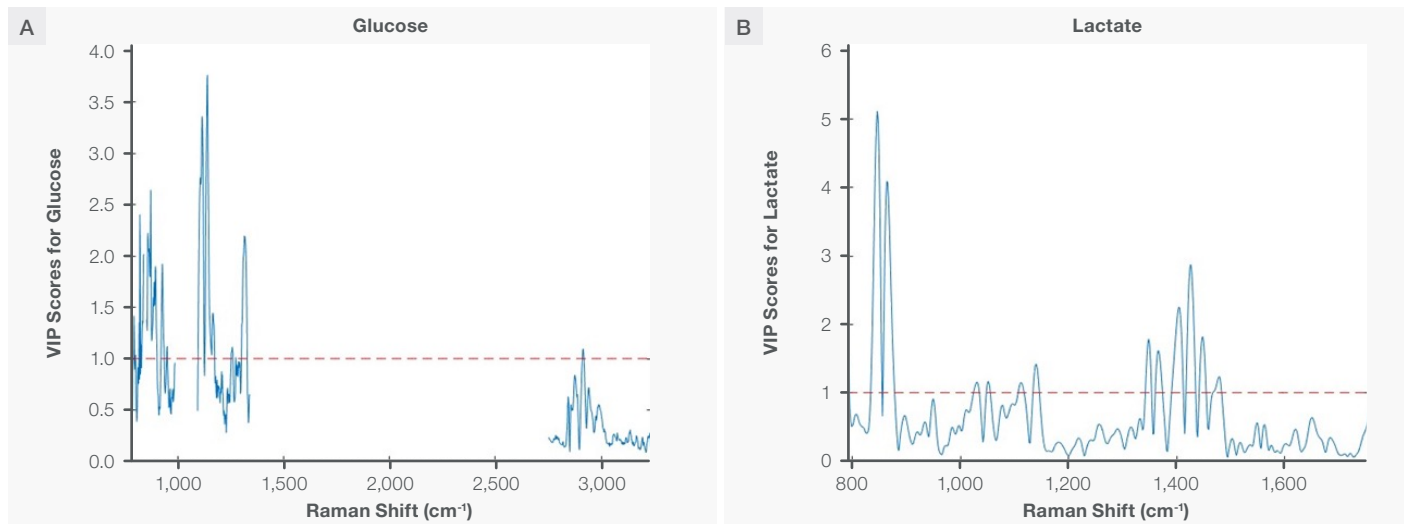Table 1. PLS model performance statistics for glucose and lactate.

Figure 4. The variable importance plot (VIP) analysis for the (A) glucose and (B) lactate PLS models. A Raman shift with values greater than 1 (above the dotted line) is considered significant for the model.
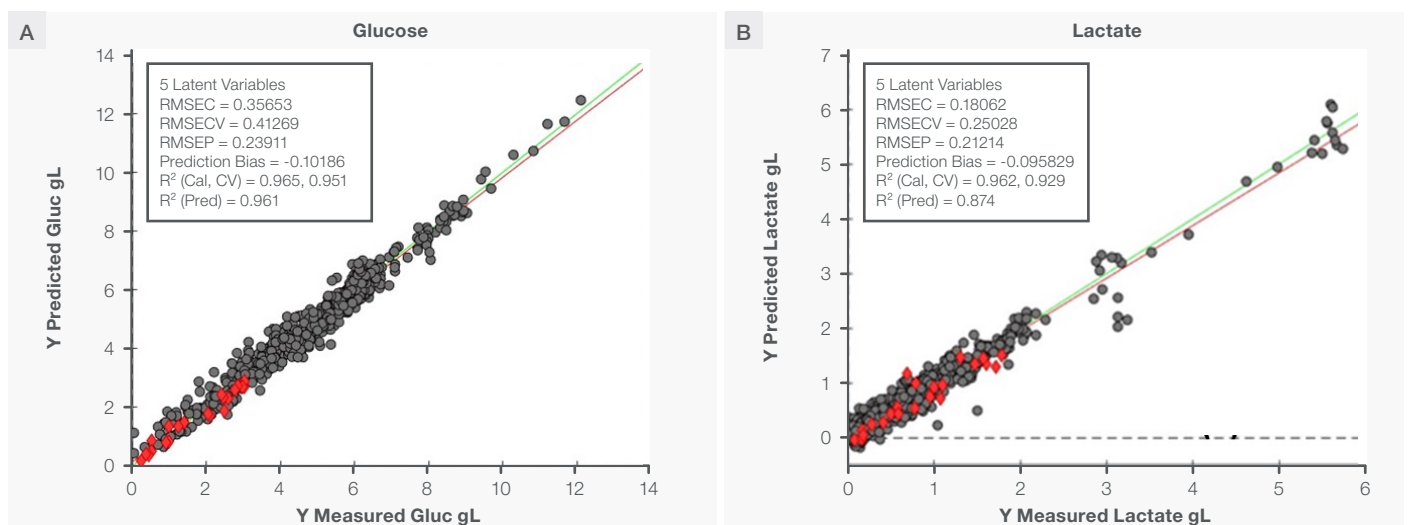

Figure 5. The correlation plot of prediction value vs. measured value for (A) glucose and (B) lactate in the bioreactor run for cultivated meat. The grey circles are training samples, and the red diamonds are the test samples.
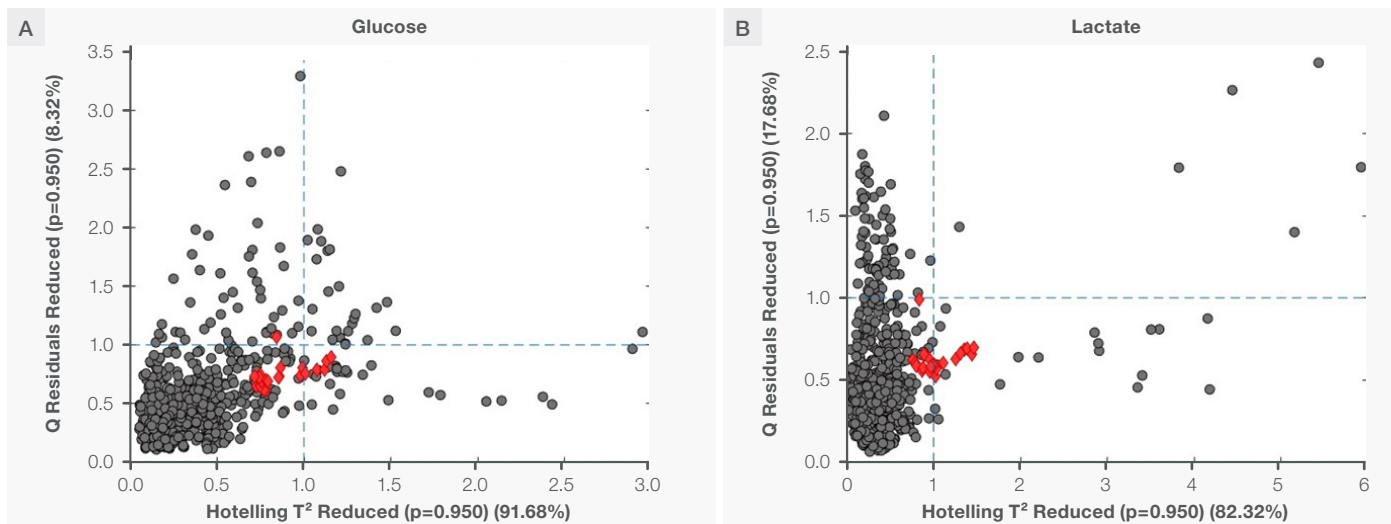
Figure 6. Q residual (reduced) vs. Hotelling T^2 (reduced) plot for (A) glucose and (B) lactate. The grey circles are training samples, and the red diamonds are the test samples.

The validity of the PLS model prediction was evaluated by analyzing the Q-residual-versus-Hotelling T^2 plots, as shown in Figure 6. Q-residuals account for the residual in the test data after projecting into the model space, while Hotelling's T^2 measures the distance of the data from the center of the distribution of calibration data. Low Q-residuals and Hotelling's T^2 scores mean the test data is within the model space. These criteria must be met before applying a model to the test data or accepting the predicted result with high confidence. In this study, the test samples (from the cultivated meat bioreactor) have Q-residual (reduced) and Hotelling T^2 (reduced) scores of ~1 when projected into the model space. This means the test samples are within the model space with 95% confidence, even though the training and test samples are two different datasets. This is only possible if the models are only analyte-specific and free of other process dependence. *Thus, this not only provides the validity of prediction from models but also substantiates the successful transferability of models.*

Conclusions

- Raman spectroscopy can offer real-time and simultaneous monitoring of glucose and lactate in cultivated meat bioreactors.

- Successful transferability of chemometric models developed for mAb productions to the cultivated meat bioreactor was demonstrated. The transferability was largely due to the specificity of the model for the analyte of interest, as illustrated by the statistical analysis.

- The model transferability across instruments was also demonstrated to reflect low inter-instrument variance. A detailed study on inter-instrument model transferability is reported in our previous work.[10]

- The synergic operation of Lykos PAT software with transferable models, in conjunction with the stable and calibration-free MarqMetrix All-In-One Process Raman Analyzer, was shown to be an efficient PAT solution for bioprocess development and monitoring.

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