Technical Overview

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Rapid, Simple, and High-Throughput Nutritional Phenotyping of Pulse Crops

Evaluating nutrients using the Agilent Cary 630 FTIR spectrometer

Abstract

This technical overview explores the application of an Agilent Cary 630 FTIR spectrometer for rapid, simple, and high-throughput phenotyping of pulse crops. Pulse crops are valued for their high nutritional content, and breeding programs require optimized workflows to enhance nutritional quality and reduce turnaround times. Traditional phenotyping methods (for example, enzymatic assays or GC/MS) are labor-intensive, costly, and time-consuming. In contrast, Fourier-transform infrared (FTIR) spectroscopy offers a promising alternative with minimal sample preparation, rapid analysis, reduced sample destruction, and simplified workflows. In this technical overview, the Cary 630 FTIR spectrometer is demonstrated to be effective in predicting protein quality and digestibility, fatty acid composition, and starch content in pulse crops. The compact design, affordability, and ease-of-use make this spectrometer an optimal tool for crop and plant breeding programs.

Introduction

Pulse crops (such as chickpeas, lentils, and dry peas) are high in nutritional content, including plant-based protein, low-digestible carbohydrates, and many micronutrients.[1,2] Pulse crops are therefore a foundational focus in agriculture, with breeding programs requiring optimized workflows to improve turnaround times and nutritional quality. Furthermore, the identification of quantitative trait loci (QTL) and gene pathways for use in pulse crop breeding requires extensive amounts of phenotypic data.[3,4] Workflow optimization is key to ensure lower costs and rapid data acquisition, and therefore, the potential for more efficient crop generation. Compared to traditional methods of nutritional phenotyping, high-throughput phenotyping (HTP) aims to be less expensive, simpler to use in the field, and can provide significantly more data for predictive decision making.

FTIR spectroscopy has emerged as a highly promising, high-throughput method for nutritional phenotyping of pulse crops. FTIR requires minimal labor and sample preparation with rapid turnaround time (one to two minutes). This is in contrast to the complexity, expense, and longer turnaround times of traditional techniques such as GC/MS or enzymatic methods. Moreover, sample destruction is greatly reduced in FTIR, better facilitating reruns when required.[5,6]

Studies have shown that the Agilent Cary 630 FTIR spectrometer (Figure 1) can successfully be used for pulse crop phenotyping, providing a simple, high-throughput, and low-cost means of obtaining phenotypic data.[5–8] Operation of the system is easy and intuitive, and the need for data analysis and interpretation is minimal, reducing the requirement for skilled personnel and training cost.

This overview demonstrates the promising value of the Cary 630 FTIR spectrometer in pulse crop phenotyping for improved breeding program workflows. When combined with an Agilent Cary 630 FTIR diamond attenuated total reflectance (ATR) module, this spectrometer was successfully used to robustly and reliably predict protein quality and digestibility, as well as fatty acid and starch composition, in pulse crops.

Figure 1. An Agilent Cary 630 FTIR spectrometer with a diamond ATR module is the ideal tool for the fast, easy analysis of nutrients in crop samples.

Experimental

Sample preparation

While traditional GC/MS and enzymatic approaches require extensive sample preparation and often rely on hazardous chemicals, FTIR analysis requires little to no sample preparation (see the example in Figure 2).

Instrumentation and software

All FTIR spectroscopic data in these studies were collected using a Cary 630 FTIR spectrometer with a diamond ATR module. Spectroscopic data were analyzed in the Agilent MicroLab Expert software.

Traditional analytical methods for nutritional phenotyping in pulse crops were performed to determine actual sample parameters. These values were then used to build and validate partial least squares regression (PLSR) models for FTIR spectroscopy (chemometric modeling).

These traditional methods were carried out using the following platforms and methodologies:

- Protein analysis
  - Combustion nitrogen analyzer
  - Agilent 1100 series HPLC system
  - Protein digestibility corrected amino acid score (PDCAAS) enzyme assay
- Fatty acid analysis
  - Agilent 8860 GC system with Agilent 5977B mass spectrometric detector
- Starch analysis
  - Modified Megazyme resistant starch assay method

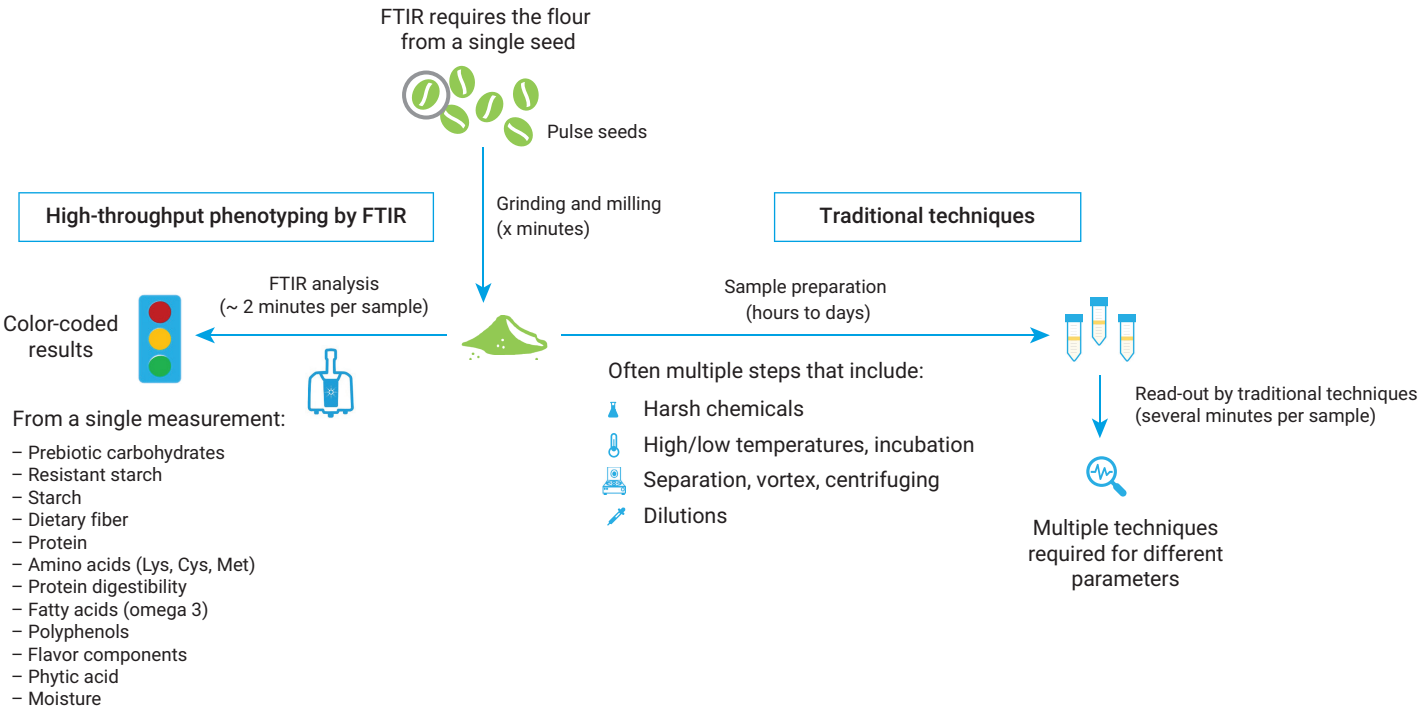

Figure 2. Comparison of sample preparation for traditional techniques versus FTIR spectroscopy for pulse crop phenotyping.

Chemometric modeling

PLSR modeling was used to establish the relationship between the predictive measurements of FTIR spectral data and the actual values obtained from more traditional methods (e.g., HPLC, GC, or PDCAAS).

Chemometric models for the different parameters, correlating nutrient parameters with FTIR spectra characteristics, were generated in the MicroLab Expert software – an FTIR software for sophisticated spectral analysis. The MicroLab Expert software uses a "Calibration Wizard" that guides users through the generation of chemometric models (Figure 3).

The generated chemometric models can then be implemented into the Agilent MicroLab software for routine analysis of crop samples.

The MicroLab software uses picture-guided workflows to lead users through each step of the analysis and provides the final results with color-coding, making the use of the system and the interpretation of results as easy as 1, 2, 3 – reducing training needs and handling errors (Figure 4).

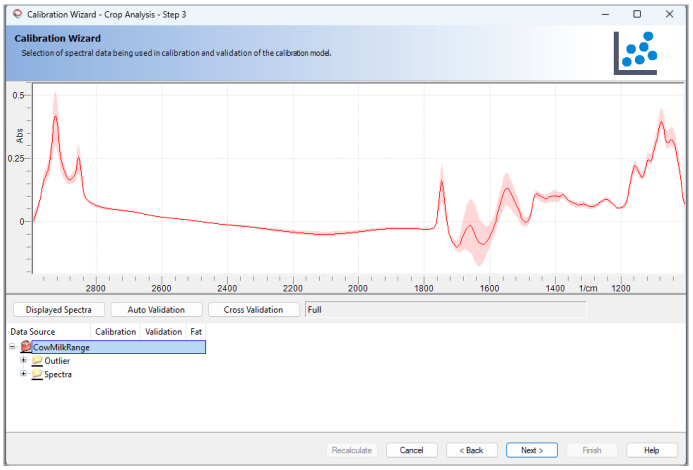

Figure 3. Use of the Calibration Wizard for generating chemometric models.

1 Start the analysis.

2 Follow the picture-driven software guide.

3 Instantly receive color-coded, actionable results.

Figure 4. Three simple steps using Agilent MicroLab software and Agilent FTIR spectrometers make performing an analysis straightforward, removing training needs.

Quantification model development for nutritional phenotyping in pulse crops (selected examples)

Protein quality and digestibility in chickpea, dry pea, and lentil flours

Pulse crops are an increasingly popular source of plant-based protein. Therefore, protein quality and digestibility are key phenotypic traits targeted by breeding programs. Pulse crops are known to be low in sulfur-containing amino acids (SAAs) L-methionine and L-cystine.[9] To improve nutritional content, there is a focus on producing varieties that are higher in SAAs. In addition, breeding programs require data on protein digestibility, or the fraction of protein that can be digested to release essential amino acids. Digestibility is influenced by secondary structures (alpha and beta sheets), with a lower fraction of beta sheets tied to higher digestibility.[10]

Typical methods for amino acid analysis often require two to three days for sample digestion followed by quantification using HPLC. Digestibility is generally determined using the PDCAAS assay, which involves extensive sample preparation and expensive laboratory procedures. These analysis techniques require highly trained personnel and create bottlenecks in breeding programs that prevent high-throughput data acquisition.

The use of a Cary 630 FTIR spectrometer was demonstrated to be a successful and efficient approach for analyzing protein, including SAAs and digestibility. Multivariate models for protein analysis show good predictive accuracy and fit (R^2 ≥ 0.815; Table 1).[7] Additionally, t-tests revealed no significant differences between actual and predicted protein and SAA concentrations (α = 0.05).[7]

Table 1. Multivariate model statistics for protein analysis in pulse crops.[7]

Model Name	R^2	RMSEC	RMSECV	RMSEP	SEP	Bias
Chickpea Total Protein	0.948	0.093	0.093	0.10	0.10	−0.0057
Dry Pea Total Protein	0.845	0.096	0.096	0.093	0.091	0.0039
Lentil Total Protein	0.845	0.13	0.13	0.11	0.11	0.016
Lentil SAA	0.827	0.014	0.014	0.022	0.021	−0.0066
Lentil Methionine	0.815	0.0075	0.0075	0.014	0.014	0.0011

RMSEC = Root mean square error of calibration
RMSECV = Root mean square error of cross-validation
RMSEP = Root mean square error of prediction
SEP = Standard error of prediction

For protein digestibility, models achieve high R^2 values (≥ 0.88) for actual versus predicted measurements (Figure 5).[8]

Two-tailed t-tests and F-tests (α = 0.05) show no significant differences between actual and predicted digestibility measurements.[8]

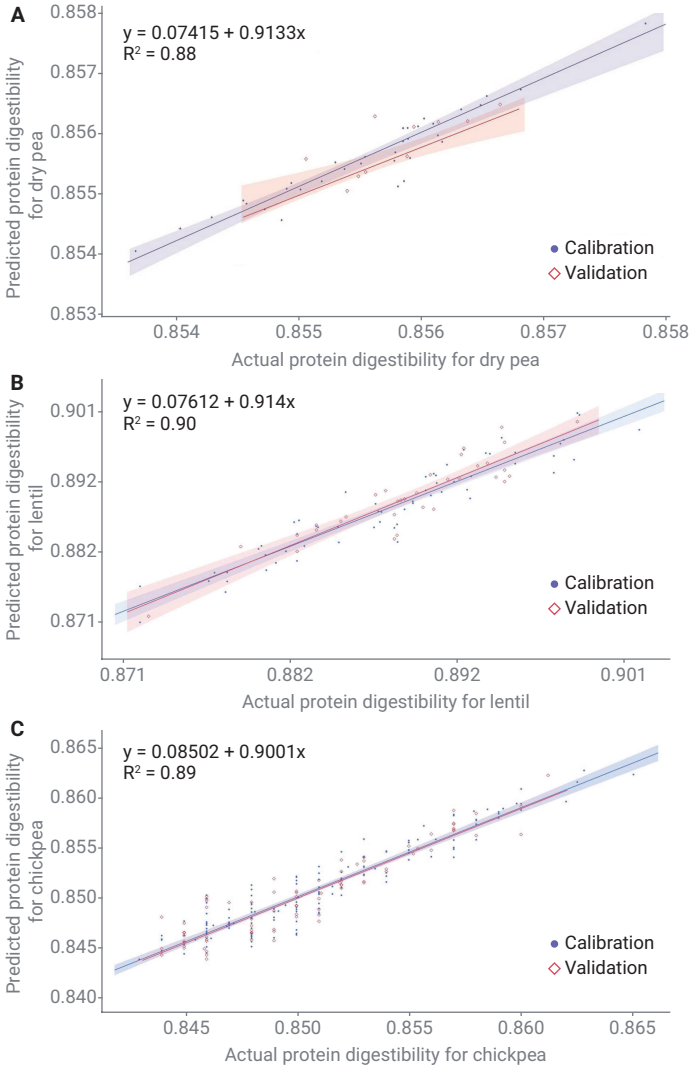

Figure 5. Actual protein digestibility of samples versus the predicted protein digestibility using FTIR analysis. Both calibration and validation sample sets were included in these analyses to help ensure accuracy and reliability. (A) PLSR model performance for undigested dry pea flour. (B) PLSR model performance for undigested lentil flour. (C) PLSR model performance for undigested chickpea flour.[8]

Fatty acids in chickpea flour

Chickpeas have a high fat content compared to other pulse crops (4 to 10%).[11] Chickpea fatty acids may be saturated or unsaturated, with breeding programs targeting varieties with higher levels of beneficial fats such as linoleic acid (LA) and oleic acid (OA).

The Cary 630 FTIR spectrometer was identified as a promising method for fast and easy chickpea fatty acid analysis, with the ability to successfully predict concentrations of total fatty acids (TFAs), total unsaturated fatty acids (TUSFAs), and total saturated fatty acids (TSFAs). All three models achieve R^2 values greater than 0.90 (Figure 6).[5] Two-tailed t-tests ($\alpha = 0.05$) show no significant differences between actual values and values predicted using FTIR.[5]

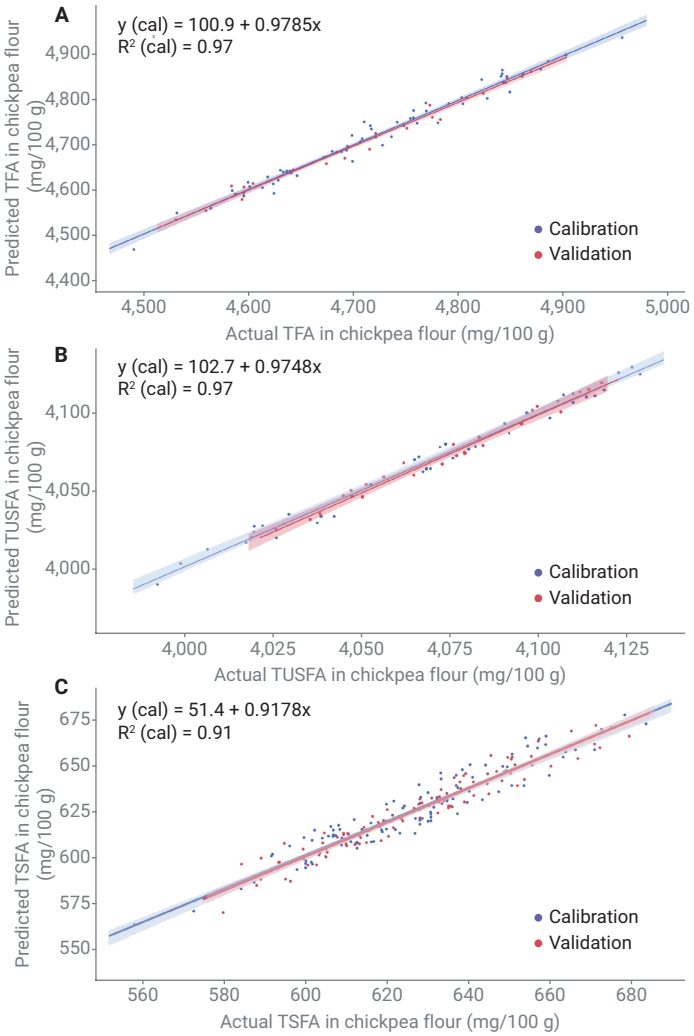

Figure 6. Actual fatty acid measurements versus the predicted fatty acid measurements using FTIR analysis. Both calibration and validation sample sets were included in these analyses to help ensure accuracy and reliability. PLS regression models for (A) total fatty acids (TFAs), (B) total unsaturated fatty acids (TUSFAs), and (C) total saturated fatty acids (TSFAs).[5]

Starch analysis in chickpea, dry pea, and lentil flours

Approximately 70 to 80% of carbohydrate content in pulse crops is starch, and most notably, resistant starch (RS).[12] RS has many known human health benefits as a dietary fiber, including its promotion of a healthy gut biome. Traditional methods for the quantification of starch, such as colorimetric assays and enzyme hydrolysis, are expensive with long turnaround times. Therefore, the use of a Cary 630 FTIR spectrometer offers a promising alternative for rapid starch analysis.

Figure 7 illustrates the actual versus predicted starch concentrations in these crops.[6]

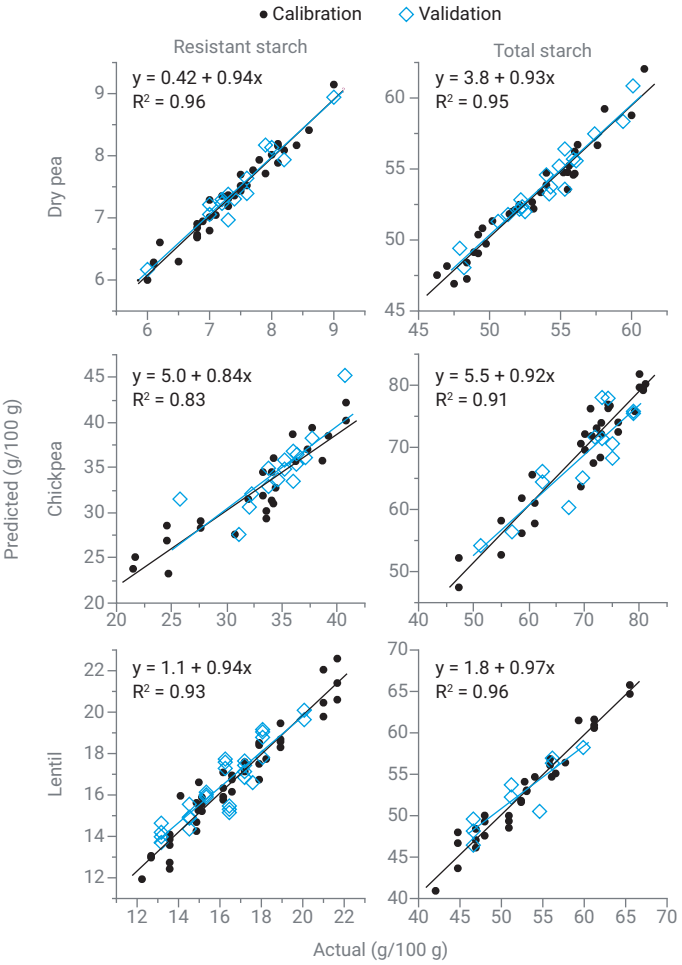

Figure 7. Actual versus model-predicted resistant and total starch concentrations for dry pea, chickpea, and lentil flours. Both calibration and validation sample sets were included in these analyses to help ensure accuracy and reliability. Equations and R^2 values are given for the calibration data.[6]

Predictive measurements using the FTIR models perform well, with high multivariate R^2 values and satisfactory RMSEC values (Table 2).[6]

Table 2. Multivariate model statistics for resistant and total starch (RS and TS) analysis in pulse crops.[6]

Model Name	R^2	RMSEC	RMSECV	RMSEP	SEP
Dry Pea RS	0.96	0.16	0.15	0.16	0.16
Dry Pea TS	0.95	0.95	0.89	0.78	0.78
Chickpea RS	0.96	0.72	0.59	0.98	0.80
Chickpea TS	0.91	3.2	2.9	4.0	3.9
Lentil RS	0.93	0.84	0.70	0.84	0.77
Lentil TS	0.94	1.8	1.4	2.4	1.7

RMSEC = Root mean square error of calibration
RMSECV = Root mean square error of cross-validation
RMSEP = Root mean square error of prediction
SEP = Standard error of prediction

Fast and easy routine analysis of crop samples

Prediction models developed and evaluated using the sophisticated MicroLab Expert Chemometric Model engine can be implemented into the MicroLab software for fast and easy analysis of crop samples (Figure 8).

Multiple models, and therefore nutrient parameters, can be applied in one single method, giving a full picture of the crop's characteristics in one measurement. This reduces operator training needs, the risk of user mistakes, and the time to results.

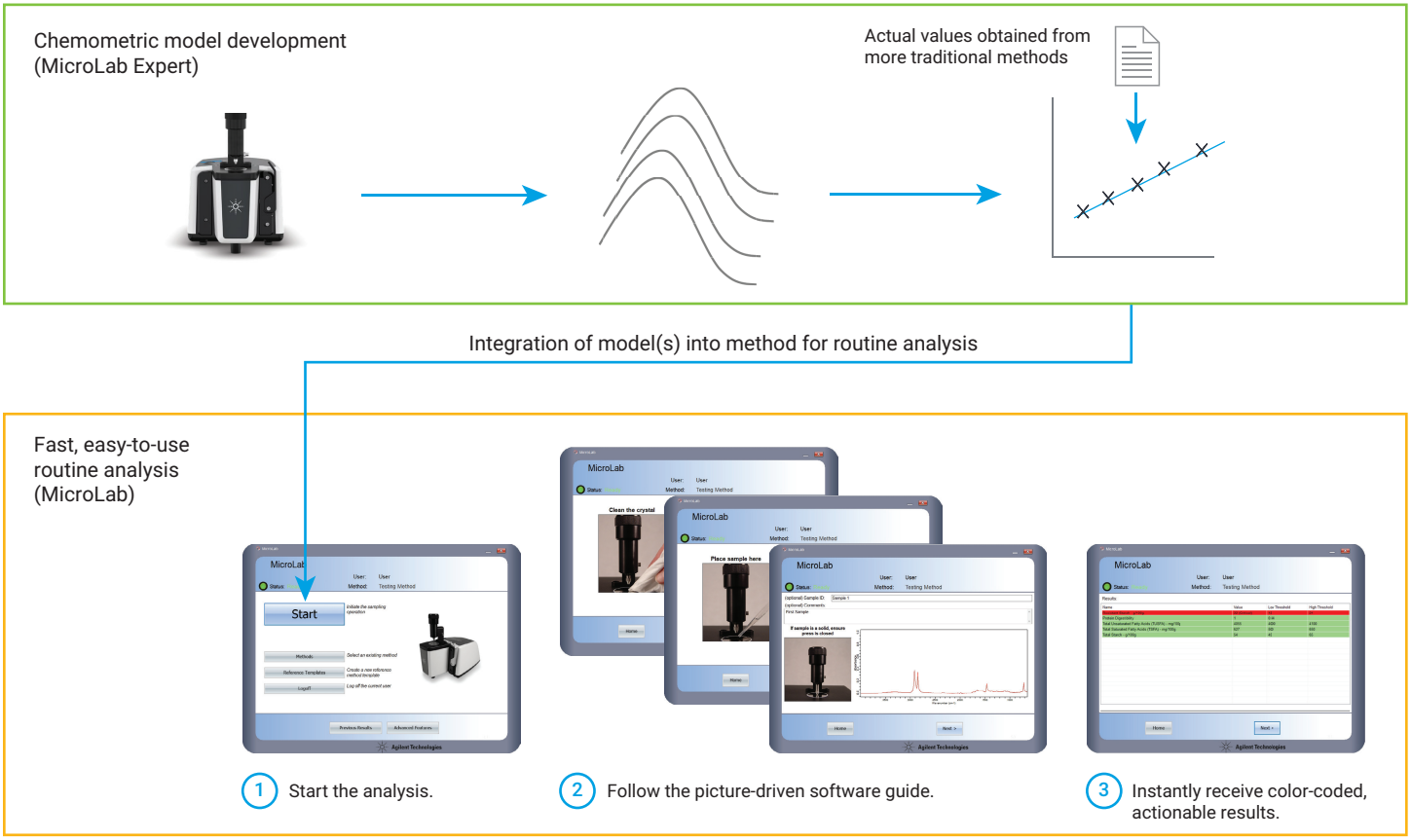

Figure 8. The path to fast, easy, routine analysis using an Agilent Cary 630 FTIR spectrometer. The Agilent MicroLab Expert software and MicroLab Expert Chemometric Model engine allow the development of comprehensive chemometric prediction models that can then be implemented into the simple-to-use MicroLab software, removing complexity from the analysis.

Conclusion

With the increasing worldwide demand for pulse crops, nutritional phenotyping of these food sources is vital to ensure efficient and effective breeding programs. Furthermore, with the advent of molecular breeding technologies (for example, marker-assisted backcrossing and genome-wide association studies), there is a need for the efficient collection of large phenotypic datasets. However, traditional phenotyping methods often require complex and time-consuming workflows that involve expensive equipment and chemicals. This leads to low-throughput results that are not suitable to the needs of pulse breeding programs.

Fourier-transform infrared (FTIR) spectroscopy is emerging as a promising alternative to these traditional methods. The Agilent Cary 630 FTIR spectrometer is designed to deliver high-throughput results in an ultra-compact, affordable, and simple-to-use package. Operation requires little to no technical training, and analysis is made simple with the powerful MicroLab software, which includes visually guided workflows and color-coding for rapid and easy interpretation of results.

Studies have shown that the Cary 630 FTIR can successfully analyze and predict nutritional phenotypic traits in pulse crops, including proteins [7,8], fatty acids[5], and starches[6].By implementing more rapid and cost-effective analyses into workflows with a Cary 630 FTIR spectrometer, breeding programs have the potential to accelerate genomic discoveries and decrease generation times. Moreover, these solutions can also provide opportunities for the expansion of programs into under-resourced countries for improved nutritional gains.

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References

1. Johnson, N.; Johnson, C. R.; Thavarajah, P.; Kumar, S.; Thavarajah, D. The Roles and Potential of Lentil Prebiotic Carbohydrates in Human and Plant Health. *Plants* **2020**. DOI: 10.1002/ppp3.10103.
2. Foyer, C. H.; Lam, H.-M.; Nguyen, H. T.; Siddique, K. H. M.; Varshney, R. K.; Colmer, T. D.; Cowling, W.; Bramley, H.; Mori, T. A.; Hodgson, J. M.; *et al.* Neglecting Legumes Has Compromised Human Health and Sustainable Food Production. *Nature Plants* **2016**, *2*, 16112. DOI: 10.1038/nplants.2016.112.
3. Liu, X.; Qin, D.; Piersanti, A.; Zhang, Q.; Miceli, C.; Wang, P. Genome-Wide Association Study Identifies Candidate Genes Related to Oleic Acid Content in Soybean Seeds. *BMC Plant Biol.* **2020**, *20(1)*, 1–14. DOI: 10.1186/s12870-020-02607-w.
4. Sab, S.; Lokesha, R.; Mannur, D. M.; Somasekhar, J. K.; Mallikarjuna, B. P.; Laxuman, C.; Yeri, S.; Valluri, V.; Bajaj, P.; *et al.* Genome-Wide SNP Discovery and Mapping QTLs for Seed Iron and Zinc Concentrations in Chickpea (Cicer arietinum L.). *Front. Nutr.* **2020**, *7*, 559120. DOI: 10.3389/fnut.2020.559120.
5. Madurapperumage, A.; Johnson, N.; Thavarajah, P.; Tang, L.; Thavarajah, D. Fourier-Transform Mid-Infrared (FT-MIR) Spectroscopy as a High-Throughput Phenotyping Tool for Measuring Total Fatty Acids in Chickpea (Cicer arietinum L.) Flour. *ACS Food Sci. Technol.* **2023**, *3(9)*, 1568–1576. DOI: 10.1021/acsfoodscitech.3c00239.
6. Johnson, N.; Thavarajah, P.; Madurapperumage, A.; Windsor, N.; Tang, L.; Thavarajah, D. Fourier-Transform Infrared Spectroscopy: An Inexpensive, Rapid, and Less-Destructive Tool for Starch and Resistant Starch Analysis from Pulse Flour. *Plant Phenome J.* **2023**, *6(1)*, 1. DOI: 10.1002/ppj2.20086.

7. Madurapperumage, A.; Johnson, N.; Thavarajah, P.; Tang, L.; Thavarajah, D. Fourier-Transform Infrared Spectroscopy (FTIR) as a High-Throughput Phenotyping Tool for Quantifying Protein Quality in Pulse Crops. *Plant Phenome J.* **2022**, *5*, e20047. DOI: 10.1002/ppj2.20047.
8. Madurapperumage, A.; Windsor, N.; Johnson, N.; Thavarajah, P.; Tang, L.; Thavarajah, D. Fourier-Transform Mid-Infrared Spectroscopy for In Vitro Protein Digestibility Measurement of Pulse Crops. *Crop Sci.* **2024**, 1–12. DOI: 10.1002/csc2.21300.
9. Boye, J.; Wijesinha-Bettoni, R.; Burlingame, B. Protein Quality Evaluation Twenty Years After the Introduction of the Protein Digestibility Corrected Amino Acid Score Method. *Br. J. Nutr.* **2012**, *108 (2, Suppl)*, S183–S211. DOI: 10.1017/S0007114512002309.
10. Carbonaro, M.; Maselli, P.; Nucara, A. Relationship Between Digestibility and Secondary Structure of Raw and Thermally Treated Legume Proteins: A Fourier Transform Infrared (FT-IR) Spectroscopic Study. *Amino Acids* **2012**, *43(2)*, 911–921. DOI: 10.1007/s00726-011-1151-4.
11. USDA: Food Data Central. https://fdc.nal.usda.gov/fdc-app.html#/food-details/174288/nutrients (accessed 2023-06-01).
12. Ren, Y.; Yuan, T. Z.; Chigwedere, C. M.; Ai, Y. A Current Review of Structure, Functional Properties, and Industrial Applications of Pulse Starches for Value-Added Utilization. *Compr. Rev. Food Sci. Food Saf.* **2021**, *20(3)*, 3061–3092. DOI: 10.1111/1541-4337.12735.

Further information

- Agilent Cary 630 FTIR Spectrometer
- Agilent MicroLab Software
- Agilent MicroLab Expert Software
- FTIR Analysis & Applications Guide
- FTIR Spectroscopy Basics – FAQs

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