Optimizing plant-based proteins using Raman spectroscopy

Key Issues

- Plant-based proteins are emerging protein sources in food, beverages, and nutritional supplements
- Molecular properties of plant-based proteins affect nutrition, functionality, flavor, and sensory attributes
- Raman spectroscopy provides rapid, non-destructive, analysis of plant-based proteins for applications ranging from product quality understanding to inline process monitoring and control applications

Introduction

Plant-based proteins are an emerging source of sustainable and nutritious protein with uses in foods, beverages, and nutritional supplements. The molecular properties of plant-based proteins, such as composition, molecular structure, viscoelasticity, and solubility are different from those of animal-based proteins. Those differences provide a different sensory experience, which can lead to consumer rejection of the plant-based product. Thus, plant-based proteins often require specialized extraction, functionalization, and formulation processing to optimize their nutrition and sensory properties. One of those processes is fermentation to quickly acidify the protein, control flavor profiles, and improve emulsification. Throughout the plant-based protein production, detailed knowledge of the composition and molecular structure is needed to keep a tight control on the protein's characteristics and to monitor for process-induced transformations. For this reason, many plant-based protein manufacturers are adopting new approaches to process analysis, including Raman spectroscopy.

Raman advantages

Raman spectroscopy has the ease of near-infrared (NIR) measurements, but with much more specificity. Because of that specificity, Raman offers the possibility of simultaneously analyzing multiple components or aspects with a single measurement. In recent years, Raman has opened new avenues to analyses for understanding, monitoring, and controlling plant-based proteins and their manufacturing processes. In this application note, we provide examples relevant to plant-protein manufacturing: fermentation monitoring and protein structure analysis. These studies show the process monitoring and product quality measurement capabilities of Raman.

Materials and methods

Two relevant studies show feasibility of Raman spectroscopy for plant-based proteins: 1) monitoring fermentation processes, and 2) measuring protein composition and molecular structure. In the fermentation monitoring experiment, a Raman probe was immersed directly into a fermentation process and used to quantify sugars and alcohols *in situ*. Raman signal was collected by a Raman analyzer. In the protein composition experiment, consumer samples of plant-protein supplements were measured in a laboratory using a non-contact large volumetric probe. Protein band assignments were made based on literature in Raman spectroscopy of biological molecules.[1, 2]

Results and discussion

Figure 1 shows a representative time course of a fermentation, where sugars are converted into alcohols. Raman bands for sugars are well resolved from alcohol bands, enabling simultaneous measurement of these two important components. The specificity of Raman spectroscopy enables simultaneous measurement of multiple fermentation components without needing to pull a sample from the fermenter tank. This aspect reduces operational complexity of Raman spectroscopy in comparison

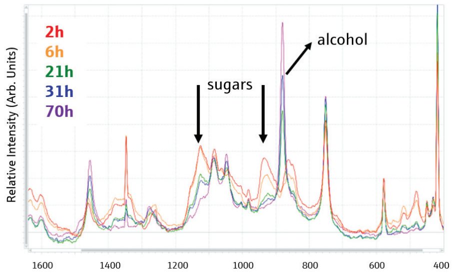

Figure 1: Raman spectroscopy enables simultaneous measurement of multiple components. In this example fermentation, sugars and alcohols can be quantified in the same *in situ* measurement.

ⓘ All Raman analyzers and probes referenced in this application note are Endress+Hauser products powered by Kaiser Raman technology.

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with single attribute process sensors. Other studies have shown Raman is equally well suited for monitoring bacterial or yeast fermentations (data not shown).

Figure 2 shows a representative Raman spectrum of plant-based proteins in commercially available nutritional supplements. The well-resolved peaks report on protein backbone and side chain groups. These bands can be used to characterize the chemical environment of the protein amino acids (marked with * in Figure 2) and assess higher order structure including the presence of α-helix, β-sheet, and random coil structures (marked with ‡ in Figure 2). Moreover, Raman measurements of proteins can be accomplished in an aqueous environment, in solid form, and in the final formulated product. As a proven technique in biophysics, Raman is used to understand structural modifications and is compatible with isotope exchange studies.

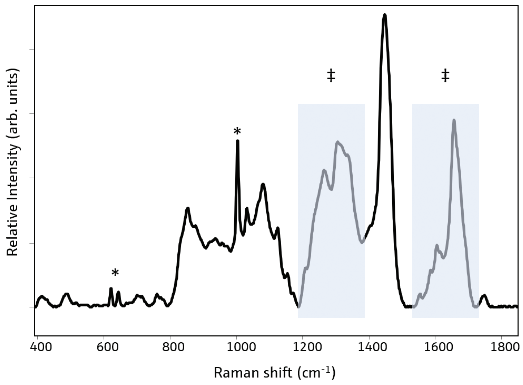

Figure 2: The Raman spectrum of a plant-based protein provides information on composition (*) and molecular structure (highlighted in grey).

Conclusions

The capability of analytical technologies to keep pace with sophisticated food manufacturing processes becomes increasingly more important in order to ensure a consistent product. Raman spectroscopy provides detailed chemical information on a sample without needing to prepare, extract, or destroy it. These features have enabled Raman spectroscopy in biochemistry, pharmaceutical, and food industries for applications ranging from laboratory analysis to quality assurance and inline product quality control. As a measurement technique for proteins, Raman imparts many benefits including a long history of measurement success, direct measurements in aqueous solutions, highly specific information on backbone and side chain groups, and translation of the measurement from the laboratory into process monitoring and manufacturing applications. Raman's specificity and compatibility with existing process hardware have yielded successes in optimizing product functionality, feedback control of bioprocesses, and monitoring unit operations such as hydrolysis, blending, freeze drying, granulation, and extrusion.

Raman shift (cm^{-1})	Band assignment	Component
620,640	Doublet	Tyrosine, Phenylalanine
940	C-C protein backbone	Protein
1001	Ring breathing	Phenylalanine
1080	C-N, C-C stretch	Protein, polysaccharides
1125	C-C, C-OH C-N stretch C-O-C glycosidic linkage	Protein, polysaccharides
1235-1270	Amide N-H, α-helix	Protein structure
1270	Amide N-H, random coil	Protein structure
1340	CH_2/CH_3 wag	Protein
1446	CH_2/CH_3 deformation	Proteins, lipids, carbohydrates
1655	Amide C=O, α-helix	Protein structure
1670	Amide C=O, random coil	Protein structure
1687	Amide C=O, β-sheet	Protein structure

Table 1: Raman band assignments in plant-based protein supplements show contributions from protein, lipids, and carbohydrates that can be used to understand the composition and molecular structure of macronutrients.

Raman is a technology that is robust, scalable, and works in aqueous environments without needing to use exogeneous labels. Raman models developed in the laboratory can be transferred to a process, providing multi-component knowledge and enabling real-time process corrections. These applications and related examples in biotechnology show the value of Raman spectroscopy in protein analysis of food products for research, quality control, and process monitoring purposes.

References

1. Koenig JL **(1972)** Raman spectroscopy of biological molecules: A review. J Polym Sci Macromol Rev 6:59–177
2. De Gelder J, De Gussem K, Vandenabeele P, Moens L **(2007)** Reference database of Raman spectra of biological molecules. J Raman Spectrosc 38:1133–1147