Identification of Dairy and Plant-Based Milks by Agilent Resolve Raman Spectroscopy

Fast, direct analysis of milk products and quantification of fat content in milk samples

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Abstract

Raman spectra for three dairy and nine plant-based milks were collected using the Agilent Resolve handheld Raman analyzer. The samples included skimmed, semi-skimmed, and whole (full fat) dairy milks, three variants of oat milk, and soy, almond, hazelnut, coconut, rice, and pea milks.

The Raman spectra for each sample were used to create a "Milk" spectral library in the Agilent Command Fleet Management software package. The new library was then added to the list of libraries that are accessible from the Resolve handheld system. All milk types were tested using Resolve and excellent matches were achieved, confirming the accuracy of the identification method.

The high quality of the Raman spectra also enabled the identification of functional groups that could be attributed to the main types of macronutrients present in the samples. This distinction enabled the fat content to be modelled using multivariate analysis-partial least squares with one latent variable (MVA-PLS1), enabling the quantification of fat in dairy milk using the handheld analyzer.

Introduction

The global dairy market was valued at approximately 900 billion USD in 2023.[1] Dairy milk's share of this market constituted an estimated 185 billion USD in 2022.[2] Plant-based milk products have seen a rapid rise in popularity over recent years, accounting for an estimated market valuation of 18 billion USD.[3] Milk is an excellent source of carbohydrates, fats, proteins, vitamins, and minerals, making it an important staple food for people of all ages. The proportions of these constituents depend on the type of milk, and the level of processing used to produce milk variants such as full-fat (whole), reduced fat (semi-skimmed), and fat-free (skimmed).

The Agilent Resolve handheld Raman analyzer enables the acquisition of high-quality Raman spectra of solids, powders, liquids, and mixtures. In this study, the Resolve analyzer was used in surface mode to collect spectra for 12 diverse fluid milk products, requiring less than 30 seconds of analysis time per sample. PC-based Agilent Command Fleet Management software was then used to create a spectral library for "Milk", based on the spectra of the known samples. The Command software then sent the newly created spectral library to the Resolve analyzer, enabling it to identify each milk type with a high degree of accuracy. Command can also send spectral libraries to multiple (a fleet of) Resolve analyzers. Once the library has been added to the spectral library database on the handheld device, Resolve can identify or confirm the identity of milk type directly in the manufacturing process stream and in storage vessels.

Also, Resolve produces high-quality Raman spectra that can be used to create quantitative models. In this study, a quantitative method for the determination of dairy milk fat was developed based on MVA-PLS1. Once established, the quantitative model was able to be used to accurately predict the fat content of dairy milk products.

Experimental

Samples

Cartons or plastic bottles containing three dairy milk and nine seed or nut-based milk products were bought in a supermarket in the UK. Each carton or bottle was shaken well before the fluid was decanted into a glass or plastic vessel, ready for analysis. It was especially important to shake the plant-based milk products as they had not been homogenized under high pressure, unlike the dairy-based milks. All the samples were opaque, white liquid emulsions with water content varying from 88.5 to 96.6 g per 100 mL.

Instrumentation

The Resolve handheld Raman analyzer, shown in Figure 1, is a flexible spectrometer that is widely used for the identification of chemicals, including hazardous materials. Depending on the aims of the application, the Resolve can be operated in three different measurement modes, including:

- **Surface scan:** Line-of-sight measurements, similar to conventional point-and-shoot Raman—selected when there is no barrier between the sample and the analyzer.
- **Vial Holder mode:** Selected when the sample is placed into a standard vial (such as Agilent storage vials, 100/pk, 13-425 cap, part number 5183-4311).
- **Through-barrier mode:** Uses Agilent proprietary spatially offset Raman spectroscopy (SORS) technology—selected when the sample is packaged or contained. Typical barriers include a wide variety of colored and opaque plastics, glass, paper, cardboard, sacks, and fabrics.

In this study, the Resolve Raman analyzer was operated in standard surface scan mode by selecting "clear bag or none" on the Resolve screen and vial mode by selecting "Vial" on the Resolve screen.

Figure 1. Agilent Resolve handheld Raman analyzer.

Measurement mode

First, Resolve was used to acquire spectra for all 12 milks in standard 5 cm clear glass vials in Vial Holder mode. However, due to the colloidal nature of the emulsions, vial measurements displayed enhanced signals for the sample due to the glass vial, as shown by the red spectrum in Figure 2. The green spectrum shows peaks generated by the empty glass vial.

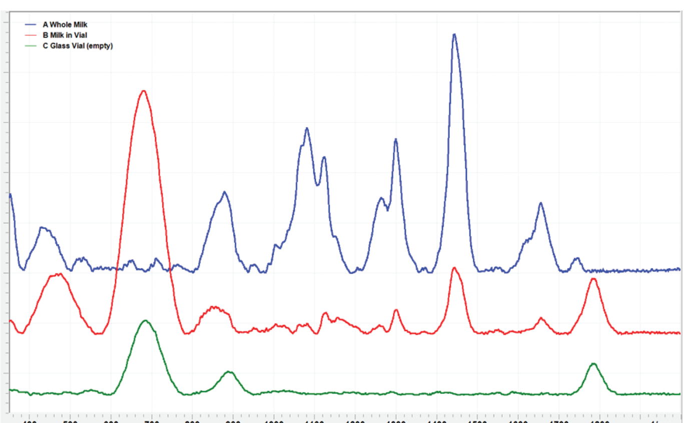

Figure 2. Stacked Raman Resolve spectra. Green: empty clear glass vial; red: whole milk in a glass vial (vial mode); blue: spectrum that was collected directly from the whole milk sample in surface scan (barrier-free) mode.

To avoid the effects of the glass vial, each of the 12 milk samples was decanted into a plastic container and Resolve scans were collected in surface (barrier free) mode. The analyzer was held vertically above the container and the end of the cone was allowed to contact the surface of the milk. This method provided detailed Raman spectral bands that were free of glass peaks, as shown by the blue spectrum in Figure 2. The same whole milk sample was used for both the milk in vial (red spectrum) and surface mode (blue spectrum) measurements shown in Figure 2, while the empty vial is represented by the green spectrum.

All further work presented in this study was performed in surface scan mode.

Workflow using Command software

Creating new spectral libraries using Resolve and PC-based Command software is quick and easy. All the surface mode scans were transferred from the Resolve analyzer to Command software by use of a USB connection. Command was then used to create a new "Milk" library based on the average of five scans per milk sample. The 12 milk types listed in Table 1 were added to the Milk spectral library. This newly created library was then exported back to the Resolve analyzer, ready for use. Command was also used to export all the individual Raman spectra as .spc files to Agilent MicroLab Expert software, which was also installed on the PC, for quantitative multivariate modeling of the fat content of the samples.

Table 1. Nine common plant-based milks and three common types of dairy milk with their macronutrient and fiber values per 100 mL of sample.

	Mass of Nutrient (g) per 100 mL			
Milk Sample	Fat	Protein	Carbohydrate (Sugar)	Fiber
Oat Milk (Barista)	3.0	1.1	7.1 (3.4)	0.8
Almond Milk	1.2	0.5	2.6 (2.5)	0.3
Soy Milk	1.1	2	0.1 (0.1)	0.2
Coconut Milk	1.1	0.3	2.0 (1.5)	0.4
Oat Milk/Drink	1.5	1.1	7.1 (3.4)	0.8
Hazelnut Milk	1.6	0.4	3.2 (3.2)	0.3
Pea Milk	1.4	2.2	0	1.8
Rice Milk	1.0	0.1	10 (7.1)	0
Oat and Hemp Milk	1.0	1.1	8.7 (3.3)	0
Skimmed Milk	0.1	3.6	4.9 (4.9)	0
Semi-Skimmed	1.7	3.6	4.8 (4.8)	0
Whole Milk	3.6	3.3	4.6 (4.6)	0

Results and discussion

Identifying milk types with Resolve

The Raman spectra of both the dairy and plant milks contain spectral peaks that are related to the fat, protein, carbohydrate, and fiber content of the samples. The stacked spectra of all 12 milks in Figure 3 show clear differences between the samples.

To test the accuracy of the Resolve to identify different types of milk, five scans were performed for each milk sample, and the spectra were compared to the Milk spectral library. Resolve correctly identified each milk type in every instance (five times out of five) with match quality levels ranging from 87 to 99%.

The differences between all the milks relate predominantly to the presence or absence of the three main macronutrient (fat, protein, and carbohydrate) groups and the fiber content. Figure 4 shows example match results for semi-skimmed milk, soy milk, oat milk, and water. Soy milk comprises around 97% water and has the least amount of milk solids (the sum total of the macronutrients and fiber). Despite the high water content, the Resolve Raman analyzer positively identified soy milk from the other milks.

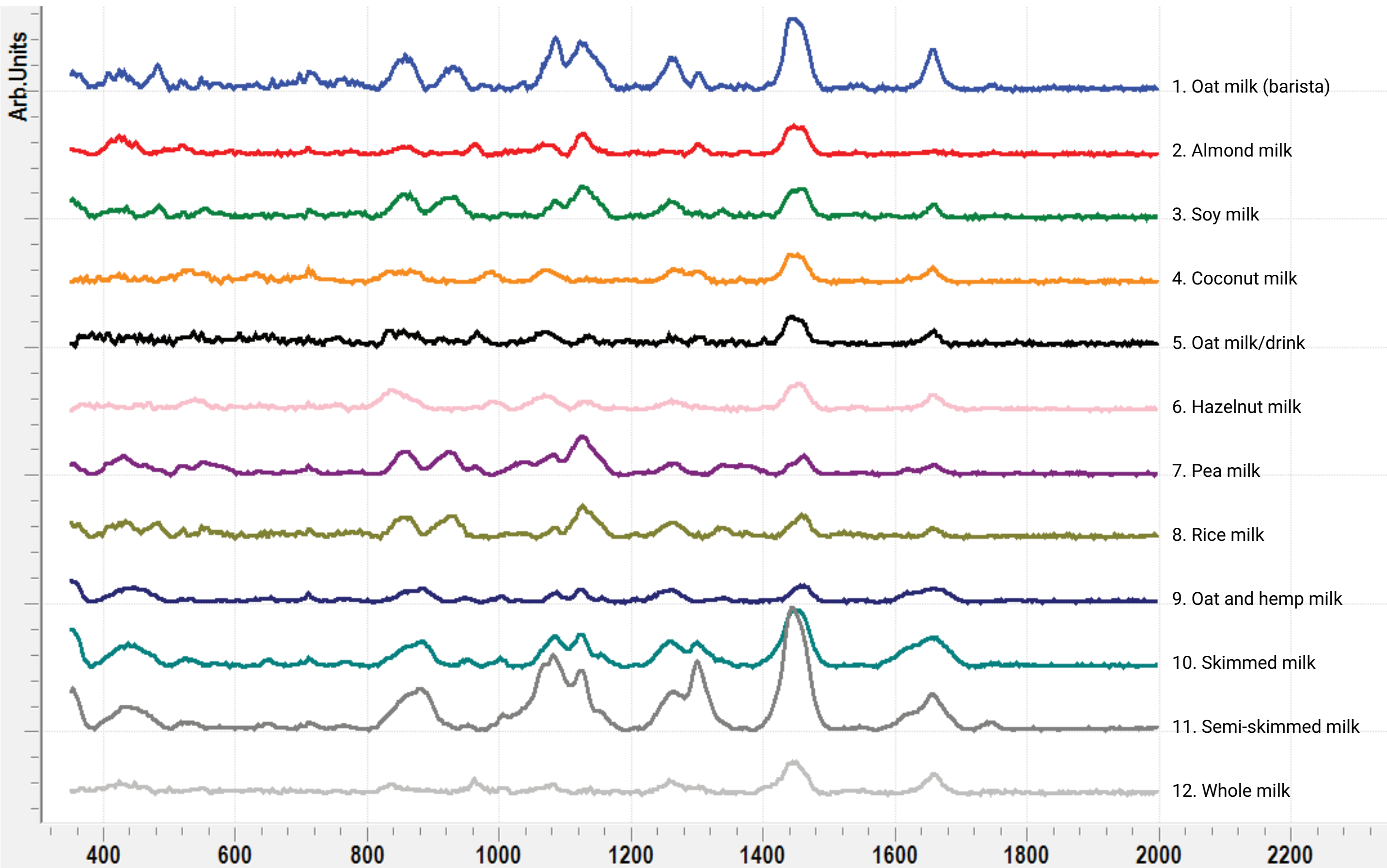

Figure 3. Raman spectra of 12 types of milk collected in surface scan mode using the Agilent Resolve Raman handheld analyzer.

Figure 4. (A) Agilent Resolve Raman handheld analyzer and small plastic container of semi-skimmed milk. (B) Scan of semi-skimmed milk with a match quality of 98%. (C) Scan of soy milk with a match quality of 90%. (D) Scan of oat milk with a match quality of 97%. e) Scan of water (no match 0%).

Macronutrient analysis

The macronutrient and fiber composition of the 12 milks examined in this study are listed in Table 1.

Fats and proteins are complex compounds that display distinctive spectral features related to the structure of the macronutrient. Since the fat and protein content of milk vary between the different types of milk (as detailed in Table 1), the milks can be distinguished based on the size and shape of any identified fat and protein bands. The peak bands for fat and protein in the Resolve Raman spectra for soy, oat, pea, rice, and skimmed dairy milks are shown in Figure 3.

- **Proteins** are made up of polypeptides, which are in turn made up of amino acids. In plant or dairy milk, the proteins contain many peptide bonds, resulting in a main peak at ~1,650 cm^{-1} and a minor peak at 1,600 cm^{-1} for aromatic amino acids. Protein molecules are much larger than fat compounds.
  - Dairy protein contains a complete amino acid profile, so the peaks are broader.
  - Nut or grain proteins do not include all amino acids, so the peaks are sharper.
- **Fat compounds** in plants, nuts, or dairy milk are a mixture of different triglyceride fatty acids—where three fatty acid groups are bound to a glycerol—which contain many methylene groups (CH_2) and a terminal methyl group (CH_3). These groups result in the bands at ~1,450 cm^{-1}. When the fatty acid is unsaturated, extra bands appear at 1,650 and 1,300 cm^{-1}. The 1,300 and 1,100 cm^{-1} peaks relate to the ester group of the triglyceride fatty acid ester, which is a relatively minor contributor.
- **Carbohydrates**, also often called saccharides, are the most diverse class of compounds spectrally. The main types of carbohydrates in milks are simple sugars (mono- and di-saccharides), polysaccharides, and cellulose fiber. Fiber is often included as a separate item in the list of ingredients on foods.

  Due to the range of compounds that qualify as a carbohydrate, plus some degree of overlap with the fat content, the carbohydrate-related bands are slightly more complex. The selectivity of the Resolve Raman spectra reveals some clear bands in the spectral region of 500 to 1,200 cm^{-1} due to the different types of carbohydrates and fiber in the selected milks (Figure 6 and Table 1). Selected representative milks shown in Figures 5 and 6 display the following features:
  - **Soy milk** contains the lowest combined carbohydrate and fiber content (0.3 g) and exhibits the least number of features and least intense peaks.
  - **Oat milk** contains the second highest combined carbohydrate content (7.9 g) with the second largest peak area for the whole spectral region. The carbohydrate content of oat milk comprises both simple and complex sugars. The simple sugar is principally disaccharide maltose.

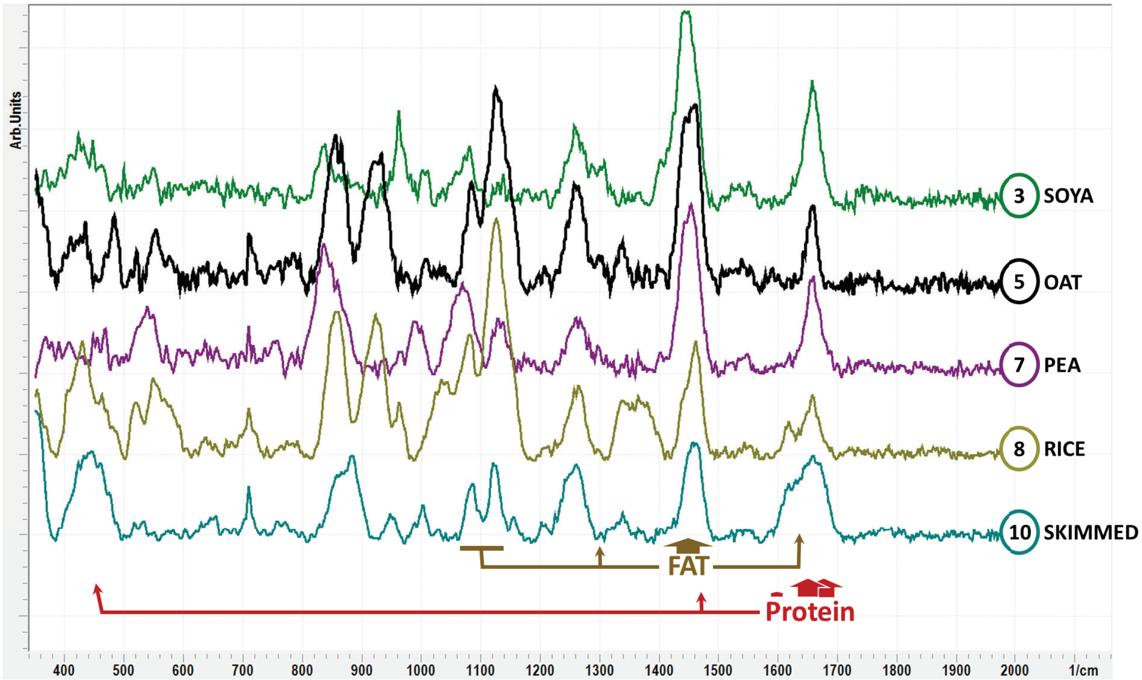

Figure 5. Raman spectra of selected milks (soy, oat, pea, rice, and skimmed dairy milk), highlighting the peak band assignments for fat and protein. The large arrows represent a major contributor, while the smaller arrows indicate a less significant contribution. The flat bars represent a variable contribution.

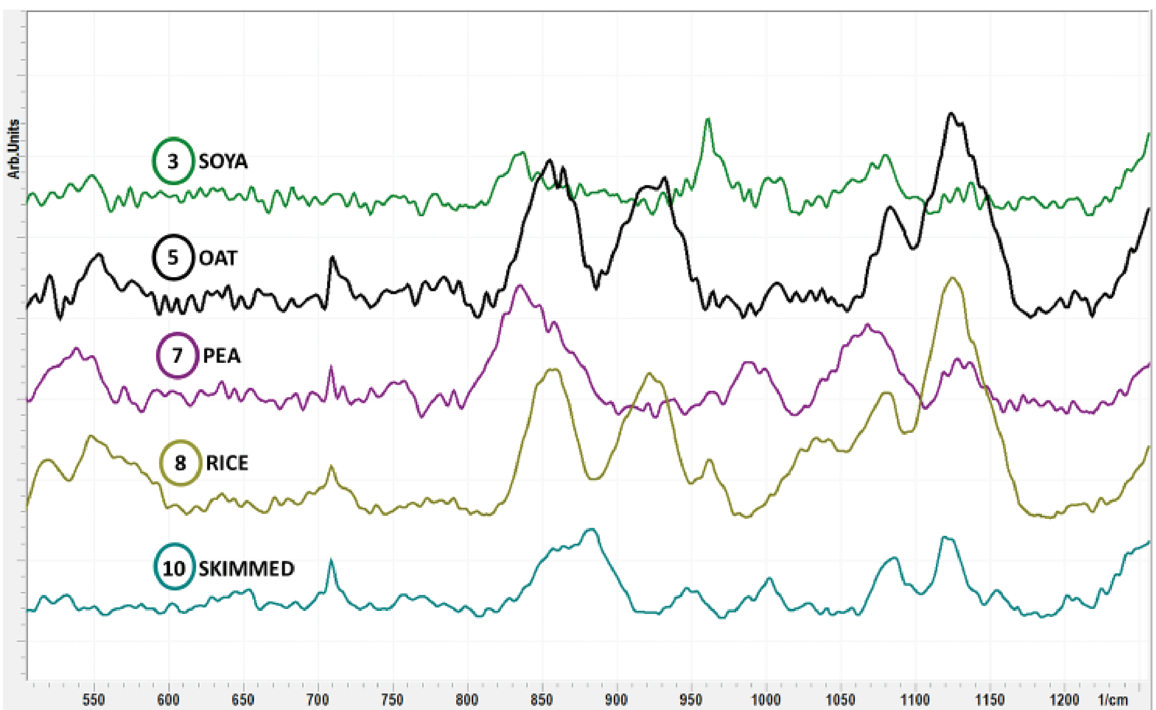

Figure 6. The main carbohydrate region of Raman spectra (500 to 1,200 cm^{-1}) for selected plant and dairy milks.

- **Pea milk** contains no sugar and high relative fiber (1.8 g), which accounts for a main peak at 835 cm^{-1} related to beta(1–4) linkage.
- **Rice milk**, which contains 7.1 g of simple sugars and 2.9 g of complex sugar with zero fiber, has the highest carbohydrate content and the largest spectral features. It has a similar profile to oat milk, with some minor differences.
- **Skimmed dairy milk** – all the carbohydrates belong to the disaccharide class of simple sugars, lactose (glucose and galactose disaccharide), with zero fiber. Therefore, most of the features in the 500 to 1,200 cm^{-1} region relate to lactose with some minor contributions from the fat and protein content of the sample.

Fat analysis of dairy milk

Milk derived from mammals is naturally high in fat. Before treatment, the fat content of cow's milk ranges from 3.25 to 5% with some breeds of cow able to produce milk with a fat content above 5%. To produce milk products with different fat content, such as skimmed (nonfat), semi-skimmed (low or reduced fat), and whole (full-fat), milk is often blended and homogenized. Although there is some variation by country or geographic area, the fat content of skimmed milk is typically 0.1 to 0.3%, 1.7% for semi-skimmed, and approximately 3.6% for whole milk. In a milk emulsion (plant or diary) the fat forms micelles, which are metastable fat globules that stay in solution.

The enhanced scatter that is evident in the Raman spectra for the three types of dairy milks at ~1,450 cm^{-1} is mainly due to fatty acids, with a minor contribution from lactose and protein. The intensity of the peak at 1,450 cm^{-1} correlates to the fat content of the sample, and, as the fat content increases, the peak position shifts to lower wavenumbers. In fact, the intensity of the signal throughout the spectra is strongest for whole milk and weakest for the lowest fat milk, skimmed milk, as shown in Figure 7.

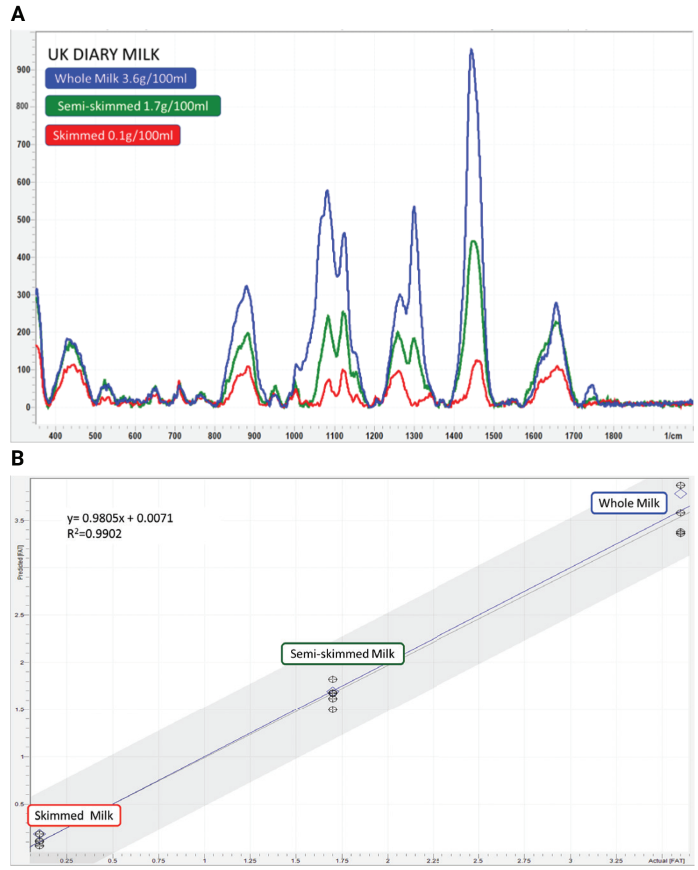

Figure 7. (A) Raman spectra collected in barrier-free surface scan mode of three common UK grades of milk: skimmed (red, 0.1 g/100 mL fat), semi-skimmed (green, 1.7 g/100 mL fat), and whole milk (blue, 3.6 g/100 mL fat). (B) Actual versus predicted MVA-PLS1 model for the milk fat for the three common classes of dairy milk. The training calibration spectra are shown by the black markers and the validation spectra are shown by the blue diamonds.

The bulk of the protein of dairy milk is composed of two main types: casein and whey, with a ratio 83 to 1. Casein and whey produce unique amide bands around 1,650 cm^{-1}. The shape of the dairy protein is broader than the plant protein, which is sharper, as shown in Figure 5. The dairy milks have a relatively high protein content compared to the plant-based milks of 3.6 g per 100 mL for the skimmed and semi-skimmed milks and 3.3 g per 100 mL for whole milk (Table 1).

The price that wholesalers pay primary dairy producers for milk depends on the fat content, so having access to a quick means of checking the fat content could help to establish a fair price. As shown in Figure 7B, the fat content of dairy milk can be quantified using an MVA-PLS1 model that was developed with both calibration and validation data. The linearity of the regression model (R^2 of 0.9902) demonstrates that the multivariate model can be used to accurately predict the fat content of unknown milk samples.

Conclusion

A variety of dairy and plant-based milks were investigated using the Agilent Resolve handheld Raman analyzer. Highlights of the study include:

- Generation of high-quality Raman spectra of milk obtained using the Resolve in surface mode.
- The simplicity of Agilent Command Fleet Management software to build a new spectral library for Milk, enhancing the choice of libraries on the Resolve analyzer. Further milk variants can be added with ease, e.g., organic, lactose-reduced, and lactose-free.
- Accurate identification of the milk type during library testing of the 12 milk types against the new Milk library.
- Selectivity and sensitivity of the spectral features allowed for the analysis of macronutrients present in each of the milks. The position of bands relating to the macronutrients will also be present in other foods, so the Resolve could be used to investigate other foods and beverages.
- Modeling of the fat content of dairy milks for the quantification of fat in full-fat, semi-skimmed, and skimmed milk. Models for plant-based milks and other nutrients could easily be established using the same methodology.

The high spectral quality of the portable Raman Resolve makes it a powerful tool for fast, real-time measurements of foods for identification, quality, and/or composition purposes, including of the macronutrient values.

References

1. Estimated Dairy Market Value Worldwide in 2020 and 2028, *Statista* April **2023**.
2. Fluid Milk Market: Global Industry Trends, Share, Size, Growth, Opportunity and Forecast 2023–2028, *Research and Markets* July **2023**.
3. Global Plant-Based Milk Market 2023–2032, *Custom Market Insights* January **2023**.

Further information

Resolve Handheld Raman Analyzer

Command Fleet Management Software

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