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Raman spectroscopy enables non-invasive and quantitative assessment of macronutrients in baked foods

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Abstract

Fast and accurate assessments of macro and micronutrients in food can be used to balance the diet of millions of people around the world. This can help to prevent the development of chronic diseases such as obesity and diabetes. Modern analytical methods that can be used to reveal information about the nutritional composition of food are destructive and laborious. In this study, we examine the potential of Raman spectroscopy in the non-invasive, non-destructive, and quantitative assessment of carbohydrates, gluten, carotenoids, and fats in baked foods, including bread and crackers. The Raman effect is based on the phenomenon of inelastic light scattering. Our results demonstrate that Raman spectroscopy could be used to quantify the amount of carbohydrates, gluten, carotenoids, and fats in baked foods. Furthermore, unique spectroscopic signatures of bread and crackers could be used for their automated identification. These findings demonstrate that Raman-based sensors can be used to personalize nutrition and control the quality of consumed food products.

KEYWORDS

baked foods, gluten, PLS-DA, precision nutrition, Raman spectroscopy

1 | INTRODUCTION

Overweight and obesity are chronic diseases that are caused by unbalanced carbohydrate-rich diets. In the United States, nearly one in three adults is overweight (30.7%), whereas more than two of every five adults have obesity (42.4%).[1] Both overweight and obesity are emerging problems for children. Currently, one out of every six children and adolescents ages 2 to 19 years are overweight (16.1%), whereas one out of five children and adolescents of the same age has obesity.[1] If not mitigated by healthy diets, these diseases trigger other deadly pathologies, such as diabetes, cardiovascular disease, and cancer.[2]

Healthy diets require precise and direct assessment of nutrients in the consumed food that should be label-free, minimally costly, and laborious. Currently used analytical techniques that can be used for the quantitative assessment of proteins and carbohydrates in foods are extremely laborious. For instance, megazyme assay that is used to determine the concentration of carbohydrates requires extraction and purification of food materials,

development of calibration curves, and utilization of spectrophotometry to enable quantification of the concentration of carbohydrates in the sample.[3] The Dumas combustion assay is often used to quantify the concentration of proteins in the food.[4] Similar to the megazyme assay, this technique is destructive, costly, and laborious. One can expect that this problem can be overcome by the use of hand-held sensors that can be used to quantify the amount of macronutrients in the food.[5–7] To be broadly used, such sensors have to be non-invasive, non-destructive, label-free, minimally laborious, and inexpensive.

A growing body of evidence suggests that Raman spectroscopy (RS), an emerging analytical method, can be used to probe and quantify the concentration of carbohydrates, proteins, and other macronutrients in foods.[8–10] For instance, Krimmer and co-workers showed that RS could be used to probe relative concentrations of proteins, carbohydrates, carotenoids, and fiber in maize kernels.[9] For this, Krimmer and co-workers acquired 50 spectra with 830 nm excitation from six corn varieties. Next, the researchers determined the vibrational bands that originated from these macronutrients. Finally, using ANOVA, Krimmer and co-workers identified maize varieties with low and high concentrations of proteins, carbohydrates, carotenoids, and fiber.[9] Expanding upon this, Morey and co-workers utilized RS to determine the relative concentration of starch in nine different varieties of potato tubers.[10] Furthermore, the researchers built the calibration curve by measuring suspension with different concentrations of starch. Morey and co-workers showed that this calibration plot could be used for the direct quantification of starch in potato tubers.

Our group also demonstrated that different plant species and their varieties could be identified by RS via their unique vibrational fingerprints.[8,11] For instance, Farber and co-workers showed that RS could be used to identify poison ivy and other plant species, as well as differentiate between isogenic varieties of peanuts.[8,11] To achieve this, researchers employed partial least-squared discriminant analysis (PLS-DA) of ~50 spectra acquired from leaves of different plant varieties. In parallel, Abreu and co-workers found that by using RS, coffee quality can be examined. Specifically, changes in the vibrational signatures of kahweol, the major ingredient of coffee, could be used to determine the storage conditions and the duration of storage.[12,13] Recently, our group demonstrated that the relative concentration of carbohydrates could be probed in ramen noodles using RS. Furthermore, it was found that RS could be used to differentiate between gluten-free and gluten-rich ramen with ~100% accuracy.[14]

Expanding upon this, we propose to determine whether RS could be used for the quantitative assessment of macronutrients in baked foods, including bread and crackers. For this, we acquired Raman spectra from six different varieties of baked foods. We also performed chemometric analyses of these spectra to investigate the accuracy of Raman-based quantification of carbohydrates, gluten, and fats in these food products.

2 | MATERIALS AND METHODS

2.1 | Materials

Baked foods were purchased from local grocery stores in College Station, TX (Figure S1).

2.2 | RS

Raman spectra from all the baked foods were collected using a hand-held Agilent Resolve spectrometer equipped with an 830-nm laser (beam diameter ~ 2 mm). We used the following experimental parameters to acquire the spectra: 1-s collection time and 495-mW laser power. The baseline spectral subtraction was performed by Agilent Resolve software. In total, 20–25 spectra were collected from each type of the backed foods. Spectra were acquired at different locations from the surface of the bread or crackers. On average four to five spectra were taken from each piece of the baked food. Thus, four to five pieces of bread or crackers were analyzed to collect 20–25 spectra.

2.3 | Spectral processing and statistical analysis

Spectral processing and averaging were conducted using Matlab (Mathworks) equipped with PLS_Toolbox 9.0 (Eigenvector Research, Inc., Manson, WA). For PLS-DA, spectra were MSC normalized and filtered through smoothing (SavGol). The reported PLS-DA models had nine latent variables. For ANOVA, the first derivative was taken from the spectra. Then, spectra were mean centered and area normalized.

3 | RESULTS AND DISCUSSION

Raman spectra acquired from baked foods have the vibrational bands that could be assigned to C–O–C and C–O–H vibrations of sugars (441, 481, 580, 714, 768, 857,

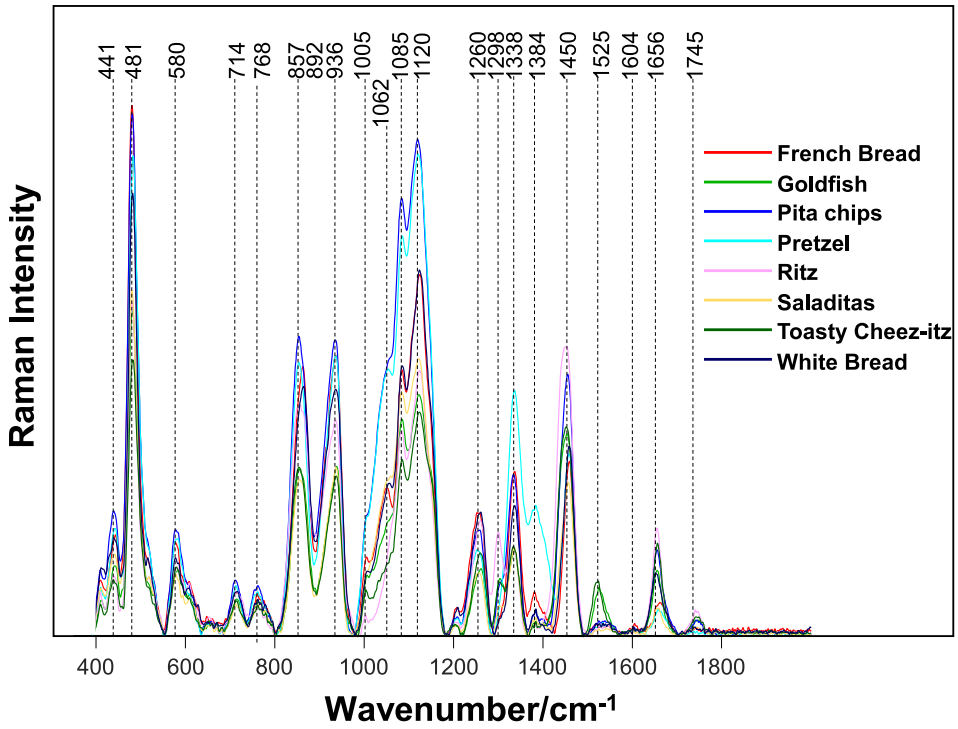

FIGURE 1 Averaged Raman spectra acquired from French bread, "Goldfish," "Pita chips," pretzel, "Ritz," "Saladitas," "Toasty Cheez-itz," and white bread. [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Vibrational bands and their assignments for Raman spectra acquired from different baked foods.

Band	Vibrational mode	Assignment
1743	C=O stretching	Lipids[14,15]
1656	C=O stretching (amide I)	Proteins[14,16,17]
1604	Aromatic ring	Proteins[16,17]
1525	–C=C–	Carotenoids[18,19]
1450	δ (CH) + δ (CH_2)	Aliphatic[20]
1384	δ(C–O–H) coupling of the CCH and COH deformation modes	Carbohydrates[20]
1338	δCH_2 bending vibration	Aliphatic[20]
1298	CH_2 twisting	Lipids[15]
1260	δ(C-C-H) + δ(O–C–H) + δ(C–O–H)	Carbohydrates[20,21]
1124	ν (C-O) + ν(C–C) + δ(C–O–H)	Carbohydrates[20]
1085	ν(C-O) + ν(C–C) + δ(C–O–H)	Carbohydrates[20]
1062	ν (C–O–C)	Lipids
1005	Phenylalanine ring stretching mode	Proteins[16]
936	δ(C–O–C) + δ(C–O–H) + ν(C–O) α-1,4 glycosidic linkages	Carbohydrates[20]
892	CH_2 wagging	Lipids[15]
857	δ(C–C–H) + δ(C–O–C) glycosidic bond; anomeric region	Carbohydrates[20]
768	δ(C–C–O) related to glycosidic ring skeletal deformations	Carbohydrates[20]
714	δ(C–C–O) related to glycosidic ring skeletal deformations	Carbohydrates[20]
580	δ(C–C–O) + τ(C–O)	Carbohydrates[20]
481	CCO and CCC deformations; Related to glycosidic ring skeletal deformations δ(C–C–C) + τ(C–O) Scissoring of C–C–C and out-of-plane bending of C–O	Carbohydrates[20]
441	Skeletal modes of pyranose ring	Carbohydrates[20]

892, 936, 1062, 1085, 1120, 1260, 1338, and 1384 cm^{-1}), as well as amide I of gluten and other proteins (1656 cm^{-1}) (Figure 1 and Table 1).

In some of the acquired spectra we also observed the carbonyl vibration of fats (1745 cm^{-1}),[14] as well as carotenoids (1525 cm^{-1})[18] (Figure 1 and Table 1). Finally, we found that Raman spectra acquired from baked foods exhibit CH and CH_2 bands at 1450 cm^{-1}, which could not be unambiguously assigned to any of the discussed above classes of macronutrients (Table 1 and Figure S2).

Previously reported results by our research group demonstrate that changes in the intensities of C–O–C and C–O–H vibrations could be used for the quantification of the carbohydrates in the sample.[14] Our current results showed that the relative intensity of a 1122 cm^{-1} band was lower in the spectra acquired from French bread, "Goldfish," "Ritz," "Salditas," "Toasty Cheez-itz," and wheat bread compared to the Raman spectra collected from "Pita chips" and "Pretzel." These results demonstrate that "Pita chips" and "Pretzel" possess the highest amounts of carbohydrates compared to other baked foods.

We also found that RS could be used to quantify the amount of protein, such as gluten in these pastry products. Specifically, we found that Pretzel, French bread, and "Saladitas" possessed the lowest amounts of gluten compared to other products, whereas the concentration of proteins was the highest in "Ritz" and "Toasty Cheez-itz" (Figure S2).

In the Raman spectra acquired from crackers, we found the vibrational band centered at 1745 cm^{-1} (Figure 1). Previously reported spectroscopic analysis of triacylglycerols by our group demonstrated that this band originated from the C=O vibration of lipids.[14] Based on these results, we can conclude that nearly all crackers possess fats, which likely came from the baking process. At the same time, this band was not evident in the Raman spectra acquired from bread and pretzel. Thus,

TABLE 2 Misclassification table of cross-validation for the PLS-DA model of fat-free and fat-rich baked foods.

	Accuracy, %	Fat-free	Fat-rich
Fat-free	**99.2**	**75**	**1**
Fat-rich	**100**	**0**	**120**

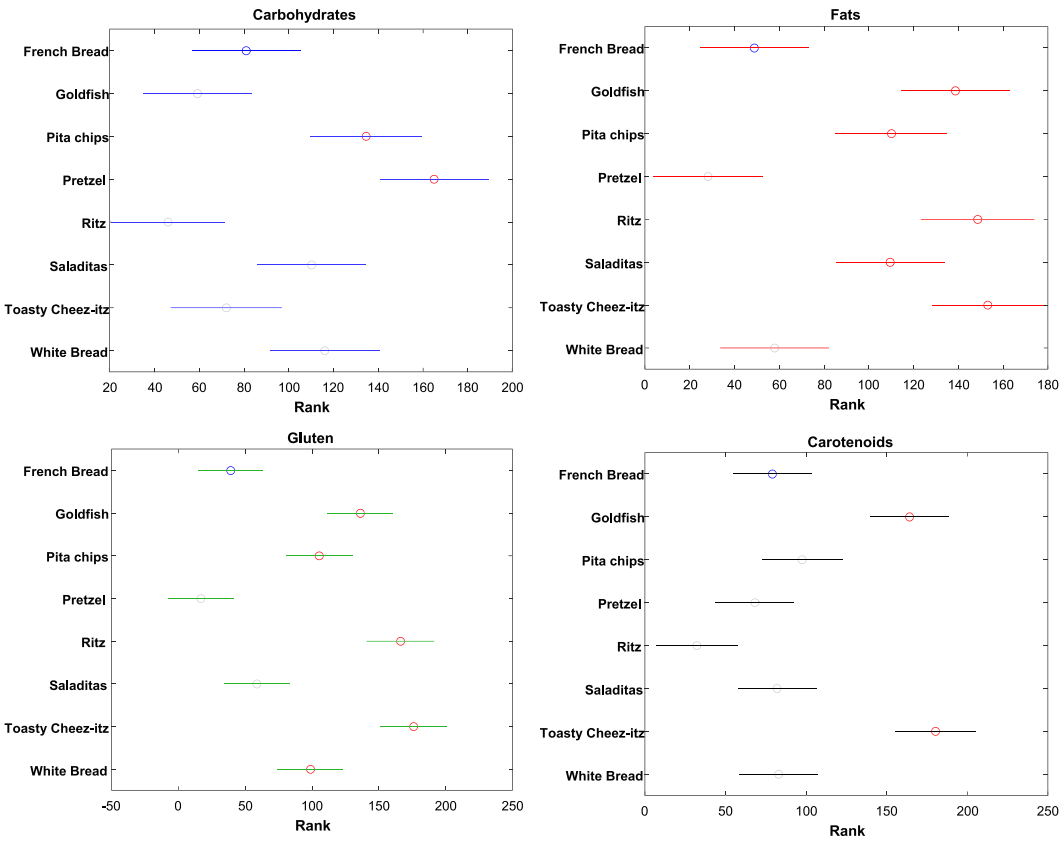

FIGURE 2 Means (circles) and confidence intervals for the intensities of 1120 cm^{-1} (carbohydrates), 1656 cm^{-1} (gluten), 1745 cm^{-1} (fats), and 1525 cm^{-1} (carotenoids) in the spectra acquired from different baked foods. [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Misclassification table of cross-validation for the PLS-DA model of different brands of baked foods.

	Accuracy, %	French bread	Goldfish	Pita chips	Pretzel	Ritz	Saladitas	Toasty Cheez-itz	White bread
French bread	80.0	20	0	0	0	0	3	0	0
Goldfish	92.0	0	23	0	0	0	0	0	0
Pita Chips	87.5	0	0	21	0	0	0	0	0
Pretzel	100.0	1	0	0	25	0	0	0	0
Ritz	100.0	0	0	2	0	23	0	0	0
Saladitas	88.0	4	1	1	0	0	22	0	0
Toasty Cheez-itz	100.0	0	1	0	0	0	0	24	0
White bread	100.0	0	0	0	0	0	1	0	25

our results showed that RS could be used to probe the presence of fats in pastry products.

The question to ask is how accurate such predictions can be made using a hand-held Raman spectrometer. To answer this question, we utilized partial least squares discriminant analysis (PLS-DA). Our results showed that RS enabled 100% accurate identification of fat-free and 99.2% accurate identification of fat-rich baked foods, Table 2.

Our results also revealed that some of the acquired spectra exhibit the vibrational band (1525 cm^{-1}) that could be assigned to the polyene moiety of carotenoids (Figure 2).[18] These compounds are broadly used as organic colorants in the food industry. For instance, their presence in the salmon enables the orange-red appearance of the fish meat. These findings demonstrate that RS could be used to detect and quantify the carotenoid concentration in baked foods. Specifically, we found that "Goldfish" and "Toasty Cheez-itz" possessed the highest concentration of carotenoids compared to other baked foods. These results are consistent with the yellow appearance of these crackers.

Finally, we ask the question about the extent to which the spectroscopic signatures of baked foods can be used for their identification. Our PLS-DA results demonstrate that RS enables highly accurate identification of different pastry products. Specifically, Pretzel, "Ritz," "Toasty Cheez-itz," and white bread could be identified with 100% accuracy, whereas other products could be confirmatory identified with 80%–92% accuracies (Table 3).

4 | CONCLUSIONS

Our results show that RS can be used to differentiate between fat-free and fat-rich baked foods with nearly 100% accuracy. Furthermore, RS could be used to quantify the amount of gluten in baked foods. We also demonstrated that RS was capable of quantifying the amounts of carbohydrates and carotenoids in baked foods, as well as allowing for the identification of different pastry products based on their spectroscopic signatures. Considering the non-invasive, non-destructive, and label-free nature of RS, one can expect that hand-held Raman sensors could be used in daily life for quality control of consumed food in both restaurants and grocery stores.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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