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**Research Paper**

# Application of Fourier Transform Infrared (FTIR) spectroscopy for the analysis of legume seeds

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## ABSTRACT

**Legume seeds are highly valued for their rich nutritional profile and many culinary applications, making them essential to human diets in many civilizations worldwide. Legume seeds have lower protein content comparable to that found in animal sources. Legumes have many health benefits, such as improved blood sugar control, weight management, cardiovascular health, and a reduced risk of developing chronic diseases, including diabetes, cancer, and heart conditions. The functional groups in legume seeds such as black chickpea, cowpea, white chickpea, small red bean, and red lentil were analyzed using FTIR spectroscopy combined with principal compound analysis. The findings indicated that five samples had O-H stretching, C-H stretching, and N-H stretching bonds in the wave number range of 2924 cm-1, which implies the presence of alcohol, carboxylic acid, amine salt, and alkane. The nitro compound having N-O stretching was observed in all samples except white chickpea. All the samples have C-F stretching bonds, representing the presence of fluoro compound in the wave range of 1002-1115 cm-1, except for red beans. It is worth noting that the properties of red lentils differ significantly from those of black chickpeas and cowpeas, and the properties of cowpeas differ from those of small red beans.**

*Key words:* Legume, FTIR, Minerals, Vitamin, Principal compound analysis, Nutrients

## INTRODUCTION

A legume is a kind of plant belonging to the family Fabaceae, which also includes several well-known crops, including beans, peanuts, lentils, and peas. These plants have a reputation for fixing nitrogen in the soil by living symbiotic with bacteria that fix nitrogen. Legumes are prized for their dietary content, high protein content, and other vital elements. They are staple foods for many people worldwide and are essential to agriculture and human nutrition. Legumes are also frequently utilized in crop rotation to increase soil sustainability and fertility. Legumes are rich in complex carbohydrates, dietary fiber, and protein as well as large levels of vitamins and minerals (Costa *et al.* 2006). In addition to their nutritional value, legume seeds contain anti-nutritional factors such as glucosides, tannins, alkaloids, protease inhibitors, lectins, and other harmful physiologically active chemicals. Due to various dietary fibre fractions in legume seeds, essential for digestion and help combat lifestyle and cancer-related illnesses, nutritionists emphasize the importance of incorporating these seeds into one's diet (Johnson *et al.* 2020). According to (Khattab *et al.* 2009), protein content in legume seeds is comparatively lower than that of animal protein and higher than cereal. A study conducted by Iqbal *et al.* (2006) found that four types of legumes, namely chickpea, cowpea, green pea, and lentil, are excellent sources of essential minerals such as potassium, copper, calcium, and zinc, as well as phosphorus and iron. Cowpea was also found to be rich in ash content. The study further concluded that these legumes, except for amino acids like tryptophan and sulfur-containing amino acids, are abundant in essential amino acids like arginine, leucine, and lysine, which can fulfill human amino acid requirements. Legumes are cholesterol-free and low in fat since they come from plants. Several studies have shown that legumes have beneficial properties that can help reduce weight, lower blood pressure and cholesterol levels, and decrease inflammation that can lead to cardiovascular diseases (CVD) (Abeysekara *et al.* 2012, Flight and Clifton 2006, Hermsdorff *et al.* 2011, Nilsson *et al.* 2013). Research has shown that consuming legumes like beans and lentils is useful in reducing he risk of coronary heart disease (CHD). The presence of high levels of saponins and phytosterols found in legumes, which are useful in lowering the low-density lipoprotein cholesterol (LDL-C) and serum cholesterol significant factor associated with CHD mortality. Studies have suggested that consuming dried legumes can promote lipid homeostasis and lower saturated fat intake, thus further reducing the risk of developing cardiovascular disease (CVD). To promote the consumption of legumes in human nutrition, the United Nations designated 2016 as the International Year of Pulses (IYP) in their 2014 resolution. The FTIR spectroscopy technique, as described by Jamme et al. (2008) and Mills *et al.* (2005), was utilized to identify the functional groups present in the sample and provide relevant information in the form of peaks in combination with principal compound analysis. By analyzing the peaks obtained, it was possible to determine the levels of protein, fat, carbohydrates, ash, moisture, and minerals in the sample.

The present research paper focuses on the determination of functional groups in five selected legume grains (black chickpea, cowpea, white chickpea, small red bean, and red lentil) using the FTIR spectroscopy (400-4000cm$^{-1}$) in combination with principal compound analysis to determine the protein, fat, starch, and moisture contained in legume seeds.

## MATERIALS AND METHODS

### *Sample collection and preparation*

Five different dry legume seeds namely black chickpea, cowpea, white chickpea, small bush bean, and red lentils are collected from Balagi shop, Green Valley, Punjab. All the grains were cleaned physically to remove dust particles, damaged seeds, seeds of other grains, and impurities before performing the analysis.

### *Fourier transform infrared spectroscopy analysis for the legume seeds*

Fourier Transform Infrared spectroscopy is a highly effective and emerging technique for analyzing samples of various quality parameters as traditional techniques for analyzing the nutritional content of legumes are expensive, time-consuming, and require hazardous chemicals and solvents. Additionally, the samples are often destroyed, making them unsuitable for large-scale studies. It is a rapid and sensitive technique that employs a wide range of sampling methods. FTIR operates based on functional groups and provides information through peaks. Information related to moisture, protein, fat, ash, carbohydrates, and grain hardness can be obtained by analysing the peaks. For instance, peaks for water can be observed in the range of 1,640 cm$^{-1}$ and 3,300 cm$^{-1}$ (Manley *et al.* 2002), based on functional groups H and OH. Protein can be observed in the ranges of 1,600 per cm to 1,700 per cm and 1,550 per cm to 1,570 per cm, as reported by Manley *et al.* (2002), based on bond amide I and amide II, respectively. Fat can also be observed within these ranges based on the C-H bond. Starch can be observed between 2,800 and 3,000 per cm (C-H stretch region) 3,000 and 3,600 per cm (O-H stretch region). The FT-IR critical bands method was used to capture starch spectra. Spectra were recorded in three regions - the fingerprint region (800-1500 cm−1), the C–H stretch region (2800-3000 cm−1), and the O–H stretch region (3000-3600 cm−1), as described in the approach by Belton *et al.* (1991).

## RESULTS AND DISCUSSION

It has been noted (Figure 1) that when the wavenumber increases, the transmittance of every sample decreases. Sample 1 (black chickpea) has the lowest transmittance at the wavenumber below 1091 per centimeter, whereas sample 2 (cowpea) has the lowest transmittance of all the samples at the wavenumber above 1091 per centimeter. The highest transmittance is seen in sample 3 (white chickpea) at a wavenumber below 2415 centimeters, while sample 5 (red lentil) exhibits the highest transmittance at a wavenumber above 2415 per centimetres.

Spectra (Table 1) of five legume seeds (black chickpea, cowpea, white pea, small red string bean, and red lentil) were obtained using FTIR (Perkin Elmer) and almost identical peaks were found at the same wave number. One gram of each sample was added to the absorption chamber and the sample was tested in triplicate in the mid-infrared range from 4000 cm$^{-1}$ to 450 cm$^{-1}$. Peaks between 1000 - 1400 cm$^{-1}$ indicate the presence of fluorine compounds for C-H stretching (Naumann 2001). Fluorine compounds are more stable due to the strength of the carbon-fluorine bond. It also shows that halogenated compounds in the range of 500-600 cm$^{-1}$ cause C-L stretching and strong peaks. The fact that the wave number is between 1500 and 1550 cm$^{-1}$ and the presence of N-O stretching and C-H bending groups indicate the presence of nitro compounds and alkanes. Strong peaks were

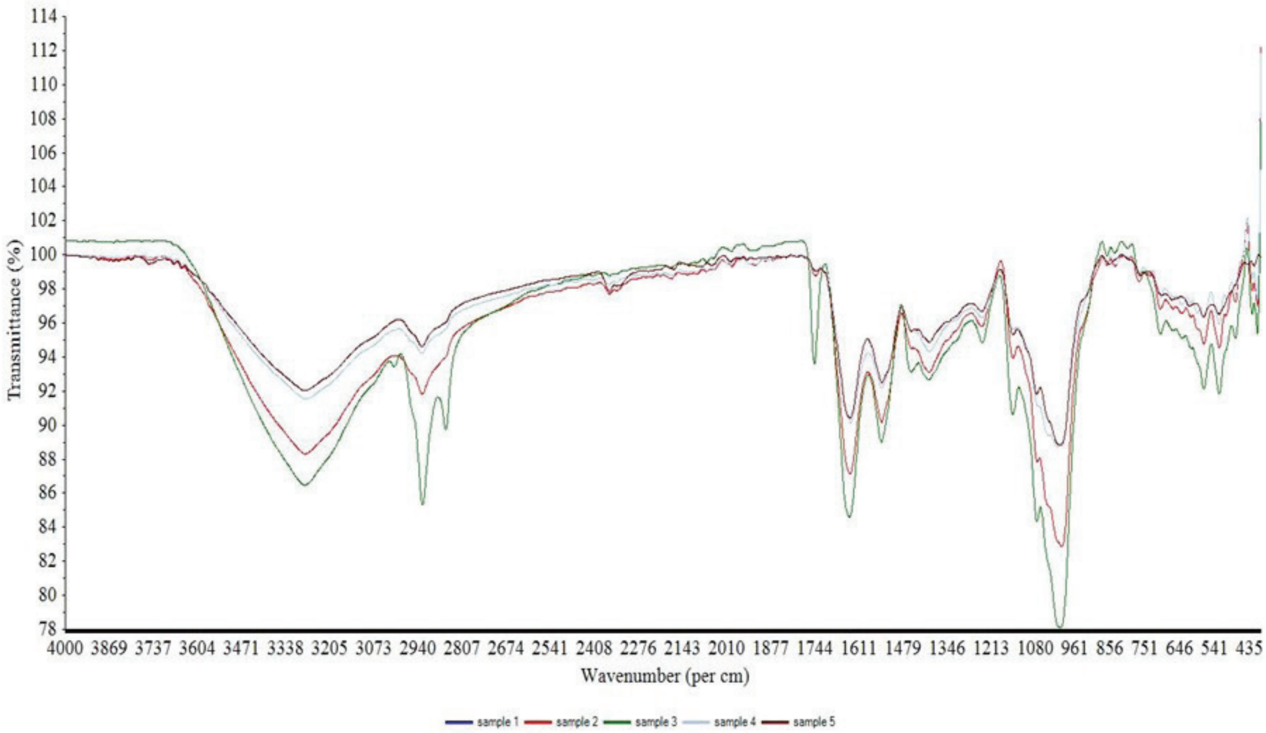


**Fig. 1.** The Fourier Transform Infrared Spectra of the five legumes in transmittance mode

**Table 1.** The Transmittance valleys for the five legumes in the Fourier Transform Infrared Spectra.

| Black chickpea | Cow pea | White chickpea | Red bean | Red lentil |
|---|---|---|---|---|
| | | 3262.01 | | |
| 3279 | 3278.01 | | 3275.83 | 3277.68 |
| 2924 | 2925.22 | 2924.57 | 2926.65 | 2926.32 |
| | 2361.38 | | | 2361.76 |
| 2854 | | | | |
| 1744 | | | | |
| 1639 | 1636.77 | 1636.04 | 1635.54 | 1636.58 |
| 1542 | 1541.8 | | 1541.89 | 1540.89 |
| 1454 | | | | |
| 1400 | 1398.92 | 1403.76 | 1398.06 | 1398.19 |
| 1239 | 1241.05 | | 1240.04 | |
| 1147 | 1146.28 | | | |
| 1074 | | | 1013.3 | |
| 1002 | 999.21 | 1014.46 | | 1003.3 |
| 863 | 839.61* | | 838.79* | 857.28* |
| | 765.61* | | 702.35 | |
| 702 | 702.2 | | | |
| 571 | 570.95 | | 570.91 | 572.7 |
| 525 | 524.86 | | 525.91 | 526.15 |
| 476 | 476.32 | | 475.75 | |
| 426 | | | | |
| 410 | 410.3 | | 410.21 | |

observed for nitro compounds and moderate peaks were observed for alkanes. As stated in (Naumann 2001, Bhattacharjee *et al.* 2005), functional groups such as alkanes, carboxylic acids, ketones, and aldehydes are found in the wave number range of 1500-1000 cm$^{-1}$. The functional group like O-H stretching was found only in white chickpeas in the range of 3262.01 cm$^{-1}$, which indicates the presence of alcohol and a carboxylic acid, whereas bonds like O-H stretching, C-H stretching, and N-H stretching were found only in other legume seeds like black chickpea, cowpea, red bean and red lentils in the wave number range 3279 cm$^{-1}$, 3278.01 cm$^{-1}$, 3275.83 cm$^{-1}$ and 3277.68 cm$^{-1}$ that indicate the presence of alkyne, alcohol and carboxylic acids.

The study found that the functional group O-H stretching was only present in white chickpeas, indicating the presence of alcohol and carboxylic acid. However, bonds such as O-H stretching, C-H stretching, and N-H stretching were found in black chickpeas, cowpeas, red beans, and red lentils, indicating the presence of alkyne, alcohol, and carboxylic acids. All five samples had O-H stretching, C-H stretching, and N-H stretching bonds in the wave number range of 2924 cm-1, indicating the presence of alcohol, carboxylic acid, amine salt, and alkane. The same bond was only found in black chickpeas in the range of 2854 cm$^{-1}$. The C-C stretching and N-H bending bonds were also present in all the samples, indicating the presence of amine and alkene. The C-H bending bond was only observed in black chickpeas in the range of 1454 $cm^{-1}$, indicating the presence of alkane. The nitro compound having N-O stretching was observed in all samples except white chickpeas. The esters, δ-lactone, and cyclopentanone compounds having the C=O stretching were observed only in black chickpeas in the range of 1744 $cm^{-1}$. The fluoro compound, sulfate, alcohol, and alkane were observed in all the samples, indicating the C-F stretching bonds, S=O stretching bonds, and O-H bending bonds in the wave number range 1400 $cm^{-1}$. The fluoro compound, alkyl aryl ether, and the amine having the C-F stretching, C-O stretching, and C-N stretching bonds were observed in black chickpeas, cowpeas, and red beans but not in white chickpeas and red lentils. The function groups such as C-F stretching, C-N stretching, and C-O stretching were only observed in black chickpeas and cowpeas in the range of 1147 $cm^{-1}$ and 1146.28 $cm^{-1}$, respectively, indicating the presence of fluoro compound, amine, alcohol, aliphatic ether. The C-F, C-N, and C-O stretching were observed in black chickpeas in wave number 1074 $cm^{-1}$ and red beans in wave number 1013.3 $cm^{-1}$, indicating the presence of fluoro compound, amine, and primary alcohol. In contrast, the other three samples, cowpeas, white chickpeas and red lentils, did not present the above compounds. All the samples have C-F stretching bonds, representing the presence of fluoro compound in the wave range of 1002-1115 $cm^{-1}$, except for red beans. The halo compound having a C-CL stretching functional group was observed in cowpeas and red beans. C-CL stretching and C=C bending bonds were observed in black chickpeas and cowpeas, indicating the presence of the halo compound. The C-Cl stretching bonds, C-Br stretching bonds, and C-I stretching bonds were observed in all four samples except white chickpeas, indicating the presence of halo compounds. The transmittance was highest in red beans, followed by cowpeas, black and white chickpeas, and red lentils.

***Principal compound analysis***

Principal component analysis is one statistical method for decreasing the dimensionality of large data sets (Wiley 1981). The primary variable is a set of major variables that best explains the variation in value. The second principle, which is perpendicular to the first, is the one that accounts for the greatest residual change in the data. No information is unnecessary because all the factors are equal to each other (Dona *et al.* 2009).

(Figures 2 and 3) represent the loadings and scores of two principal components (PC-1 and PC-2) of some data's principal component analysis. PC-1 captures most of the variation in the data, as it has a variance of 96%. The loadings for PC-1 range from -1 to 1 and represent the correlation coefficients between the original variables (X-variables) and PC-1. The correlation coefficient varies from -1 to 1. A correlation coefficient value of 1 or -1 represents perfect correlation, 0.1 to 0 represents no or minimal correlation. The loading for the first variable is 0.96, indicating a strong positive correlation with PC-1. The loading for the second variable is 0.58, indicating a moderate positive correlation with PC-1. The loadings for the remaining variables are close to zero, indicating weak or no correlation with PC-1. The scores for PC-1 range from -0.8 to 1, representing the projections of the original data points onto PC-1. A score of -0.8 indicates that the corresponding data point contributes negatively to PC-1, while a score of 1 indicates that the data point contributes positively to PC-1. PC-2, on the other hand, captures only a tiny portion of the variation in the data, with a variance of 4%. The loadings for PC-2 range from -1 to 1 and represent the correlation coefficients between the original variables and PC-2. The loading for the first variable is -0.27, indicating a weak negative correlation with PC-2. The loading for the second variable is -0.83, indicating a strong negative correlation with PC-2. The loadings for the remaining variables are close to zero, indicating weak or no correlation with PC-2.

The scores for PC-2 range from -30 to 15, representing the projections of the original data points onto PC-2. The loadings help us understand the meaning of the principal components and the relationships between the original variables. The scores allow us to visualize the distribution of the data points in the principal components' reduced space. The graph shows that PC-1 has a more comprehensive range and more variation in scores than PC-2. The distribution of PC-1 scores suggests that there are two groups of scores, one with lower scores of around -10 and another with higher scores of around 50. The distribution of PC-2 scores is more consistent, with most scores falling between -10 and 10. The samples for PC-2 scores indicate that samples 4 and 5 have lower scores, while samples 2 and 3 have higher scores. Using principal component analysis, the graph shows the clustering of different legume seeds based on the FTIR spectrum. Figure 2 is the result obtained from the principal component analysis. The legume seeds are clustered into four groups based on the clustering result. Samples 1 and 3 (black chickpea and white chickpea) were clustered in the 2nd quadrant, indicating similar

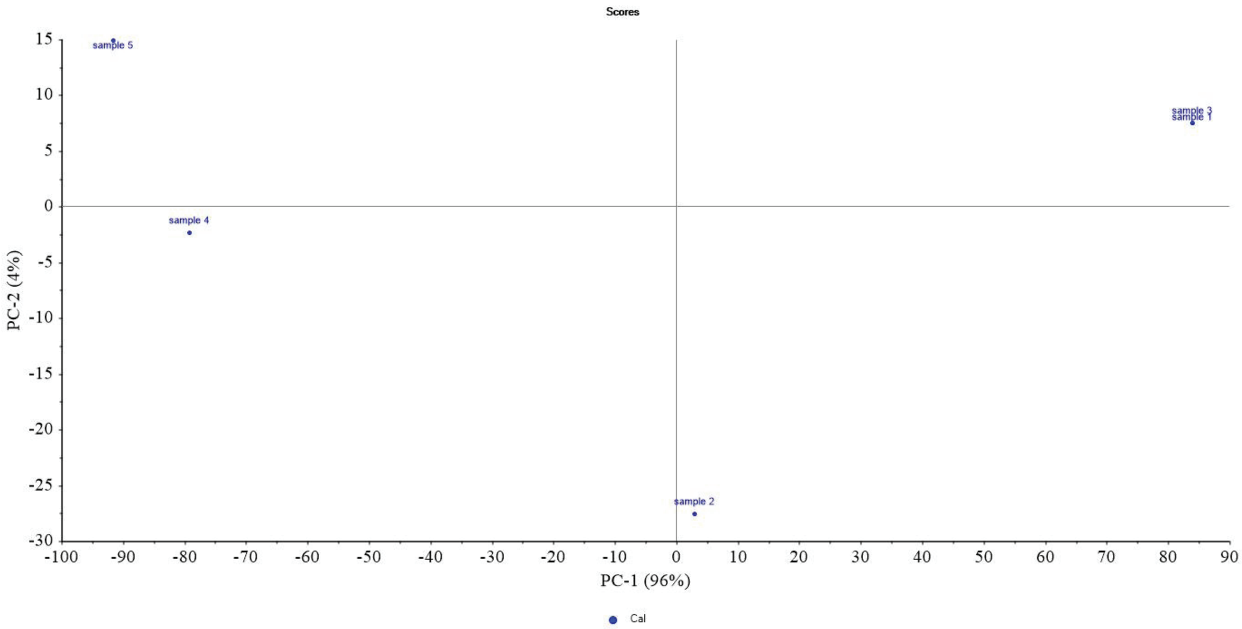


**Fig. 2.** Principal Component Analysis Score plot showing the relation between the 5 legumes on the basis of Fourier Transform Infrared Spectra

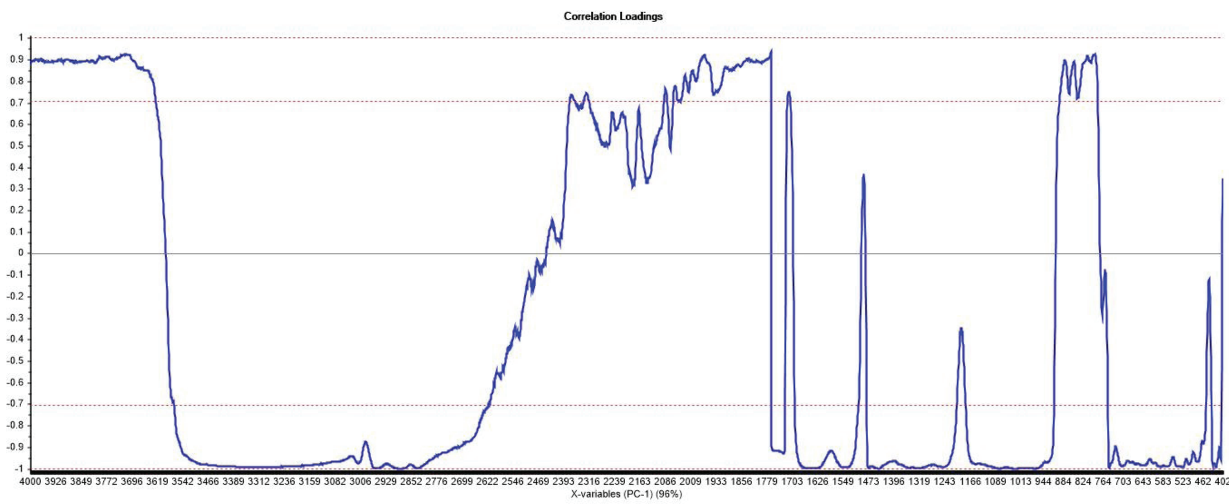


**Fig. 3.** Principal Component Analysis Loading plot on the basis of Fourier Transform Infrared Spectra

properties. Sample 2 (cowpea) is clustered in the 4th, sample 4 (small red bean) in the 3rd and sample 5 (red lentil) in the 1st quadrant. The properties of sample 5 (red lentil) are entirely different from samples 1 (black chickpea) and 2 (cowpea) and the properties of sample 2 (white chickpea) differ from sample 4 (small red bean).

## CONCLUSION

Legume seeds are an excellent source of plant-based protein, making them a great option for individuals following vegan diets. They typically contain high levels of protein, ranging from 20% to 40% of their dry weight, and have an exceptional amino acid profile. However, it's worth noting that some varieties may lack specific critical amino acids such as lysine or methionine. Legume seeds are an excellent source of essential minerals like iron, zinc, and magnesium. They are also rich in dietary fibre, complex carbohydrates, vitamins (especially folate, vitamin B6, and vitamin K), and phytochemicals, such as flavonoids and phenolic compounds. Legume seeds can remarkably increase soil fertility and reduce the need for synthetic fertilizers by partnering with nitrogen-fixing bacteria to fix atmospheric nitrogen. In addition, legume crops are highly adaptable to various agroecological settings and can thrive in drought conditions, making them an ideal choice for farmers facing the challenges of climate change. Several epidemiological studies have found that consuming legumes regularly can protect against various disorders, including diabetes, colon cancer, and coronary heart disease (Pasquale *et al.* 2016).

In this study, Fourier transform infrared (FTIR) spectroscopy was used to identify and predict the functional groups present in five selected legume seeds. The results showed that only white chickpeas had O-H stretching in the range of 3262.01 cm-1, indicating the presence of alcohol and carboxylic acid. Black chickpeas, cowpeas, red beans, and red lentils had O-H stretching, C-H stretching, and N-H stretching in the wave number range of 3279 cm-1, 3278.01 cm-1, 3275.83 cm-1, and 3277.68 cm-1, suggesting the presence of alkyne, alcohol, and carboxylic acids. All five samples had O-H stretching, C-H stretching, and N-H stretching bonds in the wave number range of 2924 cm-1, indicating the presence of alcohol, carboxylic acid, amine salt, and alkane. The nitro compound having N-O stretching was observed in all samples except white chickpeas. Black chickpeas, cowpeas, and red beans showed the presence of fluoro compound, alkyl aryl ether, and amine having C-F stretching, C-O stretching, and C-N stretching bonds, while white chickpeas and red lentils did not have these compounds. The highest transmittance was observed in red beans, followed by cowpeas, black and white chickpeas, and red lentils. The properties of sample 5 (red lentil) were entirely different from samples 1 (black chickpea) and 2 (cowpea), and the properties of sample 2 (white chickpea) differed from sample 4 (small red bean).

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