Functional Group Analysis of Germinated Millets and Legumes

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ABSTRACT
Germinated millets and legumes are rich in nutritional significance like calcium, iron, proteins, vitamins, etc. This study aims to find the active functional groups and compounds present with their bond characteristics based on the peak obtained from FT-IR for germinated millets and legumes that are subjected for shade drying and tray drying at 70°C. Generally, Millets and legumes are used for specialty foods like health mix, etc or as a part of generally food products due to their increased health significance. In this study, the findings are the presence of characteristic functional groups like carboxylic acids, aromatic rings, disulphide, esters, alkanes, amines, nitro compounds, sulphates, oxime, phosphine, thiocarbonyl, silane, amides, sulfate, alkenes, etc. There is some difference in functional groups based on the method of drying. These results help identify the bioactive compound which places a major role in our nutritional health aspects. These germinated products could add value to our daily diet.

Keywords: Functional groups, Millets and legumes, Shade drying, Tray drying.

Asian Journal of Dairy and Food Research (2019)

INTRODUCTION
Millets are a group of highly variable small-seeded grasses, widely grown around the world as cereal crops or grains for fodder and human food. The most widely grown millets are pearl millet and finger millet, which are important crops in India. Legume seeds are employed as a protein source for animal and human nutrition not only for their nutritional value (high in protein, lipids, and dietary fiber) but also their adaptability to marginal soils and climates. Human consumption of legumes has been increased in recent years, being regarded as beneficial food ingredients. Legume seeds contain a great number of compounds which qualify as bioactive compounds with significant potentials benefits to human health. These compounds vary considerably in their biochemistry and they can be proteins, glycosides, tannins, saponins, alkaloids, etc. Germination is the process of reactivation of metabolic machinery of the seed resulting in the emergence of radicle and plumule. Mungbean (Vigna radiata L. Wilczek) is one of the important Kharif pulses and is widely grown in summer and rainy seasons in several states of India (Salam, 2009). Under proper conditions, seeds begin to germinate and the embryonic tissues resume growth. Germination depends on both internal and external conditions. Important external factors are temperature, water, oxygen or air and sunlight or darkness. Water activates cellular metabolism and growth resumes. Oxygen provides energy to grow. Temperature affects cellular metabolism and growth rate. Germination generally improves the nutritional quality of food by increasing their nutrient content and digestibility. It can bring about a two-fold increase in bioavailability of iron, whereas malting does the same with five to ten-fold increases. It improves the presence of vitamin C and fiber in particular during germination (Yuvi, 2013), reduces the viscosity of the food through the amylolytic breakdown of starch, thus reducing bulk. Bioactive compounds are important in human health since it exhibits many health benefits for the human consumption, it is recommended that further studies be done on bioactive compounds in identifying their biological activities and characterization for the medicinal purposes as well as food application. The application of fourier transform infrared spectroscopy method (FTIR) can be further enhanced to produce better identification and characterization of bioactive compounds for the human health benefits. FTIR is the main tool to identify the functional compounds present in foods.

SAMPLE PREPARATION
Soaking: Millets like pearl and finger with legumes like mung bean and chickpea are selected as the samples which are weighed and soaked in water for 8 hours to initiate the germination. Germination increases the bioavailability and nutritional significance of the sample. Samples were weighed equally and tied in a muslin cloth for germination process total 72 hours were given for germination.

Drying: In this study, we employed two types of drying method to differentiate the drying characteristics and their influence in the bioavailability of the samples. The two types of drying include Tray drying and Shade drying. In tray drying, the temperature maintained is 70°C until complete drying.
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of the sample. Shade drying is another method followed which include drying under the shade with atmospheric temperature. This method is carried until complete drying of the sample.

**FT-IR Analysis:** The samples are analyzed to get the FT-IR Spectra using FT-IR Spectrophotometer (Thermo Nicolet, USA) equipped with software OMNIC Version 6.0.a. The FT-IR Spectra of the Millets and Legumes of different drying methods analyzed by Chemo-metrics, principle compounds analysis (PCA) to evaluate identification, classification, and differentiation using Perkin Elmer Application software (AOCS 1989).

**Result and Discussion**

FTIR is an important quality assessment of many food materials. FTIR spectroscopic study which reveals the presence of different functional groups present in germinated dried legumes and millets. The various germinated legumes and millets dried at two types of drying is shown in Graph.1. The germinated legumes and millets are a very important source of functional compounds for the main development of chemotherapeutic agents. The various functional groups present in the dried products which appearing in form of bands due to molecular vibrations (Arnold et al. 1971). The peaks at 2931 cm\(^{-1}\) is associated with asymmetric and symmetric stretching modes of alkane C-H. In the fingerprint region 860–1080 cm\(^{-1}\) shows the alkane C=C, anhydrides C = O in both the type of drying. On comparing both drier 1539.20 cm\(^{-1}\) corresponding to aromatic C=C bending was found in a tray drier for finger millets (Reginold et al. 2015).

When comparing the germinated pearl millets it was identified 18 functional compounds in a tray drier. The asymmetric stretching frequencies for aqueous carboxylates are generally between 1540 to 1650 cm\(^{-1}\). The stretching vibration of amines (K-N) substituents is in the region from 1080,14, carboxylic acids (C-O) groups between 1 155 to 1244. Shade dried samples result in loss of carboxylic acids (Starlin et al., 2012). The absorption bands between 1000 cm\(^{-1}\) and 1200 cm\(^{-1}\) 245 were characteristic of the C-O stretching of polysaccharide skeleton. Mortlock et al, 1989 reported that the bands at 1417 cm\(^{-1}\), 1600 cm\(^{-1}\) 250, and 3400 cm\(^{-1}\) also changed during the retrogradation process, suggesting an amorphous region (Wilson and Betlon, 1988).

The mug bean contains total number 14 of functional groups on tray drying which shows an increase compare to sun drying. The frequencies 1080, 155,1543 cm\(^{-1}\) shows silane thiocarbonyl and nitro compound. The mung bean after germination shows the peaks at 950 to 1200 cm\(^{-1}\) suggested the presence of C–O–C and C–OH link bonds in both tray dried and sun-dried powder. This result has a significant

Graph 1: (A) FTIR- spectrum tray dried finger millet; (B) FTIR- spectrum shade dried finger millet

Graph 2: (A) FTIR- spectrum of tray dried pearl millet; (B) FTIR- spectrum shade dried pearl millet
Graph 3: (A) FTIR- spectrum of tray dried mung bean; (B) FTIR-spectrum of shade dried mung bean

Graph 4: (A) FT-IR Spectrum for Tray dried Chickpea; (B) FT-IR spectrum for Shade dried Chick Pea

Table 1: Functional groups identified using FT-IR Spectroscopy of Tray dried and Shade dried Finger Millets

| S. no | Tray dried | Shade dried |
|-------|------------|-------------|
|       | Peak | Intensity | Compounds            | Peak | Intensity | Compounds            |
| 1     | 439.77 | 25.15     | Aryl disulfide (S=S) | 437.84 | 26.56     | Aryl disulfide (S=S) |
| 2     | 530.42 | 18.53     | Disulfide (S-S)      | 528.50 | 20.38     | Disulfide (S-S)      |
| 3     | 574.79 | 17.30     | Aliphatic iodo (C-I) | 576.72 | 18.72     | Aliphatic iodo (C-I) |
| 4     | 709.80 | 25.24     | Amines (NH2&N-H)     | 709.80 | 27.33     | Amines (NH2&N-H)     |
| 5     | 765.74 | 29.42     | Amines (NH2&N-H)     | 765.74 | 31.51     | Amines (NH2&N-H)     |
| 6     | 860.25 | 39.46     | Alkane(C-C)          | 860.5  | 42.64     | Alkane (C-H)         |
| 7     | 927.76 | 30.07     | Esters (P-OR)        | 927.76 | 33.87     | Esters (C=O)         |
| 8     | 1080.14| 10.81     | Anhydrides (C=O)     | 1018.41| 10.00     | Amines (C-N)         |
| 9     | 1155.36| 11.86     | Alcohol (C-O)        | 1080.14| 11.37     | Anhydrides (C=O)     |
| 10    | 1244.09| 28.74     | Carboxylic acids (C- O ) | 1155.36| 12.67     | Alcohol (C-O)        |
| 11    | 1367.53| 23.19     | Nitro (N=O)          | 1242.16| 32.65     | Amines (C-N)         |
| 12    | 1419.61| 23.84     | Sulfate (S=O)        | 1367.53| 26.59     | Sulfonyl chloride (S=O) |
| 13    | 1539.20| 44.65     | Aromatic (C=C)       | 1419.61| 27.26     | Sulfate (S=O)        |
| 14    | 1649.14| 27.27     | Alkane (C=C)         | 1539.20| 48.94     | Nitro (N=O)          |
| 15    | 2368.59| 95.30     | Phosphine (P-H)      | 1649.14| 30.69     | Amines (NH2)         |
| 16    | 2931.80| 20.07     | Alkane (C-H)         | 2360.87| 93.91     | Phosphine (P-H)      |
Table 2: Functional groups identified using FT-IR spectroscopy of tray dried and shade dried pearl millets

| S. no | Pearl millet | Tray dried | Shade dried |
|-------|--------------|------------|------------|
|       | Peak         | Intensity  | Compounds   | Peak         | Intensity  | Compounds   |
| 1     | 437.84       | 63.72      | Aryl disulfide (S-S) | 410.84       | 69.63      | Aryl disulfide (S-S) |
| 2     | 528.50       | 46.92      | Disulfide (S-S)     | 433.98       | 67.79      | Aryl disulfide (S-S) |
| 3     | 576.72       | 42.44      | Aliphatic iodo (C-I)| 530.42       | 42.65      | Disulfide (S-S)     |
| 4     | 707.88       | 57.66      | Esters (S-OR)       | 576.72       | 36.04      | Aliphatic iodo (C-I) |
| 5     | 765.74       | 65.47      | Esters (S-OR)       | 709.80       | 49.26      | Esters (S-OR)       |
| 6     | 860.25       | 76.96      | Amines (NH₂&N-H)    | 765.74       | 55.50      | Amines (NH₂&N-H)    |
| 7     | 926.69       | 61.07      | Esters (P-OR)       | 860.25       | 69.06      | Esters (NH₂&N-H)    |
| 8     | 1018.41      | 10.34      | Amines (C-N)        | 926.69       | 48.77      | Amines (C-N)        |
| 9     | 1080.14      | 15.13      | Amines (K-N)        | 1080.14      | 13.95      | Thiocarbonyl (C=S)  |
| 10    | 1155.36      | 20.19      | Carboxylic acids (C-O) | 1157.29     | 15.23      | Thiocarbonyl (C=S)  |
| 11    | 1244.09      | 50.18      | Carboxylic acids (C-O) | 1246.02     | 33.41      | Phosphonate (P=O)   |
| 12    | 1369.46      | 43.05      | Sulfate (S=O)       | 1416.91      | 26.05      | Sulfate (S=O)       |
| 13    | 1458.18      | 44.01      | Sulfate (S=O)       | 1460.11      | 26.47      | Aromatic (C=C)      |
| 14    | 1543.05      | 11.02      | Amines (NH₂&N-H)    | 1541.12      | 18.12      | Nitro (N=O)         |
| 15    | 1649.14      | 36.21      | Phosphonate (P=O)   | 1649.14      | 25.39      | Amines (NH₂&N-H)    |
| 16    | 2059.98      | 94.91      | Aromatic combination band | 2358.94     | 36.13      | Alkane (C=H)        |
| 17    | 2374.37      | 95.60      | Phosphine (P-H)     | 2933.73      | 21.21      | Phosphine (P-H)     |
| 18    | 2935.66      | 31.47      | Alkane (C=H)        | 1653.00      | 24.54      | Alkane (C=H)        |

Table 3: Functional groups identified using FT-IR Spectroscopy of tray dried and shade dried mung bean

| S. no | Mung bean | Tray dried | Shade dried |
|-------|-----------|------------|------------|
|       | Peak      | Intensity  | Compounds   | Peak         | Intensity  | Compounds   |
| 1     | 435.91    | 37.35      | Aryl disulfide (S-S) | 433.98       | 25.90      | Aryl disulfide (S-S) |
| 2     | 526.57    | 28.83      | Disulfide (S-S)     | 522.71       | 20.01      | Disulfide (S-S)     |
| 3     | 574.79    | 27.55      | Aliphatic iodo (C-I)| 574.79       | 19.36      | Aliphatic iodo (C-I) |
| 4     | 761.88    | 44.14      | Esters (S-OR)       | 763.81       | 33.52      | Esters (S-OR)       |
| 5     | 858.32    | 53.50      | Amines (NH₂&N-H)    | 858.32       | 43.67      | Amines (NH₂&N-H)    |
| 6     | 927.76    | 41.88      | Esters (P-OR)       | 927.76       | 31.22      | Esters (P-OR)       |
| 7     | 1080.14   | 15.13      | Silane (Si-OR)      | 1157.29      | 16.62      | Phosphate (P=O)     |
| 8     | 1155.36   | 22.16      | Thiocarbonyl (C=S)  | 1246.02      | 26.58      | Phosphoramde (P=O)  |
| 9     | 1242.16   | 36.21      | Phosphonate (P=O)   | 1409.96      | 21.11      | Sulfate (S=O)       |
| 10    | 1543.05   | 24.74      | Nitro (N=O)         | 1541.12      | 18.12      | Nitro (N=O)         |
| 11    | 1649.14   | 11.02      | Amines (NH₂)        | 1649.14      | 10.68      | Amines (NH₂)        |
| 12    | 2059.98   | 94.91      | Aromatic combination band | 2358.94     | 93.98      | Phosphine (P-H)     |
| 13    | 2374.37   | 95.60      | Phosphine (P-H)     | 2933.73      | 21.21      | Phosphine (P-H)     |
| 14    | 2935.66   | 31.47      | Alkane (C=H)        | 1653.00      | 24.54      | Alkane (C=H)        |

variation which is given in water-soluble polysaccharide extracted from mung bean (Bellamy, 1980).

Estimating the functional properties of pulses and health beneficial properties of the pulses towards the wider usage of its fractions in regular foods according to the FAO/WHO/UNU reference pattern for school children and adults chickpea has an accept level of essential amino acids among all the legumes (Bernard, 1980). The broadband observed at \( \sim 2900 \) cm\(^{-1}\) corresponds to O–H stretching vibrations, arising mainly from water, protein, and carbohydrates. From chickpea germinated flour 2927 cm\(^{-1}\) (C–H stretching of neutral lipids, proteins, and carbohydrates) and 1745 cm\(^{-1}\) (C O stretching of lipid ester groups) have been used to indicate lipid characteristics. Similar results were identified in soy and pea (Braue, 1987). Hemicelluloses and cellulose polysaccharides (1456–1240 cm\(^{-1}\)) 15 were also observed in both types of drying (Coates et al., 2015). From this study, it is found that most of the functional properties are retained...
Table 4: Functional groups identified using FT-IR Spectroscopy of tray dried and shade dried chickpea

| S. no | Tray dried | Shade dried |
|-------|------------|-------------|
|       | Peak (cm⁻¹) | Intensity  | Compounds       | Peak (cm⁻¹) | Intensity  | Compounds       |
| 1     | 437.84      | 62.49       | Aryl disulfide (S-S) | 435.91      | 61.35       | Aryl disulfide (S-S) |
| 2     | 526.57      | 47.11       | Disulfide (S-S)    | 526.57      | 50.21       | Disulfide (S-S)    |
| 3     | 574.79      | 43.39       | Aliphatic iodo (C-I) | 574.79      | 48.00       | Aliphatic iodo (C-I) |
| 4     | 705.95      | 56.42       | Ester (P-OR)       | 707.88      | 58.98       | Ester (S-OR)       |
| 5     | 763.81      | 43.39       | Amines (NH₂&N-H)   | 763.81      | 69.27       | Ester (S-OR)       |
| 6     | 860.25      | 58.02       | Ether (C-O)        | 860.25      | 82.70       | Amines (NH₂&N-H)   |
| 7     | 931.62      | 58.02       | Alkane (C-H)       | 926.96      | 66.62       | Esters (P-OR)      |
| 8     | 991.41      | 15.93       | Alkane (C-H)       | 991.41      | 20.44       | Esters (P-OR)      |
| 9     | 1082.07     | 19.87       | Ether (C-O)        | 1082.07     | 26.52       | Thiocarbonyl (C=S) |
| 10    | 1159.22     | 23.97       | Amines (C-N)       | 1159.22     | 28.63       | Sulfoxide (S=O)    |
| 11    | 1244.09     | 45.45       | Amines (C-N)       | 1244.09     | 52.34       | Phosphonate (P=O)  |
| 12    | 1415.75     | 38.30       | Sulfate (S=O)      | 1458.18     | 44.85       | Aromatic (C=C)     |
| 13    | 1458.18     | 38.33       | Aromatic (C=C)     | 1544.98     | 45.89       | Nitro (N=O)        |
| 14    | 1543.05     | 37.73       | Nitro (N=O)        | 1654.92     | 29.10       | Oxime (=NOH) (C=N) |
| 15    | 1653.00     | 20.68       | Oxime (=NOH) (C=N) | 1743.65     | 50.47       | Esters (C=O)       |
| 16    | 1745.58     | 47.92       | Esters (C=O)       | 2370.51     | 83.39       | Phosphine          |
| 17    | 2374.37     | 85.93       | Phosphine (P-H)    | 2929.87     | 18.32       | Carboxylic acid (O-H) |
| 18    | 2927.94     | 16.36       | Alkane (C-H)       |             |             |                  |

in both germinated legumes and millets. However, the use of legume flours in various food formulations is dependent on their nutritional and functional properties (Bhokre 2015). Consuming energy-rich germinated products helps in retaining the minor nutrients in our body.

**Conclusion**

The extensive work on this project for identifying the functional groups from germinated millets and legumes were evaluated and analyzed. The importance of germinated millets and legumes are not known by the people. By analyzing this functional compound we can develop health functional foods for consumers (Compton, 1987). The results of FTIR revealed that the on comparing both the drying technique tray dried samples revealed a higher number of aromatic, aliphatic and O-H group which states that the presence of phenol compounds and primary amines (Afran, 1991). Finally, these results suitable germination process and drying condition could provide good sources of bioactive compounds from legumes and their germinated products for nutraceutical applications (Emmous et al., 1990) Same results were shown in fruit drying in effect of temperature on drying kinetics (Rigi, 2014). Thus, the functional groups denote that carbohydrate-rich food maintains glycemic homeostatic, gastrointestinal integrity and also serves as the vehicle for important micronutrients and phytochemicals (Jain et al., 2012).

**Acknowledgment**

Authors are thankful to the Department of Food Technology, Kalasalingam Academy of Research and Education, India for providing infrastructural facilities to carry out this study.

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