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Functional groups and phytochemical characterization of traditional paddy cultivars through fourier transform infrared spectroscopy analysis

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Abstract

Characterizing and identifying the functional group of paddy cultivars are important features that determine the nutritional qualities of rice for human consumption. In the current study, traditional paddy cultivars were collected based on the preference for cultivation of Tamil Nadu paddy growers, and the functional group of each rice cultivar was investigated using the fourier transform infrared spectroscopy technique. Our work is confined to eleven traditional paddy cultivars of *Oryza sativa* L. (Arubatham kuruvai, Mappillai samba, Illupaipoo samba, Thanga samba, Poonkar, Thooyamalli, Sigappu kavuni, Kalabath, Kothamalli samba, Karrupu kavuni, and Karunkuruvai). Among the cultivars of *O. sativa* (Mappillai samba) recorded the presence of a higher level of functional groups denoting the presence of aliphatic hydrocarbons, aliphatic primary amides, alkyl monosubstituted, inorganic phosphate, and primary aliphatic alcohol. *O. sativa* (Mappillai samba, Poonkar, Thooyamalli, Sigappu kavuni, Kalabath, Kothamalli samba, and Karrupu kavuni) had the hydroxyl group (-OH) extended at 3643-3630 cm^{-1} (primary aliphatic alcohol). It is interesting to note that furan, which has anti-inflammatory and antioxidant properties, was expressed in Kalabath in the 3000 cm^{-1} spectral band. According to the study, the selection of traditional rice cultivars with higher functional properties could benefit the farming community by boosting the health of consumers as well as enriching the farming economy.

1. Introduction

Rice is one of the most important staple cereals for almost half of the world's population, particularly in Asia. China leads the world in rice production with 211 million tonnes, followed by India with 177 million tonnes. In India, rice is farmed in about 44 million hectares with an average yield of 2.78 t ha^{-1} (Agricultural Statistics, 2018). About 14.88 million hectares of land are currently under organic farming throughout 162 nations (Yadav, 2013). Asia is home to 34 % of the world's organic farmers, with India having the 33rd largest area under organic production. Currently, farmers are using traditional rice varieties while transitioning to organic production. The Green Revolution of the 1960s focused on creating high-yielding rice varieties with higher yields, shorter cropping seasons, and higher cropping intensities by cultivating two to three crops in one year. The loss of old rice varieties was one of the main ecological effects of the Green Revolution's adoption of new, high-yielding varieties (Ashraf and Lokanadan, 2017; Arti Ghabru *et al.*, 2022). Roughly, 400 traditional rice cultivars are available for cultivation in Tamil Nadu. Only a small number of these landraces, nevertheless, have had their nutritional qualities, potential medicinal benefits, and grain end-use quality thoroughly studied (Udhaya Nadhini *et al.*, 2023).

Nutrient-rich cereals play an important role in reducing malnutrition and developing immunity (Mounika and Hymavathi, 2021). A healthy diet, nutrient-rich food, and functional properties are accountable for a potent immune system (Kritika Kaushal *et al.*, 2022; Indumathi and Sharma, 2022), which protects our body from entering pathogens and also cures several human diseases (Dolly *et al.*, 2022). Hunger, malnutrition, and obesity are the biggest problems in the future (Priya Sundarajan, 2023). Hence, healthy food, diets, and improving food production with high nutritive value are the needs of the hour (Rani *et al.*, 2023). Farmers are now gradually discovering that traditional rice cultivars naturally have a valuable gene pool to adapt to climate change (Jayashree *et al.*, 2021). In recent years, traditional rice has become more and more popular for organic farming. Farmers are being drawn to cultivate due to the significant aspects of traditional cultivars, such as their medicinal and nutritional qualities and customer preferences.

Chemical compounds and their physical properties are defined and described by their functional groups. Several techniques are employed to investigate the differences in the functional characteristics of the grains of various traditional rice types. Fourier transform infrared spectroscopic analysis, SDS-PAGE, and atomic absorption spectroscopy are a few of these techniques (Jamme *et al.*, 2008), along with a few additional widely used strategies (Cubadda *et al.*, 2004). Several agricultural goods, such as flax stem, wheat, maize, oat, rye, grains, and soybean seed, have been effectively chemically mapped. Fourier transform infrared (FTIR) spectroscopy has also been utilized to identify functional groups, because of the distinct vibrational frequencies that are linked to each group in these products (Himmelsbach *et al.*, 1998).

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FTIR spectroscopic analysis is an efficient method for compositional research that has been widely utilized to investigate the composition of hard and soft milling rice, as well as the cell walls of cereal grains, in conjunction with multivariate statistical analysis. This method is sensitive, faster, non-destructive, and time-saving to alterations in molecular structure and may identify a variety of functional groups. FTIR has been used to measure a variety of rice quality-related parameters. These days, FTIR has several indisputable benefits over conventional methods in the pharmacological, biological, and transdisciplinary domains, including molecular investigation of main cell wall characteristics, in pharmaceutical, biomedical, and clinical fields (Khairi *et al.*, 2022). Furthermore, the sensitivity and precision of FTIR detectors, along with a variety of software algorithms, have greatly increased the practical use of infrared in quantitative analysis (Dowell *et al.*, 2006). In this context, it is critical to attempt an investigation of the phytochemicals and functional group prediction

of traditional paddy cultivars to reveal their nutritional importance for the control of human disorders.

2. Materials and Methods

2.1 Sample collection

Commonly grown traditional rice grain was collected from different parts of Tamil Nadu, India. These cultivars include *O. sativa* (Arubatham kuruvai, Mappillai samba, Illupaipoo samba, Thanga samba, Poonkar, Thooyamalli, Sigappu kavuni, Kalabath, Kothamalli samba, Karrupu kavuni, and Karunkuruvai) possess several health benefits (Table 1). For functional group analysis, the healthy grains were further cleaned and stored at room temperature, approximately 25 to 28°C. The samples are collected from organic traditional rice growers. Soils are neutral in reaction (7.5-7.9), electrical conductivity (0.9-1.4 dSm⁻¹), and high in organic carbon content (0.8-1.2 %), available nitrogen (340-420 kg ha⁻¹), phosphorus (17-24 kg ha⁻¹) and potassium status (390-718 kg ha⁻¹).

Table 1: Details on the collection of different paddy cultivars and their significance

S. No.	Name of the cultivar	Region	Important features	References
1.	<i>O. sativa</i> (Arubatham kuruvai)	Tiruchirapalli	Strengthen bone and immune system, delaying aging, and protecting against chronic diseases. Prevents the onset of diabetes. Optimizes the digestion process.	Balasubramanian <i>et al.</i> , 2019
2.	<i>O. sativa</i> (Mappillai samba)	Karur	Suitable for those with diabetes and has a low glycemic index. High in fiber and iron. Contains vitamin B1, which helps in healing the stomach and intestines disease.	Rajendran and Chandran, 2020; Radha <i>et al.</i> , 2022
3.	<i>O. sativa</i> (Illupaipoo samba)	Tanjore	Strengthens bones, contains antioxidants, and antiviral properties, and improves the immune system.	Forshed Dewan <i>et al.</i> , 2023
4.	<i>O. sativa</i> (Thanga samba)	Villupuram	It increases the longevity of human life. Contains a high in total ash, fat, and protein. Rich in calcium, potassium, iron, magnesium, zinc, and phosphorus.	Balasubramanian <i>et al.</i> , 2019
5.	<i>O. sativa</i> (Poongar)	Nagapattinam	Contains antioxidants and antiviral properties, improves the immune system, and is good for pregnant women. It induces good secretion of breast milk.	Rathna Priya <i>et al.</i> , 2019; Radha <i>et al.</i> , 2022
6.	<i>O. sativa</i> (Karupu kavuni)	Ramanathapuram	Rich in fat, ash, and protein. Phosphorous, zinc, calcium, magnesium, iron, and potassium are all abundant.	Valarmathi <i>et al.</i> , 2015
7.	<i>O. sativa</i> (Thooyamalli)	Perambalur	Contains high fiber and protein. Very high in fat. Contains more iron, magnesium, and zinc.	Balasubramanian <i>et al.</i> , 2019
8.	<i>O. sativa</i> (Sigappu kavuni)	Puthukottai	It has high levels of iron, calcium, magnesium, potassium, zinc, and phosphorus among other minerals. High in fat and total ash.	Kalaivani <i>et al.</i> , 2020
9.	<i>O. sativa</i> (Kalabath)	Karur	It has significant levels of iron, calcium, zinc, and phosphorus among other minerals. High in fat and protein.	Majumder <i>et al.</i> , 2019
10.	<i>O. sativa</i> (Kothamalli samba)	Thiruvarur	High in fat and protein. It has higher levels of phosphorus, calcium, zinc, and iron.	Balasubramanian <i>et al.</i> , 2019
11.	<i>O. sativa</i> (Karunkuruvai)	Cuddalore	Treat skin conditions, elephantiasis, and urogenital infections, dilute bad cholesterol, toxic stings and bites, and enhance overall well-being. It has a low glycemic index. It contains more levels of zinc, calcium, and iron.	Balasubramanian <i>et al.</i> , 2019; Pushpam <i>et al.</i> , 2019

2.2 Rice grain sample preparation

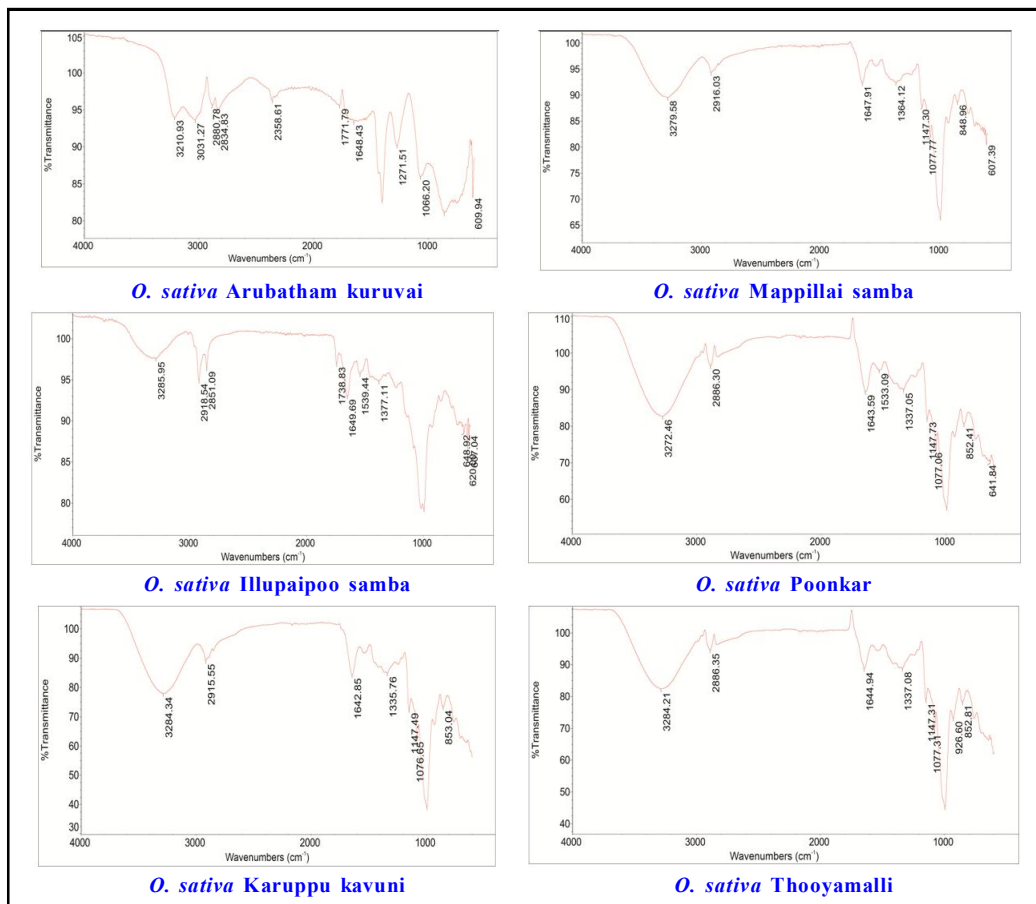
To create uniform flour and minimize size, samples of dried rice grains were ground in a Brabender Quadrumate Senior Mill (C.W. Brabender Instrument, Inc.). Subsequently, the samples were placed on infrared plates.

2.3 Fourier Transform Infrared analysis of rice grain

The functional group for each paddy cultivar was determined by FTIR (Nicolet iS10 spectrometer) analysis of the prepared rice grain samples (Figure 1). The absorption spectra of each sample were acquired between 400 and 4000 cm^{-1} (Malik *et al.*, 2023).



Figure 1: Image of the fourier transform infrared spectroscopy (Nicolet iS10 spectrometer) used for analysis.



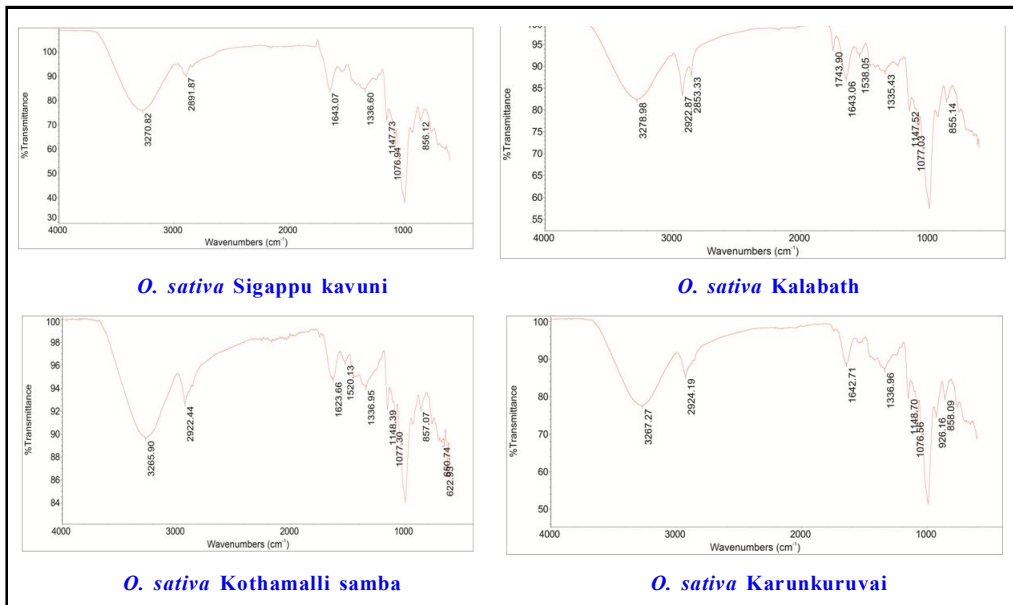
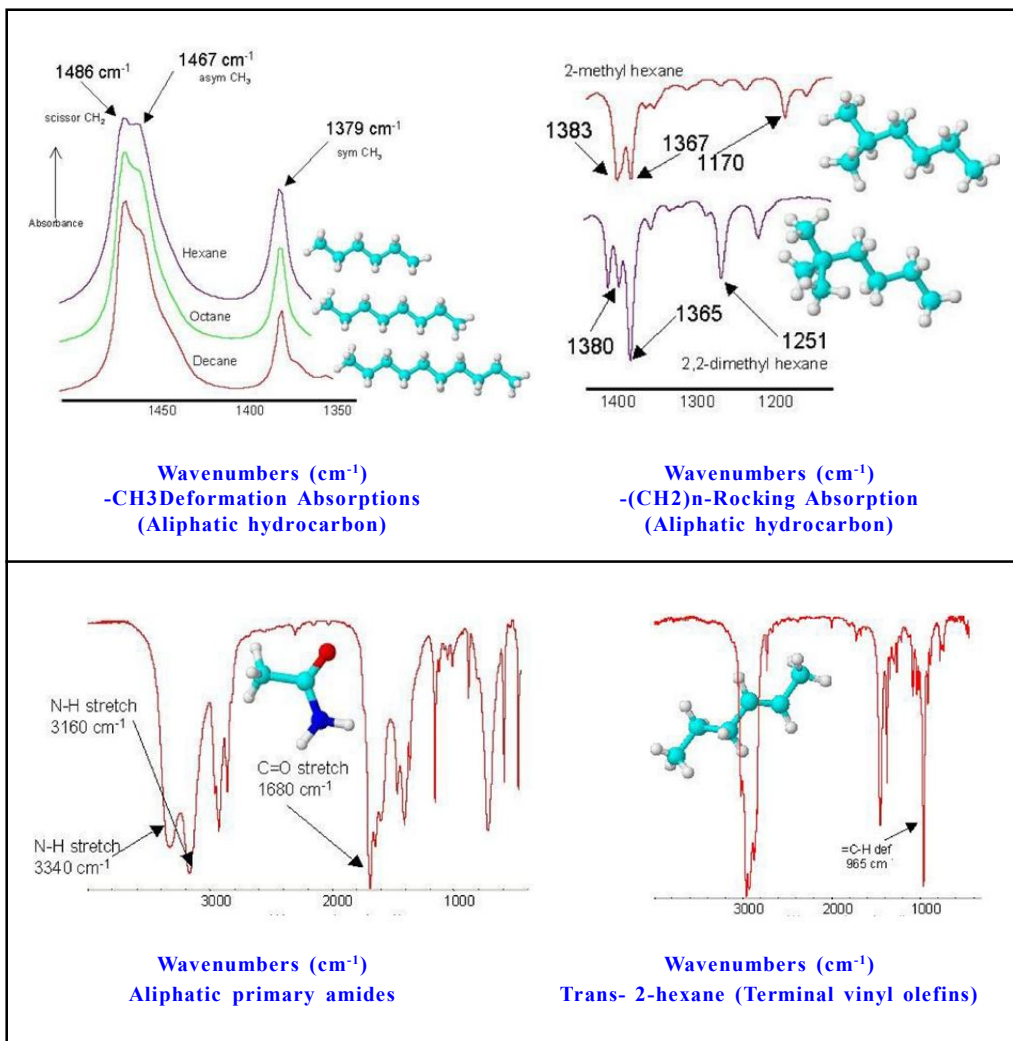


Figure 2: Fourier transform infrared spectroscopy spectra of traditional rice cultivars.



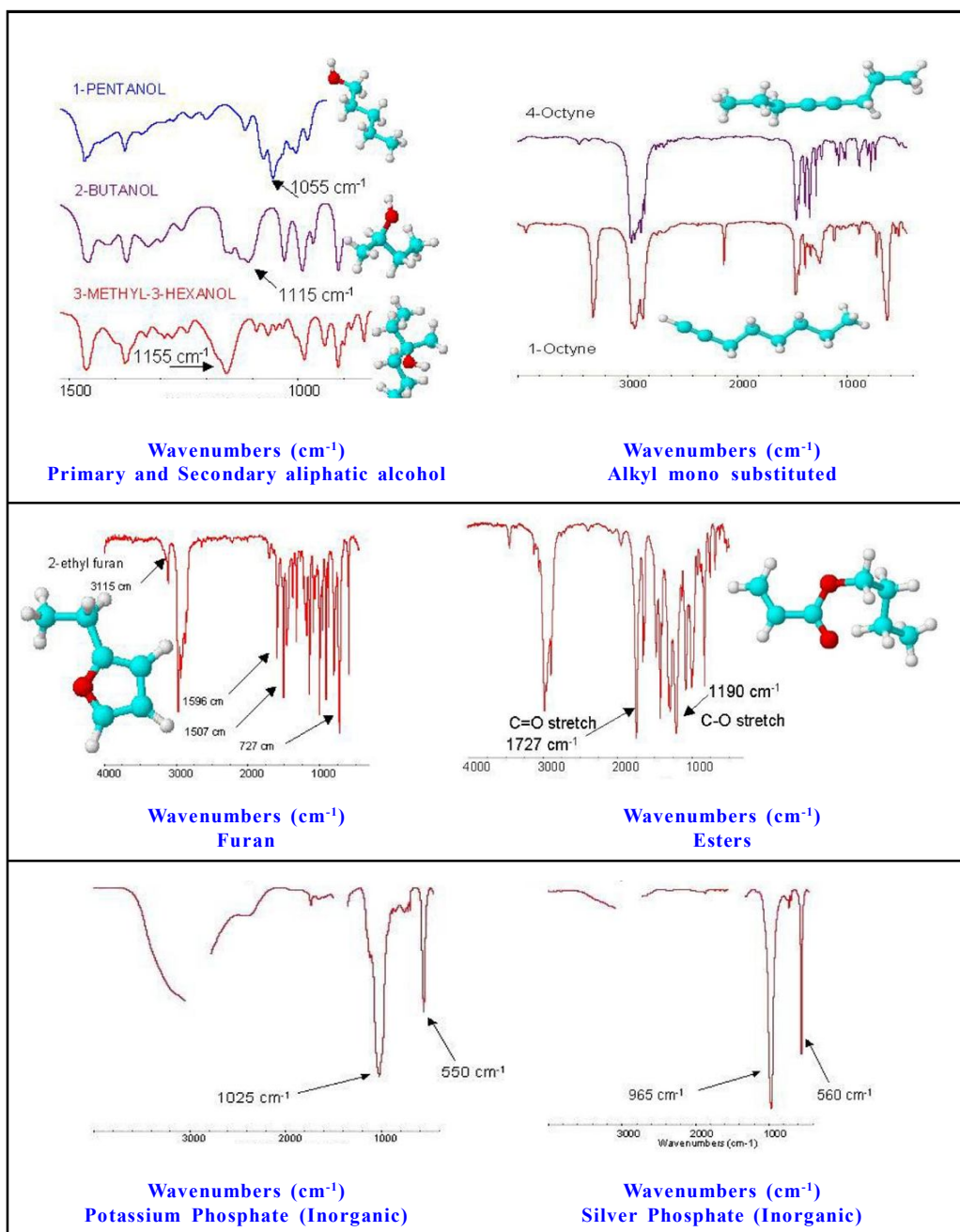


Figure 3: An infrared spectra and identified functional group in traditional paddy cultivars *O. sativa*.

3. Results

In this current study, FTIR analysis was conducted to identify the functional groups of different rice cultivars (Figure 2). The functional group of eleven rice cultivars was evaluated by observing their infrared spectra and given in Table 2. The spectra of each cultivar were recorded in the range of 4000-400 cm⁻¹ (Figure 3). The spectra of *O. sativa* (Arubatham kuruvai) consist of two spectra that indicate the presence of aliphatic hydrocarbon and esters. The band C=O stretching (esters) occurs in the range of 1750-1200 cm⁻¹ as reported by Colom *et al.* (2003). The aliphatic hydrocarbon (CH₃ and CH₂) group, which is present in *O. sativa* (Mappillai samba, Illupaipoo

samba, Thooyamalli, Kalabath, Kothamalli samba, and Karrupu kavuni) was also identified as the source of the absorption peak at 2975-2950 cm⁻¹. The asymmetric stretching vibration of the aliphatic group, such as the CH₃ vibration, occurs at 2975-2950 cm⁻¹ in these rice cultivars, while the symmetric CH₃ vibration (2885-2865 cm⁻¹) and the CH₂ absorption occur at 2870-2840 cm⁻¹. The band 3350-3180 cm⁻¹ indicated the presence of aliphatic primary amides which is NH- primary stretching produced by all the rice cultivars except the cultivar of *O. sativa* (Arubatham kuruvai, Illupaipoo samba, and Thanga samba) indicating the absence of an aliphatic primary amide group. While the NH- secondary amides stretching occur between 3320 and 3070 cm⁻¹. The aliphatic alkyne monosubstituted groups

(-C: C-H strong stretching), which are only identified in *O. sativa* (Mapillai samba and Karrupu kavuni), were identified as the source of the absorption peak at 3300 cm^{-1} . There are no aliphatic alkynes mono substituted groups found in *O. sativa* (Arubatham kuruvai, Illupaipoo samba, Thanga samba, Poonkar, Thooyamalli, Sigappu kavuni, Kalabath, Kothamalli samba, and Karrupu kavuni). The characteristics spectral band between $1000\text{-}550\text{ cm}^{-1}$ has been marked for the presence of inorganic phosphate which is denoted in *O. sativa* (Mapillai samba, Illupaipoo samba, Karuppu kavuni, and Thooyamalli).

The hydroxyl group (-OH) characteristics were present in *O. sativa* (Mappillai samba, Poonkar, Thooyamalli, Sigappu kavuni, Kalabath, Kothamalli samba, and Karrupu kavuni) which are attributed to alcoholic groups. This primary aliphatic alcohol stretched at $3643\text{-}3630\text{ cm}^{-1}$ spectra. Similarly, the secondary aliphatic alcohol group (-

OH stretches) was expressed at the band between $3635\text{-}3620\text{ cm}^{-1}$ which signifies *O. sativa* (Illupaipoo samba and Kothamalli samba). The spectral band at $1670\text{-}1630\text{ cm}^{-1}$ indicates the presence of the CO amide group (aliphatic tertiary amides) in the *O. sativa* (Illupaipoo samba). In comparison, other rice cultivars recorded the absence of aliphatic tertiary amides. Because the furan compound is present in the *O. sativa* (Kalabath) rice cultivar, the strong band expressed above 3000 cm^{-1} indicated the heterocyclic organic compound (=C-H stretching). Research has demonstrated the anti-inflammatory and antioxidant properties of furan found in rice. *O. sativa* (Arubatham kuruvai, Mappillai samba, Illupaipoo samba, Thanga samba, Poonkar, Karuppu kavuni, Thooyamalli, Sigappu kavuni, Kothamalli samba, and Karunguruvai) did not indicate heterocyclic organic compounds. The terminal vinyl olefins group -CH=CH₂ stretched at $3095\text{-}3075\text{ cm}^{-1}$ which is produced by the *O. sativa* (Kothamalli samba) rice cultivar (Table 2).

S.No.	Cultivar	Aliphatic hydrocarbon	Aliphatic primary amides	Alkylmono substituted	Inorganic phosphate	Primary aliphatic alcohol	Aliphatic tertiary amides	Secondary aliphatic alcohol	Furan	Terminal vinyl olefins	Esters
1.	<i>O. sativa</i> (Arubatham kuruvai)	+	-	-	-	-	-	-	-	-	+
2.	<i>O. sativa</i> (Mappillai samba)	+	+	+	+	+	-	-	-	-	-
3.	<i>O. sativa</i> (Illupaipoo samba)	+	-	-	+	-	+	+	-	-	-
4.	<i>O. sativa</i> (Thanga samba)	-	-	-	-	-	-	-	-	-	-
5.	<i>O. sativa</i> (Poongar)	-	+	-	-	+	-	-	-	-	-
6.	<i>O. sativa</i> (Karupu kavuni)	-	+	-	+	+	-	-	-	-	-
7.	<i>O. sativa</i> (Thooyamalli)	+	+	-	+	+	-	-	-	-	-
8.	<i>O. sativa</i> (Sigappu kavuni)	-	+	-	-	+	-	-	-	-	-
9.	<i>O. sativa</i> (Kalabath)	+	+	-	-	+	-	-	+	-	-
10.	<i>O. sativa</i> (Kothamalli samba)	+	+	-	-	+	-	+	-	+	-
11.	<i>O. sativa</i> (Karunkuruvai)	+	+	+	-	+	-	-	-	-	-

(+) sign represent the presence of functional group; (-) sign represents the absence of functional group.

Based on the result of FTIR analysis, the presence of an alcoholic group in *O. sativa* (Mappillai samba, Illupaipoo samba, Poonkar,

Karuppu kavuni, Thooyamalli, Sigappu kavuni, Kalabath, Kothamalli samba, and Karrupu kavuni) was shown to have high cellulose and hemicellulose content as compared to other cultivars.

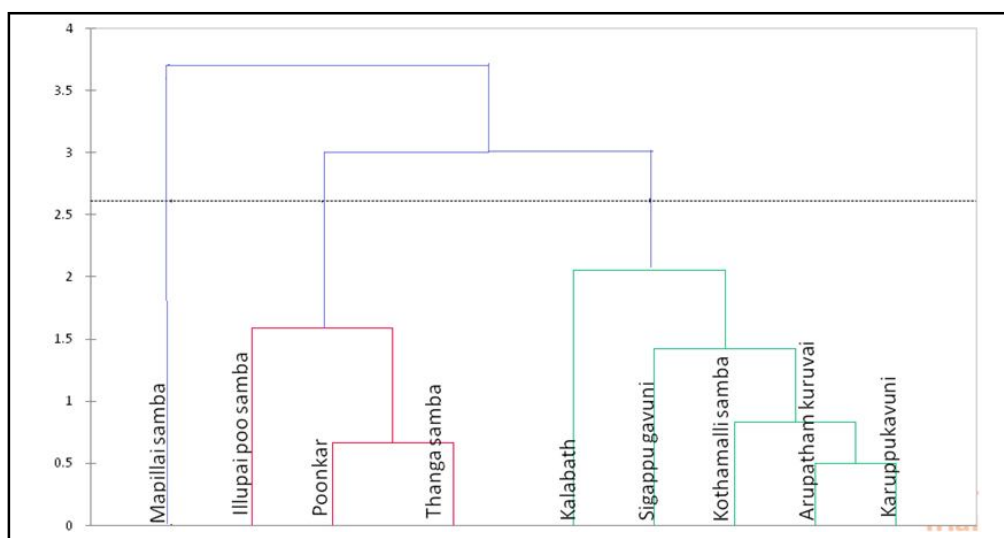


Figure 4: Dendrogram illustrating the similarity of functional groups among the rice cultivars of *O. sativa*.

4. Discussion

Traditional paddy cultivars are one of the major nutritious cereals that possess enormous therapeutic values, and their biomolecules protect the human disorders. The current research is confined to the FTIR analysis of *O. sativa* (Arubatham kuruvai, Mappillai samba, Illupaipoo samba, Thanga samba, Poonkar, Karuppu kavuni, Thooyamalli, Sigappu kavuni, Kalabath, Kothamalli samba, and Karunkuruvai) for prediction of their nutritional properties. FTIR technique is widely used for the identification and characterization of the functional groups exhibited in crops, microbes, and food samples (Papadopoulou *et al.*, 2021). The traditional rice cultivars, such as *O. sativa* (Mappillai samba, Illupaipoo samba, Thooyamalli, Kalabath, Kothamalli samba, and Karrupu kavuni), were shown as an aliphatic hydrogen group at absorption peaks of 2975-2950 cm^{-1} . Similar peak abundance representing the amide functional group was reported in coffee oils using FTIR techniques (Bedoya *et al.*, 2017; Sujka *et al.*, 2017). The hydroxyl group (-OH) characteristics were found to be present in *O. sativa* (Mappillai samba, Poonkar, Thooyamalli, Sigappu kavuni, Kalabath, Kothamalli samba, and Karrupu kavuni), which are attributed to alcoholic groups. The current study agrees with Fan *et al.* (2012) and Malik *et al.* (2023). Based on the result of FTIR analysis, the presence of an alcoholic group in *O. sativa* (Mappillai samba, Illupaipoo samba, Poonkar, Karuppu kavuni, Thooyamalli, Sigappu kavuni, Kalabath, Kothamalli samba, and Karunkuruvai) was shown to have high cellulose and hemicellulose content as compared to other cultivars as reported for paddy straw (Bhattacharyya *et al.*, 2020; Giang *et al.*, 2020; Thakur *et al.*, 2020).

In traditional rice cultivars, the contents of phytochemicals depend on the presence of functional elements. Functional elements were recorded, and similar results were compared using a dendrogram (Figure 3). The rice cultivars such as *O. sativa* (Arubatham kuruvai, Sigappu kavuni, Kothamalli samba, Kalabath, and Karrupu kavuni) were represented in the same cloud due to the expression of similar functional groups, while *O. sativa* (Illupaipoo samba, Thanga samba, and Poonkar) were occupied in another cloud. Among the rice cultivars, *O. sativa* (Mappillai samba) exhibited the presence of more functional groups as compared to other cultivars. Similar trends in traditional rice cultivars through FTIR and the distribution of different functional groups, *viz.*, hydroxyl, amide groups, carbohydrates, and phenols were observed by Udhaya Nandhini *et al.* (2023).

Rice flour's fourier transform infrared spectra and found that the flour exhibited five spectrum peaks, each of which may have represented a functional group in the sample. The N-H bonds at peak 2019.54 cm^{-1} , 1546.96 cm^{-1} , and 785.05 cm^{-1} infrared spectrum absorption revealed the existence of an amine functional group, which is suggestive of protein content. An important component of flour is their starch content. Since amylase and amylopectin are the primary components of starch, their vibrational modes account for most of the spectrum of the starch band. Peaks were detected in regions below 800 cm^{-1} and 800 to 1500 cm^{-1} (Abishek Thakur *et al.*, 2020). The spectrum revealed the existence of O-H groups at wavelengths between 3385.64 and 3393.27 cm^{-1} , C-H groups at 2929.29 and 2930.50 cm^{-1} , N-H groups at 2150.71-2154.20 cm^{-1} , C=O groups at 1650.01 and 1655.52 cm^{-1} , and C-N groups at 1155.48 and 1155.22 cm^{-1} , respectively (Abhishek *et al.*, 2020; Raharja *et al.*, 2018). The crystalline and amorphous starch indices in the sample were defined by bands at 1047 and 1022 cm^{-1} , respectively (Sevenou *et al.*, 2002).

Bioactive compounds present in traditional rice have several physiological effects on human health. Phenolics with one or more aromatic rings and one or more hydroxyl groups are linked to several health benefits for people, including the ability to reduce inflammation, hypoglycemia, cancer, allergies, and atherosclerosis (Tan and Norhaizan, 2017). Phosphorus is necessary for bone, teeth, energy metabolism, synthesis of amino acids and protein. It is a component of nucleic acids, involved in cellular metabolism and enzyme systems (Cashman, 2006). The aliphatic compounds act as a substitute for sugars (Piebiep and Henrique, 2014) and esters that have fragrant odors reason for essential oils and food flavor. Antibacterial action of furan was achieved by altering enzymes and selectively inhibiting microbial growth (Alizadeh *et al.*, 2020). Numerous life forms have been found to naturally contain olefins. For instance, carrots naturally contain an olefin known as beta-carotene, a vitamin. It is well known that olefins are used in many different aspects of daily human life. Ethylene is an olefin, and is frequently utilized to encourage fruit ripening, hence cutting down on the amount of time needed to wait before eating the fruit. Several traditional rice's contain amines and amides which are a main component of nucleic acids, proteins, enzymes, *etc.* Numerous bioactive components, including phenolic compounds, flavonoids, tannins, anthocyanins, proanthocyanidins, phytic acids, and γ -oryzanol, are abundant in traditional rice. 32 phenolic acids, including derivatives of hydroxyl cinnamic acid, and 7 distinct flavonoids have been found in rice cultivars. Furthermore, rice grains show promise as a nutraceutical for their anticancer, antibacterial, anti-inflammatory, antiarthritic, and antidiabetic properties (Forshed Devan *et al.*, 2023) and antioxidant activity (Udhaya Nandhini *et al.*, 2023).

Traditional paddy cultivars were reported to treat human diseases and involved in the induction of antioxidant activity. Malnutrition is a major disorder in developing countries and few scientists have made several attempts to develop new cultivars incorporating nutrition-rich paddy cultivars to feed the increasing population. Traditional rice cultivars were reported to cure deficiencies like protein-energy malnutrition (PEM) and treat biological diseases like nephrological disorders, diabetes, cardiovascular disease, and neurological disorders (Kowsalya *et al.*, 2022).

5. Conclusion

A healthy diet, nutrient-rich food, and functional properties play an important role in reducing malnutrition and developing immunity. To overcome the biggest problems like hunger, and obesity; healthy food, diets, and improving food production with high nutritive value are the needs of the hour. Under such circumstances, traditional rice cultivars have rich sources of biological properties that help the growing population protect themselves. The current study's goal was to investigate the various functional characteristics of traditional rice using FTIR technology. The findings indicate that aliphatic hydrocarbons, aliphatic primary amides, alkyl mono substituted, inorganic phosphate, primary aliphatic alcohol, aliphatic tertiary amides, secondary aliphatic alcohol, furan, terminal vinyl olefins, and esters are present in the functional characteristics of traditional rice varieties. The presence of these functional groups suggests the presence of furan, hemicelluloses, and cellulose, all of which have several antioxidant and anti-inflammatory qualities. Hence, to gain importance, extensive research on the identification of functional groups in traditional paddy cultivars may be useful in exploring

therapeutic value and discovering functional compounds and will also help design drugs for medical treatment. However, few studies have been carried out on the quantification of each functional property, and in-depth studies on the quantification of each functional property, and their medicinal values must be thoroughly studied for human and animal consumption. The study suggests that selecting traditional rice cultivars with improved functional traits may aid in expanding the farming community's cultivated area as well as improving the agricultural economy and health advantages for consumers.

Future research on traditional rice

Future research should be focused on the laboratory and field level to explore the significance of biological and functional properties in traditional rice, which plays a major role in human health. Currently, traditional rice is regarded as a nutraceutical and functional food besides being a staple food and primary source of carbohydrates or starch. The research should aim to meet the following requirements in the future.

- In-depth studies on the quantification of each functional property must be thoroughly studied.
- The medicinal properties of traditional rice have to be evaluated, and their side effects need to be studied well.
- The anticancer property of traditional rice should be studied because it is now an emerging problem in all stage groups of people.
- Research into the bioassay and purification of each compound and the procedures required to define each compound is essential.
- The impact of traditional rice and its extracts on the overconsumption of traditional rice should be studied to find out toxicity or allergic reaction.
- The biological and functional properties of paddy straw have to be studied well, as it is used as animal fodder.
- A study of the different properties of rice bran is also needed because it is now popular as an alternative to other cooking oils.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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