Differential Analysis of Carbohydrate Content in Selected Rice Varieties Using the Anthrone Spectrophotometric Method

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Abstract

Carbohydrates are essential macronutrients besides proteins and fats, by functioning as the primary source of energy and maintains the normal physiological processes. Both overconsumption or insufficient consumption of carbohydrates results in serious ailments such as diabetes, chronic kidney disease, etc. Therefore, evaluating carbohydrate content in staple foods like rice is essential for disease prevention and management. This study focuses on the estimation of carbohydrate content in several varieties of rice (Oryza sativa), namely brown rice, jasmine rice, sona masoori rice, basmati rice, black rice, fortified rice, red rice and quinoa. The qualitative estimation of carbohydrates was performed by the Molisch test and iodine test which aid in establishing the presence of carbohydrates in various sample extracts. Whereas the quantitative determination was executed by the anthrone method using colorimeter. Additionally, all of the samples were analysed by IR spectroscopy to further validate the efficiency of the tests performed. The anthrone method estimated the carbohydrate content as follows: (basmati rice-0.275 mg/ml, Sona Masoori-0.325 mg/ml, jasmine rice-0.613 mg/ml, fortified rice-0.5mg/ml, quinoa-0.475 mg/ml and brown rice-0.238 mg/ml)**.** The IR spectra of the samples exhibited peak values at wavelengths that correspond to the functional groups associated with carbohydrates. The results of the anthrone method signified the nutritional diversity of the samples. These results were further validated by the findings of the IR spectra. Several health conditions can thus be managed by incorporating the suitable rice variety in accordance to their carbohydrate content.

Keywords: Carbohydrates; Diabetes; Rice; Anthrone; Colorimeter; IR spectroscopy.

1. Introduction

Carbohydrates constitute one of the most fundamental macronutrients in human nutrition, exerting a profound and multifaceted influence on physiological homeostasis alongside proteins and fats. Structurally, carbohydrates are categorized into monosaccharides, disaccharides, oligosaccharides, and polysaccharides, and functionally classified as simple and complex carbohydrates. Chemically, they are commonly represented by the empirical formula $(CH_2O)_n$.

Carbohydrates, although chemically less complex than other macronutrients, are fundamental to normal physiological functioning in humans. They serve as the primary source of metabolic energy and are critically involved in the homeostasis of blood glucose levels, insulin and lipid metabolism (Holesh JE, Aslam S, 2025).

Given their pivotal physiological functions, both insufficient and excessive carbohydrate intake have been associated with serious health consequences. Carbohydrate deficiency can precipitate metabolic disturbances such as ketonemia, dehydration, and myasthenia, reflecting impaired energy homeostasis (Karri Prasanna et al, 2025). Conversely, chronic overconsumption of

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carbohydrates has been strongly implicated in the pathogenesis of cardiovascular diseases, type 2 diabetes mellitus, chronic kidney failure, neuropsychiatric disorders, and various forms of cancer (Clemente-Suárez VJ, Mielgo-Ayuso J, 2022).

To maintain metabolic equilibrium and minimize diet-related health risks, an optimal daily carbohydrate intake of approximately 200–300 g has been recommended. Staple cereals such as rice and wheat represent major dietary sources of carbohydrates, predominantly in the form of starch, highlighting their vital role in global dietary and nutritional patterns (Holesh JE, Aslam S, 2025).

Given the dual nutritional significance of carbohydrates—encompassing both their physiological benefits and potential adverse effects—accurate qualitative and quantitative assessment of carbohydrate content in food matrices is imperative. The present study systematically evaluates both reducing and non-reducing sugars in selected cereal and pseudo-cereal grains, including multiple varieties of rice (*Oryza sativa*)—Sona masoori, Jasmine rice, Basmati rice, brown rice, fortified rice, black rice, and red rice—as well as quinoa (*Chenopodium quinoa*).

Reducing sugars such as monosaccharides and disaccharides, are the carbohydrates that have a strong reducing action due to the presence of free aldehyde and ketone groups. On the counterpart, non reducing sugars such as polysaccharides, do not possess reducing action due to lack of free aldehyde and ketone groups (Durvesh, Burhade, 2025). The qualitative characterization was proceeded with the iodine test and the Molisch test, as these sources primarily comprise starch, with no availability of free reducing sugars to be depicted by other tests for carbohydrates. The Molisch reaction results in the production of a violet ring at the interface of both the liquid phases, whereas the iodine assay produces a deep blue-black coloration upon interaction with starch polymers. The quantitative determination was conducted by the anthrone reagent, a frequently employed method for the evaluation of total carbohydrates, whose principle relies on the production of a blue green complex due to the fusion of the dehydrated sugars with the anthrone reagent (Karri Prasanna et al, 2025).

2. Materials and methods

2.1. Preparation of extracts

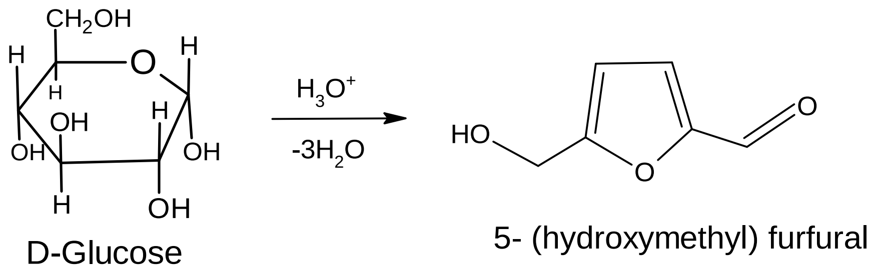
100 grams of various samples were accurately weighed and cooked by pressure cooker method. The cooked samples were made to a fine paste by mashing. 10 grams (10,000 mg) of the mashed samples were accurately weighed and transferred to beakers, to which 10 ml of 2.5N HCL was added and dissolved with the help of a stirring rod. The resultant mixtures were subsequently boiled in a water bath for 30 minutes. After boiling, the mixtures were cooled to room temperature, and sufficient amount of sodium carbonate was added until effervescence ceases, to neutralise the acid present. The neutralized sample mixtures were transferred into 100 ml volumetric flasks, and the final volume was made to 100 ml with distilled water to prepare the extracts required for analysis (Agrawal, Neeru, 2011) (Suman, and P. Boora, 2016) given in fig 1.

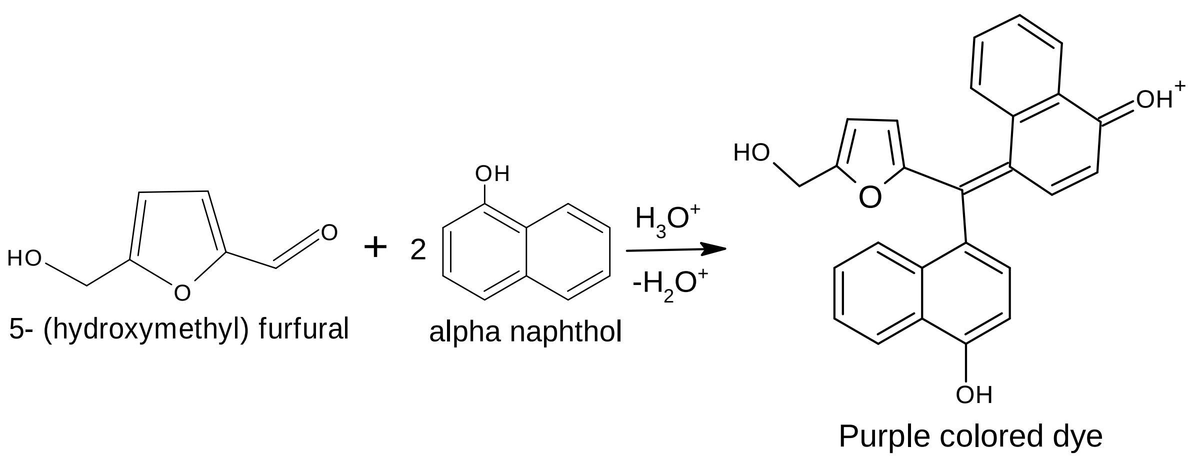
Fig 1: The prepared extracts of various types of rice and millet

2.2. Qualitative estimation of carbohydrates

2.2.1. Molisch test

It is a preliminary test used to determine the presence of carbohydrates in the samples, and to differentiate between carbohydrates and non-carbohydrates. This test relies on the carbohydrate's dehydration reaction and the subsequent detection of carbohydrates based on the colour. Concentrated H_2SO_4 is added to dehydrate the carbohydrates such as pentoses and hexoses into aldehydes such as furfural and hydroxy methyl furfural respectively. α-naphthol present in the Molisch reagent condenses with the aldehyde to generate a purple-coloured ring at the interface of the solutions (Shukla Pooja, Meghani Sonali, 2022).





1 drop of Molisch's reagent (10 percent-naphthol in ethanol) was added to a test tube containing 2 ml of the extract. To this 1ml of concentrated H_2SO_4 was added along the walls of the test tube, resulting in the formation of a layer at the bottom of the tube. The reaction was monitored closely, for the formation of a purple-coloured complex at the junction of the two liquids (Shukla Pooja, Meghani Sonali, 2022) (Setiawan, Dedy, 2024).

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2.2.2. Iodine test

This test is used for the determination of starch in a sample, and for differentiation of various carbohydrates such as glycogen, monosaccharides by producing different colours. The reaction of starch with iodine produces an adsorbed entity which is blue in colour. The produced colour is lost upon heating or addition of alkali such as NaOH. The required bluish black colour is a result of the noncovalent interactions of the iodine molecule which binds to the coiled structure of the polysaccharide. Whereas upon heating or addition of an alkali, the coiled structure changes into a linear structure, resulting in the decreased absorptive power of starch, which releases the iodine from the coiled shape of the polysaccharide, causing the colour to disappear. The presence of starch is reconfirmed by the addition of HCL which causes the reoccurrence of the colour (Shukla Pooja, Meghani Sonali, 2022).

$$3I_2 + NaOH \longrightarrow 5NaI + NaIO_3 + 3H_2O$$

$$NaIO_3 + HCl \longrightarrow HIO_3 + NaCl$$

$$5\ NaI + 5\ HCl \longrightarrow 5HI + 5\ NaCl$$

$$HIO_3 + 5\ HI \longrightarrow 3\ I_2 + 3\ H_2O$$

3 ml of each extract was accurately measured into clean test tubes and 1 ml of 40% NaOH was added followed my thorough mixing. A few drops of 0.1 N iodine solution were added to the mixed solutions. Later, 1 ml of concentrated HCL was accurately measured and transferred to each test tube. The colour changes produced for each sample is observed and noted.

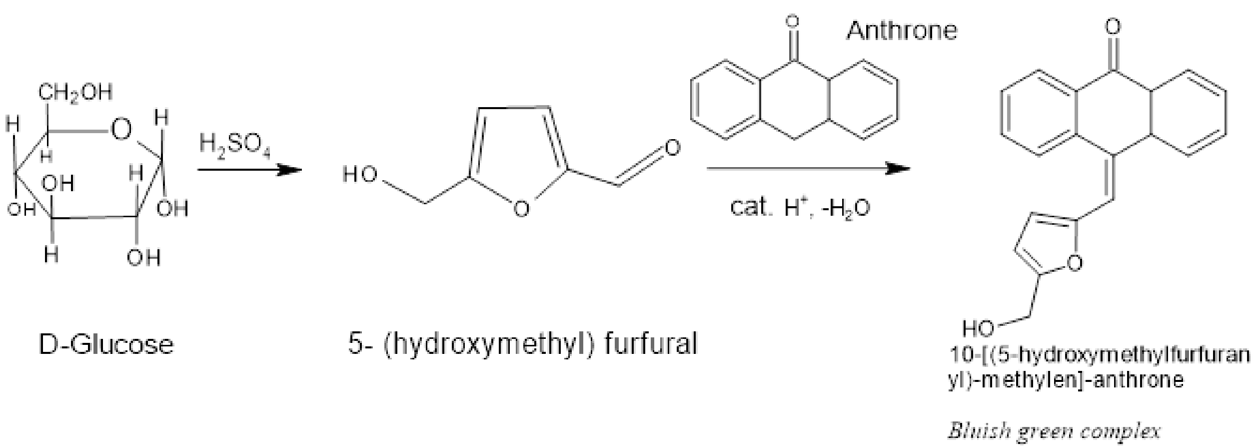
2.3. Quantitative estimation of carbohydrates

2.3.1. Estimation of carbohydrates using anthrone reagent by colorimetry

This is the commonly employed method for the estimation of total carbohydrates (monosaccharides, disaccharides, oligosaccharides and polysaccharides) present in the samples.

This method involves the conversion of non reducing sugars to reducing sugars by the action of concentrated H_2SO_4 (Karri Prasanna et al, 2025).

The principle is based on the formation of a blue green complex due to action of concentrated H_2SO_4 present in the anthrone reagent. The acid present cleaves the polysaccharides into monosaccharides. The resultant monosaccharides, hexoses and pentoses, are then dehydrated into aldehydes, furfural and hydroxy methyl furfural respectively. The produced aldehydes condense with the anthrone reagent, resulting in the production of a blue-green complex, which is quantified by colorimetry at a wavelength of 620 nm (Karri Prasanna et al, 2025).



A. Reagent preparation: 200 mg of anthrone was dissolved in a volume of 100 ml sulphuric acid, prepared by dissolving 500 ml of concentrated sulphuric acid in 200 ml of distilled water. The freshly prepared anthrone reagent should be used within 12 hours (YEMM EW, WILLIS AJ, 1954).

B. Glucose stock solution: 1 gram of glucose was accurately weighed and dissolved in 500 ml of distilled water.

C. Procedure

Preparation of the standard curve: Different volumes of the standard stock solution (100 µL, 150 µL, 200 µL, 250µL, 300µL) were taken into test tubes. The volume was made to 1ml with distilled water in all the test tubes. A blank was prepared with 1 ml of distilled water without samples.

Reaction with anthrone reagent: To the above test tubes, a volume of 5 ml anthrone reagent was added, and was mixed well by vortexing. Later, 1 ml of the sample extracts were pipetted out into test tubes and 2 ml of freshly prepared anthrone reagent was added into each test tube of the extracts. A reference was prepared by taking 2 ml of the anthrone reagent into a test tube and diluted with distilled water to make up the volume up to 1 ml and mixed well.

Incubation: All the test tubes of the standard were covered and heated in a boiling water bath (100ºC) for 10 minutes. After that, all tubes were cooled to room temperature.

Absorbance measurement: The absorbance of the blue green complexes of the standard, sample and reference were measured using a colorimeter at a wavelength of 620 nm and the values were noted. The quantitative determination of all the samples was performed in triplicates.

Plotting of standard curve: The curve was prepared by plotting the obtained absorbance values on y axis against the concentrations of standard glucose solution on x axis. This graph was later used for determining the concentration of carbohydrates in the samples (Karri Prasanna et al, 2025).

3. IR spectral characterization of carbohydrates

The Fourier transform infrared spectroscopy (FTIR) analysis was performed by the Shimadzu equipment for the characterization of starch present in various samples. The various types of rice (jasmine rice and red rice) and quinoa grains were powdered and two mg of each sample were mixed with 200 mg of FTIR grade KBr and compressed into a pellet. (Jaiganesh V, 2025) The FTIR spectral was set at a wavelength of 4000-400 1/cm, with 10 scans and a resolution of

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4(1/cm). The chemical moieties corresponding to starch were determined on the basis of the peak value and wavelength range of IR (Jaiganesh V, Kannan C, 2025).

4. Results

The results obtained from the qualitative analysis of carbohydrates (Table I), indicated the presence of starch in all of the extracts, by producing the desired colour or response in both the tests.

Table I: Results of the qualitative analysis of carbohydrates in the extracts

Test	Observation	Inference
Molisch test	A purple-coloured ring was formed between the acid and the test layer	Presence of carbohydrates is confirmed
Iodine test	No colour has been observed upon addition of iodine to alkaline solution Bluish-black colour has been observed on addition of concentrated HCL to the above sample	Presence of starch is confirmed

The results of the quantitative estimation were interpreted based on the intensity of the blue-green colour which indicates the carbohydrate concentration. The absorbance values of the unknown samples are compared with the standard curve to estimate the carbohydrate content present in the samples by implementing the formula:

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$$C_{unknown} = \frac{A_{unknown} - b}{m}$$

Where,

$A_{unknown}$= absorbance of the test sample at 620 nm

m= slope of the standard curve of glucose stock solution

b= y-intercept of the calibration curve

$C_{unknown}$= concentration of carbohydrates in the sample.[2,8]

The standard curve obtained by plotting the absorbance values of the standard stock solution against the concentrations is given in Fig 1. And the standard values are given in table II.

Table II: Standard values of the glucose stock solution

S No	Glucose solution (µL)	Glucose(mg)	Distilled water (ml)	Anthrone reagent (ml)	Absorbance values at 620 nm
1	100	0.2	0.90	5	1.64
2	150	0.3	0.85	5	1.71
3	200	0.4	0.80	5	1.78
4	250	0.5	0.75	5	1.91
5	300	0.6	0.70	5	1.95

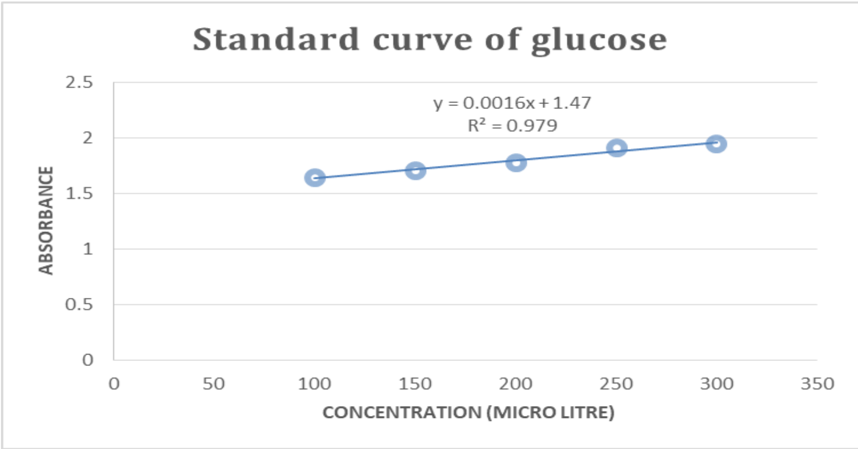

Fig 2: Standard curve of glucose stock solution

The estimated amount of carbohydrates in all the samples determined by the above-mentioned formula are given in Table III. Triplicate readings for Absorbance were determined for each sample. The Mean values were calculated for each sample to get accurate results.

Table III: Estimated concentration of carbohydrates in the samples

Sample	Absorbance at 620 nm		Estimated amount of carbohydrate (mg/ml)
	Mean	Std Deviation (±)	
Sona Masoori	1.73	0.325	0.325
Fortified rice	1.05	0.525	0.525
Quinoa	1.83	0.450	0.450
Brown rice	1.64	0.212	0.212
Jasmine rice	1.96	0.613	0.613
Basmati rice	1.69	0.275	0.275
Black rice	1.93	0.575	0.575
Red rice	1.87	0.500	0.500

The FTIR spectra for various samples analysed by KBr pellet method at a wavelength of 4000-400 1/cm are given in fig 3,4 and 5. The infrared spectra of the black rice, jasmine rice and quinoa are shown in table IV.

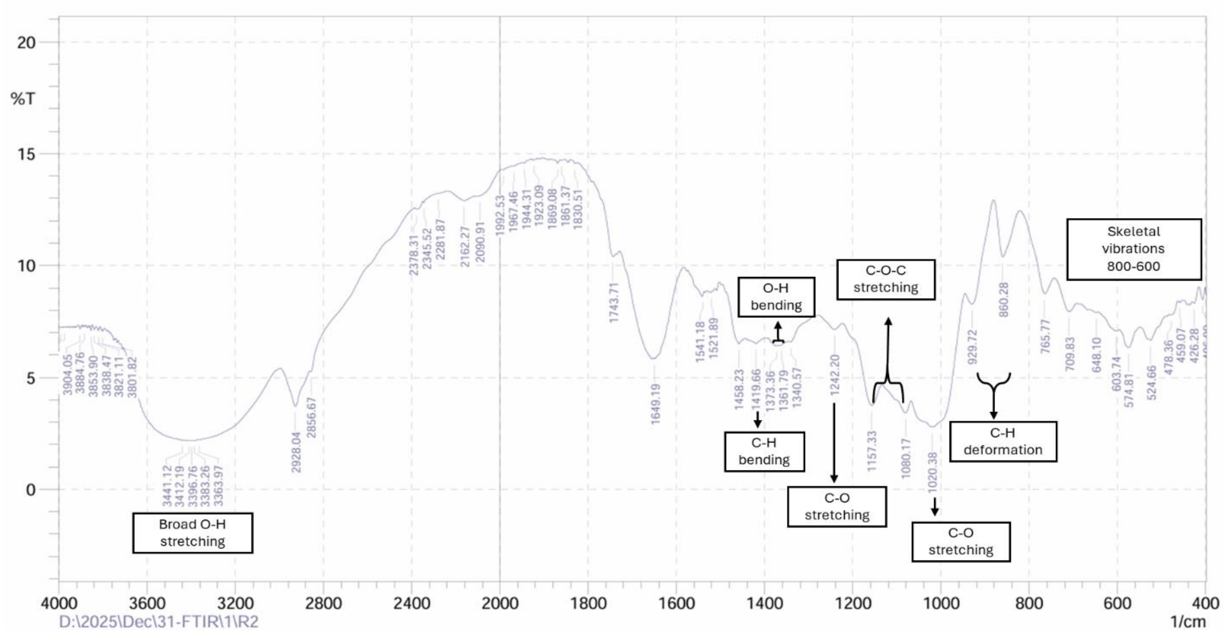

Fig 3: FTIR spectra of black rice

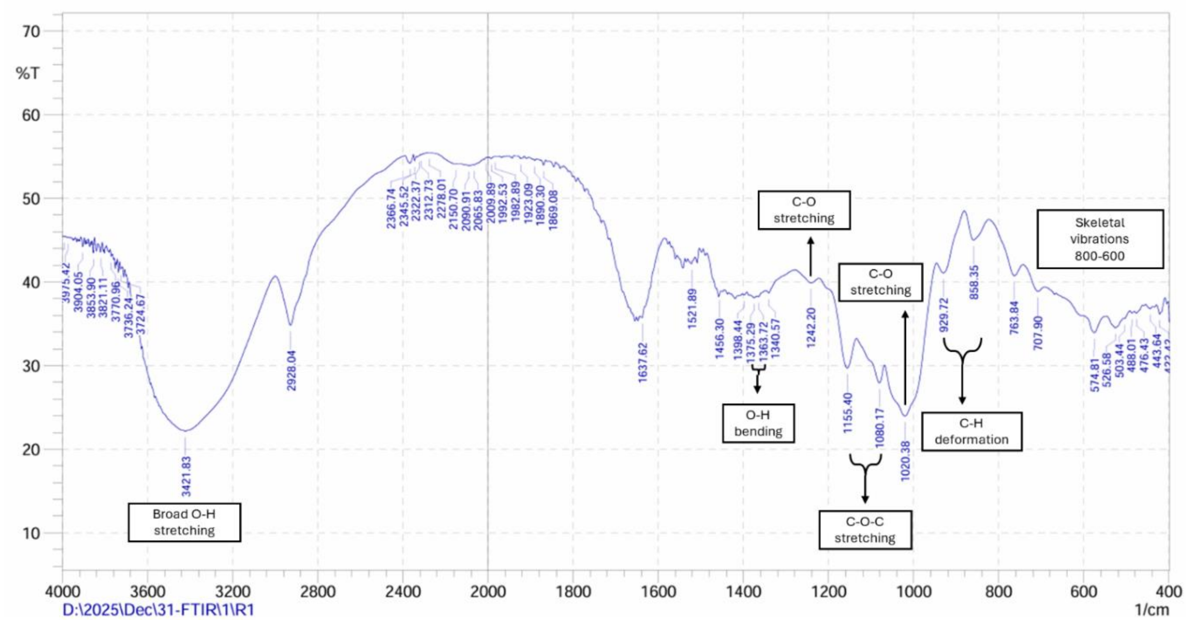

Fig 4: FTIR spectra of jasmine rice

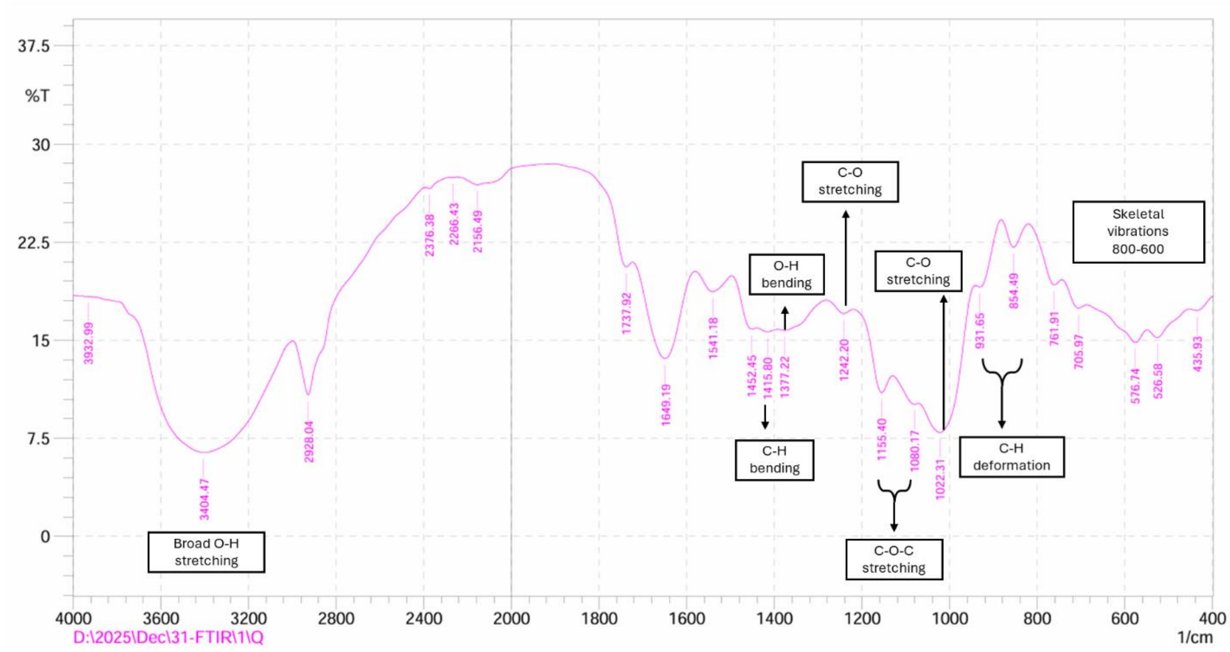

Fig 5: FTIR spectra of quinoa

The chemical groups present in the samples were characterized based on the peak values obtained within a wavelength range which are correlated to determine the macronutrients and constituents present such as carbohydrates, proteins, lipids, fatty acids etc are given in table IV.

Table IV: Correlation of wavelength of peak value and corresponding chemical group

Wavelength range of observed peak value (1/cm)	Corresponding chemical group
3600-3200	Broad O-H stretching
1450-1410	C-H bending
1380-1360	O-H bending
1260-1230	C-O stretching
1160-1080	C-O-C stretching
1050-1020	C-O stretching
930-850	C-H deformation
800-600	Skeletal vibrations

5. Discussions

The qualitative and quantitative tests performed for the determination of carbohydrates in various samples such as Sona Masoori, brown rice, basmati rice, fortified rice, jasmine rice and quinoa, have been successful in yielding positive results. The qualitative tests performed in prior to the conduction of quantitative tests, have successfully established the presence of the carbohydrates (by both Molisch and iodine test) and especially starch (by iodine test). The quantitative estimation performed by the utilization of the most common anthrone method, has aided in the estimation of the carbohydrate concentration by providing accurate results that align with the actual carbohydrate content in the samples. The results thus obtained, (basmati rice-0.275 mg/ml, Sona Masoori-0.325 mg/ml, jasmine rice-0.613 mg/ml, fortified rice-0.525 mg/ml, quinoa-0.450 mg/ml, black rice-0.575 mg/ml, red rice-0.500 mg/ml and brown rice-0.212 mg/ml) help in determining the carbohydrate composition of the samples, depending upon the extent of carbohydrate content present in the samples.

Highest carbohydrate concentration: Jasmine rice has been observed to contain the highest carbohydrate concentration (0.613 mg/ml) amongst the other samples, which is in accordance to its usual total carbohydrate content, it is followed by black rice with 0.575 mg/ml of carbohydrate content.

Moderate carbohydrate content: Fortified rice and red rice have been observed to have a carbohydrate concentration of (0.525 and 0.500 mg/ml) respectively.

Lower carbohydrate concentration: Sona Masoori, Quinoa and Basmati rice have shown lower concentration of carbohydrate, (0.325, 0.450 and 0.275 mg/ml) respectively.

Lowest carbohydrate concentration: Brown rice reported the lowest content of 0.212 mg/ml, which is in accordance to its usual carbohydrate content.

The infrared spectra of black rice, jasmine rice and quinoa depicted a strong absorption band in the wavelength range of 3600-3200 cm^{-1}, corresponding to the O-H stretching vibrations, which indicates the presence of starch and cellulose derivatives. The peaks observed in the 1260-1230 cm^{-1} and 1050-1020 cm^{-1} wavelength ranges are associated with C-O stretching, indicating the presence of carbohydrates (especially starch). Additionally, the peaks observed at the 1380-1360 cm^{-1}, 1160-1080 cm^{-1}, 930-850 cm^{-1}, 800-600 cm^{-1} ranges are associated with O-H bending, C-O-C stretching, C-H deformations and skeletal vibrations. These particular functional groups indicate the presence of carbohydrates, especially starch (amylopectin and amylose) and polysaccharides. Furthermore, the peaks obtained in the 1450-1410 cm^{-1} range indicate the presence of C-H bending vibrations, confirming the presence of carbohydrates, specifically starch.

Based on this, the various types of rice and millet can be incorporated in a daily diet for efficient results, especially in individuals with type 2 diabetes, cardiovascular diseases, cancer, allergies, chronic kidney disease, etc which are generally associated with an excessive consumption of carbohydrates. The results obtained suggest that the consumption of foods with lowest carbohydrate content such as brown rice, can be beneficial in managing the above mentioned diseases, and the staple foods with moderate carbohydrate content can be consumed for preventing carbohydrate deficiency which has been associated with conditions such as ketosis, muscle weakness and energy loss, and those with highest carbohydrate content are better to be consumed occasionally, due to their tendency to cause a blood glucose spike. On a general note, the above tested methods for the estimation of carbohydrates provide an informative insight for the healthy consumption of several staple foods.

Conflict of interest

None

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