



## ORIGINAL RESEARCH ARTICLE

## Evaluation of mineral and near-infrared forecasting of wheat yield varieties using spectrophotometric techniques

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## ABSTRACT

**BACKGROUND AND OBJECTIVES:** Iron, an essential micronutrient, significantly contributes to growth, immune health, and cognitive development in human health. Inadequate dietary iron intake leads to iron deficiency anemia, affecting nearly 1.6 billion people, especially pregnant women and preschool children. Biofortification and fortification of iron in wheat is an acceptable and cost-effective strategy to alleviate iron deficiencies. This study aims to address iron deficiencies through the strategy of fortification and biofortification of wheat varieties. The study places specific emphasis on the proximate composition and iron/mineral content of different wheat varieties. To achieve these objectives, different spectrometric methods were employed to analyze the wheat samples.

**METHODS:** Proximate and mineral quantification were carried out following standard Association of Official Analytical Chemists methods using ultraviolet-visible spectrophotometry, atomic absorption spectrometry, inductive coupled plasma-mass spectroscopy, and prediction was carried out using near-infrared spectra combined with chemometrics.

**FINDINGS:** The samples had moisture content (1.1- 4.5 percent), protein (18.0- 22.6 percent), fat (0.3 - 0.6 percent), gluten (6.3- 10.3 percent), fiber (0.3 - 1.4 percent), alcoholic acidity (0.04- 0.08 percent), ash (0.9- 1.7 percent), and carbohydrate (71.1- 75.2 percent). Iron was determined and compared by spectrophotometric methods. Iron concentration ranged from (0.7 to 6.3 milligrams/100 grams) in ultraviolet-visible analysis, (0.7 to 6.74 milligrams/100 grams) in atomic absorption spectrometry, and (0.81 to 6.8 milligrams/100 grams) in inductive coupled plasma-mass spectroscopy. The obtained results were compared with the standard "Food Composition and Food Safety Standard Authority of India" and predicted using near-infrared spectra combined with chemometrics.

**CONCLUSION:** The work aims to investigate the nutritional content of various wheat varieties, particularly focusing on iron content, which could potentially have implications for improving dietary strategies and addressing nutritional deficiencies. The biofortified varieties (HI-8663 and HI-1605) were found to have high iron content when compared to normal wheat. The acquired results bridge the intricate relationship between plant-based diets, micronutrient deficiencies, providing valuable insights into combating iron deficiencies in public health with the potential achievement of improved nutritional understanding, optimized wheat selection, advanced analytical techniques, education, awareness, and iron deficiency mitigation.

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## INTRODUCTION

Iron is an essential micronutrient that significantly contributes to growth, immune health, and cognitive development. As the world's population exceeds billions, malnutrition in public health remains a grave concern, with health issues often going unaddressed. In India, the focus of most health programs remains on children under five, women of reproductive age, and adolescents, while other health issues are often neglected (Vijayshree et al., 2023). Inadequate dietary iron intake, especially during critical periods of growth, development, infancy, and pregnancy, can lead to chronic blood loss, resulting in iron deficiency and increased perinatal risks for mothers and newborns (Chouraqui, 2022; Allen et al., 2018; Chaparro and Suchdev, 2019; Narozhnykh, 2023). Iron deficiency and associated anemia are widespread, affecting nearly 1.2 billion people, especially pregnant women (41.8%) and preschool children (47.4%) (Bruno and Egli, 2008; McLean et al., 2009; White and Broadley, 2009; Chekraverthy et al., 2023). Iron plays a crucial role in various physiological processes within the human body, making the identification of iron deficiency paramount in the biomedical field. Early detection and intervention can prevent the progression of anemia and its associated health complications. Various methods, including blood tests such as serum ferritin, serum iron, transferrin and total iron-binding capacity, hemoglobin, hematocrit levels, and complete blood count, are used to identify iron deficiency. These methods, combined with thorough clinical evaluation, help healthcare professionals diagnose and monitor iron deficiency, enabling timely intervention through dietary changes or addressing underlying causes. Regular monitoring of iron status is crucial, especially in vulnerable populations such as pregnant women, infants, and individuals with chronic diseases (Fernando and Santiago, 2009). Iron deficiency can be related to environmental factors in several ways. Firstly, the availability of iron in the environment can directly affect human intake. In areas where soil and water are low in iron content, there is a higher prevalence of iron deficiency, which can be problematic in agricultural regions where crops may fail to accumulate sufficient iron. This, in turn, can impact soil quality and plant growth. Low iron levels in soil can lead to reduced nutrient availability and poor plant growth, potentially affecting the overall nutritional intake of the

population. Iron deficiency can be addressed through various environmental strategies, including enriching soil with micronutrients/minerals to facilitate nutrient uptake by crops or improving agricultural practices (Rüdiger and Udo, 2003). Despite various supplementation efforts to treat malnutrition in India, the problem persists. In response, cost-effective dietary intervention strategies, such as fortification (physical mixture of ferrous forms) (Uauy et al., 2002) and biofortification (genetic manipulation) (Khush et al., 2012) have been developed to alleviate deficiencies. In recent years, dietary interventions, including the use of iron-fortified and biofortified functional foods, have played a vital role in addressing iron deficits. Among these staple varieties, Wheat (*Triticum aestivum*) is one of the most significant cereal crops grown worldwide, serving as a staple for nearly 2.5 billion people. It is of great interest due to its high consumption as a nutritional food with greater iron bioavailability compared to other staple foods (Arif et al., 2010). Special efforts have been initiated by the Indian Council of Agricultural Research (ICAR) to enrich wheat varieties like HI-8663 and HI-1605 with iron, reaching levels of about 40-43 parts per million (ppm), significantly higher than normal varieties. This biofortified form assumes great significance in achieving the nutritional security of the country (Kumar et al., 2020). When routinely consumed, fortified/biofortified foods can safely and effectively increase iron levels in the body, promoting human health through nutritional advantages. (Bouis and Saltzman, 2017). The Food Safety and Standards Authority of India (FSSAI) recommends daily iron intake of approximately 17 mg for adult men, 21-35 mg for women, 5 mg for infants, and 9 to 32 mg for boys and girls (ICMR, 2010; Chekraverthy et al., 2023). Therefore, there is a need to quantify the iron content in food products such as cereal crops, which are consumed daily. The fortification and biofortification strategies applied to various wheat varieties are expected to significantly improve the mineral content, specifically iron content. It is hypothesized that these strategies will lead to an increase in the iron/mineral content of wheat varieties, potentially contributing to a reduction in iron deficiency anemia and promoting overall human health. In this context, several techniques have been evaluated, including ultraviolet-visible spectrophotometry (UV/Vis), Atomic absorption spectrophotometry (AAS),

Table 1: Overview of the literature for assessment of quality parameters and minerals in wheat and wheat flour

SN	Methodology	Sources
1	Determination of iron in different types of wheat flours	Ihsanullah <i>et al.</i> , 2002
2	Quality assessment of different iron-fortified wheat flours	Arif <i>et al.</i> , 2010
3	Determination of iron species in samples of iron-fortified food	Niedzielski <i>et al.</i> , 2014
4	A comparative assessment of several quality parameters of normal commercial wheat flour in Bangladesh	Saeid <i>et al.</i> , 2015
5	Determination of iron content in wheat flour of organized and unorganized sector	Neerja Usha Kujur <i>et al.</i> , 2019
6	Profiling of nutritional traits in indigenous wheat cultivars	Parvez <i>et al.</i> , 2019
7	Evaluation of iron content in bakery flour samples of Tehran, Iran	Sara Mohamadi <i>et al.</i> , 2023
8	Proximate values and elemental analysis in wheat and soybean using inductively coupled plasma mass spectrometry	Kowmudi <i>et al.</i> , 2023

Inductive coupled plasma- mass spectrophotometry (ICP-MS) for mineral quantification, and near-infrared spectroscopy (NIR) for predicting the mineral content and proximate composition of fortified, biofortified, and normal wheat flours. Although extensive studies have been conducted on the proximate properties of functional wheat varieties globally, limited information is available on Indian biofortified and fortified wheat varieties (Raquel *et al.*, 2013; Poudel and Bhatta, 2017). An overview of the literature for the assessment of quality parameters and minerals in wheat and wheat flour is presented in Table 1.

NIR, a rapid, non-destructive tool, is used as a secondary method for predicting the proximate and mineral composition in wheat samples by developing accurate models. NIR spectrum combined with chemometric regression models, such as the partial least square regression model (PLSR), provides accuracy, with a coefficient of determination ( $r^2$ ), for predicting sample properties (Shun *et al.*, 2022). Limited research has been carried out to compare biofortified and fortified wheat varieties, and there is a gap in the scientific literature concerning NIR calibration development and the prediction of wheat flour composition (Iván *et al.*, 2023). This research work signifies a nutritional strategy to combat iron deficiencies by incorporating different wheat varieties into the diet and focuses on comparing and predicting the composition in terms of proximate and mineral content of various wheat varieties. The current study emphasizes proximate composition along with a comparative analysis of iron content in fourteen different wheat samples (i.e., biofortified, fortified, and normal wheat) using various spectrometric methods. Prediction of mineral and proximate composition is achieved using NIR

spectroscopy combined with chemometrics. The study places specific emphasis on the proximate composition and iron/mineral content of different wheat varieties. To achieve these objectives, various spectrometric methods were employed to analyze the wheat samples. The study was conducted at JSS College of Pharmacy, Ooty, Tamil Nadu, and DRDO-Defence Food Research Laboratory, Mysuru, India, during 2022-2023.

## MATERIALS AND METHOD

### *Samples collection and preparation*

Biofortified (BW) wheat varieties, specifically HI-8663 and HI-1605, as well as normal whole wheat varieties (NW) including HI-803, GW-H57, H-8753, LOK-303, and LOK-807, were sourced from various cultivars across India. Additionally, commercially available fortified (F) and normal wheat flours (NF) were obtained from a local market in Tamil Nadu. The research was conducted collaboratively at JSS College of Pharmacy in Ooty, Tamil Nadu, and the DRDO-Defence Food Research Laboratory in Mysuru, India. The collected samples were meticulously cleaned and allowed to air-dry for 48 hours before being processed using a laboratory pulverizer machine (sieve size - 1). Each flour sample was carefully stored in airtight containers at -4 degrees Celsius ( $^{\circ}\text{C}$ ) until further use. All chemicals utilized in this study were of analytical grade and procured from Sigma-Aldrich, India. Deionized and double-distilled water were employed throughout the entire research process. The proximate composition and mineral quantification were determined using established methods provided by the Association of Official Analytical Chemists (AOAC). For the prediction

of wheat flour composition, near-infrared spectroscopy (NIR) combined with chemometric techniques was employed.

#### *Instruments*

The essential mineral contents were determined using UV/Visible spectrophotometry (Shimadzu UV 1700), Atomic absorption spectrophotometry AAS (Shimadzu Atomic Absorption Spectrophotometer AA 6300), Inductive coupled plasma-Mass spectrophotometry (Nexion 300/350 ICP-MS) and NIR spectrometer (Brimrose luminar 5030) for prediction of wheat flour composition.

#### *Determination of proximate composition*

The analysis of wheat samples encompassed the assessment of moisture, ash, fiber, fat, protein, alcoholic acidity, gluten, and carbohydrate. In brief, the moisture content of the samples was determined through oven drying at 130°C for 2 hours until a constant weight was achieved, expressed as a percentage (Parvez, 2019). Following the determination of moisture content, the oven-dried samples were incinerated in a muffle furnace and maintained at a temperature of 550-600°C until grey ash was obtained. The ash value was then weighed according to the AOAC method (AOAC, 1995). Subsequently, the gluten content was calculated in its dried form, adhering to the AOAC procedure (AOAC, 2002; Kowmudi *et al.*, 2023). Protein extraction was performed using an alkaline extraction method, and quantification was achieved using Bovine serum albumin as a protein standard (Raghuramulu *et al.*, 1983; Plummer, 1988; Mæhre *et al.*, 2018; Samimi and Validov, 2018). The determination of fat content was conducted using the Soxhlet apparatus, employing petroleum ether as an extraction solvent. The extraction process was continued for 6 hours, followed by solvent evaporation and subsequent fat content calculation (AOAC, 2000). Fiber content was determined after fat extraction, utilizing petroleum ether as a solvent, and followed by acid and alkali condensation. Alcoholic acidity was determined following AOAC methods (AOAC, 2005). Lastly, carbohydrate content was calculated using a distinct method, namely: Total Carbohydrate = 100 - (moisture% + ash% + fiber% + fat% + protein%) (Owusua *et al.*, 2022).

#### *Determination of mineral content*

Iron analysis was conducted on 14 samples using Atomic Absorption Spectrophotometry (AAS) with hollow cathode lamps and an air-acetylene mixture. For this analysis, the samples were dry ashed in a muffle furnace at 525°C for 6 hours until white or grey ash was formed. The ash was then dissolved in 0.1N HCl, diluted to 50ml with deionized water, and the analysis was carried out. The fourteen wheat samples underwent mineral estimation, including Iron (Fe), Potassium (K), Magnesium (Mg), Calcium (Ca), Sodium (Na), Zinc (Zn), Manganese (Mn), Copper (Cu), Chromium (Cr), and Selenium (Se), using Inductive Coupled Plasma-Mass Spectrometry (ICP-MS). The following instrumental conditions were maintained: 1L/min Ar gas flow, He nebulizer gas flow of 0.98 L/min, a spray chamber temperature of 2°C; plasma power of 1600W; sample aspiration rate of 300µl/min (Kowmudi *et al.*, 2023). The method was optimized using a National Institute of Standards and Technology (NIST®) 1567b wheat flour as a standard reference material. Given that the selected biofortified and fortified wheat samples are enriched with iron, a comparative assessment of iron content was conducted and reported using different spectrometric techniques in accordance with AOAC official methods (Elemental Analysis Manual, 2015). Microwave digestion was also performed for the sample digestion using 5ml of nitric acid and 2ml of hydrogen peroxide following the digestion program as mentioned in Table 2. The samples underwent 30 minutes of heating followed by 15 minutes of cooling and were then diluted to 50ml using deionized water (Elemental Analysis Manual, 2015). The present work involved a comparative assessment of iron content in different wheat samples using three different spectrometric techniques. Iron quantification was achieved using UV spectrophotometry (Shimadzu UV 1700) in reaction with 2, 2'-bipyridyl in an acetate buffer environment at pH 4.5, employing a photometry wavelength of 520 nanometers (nm). This was followed by Flame Atomic Absorption Spectrophotometry (AAS) (Shimadzu Atomic Absorption Spectrophotometer AA 6300) equipped with standard hollow cathode lamps as a radiation source with air-acetylene flames, Inductive Coupled Mass Spectrometry (NexION 300/350 ICP-MS), and NIR for prediction. The aforementioned

Table 2: Microwave digestion program for iron estimation

Step	Power (W)	Ramp (min.)	Hold (min.)
1	500	01	04
2	1000	05	05
3	1400	05	10
4	00	-	15

spectrometric techniques have significant implications for the agricultural industry, particularly in terms of crop yield forecasting. These techniques enable the analysis of various minerals based on their spectral characteristics. In the context of crop yield forecasting, they provide valuable insights into plant health, growth, and crop quality, allowing for early detection of mineral shortages and quality assessments. These techniques empower farmers to make data-driven decisions that can lead to higher yields and sustainable farming practices

#### *NIR spectroscopy and chemometric methods*

NIR spectra were collected from a total of 15 wheat flour samples, which included NIST 1567b, biofortified, fortified, and normal wheat flours, for calibration development. The samples were analyzed using a Brimrose Lumina 5030 NIR spectrometer. NIR spectroscopy is a rapid and non-destructive technique (Shun *et al.*, 2022) employed for predicting wheat flour composition. The selected spectral range was between 1100-2100nm intervals, and the diffuse reflectance signal of the NIR spectrum is represented as  $\log(1/R)$ , where (R=reflectance) (Uma *et al.*, 2020). Calibration was carried out using the spectra of the samples and their respective proximate and mineral content, which had been analyzed in the laboratory. The obtained spectra were complex due to overtone and combinational vibration bands. To comprehend the intricate nature of the NIR spectra and predict the mineral and proximate composition, Chemometric analysis was employed. The spectral data underwent Chemometric treatment using The Unscrambler X 10.5 (64-bit) software. The Savitzky-Golay Algorithm was applied to minimize noise in the spectrum data, smoothen and enhance signals containing chemical information associated with the samples, and improve the calibration model building (Zhenjiao *et al.*, 2022). From the NIR data, in combination with the reference data, predictive models were developed using Partial Least Squares Regression (PLSR) to

assess the performance of the developed models. The impact of wavelength range and spectral pre-processing methods on the predictive ability of the model is discussed for individual parameters, and the best models were selected.

#### *Statistical analysis*

All measurements were carried out in triplicate for each of the samples and results were expressed as mean  $\pm$  values of standard deviations using Microsoft Excel, 2016, IBM SPSS statistic viewer, and the Unscrambler X 10.5 (64-bit) software.

## RESULTS AND DISCUSSIONS

### *Proximate composition of BW, NW, F, and NF wheat varieties*

The proximate composition of the wheat samples are represented in (Table 3) as Mean $\pm$ SD, and statistical analysis with positive correlation revealed significance at  $p < 0.01$ . The moisture content of the wheat samples ranged from 1.1% to 4.5%, with BW having the highest moisture content at 4.5% and Fortified flours showing the lowest moisture content of 1.1%. These results align with the standard FSSAI value for wheat and flour, which is  $< 14\%$  by weight. Moisture content is critical for maintaining the quality and shelf life of finished products, as lower moisture levels enhance storage stability, prevent mold formation, and inhibit other biochemical processes. The protein content ranged from 18.0% to 22.6%, with FF having the highest protein content at 22.6%, while BW had a protein content of 18.0%. Higher protein content indicates firmer dough, while lower protein content results in softer dough. The protein content found in the wheat flour samples was within acceptable limits, although it exceeded the average amount stated in the Indian Food Composition Table (IFCT, 2017). This discrepancy may be attributed to the higher protein content in the endosperm compared to wheat bran (Uauy *et al.*, 2006). Gluten content in the wheat samples varied from 6.3% to 10.3%, with

Table 3: Proximate composition of different wheat samples (%)

SN (Lab code)	Moisture	Ash	Fibre	Fat	Protein	Alcoholic acidity	Gluten	Carbohydrate
NW1	4.3± 0.05	1.5±0.05	1.36±0.05	0.56±0.05	20.43±0.05	0.08± 0.011	7.56± 0.05	71.6±0.05
NW2	3.66±0.05	1.2±0.01	1.13±0.05	0.60±0.01	21.4±0.05	0.08± 0.005	6.86± 0.05	72.0±0.05
NW3	4.4± 0.05	1.4± 0.05	0.83±0.05	0.26±0.05	20.1±0.05	0.08± 0.005	6.6±0.05	73.0±0.05
NW4	3.5±0.05	1.7±0.05	1.36±0.05	0.36±0.05	18.4±0.05	0.08±0.005	7.2±0.05	74.2±0.05
NW5	4.4±0.05	1.5±0.05	1.1±0.05	0.33±0.05	20.4±0.05	0.08±0.005	7.5±0.05	72.3±0.05
BW1	4.1±0.05	1.4±0.02	1.21±0.01	0.31±0.01	18.0±0.05	0.08±0.005	6.33±0.05	75.2±0.05
BW2	4.5±0.01	1.41±0.01	1.34±0.03	0.31±0.01	21.5±0.01	0.08±0.005	7.0±0.05	71.1±0.05
NF1	4.4±0.05	0.90±0.005	1.40±0.011	0.40±0.011	21.3±0.05	0.08±0.005	8.1±0.05	71.4±0.05
NF2	3.31±0.05	0.90±0.005	1.4±0.011	0.40±0.011	21.3±0.05	0.08±0.005	8.1±0.05	71.4±0.05
NF3	3.03±0.05	1.16±0.05	0.66±0.05	0.36±0.05	21.5±0.05	0.08±0.005	6.3±0.05	73.2±0.05
NF4	3.3±0.05	1.53±0.05	0.36±0.05	0.36±0.05	22.1±0.05	0.05±0.005	10.1±0.05	72.4±0.05
F1	1.13±0.05	1.5±0.05	0.33±0.05	0.33±0.05	22.6±0.05	0.05±0.005	10.3±0.05	74.1±0.05
F2	1.8±0.05	1.3±0.05	0.33±0.05	0.36±0.05	22.3±0.05	0.07±0.005	8.3±0.05	73.8±0.05
F3	1.8±0.05	1.3±0.05	0.36±0.05	0.33±0.05	21.6±0.05	0.04±0.005	6.6±0.05	74.4±0.05

\*NW-Normal wheat, BW- Biofortified wheat, NF-Normal flour, FF- Fortified flour

Values were presented as mean± standard deviation, n = 3. The data show a significant difference ( $p < 0.01$ ) between the different wheat varieties.

BW having the lowest content at 6.3% and NF at 10.3%. Gluten is responsible for the elastic properties and elongation of dough. The research findings comply with regulations stating that the weight of gluten in wheat flour should not be less than 6.0%. Fat content in wheat flour ranged from 0.3% to 0.6%, with NW having the highest fat content at 0.6%, while other samples had a content of 0.3%. These values fall within the acceptable range according to Indian Food Composition Table (IFCT) standards. Fat content is higher in whole wheat bran and germ but lower in flour, and variations can occur due to milling processes and differences in extraction rates (Adams *et al.*, 2002; Nisar *et al.*, 2020). The ash content in wheat varied from 0.9% to 1.7%, with NW having the highest ash content at 1.7% and NF at 0.9%. The total ash content of wheat flour should not exceed 2.0%

by weight, and the present data aligns with FSSAI regulations. Ash content is nutritionally significant as it contains minerals, but excessive ash levels can lead to undesirable darkening of dough and food products. Dietary fiber plays a crucial role in slowing the rate of glucose breakdown and absorption, maintaining glucose levels, and promoting continuous carbohydrate breakdown. The crude fiber content in different wheat flours ranged from 0.3% to 1.4%, with FF having the lowest fiber content of 0.3% and BW at 1.4%. National guidelines dictate that crude fiber in wheat flour should not exceed 2.5%. The alcohol acidity of wheat flour samples ranged from 0.04% to 0.08%, with NF and FF having the highest alcohol acid content at 0.08%. These values are within the permissible limit of 0.18 percent by weight set by FSSAI regulations. Carbohydrates are a significant source of

Table 4: Determination of iron content in wheat samples by UV/Vis, AAS, and ICP MS

Sample lab code	UV/ visible	AAS	ICP MS
	Expressed in mg/100g	Expressed in mg/100g	Expressed in mg/100g
NW1	4.4±0.05	4.2±0.02	4.2±0.05
NW2	1.6±0.05	1.7±0.05	2.0±0.05
NW3	0.7±0.05	0.7±0.005	0.84±0.005
NW4	0.7±0.05	0.74±0.005	0.81±0.005
NW5	2.90±0.005	2.24±0.005	2.86±0.05
BW1	6.33±0.05	6.74±0.011	6.86±0.05
BW2	6.13±0.05	6.24±0.005	6.27±0.01
NF1	2.16±0.05	2.23±0.05	2.36±0.05
NF2	2.23±0.05	2.06±0.05	2.46±0.05
NF3	1.4±0.05	1.24±0.01	1.23±0.005
NF4	1.4±0.05	1.43±0.05	1.53±0.05
F1	1.66±0.05	1.63±0.05	1.72±0.011
F2	4.56±0.05	5.1±0.01	5.33±0.05
F3	2.76±0.05	2.56±0.05	3.16±0.05

\*NW-Normal wheat, BW- Biofortified wheat, NF-Normal flour, FF- Fortified flour

Values were presented as mean± standard deviation, n = 3. The data show a significant difference ( $p < 0.01$ ) between the different wheat varieties.

energy and should be present in high concentrations in breakfast and weaning recipes. Carbohydrate content in various flours ranged from 71.0% to 75.2%, with BW showing the highest carbohydrate content at 75.2% and NF at 71.1%. National standards have not yet established specific criteria for wheat flour's carbohydrate content, although regular wheat flour typically contains around  $64.17 \pm 0.32$  or  $64.72 \pm 1.7$ , as stated in IFCT, 2017.

#### Comparative Iron quantification using three different instrumentation techniques

Mineral analysis using spectrophotometric methods plays a crucial role in predicting wheat composition by measuring the absorbance of light at different wavelengths. When applied to mineral analysis in agriculture, it provides insights into nutrient content in plants, which are crucial for affecting crop yield, nutrient assessment, optimal nutrient management, and early detection of deficiencies. Following the methodology described above, the iron content in fourteen samples of wheat and wheat flour has been presented (Table 4). Considering the samples analyzed by UV, AAS, and ICP-MS, the overall iron concentration ranged from 0.7 mg/100g to 6.8 mg/100g in UV, 0.75 mg/100g to 6.8 mg/100g in AAS, and 0.81 mg/100g to 6.8 mg/100g in ICP-MS. Both BW and FF were found to have high iron content compared to NW and NF. For example, BW1 had iron content of 6.3 mg/100g, 6.74 mg/100g, and 6.86 mg/100g in UV, AAS, and ICP- MS, respectively. BW2 had iron content

of 6.1 mg/100g, 6.24 mg/100g, and 6.27 mg/100g in UV, AAS, and ICP-MS, respectively. FF2 showed iron content of 4.56 mg/100g, 5.1 mg/100g, and 5.3 mg/100g in UV, AAS, and ICP-MS, respectively. As per IFCT standards, wheat and wheat flour should contain  $4.10 \pm 0.67$  mg/100g and  $3.97 \pm 0.78$  mg/100g, respectively. In comparison to these standards, the functional foods BW and FF had higher iron content than NF. Moreover, all the methods/techniques used in this study to quantify iron content were positively correlated, and the correlation was significant at  $p < 0.01$ . The samples were subjected to ICP-MS for mineral estimation because the presence of other minerals may promote or inhibit iron absorption, which also plays a vital role in human health and helps in the prevention of various deficiency disorders. Mineral analysis of NIST®1567b wheat flour was carried out to optimize the quantification method, and the limit of detection (LOD) and limit of quantification (LOQ) were determined and compared with the certified reference values, as shown in (Table 5). A study of the mean elemental composition of wheat samples showed that K, Mg, Ca, and Na were the most abundant elemental species. K values were higher in the flour samples, whereas Cr and Se values were lower in all the samples. All varieties of wheat flour exhibited maximum mineral content, but in the case of NF4, mineral content was found to be in trace amounts, and some minerals were not detected. These variations may be attributed to wide variations in soil and environmental conditions during crop cultivation

Table 5: Mineral analysis of NIST®1567b wheat flour using ICP-MS

	LOD $\mu\text{g/g}$	LOQ $\mu\text{g/g}$		
Na	0.010	0.500	6.71±0.21	6.83±0.07
Ca	0.010	0.500	191±3.3	200.01±3.09
Mg	0.010	0.500	398±12	407±6.34
K	0.010	0.500	1325±20	1334±11.8
Cr	0.001	0.008	NA*	0.04±0.01
Mn	0.001	0.008	9.00±0.78	8.95±0.37
Cu	0.050	0.100	2.03±0.14	1.92±0.06
Zn	0.050	0.100	11.6±0.26	11.8±0.53
Fe	0.050	0.100	14.1±0.33	13.8±0.37

\*NA: Not available

and warrant further investigation. This study revealed that the concentration of minerals followed the order of  $\text{K} > \text{Mg} > \text{Ca} > \text{Na} > \text{Fe} > \text{Zn} > \text{Mn} > \text{Cu} > \text{Cr} > \text{Se}$ . The data on mineral contents in fourteen wheat samples are presented in Table 6. Mineral quantification by ICP-MS was found to be statistically significant at  $p < 0.01$ . As per standard procedures, the present study was conducted to estimate the proximate composition along with iron determination in all fourteen wheat samples. Components like carbohydrates, protein, fat, moisture, etc., were determined and then compared to Indian food standard data (IFCT, 2017). To date, very few analytical techniques have been reported using UV-spectrometry and Atomic Absorption Spectrometry for iron estimation and proximate composition analysis. Limited information is available for a comparative approach to the nutritional and iron composition of fortified and biofortified Indian- based wheat cultivars. For instance, Saeid *et al.*, (2015) investigated different nutritional parameters comparatively in various commercially available wheat flours in Bangladesh, following AOAC protocols. Similarly, Niedzielski *et al.*, (2014) determined iron levels in food samples using UV and AAS techniques. However, there is limited research on the comparative analysis of mineral content in cereal crops. In another study, Akinyele and Shokunbi (2015) conducted a comparative extraction study comparing dry ashing and wet digestion methodologies. Additionally, Arif *et al.*, (2010) emphasized the importance of fortification in the quality assessment of iron in different fortified wheat flours, yielding promising results. Furthermore, Hailu Kassegn (2018) determined the proximate composition and bioactive components of Abyssinian purple wheat. However, no quantification was performed to compare BW

or FF wheat samples in this study. In this study, a comparative assessment of iron estimation was carried out in different functional wheat varieties using higher and more sophisticated instruments. The current study also showed better recovery and less contamination during digestion methods for analysis in wheat samples. Furthermore, it was observed that BW and FF showed significantly higher iron content when compared to the normal samples. The present analytical procedure is very reliable and sensitive in iron analysis, allowing for easy and quick assessment of bioactive components. The results of the analyte were compared with standard regulatory data, and they agreed with the standard reported values. This study highlights the effectiveness of BW and FF wheat by promising an increase in nutritional levels upon human consumption.

#### NIR spectrum and chemometric analysis

NIR spectroscopy is a powerful analytical technique for predicting mineral and wheat composition due to its non-destructive and rapid nature. The samples were scanned to record the reflectance spectra over a range of NIR wavelengths. A calibration model was developed that correlates with the known mineral content of the wheat samples, allowing the prediction of the mineral content of unknown samples by analysing their NIR spectra. When compared with traditional methods of analysis, NIR spectroscopy offers speed, efficiency, non-destructiveness, simultaneous multiple compound analysis, and accuracy. It can provide accurate predictions for various wheat yield components, which are mainly influenced by the quality of the calibration and instrument performance. The accuracy of the prediction was influenced by advanced chemometric



Table 6: Determination of mineral contents in wheat samples by ICP-MS (ppm)

SL. No.	Na	Ca	Mg	K	Cr	Mn	Cu	Zn	Se
NW1	5.1±0.05	1.27±0.005	3.34±0.005	20.63±0.005	0.01±0.005	0.113±0.005	0.03±0.005	0.113±0.005	0.02±0.005
NW2	5.52±0.005	4.95±0.005	26.3±0.011	90.46±0.005	0.021±0.001	0.41±0.005	0.061±0.001	0.40±0.011	0.02±0.005
NW3	5.64±0.005	5.06±0.005	28.9±0.011	110.5±0.005	0.02±0.005	0.40±0.011	0.06±0.001	0.45±0.005	0.02±0.002
NW4	6.52±0.005	10.6±0.01	54.6±0.011	196.9±0.005	0.03±0.001	1.23±0.005	0.14±0.005	1.04±0.005	ND
NW5	6.05±0.01	5.31±0.005	25.8±0.01	100.3±0.005	0.021±0.001	0.41±0.005	0.060±0.001	0.48±0.005	ND
BW1	6.32±0.005	5.32±0.005	25.7±0.005	90.12±0.005	0.02±0.005	0.45±0.005	0.05±0.005	0.40±0.011	ND
BW2	3.01±0.005	3.03±0.005	24.4±0.005	113.3±0.01	0.033±0.005	0.33±0.005	0.07±0.005	0.54±0.005	0.020±0.001
NF1	6.7±0.005	7.15±0.005	29.3±0.005	101.8±0.005	0.030±0.001	0.823±0.005	0.066±0.005	0.443±0.005	0.020±0.001
NF2	5.93±0.005	5.54±0.005	25.5±0.005	95.5±0.005	0.020±0.0011	0.65±0.005	0.07±0.005	0.44±0.005	0.020±0.001
NF3	6.84±0.01	10.7±0.005	19.9±0.005	279.8±0.005	0.04±0.005	0.27±0.005	0.090±0.0011	0.17±0.005	0.020±0.005
NF4	0.07±0.005	0.36±0.005	0.103±0.005	0.25±0.005	ND	0.02±0.005	ND	0.036±0.005	0.033±0.005
F1	0.74±0.005	0.28±0.005	0.56±0.005	5.81±0.005	ND	0.02±0.005	ND	0.010±0.005	ND
F2	0.61±0.01	0.92±0.005	1.98±0.005	46.7±0.005	ND	0.02±0.005	0.010±0.005	0.010±0.005	ND
F3	0.74±0.005	1.17±0.005	1.97±0.005	48.56±0.005	ND	0.030±0.0011	0.010±0.005	0.010±0.005	ND

\*NW-Normal wheat, BW-Bio-fortified wheat, NF-Normal flour, FF-Fortified flour, ND- Not detected

Values were presented as mean± standard deviation, n = 3. The data show a significant difference ( $p < 0.01$ ) between the different wheat varieties.

statistical analysis. The NIR spectrum of the samples was observed in the range of 1100-2100nm. The peak absorbance spectrum of the samples was obtained by the system, and chemometrics were applied for statistical analysis of the spectrum and laboratory-analyzed data. The obtained raw data was plotted in Fig. 1 as a line graph, which was further processed using the Savitzky Golay method for spectrum smoothing (Zhenjiao et al., 2022). Descriptive statistics of the data, as shown in Figs. 2 and 3 and

Table 7, indicated a higher standard deviation in K, Carbohydrate, Protein, and Mg compared to other parameters' data. Furthermore, PLSR analysis was applied along with the Nonlinear Iterative Partial Least Squares (NIPLAS) algorithm. It was observed that the first three factors were sufficient to predict the mineral and proximate composition of the wheat flour, as represented in Figs. 4 and 5. The PLSR analysis was carried out by considering factor 1 and 2 scores. NBW4, BW2, NB5, and NBW2 showed a positive

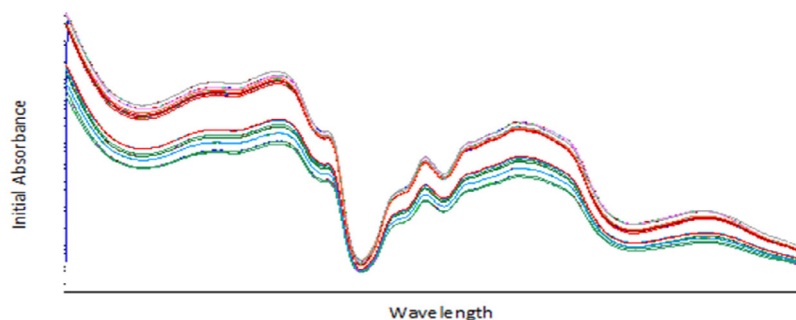


Fig. 1: Line plot of the raw sample spectrum data

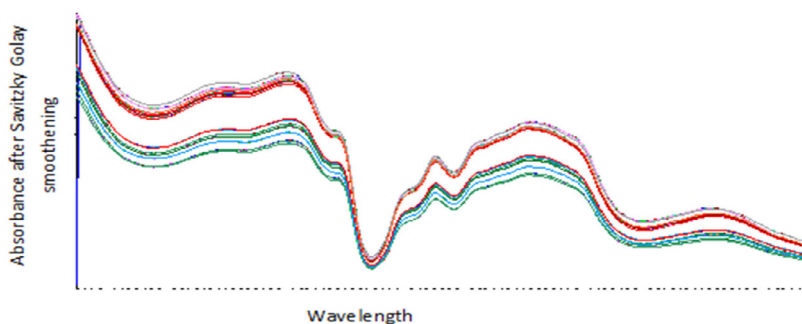


Fig. 2: Line plot after smoothing of the raw data by Savitzky Golay

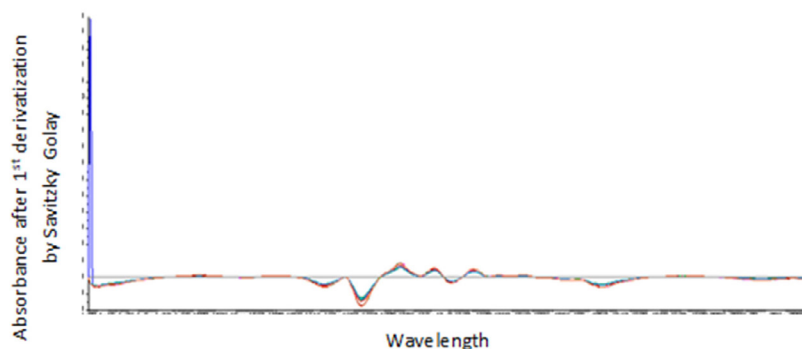


Fig. 3: Line plot after 1<sup>st</sup> derivatization by Savitzky Golay

correlation with each other and had coefficient loadings that predicted higher amounts of Moisture, Ash, Fiber, Carbohydrate, Fat, Alcoholic acidity, Zn, Mg, Fe, Ca, Na than other parameters. On the other hand, NB3, BW1, NBW1 showed a positive correlation with each other and were negatively correlated with other samples. They predicted higher amounts of Mn, Gluten, and Protein than other parameters. NFW1, NF3, NF4, FW3 showed a good correlation with high Moisture, Ash, Fiber, Carbohydrate, Fat, Alcoholic acidity, Zn, Mg, Fe, Ca, Na. NF2, FW1, 1567b, FW2 had a good correlation with high Protein, Gluten, Mn and were negatively correlated with the other independent variable samples.

The correlation coefficients between NIR predictions and reference analysis varied across different parameters. The lowest correlation

coefficient was observed in the prediction of fat content, with an  $r^2$  value of 0.826770, while a notably higher correlation coefficient of  $r^2 = 0.98385$  was found for carbohydrates, as detailed in Table 6. It is generally considered that NIR estimation is robust when the correlation coefficient between NIR readings and the reference method exceeds 0.75. Based on this criterion, it can be concluded that there was an acceptable relationship between observed and predicted values for all calibration parameters. Notably, the NIR data indicated that BW2 and FW3 predicted the highest iron content compared to the other samples, which aligns with the quantification results obtained using UV, AAS, and ICP-OES. These results demonstrate that the developed models are capable of accurately predicting the composition of the wheat samples.

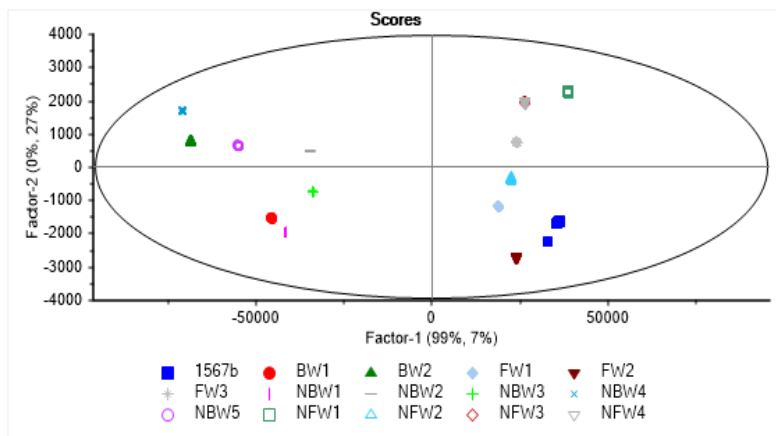


Fig. 4: Scores correlation between the different wheat flour samples

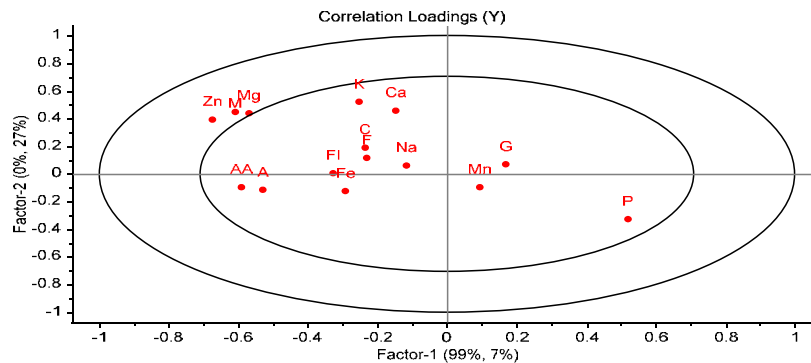


Fig. 5: Correlation loadings of the samples representing different parameters in the sample

Table 7: Standard deviation and correlation coefficient of the sample data by descriptive statistics.

Sl no.	M	A	FI	F	Pr	AA	G	Cbh	Fe	Na	Ca	Mg	K	Mn	Zn
Mean	3.0	1.3	0.9	0.3	22.2	0.07	7.5	72.1	2.26	4.46	4.26	15.8	81.1	0.50	0.26
Max	4.5	1.8	1.75	0.6	31.7	0.095	10.3	75	5.51	9.05	10.76	54.65	279.84	2	1.04
Min	1.1	0.9	0.3	0.3	18	0.04	5.5	63.89	0.11	0.08	0.29	0.1	0.26	0.02	0.01
Range	3.4	0.9	1.45	0.3	13.7	0.055	4.8	11.11	5.4	8.97	10.47	54.55	279.58	1.98	1.03
SD	1.23	0.23	0.50	0.09	3.34	0.01	1.42	2.93	1.49	2.95	3.32	14.8	76.9	0.58	0.26
r <sup>2</sup>	0.969	0.961	0.961	0.8267	0.895	0.9178	0.8494	0.9838	0.9359	0.9117	0.9505	0.927586	0.94208	0.842214	0.92188

M- moisture; A- ash; FI- fiber; F- fat; Pr- protein; AA- alcoholic acidity; G- gluten; Cbh- carbohydrate; Fe- iron; Na- sodium; Ca- calcium; Mg- magnesium; K- potassium; Mn- manganese; Zn- zinc; SD- standard deviation.

## **CONCLUSION**

Micronutrient deficiency, often referred to as hidden hunger, remains a widespread concern in populations, particularly among those who rely on undiversified plant-based diets. Adequate nutrition, including essential micronutrients, is pivotal for addressing malnutrition, particularly in vulnerable populations such as children and women who primarily consume plant-based diets. Iron, a crucial nutrient, plays a fundamental role in various biological processes. Nutritional strategies such as fortification and biofortification hold significant potential not only in alleviating micronutrient deficiencies but also in contributing to broader goals of sustainable agriculture and environmental stewardship. This research aims to make a meaningful contribution to the fight against iron deficiencies by thoroughly investigating the nutritional profiles of various wheat varieties, with a particular focus on their iron content. Such investigations have the potential to inform dietary strategies and effectively address nutritional deficiencies. Existing research on fortified and biofortified foods has generally yielded promising outcomes, indicating improvements in the nutritional status of participants. Regular assessment and analysis of mineral content in cereals and food products are essential for gaining a deeper understanding of the effectiveness of biofortification and fortification. To the best of our knowledge, this study represents a pioneering effort, especially in the comparative assessment of iron content in regular, fortified, and biofortified wheat varieties using various spectrophotometric techniques. Additionally, it involves the prediction of proximate and mineral composition through NIR spectroscopy and chemometric analysis. Among the spectrophotometric techniques employed, ICP-MS demonstrated exceptional sensitivity in quantifying iron and other trace elements, followed by AAS and UV-Visible spectrophotometry. The study also comprehensively investigated the proximate components in all wheat varieties, which were found to align with Indian dietary requirements. Notably, the biofortified varieties, namely HI8663 and HI1605, exhibited significantly higher iron content compared to fortified and normal wheat varieties. The calibration evaluation parameters and the development of PLSR models resulted in correlation coefficients between reference values and NIR

measurement values falling within the acceptable range. This demonstrates the efficacy of NIR technology in predicting the composition of samples, and these models can be readily applied to unknown samples, providing results comparable to laboratory determinations. The preliminary comparative analysis and NIR data prediction of biofortified and fortified wheat varieties offer valuable insights for identifying suitable varieties that can contribute to mitigating iron deficiencies and related anemia. The incorporation of spectrophotometric techniques into farming practices has the potential to revolutionize decision-making processes, enabling farmers to gain a deeper understanding of their crops' health, growth, and nutritional needs. This, in turn, can lead to more efficient resource management, reduced environmental impact, and ultimately, higher yields and improved economic returns. It is crucial to maintain vigilance regarding micronutrient intake, as some minerals may have adverse effects and be linked to various health problems. Raising awareness of nutrition and fitness needs is essential. In addition to addressing the single-nutrient deficiency at the heart of this research, biofortification and fortification represent sustainable solutions to tackle a range of micronutrient deficiencies that could affect future generations. Therefore, the findings of this study help bridge the intricate relationship between plant-based diets, micronutrient deficiencies, and sustainable agricultural practices, providing valuable insights for enhancing public health.

## **AUTHOR CONTRIBUTIONS**

H.A. Pardhe conducted the research, performed data analysis, and drafted the manuscript. N. Krishnaveni, the corresponding author, made significant contributions to the conceptualization and design of the study, data analysis and interpretation, and manuscript revision. B.K. Chekraverthy contributed to data analysis and manuscript drafting. S. Patel assisted in the procurement of samples and data analysis. S. Naveen provided technical support for the analysis of samples, and V. Rashmi offered technical assistance for spectrometric analysis. P.C. Govinden provided instrumental support

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#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

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#### ABBREVIATIONS

%	Percent
<	Less than
°C	Degree Celsius
µg/g	Microgram/gram
µl/min	Microliter/minute
AAS	Atomic absorption spectrophotometry

AOAC	Association of official analytical chemists
Ar	Argon
BW	Biofortified wheat
Ca	Calcium
Cr	Chromium
Cu	Copper
F	Fortified
FSSAI	Food safety and standards authority of India
H <sub>2</sub> SO <sub>4</sub>	Sulphuric acid
HCl	Hydrochloric acid
He	Helium
ICAR	Indian council of agricultural research
ICP-MS	Inductive coupled plasma-mass spectrophotometry
IFCT	Indian food composition table
K	Potassium
L/min	Litre/minute
LOD	Limit of detection
LOQ	Limit of quantification
Mean±SD	Mean standard deviation
Mg	Magnesium
mg/100g	Milligram/100 gram
mg/day	Milligram/day
Min	Minute
Mn	Manganese
Na	Sodium
nm	Nanometer
NF	Normal flours
NIPLAS	Nonlinear Iterative partial least squares
NIR	Near-infrared spectroscopy
NIST	National institute of standards and technology
NW	Normal wheat
PLSR	Partial least square regression model
PPM	Parts per million
R	Reflectance
r <sup>2</sup>	coefficient of determination
RDA	Recommended daily allowance
Se	Selenium
UV- VIS	UV/Visible spectrophotometry
W	Watts
Zn	Zinc

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