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CASE STUDY

Proximate values and elemental analysis in wheat and soybean using inductively coupled plasma mass spectrometry

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ABSTRACT

BACKGROUND AND OBJECTIVES: Determining food composition and bioactivity is critical to both theoretical and applied research in food science and technology. This is frequently used to determine the nutritional value and overall acceptance of the food by consumers. The current study's goal is to determine the macronutrient, mineral and antioxidant activity of selected wheat and soybean varieties with the potential to be useful in the food industry to develop value-added products that are safe for consumption.

METHODS: The proximate and mineral composition, gluten characterization, total phenolic contents, and antioxidant activity of three wheat cultivars and five soybean cultivars that are indigenous to India were studied.

FINDINGS: Wheat varieties were found to be rich in carbohydrates (65.8-68.8 percent) and gluten (27.2-28.6 percent), whereas soybean varieties were found to be the richest source of protein (32.8-33.7 percent), fat (17.1-17.6 percent), fiber (21.7-28.8 percent), polyphenols (2.76-3.59 milligram gallic acid equivalent per gram, and antioxidant activity (97-123 microgram ascorbic acid equivalent per gram). These samples were also found to have significant content of essential minerals.

CONCLUSION: The tested samples had a high nutritional value and energy content and could be a good source of nutrition for a large population. A comprehensive report on the proximate and mineral composition, total phenolic content and antioxidant activity of the wheat and soybean varieties collected from the Rewa district, India, was reported.

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
68	2	5

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INTRODUCTION

Wheat (*Triticum aestivum)* is among the most significant cereal crops grown worldwide and a staple for nearly 2.5 billion people (Reynolds and Braun, 2022). Wheat is a major source of protein in low- and middle-income countries, as well as a good source of calories and dietary fiber. Wheat consumption lowers the risk of chronic diseases such as obesity, diabetes, cardiovascular disease, and cancer due to bioactive components (Liu *et al*., 2020). Flavonoids, phenolic acids, alk(en) resorcinol's (Ars), benzoxazinoids (Bxs), phytoestrogens, tocopherols and tocotrienols, carotenoids, and other minor components, along with dietary fiber in wheat, have been found to benefit human health (Tian *et al*., 2022). Soybeans (*Glycine max*) have emerged as the "golden bean of the twenty-first century" due to the potential health benefits obtained from consumption (Nwosu *et al.*, 2019). It is an East Asian legume with high protein levels comparable to animal proteins because it is the only vegetable source that contains all essential amino acids (Singh *et al*., 2019). Soybean bioactive components such as isoflavones, peptides, saponins, and phytosterols have been shown to be beneficial to health (Swallah *et al*., 2022). The availability of phytoconstituents and nutritional quality of wheat (Tian *et al*., 2022) and soybeans (Alamu *et al*., 2019) influenced by a variety of factors including genotype, pedoclimatic, processing, and consumption pattern. India is one of the leading producers of wheat and soybeans (MAFW, 2022). Wheat accounts for a significant portion of the food basket in a vastly populated country like India, accounting for 36% of total food grains produced, ensuring not only food security but also nutritional security (Sendhil *et al.*, 2019). Soybean production and consumption in India is expected to increase due to its nutritional value and favorable agro-climatic conditions (Kolar *et al.*, 2021). Given the various health benefits attributed to the bioactive components in wheat and soybeans, there is a growing interest in exploiting wheat and soybeans to develop functional foods, which is facilitated by a thorough understanding of the nutrient profile (macro and micronutrients) variability among different genotypes of wheat and soybean cultivars, which could aid in the identification of raw materials for improved product development. Significant and increasing consumption of wheat and soybean, as well as widespread public distribution in India, is also associated with major concerns such as increased exposure to toxic heavy metals that affects human health (Suman *et al.,* 2020). Indian soybean cultivars are found to be sensitive to phytotoxic ozone levels (Mukherjee *et al*., 2021) and crop heat stress (Sun *et al*., 2019), resulting in lower yield and nutritional status. Mineral composition is as important to human health as bioactive constituents in the maintenance of the enzymatic system, vitamin synthesis, and haemoglobin formation (Kashyap *et al.*, 2022). Wheat and soybeans are rich in essential minerals such as zinc, copper, iodine, manganese, sodium, potassium, calcium, magnesium, iron, and phosphorus (Noori *et al*., 2022). Plants typically absorb different elements from soil and water, with different rates of absorption for each element. Soil is an important component of the biosphere that is exposed to a variety of pollutants, including heavy metals released into the atmosphere by both natural and anthropogenic activities (Kumar *et al*., 2019). The increase in heavy metal content has a biomagnification effect on all organisms. Heavy metals with high transfer coefficients, such as cadmium and lead, are easily absorbed by plants because of technogenic emissions (Chaplygin *et al*., 2018) and mining activities (Wu *et al*., 2021). Madhya Pradesh is one of the leading states in India in terms of wheat and soybean production (DES, 2021), and it also has the second highest number of mines in the country as per the report the Ministry of Mines, Government of India in 2018 (Kumar *et al*., 2019). Heavy metal estimation, as well as elemental composition estimation, is critical in Madhya Pradesh wheat and soybean cultivation, using sensitive and accurate analytical techniques that could help to demonstrate the impact of environmental pollution on the possible bioaccumulation of heavy metals in toxic levels in agricultural produce. Unsustainable agricultural practices, such as excessive nitrogen fertilization, endanger soil fertility and deprive plants of essential elements. To draw possible conclusions on the aspect of functional food development and the influence of genetic variability and environment on the availability of bioactive constituents, it is critical to understand the status of nutrient and elemental composition in wheat and soybeans using well-established protocols (AOAC, 2000). The nutritional quality of a food substance can be determined by evaluating its proximate composition (moisture, ash, protein, fat, fiber, carbohydrates, and total energy) for macro and micronutrient content. To determine the level of heavy metal contamination, the safety of a specific food substance for consumption and consideration for further development could be evaluated using highly sensitive and accurate analytical methodology. Gluten, a protein fraction rich in the multifunctional gliadin and glutenin found in wheat, is an important determinant of wheat quality, imparting cohesiveness, and elasticity to wheat flour (Zhang *et al*., 2022). The elasticity and extensibility of wheat dough are influenced by the quality and quantity of gluten in wheat, which is an important aspect of the baking industry. Gluten content is directly related to wheat grain protein, which is greatly influenced by the soil microclimate, which combines the effects of temperature, water content, and aeration (Schopf *et al*., 2021). The genetic variation among wheat cultivars has a significant impact on the qualitative characteristics of gluten (Amiri *et al*., 2018). Soybeans are the richest source of polyphenols, and since soybean polyphenols have antioxidant properties (Khosravi and Razavi, 2021), the phenolic content of soybeans, and their ability to exhibit anti-oxidant activity, is a major determinant of their nutritional value (Choi *et al*., 2020). Out-of-limit heavy metal concentrations, such as cadmium, copper, lead, and zinc, are determinants of heavy metal contamination and potential risk to human health (Sobhanardakani *et al*., 2016). The current study's aims are to determine the macronutrient, mineral and antioxidant activity of selected wheat and soybean varieties with the potential to be useful in the food industry to develop value-added products that are safe for consumption. This study was conducted in 2021 at the DRDO-Defence Food Research Laboratory in Mysuru, India.

MATERIALS AND METHODS

Samples

Three wheat cultivars: LOK-803, HI-1544, and LOK-273, and five soybean cultivars: Ahilya 1 (NRC-2), Ahilya 2 (NRC-12), Ahilya 3 (NRC-7), Pratap Soya (RKS-18), and Shakti (MAUS-81) were collected from local farms cultivated using conventional farming methods in the Rewa district, Madhya Pradesh, India. collected samples were stored at -4°C in a temperature and humidity controlled cold room (Bluestar, India) until further analysis. Collected samples were powdered using laboratory flour mill (Softel, India) prior to the analysis.

Chemicals and equipment

Sulfuric acid (98%, H_2SO_4), Sodium hydroxide (NaOH), Boric acid (H_3BO_3), 0.1 N Hydrochloric acid (HCl) solution, 6N HCl, sodium chloride (NaCl) and sodium carbonate buffer are were procured from Merck Life Sciences Pvt. Ltd., Mumbai, India. For the elemental analysis, 60% ultra-pure nitric acid, and 31% ultra-pure hydrogen peroxide solution were purchased from Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India. Proximate analysis of the collected samples was performed using the following equipment: hot air oven (Heratherm, Thermo Scientific, Germany), fiber extractor (Fibrethem, Gerhardt, Germany), muffle furnace (Thermotek electric furnace, Mysore), fat extractor (Soxtherm, Gerhardt, Germany), and fully automated kjeldahl analyzer (Kjeltech™ 8400, Foss, Sweden) equipped with a block digestor, neutralizer, and autosampler. The elemental analysis was performed using a Titan MPS Microwave Sample Preparation System (Perkin Elmer, Inc., Waltham, MA, USA) and a NexION Inductively Coupled Plasma Mass Spectrometer (Perkin Elmer, Inc., Waltham, MA, USA).

Gluten content of different wheat cultivars

The gluten extraction was performed in accordance with the protocol as mentioned in American Association of Cereal Chemists (AACC, 2005). About 25 gram (g) of wheat flour were used to make the dough with the 3% sodium chloride solution. For 40 minutes, the dough was immersed in water. The dough was washed with a stream of running water to remove the starch and other soluble, insoluble, and lipid components until a clear wash water was obtained. The isolated gluten was allowed to stand in water for 1 hour before being dried to the maximum extent possible before being weighed to determine the wet gluten content (weight of wet gluten × 100/weight of flour). To determine the dry gluten content (weight of dry gluten × 100/weight of dry gluten), the moist gluten was freeze-dried at -46°C for 48 hours. The minimum gluten content of wheat flour should be around 24% (wet) and 8% (dry) (Singh and Singh, 2006). The physicochemical properties of extracted gluten, such as moisture content (wet and dry gluten), water absorbing capacity (dry gluten), and oil absorbing capacity (dry gluten), were determined. The moisture content (MC, % = Final weight/Initial weight x 100) of wet (MCWG, %) and dry (MCDG,

%) gluten samples was determined by drying them in an oven at 120°C for 4 hours (AACC, 2005). The water absorbing capacity (WAC) of dry gluten was determined by dispersing dry gluten (500 mg) in 10 mL of distilled water, thoroughly mixing, and agitating for 1 hour, and centrifuging the sample at 5000 rpm for 30 minutes (Sosulski, 1962). The supernatant was removed, and the sediment was weighed to determine the water absorbing capacity (WAC, % = weight of sediment/weight of sample x 100). The oil absorption capacity (OAC) of dried wheat gluten samples was determined by reconstituting the dried gluten (500 mg) in 10 mL of mineral oil, mixed thoroughly, and agitated for 1 hour (Lin *et al*., 1974). Then samples were centrifuged at 5000 rpm for 30 min. The supernatant was removed and sediment was weighed to determine the oil absorption capacity (OAC, % = weight of sediment/weight of sample x 100).

Total phenolic content and DDPH antioxidant assay

The Folin-Ciocalteu method was used to determine the total phenolic content of wheat and soybeans (Kumar *et al*., 2009). 0.1 mL of aqueous-acetone extract (70%) was increased made up to 0.5 mL with distilled water. 0.25 mL of Folin-Ciocalteu reagent and 1.25 mL of aqueous sodium carbonate solution (20%) were added, and the solution was vortexed for 1 minute before being left to stand in the dark for 40 minutes. The absorbance at 725 nm of the resulting blue coloration was compared to a blank. The total phenolic content was calculated as a gallic acid equivalent (GAE) and expressed as µg GAE/g dry material by interpolating the calibration curve range of gallic acid standard solutions (0.1 to 1.0 mg/mL). The antioxidant activity of soybeans was evaluated using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical assay (Rai *et al*., 2011). 0.2 mL of aqueous-methanolic (50%) soybean extract was mixed with 2 mL of DPPH solution (0.16 mM) and vortexed for 3 minutes before agitating for 30 minutes at room temperature in the dark. After 30 minutes of incubation, the absorbance at 517 nm was measured with a spectrophotometer, and the DPPH free radical scavenging activity (% = [1-(absorbance of sample - absorbance of blank)/absorbance of control] x100) was expressed as µg of ascorbic acid equivalent (AAE/g) of soybean.

Methodology to perform proximate analysis

The moisture content, ash content, carbohydrate content, nitrogen content, protein content, fiber, total fat, and total available energy of selected wheat and soybean samples were evaluated to determine the proximate composition. The moisture content (%) in the samples was determined using a hot air oven (Heratherm, Thermo Fisher Scientific, Germany) by drying the samples (10 to 12 hours at 90°C) until they reached a constant weight (AOAC, 2000). Fat content (%) in the samples was determined by extraction using petroleum ether in a software controlled Automatic Soxtherm apparatus (C. Gerhardt GmbH and Co. KG, C., Germany) for 4 hours at 60°C-70°C (Noureddini and Byun, 2010). The crude protein was determined using the Kjeldahl method, as described by Nielsen (Nielsen, 2003). The protein content of the samples was determined using a nitrogen-to-protein conversion factor of 5.83 for wheat samples and 5.71 for soybean samples according to 'Jones factors' for conversion of nitrogen to protein (Longvah *et al*., 2017). The crude ash content was determined using a dry ashing method (Thiex *et al*., 2012). The samples were incinerated at 550° C in a furnace (Thermotek electric furnace, Mysore, India) until carbon residue disappeared. The obtained ash was cooled, weighed, and used to determine the total ash content (%). Crude fiber was determined for the digestible portion of the fat free extract which was hydrolyzed away by serially heating it with dilute acid and dilute alkali (AOAC, 2005). The obtained residue was dried and weighed to determine the fiber content (%). The carbohydrate content (%) of a sample was calculated based on the content of protein, fat, fiber, ash, and moisture (AOAC, 2005). The caloric value was calculated by multiplying the percentages of proteins and carbohydrates by a factor of four (kJ/g) and total lipids by a factor of nine (kJ/g).

Protocol to perform elemental analysis using Inductively coupled plasma – mass spectrometry

The Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) (NEXION, Perkin Elmer, Inc., Waltham, MA, USA) with a microwave sample digestor (TITAN MPS, Perkin Elmer, Inc., Waltham, MA, USA) was used in this study. The instrumental conditions: plasma (Ar) gas flow of 15 Liters per minute (L/min); carrier (Ar) gas flow of 1 L/min;

nebulizer (He) gas flow of 0.98 L/min; spray chamber temperature of 2°C; sampler and skimmer cones with nickel tips; plasma power of 1600 W; sample uptake rate of 300 µL/min were maintained throughout the experimentation. NIST® 1567b wheat flour and NIST® 3234 soybean flour standard reference materials (SRMs) were used in this study to validate the method. Prior to ICP-MS analysis, samples were digested as a preliminary step using the previously described method (Bosnak and Pruszkowski, 2014). 1 g sample was weighed accurately and transferred to a pre-cleaned polytetrafluoroethylene (PTFE) sample vessel. Then, 5 mL of 60% ultra-pure nitric acid and 2 mL of 31% ultra-pure hydrogen peroxide solution was added to the PTFE vessel containing sample within to facilitate the digestion of organic matter. The PTFE vessels are loaded onto a microwave digestor operated at high temperature for the sample digestion. At an operating power range of 0-1400 W, the digestion programme included 30 minutes of heating and 15 minutes of cooling. All samples were completely digested, yielding clear solutions that were diluted to a final volume of 50 mL with ultra-pure deionized water. Gold was added to all solutions at a final concentration of 200 µg/L. Blank samples were prepared by microwave digestion of the acid mixture, as previously described. The PerkinElmer pure single and multielement standards were used to calibrate the ICP-MS method (diluted into 10% HNO_3). The calibration standard ranges were determined based on an element's expected nutritional level. Calibration ranges of 0-300 µg/g were considered for high-level nutritional elements such as potassium (K) or sodium (Na); 0-20 µg/g for low/medium-level essential elements such as manganese (Mn) or iron (Fe); and 0-200 ng/g for heavy metals such as lead (Pb) or mercury (Hg). A multielement standard solution containing indium (In), scandium (Sc), germanium (Ge), lithium-6 (^{6}Li), and terbium (Tb) was also introduced online for internal standardisation across the entire mass range. Acetic acid was added to the internal standard solution to compensate for residual carbon from sample digestion.

RESULTS AND DISCUSSION

Gluten content and characterization of dried gluten

The data concerning protein content (%), wet gluten (%), dry gluten (%), moisture content (%), water absorbing capacity (%) and oil absorbing capacity (%) of wheat samples LOK-803, HI-1544 and LOK-273 are presented in Table 1. Gluten content and characteristics are critical factors in determining wheat flour quality (Kulkarni *et al*., 1987). The wet gluten (WG) and dry gluten (DG) contents of the flour from the analyzed wheat cultivars ranged from 27.25% to 28.44% (WG) and 8.08% to 8.51% (DG) with no statistically significant difference (CV = 2.66% and 3.28%) between the cultivars. The WG and DG contents are in accordance with Kaushik *et al.,* 2015, Kaushik *et al.,* 2013, Kumar *et al.,* 2013 and Singh and Singh 2006. The WG and DG contents of wheat cultivars are correlated with the protein content with higher correlation coefficients (r^2 = 0.998 and 0.992), which is consistent with Cho *et al.,* 2018, Başlar and Ertugay 2011, and Kulkarni *et al.,* 1987. Wheat varieties and sowing time have been found to influence gluten content (Coventry *et al*., 2011). Traditional wheat processing methods such as soaking, frying, and parboiling reduce the gluten content (Balamurugan *et al*., 2018). Increased nitrogen treatment was also found to increase gluten content (Cho *et al.,* 2018). The WG content of a wheat flour determines its protein quality and baking quality, while the DG content determines its water absorption capacity. Gluten vitality, an essential aspect of gluten functionality in the baking industry, has been linked to the water absorption capacity (Frederix *et al*., 2004). Freeze-dried gluten extracted from wheat cultivars had water absorbing capacities (WAC) ranging from 257.1% to 286.7%. The WAC of wheat cultivars is highly correlated with the DG content (r^2 = 0.965). The oil absorption capacity (OAC) of wheat flour is an important factor to consider when frying and cooking noodles. The OAC of freeze-dried gluten extracted from wheat cultivars ranged from 260.2% to 283.1% and was found to be correlated with the DG content (r^2 = 0.905). The similarity of WAC and OAC values (r^2 = 0.985) is in accordance with Kaushik *et al.,* 2015; Kaushik *et al.,* 2013; Singh and Singh 2006. When compared to the minimal levels reported by Singh and Singh 2006, the gluten contents (WG and DG) of the examined wheat cultivars are significant, implying their potential industrial application.

Proximate composition of soybean and wheat cultivars

The data concerning the contents of moisture (%), ash (%), protein (%), total fat (%), total fiber (%),

Fig. 1: Wheat varieties collected from the local farms in Rewa district, Madhya Pradesh, India. (a) LOK-803. (b) HI-1544. (c) LOK-273

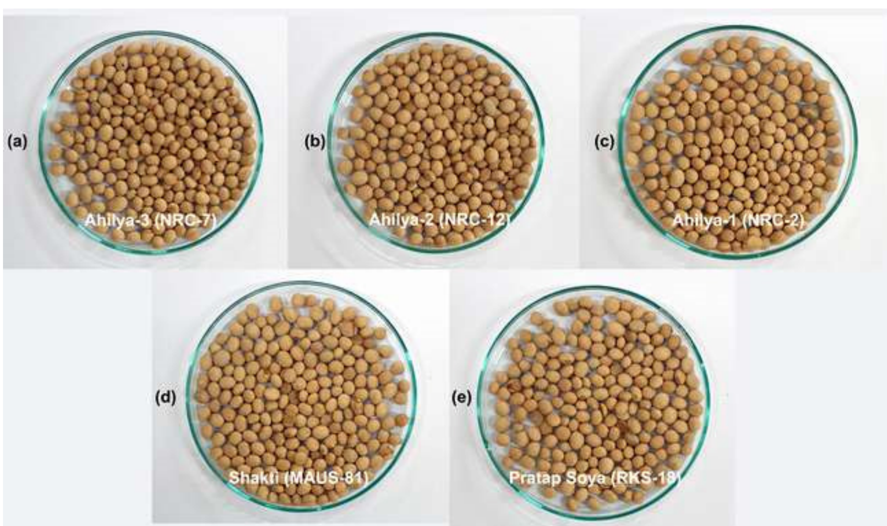

Fig. 2: Soybean varieties collected from the local farms in Rewa district, Madhya Pradesh, India. (a) Ahilya-3 (NRC-7). (b) Ahilya-2 (NRC-12). (c) Ahilya-1 (NRC-2). (d) Shakti (MAUS-81). (e) Pratap soya (RKS-18)

Table 1: Values of protein and gluten composition and gluten characteristics of wheat cultivars

Sample	PC (%)	WG (%)	DG (%)	MCWG (%)	MCDG (%)	WAC (%)	OAC (%)
LOK-803	10.53 ± 0.07	28.44 ± 0.67	8.51 ± 0.15	37.29 ± 0.65	11.56 ± 0.11	279.7 ± 4.89	274.2 ± 3.56
HI-1544	10.58 ± 0.11	28.66 ± 0.63	8.49 ± 0.17	41.75 ± 1.03	12.37 ± 0.15	286.5 ± 3.54	283.1 ± 3.33
LOK-273	10.07 ± 0.14	27.25 ± 0.52	8.08 ± 0.21	32.38 ± 0.73	11.21 ± 0.08	257.1 ± 4.60	260.2 ± 0.82

PC: protein content; WG: wet gluten; DG: dry gluten; MCWG: moisture content of wet gluten; MCDG: moisture content of dry gluten; WAC: water absorbing capacity of dried gluten; OAC: oil absorbing capacity of dried gluten.

Table 2: Proximate values of analysed soybean and wheat cultivars

Sample	Moisture %	Ash %	Protein %	Total Fat %	Dietary fibre %	Carbohydrate %	Energy KJ
Soybean samples							
Ahilya 1 (NRC-2)	4.36	1.31	32.85	17.19	22.31	21.94	1564
Ahilya 2 (NRC-12)	5.53	1.29	33.25	17.13	21.72	21.05	1554
Ahilya 3 (NRC-7)	5.02	1.37	33.49	17.16	22.47	20.46	1549
Pratap Soya (RKS-18)	5.66	1.24	33.21	17.57	28.89	13.41	1441
Shakti (MAUS-81)	5.56	1.29	32.88	17.22	27.54	15.49	1458
Wheat samples							
LOK-803	7.85	1.27	10.54	1.341	10.23	68.75	1377
HI-1544	8.40	1.30	10.58	1.508	9.31	68.88	1386
LOK-273	9.07	1.39	10.07	1.682	10.71	67.07	1354

carbohydrate (%) and energy (KJ) for the analyzed soybean samples: Ahilya-1 (NRC-2), Ahilya-2 (NRC-12), Ahilya-3 (NRC-7), Pratap soya (RKS-18), Shakti (MAUS-81) and wheat samples: LOK-803, HI-1544 and LOK-273 are presented in Table 2. The moisture content of soybean and wheat samples ranged from 4.36% to 5.66% (p<0.05) and 7.85 to 9.07 (p<0.05). The moisture contents of soybean and wheat samples are found to be in close agreement with AL-Amery *et al.,* 2019 and Kassegn, 2018. The lower moisture content of the analyzed soybean and wheat cultivars suggests a longer shelf life (Gebrezgi, 2019). The ash content (an index of inorganic content) of the soybean (1.24% to 1.37%, p<0.05) and wheat cultivars (1.27% to 1.39%, p<0.05) tested was less than the recommended maximum limit of 1.5%. The ash content of the reported soybean samples was lower than the values reported by Bayero *et al.,* 2019. Soybean cultivars (32.85% to 33.49%, p<0.05) contained more protein than wheat cultivars (10.07 to 10.58, p<0.05) tested. The protein content of the soybean samples was consistent with that reported by Gaikwad and Bharud 2017, for other Indian soybean cultivars such as KDS-837, KDS-798, JS-335, and DS-9712. Because soybeans have the highest protein content, they are an ideal candidate for processing into a variety of protein-rich foods such as soy milk, tofu, whey protein isolates, and other products that are becoming increasingly popular as a component of the vegan diet around the world (Hess, 2022). Soya bean meal (SBM) is widely regarded as one of the most important sources of protein and essential amino acids in non-ruminant diets around the world (Ibáñez *et al.*, 2020). The total fat content of soybean and wheat samples ranged from 17.14% to 17.57% (p<0.05) and 1.34% to 1.68% (p<0.05), respectively. The lower content of wheat samples implies that wheat can be stored for an extended period without producing unpleasant odours or oxidizing some fatty acids. Soybeans are found to be rich in carnitine, a bioactive essential fatty acid that has been studied for its role in fat metabolism (Mogensen, 2017). The fiber content of the soybean cultivars tested significantly varied from 21.72% to 28.89%, with Ahilya-2 (NRC-12) having the lowest content and Shakti having the highest (MAUS-81). Wheat cultivars contained less fiber (9.31% to 10.70%, p<0.05) than soybeans. Regular soy consumption has also been shown to help lower total cholesterol and low-density lipoprotein cholesterol (Jenkins *et al.*, 2019). Wheat cultivars had a significantly higher carbohydrate content (67.08% to 68.89%, p<0.05) than soybeans (13.4 to 21.95, p<0.05) in accordance with Ocheme *et al.,* 2018, indicating that wheat is a good source of carbohydrates.

Macro and micro mineral composition of soybean and wheat cultivars

The quantitative data for the microwave digested samples of wheat (LOK-803, HI-1544, and LOK-273) and soybean cultivars (Ahilya 1 (NRC-2), Ahilya 2 (NRC-12), Ahilya 3 (NRC-7), Pratap soya (RKS-18), and Shakti (MAUS-81)) analysed for the content of macro and microminerals: sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), chromium (Cr), manganese (Mn), Iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pd) were presented in Tables 3 and 4. The calibration ranges specified in the previous section for various levels of elements were reported to show linear responses with excellent coefficient of regression values (r^2 > 0.9998). The macro and micromineral content of wheat cultivars was compared to certified values of standard reference material (NIST® 1567b Wheat flour). Na, Mg, K (LOK-273), Ca (LOK-803 and LOK-273), Mn, Fe, Cu, and Zn levels were found to be significantly higher ($p<0.05$) than certified levels. Mineral content of soybean cultivars was found to be in significant agreement ($p<0.05$) with NIST® 3234 soybean flour standard reference material certified values. The

Table 3: Elemental analysis of NIST®1567b wheat flour and wheat varieties LOK-803, HI-1544, and LOK-273 using ICP-MS

Element	Sensitivity		NIST® 1567b		LOK-803	HI-1544	LOK-273
	LOD	LOQ	Reference	Observed			
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
^{23}Na	0.010	0.500	6.71 ± 0.21	6.84 ± 0.07	17.5 ± 0.61	25.9 ± 2.4	20.2 ± 1.4
^{24}Mg	0.010	0.500	398 ± 12	408 ± 6.34	498 ± 31	605 ± 9.8	485 ± 13
^{39}K	0.010	0.500	1325 ± 20	1335 ± 11.8	1135 ± 12	1076 ± 10	1712 ± 37
^{44}Ca	0.010	0.500	191 ± 3.3	200 ± 3.09	285 ± 11	175 ± 5.8	327 ± 20
^{52}Cr	0.001	0.008	NA*	0.05 ± 0.01	ND**	ND	ND
^{55}Mn	0.001	0.008	9.00 ± 0.78	8.96 ± 0.37	27.7 ± 0.95	34.9 ± 1.6	39.2 ± 2.9
^{56}Fe	0.050	0.100	14.1 ± 0.33	13.5 ± 0.37	36.4 ± 2.4	42.2 ± 2.6	45.9 ± 4.1
^{60}Ni	0.050	0.100	NA	0.11 ± 0.01	ND	ND	ND
^{63}Cu	0.050	0.100	2.03 ± 0.14	1.93 ± 0.06	4.36 ± 0.41	4.56 ± 0.37	5.16 ± 0.26
^{66}Zn	0.050	0.100	11.6 ± 0.26	11.9 ± 0.53	25.5 ± 1.2	24.2 ± 1.9	25.7 ± 2.3
^{111}Cd	0.001	0.005	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
^{208}Pb	0.001	0.005	0.01 ± 0.00	0.01 ± 0.00	ND	ND	ND

*NA: Not Available
**ND: Not Detected

Table 4: Elemental analysis of NIST®3234 soybean flour and soybean varieties Ahilya 1 (NRC-2), Ahilya 2 (NRC-12), Ahilya 3 (NRC-7), Pratap soya (RKS-18), and Shakti (MAUS-81) using ICP-MS

Element	Sensitivity		NIST® 3234		NRC-2	NRC-12	NRC-7	RKS-18	MAUS-81
	LOD	LOQ	Reference	Observed					
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
^{23}Na	0.010	0.500	2.52 ± 0.45	2.32 ± 0.39	2.44 ± 0.23	5.75 ± 0.20	4.06 ± 0.17	4.94 ± 0.25	3.74 ± 0.19
^{24}Mg	0.010	0.500	NA	0.03 ± 0.00	3382 ± 67	2830 ± 65	2633 ± 82	3134 ± 41	2893 ± 50
^{39}K	0.010	0.500	25010 ± 560	24960 ± 436	24436 ± 423	24297 ± 446	21311 ± 441	22642 ± 506	23290 ± 418
^{44}Ca	0.010	0.500	3191 ± 56	3025 ± 59	3097 ± 58	2780 ± 23	3055 ± 48	2975 ± 54	2891 ± 51
^{52}Cr	0.001	0.008	NA	0.05 ± 0.00	4.26 ± 0.21	5.00 ± 0.24	4.40 ± 0.37	4.23 ± 0.20	4.83 ± 1.2
^{55}Mn	0.001	0.008	36.8 ± 0.88	35.3 ± 0.40	36.1 ± 0.56	42.6 ± 2.4	32.1 ± 2.1	58.7 ± 4.1	46.7 ± 2.4
^{56}Fe	0.050	0.100	80.3 ± 2.7	81.0 ± 3.6	85.9 ± 3.3	158 ± 4.5	98.5 ± 3.9	86.5 ± 3.2	101 ± 5.0
^{60}Ni	0.050	0.100	NA	0.05 ± 0.00	9.93 ± 0.49	17.4 ± 0.67	9.97 ± 0.37	10.5 ± 0.40	5.51 ± 0.36
^{63}Cu	0.050	0.100	15.3 ± 0.26	14.8 ± 0.15	14.1 ± 0.96	10.9 ± 0.51	17.3 ± 0.72	28.1 ± 2.9	26.7 ± 2.7
^{66}Zn	0.050	0.100	48.9 ± 1.1		51.3 ± 2.6	55.1 ± 3.1	63.5 ± 3.5	49.8 ± 2.6	80.4 ± 5.1
^{111}Cd	0.001	0.005	NA	ND	ND	ND	ND	ND	ND
^{208}Pb	0.001	0.005	NA		0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.04 ± 0.00	ND

Table 5: Total phenolic content and antioxidant activity of soybean and wheat cultivars

Sample	Total phenolic content (mg GAE/g sample)	Antioxidant activity (µg AAE/g sample)
Soybean samples		
Ahilya 1 (NRC-2)	3.13 ± 0.11	112 ± 2.39
Ahilya 2 (NRC-12)	3.59 ± 0.09	123 ± 1.75
Ahilya 3 (NRC-7)	2.76 ± 0.13	97.1 ± 2.56
Pratap soya (RKS-18)	3.29 ± 0.09	115 ± 2.44
Shakti (MAUS-81)	3.04 ± 0.10	105 ± 2.01
Wheat samples		
LOK-803	1.73 ± 0.02	57.3 ± 1.04
HI-1544	2.21 ± 0.03	62.1 ± 0.86
LOK-273	2.05 ± 0.02	59.6 ± 1.53

results were consistent with those reported by Longvah *et al.,* 2017. According to Pandey *et al.,* 2016 and AL-Amery *et al.,* 2016, the content of macro minerals such as Na, K, Mg, and Ca in wheat and soybean cultivars are higher. Heavy metal levels for wheat and soybean cultivars selected for this study are within recommended dietary allowances for Fe, Mn, Ni, Zn, Cd, and Pb (FSSAI, 2020) suggesting that there is no heavy metal contamination. The findings of this study suggest that consuming soybeans and wheat may help maintain electrolyte balance, which is important for regulating many physiological functions such as acid-base homeostasis, neuronal processes, oxygen transport, bone composition and function, muscular physiology, and so on.

Total phenolic content and antioxidant activity of soybean and wheat cultivars

Polyphenols are important bioactive compounds found in foods that have anti-oxidant properties. The total phenolic content (TPC; mg GAE/g) and the total antioxidant activity (µg AAE/g) of the soybean and wheat cultivars are presented in Table 5. The TPC of soybean and wheat cultivars tested did not differ significantly ($p<0.05$) from one another, ranging from 1.73 to 2.21 mg GAE/g and 2.76 to 3.59 mg GAE/g, respectively. The results were comparable with Yusnawan, 2018 and *Punia et al.,* 2019, respectively. The total antioxidant activity of soybean and wheat cultivars tested is significantly correlated ($r^2 = 0.966$) with the total phenolic content. The antioxidant activity of the wheat and soybean samples was in accordance with Yusnawan, 2018 and Punia *et al.,* 2019. The phenolic content and antioxidant activity of soybean cultivars tested were comparable with Mastura *et al.,* 2017; Marathe *et al.,* 2011; Sakthivelu *et al.,* 2008. The phenolic content and antioxidant activity of wheat cultivars tested were comparable with Bhat *et al.,* 2020; Karwasra *et al.,* 2018; Narwal *et al.,* 2014; Revanappa and Salimath 2011.

CONCLUSION

The wheat and soybean samples screened positive for nutritional qualities, particularly protein, fat, and fiber content. Gluten content, an indicator of flour quality, was found in wheat but not in soybean cultivars. Wheat cultivar HI-1544 has the highest gluten content (WG: 28.6%; DG: 8.49%) as well as characteristics such as WAC (286%) and OAC (283%). Besides the high gluten content, wheat cultivars are found to be higher in carbohydrate content than soybean cultivars, with HI-1544 having the highest carbohydrate content (68.8%). Overall, the proximate composition and gluten content of the wheat cultivars did not differ significantly. Soybean cultivars contain more protein (NRC-7: 33.5%), fat (RKS-18: 17.5%), and fiber (RKS-18: 28.8%) than wheat cultivars. The macro and micro mineral compositions of the soybean and wheat cultivars were efficiently analysed with high accuracy and precision, using ICP-MS analysis. The mineral content of most of the analyzed samples was found to be in close agreement with the certified SRM values, with significant levels of macro minerals and heavy metal content falling within acceptable limits. Soybean cultivars had the highest TPC content (NRC-12: 3.59 mg GAE/g), which was found to be directly proportional to the highest antioxidant activity (NRC-12: 123 g AAE/g). The use of SRMs to standardise analyte response in an analytical method also aids in improving the analysis's accuracy and overall performance. Palatability, digestibility, toxicity, and nutritional value of foods, on the other hand, are important research topics in the development of functional foods. Exploring nutritional aspects aids in the quality improvement of wheat and soybean cultivars for potential use in the development of functional foods. The analyzed soybean (Ahilya 1 (NRC-2), Ahilya 2 (NRC-12), Ahilya 3 (NRC-7), Pratap soya (RKS-18), and Shakti (MAUS-81)) and wheat cultivars (LOK-803, HI-1544 and LOK-273) are found to be rich in macronutrients, macro and micro minerals, bioactive constituents, and free of heavy metal contamination. The nutritional quality of wheat and soybean cultivars could be used to make scientific decisions about the applicability and safety of the nation's food supply.

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AUTHOR CONTRIBUTION

G. Kowmudi performed the experiment execution, data analysis, and manuscript drafting; V. Rashmi assisted technically and performed Inductively coupled plasma mass spectrometric analysis; K. Anoop integrated the data and provided instrumental assistance. N. Krishnaveni, the corresponding author, conceptualised the work, assisted with sample collection, and proofread the manuscript. S. Naveen assisted in evaluating the experimental integrity.

CONFLICT OF INTEREST

The authors declares that there is no conflict of interests 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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PUBLISHER'S NOTE

ABBREVIATIONS

AACC	American Association of Cereal Chemists
AAE	Ascorbic Acid Equivalent
AOAC	Association of Analytical Chemists
Ar	Argon
As	Arsenic
°C	Centigrade
Ca	Calcium
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
CV	Coefficient of Variance
DG	Dry Gluten
DPPH	2,2-diphenylpicrylhydrazyl
FAO	Food and Agriculture Organization
Fe	Iron
FSSAI	Food Safety and Standards Authority of India
g	Gram(s)
GAE	Galic Acid Equivalent
Ge	Germanium
H_3BO_3	Boric acid

HCl	Hydrochloric acid
H_2SO_4	Sulfuric acid
Hg	Mercury
ICP-MS	Inductively Couple Plasma Mass Spectrometry
In	Indium
K	Potassium
KJ/g	Kilo Joules per gram
L	Liter(s)
Li	Lithium
LOD	Limit of detection
LOQ	Limit of quantification
MCDG	Moisture Content of Dry Gluten
MCWG	Moisture Content of Wet Gluten
Min	Minute(s)
Mn	Manganese
N	Normality
Na	Sodium
NA	Not available
ND	Not detected
Ng	Nanogram(s)
ng/g	Nanogram per gram
Ni	Nickle
NIST	National Institute of Standards and Technology
NaOH	Sodium hydroxide
OAC	Oil Absorption Capacity
p	Level of significance
PC	Protein Content
Pb	Lead
PTFE	Polytetrafluoroethylene
r^2	Coefficient of regression
Sc	Scandium
SBM	Soybean meal
SRM	Standard Reference Material
Tb	Terbium
TPC	Total Phenolic Content
QC	Quality Control
µL	Microliter(s)
µg/g	Micro grams per gram
µg/L	Micro grams per Liter
µg/mL	Micro grams per milliliter
W	Watts
WAC	Water Absorption Capacity
WG	Wet Gluten
Zn	Zinc

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