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Infrared Heating Influences the Amylose and Bioactive Compounds of Kodo Millet (*Paspalum scrobiculatum*)

Mounika Edde[1,2], Debabandya Mohapatra[3]*, Adinath Kate[1], Ravindra Naik[4], Manoj Kumar Tripathi[1] & Krishna Pratap Singh[5]

[1]Agro Produce Processing Division, ICAR-Central Institute of Agricultural Engineering, Nabibagh, Berasia Road, Bhopal 462 038, India

[2]The Graduate School, IARI, Pusa Campus, New Delhi 110 012, India

[3]Automation and Plant Engineering Division, ICAR-National Institute of Secondary Agriculture, Namkum, Ranchi 834 010, India

[4]Regional Station, ICAR-Central Institute of Agricultural Engineering, Coimbatore 641 007, Tamil Nadu, India

[5]Agricultural Engineering, SMD, KAB-II, Pusa Campus, New Delhi 110 012, India

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Kodo (*Paspalum scrobiculatum*), a minor millet, has immense nutritional potential, but its processing poses challenges in maintaining quality parameters. This study investigates the novel use of infrared radiation to assess the amylose content and bioactive compounds of dehusked Kodo millet. The millet was subjected to infrared (IR) treatment at different IR power densities (11, 13, 15, 17 kW/m^2), initial grain moisture content (10, 12, 14, 16% w.b) and treatment duration (30, 90, 150, 210 s). The results indicated that IR treatment significantly changed the amylose content and bioactive compounds. IR-treated samples exhibited increased phenolic, flavonoid content, and DPPH scavenging activity compared to the untreated millet. Increased amylose content and reduction in the tannin contents were observed at the initial moisture content of 12% (w.b), IR power density of 13 kW/m^2 and 90 s IR treatment. IR treatment of kodo millet is a promising and innovative method for improving its quality and nutritional value. The ability to selectively preserve bioactive compounds, coupled with energy-efficient and precise processing, underscores its potential to revolutionize traditional methods.

Keywords: Dehusked millet, DPPH scavenging activity, Flavonoids, Phenolics, Minor millet

Introduction

Kodo millet (*Paspalum scrobiculatum*), a small seeded cereal cultivated particularly in semi-arid regions of Asia, has gained recognition for its nutritional profile. Kodo millet exhibits an interesting bioactive profile, including significant levels of total phenolics and flavonoids, contributing to its antioxidant properties, mitigating oxidative stress and reducing chronic disease risk.[1,2] Its DPPH radical scavenging activity underscores its ability to neutralize free radicals. The amylose content determines its cooking properties and texture. However, the presence of anti-nutritional components like tannins, phytic acid may interfere with nutrient absorption and protein digestion, causing digestive distress and allergic reactions.[3]

The kodo millet is traditionally consumed as rice, obtained by dehusking the grains. The dehusked grain is milled to produce grits or flour with varied culinary uses. Several conventional thermal treatments, such as hydrothermal treatment (parboiling, soaking, steaming) and electromagnetic radiation heating (microwave, infrared heating), have been recommended for food grains to improve texture, flavour, and palatability.[4,5] Hydrothermal treatments, including parboiling and soaking, have several drawbacks, such as nutrient loss, time and energy consumption, uneven heating, and they also diminish water-soluble bioactive compounds, including vitamins and antioxidants.[5] Moreover, high temperature exposure during steaming, degrades the heat-sensitive compounds, impacting the nutritional quality.[6–8]

Infra-Red (IR) heating offers faster heating times, resulting in shorter cycle times and increased productivity in terms of resource utilization and cost-effectiveness. It also improves the availability of bioactive compounds compared to other heating methods.[9] IR radiation penetrates the surface of food materials by utilizing electromagnetic frequencies in the IR range of 1.8 and 3.4 μm and generates thermal energy rapidly and uniformly. This non-contact, energy-efficient heating method has been proven to maintain bioactive compounds during the processing of legumes.[10] The anti-nutritional factors were also reduced

*Author for Correspondence
E-mail: Debabandya.Mohapatra@icar.gov.in, debabandya@gmail.com

with the IR heating of sorghum and barley samples.[11,12] There is evidence to indicate that IR waves interact with the polysaccharides present in cereals and pulses to induce a range of improvements in food processing and product quality through modifications of bioactive content, antioxidants, macronutrients, altering their digestibility and bioaccessibility.[8,13,14] Due to short penetration depth (a few μm to mm), IR heating has been used for parboiling and drying small-size grains like rice[4] and as a pre-milling treatment for maize and pigeon pea.[9,13] To the best of our knowledge, there is no information on the impact of IR heating on amylose and the bioactive compounds of kodo millet. Therefore, this investigation was undertaken to understand the effect of IR heating on the bioactive compounds, amylose, and tannin content of kodo millet.

Materials and Methods

Raw Materials and Reagents

Kodo millet (*c.v.*:JK 48), a monocot seed encased in hard, persistent husks with dark brown to grey color, harvested during the Kharif season in 2022, was sourced from the eastern region of Madhya Pradesh, India. The millet was cleaned and stored under clean, dry conditions at room temperature. The initial moisture content was determined by AACC 44–17 (2013) method. The grains were pre-conditioned to different moisture contents [12, 14 and 16%, wet basis (w.b)] by adding a calculated amount of water, followed by sealing them in low-density-polyethylene ziplock pouches and keeping them at 4°C for 24 h in a cold room. Samples were allowed to reach room temperature prior to experimentation. All the chemicals used to analyze quality parameters were procured from HiMedia Laboratories, India.

Infrared Radiation Heating

The laboratory IR heating system used for controlled heating of the kodo millet (Fig. 1) comprised of the following parts: (1) IR generator (IR quartz bulb: 250 W, input voltage:0–270 V, Philips India Limited), (2) sample holder, (3) stirrer and (4) voltage control variac transformer (Graff Electro, Delhi, India).

Based on the preliminary trials, the following levels were selected. The millet (100 g) having a bed thickness of a single grain layer, with different Initial Moisture Contents (IMC) (10, 12, 14 &16% w.b) was exposed to IR treatment at various Infrared Power Densities (IRPD) (11, 13, 15 & 17 kW/m^2) for different levels of IR

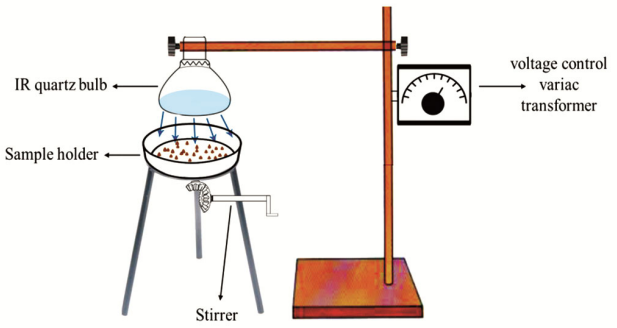

Fig. 1— Schematic diagram of laboratory IR setup for treating kodo millet

duration (30, 90, 150 & 210 s). During the IR exposure, the samples were continuously agitated with a stirrer attached to the sample holder while maintaining a constant distance of 60 mm from the IR heating source. The surface temperature of the grains was measured using an infrared (IR) thermometer (RS PRO RS-8868, UK) immediately after treating the millet. Different combinations of processing parameters with IR treatment resulted in a range of grain temperatures of 64.3–106.5°C.

The IR-treated and untreated (control) kodo millet were dehusked through multiple passes using a laboratory rubber roll sheller (Indosaw Pvt, Ltd, India). The unhusked and broken grains were separated from the husked grains. Husked grains were ground using a laboratory mill (IKA basic analytical mill, USA) to analyze the quality parameters. The sample extracts were prepared by combining the dehusked millet powder (1 g) with 80% methanolic solution (20 mL), followed by centrifugation (C 24, REMI Group Laboratory Instruments, India) for duration of 20 min at a rotational speed of 3000 rpm. The resultant supernatant was used for analysis of bioactive compounds such as total phenolics, total flavonoids and DPPH activity.

Total Phenolic Content

The extract sample of 200 μL was treated with 10 mL of Folin-Ciocalteu Reagent (FCR)(1:10 v/v of FCR and distilled water) and 8 mL of 7.5% Na_2CO_3 solution. The samples were incubated at room temperature in a light-free environment for 90 min, and the absorbance was taken at 750 nm using a UV visible spectrophotometer (A116352, Shimadzu Corp., Japan). Gallic acid was used (0–200 mg/mL) as a reference standard, and the Total Phenolic content (TPC) was expressed as milligrams of gallic acid equivalent per gram of sample (mg GAE/g sample).[15]

Total Flavonoid Content

The sample extract of 1 mL was diluted into distilled water (4 mL), followed by the addition of 0.5 M $NaNO_2$ (0.3 mL), $AlCl_3.6H_2O$ (0.3 mL), 1M NaOH (2 mL), and was finally diluted to 10 mL with distilled water. After vortexing the mixture, the absorbance value at 510 nm was measured spectrophotometrically. Quercetin was used as a standard and the Total Flavonoid Content (TFC) was expressed as milligrams of quercetin equivalent per gram of sample (mg QE/g sample).[16]

Amylose Content

The millet was ground into powder using a mortar and pestle. A sample weighing 100 mg was added with the 9 mL of 1 M NaOH. The mixture was boiled for a period of 10 min and diluted to a volume of 100 mL. An aliquot solution (2.5 mL) was mixed with 1 N acetic acid (0.5 mL) and 0.2% iodine solution (1 mL). This solution was made up to 50 mL, then vortexed (VS-1108, Sunline) and left to stand for 20 min. The absorbance value was read at 720 nm using Ultra Violet (UV) visible spectrophotometer and Amylose Content (AC) was calculated using the standard amylose content as a reference and expressed in percentages.[17]

DPPH Scavenging Activity

The aliquot (1 mL) was mixed with methanol (1 mL) and 0.4mM methanolic DPPH solution (1 mL). The control was prepared with an identical amount (2 mL) of methanol, and DPPH each and incubated for 30 min in the shade. The absorbance value was taken at a wavelength of 517 nm using UV visible Spectrophotometer. The % of DPPH activity was calculated using the following equation.[18]

$$\%\ \text{DPPH inhibition} = \left[1-\frac{\text{Absorbance value of sample}}{\text{Absorbance value of control}}\right]\times 100$$

Tannin Content

The mixture of 0.5 g of powdered material and 25 mL of water was boiled for 30 min. It was subsequently centrifuged at 5,000 rpm for 20 min to separate the supernatant. The supernatant (0.1 mL) was added to 1:1N FCR (0.5 mL), 35% sodium carbonate (1 mL), and diluted to 10 mL before incubating for 30 min. The absorbance value was noted at 700 nm, and Tannin Content (TC) was determined using a calibration curve with tannic acid as a reference standard and expressed as milligrams of tannic acid equivalent per gram of sample (mgTAE/g sample).[19]

Statistical Analysis

The quality parameters of different treatment combinations were evaluated by Analysis of Variance (ANOVA) using Tukey's Honest Significant Difference (HSD) tests at a 5% significance level. The statistical analysis was done using the SAS software (SAS version 9.3).

Results and Discussion

Effect of IR Heating on Total Phenolic Content

The TPC of IR-treated and untreated kodo millet is presented in Fig. 2. The TPC of the control sample was 141.17 ± 0.23 mg GAE/100 g sample, whereas that of the IR-treated sample varied between 158.60 to 188.12 mg GAE/100 g sample. The TPC significantly increased in the IR-treated sample compared to that of the untreated millet ($p < 0.05$) (Tables 1 and 2).

The fluctuation in TPC was observed during the IR treatment, where it exhibited an increasing trend with an increase in the IRPD from 11 to 13 kW/m², followed by a gradually decreasing trend (Fig. 2 a–d). The increase in the TPC during the lower IRPD is likely from an initial boost in the biochemical pathways and enzyme activity at moderate IR treatment levels.[20] Nevertheless, as IRPD increased, the temperature subsequently rose, causing the denaturation of enzymes, leading to a reduction in the production of phenolic compounds, which could potentially cause a decline in the TPC of the grain.[21] The results align with the increased bioactive compounds, antioxidants and their bio accessibility on IR heating of maize.[9]

There was a marked increase in the TPC as treatment time increased from 30 to 90s and later on reduced with an increase in treatment time from 90 to 210s ($p < 0.05$) (Table 1), as shown in Fig. 2 (a–d). IR heating for 30 to 90s likely caused biochemical reactions that activated enzymes that produced and released phenolic compounds. Heat could also have broken down the complex molecules, releasing bound phenols[22], consequently increasing the extractability of phenols. On the other hand, prolonged heating with a longer exposure time could have led to the degradation of phenols, which is associated with the destruction of thermo labile compounds at high temperatures.[23] A similar trend of reducing the TPC of soybeans with increasing IR treatment and heating durations has been reported.[24]

Fluctuations in the TPC of grains with changing moisture levels were influenced by absorbability and

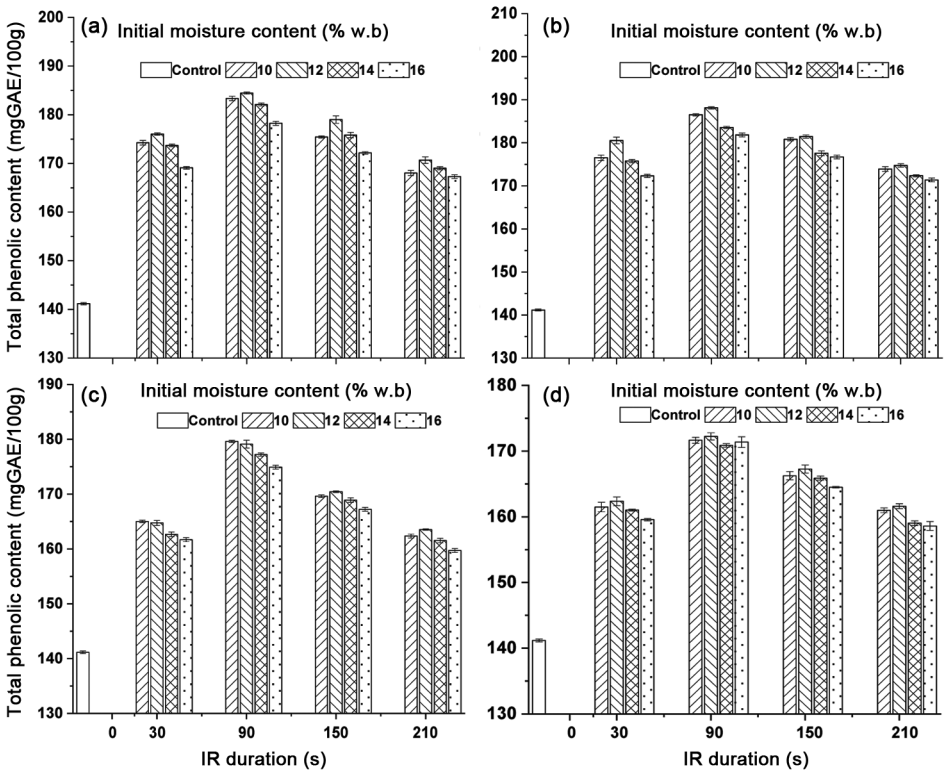

Fig. 2 — Effect of IR duration and IMC at varying IRPD: (a) 11 kW/m^2, (b) 13 kW/m^2, (c) 15 kW/m^2, (d) 17 kW/m^2 on TPC
*Experiments were conducted in triplicates; **Control represents untreated kodo millet with 10% IMC

Table 1 — Full factorial ANOVA results for the effects of IRPD, IR duration and IMC on bio active compounds of infrared pre-treatment of kodo millet

Source	DF	Sum of squares	Mean square	F value	Pr> F
Total phenolic content (TPC)					
IRPD	3	5689.8988	1896.6329	4799.23	<0.0001
IR duration	3	4683.6895	1561.2298	3950.53	<0.0001
IRPD × IR duration	9	167.7399	18.6378	47.16	<0.0001
IMC	3	487.4413	162.48044	411.14	<0.0001
IRPD × IMC	9	66.3077	7.3675	18.64	<0.0001
IR duration × IMC	9	18.8827	2.0981	5.31	<0.0001
IRPD×IR duration × IMC	27	52.7487	1.9537	4.94	<0.0001
Error	131	50.6908	0.3870		
Total flavonoid content (TFC)					
IRPD	3	229003.4275	76334.4758	238426	<0.0001
IR duration	3	967665.2083	322555.0694	1007481	<0.0001
IRPD × IR duration	9	15592.4143	1732.4905	5411.33	<0.0001
IMC	3	843935.5002	281311.8334	878660	<0.0001
IRPD × IMC	9	6127.1385	680.7932	2126.42	<0.0001
IR duration × IMC	9	80175.1141	8908.3460	27824.7	<0.0001
IRPD × IR duration × IMC	27	18824.8261	697.2158	2177.71	<0.0001
Error	131	41.240	0.315		
Amylose content (AC)					
IRPD	3	70.6113	23.5371	83.66	<0.0001
IR duration	3	47.1244	15.7081	55.83	<0.0001
IRPD × IR duration	9	1.3498	0.1499	0.53	0.8483
IMC	3	158.9301	52.9767	188.30	<0.0001

(*Contd*.)

Table 1 — Full factorial ANOVA results for the effects of IRPD, IR duration and IMC on bio active compounds of infrared pre-treatment of kodo millet

Source	DF	Sum of squares	Mean square	F value	Pr> F
IRPD × IMC	9	28.3327	3.1481	11.19	<0.0001
IR duration × IMC	9	7.4414	0.8268	2.94	0.0033
IRPD × IR duration × IMC	27	8.2343	0.3050	1.08	0.3685
Error	131	36.0009	0.2748		
DPPH scavenging activity (DPPH)					
IRPD	3	76.7958	25.59861	96.79	<0.0001
IR duration	3	23.9069	7.9690	30.13	<0.0001
IRPD × IR duration	9	1.3751	0.1528	0.58	0.8134
IMC	3	12.2110	4.0703	15.39	<.0001
IRPD × IMC	9	0.3262	0.0362	0.14	0.9986
IR duration × IMC	9	0.4718	0.0524	0.20	0.9940
IRPD×IR duration × IMC	27	0.53058628	0.0197	0.07	1.0000
Error	131	34.2385	0.2614		
Tannin content (TC)					
IRPD	3	354.2712	118.0904	373.33	<0.0001
IR duration	3	1877.0000	625.6667	1977.98	<0.0001
IRPD × IR duration	9	29.2306	3.2478	10.27	<0.0001
IMC	3	102.9344	34.3115	108.47	<0.0001
IRPD × IMC	9	5.4825	0.6092	1.93	0.0538
IR duration × IMC	9	6.7068	0.7452	2.36	0.0170
IRPD×IR duration × IMC	27	28.8431	1.0683	3.38	<0.0001
Error	131	40.9117	0.3123		

Table 2 — Post hoc test results all the combination of processing parameters on the bioactive compounds of IR treated kodo millet

Treatment combinations			Bioactive compounds				
IRPD	IR duration	IMC	TPC	TFC	AC	DPPH	TC
	Control		141.17 ± 0.23^{g}	1307.25 ± 0.36^{r}	12.20 ± 0.45^{y}	59.76 ± 0.44^{p}	31.05 ± 0.46^{a}
11	30	10	174.25 ± 0.48^{npo}	1428.35 ± 0.54^{w}	$14.90 \pm 0.36^{bcdefghijklmn}$	$65.11 \pm 0.12^{abcdefghijk}$	8.58 ± 0.36^{vwxyz}
11	30	12	176.04 ± 0.23^{nm}	1476.67 ± 0.49^{q}	$15.22 \pm 0.39^{bcdefghij}$	$65.56 \pm 0.23^{abcdefghi}$	8.33 ± 0.22^{vwxyzb}
11	30	14	173.70 ± 0.25^{qpo}	1400.89 ± 0.22^{b}	$13.25 \pm 0.23^{lmnopqrstuvwx}$	$65.18 \pm 0.12^{abcdefghijklm}$	9.73 ± 0.37^{stuvwx}
11	30	16	169.06 ± 0.27^{tvu}	1315.67 ± 0.65^{q}	12.68 ± 0.22^{stuvwx}	$65.03 \pm 0.12^{abcdefghijklmn}$	11.64 ± 0.23^{nopqrs}
11	90	10	183.34 ± 0.45^{dc}	1543.46 ± 0.55^{j}	15.38 ± 0.26^{bcdefgh}	65.85 ± 0.13^{abcde}	7.24 ± 0.26^{zb}
11	90	12	184.46 ± 0.22^{bc}	1698.00 ± 0.53^{b}	16.39 ± 0.29^{bc}	66.03 ± 0.27^{abc}	6.85 ± 0.27^{b}
11	90	14	182.30 ± 0.32^{de}	1508.56 ± 0.69^{m}	$14.38 \pm 0.12^{defghijklmnopqrs}$	65.65 ± 0.11^{abcdefg}	7.62 ± 0.13^{yzb}
11	90	16	178.24 ± 0.42^{kjhi}	1454.00 ± 0.61^{u}	$13.75 \pm 0.29^{ghijklmnopqrstuvw}$	$65.42 \pm 0.24^{abcdefghijk}$	8.87 ± 0.11^{uvwxyz}
11	150	10	175.43 ± 0.22^{lnmo}	1512.32 ± 0.55^{l}	$15.06 \pm 0.12^{bcdefghijkl}$	$65.44 \pm 0.12^{abcdefghijk}$	12.25 ± 0.48^{mnop}
11	150	12	179.01 ± 0.77^{gjhi}	1587.33 ± 0.68^{f}	15.58 ± 0.29^{bcdef}	65.82 ± 0.10^{abcde}	11.96 ± 0.55^{nopq}
11	150	14	175.82 ± 0.56^{lnmo}	1475.33 ± 0.39^{q}	$13.31 \pm 0.36^{klmnopqrstuvwx}$	$65.23 \pm 0.22^{abcdefghijklm}$	12.48 ± 0.11^{lmnop}
11	150	16	172.17 ± 0.25^{qpr}	1350.02 ± 0.38^{j}	12.71 ± 0.39^{rstuvwx}	$65.07 \pm 0.22^{abcdefghijklmn}$	13.93 ± 0.55^{ghijklm}
11	210	10	168.03 ± 0.54^{wvu}	1398.21 ± 0.69^{c}	$14.69 \pm 0.32^{cdefghijklmnop}$	$65.05 \pm 0.23^{abcdefghijklmn}$	14.40 ± 0.23^{ghijkl}

(*Contd*.)

Table 2 — Post hoc test results all the combination of processing parameters on the bioactive compounds of IR treated kodo millet

Treatment combinations			Bioactive compounds				
IRPD	IR duration	IMC	TPC	TFC	AC	DPPH	TC
	Control		141.17± 0.23^{g}	1307.25 ± 0.36^{r}	12.20 ± 0.45^{y}	59.76 ± 0.44^{p}	31.05 ± 0.46^{a}
11	210	12	170.69 ± 0.68^{tsr}	1415.69 ± 0.22^{y}	15.11 ± 0.32^{bcdefghijk}	65.45 ± 0.22^{abcdefghijk}	14.32 ± 0.36^{ghijkl}
11	210	14	169.01 ± 0.31^{tvu}	1376.67 ± 0.33^{h}	13.09 ± 0.31^{nopqrstuvwx}	64.81 ± 0.33^{abcdefghijklmno}	14.55 ± 0.25^{ghij}
11	210	16	167.28 ± 0.39^{wv}	1277.33 ± 0.26^{u}	12.36 ± 0.38^{x}	64.50 ± 0.28^{bcdefghijklmno}	14.62 ± 0.55^{ghij}
13	30	10	176.53 ± 0.64^{lkm}	1468.49 ± 0.39^{s}	15.28 ± 0.27^{bcdefghi}	65.35 ± 0.29^{abcdefghijk}	8.69 ± 0.44^{vwxyza}
13	30	12	180.53 ± 0.79^{gef}	1490.26 ± 0.62^{p}	16.20 ± 0.3^{bcd}	65.88 ± 0.26^{abcd}	7.90 ± 0.32^{xyz}
13	30	14	176.90 ± 0.36^{lkjm}	1435.33 ± 0.56^{v}	14.34 ± 0.48^{efghijklmnopqrs}	65.36 ± 0.13^{abcdefghijk}	8.93 ± 0.52^{uvwxyz}
13	30	16	172.31 ± 0.36^{qpr}	1341.33 ± 0.53^{k}	13.23 ± 0.46^{mnopqrstuvwx}	65.18 ± 0.11^{abcdefghijkl}	10.12 ± 0.38^{qrstuv}
13	90	10	186.50 ± 0.26^{ab}	1588.56 ± 0.63^{f}	15.78 ± 0.68^{bcde}	66.05 ± 0.23^{ab}	6.46 ± 0.66^{b}
13	90	12	187.81 ± 0.27^{a}	1712.21 ± 0.39^{a}	18.90 ± 0.12^{a}	66.51 ± 0.23^{a}	4.11 ± 0.33^{c}
13	90	14	183.56 ± 0.23^{dc}	1549.33 ± 0.42^{h}	14.88 ± 0.11^{bcdefghijklmno}	65.88 ± 0.32^{abcd}	7.41 ± 0.15^{yzb}
13	90	16	181.83 ± 0.44^{de}	1482.00 ± 0.67^{q}	13.96 ± 0.12^{efghijklmnopqrstu}	65.59 ± 0.25^{abcdefghi}	7.62 ± 0.22^{yzb}
13	150	10	180.83 ± 0.38^{gef}	1570.12 ± 0.52^{g}	15.33 ± 0.69^{bcdefgh}	65.62 ± 0.12^{abcdefgh}	12.24 ± 0.52^{mnop}
13	150	12	181.46 ± 0.37^{def}	1598.12 ± 0.55^{e}	16.63 ± 0.13^{b}	66.12 ± 0.25^{ab}	11.96 ± 0.23^{nopq}
13	150	14	177.57 ± 0.56^{lkjhi}	1465.33 ± 0.35^{t}	14.52 ± 0.13^{defghujklmnopqr}	65.42 ± 0.27^{abcdefghijk}	12.48 ± 0.37^{lmnop}
13	150	16	176.72 ± 0.42^{lkm}	1377.33 ± 0.33^{h}	13.45 ± 0.41^{jklmnopqrstuvwx}	65.31 ± 0.12^{abcdefghijkl}	12.98 ± 0.46^{ijklmno}
13	210	10	173.91 ± 0.54^{npo}	1406.67 ± 0.65^{a}	15.06 ± 0.31^{bcdefghijkl}	65.30 ± 0.07^{abcdefghijkl}	14.26 ± 0.48^{ghijkl}
13	210	12	174.76 ± 0.39^{nmo}	1425.25 ± 0.25^{x}	15.02 ± 0.61^{bcdefghijklm}	65.64 ± 0.12^{abcdefg}	13.96 ± 0.56^{ghijklm}
13	210	14	172.35 ± 0.25^{qpr}	1387.00 ± 0.31^{f}	14.22 ± 0.78^{efghijklmnopqrst}	64.99 ± 0.25^{abcdefghijklmn}	14.44 ± 0.46^{ghijk}
13	210	16	171.37 ± 0.43^{sr}	1333.33 ± 0.32^{l}	13.08 ± 0.56^{nopqrstuvwx}	64.88 ± 0.14^{abcdefghijklmno}	14.99 ± 0.33^{ghf}
15	30	10	165.03 ± 0.22^{yx}	1412.10 ± 0.63^{z}	13.18 ± 0.54^{nopqrstuvwx}	64.73 ± 0.23^{bcdefghijklmno}	10.01 ± 0.42^{rstuvw}
15	30	12	164.76 ± 0.47^{yxz}	1429.65 ± 0.37^{w}	14.69 ± 0.46^{bcdefghijklmno}	65.26 ± 0.28^{abcdefghijkl}	9.61 ± 0.44^{tuvwx}
15	30	14	162.67 ± 0.41^{cabz}	1364.67 ± 0.54^{i}	13.92 ± 0.45^{fghijklmnopqrstuv}	64.73 ± 0.28^{bcdefghijklmno}	10.12 ± 0.23^{qrstuv}
15	30	16	161.68 ± 0.37^{cbd}	1286.67 ± 0.34^{t}	12.54 ± 0.63^{vwx}	64.49 ± 0.23^{bcdefghijklmno}	12.58 ± 0.35^{klmno}
15	90	10	179.63 ± 0.21^{ghf}	1529.45 ± 0.61^{k}	13.99 ± 0.60^{efghijklmnopqrstu}	65.53 ± 0.23^{abcdefghij}	8.09 ± 0.44^{wxyzb}
15	90	12	179.12 ± 0.22^{ghi}	1648.23 ± 0.36^{c}	15.56 ± 0.68^{bcdefg}	65.76 ± 0.32^{abcdef}	8.09 ± 0.44^{vwxyzb}
15	90	14	177.22 ± 0.29^{lkji}	1507.33 ± 0.54^{mn}	14.62 ± 0.69^{cdefghijklmnopq}	65.53 ± 0.25^{abcdefghij}	8.11 ± 0.23^{vwxyzb}
15	90	16	174.94 ± 0.35^{nmo}	1423.63 ± 0.49^{x}	13.25 ± 0.31^{lmnopqrstuvwx}	65.20 ± 0.29^{abcdefghijkl}	9.24 ± 0.12^{uvwxy}

Table 2 — Post hoc test results all the combination of processing parameters on the bioactive compounds of IR treated kodo millet

Treatment combinations			Bioactive compounds				
IRPD	IR duration	IMC	TPC	TFC	AC	DPPH	TC
	Control		141.17± 0.23^{g}	1307.25 ± 0.36^{r}	12.20 ± 0.45^{y}	59.76 ± 0.44^{p}	31.05 ± 0.46^{a}
15	150	10	169.62 ± 0.25^{su}	1506.33 ± 0.54^{n}	13.35 ± 0.36^{klmnopqrstuvwx}	64.67 ± 0.28^{bcdefghijklmno}	13.55 ± 0.63^{ghijklmn}
15	150	12	170.46 ± 0.14^{tsr}	1546.01 ± 0.22^{i}	14.90 ± 0.38^{bcdefghijklmn}	65.26 ± 0.29^{abcdefghijkl}	12.81 ± 0.23^{jklmno}
15	150	14	168.91 ± 0.41^{tvu}	1435.33 ± 0.63^{v}	14.22 ± 0.56^{efghijklmnopqrst}	64.88 ± 0.22^{abcdefghijklmno}	14.32 ± 0.23^{ghijkl}
15	150	16	167.23 ± 0.35^{wv}	1321.33 ± 0.29^{o}	13.01 ± 031^{pqrstuvwx}	64.73 ± 0.32^{bcdefghijklmno}	14.92 ± 0.55^{fghi}
15	210	10	162.36 ± 0.36^{cab}	1379.33 ± 0.62^{g}	12.84 ± 0.36^{qrstuvwx}	64.09 ± 0.21^{efghijklmno}	17.02 ± 0.23^{de}
15	210	12	163.53 ± 0.12^{yabz}	1402.45 ± 0.27^{b}	13.74 ± 0.38^{ghijklmnopqrstuvw}	64.57 ± 0.29^{bcdefghijklmno}	16.67 ± 0.35^{def}
15	210	14	161.54 ± 0.41^{cbd}	1331.33 ± 0.46^{m}	13.41 ± 0.56^{jklmnopqrstuvwx}	64.02 ± 0.21^{fghijklmno}	17.29 ± 0.49^{cd}
15	210	16	159.74 ± 0.35^{fed}	1289.23 ± 0.59^{s}	12.55 ± 0.26^{wx}	63.91 ± 0.25^{ghijklmno}	18.02 ± 0.55^{cd}
17	30	10	161.49 ± 0.72^{cbd}	1389.63 ± 0.58^{e}	12.81 ± 0.16^{qrstuvwx}	63.54 ± 0.26^{lmno}	11.90 ± 0.63^{nopqr}
17	30	12	162.36 ± 0.66^{cab}	1401.01 ± 0.65^{b}	14.29 ± 0.69^{eghijklmnopqrst}	64.18 ± 0.23^{defghijklmno}	11.25 ± 0.44^{opqrst}
17	30	14	161.01 ± 0.15^{ced}	1318.23 ± 0.62^{p}	13.20 ± 0.65^{mnopqrstuvwx}	63.78 ± 0.25^{jklmno}	11.99 ± 0.55^{nopq}
17	30	16	159.57 ± 0.19^{fed}	1255.33 ± 0.42^{v}	12.47 ± 0.39t^{uvwx}	63.69 ± 0.13^{klmno}	13.24 ± 0.42^{ghijklmn}
17	90	10	171.66 ± 0.46^{qsr}	1507.48 ± 0.32^{mn}	13.41 ± 0.52^{klmnopqrstuvwx}	64.44 ± 0.24^{bcdefghijklmno}	9.69 ± 0.63^{tuvwx}
17	90	12	172.24 ± 0.53^{qpr}	1615.89 ± 0.35^{d}	15.56 ± 0.36^{bcdefg}	64.86 ± 0.23^{abcdefghijklmno}	10.18 ± 0.52^{qrstuv}
17	90	14	170.87 ± 0.28^{tsr}	1468.92 ± 0.29^{s}	14.21 ± 0.32^{efghijklmnopqrst}	64.38 ± 0.25^{bcdefghijklmno}	8.93 ± 0.44^{uvwxyz}
17	90	16	171.38 ± 0.22^{sr}	1411.33 ± 0.44^{z}	13.11 ± 0.35^{nopqrstuvwx}	64.08 ± .27^{efghijklmno}	10.64 ± 0.36^{pqrstu}
17	150	10	166.26 ± 0.62^{wx}	1396.24 ± 0.34^{d}	12.96 ± 0.65^{pqrstuvwx}	64.02 ± 0.27^{fghijklmno}	14.25 ± 0.12^{ghijkl}
17	150	12	167.27 ± 0.62^{wv}	1502.56 ± 0.62^{o}	15.02 ± 0.26^{bcdefghijklm}	64.27 ± 0.36^{cdefghijklmno}	13.96 ± 0.54^{ghijklm}
17	150	14	165.888 ± 0.32^{wx}	1325.56 ± 0.59^{n}	13.73 ± 0.24^{hijklmnopqrstuvw}	63.87 ± 0.21^{hijklmno}	15.18 ± 0.12^{efg}
17	150	16	164.50 ± 0.12^{yaxz}	1286.67 ± 0.64^{t}	12.65 ± 0.25^{stuvwx}	63.73 ± 0.25^{klmno}	17.5 ± 0.28^{cd}
17	210	10	160.99 ± 0.36^{ced}	1341.57 ± 0.38^{k}	12.65 ± 0.31^{stuvwx}	63.47 ± 0.36^{mno}	18.26 ± 0.36^{cd}
17	210	12	161.60 ± 0.38^{cbd}	1376.46 ± 0.49^{h}	13.49 ± 0.36^{ijklmnopqrstuvwx}	63.83 ± 0.29^{ijklmno}	17.96 ± 0.36^{cd}
17	210	14	159.04 ± 0.35^{fe}	1309.57 ± 0.29^{q}	13.06 ± 0.69^{opqrstuvwx}	63.32 ± 0.26^{no}	19.11 ± 0.21^{bc}
17	210	16	158.60 ± 0.68^{f}	1223.89 ± 0.36^{w}	12.41 ± 0.26^{wx}	63.17 ± 0.19^{o}	20.64 ± 0.32^{b}

*Note: Values are mean of 3 replications; Means with the same letter are not significantly different in a column at 95% confidence interval

polarization of IR radiation. In the IMC range (10 to 12% w.b), an increase in the absorbability of IR radiation promoted conditions favoring phenolic synthesis. At the same time, polarization changes might have impacted the stress responses, thereby enhancing the extractability of phenolics.[24] However, when IMC levels reached 12 to 16% (w.b), both absorbability and polarization were amplified, which interfered with the detection and stability of phenolics, causing a decline. The TPC was also observed to be changed with the increase in moisture content levels of rice flour during the heat moisture treatment.[25]

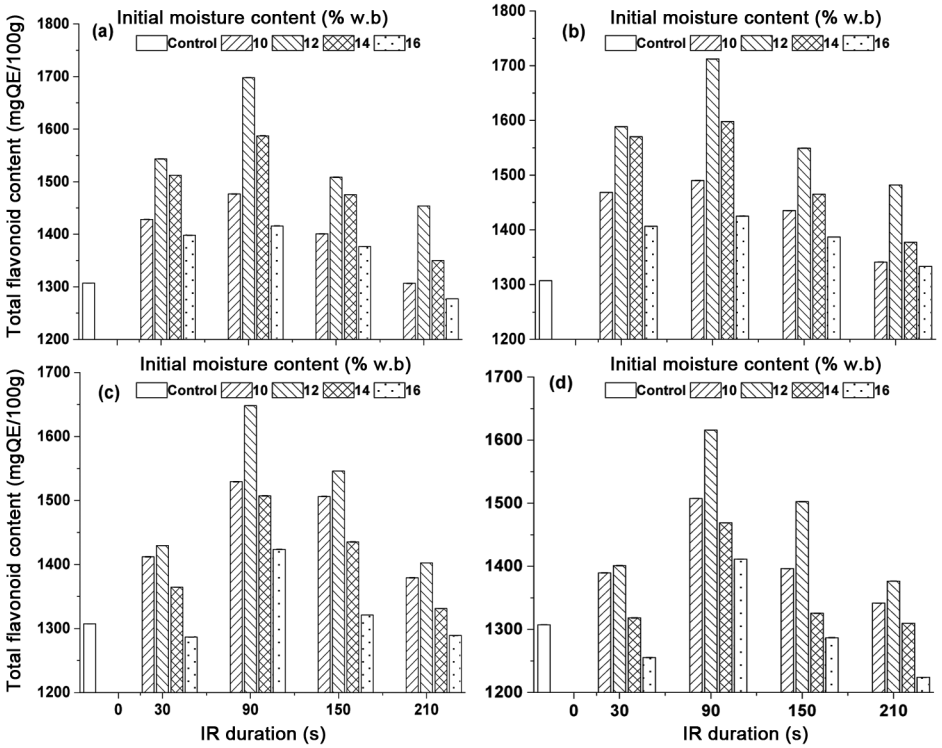

Fig. 3 — Effect of IR duration and IMC at varying IRPD: (a) 11 kW/m^2, (b) 13 kW/m^2, (c) 15 kW/m^2, (d) 17 kW/m^2 on TFC
*Experiments were conducted in triplicates; **Control represents untreated kodo millet with 10% IMC

Effect of IR heating on Total Flavonoid Content

The TFC of the IR-treated kodo millet and control samples is shown in Fig. 3. The TFC of the control sample was 1307.25 ± 0.36 mg QE/100g sample, while that varied between 1223.89 and 1712.21 mg QE/100g for the IR-treated sample.

The TFC of kodo millet in response to different IRPD is shown in Fig. 3 (a, b, c, d). The increase in TFC with the increase in the IRPD from 11 to 13 kW/m^2 may be due to the thermal energy provided, particularly for compounds such as quercetin, kaempferol and hormetic stress response, in which moderate IR heating stimulates secondary metabolism, which includes flavonoid synthesis.[23] Conversely, a decline in TFC from 15 to 17 kW/m^2 IRPD can be attributed to excessive heat stress disrupting the metabolic pathways involved in flavonoid synthesis, reducing the flavonoid levels. Our observations are in accordance with that of IR-treated rice.[20]

The results showed significant changes in the TFC with the IR treatment duration ($p < 0.05$) (Tables 1 and 2). After heating for 30 to 90 s, flavonoids could be released or biosynthesized, possibly through activating enzymes or breaking down flavonoid precursors, increasing flavonoid content. In contrast, the prolonged heat exposure during the 90 to 210 s treatment could have resulted in the degradation of flavonoids, possibly due to the thermal instability of certain flavonoids.[24] The results corroborated with the observations of Ghimeray *et al*.[23], which indicated an initial increase in TFC and further decrease with an increase in treatment time and temperature during far-IR treatment of buckwheat sprout powder. The results of Tangkhawanit *et al*.[22] indicated that far IR-hot air drying increased the number of bioactive components in soybean seeds.

During IR heating, the increase in TFC in the IMC range of 10–12% (w.b) of kodo millet could be attributed to enhanced accessibility and release of previously bound flavonoids as the higher moisture facilitates cellular structure breakdown.[24] However, as IMC further rose from 12 to 16%, potential thermal degradation, oxidation, and other chemical reactions driven by prolonged exposure to elevated moisture levels might have led to a subsequent decrease in flavonoid content ($p < 0.05$) (Table 2).[25,26] Generally, flavonoids like quercetin and kaempferol, commonly found in cereal grains and known for their antioxidant properties, might respond to increased moisture by becoming more soluble and accessible for extraction during heating, potentially contributing to the observed rise in flavonoid content.[26] Ziegler *et al*.[27]

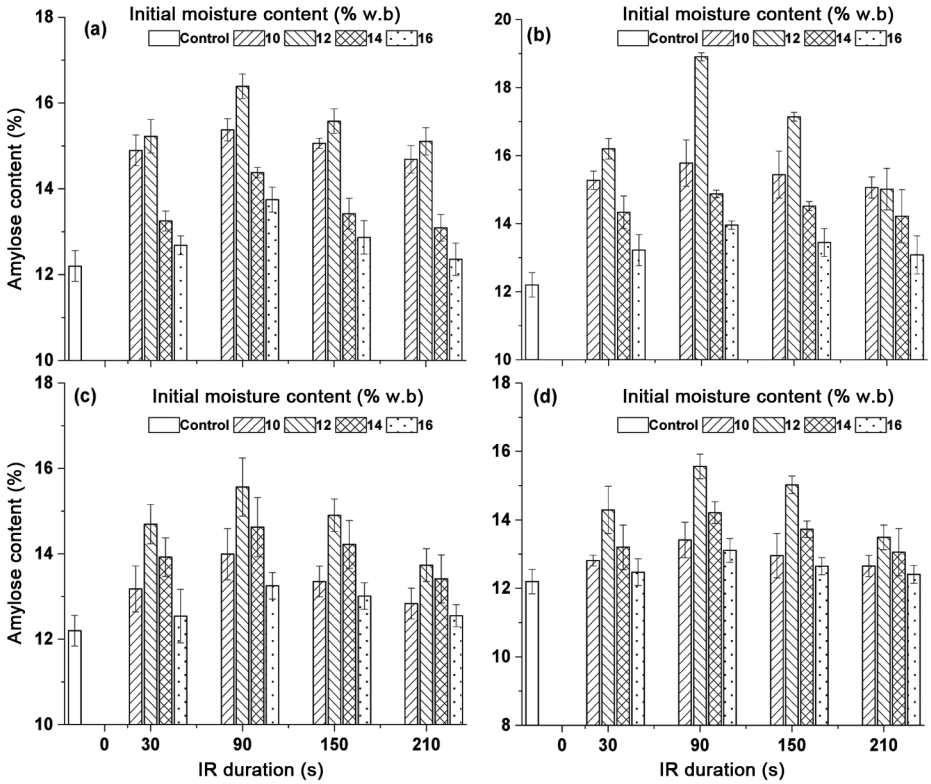

Fig. 4 — Effect of IR duration and IMC at varying IRPD: (a) 11 kW/m^2, (b) 13 kW/m^2, (c) 15 kW/m^2, (d) 17 kW/m^2 on AC
*Experiments were conducted in triplicates; **Control represents untreated kodo millet with 10% IMC

also found significant changes in the TFC of soybean with different moisture levels during IR treatment.

Effect of IR Heating on Amylose Content

The AC of IR-treated and control samples is presented in Fig. 4. The AC of the control was 12.20 ± 0.45%, whereas that of the IR-treated sample varied from 12.36 to 18.91%. The IR-treated sample had higher AC than the control sample ($p < 0.05$) (Table 1 and 2).

During the IR treatment, the AC increased as the IRPD increased from 11 to 13 kW/m^2, as shown in Fig. 4 (a–d) ($p < 0.05$) (Table 2). This rise is likely due to the swelling and partial gelatinization of starch granules. During this gelatinization, the hydrogen bonds holding the starch molecules together break leading to the increase of AC. However, as the IRPD further rose to 17 kW/m^2, the increased heat exposure appeared to cause a more pronounced disruption of starch granules, leading to structural changes and retro gradation processes. Rather than facilitating the release of amylose molecules, these processes may promote their degradation or insolubilization, ultimately contributing to the observed decrease in AC.[20] This contrasting behavior showcased the intricate effects of heat on the millet's starch composition during IR treatment. Arce Arce *et al.*[28] also observed the degradation of amylose and amylopectin in common beans with the severity of IR treatment conditions.

During 30 to 90s of IR treatment, there was an increase in the AC, most likely due to starch gelatinization, where heat disrupts the crystalline structure, releasing amylose. However, the trend shifted during the 90 to 210s treatment, showing a decrease. This was likely due to the hydrolysis of the alpha-1-4 glucosidic bonds in amylose, resulting in structural changes that reduced amyloidosis levels at the elevated grain temperatures.[28] IR heating reaches a critical point at which amylose molecules are thermally degraded and decrease in abundance. The overall changes in AC might be attributed to the thermal depolymerization of amylose (possibly amylopectin, too), which was also evinced by the work of Liu *et al*.[29] on lentil seeds.

As the IMC of kodo millet increased from 10 to 12% (w.b), the IR treatment might have facilitated a gradual breakdown of starch granules, leading to an increased release of amylose.[20] However, when the IMC further increased from 12 to 16%, the extended exposure to the

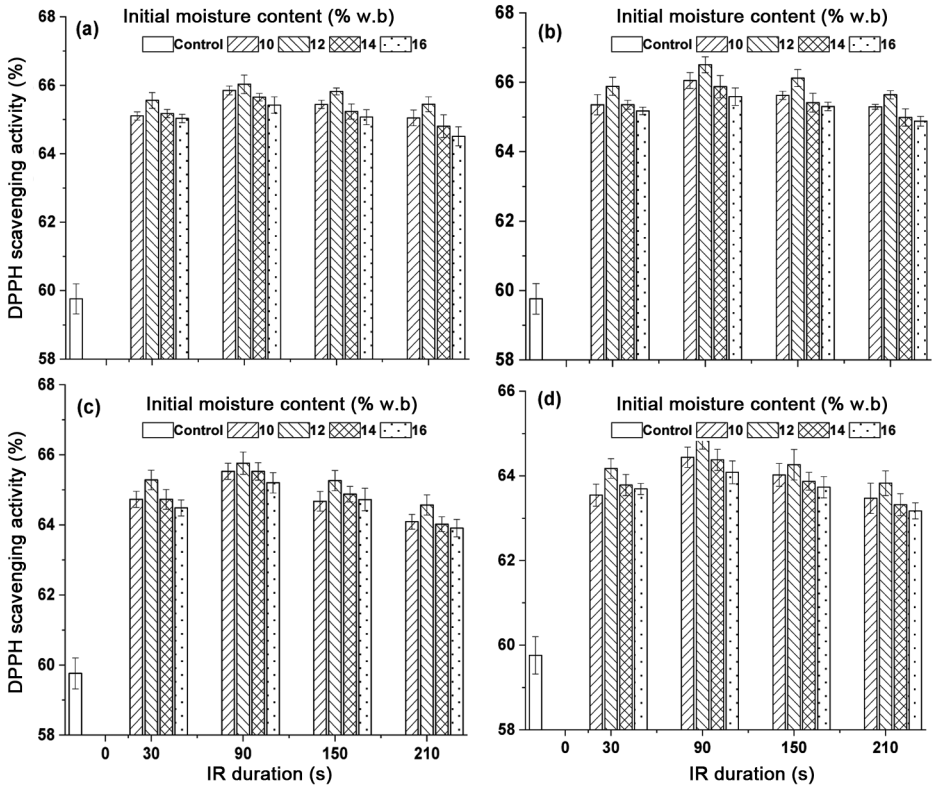

Fig. 5 — Effect of IR duration and IMC at varying IRPD: (a) 11 kW/m^2, (b) 13 kW/m^2, (c) 15 kW/m^2, (d) 17 kW/m^2 on DPPH scavenging activity; *Experiments were conducted in triplicates, **Control represents untreated kodo millet with 10% IMC

IR radiation could have led to over-processing of the starch, causing a more pronounced degradation of linear- and long chain amylose molecules and a decrease in AC. Therefore, the combined effects of IMC and treatment duration contributed to the observed variations in AC during the IR treatment of kodo millet. At the same time, higher IMC made the amylose molecules more soluble, and less likely to form into longer chains.[29]

Effect of IR Heating on DPPH Scavenging Activity

The DPPH activity of IR-treated and control samples is shown in Fig. 5. The IR-treated samples had a significantly higher activity than the control ($p < 0.05$) (Tables 1 & 2). The DPPH% inhibition of untreated millet (control) was 59.76 ± 0.44%, and the treated samples varied from 63.17 to 66.51%.

The results from Table 2 and Fig. 5 (a–d) revealed an increase in DPPH in the IRPD ranging from 11 to 13 kW/m^2; after that, it declined significantly. The increased DPPH scavenging activity during the lower IRPD could be attributed to the improved generation and transfer of heat energy within this IRPD range. As a result, antioxidants and other bioactive compounds may have been released from the sample matrix more efficiently, thereby significantly increasing their ability to neutralize DPPH radicals.[21] However, the subsequent decrease in scavenging activity in the 15 to 17 kW/m^{2}IRPD range might be due to excessive heat generated at higher power densities, which may have resulted in the degradation of sensitive antioxidants or alteration of the structural integrity of the compounds responsible for scavenging DPPH radicals, resulting in reduced antioxidant efficacy within this range.[20] DPPH activity of soybeans also reportedly increased with the IR treatment against control and a minor reduction in DPPH activity as the IR power increased.[21]

A significant increase in DPPH activity in the treatment duration from 30 to 90s and a decrease during 90 to 210s treatment duration were observed ($p < 0.05$) (Tables 1 and 2), as shown in Fig. 5 (a,b,c,d). The increase in DPPH scavenging activity between 30 and 90s of IR treatment could be due to enhanced interaction, which releases antioxidants. The decrease in DPPH in the later stage could be attributed to the degradation of susceptible antioxidants or the formation of undesirable byproducts during prolonged exposure to IR heating. This is in conformation with the IR heating of maize and rice.[8,20]

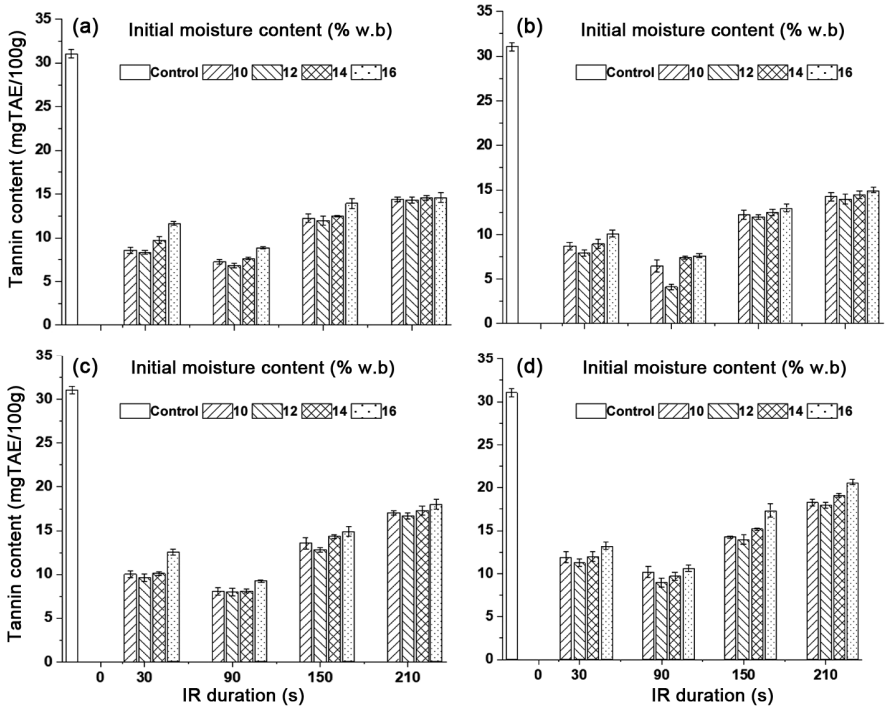

Fig. 6 — Effect of IR duration and IMC at varying IRPD: (a) 11 kW/m^2, (b) 13 kW/m^2, (c) 15 kW/m^2, (d) 17 kW/m^2 on TC
*Experiments were conducted in triplicates; **Control represents untreated kodo millet with 10% IMC

DPPH scavenging activity increased in the IMC range of 10 to 12% (w.b), followed by a decrease in the 12 to 16% w.b ($p < 0.05$) IMC range. The increase in DPPH may be attributed to the optimal moisture range that stimulates antioxidant release. As a result of IR treatment, bioactive compounds may be released, strengthening their capability to neutralize DPPH radicals.[8] As moisture content increased from 12 to 16%, scavenging activity decreased, possibly due to the higher absorbability of IR radiation, which builds up more thermal energy, bringing about structural and molecular degradation of heat-sensitive compounds. Consequently, antioxidants are less effective at neutralizing DPPH radicals at this moisture content level. Chuwech *et al.*[26] also observed a significant change in the DPPH activity of purple rice flour at different moisture levels.

Effect of IR Heating on Tannin Content

The TC of the IR-treated and control sample is presented in Fig. 6. The TC of the control was 31.04 ± 0.46 mg TAE/100 g sample, whereas, in the case of the IR-treated sample, it varied from 4.10 to 20.64 mg TAE/100 g sample. The IR-treated sample had significantly lower TC than the control sample ($p < 0.05$) (Tables 1 & 2).

A decreasing trend of TC (Fig. 6 a–d) in the IRPD range of 11–13 kW/m^2 was followed by an increase in the 15 to 17 kW/m^2 range. At the lower IRPD range, the IR heating could have caused the breakdown of larger tannin molecules into smaller and more soluble compounds, reducing the measured TC.[11] A high IRPD can raise millet grain temperatures. Some tannins may polymerize at this higher temperature, combining to form bigger, more complex molecules and being less extractable. Furthermore, higher IRPD can induce molecular rearrangements in tannins due to increased energy. Consequently, new tannin derivatives or isomers may be produced that are difficult to extract from the cellular matrices.[30] As per the study of Rousta *et al.*[12], IR treatment significantly reduced the TC in sorghum compared to control. The tempering and IR treatment caused a significant reduction in the tannins of both desi chickpea and hull-less barley flours.[11]

For 30 to 90s of IR treatment, the decline in TC may be due to thermal degradation and the release of tannin components.[30] Higher exposure time might lead to structural changes in millet grains, making tannin extraction more difficult.[12] Our results corroborated those of Bai *et al.*[11] who noticed a significant reduction in TC during the IR treatment of barley and chickpea flour samples.

The decrease in TC in the IMC range of 10 to 12% (w.b) during IR treatment might be due to the

breakdown of larger tannin molecules into smaller fragments as a result of the heat and energy exposure ($p < 0.05$). This could reduce the measured TC as certain tannin compounds become altered or fragmented.[18] Conversely, the subsequent increase in TC could be attributed to the fact that higher IMC may influence the complex formation of tannin with macromolecules, affecting their extractability.[31] Besides, the increase in moisture content binds tannins together, making them harder to break down.[11]

Conclusions

The effect of infrared heating on phenolic content, flavonoid content, DPPH scavenging activity, amylose content, and tannin content of kodo millet provides valuable insights into the nutritional and antioxidant properties of this food processing technique. Moderate levels of infrared heating showed promising results in enhancing the grain's antioxidant capacity, as evinced by increased total phenolic and flavonoid content, as well as improved DPPH scavenging activity, signifying enhanced free radical scavenging ability and amylose content. However, it also emphasized the importance of careful temperature control, as excessive heating leads to the reductions in flavonoid and phenolic content, potentially compromising the overall nutritional value of kodo millet. These findings offer valuable implications for optimizing infrared heating conditions to produce nutritionally enriched kodo millet-based products, promoting healthier dietary choices for consumers. Further research is needed to explore the mechanisms underlying these changes and to optimize the infrared heating conditions of kodo millet.

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Conflict of interest

The authors declare there is no conflict of interest among them.

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