In vitro nutrient digestibility and antioxidative properties of flour prepared from sorghum germinated at different conditions

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Revised: 23 April 2019 / Accepted: 30 April 2019 / Published online: 21 May 2019
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Abstract Germination can be used as a bio-processing practice to enhance the digestibility of nutrient and improve the bioactive compounds and rheological properties of food grains. In the present study, effect of germination time 12, 24, 36 and 48 h and temperature 25, 30 and 35 °C on carbohydrate profile, enzyme activity, in vitro nutrient digestibility, antioxidant activity, bioactive components and rheological characteristics of sorghum was examined. As time and temperature for germination progressed, it considerably enhance the activity of diastase enzyme and also the sugar content by hydrolysis of starch and further enhance the in vitro digestibility of starch by 10.50–36.25%. Germinated sorghum had high in vitro protein digestibility and it ranges from 57.50 to 77.91% as compared to native sorghum (54.09%). Germination of sorghum for longer time period at elevated conditions appreciably improve the antioxidant activity by 4.24–52.96%, total phenolic content and flavonoid content by 1.60–4.09 mgGAE/g and 60.30–94.03 mgQE/100 g, respectively Similarly reducing power increased from 29.27 to 47.19 µg AAE/g and metal chelating activity

enhanced 19.48–52.09% as period for germination goes from 12 to 48 h and temperature from 25 to 35 °C. Increased enzyme activity during germination degrades the starch and thus lowers down the peak and final viscosity of sorghum. Increased enzymatic activity and higher antioxidant activity also lower down the lightness value by 12.48% while a* was increased by 6.78%. Germination of sorghum thus offers a tool to increase the nutrient digestibility and bioactive potential of sorghum without any chemical or genetic engineering.

Keywords Germination · Sorghum · In vitro digestibility · Antioxidant activity · Bioactive components · Paste viscosity

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench), the most drought-tolerant coarse cereal is essential food commodity for majority of populations in semi arid regions of Asia and Africa (Mohapatra et al. 2019). Sorghum is the primary source for weaning foods in developed and developing nations due to its better nutritional compositions as they are served as staple food providing energy supply and nutrients in poor people's diet (Afify et al. 2012b; Singh et al. 2017). Traditionally, sorghum is used for preparation of alcoholic and non-alcoholic beverages, porridge, flat bread and weaning food (Claver et al. 2010; Hassani et al. 2013).

In the present scenario, sorghum gained a perceptive attention amongst health foods owing to its pheloids for healthful life among developed countries (Mohapatra et al. 2019). Peoples suffering from gluten intolerance, sorghum can be used to replace the flour containing the gluten, as it is suggested safe for consumption by the people suffering

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from celiac disease (Phattanakulkaewmorie et al. 2011). Sorghum thus, offers a good quality source for development of gluten free products like breads, cakes and cookies, snacks and pasta (Sharanagat et al. 2019). It also gained popularity as a nutraceutical and functional food, owing to the presence of significant levels of phenolic acids and its derivatives which exhibits health promoting properties due to their antioxidant nature (Mohapatra et al. 2019). Sorghum is an important source of protein and minerals (Correia et al. 2010), functional health promoting components such as vitamin B group, fibres, antioxidant phenolics and cholesterol-lowering waxes (Hassani et al. 2013).

Germination is a natural, biological processing technique in which seed come out of its legacy state which induced the activity of dormant enzymes, thus improve the functionality of cereal grains (Noda et al. 2004; Hefni and Witthöft 2011). Germination has been used as an alternative to mask undesirable tastes and smells, as well as reduce the presence of anti-nutritional compounds (Sangronis and Machado 2007; Hubner and Arendt 2013), increase the micronutrients, enhance the nutritional, chemical and sensorial characteristics of seeds (Hefni and Witthöft 2011; Adedeji et al. 2014). Several researches showed massive transformation in contents of phytochemical during germination, which reveals remobilisation, breakdown and deposition of nutrients (Donkor et al. 2012). Various biochemical activities occurs during germination generate various antioxidants and bio-functional compounds due to enzyme action of the cell wall for instance tocopherols, phenolic acids, Vitamin C and γ -aminobutyric acid (GABA) and results in enhanced antioxidant capacity (Singh and Sharma 2017). During germination various hydrolytic enzymes were activated (Correia et al. 2010), leading to the degradation of complex starch, polysaccharides, proteins and fats into simpler forms, thereby increasing nutrient accessibility, and starch and protein digestibility and bio-availability (Afify et al. 2012a; Sharma et al. 2018). The increased enzymatic activities also results in degradation of starch which lowers the viscous properties of the flour.

As sorghum gained a lot of popularity in the development of gluten free products and for its better use in the development of variety food products with health enhancing components for all the age groups, there is a need to increase its antioxidant compounds and nutrient digestibility through natural processing technology. Germination enhances the antioxidant properties of several food grains. Singh and Sharma (2017) reviewed and reported a prominent increase in antioxidant activities, phenol and flavanoid compounds and other bioactive components of wheat, brown rice, barley, buckwheat and millets. However, scanty literature is available on changes in bioactive components and nutrient digestibility of sorghum during

germination conditions. Hence, the study was carried out to examine the changes and enhancement in the nutrient digestibility, carbohydrate profile, bioactive components and pasting properties of sorghum at various germination time and temperature conditions.

Materials and methods

Germination of sorghum

Sorghum cultivar SL 44 was procured from Department of Plant Breeding and Genetics, PAU Ludhiana, Punjab (India). The seeds were sorted, washed followed by dipping in distilled water at 25 °C for 10 h, care was taken to change the water in every 5 h interval. Soaked grains were placed on a tray encrusted and covered with a piece of fabric and placed in an incubator (Narang Scientific Works, New Delhi). The grains were germinated for 12–48 h at 25, 30 and 35 °C (90–95% RH). After each germination treatment, grains were taken out of the incubator and dried in a tray drier to a moisture content of $7 \pm 2\%$ to restrict germination. Dried germinated and non-germinated grains were then packed in airtight containers placed at cool and dark place (4 °C) for further investigation.

Carbohydrate profile

Starch and sugar content

Starch present in the given sample converted to sugars by acid hydrolysis and sugars thus released was calculated by measuring the volume of non-reduced sugar solution necessary to absolutely reduce a known volume of Fehling's solution to red insoluble cuprous oxide (AACC 2000). Sugars (total and reducing) present in the samples were measured by amount of ferricyanide reduced by sugar extract under favourable conditions as per standard AACC (2000) method. Briefly, 5.675 g of sample flour was mixed with 95% ethyl alcohol (2 ml) and 50 ml of acetic acid buffer solution. Shake the contents to bring the flour into suspension and add of 12% sodium tungstate solution (2 ml). Filter the contents and use aliquot for subsequent sugars analysis.

Diastase activity

Diastase enzyme activity which represents the combined action of α and β amylases present in the flour was determined under standard reaction conditions. Enzyme extract was extracted with buffer solution (dissolve 3 ml acetic acid and 4.1 g anhydrous sodium acetate in 1 L distilled water) at 40 °C and reacted with alkaline

ferricyanide and the amount of reducing sugars thus formed by amylolytic enzyme action was then estimated by standard AACC (2000) method. Results are expressed as mg maltose produced by 10 g flour in 1 h at 30 °C using reference table.

In vitro nutrient digestibility

Starch digestibility

The protocol of Bernfeld (1955) as described and modified by Sharma et al. (2018) was followed to measure the content of in vitro digestibility of starch, by estimating the amount of maltose produced by the action of dinitrosalicylic acid. Briefly 0.1 g of finely ground sorghum was mixed with phosphate buffer (20 ml/pH 6.9) and then 100 mg of powdered α -amylase was added followed by 2 h incubation of mixture at 37 °C and then centrifugation (3000 rpm/15 min). 1 ml of clear aliquot and 2 ml of dinitrosalicylic acid were mixed together bring to boil (5 min) followed by cooling and volume makeup to 50 ml with distilled water. The OD of the mixture was measured at 540 nm and maltose standard curve was prepared to calculate mg of maltose released equivalent to sample OD.

Protein digestibility

The procedure of Akeson and Stachman (1964) as described and modified by Sharma et al. (2018) was used measure in vitro proteins digestibility. 500 mg of sorghum sample was mixed with 50 ml of pepsin sol. (pH 1.9) made in 0.1 N HCL and incubated at 37 °C for 2 h for first, followed by neutralization of the contents with 0.2 N NaOH. Toluene was added to maintain aseptic conditions. The contents were again mixed with pancreatin solution (pH 8) and incubated at 37 °C for 2 h for 2nd stage proteolysis, followed by centrifugation of the contents * 3000 rpm/15 min). Aliquot was discard, sediments were dried Nitrogen present in the sediments was measured using Macrokjedahl method and in vitro proteins digestibility was computed by ratio of the protein in the sediments to the ratio of protein content in sample before digestion.

Antioxidant activity (DPPH free radical scavenging activity)

Procedure of Brand-Williams et al. (1995) with slight modification was followed to measure the antioxidant activity which require the use of free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanolic solution. 0.1 ml of acidified methanol extract (80%) was mixed with 3.9 ml of DPPH solution (0.2 mM) prepared in pure methanol and

the contents were mixed well, kept for 30 min in dark followed by immediately measurement of the absorbance of the contents at 515 nm. The antioxidant activity was calculated as % discoloration:

$$\text{Antioxidant activity (AOA) (\%)} \\ = 1 - \frac{\text{Absorbance of sample } t = 30}{\text{Absorbance of control } t = 0} \times 100$$

Total phenolic contents

Method of Gujral et al. (2012) which involve the use of Folin–Ciocalteu (FC) reagent was followed to measure the total phenolic content (TPC). 1000 mg of sorghum was mixed with acidified methanol solution containing 0.1% HCl for 2 h at 43 ± 2 °C, followed by centrifugation of the contents. Extraction of the contents was repeated and contents were mixed and final volume is made to 50 ml with acidified methanolic solution (80%). From the volume, 0.5 ml of aliquot and 0.5 ml of methanol is mixed with 10-folds freshly dilute FC reagent (5 ml) followed by addition of saturate solution of NaHCO₃ (4 ml) after 4 min. The reaction mixture was shaken well and kept aside for 2 h at 25 °C followed by measurement of the OD of the contents at 765 nm using spectrophotometer. Results were expressed as mg of Gallic acid equivalents (GAE)/g on dry weight basis.

Total flavanoid contents

Procedure as described by Woisky and Salatino (1998) was employed to measure the total flavanoid content in the samples with minor alterations. 1.0 g of sorghum was mixed with acidified methanol solution containing 0.1% HCl for 4 h at 43 ± 2 °C, followed by centrifugation (3000 rpm/15 min) of the contents. Take aliquot (2.0 ml) and mix it with 10% solution of aluminium chloride solution (0.1 ml) followed by 1.0 M/L solution of potassium acetate (0.1 ml) and 2.8 ml of distilled water. Mix the contents properly and place them in dark form for half hour min and take the OD at 415 nm. Results were expressed as mg Quercetin Equivalent (QE)/100 g on dry weight basis.

Metal chelating (Fe⁺²) activity

The procedure as described by Sharma and Gujral (2011) was employed to calculate the Fe⁺² chelating activity. Sorghum sample (500 µg) was mixed with 80% methanol (5 ml) and extracted for 120 min at orbital shaker (Narang Scientific Works, New Delhi), followed by centrifugation of the contents (10,000 rpm/10 min). Take 0.5 ml of aliquot and put of solution of ferrous chloride (0.05 ml) and then add 80% methanol (1.6 ml) and shake well. After

5 min add 0.1 µl of ferrozine solution, shake the contents and allow them to rest for 10 min at room temperature followed by measurement of OD of the reaction mixture at 562 nm. Metal chelating activity was calculated as followed using methanol as blank:

$$\text{Iron Fe chelating activity (\%)} = 1 - \frac{\text{Absorbance of sample at 562 nm}}{\text{Absorbance of control at 562 nm}} \times 100$$

Reducing power

The procedure as described by Sharma and Gujral (2011) was employed to calculate the reducing power of the sorghum samples. Sorghum sample (500 µg) was mixed with 80% methanol (6.6 ml) and extracted for 240 min at orbital shaker (Narang Scientific Works, New Delhi), followed by centrifugation of the contents (10,000 rpm/10 min). Take 1 ml aliquot, 2.5 ml of 0.2 M pH 6.6 phosphate buffer and 2.5 ml of potassium ferricyanide solution (1%), mix the contents and incubate at 50 °C for 20 min. Add 10% TCA solution (2.5 ml) in the mixture followed by centrifugation of the contents (10,000 rpm/10 min). Take clear aliquot (2.5 ml) and followed by addition of distilled water (2.5 ml) and ferric chloride solution (0.1%) (0.5 ml) and read the OD at 700 nm. Results were expressed as µg ascorbic acid equivalents (AAE)/g on dry weight basis.

Rheological properties

The changes taking place in the rheological properties of sorghum during germination was assessed with the help of RVA (Rapid Visco Analyzer) (Newport Scientific, Warriewood, Australia), using standard 13 min profile. 3.5 g of sample corrected to 14% moisture was put in aluminium canister and then add 25 ml distilled water and paddle was placed in the re mixed test was performed. The pasting properties viz. pasting temperature, peak, holding, breakdown, setback and final viscosity was noted.

Color characteristics

L^* (lightness; 0-black, 100-white) a^* (+ a^* redness, – a^* greenness) and b^* (+ b^* yellowness, – b^* blueness) values of the sorghum samples was carried out using Hunter lab colorimeter (CR-300 Minolta Camera, Japan). Following equations was used to calculate the hue angle (h^*) and chroma (C) values:

$$h^* = \arctan \left(\frac{b^*}{a^*} \right)$$

$$C = \sqrt{a^{*2} + b^{*2}}$$

Statistical analysis

The data given in the tables was mean of three different values along with standard deviation. The collected data was subjected to analysis of variance (ANOVA) and Tukey's posthoc test with SPSS statistical software (version 16.0, SPSS Inc., Chicago, Illinois, USA).

Results and discussion

Carbohydrate profile of sorghum

Total starch

Data in Table 1 shows the effect of germination conditions on the starch content of sorghum. Germination time (12–48 h) and temperature (25–35 °C), had considerable ($p < 0.05$) impact on the sorghum starch content. Starch the main reservoir of energy for biosynthesis undergoes degradation during germination which leads to the formation of small dextrin and sugars. The degradation rate of starch was observed to increase as germination period progressed from 12 to 48 h and temperature increased commencing 25–35 °C. Native sorghum has 72.27% total starch content which undergoes hydrolysis as roots starts emerging with increase in metabolic activity of enzymes. Starch was extensively ($p < 0.05$) decreased by 9.17, 14.12 and 20.26%, when sorghum was germinated for 48 h at 25, 30 and 35 °C, respectively, due to the increased activity of amylases enzyme which hydrolyses the starch, to yield 6-carbon sugars for new germinating seedling (Ayernor and Ocloo 2007). The extent of starch hydrolysis also enhanced as period for germination and temperature elevated owing to higher enzymatic activity.

Total and reducing sugars

Starches and complex sugars undergo enzymatic hydrolysis to form free sugars. Data in Table 1 presents the changes in the total sugars and reducing sugars of native sorghum and germinated sorghum. Period and temperature for germination had a significant ($p < 0.05$) positive influence on the total and reducing sugars of sorghum. Native sorghum has 3.64% total and 2.22% reducing sugars, which showed significant ($p < 0.05$) increase as germination period increase from 12 to 48 h and temperature from 25 to 35 °C. In the course of varying germination conditions total sugars of sorghum increased significantly ($p < 0.05$) and ranged between 5.12 and 7.71%, while reducing sugars varied between 3.07 and 9.00%. After 12 h of germination the mean total sugars of sorghum was 6.11% which

Table 1 Effect of germination conditions on the carbohydrate profile and diastase enzyme activity of sorghum flour

Treatment	Temperature (°C)	Time (h)	Carbohydrate profile			Diastase activity (mg maltose/10 g)
			Total starch (%)	Total sugars (% glucose)	Reducing sugars (% glucose)	
Control	–	–	72.27 ± 0.76 ^a	3.64 ± 0.18 ^k	2.22 ± 0.09 ^j	273.44 ± 19.66 ^j
Soaked	25	10	71.39 ± 0.21 ^{ab}	4.94 ± 0.18 ^j	2.56 ± 0.00 ^j	341.67 ± 16.21 ⁱ
Germination conditions	25	12	70.34 ± 0.38 ^{Pb}	5.12 ± 0.00 ^{Sj}	3.07 ± 0.03 ^{Ri}	372.53 ± 0.00S ^{hi}
		24	68.48 ± 0.04 ^{Qc}	6.33 ± 0.18 ^{Rhi}	3.41 ± 0.10 ^{QRhi}	567.68 ± 16.38 ^{Rg}
		36	67.28 ± 0.21 Rd	8.76 ± 0.34 ^{Qf}	3.91 ± 0.07 ^{Qg}	709.83 ± 13.04 ^{Qf}
		48	65.64 ± 0.18 ^{Se}	10.20 ± 0.00 ^{Pe}	5.20 ± 0.29 ^{Pe}	962.65 ± 16.25 ^{Pe}
	30	12	68.87 ± 0.21 ^{Pc}	5.82 ± 0.05 ^{Sij}	3.67 ± 0.00 ^{Sgh}	446.24 ± 0.00 ^{Sh}
		24	66.90 ± 0.13 ^{Qd}	7.23 ± 0.19 ^{Rgh}	4.32 ± 0.00 ^{Rf}	742.34 ± 29.43 ^{Rf}
		36	63.63 ± 0.15 ^{Rf}	11.50 ± 0.09 ^{Qd}	6.76 ± 0. + 00 ^{Qc}	1385.84 ± 35.75 ^{Qd}
		48	62.06 ± 0.05 ^{Sg}	16.41 ± 0.00 ^{Pb}	8.27 ± 0.10 ^{Pb}	1667.65 ± 19.28 ^{Pc}
	35	12	65.66 ± 0.20 ^{Pe}	7.40 ± 0.00 ^{Sg}	3.84 ± 0.00 ^{Sg}	740.36 ± 16.11 ^{Sf}
		24	62.82 ± 0.05 ^{Qgf}	9.12 ± 0.02 ^{Rf}	5.97 ± 0.10 Rd	1677.76 ± 19.40 ^{Rc}
		36	60.32 ± 0.42 ^{Rh}	13.36 ± 0.00 ^{Qc}	8.17 ± 0.00 ^{Qb}	2300.95 ± 42.18 ^{Qb}
		48	57.63 ± 0.25 ^{Si}	17.71 ± 0.67 ^{Pa}	9.33 ± 0.00 ^{Pa}	2415.83 ± 0.00 ^{Pa}
Mean [#]						
Germination temperature (°C)		25	67.93 ^P	7.60 ^r	3.89 ^r	653.16 ^r
		30	65.36 ^Q	10.28 ^q	5.76 ^q	1060.50 ^q
		35	61.61 ^r	11.89 ^P	6.83 ^P	1783.70 ^P
Germination time (h)		12	68.28 ^P	6.11 ^s	3.53 ^s	519.68 ^s
		24	66.06 ^Q	7.56 ^r	4.57 ^r	959.92 ^r
		36	63.74 ^r	11.21 ^q	6.28 ^q	1465.50 ^q
		48	61.77 ^s	14.77 ^P	7.60 ^P	1682.00 ^P

Values represent mean ± standard deviation

Values having different superscripts from a, b, c to k differ significantly ($p < 0.05$) from each other column wise among different treatments

*Values having different superscripts P, Q, R and S show significant ($p < 0.05$) differences during different germination time at each germination temperature respectively

[#]Means (germination temperature and time) having different superscripts from p, q, r and s differ significantly ($p < 0.05$) separately for mean values of germination temperature and time

significantly ($p < 0.05$) increased by 31.80, 61.75 and 111.98% after 24, 36 and 48 h of germination, respectively. Similar trend for mean noteworthy enhancement in total sugars was also observed when temperature for grain germination elevated from 25 to 35 °C. Reducing sugar content of sorghum germinated at different condition ranged from 3.07 to 9.33%. An increased of 64.44 and 186.67% in reducing sugar was assessed after germination period of 24 and 48 h at 25 °C. When temperature for germination was extended to 30 and 35 °C, reducing sugar content further increased significantly. Saman et al. (2008) as well reported enhancement in total and reducing sugar content of germinated cereals with increase in germination time. The increase in the sugar after germination was due to hydrolysis of starch by amylase enzyme along with combined action of invertase which helps out in the complete hydrolysis of starch and complex sugars to simple and

reducing sugar, to yield glucose and fructose (Mohan et al. 2010; Moongngarm and Saetung 2010).

Diastase activity

Diastase activity which combined action of alpha and beta amylases was significantly ($p < 0.05$) effected by conditions for grain germination (Table 1). Raw sorghum exhibit enzyme activity of 273.44 mg maltose/10 g, which was significantly ($p < 0.05$) increased as the germination period increased from 12 to 48 h and temperature from 25 to 35 °C and it ranged between 372.53 and 2415.83 mg maltose/10. Interactions among variable conditions for grain germination also had significant influence on enhanced enzymatic activity. Ayernor and Ocloo (2007) also state that the diastase activity enhanced significantly with progress in the germination period.

In vitro nutrient digestibility

Starch digestibility

Germination conditions had significant ($p < 0.05$) influence on in vitro digestibility of starch. Enhancement in period of germination from 12 to 48 h and temperature from 25 to 35 °C significantly ($p < 0.05$) enhanced the digestibility of starch in sorghum as compared to control (Table 2). In vitro digestibility of starch of native sorghum was 34.76% and it enhanced appreciably by 4.32% after 10 h of soaking. During germination at variable conditions, in vitro digestibility of starch of germinated sorghum ranged from 38.37 to 47.68% and exhibited a significant ($p < 0.05$) enhancement of 10.50–36.25% as compared to native sorghum. Interaction between conditions for germination also exhibited a significant ($p < 0.05$) influence on germinated sorghum starch in vitro digestibility.

Germination induces metabolic and structural transformation in the starch molecules and in crystalline form and the remaining components after germination exhibited enhanced inclination to enzyme attack (Srichuwong and Jane 2007), which results in enhanced starch digestibility of germinated sorghum in the present study. Similar results for increase in in vitro digestibility of starch was also reported by Chung et al. (2012) and Xu et al. (2012) in rice due to degradation of starch molecules by action of amylase enzymes.

Protein digestibility

Data showing the in vitro digestibility of protein values in grain sorghum as influenced by germination conditions was presented in Table 2. Native sorghum shows an in vitro protein digestibility value of 54.09%. Variable germination conditions hold significant ($p < 0.05$) positive influence on

Table 2 Effect of germination conditions on the in vitro nutrient digestibility of sorghum flour

Treatment	Temperature (°C)	Time (h)	In vitro nutrient digestibility	
			Starch digestibility (%)	Protein digestibility (%)
Control	—	—	34.76 ± 0.76 ^j	54.09 ± 0.96 ^h
Soaked	25	10	36.25 ± 0.57 ⁱ	54.98 ± 0.84 ^h
Germination conditions	25	12	38.37 ± 0.42 ^{Rgh}	57.50 ± 0.76 ^{Rg}
		24	39.86 ± 0.23 ^{Rg}	59.33 ± 0.17 ^{Qef}
		36	41.14 ± 0.31 ^{Qd_e}	60.22 ± 0.91 ^{Qef}
		48	42.26 ± 0.12 ^{Pd}	63.03 ± 0.32 ^{Pde}
	30	12	40.67 ± 0.60 ^{Ref}	60.27 ± 0.92 ^{Sef}
		24	42.86 ± 0.67 ^{Qd}	63.43 ± 1.07 ^{Rde}
		36	44.07 ± 0.81 ^{Pbc}	66.47 ± 0.07 ^{Qcd}
		48	45.08 ± 0.58 ^{Pb}	69.96 ± 0.18 ^{Pc}
	35	12	40.82 ± 0.34 ^{Ref}	67.19 ± 0.23 ^{Scd}
		24	44.45 ± 0.21 ^{QRbc}	70.03 ± 0.88 ^{Rc}
		36	45.22 ± 0.18 ^{Qb}	73.52 ± 0.44 ^{Qb}
		48	47.68 ± 0.15 ^{Pa}	77.91 ± 0.28 ^{Pa}
Mean [#]				
Germination temperature (°C)		25	40.41 ^r	60.02 ^r
		30	43.17 ^q	65.03 ^q
		35	44.54 ^p	72.16 ^p
Germination time (h)		12	39.95 ^r	61.65 ^s
		24	42.39 ^q	64.26 ^r
		36	43.47 ^{pq}	66.74 ^q
		48	45.01 ^p	70.30 ^p

Values represent mean ± standard deviation

Values having different superscripts from a, b, c to i differ significantly ($p < 0.05$) from each other column wise among different treatments

*Values having different superscripts P, Q, R and S show significant ($p < 0.05$) differences during different germination time at each germination temperature respectively

[#]Means (germination temperature and time) having different superscripts from p, q, r and s differ significantly ($p < 0.05$) separately for mean values of germination temperature and time

Table 3 Effect of germination conditions on the antioxidant activity, total phenolic and flavonoid content of sorghum flour

Treatment	Temperature (°C)	Time (h)*	Antioxidant activity (% inhibition of DPPH)	Total phenolic content (mg GAE/g)	Total flavanoid content (mg QE/100 g)
Control	–	–	61.11 ± 1.57 ⁱ	1.30 ± 0.08 ^{jk}	57.68 ± 1.46 ⁱ
Soaked	25	10	60.22 ± 1.10 ⁱ	1.19 ± 0.04 ^{jk}	52.68 ± 1.52 ^j
Germination conditions	25	12	63.70 ± 1.45 ^{Rgh}	1.60 ± 0.14 ^{Rij}	60.30 ± 1.08 ^{Sh}
		24	64.61 ± 0.55 ^{Rgh}	2.24 ± 0.28 ^{QRefg}	63.85 ± 0.37 ^{Rgh}
		36	71.11 ± 1.18 ^{Qef}	2.58 ± 0.14 ^{PQdef}	67.56 ± 0.92 ^{Qfg}
		48	76.81 ± 1.37 ^{Pcd}	3.21 ± 0.28 ^{Pbcd}	79.22 ± 0.45 ^{Pbc}
	30	12	68.06 ± 1.96 ^{Sfg}	1.76 ± 0.11 ^{Rgh}	63.47 ± 1.47 ^{Sgh}
		24	74.31 ± 0.98 ^{Rde}	2.38 ± 0.08 ^{QRdef}	67.30 ± 1.16 ^{Rfg}
		36	77.64 ± 0.20 ^{Qcd}	2.64 ± 0.28 ^{Qcde}	74.32 ± 0.47 ^{Qde}
		48	86.81 ± 1.37 ^{Pb}	3.59 ± 0.21 ^{Pb}	81.63 ± 2.23 ^{Pbc}
	35	12	73.19 ± 0.20 ^{Sde}	1.84 ± 0.28 ^{Sgh}	69.58 ± 1.79 ^{Sef}
		24	81.11 ± 1.18 ^{Rc}	2.24 ± 0.28 ^{Refg}	77.36 ± 0.69 ^{Rcd}
		36	86.53 ± 0.98 ^{Qb}	3.44 ± 0.28 ^{Qbc}	83.73 ± 1.10 ^{Qb}
		48	93.47 ± 0.98 ^{Pa}	4.09 ± 0.21 ^{Pa}	94.03 ± 0.26 ^{Pa}
Mean [#]					
Germination temperature (°C)		25	69.05 ^r	2.41 ^r	67.73 ^r
		30	76.70 ^q	2.60 ^q	71.68 ^q
		35	83.57 ^p	2.91 ^p	81.18 ^p
Germination time (h)		12	68.31 ^s	1.73 ^s	64.45 ^s
		24	73.34 ^r	2.29 ^r	69.50 ^r
		36	78.43 ^q	2.87 ^q	75.20 ^q
		48	85.73 ^p	3.63 ^p	84.96 ^p

Values represent mean ± standard deviation

Values having different superscripts from a, b, c to k differ significantly ($p < 0.05$) from each other column wise among different treatments

*Values having different superscripts P, Q, R and S show significant ($p < 0.05$) differences during different germination time at each germination temperature respectively

[#]Means (germination temperature and time) having different superscripts from p, q, r and s differ significantly ($p < 0.05$) separately for mean values of germination temperature and time

the in vitro digestibility of sorghum protein and it amplified appreciably as the germination duration progressed from 12 to 48 h and temperature increased from 25 to 35 °C (Table 2). Sorghum germinated at 35 °C for 48 h showed maximum in vitro protein digestibility value (77.915%), while lowest (57.50%) was observed after 12 h of germination at 25 °C. This shows that time and temperature have significant influence on the in vitro digestibility and it was also confirmed from statistically significant interaction effect on protein digestibility. Comparable outcomes were also observed by Elkhailil et al. 2001 and Correia et al. 2010, while working with sorghum. They reported that during germination process proteins were denatured by action of inherent protease enzymes, causing structural changes in sorghum storage protein, which expose the sites of denatured protein and make it more susceptible to enzymatic hydrolysis (pepsin attack) thus increasing the in vitro protein digestibility. The enhanced nutrient

digestibility of the germinated sorghum flour increased the market application and utilization of such flour in formulations of processed weaning foods.

Antioxidant activity (DPPH free radical scavenging activity)

Germination conditions significantly ($p < 0.05$) influenced the antioxidant activity of sorghum as per data shown in Table 3. Statistically significant ($p < 0.05$) difference was detected in antioxidant activity (DPPH scavenging ability) of native, soaked and germinated sorghum samples. Antioxidant activity of sorghum prior to soaking was 61.11% which reduced marginally to 60.22% after 10 h of soaking. Germination of sorghum for 12–24 and 48 h, showed a significant ($p < 0.05$) enhancement in mean antioxidant activity by 11.78–20.01 and 40.29%, respectively, as compared to native sorghum. Similar effect was

observed in case of change in temperature. Mean antioxidant activity of sorghum was observed to be 69.05% when germination was carried at 25 °C and it increased significantly to 76.70 and 83.57% at 30 and 35 °C of germination temperature. This shows that longer is the germination duration and higher the temperature, superior is the antioxidant activity. Gupta et al. (2013) reported that antioxidative enzymes such as superoxide-dismutases, glutathione-S-transferase, peroxidases and catalases having high antioxidant potential were induced to higher levels during germination at high temperature, which results in enhancement in antioxidant activity of sorghum in the present study as their germination temperature increased from 25 to 30 °C and 35 °C. Similar outcome were also conveyed by studies of Cáceres et al. (2014), they reported germination of brown rice at 34 °C exhibited the elevated antioxidant activity in comparison to when germination at 28 °C.

Total phenolic content

Data for the total phenolic contents (TPC) of raw, soaked and sorghum germinated for different conditions is given in Table 3. Native sorghum has TPC of 1.30 mg/g GAE, which reduced slightly during soaking due to leaching or solubilisation of phenolic compounds in soaking water (Lu et al. 2007). Germination of sorghum afterwards brought a significant ($p < 0.05$) perceptible enhancement in TPC and this increase was time and temperature dependent as observed from data presented in Table 3. Sorghum germination for 12 h had 33.07% high TPC in comparison to control and TPC levels was additionally enhanced by 76.15–179.23% when period of germination amplified to 24 and 48 h respectively. Similarly when temperature was elevated from 25 to 30 and 35 °C, significant ($p < 0.05$) enhancement in the TPC was also observed. Similar results were also reported by Pal et al. (2016) during germination of brown rice. Cáceres et al. (2014), also confirms that germination temperature also significantly influenced the accumulation of total phenolic contents in germinated grains. They reported that germination at 34 °C display a higher TPC content than those germinated at 28 °C in different brown rice cultivars, as during germination saccharolytic enzymes hydrolyse the cell wall to release the bound phenolic compounds and thus increasing free phenolics forms which results in higher TPC in germinated grains (Sharma et al. 2012).

Total flavanoid content

Flavonoids having health promoting effects was associated with antioxidant properties and have synergistic consequence with other antioxidants, was significantly

($p < 0.05$) influenced by variable germination conditions (Table 3). The total flavonoid content (TFC) of native sorghum was 57.68 mg QE/100 g and it ranged from 60.30 to 94.03 mg QE/100 g at different germination process conditions. Progress in germination period from 12 to 48 h and temperature from 25 to 35 °C, the mean TFC in sorghum was increased by 11.88–47.30% and 17.42–40.74%, respectively. This reveals that progress in germination period and temperature drastically enhanced the TFC of sorghum. Interaction between conditions for germination also had noteworthy influence on the TPC of sorghum. During germination the flavonoid pathway was synthesised by activation of metabolic phenylpropanoid pathway, during which acetyl coenzyme A esters (CoA) was generated by the intermediates which are further converted to flavonoids. Diverse kinds of enzymes and co-factors are manufactured during germination which also show the way for production of the flavonoids (Singh and Sharma 2017).

Metal chelating activity

Data in Table 4 shows the % metal chelating activity exhibited by native and germinated sorghum samples at different germination conditions. Native and soaked sorghum exhibits metal chelating activity of 14.91 and 15.67%, respectively. The metal chelating activity of sorghum was appreciably ($p < 0.05$) affected by variation in germination temperature and time and it ranged from 19.48 to 52.09%. 12 h germination sorghum exhibits 24.43% mean metal chelating activity which was 63.83% more in comparison to raw sorghum and it further enhanced by 104.02–175.57% after 24–48 h of germination respectively. The mean metal chelating activity of sorghum germinated at 25 °C was 23.25% which was significantly ($p < 0.05$) enhanced to 34.67 and 40.60% when temperature for germination was elevated to 30 and 35 °C. This illustrate that elevated germination temperature and prolonged time results in significantly ($p < 0.05$) higher metal chelating activity of sorghum. Among all the sorghum samples germinated at variable conditions, highest metal chelating activity (52.09%) was observed after 48 h of germination at 35 °C, while lowest (19.48%) after 12 h of germination at 25 °C. Enhancement in period and temperature for germination cause degradation and structure alteration of the phenolic compounds to different products of maillard reaction that may works as antioxidant and exhibited higher metal chelating activity (Sharma et al. 2012).

Reducing power

Germination period and temperature had a significant ($p < 0.05$) influence on the reducing power of sorghum

Table 4 Effect of germination conditions on the metal chelating activity and reducing power of sorghum flour

Treatment	Temperature (°C)	Time (h)	Metal chelating activity (%)	Reducing power (μg AAE/g)
Control	–	–	14.91 ± 0.31 ^j	25.24 ± 0.53 ^k
Soaked	25	10	15.67 ± 0.77 ^j	27.05 ± 0.37 ^{jk}
Germination conditions	25	12	19.48 ± 1.23 ^{Pi}	29.27 ± 0.74 ^{Rij}
		24	21.54 ± 0.15 ^{QRgh}	34.17 ± 0.54 ^{Qgh}
		36	22.96 ± 0.61 ^{QRg}	38.88 ± 1.03 ^{Pe}
		48	29.04 ± 1.23 ^{Pde}	40.24 ± 0.74 ^{Pde}
	30	12	24.15 ± 1.08 ^{Sfg}	31.71 ± 0.42 ^{Shi}
		24	31.87 ± 0.61 Rd	35.02 ± 0.00 ^{Rf}
		36	36.11 ± 0.46 ^{Qc}	41.66 ± 0.13 ^{Qcd}
		48	46.54 ± 1.08 ^{Pb}	44.75 ± 0.19 ^{Pb}
	35	12	26.65 ± 0.61 ^{Sef}	36.15 ± 0.05 ^{Sf}
		24	37.85 ± 1.05 ^{Rc}	39.59 ± 0.48 ^{Rde}
		36	45.89 ± 0.46 ^{Qb}	42.95 ± 1.18 ^{Qbc}
		48	52.09 ± 0.61 ^{Pa}	47.19 ± 1.04 ^{Pa}
Mean [#]				
Germination temperature (°C)	25	23.25 ^r	35.64 ^r	
	30	34.67 ^q	38.29 ^q	
	35	40.62 ^p	41.47 ^p	
Germination time (h)	12	24.43 ^s	23.38 ^s	
	24	30.42 ^r	36.28 ^r	
	36	34.99 ^q	41.17 ^q	
	48	42.56 ^p	44.06 ^p	

Values represent mean ± standard deviation

Values having different superscripts from a, b, c to k differ significantly ($p < 0.05$) from each other column wise among different treatments

*Values having different superscripts P, Q, R and S show significant ($p < 0.05$) differences during different germination time at each germination temperature respectively

[#]Means (germination temperature and time) having different superscripts from p, q, r and s differ significantly ($p < 0.05$) separately for mean values of germination temperature and time

which is associated reductones and their antioxidant activity is based on the free radical chain reaction cessation by contributing a hydrogen atom and inhibiting peroxide formation (Sharma et al. 2012). Reducing powers of sorghum significantly ($p < 0.05$) amplified as germination period increase from 12 to 48 h and temperature from 25 to 35 °C (Table 4). Native sorghum shows reducing power of 25.24 μg AAE/g, which after germination for 12–48 h at 25–35 °C ranged from 29.27 to 40.24 μg AAE/g, showing an increase of 15.97–59.42%, as compared to native sorghum. Interaction between period and temperature of germination also exhibited a noteworthy influence on the reducing power of sorghum. Among different germination condition, highest reducing power was reported after germination of 48 h at each germination temperature condition. During germination some compounds of sorghum donates electron to free radicals to help to stabilize radical chain reactions which in reply results in higher reducing power. Sharma and Gujral (2011) reported that reducing

power is mainly due to the phenolics and flavonoids as they possess the capacity to serve as reductones by donating electrons. Therefore, the increase in generation of more phenolic and flavonoids compounds can also be the cause for increment in reducing power after germination in comparison to native sorghum in present study.

Rheological properties

Germination conditions at varying period and temperature significantly ($p < 0.05$) influence the rheological properties of sorghum (Table 5). Pasting temperature (PT), which provide an indication of the gelatinization time of starch during swelling, was significantly ($p < 0.05$) influenced by variation in temperature and period of germination. Lowest PT was recorded for native followed by soaked sorghum, which increased considerably ($p < 0.05$) as germination period and temperature progressed and reached to max of 91.7 after 48 h of germination at 35 °C. When period of

Table 5 Effect of germination conditions on the rheological properties of sorghum flour

Treatment	Temperature (°C)	Time (h)*	Pasting Temperature (°C)	Viscosity (cP)				
				Peak	Hold	Final	Breakdown	Setback
Control Soaked Germination conditions	–	–	81.2 ± 0.6 ^e	816.5 ± 19.4 ^{bc}	814 ± 18.4 ^{bc}	2752.5 ± 30.4 ^b	364.5 ± 5.30 ^{ab}	1838.5 ± 37.1 ^b
	25	10	81.4 ± 0.6 ^e	1024.0 ± 24.7 ^a	968 ± 21.2 ^a	2889.5 ± 9.2 ^a	390 ± 5.66 ^a	2021.5 ± 44.9 ^a
	25	12	81.4 ± 0.8 ^{Re}	901.0 ± 2.1 ^{Pb}	818.5 ± 5.3 ^{Pb}	2412.5 ± 13.4 ^{Pc}	350.5 ± 5.30 ^{Pbc}	1556.5 ± 25.1 ^{Pe}
		24	81.9 ± 0.3 ^{Rde}	869.5 ± 16.6 ^{PQb}	730 ± 0.7 ^{Qd}	1915.5 ± 7.8 ^{Qf}	212 ± 6.36 ^{Qd}	1185.5 ± 4.6 ^{Qe}
	30	36	84.4 ± 0.3 ^{Qe}	823.5 ± 1.8 ^{Qbc}	514.5 ± 7.4 ^{Re}	1222 ± 5.7 ^{Rg}	93.5 ± 1.06 ^{Rf}	677.5 ± 11.0 ^{Rg}
		48	89.7 ± 0.4 ^{Pb}	721.0 ± 3.5 Rd	444 ± 14.1 ^{Sf}	1127 ± 26.9 ^{Sh}	82.5 ± 3.18 ^{Rfg}	624.5 ± 42.8 ^{Rg}
	30	12	81.9 ± 0.6 ^{Rde}	897.0 ± 5.7 ^{Pb}	821.5 ± 6.7 ^{Pb}	2384 ± 0.7 ^{Pd}	330.5 ± 3.18 ^{Pc}	1562.5 ± 20.2 ^{Pe}
		24	83.6 ± 0.1 ^{Rcd}	756.5 ± 1.8 ^{Qcd}	744.5 ± 4.6 ^{Qcd}	1255.5 ± 16.3 ^{Qg}	200 ± 0.00 ^{Qd}	711.0 ± 12.7 ^{Qf}
	35	36	89.4 ± 0.6 ^{Qb}	321.5 ± 31.8 ^{Rj}	69.5 ± 3.2 ^{Rh}	434.0 ± 2.1 ^{Rf}	80.5 ± 1.06 ^{Rfg}	244.0 ± 3.5 ^{Ri}
		48	90.0 ± 0.2 ^{Pab}	314.5 ± 14.8 ^{Rj}	56 ± 1.4 ^{Sh}	401.5 ± 12.4 ^{Rf}	75.5 ± 1.06 ^{Rfg}	250.5 ± 6.7 ^{Ri}
		12	81.5 ± 0.3 ^{Re}	865.0 ± 2.1 ^{Pb}	784.5 ± 3.2 ^{Pcd}	2188.5 ± 0.7 ^{Pe}	206.5 ± 3.89 ^{Pd}	1404.0 ± 3.5 ^{Pd}
		24	89.3 ± 0.3 ^{QRb}	514.0 ± 8.5 ^{Qd}	163.5 ± 3.2 ^{Qg}	603.5 ± 10.6 ^{Qi}	147 ± 5.66 ^{Qe}	340.0 ± 2.1 ^{Qh}
36		90.2 ± 0.2 ^{Qab}	200.5 ± 0.4 ^{Rg}	58 ± 2.3 ^{Ri}	237 ± 5.7 ^{Rk}	22.5 ± 1.06 ^{Sg}	36.5 ± 3.2 ^{Rj}	
48		91.7 ± 0.1 ^{Pa}	147.0 ± 5.7 ^{Sh}	31 ± 3.1 ^{Ri}	154.5 ± 0.7 ^{Sk}	56 ± 3.54 ^{Rg}	13.0 ± 2.1 ^{Sk}	
Mean [#]								
Germination temperature (°C)	25		83.75 ^s	828.75 ^p	626.75 ^p	1649.20 ^p	184.62 ^p	1011.0 ^p
	30		84.07 ^r	622.25 ^q	422.88 ^q	1059.50 ^q	171.62 ^q	692.00 ^q
	35		86.32 ^p	431.62 ^r	237.50 ^r	685.88 ^r	108.00 ^r	448.87 ^r
Germination time (h)	12		81.61 ^s	887.67 ^p	808.17 ^p	2315.8 ^p	295.83 ^p	1507.7 ^p
	24		82.35 ^r	698.00 ^q	546.00 ^q	1224.8 ^q	186.33 ^q	745.50 ^q
	36		85.06 ^q	472.67 ^r	195.00 ^r	524.50 ^r	71.33 ^r	319.33 ^r
	48		89.85 ^p	451.83 ^s	167.00 ^s	461.00 ^s	65.50 ^s	294.00 ^s

cP centi Poise

Values represent mean ± standard deviation

Values having different superscripts from a, b, c to k differ significantly ($p < 0.05$) from each other column wise among different treatments*Values having different superscripts P, Q, R and S show significant ($p < 0.05$) differences during different germination time at each germination temperature respectively#Means (germination temperature and time) having different superscripts from p, q, r and s differ significantly ($p < 0.05$) separately for mean values of germination temperature and time

Table 6 Effect of germination conditions on the colour characteristics of sorghum flour

Treatment	Temperature (°C)	Time (h)*	Colour characteristics			Hue angle (°)	Chroma
			L	a*	b*		
Control	—	—	57.65 ± 0.35 ^a	19.90 ± 0.00 ^f	4.01 ± 0.07 ^g	10.95 ± 0.59 ^{fg}	20.27 ± 0.04 ^{ef}
Soaked	25	10	57.35 ± 0.21 ^a	19.80 ± 0.00 ^f	3.35 ± 0.03 ^h	9.32 ± 0.20 ^{gh}	20.07 ± 0.01 ^f
Germination conditions	25	12	56.75 ± 0.35 ^{Pb}	20.15 ± 0.07 ^{Ref}	4.14 ± 0.04 ^{Rfg}	11.36 ± 0.15 ^{Rdef}	20.55 ± 0.08 ^{Sde}
		24	56.30 ± 0.28 ^{Pb}	20.35 ± 0.01 ^{QRcde}	4.02 ± 0.01 ^{Sg}	11.26 ± 0.08 ^{defR}	20.75 ± 0.22 Rd
		36	54.30 ± 0.14 ^{Qc}	20.65 ± 0.03 ^{PQbc}	5.01 ± 0.02 ^{Qcd}	13.87 ± 0.42 ^{Qb}	21.27 ± 0.03 ^{Qbc}
		48	51.35 ± 0.07 ^{Rf}	20.85 ± 0.07 ^{Pb}	5.72 ± 0.00 ^{Pa}	15.29 ± 0.05 ^{Pa}	21.62 ± 0.07 ^{Pab}
	30	12	57.60 ± 0.28 ^{Pa}	20.35 ± 0.05 ^{Qcde}	3.14 ± 0.03 ^{Rh}	8.94 ± 0.42 ^{Ri}	20.60 ± 0.05 ^{Rde}
		24	56.25 ± 0.21 ^{Qb}	20.45 ± 0.07 ^{Qcd}	4.16 ± 0.02 ^{Qfg}	11.61 ± 0.42 ^{Qcdef}	20.88 ± 0.04 ^{Qd}
		36	54.05 ± 0.21 ^{Rc}	20.85 ± 0.02 ^{Pb}	5.38 ± 0.01 ^{Pbc}	14.00 ± 0.41 ^{Pab}	21.49 ± 0.03 ^{PQabc}
		48	52.95 ± 0.21 ^{Se}	20.95 ± 0.07 ^{Pab}	5.38 ± 0.02 ^{Pab}	14.33 ± 0.14 ^{Pab}	21.62 ± 0.09 ^{Pab}
	35	12	56.50 ± 0.85 ^{Pb}	20.45 ± 0.05 ^{Rcd}	3.97 ± 0.04 ^{Sh}	10.53 ± 0.42 ^{Qfg}	20.80 ± 0.04 Rd
		24	53.90 ± 1.13 ^{PQd}	20.70 ± 0.14 ^{QRb}	4.94 ± 0.02 ^{Pdef}	12.93 ± 0.64 ^{PQbc}	21.24 ± 0.09 ^{Qc}
		36	52.50 ± 0.42 ^{QRc}	21.00 ± 0.14 ^{PQab}	4.55 ± 0.05 ^{Ref}	12.10 ± 0.08 ^{Pcde}	21.48 ± 0.14 ^{PQabc}
		48	50.45 ± 0.35 ^{Rg}	21.25 ± 0.07 ^{Pa}	4.71 ± 0.07 ^{Qc}	12.47 ± 0.04 ^{Pcd}	21.76 ± 0.07 ^{Pa}
Mean [#]							
Germination temperature (°C)		25	55.21 ^P	20.50 ^r	4.73 ^r	12.94 ^P	21.05 ^q
		30	54.67 ^q	20.65 ^q	4.49 ^q	12.21 ^q	21.14 ^q
		35	53.33 ^r	20.85 ^P	4.43 ^P	12.00 ^q	21.32 ^P
Germination time (h)		12	56.95 ^P	20.31 ^s	3.68 ^s	14.30 ^P	20.65 ^s
		24	55.48 ^q	20.50 ^r	4.33 ^r	13.33 ^q	20.95 ^r
		36	53.62 ^r	20.83 ^q	4.93 ^q	11.92 ^r	21.41 ^q
		48	51.52 ^s	21.02 ^P	5.25 ^P	10.27 ^s	21.67 ^P

Values represent mean ± standard deviation

Values having different superscripts from a, b, c to i differ significantly ($p < 0.05$) from each other column wise among different treatments

*Values having different superscripts P, Q, R and S show significant ($p < 0.05$) differences during different germination time at each germination temperature respectively

[#]Means (germination temperature and time) having different superscripts from p, q, r and s differ significantly ($p < 0.05$) separately for mean values of germination temperature and time

germination was upsurge from 12 to 48 h and temperature from 25 to 35 °C, paste temperature increased from 81.5 to 91.7 °C due to gelatinization and fragmentation of starch and protein. Rheological properties of native sorghum differ significantly ($p < 0.05$), in terms of peak viscosity, hold, final and setback viscosity from sorghum germinated at different conditions. Germination, significantly decrease the peak and final viscosity of sorghum. The decrease was higher as period of germination enhanced from 12 to 24 and 48 h, and temperature increased from 25 to 30 and 35 °C. Native sorghum has intact starch granules which were breakdown into small fragments by enzyme resulting in low peak and final viscosities. The substantial decrease in peak viscosity with increasing germination time and temperature may be attributed to degradation of starch and proteins to simpler units, and higher enzymatic activity (Pal et al. 2016). Native and soaked sorghum has highest hold viscosity which was drastically influenced by

germination conditions. Elevation in period of germination from 12 to 48 h and temperature from 25 to 35 °C substantially decreased the hold viscosity of germinated sorghum (Table 5). The hold viscosity of sorghum after 48 h of germination at each temperature was dropped off significantly ($p < 0.05$) to 444.0, 56.5 and 1.0cp, respectively. During germination starch hydrolyzing enzymes are activated which results in starch fragmentation and thus displayed the reduced viscosity (Zeeman et al. 2007). Final viscosity of sorghum germinated over different germination conditions varied significantly ($p < 0.05$). Native sorghum had final viscosity of 2652.5 cP which was appreciably higher in comparison to germinated samples. Among all the germinated sorghum samples, the 48 h germinated sorghum had lowest; while 12 h germinated sorghum had highest viscosity at each temperature (Table 5). Decrease in breakdown and setback viscosities were also observed among the sorghum germination at

varying conditions as compared to control and lower setback values of the flour samples are useful in the preparation of frozen foods.

Color characteristics

The color characteristics as well as hue angle and chroma values of germinated sorghum flour as influenced by germination period and temperature are presented in Table 6. Germination significantly ($p < 0.05$) affected the all colour characteristics. Non-germinated sorghum has highest L value, which decreased notably as period of germination progressed from 12 to 48 h and temperature increased from 25 to 35 °C. However, with enhanced germination temperature and period increased the redness (a^*) and yellowness (b^*) values of the sorghum increased appreciably. Pal et al. (2016) in their study also reported that germination increased the values of redness and yellowness of brown rice flour while it decreased the Lightness values. Hue and chroma values also increased significantly ($p < 0.05$) as the germination time and temperature increased. The increase may be due to increase in the pigmentation of the flour due to enzymatic activity and decrease in the L^* value of flour samples. Each germination condition affects the colour characteristics of sorghum in different way as sorghum samples germinated at 35 °C exhibits less yellowness in comparison to grains germinated at lower temperature. Similar end results were also reported by Chung et al. (2012), which was due to enhanced activity of polyphenol oxidase and peroxidase enzymes, as during germination oxidative products act together with denatured proteins which assist the enzymatic browning reaction and also due to synthesis of GABA (Moongngarm and Saetung 2010), which improves the formation of pigments and thus lowered down the lightness values.

Conclusion

The results showed that germination conditions appreciably enhanced the nutrient digestibility, bioactive components and lower down the pasting viscosities of the sorghum. Germination at higher temperature and duration accelerates the hydrolytic enzymes which results in enhanced starch and protein digestibility in comparison to germination for shorter duration at low temperature. Elevated temperature and germination duration results in the generation of bioactive compounds which results in enhancement in the antioxidant activity, phenolic content and reducing power. Both germination time and temperature are important factor affecting the nutrient digestibility, antioxidative properties, bioactive components and

pasting characteristics of sorghum. Results from the current study will help in delivering the collective information for the selection of optimum time and temperature of germination for the desired quality end product at both commercial and home scale level.

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