The Pharma Innovation Journal 2021; 10(11): 246-250

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(11): 246-250

www.thepharmajournal.com
Received: 13-09-2021
Accepted: 23-10-2021

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Effect of hydrothermal pretreatment on sprouting of sorghum

Anagha Deore and KA Athmaselvi

Abstract
The study seeks to evaluate the effect of hydrothermal treatment on sprouting of sorghum. The sorghum grains were soaked in water at 40 and 50 ºC for 15 minutes. Sorghum treated at 40 ºC reached maximum sprouting rate of 96.5% within 12 hours while the untreated control sample reached 90% in 20hrs. Germination being an important process for developing nutritionally beneficial food products is influenced by the hydration. Sufficient hydration due to increased temperature might have paved way for improved sprouting rate.
The pasting properties like peak viscosity, final viscosity and breakdown viscosity increased as the temperature of treatment increased. There was significant decrease in protein, and fat content. The water absorption capacity and oil absorption capacity ranged from 0.78 to 0.79 g/g and 0.64 to 0.65 g/g respectively which showed a decreasing trend from control. Sprouting is a time-consuming process thus, physical pre-treatment like soaking in warm water could accelerate the process. Also, the process doesn't require high investments making it suitable for cottage industrial applications.

Keywords: Sorghum, sprouting, hydrothermal, hydration rate

1. Introduction
Traditionally known as Jowar in India, sorghum is heat and drought resistant making it suitable for cultivation in semi-arid or tropical regions and is world's fifth important crop (Rooney, 2014; Serna-saldivar *et al*., 2019) [17, 18]. Around 3.47 MT of production was recorded in India for the year 2019 (FAO, 2019). Sorghum has higher levels of carbohydrate followed by protein and fat. It is also rich in phenolic compounds especially phenolic acids, flavonoids, and tannins (Chhikara *et al*., 2018) [2]. Its greater antioxidant activity is known to be useful in reducing risk of cancer and other lifestyle diseases (de Morais Cardoso *et al*., 2017) [5]. Along with being a good source of nutrients, sorghum is also gluten free which provides an alternative ingredient in different products that are conventionally made using wheat and its milled products. The upsurge in demand for gluten free foods is helping to bring back sorghum in the market (Woomer & Adedeji, 2021) [25].
Germination claims to improve the nutritional quality and is also important part in preparing several products and is responsible for synthesizing hydrolytic enzymes that are inactive in raw grains (Singh *et al*., 2015) [21], modifying structures and synthesizing bioactive compounds (Kaukovirta-Norja, A. *et al.*, 2004) [11]. However, sprouting can be affected by various internal as well as external stimulations (Wang *et al*., 2019) [24]. One such intrinsic factor of seeds that slows down germination is dormancy (Bewley, lack and Halmer, 2006). Various techniques like seed stratification, scarification alteration of growth hormones could be employed to breakdown seed dormancy (Rifna *et al*., 2019) [16]. Along with this, use of heat, extreme temperatures, hot water dips and exposure to radiations could prove useful in breaking seed dormancy (Tung & Serrano, 2011) [23].
Present study would help to understand the effect of hydrothermal pretreatments like soaking the grains in water at 40 ºC and 50 ºC for 15 minutes on hydration and sprouting rate of sorghum and also evaluate their effect on nutritional, functional and rheological properties.

2. Materials and Methods
Sorghum grains were purchased from a local farmer in Maharashtra. Any dirt, stones, or other undesired material was removed from the grains. Before being used in investigations, the grains were kept in airtight containers.

2.1 Hydrothermal pretreatment
100 nos. of known weight sorghum grains were placed in a net bag. 500 ml of distilled water was brought up and maintained at 40, 50 ºC in a hot water bath respectively. The grains were dipped and treated for 15 minutes at respective temperatures. Afterwards, the grains were soaked in 1L of distilled water for 12 hours followed by germination for 24 hours. The grains were allowed to germinate on filter paper in a petri plate.

2.2 Hydration Study with warm water at different soaking time
On the basis of preliminary studies 12 hours of soaking was considered for hydration study. 10 g of sorghum grains were hydrothermally treated at 40, 50 ºC for 15 minutes as mentioned above. Hydration study was performed for the treated sample soaked in distilled water maintained at 27 ± 1 ºC for 12 hours. The grains were periodically drained every 1 hour, superficially dried and the moisture content was checked by mass balance methods. The grains were soaked again, and the process was repeated. In order to study the hydration rate graph of moisture content versus time was plotted.

2.3 Determination of Germination Percentage
100 nos. of sorghum grains that were treated and soaked were germinated as mentioned above after the treatments. The germination percentage was observed every hour until maximum number of seeds were sprouted.

$$Sprouting\ Percentage\% = \frac{Total\ number\ of\ sprouted\ seeds}{Total\ number\ of\ soaked\ seeds} \times 100$$

2.4 Proximate Analysis
The proximate composition of the control and treated samples includes carbohydrate, protein, fat, fiber, and ash content. The protein is measured by calculating total nitrogen content by Kjeldhal's method (AOAC). All other compositions were estimated by following (AOAC). The total carbohydrate content was calculated by difference method as,

$$\text{Total Carohydrate (\%)} = (100 - \text{moisture} + \text{fat} + \text{crude protein} + \text{ash} + \text{crude fiber})$$

2.5 Color
The color value of pretreated samples was observed by Hunter Lab (Hunter Lab ColorFlex EZ Spectrometer). The values of lightness (L*), redness (a*), and yellowness (b*) were recorded for each sample (Dattatray *et al*., 2020) [4].

2.6 Pasting Properties
The pasting properties of flour include pasting temperature (PT), peak viscosity (PV), final viscosity (FV), breakdown viscosity (BV), and setback viscosity (SV). Rapid Visco Analyzer (Anton Paar MCR 52, Austria) was used to determine the pasting properties of sorghum flour. A 3 g sample along with 25mL distilled water was added to the canister. After being brought up and kept at 50 °C for 1 minute, each sample mixture was heated to 95 °C at a rate of 12 °C/min for 2.5 minutes. (Yi *et al*., 2017) [26].

2.7 Water Absorption Capacity and Oil Absorption Capacity
The water absorption capacity (WAC) and oil absorption capacity (OAC) was calculated following the method by (Sharanagat *et al*., 2019) [19]. 3 g of treated sample flours were mixed with 25 mL distilled water and left to rest at 25ºC for 30 minutes in a centrifuge tube. Further it was centrifuged for 25 min at 3000 g. To eliminate excess moisture the sediment-filled tubes were dried in a hot air oven at 50°C for 25 minutes.

In a pre-weighed centrifuge tube, 6 mL of soybean oil was added to 0.5 g of flour sample. After that, the mixture was left to settle for 30 minutes prior centrifuging it at 3000 g for 25 minutes to obtain a distinct layer of oil. Before weighing, the tubes were turned upside down for 25 minutes to remove any excess oil.

2.8 Total phenolic content and antioxidant activity
The method mentioned by (Sharanagat *et al*., 2019) [19] with small alterations was used to determine total phenolic content (TPC) and antioxidant activity (AOA) of treated sorghum samples. The samples were extracted in 80% methanol solution. The mixture was shaken for 1 hour at 50 ºC, 200 rpm in a water bath shaker, then centrifuged for 10 min at 5000 rpm. To determine TPC, 100 mL of extract was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of 7.5 percent sodium carbonate solution. A UV spectrophotometer was used to examine the absorbance at 765 nm after 1 hour of incubation.

AOA was determined using the same extract by measuring the percent inhibition of DPPH (1,1-diphenyl-2 picrylhydrazyl). 2 ml of DPPH (0.4 mM) methanolic solution was added to 2 ml of sample extract. The mixture was let to rest in the dark for 30 minutes. The absorbance at 517 nm was then measured using a UV spectrophotometer.

2.9 Statistical Analysis
All the experiments and analysis were performed in triplicates. One-way ANOVA followed by post-hoc method Tukey were used to compare the means. Minitab software (version Minitab 17.3.1) was used for this statistical analysis.

3. Result and Discussion
3.1 Hydration Study
The hydration rate of warm water treated samples are presented in figure1. The moisture content of the sample increased with temperature of treatment as soaking duration proceeds. The increase was rapid at the beginning and later it slowed down as equilibrium was approached. The increase in water absorption is due to increase in water diffusion at higher temperature (Kashiri *et al*., 2010) [10]. High initial water absorption may be due to effect of capillarity which reduces as the concentration of water in grain increases (Silva *et al*., 2019) [20]. The slowing down of hydration could be observed around 6-8 hours. By the end of 12 hours the moisture content for warm water treated samples WW40 and WW50 was 36.48% and 39.23%. The temperature helps to increase water absorption as it tends to soften and expand the grain (Kashaninejad *et al*., 2009) [9].

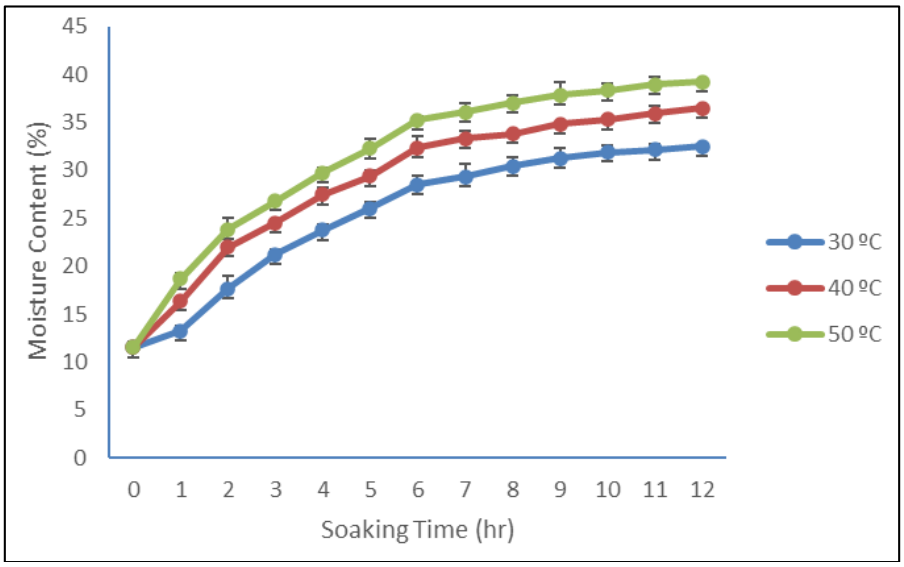

Fig 1: Effect of hydrothermal treatment on hydration rate of sorghum

3.2 Sprouting Rate

Figure 2. represents the sprouting rate of treated and control sorghum. WW40 has the highest sprouting rate of 96.5% while WW50 has of 95%. WW40 reached the maximum sprouting rate 14 hours while it took 18 hours to reach the maximum sprouting percentage. The control had 90% of sprouting rate by 20 hours. This indicates that warm water treatment was able to enhance the germination rate. The findings can be supported with observations made by Tung & Serrano, 2011 [23]; where warm water treatment at 50 ºC for 15 minutes enhanced the germination of rice seeds along with improving respiration rate and α-amylase activity.

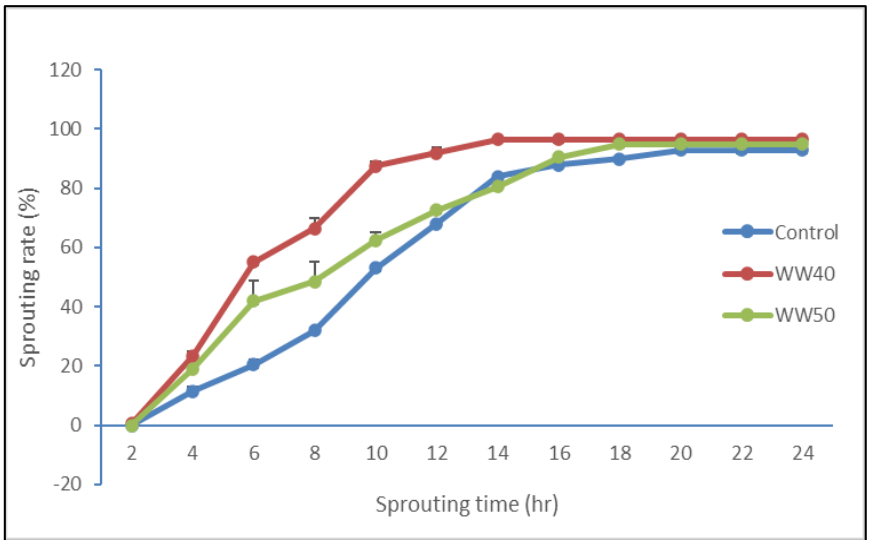

Fig 2: Effect of hydrothermal treatment on sprouting rate of sorghum

3.3 Proximate Composition

The proximate composition of control and treated sorghum is presented in Table.1. A significant difference ($p< 0.05$) between the proximate composition of warm water treated samples and the untreated control. However, the difference in the temperature of treatment did not affect the nutritional composition significantly ($p > 0.05$). The protein content was reduced to 6.25% and 5.807% for WW40 and WW50 samples respectively. Activation of protease enzymes during germination reduces the protein (Afify *et al*., 2012) [1]. This result was in accordance with Tamilselvan & Kushwaha, 2020 where malted sorghum had decreased protein content as it was utilized during development of embryo. Similarly, the fat content was reduced as it ranged between 1.70 to 1.80%. This is a result of increased lipolytic enzyme activity during sprouting (Hassan *et al*., 2017) [7]. There was not much decrease in the carbohydrate content of treated samples and ranged from 75.84 to 77.38%. The utilization of soluble sugar for respiratory activity and still prominent amylase inhibitory activity could be reason behind no significant difference between the carbohydrate content at earlier stages of germination (Mbithi-Mwikya *et al*., 2000) [14].

3.4 Pasting Properties

Changes that occur inn food on applying heat in presence of water is known as pasting properties. These changes ultimately affect the texture and use of food product (Ocheme *et al*., 2018) [15]. The pasting temperature ranged from 74.21 º C to 75.10 º C in the samples. WW50 treated sample shown the highest peak viscosity (1,293 cP), breakdown viscosity (1111 cP) and final viscosity (552 cP). There was significant decrease ($p > 0.05$) in the setback (281.05 cP) of WW50 treated sample. Lowering of setback indicates less syneresis and lower starch retrogradation (Sharanagat *et al*., 2019) [19].

Above results are in par with Dattatray *et al*., 2020 [4], where warm water treated and radiofrequency dried green gram malt showed highest values of pasting parameters. The WW40 treated sample showed lower values of peak viscosity (635 cP)

Table 1: Proximate composition and bioactive composition of sprouted sorghum

Sample	Total Carbohydrate	Protein	Fat	Fiber	Ash	TPC (mg GAE/100g)	AOA
Control	69.59±1.23 ^{b}	10.140±0.25^{a}	6.83±0.62^{a}	1.33±0.66^{b}	1.73±0.21^{a}	17.897±0.46^{a}	91.67±0.43^{a}
WW40	75.84±0.70^{a}	6.250±0.11^{b}	1.70±0.50	3.00±1.08^{a}	1.367±0.26^{a}	11.860±0.79^{b}	63.14±0.08^{b}
WW50	77.38±0.43^{a}	5.807±0.25 ^{b}	1.80±0.16	1.23±0.21^{b}	1.600±0.14^{a}	10.890±0.59^{b}	63.28±0.20^{b}

Table 2: Pasting properties of sprouted sorghum

Sample	Pasting temperature (ºC)	Peak viscosity (cP)	Final Viscosity (cP)	Breakdown Viscosity (cP)	Setback (cP)
Control	75.10±0.13^{a}	791.9±0.45^{b}	452.3±0.20 ^{c}	654±0.8 ^{b}	314.86±0.36^{b}
WW40	74.81±0.45 ^{a}	635 ±0.25^{c}	547.9±0.60^{b}	549±0.75^{c}	576.65±0.45^{a}
WW50	74.21±0.27 ^{a}	1293±1.00^{a}	552.2±0.30^{a}	1111±1^{a}	281.05±0.75^{c}

and final viscosity (457 cP). The lower value of peak viscosity is resultant of starch degradation during germination while lower final viscosity is an indication of amylose leaching (Harasym *et al*., 2020) [6].

3.5 Color

Table3. represent the hunter Lab color values of all control and treated sorghum samples. The L^*, a^* and b^* values of control and warm water treated samples ranged from 81.54 to 83.55, 1.32 to 1.62 and 9.18 to 10.71 respectively. The higher values of L^* indicates whiteness of the samples. While positive values of a^* and b^* represents redness and yellowness of the samples respectively (Ma *et al*., 2011). The re results observed are in agreement with the results presented by (Kaur & Gill, 2021). The change in color value were observed in sorghum among other cereal grains during different duration of germination. They reported that activation of oxidative enzymes during germination was responsible for decrease in L^* value and increase in a^* and b^* values.

3.6 Water Absorption Capacity and Oil Absorption Capacity

The WAC of control and warm water treated sample is presented in Table.3. Water absorption is the ability of a gram of sample to hold water (Ma *et al*., 2011) [13]. A significant decrease ($p< 0.05$) was observed in the WAC of warm water treated sorghum and the control. These results agree with the water holding capacity of sprouted durum wheat flour as studied at different duration of germination (Jribi, 2019) [8]. Germination resulting in starch degradation is responsible for the release of water from granules as well as produces crosslinking between starch chains. This leads to reduction in the ability of starch to swell and thus consequently lowering water holding capacity (Cornejo & Rosell, 2015) [3].

The oil absorption capacity of warm water treated samples was lower than that of the control. The absorption of oil depends on its ability to bind to non-polar protein chains, lipophilicity, and the amino acid composition among others. Thus, reduction in the oil absorption capacity might be due to change in polarity, protein denaturation or reduction in non-polar amino acids (Sharanagat *et al*., 2019) [19].

3.7 Total phenolic content and antioxidant activity

The total phenolic content was measured for extracts from hydrothermally treated samples and the control mentioned in Table.1. The total phenolic content was reduced for the treated sample to 11.860 ± 0.79 mg GAE/100g and 10.89 ± 0.59 mg GAE/100g for WW40 and WW50 sample. Similarly, the antioxidant activity also decreased by about 28% in the treated samples. The decrease in antioxidant activity can be associated with reduction in the phenolic activity. There was no significant difference observed for total phenolic content and antioxidant activity among different temperatures of hydrothermal treatment. These results agreed with studies on hydrothermal stability of phenolic extract obtained from brown.

Table 3: Color profile and functional properties of sprouted sorghum

Sample	L*	a*	b*	E	WAC	OAC
Control	83.55±0.00^{a}	1.32±0.00^{b}	9.18±0.00^{b}	-	1.12±0.04^{a}	1.1±0.13^{a}
WW40	81.54±0.10^{b}	1.623±0.02^{a}	10.71±0.04^{a}	2.54±0.06^{a}	0.79±0.001^{b}	0.65±0.07 ^{b}
WW50	81.79±0.22^{b}	1.617±0.01^{a}	10.59±0.087 ^{a}	2.27±0.22^{a}	0.78±0.02 ^{b}	0.64±0.02 ^{b}

Where the heat treatment had little reducing effect on the phenolic and antioxidant activity however showed no significant difference among the holding temperatures (Zeng *et al*., 2019) [27].

4. Conclusions

Sprouting rate was affected by the temperature of soaking during the pretreatment. The temperature improved the water absorption of grains by expanding and softening it, thus consequently increased the sprouting rate. Although there was not much influence of different temperature on the final sprouting percentage, but the time taken to reach it was greatly influenced. The pasting properties and nutritional profile also improved due to the pre-treatment and would be helpful in developing new products from sprouted sorghum flour.

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