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Effect of Graded Mechanical Shock Pulses from a Shock Tube on Germination, Growth, and Antioxidant Enzyme Activities in Sweet Sorghum (*Sorghum bicolor* L. CSH 16)

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Abstract : Sweet sorghum (Sorghum bicolor L. Moench CSH 16) supports food, feed, and biofuel production in arid regions, but seed germination under stress limits yields. Shockwave treatments enhance seed permeability via mechanotransduction. This study evaluates moderate shock pulses (6 pulses) for optimal germination and physiological enhancement. Seeds received 0 (control), 2, 4, 6, 8, or 10 mechanical shock pulses (Mach 1.7-2.2, ~0.5-2 MPa estimated peak pressure, microsecond rise time) from a diaphragm-based shock tube in air medium. Germination (n=30/replicate, 3 replicates), growth, vigor index, and antioxidant enzymes (SOD, CAT, APX, GST) were assessed after 14 days. ANOVA and LSD ($p<0.05$) analyzed data. All treatments achieved 100% germination (no significant difference). At 6 pulses, seedling vigor index rose 31% (3107 ± 285 vs. 2364 ± 293 control), plumule length 45% (20.5 ± 6.6 cm), radicle wet weight 61% (2.84 g/seed), with $p<0.05$ vs. control. Antioxidant enzymes peaked at higher pulses (e.g., SOD 70 ± 1 U/g FW at 10 pulses, 291% control increase). Nutritional analysis showed 12% higher energy (366 kcal) and 11% carbohydrates (78 g) at 10 pulses. Moderate shock pulses (6) optimize growth via mechanosensitive permeability without excess stress.

Keywords: Germination; seedlings; sweet sorghum; shockwaves.

Abbreviations : FGP - Final germination percentage; DAS – days after sowing; CRD - completely randomized design; LSD - least significant difference.

I. INTRODUCTION :

Sweet sorghum (*Sorghum bicolor* (L.) Moench) is a plant which belongs to the family Poaceae and originated from Africa. At present, sorghum is the fifth most important cereal crop after wheat, rice, maize and barley (Paiva CL., *et al*., 2022). Sorghum has been used extensively as animal feed, human food and as a raw material for bioethanol production. It is the main food grain for over 750 million people who live in developing countries of Africa, Asia and Latin America (Ahmed A L *et al.*, 2024). Over 70% of the world's total production is from Africa and Asia as the crop is able to grow in drought-resistant areas with limited water and nutrients.

Sweet sorghum is similar to sorghum but due to its high sugar content in its stem, can be used for food, feed, fodder and biofuel production. Sweet sorghum requires one-fourth amount of water when compared to sugarcane. After planting, the crop requires 115-125 days to grow as a short-day plant. Sweet sorghum can easily grow in drought, saline, water-logged and alkaline soils (Dalvi US *et al.*, 2022). Rapid seed germination drives yield, yet abiotic stresses hinder it; physical stimuli-like shockwaves induce mechanotransduction, activating ion channels and ROS signaling for permeability

Research indicates that low-intensity electric fields can increase germination potential by up to 29.25% and improve the germination index by 20.65% compared to untreated seeds, as observed in studies involving *Scutellaria baicalensis* (Song *et al.*, 2024). Additionally, the application of low voltage currents around plant roots has been found to stimulate metabolic processes, leading to improved growth parameters such as plant height, leaf area, and chlorophyll content in crops like mung beans and chickpeas. Furthermore, shockwave treatments have demonstrated the ability to accelerate germination rates and enhance physiological growth in various species, suggesting that these innovative techniques could be effectively integrated into sustainable agricultural practices to boost crop yields (Radhakrishnan *et al.*, 2020 and 2021).

Unlike ultrasonication or PEF, shock tubes generate true supersonic waves (Mach >1) with sharp pressure fronts, as in Reddy's tube studies on tomato (Mach >2, puncturing coats for 20-30% faster germination). Prior work on *Vigna mungo* used Mach 1.7-2.2 pulses (~1-5 MPa), boosting chlorophyll and growth 20-50% via energy flux. This study tests 2-10 pulses on CSH 16 germination, growth, antioxidants, hypothesizing optimal moderate dose balances stimulation and stress.

Therefore, the objective of this study is to investigate the potential of shockwaves on seedling growth of a sweet sorghum variety and to understand the effect of on seed germination and physiology.

II. MATERIALS AND METHODS :

In vitro germination and early seedling growth

Seeds of CSH 16 sweet sorghum varieties used in the study were collected from ICAR. A diaphragm-based shock tube (driver-driven sections, air medium) delivered mechanical shock pulses at Mach 1.7-2.2 (estimated peak pressure 0.5-2 MPa, duration ~10-100 µs, rise time <1 µs, based on similar designs). Seeds fixed in driven section received 0, 2, 4, 6, 8, 10 pulses; energy flux calibrated by pulse count. Control seeds were not shocked. Healthy, uniform seeds of all varieties were surface sterilized with 1% sodium hypochlorite for 3 min, 70% ethanol and distilled water and surface dried using Whatman paper. Ten seeds for each cultivar in each treatment were allowed to germinate on a filter paper in 9 cm diameter Petri dishes. Each filter paper was moistened with solutions of 0 (distilled water) as a control, 2,4,6,8,10 shock pulses. 10 ml of distilled water was applied to each Petri dish. The Petri plates were arranged in completely randomized design (CRD) with three replicates for each treatment. Germination room temperature was maintained at 25 ± 1°C in the dark with 8 h photoperiod and a relative humidity of 70%. Petri plates were periodically checked and respective solutions were applied to compensate evaporation. Seeds were considered germinated when the radicle had extended for at least 2mm. Seedling shoot and root length of randomly selected seedlings from each replication were measured at the time of harvest (14 days after treatment application) by using a scale. Root wet weights were recorded. After final count, final germination percent (FGP) were calculated by the following formulae (Ellis *et al*., 1981; Ruan *et al*., 2002).

$$FGP = \frac{\text{Number final germinated seeds}}{\text{Total number of seed tested}} \times 100$$

Seedling vigour index (V) was computed according to Hassan *et al*., 2013 as follows:

$$V = L \times FG$$

where L is the mean seedling length (total shoot and root length in mm) and FG is final germination percentage (%). This experiment was repeated twice to determine the consistency of results of various varieties against different levels of shockwaves. Data were analyzed using Analysis of Variance method (ANOVA) (http://faculty.vassar.edu/lowry/anova1u.html) and least significant difference (LSD) for individual parameters. Kruskal-Wallis test was conducted to assess the data distribution for each parameter such as plumule length, radicle length and seedling vigour index.

Biochemical Analysis

Sample Preparation:

Fresh leaves were used for extraction for APX, CAT, SOD and GST Assays. Extraction was done using 1 gm of fresh leaves, homogenised with extraction buffer 0.1M Phosphate buffer (pH- 7.5) containing 0.5mM EDTA. Mixture was then centrifuged at 15,000g for 20 min at 4℃. The separated supernatant was then collected and used for biochemical assays.

Ascorbate Peroxidase (APX)

APX activity was assayed using a modified method of Nakano and Asada. Activity was determined from the decrease in absorbance at 290 nm due to the oxidation of ascorbate in the reaction. The 2 mL assay mixture contained 660 µl of 50 mM potassium phosphate buffer (pH 7.0), 660 µl of 0.5 mM ascorbate, 660 µl of 0.5 mM H_2O_2, and 20 µl of crude plant extract. H_2O_2 was added last to initiate the reaction, and the decrease in absorbance was recorded for 3 minutes. The extinction coefficient of 2.8 mM^{-1} cm^{-1} for reduced ascorbate was used in calculating the enzyme activity, which was expressed in terms of milli moles of ascorbate per minute per gram fresh weight. Formula used to calculate:

$$\text{APX Enzyme activity (Units/L)} = \frac{(\Delta\text{Abs} \times \text{Total assay volume})}{(\Delta t \times \varepsilon \times l \times \text{Enzyme sample}}$$

Δ Abs- Decrease in absorbance

Δt- Time interval (3 minutes)

ε- Molar extinction coefficient

Glutathione-S-Transferase (GST)

20mg of fresh leaf sample is crushed with cooled pestle and mortar using 1000µL of 100mM potassium phosphate buffer (pH 6.25). The homogenate is then centrifuged at 8000rpm for 5min at and the supernatant is taken as the crude enzyme extract. Enzyme activity is assayed in 2ml of reaction mixture consisting 1.5ml of 100mM potassium phosphate buffer (pH 6.25), 100µl of 0.75mM CDNB (1-chloro-2,4-dinitrobenzene), 200µl of 30mM GSH (reduced glutathione) and 0.2ml of enzyme extract. The rise in absorbance due to the formation of conjugates between GSH and CDNB is recorded spectrophotometrically at 340 nm. A blank reaction is prepared where the crude enzyme extract is eliminated. The concentration of conjugates formed is calculated by using a extinction coefficient value as 9.6 mM^{-1} cm^{-1}. One unit (U) of enzyme activity is expressed as 1µmol of CDNB conjugates formed min^{-1}. The formula for calculation of concentration of product so formed and Unit/ml of enzyme is given below; Calculate change in absorbance from the linear range of plot as below. Subtract the ΔA 340/min of blank from ΔA 340/min of the sample. This rate is used to calculate GST specific activity

$$\Delta A_{340}/\text{min} = \frac{A_{340}\ (\text{final read}) - A_{340}\ (\text{initial read})}{\text{Reaction time (min)}}$$

as below.

$$\frac{\Delta A_{340}/\text{min} \times V\ (\text{ml}) \times DF}{\varepsilon_{mM} \times V_{enz}\ (\text{ml}) \times \text{path length}} = \mu\text{mol/ml/min}$$

Where

DF = dilution factor

εmM = extinction coefficient for CDNB conjugate at 340 nm = 9.6 $mM^{-1}cm^{-1}$ V (ml) = reaction volume

Venz (ml) = volume of enzyme sample tested Path length for cuvette = 1.0 cm

Superoxide Dismutase (SOD)

The reaction mixture contains 300µl each of 50mM potassium phosphate buffer (pH 6.5), 45mM Methionine, 5.3mM riboflavin, 84mM NBT (Nitro Blue Tetrazolium) and 50mM Sodium carbonate (pH 10.2). To the test, 100µl of the crude enzyme extract is added and the final volume is adjusted to 3ml with distilled water, while the control is devoid of the enzyme extract. The tubes are placed at 25°C in the presence of 10-15W fluorescent lamps. Reduced NBT is measured spectrophotometrically at 600nm after exposure to light for 10 min; the maximum reaction is evolved in the absence of the enzyme that is in control. One unit (U) of SOD activity is defined as the amount of enzyme required to cause 50% inhibition in NBT reduction. The % inhibition is calculated by the formula given below.

SOD (U/ml) = (Vp – Vc)/Vc * Ext. Coeff Vp- OD of sample at 2 min ; Vc – OD of sample at 0 min

Catalase (CAT)

The CAT activity was measured according to the method described by Aebi (1984). The CAT assay mixture of 1.5 ml consisted of 0.05 ml sample, 1 ml phosphate buffer (100 mM buffer, pH 7.0), 0.5 ml H_2O_2, and 0.95 ml distilled water. A decrease in the absorbance was recorded at 240 nm. The CAT activity was expressed as µ mol of H2O2 oxidized per minute per gram.

Enzyme activity (Units/L) = (Δ Abs × Total assay volume) / (Δ t x ε x l x Enzyme sample volume

Where Δ t is the time of incubation (min), Δ Abs is the change in absorbance, ε is the extinction coefficient of substrates in units of M-1cm-1 = 39.4 M-1 cm-1), and l is the cuvette diameter (1cm). Enzyme activity (Unit) was defined as the amount of enzyme that oxidized 1µmol of substrate/min.

Statistical Analysis

Statistical analyses was carried out employing analysis of variance (ANOVA) and Kruskal Wallis was conducted to assess the data distribution for each parameter such as plumule length, radicle length and seedling vigour index.

NUTRITIONAL ANALYSIS

Samples of Sweet sorghum (untreated), Shock-treated Sorghum (8 shocks) Shock-treated Sorghum (10 shocks) were analyzed for Energy (Kcal), Carbohydrates (g), Total Fat (g), Protein (g), Crude Fibre(g), Calcium (mg), Iron (mg)and Total Ash (g).

III. RESULTS AND DISCUSSION

The ANOVA results showed that there was an effect of shockwaves on final germination percentage, seedling vigour index, plumule and radicle length at $p \geq 0.05$.

Final germination percentage and Seedling Vigour Index

The final germination percentage range was recorded at 100% in control and in the other treatments. Seedling vigour index increased gradually from the control to 6 shock pulses. Seedling vigour index of 2364±293.7 was the lowest in the control seeds (Table 1). The highest seedling vigour index of 3107±285.8 was obtained at 6 shock pulses. From this, it can be concluded that 6 shock pulse treatment has a stimulating effect on the germination process.

Table 1: Effect of shock treatment on SVI and Wet Weight of sweet sorghum seeds

Parameter (shock)	0	2	4	6	8	10
SVI	2364±293.7	2797±304.2	2695±801.4	3107±285.8	2814±490.9	2791±363.4
Wet Weight (g/seed)	1.76	2.09	2.33	2.84	2.85	2.35

Values are mean ± standard deviation.

Table 2 : Effect of shock treatment on Plumule and Radicle lengths (in cms) of sweet sorghum seeds
Values are mean ± standard deviation.

Plumule Length

Parameter	0	2	4	6	8	10
Plumule length (cm)	14.12±4.74	17.02±6.13	15.69±6.13	20.45±6.56	19.22±6.13	17.28±6.60
Radicle length (cm)	9.52±2.50	10.95±2.77	11.26±3.36	10.62±3.70	8.72±2.87	10.63±3.83

After 14 days of sowing, the plumule length of the Control seeds averaged at 14.12 cm. The highest shoot length was recorded at 20.49 cm with 6 shock pulses (Table 1, Figure 1). This was followed by plumule length at 19.22 cm at 8 shock pulses and 17.28 cm at 10 shock pulses.
This confirms that the shock wave pulses enhanced the shoot length and that significant growth difference was observed after the shock wave treatment.

Radicle Length

After 14 days of sowing, the radicle length of the Control seeds averaged at 9.70 cm. The highest root length was recorded at 11.26 cm with 4 shock pulses (Table 1, Figure 2). This was followed by radicle length at 10.95 cm at 2 shock pulses and 10.62 cm at 6 and 10.63cm at 10 shock pulses. This confirms that the shock wave pulses enhanced the root length and that there was a significant growth difference after the shock wave treatment.

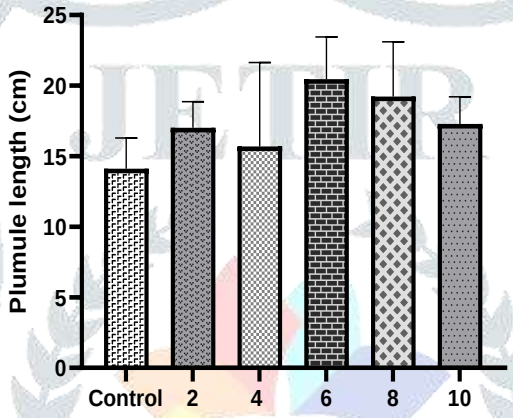


Figure 1 Effect of shock waves on plumule length (mm) on CSH16 sweet sorghum

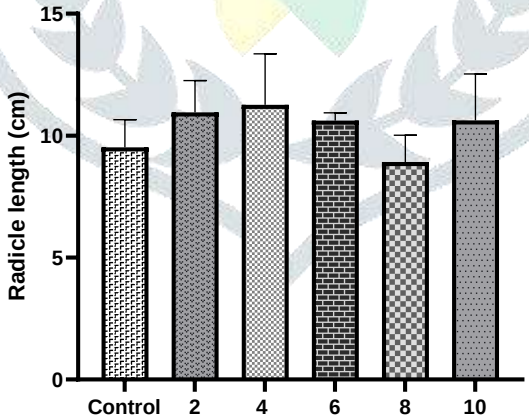
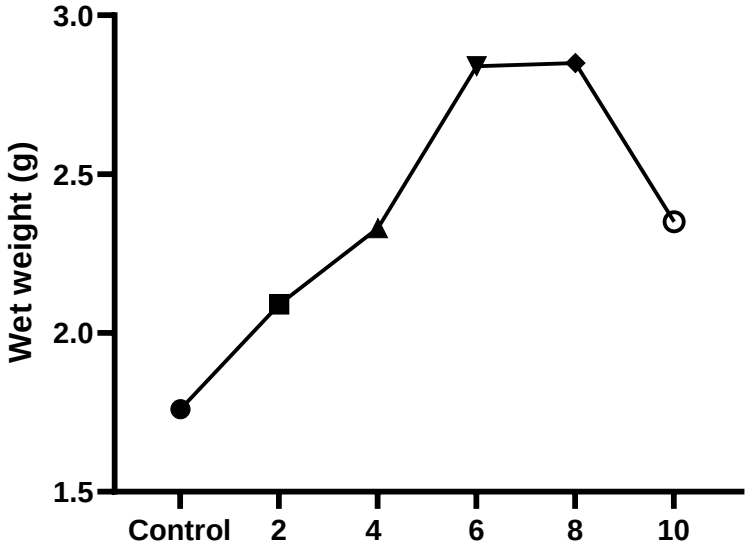


Figure 2 Effect of shockwaves on radicle length (mm) on CSH16 sweet sorghum

Radicle wet weight

Radicle wet weight showed a direct relationship with increasing shockwaves (Figure 3). Both the shoot and root dry weight was measured after 14 days of sowing.
It can be concluded that shock waves are effective in promoting growth of sorghum varieties. Further experiments can be carried out to determine the potential of shockwaves on the growth promotion of other crops. Furthermore, the soil microbial communities which are suitable for plant growth could also be investigated (Swathi G. *et al*., 2017).



Biochemical Analysis

Figure 3 Effect of wet weight after shockwave treatment on CSH16 sweet sorghum

A steady increase in enzymatic activity is observed across all assays with increasing shock concentrations, showing maximum activity at Shock 10. The Control sample shows the lowest activity in all cases, indicating that Shock 10 is the most effective treatment for maximizing antioxidant enzyme activity. However, it is important to determine the optimal shock concentration for achieving the desired antioxidant activity. While the trend shows increasing activity, identifying a saturation point or maximum effective dose is essential, as excessively high concentrations may not always be beneficial. Shock 6 exhibits better growth results (from plumule length, radicle length, wet weight and SVI data) compared to Shock 8, which shows a marginal decline in plumule/radicle lengths and SVI. This shows potential inhibitory effects of higher shock concentrations on growth. the radicle length at Shock 10 increases again after the decline observed at Shock 8. Despite the growth decline in Shock 8, enzymatic activities for SOD, CAT, GST, and APX remain unaffected and continue to increase from Shock 6 to Shock 10. This could be due to the following reasons: (i) Growth parameters may not have a direct linear correlation with enzymatic antioxidant activity. (ii) Differential sensitivity between growth and antioxidant systems could respond differently
the same treatments, thereby leading to imbalances at higher concentrations (iii) Excessive enzymatic activity in Shock 8 and Shock 10 might have disrupted normal oxidative signaling pathways critical for optimal growth. This suggests that Shock 6 may represent an optimal treatment concentration, balancing growth and antioxidant defenses effectively.
Growth and enzymatic results were not linear and therefore showed shock 6 for optimal growth and increasing AO in the plant might have reduced its growth in shock 8 and shock 10 (Table 3).

Table 3: Calculated SOD, GST, CAT and APX in samples Control with varying shock treatments

Code	SOD (U/g. FW) Mean ± SD	GST (U/g. FW) Mean ± SD	CAT (U/g. FW) Mean ± SD	APX (U/g. FW) Mean ± SD
Control	17.971 ± 1.212	0.112 ± 0.007	79.188 ± 5.749	0.099 ± 0.030
Shock 2	55.629 ± 1.204	0.629 ± 0.195	176.650 ± 3.489	0.139 ± 0.030
Shock 4	11.952 ± 1.899	0.123 ± 0.023	124.873 ± 6.979	0.103 ± 0.030
Shock 6	19.509 ± 0.605	0.357 ± 0.019	109.645 ± 6.853	0.173 ± 0.030
Shock 8	41.454 ± 1.520	0.761 ± 0.019	140.102 ± 7.343	0.425 ± 0.020
Shock 10	70.327 ± 1.009	0.826 ± 0.193	259.645 ± 12.581	1.389 ± 0.030

**Experiments carried out in triplicates; Readings expressed as mean ± Standard deviation (SD)*

Nutritional Analysis

Enhanced Energy and Carbohydrate Content

Shock wave treatment resulted in a notable increase in energy (from 328 to 366 Kcal, Table 4) and carbohydrate content (from 70.6 g to ~78 g, Figure 5). This is likely due to the disruption of cell wall structures, enhancing the accessibility of starch granules. Jiang *et al.* (2020) reported that non-thermal treatments such as ultrasonication and shock waves can improve starch gelatinization and extraction efficiency. The observed increase in calorific value further supports this mechanism of enhanced macronutrient availability.

Total Fat and Crude Fibre Improvements

An increase in total fat content (from 5.27 g to 6.3 g, Table 4) was observed. Shock wave-induced cavitation and microjet formation likely enhanced lipid extractability (Petrušić *et al*., 2021). Similarly, crude fibre content increased (from 1.41 g to 1.67 g), suggesting

improved detection or release of structural fibre components such as cellulose and hemicellulose (Wang *et al*., 2023). The micro fracturing of plant cell walls during shock treatment may have liberated these fibre fractions.

Protein Reduction

Protein content decreased post-treatment (from 13.9 g to ~10.5 g, Table 4). This decline may result from protein denaturation or aggregation during the physical process, affecting solubility and subsequent assay results (Gharibzahedi & Chronakis, 2018). Despite the apparent reduction, previous studies suggest that such denatured proteins may exhibit improved digestibility (Zhao *et al*., 2019).

Variability in Mineral Content

Calcium and iron exhibited inconsistent trends. The first shock-treated sample (8 shocks) showed marginal improvement, while the second sample (10 shocks) exhibited a reduction, particularly in calcium (from 96.6 mg to 30.9 mg, Table 4) and iron (from 3.22 mg to 1.78 mg). Variability may arise from uneven mineral redistribution or leaching during treatment (Chandrasekaran *et al*., 2013). Such outcomes suggest that treatment intensity and matrix composition critically influence mineral retention.

Total Ash Content

A slight increase in total ash content (from 1.80 g to 1.86 g, Table 4) suggests modest overall mineral retention. As ash content serves as an indirect marker of total mineral load, these results are encouraging, though further investigation is needed to assess the bioavailability of individual minerals.

Table 4 : Nutritional Analysis

Parameter	Sweet Sorghum (Untreated)	Sorghum (8 shocks)	Sorghum (10 shocks)
Energy (Kcal)	328	363	366
Carbohydrates (g)	70.6	78.2	78.1
Total Fat (g)	5.27	6.22	6.3
Protein (g)	13.9	10.3	10.6
Crude Fibre (g)	1.41	1.66	1.67
Calcium (mg)	96.6	97.3	30.9
Iron (mg)	3.22	3.29	1.78
Total Ash (g)	1.80	1.84	1.86

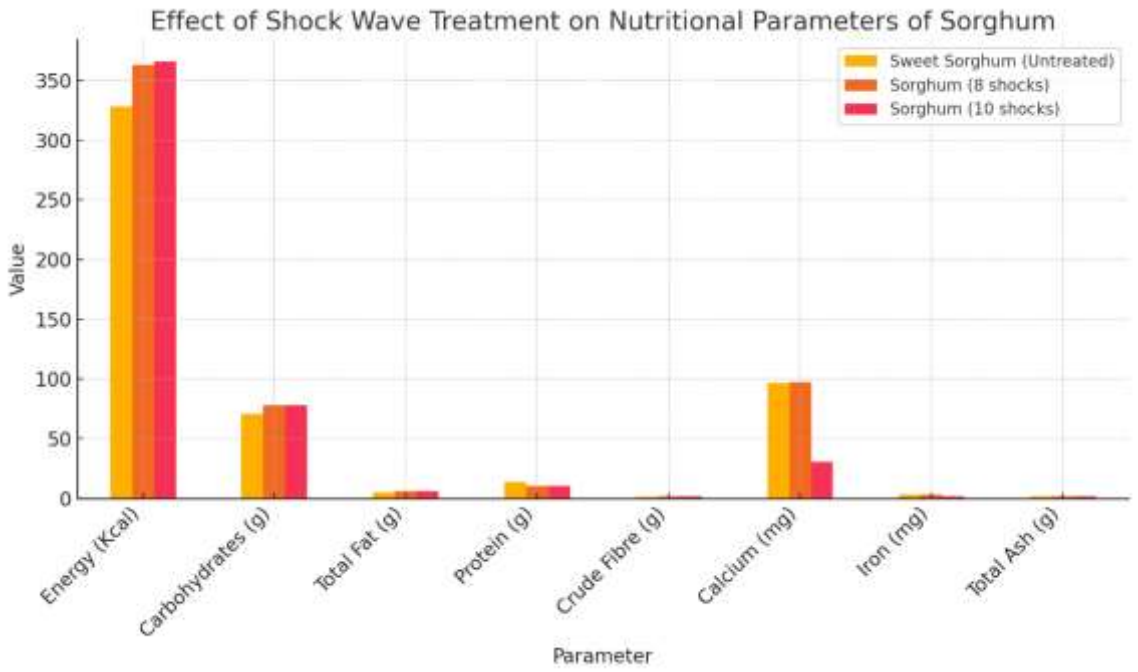


Figure 5. Effect of Shock Wave Treatment on key nutritional parameters of sorghum samples

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