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Appendix - I

Institutional Human Ethical Approval Certificate

INSTITUTIONAL HUMAN ETHICS COMMITTEE

Avinashilingam

Institute for Home Science and Higher Education for Women (Deemed to be University under Category 'A' by MHRD, Estd. u/s 3 of UGC Act 1956) Re-accredited with 'A++' Grade by NAAC. Recognised by UGC Under Section 12 B Coimbatore-641 043, Tamil Nadu, India

Chairman
Dr. SudhaRamalingam
Director-Research & Innovation,
Professor-Community Medicine,
PSG Institute of Medical Sciences & Research, Coimbatore

Member Secretary
Dr.S.Uma Mageshwari
Professor and Head,
Department of Food Service Management & Dietetics

Members
Mr. K.Arunmoli (Legal Expert)
Dr.Subhashini K. Sripathi
Dr.A.Saraswathy (Medical Officer)
Ms.D.Kavitha
Dr.A.R.Sudamani Ramasamy
Dr.G.Victoria Naomi
Dr. Judith Justin
Dr.AnithaSubash

18th April 2022

To
Ms.S.M.Devatha
Department of Food Science and Nutrition
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore – 641 043

Dear S.M.Devatha,

Ref: Your proposal No. IHEC/21-22/FSN-31entitled "Formulation and Development of Edible Cutlery and Crockery" submitted for approval of IHEC on 23.11.2021.

The Institutional Human Ethics Committee of our University hereby grants approval to your research proposal No. IHEC/21-22/FSN-31 entitled "Formulation and Development of Edible Cutlery and Crockery" submitted by you. The Approval number for the same is AUW/IHEC/ FSN-21-22/XPD-31.

We wish you all the best in your research endeavours.

Regards,

Dr.S.Uma Mageshwari
Member Secretary

Appendix – II

Plant Authentication

Selected Major Millet

II a

PEARL MILLET

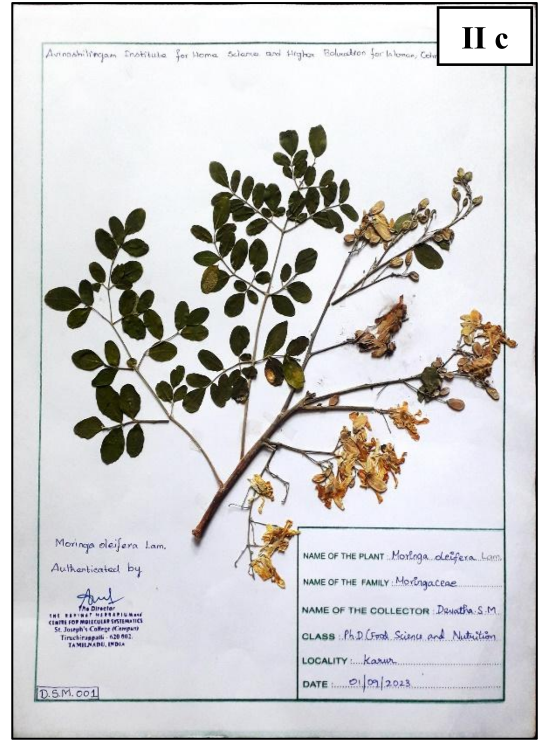
(*Cenchrus americanus* (L) Morrone)

II b

SORGHUM

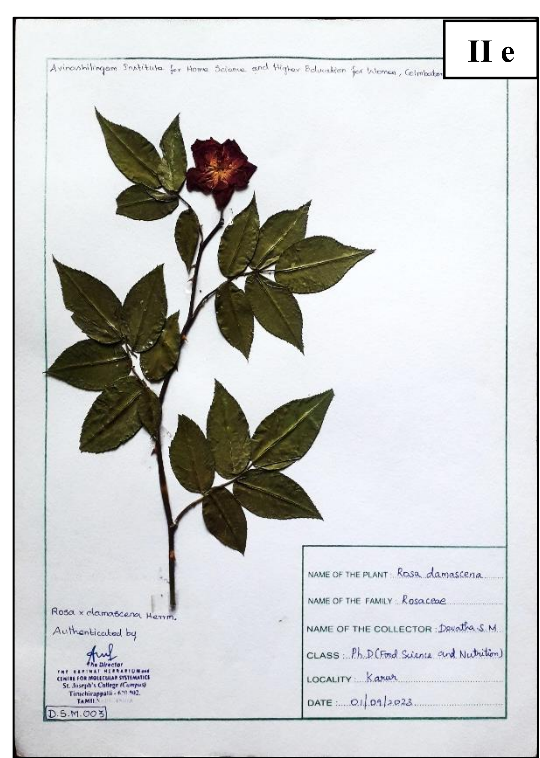
(*Sorghum bicolor* (L) Moench)

SELECTED EDIBLE FLOWERS

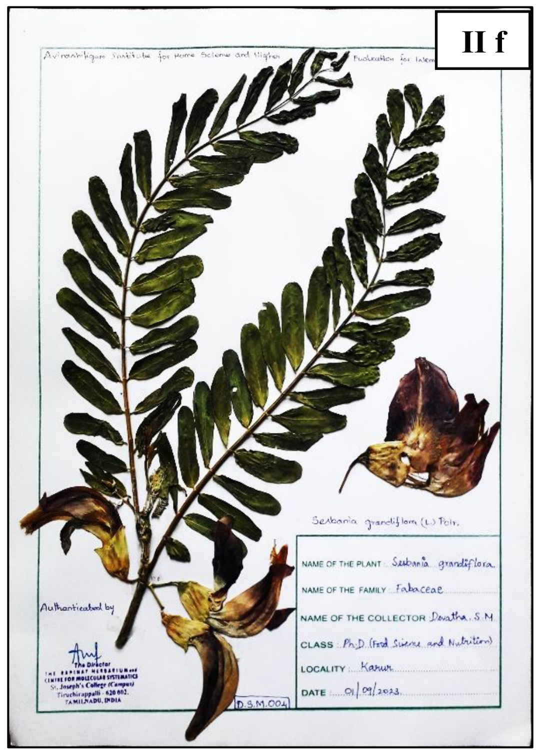


MORINGA
(*Moringa oleifera* Lam.)

HIBISCUS
(*Hibiscus rosa sinensis* L.)



ROSE
(*Rosa damescena* Herrm.)



AGATHI
(*Sesbania grandiflora* (L) Poir.)

Appendix III

Estimation of Technological Properties

Bulk density

The bulk density was calculated from the bulk volume of 100 g of samples in a 250 ml graduated cylinder. The flour was gently poured into the cylinder and the cylinder was then tapped on the table for uniform packing. Average of 5 replications was taken. The bulk density was expressed in g/ml.

$$\text{Bulk Density} = \frac{\text{Weight of the sample (g)}}{\text{Volume of the sample (ml)}}$$

Swelling Capacity

Five hundred milligram (W1) of the sample was weighed, placed into centrifuge tube and the centrifuge tube with sample was weighed (W2). Twenty ml of distilled water was added (VE) and heated for 30 min in a water bath at 90°C, with occasional stirring, the tubes were cooled and centrifuged at 5000rpm for 10 min. The supernatant was decanted into a pre-weighed (W4) petriplate and dried at 105°C and weighed (W5). The inner side of the centrifuge tube was wiped, dried and weighed (W3). Per cent swelling power was calculated using the following formulae:

$$\text{Swelling Capacity (\%)} = \frac{w_3 - w_2}{w_1} \times 100$$

Water Holding Capacity (ml/g)

15 ml of distilled water was added 100 g of the flour in a weighed 25 ml centrifuge tube. The tube was agitated on a vortex mixer for 2 min. It was centrifuged at 4000 rpm for 20 minutes. The clear supernatant was decanted and discarded. The adhering drops of water was removed and the reweighed. Water absorption capacity is expressed as the weight of water bound by 100 g of dried flour.

Oil Holding Capacity (ml/g)

Oil absorption capacity (OAC) of the millet flour was determined using the method of (Sathe and Salunkhe, 1981). 10 ml of refined com oil was added 100 g of the flour in a weighed 25 centrifuge tube. The tube was agitated on a vortex mixer for 2min. It was centrifuged at 4000 rpm for 20 min. The volume of free oil was recorded and decanted. Oil absorption capacity is expressed as ml of oil bound by 100 g of dried flour.

Appendix - IV

Estimation of Total Phenolic Content

Weigh exactly 0.5 – 1.0 g of the sample and grind it with a pestle and mortar in 10-times volume of 80% ethanol. Centrifuge the homogenate at 10,000 rpm for 20 min. Save the supernatant. Re-extract the residue with five times the volume of 80% ethanol, centrifuge and pool the supernatants. Evaporate the supernatant to dryness. Dissolve the residue in a known volume of distilled water (5 mL). Pipette out different aliquots (0.2-2 mL) into test tubes. Make up the volume in each tube to 3mL with water. Add 0.5 mL of Folin – Ciocalteau reagent. After 3 min, add 2 mL of 20% Na_2CO_3 solution to each tube. Mix thoroughly. Place the tubes in a boiling water for exactly one min, cool and measure the absorbance at 650 nm against a reagent blank. Prepare a standard curve using different concentrations of catechol. From the standard curve find out the concentration of phenols in the test sample and express as mg phenols/100 g material.

Appendix - V

Estimation of Antinutritional Factors

Estimation of Phytic acid

Phytic acid was analyzed according to Haug and Lantzech (1983) method. According to this method an appropriate amount (0.8-1.0 gram) of sample was extracted with 0.2N HCl by taking 25 ml of 0.2N HCl in conical flask and shake for 1 h on shaker at 30 C and 80 revolutions per minute. 0.5 ml o extract was taken into test tube fitted with a ground glass stopper. 1 ml of acidic ammonium iron-III sulphate solution of known iron content was added and the tubes were covered with a stopper and fixed with a clip. Tubes were heated in a boiling water bath for 30 min after cooling in ice water for 15 min; tubes were allowed to reach at room temperature. Contents of the tube were mixed and centrifuged for 30 min at 3000 revolutions per minute. 1 ml of the supernatant was transferred to another test tube and 1.5 ml of 2,2-bipyridine solutions was added, light pinkish color appeared. The absorbance was measured at 519 nm against distilled water. Decrease in iron, in the supernatant was measure for Phytic acid contents. Standards of known Phytic acid concentration were prepared and absorbance was measured at 519 nm. Standard curve was made taking phytic acid concentration in micrograms on X-axis and Optical Density (O.D) on Y axis.

Determination of Tannin Content

Weigh 0.5 g of the powdered material and transfer to a 250 mL conical flask. Add 75 mL water. Heat the flask gently and boil for 30 min. Centrifuge at 2,000 rpm for 20 min and collect the supernatant in 100 mL volumetric flask and make up the volume. Transfer 1 mL of the sample extract to a 100 mL volumetric flask containing 75 mL water. Add 5 mL of Folin-Denis reagent, 10 mL of sodium carbonate solution and dilute to 100 mL with water. Shake well. Read the absorbance at 700 nm after 30 min. If absorbance is greater than 0.7, make a 1+4 dilution of the sample. Prepare a blank with water instead of the sample. Prepare a standard graph by using 0-100 µg tannic acid

Determination of Saponin Content

A calibration curve was elaborated using saponins as the standard, with a purity percentage of 100%. In analytical balance, 2 mg of the standard was weighed and dissolved in 70% ethanol. 1 ml of the standard solution and 3.5 ml of Liebermann-Burchard reagent (16.7% acetic anhydride in concentrated sulfuric acid) were added to scan between 325 and 600 nm in the spectrophotometer and to determine the wavelength of the maximum absorption. From the concentrated standard solution, dilutions (0-0.4 mg/ml) were made with the same solvent. 1 ml of the reagent was added to each of these solutions, and after 30 min, the absorbance was measured. The calibration curve was performed in triplicate. The measured absorbance was the result of the absorbance of saponin.

Appendix VI

Estimation of Total Plate or Microbial Count

Serial Dilution Technique

Using separate sterile pipette make decimal dilutions of 10^{-2},10^{-3}, 10^{-4},10^{-5} and 10^{-6}. Add 10 g of sample and homogenate to 90 ml of diluents. Serially dilute in flasks containing 90 ml of diluents and soon up to 10^{-6}. Pipette out 1ml from each dilution into separate sterile Petri dish. Add 1ml of the molten nutrient agar in each petridish. Mix the inoculums with the agar by swirling the plate. Allow to solidify for 30 minutes. Incubate at 37ºC for 24-4 hours. Count the colonies and select the plates containing not more than 100 colonies and not less than 15 colonies in two consecutive dilutions.

(Standard plate count/g = Number of colonies = dilution factor)

Appendix VII

Estimation of Nutrient Content

Estimation of Moisture

Take sterilized petri plates, weigh them and note the initial reading (W1). Place 5 g of the given sample in the petri plate, weigh them and note the reading (W2). Keep it in hot air oven at 100°C for 4 hours. After the sample has been dried take out the petri plate from hot air oven and keep it inside the desiccator until it cools. Weigh the petri plate and note the final reading (W3).

% of moisture= weight of petri plate+ weight of sample – final weight of the petri plate × 100

Weight of sample

Determination of Ash Content

About 5g of the sample was weighed accurately into a tarred platinum or porcelain crucible (which had previously been heated to about 600 c and cooled). The crucible was then placed on a clay pipe triangle and heated in a muffle furnace for about 3-5hors at 600 c. the crucible was then cooled in a desiccator and weighed. To ensure completeness of aching heated in a muffle furnace for half an hour, cooled and weighed this was repeated till two consecutive weights were the same and ash was almost white or greyish white in color.

Estimation of Carbohydrate

Homogenize 0.1 – 0.5 g of the sample in hot 80 % ethanol to remove sugars. Centrifuge and retain the residue. Wash the residue repeatedly with 80% ethanol till the washings do not give color with anthrone reagent. Dry the reside well over a water bath. To the residue add 5.0 mL of water and 6.5 mL of 52% perchloric acid. Extract at 0ºC for 20 min. Centrifuge and save the supernatant. Repeat the extraction using fresh perchloric acid. Centrifuge and pool the supernatant and make up to 100 mL. Pipette out 0.1 or 0.2 mL of the Supernatant and make up the volume to 1 mL with water. Prepare the standards by taking 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard and make up the volume to 1 mL in each tube with water. Add 4 mL of anthrone reagent to each tube. Heat for eight minutes in a boiling water bath. Cool rapidly and read the intensity of green to dark green color at 630 nm.

Calculation

(multiply the value by a factor of 0.9 to arrive at the starch content)

% of carbohydrate= Concentration of solution × 100

Volume of sample

Estimation of Protein

Weigh about 1-2 g of the sample and transfer to a 500 or 800 ml Kjeldahl flask taking care to see that no portion of the sample clings to the neck of the flask. Add 0.7 g of Mercuric oxide, 15 g of Potassium Sulphate and 40 ml of concentrated sulphuric acid (Mercuric oxide is added to increase the rate of organic breakdown during acid digestion). Because of environmental/safety concerns over handling and disposal of mercury, copper sulphate can be used. This is important from safety point of view as mercury vapors might escape into the environment during the distillation process. Missouri catalyst tablets known as Kjeldahl tablets (Composition: 48.8% Sodium sulphate & 48.9% Potassium sulphate & 0.3 % copper sulphate can also be used). Add two to three glass beads. Place the flask in an inclined position on the stand in the digestion chamber and digest. Heat the flask gently at low flame until the initial frothing ceases and the mixture boils steadily at a moderate rate. During heating rotate the flask several times. Continue heating for about an hour or more until the

colour of the digest is pale blue. If black specs are present after 30 minis of digestion, wrap the vessel with aluminum foil and keep for 2-3 minis. By doing this black specs would move down from the walls in the digestion mixture. If the specs are still present, remove the vessel from heat and allow to cool for 10 mins. Do not modify the heat intensity in the whole process. Alternatively, few drops of water may also be poured down across the side of the flask. Cool the digest and add slowly 200 ml of water. Cool, add a piece of granulated zinc or anti bump granules and carefully pour down the side of the flask sufficient NaOH solution (450 gm/liter) to make the contents strongly alkaline (about 110 ml) before mixing the acid and alkaline layer.

Connect the flask to a distillation apparatus incorporating an efficient flash head and condenser. To the condenser fit a delivery tube which dips just below the surface of the pipette volume of standard acid contained in a conical flask receiver. (Precaution: The receiving solution must remain below 45ºC to prevent loss of ammonia). Mix the contents of the digestion flask and boil until 150 ml have distilled into the receiver. Add 5 drops of methyl red indicator and titrate with 0.1 N NaOH solutions.

Carry out a blank titration simultaneously. 1 ml of 0 .1 N (H_2SO_4) = 0.0014gm N.

Calculation

$$\text{Nitrogen content (N) in \%} = \frac{\text{(Blank – Titre value) x Normality x 1.4}}{\text{Sample weight}}$$

Calculate protein %: N x Conversion factor (6.25)

Estimation of Fat

Accurately weigh 3 - 4 g of sample into a thimble lined with a circle of filter paper and containing a small amount of sand. Place thimble and contents in 50 mL beaker and keep it in Soxhlet apparatus for 1½ hours ± 10 minutes at 125 ± 1 °C. Upon completion of the extraction, separate the unit and pour off the ether (and thimble) from the extractor into a large filter (to collect the thimbles) positioned on a container. Repeat until most of the ether is removed and the flask has very little ether left. Take apart the Soxhlet unit and place flask on a steam bath to evaporate the remaining petroleum ether. Dry flask and its contents in a mechanical convection oven at 100 - 102 °C for time required to obtain constant weight. Cool to room temperature.

$$\text{Fat content, percent} = \frac{100\ (B - C)}{A}$$

Where, A = Sample weight, B = Weight of flask after extraction and C = Weight of flask prior to extraction

Determination of Crude Fibre Content

5g of the sample was weighed into a 500ml beaker and 200ml of boiling 0.255N sulphuric acid was added. The mixture was boiled for 30 minutes keeping the volume constant by adding water at frequent intervals (a glass rod inserted in the beaker helps smooth stirring and boiling). At the end of the period, the mixture was filtered through a muslin cloth and the residue was washed with hot water till free from acid. The mixture was then transferred to a beaker containing 200ml of boiling 0.313N sodium hydroxide. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a muslin cloth. The residue was washed with hot water till free from alkali following by washing with some alcohol and ether. It was then transferred into a crucible, dried overnight at 80-100^{0}c and weighed. The crucible was heated in a muffle furnace at 600^{0}c for 2-3 hours. Cooled and weighed again. The difference in the weight represents the weight of the fibre.

Estimation of Vitamin C

Pipette out 5ml of the working standard solution in a 100 mL conical flask. Add 10 mL of 4% of oxalic acid and titrate against dye in a burette (V_1). End point is the appearance of pink color which persists for a few minutes. The amount of the dye consumed is equivalent to the amount of ascorbic acid. Extract the sample (0.5 – 5 g sample) in 4% oxalic acid and make up to a known volume (100 mL) and centrifuge. Pipette out 5ml of the supernatant, add 10 mL of oxalic acid and titrate against dye (V2).

$$\text{Vit C \%} = \frac{0.5}{V_1} \times \frac{V_2}{5} \times \frac{100}{\text{Sample weight}} \times 100$$

Where, V_1= Volume of standard, V_2=Volume of sample

Estimation of Calcium

Two ml of sample is taken into a 15 ml centrifuge tube. Add 2 ml of distilled water and 1ml of 4% ammonium oxalate solution and mix thoroughly and leave overnight. Again the contents are mixed and centrifuged for 5min at 1500 rpm. The supernatant liquid is poured off and the centrifuge tube drained by inverting the tube for 5 min on a rack (care should be taken not to disturb the precipitate). The mouth of the centrifuge tube is wiped with a piece of filter paper. The precipitate is stirred and the sides of the tubes are washed with 3 ml of dilute ammonia. It is centrifuged again and drained as before. The precipitate is washed once more with dilute ammonia to ensure the complete removal of ammonium oxalate. The precipitate is dissolved in 2 ml of 1N $H2SO_4$. The tube is heated by placing in it a boiling water bath for 1 min and titrated against 0.01N $KMno_4$ solution to a definite pink colour persisting for at least 1 min.

Calculation

1 ml of 0.01N $KMno_4$ is equivalent to 0.2004 mg of calcium

1 mg of calcium / 100 ml serum= (x-b) x 0.2004 x $\frac{100}{2}$

where,

X=number of ml of 0.01N $KMno_4$ required to titrate of sample,

b= number of ml of 0.01N $KMno_4$ required to titrate 2 ml of H2SO4 (Blank)

If the normality of $KMno_4$ is N, the value obtained in the above formula should be multiplied by the factor N/0.01.

Estimation of Iron

2g of the sample was ached by ignition when ashing had been com pelted 5ml of hydrochloric acid was added and made up to 100ml in a volumetric flask. Took different aliquots of the standard solution (1ml-5ml) to corresponding to 10-50 gammas in a series of the test tube. Added 1ml of 30% H_2So_4 1ml of potassium per sulphate and 1.5ml of potassium thiocyanate to all the test tubes. This was made up to 10ml with water. A blank was prepared by adding the reagents except the standard or the unknown solution. Allowed the colour to develop for 20minutes and the intensity was read at 530-540μ filters in the colorimeter.

Estimation of Phosphorus

One ml of the ash solution was taken in two test tubes. 1 ml of molybdate II and 0.4 ml of ANSA were added and the volume was made upto 10 ml with distilled water. To 1 ml, 2ml, 3 ml, 4 ml and 5 ml of standard solution, 1 ml of molybdate I solution and 0.4 ml of ANSA were added and made upto 10 ml. All the tubes containing 10 ml of the solution were mixed well and allowed to stand for 15 minutes. Simultaneous, a blank was prepared by

mixing 8.6 ml of water, 1 ml of molybdate II and 0.4 ml of ANSA. The color developed was read in the colorimeter using red filter of wavelength 660 nm.

Appendix - VIII

Sensory Evaluation Form

Name : **Designation :**
Department : **Product :**

Attributes	Scores	Sample	Sample	Sample	Sample	Sample	Sample
Shape or Appearance	9 - Like extremely 8 - Like very much 7 - Like Moderately 6 - Like Slightly 5 - Neither like nor dislike 4 - Dislike slightly 3 - Dislike moderately 2 - Dislike Very much 1- Dislike extremely						
Colour							
Taste							
Flavour							
Crispiness							
Overall acceptability							

(Signature)

Appendix – IX

Qualitative analysis of Phytochemical Constituents

Sample preparation:

7g of sample was measured in a conical flask and added 70ml of water. The conical flask was then kept in a mechanical shaker for 5 hours. It was then kept in a refrigerator for an overnight. Then it was filtered using a whatman filter paper. The extract was used for the analysis of phytonutrient analysis.

1. Test for alkaloids

Mayer's test - Take few ml of sample and add 2 ml of mayer's reagent. White creamy layer indicates the presence of alkaloid

Wagner's test- Take few ml of sample and add few drops of wagner's reagent. Reddish brown colour precipitate indicates the presence of alkaloid.

2. Test for flavonoid

Lead acetate test - To 50 mg of sample added 50 ml of water and 3 ml of 10% lead acetate. White precipitate indicates the presence flavnoid.

3. Test for glycosides - Add 2 ml of extract with 3 ml of chloroform. Chloroform separates into two layer and add 10 % ammonia. Appearance of pink colour indicates the presence of glycosides.

4. Test for Amino acids

Ninhydrin test - Add 2 drops of Ninhydrin to 2 ml of sample. Appearance of purple colour indicates the presence of amino acids.

5. Test of Carbohydrate

Molisch test - To 2 ml of sample added 2 drops of alcoholic solution (naphthol). Mix well and add few drops of Conc.H_2SO_4. Violet ring formed indicates the presence of Carbohydrate.

Benedict's test - To 0.5 ml of sample 0.5 ml of benedict's reagent is added and kept in boiling water bath for 2 minutes. Colour precipitate indicates the presence of Carbohydrate.

6. Test for tannins - 3 mg of sample, 5 ml of water and few drops of 5% ferric chloride are added to a test tube. Dark green colour indicates the presence of phenolic compounds and tannins.

Gelatin test- To 50 mg of sample added 1% gelatin. White precipitate indicates the presence of phenolic compounds and tannins.

Alkaline reagent test- To 2 ml sample added 2 ml of 10% ammonium hydroxide. Yellow fluorescence indicates the presence of phenolic compounds.

7. Test for Phenol

Ferric chloride Test-3 mg of sample, 5 ml of water and few drops of 5% ferric chloride are added to a test tube. Dark green colour indicates presence of phenols

Gelatin Test - To 50 mg of sample added 1% gelatin. Presence of yellow fluorescence indicates the presence of phenols.

8. Test for saponins - To 1 ml of sample 5 ml of distilled water is added and shake. 2-layer separation indicates the presence of saponins.

9. Test for Terpenoids - 1ml chloroform is added to 2ml of sample and add few drops of Conc. H_2SO_4. Reddish brown precipitate shows the presence of Terpenoids.

Appendix - X

Estimation of Antioxidant activity

Diphenyl Picrydyl Phenyl Hydrazine (DPPH) Test

4mg of DPPH is made upto 100ml with methanol. 10mg of Butylated Hydroxy Tolune is made upto 100ml with methanol. 10ml of Stock solution is made upto 100ml with methanol**.** 1gm of sample is added with 10ml of methanol and filtered through filter paper**.** Then the volume of solution and concentration in standards and samples are as follows,

<table>
<tr><th>S.no</th><th>Solution</th><th>Volume of Solution (ml)</th><th>Volume of DPPH (ml)</th><th></th></tr>
<tr><td>1</td><td>Standard1</td><td>0.1</td><td>2.9</td><td rowspan="10">Note the reading at 517nm against blank in a spectrophotometer</td></tr>
<tr><td>2</td><td>Standard2</td><td>0.2</td><td>2.8</td></tr>
<tr><td>3</td><td>Standard3</td><td>0.3</td><td>2.7</td></tr>
<tr><td>4</td><td>Standard4</td><td>0.4</td><td>2.6</td></tr>
<tr><td>5</td><td>Standard5</td><td>0.5</td><td>2.5</td></tr>
<tr><td>6</td><td>Sample 1</td><td>0.1</td><td>2.9</td></tr>
<tr><td>7</td><td>Sample2</td><td>0.2</td><td>2.8</td></tr>
<tr><td>8</td><td>Sample3</td><td>0.3</td><td>2.7</td></tr>
<tr><td>9</td><td>Sample4</td><td>0.4</td><td>2.6</td></tr>
<tr><td>10</td><td>Sample5</td><td>0.5</td><td>2.5</td></tr>
</table>

Calculation

$$\% \text{ of inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

A_c= Absorbance of control , A_s= Absorbance of Sample

Appendix - XI

Brine Shrimp Lethality Assay

Preparation of Stock solution:

In this study, we prepared a stock solution for analysis using samples denoted as CP. The sample was taken in varying volumes of 100µl, 250µl, 500µl, 1000µl, and 1500µl. To create the stock solution, the sample was subjected to dilution with distilled water. The concentration of the original samples was determined prior to the dilution process. The final concentration of the stock solution was predetermined based on the experimental requirements and the desired concentration range for the saline solution.

The dilution factor for the sample was calculated by dividing the final volume of the stock solution by the initial volume of the respective sample. The final volume of the stock solution was obtained by adding the volume of the sample to the volume of distilled water used for dilution. Following accurate measurements of the appropriate volumes, the dilution process was carried out, and each stock solution was thoroughly mixed to ensure homogeneity. Subsequently, aliquots of the prepared stock solutions were transferred to individual beakers, each containing saline solution. These beakers were then ready for further analysis using the respective stock solution concentrations. Throughout the preparation process, adherence to scientific rigor was maintained to ensure reliable and reproducible results for the analysis of the saline solutions with the prepared stock solutions.

Procedure

In this scientific investigation, we conducted an experiment to assess the potential toxicity of different concentrations of a sample solution on shrimp. The experiment involved introducing 30 shrimps into a mixture of saline solution (25ml) and various concentrations of the sample solution.

To establish a baseline for comparison, a blank solution was also prepared, consisting of 30 shrimps in a brine solution. Additionally, a positive control was included in the study, which involved using a known toxic substance, potassium dichromate, at a concentration of 1mg/ml. The shrimps' behavior and mortality rates were monitored at specific time intervals: 1, 2, 4, 6, and 24 hours after exposure to the solutions. The mortality rate was recorded after the 24-hour observation period for both the sample solutions and the control solutions (blank and positive control).

The purpose of this experiment was to determine whether the sample solution had any adverse effects on the shrimps' survival and behavior compared to the blank solution (no toxicant) and the positive control (known toxicant). The findings from this study will contribute to the understanding of the potential environmental impact of the sample solution and its safety implications.

It is important to note that all experimental procedures were conducted following scientific protocols and guidelines to ensure accuracy, reproducibility, and reliability of the results. The welfare and ethical considerations of the test organisms were also taken into account throughout the experiment.

Appendix - XII

Fellowship Certificate

सामाजिक न्याय और अधिकारिता विभाग
Department of Social Justice & Empowerment
सामाजिक न्याय एवं अधिकारिता मंत्रालय, भारत सरकार
Ministry of Social Justice & Empowerment, Government of India
शास्त्री भवन, नई दिल्ली - 110001
Shashtri Bhawan, New Delhi - 110011

NATIONAL FELLOWSHIP FOR BACKWARD CLASSES STUDENTS
FELLOWSHIP AWARD LETTER

No. NBCFDC/E-70291 Dated: **28.03.2023**

DEVATHA S M
Son/Daughter of **SELVI M**
and **MANIVEL K**
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Subject: **HOME SCIENCE**
NBCFDC Ref. No.: **220510570109**

Dear Candidate,

I am pleased to inform you that you have been selected in the **December 2021 & June 2022 (merged session) National Eligibility Test (UGC–NET)** for award of Fellowship under the scheme of National Fellowship for Backward Classes Students (NFOBC).

The Fellowship will be provided as per the scheme guidelines of National Fellowship for Backward Classes Students (NFOBC) issued by Ministry of Social Justice and Empowerment (MoSJE), Govt. of India available at https://socialjustice.gov.in/writereaddata/UploadFile/SHREYAS%20Scheme.pdf. The eligibility of the candidate for availing the fellowship is to be ensured by the University / Institution.

With best wishes,

(Suresh Kumar)
General Manager, NBCFDC

5th Floor, NCUI Building, 3, Siri Institutional Area, August Kranti Marg, New Delhi-110016 | Toll Free : 18001023399 (10:00 AM to 5:00 PM On Weekdays) | Email : info@nbcfdc.gov.in

Appendix – XIII

Published Patent

(12) PATENT APPLICATION PUBLICATION
(19) INDIA
(22) Date of filing of Application :27/04/2024

(21) Application No.202441033644 A
(43) Publication Date : 03/05/2024

(54) Title of the invention : A PROCESS OF PREPARATION OF SUSTAINABLE DINING WITH PEARL MILLET MENAGERE AND PRODUCT THEREOF

(51) International classification	:A21D0002360000, A61K0035618000, A21D0008040000, A23L0007109000, A21D0013330000
(86) International Application No Filing Date	:NA :NA
(87) International Publication No	: NA
(61) Patent of Addition to Application Number Filing Date	:NA :NA
(62) Divisional to Application Number Filing Date	:NA :NA

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(57) Abstract :
TITLE: A PROCESS OF PREPARATION OF SUSTAINABLE DINING WITH PEARL MILLET MENAGERE AND PRODUCT THEREOF APPLICANT: AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN ABSTRACT The present invention discloses a process of preparation of pearl millet menagere for sustainable dining comprises of a. mixing 50-70 parts of pearl millet flour, 20-35 parts of wheat flour, 10-15 parts of jaggery, 3-5 parts of edible oil, 0 – 10 parts of edible flowers with 30-75 parts of water to form a crumbly dough; b. compressing the crumbly dough in a mold and baking at 70-90 °C for 10-15 minutes to form a baked product with thickness of 2 to 5 mm; c. melting edible grade carnauba wax at 85 °C followed by coating on the baked product and spraying 2-5% of citric acid on all sides to form pearl millet menagerie.

No. of Pages : 39 No. of Claims : 9

Appendix – XIV

Appendix 1.2 List of Publication Details

Avinashilingam Institute for Home Science and Higher Education for Women
(Deemed to be University Estd. u/s 3 of UGC Act 1956, Category 'A' by MHRD Re-accredited with A++ Grade by NAAC. CGPA 3.65/4, Category I by UGC
Coimbatore - 641 043, Tamil Nadu, India

Appendix L2

(Item No 5 of Check List)

Details of Research Publications

S.No	Article	Journal	Other Details Vol/No/Page No/ Year	Published in UGC-CARE / Scopus Indexed/ Web of Science
1	A Comparative Study on Characterization of Processed and Unprocessed Sorghum Flours	The Journal of Research ANGRAU	*52*(1) 76-84	CARE I
2	Formulation and Quality Assessment of Edible Cups from Sorghum bicolor.	Indian Journal of Nutrition and Dietetics,	*60*(4) *2023*	CARE I
3	Sorghum spoons enriched with selected edible flowers: A sustainable alternative to conventional cutlery in the food and tourism sectors.	Biomass Conversion and Biorefinery	1-14. 2024	CARE II

*Proof of list of Journals from Internet to be attached along with copies of reprints.

Scholar : S.M.Devatha

Supervisor : [signature] 2/12/24.

[signature] 02/12/24

Checked By:

HoD / Dean of Respective School

The scholar Miss. Devatha, SM (20PHFNF003) has published her research articles in the following journals:
1. The Journal of Research ANGRAU - indexed in UGC Care Gp. I
2. Biomass Conversion and Biorefinery - indexed in scopus.
This may be considered.

[signature] 02.12.24
Asst. Librarian

DoI: https://doi.org/10.58537/jorangrau.2024.52.1.09
J. Res. ANGRAU 52 (1) 76-84, 2023

A COMPARATIVE STUDY ON CHARACTERIZATION OF PROCESSED AND UNPROCESSED SORGHUM FLOURS

S.M. DEVATHA* and RAAJESWARI PARAMASIVAM

Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore – 641 043

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ABSTRACT

The effect of physical, technological and functional properties of unprocessed, germinated and dry roasted grains and flours of sorghum were studied in the year 2023. The physical properties of the grains such as length, breadth, length/breadth ratio, thousand grain weight showed that, germinated grains measured minimal results as the stores were utilized for sprouting. Germinated sorghum yielded 94.6%, whereas, unprocessed and roasted yielded 91% of the total flour yield. The technological properties of the flours were analyzed as quality indicator. Bulk density, oil and water holding capacity, and swelling capacity of the roasted flour were higher in quality when compared to unprocessed flour. Optical property indicated that roasted flour was darker and germinated sorghum flour was lighter than unprocessed flour. FTIR of germinated sorghum flour showed stronger peaks indicating the presence of polysaccharides. TGA of germinated sorghum flour exhibited maximum reaction zone due to changes in saccharides and dextrin. Roasted sorghum flour showed better properties that increase hardness, whereas, germinated flour soften the food product. Sorghum flour showed less than 7 x 10^{-1} CFU/g at day 180.

Keywords*:* Germination, Property analysis, Roasting, Sorghum flour and grain

INTRODUCTION

India is the prime capital for consuming and producing millets and sixth largest producer of sorghum (*Sorghum bicolor* [L.] *Moench*) globally. Millet is a staple food in Indian food basket. Consumption of millet in daily diet have been decreased over the period of time due to lack of knowledge, increased cooking time, and insufficientnumber of processed food items (Llopart and Drago, 2016). Government and food manufacturers became more aware to increase the consumption and utilization of millets, particularly sorghum, to help farmers to cultivate more resilient crops and make population to overcome hunger, metabolic syndrome and climate change (Kane-Potaka *et al.* 2021). Sorghum is a gluten-free cereal substitute with high nutrient profile packed with micronutrients and a good source of fiber, protein, antioxidants, and bioactive compounds, but anti-nutritional factors such as phytic acid and tannin, prevent from reaching its full

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nutritional potential. The effects of several wet and dry processing techniques, including steeping, fermentation, germination, roasting, and popping at different temperature and time span on these antinutritional factors of sorghum flour were proved to have impact on the structural and chemical characteristics (Paliwal and Sharma, 2022 ; Anjitha *et al*., 2021). The primary phenolic compounds in sorghum are tannins, phenolic acids and 3-deoxyanthocyanidins with good potential of antioxidant activity. Consuming whole sorghum improves gut health, lowers the risk of chronic diseases, and is used as a promising natural multifunctional additive in a wide range of food applications. (Birhanu, 2021). This study was carried out with an attempt to improve the physical, technological, and functional qualities of white sorghum grains through soaking, germination, and roasting to utilize in food processing and production industry.

MATERIAL AND METHODS

Selection of sorghum grains

White variety of sorghum is highly available and consumed largely as compared to red or yellow colored sorghum. Good quality of white variety sorghum grains were purchased from supermarket in Coimbatore, Tamil Nadu.

Processing of selected sorghum grains

The whole sorghum grains were washed, cleaned and sorted to remove husk and other impurities and dried below 12 ºC. Conventional preliminary processing like soaking, germination and roasting were done to enrich the physical, technological and functional properties. Sorted sorghum grains were soaked for eight hours and dried by spreading in muslin cloth to remove the excess moisture. Then dried sorghum grains were tied in white muslin cloth for germination for 35 h as suggested by Olamiti *et al.* (2020). Then the germinated grains were shade dried for 6 h till the moisture attained 12 percent and ground into fine flour in milling machine and kept in air-tight container for further investigation. Clean dried white sorghum grains were dry roasted in iron pan for 12 minutes at 90 °C till few grains popped and the roasted grains were kept undisturbed till it reaches 25 °C to facilitate grinding. Then the roasted grain was milled and sieved through 3 mm mesh as recommended by Ranganathan *et al.* (2014) and preserved in an airtight container for future study.

Physical properties of grains

Physical properties of the selected and processed grains were analyzed to measure the quality. Thickness (mm), length (mm), breadth (mm), length / breadth ratio, thousand grain volume (ml) and thousand grain weight (g) were measured by the standardized procedure of Khatoniar and Das (2020).

Technological properties of flour

Technological properties of flour was determined to identify the difference among unprocessed, germinated and roasted sorghum flour. Bulk density (g/ml), swelling capacity (ml/g), water holding capacity (ml/g) andoil holding capacity (ml/g) were determined by usingthe procedure that was followed by Akinola*et al.* (2017). Standard AOAC procedure was used to analyze moisture by AOAC 930.15 and ash by AOAC 942.05 standards.

Optical properties of sorghum flour

The colour of unprocessed, germinated and roasted sorghum flour was analyzed through Laboratory Scale Food Colorimeterand different values of L*, a* and b* were obtained. L" values, 0 - 100 indicates lighter to darker shades of the taken sample. Positive value of a* refers to greenness and negative shows the presence of redness, whereas, positive and negative values of b* indicates yellowness and blueness.

Functional properties of the flour

The functional groups of the unprocessed and processed sorghum flour were determined through the peaks obtained from Shimadzu Miracle 10 Fourier Transform Infrared spectrophotometer. 0.5 g of sample was utilized to determine the spectra and the peaks were found between 4000 cm^{-1} to 450 cm^{-1} wave numbers at 16 runs per scan.

The crystalline or amorphous nature of sorghum flour was measured by patterns through X – Pert Pro, PANalytical model X-Ray Diffraction (XRD). 5 g of fine sample was loaded and operated at 30 mA and 45 kV between the scanning regionsfrom 10° to 79° 2 theta with continuous step size of 0.01 at 5.71 seconds. The Debye-Scherrer formula, D = K ë / â Cosè, has been applied to estimate the average crystallite size of sorghum flour, where, D is the crystalline size, ë is X-ray wave length of Cu, â is full width at its half maximum and k is dimension less shape factor with fixed value of 0.94.

Thermal properties of the flour

Thermal property analysis of unprocessed, germinated and roasted sorghum flour was identified by Thermo Gravimetric Analyser (EXSTAR/C300) to analyze the percentage of thermal degradation as the flour undergoes certain physiochemical changes with change in temperature. Sample taken was treated from 20 ºC to 1000 ºC with increase in 20 ºC per minute in alumina pan.

Microbial evaluation of the flour

Total Plate or microbial count was analyzed by modified procedure of Makawi *et al*. (2019). 9 ml of sterile peptone water (0.1%) and 1 g of sample were mixed. To create sequential 10 fold dilutions, 1 ml of the homogenate was diluted with 9.0 ml of water that contained 0.1% peptone, by pour plate method suitable serial dilutions in duplicate with plate count agar to make 10^1 to 10^5 dilutions and incubated at 35 °C for 48 ± 2 h. Colonies were counted when less than one-quarter of the dish is overgrown by spreading that is unaffected and multiply that number by the dish's total area.

RESULTS AND DISCUSSION

Grain yield of sorghum

500 g of cleaned and sorted sorghum were milled which yielded 91 percent of fine flour. Ninepercent were coarse flour when sieved in 3 mm mesh. 500 g of sorghum were germinated and the sprouts were grown upto 1.5 to 1.8 cm after 35 h of germinating and the weight of the grains were increased by absorbing water in the initial stage. After shade drying for six hours, the weight was reduced as the stores were utilized during germination and yielded 94.6 percent. Whereas, 500 g of roasted sorghum

Table 1. Grain yield of sorghum

S.No.	Sample	Sample Taken (g)	Processing Time	After Proces-sing (g)	Flour yield after drying(g)	% of Yield
1	Sorghum - Unprocessed	500	Unprocessed	500	475	91
2	Sorghum – Germinated	500	8 h soaking and 35 h Germination	548	473	94.6
3	Sorghum – Roasted	500	12 mins	450	455	91

yielded 91 percent due to increase in hardness of endosperm while browning and change in gelatinization of starch while popping. (Anjitha *et al*. 2021). After milling, germinated sorghum flour was soft in nature as compared to unprocessed and roasted flour and the total grain yield was depicted in Table 1.

Physical properties of sorghum grains

Physical properties of sorghum were determined through standardized procedures to check the quality of selected and processed sorghum grains and the results were presented (Table 2). The length, breadth and length to breadthratio of unprocessed and roasted sorghum were similar and those of germinated sorghum were reduced in size. Slight loss in thickness of the grains during germination was also noticed. Maximum thousand grain weight was observed in unprocessed sorghum, whereas, minimum was obtained in germinated sorghum. Thousand grain volume was ranged between 36 ml and 44 ml and the highest was noticed in germinated sorghum. Khatoniar and Das (2020) observed the physical dimensions of major millets and among them proso millet exhibited better properties.

Technological properties of flour

Technological properties namely, bulk density, swelling capacity, water and oil holding capacity of the flours were analysed and tabulated (Table 3). Bulk density and swelling capacity washigher in unprocessed sorghum flour than germinated and roasted flour. Water and oil holding capacity of roasted sorghum flour is higher followed by germinated and unprocessed flour as it is considered as the important factor for the formulation offood products as well as a thickening agent. Akinola *et al*. (2017) found that bulk density and water holding capacity was reduced during malting and fermentation as compared to unprocessed pearl millet. Germinated and roasted sorghum flour exhibited better technological properties as compared to other major millets (Khatoniar and Das, 2020). Germinated sorghum flour contains more moisture and ash content followed by roasted and unprocessed flour.

Optical properties of the sorghum flour

Optical property of sorghum flours were evaluated(Table 4). The properties were evaluated thrice, and presented as mean and standard deviation.Analysis was done in triplicates, reported as mean ± standard deviation and the results were tabulated in Table 4. The *L were ranging from 42.63 to 47.83, whereas, a* and b* were 4.81 to 9.5 and 3.05 to 10.49, respectively. The lightness (*L) is highest

Table 2. Physical properties of the grain (n=3)

S.No.	Quality Parameters	Sorghum Unprocessed	Sorghum Germinated	Sorghum Roasted
1	Length (mm)	0.61±0.012	0.52±0.12	0.61±0.20
2	Breadth (mm)	0.69±0.01	0.57±0.20	0.69±0.04
3	Length / Breadth ratio	0.88±0.40	0.91±0.54	0.88±0.40
4	Thickness (mm)	0.59±0.02	0.57±0.03	0.59±0.10
5	Thousand grain Weight (g)	29.6±0.32	23.2±1.09	26.4±0.82
6	Thousand Grain Volume (ml)	36±1.12	44±2.32	41±2.01

* Mean ± SD Values

Table 3. Technological properties of the sorghum flour (n=3)

S.No.	Parameters	Sorghum Unprocessed	Sorghum Germinated	Sorghum Roasted
1	Bulk Density (g/ml)	0.17 ± 0.01	0.14 ± 0.04	0.16 ± 0.07
2	Swelling Capacity (ml/g)	3.6 ± 0.17	2.4 ± 0.09	3.2 ± 0.12
3	Water Holding Capacity (ml/g)	1.2 ± 0.02	1.4 ± 0.05	1.8 ± 0.04
4	Oil Holding Capacity (ml/g)	0.8 ± 0.01	1.3 ± 0.12	1.0 ± 0.08
5	Moisture (%)	10.3 ± 1.15	15.7 ± 1.57	9.3 ± 0.98
6	Ash (%)	19.6 ± 1.28	20.0 ± 1.92	19.8 ± 1.03

Mean ± SD

in sorghum geminated flour with 47.83 ± 0.35 value which showed lighter than unprocessed and roasted flour. Germinated sorghum flour showed the most reddish and yellowish shade with 4.83 and 3.05 values, respectively. The product developed with roasted sorghum flour provides intense optical property as compared to unprocessed and germinated flour. Olamiti *et al*. (2020) declared that prolonged malting and fermentation times resulted in lighter flour, while shorter malting and fermentation times resulted in denser flour. Sorghum exhibited high L* content around 72.02 and 73.72, a* content within 2.50 and 3.30, and chrome content between 13.10 and 14.82.

Functional properties of the sorghum flour

Functional properties of the unprocessed, germinated and roasted sorghum flours were determined by Fourier transform infrared (FTIR) spectroscopy from 4000 cm^{-1} to 450 cm^{-1}. Unprocessed sorghum flour contains medium O-H peaks at 3873.06 to 3718.76 cm^{-1} and weak C –H band at 3502.08 to 3363.86 cm^{-1} , whereas, geminated sorghum flour contains strong peaks at 3834.40 cm^{-1} and roasted sorghum flour shows medium peaks at 3718.76 to 3834.40 cm^{-1} showed the presence of vibrational peaks due to bound water and moisture content in flour (Olamiti *et al*. (2020). Medium C–H peaks at 2306.86 to 2970.38 cm^{-1} region were present in all the three flours. Medium carbonyl stretch peak at 1697 to 1643 cm^{-1} for unprocessed andweak peak at 1689 cm^{-1} region shows the presence of lipids and germinated flour doesn't contain carbonyl stretch. Strong C=C stretching peaks at 1519 cm^{-1} for unprocessed and 1527 cm^{-1} for germinated and roasted sorghum flour shows the presence of the amide II from N – H bending that confirms the presence of protein. Unprocessed sorghum flour contains strong peak at 1519 cm^{-1} and weak peaks at 1080 to 1458 cm^{-1} shows the

Table 4. Optical properties of the sorghum flour (n=3)

S.No.	Sample	L*(0 - black, 100 - white)	a*(+ red, - green)	b*(+ yellow, - blue)
1	Sorghum Unprocessed	45.77 ± 1.48	4.81 ± 0.71	4.59 ± 0.75
2	Sorghum Germinated	47.83 ± 0.35	4.83 ± 0.5	3.05 ± 0.21
3	Sorghum Roasted	42.63 ± 0.18	9.05 ± 0.22	10.49 ± 0.65

Mean ± SD

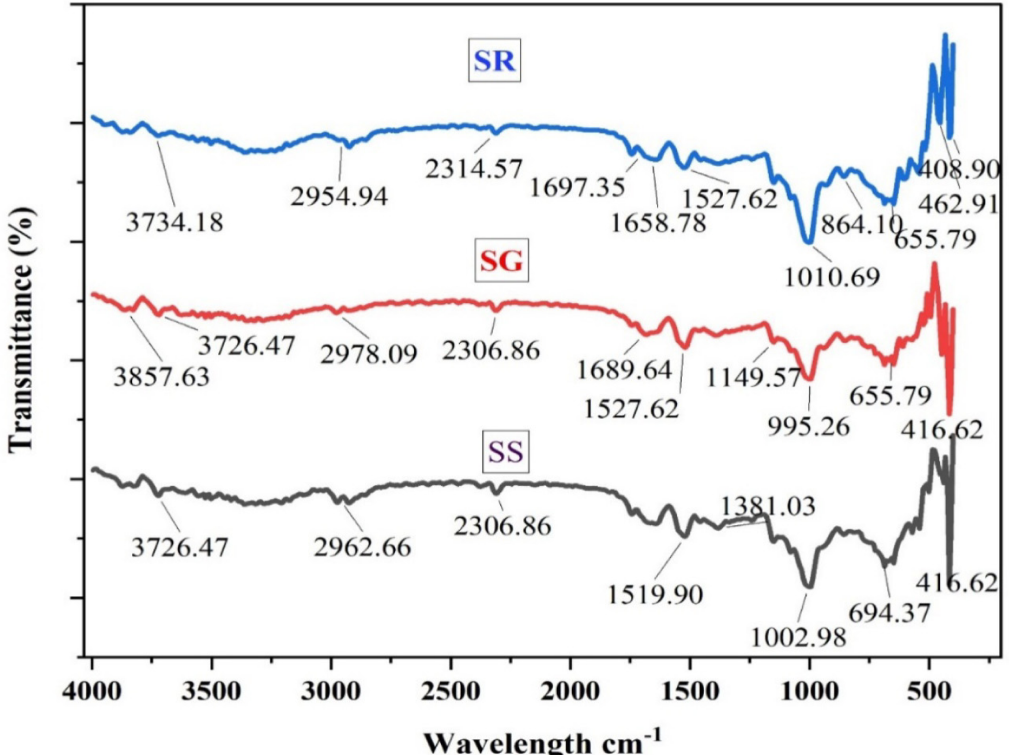

Figure 1. FTIR Peak of the sorghum flours

***SS – Unprocessed Sorghum Flour, SG – Germinated Sorghum Flour, SR – Roasted Sorghum Flour**

presence of O - H, C - O and C - C bonds which is saturated primary alcohol ring and germinated and roasted flour showed minimum weak peak at these region shows the denaturation of organic compounds. Unprocessed sorghum flour showed more peaks at 416 to 995 cm^{-1} , whereas, geminated flour showed at 416 to 725 cm^{-1} and roasted showed between 424 and 810 cm^{-1} confirming the presence of amylose and amylopectin.

A medium peak at 1743.65 $cm^{-1.}$ present in unprocessed sorghum flour showed the presence of tannin and there are no peaks at 1700 to 1400 cm^{-1} confirming the absence of tannin in germinated and roasted flour (Lin *et al.,* 2021). Steeping and fermentation of sorghum flour does not have impact on structural property but phytic acid and tannin content were reduced upto 21–52 percent due to first-order degradation kinetics due to conventional processing (Paliwal and Sharma, 2022). Malted and fermented pearl millet flour showed increased protein content due to protein synthesis as compared to unprocessed flour (Akinola *et al*., 2017).

Crystalline nature and crystal size of the processed and unprocessed sorghum flour was determined through X-Ray diffraction. Unprocessed sorghum flour showed main reflection at 11.8, 13.6, 15.3.8, 16.1, 19.1, 20.9, 21.3, 22.7, 23.7, 25.1, 31.2, 38.7, 40.6, 41.7, 44.0, 60.8, 61.0 and exhibited sharp peaks that indicated crystalline structure of the flour with a crystal size of 88.92 nm calculated by Debye-Scherrer formula, whereas, germinated sorghum flour exhibited peaks at 12.0, 15.3, 19.1, 21.1, 22.4, 24.9, 25.5, 27.6, 31.3, 32.1, 36.3, 38.5, 54.3regions with 55.91 nm crystal size and the

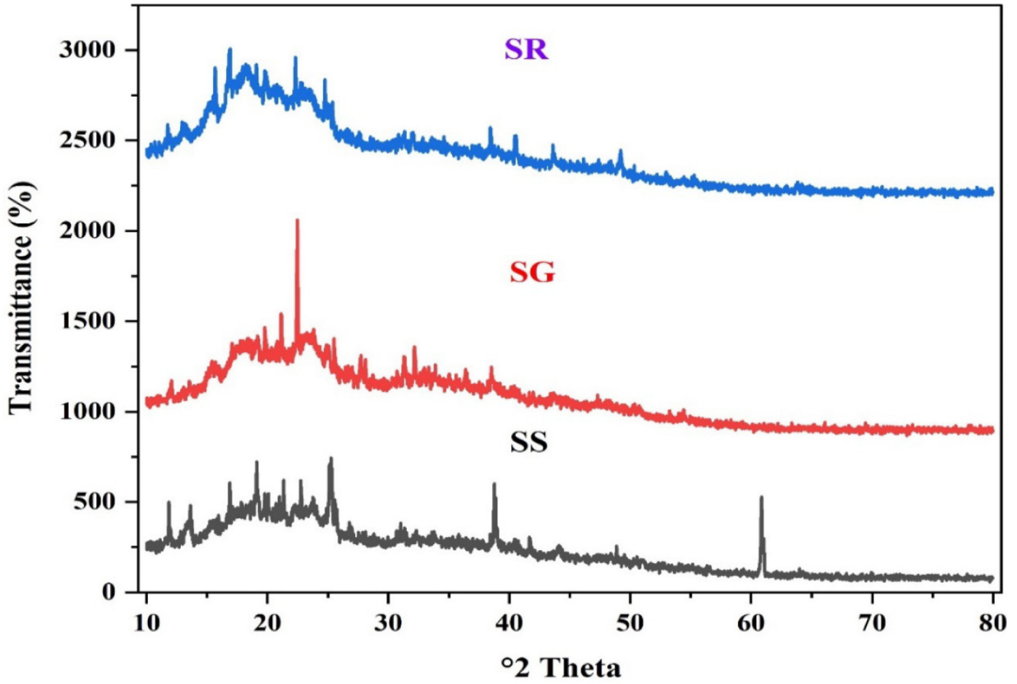

Figure 2. X-Ray Diffraction peak of the sorghum flours

***SS – Unprocessed Sorghum Flour, SG – Germinated Sorghum Flour, SR – Roasted Sorghum Flour**

peaks were low and diffused due to crystalline disruption of the double helices that influences the nature of flour.

Roasted sorghum flour showed sharp peaks at 13.1, 15.6, 16.8, 19.8, 22.3, 24.7, 25.3, 32.0, 38.4, 40.4, 43.6, 40.1 exhibited 65.25 nm crystal size. The changes in peak was noted in fermentation and malting of sorghum and pearl millet (Olamiti *et al*., 2020). Inclusion of water and other ingredients is determined by the particle size of flour. The smaller particle size, increases the water absorption property (Akinola *et al*., 2017).

Thermal properties of the flour

Thermal properties of unprocessed, germinated and roasted sorghum flours were analyzed through Thermo Gravimetric analyzer to evaluate the physicochemical changes and thermal degradation. 3.66 mg, 5.42 mg and 4.67 mg of unprocessed, germinated and roasted sorghum flour was taken in the alumina pan and thermally degraded from 24 ºC to 1000 ºC. No change was found till 50 ºC. Gradual weight loss was found till 100 °C shows the thermal degradation of moisture. At 300 ºC, first zone of thermal degradationin unprocessed, germinated and roasted sorghum flour wereobserved as a weight loss of 40.4 percent, 33.3 percent and 33.1 percent, respectively. The second zone of changes were enormous which showed a weight loss of 36.7 percent, 42.3 percent and 49.4 percent, respectively in unprocessed, germinated and roasted sorghum flour. A total loss of 94.3 percent, 91.5 percent and 93.8 percent was noticed with a residue of 0.20 mg, 0.46 mg and 0.28 mg, respectively in unprocessed, germinated and roasted sorghum flour. Wang *et al*. (2021) observed the physicochemical changes in cereal and tubers starch between 100 °C to 320 °C due to changes in saccharides and dextrin compounds.

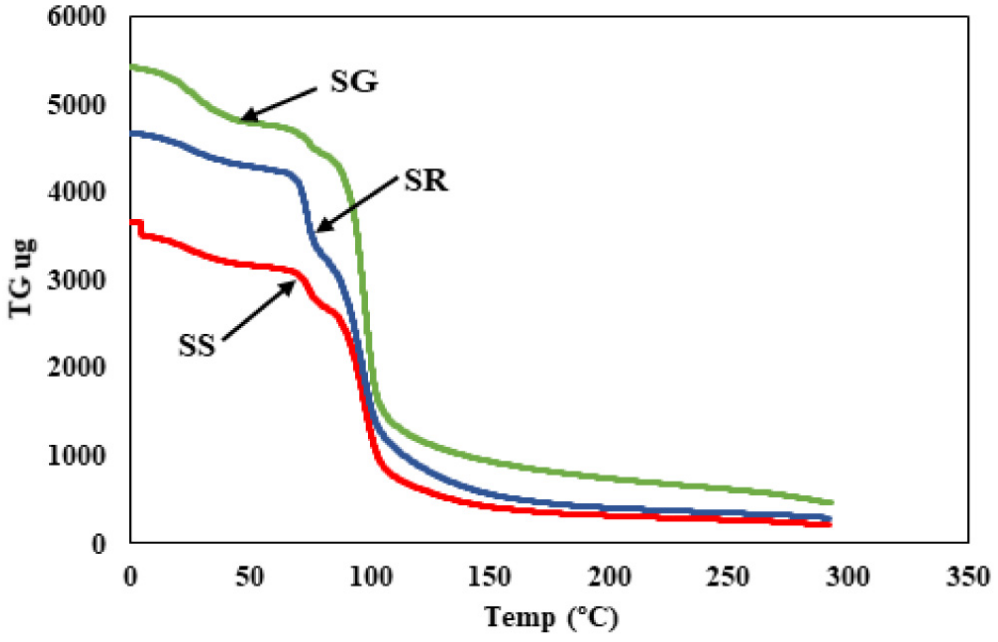

Figure 3.TGA of sorghum flour

***SS – Unprocessed Sorghum Flour, SG – Germinated Sorghum Flour, SR – Roasted Sorghum Flour**

Microbial evaluation of the flour

Microbial evaluation was done by total plate count for 180 days in the interval of 45 days. Sorghum flour grind in sterile and aseptic environment showed zero CFU/g at day 1 and minimal colony counts at 180 day. Unprocessed, germinated and roasted sorghum flour showed 1 x 10^{-1}, 2 x 10^{-1} and 0 x 10^{-1} on the day 45, 2 x 10^{-1}, 4 x 10^{-1} and 1 x 10^{-1} on the day 90 and 4 x 10^{-1}, 5 x 10^{-1} and 1 x 10^{-1} on the day 135. Unprocessed, germinated and roasted sorghum flour showed 5 x 10^{-1} CFU/g, 7 x 10^{-1} CFU/g and 3 x 10^{-1} CFU/g at the end of the 180 days. Germinated sorghum flour showed gradual increase in colony count as per the increased level of moisture content and water activity, whereas, roasted sorghum flour showed the minimal colony count. According to the Food Safety and Standards (Labelling and Display) Regulations (2020) total plate count of millet and millet products should not be more than 1,000 CFU/g.

CONCLUSIONS

This research studied the properties of sorghum flour by conventional processing methods so that one can develop products and increase the usage of this underutilized millet. Processed sorghum grains and flours influenced the functional property, optical property and technological property to reinforce food processing companies for standardizing baked and extruded products. Germinated flour might endure the preparation of smooth baked products, whereas, roasted flour can be used in the preparation of extruded crispy products when compared to unprocessed sorghum flour.

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ORIGINAL ARTICLE

Sorghum spoons enriched with selected edible flowers: A sustainable alternative to conventional cutlery in the food and tourism sectors

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Abstract
Sorghum, a widely cultivated grain, shows great potential for eco-friendly and innovative applications. This study focuses on developing sorghum-based spoons enhanced with edible flower powders (hibiscus and rose) and roasting techniques, offering a sustainable alternative to single-use plastic (SUP) spoons in the food and tourism industries. The spoons were standardized using a closed mold, resulting in a 2-mm thickness, 13-cm length, and 8-g weight. Adding flower powders and roasting significantly improved the spoons' optical properties (L*, a*, b* values) and their micronutrient and fiber content compared to unprocessed versions. Sensory evaluation indicated high consumer acceptability, with flower-enhanced spoons scoring above 8, and unprocessed versions scoring 7 on a 9-point hedonic scale for appearance, taste, flavor, texture, and hardness. Texture analysis demonstrated superior strength in flower-enriched spoons (56.21 N) compared to roasted (34.5 N) and unprocessed (31 N) versions. Roasted spoons retained structural integrity in hot soups for up to 30 min, while flower incorporation did not affect durability or water absorption, and all spoons withstood a 50-cm drop test. Microbial loads were within safe limits (13×10^1 cfu) after 120 days, with minimal cytotoxicity (3 to 10%) in the brine shrimp lethality assay. Additionally, the spoons were fully biodegradable within 12 days. This study underscores the potential of sorghum-based spoons as a sustainable, biodegradable alternative to conventional cutlery, supporting environmental conservation and reducing plastic waste.

Keywords Edible and eco-friendly spoon · Sorghum · Roasting · Flower powder · Characterization · Less toxic

1 Introduction

Plastic waste poses a major environmental threat due to its non-biodegradable nature and recycling challenges, leading to harmful microplastics in land and water ecosystems. The massive production and disposal of plastic items, including over 120 million pieces of disposable cutlery annually in India, have intensified this global issue [1, 2]. The widespread use of single-use plastics (SUP) in the food industry has severely impacted marine life, soil health, and ecosystems [37]. With approximately 8 million tons of plastic waste entering oceans annually, the global crisis demands urgent action, especially as Europe alone generates over 25 million tons of plastic waste annually but recycles less than 30% [38].

The regulatory landscape surrounding SUP is rapidly evolving, with many countries enforcing stricter measures and promoting biodegradable and edible alternatives [39]. Sorghum (*Sorghum bicolor* L. Moench), among the many substitutes investigated, including rice and wheat-based materials, stands out as the superior alternative because of its durability, sustainability, and nutritional advantages. Sorghum, a staple commonly grown crop in semi-arid parts of Asia and Africa, is rich in vital elements including energy, protein, fiber, vitamins, minerals (such as iron and zinc), and bioactive compounds which are also drought-resistant [3–5]. Recent studies highlight sorghum's bioactive compounds, such as 3-deoxy anthocyanidins, ferulic acid, and apigenin, which exhibit potential health benefits, including antioxidant, anti-inflammatory, anti-cancer, and anti-diabetic properties [5–9]. Roasting sorghum enhances its functional properties by significantly increasing its total phenolic and

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flavonoid content, improving its antioxidant capacity, and enhancing its pasting characteristics. This makes roasted sorghum an ideal candidate for developing functional and health-oriented bakery products [9–11].

The food industry, driven by consumer demand for nutritious and functional products, has seen an increase in the consumption of novel ingredients such as fruits, vegetables, and edible flowers into new product formulations [11, 12]. One innovative approach to addressing the environmental impact of single-use plastics is the development of edible spoons made from sorghum. Enhancing the value of sorghum through processing and the incorporation of flower powders such as rose and hibiscus not only improves the functional properties of the edible spoons but also contributes additional health benefits. Rose flowers are a rich source of dietary phytochemicals, carotenoids, and phenolic acids, making them potent antioxidants with anti-inflammatory, anti-cancer, anti-aging, antimicrobial, hepatoprotective, and neurogenic properties. Edible rose flowers have long been used in traditional foods and Ayurvedic medicine [13]. Similarly, hibiscus flowers, rich in organic acids and phenolic compounds such as myricetin, quercetin derivatives, kaempferol, and anthocyanins, exhibit strong nutraceutical potential without cytotoxic effects [14].

While several studies have explored biodegradable and edible alternatives to plastic cutlery [1, 39–44], this research stands out by utilizing roasted sorghum and flower powders (hibiscus and rose) to develop an edible spoon that is not only biodegradable but also nutrient-dense and functionally superior through a spoon-making machine. In addition to addressing plastic waste, the present research contributes to the worldwide transition to sustainable food systems by improving the technical, sensory, and structural qualities of spoons made from sorghum and edible flower enhancement. This invention is useful for hot and cold food applications in an array of contexts, from home usage to large-scale events in the tourist sector, because it combines better mechanical durability with greater micronutrient content, an advantage over existing options. This article focused on standardizing sorghum edible spoons as an innovative approach to fostering the cultivation of resilient crops ensuring food safety and security consuming traditional millet in a modern way and introducing millets in daily diet. By promoting the cultivation of millets and reducing plastic pollution, these innovative utensils contribute to a more resilient and sustainable food system and the impact of edible sorghum spoons on food security and millet cultivation extends beyond environmental sustainability to encompass nutritional resilience, agricultural diversity, and support for sustainable practices.

2 Materials and methods

2.1 Selection and processing of sorghum grains and edible flowers

Premium quality white sorghum grains were purchased from a departmental store in Coimbatore, Tamil Nadu, India. Pink shade roses (*Rosa damascena mill* L) and red-colored five-petaled hibiscus flowers (*Hibiscus rosa- sinensis*) were selected and collected around Karur district from January to March 2023. The Rapinat Herbarium, St. Joseph's College, Thiruchirapalli, Tamil Nadu verified the authenticity of sorghum, rose, and hibiscus. Sorghum grains were sorted, cleaned, and washed to remove impurities and dried. Dried sorghum was powdered and stored in airtight containers. White sorghum grains were dry roasted in an iron pan for 12 min at 90 °C, till a few grains popped and finely ground into flour and stored in a stainless-steel airtight container to enhance the nutrient profile, sensory attributes, appeal, and functional properties of the sorghum spoon and to decrease the anti-nutritional factors. Roasting improved organoleptic properties, physical and functional qualities, edibility, and digestibility [10]. Petals from rose and hibiscus were separated, shade-dried, finely powdered, and kept in an airtight container with less than ten percent moisture. Technological and functional properties of unprocessed and roasted sorghum grains [15] and selected edible flowers [16] were analyzed before the fabrication and standardization of sorghum spoons. All the chemicals and solvents used for nutrient analysis and functional properties were purchased from Sigma Aldrich, India from a chemical vendor in Coimbatore, Tamil Nadu.

2.2 Standardization of the flowers infused sorghum spoon

The sorghum spoon is fabricated with 6 g of unprocessed or roasted sorghum flour, 1 g of wheat flour for binding, 1 g of jaggery for flavor, and 7 to 9 mL of water. Then, the finalized compositions were blended into a thick batter and baked at 70 °C for five minutes in a spoon-shaped closed mold to fabricate a standardized sorghum spoon. 1.5 g of shade-dried hibiscus, or rose flower powder was incorporated to enhance the roasted sorghum spoon functionally. The standardized sorghum spoon displayed in Fig. 1, coded as (SUS) for Sorghum Unprocessed Spoon, whereas (SRS) stands for Sorghum Roasted Spoon, (SRHS) as Sorghum Roasted Hibiscus incorporated Spoon and Sorghum Roasted Rose incorporated Spoon as (SRRS), respectively. The baking temperature, selection of quality ingredients,

Fig. 1 Standardized sorghum spoon

and less water usage impact the texture of edible spoons which can be standardized through baking, injection, or extrusion techniques [17]. Considering the distinct viscoelasticity of gluten, it is frequently employed in the baking process as the gluten content was lowered, the product's textural qualities were also reduced [18].

2.3 Physical and optical properties of sorghum spoon

The height, width, and thickness of the sorghum spoon were measured using a laboratory scale vernier caliper (Stanley Black & Decker India Private Ltd, Bangalore, India). A digital weighing balance (Saffron, Gujarat, India) was used to analyze the weight. L*, a*, b*, and Δ E were measured using a laboratory-scale Food Colorimeter (MiniScan XE plus) to analyze the optical properties of the standardized sorghum spoon on day 1 and day 120. L*, which is a measurement of lightness to darkness in the range of zero to hundred. Positive values of a* and b* show red and yellowness whereas negative values of a* and b* indicate green and blueness. Δ E is the measure of chroma and hue differences [18, 19].

2.4 Sensory evaluation of sorghum spoon

The standardized sorghum spoon's sensory attributes, including shape or appearance, color, taste, flavor, crispiness or hardness, and overall acceptability, were assessed using a 9-point hedonic scale. One being disliked extremely and nine were liked extremely. To determine the changes in sensory characteristics over storage, thirty semi-trained panelists compared the standardized sorghum spoons' substantial similarities on day 1 and day 120. After the spoon was immersed in hot clear soup, ice cream, and smoothies for 30 min at several degrees, its sensory qualities were assessed. The sensory characteristics of the food items were also examined to observe the interaction of sorghum spoons with the food at various temperatures [20].

2.5 Proximate analysis of sorghum spoon

Proximate analyses, such as moisture, ash, carbohydrates, protein, fat, and crude fiber were analyzed by AOAC standards [21]. Vitamin C, iron, calcium, and phosphorus were analyzed by a standardized procedure including ascorbic acid assay, Folin Ciocalteau reagent method, and titration against $KMnO_4$ for the standardized sorghum spoon [22].

2.6 Antioxidant activity of sorghum spoon

Antioxidant activity of sorghum spoon was examined using ascorbic acid as a standard in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity assay and determined IC50 values using linear regression. Strong antioxidant activity is defined as having an IC50 value between 10 and 50 mg/mL, intermediate antioxidant activity between 50 and 100 mg/mL, and poor antioxidant activity over 100 mg/mL [23, 24].

2.7 Mechanical properties of sorghum spoon

2.7.1 Texture analyzer of sorghum spoon

A texture analyzer (Shimadzu EZ-SX) examined the standardized spoon's hardness, elastic force, and break force using a three-point bending test. The spoon was measured at a speed of 1 mm per second until it fractured into pieces due to compression, shear, and puncture peaks [20].

2.7.2 Exposure and drop test of sorghum spoon

The sorghum spoon's structural integrity in terms of the ability to endure temperature was assessed by subjecting it to ambient, hot, and cold temperatures for an hour at 35 °C, 80 °C, and 4 °C [1]. A drop test was performed to determine the endurance of accidental falls and the free-fall nature of the spoon from 10 to 50 cm on a smooth plain surface.

2.7.3 Water absorption test of sorghum spoon

The water absorption test determines the quantity of water absorbed by the sorghum spoon during the exposure of 30 min. A known weight of the sorghum spoon (W1) was placed in a beaker containing 50 mL of ambient, hot, or cold liquids and allowed to remain stationary for 30 min. Then, the final weight was noted (W2) and the percentage of water absorption was computed by the formula (1), after using tissue paper to remove the surface water [1, 2].

$$\text{Percentage of Water Absorption (\%)} = \frac{W2 - W1}{W1} \text{x } 100 \tag{1}$$

2.8 Functional properties of sorghum spoon

2.8.1 FTIR of sorghum spoon

The peaks from the Fourier Transform Infrared spectrophotometer (Shimadzu MIRacle10) were used to identify the functional groups of the standardized sorghum bowls. The spectra of 0.5 g of sample were obtained, at 16 runs per scan, and the peaks were identified between 4000 and 450 cm^{-1} wavenumbers [35].

2.8.2 Thermogravimetric analysis of sorghum spoon

A thermogravimetric analyzer (EXSTAR/C300) was used to identify the thermal properties and to calculate the percentage of thermal deterioration as the spoon endures certain physiochemical changes in response to the controlled rise in temperature. The sample was heated in an alumina pan from 20 to 1000 °C at 20 °C increase per minute [34].

2.9 In vitro cytotoxicity analysis of sorghum spoon

Brine shrimp lethality assay assessed the potential toxicity of SUS, SRS, SRHS, and SRRS on shrimp by exposing them to various concentrations in a saline mixture, with saline water as blank and potassium dichromate (1 mg/mL) as a positive control. Similarly, 30 brine shrimp nauplii (after 24 h of hatching) were exposed to different concentrations (100, 250, 500, 1000, and 1500 mg/mL), to record the mortality rate at 1, 2, 4, 6, and 24 h. The mortality percentage of brine shrimp in the samples, blank, and positive control was calculated as follows, Eq. (2) [25, 26].

$$\% \text{ of Mortality } = \frac{\text{Number of brine shrimp mortal}}{\text{Numebr of brine shrimp introduced}} \text{x100} \quad (2)$$

2.9.1 Shelf-life evaluation of sorghum spoon

The total plate count of the sorghum spoon was examined by dispensing one gram of the spoon sample in 9 mL of sterile peptone water (0.1%). Serial dilutions from 10^1 to 10^9 in duplicates were made using homogenate water with 0.1% peptone and poured in plate count agar. The dilution was incubated at 35 °C for 48 h and the colonies were counted [27]. The change in weight of the sorghum spoon for 120 days was analyzed to measure the atmospheric reaction as it is an important factor to alter the shelf life of the product.

2.9.2 Soil degradation analysis of sorghum spoon

A 2-g spoon was buried in a beaker with the collected wet sandy topsoil from the star garden at Avinashilingam Institute in Coimbatore, Tamil Nadu, to a depth of 5 cm to ensure the total degradation. On the third, sixth, and ninth days, the samples were taken from the soil and dried for 24 h at 70°C in a hot air oven. The weight change was then calculated using a modified approach [1, 19].

2.9.3 Statistical analysis of sorghum spoon

Data analysis was carried out using the version of IBM SPSS Statistics 25 software. Two-way ANOVA with Duncan's post hoc analysis was done for the optical properties of the sorghum spoon for days 1 and 120. An Independent sample '*t*' test was conducted for the remaining properties to find out the statistical difference of the sorghum spoon in terms of unprocessed, roasted, and enriched with flower powder at a 95% ($p < 0.05$) level of significance.

3 Results and discussion

3.1 Physical and optical properties of sorghum spoon

The total length of the spoon is 13 cm and the head length is 4.5 cm with a breath at the top of 1 cm, middle of 3.2 cm and bottom of 1.6 cm, and hand width is 1.1 to 1.3 cm. The average weight of the spoon is 8 g with 2-mm thickness. In addition to replacing single-use plastics in culinary and recreational endeavors, the sorghum spoon is employed to spread jam or butter on toast or sandwiches, mix liquids, and consume hot soup, ice cream, and cakes.

The optical properties of the standardized sorghum spoon on day 1 and day 120 are displayed in Table 1. On Day 1, the L* values of SUS and SRS were similar between 10.51 and 10.59, and SRHS and SRRS exhibited significant differences ranging from 6.54 to 7.95. On day 120, SUS, SRS, SRHS, and SRRS showed slightly increased lightness and remained lighter in shade. On day 1, a* of SUS, SRS, SRHS, and SRRS were positive values depicted redder shade between 0.27 and 2.62, and all the spoons depicted significant differences. On day 120, the presence of bioactive chemicals and storage caused the a* values of SUS and SRS to exhibit a slight rise in red hue, while SRHS and SRRS showed a considerable increase in red hue. Unprocessed, roasted, hibiscus, and rose-incorporated spoons' b* values displayed a yellow tint with statistically significant variations between days 1 and 120. SRRS and SRHS demonstrated a substantial rise throughout the course of storage. SUS and SRS show the least change in color over time, indicating good color

Table 1 Optical properties of sorghum spoon

Optical property	L*	a*	b*	Δ E
At Day 1				
SUS	10.59 ± 0.41^{c}	1.27 ± 0.11^{a}	7.46 ± 0.42^{c}	6.82 ± 0.47^{b}
SRS	10.51 ± 0.25^{c}	1.48 ± 0.21^{a}	6.34 ± 0.41^{b}	6.43 ± 0.44^{b}
SRHS	6.54 ± 0.31^{a}	2.62 ± 0.33^{a}	3.33 ± 0.19^{a}	6.54 ± 0.31^{b}
SRRS	7.95 ± 0.48^{b}	1.29 ± 0.20^{b}	4.24 ± 0.19^{a}	5.13 ± 0.18^{a}
At Day 120				
SUS	11.05 ± 0.33^{c}	1.01 ± 0.78^{a}	7.01 ± 0.97^{c}	6.41 ± 0.47^{b}
SRS	11.11 ± 0.4^{c}	1.32 ± 0.34^{a}	6.28 ± 0.37^{b}	6.85 ± 0.73^{b}
SRHS	7.19 ± 0.28^{a}	2.57 ± 0.39^{a}	3.17 ± 0.40^{c}	6.35 ± 0.63^{b}
SRRS	8.30 ± 0.23^{b}	1.14 ± 0.13^{b}	3.06 ± 0.02^{c}	1.88 ± 0.35^{a}

($n = 3$, $df = 4$), Mean ± SD, *—Significant, **—Not Significant, *SUS*, Sorghum unprocessed Spoon; *SRS*, Sorghum roasted Spoon; *SRHS*, Sorghum Roasted Hibiscus Spoon; *SRRS*, Sorghum Roasted Rose Spoon; Values in superscript in each column denote the significant difference ($p < 0.05$)

stability, and remain relatively light and yellow over time. SRHS and SRRS remain darkest and reddest over time. These findings align with previous studies, which demonstrated the addition of vegetable extracts and flower powders can increase the intensity of color during storage, as seen in cookies with similar ingredients [10, 12]. As previously noted in the study [11], roasting sorghum also contributed greatly to changes in its optical qualities by increasing the total color change and browning index significantly reducing brightness. The study demonstrates that roasting sorghum and adding bioactive flower powders cause noticeable and dynamic color changes.

3.2 Sensory evaluation of the sorghum spoon

A radar chart (Fig. 2) displays the results of a sensory evaluation of SUS, SRS, SRHS, and SRRS on day 1, day 120, as well as the sensory qualities of the spoon and food items after exposure to ambient (28 °C), hot (70 °C), and cold (4 °C) temperatures in juice or smoothie, hot soup, and ice cream for 30 min through 30 semi-trained panel members. The highest overall acceptability score on day

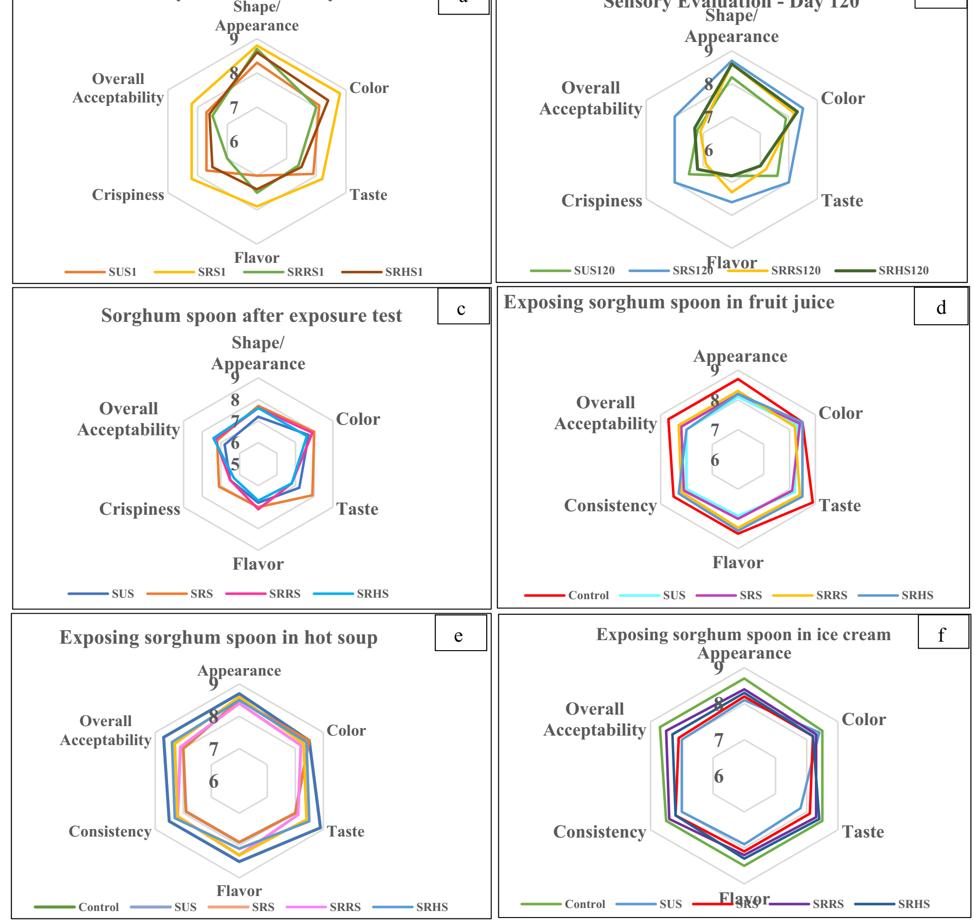


Fig. 2 Sensory evaluation of sorghum spoon (a) on day 1, (b) on 120 days of storage, (c) sorghum spoon after exposing in food sample for 20 min, (d) exposing of sorghum spoon in lemon juice at 28°C, (e) exposing of sorghum spoon in hot soup at 70 °C, (f) exposing of sorghum spoon in ice cream at 4°C

1, was 8.2 points for SRS, while the lowest overall sensory preference score was 7.5 points for SRHS. The shape and color attributes of the four spoons were very much liked by sensory evaluators. The taste, flavor, and crispiness of SRS were 8.2, 7.9, and 8.2 mean scores which is more than SUS, SRHS, and SRRS. Hibiscus and rose flower powder influence the sensory attributes and slightly decrease the taste preference but all four standardized spoons scored above 7 points indicating like many categories (Fig. 2a). On day 120, the flavor, and crispiness of SUS, SRS, SRHS, and SRRS exhibited differences, and the shape, color and overall acceptability of the spoon remained the same. After exposure to 30 min at different temperatures, the sensory attributes score of all variations was decreased (Fig. 2b). The spoon's appearance, color, and taste scored 8 points, which were reduced to 7 points, and flavor, crispiness, and overall acceptability were lowered from 8 to 6.5 to 7 after exposing the spoon for 30 min at different temperatures in fruit juice, smoothie, and hot soup that proved sensorily acceptable (Fig. 2c). Sensory attributes of fruit juice or smoothie, hot soup and ice cream were also evaluated after exposure in SUS, SRS, SRHS and SRRS. After exposure, the evaluation revealed that none of the food items' sensory qualities changed, and every characteristic received a score of at least 8, comparable to the control sample (Fig. 2d–f). Similar trends were observed in, standardized edible spoons from raw tapioca and banana, which scored 7 points in overall sensory attributes [2]. Kappa carrageenan edible spoon scored a mean average overall acceptability of 6 points [28]. Sensory evaluation of the dietary cookies started to deteriorate at 180 days of storage due to high fiber content [29]. The rose and hibiscus flowers inclusion prohibit the degradation rate in the product [12]. The alginate and crab shell flour spoon scored between 7 and 8 in terms of appearance, color, taste, and textural properties [30].

3.3 Proximate analysis of sorghum spoon

Proximate analysis of the sorghum spoon is evaluated in triplicates and the results are tabulated in Table 2. The roasted sorghum spoon exhibited the lowest moisture content at 2.9%, significantly lower than SUS, SRHS, and SRRS, with notable differences observed among all four standardized spoons. Ash content of SRS and SRHS exhibited significant differences and SUS and SRHS showed similarity. The inclusion of flower powder indicated an increase in moisture and ash content. Macronutrients like carbohydrates, protein, and fiber increase after roasting and flower powder enrichment. SUS, SRS, and SRHS showed significant differences in carbohydrate, protein, and fiber. Fat content was reduced in SRS (0.89 g) due to roasting whereas SUS (0.99 g) resulted more and showed significant similarities. Vitamin C and iron did not significantly increase after enrichment of flower powder and roasting of sorghum in the standardized sorghum spoon and sorghum spoons exhibited 0.01 to 0.04 mg of vitamin C and 0.39 to 0.50 mg of iron. SUS, SRS, and SRHS showed significant differences in calcium content and all four standardized spoons revealed significant differences in phosphorous content. SRHS showed high calcium and phosphorous content. The results are consistent with earlier research, when sorghum grain was ground and processed, sorghum flour produced comparable findings [31]. The moisture level of sorghum edible spoons was 5.32%, and the percentage rose when vegetable extract was incorporated [19]. Alginate and crab shell flour spoons contain 2.65% and 3.66% moisture and ash content, 83.64%, 8.31%, and 1.42% carbohydrate, protein, and fat content,

Table 2 Proximate analysis of sorghum spoon

Proximate analysis	SUS	SRS	SUS and SRS	SRS			
				SRHS		SRRS	
	Mean ± SD	Mean ± SD	*t*-value	Mean ± SD	*t*-value	Mean ± SD	*t*-value
Moisture (%)	3.42 ± 0.03	2.91 ± 0.05	14.299*	3.2 ± 0.02	9.116*	3.47 ± 0.04	14.26*
Ash (%)	0.32 ± 0.005	0.35 ± 0.03	1.76**	0.43 ± 0.03	2.67*	0.41 ± 0.02	2.56**
Carbohydrate (g)	6.7 ± 0.02	6.88 ± 0.07	4.03*	6.85 ± 0.07	0.50**	6.81 ± 0.07	1.33**
Protein (g)	0.81 ± 0.03	0.91 ± 0.02	4.10*	0.96 ± 0.01	2.98*	0.87 ± 0.07	0.92**
Fat (g)	0.27 ± 0.01	0.25 ± 0.03	1.01**	0.26 ± 0.03	0.37**	0.27 ± 0.06	0.59**
Fiber (g)	0.17 ± 0.01	0.23 ± 0.03	2.92*	0.37 ± 0.06	3.52*	0.3 ± 0.03	2.39**
Vitamin C (mg)	0.01 ± 0.005	0.02 ± 0.02	0.72**	0.04 ± 0.025	1.01**	0.04 ± 0.02	1.11**
Iron (mg)	0.39 ± 0.02	0.41 ± 0.03	1.8**	0.50 ± 0.03	3.51**	0.41 ± 0.03	0.00**
Calcium (mg)	2.73 ± 0.01	2.77 ± 0.02	2.64*	2.92 ± 0.04	5.01*	2.81 ± 0.07	0.91**
Phosphorous (mg)	27.42 ± 0.04	27.64 ± 0.03	7.68*	28.16 ± 0.02	22.90*	27.92 ± 0.06	7.09*

($n = 3$, df = 4), Mean ± SD, *—Significant, **—Not Significant, *SUS*, Sorghum unprocessed Spoon; *SRS*, Sorghum roasted Spoon; *SRHS*, Sorghum Roasted Hibiscus Spoon; *SRRS*, Sorghum Roasted Rose Spoon

respectively [30]. Foxtail millet and wheat flour-based cutlery delivered substantial nutritional benefits, with carbohydrate, protein, fat, and crude fiber levels of 76 g, 10 g, 1.2 g, and 1.9 g, respectively [45].

3.4 Antioxidant activity of sorghum spoon

The percentage of inhibition for SUB, SRB, SRHB, and SRRB at different concentrations (10, 50, 150, 250, 350, 500, and 750 µL) along with the IC 50 value is displayed (Fig. 3). The percentage of inhibition in SUB rises with concentration and reaches a high of 66.43% at 350 µL. The inhibition diminishes at 500 and 750 µL (63.67% and 60.16%, respectively). In SRB, the inhibition percentage peaks at 350 µL (60.12%) and increases progressively with concentration. There is a small reduction in inhibition (59.02% and 58.67%, respectively) at higher doses (500 and 750 µL). SRHB shows a consistent increase in inhibition percentage with increasing concentration, achieving the highest inhibition at 350 µL (73.97%). The inhibition percentage remains relatively high at 500 µL and 750 µL (71.5% and 69.98%, respectively). SRRB shows the inhibition percentage steadily increases with concentration, at 750 µL with 64.9%. IC 50 value of SUS was 160.12 µL/mL whereas the SRS was 85 µL/mL, SRHS was 70 µL/mL and SRRB was 75 µL/mL. SRHB is the most effective sample with the lowest IC50 (70 µL/mL), indicating that it requires the least concentration to achieve 50% inhibition. SRRB and SRHB show increased inhibition with higher concentrations, and SUB and SRB show a slight decrease in inhibition percentage at 500 and 750 µL, suggesting a possible saturation effect. SRHB maintains high inhibition percentages even at higher concentrations, making it a consistent inhibitor. Parallel results were reported by [12], a 5% incorporation of rose flower powder in wheat cookies had greater levels of total phenolic and anthocyanin contents and stronger antioxidant activity during storage. Foxtail millet cutlery also demonstrated notable antioxidant properties, including catechins, ferulic acid, lignin, saponin, and betacyanin [45].

3.5 Mechanical properties of sorghum spoon

3.5.1 Texture property of the sorghum spoon

Texture property is the important mechanical attribute that measures the breakability of the standardized sorghum spoon enriched with rose and hibiscus flower powder and roasting. The hardness force and elastic force of the sorghum spoon were tabulated in Table 3. SRHS and SRRS showed the highest hardness and break force with 52.05 N and 59.5

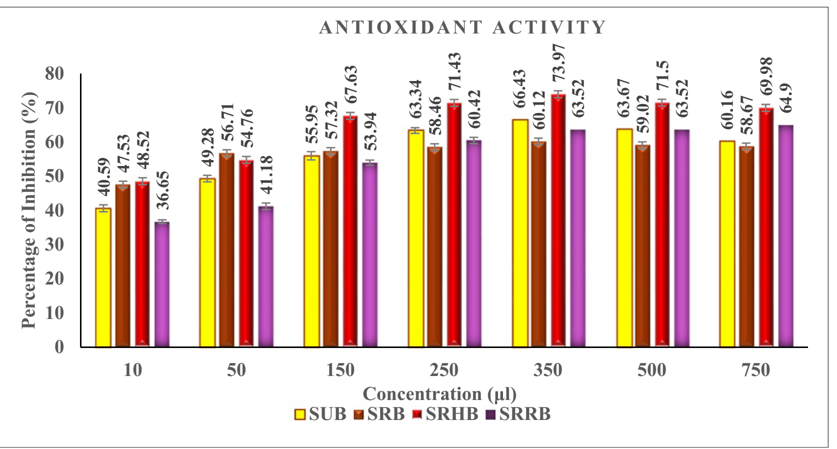


Fig. 3 Antioxidant activity of sorghum spoon

Table 3 Texture analysis of sorghum spoon

Texture Analysis	SUS	SRS	SUS and SRS	SRS			
				SRHS		SRRS	
	Mean ± SD	Mean ± SD	*t*-value	Mean ± SD	*t*-value	Mean ± SD	*t*-value
Hardness and Break Force (N)	31.006 ± 1.7	34.5 ± 1.32	2.80*	52.92 ± 0.23	23.78*	59.5 ± 2.18	16.98*
Elastic force (N/mm^2)	113.78 ± 5.13	97.76 ± 0.84	5.33*	68.69 ± 0.9	40.61*	77.5 ± 0.54	34.96*

(*n* = 3, df = 4), Mean ± SD, *—Significant, **—Not Significant, *SUS*, Sorghum unprocessed Spoon; *SRS*, Sorghum roasted Spoon; *SRHS*, Sorghum Roasted Hibiscus Spoon; *SRRS*, Sorghum Roasted Rose Spoon

N and SUS showed the lowest break force. The elastic force is inversely proportional to the harness and break force. SUS scores more elastic force and SRHS showed less with 69.11 N/mm^2. SUS, SRS, SRHS, and SRRS showed significant differences in hardness and elastic force. The hardness and break force of the sorghum spoon showed that the flower-incorporated spoon required more force to break than SUS and SRS. The sorghum spoon produced a superior outcome comparable to earlier findings. The kappa carrageenan edible spoon exhibited 5.15 kgf of fracturability and 5.26 kgf of hardness force [28]. The hardness force of a sorghum spoon coated with rice and wheat flour was 7.95 N; adding beetroot and jamun extract raised the hardness force to 15.15 N; adding spinach extract lowered the hardness force to 4.76 N [19]. Alginate and crab shell flour spoons showed 10.32 kgf hardness force [30].

3.5.2 Exposure of the sorghum spoon

Sorghum spoons were exposed to juice, smoothie, hot soup, and ice cream for 60 min. (Fig. 4). Spoons have a holding capacity of up to 50 min in lemon juice and mixed fruit smoothie, 30 min in hot soups, and 60 min in ice cream without losing functionality. Sorghum spoons can also be utilized in spreading jam or butter on bread and sandwiches without any functional change and withstand break force. The sorghum spoon is a sustainable and convenient solution for a wide range of culinary delights as the versatility of the sorghum spoon proves to be an ideal choice for both hot soups, and indulgent servings of ice cream and in consuming smoothies or mixing of juices. Parallel results were reported by [2], Raw tapioca and banana spoons hold hot, cold, and ambient food serving temperature conditions for 30 min.

3.5.3 Drop test of the sorghum spoon

The spoon was dropped from 10 to 60 cm to measure the free fall ability (Fig. 5). SUS withstands up to 30 cm and exhibited first crack at 40 cm and completely broken at 50 cm. SRS showed first crack at 50 cm and completely broken at 60 cm. A sorghum spoon enriched with hibiscus and rose powder showed a free fall impact of 40 cm and started to break into pieces when fell from 50 cm. Flower incorporation unchanged the free fall impact of the sorghum spoon.

3.5.4 Water absorption rate of sorghum spoon

The water absorption rate of sorghum spoons is crucial for determining their practical applications and limitations in various food service scenarios and the results are tabulated in Table 4. Food absorption (thin, clear soup) in a hot medium is closely correlated with temperature rise. The spoon indicated higher absorption between 70 and 55 °C, and after 44 °C, the percentage of absorption decreased. All spoon types absorb between 33 and 37% of the water at 70 °C. At the end of 30 min; SUS spoons absorb the highest percentage of water, due to unprocessed sorghum flour. In cold conditions (4 °C), water absorption rates are significantly lower, with all spoon types absorbing less than 13%, indicating that lower temperatures slow the absorption process. At ambient

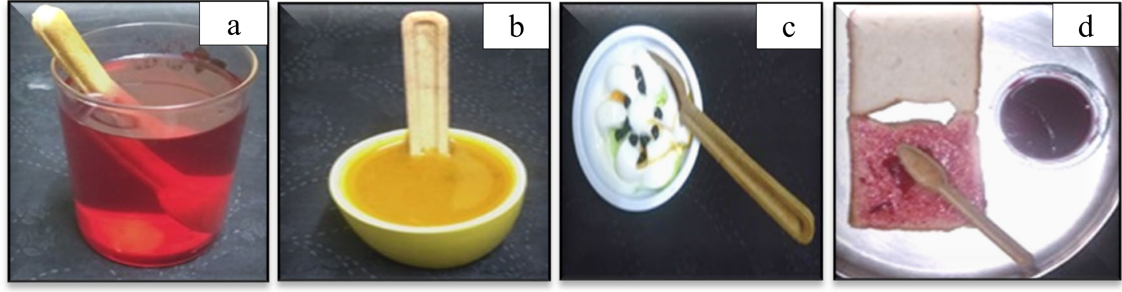


Fig. 4 Exposure test of sorghum spoon, **a** fruit juice thin soup, **b** thin soup ice cream, **c** ice cream fruit juice, **d** spreading jam on bread

Fig. 5 Drop test of sorghum spoon. **a** 40 cm of SRS, **b** 50 cm of SRRS, **c** 50 cm of SRHS

Table 4 Water absorption rate of sorghum spoon

Duration		Duration (min)						
	Temperature	0	5	10	15	20	25	30
Hot (70 °C) (Thin and clear soup)	**Temp (°C)**	**70**	**62**	**55**	**50**	**44**	**40**	**30**
	SUS (%)	0	15.85	22.54	29.12	33.28	35.14	36.75
	SRS (%)	0	13.67	17.18	24.68	29.76	32.76	34.12
	SRHS (%)	0	12.95	16.83	23.45	28.12	31.45	33.79
	SRRS (%)	0	13.14	17.08	23.75	29.05	30.92	33.52
Cold (4 °C) (Ice cream)	**Temp (°C)**	**4**	**4**	**5**	**6**	**7**	**8**	**9**
	SUS (%)	0	3.87	5.37	8	9.62	10.62	12.5
	SRS (%)	0	3.12	5.72	6.75	7.12	7.87	9.25
	SRHS (%)	0	3.62	4.37	6.12	6.75	7.62	9.5
	SRRS (%)	0	3.37	4.62	6.25	6.5	7.65	8.87
Ambient (28 °C) (Fruit Juice/ Smoothie)	**Temp (°C)**	**28**	**28**	**28**	**29**	**29**	**30**	**30**
	SUS (%)	0	9.87	16.35	23.12	25.87	27.75	29.87
	SRS (%)	0	9.12	12.12	16.5	19.12	22.12	25.01
	SRHS (%)	0	8.87	11.75	15.5	20.12	21.5	24.38
	SRRS (%)	0	9.01	11.87	15.37	19.87	21.25	24.25

temperature (28 °C), water absorption increases steadily, with SUS spoons reaching nearly 30%, while SRS, SRHS, and SRRS spoons show slightly lower absorption rates. Overall, SUS spoons generally absorb the most water across all temperature conditions, with the highest rates in hot conditions and the lowest in cold conditions. SRS spoons consistently show slightly lower water absorption than SUS but higher than SRHS and SRRS in most conditions, while SRHS and SRRS have comparable absorption rates, with SRHS showing slightly higher absorption in cold conditions and SRRS in hot and ambient conditions. The longer a sorghum spoon is used before it disintegrates or loses its shape and ability to act as a spoon when the water absorption is low. The results are supported by prior research, a non-edible wheat spoon absorbs between 38 and 50%, 10 to 35%, and 15 to 41% when exposed to hot, cold, and ambient temperatures [2]. The edible spoon composed of wheat flour, and barnyard flour has water absorption values of 49.76%, 35.93%, and 41.09% at 10 °C, 29 °C, and 50 °C, respectively [32].

3.6 Functional properties of sorghum spoon

3.6.1 FTIR of sorghum spoon

SUS, SRS, SRHS, and SRRS showed similar mediate to strong peaks at 416.93, 440.87, 472.56, 545.32, 590.14, 648.14 cm^{-1} and 730.45 cm^{-1} exhibiting the presence of amylose and amylopectin content. Peaks at 810.72, 957.34, 1150.49, 1380.14, and 2980.17 cm^{-1} confirmed the presence of the amide II from N–H bending. The four spoons all had peaks between 3700 and 3900 cm^{-1}, however, the presence of vibrational peaks attributed to bound water was particularly noticeable at 3714.45, 3830.12, and 3920.76 cm^{-1}. Phenolic compounds were present in SRHS and SRRS at peaks, particularly 3287.93 and 3316.51 cm^{-1}, but were undetectable in SUS and SRS. Intensive peaks at the region around 1500 to 2500 cm^{-1} were found in sorghum flour which was absent in sorghum spoon which may be due to processing [15]. The peaks at 3600–3200 cm^{-1} indicate the presence of -OH groups, characteristic of phenolic

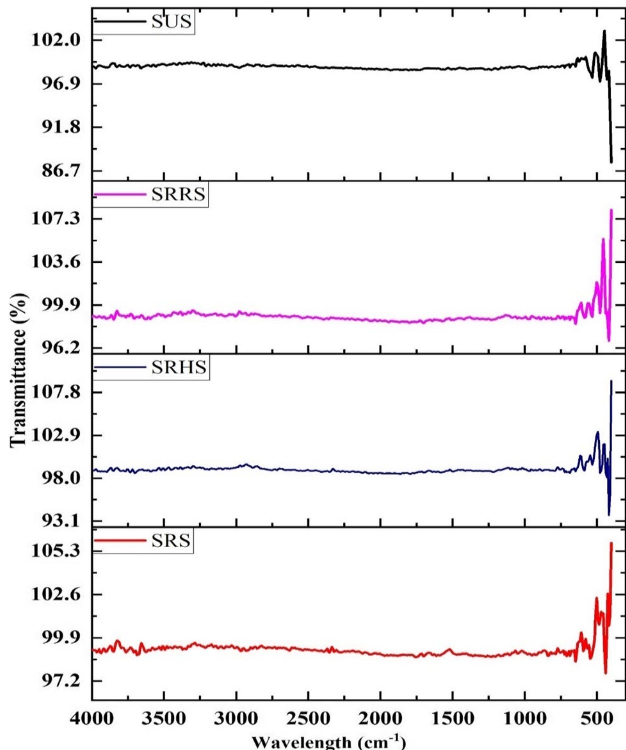


Fig. 6 FTIR peaks of Sorghum spoon

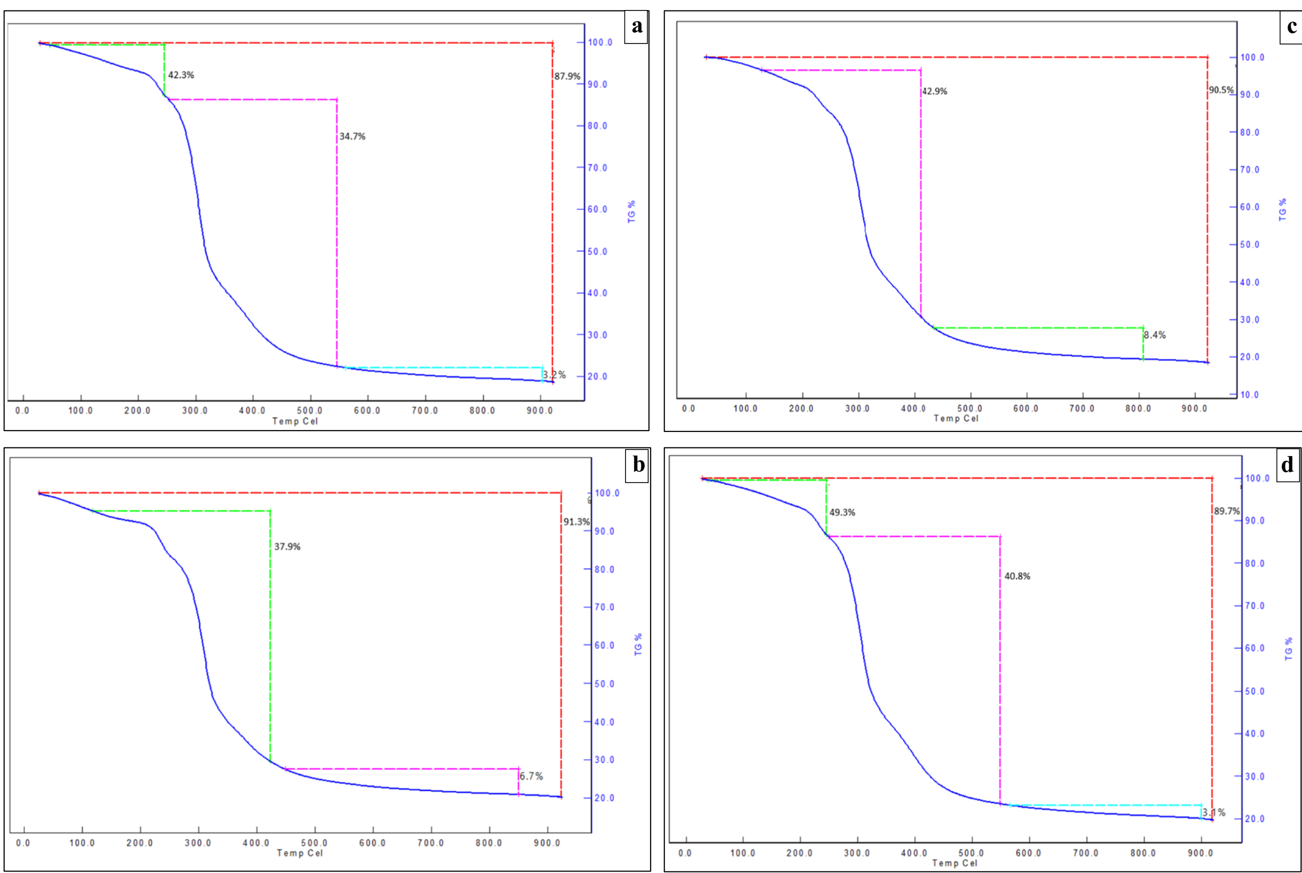


Fig. 7 TGA peaks of Sorghum spoon, **a**. SUS, **b**. SRS, **c**. SRHS, **d**. SRRS

compounds. Bands in the 2800–3000 cm^{-1} range exhibit -(CH)n groups, typical of alkyl, aliphatic, and aromatic compounds. The lower intensity at 2954 to 2852 cm^{-1} is attributed to C-H bond stretching. The strong bands at 1215 and 740 cm^{-1} correspond to the C-O bond stretching in phenolic compounds and the C-H bond stretching in aromatic compounds, respectively [35, 36] Fig.6.

3.6.2 Thermogravimetric analysis of sorghum spoon

TGA of sorghum spoon shows the thermal degradation of organic, and inorganic compounds during the temperature rise (Fig. 7). SUS, SRS, SRHS, and SRRS showed 42.3%, 46.1%, 47.6%, and 49.3%, respectively, of thermal degradation between 100 and 250 °C, exhibiting the initial mass loss is primarily attributed to the evaporation of moisture or the decomposition of low molecular weight constituents. SRRS exhibits the highest mass loss at 49.3%, exhibiting

Table 5 Invitro cytotoxicity analysis of sorghum spoon

Concentration (µg/mL)	Mortality of brine shrimp (no. of shrimps dead) / (24 h)							
	SUS		SRS		SRHS		SRRS	
	No. of shrimp mortal	% Mortality	No. of shrimp mortal	% Mortality	No. of shrimp mortal	% Mortality	No. of shrimp mortal	% Mortality
100	0	0	0	0	0	0	0	0
500	0	0	0	0	0	0	0	0
1000	1	3	0	0	1	3	1	3
1500	2	7	1	3	3	10	3	10

Table 6 Shelf-life Analysis of Sorghum Spoon

Spoon	Day 1	Day 30		Day 60		Day 90		Day 120	
	Average Weight (g)	Total Plate Count (CFU/g)	Change in Weight (g)	Total Plate Count (CFU/g)	Change in Weight (g)	Total Plate Count (CFU/g)	Change in Weight (g)	Total Plate Count (CFU/g)	Change in Weight (g)
SUS	7.9 ± 0.07	2×10^1	8.06 ± 0.09	4×10^1	8.15 ± 0.11	9×10^1	8.2 ± 0.16	13×10^1	8.39 ± 0.19
SRS	7.09 ± 0.03	0×10^1	7.22 ± 0.04	0×10^1	7.31 ± 0.04	0×10^1	7.42 ± 0.05	1×10^1	7.53 ± 0.06
SRHS	7.47 ± 0.41	2×10^1	7.6 ± 0.39	4×10^1	7.71 ± 0.34	9×10^1	7.84 ± 0.31	11×10^1	7.97 ± 0.36
SRRS	7.81 ± 0.03	3×10^1	7.99 ± 0.08	5×10^1	8.12 ± 0.08	9×10^1	8.26 ± 0.09	10×10^1	8.40 ± 0.11

n = 3, Mean ± SD, *SUS*, Sorghum unprocessed Spoon; *SRS*, Sorghum roasted Spoon; *SRHS*, Sorghum Roasted Hibiscus Spoon; *SRRS*, Sorghum Roasted Rose Spoon

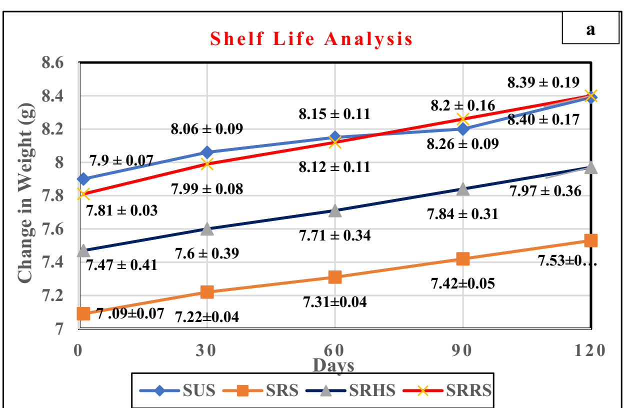
the greater presence of moisture content. In the 250–750 °C range, substantial material decomposition occurs, likely involving the degradation of organic compounds. SRHS shows the greatest mass loss in this range at 42.9%. As the temperature increases to 750–1000 °C, further decomposition is observed, which typically involves the breakdown of more inorganic compounds. SUS demonstrates the highest mass loss in this range at 15.9%, indicating the presence of components that decompose at elevated temperatures. Overall, SRS records the highest total mass loss during thermal degradation, reaching 91.3%. Thermal degradation of four samples was high at the initial phase (20 to 250 °C) and minimal at the last phase between 750 and 1000 °C showing high moisture content [15, 34, 35].

3.7 In vitro cytotoxicity analysis of sorghum spoon

Brine shrimp lethality assay of SUS, SRS, SRHS, and SRRS for 24 h was evaluated, and the result showed minimal cytotoxicity (Table 5). At 100 and 500 mg/mL concentrations of SUS, SRS, SRHS, and SRRS, all the brine shrimp were alive after 24 h. At 1000 mg/mL concentration of SRS, all the brine shrimp were alive and in SUS, SRHS, and SRRS, 3% of brine shrimp were mortal after 24 h of observation. At 1500 mg/mL concentration, SRS showed 3% mortality, SUS showed 7% mortality, and SRHS and SRRS showed 10% mortality after 24 h of exposure. SUS, SRS, SRHS, and SRRS brine shrimp lethality assay revealed a minimal cytotoxic effect on brine shrimp nauplii and increasing concentrations increases the mortality rate. Similar patterns were reported in, extracts from sorghum, hibiscus, and clove flowers strongly showed a cytotoxic effect on brine shrimp due to its bioactive and phenolic compounds [25, 26].

3.8 Shelf life of the sorghum spoon

The total microbial load of the sorghum spoon was analyzed by serial dilution technique and weight change is yet another important shelf-life indicator and the observations were specified in Table 6. On the 120th day, SUS showed 7×10^1 cfu, SRS showed 2×10^1 cfu, and SRHS and SRRS showed 13×10^1 cfu and 7×10^1 cfu. SRS showed minimal total bacterial growth that has a prolonged shelf life among SUS, SRHS, and SRRS. Kappa carrageenan edible spoon showed below 6.5×10^2 cfu [28].



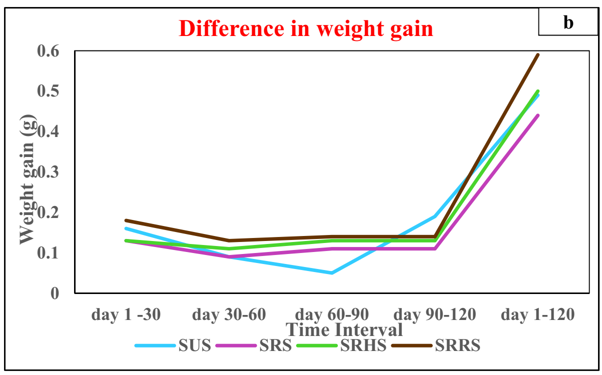
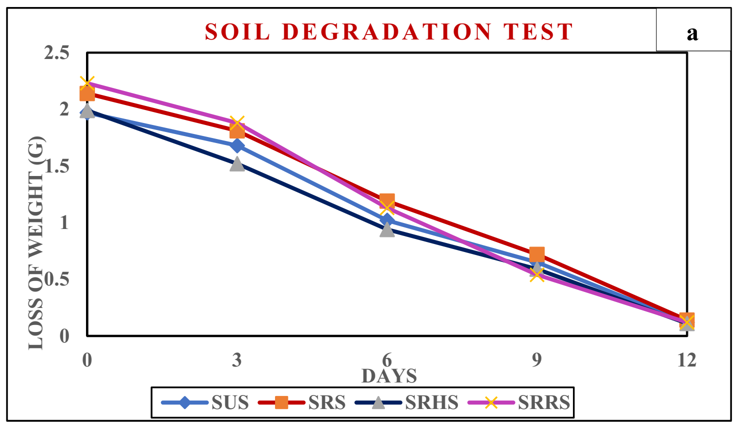


Fig. 8 **a** Change in weight of sorghum spoon on consecutive months to assess absorption of environmental moisture during storage as shelf-life analysis parameter. **b** Difference in weight gain of sorghum spoon in consecutive months



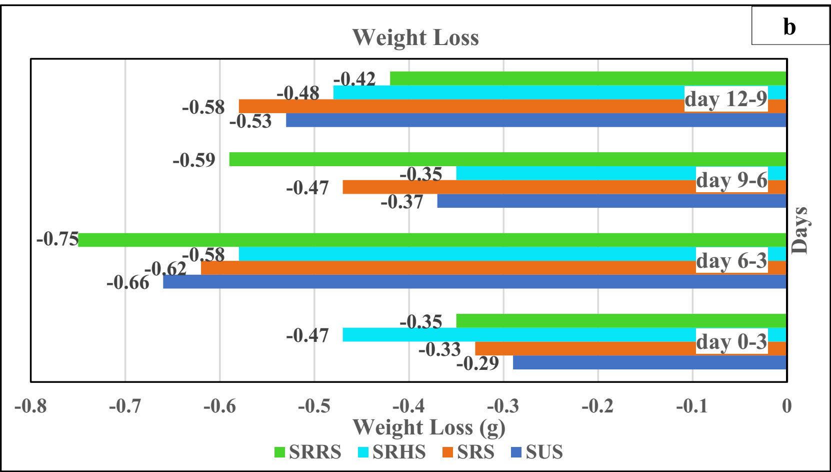


Fig. 9 **a** Soil degradation test of sorghum spoon. **b** Weight loss percentage for every 3 days to observe the degradation of sorghum spoon

The change in weight of the spoon was monitored for 120 days with an interval of 30 days and the results were depicted (Fig. 8a and b). On the 120th day, SUS increased by 0.49 g from 7.9 to 8.39 g, respectively. SRS exhibited a total increase of 0.44 g with a stable rise of 0.09 g, 0.13 g, and 0.11 g on the 30th, 60th, and 90th days from 7.09 g on day 1 and 7.53 g on the 120th day. SRHS showed that 0.50 g was increased from 7.47 to 7.97 g whereas SRRS by 0.59 g from 7.81 to 8.4 g revealed that the incorporation of flowers affects the shelf life of spoon and roasting improves the shelf life**.**

3.9 Soil degradation analysis of sorghum spoon

Soil degradation is an important parameter, as the spoon is disposed of or thrown away when not consumed, it will readily decompose in soil due to the humidity and soil microorganisms that decrease the carbon load due to single-use plastic cutlery usage. The sorghum spoon was taken out from the soil every third day and the wet pieces were dried at 70 °C for 24 h to measure the consecutive weight loss (Fig. 9a and b). 1.97 g of SUS, 2.14 g of SRS, 1.99 g of SRHS, and 2.23 g of SRRS of sorghum spoon were buried in the soil at 5 cm depth in a soil-containing glass beaker. SUS degraded 1.68 g, 1.02 g, and 0.65 g on the third, sixth, and ninth day. 1.81 g, 1.19 g, and 0.72 g of weight loss were noted in SRS. SRHS exhibited a weight loss of 1.52 g, 0.94 g, and 0.59 g and SRRS lost 1.88 g, 1.13 g, and 0.54 g every third day up to 9 days. On the twelfth day, SUS was degraded to 0.12 g and SRS, SRHS, and SRRS to 0.14 g, 0.11 g, and 0.12 g, respectively, with stable degradation loss and degradation cannot be measured further. Edible and ecofriendly sorghum spoons were degraded by the action of soil microorganisms present abundantly within 12 days and flower powder incorporation or roasting of sorghum doesn't significantly alter the percentage of degradation. Figure 9b shows that the degradation was higher during 3 to 6 days and during the initial 3 days, it was minimal. According to the results, the brewer's leftover grain bowl broke down by 30% in 10 weeks as soil microorganisms used enzymatic or metabolic processes to transform the bio-based material into carbon and energy [1]. The edible spoon sheet was decomposed within 5 to 7 days [19] and the moringa pod spoon degraded in 20 days [33].

4 Conclusion

The development of sorghum-based edible spoons enriched with hibiscus and rose powders offers an innovative, eco-friendly solution to the environmental challenges posed by plastic waste. Sorghum usage promotes the growth of drought-tolerant crops and lessens dependency on single-use plastics, improving food security and sustainability. The standardized flower powder incorporated edible spoons demonstrate strong structural integrity, with break forces between 31 and 59 N. It withstands falls up to 40 cm, making it a durable, safe alternative for daily use. Enhanced through roasting and flower incorporation, the spoons maintain functionality for 30 min in hot or cold foods and beverages while offering improved sensory appeal and nutritional value. The sorghum spoons demonstrated excellent safety and environmental performance, with microbial loads remaining within safe limits (13×10^1 cfu) after 120 days and minimal cytotoxicity (3 to 10%) in the brine shrimp lethality assay. Moreover, the spoons were fully biodegradable within 12 days, reinforcing their eco-friendly nature and suitability as a sustainable alternative to conventional cutlery. Future research should focus on scaling production, investigating the integration of additional bioactive ingredients, and optimizing shelf life and performance in diverse environments. Overall, by addressing the adverse impacts that plastic waste has on the environment and human health, sorghum spoons play a key role in the global transition towards sustainability. With further development, edible sorghum spoons have the potential to become a widely used, biodegradable substitute in the food and tourism industries.

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Author contribution Devatha Manivel: Investigation, Conceptualization, Formal analysis, Methodology, Data curation, Writing—Original draft. Raajewari Paramasivam: Supervision, Reviewing, and Validation.

Declarations

Ethics approval This study does not involve any harm to the participation of human subjects or animals conducted by any authors. The research has been conducted in a way that respects the dignity rights and welfare of all the participants.

Conflict of interest The authors declare no competing interests.

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