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OPTIMIZATION OF WHEAT AND SORGHUM SEERA (SORGWHEAT SEERA): EFFECT ON FUNCTIONAL, PHYSICOCHEMICAL AND STRUCTURAL PROPERTIES

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KEY WORDS: wheat seera, sorghum seera, physicochemical property, functional property, structural

property

ABSTRACT

While many fermented products grace the market, seera stands out as a traditional delicacy from the hilly regions of Himachal Pradesh. The microflora found in seera includes yeasts such as Saccharomyces cerevisiae and Cryptococcus laurentii, as well as bacteria such as Lactobacillus amylovorus and Bacillus spp., which are crucial due to their distinct characteristics. Our research focuses on optimizing seera made from wheat and sorghum millet to enhance its quality. We used a mixture design approach to explore the effects of three independent variables: wheat (X1, 30-90 g), sorghum (X2, 30-90 g), and temperature (X3, 25-45 °C). The key performance indicators were bulk density (Y1), water absorption capacity (Y2), and oil absorption capacity (Y3). The optimal formulation, comprising 90 g of wheat, 30 g of sorghum, and a fermentation temperature of 25 °C, achieved impressive results: a bulk density of 538 kg/m^3 , water absorption capacity of 1.605 g/g, and oil absorption capacity of 1.98 g/g. Compared to the control sample, sorgwheat seera exhibited higher protein and crude fiber content, while moisture levels were lower. Additionally, the morphology of the sorgwheat seera revealed fragmented particles, a testament to the fermentation process. This study highlights the potential of optimizing traditional fermented foods to improve their nutritional profile and functional properties.

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ОПТИМИЗАЦИЯ СИРЫ ИЗ ПШЕНИЦЫ И СОРГО: ВЛИЯНИЕ НА ФУНКЦИОНАЛЬНЫЕ, ФИЗИКО-ХИМИЧЕСКИЕ И СТРУКТУРНЫЕ СВОЙСТВА

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сира из пшеницы, сира из сорго, физикохимические свойства. функциональные свойства,

структурные свойства

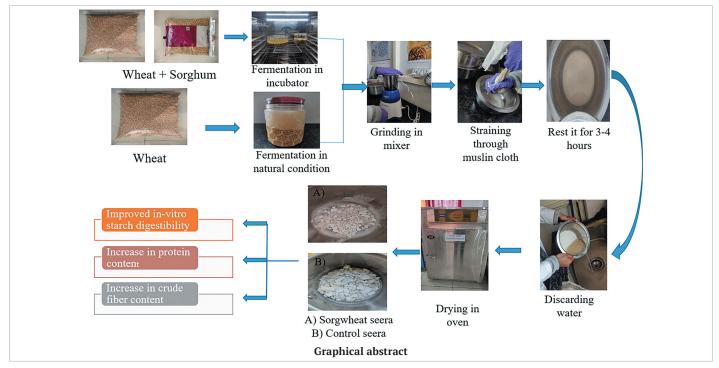
КЛЮЧЕВЫЕ СЛОВА: АННОТАЦИЯ

Хотя многие ферментированные продукты украшают рынок, сира выделяется как традиционный деликатес из высокогорных районов штата Химачал-Прадеш. Микрофлора, встречающаяся в сира, включает дрожжи, такие как Saccharomyces cerevisiae и Cryptococcus laurentii, а также бактерии, такие как Lactobacillus amylovorus и Bacillus spp., которые критически важные для её отличительных характеристик. Наше исследование сфокусировано на оптимизации сиры, изготовленной из пшеницы и сорго для повышения её качества. Мы использовали смешанную экспериментальную схему для изучения влияния трех независимых переменных: пшеница (Х1, 30-90 г), сорго (Х2, 30–90 г) и температура (X3, 25–45°C). Ключевыми показателями эффективности были объемная плотность (Y1), водопоглощающая способность (Ү2) и маслопоглощающая способность (Ү3). Оптимальная рецептура, включающая 90 г пшеницы, 30 г сорго и температуру ферментации 25°С, показала впечатляющие результаты: объемная плотность 538 кг/см³, водопоглощающая способность 1,605 г/г и маслопоглощающая способность 1.98 г/г. По сравнению с контрольным образцом сира из сорго и пшеницы имела более высокое содержание белка и сырой клетчатки, в то время как уровни влаги были ниже. Кроме того, морфология сиры из сорго и пшеницы показала фрагментированные частицы — свидетельство процесса ферментации. Данное исследование подчеркивает потенциал оптимизации традиционных ферментированных пищевых продуктов для улучшения их пищевого профиля и функциональных свойств.

БЛАГОДАРНОСТИ: Авторы выражают благодарность и искреннюю признательность Кафедре пищевых продуктов и нутрициологии, университета Бабасахеба Бхимрао Амбедкара, Лакхнау, за предоставление лабораторного комплекса для проведения экспериментов и выполнения данного исследования.

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1. Introduction

A typical fermented foodstuff found primarily in the rural and hilly regions of Himachal Pradesh is fermented wheat flour or seera. It is produced by fermenting wheat grains. Fermentation is a conventional, natural, and cost-effective bioprocessing technology that has been extensively utilized in the contemporary food business to enhance the nutritional and sensory attributes of food [1]. Fermented food provides a plethora of health advantages. In addition to its health benefits, seera also significantly contributes to enhancing flavor, nutritional content, and improving stability while reducing cooking time. In India, there are various types of fermented food made from cereals and legumes, such as bread, idli, dhokla, pinni, dosa, and papad. On the other hand, there is little information about a similar meal known as seera. Seera contains a diverse range of microflora that performs a crucial part in the process of fermentation. These include yeasts such as Saccharomyces cerevisae, Cryptococcus laurentii, and Torulospora delbrukeckii, as well as bacteria such as Lactobacillus amylovorus, Bacillus spp. and Leuconostoc spp.

Seera is often created through a spontaneous fermentation process that involves wheat grains being submerged, ground, and fermented. Water is poured over the grains for a period of 2–3 days to facilitate fermentation by the naturally occurring bacteria. After a period of 2–3 days, the grains are pulverized and the soaking process is finished to facilitate the settling of starch granules and certain nitrogenous substances, while the covering of the grain is eliminated. The solid material that settles is subsequently dehydrated by exposure to sunlight, resulting in a substance known as seera. In its fresh state, it appears to be bright and white. The way it looks is similar to semolina halva, but the texture is softer and smoother [2]. To make seera, the dried ingredients are put into water, which is then poured into hot ghee. The dried ingredients are combined with water and added to hot ghee to make seera. The right amount of sugar is then added to make a mush; it is then done [3].

Food epitomizes communal culture by conveying insights about dietary practices, preferences, consumption trends, nutritional security, community health, agricultural and animal systems, as well as socioeconomic, ethnic, and religious taboos [4]. Traditional knowledge is swiftly diminishing due to rapid urbanization, the homogenization of dietary practices, rural outmigration, deforestation, scarcity of plant resources, increased accessibility to allopathic medicine, and inadequate dissemination of information and expertise from older to younger individuals [5].

Traditional knowledge comprises information intertwined with local cultural values and ethics, facilitating the sustainable management of natural resources by indigenous populations and local organizations [6,7]. The Himalayan republics harbor thousands of varieties of fermented and ethnic dishes that are consumed. The predominant sustenance ingested by the populace in the country's remote regions comprises an array of ethnic cuisines from the Indian Himalayas, including Sepu Bari, Seera, Salori, Bhaturu, Aenkali, Childa-bhala, Gundruck, Dhulliachar, Jaanr, Angoori, Chuli, Auria, Jhol, Chhang, and Churpi. These foods have

rarely been investigated for their potential to provide novel and healthenhancing microbes [8]. Foods such as seera are incredibly healthful. In sometimes, other grains like buckwheat, millet, and barley are added to make it even healthier. Some well-known traditional fermented foods that contain probiotics are seera, bhaturu, bari, jhol, chhang, and others. These foods are not only fermented in the traditional way, but they are also useful foods. This means that they are good for people's health in the state and across the country [9]. Fermented cuisine is becoming more and more popular worldwide. In Himachal Pradesh's remote marketplaces, it is distributed in tiny packets and provides a source of income for the local community. The inhabitants derive numerous health and economic advantages from it. In addition to its nutritious benefits, the local cuisine also showcases a dynamic diversity influenced by race and geography. It is advisable for individuals with jaundice and hepatitis. Postnatal women are also advised to use it. The data demonstrates that pregnant women in Himachal Pradesh engage in food preparation, including the making of seera, in order to prevent miscarriages [10,11].

Seera is mostly made from wheat grains, but the addition of millet boosts the nutritional content of seera. Fermented meals primarily consist of a complex amalgamation of proteins, carbohydrates, lipids, and other components, which are simultaneously or sequentially altered by various microbes.

An overall decline in antinutritional variables was noted, accompanied by an increase in nutrient bioavailability and bioaccessibility, antioxidant activities, and sensory attributes of cereal-based foods and beverages [12–14].

The ancient proverb "Let food be medicine" remains relevant today, especially considering the situation after the COVID-19 pandemic. The epidemic has compelled humanity, which reassess their dietary patterns, particularly due to the heightened desire for traditional and functional foods, which offer numerous health advantages. Nevertheless, many opportunities remain untapped as a result of insufficient technological capabilities for widespread manufacturing [15].

Although there are many studies on making seera, its functional properties, effect of different drying techniques on various properties of seera, optimization of multigrain seera with added values and multigrain fermented noodles using seera, there remains a significant gap in identifying the ideal fermentation temperature and wheat-to-sorghum ratio necessary for industrial scale processing. This research focuses on determining the optimal fermentation temperature and grain ratio, and evaluating their impact on the functional, physico-chemical and structural properties of sorgwheat seera. This study seeks the enhancement of nutritional value, digestibility and bioavailability of nutrients while optimizing the necessary parameters. The research also seeks to contribute to the revival of traditional food practices, improve nutritional intake and highlight their potential of health promotion and food diversification. Moreover, this study provides insights into process standardization, support of technological advancement for commercial-scale production, and ultimately, heritage and economic opportunities.

2. Objects and methods

2.1. Materials

Wheat grains (*Triticum aestivum* L.) were purchased from Lulu hypermarket on Shaheed path road Lucknow. Sorghum grains (*Sorghum bicolor* L. Moench) were purchased from online big basket application.

2.2. Control and sorgwheat seera preparation

The control seera was made using the technique described by [2]. In the traditional method, wheat grains were first soaked with water in the ratio of 1:2 at room temperature for 2–3 days to allow natural fermentation of grains. Following fermentation, grains were milled and steeped to enable the sedimentation of starch granules and some proteins, after which the bran was eliminated. Then the starch solids were sun dried, and the final product was called control seera. In sorgwheat seera, wheat and sorghum grains (according to the Central Composite Design formulations) were soaked with tap water at a ratio of 1:2 in an incubator for 48 hours. After soaking for 12 hours, the water was discarded (to avoid unintended fermentation) and fresh water was added right away. After fermentation, grains were ground in a mixture grinder, and wheat and sorghum bran were separated using muslin fabric. After extraction of starch, steeping was used to give the kernels of starch and some proteins sufficient time to set. Later on, water was decanted from the mixture. After that, settled solids were oven dried at 60 °C for 6-8 hours until a constant moisture was obtained.

2.3. Experimental design of sorgwheat seera

Response Surface Methodology software was implemented to optimize the wheat and sorghum seera. In this study, the experiments were performed using a quadratic model in conjunction with the central composite design. The three independent variables were wheat (X1), sorghum (X2,) and temperature (X3). The dependent variables were bulk density (Y1), water absorption capacity (Y2) and oil absorption capacity (Y3). Seera was prepared by using 20 experimental formulations with three independent variables. Table 1 lists the CCD levels along with their coded values.

To find the ideal quantity of components, the results of responses from the quadratic model were analyzed. All responses were fitted using a polynomial equation of second order and this equation is:

$$Yi = B_{i1}X_1 + B_{i2}X_2 + B_{i3}X_3 + B_{i12}X_1X_2 + B_{i13}X_1X_3 + B_{i23}X_2X_3 + B_{i123}X_1X_2X_3 + \text{Error}$$
 (1

where, Y_1 = attributes, X_1 = whole wheat, X_2 = whole sorghum, X_3 = temperature; B_1 , B_2 , B_3 = linear coefficients; B_{12} , B_{13} , B_{23} = quadratic coefficients; B_{123} = cubic coefficient.

Table 1. Levels of independent variables for experimental design Таблица 1. Уровни независимых переменных для схемы эксперимента

Symbol	Independent variables	Low level	Mid-level	High level
A	Whole wheat (g)	10	60	110
В	Whole sorghum (g)	10	60	110
С	Temperature (°C)	18	35	52

2.3.1. Bulk density

Bulk density technique has been described by [2]. Ten milliliter graduated polycarbonate cylinders were carefully filled with the samples (control and optimized seera). The bottoms of the cylinders were slightly shaken several times on a lab table coated in foam until the sample level stopped dropping. The weight of the samples was computed. The weight of the samples divided by their volume (kg/m³) was used to compute the bulk density. The weight of the samples divided by their volume after tapping (kg/m³) was used to compute the tapped bulk density.

2.3.2. Water absorption capacity

Water absorption capacity technique has been described by [2]. In pre-weighed centrifuge tubes, 12.5 mL of distilled water was mixed with 1.5 g of each sample (seera), calculated on the basis of dry mass. After 30 minutes of ambient temperature stirring, dispersions were rotated for 15 minutes at 3000 rpm using a Sigma 3–18KS centrifuge (Sigma Laboezentrifugen, Germany). After draining the surplus moisture from the centrifuge tubes with the samples and decanting the supernatants, the tubes were weighed again. Grams (g) of water retained per grams (g) of seera were used to indicate the weight increase.

2.3.3 Oil absorption capacity-

This method of oil absorption capacity has been described by [2]. In centrifuge tubes that had been previously weighed, six milliliters of mustard oil and one gram of each sample (seera) were mixed. To distribute the sample throughout the oil, the contents were vortexed for one minute. After being stored vertically for 30 minutes, samples were rotated for 15 minutes at 3000 rpm (Sigma 3–18KS centrifuge, Sigma Laboezentri-

fugen, Germany). Pipettes were used to remove the oil coating, and the tubes were left upside-down for ten minutes to allow the oil to drain before being weighed again. The weight (wt.) increase was stated as grams of absorbed oil per grams of seera.

2.4. pH

The method of pH was described by [16]. After standardizing at 25 °C using pH 4.0 and 7.0 buffers, samples were equally combined with purified water in a 1:1 ratio (w/v). The pH of the samples was then measured using a digital pH meter (MAC-1600 pH Meter, DKK-TOA Corporation, Japan).

2.5. Color

A colorimeter (Chromameter, CR-400, Konica Minolta Optics, Japan) was used to assess the color of the seera samples using the L*, a*, and b* readings. The L* value, which has a range of 0 to 100, denotes brightness. The a* value indicates the level of the reddish-green color. A larger positive a* value shows more red. A degree of the yellow-blue color is represented by the b* value. Higher positive b* values indicate more yellow [2].

2.6. Proximate analysis

Moisture, ash, fat, protein, crude fiber and carbohydrate content were determined as per the AOAC standards.

2.7. Texture analyzer

The texture profile was determined by using a TA HD plus Texture Analyzer (Stable Micro Systems, United Kingdom) with a 5 kg load cell. A test using two-bit compression was employed. A 10-millimeter cube was prepared. The samples were crushed using a cylinder-shaped plunge (P/5, dia = 5 mm) at a rate of 0.5 mm/s up to a distance of 10.0 mm. A pair of biting force—time curves was generated, facilitating the measurement of all textural attributes [17].

2.8. Structural properties

2.8.1. Fourier Transform Infrared Spectroscopy (FTIR)

Using an FTIR spectrophotometer Nicolet 6700 (Thermo Scientific, USA), the Fourier transform infrared (FTIR) spectrum of flour was obtained at room temperature. After mixing the samples with potassium bromide powder, the mixture was compressed into tablets. Potassium bromide was used as a blank during calibration, and the collected spectrum fell between 600 and 4,000 cm⁻¹ [18,19].

2.8.2. X-Ray Diffraction (XRD)

Examination of the XRD patterns of the sorgwheat and control seera was done with an XRD (D8 Advance Eco, Bruker, Germany). The angle of scattering (20) was varied at a rate of 0.02° per angle between 5–60°C. The operational current and voltage was adjusted to 35 mA - 40 kV during the test, respectively [20]. Using the amorphous area and crystalline area (AC) calculus, equation (2) was utilized to determine the crystallinity index (Ci) of the samples.

$$C_i = \frac{A_c}{A_c + A_a} \times 100. \tag{2}$$

2.8.3. Scanning Electron Microscope (SEM)

Morphological outer layer characteristics for the samples were analyzed using a scanning electron microscope (SEM) model (JSM 6490 LV, JEOL, Japan). Multiple magnifications were used to examine the sample, including $\times 550,\,\times 1000,\,$ and $\times 2000.$ Before scanning, a small coating of platinum was applied to the control and sorgwheat seera samples to produce electricity. A distance of 7.4 mm was chosen as the working distance, and 15 kV was chosen as the acceleration voltage. The SEM apparatus was operated at a current of 58 μA [18].

2.9. Antimicrobial activity

2.9.1. Isolation of lactic acid bacteria

Lactic acid bacteria (LAB) were isolated using the serial dilution agar method. An initial dilution of 1:10 was prepared by dissolving 1 g of each sample in 9 ml of sterile distilled $\rm H_2O$ and vortexed thoroughly. The subsequent dilutions of 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , and 10^{-6} were made in succession by transferring 1 ml from the previous tubes to the next tubes with 9 ml of water.

After seeding MRS agar plates and plates with nutrient agar with 0.1 ml of each dilution, they were left to incubate at 37 °C for 24 hours. Plate counts were determined on MRS agar for lactic acid bacteria and on nutrient agar for total aerobic mesophilic bacteria and presumptive *Bacillus* spp. (identified and enumerated based solely on macroscopic appearance in colonies); dedicated yeast isolation on selective yeast media (e. g., SDA/YPD with chloramphenicol) was not performed as part of the culture-dependent assays in this study. No obvious yeast colonies were

observed on the media used, and therefore yeasts are not reported among the predominant microflora.

2.9.2. Strains

The test strains of *Staphylococcus aureus* and *Escherichia coli* were taken from the culture collection of the Department of Microbiology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh (India).

2.9.3. Preparation of the supernatant of bacterial isolates

The strains were individually inoculated in sterile MRS broth at an initial bacterial concentration of 10^6 CFU/ml. Following incubation at $37\pm1\,^\circ\text{C}$ for 24 and 48 hours, the pH of each sample was determined in triplicate using a Jenway 3510 pH meter (Barloworld Scientific Ltd., Staffordshire, UK) before extraction. After specific incubation periods of 24 and 48 hours, the supernatants of each strain were obtained using centrifugation at 6000 rpm for 20 minutes at 4 °C and then sterilized using 0.22-µm pore size filters (MF-Millipore®, Merck KGaA, Darmstadt, Germany). All supernatants were preserved at $-20\,^\circ\text{C}$ until utilized [21].

2.9.4. Screening of selected isolates for antimicrobial activity

Isolates were inoculated into MRS broth and allowed to incubate for 24 to 48 hours at 37 °C. Following incubation, the antibacterial activity was tested using the agar well diffusion technique as per Goa et al. [22]. *Escherichia coli* and *Staphylococcus aureus* were tested against the supernatant of bacterial isolates.

2.9.5. Determination of inhibitory activity of LAB and Bacillus isolates

The inhibitory potential of lactic acid bacteria (LAB) and *Bacillus* spp. was evaluated using the well diffusion assay. The test pathogen was grown in nutrient broth to the logarithmic phase and then spread uniformly on the surface of nutrient agar plates using a sterile cotton swab. Wells of 6 mm diameter were aseptically punched into the agar using a sterile cork borer. Cell-free culture supernatants of LAB and *Bacillus* isolates were prepared by centrifugation at 10,000 rpm for 10 min, followed by filtration through a 0.22 µm membrane filter to remove residual cells.

Aliquots (100 μ L) of these sterile supernatants were added into the wells, while sterile broth served as the negative control and the antibiotic as the positive control (vancomycin and ampicillin) for *Staphylococcus aureus* and *Escherichia coli*, respectively. Plates were incubated at 37 °C for 24 h, and antimicrobial activity was assessed by measuring the diameter of the clear inhibition zones around the wells.

2.10. Statistical analysis

For optimization and experimental design, central composite design of Design-Expert (version 13) was applied. The one-way ANOVA test was performed in the SPSS program, and Duncan's multiple range test was employed to ascertain significance of each term.

3. Result and discussion

The state of Himachal Pradesh has a vast basket of traditional foods of its own. People of the state have been following these methods of preparation from their ancestors and these dishes continue to be a tradition in most parts of Himachal. Some of the traditional foods still form a staple diet while the rest are prepared during special occasions. The food patterns of the state are highly influenced by the availability of raw materials as well as geographical and climatic conditions. The traditional fermented items prepared by the local people are generally associated either with festivals or with the seasons.

3.1. Optimization of seera

RSM software was used to optimize an effect of the independent variables of seera on the responses such as bulk density, water absorption capacity and oil absorption capacity. The experimental results of all combinations of mixture design are presented in Table 2. In addition, ANOVA test was performed to assess the model suitability. All responses were statistically significant and are shown in Table 3. As a result, the second order regression equation appropriately described the effect of independent variables on the responses of the sorgwheat seera. A linear and interactive effect of each independent variable on responses was studied using the linear and 3D response surface plots shown in Figure 1.

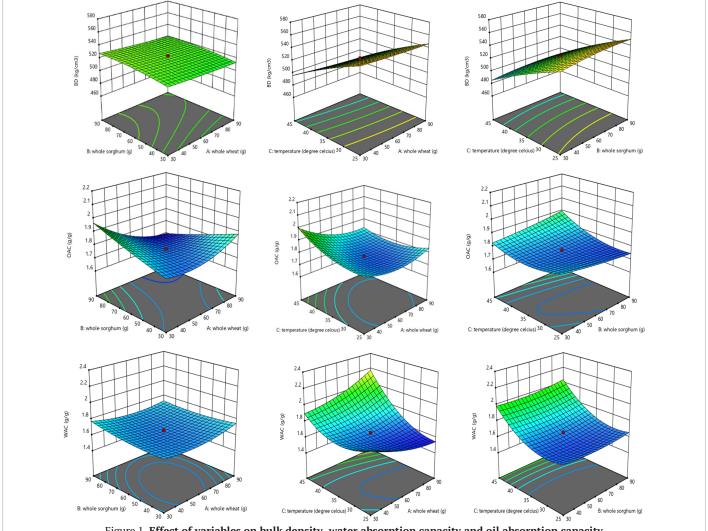


Figure 1. Effect of variables on bulk density, water absorption capacity and oil absorption capacity
Рисунок 1. Влияние переменных на объемную плотность, водопоглощающую способность и маслопоглощающую способность

Table 2. Central Composite Design for variables: A (whole wheat), B (whole sorghum) and C (temperature) and their responses Y1 (bulk density (kg/m³), Y2 (water absorption capacity (g/g)) and Y3 (oil absorption capacity (g/g))

Таблица 2. Центральный композиционный план для переменных: А (цельная пшеница), В (цельное сорго) и С (температура) и их отклики Y1 (объемная плотность (kg/м³)), Y2 (водопоглощающая способность (г/г)) и Y3 (маслопоглощающая способность (г/г))

	(г/г)) I	и Ұ3 (маслопо	глощающая	способнос	ть (г/г))	
Run	Whole wheat, g	Whole sorghum, g	Tempera- ture, °C	Y1, (kg/m ³)	Y2, g/g	Y3, g/g
1	90	30	45	482	2.21	1.899
2	60	60	35	522	1.651	1.764
3	60	60	35	524	1.657	1.764
4	30	45	45	490	1.88	1.88
5	60	60	18	566	1.786	1.841
6	30	90	25	552	1.869	1.9
7	30	30	25	541	1.82	1.79
8	110	60	35	520	1.8	1.856
9	10	60	35	532	1.802	1.998
10	60	60	35	523	1.652	1.764
11	60	110	35	524	1.65	1.76
12	30	90	45	496	1.978	2.14
13	60	60	35	519	1.649	1.77
14	90	30	25	538	1.605	1.98
15	60	10	35	510	1.701	1.782
16	90	90	45	487	2.12	1.78
17	60	60	52	465	2.389	1.976
18	60	60	35	521	1.659	1.764
19	90	90	25	547	1.55	1.72
20	60	110	35	522	1.766	1.78

Table 3. Results of regression coefficient and analysis of variance for an effect of a variable on bulk density, water absorption capacity and oil absorption capacity

Таблица 3. Результаты коэффициента регрессии и дисперсионного анализа для эффекта переменной на объемную плотность, водопоглощающую способность и маслопоглощающую способность

Source	BD	WAC	OAC
Model Intercept	522.25	1.65	1.76
A	-3.27	0.0019	-0.0419
В	3.76	0.0150	-0.0009
С	-28.64	0.1785	0.0391
AB	-0.3750	-0.0251	0.0936
AC	-1.13	0.1374	-0.0439
BC	-1.13	0.0132	0.0304
A2	0.7964	0.0544	0.0602
B2	-2.73	0.0301	0.0076
C2	-2.77	0.1514	0.0514
p-value	< 0.0001	< 0.0001	< 0.0001
F-value	275.88	2459.39	1022.12
Lack of fit	0.3693	0.0672	0.0877
Std. dev.	2.19	0.0066	0.0049
Mean	519.03	1.81	1.85
\mathbb{R}^2	0.9960	0.9995	0.9989
Adj. R ²	0.9924	0.9991	0.9931

3.1.1. Effect of independent variables on bulk density

The bulk density of optimized seera ranged from 465 to 566 cm³, subjected to the level of independent variables. The maximum bulk density was observed for run 17 (60 g whole wheat, 60 g whole sorghum and a temperature of 52 °C and minimum for run 5 (60 g whole wheat, 60 g whole sorghum and 18 °C temperature). The quadratic model was established for bulk density. It was found that the independent variables (whole wheat, whole sorghum and temperature) had a significant impact on the multigrain seera flour (P < 0.05, R^2 0.99).

The optimized bulk density of optimized seera in run 14 (90 g whole wheat, 30 g whole sorghum and a temperature of $25\,^{\circ}$ C) was found to be 538 cm³. The major impact on the bulk density is due to the temperature factor because at the lowest temperature there is less breakdown of starch particles during fermentation.

3.1.2. Effect of independent variables on water absorption capacity

The water absorption capacity of optimized seera ranged from 1.55 to 2.389 g/g, subjected to the level of independent variables. The maximum water absorption capacity was observed for run 17 (60 g whole wheat, 60 g whole sorghum and a temperature of 52 °C and minimum for run 19 (90 g whole wheat, 90 g whole sorghum and a temperature of 25 °C). The quadratic model was established for bulk density. It was found that the independent variables (whole wheat, whole sorghum and temperature) had a significant impact on the multigrain seera flour (P < 0.05, R^2 0.99).

The optimized water absorption capacity of optimized seera in run 14 (90 g whole wheat, 30 g whole sorghum and a temperature of $25\,^{\circ}$ C) was found to be $1.605\,\text{g/g}$.

3.1.3. Effect of independent variables on oil absorption capacity

The oil absorption capacity of optimized seera ranged from 1.72 to 2.14 g/g, subjected to the level of independent variables. The maximum oil absorption capacity was observed for run 12 (30 g whole wheat, 90 g whole sorghum and a temperature of 45 °C) and minimum for run 19 (90 g whole wheat, 90 g whole sorghum and a temperature of 25 °C). The quadratic model was established for bulk density. It was found that the independent variables (whole wheat, whole sorghum, and temperature) had a significant impact on the multigrain seera flour (P < 0.05, $R^2 = 0.99$).

The optimized oil absorption capacity of optimized seera in run 14 (90 g whole wheat, 30 g whole sorghum and a temperature of 25 °C) was found to be 1.98 g/g.

3.1.4. Optimized values of independent variables

Design-Expert Software (version 13) was used to determine the numerical optimization. The responses used were bulk density, water absorption capacity and oil absorption capacity. Numerical optimization recommended the maximum acceptability (0.877), which can be achieved by preparing of sorgwheat seera with whole wheat (90 g), whole sorghum (30 g) and temperature (25 °C), respectively. Bulk density, oil absorption capacity, and water absorption capacity were all optimum amounts of the projected values responses, respectively. Validation of the model was done by conducting experiments at an optimum level of independent variables: whole wheat (90 g), whole sorghum (30 g) and temperature (25 °C). The experimental data showed comparability with the control seera. The optimum levels of the concentration of whole wheat and whole sorghum, and temperature were 90 g, 30g, m and 25 °C, respectively.

3.2. Water and oil absorption capacity

Water absorption capacity (WAC) in sorgwheat seera and control seera was found to be 1.688 (g/g) and 1.639 (g/g), respectively. The increase in WAC could be attributed to the low moisture content in the samples and formation of simple sugars, such as maltose, dextrin, glucose and so on, having higher affinity for water during fermentation [23,24].

Oil absorption capacity (OAC) of sorgwheat seera and control seera was found to be 1.941 (g/g) and 1.838 (g/g), respectively. As the native seera contains high amounts of starch and does not have polar groups, oil absorption merely depends upon the physical entrapment, size and shape of the starch [24,25,26]. The increase in OAC in sorgwheat seera can also be connected with the less deformed structure of starch and amino acids present in the final composition.

3.3. pH

The pH of sorgwheat seera and control seera was 3.34 and 3.25, respectively. The pH of the samples decreased and showed the acidic nature. The low pH supports the growth of various yeasts and lactic acid bacteria important in food fermentation [27]. The pH value observed in earlier research by Savitri et al. [16] was 3.45, which corresponds closely to the present study.

3.4. Proximate analysis

The proximate analysis of sorgwheat seera and control seera is represented in Table 4. Optimized sorgwheat seera depicted higher amount of protein (7.59%), crude fiber (1.52) and fat (3.77) as compared to the control seera, which contained 6.27% of protein, 1.04% of crude fiber and 0.25% of fat. The results are in agreement with the previous study by Ahmad et al. [17]. Mainly, the protein content, crude fiber and fat content were higher as compared to the control seera. Jeyakumar et al. [28] reported that fermentation improves protein digestibility by diminishing anti-nutritional agents and liberating free amino acids, without

necessarily elevating the total protein concentration. The increase in protein and crude fiber is due to the addition of sorghum in sorgwheat seera [29]. The moisture content in the sorgwheat and control seera was 8.99 and 11.10%, respectively. The decrease in the moisture content of sorgwheat seera is due to the drying method used as compared to the control seera. Similar result was also shown by Ahmad et al. [2].

Table 4. The proximate analysis of sorgwheat seera and control seera

Таблица 4. **Приближённый анализ сиры из пшеницы** и сорго и контрольной сиры

		_
Parameters	Control	Sorgwheat seera
Carbohydrate, %	78.46 ± 0.12^{a}	79.31 ± 0.20^{b}
Fat, %	0.25 ± 0.02^{b}	3.77 ± 0.20^a
Protein, %	6.27 ± 0.02^{b}	7.58 ± 0.02^{a}
Ash, %	0.41 ± 0.01^{b}	0.35 ± 0.01^a
Moisture, %	11.36±0.01a	8.96±0.02a
Crude fiber, %	1.04±0.01 ^b	1.53±0.02a

Values expressed as mean \pm S. D. Mean in rows with different superscript a, b, c is significant (p < 0.05).

3.5. Texture analyzer

The hardness of the control seera and sorgwheat seera was found to be 450 g (4.41N) and 890 g (8.73 N), respectively. Gumminess is often measured for semi-solid meals; hence, we have chosen to evaluate this characteristic instead of chewiness, which is more applicable to solid foods. The gumminess value for the sorgwheat seera was determined to be 105.50 ± 3.1 gf. The springiness (elasticity) of sorgwheat seera was determined to be 0.275, which is much lower than that of the control seera. The reduction in springiness can be considered beneficial, as food with reduced springiness will necessitate less energy for chewing [30]. The adhesive property of the sorgwheat seera was also observed to be lower in comparison to the control seera [26].

3.6. Structural properties

3.6.1. Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR of the control seera and sorgwheat seera is shown in Figure 2. A broad peak was observed at 3406 cm⁻¹ and 3400 cm⁻¹ for the control seera and sorgwheat seera, respectively. That might be O-H stretch vibrations. A small peak at 2905 cm⁻¹ and 2900 cm⁻¹ for both of the samples can be attributed to C-H bond stretching associated with the ring methane hydrogen atoms. The presence of carbohydrates can be confirmed from the fingerprint region of 1200–800 cm⁻¹. The protein region could be confirmed by the sharp peak at 1652 cm⁻¹ (amide 1), attributed to N-H amide bond stretching. This may be due to the addition of sorghum in the sorgwheat seera. In the control seera the peak was noticed at 1655 cm⁻¹. A small peak at 1302 cm⁻¹ and 1300 cm⁻¹ was ascribed to O-C-H, C-C-H and C-O-H bending vibrations. The peak at 1150 cm⁻¹ indicated the C-O

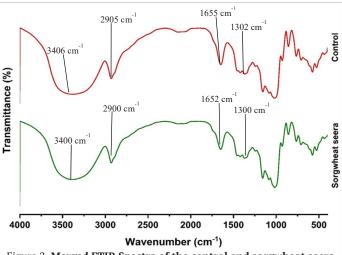


Figure 2. Merged FTIR Spectra of the control and sorgwheat seera Рисунок 2. Совмещенные спектры FTIR контрольной сиры и сиры из пшеницы и сорго

and C-O-H vibrational modes [26,31,32]. The band at 997 cm⁻¹ represented the anhydrous C=O stretch of glucose ring and this region reflects the secondary structure of proteins [33]. The crucial band observed at 860 cm⁻¹ and minor peaks at 760 and 670 cm⁻¹ are known as the fingerprint region and ascribed to skeletal modes of pyranose ring of glucose units (starch).

3.6.2. X-ray Diffraction (XRD)

X-ray Diffraction (XRD) patterns of the control seera and sorgwheat seera are given in Figure 3. In both samples, the sharp peak identified at

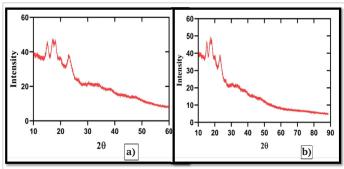


Figure 3. X-ray diffraction of a) Control seera b) Sorgwheat seera Рисунок 3. Рентгеновская дифракция: а) контрольная сира b) сира из пшеницы и сорго

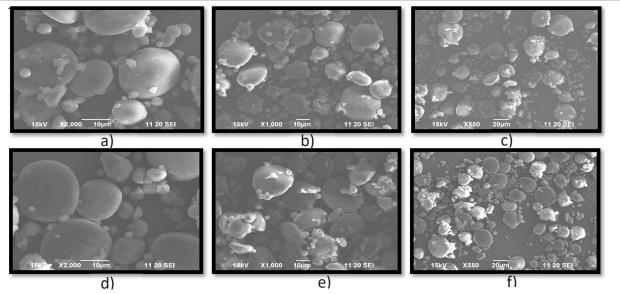


Figure 4. Scanning Electron Microscopy of i) Control seera at a) X2000, b) X1000 c) X550 and ii) sorgwheat seera d) X2000, e) X1000 f) X550 Pисунок 4. Сканирующая электронная микроскопия i) контрольная сира при a) X2000, b) X1000 c) X550 и ii) сира из пшеницы и сорго Sorgwheat seera d) X2000, e) X1000 f) X550

15° and 23° was observed. In addition, two more peaks were observed at 17° and 18°. The degree of crystallinity of the control seera and sorgwheat seera was found to be 33% and 25%, indicating that the amorphous region has improved. Furthermore, adding sorghum in the sorgwheat seera influences the crystallinity pattern of seera [31,34].

3.6.3. Scanning Electron Microscopy (SEM)

SEM analysis of the control seera and sorgwheat seera as is shown in Figure 4. SEM analysis reveals the structural orientation of macromolecules. The starch granules of seera were broken, and the fermentation phenomenon leads to superficial corrosion [17]. Zhao et al. [35] also reported small cracks and spots in fermented wheat starch. However, the crack size increased with an increase in the fermentation period. Starch granules of various sizes were visible on the sorgwheat seera and surface become rough and loosely attached to protein. It could be attributed to the breakdown of the starch protein network, which will eventually reduce hardness and accelerate water penetration. Fermentation improved the surface structure, which helped in food product development by improving its ability to rehydrate and absorb water [36].

3.7. Antimicrobial activity

The microbial consortium in the developed seera was predominantly composed of lactic acid bacteria and presumptive *Bacillus* spp. The number of colonies that grew on the plates (Figure 5) was counted and reported as CFU $\rm g^{-1}$ of the specimen. Total CFU count of all bacteria responsible to bring about characteristic fermentation in the samples ranged between 52×10^6 and 67×10^6 CFU/g as shown in Table 5.

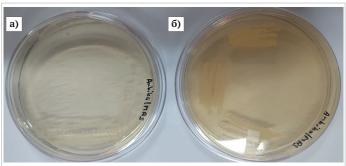


Figure 5. **Bacteria isolated from seera:** a) *LAB* b) Presumptive *Bacillus* spp.

Рисунок 5. **Бактерии**, **выделенные из сиры**: а) МКБ b) Предполагаемые *Bacillus* spp.

LAB/Presumptive *Bacillus* spp. was found to be predominant microflora in developed seera. Many LAB and presumptive *Bacillus* spp. were found to inhibit foodborne pathogenic bacteria, and the antagonistic potential of these microbes is of special interest to suppress the growth of many foodborne pathogens. The inhibitory action of these lactic acid bacteria and presumptive *Bacilli* spp. was explored.

Table 5. Microbial profile of sorgwheat seera

Таблица 5. **Микробиологический профиль сиры из пшеницы и сорго**

Samples	Total Count (CFU/g)	Predominant microorganism
Control	$52\!\times\!10^{6a}$	Lactobacillus fermentum, Lactobacillus spp. F1.
Sorgwheat seera	67×10 ^{6b}	Lactobacillus fermentum, Lactobacillus spp. F1. and presumptive Bacillus spp.

Data are means ± standard deviation of six replicates. All data were calculated on a dry weight basis.

a, b: Values in the same column with different superscripts are significantly different (P < 0.05) according to one-way ANOVA followed by Tukey's test.

3.7.1 Inhibitory activity of LAB and Bacillus isolates

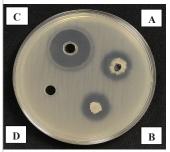
The antibacterial efficacy of LAB and presumptive *Bacillus* isolates was confirmed by the well diffusion assay. The wells that contained the cell-free culture supernatants of the isolates showed distinct zones of inhibition, whereas the negative control wells showed no inhibition (Figure 6). In comparison to presumptive *Bacillus* species, LAB had relatively greater zones of inhibition among the tested isolates, suggesting stronger antagonistic activity. The results validate that both bacterial groups can generate extracellular inhibitory compounds that impede the growth of

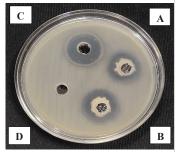
the test pathogen. The supernatant of presumptive *Bacillus* species inhibited *S. aureus* and *E. coli* with zones of 21 mm and 22 mm, respectively, as indicated in Table 6. In contrast, the supernatant of LAB species showed somewhat greater inhibition zones, especially against *E. coli* (24 mm) and *S. aureus* (21.5 mm). The positive control exhibited the largest inhibition zones (28 mm for *S. aureus* and 24.5 mm for *E. coli*), thereby confirming the experiment, while the negative control showed no inhibition. The results indicate that LAB isolates demonstrate more potent antagonistic effects than presumptive *Bacillus* spp., with both significantly enhancing the antibacterial potential of the produced seera.

Table 6. Inhibition zones against Staphylococcus aureus and Escherichia coli

Таблица 6. Зоны ингибирования Staphylococcus aureus и Escherichia coli

Sample	I donala.	Zone of inhibition (mm)		
	Identity	Staphylococcus aureus	Escherichia coli	
A	Presumptive <i>Bacillus</i> spp. supernatant	21	22	
В	LAB spp. supernatant	21.5	24	
С	Positive control	28	24.5	
D	Negative control	_	_	





Staphylococcus aureus

Escherichia coli

Figure 6. Well diffusion assay showing inhibitory activity of LAB and *Bacillus* isolates against the test organisms Рисунок 6. Анализ методом диффузии в агар, показывающий

Рисунок 6. Анализ методом диффузии в агар, показывающий ингибирующую активность изолятов МКБ и *Bacillus* против тест-микроорганизмов

4. Conclusion

Sorgwheat seera, which has various health benefits to tackle diseases, was optimized using the statistical tool RSM. The mixture design was used for the experiments. A total of 20 experiments were carried out. After these experiments were analyzed, the most desirable formulation was predicted by software, which was analyzed to characterize and compare sorgwheat seera with the control sample. The responses were bulk density, water absorption capacity and oil absorption capacity, which were found to be somewhat higher in sorgwheat seera than in the control sample. Sorgwheat seera had higher protein, crude fiber and fat content and was lower in moisture than the control sample. The lower pH of sorgwheat seera indicates the development of an acidic environment, which is characteristic of spontaneous cereal fermentations. Such an acidic condition is well-known to favor the growth and metabolic activity of lactic acid bacteria (LAB) and presumptive *Bacillus* species, both of which play a crucial role in cereal-based food fermentation.

In the present study, the isolation of *Lactobacillus* spp. and presumptive *Bacillus* spp. further validated the role of beneficial microflora, while antimicrobial assays confirmed their ability to produce extracellular inhibitory metabolites, thereby suppressing foodborne pathogens such as *Staphylococcus aureus* and *Escherichia coli*. Our findings reinforce the value of traditional, microbiome-driven processing in augmenting the nutritional benefits of cereal foods. While the Western World has the financial means to enhance its food with artificial vitamins, the poor world must depend on natural richness for its vitamins and critical amino acids. Seera is physiologically fortified with vitamins and amino acids through fermentation, making it a highly nutritious source of food for those who consume it. As seera is a traditional food product and is also limited to a particular region, this work may suggest a way forward to produce sorgwheat seera at commercial level with optimized parameters.

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