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Optimization of process parameters for development of instant pearl millet *raab* mix

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Abstract
Pearl millet (*Pennisetum glaucum*), a staple cereal renowned for its nutritional density and resilience, remains underutilized in the modern food industry primarily due to its susceptibility to enzymatic degradation. This research aimed to address these challenges by developing a shelf-stable, instant *raab* mix from pearl millet through the optimization of ingredients with key processing parameters. The study employed Response Surface Methodology (RSM) with a Box-Behnken Design (BBD) to investigate the effects of three independent variables *ghee* proportion, roasting temperature and roasting time on critical product quality attributes, including Free Fatty Acid (FFA) content, lipase activity, peroxidase activity and moisture content. The results demonstrated a highly significant negative effect of roasting temperature and time on all measured parameters, indicating effective thermal inactivation of enzymes and moisture reduction. The model successfully identified a synergistic relationship between ghee proportion, roasting temperature and roasting time, which collectively minimized enzymatic activity and lipid hydrolysis. The statistical models for all responses were found to be highly significant and predictive, with coefficient of determination (R^2) values ranging from 0.9256 to 0.9943. The study's most significant finding was the successful validation of an optimized set of processing conditions 12.19% *ghee* proportion, 186.92 °C roasting temperature and 15 min roasting time yielded a product with minimal quality degradation.

Keywords: Pearl millet, Instant, *raab* mix, Optimization, Response surface methodology (RSM).

1. Introduction
Pearl millet, in particular, is a nutritionally dense food source. According to nutritional data, it provides a high amount of energy, roughly 361 kcal per 100g, a value comparable to brown rice and higher than sorghum, primarily because of its greater lipid content (Gopalan *et al.*, 2004; Nambiar *et al.*, 2011) [12].
Beyond energy, pearl millet is rich in essential macronutrients and micronutrients. A 100g serving contains 11.6 g of protein and 1.2 g of dietary fiber, which are crucial for a balanced diet. It is also an excellent source of essential minerals, including magnesium (137 mg), iron (8.0 mg) and zinc (3.1 mg) (Gopalan *et al.*, 2004) [12]. This rich composition makes pearl millet a valuable food for addressing nutrient deficiencies, particularly in regions where it is a staple.
This nutritional superiority underpins the importance of developing new, convenient products from pearl millet, such as an instant *raab* mix. By leveraging its inherent health benefits, such products can enhance food security and promote better health outcomes for a broader consumer base.
Despite its significant nutritional value and agronomic importance, pearl millet remains underutilized in the modern food industry. Its limited acceptance is primarily due to challenges related to its poor shelf life and the non-ideal quality of dough produced from its flour (Goswami *et al.*, 2023) [13]. These limitations hinder its widespread adoption and processing into value-added products, despite growing consumer demand for functional and health-conscious foods.
The market for ready-to-cook products in India has shown significant growth, driven by changing consumer lifestyles and the demand for convenience (Anon., 2024c). This trend presents an opportunity to develop convenient, shelf-stable and nutritious food products from millets. Pearl millet *raab*, a traditional beverage known for its soothing and digestive properties, is a promising candidate for such a product. By converting pearl millet into an

instant *raab* mix, its practical utility can be enhanced, offering a quick and healthy option for busy individuals while simultaneously adding value to the raw crop. The development of such a product can bridge the gap between traditional food practices and modern consumer needs, leveraging the inherent nutritional benefits of pearl millet.

2. Materials and Methods

2.1 Raw Materials

Pearl millet grains, *ghee* and other ingredients for experiment were procured from local wholesale market of *Danapith*, Junagadh.

2.2 Roasting Treatment

Preliminary filler trials were conducted to support the initial development and standardization of the pearl millet *raab* mix recipe and its characteristics, with a focus on determining appropriate ranges for *ghee* and flour proportions to establish consistent mixing and roasting procedures.

The effect of three independent variables namely *ghee* proportion (X_1), temperature of roasting (X_2) and time of roasting (X_3) optimized based on seventeen runs of roasting process. The experiment conducted to optimize the independent parameters of roasting operation on mixture of pearl millet flour and *ghee*.

The dried grains were then milled into fine flour using a 180 µm mesh sieve to achieve uniform particle size. A fixed proportion of *ghee* (5%, 10% and 15%) was added to the flour and thoroughly mixed using a flour mixer (Mangal Bakery Machineries). The blend was roasted in a baking oven (BABA Ramdev Engineering) at 170 °C, 180 °C and 190 °C for 9, 12 and 15 min, roasting temperature and roasting time respectively, ensuring consistent heat application. After roasting, the mix was cooled to ambient temperature and pearl millet *raab* mix was subjected to chemical analysis for optimization.

2.3 Chemical Analysis

The dependent parameters such as enzyme activity, biochemical and proximate parameters were analysed. Free Fatty Acid (FFA) of pearl millet flour was determined by titrating it against potassium hydroxide (KOH) in the presence of phenolphthalein indicator and Deeth *et al.* (1975) [11] suggested the method was used with slight modifications. Lipase activity is determined by measuring the amount of fatty acid released over time and Baron (1979) suggested the method. The peroxidase assay is a method used to measure the activity of the enzyme peroxidase and Guibault (1976) [14] suggested the method. Moisture content was determined using the standard oven drying method, as outlined by AOAC (2012a) and Yadav *et al.* (2025) [37].

2.4 Statistical Analysis

Optimization was done using Response Surface Methodology (RSM), Box Benken Design (BBD) for designing the experiment. A Box Benken Design (BBD) of three variables at three levels each with five centre point combinations used to optimize the roasting operation of pearl millet flour using Design Expert (version 13.0) (Khuri and Cornell, 1987).

2.5 Validity Test

By carrying out the experiment in triplicate, the ideal condition discovered by statistical analysis was confirmed. The mean experimental value of various response factors were employed to assess the reliability and suitability of the anticipated models.

3. Results and Discussion

3.1 Effect of Roasting Treatment on Free Fatty Acid (FFA)

Table 1 presents the free fatty acid (FFA) values in pearl millet raab mix prepared under varying ghee proportions, roasting temperatures, and roasting times. FFA is a critical marker of lipid hydrolysis and oxidative deterioration, influencing shelf life, nutritional quality, and sensory acceptance of fat-rich foods. The observed FFA ranged between 0.19% and 1.03%, with the lowest level at 15% ghee, 190 °C, and 12 min, and the highest at 10% ghee, 170 °C, and 9 min.

The combined effects of ghee proportion, roasting temperature, and roasting time showed consistent trends in lowering FFA levels with increases in each parameter. For instance, at fixed roasting times, raising ghee proportion and roasting temperature reduced FFA, with the minimum predicted value of 0.17% at 14.9% ghee and 187 °C. This was attributed to enzyme denaturation at higher temperatures and lipid-induced hydrophobic barriers reducing moisture entry and enzyme activity (Ananthu *et al.*, 2023; Zhang *et al.*, 2025) [2, 39].

Similarly, under constant roasting temperature, both FFA levels and predicted values confirmed a decreasing trend with longer roasting times, reaching a minimum of 0.23% at 15% ghee and 12 min. These findings align with Dang *et al.* (2022) [10], who highlighted roasting time as a key factor in enhancing oxidative and hydrolytic stability of cereal-based systems.

Table 1: Effect of Roasting variables on biochemical, functional, enzymatic, moisture content and sensory parameters on pearl millet *raab* mix

Sr. No.	*Ghee* proportion (%)	Temperature of roasting (°C)	Time of roasting (min)	Peroxidase (µmol/min /g)	Lipase (µmol FFA /h)	FFA (%)	Moisture content (%)
1.	5	170	12	52.2	27.3	0.98	3.72
2.	15	170	12	45.1	26.3	0.53	3.68
3.	5	190	12	33.3	17.6	0.31	1.23
4.	15	190	12	27.5	15.7	0.19	1.16
5.	5	180	9	43.7	18.6	0.87	2.78
6.	15	180	9	40.3	19.1	0.4	2.75
7.	5	180	15	39.3	11.7	0.44	1.96
8.	15	180	15	29.54	9.8	0.31	1.6
9.	10	170	9	50.3	28.6	1.03	3.95

10.	10	190	9	35.3	18.3	0.45	1.31
11.	10	170	15	43.5	24.1	0.76	3.26
12.	10	190	15	26.4	12.6	0.29	1.11
13.	10	180	12	39.6	21.3	0.38	2.61
14.	10	180	12	37.7	22.4	0.35	2.6
15.	10	180	12	39.3	21.5	0.37	2.67
16.	10	180	12	38.8	22.5	0.39	2.5
17.	10	180	12	39.2	22.6	0.36	2.4
18.	Control			57.4	20.6	0.2	7.04

Table 2: Analysis of variance (ANOVA) for surface quadratic model of different biochemical, proximate, enzyme activity of developed pearl millet *raab* mix

Source	Peroxidase	Lipase	FFA	Moisture Content
Intercept	38.92	22.06	0.37	2.56
Linear terms				
X_1	-3.26***	-0.537	-0.146***	-0.063
X_2	-8.587***	-5.262***	-0.257***	-1.225***
X_3	-3.86***	-3.3***	-0.118***	-0.357***
Interaction terms				
X_1X_2	0.325	-0.225	0.0825***	-0.0075
X_1X_3	-1.59**	-0.6	0.0850***	-0.0825
X_2X_3	-0.525	-0.3	0.0275*	0.1225
Quadratic terms				
$(X_1)^2$	-0.03	-3.2175***	0.0025	-0.1217
$(X_2)^2$	0.636	2.883***	0.130***	0.1325
$(X_3)^2$	-0.68	-4.043***	0.133***	-0.1618
Indicators for model fitting				
R^2	0.9943	0.9375	0.9256	0.9847
Adj-R^2	0.9869	0.9122	0.8997	0.9650
Pred-R^2	0.9472	0.8839	0.8712	0.8037
Adeq. Precision	41.2042	18.203	17.772	23.99
F-Value	134.95	54.29	343.86	50.09
Lack of Fit	NS	NS	NS	NS
C.V.%	2.1	4.84	5.1	7.08

X_1 = *Ghee* proportion (%), X_2 = Roasting temperature (°C), X_3 = Roasting time (min) ***Significant at $P<0.001$, **Significant at $P<0.01$, *Significant at $P<0.05$, NS = Non-significant

A parallel effect was observed with roasting temperature and time when ghee proportion was fixed at 10% (Fig. 1). Predicted and observed trends showed a sharp reduction in FFA as temperature rose from 170 °C to 190 °C and roasting time from 9 to 15 min, with the minimum of 0.23% at 190 °C and 13 min. This trend corroborates Pradeep *et al.* (2014) [32], who demonstrated improved lipid stability in millet-based systems through thermal processing.

The statistical model confirmed the robustness of these findings. The quadratic FFA model (Table 2) was highly significant (F-value 343.48, $p<0.001$) with non-significant lack of fit ($p > 0.05$), validating its adequacy. The coefficient of determination ($R^2 = 0.9256$) explained 92.56% of variability, supported by strong adjusted R^2 (0.8997) and predicted R^2 (0.8712), both exceeding empirical model validity thresholds (Koocheki *et al.*, 2010) [19]. Adequate precision of 17.772, well above the cut off four, and a coefficient of variation (CV = 5.1%) further emphasized statistical reliability and experimental precision (Akesowan and Choonhahirun, 2013; Little and Hills, 1978; Mendenhall, 1975; Haber and Runyon, 1977) [1, 20, 22, 15].

The derived model, giving the empirical relation between the free fatty acid and the test variables in coded units, was obtained as under:

$$FFA = 0.37 - 0.1463 * X_1 - 0.2575 * X_2 - 0.1188 * X_3 + 0.0825 * X_1X_2 + 0.085 * X_1X_3 + 0.0275 * X_2X_3 + 0.0025 * (X_1)^2 + 0.13 * (X_2)^2 + 0.1325 * (X_3)^2 \quad \text{…Eq. 1}$$

Where, X_1, X_2 and X_3 are the coded factors of *ghee* proportion, roasting temperature and roasting time, respectively.

In Table 2, among the linear terms all three variables *ghee* proportion, roasting temperature and roasting time had highly significant effects ($p < 0.001$) with negative values. The interaction effects were all positive and statistically significant, with *ghee* proportion with roasting temperature and *ghee* proportion with roasting time showing high significance at $p < 0.001$, while the interaction between roasting temperature with roasting time was significant at $p < 0.05$. Regarding the quadratic terms, *ghee* proportion was found non-significant, whereas both roasting temperature and roasting time were positive and highly significant ($p < 0.001$).

3.2 Effect of Roasting Treatment on Lipase Activity

Table 1 and Fig. 2 reveals that lipase activity in pearl millet raab mix varied with ghee proportion, roasting temperature, and time, ranging from 28.6 µmol FFA/h at 10% ghee, 170 °C, and 9 min to 9.8 µmol FFA/h at 15% ghee, 180 °C, and 15 min. Lipase catalyses triglyceride breakdown into free fatty acids, so its inactivation is crucial to prevent rancidity and quality loss in lipid-rich foods.

Lipase activity consistently decreased with higher roasting temperatures, longer roasting times, and greater ghee proportions, with predicted minima around 15.75 µmol FFA/h at 15% ghee and 190 °C, and 10.48 µmol FFA/h at 15 min roasting time. Thermal denaturation is the main reason for enzyme inactivation, with temperature playing a more significant role than ghee. Although ghee may provide some thermal insulation, its effect was limited and statistically insignificant. These findings align with studies reporting irreversible lipase inactivation during thermal processing of cereal products (Mudgil *et al.*, 2023; Yarrakula *et al.*, 2022; Pathare *et al.*, 2024; Irondi *et al.*, 2019) [24, 38, 29, 17].

The combined effect of higher temperature and roasting time produced a strong synergistic decline in lipase activity, indicating the importance of cumulative heat exposure for enzyme suppression. Predicted minimum activity reached 12.06 µmol FFA/h at 190 °C and 15 min, corroborating reports that sufficient roasting intensity is needed to improve cereal-based mix stability by enzyme inactivation (Liu *et al.*, 2023; O'Connor *et al.*, 1992) [21]. Activity reduction was most pronounced at lower ghee levels, confirming the effectiveness of roasting-induced thermal denaturation in reducing lipolytic activity (Hema *et al.*, 2022; Irondi *et al.*, 2019) [16, 17].

The statistical model was highly significant ($F = 54.29$; $p < 0.0001$), with good fit ($p = 0.089$), explaining 93.75% of variability (R^2) and supported by high adjusted and predicted R^2 values. Adequate precision (18.203) and low CV (4.84%) confirmed model reliability and experimental precision

The analysis of variance (ANOVA) for lipase activity (Table 2) indicated that the quadratic model was highly significant (F-value = 54.29; $p < 0.0001$), with a non-significant lack of fit ($p = 0.089$), confirming the model's adequacy for representing the observed data. The coefficient of determination (R^2 = 0.9375), adjusted R^2 (0.9122) and predicted R^2 (0.8839) were all well above 0.8, suggesting excellent model reliability and internal consistency. The small gap between adjusted and predicted R^2 indicates that the model was not over fitted. Moreover, the adequate precision value of 18.203 was much higher than the required threshold of 4.0, reflecting a strong signal-to-noise ratio. The coefficient of variation (CV = 4.84%) also supports the high precision and reproducibility of the experimental data.

The derived model, giving the empirical relation between the Lipase activity and the test variables in coded units, was obtained as under:

$$\text{Lipase activity} = 22.06 - 0.5375 * X_1 - 5.2625 * X_2 - 3.3 * X_3 + 0.225 * X_1X_2 + 0.6 * X_1X_3 + 0.3 * X_2X_3 + 3.2175 * (X_1)^2 + 2.8825 * (X_2)^2 + 4.0425 * (X_3)^2 \quad \text{...Eq. 2}$$

Where, X_1, X_2 and X_3 are the coded factors of *ghee* proportion, roasting temperature and roasting time, respectively.

Regression coefficients in Table 2 revealed that linear terms, roasting temperature and roasting time had highly significant and negative effects on lipase activity ($p < 0.001$), while the effect of *ghee* proportion was found non-significant. The interaction terms were all found non-significant. All the quadratic terms were highly significant ($p<0.001$) with *ghee* proportion, roasting time being negative and roasting temperature affirming positive values.

3.3 Effect of Roasting Treatment on Peroxidase Activity

Table 1 shows peroxidase activity in pearl millet raab mix subjected to varying ghee proportions, roasting temperatures, and times. Peroxidase activity is an important enzymatic marker for assessing thermal processing effectiveness and enzyme stability in cereal matrices. Observed activity ranged from a maximum of 52.2 µmol/min/g at 5% ghee, 170 °C, and 12 min to a minimum of 26.4 µmol/min/g at 10% ghee, 190 °C, and 15 min.

The combined effect of ghee proportion and roasting temperature (Fig 3(a)), with fixed roasting time, revealed a steep decline in peroxidase activity as temperature increased. Minimum predicted activity (28.07 µmol/min/g) occurred at 15% ghee and 190 °C, aligning with the known temperature sensitivity of peroxidase, which rapidly denatures at high heat (Poutanen, 1997; Zhao *et al.*, 2020; Yarrakula *et al.*, 2022) [31, 38, 40]. Activity remained highest at lower roasting temperatures and minimal ghee levels.

When observing the interaction of ghee and roasting time (Fig 3(b)) at fixed temperature (180 °C), peroxidase activity decreased steadily with longer roasting durations, reaching a predicted minimum of 29.56 µmol/min/g at 15% ghee and 15 min. This reflects protein unfolding and enzyme deactivation during extended thermal exposure, consistent with reports on roasting duration effects in food systems (Wang *et al.*, 2022; Mudau *et al.*, 2022) [36, 23]. Activity declines were especially prominent at lower ghee levels.

The effect of roasting temperature and time in fig 3(c), holding ghee at 10%, showed the lowest predicted peroxidase activity of 25.96 µmol/min/g at 190 °C and 15 min, indicating that extended exposure at high temperatures causes irreversible enzyme structure disruption and loss of catalytic function (Padmaja *et al.*, 2024) [28].

The analysis of variance (ANOVA) for peroxidase activity (Table 2) revealed that the quadratic model was statistically significant, with an F-value of 134.95 ($p < 0.001$) and a non-significant lack of fit ($p > 0.05$), confirming the adequacy of the model. The coefficient of determination (R^2 = 0.9943) along with adjusted R^2 (0.9869) indicated a very strong fit, explaining a substantial proportion of the variability in the response. The predicted R^2 value (0.9472) was in reasonable agreement with the adjusted R^2, showing a difference of less than 0.2 and reflecting good model predictability. Additionally, the adequate precision value of 41.20, being far above the threshold of 4.0, signified an excellent signal-to-noise ratio and thus, the model's strong reliability. The coefficient of variation (CV = 2.10%) was within acceptable limits, confirming the reproducibility and precision of the experimental observations.

The derived model, giving the empirical relation between the Peroxidase activity and the test variables in coded units, was obtained as under:

$$\text{Peroxidase activity} = 38.88 - 3.26 * X_1 - 8.587 * X_2 - 3.86 * X_3 + 0.325 * X_1X_2 - 1.59 * X_1X_3 - 0.525 * X_2X_3 - 0.03 * (X_1)^2 + 0.636 * (X_2)^2 - 0.525 * (X_3)^2 \quad \text{...Eq. 3}$$

Where, X_1, X_2 and X_3 are the coded factors of *ghee* proportion, roasting temperature and roasting time, respectively.

In Table 2, the linear terms of *ghee* proportion, roasting temperature and roasting time all exhibited negative, highly significant effects ($p < 0.001$) indicating their strong individual influence on peroxidase activity. In contrast, all the interaction terms except *ghee* proportion with roasting temperature (negative, significant at $p<0.01$) and the quadratic terms were statistically found non-significant.

3.4 Effect of Roasting Treatment on Moisture Content

Table 1 and fig 4 summarizes the moisture content of pearl millet raab mix prepared under various ghee proportions, roasting temperatures, and times. Moisture content is critical for shelf life and microbial safety, as higher water levels promote microbial growth and enzymatic action. Observed values ranged from 1.11% to 3.95%, with the lowest moisture recorded at 10% ghee, 190 °C, and 15 min, and the highest at 10% ghee, 170 °C, and 9 min.

Raising the roasting temperature (with time fixed) sharply decreased moisture, with predicted minima of 1.16% at 15% ghee and 190 °C, matching the trend of temperature-driven water loss across all ghee proportions. Increasing roasting time (with temperature fixed at 180 °C) reduced moisture further, reaching a minimum of 1.78% at 15% ghee and 15 min, though no clear trend was seen for ghee's effect. These results reflect the role of time in facilitating moisture removal (Bhattacharya, 2014) [19].

Combining higher roasting temperature and extended time led to even greater reductions, with minimum moisture content predicted at 0.95% for 190 °C and 15 min at 10% ghee, highlighting the effectiveness of sustained thermal input. Conversely, maximum moisture was retained (3.95%) at lower roasting temperature (170 °C) and shorter time (9 min). These trends align with research noting that high temperatures accelerate matrix breakdown and water mobility, moving moisture to the surface for evaporation (Nantanga *et al.*, 2008; Shang *et al.*, 2021) [26, 34]. The interaction of roasting variables thus plays a decisive role in engineering moisture levels for shelf-stable products, with results matching other studies on instant cereal mixes where optimized roasting produced maximal moisture reduction (Perraulta *et al.*, 2021; Vinutha *et al.*, 2022) [30, 35].

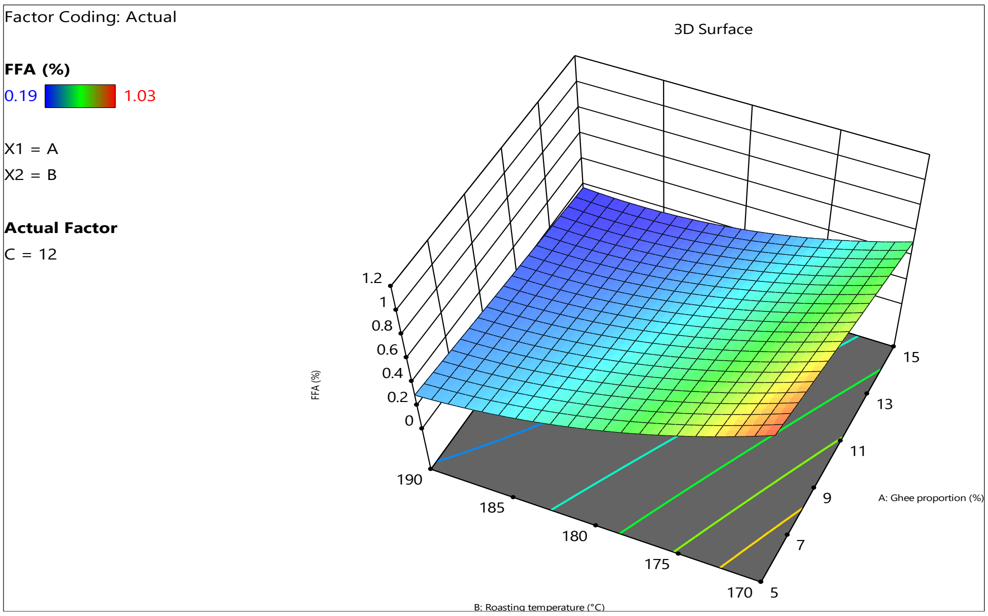
The analysis of variance (ANOVA) for moisture content (Table 2) revealed that the developed quadratic model was statistically significant (F-value = 50.09, $p < 0.001$), with a non-significant lack of fit ($p > 0.05$), confirming the model's adequacy in explaining the variability in the response. The coefficient of determination (R^2 = 0.9847), adjusted R^2 (0.9650) and predicted R^2 (0.8037) were all above the 0.8 threshold, reflecting a strong model fit and predictive reliability. The relatively close agreement between adjusted and predicted R^2 values further supports the internal consistency of the model and its ability to make valid predictions within the defined experimental space. In addition, the model showed an adequate precision value of 13.102, which is considerably above the critical value of 4.0, indicating a strong signal-to-noise ratio for navigating the design space efficiently. The coefficient of variation (CV = 7.08%) suggested high precision and acceptable reproducibility across the experimental runs.

The derived model, giving the empirical relation between the moisture content and the test variables in coded units, was obtained as under:

Moisture content =	$2.556 - 0.0625 * X_1 - 1.23 * X_2 - 0.3575 * X_3 - 0.0075 * X_1X_2 - 0.0825 * X_1X_3 + 0.1225 * X_2X_3 - 0.1218 * (X_1)^2 + 0.133 * (X_2)^2 - 0.1617 * (X_3)^2$	…Eq. 4

Where, X_1, X_2 and X_3 are the coded factors of *ghee* proportion, roasting temperature and roasting time, respectively.

Among the linear terms, roasting temperature and roasting time had negative and highly significant effects at $p < 0.001$, while *ghee* proportion was non-significant. All interaction terms and quadratic terms were found statistically non-significant.



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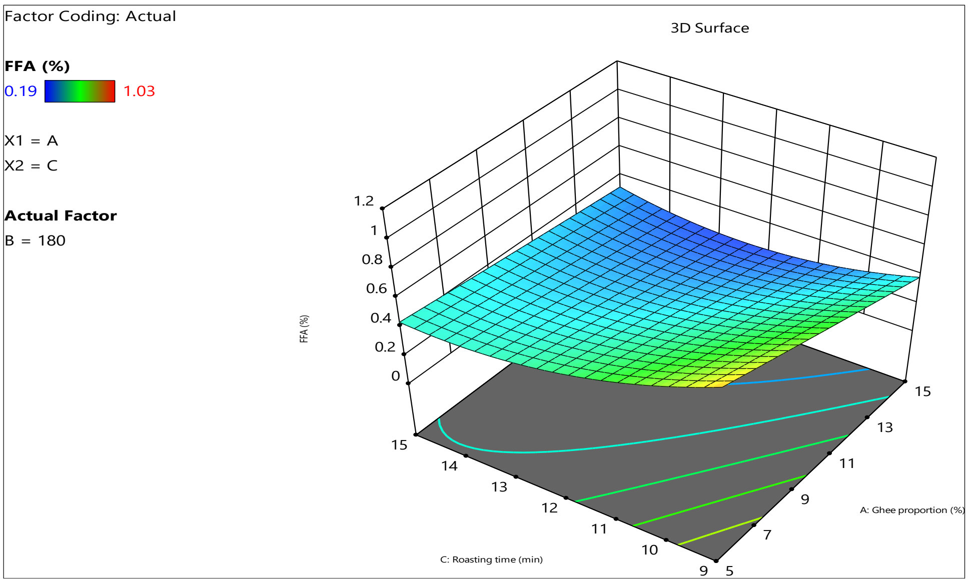

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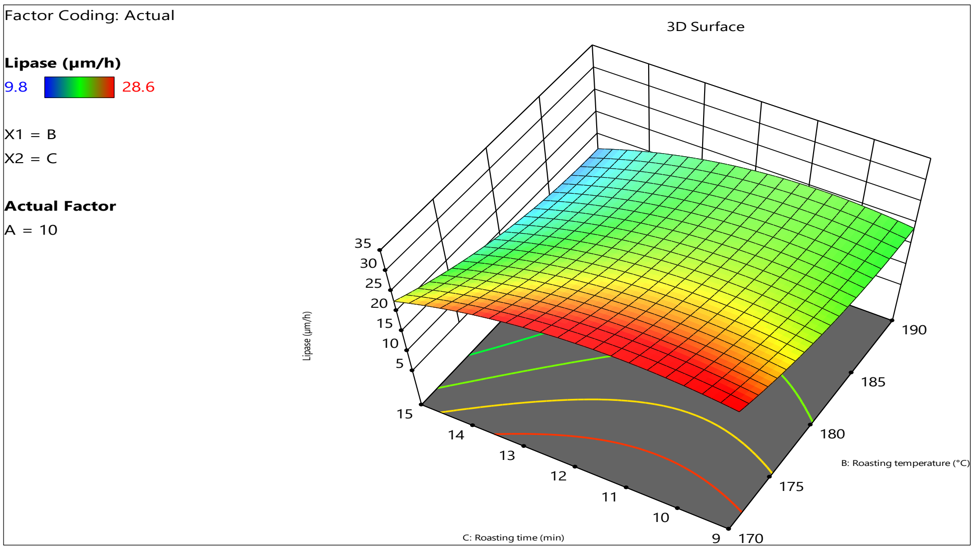
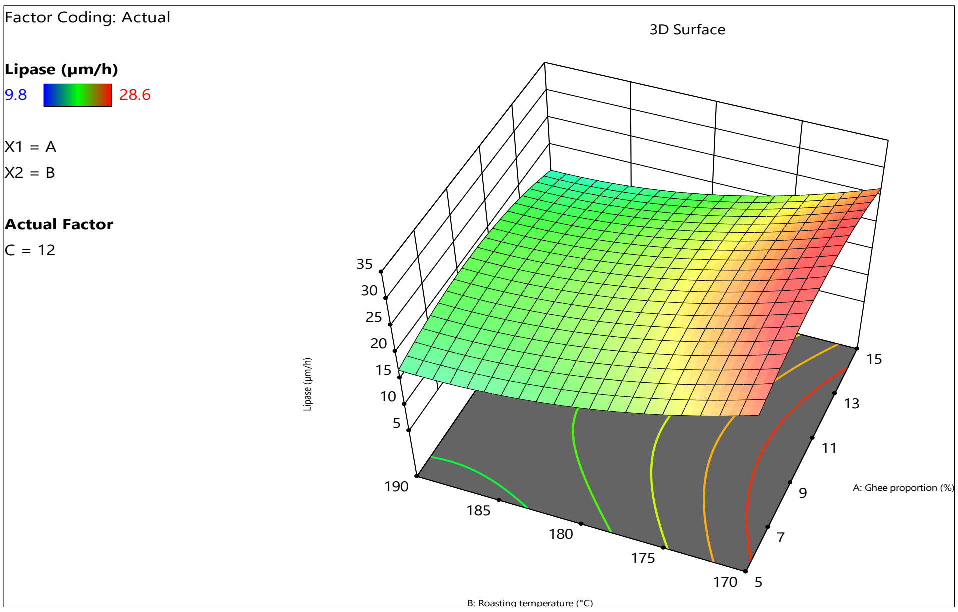


(c)

Fig 1: Effect of ghee proportion, roasting temperature and roadting time on free fatty acid



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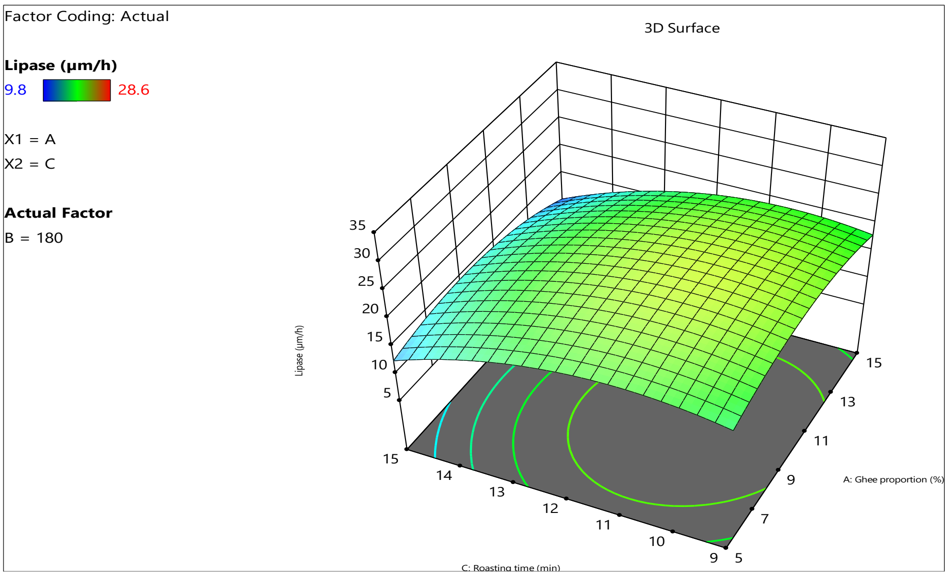

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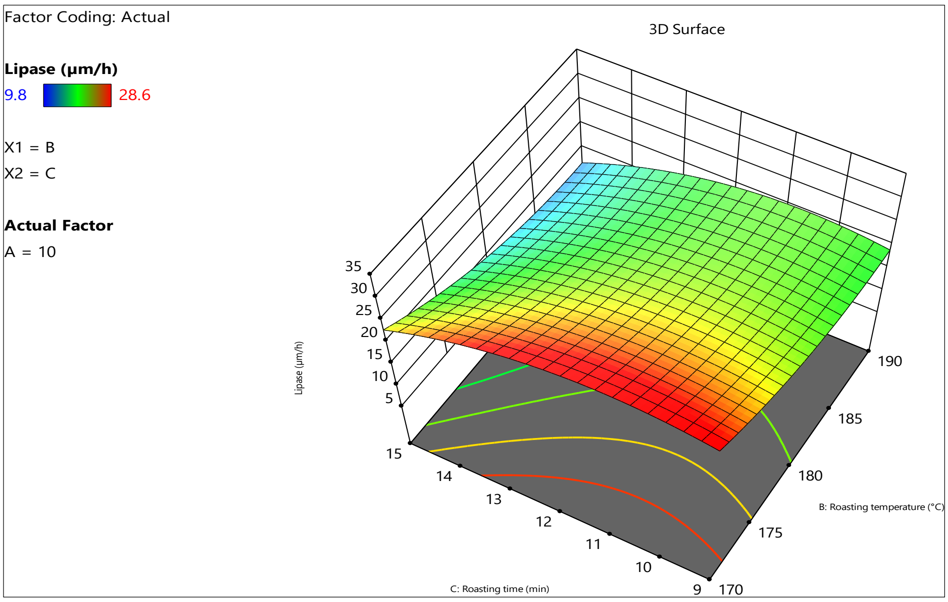
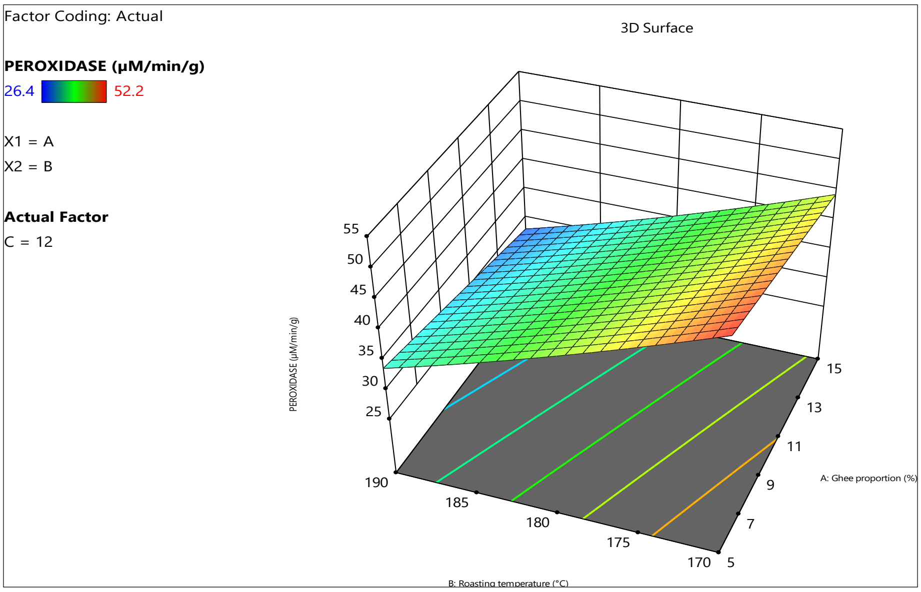


(c)

Fig 2: Effect of ghee proportion, roasting temperature and roadting time on lipase activity



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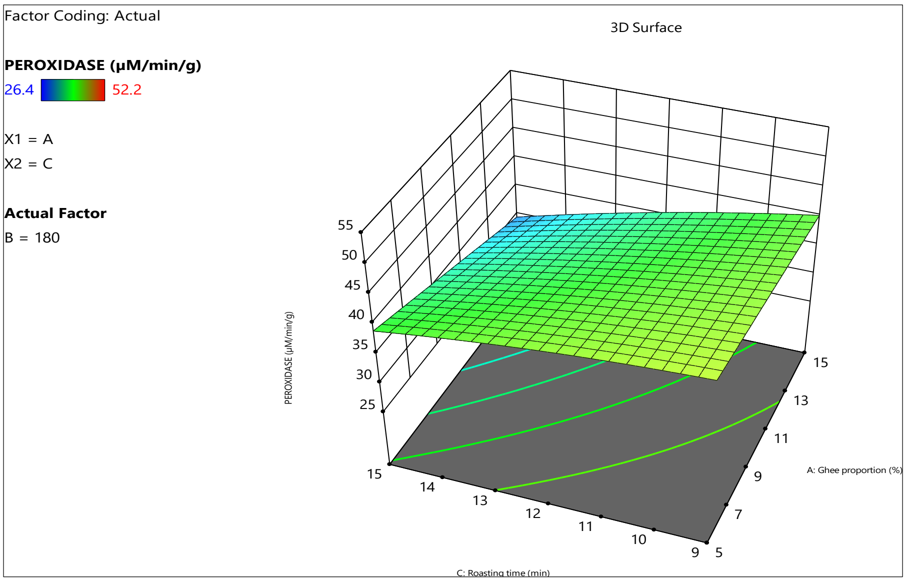

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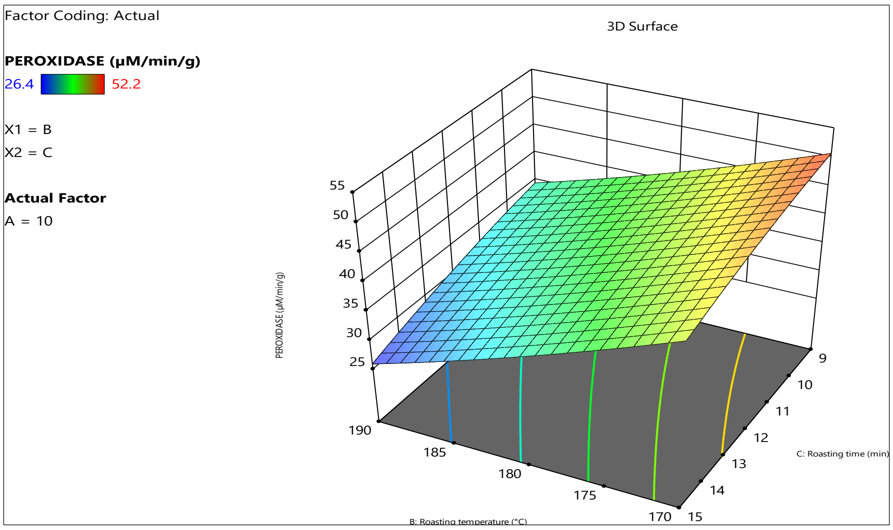
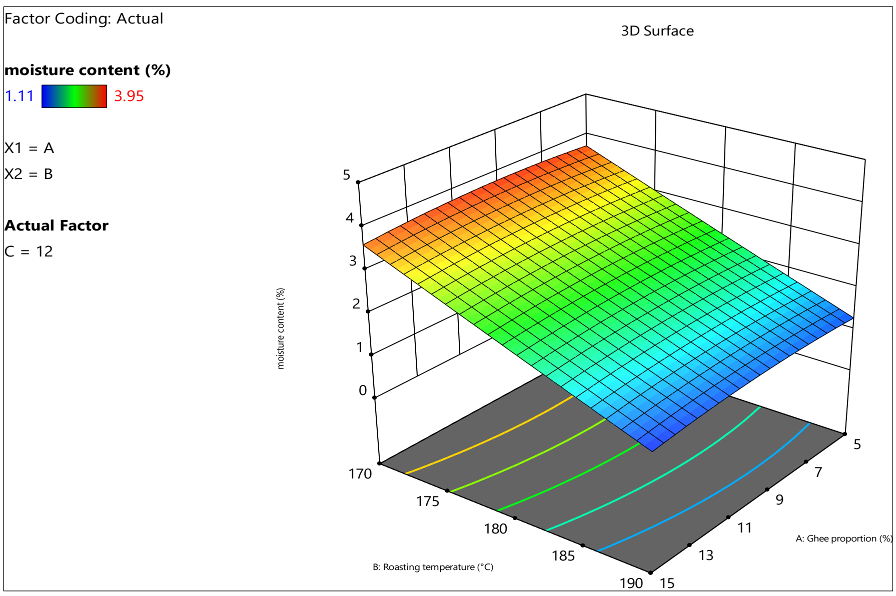


(c)

Fig 3: Effect of ghee proportion, roasting temperature and roadting time on peroxidase activity



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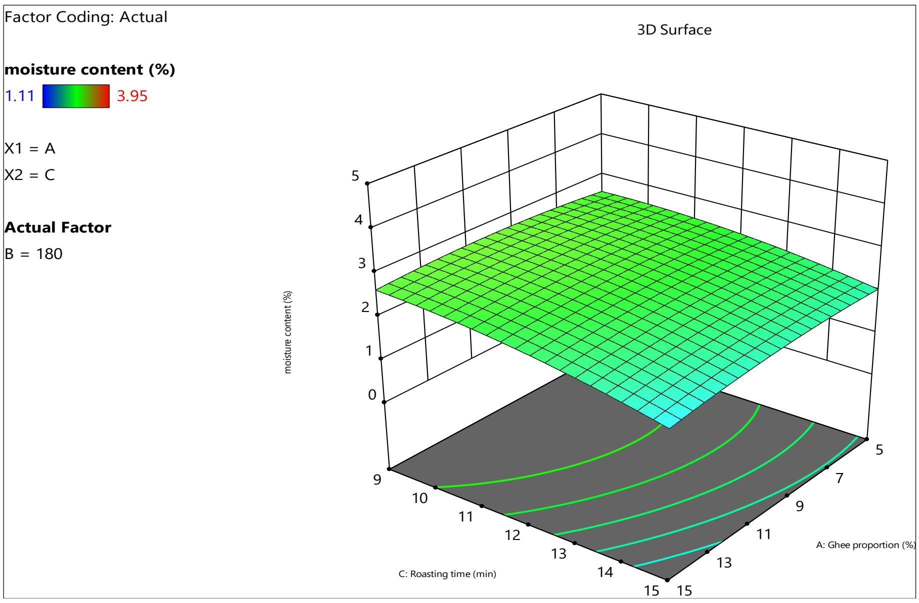

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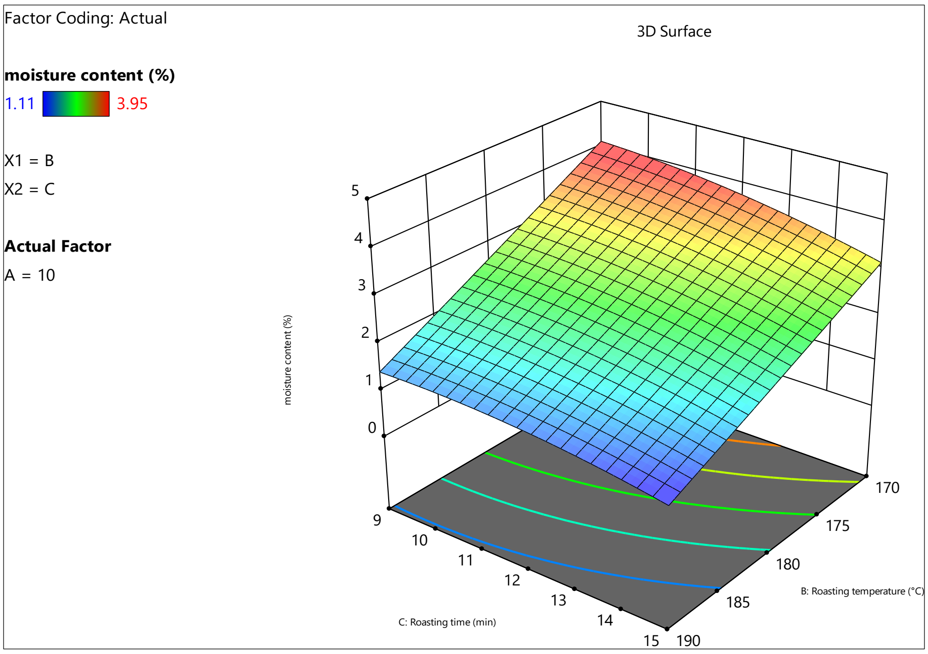

(c)

Fig 4: Effect of ghee proportion, roasting temperature and roadting time on moisture content

4. Optimization and Validation of Processing Variables

The optimization of the pearl millet *raab* mix was thoroughly conducted using Response Surface Methodology (RSM) via Design Expert software version 13, aiming to establish ideal processing conditions that balance functional, biochemical and sensory qualities. The independent parameters: *ghee* proportion (%), roasting temperature (°C) and roasting time (min) were explored using a Box-Behnken Design to understand their interactive influence on eleven critical response variables. The software generated optimal values of 12.19% *ghee*, 186.92 °C roasting temperature and 15 min roasting time. These were experimentally validated using the nearest feasible levels of *ghee* proportion, roasting temperature and roasting time (12%, 187 °C and 15 min) and the outputs were compared against the predicted values. The resulting deviations for all responses were within an acceptable range (≤5.7%), which demonstrates the predictive accuracy and reliability of the statistical model employed.

5. Conclusion

This study successfully applied Response Surface Methodology (RSM) with a Box-Behnken Design (BBD) to optimize the processing parameters for a shelf-stable, instant pearl millet *raab* mix. The research demonstrated that roasting temperature and time had a highly significant negative effect on key quality indicators, including Free Fatty Acid (FFA) content, lipase activity and peroxidase activity. This confirmed that the thermal treatment was effective in inactivating the enzymes responsible for product degradation. Additionally, increased roasting temperature and time were crucial for reducing moisture content, which is essential for ensuring long-term shelf stability. The study successfully identified an optimal set of processing conditions: 12.19% *ghee*, 186.92 °C roasting temperature and 15 min roasting time. The experimental validation confirmed the predictive accuracy of the statistical models, with a minimal deviation of ≤5.7% between predicted and experimental values.

References

1. Akesowan A, Choonhahirun A. Effect of enzyme treatment on guava juice production using response surface methodology. The Journal of Animal and Plant Sciences. 2013;23(1):114-120.
2. Ananthu R, Buvaneswaran M, Meena L, Sunil CK. Microwave treatment effect on functional and pasting properties and storage stability of white finger millet. Journal of Food Process Engineering. 2023;46(7):e14341.
3. Anonymous. Millets to be consumed in the winter season. 2024. Available from: https://apeda.gov.in/milletportal/Pearl_millet.html [Accessed 2024 Jun 11].
4. Anonymous. Agricultural and Processed Food Products Export Development Authority, Government of India. 2024. Available from: https://apeda.gov.in/milletportal/Production.html [Accessed 2024 Jun 19].
5. Anonymous. U.S. Department of Agriculture, Foreign Agricultural Service. 2025. Available from: https://www.fas.usda.gov/data/production/commodity/0459100 [Accessed 2025 Feb 19].
6. AOAC International. AOAC Official Method 930.15: Moisture content in feeds. In: Official Methods of Analysis. 19th ed. Virginia (USA); 2012.
7. Balasubramaniam VM, Barbosa-Cánovas GV, Lelieveld H, editors. High pressure processing of food: Principles, technology and applications. Springer; 2016.
8. Barron EJ. Plant lipase. In: Paech K, Tracey MY, editors. Modern Methods of Plant Analysis. Vol 7. Springer; 1979. p. 448.
9. Bhattacharya S. Conventional and advanced food processing technologies. John Wiley and Sons; 2014.
10. Dang B, Zhang WG, Zhang J, Yang XJ, Xu HD. Effect of thermal treatment on the internal structure, physicochemical properties and storage stability of whole grain highland barley flour. Foods. 2022;11(14):2021.
11. Deeth HC, Fitz-Gerald CH, Wood AF. A convenient method for determining the extent of lipolysis in milk. Australian Journal of Dairy Technology. 1975;30(3):109-111.
12. Gopalan C, Rama Sastri BV, Balasubramanian SC. Nutritive value of Indian foods. Indian Council of Medical Research; 2004. p. 59-67.
13. Goswami S, Vinutha T, Kumar RR, Singh T, Ali A, Meena MC, *et al*. Recent techniques for improving shelf life and value addition in pearl millet flour. Annals of Arid Zone. 2023;62(2):103-108.
14. Guibault GG. Handbook of Enzymatic Methods of Analysis. Marcel Dekker; 1976. p. 147-149.
15. Haber A, Runyon R. General statistics. 3rd ed. Addison-Wesley; 1977.
16. Hema V, Ramaprabha M, Saraswathi R, Chakkaravarthy PN, Sinija VR. Millet food products. In: Handbook of Millets—Processing, Quality and Nutrition Status. 2022. p. 265-299.
17. Irondi EA, Adegoke BM, Effion ES, Oyewo SO, Alamu EO, Boligon AA. Enzymes inhibitory property, antioxidant activity and phenolics profile of raw and roasted red sorghum grains in-vitro. Food Science and Human Wellness. 2019;8(2):142-148.
18. Khuri AI, Cornell JA. Response surface design and analysis. Marcel Dekker; 1987.
19. Koocheki AS, Mortazavi A, Shahidi F, Razavi SMA, Kadkhodaee R, Milani JM. Optimization of mucilage extraction from *Alyssum homolocarpum* using response surface methodology. Journal of Food Process Engineering. 2010;33:861-882.
20. Little TM, Hills FJ. Agricultural experimental design and analysis. John Wiley; 1978. p. 170.
21. Liu Y, Li M, Jiang D, Guan E, Bian K, Zhang Y. Superheated steam processing of cereals and cereal products: A review. Comprehensive Reviews in Food Science and Food Safety. 2023;22(2):1360-1386.
22. Mendenhall W. Introduction to probability and statistics. 4th ed. Duxbury Press; 1975. p. 273.
23. Mudau M, Ramashia SE, Mashau ME. Mineral content, functional, thermo-pasting and microstructural properties of spontaneously fermented finger millet flours. Foods. 2022;11(16):2474.
24. Mudgil P, Alblooshi M, Singh BP, Devarajan AR, Maqsood S. Pearl millet protein hydrolysates exhibiting effective in-vitro antioxidant, antidiabetic and anti-lipidemic properties. International Journal of Food Science and Technology. 2023;58(6):3264-3272.
25. Nambiar VS, Dhaduk JJ, Sareen N, Shahu T, Desai R. Potential functional implications of pearl millet (*Pennisetum glaucum* L.) in health and disease. Journal of Applied Pharmaceutical Science. 2011;1(10):62-67.
26. Nantanga KK, Seetharaman K, De Kock HL, Taylor JR. Thermal treatments to partially pre-cook and improve the shelf-life of whole pearl millet flour. Journal of the Science of Food and Agriculture. 2008;88(11):1892-1899.
27. O'Connor J, Perry HJ, Harwood JL. A comparison of lipase activity in various cereal grains. Journal of Cereal Science. 1992;16(2):153-163.
28. Padmaja PG, Venkateswarlu R, Singh SP, Tonapi VA. Enhancing shelf life of pearl millet flour. In: Pearl Millet in the 21st Century. 2024. p. 549-566.
29. Pathare AM, Singhal RS, Rao BD, Gokhale JS. Extrusion inhibits lipolytic enzymes in pearl millet (*Pennisetum glaucum*) flour. Innovative Food Science and Emerging Technologies. 2024;93:103605.
30. Perraulta Lavanya J, Gowthamraj G, Sangeetha N. Effect of heat-moisture treatment on physicochemical, functional and antioxidant characteristics of white sorghum (*Sorghum bicolor* L.) grains and flour. Journal of Food Processing and Preservation. 2021;45(12):160-179.
31. Poutanen K. Enzymes: An important tool in the improvement of the quality of cereal foods. Trends in Food Science and Technology. 1997;8(9):300-306.
32. Pradeep PM, Dharmaraj U, Rao BVS, Senthil A, Vijayalakshmi NS, Malleshi NG, *et al*. Formulation and nutritional evaluation of multigrain ready-to-eat snack mix from minor cereals. Journal of Food Science and Technology. 2014;51:3812-3820.
33. Rose DJ, Ogden LV, Dunn ML, Pike OA. Enhanced lipid stability in whole wheat flour by lipase

inactivation and antioxidant retention. Cereal Chemistry. 2008;85(2):218-223.
34. Shang J, Li L, Liu C, Hong J, Liu M, Zhao B, *et al*. Relationships of flour characteristics with isolated starch properties in different Chinese wheat varieties. Journal of Cereal Science. 2021;99:103210.
35. Vinutha T, Kumar D, Bansal N, Krishnan V, Goswami S, Kumar RR, *et al*. Thermal treatments reduce rancidity and modulate structural and digestive properties of starch in pearl millet flour. International Journal of Biological Macromolecules. 2022;195:207-216.
36. Wang Y, Zhao H, Song X, Zhang W, Yang F. Drying kinetics, physicochemical properties and sensory quality of instant foxtail millet as affected by drying methods. Polish Journal of Food and Nutrition Sciences. 2022;72(1):69-78.
37. Yadav S, Kumar M, Kaur P, Sidhu GK, Singh S. Comparative analysis of hermetically and conventionally stored paddy (Basmati-1121). Journal of Biosystems Engineering. 2025;1-10.
38. Yarrakula S, Mummaleti G, Pare A, Vincent H, Saravanan S. Hot-air assisted radio-frequency hybrid technology for inactivating lipase in pearl millet. Journal of Food Processing and Preservation. 2022;46(10):161-178.
39. Zhang J, Liu Y, Wang P, Zhao Y, Zhu Y, Xiao X. The effect of protein-starch interaction on the structure and properties of starch and its application in flour products. Foods. 2025;14(5):778.
40. Zhao B, Shang J, Liu L, Tong L, Zhou X, Wang S, Zhou S. Effect of roasting on enzyme inactivation and starch properties of highland barley. International Journal of Biological Macromolecules. 2020;165:675-682.