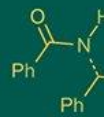


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Optimization of microwave treatment for storage life enhancement of pearl millet flour

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Abstract

Pearl millet contains higher amount of fat content among all other millets. It can be stored for longer duration like months to years without the development of rancidity in whole grain form, but storing in the form of flour is difficult as it has very short shelf life unlike other commercial flours. The development of rancidity is due to high fat content and lipolytic action of enzymes. Apart from enzymatic degradation, oxidative degradation promotes oxidation of free fatty acid (FFA) which leads to the production of peroxides which gives off flavours and bitterness. The study aimed to control both enzymatic and oxidative degradation of flours by giving microwave treatment to grains. The three independent variables such as moisture content (14, 18, 22%), microwave power (600, 800, 1000 W) and time of exposure (75, 85, 95 s) were used for optimizing microwave treatment of pearl millet grains on various attributes of rancidity developing compounds. The optimized condition for microwave processing of pearl millet grains was 1000 W power level, 18% moisture content and 93 s time of exposure based on the dependent variables such as lipase, peroxidase, FFA, total phenol were found to be 12.018 μM FFA/h/g, 32.843 $\mu\text{M}/\text{min}/\text{g}$, 0.29%, 0.569%, respectively.

Keywords: Pearl millet grains, tempering, microwave treatment, optimization

1. Introduction

Millets are one of the first small seeded grasses, can survive in water-scarce environments on infertile soils where no other cereal crops can grow. They possess long appreciated tag of “poor man’s food grain” due to its fine affordability. As a cereal crop for the advancing civilizations, they were originally domesticated in Asia and Africa and eventually spread to other parts of the world (Anon., 2023) [2].

Pearl millet (*Pennisetum glaucum* L.) also known as *bajra* is one of the earliest foods known to humans and has been a staple food for thousands of years in many parts of the world. Pearl millet belongs to the family of *Poaceae* (Punia *et al.*, 2020; Deepak *et al.*, 2012) [15, 6].

In spite of having a strong nutritional profile and strengthened agronomic features, pearl millet has not gained widespread popularity when compared to cereals across the world. Pearl millet seed is a caryopsis, which consists of the seed coat or pericarp, aleurone layer, starchy endosperm and germ. The aleurone layer of seed includes lipase, which is the earliest and most abundant enzyme responsible for fat breakdown. In intact seed, both the lipase enzyme and lipids are present in distinct compartments of the grain. Once the high fat germ of pearl millet is milled into flour, it induces hydrolysis of lipid (5.1% on a d.b.) (Meera *et al.*, 2011) [13] to free fatty acids, an action named hydrolytic rancidity (Rani *et al.*, 2018) [16]. Oxidative rancidity results in hydroperoxides and generates off-odour causing secondary metabolites. Overall, the hydrolytic and oxidative rancidity causes the formation of off-odour and low shelf-life of the pearl millet flour.

Generally, pearl millet flour should be stored under normal condition, if stored in high moisture and oxygen condition, it becomes bitter and rancid. Heat treatment of pearl millet grains prior to milling into flour increases the flour's keeping quality without affecting its acceptability (Arora *et al.*, 2002) [3].

As thermal treatments reduce rancidity, microwave treatment is preferred because it saves time and energy compared to other methods. Microwave heating is based on the transformation of alternating electromagnetic field energy into thermal energy by affecting

polar molecules of material. Poor keeping quality of flour because of the development of off flavour shortly after grain milling is a major restriction to its wider acceptability by consumers and its quantitatively high utilization in the food industry.

Therefore, the aim of this study is to examine the effects of microwave treatments on various attributes of rancidity developing compounds of pearl millet flour, particularly focusing on lipid degradation during storage. Thus, by considering the above aspects, the research work will be undertaken.

2. Materials and Methods

2.1 Raw materials

The cleaned stored pearl millet grains (var.GHB 1129) were procured from the Pearl millet Research Station, Jamnagar and also, its production is more and popular for consumption in Gujarat.

2.2 Microwave treatment

The pearl millet grains were cleaned and the sample of desired moisture levels were prepared by adding the required amount of distilled water as calculated by following equation.

$$Q = \frac{W_i(M_f - M_i)}{(100 + M_i)}$$

Where, Q = Mass of water to be added (g), W_i = initial mass of sample (g), M_i = initial moisture content of sample (12.2% d.b.) and M_f = final moisture content of sample (% d.b.)

The samples were mixed thoroughly with calculated amount of water (10.46, 35.36, 61.97 ml) by adding distilled water to meet the required moisture content such as 14, 18, 22% and then kept in glass jars for tempering. The grains were mixed often at regular intervals to avoid the moisture

deposition at bottom and tempered for 8 hours to achieve even moisture distribution. The conditioned grains were treated with microwaves as per the treatment combinations and dried to milling moisture content (10 ± 2 °C) using tray dryer. The microwave treated grains were milled into flour using disc type flour mill and the dependent parameters such as enzyme activity, biochemical and proximate parameters were analysed. The analysed data were optimised with Response surface methodology (RSM) – Box Benken Design (BBD) design and the optimised conditions were used for further storage study.

2.3 Chemical analysis

Free fatty acid of pearl millet flour was determined by titrating it against potassium hydroxide (KOH) in the presence of phenolphthalein indicator and the method was suggested by Deeth *et al.* (1975) [7] was used with slight modifications. Total phenol content was estimated by the method suggested by Malick and Singh (1980) [12]. Lipase activity is determined by measuring the amount of fatty acid released over time and the method was suggested by Baron (1979) [4]. The peroxidase assay is a method used to measure the activity of the enzyme peroxidase and the method was suggested by Guibalt (1976) [8].

2.4 Statistical analysis

The standardization of dependent variables was conducted by Response surface methodology (RSM) software and statistical analysis will be analyzed by Box Benken Design (BBD) (Khuri and Cornell, 1987) [10] with six central points.

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.

Table 1: Effect of microwave treatment on biochemical, proximate and enzyme activity of pearl millet flour

Sr. No.	Moisture content	Power	Time	Lipase activity (μ M FFA/h/g)	Peroxidase activity (μ M/min/g)	FFA (%)	Total phenol (%)
1.	18	1000	95	15.94	33.05	0.35	0.60
2.	14	800	95	32.99	77.03	1.46	1.04
3.	18	600	95	42.34	72.50	1.51	1.00
4.	14	800	75	48.27	112.82	1.78	1.50
5.	22	800	95	42.97	79.43	1.54	1.08
6.	18	1000	75	18.02	66.83	0.49	0.92
7.	22	600	85	90.09	109.31	2.18	1.45
8.	18	800	85	19.84	47.29	1.50	0.68
9.	22	800	75	70.81	100.11	2.05	1.33
10.	14	1000	85	29.18	87.93	0.63	1.19
11.	18	600	75	69.87	76.04	1.96	1.04
12.	18	800	85	20.07	51.12	1.12	0.73
13.	18	800	85	22.07	53.10	1.26	0.75
14.	18	800	85	21.12	45.03	1.33	0.81
15.	14	600	85	59.37	106.34	1.90	1.41
16.	18	800	85	20.02	48.57	1.06	0.79
17.	22	1000	85	33.25	70.94	0.55	0.97
18.	18	800	85	25.60	50.55	1.34	0.71
19.	control			107.44	157.59	2.58	1.66

Table 2: Analysis of variance (ANOVA) for surface quadratic model of different biochemical, proximate, enzyme activity of microwave treated pearl millet flour

Source	Lipase activity	Peroxidase activity	Total phenol	Acid value/FFA
Intercept	21.45	49.28	0.75	1.27
Linear terms				
X ₁	8.41***	-3.04*	-0.039	0.069
X ₂	-20.66***	-13.18***	-0.153***	-0.693***
X ₃	-9.09***	-11.72***	-0.135***	-0.178**
Interaction terms				
X ₁ X ₂	-6.66**	-4.99*	-0.065	-0.090
X ₁ X ₃	-3.14*	3.78	0.052	-0.046
X ₂ X ₃	6.36**	-7.56**	-0.070	0.078
Quadratic terms				
(X ₁) ²	21.87***	37.30***	0.43***	0.338***
(X ₂) ²	9.65***	7.06**	0.082*	-0.291**
(X ₃) ²	5.44**	5.77**	0.063	0.102
Indicators for model fitting				
R ²	0.9929	0.9903	0.9763	0.9710
Adj-R ²	0.9849	0.9793	0.9497	0.9385
Pred-R ²	0.9302	0.9052	0.7536	0.9042
Adeq. Precision	37.8590	31.1574	19.7536	18.3926
F-Value	124.49	90.43	36.66	29.81
Lack of fit	NS	NS	NS	NS
C.V%	7.07	4.89	6.31	10.28

X₁ = Moisture content, X₂ = Power, X₃ = Time, ***Significant at $p < 0.001$, **Significant at $p < 0.01$, *Significant at $p < 0.05$, NS = Non-significant

3. Results and Discussion

3.1 Effect of microwave treatment on lipase activity of pearl millet flour

The effect of process variables on lipase activity of pearl millet flour has been presented in the Fig. 1. The graphical representation using RSM demonstrates that increase in moisture content and power leads to a decline in lipase activity. However, it is observed that a further increase in moisture content there is less reduction in lipase activity because of lower temperature achieved due to evaporative cooling in pearl millet grains. For longer time of exposure, higher power level and controlled moisture content there is a highest reduction in lipase activity. The lipase activity ranged from 90.09 to 15.94 $\mu\text{M FFA/h/g}$ for all the treatment combinations from the Table 1. The highest lipase activity was observed for the combination of 22% moisture content, 600 W power and 85 s time, while the lowest bulk density was found for the combination of 18% moisture content, 1000 W power and 95 s time. Yadav *et al.* (2012) [21] reported the decreasing lipase activity on increasing time of exposure at all moisture levels in comparison with raw pearl millet. Xu *et al.* (2013) [20] reported similar trends of

lipase activity of microwave treated wheat germ and it was decreased with increase in duration of microwave exposure and Mohajer khorasani *et al.* (2019) [14] reported the inactivation of lipase activity were increased with increase in microwave exposure and moisture levels. The decreased lipase activity of flour was due to high temperature attained by the sample through transformation of microwave energy into thermal energy. Keying *et al.* (2009) [9] also observed lipase inactivation in naked oat kernels during microwave heating and correlated with temperature and exposure time. Table 2. illustrates the findings of regression analysis and ANOVA for the lipase activity of microwave treated pearl millet flour. The table demonstrates, that moisture content showed positive linear effect which there were significant at $p < 0.0001$ but power and time of exposure showed negative linear effect on lipase activity which was significant at $p < 0.0001$ respectively. At the same time, the interaction effect of moisture content and power was significantly negative at $p < 0.05$ and power and time of exposure was significantly positive at $p < 0.005$. The moisture content, power and time of exposure showed positive quadratic effect which there were significant at $p < 0.0001$ and $p < 0.05$.

Lipase activity =	$21.45 + 8.41 * X_1 - 20.66 * X_2 - 9.09 * X_3 - 6.66 * X_1X_2 - 3.14 * X_1X_3 + 6.36 * X_2X_3 + 21.87 * (X_1)^2 + 9.65 * (X_2)^2 + 5.44 * (X_3)^2$
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The calculated F-value for lipase activity (124.49) was significant at $p < 0.0001$. At the same time, it possessed non-significant lack of fit ($p > 0.05$). These values indicated that the model for lipase activity was fitted and reliable. The R² value and Adj-R² value for the lipase activity were 0.9929 and 0.9849 respectively, which were higher than the 0.8, indicating the adequacy, good fit and high significance of the model. The Pred-R² (0.9302) was in reasonable agreement with the Adj-R². The high Adeq. Precision value (>4) again supported the significance of the model for lipase activity. The lower value of coefficient of variation (7.07%)

for lipase activity explained that the experimental results were precise and reliable (Table 2.).

3.2 Effect of microwave treatment on peroxidase activity of pearl millet flour

The changes in peroxidase activity of pearl millet flour after microwave treatment are presented in Fig. 2. The peroxidase activity decreased upon moisture content and power of microwave treatment. Further increase in moisture content leads to less reduction in peroxidase activity and maximum reduction in peroxidase activity up to the maximum level of power and time of microwave exposure. The peroxidase

activity ranged from 33.05 to 112.82 $\mu\text{M}/\text{min}/\text{g}$ across all treatment combinations (Table 1). The highest peroxidase activity was observed for the combination of 14% moisture content, 800 W power and 75 s time, while the lowest peroxidase activity was found for the combination of 18% moisture content, 1000 W power and 95 s time. Tao *et al.* (1993) [19] used peroxidase activity a rapid indicator of treatment effect immediately after microwave heating because this assay is faster and simpler than lipase activity determination. Also, they reported that microwave treatment reduced the peroxidase activity and found all the peroxidase activity of microwave heat treated bran samples was lower than that of control samples. This was due to relatively longer time of exposure with microwave treatment. The

lower the peroxidase activity, the more effective was the treatment.

Table 2 showed the results regression analysis and ANOVA for the peroxidase activity of microwave treated pearl millet flour. The table indicates, that moisture content, power and time of exposure showed negative linear effect which were significant at $p < 0.05$, $p < 0.0001$ and $p < 0.0001$ respectively. Concurrently, the interaction effect of moisture content and power, power and time of exposure was significantly negative at $p < 0.05$ and $p < 0.01$ respectively. The moisture content, power and time of exposure showed positive quadratic effect which there were significant at $p < 0.0001$, $p < 0.01$ and $p < 0.05$. The interaction effect of moisture content and time of exposure, showed a non-significant effect on peroxidase activity.

Peroxidase activity =	$49.28 - 3.04 * X_1 - 13.18 * X_2 - 11.72 * X_3 - 4.99 * X_1X_2 + 3.78 * X_1X_3 - 7.56 * X_2X_3 + 37.30 * (X_1)^2 + 7.06 * (X_2)^2 + 5.77 * (X_3)^2$
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The computed F-value for peroxidase activity (90.43) was significant at $p < 0.0001$. At the same time, it possessed non-significant lack of fit ($p > 0.05$). These figures highlighted that the model for peroxidase activity was correctly fitted and reliable. The R^2 value and Adj- R^2 value for the peroxidase activity were 0.9903 and 0.9793 respectively, which exceeded 0.8, indicating that the model was adequate, well fitting and highly significant. The Pred- R^2 (0.9052) was in reasonable agreement with the Adj- R^2 . The higher Adeq. Precision value (>4) further supported the significance of the model for peroxidase activity. The lower value of coefficient of variation (4.89%) for peroxidase activity explained that the experimental results were valid and determinative (Table 2.).

3.3 Effect of microwave treatment on free fatty acid of pearl millet flour

The changes in acid value/FFA of pearl millet flour after microwave treatment are presented in Fig. 3. There is a reduction in acid value/FFA for increase in moisture content and power. Further increase in moisture content and power there is less reduction in acid value/FFA. The maximum reduction was upto the maximum level of power and time. The acid value/FFA ranged from 0.35 to 2.18% (Table 1). The highest FFA was observed for the combination of 22%

moisture content, 600 W power and 85 s time, while the lowest FFA was found for the combination of 18% moisture content, 1000 W power and 95 s time. Liu *et al.* (2021) [11] reported microwave treatment of wheat bran significantly decreased the free fatty acid (FFA) content and it was lower than raw sample. The FFA content decreased with increase in treatment time and microwave power. Similarly, Adebawale *et al.* (2020) [1] reported that the FFA content of microwave treated whole grain sorghum flour was 50% lesser than that of an untreated sample. This was due to more FFA degraded into hydroperoxides at higher microwave power. The unstable peroxides decomposed swiftly into volatile compounds, which reduced the FFA content of samples.

Table 2 displays the findings of the regression analysis and ANOVA for the FFA of microwave treated pearl millet flour. The table indicates, that power and time of exposure showed negative linear effect which were significant at $p < 0.0001$ and $p < 0.01$, respectively. The moisture content showed positive quadratic effect which was significant at $p < 0.001$ and power showed negative quadratic effect which there was significant at $p < 0.01$, respectively. The all interaction and quadratic effect of time of exposure showed non-significant effect on acid value/FFA.

Acid value/FFA =	$3.765 + 0.064 * X_1 - 0.69 * X_2 - 0.161 * X_3 - 0.108 * X_1X_2 - 0.04 * X_1X_3 + 0.073 * X_2X_3 + 0.345 * (X_1)^2 - 0.283 * (X_2)^2 + 0.085 * (X_3)^2$
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The computed F-value for acid value/FFA (29.81) was significant at $p < 0.0001$. At the same time, it possessed non-significant lack of fit ($p > 0.05$). These figures highlighted that the model for acid value/FFA was fitted and reliable. The R^2 value and Adj- R^2 value for the acid value/FFA were 0.9710 and 0.9385 respectively, which exceeds 0.8, indicating that the model was adequate, well fitting and highly significant of the model. The Pred- R^2 (0.9042) was in reasonable agreement with the Adj- R^2 . The high Adeq. Precision value (>4) further supported the significance of the model for acid value/FFA. The lower value of coefficient of variation (3.43%) for acid value/FFA explained that the experimental results were precise and reliable (Table 2).

3.4 Effect of microwave treatment on total phenol content of pearl millet flour

The changes in total phenol of pearl millet flour after microwave treatment are presented in Fig. 4. There is a reduction in total phenol with increase in moisture content and power level and further increase in moisture content there is less reduction. The total phenol decreased upto the maximum level with power and time. The total phenol content ranged from 0.60 to 1.50% from the Table 1. The highest total phenol was observed for the combination of 14% moisture content, 800 W power and 75 s time, while the lowest total phenol was found for the combination of 18% moisture content, 1000 W power and 95 s time. Chandrasekara *et al.* (2012) [5] reported that after the process of cooking of pearl millet the total phenolic content was

decreased. They concluded that the reduced total phenolic content after boiling in millet grains could be due to the degradation of phenolics upon heat treatment or leaching into the endosperm to form complexes with proteins and other macromolecules, thus making phenolics less extractable. Also, Siah *et al.* (2014) [18] reported boiling reduces the total phenol content of faba bean cultivars. Sharma *et al.* (2011) [17] reported microwave heat treatment reduced the total phenolic content of barley.

Total phenol =	$1.06 - 0.04 * X_1 - 0.15 * X_2 - 0.14 * X_3 - 0.06 * X_1X_2 + 0.05 * X_1X_3 - 0.07 * X_2X_3 + 0.43 * (X_1)^2 + 0.078 * (X_2)^2 + 0.06 * (X_3)^2$
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The computed F-value for total phenol (36.66) was significant at $p < 0.0001$. At the same time, it possessed non-significant lack of fit ($p > 0.05$). These figures highlighted that the model for total phenol was correctly fitted and reliable. The R^2 value and Adj- R^2 value for the total phenol were 0.9763 and 0.9497 respectively, which exceeded 0.8, indicating that the model was adequate, well fitting and

Table 2 displays the findings of the regression analysis and ANOVA for the total phenol of microwave treated pearl millet flour. The table indicates, that power and time of exposure showed negative linear effect which were significant at $p < 0.001$ respectively. The moisture content and power showed positive quadratic effect which there were significant at $p < 0.0001$ and $p < 0.05$. All the interaction effect and linear effect of moisture content showed non-significant effect on total phenol.

highly significant. The Pred- R^2 (0.7536) was in reasonable agreement with the Adj- R^2 . The high Adeq. Precision value (>4) further supported the significance of the model for total phenol. The lower value of coefficient of variation (6.31%) for total phenol explained that the experimental results were valid and determinative (Table 2)

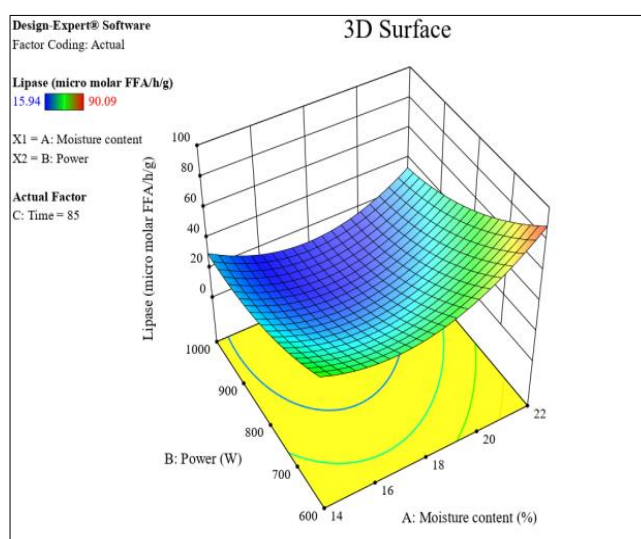


Fig 1(a): Lipase activity as a function of moisture content & power

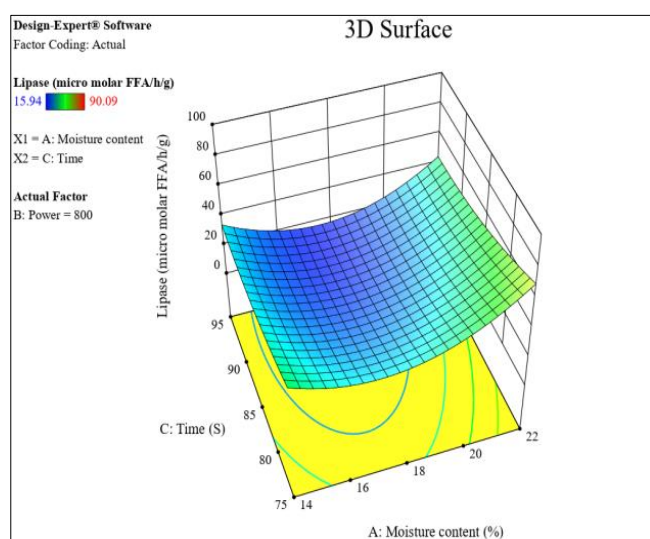


Fig 1(b): Lipase activity as a function of moisture content & time

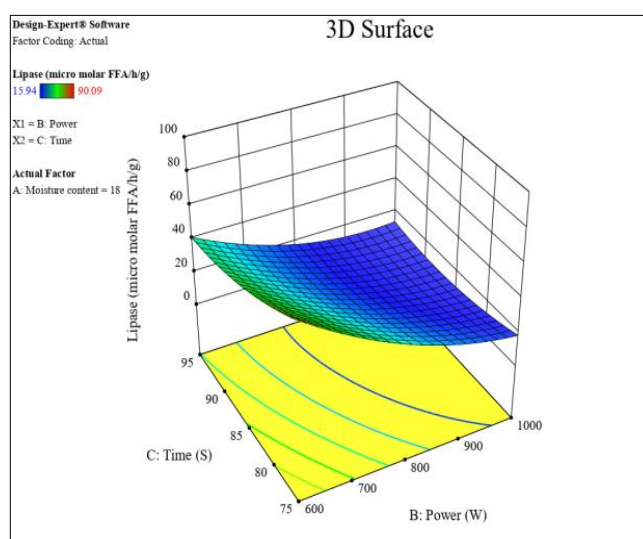


Fig 1(c): Lipase activity as a function of power & time

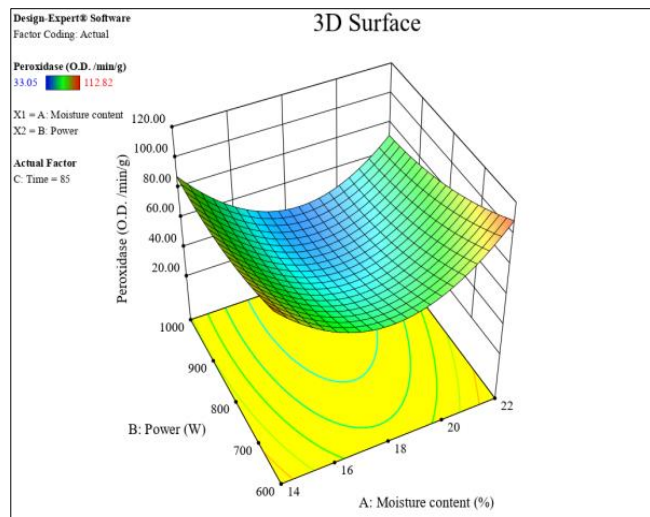


Fig 2(a): Peroxidase activity as a function of moisture content & power

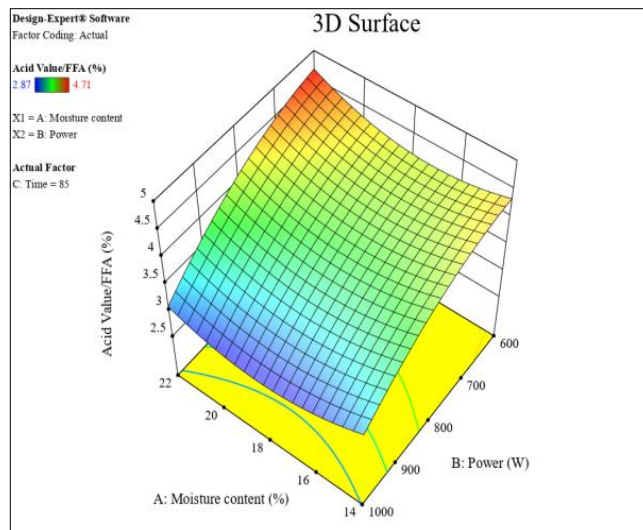


Fig 3(a): Acid value/FFA as a function of moisture content & power

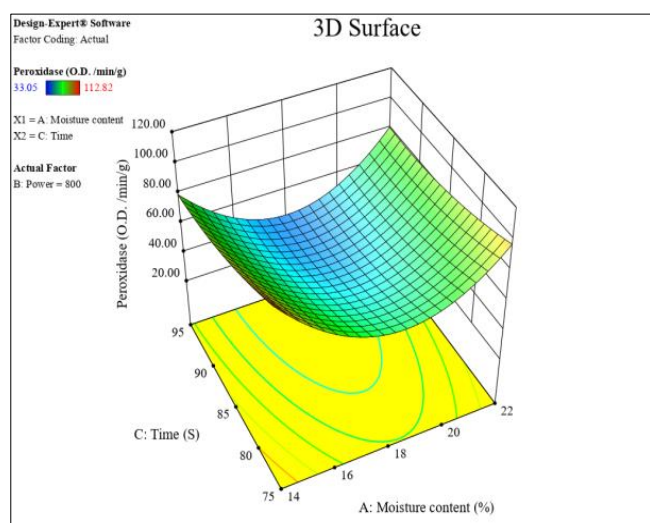


Fig 2(b): Peroxidase activity as a function of moisture content & time

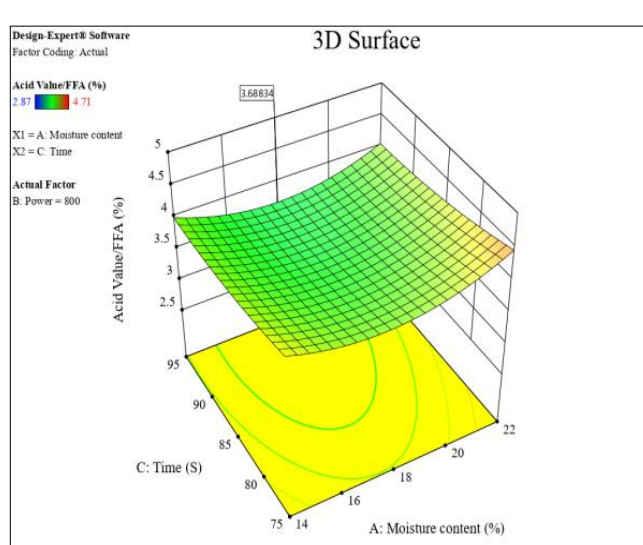


Fig 3(b): Acid value/FFA as a function of moisture content & time

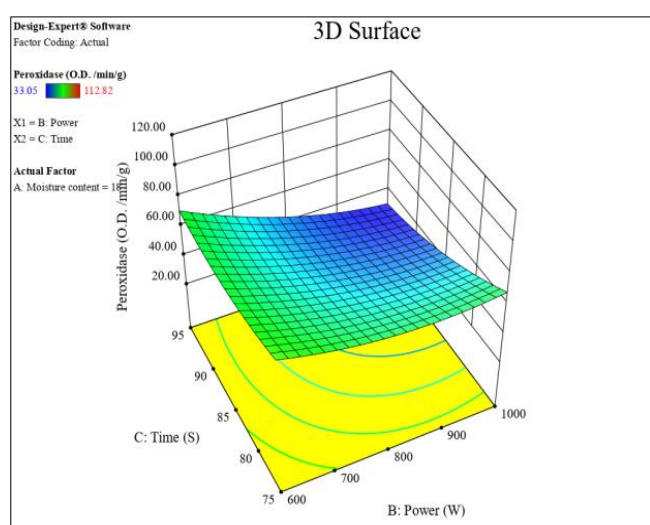


Fig 2(c): Peroxidase activity as a function of power & time

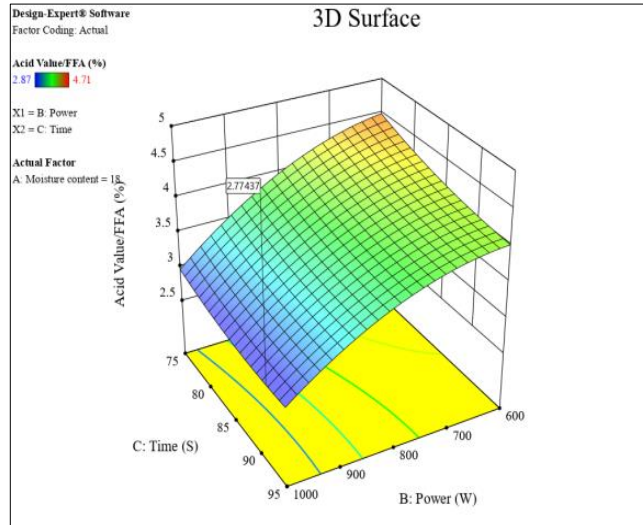


Fig 3(c): Acid value/FFA as a function of power & time

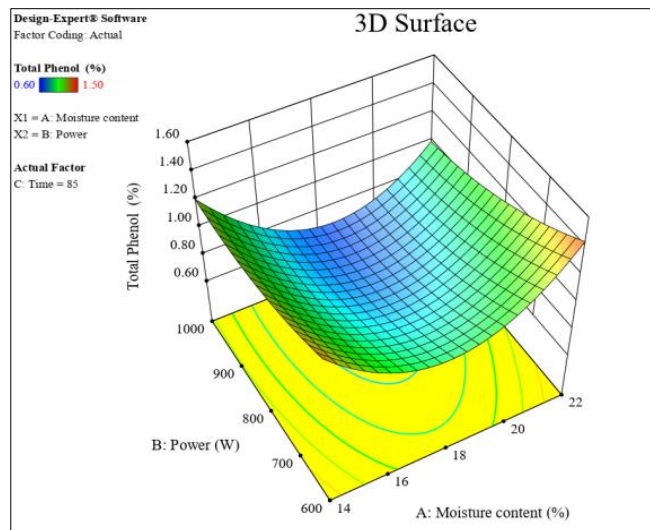


Fig 4 (a): Total phenol as a function of moisture content & power

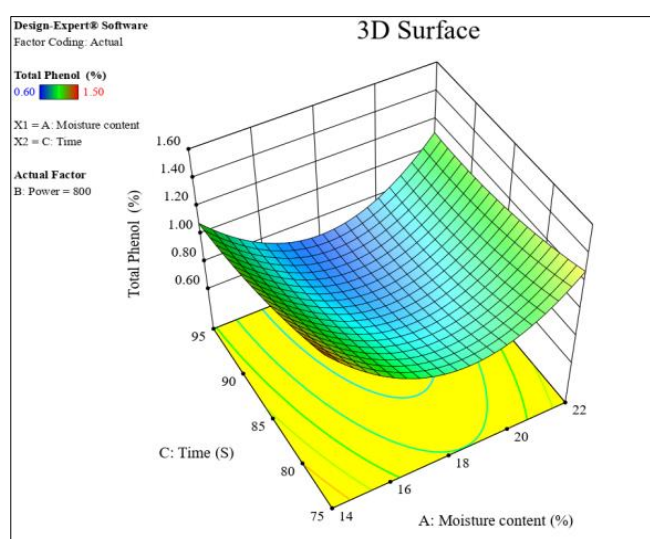


Fig 4(b): Total phenol as a function of moisture content & time

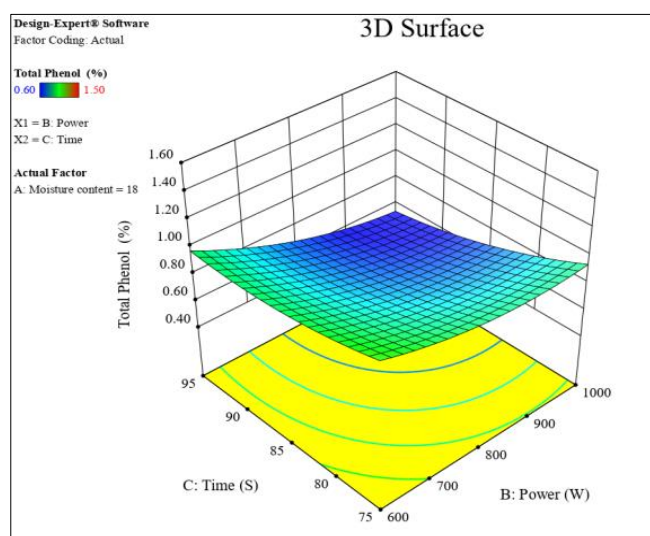


Fig 4(c): Total phenol as a function of power & time

3. Optimization and validation of process variables

The optimum condition for the microwave treatment of pearl millet grains was determined by numerical optimization technique, using Design Expert software version 13. The main criteria applied for the constraints

optimization in study were: (a) lipase activity: minimum, (b) peroxidase activity: minimum, (c) acid value/FFA: minimum, (d) total phenol: minimum. The optimum treatment conditions were found to be 17.5% (w.b) moisture content, 997.24 W power and 92.84 s time of exposure. The analysis showed that at this combination of moisture content, power and time of exposure with microwave, it would be possible to make microwave treated flour with a lipase activity of 12.018 μM FFA/h/g, peroxidase activity of 32.843 $\mu\text{M}/\text{min}/\text{g}$, acid value/FFA of 0.29%, total phenol of 0.569% with a desirability of 1. By considering the practical feasibility, the optimized conditions were slightly modified as 18% moisture content, 1000 W power level and 93 s time of exposure which resulted 12.5 μM FFA/h/g (4.01% deviation), peroxidase activity as 31.61 $\mu\text{M}/\text{min}/\text{g}$ (3.75% deviation), FFA content as 0.28% (3.44% deviation) and total phenol as 0.55% (1.78% deviation).

4. Conclusion

Based on the above study, it is suggested that to enhance the storage life of pearl millet flour, the optimized conditions are a moisture content of 18% (w.b.), a power of 1000 W and a time of 93 s. These conditions resulted the experimental values of lipase activity as 12.5 μM FFA/h/g, peroxidase activity as 31.61 $\mu\text{M}/\text{min}/\text{g}$, FFA content as 0.28% and total phenol as 0.55%.

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