Arresting Rancidityin Pearl Millet Flour through Halogen Air Frier Treatment

Abstract

Background: Pearl millet (*Pennisetum glaucum*), a highly nutritious and climate-resilient cereal, is constrained in its utilization due to rapid rancidity and bitterness in its flour. These issues stem from lipid oxidation and the enzymatic activities of lipase and lipoxygenase, which significantly limit the shelf life and consumer acceptability of the flour.

Aim: This study investigates the effect of halogen air fry treatment on the enzyme activity and color parameters of pearl millet flour.

Study design: The study was carried out at College of Food Processing Technology and Bio Energy, Anand, in the year 2024.

Methodology: Pearl millet grains were subjected to halogen air fry treatment at temperatures ranging from 110°C to 150°C for durations of 1 to 4 minutes with a bed thickness of 0.5 cm. Results:The lipase activity was significantly reduced in treated samples compared to the control, reaching negligible levels at 150°C for 4 minutes. Similarly, lipoxygenase activity was completely inactivated at 140°C and 150°C for 4 minutes. In addition, treated flour exhibited increased redness and yellowness, indicating alterations in color values due to heat treatment. These findings suggest that halogen air frying is an effective pre-milling treatment for inactivating rancidity-causing enzymes in pearl millet flour.

Conclusion:This intervention not only addresses the issue of rancidity but also extends the shelf life of the flour, improving its overall quality and acceptability. Halogen air frying offers a promising, efficient approach to enhancing the functional properties of pearl millet flour for wider utilization in food applications.

Keywords: Pearl millet, lipase, lipoxygenase, rancidity, Shelf life

Introduction

Pearl millet (*Pennisetum glaucum*), a resilient cereal crop, has been a staple in arid and semi-arid regions for millennia. It has gained significant attention due to its resilience to harsh environmental conditions and high nutritional value. Its origins trace back to the Sahel zone of West Africa. Archaeological evidence indicates its domestication around 2500–2000 BCE

in West Africa before introducing to the Indian subcontinent and other parts of Asia (Singh, 2023). Its ability to thrive in regions with high temperatures, low rainfall, and nutrient-poor soils underscores its importance as a climate-resilient crop (Djanaguiraman*et al*., 2024).

Globally, pearl millet is primarily cultivated in Africa and South Asia. In Africa, countries such as Nigeria, Niger, Mali, and Burkina Faso are leading producers, while in India, states like Rajasthan, Uttar Pradesh, Haryana, Gujarat, and Madya Pradesh dominate its production. India alone contributes to over 40% of the global pearl millet production by producing 9.62 million tonnes, owing to its adaptability to the semi-arid tropical climate(Directorate of Economics & Statistics, 2022). Its role in ensuring food security for millions in these regions is particularly critical, especially for marginal and resource-poor farmers.

Pearl millet is recognized for its high nutritional profile, which includes high levels of protein, dietary fiber, essential amino acids, and micronutrients like iron and zinc (Vinay and Singh, 2024). Despite these benefits, its consumption faces several barriers, primarily due to issues with the quality and acceptability of processed products. One of the most significant challenges is the rapid onset of rancidity in pearl millet flour, which severely limits its shelf life (Bhargavi, 2024). Rancidity in pearl millet flour arises from the high lipid content of the grain, particularly in its germ, coupled with the activity of lipolytic and oxidative enzymes such as lipase and lipoxygenase (Ravi and Rana, 2024).

The mechanism of rancidity involves two major pathways: enzymatic and oxidative. Enzymatic rancidity is triggered by lipase, which hydrolyzes triglycerides into free fatty acids. These free fatty acids are further oxidized, leading to the formation of hydroperoxides and secondary volatile compounds like aldehydes and ketones that impart unpleasant odors and flavors (Sruthi and Rao, 2021). Similarly, oxidative rancidity occurs due to the action of lipoxygenase, which catalyzes the oxidation of unsaturated fatty acids, resulting in a deterioration of flavor and quality. The development of bitterness in pearl millet flour is often associated with these processes, compounded by the activity of polyphenol oxidase, which contributes to enzymatic browning and further affects consumer acceptability ((Ali*et al*., 2023).

Processing limitations add to the challenges of utilizing pearl millet in food applications. Traditional methods of milling, such as stone grinding, expose the grain to oxygen and moisture, accelerating rancidity. Moreover, the lack of standardized processing techniques in many production areas limits the development of consistent and high-quality pearl millet

products.Efforts to mitigate these challenges have focused on improving processing methods and storage techniques. Thermal treatments, such as hydrothermal, blanching, steaming roasting and extrusion, have been shown to inactivate lipase and lipoxygenase enzymes, thereby enhancing the storage stability of pearl millet flour (Kumar*et al.*, 2024; Pathare*et* al., 2024). Similarly, novel techniques such as microwave treatment and controlled-atmosphere packaging have demonstrated potential to delay rancidity and preserve the sensory quality of pearl millet products (Yadav*et al*., 2012). However, the scalability and economic feasibility of these interventions remain areas of active research.

The present investigation aims to inhibit rancidity-causing enzymes through halogen air frier treatment with minimal color changes in the pearl millet flour.

Materials and Methods

The pearl millet of variety Shree Sagar 8484 was procured from the Jibratha Seed Tech LLP, Ahmedabad. The seeds were cleaned to remove immature seeds, stalks, and foreign materials. The standard oleic and linoleic acids were purchased from Sigma Aldrich and the rest of the chemicals were procured from Hi-Media, SRL and Loba Chemie.

Halogen air fry treatment

The Halogen air frier (Halogen oven infiniticook3514I, Usha International Ltd, New Delhi)of 12 L capacity with 1300 Wwas pre-heatedfor 1 minute. The pearl millet grains were spread on the Teflon plate with a bed thickness of 0.5 cm. Later it was placed in a halogen oven and treatment was given at a temperature ranging from 110-150□ for 1-4minutes. The treated grains were allowed to cool in a desiccator to minimize the moisture gain. After cooling the grains were milled in a laboratory mixer grinder to a fine flour form and sieved in 250 μsize sieve. The flour was packed in metallized laminates (thickness: 110 μ) until further analysis.

Preparation of enzyme extract

The one gram of flour was homogenized with 10 ml of 0.2 M potassium phosphate buffer in a prechilled pestle and mortar. The mixture was centrifuged in a refrigerated centrifuge at 10,000 rpm for 20 min at 4□. The supernatant was decanted carefully into another test tube and it was used as crude enzyme extract to determine the enzyme activities.

Lipase assay

The lipase assay was determined as per the method of Pathare*et al*. (2024). An aliquot of 100 µL enzyme extract was combined with 1 mL of substrate solution consisting of 0.98% (w/v) NaCl, 200 µL of Tween 20, and an olive oil-water mixture in a 1:3 ratio. The reaction mixture was incubated at 37 °C for 15 minutes, followed by heat inactivation in a water bath at 90 °C for 5 minutes. Subsequently, 5 mL of chloroform was added to the mixture, and it was allowed to incubate at room temperature for 30 minutes. To the lower phase, 2 mL of copper triethanolamine reagent, comprising 1 M triethanolamine, 1 N acetic acid, and 6.45% (w/v) copper nitrate, was thoroughly mixed. This resulted in two distinct color layers: an upper blue layer and a lower yellow layer. Next, 100 µL of 11 mM diethyl dithiocarbamate (DDC) was added to the lower yellow layer, and its absorbance was measured at 440 nm. A standard curve using oleic acid was prepared, and enzyme activity was determined in terms of mM free fatty acids released per minute per mg of soluble protein.

Lipoxygenase assay

The assay was performed according to the method mentioned by Goyal and Chugh (2017), using linoleic acid as the substrate. The substrate solution was prepared by gradually adding 0.5 mL of linoleic acid dropwise into 0.5 mL of Tween 20 dissolved in 10 mL of borate buffer at pH 9.0, ensuring thorough mixing to form a fine emulsion. Following this, 1.3 mL of 1 N NaOH was added with continuous stirring to maintain dispersion. Subsequently, 90 mL of borate buffer was mixed into the solution, and the final volume was adjusted to 200 mL with distilled water, yielding a linoleic acid substrate concentration of approximately 7.5 mM.

For the assay, a reaction mixture with a total volume of 2.66 mL was prepared. This mixture consisted of 2.5 mL of 0.05 M acetate buffer (pH 4.2), 60 µL of the linoleic acid substrate solution (7.5 mM), and 0.1 mL of enzyme extract. The reaction progress was monitored spectrophotometrically by measuring the increase in absorbance at 234 nm for 2 minutes, using a blank as the reference. The LOX activity was expressed as nanomoles of hydroperoxide produced per minute per milligram of soluble protein (nM HPOD min^{-1} mg^{-1} soluble protein), calculated using the molar extinction coefficient of linoleic acid (25 mM^{-1} cm^{-1}).

Color values

The color values of pearl millet flourwas measured by Lovibond colorimeter (Model: RT850i) in terms of L*(Lightness), a*(redness and greenness) and b* values (yellowness and blueness). The instrument was calibrated with white and black standards before conducting a

test. A flour is filled into a cuvette and placed against the light source (D65). Data were received through the software in terms of *L** (lightness), ranging from 0 (black) to 100 (white), *a** (redness), ranging from +60 (red) to -60 (green), and *b** (yellowness), ranging from +60 (yellow) to -60 (blue) values.

Statistical analysis

The results were recorded along with their mean and standard deviation, based on triplicate measurements. One way ANOVA was performed to test the significant difference between the treatments using Tukey (HSD) test.

Results and Discussion

Effect of air fry treatment on lipase activity

The pearl millet flour is susceptible to rancidity by enzymes such as lipase and lipoxygenase and is inactivated at high temperatures. Lipase hydrolyse fat into free fatty acids and hence onset of rancidity occurs. The control pearl millet flour (without treatment) had a lipase activity of 475 mM/min/mg protein. While after giving air fry treatment the lipase activity of the pearl millet flour decreased considerably as depicted in Figure 1. The lipase activity was decreased to 330 mM/min/mg protein at 110□ for 1 minute, further, its activity decreased to 73.76 mM/min/mg protein after 4 minutes at the same temperature. At 120□ for 1 minute, the lipase activity was found to be 285.43 mM/min/mg protein and it decreased significantly ($p<0.05$) with time and at 4 minutes the activity was noticed to be 57.89 mM/min/mg protein. The lipase activity of flour treated at 130□ for 1 minute was 226.92 mM/min/mg protein and after 4 minute it was 44.23 mM/min/mg protein. Additionally, the lipase activity of flour at 140□ for 1 minute was 181.78 mM/min/mg protein and decreased to 34.22 mM/min/mg protein after 4 minutes. Furthermore, the lipase activity at 150□/1 minute was observed to be 173.92 mM/min/mg protein and after 4 minutes, it decreased remarkably to 16.44 mM/min/mg protein. The complete inactivation was not achieved through this treatment but lipase activity was decreased to 16.44 mM/min/mg protein, which is sufficient for the shelf life extension of pearl millet flour for at least 3 months from the 15 days. A similar maximum inhibition was observed by Pathare*et al.* (2024) in extruded pearl millet flour at 160□. The enzyme activity decreased with temperature and time due to the denaturation of protein, thereby active site of enzymes was modified and finally its losses its structural integrity.

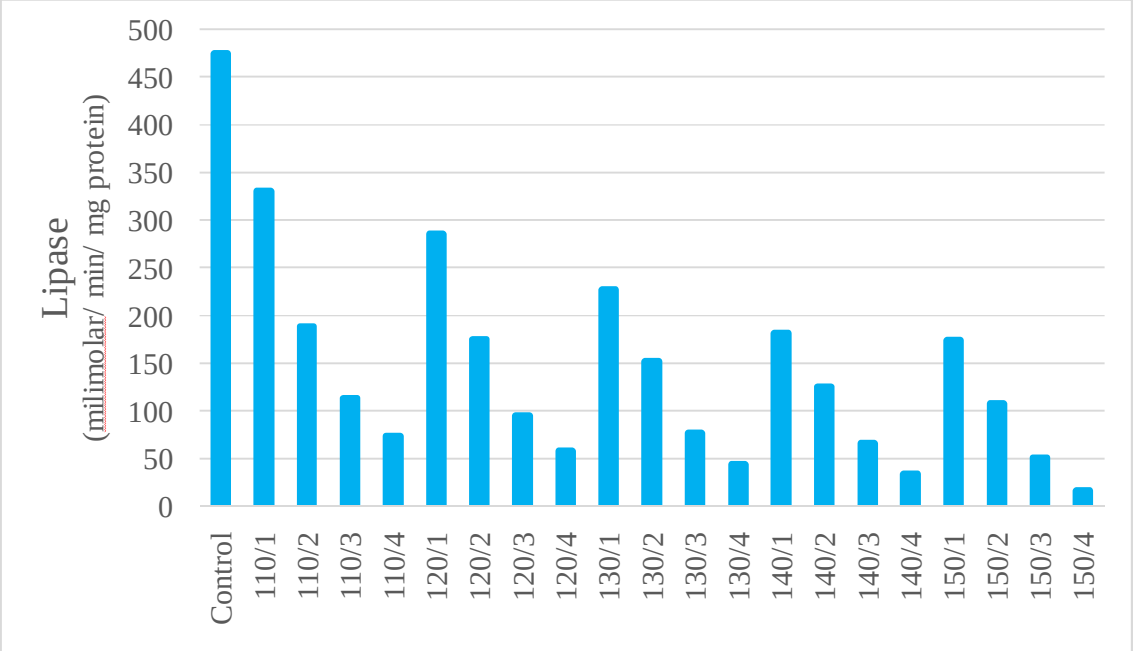

Fig 1: Lipase activity of pearl millet flour with the air fry treatment. 110, 120, 130, 140 & 150 are the temperature in □ and 1, 2,3 & 4 are the time in minutes

Effect of air fry treatment on lipoxygenase activity

The free fatty acids formed from hydrolysis of fat by lipase enzyme is further converted into aldehydes and ketones lipoxygenase enzyme. The inactivation of LOX enzyme stabilizes the pearl millet flour by preventing oxidation. The LOX activity of pearl millet flour is depicted in the Figure 2. As shown in Figure 2, the control flour had the highest LOX activity of 0.81 nM HPOD/min/mg of protein. As LOX enzyme is more sensitive to heat treatment, all ait fry treated flour showed lower enzyme activity (<0.1 nM HPOD/min/mg of protein). The LOX activity of flour at 110□/1 minute was 0.09 nM HPOD/min/mg of protein and decreased to 0.02 nM HPOD/min/mg of protein after 4 minutes. Similarly, LOX activity reduced at 120□/1 minute of 0.08 nM HPOD/min/mg of protein and after 4 minutes decreased to 0.02 nM HPOD/min/mg of protein. The LOX activity was completely not observed at 140□/4 minutes and 150□/4 minutes. A similar decrease in the LOX activity was observed by Vinutha*et al*. (2022), where they have given treatment to flour in NIR lamp (150watt X 4/240 V).

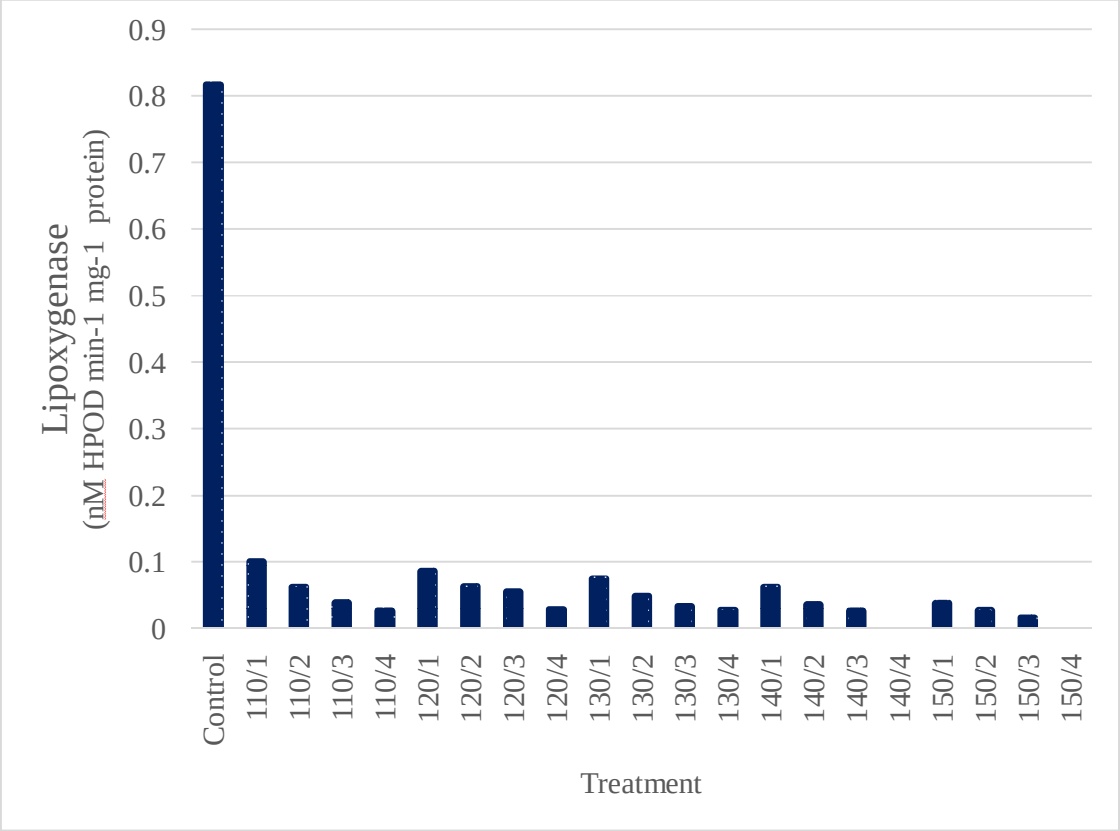

Fig 2: Lipoxygenase activity of pearl millet flour

Effect on color values

The color values indicate the changes in the appearance of pearl millet flour after undergoing halogen air fry treatment. These values, as presented in Table 1, provide insights into the impact of processing on the flour's visual characteristics. The lightness value (L*) of the untreated control sample was 61.82, which significantly decreased after treatment. However, the variations in lightness did not follow a consistent trend across different treatment conditions. The positive a* values indicate the redness of the samples; the control sample had an a* value of 1.28, while the redness of all treated samples increased significantly ($p < 0.05$) with higher temperatures and longer treatment times. Similarly, the b* values, which represent the yellowness of the flour, showed a notable increase. The untreated control had a b* value of 12.19, and this value significantly ($p < 0.05$) increased with treatment time and temperature. These changes in redness and yellowness suggest that higher temperatures induce Maillard reactions or caramelization, leading to darker, more intense color changes in the flour. Overall, the results demonstrate that high-temperature treatment not only alters the

enzyme activity but also significantly impacts the visual properties of the flour, which may influence consumer perception and product quality.

Table1: Effect of halogen air fry on color values of pearl millet flour

Temperature	Time	L*	a*	b*
Control	-	61.82 ± 0.11^{b}	1.28 ± 0.09^{bcd}	12.19 ± 0.06^{a}
110□	1 minute	67.1 ± 0.19^{de}	0.91 ± 0.02^{a}	13.38 ± 0.14^{c}
	2 minutes	69.08 ± 0.74^{gh}	2.05 ± 0.06^{e}	16.1 ± 0.05^{e}
	3 minutes	62.03 ± 0.85^{b}	5.86 ± 0.16^{j}	19.35 ± 0.04^{g}
	4 minutes	59.51 ± 0.36^{a}	5.79 ± 0.1^{j}	20.71 ± 0.34^{ij}
120□	1 minute	66.31 ± 0.43^{de}	0.94 ± 0.13^{a}	12.57 ± 0.27^{ab}
	2 minutes	69.21 ± 0.49^{gh}	1.32 ± 0.08^{cd}	14.43 ± 0.26^{d}
	3 minutes	66.47 ± 0.26^{de}	3.98 ± 0.07^{g}	19.39 ± 0.13^{g}
	4 minutes	61.59 ± 0.14^{b}	6.3 ± 0.1+k	21 ± 0.25^{ij}
130□	1 minute	68.87 ± 0.06^{gh}	1.07 ± 0.04^{abc}	13.43 ± 0.12^{c}
	2 minutes	69.94 ± 0.1^{h}	1.43 ± 0.07^{d}	14.94 ± 0.03^{d}
	3 minutes	66.59 ± 0.02^{de}	4.72 ± 0.02^{h}	20.57 ± 0.03^{hi}
	4 minutes	63.91 ± 0.17^{c}	5.15 ± 0.02^{i}	20.34 ± 0.06^{ij}
140□	1 minute	68.31 ± 0.23^{fg}	0.98 ± 0^{a}	13.22 ± 0.18^{c}
	2 minutes	69.79 ± 0.52^{h}	1.31 ± 0.07^{cd}	14.73 ± 0.29^{d}
	3 minutes	67.41 ± 0.38^{ef}	4.15 ± 0.07^{g}	19.83 ± 0.24^{gh}
	4 minutes	62.2 ± 0.18^{b}	6.04 ± 0.01^{jk}	20.9 ± 0.02^{ij}
150□	1 minute	66.24 ± 0.52^{d}	1.02 ± 0.09^{ab}	12.37 ± 0.35^{ab}
	2 minutes	67.26 ± 0.22^{def}	1.13 ± 0.01	13 ± 0.04^{bc}
	3 minutes	68.81 ± 0.13^{gh}	3.02 ± 0.18^{f}	18.05 ± 0.41^{f}
	4 minutes	63.81 ± 0.01^{c}	5.41 ± 0.03^{i}	20.85 ± 0.03^{ij}

Data are represented as mean ± SD (n=3). The different superscript of letters in a column significantly varies ($p<0.05$)

Conclusion

Pearl millet utilization is limited by the rapid onset of rancidity and bitterness in its flour due to the enzymatic activities of lipase and lipoxygenase. This study demonstrated that halogen air fry treatment effectively addresses these issues by significantly reducing lipase activity and completely inactivating lipoxygenase at 150°C for 4 minutes. The treatment also altered the flour's color properties, increasing redness and yellowness, which may enhance its visual appeal. These findings suggest that halogen air frying is a practical pre-milling treatment for

extending the shelf life and improving the sensory quality of pearl millet flour. By inactivating rancidity-causing enzymes, this method provides a simple and efficient solution for overcoming a key barrier to the broader utilization of pearl millet. The approach holds promise for enhancing the functional and economic value of pearl millet, paving the way for its greater application in food systems while maintaining its nutritional benefits. Future studies could focus on optimizing processing conditions with the moisture contentcan enhance quality and consumer acceptability.

Data Availability

The data obtained during the study are available from the corresponding author upon reasonable request.

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