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A Perspective on Addressing Rancidity in Pearl Millet

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Abstract: Pearl millet is a highly nutritious crop having the inherent capacity to grow under adverse environmental conditions without compromising with the quality and quantity of the grains. Among millets, pearl millet has many health benefits and new cultivars that are being conventionally cultivated provides a higher level of dietary iron to women. However, off-odour development in flour is one of the major limiting factors in the popularization of pearl millet all over the world. The poor keeping quality of pearl millet flour is due to the oxidative/hydrolytic rancidity caused by enzymes like lipase and lipoxygenase that hydrolyse triacylglycerol to free fatty acids and leads to rapid deterioration of flour during storage. In this review, the changes observed in nutritional value of pearl millet flour during storage, key biochemical parameters associated with the development of flour rancidity and the genetics controlling rancidity associated traits are discussed.

Key words: Pearl millet, nutritional security, rancidity, climate-resilience, shelf life, lipase, lipoxygenase

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Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is the staple food of majority of the poor and small land holders, as well as a source of feed and fodder for livestock in the rainfed regions of the country. Following wheat, rice and maize, pearl millet is the fourth most widely cultivated food grain crop in India. Rajasthan, Maharashtra, Uttar Pradesh, Haryana and Gujarat accounted for more than 90% of the total area under pearl millet and contributed to 87.7% of the total production (Jorben *et al.*, 2020). In India, average production of pearl millet during 2020-21 is 10.86 mt cultivated in an area of 7.65 mha with productivity of 1420 kg ha^{-1} (Anonymous, 2021).

Being a C4 plant with high photosynthetic efficiency and dry matter production capacity, pearl millet excels all other cereals. It is a climate-resilient crop having the inherent capacity to tolerate the vagaries of nature without compromising with the quality and quantity of the grains (Singh *et al.*, 2017). Pearl millet has the ability to grow under unfavorable agro-climatic conditions such as low soil fertility, high temperature and low rainfall (200-600 mm) and therefore can be grown as substitute crop for staple crops like rice, wheat and maize (Shivhare and Lata, 2017). In India, substituting diversified wheat-rice cropping system with millets and providing the same in the diet will help in combating the problem of food scarcity (Chandrasekara and Shahidi, 2012). Among the various millets such as foxtail millet, finger millet, barnyard millet, proso millet, kodo millet and little millet, pearl millet is the one having unlimited health benefits and is regarded as the powerhouse of nutrition (Vanisha *et al.,* 2011).

Nutritive value and health benefits (nutritional security)

Although food grain production has increased, in the present situation, nutritional security is crucial to feed growing population and meet out the good health of people. To lead an active and healthy life, intake of all essential macronutrients and micronutrients in sufficient quantities through a balanced diet is needed. Pearl millet is the primary source of energy for persons or communities inhabiting the semi-arid tropics and drought-prone regions of Asia and Africa due to its nutritionally superior grain enriched with high amounts of essential amino acids, proteins, better fat digestibility, vitamins and minerals (Patil *et al.*, 2021). Pearl millet grain has a larger germ layer than other cereals, apart from maize (Taylor, 2004), and has a higher lipid content (5-7%) characterized by high levels of unsaturated fatty acids (Sharma *et al.,* 2015). It is a good source of carbohydrate, dietary fiber (1.2/100 g), 13.6% crude protein, quality protein (8-19%), 63.2% starch, α-amylase activity, minerals (2.3 mg/100 g), vitamins A and B, and antioxidants such as coumaric acids and ferulic acid, high levels of polyphenols and other biologically advantageous compounds, thereby designating it as a "Smart food" (Tako *et al.,* 2015).

It contains about 74% of polyunsaturated fatty acids (PUFA) with nutritionally important omega-3 fatty acids such as oleic acid (25%), linoleic acid (45%) and linolenic acid (4%) which are incredibly important for overall health particularly for the brain (Goswami *et al.*, 2020). The typical fatty acid profile of pearl millet triglycerides is presented in Fig.1. Being gluten free, pearl millet is widely recommended for the patients with celiac disease who are generally allergic to the gluten content of wheat and other cereals (Singh *et al.,* 2018). It prevents constipation by acting as a probiotic diet for the microorganisms in our bodies. The niacin found in pearl millet grain has the capability to decrease cholesterol (Satyavati *et al.*, 2021).

Pearl millet consumption also controls diabetes at an early stage due to the presence of high proportion of resistant starch (RS) and slowly digestible starch (SDS) which contributes to the low glycemic index (GI) (Annor *et al.,* 2015). In terms of both macronutrients and micronutrients, pearl millet has higher concentrations of iron, zinc, magnesium, calcium, phosphorus, copper, manganese, riboflavin, and folic acid. Owing to such excellent nutritional values, it is becoming more popular and is preferred by people all over the world even in industrialised countries.

Due to its nutritional superiority, various health benefits and climate-resilience nature,

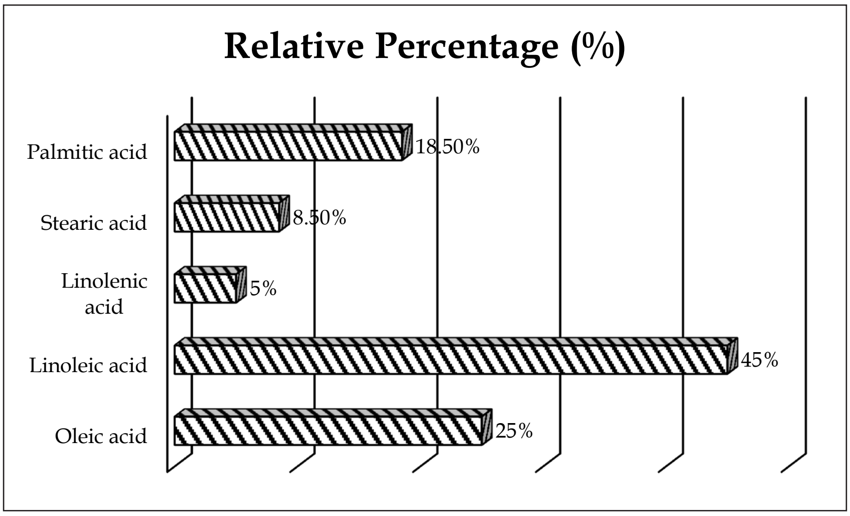

Fig. 1. Typical fatty acid composition of pearl millet triglycerides.

pearl millet has been renamed as "Nutri-cereal" (Gazette of India, No. 133 dated 13 April, 2018) (Satyavati *et al.,*2021). Recently, the year 2023 has been declared as "International Year of Millets" by the general assembly of United Nation (UN), which was further approved by Food and Agriculture Organization (FAO), Rome.

Changes observed in the nutritional value of pearl millet flour during storage:

Mostly, pearl millet is ground at home without discarding any portion of grain. Few days after milling, the flour becomes rancid due to the absorption of lipids present in the germ, increase in free fatty acids, fat acidity as well as variations in the proportions of polar and non-polar lipids (Gray, 1978). With the advancement of technology, the milling process has been industrialised which results in the lengthy storage period. During storage, moisture content and reducing sugars are increased as reported by Lai and Varriano-Marston (1980). Vinutha *et al.* (2022) observed a significant decrease in the enzyme activities of lipase (47.8%), lipoxygenase (84.8%), peroxidase (98.1%) and polyphenol oxidase (100%) in hydro treatment (HT)- hydro thermal treatment (HTh)- thermal infrared rays (thNIR) treated flour compared to the individual treated flour. A slight decrease in the concentration of oleic, linoleic and linolenic acids and relative increase in palmitic and stearic acids was observed by Carnovale and Quaglia (1973).

Enzymes contributing to flour rancidity

Despite having a nutrient punch, the full potential of pearl millet flour is limited to few specific regions all around the world due to the poor keeping quality of the flour and development of off-odour during storage. It is mainly due to the presence of high active lipases coupled with highly unsaturated fatty acids which causes hydrolysis of fats to fatty acids that leads to rapid deterioration of flour during storage.

Lipase (triacylglycerol acyl hydrolase, E.C.3.1.1.3) enzyme, is concentrated in the pericarp, aleurone layer and germ and accounts for the stepwise hydrolysis of the triacylglycerol into diacylglycerol, monoacylglycerol, glycerol and free fatty acids. Since unsaturated fatty acids are in abundance, they get oxidised in presence of moisture and oxygen, resulting in rapid deterioration in the fat quality of pearl millet flour. It has been reported that pearl millet lipase shows relatively higher activity than that of most other cereal grains (Galliard, 1999).

Pearl millet is also reported to contain oxidative enzymes such as peroxidases (Reddy *et al*., 1986) and "enzymatic browning" catalysing enzymes such as polyphenol oxidase (PPO), both of which play an important role in deterioration of pearl millet flour quality, immediately after milling of the grains, due to development of rancidity in the flour. Hence, when pearl millet is milled into flour under conditions of moderately high moisture and oxygen exposure, chemical changes manifest themselves as off odors and/or off-taste of the flour or in products made from the flour. Oxidative rancidity results in hydroperoxides (chain reaction through autoxidation) and subsequently generation of off-odour causing volatile secondary metabolites (aldehydes, ketones, acids, polymers etc.). Enzymatic rancidity results in free fatty acids by the action of lipase and further generation of bitter and mousy odour causing phenolic aglycones, by the action of Peroxidase on C-glycosyl flavones (Eskin and Przybylski, 2001). Bitter compounds are also formed due to enzymatic browning by the action of polyphenol oxidase (PPO). The schematic representation of the mechanism of onset of rancidity is presented in Fig. 2. Overall, the hydrolytic and oxidative rancidity causes the formation of off-odour and low shelf-life of the pearl millet flour.

Recently, Goswami *et al.* (2020) developed rancidity matrix for 93 pearl millet genotypes using different biochemical markers such as comprehensive peroxide value (CPV) and comprehensive acid value (CAV) along with the activity of the enzymes lipase and lipoxygenase in fresh flour (group I) and 10 days stored flour (group II). A pilot experiment performed to standardize the critical stage for rancidity measuring tests revealed that maximum CPV was reached on the 10^{th} day and thereafter its value gradually decreased indicating that the 10^{th} day is a crucial stage for measuring rancidity status in pearl millet flour. They revealed that different pearl millet genotypes behave differently for CPV and CAV both in group I and group II and therefore classified

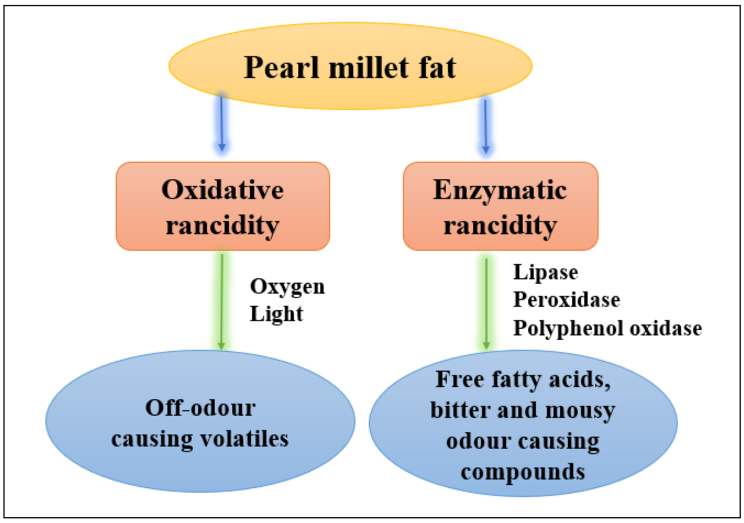

Fig. 2. Schematic representation of the rancidity mechanism.

genotypes into low, medium and high rancid categories. Further, each category was divided into two classes and hence total six classes (Table 1) were formed which can prove very useful for the pearl millet breeders to develop low rancid pearl millet lines.

Genetic improvement for low rancidity

Pearl millet landraces (Jafrabadi, Chanana Bajra-2, Chadi Bajra and Damodara Bajra) are potential genetic material to develop low rancid with nutritionally superior varieties of pearl millet. On comparison of these four pearl millet landraces with biofortified pearl millet variety (Dhanashakti) for off flavour development and nutritional quality, they showed longer and better shelf life as reported by Vinutha *et al.* (2022). The combining ability studies revealed ICMA 054444, ICMA 04999, ICMA 98222, ICMA 94444 and ICMR 074444 are the best combiners for grain yield and rancidity associated characters and can be used in development of low rancid, high yielding hybrids. Higher variance due to specific combining ability for rancidity associated characters indicated the predominance of non-additive gene action. Alcoholic acidity as a measure of rancidity showed a significant positive association with test weight, lipase and lipoxygenase in parents and negative association with test weight in hybrids. All these clearly suggest the role of enzyme lipase and peroxidase in development of rancidity in pearl millet (Pallavi *et al.*, 2023). Goyal *et al.* (2015) identified two contrasting inbreds, G73-107 (high rancid) and HBL 11 (low rancid) with respect to hydrolytic rancidity and reported that stability of contrasting nature of flour (with respect to fat content, development of fat acidity, pH of water extract of flour and enzyme activities) of these inbreds was not altered by period of storage. These inbreds are now being used to test heritability of rancidity in hybrids and in developing low rancid pearl millet hybrids/composites (Goyal *et al.*, 2015).

Aher *et al.* (2022) identified mutations in TAG lipase genes *PgTAGLip1* and *PgTAGLip2* that contribute to lower amounts of free fatty acid accumulation in seeds after milling. Therefore, allelic variations in these genes provide an opportunity for future chemical mutagenesis or CRISPR-based approaches to create loss-of-function of lipase genes *PgTAGLip1* or

Table 1. Rancidity matrix developed to classify the pearl millet genotypes

Rancidity matrix		CAV_0	CAV_{10}	CPV_0	CPV_{10}
Low rancid	Group – I	1.2-1.6	1.5-2.0	0-15	9-30
	Group – II	1.7-2.0	2.1-2.8	16-30	31-60
Medium rancid	Group – III	2.1-3.5	2.9-4.5	31-45	61-75
	Group – IV	3.5-4.5	4.6-6.0	46-60	76-90
High rancid	Group – V	4.6-6.0	6.1-7.0	61-80	91-110
	Group - VI	>6.0	>7.0	>80	>110

PgTAGLip2, thereby mitigating rancidity in elite milled pearl millet germplasm.

Processing technologies for increasing shelf life

Also, effect of rancidity not only depends on the length of storage period but also on the type of containers in which they are stored. Chaudhary and Kapoor (1984) reported that pearl millet flour becomes rancid on days 6,7,8 and 10 and inedible on days 11,12,13 and 14 of storage in gunny sacks, earthen pots, tin cans and polythene bags, respectively.

Millets are usually processed before consumption to remove the inedible portions, extend the shelf life, and improve nutritional and sensory properties. Primary processing techniques such as dehulling, soaking, germination, roasting, drying, polishing and milling (size reduction) are followed to make millets fit for consumption, while modern or secondary processing methods such as fermenting, parboiling, cooking, puffing, popping, malting, baking, flaking, extrusion, etc., are used to develop millet-based value-added processed food products (Birania *et al.,* 2020). Although these processing techniques aim to enhance the digestibility and nutrient bioavailability, a significant amount of nutrients are lost during subsequent processing (Nazni *et al.,* 2016). So, understanding changes in the nutritional composition and digestibility with respect to different mechanical processing methods (Fig. 3) helps in choosing suitable processing method to maximize nutrient availability, improve palatability, and increase shelf life (Gowda *et al.,* 2022).

In a study conducted by Datta Mazumdar *et al.* (2016) as part of ICRISAT's involvement in the CGIAR Research Program on Dry land Cereals in order to evaluate the suitability of popular Indian commercial varieties/hybrids for obtaining shelf-stable pearl millet flour, acid values and peroxide values of pearl millet flour stored under three storage conditions *i.e.,* refrigerated (4°C), room temperature (25°C) and accelerated (35°C, 70% RH) revealed the existence of diversity in the rancidity profile among the pearl millet varieties/hybrids. Peroxide value was found to increase till 10th day (reached maximum on 10th day) and gradually decreased, while the acid value increased over the storage period. This helps in identifying the genotypes least susceptible to rancidity that can be promoted for use in production of shelf-stable pearl millet flour in conjunction with appropriate pre-treatment, processing and packaging technologies.

Way Forward to overcome Rancidity

Development of off-flavour in pearl millet flour upon storage after a short time is the major hindrance for its acceptability by the consumers. The off-flavour is an old and unresolved problem associated with pearl millet and is the major hindrance for consumer acceptability. Thus, use of pearl millet in food industry and consequently its consumption by urban population is very low. Generally hydrothermal treatment and combinations of more than one technique can be applied to extend pearl

Nutritional Property / Processing Method	Energy	Carbo-hydrate	Protein	Minerals	Dietary Fiber	Fat	Vitamins	Anti-oxidants
Dehulling	←	→	←	←	←	←	←	----
Milling	----	----	←	←	←	←	←	----
Soaking	----	←	----	⇄	----	←	----	←
Germinating	→	⇄	→	→	→	⇄	→	→
Malting	----	----	----	----	→	←	----	----
Fermenting	----	→	→	→	→	←	→	----
Roasting	←	----	←	⇄	⇄	⇄	----	----
Extrusion	←	←	←	----	←	----	----	----
Cooking	←	←	⇄	----	→	←	----	----
Puffing	----	----	→	⇄	←	←	----	----

Increase → Decrease ← Either increase or decrease ⇄ Remain constant – – –

Fig. 3. Changes in the nutritional properties due to different processing methods.

millet flour shelf-life. While these techniques have been effective in minimizing rancidity to some extent, only large-scale processing would justify their economic feasibility, which may not be practical in rural agricultural communities. The improved stability of shelf life following stringent treatments suggests that biological enzymatic driven processes drive the generation of off-flavour volatiles in pearl millet flours. Thus, genetic improvement is a promising alternative to physical stabilization techniques for extending the storage life of flour.

Once if the qualitative and quantitative relationship between particular constituent(s) of grain, rancidity determinants and organoleptic evaluation is established, it is possible to develop rancid free pearl millet hybrid/ variety through conventional plant breeding by increasing inherent capacity of crop to produce low rancid flour. CRISPR-based approaches to create loss-of-function of lipase genes, thereby mitigating rancidity in elite milled pearl millet germplasm needs exploration.

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