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Regulation of lipase activity to arrest lipid hydrolysis in pearl millet (*Pennisetum glaucum* L.)

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Abstract
Food security is the major challenge being faced by the developing world, especially in the Least Developed Countries (LDCs). Diversification in terms of food habits and agricultural by incorporating millets along with other regular cereals will provide a safe option for overcoming the problem of food scarcity. Among millets, pearl millet has received major focus owing to its excellent nutritional composition. Despite nutritionally rich and high medicinal value, the full potential of pearl millet is limited, due to high rancidity as a result of lipase activity. The present study therefore aims to reduce lipolysis by inactivating lipases in pearl millet genotypes differing in seed coat colour *viz.* black, white and purple. Various physical treatments have been applied on selected genotypes like dry-heat treatment, microwave irradiation and vacuum packaging. Among, various treatments, Microwave treatment proved to be better than dry heat in reducing lipase activity. Microwaves are penetrating waves and heat up the grain evenly and reduced lipases by 26% in dhanshakti, 4% in WGI-100. Pusa purple 1 (purple) variety showed increase in lipase activity upon microwave by 23%. Decortication in dhanshakti reduced lipase activity by 6% with a loss of nutrients. Vacuum packaging increased lipase activity by 35% in dhanshakti and by 20% in WGI-100. Purple variety showed a decrease in lipase activity by 20%. Pusa purple 1 variety is best among the three varieties without treatment.

Keywords: Food security, pearl millet, rancidity, physical treatments

Introduction
Food security is based on four pillars: (1) food availability, (2) food access, (3) stability and (4) utilization (Tadele, 2019). Food security can be improved by focusing on both the major and minor (also known as an orphan) crops of the world (Fahey, 1998) [3]. Rice, wheat, and maize are major consumed cereal crops which are highly prone to various environmental stresses like drought, heat, salinity, etc. Millets are getting their due credit in the last few years because of its robust nature and balanced nutritional composition of the grains. Among all the millets, pearl millet, in particular, is critically important for food security as well as nutritional security in some of the world's hottest, driest cultivated areas. Pearl millet accounts for almost half of global millet production. In India, pearl millet is the fourth most widely cultivated crop after rice, wheat, and maize. Pearl millet is known to grow under adverse environmental conditions without compromising with the quality and quantity of the produce (Singh, 2003). Nutritional value of pearl millet is much better than the most widely consumed crops such as wheat, rice, maize, and sorghum. Pearl millet is a good source of energy; the carbohydrate composition (63.4 g/ 100 g) of pearl millet is on par with other majorly consumed cereal crops. It has quality protein (9-13%) with more balanced amino acid profile, rich in vitamins (Vit. A, Vit. B1, Vit. B2, Vit. B3, folic acid etc.), minerals (Fe, Zn, Ca, P, K, Mg, Mn etc.) soluble and insoluble dietary fibres (1.2 g/100g), antioxidants (Purple grain pearl millet is rich in anthocyanin). The fat content of pearl millet is 7-8% with better fat digestibility than other cereals. It contains up to 74% unsaturated fatty acids of total fat with a higher content of nutritionally important omega-3-fatty acids. The remaining 26% consists of saturated fatty acids such as palmitic (C16:0) and stearica acid (C18:0) (Muthamilarasan *s.*, 2016) [9]. Pearl millet is also significantly rich in slowly digestible starch (SDS) and resistant starch (RS) which is suitable for the diabetic patient (Kam *et al.*, 2016) [6]. Despite nutritionally rich and high medicinal value, the full potential of pearl millet has not been harvested yet, due to lipid oxidation (rancidity) and off-odour development during storage *i.e.*, short shelf-life. This is mainly due to the high-fat content along with highly active lipases causing hydrolysis of fats into fatty acids (Gilliards, 1983) [4]. Lipase 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 oxidized in the presence of moisture and oxygen, resulting in undesired characteristics. It has been reported that pearl millet lipase shows relatively higher activity than that of most other cereal grains (Gilliard, 1983) [4]. Lipases are highly thermostable enzymes and thus difficult to denature.

The present study focusses on destabilizing lipases and optimize a treatment using various physical methods which can be done/given at homes or industries to enhance the shelf life of pearl millet flour for consumption and marketing.

Material and Methods

Plant Materials

Three pearl millet varieties (Dhanshakti, WGI-100, and Pusa purple 1 (Purple)) were procured from Division of Genetics, IARI, New Delhi and AICRP on pearl millet, Jodhpur.

Microwave treatment

The pearl millet grains were heated in a microwave oven (LG, 28L, AC230 50Hz) for 30 sec and comprehensive acid value and lipase activity was estimated in these samples.

Vacuum treatment

Pearl millet flour was packed using a vacuum packaging machine (Swift Pack, 1260x780x960, 1500W, 1 Kpa) and stored for further biochemical analysis.

Comprehensive acid value (CAV) estimation

The comprehensive acid value is defined as the number of milligrams of potassium hydroxide/sodium hydroxide required to neutralize the free fatty acids as well as other phenolic acids present in the one gram of flour. The comprehensive acid value in the flour was estimated using the standard methods of comprehensive acid value estimation of A.O.A.C (2000).

Lipase activity assay

Lipase activity was assayed following the method as described by Itaya and Ui (1965) [5].

Result and Discussion

Microwave treatment

C.A.V and lipase activity was observed to be minimum in Pusa Purple 1 control sample. After microwave treatment the C.A.V and lipase activity were found to be decreased in Dhanshakti and white variety (WGI-100) (Fig. 1) but Pusa purple 1 (Purple) variety showed increased comprehensive acid value and lipase activity after treatment, as compared to the other two varieties. Lipase activity was found to be decreased from 49.3 to 36.8 U/mg and from 50.9 to 48.3 U/mg in Dhanshakti, and WGI-100 variety respectively, as compared to their respective controls. In Pusa purple 1 (Purple), increase in lipase activity was observed from 34.8 to 42.3 U/mg (Fig. 1a). Dhanshakti showed 26.3%, Pusa purple 1 (Purple) variety showed a 16.6% and WGI-100 showed a 12.2% decrease in comprehensive acid value (Fig. 1b). Microwaves are penetrating waves that can penetrate deeper layers through seed coat in less time. The dipolar water molecules in the rice bran are excited by the electromagnetic waves, and the water molecules are made to spin. The resulting enhanced kinetic energy, along with the friction, produces the heat that results in the even distribution of heat (Roman, 1989) [10].

Vacuum treatment

Lipase activity and the C.A.V was found to be increased after 30 days storage in all varieties except Pusa purple 1 (Purple). As improper storage attracts micro-organisms which also contribute much to the lipid hydrolysis by releasing microbial lipases. Dhanshakti showed an increase in lipase activity from 50 to 85 U/mg and WGI-100 from 49 to 88 U/mg (Fig. 2a). The lipase activity in Pusa purple 1 (Purple) was decreased from 65 to 63 U/mg. The absence of oxygen is suitable for the growth of anaerobic bacteria which contribute microbial lipases to deteriorate oil resulting in increased lipase activity and comprehensive acid value (Champagne and Hron, 1992). The C.A.V was increased from 10 to 12 mg NaOH/g in Dhanshakti (20%) and from 9.5 to 11.2 mg NaOH/g (17%) in WGI-100 variety. The Pusa purple 1 (Purple) variety showed a decrease in C.A.V from 16 to 13 mg NaOH/g (Fig. 2b). This treatment can reduce the oxidative rancidity but not the hydrolytic rancidity. However, when microwave treated flour packed in vacuum had effectively reduced both lipase activity and comprehensive acid value because microwaves killed bacteria which lead to the reduced contribution of microbial lipases during storage (Malekian, 2000) [8].

Effect of physical treatments during storage

Dhanshakti showed maximum lipase activity *i.e.*,115 U/mg on the 10th day of storage under the control condition (Fig. 3). Microwave treated dhanshakti grains showed a reduction in lipase activity throughout storage (Fig. 3a). The C.A.V was observed to be increasing throughout storage, minimum in the fresh flour and found to be increased gradually during storage (Fig. 3b). Lipase activity was found to be decreased on 30th day as compared to 10th day in a control sample of WGI-100 variety (Fig. 4a). Microwave treatment decreased both the lipase activity and C.A.V throughout the storage. Lipase activity and the comprehensive acid value was observed to be 49 U/mg and 8.9 mg NaOH/g on the 30th day and fresh flour in white variety respectively (Fig. 4b). Pusa purple 1 (Purple) variety showed maximum lipase activity on the 10th day and comprehensive acid value throughout storage (Fig. 5). Microwave treatment increased both lipase activity and C.A.V (Fig. 5a and 5b). The reason is that the purple seed coat is rich in flavonoids which are a potent inhibitor of lipase (Chen *et al.*, 2019) [2]. After microwave treatment, loss of flavonoids resulted in the high activity of lipase. All three varieties *viz*. Dhanshakti, Purple and White showed a similar pattern of enzyme activity and comprehensive acid value for 30 days in 10 days interval. Both enzyme activity and the comprehensive acid value was highest on the 10th day and decreased subsequently. Microwave treated samples retained the effect for 30 days with a reduction in lipase activity at every interval studied. Vacuum packaging is not effective in terms of lipid hydrolysis but it has a role in reducing the lipid oxidation which produces a foul smell.

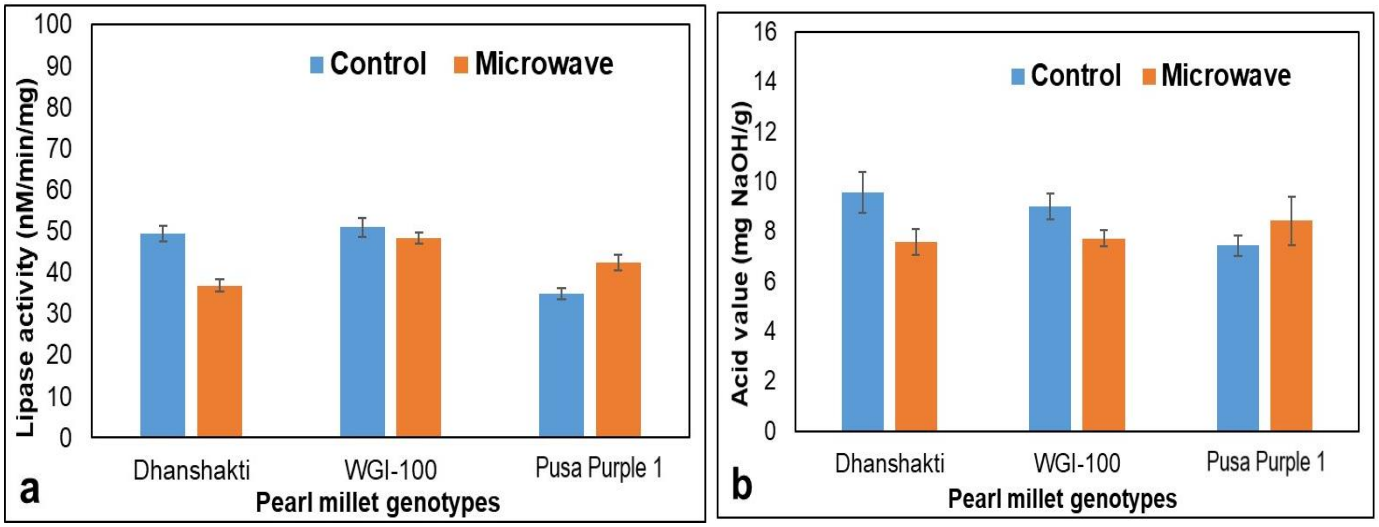

Fig 1: Comparative analysis of Lipase activity and Comprehensive Acid Value (C.A.V) in three genotypes of pearl millet; Dhanshakti (Grey), WGI-100 (White) and Pusa purple 1 (Purple); (a) Lipase activity in all three genotypes; (b) Acid Value in all three genotypes was estimated in freshly prepared flour from the grains treated with microwave for 30 sec and in their control samples; values are expressed as mean± SE.

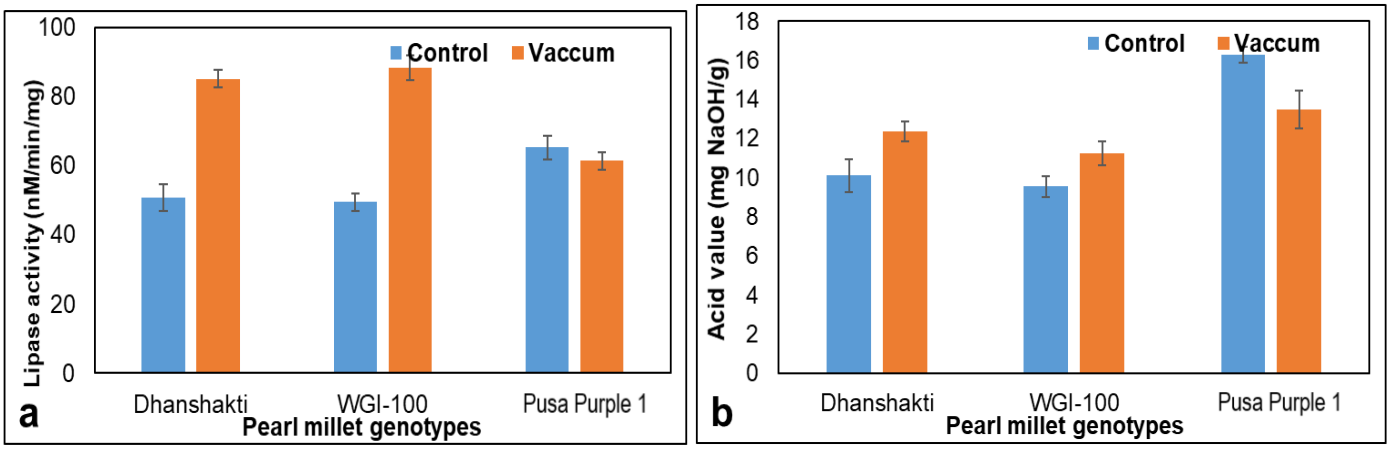

Fig 2: Comparative analysis of Lipase activity and C.A.V in three genotypes of pearl millet; Dhanshakti (Grey), WGI-100 (White) and Pusa purple 1 (Purple); Lipase activity (a) and C.A.V (b) of Dhanshakti, WGI-100 (White) and Pusa purple 1 (Purple) varieties after storing samples for 30 days in vacuum sealed packet in their control samples; values are expressed as mean± SE.

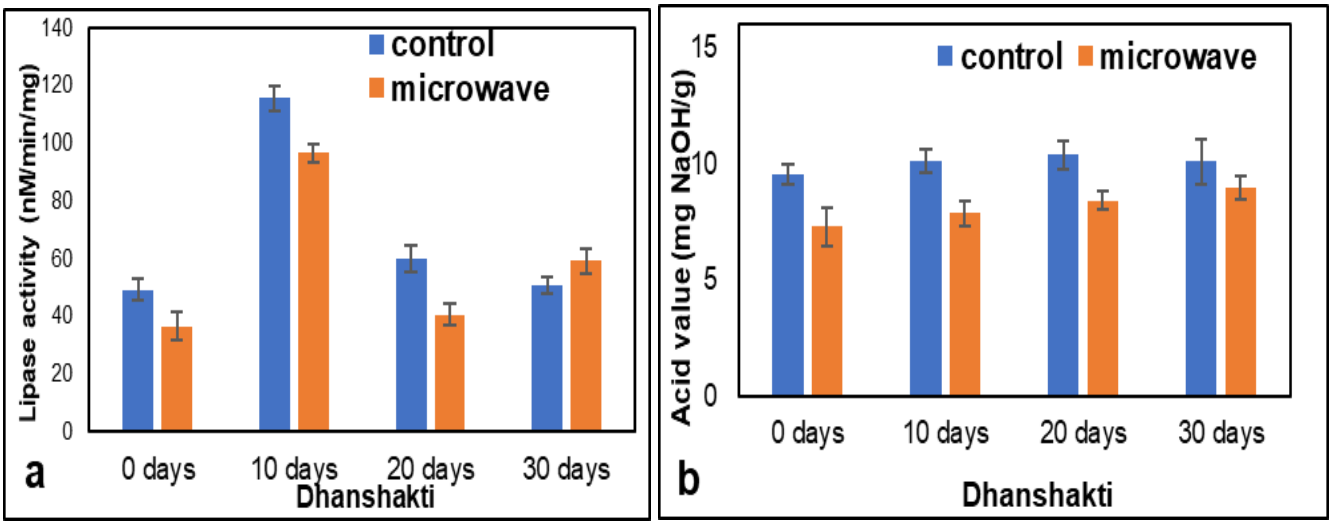

Fig 3: Estimation of Lipase activity and C.A. V in flour of Dhanshakti variety after different treatments and at various storage intervals; (a) Lipase activity (b) C. A. V was estimated in freshly prepared flour from the grains treated with microwave and stored for 10 days, 20 days and 30 days along with their control samples; values are expressed as mean± SE.

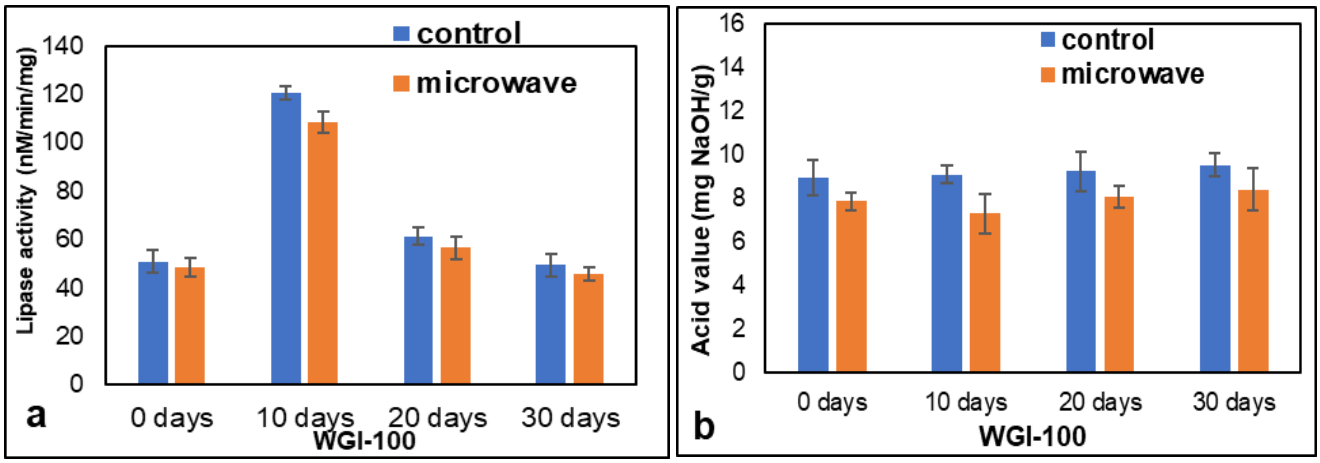

Fig 4: Estimation of Lipase activity and C.A.V in flour of WGI-100 variety after different treatments and at various storage intervals; (a) Lipase activity (b) C.A.V was estimated in freshly prepared flour from the grains treated with microwave and stored for 10 days, 20 days and 30 days along with their control samples; values are expressed as mean± SE

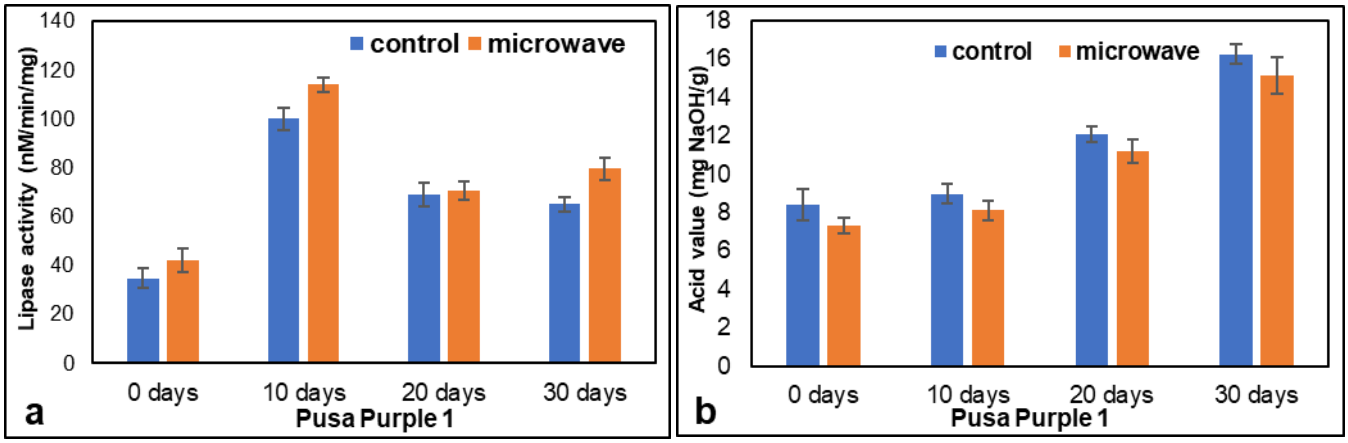

Fig 5: Estimation of Lipase activity and C.A.V of flour prepared from decorticated dhanshakti after different treatments and at various storage intervals; (a) Lipase activity (b) C.A.V was estimate in freshly prepared flour from the grains treated with microwave and stored for 10 days, 20 days and 30 days along with their control samples; values are expressed as expressed as mean± SE

Conclusion

Microwave treatment proved effective in reducing lipase activity and free fatty acid release. The treatment varies among varieties based on the biochemistry and physiology of the plant. Purple variety rich in flavonoids like anthocyanins are potent antioxidants and also inhibit lipases. Therefore, purple variety though lipid-rich had low lipase activity. Microwave treatment increases lipase activity due to the destruction of flavonoids. Proper storage is one of the main factors contributing to rancidity. Storing in airtight containers kept in cool dark place effectively increases shelf life. The vacuum created a suitable environment for anaerobic bacteria to grow and contribute microbial lipases further adding up to the rancidity. However, microwave treated flour followed by vacuum packaging showed better results.

Acknowledgement

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