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Plants Fatty Acid Esterases: A review

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ABSTRACT: There are several lipolytic enzymes in nature, and they have been identified from microbial and plant sources as well as from the tissues of mammals. In plants, they are mainly located in energy reserve tissues like seeds. Poor shelf life of whole flour of coarse cereals including pearl millet and by products of refined flour of fine cereals like wheat, rice and oats is an issue of concern for industries and consumers. Of the whole set of enzymes in bran and germ, lipase and esterase, mainly present in the outer bran fraction of the cereal kernel, hydrolyze water insoluble and soluble esters yielding free fatty acids that generate lipolytic rancidity. As "lipolytic enzymes," esterases (carboxyl ester hydrolases; EC.3.1.1.1) and lipases (triacylglycerol acylhydrolase; EC.3.1.1.3) have the ability to hydrolyze hydrophobic short and long chain carboxylic acid esters, respectively. Esterases catalyze the hydrolysis of ester bonds of water soluble substrate and show a preference for water-soluble fatty acids of less than 10 carbon atoms. Esterases catalyze the hydrolysis mainly esters composed of short chain fatty acids. Triglycerides contained in endosperm are hydrolyzed into free fatty acids and glycerol by lipolytic enzymes found in seeds. Moreover, chemical and kinetic characters of Plants fatty acid esterase are very less known. Comparison of chemical properties of Esterase and kinetic behavior with esters of different plants will give the enzyme great potential for application in the production of low molecular weight esters, in the food industry, and in chemical product. Physiochemical and kinetic properties, high stability, and low cost of production is the main advantage of esterase, it is possible to get more healthy and stable enzymes for industrial uses.

Keyword: Fatty Acid, Esterases, lipolytic enzymes, energy reserve tissues, free fatty acids

INTRODUCTION

A top goal has always been ensuring food security because of the rising need for food as the population grows. Lipase and esterase are two of the many enzymes found in bran and germ, and they are primarily found in the outer bran portion of cereal kernels. They hydrolyze water-insoluble and soluble esters, producing free fatty acids that cause lipolytic rancidity (Doblado-Maldonado *et al*., 2012). High lipids content is one of the important factors responsible for development of rancidity (Goyal *et al.,* 2015). High lipolytic enzyme esterase activity has been substantially associated with the development of hydrolytic rancidity. In moistened flour, lipoxygenase either performs non-enzymatically or enzymatically the oxidation of the free fatty acids collected during storage. By oxidising polyunsaturated fatty acids produced by lipase activity in the presence of too much moisture while the dough is being mixed, it may have a negative impact on the quality of the wheat (Delcros *et al*., 1998; Mann and Morrison, 1975). The secondary oxidation products, such as volatile substances like hexanal and various ketones, are produced by the rearrangement and decomposition of the hydroperoxide derivatives produced as a result of Lipoxygenase activity (Doblado-Maldonado *et al*., 2012). Poor bread quality is caused by hydrolytic and oxidative rancidity products (Tait and Galliard 1988), production of bitter compounds (Bin and Peterson 2016) and a decline in sensory properties (Hansen and Rose 1996). Despite being assigned different enzyme class numbers for the two distinct functions, the identical enzymes perform amidase and esterase reactions. The esterases are divided into A-, B-, and C-esterases based on how they interact with toxicologically significant organophosphates. Organophosphates are potent inhibitors of B-esterases, which accounts for their (intended selective insecticidal as well as unintentional overdose mammalian) toxicity. Organophosphates are substrates of A-esterases and are consequently detoxified by them. C-esterases do not interact with organophosphates. Since esterases can be used in a wide range of industries, including the food

industry, pharmaceuticals, fine chemistry, etc., there is increasing interest in them. Numerous characteristics of esterases, including their widespread dispersion, measurement, manufacturing, synthesis target, purification, and molecular biology, have been published. The esterases are used in the synthesis of low molecular weight esters, the creation of dairy products, wine, fruit juices, beer, and alcohol (Goyal *et al.,* 2015). Modulators as extrinsic factors, often metal ions or organic compounds of known as well as unknown function have often been employed either to identify their role as direct participation as cofactor in catalysis, identification of amino acid residues in active site, or stabilizer/de-stabilizer of intra and inter subunit interactions so as to change catalysis favourably. Negative modulation of lipids hydrolyzing or oxidizing enzymes native to grains by pre treatment of grains and/or fortification of flour with food grade additives may prove to be an effective approach for decreasing their activities in flour and thus slowing down the reactions leading to minimal production of undesirable metabolites *viz*., free fatty acids, hydroxyperxides, volatile aldehydes/ketone etc Barros and Macedo (2015). Gaining knowledge of its physico-chemical and kinetic properties is a pre-requisite for identifying modulator(s) of enzyme activity which necessitates purification of fatty acid esterases.

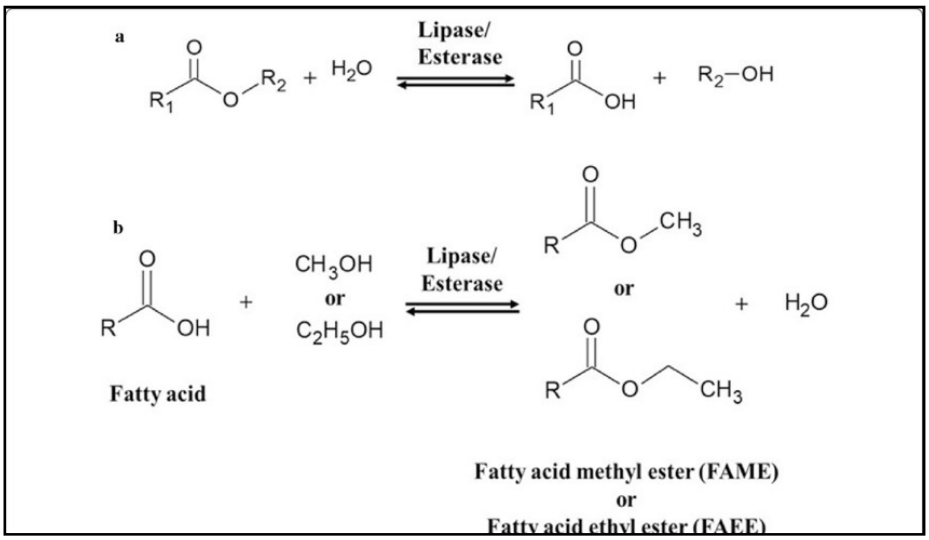

Fig. 1. Enzymatic Reaction catalysed by Esterase.

Partial purification of plant Fatty acid esterases. Extraction buffers or solutions used by the investigators for preparation of crude extract of Fatty acid esterases from various plant sources along with their activities and specific activity are listed in Table 1. Barros and Macedo (2015) extracted the enzyme from soybean seeds in1 mM $CaCl_2$ with added 5 mM EDTA, however, earlier (Barros and Macedo 2011). They used only 1 mM $CaCl_2$, 1 mM. Chen *et al*. (2019) also preferred 1mM $CaCl_2$ containing 2.5 mM DTT and 0.1% Triton X-10 for extracting the enzyme from rice bran. Therefore, mentioned researcher did not mention pH of $CaCl_2$ solutions. Tris-HCl buffer of either 50 or 100 mM strength and pH ranging from 7.0 to 8.3 was used for extraction of esterase and 50 mM phosphate buffer of pH 7.0 or 7.5 have been used by the researchers. Invariably pearl millet fatty acid esterases have been extracted in 100 mM phosphate buffer of pH 8.0 (Sheenu *et al.,* 2018). Activities and specific activities of FAEs in crude extracts varies depending upon plant species.

Levels of fold purification and recovery of enzyme activity of different plant Fatty acid esterases by fractional precipitation with ammonium sulphate and organic solvents are presented in Table 3. Recovering 92 % of the enzyme activity purified Fatty acid esterases from *Synadenium grantii*. Staubmann *et al*. (1999) used 50-80 % alcohol for fractional precipitation of *Jatropha curcas* L. FAE but had not reported recovery of the activity.

Physiochemical and kinetic properties of plant Fatty acid esterase. Earlier a good comparison of activities of p-nitrophenylpalmitate dependent esterases and lipase of many grasses was made by Mohamed *et al*. (1999). They reported high variation the level of activities not only among the genus but within species or cultivars. By conducting experiments at various temperatures in the range of 30-90°C at pH 7.0, using 0.1 M phosphate buffer, it was possible to determine the impact of temperature on the esterase activity. Most pure esterases from plants and animals were found to work best at a pH between 7.0 and 9.0. For sorghum, barley, and Mucuna seeds, a pH of 7.0 was shown to be ideal for plant esterases. Most plant esterases were stable in the pH range of 4 to 9.0. Similar to this, Caesalpania seed esterase had an optimal pH of 7, and it was stable between pH 4 and 9.0.

Table 1: Extraction medium used and levels of activity of fatty acid esterase in various tissues of plants reported in literature.

Investigator(s)	Year	Crop/Species/ Genotype	Tissue	Extraction Buffer		Substrate Used	Enzyme activity (Crude extract)	Specific activity (based on protein content)
				Type	pH			
Govindappa *et al*.,	1987	*Synadenium grantii*	Latex	50 mM Phosphate	7.0	1-NB*	0.805 µmol /ml	0.805 µmol/mg
Staubmann *et al.,*	1999	*Jatropha curcas* L.	Seeds	100 mM Tris-HCl	7.0	p-NPB	NR	0.067 µmol/ mg
Hou *et al*.,	1999	Yam	Tuber	50 mM Tris-HCl	8.3	β –NM**	NR	0.44 µmol/mg
Mohamed *et al.,*	1999	*Avena fatua*	Seeds	50 mM Phosphate	7.5	p-NPP****	2.99 units/g	0.02 units/mg
Stuhlfelder *et al.,*	2002	Tomato	Cell culture	50 mM Phosphate$	7.5	MeLeA*****	4430 pkat	1.77 pkat/mg
Fahmy *et al.,*	2006	*Cucurbita pepo*	Seeds	50 mM Tris-HCl	7.5	-	-	-
Barros and Macedo	2011	Soyabean	Seeds	1mM $CaCl_2$$$	NR	p-NPB	58 units/ml	-
Subramani *et al*.,	2012	*Jatropha curcas*	Seed	50 mM Phosphate	7.0	p-NPB	5.74 µmol/g	0.033 µmol/mg
Barros and Macedo	2015	Soyabean	Seeds	1 mM $CaCl_2$$$$	NR	p-NPB	NR	1.58 µmol/mg
Bajaj *et al.,*	2016a	Pearl millet (Hybrid HHB 234)	Flour (F_2 Grains)	100 mM Phosphate	8.0	p-NPB	50.4Units/g	2.4 Units/mg
Sheenu *et al.,*	2018	Pearl millet (Hybrid HHB197)	Flour (F_1 Seeds)	100 mM Phosphate	8.0	p-NPB	101.7Units/g	2.92 Units/mg
Sheenu *et al.,*	2018	Pearl millet (A-line ICMA 97111)	Flour (OP Seeds)	100 mM Phosphate	8.0	p-NPB	81.14 Units/g	1.87 Units/mg
Sheenu *et al.,*	2018	Pearl millet (R-line HBL 11)	Flour (OP Seeds)	100 mM Phosphate	8.0	p-NPB	39.14 Units/g	1.33 Units/mg
Chen *et al.,*	2019	Rice	Bran	1 mM $CaCl_2$$$$$	NR	p-NPB	2.20 Units/g	0.13 Units/mg
Sunil	2020	Pearl millet (Hybrid HHB 94)	Flour (F_1 seeds)	100 mM Phosphate	8.0	p-NPB	223.8 Units/g	2.14 units/mg
Sunil	2020	Pearl millet (Hybrid HHB 94)	Flour (F_2 grains)	100 mM Phosphate	8.0	p-NPB	248.3 Units/g	2.46 units/mg
Sunil	2020	Finger millet	Flour (Grains)	100 mM Phosphate	8.0	p-NPB	54.92 Units/g	3.01 units/mg

Table 2: Occurrence and activities of plant fatty acid esterases wild and cultivated species of Gramineae family.

Genus / Species	Common name	Variety	Specific activity[@@] (Units/mg protein)	
			Fatty acid esterase	Fatty acid esterase
Oryza sativa	Rice	-	2.28	0.20
Lolium perenne	English ryegrass	-	1.46	0.10
Avena fatua	Common wild oat	-	2.99	0.02
Avena sterilis	Wild oat	-	4.65	0.16
Phalaris minor	Small canary grass	-	1.49	0.21
Hordeum vulgare	Barley	Distichon	2.76	0.08
		Hexastichon	0.75	0.02
Triticum durum	Durum wheat	Sohag 1	0.75	0.03
Triticum aestivum	Bread wheat	Sacha 69	1.58	0.11
		Sacha 8	1.68	0.06
		Giza 165	0.28	0.02
Eleusine coracana	Finger millet	-	1.19	0.07
Panicum repens	Torpedo grass	-	0.99	0.13
Echinochola crusgalli	Barnyard millet	-	0.57	0.25
Setaria viridis	Wild foxtail millet	-	7.67	0.61
Sorghum bicolour	Great millet	Giza 129	1.06	0.11
		Giza 10	1.22	0.14
		Saccharatum	0.53	0.06
Zea mmexicana	Corn	-	0.53	0.07
Zea mays	Corn	Everta	1.31	0.09
		Giza 310	0.71	0.05
		Giza 161	0.79	0.04
		Saccharatum	1.16	0.06

[@]Unit of activity- l mol p-nitrophenol per hour under standard assay conditions. [@@] Unit of activity- 1 umol fatty acid /hr

Table 3: Purification fold and recovery of plant fatty acid esterases reported in literature.

Investigator (s)	Year	Crop/Species/ Genotype	Tissue	Substrate Used	NH_2SO_4 Fractionation				
					% saturation	Enzyme Activity	Specific Activity	Fold Purification	Recovery (%)
Govindappa *et al*.,	1987	*Synadenium grantii*	Latex	1-NB*	0 - 60[@]	445 µmol /ml	1.59 µmol/ mg	2	92
Staubmann *et al*.,	1999	*Jatropha curcas* L.	Seeds	p-NPB	50 - 80[@@]	NR	NR	NR	NR
Stuhlfelder *et al*.,	2002	Tomato	Cell culture	MeJ**	30 - 70	NR	NR	NR	NR
Subramani *et al*.,	2012	*Jatropha curcas*	Seed	p-NPB	40 - 80	5.05 µmol/g	41.5 µmol/mg	3.7	88
Bajaj *et al*.,	2016 a	Pearl millet (Hybrid HHB 234)	Flour (F_2 Grains)	p-NPB	30 - 60	21.7 units/ml	6.80	2.8	43
Sheenu *et al*.,	2018	Pearl millet (Hybrid HHB197)	Flour (F_1 Seeds)	p-NPB	30 - 60	49.1 units/g	7.71	2.6	48
Sheenu *et al*.,	2018	Pearl millet (A-line ICMA 97111)	Flour (OP Seeds)	p-NPB	30 - 60	42.0 units/g	5.08	2.7	52
Sheenu *et al*.,	2018	Pearl millet (R-line HBL 11)	Flour (OP Seeds)	p-NPB	30 - 60	22.0 units/g	3.13	2.4	58
Chen *et al*.,	2019	Rice	Bran	p-NPB	30 - 60	1.15 units/g	0.21	1.6	52
Sunil	2020	Pearl millet (Hybrid HHB 94)	Flour (F_1 seeds)	p-NPB	30 - 60	52.6 units/g	4.58	2.1	68
Sunil	2020	Pearl millet (Hybrid HHB 94)	Flour (F_2 grains)	p-NPB	30 - 60	40.1 units/g	5.55	2.3	38
Sunil	2020	Finger millet	Flour (Grains)	p-NPB	30 - 60	17.6 units/g	4.45	1.5	43

*1-NB (1-Napthyl butyrate),**MeJA (Methyl ester of jasmonic acid) [@]acetone, [@@] alcohol

Industrial Applications: The industrial uses of esterases provide significant contributions to environmentally friendly practises in the food, textile, agrochemical (herbicides, insecticides), and bioremediation industries (Gupta, 2016). Esterases hydrolyze ester bonds and act on a wide range of naturally occurring and synthetically produced substances, making them very helpful in the bioremediation process. It is possible to successfully determine the primary amino acid sequence and three-dimensional structure of enzymes through purification. The structure-function relationships of pure esterases may be established using X-ray investigations, which can help to better understand the kinetic mechanisms of esterase activity on the hydrolysis, synthesis, and group exchange of esters. Esterase is essential for the breakdown of hazardous compounds, polymers, and other natural and industrial contaminants such grain trash. The synthesis of optically pure chemicals, flavouring agents, fragrances, and antioxidants can all benefit from it (Panda and Gowrishankar 2005). In the oxidation and cycling of marine organic carbon, esterases play a significant role. Sea-derived halotolerant esterases might work well in high-salt industrial operations (Zhang *et al.,* 2017). Octyl Acetate (OA) or Octyl Ethanoate is a flavour ester with a fruity orange flavour that is utilised in the food and beverage industries. It is made from octanol and acetic acid. It can be found in foods like wines, wheat bread, cedar cheese, bananas, sour cherries, and oils made from citrus peel. It is a flavouring component that serves as the foundation for synthetic orange flavouring. The purification of esterase and its usage in the manufacture of octyl acetate ester are the main topics of the current research. This is the first account of octyl acetate being synthesized using esterase.

CONCLUSION

The natural substrates of the esterases, a huge, diversified, and complicated set of enzyme molecules with overlapping substrate specificities, remain mostly unknown. The most active esterases, however, were all found to react more favourably with short-chain esters that included a specific acyl group. The Km and Vmax of esterase indicated that, these enzymes exhibits higher activity towards the short chain naphthyl esters. It was concluded that comparatively higher activity of fatty acid esterase, lower Km value for its substrates and lower optimum temperature might be responsible for faster in situ hydrolysis of lipids. Indirect fortification of flour by ascorbic acid treatment of pearl millet grains was effective in negatively modulating activities of Fatty acid esterase for arresting not only in situ hydrolysis but also enzymatic oxidation of lipids.

FUTURE SCOPE

For the purpose of digesting fats and lipids, they are naturally present in the stomach and pancreas of humans and other animal species. Commercial lipases are mostly employed to process other fat-containing meals and generate flavours in dairy products. Through their action on the milk fats to form free fatty acids following hydrolysis, they can enhance the distinctive flavour of cheese. The use of this enzyme is widespread, with applications in the food, biofuel, detergent, and animal feed industries. As a biosensor in

the food business, it is also employed in leather, textile, and paper processing applications.

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Conflict of Interest. None.

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