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Bio-processing of Agro-industrial Wastes for Production of Food-grade Enzymes: Progress and Prospects

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Abstract

Background and Objectives: In the era of global industrialization, enzymes are being used extensively in the various sectors including food processing. Owing to the high price of enzymes, various initiatives have been undertaken by the R&D sector for the development of new processes or improvement in the existing processes for production of cost effective enzymes. With the advancement in the field of biotechnology, different bioprocesses are being used for utilization of different agro-industrial residues for the production of various enzymes. This review focuses on different types of agro-industrial wastes and their utilization in the production of enzymes. The present scenario as well as the future scope of utilization of enzymes in the food industry has also been discussed.

Results and Conclusion: The regulations from the various governmental as well as environmental agencies for the demand of cleaner environment have led to the advancement in various technologies for utilization of the wastes for the production of value-added products such as enzymes. Among the different types of fermentation, maximum work has been carried under solid state conditions by batch fermentation. The research has indicated the significant potential of agro-industrial wastes for production of food-grade enzymes in order to improve the economics of the process.

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1. Introduction

Enzymes are the biological catalysts, which are responsible for carrying out various biochemical reactions and, therefore, play an important role in the various aspects of life and its processes [1,2]. They are biodegradable in nature and environment friendly, act by enhancing the rate of any reaction through lowering the activation energy, and help to reduce the production cost in terms of resource requirements. All these factors add to their advantage as compared to the chemical processes [3]. Although enzymes have been exploited byhumans since the traditional times for the processing of various food products such as beer, wine, cheese, yogurt, kefir and other fermented beverages, but owing to their multiple applications, the demand in various food sectors has increased tremendously, as shown in Table 1. The natural sources of the enzymes include plants, animals and microorganisms. In contrast to the plant and animal sources, microbial sources are preferred due to economic as well as other technical benefits such as higher yields obtained with in the the shortest fermentation time.

Table 1. Applications of enzymes in the various food sectors**.**

S. No.	Enzyme	Food industry	Applications
1	Pectinase	Fruit industry	Clarification of the fruit juices
			Enhanced levels of fruit juice volume when fruit pulps treated with pectinase
			Soften the peel of citrus fruits
			Enhances the citrus oil extraction such as lemon oil
		Beverages industry	Accelerates tea fermentation
			Reduces foam forming property in instant tea powders
			Remove mucilaginous coat from coffee beans
		Wine industry	Imparts stability of red wine
2	Protease	Dairy industry	Prevent coagulation of casein during cheese production
			Flavor development
		Meat industry	Meat tenderization
		Baking industry	Assures dough uniformity
			Improve dough consistency
			Gluten development
			Improve texture and flavor
			Reduce mixing time
3	Amylase	Brewing industry	Fermentation of alcohol by converting starch to sugars
		Baking industry	Breakdown of starch into simple sugars; thereby allowing the bread to rise and impart flavor
			Dough conditioning
			Generates additional sugar in the bread, which improves the taste, crust color and toasting quality
			Anti-staling effect during bread making; improves the softness and shelf-life
		Fruit and brewery industry	Clarification of beer and fruit juices
4	Laccase	Wine industry	Removal of polyphenol, thereby providing stability to wines
			Preparation of cork stoppers of wine bottles
			Reduces cork taint generally imparted to aged wine bottles
		Brewing industry	Removal of oxygen at the end of beer fermentation process
			Prevent the formation of off-flavors (trans 2-nonenal)
		Fruit industry	Juice clarification
		Baking industry	Increase strength, stability and reduce stickiness
			Increase volume, improved crumb structure and softness of the product
5	β-galactosidase	Dairy industry	Production of low lactose/lactose free milk
			Production of prebiotics
			Prevents crystallization of lactose
			Production of ice creams, sweetened flavor and condensed milks
			Improves the scoop ability and creaminess of the product
6	Lipase	Fats and oils	Production of mayonnaise and other emulsifiers,
			Triglycerides synthesis and trans-esterification of triglycerides in non-aqueous media; specially fat production
		Milk cheese	Production of milk with slightly cured flavor for use in milk chocolates
			Aging, ripening and general flavor characteristics
			Lecithin modification
		Meat industry	Degumming during the refining of vegetable oil
			Fat removal
7	Naringinase	Fruit industry	Debittering of citrus fruit juices
		Wine industry	Enhances the aroma in the wine
			Production of pruning, a flavonoid
8	Papain	Meat industry	Tenderizing of meat
		Brewing industry	Prevents haze formation in beer; thereby resulting in shiny bright beer
		Baking industry	Breakdown gluten proteins in the dough in case of waffle and cracker production
9	Invertase	Sweetener, sugar Confectionery	Invert sugar production
			Production of high fructose syrup
			Manufacturing of soft-centered candies
			Manufacture of artificial honey
		Other	Production of alcoholic beverages, lactic acid, glycerol produced from the fermentation of sucrose
10	Tannase	Brewing	Removal of polyphenolic compounds
		Tea	Manufacture of instant tea

Source: [2, 69, 80, 91, 112, 134, 153]

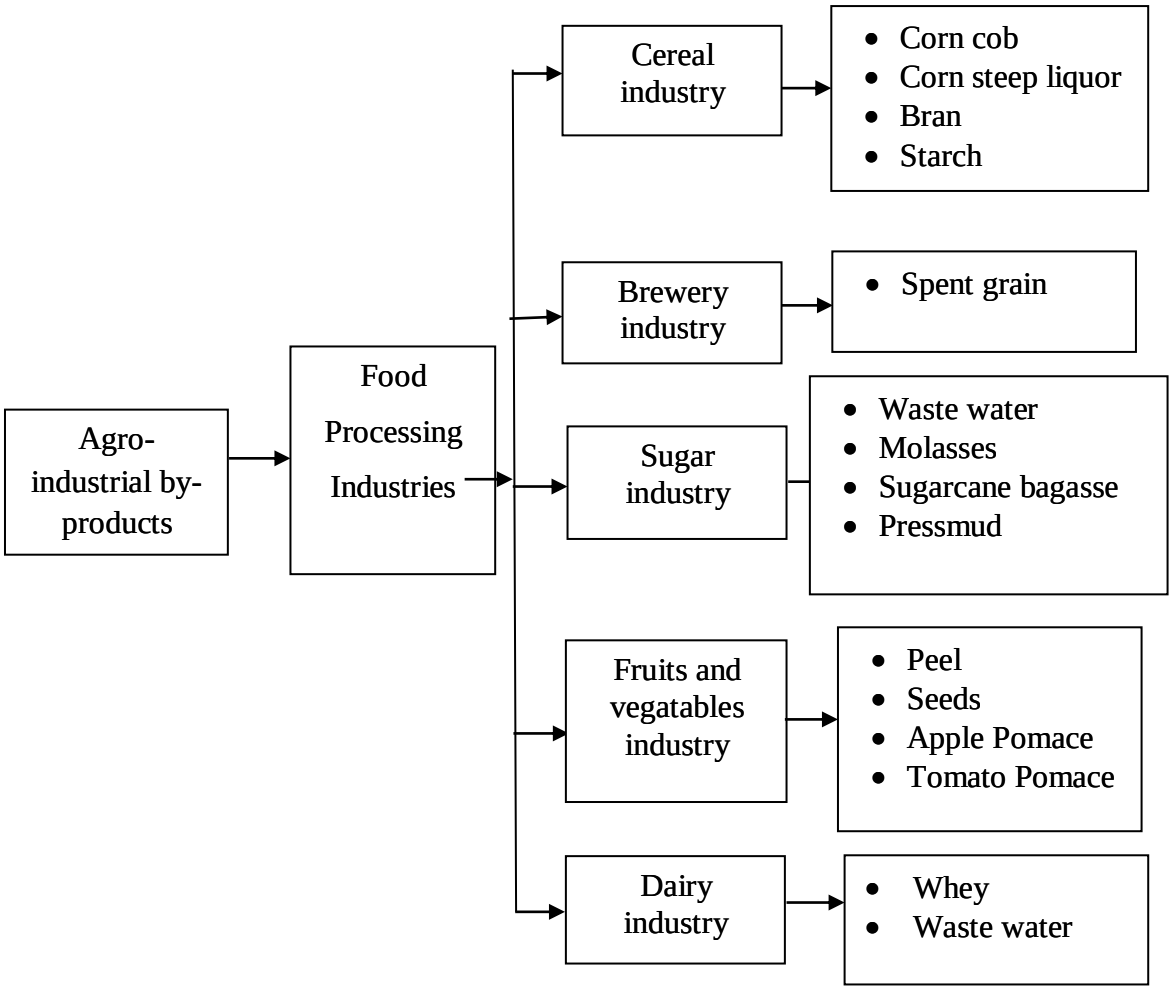

Figure 1. Generation of agro-industrial by-products from different food processing industries

Moreover, microbes can be more easily subjected to genetic manipulation in order to improve the productivity or catalytic trait of the enzyme [4]. Different microbial sources such as bacteria, yeasts and fungi have been utilized for the enzyme production by utilizing different substrates like starch, glycerol, glucose and other inorganic salts [5]. A major part of the production cost of the enzymes is mainly due to the cost of the fermentation media and processes. Therefore, to minimize the cost of production and to fulfill the industrial demands and challenges, a variety of microorganisms and cheap agro-industrial substrates have been tested to facilitate the economic production of the enzymes [6,7].

The food and agricultural industries generate excessive volumes of agro-industrial wastes worldwide. These wastes pose a serious problem of their disposal and environmental pollution [8]. Being rich in nutrients, the agro-industrial residues should not be considered as wastes, and rather can be used as raw materials to provide essential micro- and macro-nutrients for microbial cultivations for industrial production of value-added products including enzymes. In the last few years, various environmental-friendly and cost-effective technologies have been developed with the efforts made both at industrial and academic levels. These technologies aim at utilizing the waste for the development of value-added products; thereby reducing the environmental pollution and solving the issues associated with their disposal [8,9]. Furthermore, being rich in fermentable sugars and other nutrient components, microorganisms have the ability to utilize these substrates, and subsequently, convert them into various industrially important products [10]. Therefore, the conversion of renewable resources arising from the agricultural residues as well as other industries for the production of cost-effective enzyme using fermentation techniques has attracted the keen attention of researchers. Keeping this in mind, this review has focused on presenting a status report of progress on utilization of agro-industrial wastes for microbial production of food-grade enzymes and future prospects.

2. Agro-industrial wastes: Generation and composition

Agro-industrial wastes, generally, include the wastes generated during the industrial processing of agricultural or animal products or those obtained from agricultural activities in the form of straw, stem, stalk, leaves, husk, shell, peel, lint, seed, pulp, legumes or cereals (rice, wheat, corn, sorghum and

barley), bagasse from sugarcane or sweet sorghum milling, spent coffee grounds, brewer's spent grains, and many others (Fig. 1). These wastes are mainly composed of sugars, fibers, proteins, and minerals. The chief constituents of such agro-industrial wastes include cellulose, hemicelluloses and lignin, collectively being called "lignocellulosic materials" [11]. Individual wastes display a range of relative of proportion of these major constituents. This variability entails certain level of specificity/preference of their use.

Cheap agro-industrial sources such as wheat bran, soybean meal, corn steep liquor, sugarcane bagasse, whey, etc. have been used as carbohydrate as well as nitrogen sources in the lieu of synthetic ones [12]. Different types of treatments (physical, chemical, and enzymatic) can be given to these by-products in order to make them easily consumed by microbes [13]. The wastes generated from the different food industries have been discussed in the following sections.

2.1. Fruit and vegetable industries

Fruit and vegetable processing sector has expanded tremendously owing to the rapidly growing demand of packed and pre-processed foods. The growing processing of fruits and vegetables has led to the production of large amount of wastes either in the form of leaves or straw during the processing, or seeds after processing or pulp and pomace [14]. The wastes generated from these industries are diverse in nature, depending upon the processes used and the products developed [15]. The skin and seed of fruits such as mango, papaya, pineapple, and taro are obtained as the by-products during their processing [16]. Other major by-products are obtained from the citrus peel industry. Approximately, 50-60% of the citrus peel waste is generated from the citrus fruit, which acts as a potential substrate for any fermentative process [17]. Similarly, apple pomace is also a promising substrate, which is the by-product remaining after cider and juice production [18,19]. Among the vegetables, the wastes remaining after the processing of tomato into juice and puree are mainly in the form of peels and seeds and tomato pomace. Tomato processing by-products are one of the cheap and nutritional sources for the microbial growth [18]. These types of wastes are the potential substrates for microbial cultivation for targeted products as these are rich in antioxidants, antimicrobial compounds, phytochemicals and vitamins to support these operations [20,21].

2.2. Cereal industry

Due to the increasing health awareness among the people, the cereal processing sector has increased tremendously, and therefore, the wastes/by-products generated from these industries have also increased proportionally [22]. The wastes generated from this industry are, generally, the result of dry or wet milling of the cereals. Moreover, the type of waste depends upon the cereal used and on the processing techniques. The oldest and the most easiest method of disposing the by-products was their use as animal feed, but the recent trends are oriented to use them for the generation of higher value or significant products. Both cellulose and hemicellulose constitute almost 50% of the plant tissue dry biomass. These are degraded into their respective monomers (simple soluble sugars) by enzymatic hydrolysis. These hydrolytic products act as an inexpensive and renewable energy source for microbial fermentation [13].

The major by-products of cereal processing are the bran and the germ obtained during both the wet and dry milling processes. During the processing of wheat, coarse and fine middling are also obtained as the by-products. The major by-products arising from the maize processing industry, besides the germ and the bran, are the gluten meal obtained during the wet milling. Apart from these, another by-product obtained during the wet milling of corn is corn steep liquor, which is used as a nutrient source in feed and fermentation processes. It is a complex broth composed of carbohydrates, proteins, organic acids, minerals, etc. [23].

During the processing of rice, rice hulls are obtained that contain high amount of silica. De-hulled rice is further processed, which gives rice bran, rice polishing, and broken rice as by-products. Rice polishing is a good source of thiamin and crude fat, and a poor source of crude fiber. Rice bran, another by-product, has high oil content, and is a good source of vitamin B complex, amino acids and proteins [24]. De-oiled cake remaining after oil extraction from the rice bran, also constitutes a huge aggregated volume of biomass, deserving bio-transformation into value-added products.

2.3. Sugar industry

The main raw materials of sugar processing industry are sugar beet and sugar cane. Milling of the cane stalks for sugar production results in the generation of various by-products such as waste-water, molasses, bagasse, etc. [25]. The sugar mills generate about 1000 L of waste water per ton of cane crushed [26]. Another waste generated from the sugar industry is the sugarcane bagasse, which is a fibrous residue left after the crushing and extraction of juices from the cane stalk. It consists of high amount of fiber and moisture with fewer amounts of soluble solids, preferably sugars [27]. Molasses is the thick viscous dark liquid obtained during the production of raw and refined sugar from the sugarcane or sugar beet [28].

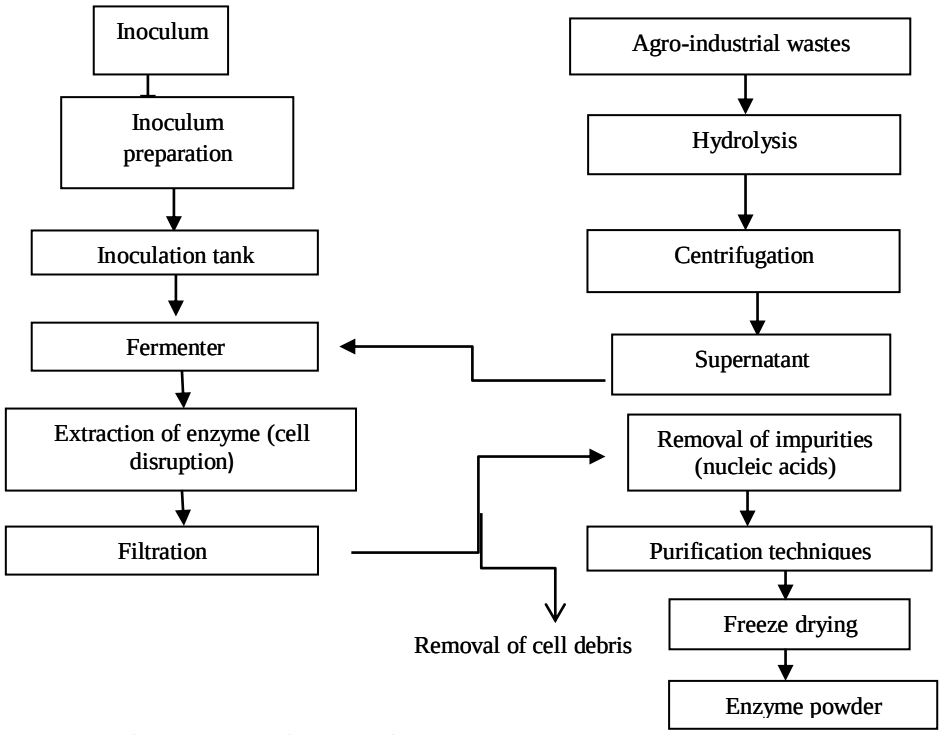

Figure 2. General scheme for production of enzymes from agro-industrial wastes

2.4. Dairy industry

Dairy industry has been one of the major industries that are responsible for the generation of large titers of effluent. Approximately 0.2-10 L of wastes are generated per liter of processed milk. Cheese whey, a by-product of the cheese processing industry remaining after the coagulation of milk and removal of casein, constitutes a major environmental problem due to its high biological oxygen. The major components of whey include lactose 4.5-5% (w v^{-1}), proteins 0.6-0.8% (w v^{-1}), and lipids 0.4-0.5% (w v^{-1}). The high percent of lactose present in the whey increases the BOD value, thus creating problems in its disposal, and thereby causing environmental problems [29,30]. However, lactose and protein present in liquid whey may also serve as a nutritious source for the growth of microbes. Furthermore, liquid whey may be processed to harvest lactose and/or proteins and nutrients for their further diversified usage.

Besides, large amount of waste water (approx. 2.5 l) is generated from the dairy industry during the washings and the processing techniques. The waste water contains casein, and inorganic salts, apart from detergents and sanitizers; thereby increasing the BOD, and COD content, and causing environmental problems [31,32].

2.5. Brewing industry

The wastes such as spent grain, and spent hops are the most common types of wastes generated from the brewing industry in large amounts. The spent grain is the important by-product of the brewing industry, contains high amount of pentosan, lignin, protein and other nutritive components [33, 34].

The conversion of such agro-industrial by-products into value-added products has recently become a highly-active area of research; therefore, various biotechnological processes and approaches are being applied to transform these wastes into the value-added products including for production of enzymes.

3. Microbial production of food grade enzymes

The microbial production of enzymes can be carried out both by submerged (SMF) and solid state fermentation (SSF); the latter being preferred method of production in the industrial sector. However, the trend has begun to shift towards SSF owing to the utilization of different agro-industrial wastes as a low cost carbon and nitrogen source; thereby reducing the cost of enzyme production [35]. Different organisms are responsible for the production of enzymes that may vary in properties such as hydrolyzing, oxidizing, or reducing enzymes. The general scheme for the industrial enzyme production is depicted in Fig. 2. Several microbial species have potential for production of commercially important enzymes. A summary list of such enzymes is presented in Table 2, whilst they are individually discussed below.

Table 2. Production of food grade enzymes using various agro-industrial residues .

Enzymes	Agro-industrial wastes	Microorganisms involved	Type of fermentation	Activity	References
Pectinase	Orange bagasse Wheat bran 10% sugarcane bagasse +90% Orange bagasse	*Thermoascus aurantiacus 179 -5*	SSF	19,320 Ug^{-1} (PL) 11,600 Ug^{-1} (PL) 40, 180 Ug^{-1} (PL)	[46]
	Lemon pulps	*Trichoderma virde* *Aspergillus niger*	Slurry state	9.01 Uml^{-1} 1.27 Uml^{-1}	[48]
	Orange bagasse: wheat bran (1:1)	*Penicillium viridicatum RFC3*	SSF	46.4 Ug^{-1} (Exo-PG) 314.4 Ug^{-1} (PL) 5.6 Ug^{-1} (Endo -PG) 71.2 Ug^{-1} (Exo-PG) 480 Ug^{-1} (PL) 5.6 Ug^{-1} (Endo-PG)	[49]
	Deseeded sunflower head with green gram husk	*Aspergillus niger DMF 27*	SMF	30.3 Uml^{-1} (Exo-PG) 18.9 Uml^{-1} (Endo-PG)	[50]
		Aspergillus niger DMF 45	SSF	45.9 Uml^{-1} (Exo-PG) 19.8 Ug^{-1} (Endo-PG)	
	Orange bagasse	*Botryosphaeria rhodina MAMB-05*	SSF	32 Uml^{-1}	[51]
	Orange bagasse: wheat bran (1:1)	*Thermomucor indicae_seudaticae*	SSF SMF	120 Uml^{-1} 13.6 Uml^{-1}	[52]
	Orange bagasse: Molokhia Stalks (1:3)	*Penicillium pinophlilum* Hedg 3503 NRRL	SSF	3270 Ug^{-1} dry solid substrate	[53]
	Orange peel	*Aspergillus niger*	SMF	117.1± 3.4 $\mu mml^{-1}min^{-1-1}$	[54]
	Orange peel	*Bacillus licheniformis* SHG 10		2.69 µg galactouronic acid $min^{-1}mg^{1}$	[55]
	Citrus pulp and sugarcane Bagasse	*Aspergillus. oryzae* CPQBA 394-12 DRM 01	SSF	45±4 Ug^{-1}	[56]
	Orange peel and groundnut oil cake	*Saccharomyces cerevisae PVK4*	SMF	6285 Uml^{-1}	[57]
Amylase	Coconut oil cake	*Aspergillus oryzae*	SSF	3388 $Ugds^{-1}$	[59]
	Wheat bran	*Thermomyces lanuginosus ATCC 58157*	SSF	4.946×10^{5} Ukg^{-1}	[60]
	Wheat bran	*Bacillus* sp. PS-7	SSF	4,64,000 Ug^{-1} dry bacterial bran	[61]
	Potato peel	*Bacillus subtilis* DM-03	SSF	532±5 Ug^{-1}	[63]
	Rice bran Rice bran:wheat bran (1:2)	*Streptomyces* sp. MSC702	SMF	373.89 $IUml^{-1}$ 549.11 $IUml^{-1}$	[65]
	Wheat bran	*Aspergillus oryzae* IIB-6	SSF	7800 $Ugds^{-1}$	[12]
	Soyabean meal	*Aspergillus.oryzae S2*	SSF	22118.34 Ug^{-1} dry substrate	[66]
	Rape seed cake, potato peel and Feather	*Bacillus subtilis* PF1	SMF	16.39±4.95 μgml^{-1}	[67]
	Brewery waste	*Bacillus subtilis* UO-01	SMF	9.35 $EUml^{-1}$	[68]
Laccase	Barley bran	*Trametes versicolor*	SMF	500-600 Ul^{-1}	[72]
	Banana leaf biomass	*Pleurotus ostreatus* *Pulmonarius sajorcaju*	SSF	1.7106 Umg^{-1} protein 1.6669 Umg^{-1}	[73]
	Groundnut seeds	*Trametes hirsuta*	SSF	600-700 $nkatl^{-1}$	[74]
	Oat husks supplemented with combined fiber and deinking sludge	*Cerrena unicolor T 71*	SSF	178 $nkatg^{-1}$ DW	[75]
	Banana peel:mandarin peel: cantaloupe peel (5:3:2)	*Pleurotus florida NCIM 1243*	SSF	6.8 Ug^{-1}	[76]
	Brewery waste	*Phanerocheate chrysosporium*	SSF	738.97 $Ugds^{-1}$	[77]
Lipase	Babassu cake	*Penicillium simplicissimum*	SSF	26.4 Ug^{-1}	[81]
	Wheat bran:gingelly oil cake (3:1)	*Aspergillus niger MTCC 2594*	SSF	384.3 Ug^{-1}	[82]
	Palm oil mill effluent	*Candida cylindracea ATCC 14830*		20.26 $IUml^{-1}$	[7]
	Wheat bran, coconut oil cake and wheat rawa	*Aspergillus niger MTCC 2594*	SSF	628.7 $Ugds^{-1}$	[83]
	Defatted rice bran	*Aspergillus fumigatus* MTCC 9657	SSF	8.13 $IUml^{-1}$	[84]
	Castor oil cake and sugarcane bagasse	*Trichoderma harzianum*	SSF	4 $Ugds^{-1}$	[85]
	Olive oil with crambe meal	Fusarium	SSF	5.08 $Ugds^{-1}$	[86]

	Crambe meal		SMF	3.0 IUml^{-1}	
	Seasame oil cake	*Candida rugosa NCIM 3462*	SSF	22.40 Ug^{-1}	[87]
	Palm oil industry waste	*Aspergillus niger*	SSF	15.41 IUml^{-1}	[89]
Tannase	Cashew apple bagasse	*Aspergillus oryzae*	SSF	4.63 Ug^{-1}	[96]
	Rice bran	*Aspergillus oryzae*	SSF	14.40 Ug^{-1}min^{-1}	[97]
	Bahera fruit powder :wheat Bran (3:7)	*Aspergillus heteromorphus* MTCC 5466	SSF	1060 Ugds^{-1}	[98]
	Tamarind seed powder	*Aspergillus flavus MTCC 3783*	SMF	139.3 Uml^{-1}	[99]
	Coffee pulp	*Penicillium verrucosum*	SSF	115.995 Ugds^{-1}	[100]
	Wheat bran	*.foetidus. terreus*	SSF	47.3 Umg^{-1}	[101]
Invertase	Red Carrot jam processing residue	*S. cerevisae NRRL Y-12632*	SSF	272.5 Ug^{-1} dry substrate	[107]
	Orange peel	*.foetidus.flavus*	SSF	25.8 IUml^{-1}	[108]
	Pressmud and spent yeast	S*accharomyces cerevisae*	SSF	430 Umg^{-1}	[109]
	Carrot peels	*Aspergillus.niger*	SSF	7.95±0.1 Uml^{-1}	[110]
Protease	Pigeon pea waste	Bacillus sp. *JB-99*	SMF	12,430±120 Uml^{-1}	[116]
	Green gram husk	*Bacillus circulans*	SSF	32000-73000 Ug^{-1}	[117]
	Green gram husk	Bacillus sp.	SSF	9550 Ug^{-1} biomass	[118]
	Potato Peel: *Imperata clyindrica* Grass (1:1)	*Bacillus subtilis DM-04*	SSF	2382 Ugds^{-1}	[119]
	Castor husk	*Bacillus altitudinis GVC11*	SSF	419,293 Ug $^{-1}$of husk	[120]
	Wheat bran	*Pseudomonas aeruginosa*	SSF	582.25±9.2 Uml^{-1}	[121]
	Dal mill waste	*Fusarium oxysporum*	SSF	8.8 µgml^{-1}	[122]
	Cotton seed cake	*Bacillus subtilis K-1*	SSF	1020 Uml^{-1}	[123]
Naringinase	Grapefruit rind	*.foetidus.foetidus*	SSF	2.58 Uml^{-1}	[130]
	Rice bran	*Aspergillus niger MTCC 1344*	SSF	58.1±1.6 Ug^{-1} dry substrate	[131]
	Sugarcane bagasse+ soyabean hulls+rice straw	*Aspergillus niger*	SSF	3.02 Uml^{-1}	[132]
	Orange rind	*Aspergillus niger*	SSF	13.89 Uml^{-1}	[133
β-galactosidase	Whey	Kluyveromyces. marxianus MTCC 1388	SMF	1.68 Umg^{-1}	[139]
	Whey	Kluyveromyces.. *marxianus* NCIM 3551	SMF		[140]
	Whey and parboiled rice Effluent	*K. marxianus* ATCC 16045	SMF	10.4 Uml^{-1}	[144]
	Acid whey	Streptococcus thermophilus	SMF	7.76 Uml^{-1}	[142]
	Whey	*Bifidobacterium animalis* ssp. lactis Bb12	SMF	6.80 Uml^{-1}	[143]
		Lactobacillus delbruckii ssp. *bulgaricus* ATCC 11842		7.77 Uml^{-1}	
	Cheese whey	Kluyveromyce. *marxianus* CBS 6556	SMF	21.99 Uml^{-1}	[145]
	Wheat bran and rice husk (1:1)	*Aspergillus oryzae*	SSF	386.6 µmoles of ONP released ml^{-1}min^{-1}	[148]
	Wheat bran	*Aspergillus tubingensis*	SSF	15,936 Ugds^{-1}	[149]
	Wheat Bran and whole wheat (7:3)	*Penicilium canescens*	SSF	5292±111 Ug^{-1}	[150]

3.1. Pectinase

Pectinases (E.C. 3.2.1.15) are a group of enzymes that hydrolyze the pectin present in vegetable cells by various mechanisms, and are divided into those that lyse glycosidic bonds along the polymer backbone-polygalacturonase, pectin lyase and pectate lyase, and those that split the methoxy groups' pectin esterase [36,37]. Pectic enzymes are, generally, used in the fruit processing industry during the process of extraction of the fruit juices to aid improved extraction and to reduce the thickness of the juice preparation. Besides the fruit industry, this enzyme is widely used in the wine, coffee and tea processing industries [38]. Microbial fermentation of pectinase had been reported to be carried using various agro-industrial residues such as wheat bran, soybean, apple pomace, cocoa, sugarcane bagasse, lemon and orange peel [39-45].

Pectinase production has been carried out using sugarcane bagasse, orange bagasse and wheat bran using thermophilic fungus *Thermoascus aurantiacus* [46]. The study revealed the higher yields of extracellular polygalactournase (Pg) and pectic lyase (Pl) enzyme production. The maximum PG activity (43 Ug^{-1}) was obtained by using wheat bran or orange bagasse as a substrate between the 2nd and 4th days of fermentation; whereas addition of sugarcane bag-asse both to orange bagasse and wheat bran substrate did not have a significant effect on the yield. As compared to the PG production, maximum Pl yield (40,180 Ug^{-1}) has been reported to be obtained between the 8th and 10th days of fermen-

tation from a mixture of 10% sugarcane and 90% orange bagasse. Orange bagasse, when used as a substrate for pectinase production, acts as an inducer for pectinase production as it contains large amount of soluble carbohydrates (fructose, glucose, sucrose and pectin) and insoluble cellulose [47]. Citrus industry by-products are well known sources for pectinase production. One such substrate, lemon pulp, a waste obtained after the extraction of lemon juice, has been used as a raw material for pectinase production using two fungal strains *Aspergillus niger* and *Trichoderma viride* during submerged fermentation. Among the two strains, maximum pectinase production of 9.01 Uml^{-1} was obtained from *T. viride* as compared to 1.27 Uml^{-1} from *A. niger* [48].

A ratio of 1:1 wheat bran and orange bagasse mixture has been used for the production of endo- and exo-polygalactouranase, pectin lyase, and pectin esterase from *Penicillium viridicatum* RFC3; the fermentation being carried out at flasks and polypropylene bags at 70% and 80% moisture content, respectively [49]. The maximum activity of endopolygalactouranase (5.6 Ug^{-1} of substrate) has been reported to be obtained from both 70% and 80% moisture; whereas exopolygalactouranase activity (71.2 Ug^{-1}) has been shown to be achieved from 80% moisture content. In a similar way, 80% moisture has been demonstrated to result in the maximum pectin lyase production (480 Ug^{-1}) in this study in contrast to the others. Moreover, maximum productivity of all the enzymes was achieved while carrying out the fermentation in the propylene bags at 80% moisture level, indicating the possibility for the scale up studies with respect to the amount of substrate to be fermented. In addition to the above enzymes, pectin esterase and other hydrolytic enzymes such as amylase, xylanase and endo-glucanase were also synthesized, as reported by Silva et al.. [49]. The comparative study conducted by Patil and Dayanand [50] on pectinase production by both submerged and solid state fermentations using deseeded sunflower head from *A. niger* DMF27 and DMF45, supplemented with green gram husk indicated higher yields of endo- (19.8 Ug^{-1}) and exo-pectinase (45.9 Ug^{-1}) from *A. niger* DMF45 in solid state fermentation, as compared to 18.9 Uml^{-1} and 30.3 Uml^{-1} of endo-pectinase and exo-pectinase, respectively from DMF27 by submerged fermentation.

Orange bagasse has also been reported as a potential raw material for the production of maximum titers of pectinase (32 Uml^{-1}) during solid state fermentation using *Botryosphaeria rhodina* MAMB-05 [51]. Similarly, orange bagasse in combination with wheat bran has been shown to result in the production of 120 $IUml^{-1}$ exo-polygalactouranase during solid state fermentation, while with submerged fermentation; a yield of 13 $IUml^{-1}$ was obtained using thermophilic *Thermomucor indicae-seudaticae*. But in comparison to SSF, the enzyme synthesized from submerged fermentation resulted in more thermostable, and demonstrated high stability at acidic pH [52]. The production of a variety of enzymes by a single strain would be beneficial as the amylase synthesized would be able to remove the starch from the banana, apple and pear juice during the clarification process. The solid state fermentation for production of exo-poly-galacturonase from the mixture of orange bagasse and molokhia stalks (1:3) resulted in the maximum productivity of 3270 Ug^{-1} dry substrate from *Penicillium pinophlilum* Hedg 3503 NRRL, as reported by Ahmed and Mostafa [53]. Although various substrates have been tested for pectinase production, molokhia stalks were used for the first time for enzyme production. The high values obtained from the mixture of orange bagasse and molokhia stalks indicate the potential of these substrates for enzyme production at low cost. Furthermore, owing to the high amount of pectic substances naturally present in the orange peel, they were used as potential substrates for enzyme production using *A. niger*, which revealed a maximum enzyme productivity of 117.1 $\mu mml^{-1}min^{-1}$ at 144 h during submerged fermentation [54].

Statistical techniques, Response Surface Methodology (RSM), and Plackett Burman Design have been used for optimization of poly-galactouranse (PGase) production using a bacterium species *Bacillus licheniformis* SHG10 from different agro-industrial wastes such as orange peel waste, lemon peel waste, banana peel waste, artichoke peel waste, and pomegranate peel waste, as well as synthetic carbon sources [55]. Among all the tested sources, maximum PGase production (2.69 µg galacturonic acid $min^{-1}mg^{-1}$) was obtained by using orange peel as a sole carbon source.

In the study by Biz et al., the microbial fermentation of pectinase was done in a packed bed reactor using 51.6% citrus pulp and 48.4% sugarcane bagasse, where the sugarcane bagasse provided the stability to the bed with high porosity, thereby preventing the problems associated with bed shrinkage and formation of agglomerates within the substrate [56]. Although fungi have been most widely used for the production of enzymes, yeast strain *Saccharomyces cerevisae* also resulted in maximum enzyme production using orange peel, groundnut oil cake and $MnSO_4$ during 48 h of fermentation period [57].

3.2. Amylases

Amylases (E.C. 3.2.1.1) are hydrolytic enzymes responsible for the complete hydrolysis of starch, and have a great industrial importance. These enzymes have potential applications in the food industry, especially in the baking, brewing, and, fruit industries [5,34]. Owing to industrial demand of these enzymes, efforts have been made to explore various technologies to obtain high yield of the enzymes at low cost. Thus, to minimize the cost of

production, various cheap, easy available agro-industrial wastes have been explored for the microbial production of amylase [58].

The use of coconut oil cake as a substrate was investigated by Ramachandran et al. [59] for production of α-amylase by *A. oryzae* under SSF conditions. Coconut oil cake has been shown to support the growth of the organism and thereby leading to the production of 1372 $Ugds^{-1}$ α-amylase in 24 h fermentation time. Supplementation of the basal medium with 0.5% starch and 1% peptone enhanced the enzyme synthesis by producing 3388 $Ugds^{-1}$ of the enzyme, and demonstrating the efficiency of coconut oil cake a promising substrate for a-amylase production. Different agro-industrial residues (millet cereal, crushed wheat, corn cobs, molasses, wheat flakes, barley bran, and rice bran) were tested for their potential during the enzyme production in SSF [60]. Among all the tested residues, wheat bran has been noted to result in maximum enzyme production, showing an enzyme activity of 261 Ug^{-1} followed by millet cereal.

Thermostable α-amylase, having the activity of about 4,64,000 Ug^{-1} bacterial bran, had been produced using wheat bran as a substrate supplemented with glycerol, L-proline, soyabean meal and vitamin B-complex from the thermophilic bacterial strain Bacillus sp. PS-7 [61]. Besides, gruel, a by-product from the wheat grinding, was used by Kammoun et al. as a medium for amylase production using *Aspergillus oryzae*, which resulted in the maximum yield of 148 Uml^{-1} during the submerged fermentation [62]. Different agro-industrial wastes (wheat bran, rice bran, mustard oil cake, *Imperata cylindrical* grass, banana leaves and potato peel) have also been tested for the microbial production of α-amylase from *Bacillus subtilis* DM-03, which indicated the maximum enzyme production of 532±5 U per gram dry substrate under optimized conditions [63]. Although various substrates have been reported for amylase production, only few substrates such as wheat bran and potato peel have the potential to induce the amylase production from microbes [64]. Besides, a mixture of rice bran and wheat bran in the ratio of 1:2 gave the maximum enzyme production of 549.11 $IUml^{-1}$ among the other agricultural residues using Streptomyces during the submerged fermentation [65].

Six different agro-industrial wastes (rice straw, rice bran, corn flakes, wheat bran, wheat flakes, and grinded wheat kernel) were used for the extracellular enzyme production from a novel isolate *A. oryzae* IIB-6 [12]. Among all the residues, wheat bran gave the maximum yield of 7800 $Ugds^{-1}$ at 80% moisture content during 72 h of fermentation. Similarly, various agro-industrial residues, viz., tuna fish powder waste, wheat gluten waste, soy bean meal as nitrogen sources, and wheat gruel as carbon source were used for the production of the enzyme under solid state conditions from *A. oryzae* S2. Although all the wastes used as nitrogen source had potential for the enzyme production, but the maximum productivity of 22118.34 Ug^{-1} dry substrate was obtained with the soyabean meal [66]. Apart from these, feather meal, rape seed cake and potato peel were utilized as a raw material for amylase production from *Bacillus subtilis* PF1, which showed the maximum production (16.39 ± 4.95 μgml^{-1}) after 96 h of incubation period [67]. Simultaneous production of protease and amylase enzyme was done from the brewery waste by *Bacillus subtilis* UO-01, which resulted in production of the maximum level of protease (9.87 $EUml^{-1}$) and amylase (9.35 $EUml^{-1}$), respectively after 15 h of incubation under the optimized conditions [68].

3.3. Laccase

Laccases, the trivial name for p-diphenol dioxygen oxido-reductase (E.C. 1.10.3.2), belonging to the family of polyphenol oxidases, are one of the oldest studied enzymes having molecular weight of about 60-90 kDa. These enzymes are widely distributed in nature, in plants such as in cabbages, pear, apples, and potato, in insects and in microbial sources such as bacteria and fungi, especially in white rot [69]. Laccases are responsible for catalyzing the oxidation of both phenolic and non-phenolic compounds [70]. They are also capable of mineralizing various synthetic dyes [71].

Laccase production from white rot fungus *Trametes versicolor* has been carried out by submerged fermentation using lignocellulosic wastes such as grape seed, grape stalks and barley bran [72]. In comparison to the others, barley bran has been shown to provide the maximum laccase activity of 500-600 Ul^{-1} from the 27^{th} to 37^{th} days. Besides, banana residual wastes, pseudostems and leaves have also been used for production of extracellular lignolytic enzymes from *Pleurotus ostreatus* and *P. sajor-caju*, which indicated the maximum production of 1.7106 $IUmg^{-1}$ protein and 1.6669 $IUmg^{-1}$ protein from leaves, owing to their larger surface area that favors the microbial growth [73]. The use of banana wastes for the production of laccase signifies their importance as a substrate to produce various enzymes as compared to the other agro-industrial residues.

Residues from the groundnut processing industry like groundnut shells and seed have been tested for their potential in laccase production from *Trametes hirsuta* in a solid state [74]. Using groundnut seed as a medium, a maximum laccase activity obtained during the 14^{th} day of fermentation has been observed to have approximately 10 times higher value obtained than that with groundnut shells. Although groundnut shells are much more suitable for enzyme production owing to their high cellulose content, porosity and roughness, still the maximum enzyme production from groundnut seed could be attributed to the fact that the seeds contain large amount of

vitamins and amino acids that would have stimulated the enzyme production.

Oat husks and wastes from paper industry viz. fiber sludge and combined sludge of fiber and de-inking were also investigated by Winquist et al. as solid supports for laccase production from white rot Basidomyces fungi *Cerrena unicolour* T71 [75]. The research group reported maximum laccase activity (178 $nkatg^{-1}$ DW) obtained from oat husks supplemented with 20% combined fiber and de-inking sludge. In contrast to the other agro-industrial wastes, oat husks proved, in their study, to be the promising substrate as it contained more nutrients and lignocellulosic material at 2-10%, which may act as an inducer for laccase enzyme production. Combined fiber and de-inking sludge contain fewer nutrients and more heavy metals, and fiber sludge contains kaoline, which causes the substrate to be dense; therefore, causing problem in the growth of fungus on the substrate. Similarly, another white rot fungus *Pleurotus florida* NCIM 1243 grown on solid supports of the various peels (banana, mandarin and cantaloupe) showed a maximum activity of 5.4 Ug^{-1} from banana peel, 3.1 Ug^{-1} from mandarin peel, and 4.0 from cantaloupe peel [76]. The combination of these peels in the ratio of 5:2:3 has been demonstrated to provide increased laccase production up to 6.8 Ug^{-1}, may be due to the synergistic action of various components present in these wastes. Solid state fermentation of the laccase using *Phanerocheate chrysosporium* from apple pomace and brewery wastes resulted in the maximum enzyme activity of 789 $Ugds^{-1}$ and 841 $Ugds^{-1}$, respectively at 80% moisture, 3 $mmolkg^{-1}$ veratryl alcohol and 1.5 $mmolkg^{-1}$ copper sulphate [77].

3.4. Lipases

Triacylglycerol-acylhidrolases or lipases (E.C. 3.1.1.3) are the most versatile biocatalysts that are capable of catalyzing several reactions such as esterification, transesterification and interesterification of lipids, as well as complete or partial hydrolysis of triacylglycerols. Due to these properties, these enzymes have a great potential in the food industry, especially for the manufacture of mono- and diglycerides, production of lipids having high amount of polyunsaturated fatty acids, flavor development, and during the maturation of cheese [78]. Therefore, to reduce the cost of enzyme for its suitability in industrial applications, intensive efforts have been made to minimize the cost of enzyme production by various agro-industrial residues using microbial sources [79]. Among the various microbial sources such as bacteria, yeast, fungi, actinomycetes, archea, etc., microbes of the genera Candida, Rhizopus, Rhizomucor, Geotrichum, Penicillium, Aspergillus, Bacillus, Pseudomonas and Staphylococcus are the most preferred sources for the commercial production of lipases [80].

Gutarra et al. compared SSF production of lipase from *Penicillium simplicissimum* by using tray-type and packed-bed bioreactors with babassu cake as the basal medium [81]. Their study revealed that lipase production in the packed-bed reactor was about 30% higher than that of tray type-bioreactors. Moreover, in the tray-type bioreactor, concentration of nitrogen did not exert any effect on the production, whereas temperature, moisture content and carbon source had a profound effect on the production. Whereas, using packed-bed reactor, the aeration rate and temperature had a negative impact on the lipase production.

Lipase production has also been done by Mala et al.. using a mixed substrate of wheat bran and gingelly oil cake in the ratio 3:1 from *A. niger* MTCC 2594. It resulted in an enzyme activity of 384.3±4.5 Ug^{-1} dry substrate, which was about 36% higher than that obtained from the submerged fermentation [82]. The scale up of lipase production on 100 g and 1 kg trays has also been shown to enhance the enzyme production by 95% and 84%, res-pectively. Another agro-industrial waste, palm oil mill effluent supplemented with 0.45% peptone and 0.65% tween 80, was used as a medium for production of lipase by submerged fermentation from *Candida cylindracea* ATCC 14830 that led to the production of 20.26 Uml^{-1} lipase [7].

Tri-substrate fermentation (TSF) technique was first developed by Edwinoliver et al. for production of lipase from *A. niger* MTCC 2594 using wheat bran, coconut oil cake and wheat rawa, which resulted in the maximum enzyme activity of 628.7±13 Ug^{-1} dry substrate. The use of TSF for the fermentation process made the process economically feasible; and therefore, this technique could easily be used for further industrial applications [83].

A comparative study was carried by Rajan and Nair [84] for production of alkaline lipase from *A. fumigatus* by both solid and submerged fermentations, where the enzyme production in both fermentation methods was reported to be similar. However, lipase production from SSF was shown to be stable up to 15 days in contrast to the enzyme synthesized by submerged fermentation.

Apart from the above wastes, various other agro-industrial residues (castor bean cake, cassava cake, sugarcane bagasse, and corn husk) have been tested for lipase production using *Trichoderma harzianum* either singly or in combination such as castor bean cake with cassava cake, castor bean cake with corn husk, and castor bean cake with sugarcane bagasse, each in the ratio of 1:1, moistened with different oils like olive oil, corn oil, sunflower oil and soyabean oil. Among all the residues, the maximum enzyme production of 4 Ug^{-1} was obtained from castor bean cake in combination with sugarcane bagasse moistened with olive oil [85]. Besides, the production of lipase from Fusarium isolate FCLA-MA-41 has been compared for both submerged and solid state fermentations using a variety of agro-industrial

residues [86]. During the submerged fermentations, a productivity of 3.0 ±0.25 Uml^{-1} lipase was noted to be obtained in the study using crambe oil supplemented with Triton X-100, ammonium sulphate and yeast extract, whereas lipase production of 5.0 Uml^{-1} was obtained during the solid fermentation using crambe meal moistened with phosphate buffer.

Different oilseed cakes (sesame oil cake, groundnut oil cake and coconut oil cake) have been used as substrates for lipase production using *Candida rugosa* NCIM 3462 as a microbial source. The maximum enzyme yield of 22.40 Ug^{-1} substrate was obtained by using sesame oil cake, and further, by optimizing the different process parameters using a statistical technique [87].

Besides the fungal species, bacteria have also been reported for production of lipase using different agro-industrial residues. Lipase has been produced using Pseudomonas strain BUP6 in a basal medium supplemented with different oil cakes viz., groundnut cake, cottonseed cake, gingerly, soyabean and coconut. Among various residues examined by Faisal et al. groundnut cake supported the growth and production of lipase [88]. Palm fiber and palm alkaline soap stock residues obtained from the palm oil industry have been used for the enzyme production from *A. niger*. In contrast to submerged fermentation, higher yield was obtained from solid fermentation, showing an activity of 15.41 $IUml^{-1}$, thereby indicating the feasibility of using palm oil industry wastes for industrial production of enzymes [89].

3.5. Tannase

Tannase, the trivial name of tannin acyl hydrolase (E.C. 3.1.1.20), catalyzes breakdown of ester and depside bonds; thereby releasing gallic or ellagic acid and glucose [90]. This enzyme has a wide range of industrial applications such as clarification of beer and beverages, manufacturing of instant tea, reducing the anti-nutritional factors of tannins, etc. [91]. The production of tannase can be done by using agro-industrial wastes as substrates either by submerged or by SSF from bacteria, yeasts and fungi [92-95].

Tannase production by using cashew apple bagasse as a substrate supplemented with tannic acid, ammonium sulphate, moisture and sucrose as an additional carbon source has been reported to reach to the yield of 4.63 Ug^{-1} dry substrate [96]. In addition, different agro-industrial wastes (paddy husk, rice bran, millet husk and groundnut shell) have been compared for their efficiency during tannase production from *A. oryzae* [97]. In contrast to all the other sources, rice bran has been reported to give the maximum enzyme production at the temperature range of 35-40ºC at pH 5.5.

Tannase production has been carried out using different substrates like bahera fruit, myrobalan, wattle powder and quebracho powder supplemented with wheat bran. Bahera fruit has been noted to be suitable substrate providing the maximum activity of 528 $Ugds^{-1}$. Moreover, addition of wheat bran as a supplement enhanced the enzyme production in this system [98]. Bahera fruit is the rich source of tannic acid, besides the other components, which makes it suitable for tannase production. RSM has been applied to achieve maximum concentrations of the enzyme using tamarind seed powder as a substrate. Under the optimum conditions of 3.22% tannic acid concentration, pH 4, temperature of 35.1ºC and fermentation time of 96 h, a maximum activity of 139.3 $IUml^{-1}$ was observed [99].

Coffee pulp, by-product of the coffee processing industry, has been used as a substrate for tannase production from *Penicillium verrucosum*, which resulted in maximum yield of 28.173±1.4 $Ugds^{-1}$ [100]. Moreover, by optimizing the various process conditions, there was a 3.93 fold rise in the productivity, showing the maximum activity of 115.995 $Ugds^{-1}$. These results indicate the potential of the coffee pulp as the chief source for the synthesis of value-added products. Moreover, SSF of tannase using *Aspergillus terreus* from different agro-industrial residues (red gram husk, green gram husk, ground nut waste, cotton seed waste, wheat bran, rice bran, coffee husk, tamarind seed powder, cashew apple bagasse, corn powder, and coconut powder) has been done [101]. Among all the tested residues, wheat bran was found to be the suitable substrate for tannase production, where the maximum tannase activity of 46.8 Umg^{-1} was observed under the optimized conditions.

3.6. Invertase

Invertase (EC 3.2.1.26), also known as β-fructofuranosidase, belongs to the GH32 family of glycoside hydrolases, and is responsible for catalyzing the conversion of sucrose into D-glucose and D-fructose [102]. Owing to these properties, this enzyme has many potential applications in the food industry, including confectionary, syrups, condensed milk, infant foods, beverages for production of artificial honey, plasticizing agents in cosmetics, and in the analytical fields for construction of sucrose biosensors [103,104]. The major sources of microbial production of invertase include Aspergillus, Bacillus, Saccharomyces and Fusarium [105]. Among all the sources, *Saccharomyces cerevisiae* or *Saccharomyces carlsbergensis* is the most widely used microbe due to its sucrose fermenting ability as sucrose is the chief source used during the fermentation [106].

Highest productivity of invertase (272.5 Ug^{-1}) has been achieved using red carrot jam processing residue as compared to the other food processing wastes (lemon pulp, orange pulp, sugarcane bagasse, grape juice residue, apple pomace and soya bean residue) used during SSF carried out by *S. cerevisae* NRRL Y-12632 after 4 days at 90% initial moisture content [107]. The potential of the different fruit

peels such as pomegranate, orange, and pineapple has been tested using *A. flavus* during the submerged fermentation [108]. Among all the tested substrates, maximum productivity of 25.8 $IUml^{-1}$ was obtained using orange peel as a raw material.

Efforts have been made to utilize the wastes generated from the sugar industry, like pressmud and the waste generated from the distillery such as spent yeast for the solid state fermentation of invertase. A maximum specific activity of 430 Umg^{-1} was found by Kumar and Kesvapillai [109] after 72 h of fermentation, when a mixture of spent yeast and cultured yeast in the ratio of 7:3 was used.

Different agro-industrial by-products (peels of orange, pomegranate, sapota, pineapple, lemon, grapes, and sugarcane bagasse) used for the enzyme synthesis from *S. cerevisae* indicated an enzyme production of 0.48±0.011 $IUml^{-1}$ from 4% orange peel. Carrot peels, by-products obtained after processing of carrot and a form of waste containing high amount of sucrose and other nutritional factors, have been utilized as a raw material for the solid state fermentation of invertase from *A. niger*, which yielded an activity of 7.95±0.1 Uml^{-1} at 90% moisture content after 72 h of fermentation [110]. Similarly, other agro-industrial wastes such as sunflower waste, cotton waste, rice husk, date syrup and molasses have been tested to get the maximum enzyme production by submerged fermentation using *A. niger* IBGE 01 [111]. Under the optimum conditions of agitation (150 $revmin^{-1}$), pH (6.0), inoculum size (5×10^6 conida), and temperature (40ºC), the highest activity of 8.23 Uml^{-1} was obtained using molasses as substrate after 72 h of fermentation.

3.7. Protease

Protease (EC 3.4.21.40) is a hydrolytic enzyme, which catalyzes the hydrolysis of the peptide bonds linking the amino acids in the polypeptide chain [112]. Among the four types of proteases (serine proteases, aspartic proteases, cysteine proteases and metallo-proteases), alkaline proteases are widely used and have a wide range of applications in detergent, leather, pharmaceutical and chemical industries [113]. These enzymes have a great potential in the various sectors of the food industry such as tenderization of meat, production of cheese, bakery products and waste management [112]. Although proteases can be extracted from a variety of natural sources, but still microbes are the preferred sources for enzyme production. Among the different microbial species (bacteria, yeasts and fungi), Bacillus is of great choice in terms of high yield of the enzyme [114,115].

Agro-industrial residues like pigeon pea waste, pineapple waste, orange peel waste, rice bran, wheat bran, raw potato starch, raw sweet potato starch, and sugarcane bagasse powder have been investigated during the solid state fermentation of protease from the thermoalkaliophilic Bacillus JB-99, where maximum enzyme production was obtained from pigeon pea waste (12,430±120 Uml^{-1}), followed by pineapple waste and orange peel [116]. *Bacillus circulans* has been used for enzyme production using green gram husk as a solid substrate and optimization of various process parameters by Taguchi orthogonal array [117]. The results indicated the maximum enzyme activity of 32000-72000 Ug^{-1} of the material under optimized conditions. Microbial production of protease has a distinct relationship with the growth of the microbial strain; thus the yield of the enzyme is correlated with the culture conditions used. Green gram husk, an inexpensive source, has been investigated for enzyme production from Bacillus, which showed the maximum enzyme activity of 9550 Ug^{-1} biomass [118]. The production of protease was affected both by the physiological and chemical characteristics of green gram husk and by the growth of the microorganism.

Cheap agro-industrial wastes such as mustard oil cake , wheat bran, rice bran , banana leaves , and kitchen wastes like used tea leaves, potato peels and *Imperata clyindrica* grass were evaluated to determine their potential during protease production by using a thermophilic bact-erial strain *Bacillus subtilis* DM 04. In contrast to all the other sources, potato peel has been reported by Mukherjee et al. as an efficient substrate resulting in maximum yield of the enzyme followed by *Imperata clyindrica* grass. Moreover, the combination of potato peel and *I. clyindrica* grass in the ratio of 1:1 (w w^{-1}) significantly enhanced the protease production as compared to the individual substrates; this technique is useful in reducing the production cost as no additional supplements of carbon and nitrogen sources are required during the production [119]. Castor husk, a by-product obtained during castor oil processing, was assessed during alkaline protease production by *Bacillus altitudinis* GVC11, which gave an enzyme activity of 419,293 Ug^{-1} of husk under the optimum conditions of moisture content, incubation period and particle size [120]. Taguchi orthogonal array was used for optimization of protease production from *Pseudomonas aeruginosa* using wheat bran as a substrate [121]. Under the optimum conditions of pH, temperature, inoculum size and agitation speed, the maximum enzyme activity of 582.25±9.2 Uml^{-1} was obtained.

Other industrial wastes like dal mill waste, oil mill waste, molasses, fruit waste and vegetable garbage have been exploited for enzyme production from *Fusarium oxysporum*, which revealed the maximum production of 8.8 μgml^{-1} after 7 days of fermentation from the dal mill waste, oil mill waste, molasses and vegetable garbage in contrast to the fruit waste [122]. Cost effective production of protease using various agricultural residues (wheat bran, cotton cake, chicken feather, mustard cake, soyabean meal, maize bran, rice husk, cane bagasse, gram husk, and corn cob) from *Bacillus subtilis* K-1

under solid state fermentation indicated the effectiveness of cotton seed oil cake during the solid state fermentation [123]. Cotton seed cake supported maximum protease production (728 Uml^{-1}), followed by gram husk (714 Uml^{-1}), mustard cake (680 Uml^{-1}) and soya bean meal (653 Uml^{-1}).

3.8. Naringinase

Naringinase, a debittering enzyme complex, constitutes both α-L-rhamnosidase (E.C.3.2.1.40) and β-D-glucosidase (E.C. 3.2.1.21). α-L-rhamnosidase is responsible for breaking down of naringin to prunin plus rhamnose; and β-D-glucosidase is mainly responsible for hydrolysis of prunin to naringenin and glucose, respectively [124,125]. The major industrial applications of this enzyme include debittering of the citrus fruit juices [126], removal of the hesperidin crystals from the orange juice [127], and enhancement of flavor, and aroma in the wine [128]. The enzyme production is carried out mostly by SSF owing to the ability of the process for utilization of various cheap agro-industrial residues, besides the other advantages [129]. Different wastes have been reported for naringinase production using SSF.

Naringinase production has been done using orange and grapefruit rind, which revealed the maximum enzyme production from grapefruit rind as a substrate [130]. The high yield of enzyme from grapefruit rind is mainly due to the presence of high narginin content (572 µmol of narginin per gram of fresh weight), which acts as an inducer for the enzyme production; thereby eliminating the use of additional naringin as a supplement. Different agro-industrial residues such as rice bran, sugar cane baggase, citrus peel and press mud in SSF were tested for their potential during enzyme production using filamentous fungi *A. niger* MTCC 1344 [131]. Among all these substrates, rice bran was found to be the most promising substrate during naringinase production, showing the maximum activity of 58.1±1.6 Ug^{-1} dry substrate. Similarly, cheap and readily available agricultural residues (sugarcane bagasse, soy bean hulls and rice straw) were assessed for the capability in enzyme production from *A. niger* strain [132]. The maximum enzyme production (1.92 Uml^{-1}) was obtained by using 0.14 g sugarcane bagasse, 1.25 g soyabean hulls and 3.05 g rice straw. Moreover, by optimizing the moisture level to 75.5% and pH to 4.0, the activity was increased to 3.02 Uml^{-1}. Statistical technique was applied to optimize the parameters to achieve a maximum concentration of the enzyme using orange rind as a substrate from *A. niger*. Under the optimal parameter conditions, the maximum enzyme activity of 13.89 Uml^{-1} was obtained [133].

3.9. *β*-Galactosidase

β-Galactosidase (*β*-D-galactoside galactohydrolase, E.C. 3.2.1.23), most commonly known as lactase, is one of the most important enzymes used in food processing, which has both hydrolytic (hydrolysis of lactose to its constituent monosaccharides, glucose and galactose) and transgalactosylation activities [134]. This enzyme has several applications in the food, dairy and fermentation industries, and thus offers nutritional, technological and environmental applications for human beings. A number of microbial sources have been used for production of *β*-galactosidase.

However, the most widely used microbial sources are Kluyveromyces sp. and Aspergillus sp. Besides synthetic medium (lactose), food industry by-products have also been tested for *β*-galactosidase production. Among the different byproducts, whey and wheat bran have shown significant potential as substrate in *β*-galactosidase production. However, most of the work has been carried out by using whey as substrate for *β*-galactosidase production.

Different yeasts, which are able to utilize lactose, can be grown on whey, and subsequently, be used for *β*-galactosidase production. The yeasts such as *Candida*. *pseudotropicalis*, *Kluyveromyces marxianus*, *Kluyveromyces. fragilis* and *Kluyveromyces. lactis* have been used by different researchers for production of this enzyme from whey-based medium. In most cases, there is a need for supplementation of whey with different nutrients for optimal production of the enzyme [134-136]. The optimal process conditions for production of the enzyme vary considerably for different yeast strains. The supplementation of yeast extract, ammonium sulfate and potassium dihydrogen orthophosphate to the whey can be useful for lactase production from *C*. *pseudotropicalis* [137]. *A. carbonarius* has also been employed for production of *β*-galactosidase grown on deproteinized cheese whey with maximal enzyme production [138].

K. marxianus MTCC 1389 has been tested for *β*-galactosidase production from whey [139]. Among the four methods tested for extraction of β-galactosidase, SDS-chlorofom method was found to be best followed by toluene-acetone, sonication and homogenization with glass beads. SDS-chloroform is a cheap and simple method for enzyme extraction from Kluyveromyces cells, which results in higher enzyme activity in contrast to the other extraction methods.

K. marxianus NCIM 3551 has been used for *β*-galactosidase production and ethanol fermentation from whey [140]. Optimum *β*-galactosidase production and ethanol fermentation were obtained over an incubation period of 20 h at pH 5.0 and at 25°C with 16 h old culture. It has been indicated that nitrogen supplementation did not have much effect on *β*-galactosidase production and ethanol fermentation.

Permeate-based medium has been used for *β*-galactosidase production. Among the different bacterial cultures tested, *L. reuteri* and

Streptococcus thermophilus were selected for *β*-galactosidase production from permeate-based medium. The maximum enzyme production (6.31 Uml^{-1}) by *L. reuteri* has been reported at lactose concentration of 6%, initial pH 5.0–7.5, ammonium phosphate as nitrogen source, and incubation temperature at 30°C after 24 h. While in case of *S. thermophilus*, maximum *β*-galactosidase production (7.85 Uml^{-1}) was achieved at 10% lactose concentration of permeate medium, at initial pH 6.0–6.5, ammonium phosphate as nitrogen source and incubation temperature 35°C after 24 h [141].

The production of an intracellular *β*-galactosidase was carried out from *Streptococcus thermophilus* grown in whey [142]. The enzyme was purified by different techniques (ammonium sulphate precipitation, dialysis, gel filtration chromatography using Sephadex G-100, and SDS-PAGE) and properties of the purified enzyme such as pH, temperature optima and kinetic parameters were determined. *S. thermophilus* A5 strain exhibited maximum enzyme productivity of 7.76 Uml^{-1} and protein content of 67 μgml^{-1} at 40ºC and pH 7.2. The V_{max} and K_m values were found to be 2.8 $IUml^{-1}$ and 3.05 mM, respectively.

The production of *β*-galactosidase (β-gal) has been carried out in deproteinized whey based medium by *Bifidobacterium animalis* ssp. *lactis* Bb12 and *Lactobacillus delbrueckii* ssp. *bulgaricus* ATCC 11842 [143]. Among the four methods (sonication, acetone toluene, SDS chloroform and lysozyme EDTA treatment) tested for *β*-galactosidase extraction, sonication gave the maximum enzyme activity (6.80 Uml^{-1}) for *B. animalis* ssp. *lactis* Bb12 while for *L. delbrueckii* ssp. *bulgaricus* ATCC 11842, lysozyme EDTA treatment was found to be the best (7.77 Uml^{-1}).

Among the seven strains of the genus Kluyveromyces tested for *β*-galactosidase activity, *Kluyveromyces marxianus* ATCC 16045 was selected for culture medium optimization on whey and parboiled rice effluent by submerged fermentation [144]. The optimization for production of *β*-galactosidase was evaluated using a factorial design.

The production of this enzyme using cheese whey and corn step liquor by *Kluyveromyces marxianus* CBS 6556 has been carried out [145] using central composite rotational design (CCRD). The highest *β*-galactosidase activity (21.99 Uml^{-1}) was achieved after 24 h and further cheese whey concentration. The results also depicted that using corn steep liquor as a supplement has a positive effect on *β*-galactosidase activity.

Apart from submerged fermentation, solid state fermentation utilizing various agro-industrial residues for production of *β*-galactosidase using fungal species such as Aspergillus, Penicillium, Fusarium, etc., has been studied by various researchers. The most common substrate used for enzyme production was wheat bran owing to the presence of high hemicellulose content, total available sugars and the availability of essential nutrients that act an inducer for *β*-galactosidase production in various microbial species [146,147]. A mixture of wheat bran and rice husk in the ratio of 1:1 was assessed for their potential during enzyme production from *A. flavus* [148]. This mixture was efficient in producing high levels of *β*-galactosidase with addition of glucose (12.5% w w^{-1}) and sodium nitrate (1%) as carbon and nitrogen sources, respectively. Moreover, activity was enhanced under the optimal conditions of mois-ture (90%), pH (5) and temperature (30ºC). Similarly, using wheat bran as a substrate, maximum enzyme production (5661.4±20.9 $Ugds^{-1}$) from *A. tubingenesis* halotolerant fungal isolate was observed when deproteinized whey was used as a moistening agent [149]. The enzyme activity was greatly influenced with the addition of corn steep liquor and magnesium sulphate, thus under the optimized conditions, the activity was increased to 15,936 $Ugds^{-1}$.

Six different agro-industrial residues (wheat bran, soya oil cake, soya meal, whole wheat and pulp beet) have been investigated for enzyme production from *Penicillin canescens* [150]. In contrast to the all the residues, maximum activity (4010 ±84 Ug^{-1}) was obtained with wheat bran, followed by whole wheat (3818 ±84 Ug^{-1}) after 7 and 9 days, respectively. Moreover, to enhance the enzyme production, combinations of wheat bran and whole wheat (7:3, 5:5 and 3:7) were taken, which indicated elevated levels of enzyme production, having the activity of about 5292±111 Ug^{-1} under the optimal conditions at (83% moisture content, 10^6 spores in per gram and at 30ºC).

4. Future prospects and industrial scope

Enzymes have a great influence in almost every industrial sector (e.g. food, feed, pharmaceutical, etc.) and thus, to meet the ever increasing demand of the consumers, the market for the industrial enzyme is also increasing tremendously. The industrial enzyme market was valued to be USD 4.2 billion in the year 2014, which is expected to rise to USD 6.2 billion dollars by the year 2020 [151]. Among the different industrial sectors, approximately more than 50% of the demand of enzymes arises from the food industry. The major food sectors include the sugar and starch processing, bakery and dairy industries. The most important food grade enzymes such as amylase, galactosidases, pectinases, proteases, lipase, etc. have a great potential, market and demand in the food sector. But the major barrier in the utilization of these enzymes in the industrial sector lies in the non-availability of the specific characteristics, cost of production, and lack of specific enzymes to carry out the industrial processes. Therefore, to reduce the above issues, novel techniques like protein engineering and genetic modification have been developed for the synthesis of enzymes having desirable properties [2].

Furthermore, with the advancement in the research and development (R&D), new biotechnological technologies are being and have been developed to minimize the cost of enzyme production. Despite of all this, the continuous pressure from the environmental agencies to minimize the pollution, the focus has been shifted to utilizing the different wastes generated from the agro-industries for enzyme production, such as Novozymes, the world leading enzyme producer that utilizes renewable sources such as soy grits, and corn starch for enzyme production [152].

5. Conclusions

This review highlights the potential of various agro-industrial residues as substrates for microbial production of food-grade enzymes. Enzymes have been an essential part of human life since the ancient times for production of various food products, and therefore, are being largely explored in various food industry sectors. Among the various natural sources, microorganisms are the preferred sources for enzyme production due to a number of advantages. Owing to the high cost of production arising from the fermentation medium and process conditions, the trend has been shifted towards the use of agro-industrial wastes for production of cost-effective enzymes. The utilization of agro-industrial wastes for microbial fermentation not only improves the process economics, but can also reduce the environmental issues related with their disposal. Although different residues have been exploited and tested for their potential as substrates for microbial fermentation of enzymes, but still the dairy and cereal industry wastes/by-products have significant potential for enzyme production. Moreover, among the different types of fermentation (solid state and submerged) tested for enzyme production, maximum work has been carried under solid state conditions by batch fermentation. Although various microbial sources have been used for enzyme production, yet new and efficient microbial sources are needed for the optimal utilization of agro-industrial wastes to achieve higher yields of the enzymes. Furthermore, the biotechnological techniques such as genetic engineering of the microorganisms, enzyme engineering, and other techniques would play an important role in the near future.

6. Conflict of interests

The authors have no conflict of interest.

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