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Review

Citric Acid Production by *Aspergillus niger* Using Solid-State Fermentation of Agricultural Processing Coproducts

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Abstract: The ability of *Aspergillus niger* strains to support citric acid production using solid-state fermentation of agricultural processing coproducts was examined in this review. Citric acid has been shown to have a number of commercial applications in the food and beverage industries. The *A. niger* strains capable of elevated citric acid production are known to contain genetic mutations that stimulate overproduction of the organic acid likely involving citric acid cycle reactions. The agricultural processing coproducts previously examined for their ability to support citric acid production by *A. niger* solid-state fermentation include fruit processing wastes, sugarcane bagasse, starch vegetable processing wastes and cereal grain processing coproducts. A comparison of citric acid production by *A. niger* strains using solid-state fermentation demonstrated that certain agricultural processing coproducts were more effective in supporting a high level of acid synthesis. In particular, fruit processing wastes, such as apple pomace, banana peels, grape pomace and orange peels, supported high levels of citric acid by the fungal strains following solid-state fermentation. On the other hand, processing coproducts of cereal grains, such as brans and ethanol processing coproducts, supported low levels of citric acid production by the *A. niger* strains using solid-state fermentation. It appeared that the cereal processing coproducts provided less available sugar content to support citric acid production by the fungal strains. It was concluded that the level of citric acid produced by the *A. niger* strains during solid-state fermentation was dependent on the sugar content of the agricultural processing coproduct utilized.

Keywords: citric acid; solid-state; *Aspergillus niger*; fermentation; processing coproducts

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1. Introduction

Citric acid is a water-soluble, specialty organic acid, which exists as a white, crystalline powder and exhibits a significant buffering capacity in water (Figure 1). The global citric acid market in 2023 is projected increase to $3.2 billion [1]. It is estimated that over a million tons of citric acid are produced globally every year [2]. Citric acid has been designated as Safe by the World Health Organization [1]. The applications of citric acid include its use as a flavor enhancer, a pH regulator, a preservative, a chelating agent, a stabilizer and an antioxidant [1]. Citric acid has a number of commercial applications in foods and beverages. It is used as an acidifier in foods plus it leaves little aftertaste [1]. In beverages, citric acid is used to balance sweetness since it imparts a tart taste [1]. Healthwise, citric acid is known to support digestion and kidney function [1]. Citric acid is used in pharmaceuticals to modify pH as well as an antioxidant in preserving vitamins. In the textile industry, citrate is used as a foaming agent to improve textile softness. Citrate is also used in the detergent industry as a replacement for phosphate in detergents to make them more environmentally friendly. It should also be noted that an organic acid, such as citric acid, could be used as a building block in the production of commodity chemicals [2].

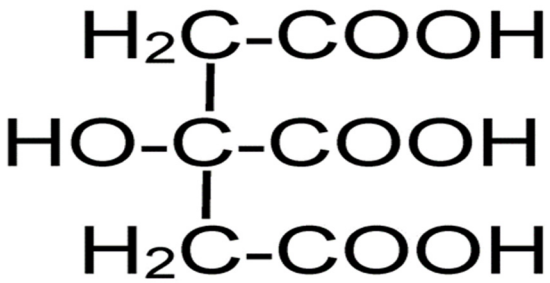

Figure 1. The structure of citric acid.

Citric acid can be isolated from citrus fruit [3–5]. Originally, citric acid was extracted from lime juice but the process was not cost effective. Similarly, chemical synthesis of citric acid using glycerol was not feasible economically. It was replaced by the microbial fermentation of citric acid, which was far more economically feasible. The majority of citric acid that is produced commercially is synthesized by microbial fermentation. Although a number of bacteria and yeast synthesize citric acid, the majority of the citric acid produced commercially is synthesized by the fungus *Aspergillus niger*. Biochemically, the synthesis of citric acid by *A. niger* occurs in its mitochondria (Figure 2). The synthesis of citric acid occurs in the citric acid cycle located in the mitochondria [3–6]. Pyruvate is formed from fermentable sugars by glycolysis. The pyruvate crosses the mitochondrial membrane and is converted to oxaloacetate by the enzyme pyruvate carboxylase. Citrate synthase catalyzes the formation of citric acid from oxaloacetate and acetyl CoA. It should be noted that not all strains of *A. niger* strains are capable of citric acid production. The citric acid-producing *A. niger* strains have been shown to be genetically modified so that they are capable of excreting high levels of citric acid [3–6]. Fermentation of citric acid is considered a green chemistry process since it does not generate any toxic products unlike during the chemical synthesis of citric acid. Most commonly, moist agricultural processing coproducts are used as substrate for a citric acid-producing *A. niger* strain to synthesize the organic acid. For example, it has been shown that the fungal stain *A. niger* ATCC 10577 was capable of producing citric acid from spent grain liquor using surface fermentation [7]. It has also been shown that the addition of phosphate or methanol to *A. niger* cultures can stimulate citric acid production by the fungus [8]. This review will focus on citric acid production by *A. niger* cultures using solid-state fermentation rather than surface or submerged fermentation. With solid-state fermentation, low value biomass from agricultural processing can be transformed into the high value commodity chemical citric acid but the type of biomass utilized is important [9].

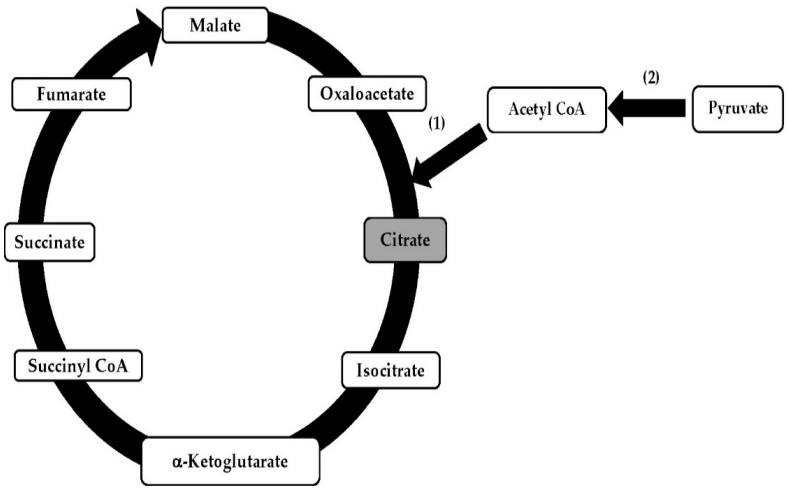

Figure 2. Citric acid synthesis by the citric acid cycle in *Aspergillus niger*. (1) Citrate synthase; (2) Pyruvate carboxylase.

2. Fruit Processing Wastes

2.1. Apple Processing Wastes

The ability of *A. niger* to synthesize citric acid using solid-state fermentation of apple processing wastes has been investigated (Table 1). Peels of African star apples (*Chrysophyllum albidum*) were used for solid-state fermentation (initial pH 3.5) by *A. niger* strains [10]. It was determined that *A. niger* strain DTO: 133-E8 produced a low citric acid concentration after 192 h at 28 °C as did *A. niger* strain DTO: 131-H5 of solid-state fermentation of the peels [10]. In the presence of 2% methanol, solid-state fermentation of the peels by strain DTO: 133-E8 or DTO: 131-H5 increased citric acid production to 1.4-fold or 1.3-fold, respectively, after 192 h at 28 °C [10]. Several studies have used apple pomace as a substrate for the fungal solid-substrate fermentation of citric acid (Table 1). An early study examined solid-state fermentation of apple pomace by *A. niger* strains ATCC 13794, ATCC 11414, ATCC 9142, ATCC 1015 and ATCC 12846 [11]. It was observed that ATCC 9142 produced the highest citric acid level following solid-state fermentation of apple pomace for 120 h at 30 °C compared to the other strains tested [11]. When 4% methanol was added to the apple pomace, solid-state fermentation by ATCC 12846 produced the highest citric acid level after 120 h at 30 °C [11]. It was 1.8-fold higher than the citric acid level produced by ATCC 9142 on the untreated apple pomace [11]. A later study found that *A. niger* MTCC 281 produced a low level of citric acid when used for solid-state fermentation of apple pomace in flasks in the presence of 4% methanol after 120 h at 30 °C [12]. Another study found that solid-state fermentation of apple pomace by *A. niger* ATCC 12846 produced a slightly higher citric acid concentration than did *A. niger* ATCC 13794 after 30 °C for 72 h when the substrate (55% initial moisture) was inoculated with 10^7 spores in flasks [13]. The addition of 3% ethanol or 4% methanol to the apple pomace resulted in about a doubling of the citric acid concentration produced by *A. niger* ATCC 12846 when the solid-state fermentation of apple pomace for 96 h at 30 °C [13]. A similar study using *A. niger* ATCC 12846 for the solid-state fermentation of apple pomace supplemented with rice husks (75% moisture and initial pH of 3.4) for 168 h at 30 °C resulted in the production of nearly 350 g citric acid/kg substrate [14]. Solid-state fermentation by ATCC 12846 of the apple pomace supplemented with rice husks increased citric acid production when either methanol or ethanol was added [14]. When solid-state fermentation of apple pomace (75% initial moisture and initial pH 3.0) by ATCC 12846 occurred in a 12-L rotating-drum-type bioreactor in the presence of 3% methanol after 120 h, a higher level of citric acid was synthesized by ATCC 12846 than if solid-state fermentation of the apple pomace occurred in a rotating-type bioreactor in the presence of 3% ethanol [15]. It should be mentioned that the use of a rotating-type bioreactor would be considered large-scale production. It is clear that the majority of solid-state fermentation studies of agricultural processing coproducts by *A. niger* have been performed using small-scale, shake flask fermentations. Apple pomace supplemented with rice husk (70% moisture) has also been used as a substrate for solid-state fermentation by *A. niger* ATCC 13794 (10^7 spores/g pomace) in 500 mL Erlenmeyer flasks for 72 h at 30 °C. Less citric acid was produced by ATCC 13794 than if solid-state fermentation of the apple pomace supplemented with rice husks was done in trays for 120 h at 30 °C [16]. It appeared that the use of apple pomace for fungal citric acid production using solid-state fermentation was highly feasible.

2.2. Banana and Plantain Peels

The solid-state fermentation of banana and plantain peels have examined as a potential substrate for citric acid production (Table 1). It was noted that solid-state fermentation of banana peels (supplemented with methanol and copper ions) by *A. niger* UABN210 produced a higher level of citric acid after 96 h at 30 °C than that level produced by *A. niger* ATCC 9142 solid-state fermentation of banana peels (initial pH 8.0) for 120 h at 30 °C [17,18]. Solid-state fermentation of banana peels (initial pH 4) by *A. niger* EU440768.1 for 240 h at 28 °C in the presence of 3% methanol resulted in citric acid production being higher than the levels synthesized by *A. niger* UABN210 or ATCC 9142 [17–19]. Solid-state

fermentation of banana peels (pH 5.0) in flasks by *A. niger* ATCC 16888 produced less citric acid after 72 h of incubation at 30 °C than when solid-state fermentation of banana peels (pH 5.0) by ATCC 16888 was performed in an aerated, air-jacketed 2 L glass column for 48 h at 30 °C [20]. Solid-state fermentation of plantain peels by *A. niger* ATCC 6275 was investigated [21]. The fermentation of plantains by ATCC 6275 resulted in a 1.5-fold higher citric acid concentration being synthesized after 96 h at 30 °C compared to its citric acid production after 144 h at 30 °C [21]. It was concluded that solid-state fungal fermentation of banana peels or plantains peels was an excellent substrate for citric acid production.

Table 1. Growth conditions and maximum citric acid production by *Aspergillus niger* strains on processing wastes of apples, bananas and plantains using solid-state fermentation.

Substrate	Strain	Sugar Addition	Growth Conditions	Methanol Addition	Citric Acid (g/kg)	Reference
Apple peels	DTO: 133-E8	15% sucrose	192 h, 28 °C	None	16	[10]
Apple peels	DTO: 133-E8	15% sucrose	192 h, 28 °C	2%	21	[10]
Apple peels	DTO: 131-H5	15% sucrose	192 h, 28 °C	None	10	[10]
Apple peels	DTO: 131-H5	15% sucrose	192 h, 28 °C	2%	13	[10]
Apple pomace	ATCC 1015	None	120 h, 30 °C	None	314	[11]
Apple pomace	ATCC 1015	None	120 h, 30 °C	4%	771	[11]
Apple pomace	ATCC 9142	None	120 h, 30 °C	None	482	[11]
Apple pomace	ATCC 9142	None	120 h, 30 °C	4%	791	[11]
Apple pomace	ATCC 11414	None	120 h, 30 °C	None	455	[11]
Apple pomace	ATCC 11414	None	120 h, 30 °C	4%	816	[11]
Apple pomace	ATCC 12846	None	120 h, 30 °C	None	259	[11]
Apple pomace	ATCC 12846	None	120 h, 30 °C	4%	883	[11]
Apple pomace	ATCC 13794	None	120 h, 30 °C	None	359	[11]
Apple pomace	ATCC 13794	None	120 h, 30 °C	4%	766	[11]
Apple pomace	MTCC 281	None	120 h, 30 °C	4%	46	[12]
Apple pomace	ATCC 12846	None	72 h, 30 °C	None	66	[13]
Apple pomace	ATCC 12896	None	96 h, 30 °C	4%	116	[13]
Apple pomace	ATCC 13794	None	72 h, 30 °C	None	61	[13]
Apple pomace	ATCC 12846	None	144 h, 30 °C	3%	342	[14]
Apple pomace	ATCC 12846	None	120 h, 30 °C	3%	221	[15]
Apple pomace	ATCC 13794	None	72 h, 30 °C	None	61	[16]
Apple pomace	ATCC 13794	None	120 h, 30 °C	3%	364	[16]
Banana peels	UABN 210	None	96 h, 30 °C	1%	82	[17]
Banana peels	ATCC 9142	None	120 h, 30 °C	None	2495	[18]
Banana peels	EU440768.1	5% glucose	240 h, 28 °C	3%	98	[19]
Banana peels	ATCC 16888	None	48 h, 30 °C	None	124	[20]
Plantains	ATCC 6275	None	96 h, 30 °C	None	29	[21]

2.3. Cocoa Pod and Coffee Husk Processing Wastes

Solid-state fermentation by *A. niger* of cocoa pods and coffee husk processing wastes to synthesize citric acid has been explored in prior studies (Table 2). Citric acid production by *A. niger* LPB BC-CCT 7717 using solid-state fermentation of cocoa pod husks was examined when 4% methanol was supplemented [22]. The strain produced the highest citric acid

concentration after growth on the husks (65% moisture, initial pH 5.5) at 30 °C for 72 h with relatively high yields [22]. It was determined that the level of citric acid synthesized by the strain on the husks after growth at 30 °C for 24 or 48 h was reduced by 31% or 72%, respectively, relative to citric acid production by the strain on the husks after 72 h at 30 °C [22]. Coffee husk processing wastes was used as a substrate for citric acid production by an isolate of *A. niger* using solid-state fermentation [23]. The addition of 3% methanol to the husks (45% moisture, initial pH 4.5) increased citric acid production and the yield by strain RCNM17 after 72 h at 30 °C by 1.6-fold or 1.3-fold, respectively, compared to when the strain was grown on unsupplemented coffee husks [23]. It appeared that both types of processing wastes were excellent substrates capable of supporting fungal citric acid production.

Table 2. Growth conditions and maximum citric acid production by *Aspergillus niger* strains on processing wastes of cocoa pods, coffee husks, figs, kiwifruit, grapes, pineapples, oranges and pomegranates using solid-state fermentation.

Substrate	Strain	Sugar Addition	Growth Conditions	Methanol Addition	Citric Acid (g/kg)	Reference
Cocoa pod husks	LPB B6–CCT 7717	None	72 h, 30 °C	4%	979	[22]
Coffee husks	RCNM 17	None	72 h, 30 °C	None	82	[23]
Coffee husks	RCNM 17	None	120 h, 30 °C	3%	188	[23]
Figs	ATCC 10577	None	360 h, 30 °C	6%	96	[24]
Kiwifruit peels	ATCC 12846	None	120 h, 30 °C	None	70	[25]
Kiwifruit peels	ATCC 12846	None	120 h, 30 °C	2%	100	[25]
Grape pomace	ATCC 1015	None	96 h, 30 °C	None	112	[26]
Grape pomace	ATCC 1015	None	96 h, 30 °C	3%	413	[26]
Grape pomace	ATCC 9142	None	96 h, 30 °C	None	304	[26]
Grape pomace	ATCC 9142	None	96 h, 30 °C	3%	498	[26]
Grape pomace	ATCC 11414	None	96 h, 30 °C	None	160	[26]
Grape pomace	ATCC 11414	None	96 h, 30 °C	3%	511	[26]
Grape pomace	ATCC 12846	None	96 h, 30 °C	None	275	[26]
Grape pomace	ATCC 12846	None	96 h, 30 °C	3%	600	[26]
Grape pomace	ATCC 13794	None	96 h, 30 °C	None	112	[26]
Grape pomace	ATCC 13794	None	96 h, 30 °C	3%	413	[26]
Grape pomace	Isolate	1.5%	24 h, 30 °C	4%	34	[27]
Pineapple peel	ATCC 9142	None	96 h, 30 °C	None	164	[28]
Pineapple peel	ATCC 9142	None	96 h, 30 °C	3%	178	[28]
Pineapple peel	ATCC 10577	None	96 h, 30 °C	None	100	[28]
Pineapple peel	ATCC 12846	None	96 h, 30 °C	None	105	[28]
Pineapple pulp	KS-7	None	120 h, 30 °C	None	17	[29]
Pineapple pulp	KS-7	None	120 h, 30 °C	3%	61	[29]
Orange peels	ATCC 9142	None	86 h, 30 °C	None	171	[30]
Orange peels	Isolate	None	144 h	None	86	[31]
Orange pulp	LPB BC	None	96 h, 30 °C	4%	261	[32]
Pomegranate peel	B60	None	192 h, 25 °C	3%	352	[33]

2.4. Figs and Kiwifruit Peels

Previous investigations have explored solid-state fermentation by *A. niger* of figs and kiwifruit peels for citric acid production (Table 2). Solid-state fermentation of dried figs (75% moisture content with initial pH of pH 7.0) by *A. niger* ATCC 10577 produced 1.5-fold less citric acid after 360 h at 30 °C than if ATCC 10577 was grown on the dried figs supplemented with 6% methanol [24]. Similarly, solid-state fermentation of kiwifruit peels by *A. niger* ATCC 12846 after 96 h at 30 °C resulted in a 1.4-lower citric acid concentration being produced compared to when the strain was grown on 2% methanol-supplemented kiwifruit peels for 96 h at 30 °C [25].

2.5. Grape Pomace

It has been reported that solid-state fermentation of grape pomace supports citric acid production by *A. niger* (Table 2). The solid-state fermentation of grape pomace by *A. niger* strains ATCC 13794, ATCC 11414, ATCC 9142, ATCC 1015 and ATCC 12846 was investigated [26]. Of the strains tested, ATCC 9142 produced the highest citric acid level following solid-state fermentation of apple pomace (moisture content 65.4% and initial pH 3.8) for 96 h at 30 °C compared to the other strains tested [26]. The addition of 3% methanol stimulated citric acid production by the strains using solid-state fermentation on grape pomace compared to no addition [26]. Methanol addition was noted to increase citric acid production by all the strains by at least 1.6-fold relative to citric acid production of grape pomace alone [26]. Solid-state fermentation by ATCC 12846 on the grape pomace supplemented with 3% methanol produced the highest citric acid level after 96 h at 30 °C compared to citric acid production by the other strains screened [26]. Although methanol addition to the grape pomace affected citric acid by ATCC 9142 using solid-state fermentation slightly, its presence increased citric acid production by ATCC 13794 by 3.7-fold higher than the citric acid level produced by ATCC 13794 in the absence of methanol [26]. In another study [27], it was shown that an *A. niger* isolate produced a significant concentration of citric acid following solid-state-fermentation of grape pomace for 24 h at 30 °C.

2.6. Pineapple Processing Waste

Pineapple processing waste has been investigated for its ability to support solid-state fermentation by *A. niger* strains (Table 2). Several *A. niger* strains, including ATCC 10577, ATCC 9142 and ATCC 12846, were examined for their ability to utilize pineapple peels for solid-state fermentation of citric acid [28]. Citric acid production by ATCC 9142 following solid-state fermentation of the pineapple waste for 96 h at 30 °C was 1.6-fold higher than citric acid production by ATCC 10577 or ATCC 12846 solid-state fermentation of pineapple waste for 96 h at 30 °C [28]. When 3% methanol was added to the pineapple waste during solid-state fermentation by all three strains, citric acid production was stimulated [28]. It was reported that solid-state fermentation by *A. niger* KS-7 of pineapple waste (65% moisture) supplemented with 2% methanol produced a 3.5-fold higher citric acid concentration after 120 h at 30 °C compared to the citric acid level synthesized by the strain grown on the unsupplemented pineapple waste for 120 h at 30 °C [29].

2.7. Orange Processing Wastes and Pomegranate Peels

Solid-state fermentation of processing wastes of oranges and pomegranates by *A. niger* have been studied for their ability to support citric acid production (Table 2). Orange peels (initial pH 4.5 and 75% moisture) were used as a substrate for solid-state fermentation by *A. niger* ATCC 9142 [30]. After 86 h at 30 °C using an inoculum of about 10^6 spores/g of dry orange peels, ATCC 9142 produced a high level of citric acid production [30]. Both the spore concentration used as the inoculum as well as the moisture content of the orange peels were critical factors in the level of citric acid produced by ATCC 9142 during solid-state fermentation. The addition of methanol to the orange peels did not enhance citric acid production by ATCC 9142 [30]. Another study explored citric acid production by an isolate

of *A. niger* grown on orange peels using solid-state fermentation [31]. When the isolate was grown on the orange peels (60% moisture content) for 144 h of growth at 35 °C more than 100 g citric acid/kg peels was synthesized [31]. Orange juice processing waste was utilized as a substrate for solid-state fermentation by *A. niger* strain LPBBC [32]. After 96 h at 28 °C of solid-state fermentation of the orange juice processing waste (initial pH 2.70 and 65% moisture content), strain LPBBC produced nearly 450 g citric acid/kg waste [32]. Pomegranate peel waste (75% moisture and initial pH 8.0) was also explored as a possible substrate for solid-state fermentation of *A. niger* [33]. Solid-state fermentation of wet and dried pomegranate peel wastes by strain B60 was investigated for citric acid production at 25 °C after 192 h [33]. Higher amounts of citric acid was produced by the strain when it was grown on the wet peel wastes than the dried peel wastes. Methanol (3%) addition to the pomegranate peel waste stimulated citric acid production by strain B60 grown at 25 °C after 192 h [33].

3. Sugarcane Bagasse

In several studies [27,32,34–41], sugarcane bagasse has been used as a substrate for solid-state fermentation by *A. niger* to produce citric acid production (Table 3). After 216 h at 30 °C, *A. niger* strain DS1 produced a 2.2-fold higher level of citric acid on sugarcane bagasse (65% moisture) in a sucrose-containing medium supplemented with 4% methanol than if the strain was grown on the unsupplemented sugarcane bagasse-containing medium [34]. Citric acid production by *A. niger* ATCC 9142 on sugarcane bagasse pretreated with urea increased citric acid production by 1.5-fold after 120 h at 30 °C compared to citric acid production by ATCC 9142 on the untreated bagasse [35]. In another investigation, it was observed that citric acid production by *A. niger* strain 318 following solid-state fermentation of sugarcane bagasse in a sucrose-containing medium for 264 h at 30 °C was about a g citric acid/kg dry bagasse [36]. A prior study showed that solid-state fermentation of sugarcane bagasse by *A. niger* strain DS1 produced a 1.4-fold higher citric acid concentration in a bioreactor (representing large-scale production) compared to the citric acid levels synthesized in shake flasks (representing small-scale production) in a sucrose-containing medium supplemented with 4% methanol for 192 h at 30 °C [37]. An isolate of *A. niger* used for the solid-state fermentation of untreated sugarcane bagasse in a sucrose-containing medium (pH 4.0) supplemented with 4% methanol in 250 mL Erlenmeyer flasks produced a relatively low citric acid concentration after growth for 24 h at 30 °C [27]. Untreated and acid-treated sugarcane bagasse in a sucrose medium containing 4% methanol was investigated for citric acid production by *A. niger* MTCC [38].

Citric acid production by *A. niger* MTCC was 2.3-fold higher on the acid-treated bagasse compared to the level of production on the untreated or treated bagasse after 240 h at 25 °C [38]. When acid-treated sugarcane bagasse supplemented with 4% methanol and urea was used as a substrate for solid-state fermentation in 250 mL Erlenmeyer flasks, *A. niger* ATCC 9142 produced a 1.2-fold higher level of citric acid after 96 h at 25 °C compared to citric acid production by the strain grown on the untreated bagasse after 120 h at 30 °C [39]. Solid-state fermentation of sodium hydroxide-treated sugarcane bagasse supplemented with 4% methanol and urea resulted in a 1.3-fold elevation of citric acid concentration relative to citric acid production by the strain grown on the untreated bagasse for 120 h at 30 °C [39]. Citric acid production by *A. niger* ATCC 9142 on acid-treated sugarcane bagasse (75% moisture content) in a sucrose medium supplemented with 4% methanol was studied and was found to produce a low level of citric acid after 144 h at 30 °C [40]. Using solid-state fermentation of untreated sugarcane bagasse (65% moisture), *A. niger* strain MCCB0201 produced a very low level of citric acid after 120 h at 30 °C [41]. If the bagasse was treated with acid, urea or heat, citric acid production by the strain increased by 3.1-fold, 2.1-fold or 1.6-fold, respectively, after 120 h at 30 °C [41]. The highest level of citric acid was produced by the strain when grown on acid-treated bagasse that was supplemented with ammonium sulfate as a nitrogen source [41].

Table 3. Growth conditions and maximum citric acid production by *Aspergillus niger* strains on sugarcane bagasse and starchy vegetable processing wastes using solid-state fermentation.

Substrate	Strain	Sugar Addition	Growth Conditions	Methanol Addition	Citric Acid (g/kg)	Reference
Sugarcane bagasse	Soil isolate	1.5% sucrose	24 h, 30 °C	4%	25	[27]
Sugarcane bagasse	DS1	15% sucrose	216 h, 30 °C	None	122	[34]
Sugarcane bagasse	DS1	15% sucrose	216 h, 30 °C	4%	206	[34]
Sugarcane bagasse	ATCC 9142	None	120 h, 30 °C	None	95	[35]
Sugarcane bagasse	318	14% sucrose	264 h, 30 °C	None	1	[36]
Sugarcane bagasse	14/20	14% sucrose	264 h, 30 °C	None	1	[36]
Sugarcane bagasse	DS1	31% sucrose	192 h, 30 °C	4%	124	[37]
Sugarcane bagasse	MTCC	31% sucrose	240 h, 25 °C	4%	3	[38]
Sugarcane bagasse	ATCC 9142	None	120 h, 30 °C	4%	76	[39]
Sugarcane bagasse	ATCC 9142	31% sucrose	144 h, 30 °C	4%	233	[40]
Sugarcane bagasse	MCCB0201	None	120 h, 30 °C	None	1	[41]
Kumara	Yang 2	None	144 h, 30 °C	None	69	[42]
Potato	Yang 2	None	144 h, 30 °C	None	3	[42]
Taro	Yang 2	None	144 h, 30 °C	None	66	[42]
Cassava bagasse	LPB 21	None	144 h, 28 °C	None	220	[43]
Cassava peels	CRL isolate	None	72 h, 32 °C	None	25	[44]

4. Starchy Vegetable Processing Wastes

4.1. Sweet Potato and Potato Processing Wastes

As shown in Table 3, processing wastes of different types of potatoes (71% moisture content) have been examined as a substrate for solid-state fermentation by *A. niger* strain Yang No. 2 [42]. Citric acid production by strain Yang No. 2 was measured following solid-state fermentation of potato waste for 144 h at 30 °C and a very low citric acid level was synthesized with a very low yield of 0.03 g citric acid/g starch used. A 23-fold higher level of citric acid was produced by strain Yang No. 2 on kumara after 144 h at 30 °C (yield of 0.58 g citric acid/g starch consumed) compared to the strain grown on the potato waste [42]. Strain Yang No. 2 produced a 22-fold higher citric acid level on taro after 144 h at 30 °C using solid-state fermentation (yield of 0.79 g citric acid/g starch consumed) compared to the strain grown on the potato waste [42]. The addition of the metal ions iron, copper, zinc or manganese was found to slightly increase citric acid production by strain Yang No. 2 on the starch substrates after 144 h at 30 °C [42]. Fungal biomass production by strain Yang No. 2 was also affected by the addition of different combinations of metal ions to the starch substrates during solid-state fermentation [42]. It was clear that the fungal citric acid production varied according to the type of starchy processing waste used during solid-state fermentation.

4.2. Cassava Processing Waste

Processing cassava waste has been explored as a substrate for solid-state fermentation by *A. niger* for the production of citric acid (Table 3). Solid-state fermentation by *A. niger* strain LPB21 of gelatinized cassava bagasse (70% moisture) after 144 h at 28 °C produced a high concentration of citric acid in 250 mL Erlenmeyer flasks [43]. In a column bioreactor (60 mL/min), strain LPB21 grown on bagasse for 144 h at 28 °C under optimized conditions produced a 1.6-fold higher citric acid concentration than the strain did under non-optimized conditions [43]. In a tray bioreactor, strain LPB21 produced about the same citric acid level as the strain produced on bagasse after 144 h at 28 °C under optimized column bioreactor

conditions [43]. In a horizontal drum bioreactor (representing large-scale production conditions), strain LPB21 produced a slightly higher concentration of citric acid on the bagasse after 120 h at 28 °C than the level of citric acid produced by the strain grown on the bagasse for 144 h at 28 °C under optimized conditions [43]. Peels of cassava were tested as a substrate for *A. niger* solid-state fermentation [44]. It was determined that solid-state fermentation of cassava peels (60% moisture) by an *A. niger* strain at 35 °C for 96 h produced relatively little citric acid [44]. In the presence of 3% ethanol, solid-state fermentation of cassava peels by the strain at 35 °C for 96 h only slightly increased its citric acid production [44].

5. Cereal Grain Processing Products

5.1. Oat and Wheat Bran

Brans have been utilized as substrates for solid-state fermentation by *A. niger* for citric acid production (Table 4). Oat bran was utilized as a substrate for solid-state fermentation by isolate C of *A. niger* [45]. When the isolate was grown on the sucrose-containing medium containing 3% methanol and oat bran, the strain produced a relatively low citric acid concentration after 72 h at 28 °C [45]. When wheat bran was utilized as a substrate, *A. niger* strain DS1 produced a 4.8-fold higher citric acid level after 72 h at 28 °C compared to the citric acid level synthesized by *A. niger* strain N1 after solid-state fermentation of wheat bran for 72 h at 30 °C [34,46]. It appeared that either bran did not sustain a high level of citric acid production by *A. niger*.

Table 4. Growth conditions and maximum citric acid production by *Aspergillus niger* strains on cereal grain processing coproducts utilizing solid-state fermentation.

Substrate	Strain	Sugar Addition	Growth Conditions	Methanol Addition	Citric Acid (g/kg)	Reference
Oat bran	Isolate C	0.1% sucrose	72 h, 28 °C	3%	70	[45]
Wheat bran	DS1	0.15% sucrose	216 h, 30 °C	None	122	[34]
Wheat bran	N1	None	72 h, 28 °C	None	4	[46]
Corncob	ATCC 10549	None	192 h, 30 °C	None	48	[47]
Corncob	KA88	None	192 h, 28 °C	None	19	[48]
Com distillers grains & solubles	ATCC 9029	None	240 h, 25 °C	None	4	[49]
Com distillers grains & solubles	ATCC 9142	None	240 h, 25 °C	None	10	[49]
Com distillers grains & solubles	ATCC 10577	None	240 h, 25 °C	None	4	[49]
Com distillers grains & solubles	ATCC 11414	None	240 h, 25 °C	None	7	[49]
Com distillers grains & solubles	ATCC 12846	None	240 h, 25 °C	None	7	[50]
Com distillers grains & solubles	ATCC 26550	None	240 h, 25 °C	None	5	[50]
Com distillers grains & solubles	ATCC 201122	None	240 h, 25 °C	None	5	[50]

5.2. Corncob

Corncobs were examined as a possible substrate for solid-state fermentation by *A. niger* (Table 4). Solid-state fermentation by *A. niger* ATCC 10549 on corncobs was capable of synthesizing a 2.6-fold higher citric acid concentration after 192 h at 30 °C compared to the level produced by *A. niger* strain KA88 after 144 h at 28 °C [47,48]. The addition of sucrose or glucose to the corncobs stimulated citric acid production by strain KA88 [48]. The addition of a nitrogen source to a medium containing sucrose and corncobs further stimulated citric acid production by strain KA88 by about double [48].

5.3. Coproducts from Corn-Based Ethanol Production

When corn-based ethanol is produced, com distillers grains and condensed com solubles are the primary coproducts that result from the process. For each bushel of com processed at ethanol plants, approximately l8 pounds of 90% corn distillers grains with solubles are produced. The corn distillers grains with solubles was explored as a substrate for solid-state fermentation by *A. niger*. Citric acid production by *A. niger* strains were analyzed on the untreated grains or autoclaved grains and was found to occur [49–52]. The highest citric acid level on the untreated grains was produced by *A. niger* ATCC 9142. It was determined that ATCC 9029 and ATCC 10577 produced about 3-fold less citric acid after 240 h relative to ATCC 9142. Similarly, citric acid production by ATCC 26550 and ATCC 201122 was noted to be half the level produced by ATCC 9142 after 240 h at 25 °C. Citric acid production by ATCC 201122 on the autoclaved grains was found to be highest after 240 h at 25 °C at a level that was 61% lower than on the untreated grains. Less citric acid was synthesized by the majority of strains screened for citric acid production on the autoclaved grains. Citric acid production by only ATCC 10577 or ATCC 12846 following solid-state fermentation of autoclaved grains after 240 h at 25 °C was more than 1.5-fold higher compared to untreated grains [49,50]. A prior study found that methanol addition to the corn distillers grains diminished citric acid production by *A. niger* ATCC 9142 using solid-state fermentation after 240 h at 25 °C while phosphate addition to the grains slightly increased citric acid production by ATCC 9142 [8]. The addition of 3% methanol to the untreated corn distillers grain with solubles using solid-state fermentation by ATCC 11414 or ATCC 26550 increased citric acid production after 240 h at 25 °C [51]. With respect to solid-state fermentation by *A. niger* ATCC 12846 or ATCC 26550 on 0.5% sulfuric acid-treated corn distillers grains for 240 h at 25 °C, it was noted that there was a 1.4-fold or 2.5-fold increase, respectively, in citric acid production by the strain compared to citric acid production on the untreated grains [52]. When the grains were treated with 1% sulfuric acid, *A. niger* ATCC 10577 produced double the concentration of citric acid on the acid-treated grains compared to the untreated grains using solid-state fermentation after 240 h at 25 °C [49]. Solid-state fermentation of acid-treated grains was found to reduce citric acid production by ATCC 11414 or ATCC 201122 compared to the untreated grains after 240 h at 25 °C [52].

6. Conclusions

The objective of the review was to specifically examine solid-state fermentation of agricultural processing coproducts as substrates to synthesize citric acid. It is clear that a variety of studies have been conducted on the solid-state fermentation of a wide range of agricultural processing coproducts to produce citric acid with varying success. It was observed that citric acid production by *A. niger* strains was dependent on the type of agricultural processing coproduct that was utilized for solid-state fermentation. It appeared that solid-state fermentation of fruit processing wastes (such as apple and grape pomace) by *A. niger* strains resulted in the highest levels of citric acid production. The lowest level of citric acid production was produced by *A. niger* strains following solid-state fermentation of potato or cereal grain processing coproducts as substrates. It was concluded that the sugar content of the substrate being utilized by the *A. niger* strain for solid-state fermentation was critical to the level of citric acid that was produced by the strain. Despite the number of *A. niger* solid-state fermentation studies investigating citric acid synthesis from agricultural processing coproducts, commercial synthesis of citric acid has relied on submerged or surface fermentation. Although this review has focused on solid-state fermentation on low value biomass by *A. niger*, any interest in comparing citric acid production by solid-state, submerged or surface fermentation from a systems efficiency has been addressed in recent reviews [53,54].

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