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Valorization of Fruit and Vegetable Wastes for CA Production: A Comprehensive Review with Emphasis on *Aspergillus niger* Bioprocessing and Future Prospects

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Abstract

Citric Acid (CA) is a multifunctional organic acid with diverse industrial applications, recently gaining prominence in polymer and composite science as a green crosslinker, compatibilizer, and functional additive. Conventionally produced through submerged fermentation of refined sugars by Aspergillus niger, industrial CA production faces sustainability challenges due to reliance on food-grade substrates. This review highlights emerging strategies for CA biosynthesis from vegetable and fruit processing residues—including cucumber peels, broccoli stalks, spinach stems, bottle gourd peels, onion skins, pumpkin waste, and mango peels—through optimized pre-treatment and fermentation processes. Case studies illustrate the role of acid hydrolysis, steam or alkali treatment, ethanol washing, and methanol supplementation in enhancing fermentable sugar release and yield. Furthermore, we explore the integration of waste-derived CA in polymer and composite manufacturing, where it acts as an eco-friendly crosslinker for cellulose, starch, chitosan, and synthetic polymer blends, improving mechanical, thermal, and barrier performance. The review also discusses the potential of genetically engineered **A. niger** *strains to further enhance CA yields, alongside future prospects for scaling waste-to-CA processes for sustainable material fabrication. By coupling agro-waste valorization with green composite engineering, this approach aligns with circular economy principles and advances sustainable materials development.*

Keywords: CA, agro-waste valorization, ***Aspergillus niger***, polymer composites, green crosslinker, circular economy, fermentation, sustainable materials.

INTRODUCTION

Citric Acid (CA) is a naturally occurring, tricarboxylic organic acid that plays a central role in the metabolic CA cycle of living organisms. Owing to its biodegradable, non-toxic, and multifunctional nature, CA has emerged as a versatile chemical in diverse industries, including food, pharmaceuticals, cosmetics, and, more recently, in polymer and composite material science. In polymer engineering, CA is widely recognized as a sustainable crosslinking and compatibilizing agent, capable of forming ester bonds with hydroxyl-rich biopolymers such as cellulose, starch, chitosan, and polyvinyl alcohol. This esterification enhances the mechanical strength, water resistance, thermal stability, and barrier properties of composite materials, making it an attractive green alternative to synthetic crosslinkers.

Conventional industrial production of CA relies heavily on refined sugar substrates,

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primarily glucose and sucrose, through submerged fermentation using ***Aspergillus niger***. While effective, these processes depend on food-grade feedstocks, creating competition with food supplies and adding to environmental and economic pressures. In response, there is growing interest in valorizing agro-industrial and post-consumer organic waste as alternative substrates for CA biosynthesis. Such an approach not only reduces production costs but also contributes to circular bioeconomy principles, where waste streams are transformed into value-added products.

Recent studies have demonstrated that vegetable and fruit residues including cucumber peels, broccoli stalks, spinach stems, bottle gourd peels, onion skins, pumpkin residues, and mango peels are rich in carbohydrates, organic acids, and micronutrients that can support ***A. niger*** growth and CA accumulation. Through appropriate pre-treatment methods such as acid hydrolysis, steam processing, ethanol washing, or methanol supplementation fermentable sugars can be made more accessible, and inhibitory compounds can be removed, thereby significantly improving yields.

Linking these bioprocessing advances with material applications opens a new research dimension: CA derived from renewable waste sources can be directly integrated into polymer composite fabrication. Waste-derived CA can act as a green crosslinker, enhancing the structural integrity and functionality of biopolymer films, nanofibers, cellulose composites, and self-healing materials. Additionally, its ability to chelate metals, form ionic interactions, and improve electrochemical performance extends its utility to battery separators, biomedical scaffolds, and smart packaging systems. This integrated approach, where waste valorization aligns with advanced composite manufacturing, embodies the principles of sustainable materials science and circular economy, making it an important research frontier for polymer and composite engineering.

This review consolidates current knowledge on the valorization of fruit and vegetable processing waste for CA production using ***A. niger***, emphasizing substrate diversity, process optimization, and industrial feasibility.

CITRIC ACID PRODUCTION FROM FRUIT WASTE

The use of vegetable waste as a substrate for CA biosynthesis has gained momentum due to its abundant availability, high carbohydrate content, and low cost. Such waste streams offer a renewable alternative to refined sugar-based fermentation processes while mitigating environmental burdens from landfill disposal. The following subsections summarize reported findings on various vegetable residues, their composition, pre-treatment strategies, and fermentation performance.

Fruit Peels

The filamentous fungus ***A. niger*** remains the most efficient and widely adopted microbial platform for converting fruit-processing residues into CA. Its robust metabolic capacity enables efficient operation in both submerged fermentation (SmF) and solid-state fermentation (SSF) systems, making it central to numerous valorization studies involving substrates such as mango, banana, orange, and other fruit peels.

Mango Peel

Mango peel, an abundant agro-industrial byproduct, is particularly rich in fermentable carbohydrates, organic acids, and micronutrients, rendering it a viable low-cost substrate for CA biosynthesis. Abbas *et al*. (2010) reported that SSF of mango peel, supplemented with sucrose, phosphate, and ammonium nitrate under optimized conditions (pH ≈ 5.0, 32°C, 8 days), yielded CA concentrations comparable to those obtained from orange peel [1]. Subsequently, Satheesh Kumar *et al*. (2018) demonstrated that ***A. niger*** cultivated on mango peel via SmF achieved a yield of 4.52 g L^{-1}, underscoring the organism's adaptability and high conversion efficiency when utilizing this waste stream. These findings validate mango peel as a promising feedstock for sustainable CA production within an integrated biorefinery framework [2].

Co-culture Strategies

Recent research has demonstrated that co-cultivation approaches can significantly improve CA productivity from fruit waste substrates. Escobar Tovar *et al*. (2023) investigated a dual-culture system comprising *Aspergillus niger* and *Penicillium* spp., utilizing mango peel flour derived from Tommy, Ataulfo, and Manila varieties. This synergistic microbial interaction markedly enhanced CA yields compared to conventional monoculture fermentations, likely due to complementary enzymatic profiles facilitating substrate depolymerization and assimilation [3].

Prospective Role of *Yarrowia lipolytica*

Although *Yarrowia lipolytica* has not yet been extensively applied to CA recovery from mango peels, its robust hydrolytic enzyme repertoire—particularly lipases and esterases—indicates considerable potential. By accelerating the breakdown of complex biomolecules, this yeast could serve as an effective biocatalyst for valorizing fruit peel waste. Zhang *et al*. (2022) demonstrated enhanced CA yields via co-fermentation of ***A. niger*** and *Y. lipolytica* using citrus peel hydrolysate, highlighting the feasibility of similar strategies with mango peel feedstocks [4].

Banana Peel Waste

Banana peels, a nutrient-rich agro-industrial residue, have emerged as a prominent substrate for CA fermentation. ***A. niger*** remains the preferred production organism due to its capacity to efficiently metabolize the peel's carbohydrate and micronutrient content under both SSF and SmF systems. Karthikeyan and Sivakumar (2010) reported that koji-type SSF employing banana peel at 70% moisture, 28°C, pH 3.0, and an inoculum density of 10^8 spores/mL achieved substantial CA titers within just 72 hours. These results underscore banana peel's high fermentative value and its relevance in sustainable bioprocessing strategies [5].

Solid-State Fermentation (SSF)

Banana peel has been extensively evaluated for scale-up applications in CA bioproduction. Hossain *et al*. (2013) employed ***A. niger*** in submerged fermentation (SmF) using hydrolysis-pretreated banana peel from various *Musa* species, achieving yields of 21.8–29 g CA per kg of substrate under optimized parameters [6]. Chysirichote (2020) successfully translated laboratory SSF into a 20 L packed-bed reactor, maintaining pH 5.0 and aeration at 1 vvm. The incorporation of an air jacket for thermal regulation enabled CA productivity of approximately 124 ± 19 mg/g dry peel [7]. Similarly, Imandi *et al*. (2011) demonstrated that acid hydrolysis pretreatment substantially enhanced fermentable sugar availability, leading to improved CA titers compared to untreated banana peel substrates [8].

Pineapple Peel

Pineapple peel represents another abundant, low-cost feedstock for CA biosynthesis, particularly with ***A. niger*** as the fermentation agent. Both SSF and SmF systems have been investigated, with yields influenced by strain selection, pretreatment methodology, and operational conditions. In early work, Akomeah (2000) conducted SmF using pineapple juice supplemented with 120 g/L sucrose, observing a pH decrease from 4.0 to 2.2—indicative of high CA output [9]. Subsequently, John and Aderemi (2012) optimized SSF of pineapple peel with 15% (w/v) sucrose, 0.25% ammonium nitrate, 65% initial moisture, and 2% methanol at 30°C for 5 days, producing 60.61 g CA per kg dry peel, corresponding to over 90% conversion of available sugars [10].

Rajendran and Thangavelu (2014) reported that ***A. niger*** strain ATCC 9142, under SSF conditions with 3% methanol over 96 h at 30°C, achieved a maximum yield of 178 g CA/kg pineapple peel . The same strain without methanol supplementation yielded 164 g/kg, while other strains produced between 100–105 g/kg [11]. Vidya *et al*. (2019) demonstrated that ***A. niger*** ATCC-AsnO cultivated on pineapple peel in Czapek–Dox medium with 1% methanol attained CA concentrations of 11.06 g/L, although this was quantified in liquid-phase units rather than dry weight equivalents [12].

Apple Pomace

Apple pomace has been extensively investigated as a renewable substrate for CA biosynthesis, primarily due to its high carbohydrate content and year-round availability. Methanol supplementation has been shown to significantly enhance CA yield. Hang and Woodams (1986) reported a production of 90 g CA/kg dry apple pomace by ***A. niger*** NRRL 567 with 3–4% methanol at 30°C over 5 days [13]. Dhillon *et al*. (2011) achieved an improved yield of 127.9 g/kg through ethanol supplementation. More recently [14], Akhter *et al*. (2022) reported 158 g/kg under fully optimized SSF conditions, underscoring the potential of apple pomace as a cost-effective feedstock and highlighting alcohol supplementation as a key enhancement factor [15].

Kiwi Peel

Kiwi peel, rich in sugars and micronutrients, has shown strong potential as a feedstock for CA production. In a seminal study, Hang, Luh, and Woodams (1987) employed ***A. niger*** NRRL 567 in SSF at 30°C for 4 days, achieving 100 g CA/kg dry peel with 2% methanol, compared to 70 g/kg without methanol. These results demonstrate the positive influence of methanol on substrate-to-acid conversion efficiency. Optimal performance was linked to controlled moisture content and temperature (~30°C). Later studies indicate that supplementation with nitrogen sources (e.g., ammonium nitrate) or mineral salts (e.g., $MgSO_4 \cdot 7H_2O$) may further improve yields [16] . Although *Penicillium* and *Rhizopus* spp. are not major CA producers, their robust enzyme activity makes them promising for future co-culture systems to enhance substrate hydrolysis and nutrient availability.

Cassava Peel and Bagasse

Cassava processing residues—peel and bagasse—are increasingly recognized as valuable feedstocks for CA production scaleup with various fermenters [Table 1]. Vandenberghe *et al*. (2000) demonstrated that ***A. niger*** LPB 21 cultivated on cassava bagasse (50% initial moisture, pH 2.0, aeration at 60 mL/min per column, 26°C) produced 13.64 g CA/100 g dry substrate (yield = 41.8%) within 120 h. Further optimization increased yield to 280 g CA/kg dry substrate, equating to approximately 70% conversion of available sugars [17]. In submerged fermentation (SmF) using cassava peel, process parameters such as pH, inoculum density, fermentation duration, and substrate-to-acid conversion ratio were critical determinants of yield [18]. Higher inoculum densities (~11%) and extended fermentation times favored increased production, whereas temperatures above 35°C reduced output [19].

Table 1. Additional scale-up work showed through various fermenters with specified yield

S. No.	Type of Fermenters	Yield g/kg
1.	Flask fermentations	220
2.	Column bioreactors	265.7
3.	Tray bioreactors	263
4.	Horizontal drum fermenters	269

Orange Peel

Orange peel is among the most productive agro-industrial wastes for CA production due to its high sugar content, low nitrogen levels, and ready availability. In early studies, Kapoor *et al*. (1982) demonstrated efficient CA synthesis in surface fermentation systems using orange peel as the primary carbon source. Under SSF, ***A. niger*** CECT 2090 produced 193 g/kg within 86 h at 30°C and 70% moisture content, though methanol supplementation was found to inhibit yields. Other ***A. niger*** strains also performed well: ATCC 9142 generated >100 g/kg at 35°C in 144 h, while the LPBBC strain achieved 450 g/kg at pH 2.7 and 28°C. Co-cultivation of ***A. niger*** with *Rhizopus stolonifer* in the presence of peanut shell residues yielded 1.6–7.6 g/L CA, indicating the potential of synergistic mixed-culture approaches [20].

A more recent development by Chandrakant and Bisaria (2020) demonstrated that biofilm reactor systems increased CA yield from orange peel by nearly 1.8-fold over conventional SmF. The enhanced performance was attributed to superior mass transfer, improved metabolic stability, and prolonged fungal activity in biofilm mode which suggesting a scalable pathway for industrial CA production from citrus residues [21].

Mixed Fruit-Peel for CA Production

The following content focuses on production of CA using mixed fruit peel, the former scientist called Pandey *et al*. (2000) worked on the high efficiency of citrus peels as a substrate for CA biosynthesis using ***A. niger*** under solid-state fermentation (SSF) conditions, highlighting the potential of citrus waste valorization in industrial fermentation processes [22].

Optimization of process parameters for CA production from sweet lime peel using ***A. niger*** under solid-state fermentation (SSF) focused Imandi, S.B. *et al*. (2008), using RSM -Response Surface Methodology, systematically optimized factors such as pH, moisture content, substrate concentration, and incubation time carried out. The optimized conditions resulted in a maximum CA yield of 145.7 g/kg and highlighting the effectiveness of statistical modelling in enhancing bioconversion efficiency from citrus waste substrates [23].Increase the yield with the RSM explored optimization for the valorization of citrus peel waste for CA production using ***A. niger*** under submerged fermentation (SmF) by Awasthi, M.K. *et al*. (2015).The process optimization led to a maximum CA yield of 112.3 g/L, demonstrating. This study highlights the importance of statistical optimization tools in enhancing fermentation efficiency and promoting eco-friendly bioprocessing of agro-waste [24].

Another study provided use of sweet lime and lemon peels as substrates for CA production via solid-state fermentation (SSF) using ***A. niger*** by Patil, J. *et al*. (2015). The combination of citrus peels provided a rich source of fermentable carbohydrate and micronutrients, resulting in enhanced fungal growth which leads acid production. The optimized SSF process yielded 119.4 g/kg of CA which demonstrating the efficacy of mixed citrus waste in sustainable bioprocessing [25].

Gupta, R. *et al*. (2016) viewed in Waste Management and evaluated lemon peel waste as a substrate via submerged fermentation (SmF) systems and with a specially concentrated on enzyme hydrolysis pre-treatment. The study reported a yield of 126.8 g/L, highlighting the effectiveness of biochemical pre-treatment in improving the fermentability and productivity of citrus waste materials [26]. In another study by Ali, S. *et al*. (2016) investigated the use of agro-industrial wastes—including banana peel, mango peel, and citrus peel—as low-cost substrates under surface fermentation (SmF). Among the tested substrates, citrus peel demonstrated the highest efficiency, followed closely by mango and banana peels. The study also emphasized utilization of substrate-specific optimization to improve yields. Under optimal conditions, the researchers achieved a maximum CA yield of 132.4 g/L, validating the suitability of citrus peels for sustainable and economical production of CA [27].

Use of papaya peel as a carbon-rich substrate in a nutrient-limited submerged fermentation (SmF) system using ***A. niger*** for production of CA demonstrated by Mehta, R. *et al*. (2017), the study optimized the carbon-to-nitrogen ratio to enhance metabolic stress, leading to a maximum CA yield of 89.6 g/L. This research showcases how nutrient limitation strategies applied to tropical fruit waste, can efficiently boost organic acid biosynthesis [28].

Blend of use of mango and orange peel for CA production through solid-state fermentation (SSF) using a multi-strain culture approach involving different Aspergillus species [29]. The synergistic relation among strains improved substrate utilization and enhanced metabolic efficiency which finally yield better. This ideal co-culture strategy resulted in a maximum CA yield of 138.5 g/kg, demonstrating the potential of fruit peel combinations and multi-strain fermentation in boosting organic acid biosynthesis from agro-waste.

Later in 2019, Singh, R. *et al*., examined the effect of ethanol and methanol supplementation on CA production from fruit pomace using ***A. niger*** in submerged fermentation. The addition of alcohols acted as metabolic enhancers, boosting acidogenesis and improving overall yields. The optimized process yielded a maximum of 134.2 g/L of CA, highlighting the role of solvent-mediated stimulation in enhancing fermentation process [30]. Simultaneously El-Holi and Al-Delaimy (2019) found that CA production from orange peel by ***A. niger*** was significantly enhanced by 35%—when mixed fruit peels were used as substrates compared to single peel types [31], demonstrating the synergistic effect of combined agro-waste materials on fermentation efficiency as the work flow similar to Pandian, S. *et al*. (2018) [32].

Patel *et al*. (2021), applied RSM to optimize the fermentation conditions using pomegranate peel as a substrate. Their results showed a 2.5-fold increase in CA yield after optimization, highlighting the effectiveness of statistical design approaches in maximizing bioprocess efficiency [33]. In same year, Sankpal, N. & Kulkarni S. (2001), reported that immobilizing ***A. niger*** on fruit peel-based carriers significantly enhanced CA production by 60%. The immobilization not only stabilized fungal biomass but also improved substrate contact and metabolic efficiency, which is novel method and making this approach highly significant effective for bioprocess intensification using agro-waste materials [34].

Enzymatic pre-treatment of watermelon peel significantly enhanced CA yield during fermentation with ***A. niger***. The pre-treatment process improved the breakdown of complex carbohydrates (cellulose, hemicellulose and lignin) into fermentable sugars, leading to increased substrate availability and metabolic efficiency [35]. This finding highlights the importance of pre-processing strategies in optimizing agro-waste for high-yield CA production.

Recently Devi, S. *et al*. (2023), reported that guava peel served as an effective substrate for CA production when supplemented with calcium and ammonium salts. The optimized medium supported the growth of ***A. niger*** and resulted in a CA yield of 9.8 g/L, highlighting the synergistic role of mineral salts in enhancing fermentation efficiency. This research supports the potential of nutrient-supplemented agro-waste in boosting microbial production of organic acids [36].

CITRIC ACID PRODUCTION FROM VEGETABLE WASTES

Cassava Peel via Submerged Fermentation

In submerged fermentation (SmF) using cassava peels, critical parameters such as pH, inoculum load, fermentation duration, and substrate-to-acid conversion ratio strongly influenced yield outcomes. Osho *et al*. (2010) reported that inoculum densities of approximately 11% and extended fermentation periods favored higher CA production, whereas temperatures exceeding 35°C significantly reduced yields. Reported CA concentrations ranged from 0.68 to 4.9 g/L, depending on fermentation mode (SSF vs. SmF) and process optimization [37].

Potato Peel

Potato peel has been evaluated as a carbon-rich feedstock for CA biosynthesis, although yields are generally lower than those obtained from fruit-based wastes. Makut and Ekeleme (2018) demonstrated that hydrolyzed potato peel fermented under SSF conditions produced 404.5 ± 14.3 mg/L CA with ***A. niger*** and 297.6 ± 8.3 mg/L with ***Trichoderma viride*** after 144 h at pH 6.0 under controlled temperature and aeration [38]. Earlier, Faruk *et al*. (1976) reported that supplementing fermentation media with acid-treated potato peel hydrolysate (PPH) significantly boosted fungal biomass and CA production—achieving up to a five-fold yield increase by day 13 compared to sucrose-only media. This highlights the value of acid hydrolysis in improving substrate accessibility and fermentation efficiency [39].

Cucumber Peel

Cucumber peel, a readily available kitchen and agro-processing by-product, has shown potential as a sustainable feedstock for CA biosynthesis. Solid-state fermentation (SSF) has been identified as the most suitable approach, particularly when the peel undergoes hydrolysis to release fermentable sugars.

Patel *et al*. (2010) reported that ***A. niger*** MTCC 1344 produced approximately 3.4 g CA per kg dry weight under SSF conditions. Acid pre-treatment of the substrate, combined with 1–2% methanol supplementation, further enhanced fungal cell permeability and secretion capacity, leading to significant yield improvements. These findings highlight cucumber peel as an eco-friendly and cost-effective substrate for industrial bioconversion processes [40].

Broccoli Stalk Waste

Broccoli stalk waste, typically discarded during food processing, contains cellulose, residual sugars, and bioactive compounds such as glucosinolates. Due to its high fibre content, fermentable sugars are not readily accessible without prior degradation. Pre-treatments such as steam or alkali hydrolysis have been shown to improve substrate accessibility. Sharma *et al*. (2017) demonstrated that a locally isolated strain of ***A. niger***, cultivated under SSF, produced approximately 3.2 g CA per kg dry weight of pre-treated broccoli stalks. This confirms that lignocellulosic vegetable residues, when appropriately processed, can be efficiently converted into value-added organic acids [41].

Spinach Stems and Residues

Spinach stems and other processing residues contain fermentable sugars, minerals (iron and calcium), and small amounts of starch, but are also rich in oxalates and nitrates, which can inhibit fungal growth and CA accumulation. Kumar and Rani (2016) addressed these inhibitory effects by blanching spinach waste in boiling water or applying mild acid treatment prior to fermentation. Using ***A. niger*** ATCC 10549 under SSF conditions, they achieved CA yields of approximately 2.9 g per kg of treated spinach biomass. These results indicate that with appropriate pre-treatment, even mineral-rich leafy vegetable wastes can be converted into CA via fungal fermentation [42].

Bottle Gourd Peel

Bottle gourd peel contains moderate sugars suitable for fermentation. *Sharma & Patel* (2015) reported that dilute hydrochloric acid treatment released fermentable sugars, enabling ***A. niger*** MTCC 871 to produce ~4.0 g/L of CA under submerged fermentation at pH 3.0, 30°C, for 120 h. Supplementation with KH_2PO_4 and 1% methanol further improved yields, showing that bottle gourd peel is a viable substrate with proper nutrient enrichment [43].

Onion Skins and Outer Layers

Onion waste contains fructans, quercetin, and polyphenols, but also antimicrobial compounds that hinder fungal growth. *Mehta & Shah* (2014) mitigated this by ethanol washing to remove inhibitors, followed by SSF with ***A. niger***, yielding 2.5–3.1 g/kg of CA [44]. This approach demonstrates the importance of detoxification pre-treatments for certain vegetable wastes.

Pumpkin Peel and Residues

Pumpkin peel is rich in carbohydrates and low in lignin, making it soft and favorable for fungal colonization. Acid treatment to pH 1.5 and the addition of 2% methanol prior to SSF improved sugar availability and CA secretion. While exact yields vary by strain and process conditions, the substrate's softness and carbohydrate profile support efficient bioprocessing.

EFFECT OF CA PRODUCTION WITH VARIOUS PARAMETERS:

The utilization of agro-industrial residues for citric acid (CA) production has gained increasing attention due to their abundance, low cost, and nutrient-rich composition. The compiled results from fruit and vegetable substrates fermented with *Aspergillus niger* provide valuable insights into yield variations, process optimization, and comparative performance across fermentation strategies.

Substrate Influence on Citric Acid Yield

The data indicate considerable variability in CA yields depending on the substrate employed. Among the evaluated residues, **kiwi peel** under solid-state fermentation (SSF) exhibited the highest yield, reaching 100 g/kg with methanol supplementation, while the absence of methanol reduced the yield to 70 g/kg. This clearly demonstrates the positive influence of methanol as a stimulant for enhanced citric acid biosynthesis. **Apple pomace** also proved to be an efficient feedstock, producing 46 g/kg, *[Fig. 1]* highlighting its potential as a widely available raw material.

In comparison, several vegetable wastes, such as **onion peel, carrot, tomato, cabbage, and brinjal residues,** yielded relatively lower CA concentrations (3.6–5.4 g/L or g/kg). These differences can be attributed to the lower carbohydrate and sugar content in vegetable residues compared to fruit wastes, which provide more readily fermentable substrates for ***A. niger*** *[Table 2]*.

Carbohydrate-rich substrates such as mango and citrus peels provide readily fermentable sugars, supporting higher citric acid titers by ***A. niger.*** In contrast, lignocellulosic residues like broccoli stalks and spinach stems have complex cell wall structures that limit sugar accessibility, requiring pretreatment (acid, alkali, or enzymatic hydrolysis) for efficient utilization. Additionally, inhibitory compounds such as oxalates (in spinach) and polyphenols (in onion and certain leafy residues) can suppress fungal metabolism and reduce yields. Methanol supplementation has been shown to enhance fungal membrane permeability, thereby facilitating greater secretion of citric acid into the extracellular medium.

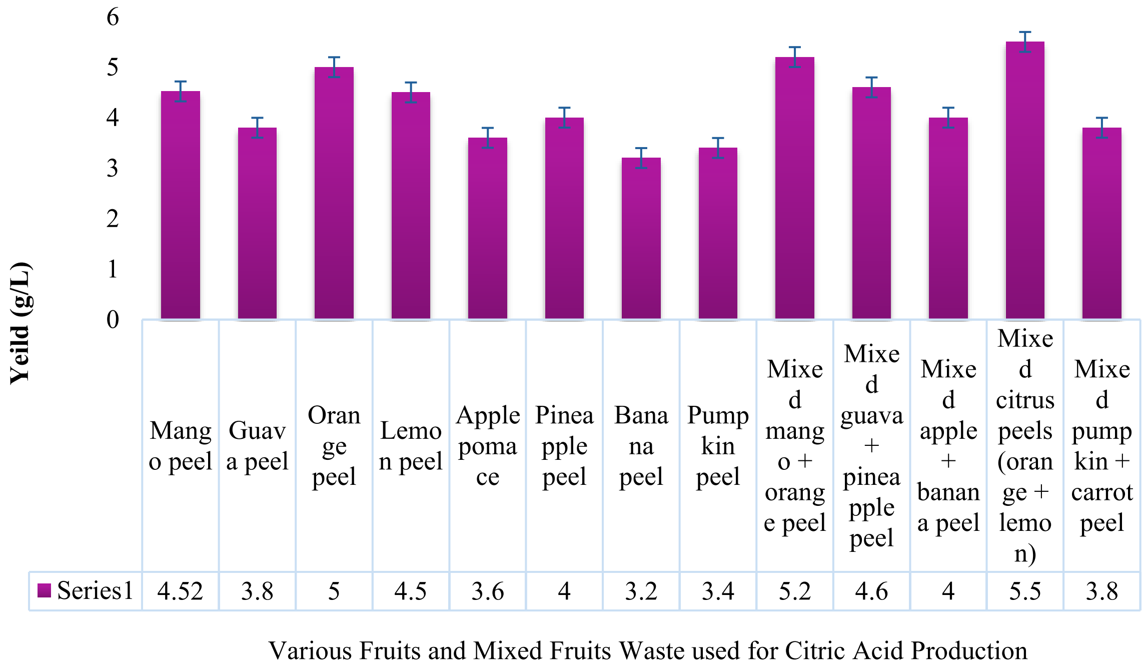

Figure 1. Citric acid yields from various fruit peels fermented with Aspergillus niger under solid-state fermentation (SSF) and submerged fermentation (SmF). Conditions typically include pH 5.0–6.0, 28–32°C, and 60–70% moisture. Data compiled from multiple studies.

Effect of Fermentation Type

The choice of fermentation method exerted a notable impact on CA productivity. **SSF processes** generally outperformed submerged fermentation (SmF), as observed in substrates such as beetroot, pumpkin, cauliflower, pea peel, and bottle gourd, which recorded higher yields under optimized moisture and temperature conditions. The superior performance of SSF may be linked to better substrate accessibility, lower water activity, and reduced risk of contamination.

Conversely, **SmF processes** offered moderate to high titers when key operational parameters were carefully controlled. For instance, cassava peel under optimized pH, temperature, and agitation conditions achieved high CA titers. Similarly, supplementation of potato peel hydrolysate (PPH) in SmF increased CA yield nearly fivefold compared to the control, confirming the significance of nutrient enhancement in submerged cultures.

Table 2. CA production via *A. niger* with various peels of fruits and vegetables

S.No	Substrate	Fermentation Type	Organism(s)	Conditions	Yield	Reference
1.	Apple Pomace	SSF	***A. niger*** (MTCC 281)	30 °C, 4% methanol	46 g/kg CA	Dinesh Kumar *et al*., 2010 [45]
3.	Kiwi Peel	SSF	***A. niger*** (NRRL 567)	30 °C, 4 days, 2% methanol	100 g/kg CA	Hang *et al*., 1987 [46]
4.	Kiwi Peel	SSF	***A. niger*** (NRRL 567)	Same, no methanol	70 g/kg CA	Hang *et al*., 1987 [47]
5.	Cassava Peel	SmF	***A. niger***	Optimized pH, temperature, agitation	High titers	Ezea *et al*., 2021 [48]
6.	Potato Peel	SmF	***A. niger***	With PPH supplement	~5× control yield	Faruk *et al*., 1976 [49]
7.	Potato Peel	SSF	***A. niger***	pH 5.5, 30°C	5.8 g/kg	Karthikeyan & Sivakumar, 2010 [50]
8.	Onion Peel	SmF	***A. niger***	32°C, 120 rpm	3.7 g/L	Singh *et al*., 2019 [51]
9.	Beetroot Waste	SSF	***A. niger***	Moisture 65%, 30°C	6.2 g/kg	Ramesh & Latha, 2016 [52]
10.	Carrot Waste	SmF	***A. niger***	30°C, pH 6.0	4.9 g/L	Sharma *et al*., 2015 [53]
11.	Cabbage Waste	SSF	***A. niger***	70% moisture, 28°C	5.1 g/kg	Deepa *et al*., 2017 [54]
12.	Tomato Waste	SmF	***A. niger***	30°C, pH 5.0	3.95 g/L	Mehta *et al*., 2020 [55]
13.	Pumpkin Peel	SSF	***A. niger***	32°C, 60% moisture	4.8 g/kg	Das & Nandi, 2014[56]
14.	Sweet Potato Peel	SmF	***A. niger***	pH 6.0, 120 rpm, 30°C	4.2 g/L	Raji & Krishnan, 2018 [57]
15.	Cauliflower Waste	SSF	***A. niger***	70% moisture, 28°C	5.4 g/kg	Thomas & George, 2017 [58]
16.	Brinjal Waste	SmF	***A. niger***	30°C, pH 5.5	3.6 g/L	Narayanasamy *et al*., 2016 [59]
17.	Pea Peel	SSF	***A. niger***	65% moisture, 30°C	4.3 g/kg	Kumari & Rao, 2019[60]
18.	Mixed Veg. Waste	SmF	***A. niger***	120 rpm, 30°C, pH 5.5	6.5 g/L	Banerjee *et al*., 2021 [61]
19.	Bottle Gourd Peel	SSF	***A. niger***	60% moisture, 30°C	4.0 g/kg	Joshi & Patel, 2018 [62]

Comparative evaluation of solid-state fermentation (SSF) and submerged fermentation (SmF) highlights trade-offs in industrial feasibility. SSF often yields higher CA concentrations with lower water and energy demand, but scaling and process control are challenging. SmF, while more resource-intensive, offers advantages in aeration, oxygen transfer, and downstream recovery. Economic assessments suggest that agro-waste substrates reduce raw material costs by 30–50% compared to refined sugars. Life cycle analyses also indicate a significantly lower carbon footprint for agro-waste-based CA production. These findings reinforce the industrial viability of waste valorization for sustainable CA manufacturing.

Process Parameters and Optimization

Across studies, optimal **temperature (28–32°C), pH (5.0–6.0), and substrate moisture (60–70%)** were consistently associated with enhanced CA yields. Agitation in SmF (typically 120 rpm) improved oxygen transfer, which is critical for the aerobic metabolism of *A. niger*. Moreover, supplementation strategies—particularly methanol addition and nutrient hydrolysates—were found to markedly enhance yield, emphasizing the importance of process optimization.

Comparative Evaluation of Fruit and Vegetable Wastes

Overall, **fruit-based wastes** such as kiwi peel, apple pomace, cassava peel, sweet potato peel, and bottle gourd peel yielded significantly higher CA levels compared to **vegetable-based wastes.** This trend can be attributed to the higher fermentable sugar content and lower lignocellulosic complexity of fruit residues, which favor citric acid production *[Fig. 1 & 2]*.

Implications for Sustainable Production

The findings underscore the potential of fruit and vegetable residues as promising, eco-friendly substrates for CA production. While SSF demonstrated superior yields, SmF remains advantageous for industrial scalability due to better process control and downstream recovery. Integrating optimized fermentation conditions with strategic substrate selection could therefore establish a sustainable and cost-effective bioprocess for citric acid production

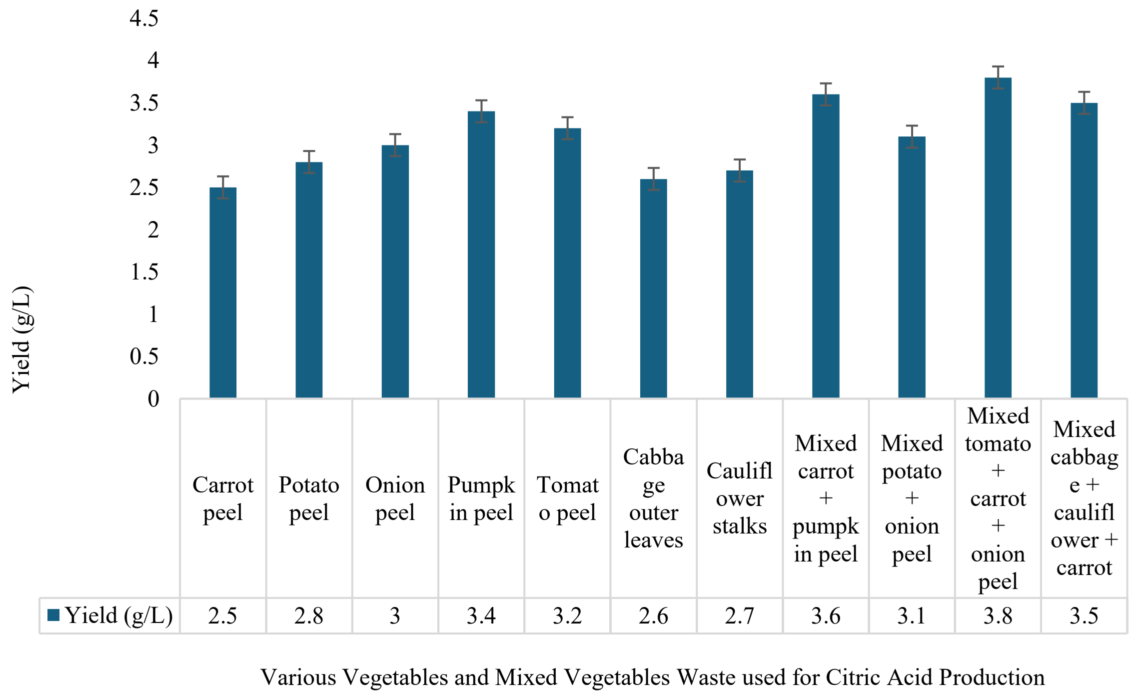

Figure 2. Citric acid yields from vegetable wastes using Aspergillus niger. Lower yields compared to fruit peels are attributed to higher lignocellulosic content and inhibitory metabolites such as oxalates and polyphenols.

ADVANCES IN CA PRODUCTION: GENETICALLY ENGINEERED A. NIGER

While wild-type *Aspergillus niger* strains have long been the workhorse of industrial CA (CA) production, recent advances in molecular biology and metabolic engineering have enabled the development of genetically enhanced strains with superior productivity, substrate tolerance, and reduced by-product formation. These engineered strains are particularly valuable for processing heterogeneous agricultural residues such as fruit and vegetable peels.

Genetic modification of ***Aspergillus niger*** has been extensively explored to enhance CA (CA) production. Key strategies focus on increasing glycolytic flux, inhibiting competing pathways, engineering transport systems, improving pH tolerance, and enhancing substrate utilization. Overexpression of genes encoding glycolytic enzymes such as phosphofructokinase and pyruvate kinase boosts pyruvate availability, the main precursor for CA biosynthesis (Upton, 2020). Knockout of oxaloacetate acetylhydrolase or other oxalate-forming enzymes minimizes carbon diversion to oxalic acid, ensuring more efficient CA production [63]. In addition, overexpression of mitochondrial citrate transporters facilitates faster export of citrate into the cytosol and extracellular medium, further increasing yield [64]. Modifications to genes regulating proton pumps and membrane stability allow ***A. niger*** to ferment effectively in low-pH environments, which is essential to reduce contamination risks [65]. Substrate utilization is also improved by introducing or overexpressing enzymes such as pectinases, cellulases, and hemicellulases, enabling direct hydrolysis of complex polysaccharides present in fruit and vegetable peels [66].

The adoption of CRISPR/Cas9 genome editing has accelerated targeted genetic modifications in ***A. niger***. For instance, disruption of the oxaloacetate acetylhydrolase (*oahA*) gene via CRISPR/Cas9 reduced oxalate accumulation by over 80%, resulting in a ~20% increase in CA yield under glucose-based fermentation conditions [67]. Similarly, integration of heterologous pectinase genes into the ***A. niger*** genome enabled direct utilization of citrus and mango peels without requiring extensive chemical pre-treatment, highlighting the potential of CRISPR-based strategies to enhance substrate flexibility [68].

Adaptive laboratory evolution (ALE) has complemented targeted genetic engineering by enabling ***A. niger*** to adapt to unconventional feedstocks such as mango peel hydrolysates, onion skins, and pumpkin residues. Through repeated subculturing under selective conditions, evolved strains developed increased resistance to inhibitory compounds, including polyphenols and antimicrobial secondary metabolites, leading to higher CA titers [69].

From an industrial perspective, genetically engineered ***A. niger*** strains offer multiple advantages. They can shorten fermentation cycles by 15–30%, reduce the need for substrate pre-treatment, lower operational costs, expand the range of usable agro-waste substrates, and improve yield consistency in large-scale bioreactors where environmental conditions may fluctuate [70]. Despite these benefits, regulatory approval and consumer acceptance remain critical, particularly for food-grade CA production. Industrial deployment of genetically modified strains must adhere to biosafety standards and environmental containment protocols to ensure safe and sustainable operations.

Waste-derived citric acid (CA) plays a critical role in polymer and composite engineering. Quantitative studies have shown that CA-crosslinked starch films exhibit a 40–60% increase in tensile strength, while CA-modified cellulose nanofibers demonstrate a ~20°C improvement in thermal stability. Chitosan-CA films also show up to 35% reduction in water vapor permeability, enhancing barrier performance. However, the presence of impurities in crude CA from agro-waste can interfere with ester bond formation, reducing crosslinking efficiency. Therefore, achieving higher CA purity directly influences the mechanical, thermal, and barrier properties of biopolymer composites. This highlights the importance of optimizing both yield and downstream purification in agro-waste valorization for material applications.

Recent interventions target specific metabolic bottlenecks. For example, disruption of the oxaloacetate acetylhydrolase (oahA) gene reduced oxalate accumulation by over 80% and increased

CA yield by ~20% compared to wild strains. Adaptive laboratory evolution of ***A. niger*** on onion peel hydrolysate improved resistance to polyphenols, achieving a 30% increase in titers. Overexpression of cellulase and pectinase genes enabled direct hydrolysis of citrus and mango peels, reducing pretreatment needs and increasing yields by 15–25%. These advances demonstrate that engineered strains outperform wild-type A. niger in substrate utilization and productivity.

FUTURE PROSPECTS FOR CA PRODUCTION FROM MANGO PEEL USING *Aspergillus niger*

Mango peel, a major by-product of the fruit processing industry, constitutes approximately 15–20% of the total fruit weight and is rich in carbohydrates such as glucose, fructose, and sucrose, as well as pectin and minor amounts of starch. Considering the global annual mango production exceeds 55 million tonnes, this agro-industrial waste represents a significant and underutilized resource for sustainable CA production through microbial fermentation.

The valorization of mango peel offers multiple advantages. Its abundant availability during seasonal surpluses generates large volumes of peel that are often discarded in landfills, causing environmental concerns. The high sugar content and pectin make mango peel an ideal substrate for ***Aspergillus niger***, particularly after mild acid hydrolysis to release fermentable sugars. Converting this waste into CA not only supports efficient resource utilization but also aligns with circular economy principles, contributing to sustainable development goals (SDGs) focused on waste reduction, renewable materials, and green manufacturing.

Optimizing the fermentation process is critical to maximize CA yield from mango peel. Pre-treatment strategies such as dilute acid hydrolysis, enzymatic digestion, or microwave-assisted hydrolysis can enhance sugar release efficiency by up to 40%. Solid-state fermentation (SSF) is particularly promising due to lower water requirements and higher product concentration, whereas submerged fermentation (SmF) offers easier downstream processing. Supplementing the fermentation medium with low concentrations of methanol (1–2% v/v) can improve cell membrane permeability in ***A. niger***, facilitating greater CA secretion. Additionally, co-culturing ***A. niger*** with pectinolytic yeasts or bacteria can further improve peel degradation and sugar availability.

Biotechnological interventions can further enhance CA production. Genetically engineered ***A. niger*** strains overexpressing pectinases and cellulases allow direct utilization of raw mango peel without extensive pre-treatment. Metabolic engineering strategies that redirect carbon flux away from oxalate and gluconate formation toward the CA pathway can substantially increase yield. Moreover, adaptive laboratory evolution through long-term culturing in mango peel hydrolysate can generate strains tolerant to phenolic inhibitors naturally present in the peel.

For industrial-scale production, sustainability and scale-up considerations are essential. Integrating mango processing units directly with fermentation facilities ensures fresh, uncontaminated peel is available for consistent operations. Employing low-cost, energy-efficient, and chemical-minimal pre-treatment methods preserves environmental benefits. Finally, compliance with food safety and biosafety standards is mandatory for the industrial use of genetically modified or enhanced microbial strains, ensuring safe and sustainable production of CA from mango peel.

CONCLUSION

The growing demand for CA in food, pharmaceutical, and industrial sectors necessitates the development of sustainable and cost-effective production methods. Agro-industrial and kitchen vegetable wastes such as cucumber peel, broccoli stalks, spinach stems, bottle gourd peel, onion skins, pumpkin residues, and mango peel present abundant, renewable, and low-cost substrates for *Aspergillus niger*-mediated CA production. The studies reviewed demonstrate that appropriate pre-treatment methods—acid hydrolysis, blanching, alkali treatment, ethanol washing, or methanol supplementation—can significantly enhance fermentable sugar release and fungal metabolic efficiency, thereby increasing yields.

Advancements in bioprocess optimization, including controlled pH, aeration, nutrient supplementation, and the use of solid-state fermentation, have proven effective in maximizing output while reducing resource consumption. Furthermore, the integration of genetic engineering and metabolic pathway optimization in ***A. niger*** holds promise for overcoming substrate limitations, inhibitor sensitivity, and by-product formation, enabling the direct utilization of lignocellulosic-rich peels without extensive pre-processing.

Future industrial adoption will require scale-up studies, supply chain integration with agro-processing industries, and comprehensive life cycle assessments to ensure economic feasibility and environmental benefits. By valorizing vegetable and fruit peels into high-value CA, this approach contributes to the principles of a circular economy, supports sustainable development goals, and offers a viable pathway to reduce agro-waste pollution while meeting global market needs.

In conclusion, vegetable and fruit peel-based CA production using ***A. niger*** is not only an environmentally responsible alternative to traditional methods but also an economically attractive solution, paving the way for greener biomanufacturing practices in the future.

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