Emirates Journal of Food and Agriculture. 2021. 33(12): 992-999
doi: 10.9755/ejfa.2021.v33.i12.2793
http://www.ejfa.me/

RESEARCH ARTICLE

Effect of waste grape pomace on citric acid bioproduction by submerged fermentation

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ABSTRACT

Citric acid is of great economic importance due to its wide range of applications. Citric acid, which is a versatile and widely used organic acid, constitutes 60% of organic acids used in the food industry and 10% of those in the pharmaceutical industry. Citric acid is produced using various agricultural wastes as a substrate through solid state or submerged fermentation. In this study waste grape pomace was proposed as a valuable substrate alternative for citric acid production using submerged fermentation. Initially screening of citric acid production capacity was conducted using 6 different *Aspergillus niger* strains (3 wildtype strains isolated from dried figs and 3 commercial ATCC strains) on synthetic medium. The wild type AN2 was determined as the highest producing strain with 14.32 mg/mL citric acid following 192 h fermentation. Then the waste grape pomace concentration effect on citric acid production was studied by five different concentrations in the range of 100-300 g of pomace in 1 L medium for 192 h fermentation. Using the wild type AN2 strain the highest production of citric acid and yield was achieved with 250 g/L grape pomace concentration as 28.26 mg/mL and 87%, respectively. Moreover, biomass production of the strains in grape pomace medium showed similar pattern with the citric acid production except biomass formation was increased with the grape pomace concentration increases. This study presented that comparing with synthetic medium waste grape pomace can be valuable substrate alternative for citric acid production using submerged fermentation. The study also contributed to the literature as waste grape pomace could be valuable substrate for future research studies.

Keywords: *Aspergillus niger*; Agricultural waste; Citric acid; Submerged fermentation

INTRODUCTION

Approximately 77 million tons of grapes were produced worldwide in 2019 harvest season (FAOSTAT, 2021). Grapes are important economical fruit in terms of production of wine, grape juice, vinegar, jam, jellies and seed oil. Massive amounts of grape are processed to produce wine which generates annually more than 9 million tons of waste products known as grape pomace (Sirohi et al., 2020). Grape pomace is composed of skins, stalks and juice, may be responsible from 20-25% of the weight of the fresh grape processed which is depend on the cultivar of the grape, harvest time, geographic origin and cultivation conditions (Sousa et al., 2014; Sirohi et al., 2020). Agricultural waste causes degradation in soil structure when disposed of directly with high acid content and is also susceptible to microbial deterioration with high moisture and sugar content. Thus, the European Union has placed strict regulations on wineries regarding the disposal of grape pomace (EC, 2010).

The pomace is a biodegradable solid waste consisted of carbohydrates, proteins, lipids, vitamins, minerals, fibres, phenolic compounds and water (tannoids, phenol carboxylic acids, anthocyanins, and resveratrol) (Sirohi et al., 2020; Sousa et al., 2014). Especially remaining white seedless grape pomace waste obtained from pressed white grapes, has sugar content in the range of 60-80% and contains 20-30% dietary fibre on dry mass basis (Zhu et al., 2015). For this reason, grape pomace is seen as a very important resource for the recovery of phytochemicals and a cost-effective fermentation raw material for the effective synthesis of surface-active biomolecules, enzymes, organic acids and biofuels (Sirohi et al., 2020).

Citric acid is found naturally in plants (particularly lemons and pineapples) and animals. This organic acid, which is colourless and safe to use, is considered environmentally friendly as it is easily biodegradable as well as dissolving in water. Citric acid and citric acid salts are employed in many applications (pH adjustment,

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Received: 30 September 2021; **Accepted:** 28 November 2021

buffering, chelation and derivatization, etc.) including agricultural, chemical, cosmetics, pharmaceutical and even construction industries, as well as in food and beverage sector. It is an indispensable additive in foods due to the preservation of foods, preventing the decomposition of fats and giving a fresh and sour taste (Show et al., 2015).

According to 2020 market data, the global citric acid market has reached 2.39 million tons and the expectations are to reach 2.91 million tons by 2026 (EMR, 2020). Citric acid was previously produced entirely from unripe lemon and pineapple for commercial purposes. After the first half of the 20th century, it started to be obtained by fermentation by microorganisms. Today, more than 90% of citric acid production for commercial purposes is produced by fermentation using *Aspergillus niger* and *Yarrowia lypolytica* (Schuster et al., 2002; Soccol et al., 2006). However, it is known that on an industrial scale *A. niger* is the major source of citric acid globally because it is more efficient in terms of citric acid production (Show et al., 2015).

Given the increasing demand and market, better manufacturing techniques and solutions need to be discovered and developed to provide effective production and to increase the yield of product recovery. To encourage large scale citric acid production, it is crucial that the production process being environmentally friendly, utilizing easily accessible and cost-effective agro-industrial waste products, at the same time ensuring high production efficiency.

Previously conducted researches which use various agricultural wastes as substrates in the production of citric acid employing *A. niger* may be exemplified as coffee, kiwi and banana peel, apple and pineapple pomace, sugar beet, sugar cane, corn cob, mulberry pomace, grape pomace, wheat straw with various types of fermentation (Hang and Woodams, 1984; Hang and Woodams, 1985; Roukas and Kotzekidou, 1997; Kumar et al., 2010; Aidynova et al., 2020; Chysirichote, 2020; Roukas and Kotzekidou, 2020).

As far as we know, it was concluded that any study on citric acid production using only grape pomace by submerged fermentation has not been conducted yet. On this basis, the most significant goal of this work is to examine the usage of waste grape pomace as a potential substrate for the production of citric acid applying the process of submerged fermentation. In order to achieve that initially, the ability of citric acid production of various native and commercial *A. niger* strains was evaluated in synthetic fermentation medium.

MATERIALS AND METHODS

Microorganisms and preparation of spore suspension

Six *A. niger* strains were used in this study. Three wild types morphologically identified strains of *A. niger* isolated from dried figs that are AN1, AN2, and AN3 were provided from Istanbul Technical University, and the rest of the strains purchased from American Type Culture Collections (USA) that are *A. niger* ATCC 9029, ATCC 10577, ATCC 10549.

The strains were subcultured every month and maintained at 4°C on potato dextrose agar (PDA) slants for future use. For spore production, strains were cultured on PDA at 30 ± 1°C for 7 days. The spores were harvested with the help of Tween-80 (10 mL, 0.1%, w/v) and elimination of mycelial growth was ensured by filtering through sterile glass wool. The spore count was calculated using an improved hemocytometer to set the count to around 1-2 x 10^6 spores per mL, then used immediately to inoculate fermentation medium.

Fermentation medium preparation

Synthetic and grape pomace fermentation mediums were prepared as follows:

The synthetic fermentation medium was prepared for preliminary screening of citric acid production capacity of the strains and determination of the optimum fermentation time. For the preparation of fermentation medium, 50 g of glucose, 2 g of NH_4SO_4, 1 g of $NaNO_3$, 0.5 g of KH_2PO_4, 0.3 g of $MgSO_4.7H_2O$, 0.01 g of $FeSO_4.7H_2O$ were dissolved in dH_2O (total volume: 1 L). After determination of the highest citric acid producer and the optimum fermentation time, the waste grape pomace concentration effect was studied as the substrate.

The waste grape pomace fermentation medium was prepared using seedless type white grapes which were obtained from local markets in July-October in Adana city in Turkey. The grapes were cleaned, stems and stalks removed and squeezed using a home-type juice extractor (HR1866/30, Philips, Holland). The waste pomace was then dried using vacuum freeze dryer (BK-FD10P, Biobase, China), grounded by a coffee grinder (75, Krups, France) and sieved to have particle size < 2 mm. The obtained grape pomace flour stored at -18°C until used.

The substrate concentration effect on production of citric acid was evaluated by preparing five different fermentation media containing 100, 150, 200, 250, and 300 g/L of grape pomace (instead of glucose in synthetic medium, described in above). The optimum grape pomace concentration was determined by the selected *A. niger* strain for citric acid production and yield.

Submerged fermentation

Shake flask fermentation studies were performed in 500 mL Erlenmeyer flasks with 200 mL medium. Both medium's pH was adjusted initially at 6.5 and sterilized with autoclave (Model HV85-L, Hirayama, Japan) at 121°C, 15 min. Inoculation was performed using 1 mL of spore suspension and incubated at 30±1°C using a shaking incubator at 150 rpm for 240 h (10 days). Incubation temperature 30±1°C and pH 6.5 used in this study were selected according to previous optimization studies (Kumar et al., 2010; Dhillon et al., 2012). During fermentation, one flask was taken every day starting from the third day until the end of incubation period for chemical analysis in order to determine optimum incubation time for highest citric acid production in synthetic medium. Once the optimum fermentation time obtained, it is used for the determination of grape pomace concentration effect, as well. Rest of the fermentation conditions such as pH of the medium, incubation temperature and agitation speed were kept the same as in synthetic medium studies.

Citric acid analysis

Citric acid concentration was quantified by using Shimadzu Class10 AVP Series HPLC system (Shimadzu, Kyoto, Japan) equipped with diode array detector set for 214 nm wavelength. The samples were collected from each flask once a day. Filtration of the supernatant was performed using Whatman No.1 filter, to eliminate solid substrate and fungal mycelia. The remaining sample was centrifuged (Allegra X-30R, Beckman Coulter, USA) at 9000×g for 20 min, and citric acid amount was determined via AOAC with some modifications using the supernatant (AOAC, 1989). C18 reversed phase column (250×4.6 mm i.d., 5 μm particle size; ACE, Aberdeen Scotland) was used at 25°C, 0.8 mL/min flow rate by applying 0.2 M KH_2PO_4 as the mobile phase. The injection volume was 20 μl. Data were recorded and processed by Class-Vp 5 software (Shimadzu, Kyoto, Japan). Five-point calibration curve was prepared using citric acid standard (Sigma Chemical Co., St Louis, USA) with a concentration range of 0.5 mg/ml to 50 mg/ml.

Biomass analysis

Biomass production was determined only from grape pomace fermentation medium following the incubation period of 192 h. The wet fungal biomass was obtained by removing the supernatant after the fermentation medium was filtered and washing the remaining cells with distilled water. It was taken into a beaker of known weight and dried in an oven adjusted to 105°C until constant weight. The beaker with weight of dried fungal biomass was determined in order to find the weight of each culture. Cell biomass (mg/mL) was calculated as dry cell weight produced per milliliter of liquid medium (Xie and West, 2009).

Total sugar analysis

Total sugar content of grape pomace powder and the residual sugar content of fermentation mediums (after incubation) were spectrophotometrically (Dubois et al., 1956).

Briefly, 5 g of sample was extracted using 25 mL of ethanol (80%, v/v) at 25°C for 15 min using ultrasonic water bath (WUC-A06H, Daihan Scientific, Korea), and repeated three times. Centrifugation of obtaining mixtures was performed at 9000×g for 10 min (Allegra X-30R, Beckman Coulter, USA). The extracts were collected, and remaining ethanol content was removed by evaporation under vacuum at 50°C (Rotavapor R-100, Büchi, Switzerland). Dried extracts were dissolved in distilled water and then spectrophotometric measurement were conducted as described below.

In order to determination of residual sugar content of the fermentation mediums, the liquid samples were taken aseptically at intervals of 24 hours started from the 3rd day of incubation. Filtration of the supernatant was performed using a glass wool in order to remove solid substrate and fungal mycelia.

2 mL of supernatant was taken into a glass tube. Then 0.05 mL of phenol solution (80%, v/v) was added and followed by the addition of 5 mL of concentrated H_2SO_4 immediately. After letting the tubes stand, they are shaken and placed in a 30°C water bath for 30 min. The absorbance was measured at 490 nm. The obtaining results were represented as glucose equivalents (g/100 g and g/100 mL).

The citric acid yield was calculated using Eq.1:

Yield (%) = 100 × [citric acid (mg/mL)/consumed sugar (mg/mL)]

Statistical analysis

Each experiment was performed in three repetitions, and the obtaining results were represented as mean ± standard deviation. Differences among mean values were tested for significance ($p<0.05$) by the Duncan's multiple range test.

RESULTS AND DISCUSSION

Six strains of *A. niger* were examined in terms of their capacity of citric acid production in synthetic medium. The influence of incubation time on citric acid production capacities of the strains are shown in Fig. 1. It can be clearly seen from the figure that the strain AN2 was determined as the highest citric acid producer. Citric acid production level was found as 14.32 mg/mL for AN2, and it is significantly ($p<0.05$) higher than all other strains. All three wildtypes'

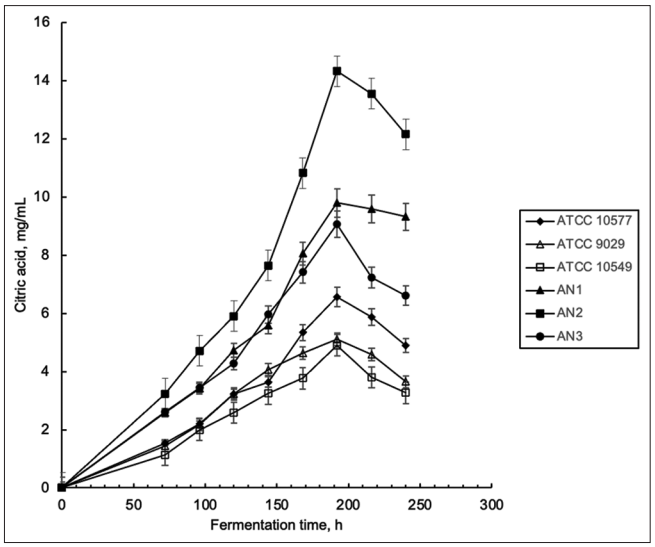

Fig. 1. Citric acid production of *A. niger* strains grown on the synthetic medium at different fermentation periods (initial pH 6.5, temperature 30°C, shaking speed 150 rpm)

strains were higher citric acid producer than commercial ones. There was a significant difference ($p<0.05$) in citric acid levels between wildtype strains and commercial ones. It is also clear that the maximum citric acid production was achieved after 192 h incubation time for all strains.

In the study conducted by Xie and West (2009), citric acid production of 7 different commercial *A. niger* strains (ATCC 9029, 9142, 10577, 11414, 12846, 26550, 201122) was investigated using wet corn distillers' grains. As a result of the study carried out with the solid-state fermentation method, the highest citric acid production was determined as approximately 10 g/kg after 10 days of incubation at 25°C with ATCC 9142 strain. Citric acid productions obtained with ATCC 9029 and ATCC 10577 *A. niger* strains were found to be approximately 3.8 and 3.9 g/kg, respectively, which were approximately half of the levels observed by this study.

In a study investigating the use of ATCC 9142 strain and banana peel in the production of citric acid, the highest production was determined as 49.9 g/L. In this study, the production of 9.8 mg/mL citric acid obtained at the end of 192 h incubation with ATCC 10577 strain was found to be higher than about 4 mg/mL produced in corn by the same strain (Amenaghawon et al., 2015). In another study conducted with kiwi peel, the highest citric acid production was determined as 70 g/kg with ATCC 12846 strain after 120 h incubation (Hang and Woodams, 1987). The reasons for the differences occurred in the results may be the differences in substrate and strain used, incubation period and fermentation method applied.

Following the determination of best citric acid producer strain, grape pomace concentration effect on citric acid and biomass production was studied for 192 h fermentation and obtained results shown in Fig. 2. The maximum citric acid concentration was observed at 250 g/L substrate concentration as 28.3 mg/mL. As it is clear from the figure the citric acid production level was increased by increasing substrate concentration until the concentration reached 250 g/L. The citric acid production on 250 g/L substrate concentration was found to be statistically significant ($p<0.05$) compared with other concentrations. However, increase in the formation of fungal biomass was observed as the substrate concentration of the fermentation medium was increased.

As described in experimental section citric acid production of all strains on grape pomace medium was examined for 192 h fermentation and the yield was calculated for each. Total carbohydrate content of waste grape pomace was found to be 35.16% in dry weight mainly as glucose and fructose. The citric acid production capacities of six *A. niger* strains in synthetic and grape pomace medium and yields are shown in Figs. 3a and 3b. As can be seen from the figures the best citric acid producer, AN2, is also provided the highest citric acid production yield, 80% and 87%, in synthetic and waste grape pomace mediums, respectively. The citric acid yield on the grape pomace medium was significantly higher ($p<0.05$) than obtained on the synthetic medium.

Research on citric acid production started in the 1950s, and the interest in this organic acid increased, especially due to its GRAS status. It is produced and sold in large quantities of citric acid abroad, most of which are in the form of citric acid monohydrate. It can be seen from the Table 1 that many studies have been carried out for investigating the citric acid production potential of commercial and wildtype *A. niger* strains using different fermentation methods on various agricultural wastes, such as sugar cane, casava pulp, kiwi, mango and banana peel, pineapple, orange and brewery waste, corncob, apple and grape pomace.

According to the previous studies, Hang and Woodams (1984) used apple paste as a substrate in the production of citric acid with five different strains of *A. niger* in their study. It was determined that *A. niger* NRRL 567 strain produced the most citric acid from apple paste in the presence of 4% methanol and the yield was found to be 88%, depending on the amount of sugar consumed. Kumar et al. (2010) optimized the production of citric acid by *A. niger* van. Tieghem MTCC 281 strain by solid state fermentation method, utilizing apple pomace as a substrate. Optimum yield was obtained with 4.6 g citric

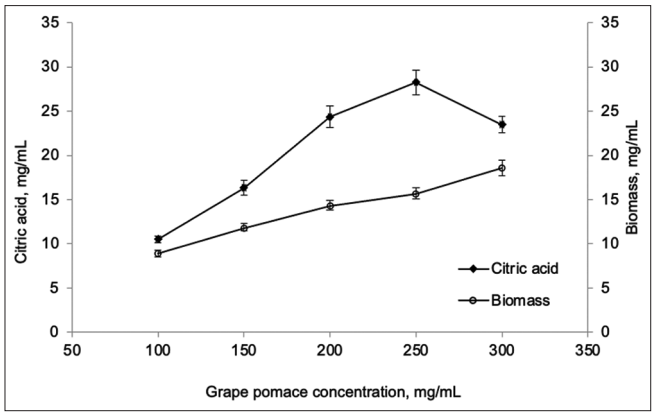

Fig. 2. Grape pomace concentration effects on citric acid production and biomass formation by AN2 (initial pH 6.5, temperature 30°C, fermentation time 192 h, shaking speed 150 rpm)

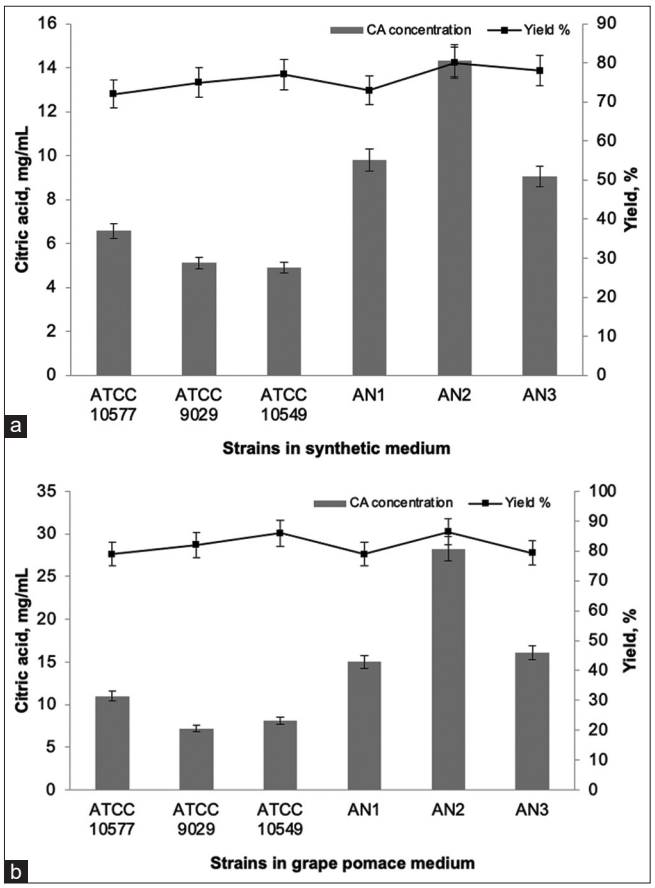

Fig. 3. Comparison of citric acid production of strains and yields (initial pH 6.5, temperature 30°C, fermentation time 192 h, shaking speed 150 rpm), a) Synthetic fermentation medium b) Grape pomace fermentation medium (250 g/L)

acid/100 g pomace and 4% methanol (v/w) for 5 days incubation at 30°C. In 2011, Dhillon et al. (2011) used apple pomace as a substrate for citric acid production and performed substrate optimization using surface response methodology. Considering the amount of carbon used, they determined the solid-state fermentation yield after 144 hours of incubation at 75% humidity, as 93% and 66% for fermentation medium containing 3% methanol and fermentation medium containing 3% ethanol, respectively. The highest amount of citric acid produced was found to be 342.41 g/kg.

In 1985, citric acid production by solid state fermentation method by *A. niger* NRRL 567 strain using grape pulp was investigated by the same researchers and the citric acid yield was determined as 60% according to the amount of sugar consumed (Hang and Woodams, 1985). Our results showed higher yield of citric acid comparing with study conducted by Hang and Woodams (1985). However, in the literature, it has been reported that the yield obtained from food industry wastes using *A. niger* varies between 20% and 90% and the yield depends on the substrate, the presence of inducers, fermentation types and strains.

Torrado et al. (2011) used orange peel as a substrate for citric acid production by solid state fermentation method using *A. niger* CECT-2090 (ATCC 9142, NRRL 599). A series of experiments were carried out by adding 1-4.8 g/L orange peel, different proportions of methanol and water to the medium. As a result, 193 mg/g citric acid production was achieved.

Deveci and Özyurt (2017) used hydrolyzate produced from orange peels and molasses as substrates in their studies in which citric acid production by *A. niger* was determined. In the study, the initial pH of the medium was set to pH 6.0, ambient temperature to 30°C, methanol ratio to 4%, shaking speed to 150 rpm, working volume to 100 mL, corn oil amount to 2%, $K_4Fe(CN)_6$ concentration to 200 mg/L. The total sugar concentration was adjusted between 100 g/L and 180 g/L. At a total sugar concentration of 100 g/L and pH 3.01, 31.8 g/L citric acid was produced, and the total sugar amount used in this process was 79 g/L. At a total sugar concentration of 140 g/L, the pH was measured as 2.36, 35.6 g/L citric acid was produced, and the total sugar concentration used was determined as 108 g/L. At a total sugar concentration of 180 g/L, pH was measured as 3.84, the amount of citric acid produced was 18.8 g/L, and the total sugar amount used in this series was measured as 132 g/L. They stated that substrate inhibition caused the decrease in citric acid production at higher sugar concentrations.

Iqbal et al. (2015) stated that the best citric acid producing strain was *A. niger* GCB117, chose reed molasses as substrate and tried citric acid production by submerged fermentation method. The maximum amount of citric acid was determined as 20.3 g/L at a sugar concentration of 150 g/L, at pH 5.5 and at 168 hours of incubation time. Abbas et al. (2016) used banana peel for the production of citric acid employing *A. niger* and optimized fermentation

Table 1: Agricultural waste materials employed for citric acid production by *A. niger*

Substrate	Strain	Fermentation	Citric acid (g/L or g/kg substrate)	Yield (%)	Reference
Apple pomace	*A.niger* NRLL 2001 *A.niger* NRLL 2270 *A.niger* NRLL 599 *A.niger* NRLL 328 *A.niger* NRLL 567	Solid state	766 816 771 798 883		Hang and Woodams, 1984
Grape pomace	*A.niger* NRLL 2001 *A.niger* NRLL 2270 *A.niger* NRLL 599 *A.niger* NRLL 328 *A.niger* NRLL 567	Solid state	413 511 498 523 600	88	Hang and Woodams, 1985
Apple pomace	*A.niger* Van Tieghem MTCC 281	Solid state	46		Kumar et al., 2010
Date syrup	*A.niger* ATTC 9142	Submerged	-	50	Roukas and Katzekidou, 1997
Pomegranate peel waste (dried-nondried)	*A.niger* B60	Solid state	278.5 306.8		Roukas and Katzekidou, 2020
Banana peel	*A.niger*	Solid state	124.0 ± 19.2		Chysirichote, 2020
Mullberry pomace	*A.niger* MT-4	Submerged	24.6		Aidynova et al., 2020
Corncobs	*A.niger* NRLL 2001 *A.niger* NRLL 2270 *A.niger* NRLL 328 *A.niger* NRLL 599	Submerged	58 ± 8.29 30 ± 2.58 58 ± 4.24 6.5 ± 0.52		Hang and Woodams, 1998
Mango peels Sweet orange peels	*A.niger*	Surface culture	7.52 11.01		Abbas et al., 2016a
Banana peels	*A.niger* ATTC 9142	Solid state	49.9		Hang and Woodams, 1987
Banana peels	*A.niger*	Surface culture	51.68		Abbas et al., 2016b
Pineapple waste	*A.niger*	Solid state	60.61		Kareem et al., 2010
Brewery wastes	*A.niger* ATTC 9142	Submerged	19	78.5	Roukas and Katzekidou, 1986
Cane molasses	*A.niger* GCBT7	Submerged	99.56 ± 3.5		Ali et al., 2002
Cane molasses Corncob	*A.niger* NCIM1055	Submerged	10.4 5.3		Shetty, 2015
Cassava peel malted sorghum	*A.niger* Mutant *A.niger*	Submerged	1.93 9.4		Adeoye et al., 2015
Kiwifruit peel	*A.niger* NRRL 567	Solid state	100		Hang and Woodams, 1987
Orange waste	*A.niger*	Solid state	46		Aravantinos-Zafiris et al., 1994
Carrot waste	*A.niger* NRRL 2270	Solid state	29	36	Garg and Hang, 1995
Cashew apple juice	*A.niger* LCFS 5	Submerged	92.8		Adoeye and Lateef, 2021

conditions. After 8 days of fermentation, they obtained maximum citric acid yield using potassium dihydrogen phosphate and ammonium nitrate with 20% banana peel medium at 32°C and pH 5.0. They also showed that citric acid production increased with the addition of 0.1% phosphate source. They obtained 11.9 g/L, 24.09 g/L and 18.8 g/L citric acid, using disodium hydrogen phosphate, potassium dihydrogen phosphate, and dipotassium hydrogen phosphate, respectively.

In 2019, Ozdal and Kurbanoglu was studied the effect of sugar beet molasses and chicken feather peptone as sole carbon and nitrogen sources, respectively, on citric acid biosynthesis by submerged fermentation using *A. niger*. They stated that citric acid production with molasses was significantly affected by chicken feather peptone concentrations (1-6 g/L). As a result they determined the maximum citric acid concentration at 4 g/L chicken feather peptone and 168 h. The maximum of 64.40 g/L citric acid production was achieved at 168 h in chicken feather peptone medium which contains 150 g/L molasses.

Adoeye and Lateef (2021) investigated the production of citric acid using waste from the processing of cashew by local strain of *A. niger*. The citric acid production of local strain in the formulated cashew apple juice medium ranged 16.0–92.8 g/L with the maximum concentration obtained on the 10th day of fermentation. This study revealed the potential application of cashew waste in high yield citric acid production in the food industry. Aidynova et al. (2020) analyzed production of citric acid employing *A. niger* on mulberry pulp which is a waste from molasses production. It was determined that optimum mulberry pulp concentration both citric acid production and cell growth is 120 g/L. As a result of the experiments 24.6 g/L was achieved as maximum citric acid production after 5 days incubation. Addition of certain amounts of $MgSO_4$ and $(NH_4)_2SO_4$ was determined to be effective for maximum

citric acid production. Considering the studies investigating the substrate effect, our study showed that the highest citric acid level was achieved with the 250 g/L of grape pomace presence in the medium. This result was lower than the previous observations by Deveci and Özyurt (2017) and Iqbal et al (2015) but it was comparable with the results achieved by Aidynova et al. (2020). As it can be seen from the previous studies, citric acid production levels vary from 1.9 to 883 g/L according to type of substrate, availability of inducers and type of fermentation and strain used. Grape pomace offers effective substrate for fermentation due to the sugar content of 60-70% mainly consists of glucose and fructose. In a study investigating the effects of sucrose, glucose, fructose, galactose and lactose on citric acid production by *A. niger* it was determined that sucrose was the best substrate followed by glucose and fructose (Hossain et al., 1984). This result also supports the efficiency of grape pomace usage in citric acid production.

CONCLUSIONS

In conclusion, grape pomace could be an up-and-coming substrate to be used in commercial production of citric acid using one of the most common fungus, *A. niger* due to its high carbohydrate contents and presence of other macro and micronutrients. Industrially, for citric acid production submerged fermentation with batch process has been preferred. In this respect this study reveals the potential of utilizing the wastes generated during fruit juice and wine production. Finally, this could lead to both citric acid production and agro-industrial waste disposal in an economical way. However further studies should be conducted in order to optimize experimental conditions for production process and in order to adapt experimental results to industrial processes pilot scale studies should be carried out.

ACKNOWLEDGEMENTS

Author is thankful to the Assoc. Prof. Dr. Funda Karbancıoğlu Güler from Istanbul Technical University for providing the three *Aspergillus niger* strains.

FUNDING

This work was financially supported by the Scientific Research Projects Coordination Unit of Adana Alparslan Türkeş Science and Technology University, Adana, Turkey (grant number: 20103009).

CONFLICT OF INTEREST

The author declare no conflict of interest.

AUTHORS' CONTRIBUTION

The author designed the study, carried out all experimental work, data analysis and interpretation, and also writing and finalising the manuscript.

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