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BIOTECHNOLOGICAL VALORIZATION OF GRAPE POMACE FOR THE PRODUCTION OF FUNGAL CELLULASES BY SOLID-STATE AND SUBMERGED FERMENTATION

VALORIZAÇÃO BIOTECNOLÓGICA DO BAGAÇO DE UVA PARA PRODUÇÃO DE CELULASES FÚNGICAS POR FERMENTAÇÃO EM ESTADO SÓLIDO E SUBMERSA

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[1]MARIA EDUARDA DE SOUSA SILVA DONATO
Universidade Federal Rural de Pernambuco, UFRPE, eduarda.donato@ufrpe.br

[2] GUILHERME GOMES DE SOUSA MAGALHÃES
Universidade Federal Rural de Pernambuco, UFRPE, guilhermegomesdesousa123@gmail.com

[3]ANDRÉ FELIPE DE MELO SALES SANTOS
Universidade Federal Rural de Pernambuco, UFRPE**,** andre.felipesantos@ufrpe.br

[4]TATIANA SOUZA PORTO
Universidade Federal Rural de Pernambuco, UFRPE, tatiana.porto@ufrpe.br

[5]LILIANA ANDRÉA DOS SANTOS
Universidade Federal Rural de Pernambuco, UFRPE, liliana.andrea.santos@gmail.com

RESUMO

A biomassa lignocelulósica é uma fonte renovável abundante e subutilizada, com potencial para a produção de energia, alimentos, produtos químicos e biotecnológicos. A produção agrícola gera grandes quantidades de resíduos lignocelulósicos, como o bagaço de uva, que podem ser aproveitados para a produção de enzimas. O bagaço de uva contém celulose, hemicelulose e lignina, sendo uma boa fonte de carbono para microrganismos. No entanto, a celulose e a lignina são de difícil degradação, e a produção enzimática de celulases é atualmente não viável economicamente devido ao alto custo das enzimas comerciais, para algumas aplicações. A busca por cepas fúngicas de alto rendimento e a otimização das condições de hidrólise são abordagens para superar essa deficiência. A celulose é o polímero natural mais abundante, usado em várias indústrias. As celulases têm amplas aplicações industriais. Dessa forma, a produção enzimática utilizando resíduos agrícolas como fonte de carbono é uma opção viável e sustentável. Fungos, especialmente os gêneros *Aspergillus*, *Trichoderma* e *Penicillium*, são bons produtores de celulases. A fermentação em estado sólido (FES) e a fermentação submersa (FS) são métodos para a produção de enzimas, cada um com suas vantagens. A FES é simples e de baixo impacto ambiental, enquanto a FS permite melhor controle dos parâmetros de fermentação. Neste estudo, foi investigada a produção de celulases fúngicas a partir do bagaço de uva por meio de FES e FS, utilizando *Aspergillus niger* e *Aspergillus tamarii*. O substrato foi padronizado, seco e triturado, sendo caracterizado quanto à umidade, sólidos totais voláteis e pH. A capacidade de absorção de água foi determinada para ajustar a umidade durante a fermentação. Os fungos foram cultivados e esporulados, com as suspensões de esporos preparadas e ajustadas para a concentração desejada. A atividade celulolítica foi medida através da atividade de CMCase, com a quantidade de glicose liberada determinada por reação com DNS. Os resultados mostraram que a maior atividade celulolítica foi obtida com *Aspergillus niger* em FS, com atividade de CMCase de 0,194 U/mL. Conclui-se que o bagaço de uva é um substrato potencial para a produção de celulases fúngicas.

Palavras-Chave: Bagaço de frutas, biomassa lignocelulósica, resíduos agroindustriais, fermentação, enzimas.

ABSTRACT

Lignocellulosic biomass is an abundant and underutilized renewable source with potential for the production of energy, food, and chemicals. Agricultural production generates large amounts of lignocellulosic residues, such as grape pomace, which can be utilized for enzyme production. Grape pomace contains cellulose, hemicellulose, and lignin, making it a good carbon source for microorganisms. However, cellulose and lignin are difficult to degrade, and enzymatic production of cellulases is currently unprofitable due to the high cost of commercial enzymes. The search for high-yield fungal strains and the optimization of hydrolysis conditions are approaches to overcome this deficiency. Cellulose is the most abundant natural polymer, used in various industries. Cellulases have broad industrial applications. Thus, enzymatic production using agricultural residues as a carbon source is a viable and sustainable option. Fungi, especially the genera *Aspergillus*, *Trichoderma*, and *Penicillium*, are good producers of cellulases. Solid-state fermentation (SSF) and submerged fermentation (SmF) are methods for enzyme production, each with its advantages. SSF is simple and has a low environmental impact, while SmF allows better control of fermentation parameters. In this study, the producing of fungal cellulases from grape pomace via SSF and SmF, using *Aspergillus niger* and *Aspergillus tamarii*, was investigated. The

substrate was standardized, dried, and ground, being characterized for moisture, volatile solids, and pH. Water absorption capacity was determined to adjust moisture during fermentation. The fungi were cultured and sporulated, with spore suspensions prepared and adjusted to the desired concentration. Cellulolytic activity was measured through CMCase activity, with the amount of glucose released determined by reaction with DNSA. The results showed that the highest cellulolytic activity was obtained with *Aspergillus niger* in SmF, with CMCase activity of 0.194 IU/mL. It is concluded that grape pomace is a potential substrate for the production of fungal cellulases.

Keywords: Fruit bagasse, lignocellulosic biomass, agro-industrial residues, fermentation, enzymes

INTRODUCTION

Lignocellulosic biomass is an underutilized, renewable resource found abundantly in nature or derived from byproducts of agro-industrial processes. It holds promise as a raw material for obtaining high-value biotechnological products. This biomass also has the potential to produce energy, food, and chemicals, facilitating the development of diverse processes and products (Kumar *et al.*, 2015). Agricultural production generates substantial amounts of waste annually, spurring interest in finding more noble and value-added uses for these agricultural lignocellulosic residues. One such use involves exploring their potential as a solid support and carbon source for microbial growth (Al Kamzari, 2023).

Among these residues, grape pomace stands out due to its large-scale production by the juice and wine industries, which are growing worldwide.This residue is the main byproduct of the wine production process and other products obtained through pressing, accounting for 20% to 30% of the dry weight of grapes (Magalhães *et al*., 2024). According to Castellanos-Gallo *et al.* (2022) and Mangiapelo *et al.* (2024), the global production of grape pomace is estimated to be approximately 8.49 million tons per year. These residues pose environmental challenges because of their limited biodegradability, potential to acidify, and the buildup of polyphenols, which can harm water and soil quality (Machado *et al.,* 2024). However, due to the large quantity generated, grape pomace can become an environmental issue, requiring effective alternatives for its utilization. Grape pomace is generated during the industrial juice extraction process and is typically used as animal feed or for extracting compounds of interest for the cosmetics industry due to its high levels of antioxidants and phenolic compounds (Amaya-Chantaca *et al*., 2022). There is also the possibility of composting the residues or incorporating them directly into agricultural soil.

Grape pomace exhibits typical characteristics of a lignocellulosic residue, such as a high concentration of cellulose (12.9-19.0%), hemicellulose (8.5-9.8%), and lignin (31.3-50.6%) (Almeida *et al.*, 2022; Selo *et al.,* 2023). Hemicellulose, present in the pomace composition, is commonly degradable and serves as the primary carbon source for microorganisms. Cellulose and lignin, however, present different degradation patterns. Lignin is recalcitrant, insoluble in water, and resistant to microbial degradation and oxidation, while cellulose is more resistant to degradation due to its crystalline structure, which impedes microbial penetration (El Achkar *et al.*, 2016).

The enzymatic saccharification of lignocellulosic biomass is currently not profitable due to the high production cost of commercial enzymes, including cellulases, produced via fermentation methods. To address this limitation, different strategies have been studied, such as searching for high-yield cellulase-producing strains and optimizing hydrolysis conditions for enzymatic production (Oksanen *et al.*, 2000; Kotchoni & Shonukan, 2002). The use of enzymes to valorize organic and agro-industrial residues is a growing field in biotechnology due to its economic and environmental benefits for production cycles. Enzymatic hydrolysis has proven to be the most advantageous pretreatment method, especially when compared to other methods, such as acid hydrolysis, which involves corrosive, toxic, and hazardous chemicals (Silva, 2019).

Cellulose is the most abundant and ubiquitous natural polymer on the planet, found in plants and widely used in ropes, candles, paper, housing wood, and many other applications. Commercially, wood is the most exploited natural cellulose resource (Eichhorn *et al.*, 2010). Cellulases have garnered significant attention from the scientific community due to their wide range of industrial applications and the complexity of the enzymatic systems they involve (Boondaeng *et al.*, 2024).

The fungal potential for enzyme production depends on the fungal species, the growth substrate (lignocellulosic biomass), and the cultivation method and conditions. Factors such as cost, yield, and stability of cellulase are essential for its commercial production, given its high market value. Costs can be reduced by using naturally available biomass as a carbon source. The availability, low cost, and abundance of lignocellulosic biomass make it a versatile substrate for enzyme production (Singh, 2021). Moreover, selecting a robust microbial agent is crucial to enhance cellulolytic enzyme production. Various microorganisms, such as bacteria, fungi, and actinomycetes, produce cellulase, but fungi are the most commonly explored due to their higher production levels (Shruthi *et al.*, 2018; Naher *et al.*, 2021). Frequently used fungi for enzyme production include the genera *Aspergillus, Trichoderma, Penicillium, Fusarium, Humicola,* and *Phanerochaete* (Ramos-Ibarra *et al.*, 2017). Notably, the filamentous fungus *Aspergillus* is an excellent producer of exoglucosidases, endoglucosidases, and high concentrations of β-glucosidases (Rodríguez-Zúñiga *et al.*, 2011).

The methods used for the degradation of lignocellulosic biomass and enzyme production are classified into two types: solid-state fermentation (SSF) and submerged fermentation (SmF).

Submerged fermentation (SmF) occurs in an excess of water, allowing for better control during the process (Santos; Orlandellir, 2019). In contrast, solid-state fermentation (SSF) involves the growth of microorganisms on solid substrates, where there is no free water, and offers an alternative for the production of various enzymes (Selo et al., 2024).

SSF is a process for in situ enzyme production. It is advantageous due to its simplicity, low energy consumption, minimal environmental impact, and high potential for large-scale enzyme production (Mansour *et al.*, 2016; Chang & Webb, 2017). SmF, on the other hand, is used for large-scale cellulase production, offering ease of handling and better control over parameters such as pH, temperature, and substrate concentration (Sirohi *et al.*, 2019).

This study aimed to analyze fungal cellulase production through SSF and SmF using grape pomace obtained from an industrial process as a lignocellulosic substrate and two fungi, *Aspergillus niger* URM5741 and *Aspergillus tamarii* URM4634.

THEORETICAL BACKGROUND

Enzyme production has gained significant interest due to the role of enzymes in the bioconversion of lignocellulosic biomass into bioproducts and energy sources (Devi *et al.*, 2022). Cellulose, a natural polysaccharide, is the main component of plant cell walls. It is a linear, unbranched polymer of cellobiose subunits, which are composed of glucose subunits. These glucose subunits can be obtained by depolymerizing plant cellulose into smaller basic blocks with the help of cellulases, a group of enzymes that includes β-glucosidases, cellobiohydrolases, and endoglucanases (Singhania *et al*., 2010).

The conversion of lignocellulosic biomass is typically a three-step process due to its low biodegradability: pretreatment, enzymatic hydrolysis, and fermentation. The first step, pretreatment, breaks the lignin-hemicellulose-cellulose complex of the biomass, making it more susceptible to enzymatic hydrolysis. During hydrolysis, cellulase and xylanase enzymes act on the cellulosic and hemicellulosic components, converting them into their respective sugars (Romero-Martínez *et al.*, 2021) and making them available for fermentation.

The cost of cellulase enzymes is a major factor contributing to the operational costs of biorefineries that produce ethanol from lignocellulosic material. Local enzyme production using low-value substrates presents an attractive option, simultaneously contributing to environmental sustainability (Gordillo-Fuenzalida, 2019). As the main residue of the wine industry, grape pomace is produced in significant quantities and represents a high disposal cost. This residue has shown potential as a substrate for bioproducts such as hydrolytic enzymes (cellulases, xylanases, and pectinases) and biofuels (Botella *et al.*, 2005; Meini *et al.*, 2021; Kurt & Cekmecelioglu, 2021).

The advantages of SSF compared to traditional SmF include higher yields, easier product recovery, absence of foam formation, and smaller reactor volumes (Raimbault, 1998). However, SmF offers the advantage of agitation during the process, which plays a dual role in

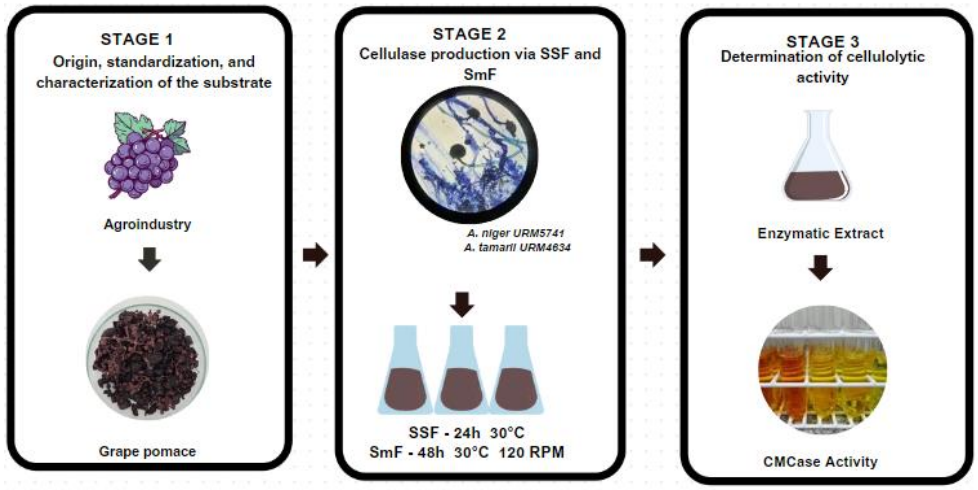
enzyme production: it promotes uniform nutrient distribution in the culture medium and provides aeration, enhancing fungal cell growth and enzyme yield (Matkar *et al.*, 2013).

Pretreatment is a critical step to break the complex structure of lignocellulosic biomass. Physical and chemical pretreatment methods are costly and can lead to secondary contamination, energy consumption, and input waste. Thus, biological pretreatment, particularly fungal pretreatment, offers advantages due to its significant impact on lignocellulosic degradation through higher lignocellulolytic enzyme production. It is also an economically viable and environmentally friendly process (Guo *et al*., 2022; Sajid *et al.*, 2022; Talwar *et al.*, 2023). Additionally, fungal pretreatment demands less energy and avoids toxic compound accumulation, leading to increased enzyme production with fewer drawbacks (Yuan *et al.*, 2014; Meini *et al*., 2021).

METHODOLOGY

The methodology developed for this study was divided into three stages: substrate standardization, enzyme production through solid-state (SSF), submerged fermentation (SmF), and cellulolytic activity assessment. Both fermentations were conducted using Cabernet Sauvignon grape pomace as the substrate and fungi from the *Aspergillus* genus, specifically *A. niger* URM5741 and *A. tamarii* URM4634, as biological treatments. The final stage involved determining cellulolytic activity by estimating endoglucanase production. Figure 1 presents the methodological sequence proposed in this study.

Figure 1. Methodological sequence proposed in the present study



Source: The authors (2024)

Stage 1: Substrate Origin, Standardization, and Characterization

The lignocellulosic substrate used for performance analysis was Cabernet Sauvignon grape pomace. It was collected from Vale das Colinas winery in Garanhuns, Pernambuco, Brazil, during the juice extraction stage following pre-fermentation. The pomace was frozen at −5 ± 1°C and transported at this temperature to the NUBIOTEC laboratory (UFRPE), where it was stored until use.

The standardization process began with moisture removal by drying the substrate in a forced-air oven (Tecnal, TE-393) at 65 ± 1°C. Weight measurements were taken every 24 hours on an analytical balance until stabilization. After drying, the substrate was ground using a blade mill (Tyler, TE-625/1) and sieved to obtain particles of Mesh 10 and Mesh 32 sizes. These were stored separately. Characterization included moisture content determination, volatile solids analysis, and pH measurement following WHO (1978) guidelines. Fiber content (cellulose, hemicellulose, and lignin) was analyzed using Senger *et al.* (2008) methodology.

Figure 2. Dry grape pomace

Source: The authors (2024)

Stage 2: Cellulase Production from the Substrate

Determination of the Water Absorption Characteristics

One gram of substrate was weighed using an analytical balance and placed in pre-weighed beakers. Incremental additions of 0.5 mL of distilled water were made using an automatic pipette. After each addition, the substrate was homogenized until free water was observed at the bottom of the beaker. The volume of water added until the appearance of free water represented the water absorption capacity of the substrate, which also corresponds to its 100% moisture level. This parameter is essential for controlling moisture during fermentation

processes.

The moisture level for both SSF and SmF was calculated using a simple proportionality rule, with the 100% moisture value for one gram of substrate set as a reference for any other desired moisture levels.

Microorganism

Two fungal strains from the *Aspergillus* genus were selected for comparing cellulolytic activity indices: *Aspergillus niger* URM 5741 and *Aspergillus tamarii* URM 4634. These microorganisms were obtained from the culture collection of the Federal University of Pernambuco (UFPE). Malt Extract Agar was used as the culture medium to maintain the fungal strains, with replication performed every thirty days. Sporulation of the cultures was carried out using Czapek medium (composed of 2.0 g/L $NaNO_3$, 0.5 g/L $MgSO_4$, 0.5 g/L KCl, 0.01 g/L $FeSO_4$, 1.0 g/L K_2HPO_4, 30 g/L sucrose, and 30 g/L agar) (Klich, 2002).

The culture media were sterilized in an autoclave at 121°C and 1 atm pressure for 20 minutes. Fungal growth occurred in an incubator (Tecnal, model TE-371) at 30°C for seven days. During microorganism selection, *A. niger* and *A. tamarii* species were inoculated into Erlenmeyer flasks containing Czapek medium and incubated at 30°C for seven days as previously described.

Inoculum standardization

Spore suspensions were prepared by adding 15 mL of sterile saline solution (0.9% NaCl w/v) with 0.01% Tween 80 to sporulated fungal cultures in Erlenmeyer flasks. The suspensions were transferred to labeled test tubes identifying the fungi present. After spore collection, spore counts were performed microscopically using a Neubauer chamber.

Spores from five grid quadrants of the chamber were counted, and the values were summed and multiplied by five. This result was then multiplied by the chamber dilution factor (10^4). The final concentration of spores (10^7 spores/mL) was achieved.

Solid-State Fermentation (SSF)

For SSF cultivation, the moisture level of the substrate was adjusted to 70% using a nutrient solution composed of 2 g/L KH_2PO_4, 1.4 g/L $(NH_4)_2SO_4$, 0.3 g/L $CaCl_2$, 0.2 g/L $MgSO_4 \cdot 7H_2O$, 5 g/L yeast extract, 0.3 g/L urea, 0.1% Tween 80, and 0.1% saline solution (containing 5 mg/L $FeSO_4 \cdot 7H_2O$, 1.4 mg/L $ZnSO_4 \cdot 7H_2O$, and 2 mg/L $CaCl_2$), enriched with 30 g/L glucose.

Three grams of substrate were placed in 250 mL Erlenmeyer flasks, and spore suspensions were added to achieve a concentration of 10^7 spores per gram of dry substrate. The cultivation process was maintained under static conditions at 30°C for 72 hours.

Submerged Fermentation (SmF)

For SmF, a nutrient solution with the same composition as described for SSF was added, using 40 mL of nutrient solution per gram of solid-fermented substrate. The culture was incubated in an orbital shaker (Tecnal, model TE-424) at 30°C with continuous agitation at 120 rpm for 72 hours.

Stage 3: Determination of Cellulolytic Activity

Cellulolytic activity was assessed based on the endoglucanase (CMCase) activity, following the method described by Ghose (1987). For this evaluation, 1% sodium carboxymethylcellulose in 0.05 M citrate buffer (pH 4.8) was used as the substrate. A volume of 0.5 mL of the substrate solution was placed in test tubes, followed by the addition of 0.5 mL of the enzymatic extract. The enzymatic reaction was carried out at 50°C for 30 minutes.

After the reaction, the amount of glucose released was measured using the dinitrosalicylic acid (DNSA) method. For both enzyme activity and substrate activity measurements, colorimetric reaction controls were included (enzyme blank and reaction blank). Absorbance readings were converted into glucose concentrations based on a previously established standard curve.

One international unit (IU) was defined as the amount of enzyme required to release 1 µmol of glucose per minute. Quantification of activity was performed using a spectrophotometer at an absorbance of 540 nm. The reducing sugar in the crude extracts was subtracted from the samples for the calculation of enzymatic activity (Equation 2). The glucose released was identified through the reaction with DNSA, following the parameters established by Miller (1959). For calculating endoglucanase (CMCase) activity, Equation 1 was used:

$$A(IU/mL) = \left(\frac{\left(\frac{RS}{180}\right)*1000}{t}\right) \div v \quad \text{(Eq. 1)}$$

Where:

A: CMCase or FPase activity (IU/mL)

RS: Reducing sugar concentration (IU/mL)

t: Reaction time (minutes)

v: Volume of enzymatic extract used (mL)

$$\text{RS (IU/mL)} = RSR - \left(\frac{RSCE}{2}\right) \quad \text{(Eq. 2)}$$

Where:

RSR: Reducing sugar concentration of the reaction (IU/mL)

RSCE: Reducing sugar crude extract (IU/mL)

RESULTS AND DISCUSSION

Characterization and Composition of the Substrate

The substrate used for SSF and SmF was characterized, as its physicochemical properties may influence the efficiency of both fermentation methods and the enzymatic production results (Table 1).

Table 1. Physicochemical characterization of grape pomace

Parameter		Grape pomace
pH		3.61±0.00
Moisture (%)		46.59±0.77
Total Volatile Solids (%)		88.00±0.47
Proteins (%)		5.0±0.00
Lipids (%)		18.9±0.00
Carbohydrates (non-lignocellulosic) (%)		20.5±0.00
Fibers (%)	Cellulose	10.71±2.65
	Hemicellulose	8.07±2.13
	Lignin	36.79±9.62

Source: The authors (2024)

The grape pomace exhibited an acidic pH (average 3.61 ± 0.00), which is characteristic of fruit residues. Similar pH values were reported by Achmon *et al.* (2019) and Castro *et al.* (2024) in their studies on grape pomace characterization (4.25 and 3.54, respectively).

Regarding moisture content, the grape pomace showed a relatively high average value (46.59% ± 0.77). Negro *et al.* (2021) reported a lower moisture content (average 9.2% ± 0.5) in their studies on grape pomace. In contrast, Castro *et al.* (2024) found a higher moisture value (66.20%). The discrepancies among these results may be attributed to various factors, such as the geographical location of grape cultivation (San Pietro Vernotico, Italy) and the processes the substrate underwent before analysis. In addition, the moisture content of grape pomace may be influenced by the grape variety and degree of ripeness (Spine and Oroian, 2021).

Concerning volatile solids, the results indicated that the grape pomace had a high value (88% ± 0.47), which is similar to the value obtained by Almeida *et al.* (2022) (90.1%). However, lower volatile solids values (71.19% and 70.30%) have been reported by other authors for grape pomace (Farru *et al.*, 2022; Ruiz *et al*., 2023).

It is essential to emphasize that volatile solids are a crucial parameter as they indirectly measure the organic matter available for microbial degradation in biotechnological processes. The value obtained in this study falls within the range reported in the literature, which varies depending on grape variety, cultivation conditions, and extraction processes.

Grape pomace presented 5.0 ± 0.00% protein, 18.9 ± 0.00% lipids, and 20.5 ± 0.00% non-lignocellulosic carbohydrates. The protein and carbohydrate contents observed were lower than those reported by Machado et al. (2024) for grape pomace, which showed 3.38% and 35.47%, respectively. In contrast, the lipid content (18.9 ± 0.00%) was higher than the value found in that study, suggesting that the differences may be associated with sampling conditions or the processing methods used.

The fiber composition of the grape pomace was found to be 36.79% ± 9.62 lignin, 10.71% ± 2.65 cellulose, and 8.07% ± 2.13 hemicellulose, classifying it as a recalcitrant lignocellulosic residue that is challenging to degrade. These findings were lower than those reported by Almeida *et al.* (2022), who observed lignin (50.6%), cellulose (19.0%), and hemicellulose (9.8%) levels in grape pomace.

Variations in the fiber composition of grape pomace may be influenced by factors such as the type of grape used as a substrate, field management practices, local environmental conditions, and the industrial extraction processes applied to the substrate.

Production of Cellulolytic Enzymes

The enzymatic activity results for fungal cellulase production through SSF and SmF, using Cabernet Sauvignon grape pomace as the substrate and two different fungi of the *Aspergillus* genus, are presented in Table 2.

Table 2. Cellulase production using grape pomace as a substrate

Microorganism	Fermentation	CMCase (IU/mL)
Aspergillus niger	SSF	0.039
	SmF	0.194
Aspergillus tamarii	SSF	0.055
	SmF	0.061

Subtitle: SSF: Solid-State Fermentation; SmF: Submerged Fermentation
Source: The authors (2024)

It was observed that *A. niger* exhibited more efficient cellulase production under SmF conditions, while *A. tamarii* showed higher cellulase production under SSF conditions using grape pomace as the substrate. This suggests that the two fungal species respond differently to cultivation conditions and the substrate used.

The highest cellulolytic activity was obtained using grape pomace as a substrate, with 0.194 IU/mL of CMCase activity by *Aspergillus niger* in SmF and 0.061 IU/mL of CMCase activity by *A. tamarii* in SSF. Similar results were reported by Kurt (2023), who achieved a maximum CMCase activity of 0.178 IU/mL in SmF using grape pomace as a substrate over seven days. However, that study was conducted with bacterial cells rather than fungal cells.

In contrast, higher cellulase production (2.0 IU/mL) by *Aspergillus* species was reported by Sosa-Martínez *et al.* (2022) using grape pomace as a substrate in SSF over four days at 30°C. Meini, Ricardi, and Romanini (2020) reported a lower cellulase production (0.00038 IU/mL) by *A. niger* using grape pomace in SmF under orbital agitation at 120 rpm and 30°C.

Comparison between the fermentation methods revealed that, while SmF provides better conditions for controlling operational parameters and nutrient distribution, SSF can be a more sustainable and cost-effective alternative, particularly for industrial-scale applications. Thus, the choice of fermentation method should consider both enzymatic production efficiency and operational, economic, and environmental factors.

CONCLUSION

This study demonstrated the promising potential of grape pomace as a lignocellulosic substrate for producing cellulolytic enzymes using two distinct fermentation processes: solid-state fermentation (SSF) and submerged fermentation (SmF). Comparative analysis showed that *Aspergillus niger*, when cultivated in SmF, exhibited the highest cellulolytic activity (CMCase of 0.194 IU/mL).

Substrate characterization revealed important physicochemical variables that directly influence the efficiency of fermentation processes. The high moisture content and significant presence of volatile solids in grape pomace, along with its cellulose and hemicellulose composition, highlight the feasibility of using this agro-industrial residue as a carbon source and solid support for microbial growth. Additionally, the high lignin concentration identified may act as a limiting factor in total biomass degradation, suggesting the need for specific optimizations in biological pretreatment to enhance enzymatic hydrolysis efficiency.

It was concluded that using lignocellulosic residues such as grape pomace in fermentation processes for cellulase production is a promising and more sustainable approach.

This emphasizes the need to continue developing more efficient and environmentally viable biotechnological processes, promoting the valorization of agro-industrial residues, adding value to production cycles, and reducing dependence on non-renewable resources.

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