PRE-TREATMENTS AND BIOTECHNOLOGICAL APPLICATIONS OF COCOA WASTE: STRATEGIES FOR OBTAINING VALUE-ADDED COMPOUNDS

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ABSTRACT

Addressing sustainability in the food industry requires innovative solutions to effectively utilize agricultural waste, reducing remnants and providing economic benefits. Cocoa waste, a byproduct of chocolate production, is generated on a large scale and holds promise for enzyme production and high-value bioproducts, including biofuels. This review examines the characteristics of cocoa waste, identifies relevant enzymes, and discusses how pre-treatment methods impact the bioprocessing of cocoa. The review aims to demonstrate the importance of integrated approaches to maximize cocoa waste utilization, addressing gaps in waste analysis standardization. Overall, this study underscores the biotechnological potential of cocoa waste and the need for cost-effective and sustainable utilization pathways to minimize waste and promote circular bioeconomy.

Keywords: Cocoa Waste. Enzymes. Agro Waste. Biorefinery. Circular bioeconomy.

1 INTRODUCTION

The food and agricultural industries have been attracting increasing investments, driving their growth[1] and industrialization. Consequently, the generation of waste has also significantly increased. However, studies indicate that this waste can be transformed into valuable raw materials for other areas due to their high nutritional value and ability to promote microbial growth. Many agro-industrial wastes are rich in lignocellulose[1], highlighting their biotechnological potential, especially in fermentation processes such as Solid State Fermentation (SSF) for enzyme production or to create specific reaction conditions for certain microorganisms.

In this context, the selection of cocoa as a feedstock is evident, given that chocolate is one of the most popular food products globally[2]. Chocolate production involves several stages, from cocoa tree cultivation to bar molding[2], generating substantial amounts of waste. According to the 2023 statistics from the International Cocoa Organization (ICCO), Brazil is the sixth-largest cocoa producer in the world, with 220 thousand tonnes annually[3]. The states of Pará and Bahia account for 96% of national production[4], providing significant employment and economic benefits. However, only 10% of cocoa is used for chocolate production, another 10% comprises the pulp, and the remaining 80% consists of waste made up of pod husk and shell. This scenario underscores the urgent need for research to explore viable, simple, and profitable ways to utilize this biomass on a large scale.

Therefore, there are two main approaches to the utilization of cocoa waste (CW): transforming it into bioproducts or using it to create optimized conditions for obtaining high-value substances. In the first case, studies have already shown the transformation of this raw material into biofuels[5] or cosmetic components. In the second, enzymes stand out as potential key players in the future of the industry. Additionally, it is important to note that CW is not merely generic waste; it includes Cocoa Pod Husks (CPH), Cocoa Bean Shells (CBS) resulting from the roasting process[4], and Cocoa Mucilage (CM), also known as pulp.

This review aims to clarify the current state of knowledge regarding CW utilization by addressing the following questions:

Which characteristics of cocoa favor or hinder enzymatic activity?

Which enzymes associated with cocoa residues are reported in the literature, and what is their origin?

What is the influence of pre-treatment and the part of cocoa used in the bioprocess?

2 MATERIAL & METHODS

This review investigates the potential of cocoa waste (CW) and enzymes for biofuel production. A literature search was conducted using PubMed, Scopus, and Science Direct, focusing on articles published within the last decade. The search employed a combination of keywords including cocoa, CW, cocoa byproducts, enzymes, microorganisms, and biofuels.Following the initial search, identified articles were uploaded to the Rayyan® web application for preliminary screening and filtering. This process aimed to select articles demonstrably aligned with the review's objectives and of primary importance for the subsequent discussion. Subsequently, the shortlisted articles were reviewed and categorized into four groups: 1) studies exploring the use of cocoa-derived enzymes; 2) research investigating CW as a feedstock for enzyme production for various applications; 3) investigations into the application of enzymes to CW for biofuel or bioproduct generation; and 4) reference articles providing supportive information on agro-industrial waste, enzymes, or biofuels beyond CW, included as necessary to strengthen the review's foundation.

3 RESULTS & DISCUSSION

Analyzing the relationship between cocoa waste and enzymes in the literature reveals two main approaches: 1. production from residual cocoa; 2. production from other sources using cocoa to enhance medium conditions. The types of enzymes identified in reviewed papers are detailed in Table 1. Both approaches show promising results but typically require significant pretreatment or supplementation. Using raw cocoa *in natura* generally does not yield sufficient endoxylanase[6], due to the lignocellulosic nature of the material. This disparity is exemplified by studies employing Solid State Fermentation of Cocoa Bean Shell supplemented with 2% (w/w) urea to produce Xylanase, Amylase, and Feruloyl Esterase, aimed at enhancing Ferulic Acid Liberation. Results indicate lower productivity in cocoa remnants compared to agro-industrial by-products such as wheat bran (WB) and brewer's spent grain (BSG)[7], which contain less lignin and more hemicellulose. Nevertheless, efforts to obtain these substances from cocoa align with bioprocessing objectives to enrich whole-wheat breads with ferulic acid, promoting health benefits against oxidative stress-related disorders[7]. This underscores the food industry's interest in investing in cocoa waste and its biotransformation.

Table 1 Enzymes related to Cocoa Waste use.

Enzyme	Part of the cocoa used	Origin*	Industry Application*
Laccase	Waste in general	White Fungus	Biodegradation of pollutants
Lipase	CPH	CPH	Bioremediation
Xylanase	CPH, CBS	Bacterian, Fungic	Sacarification
Feruloyl esterase	CBS	Fungic, Bacterian	Ferulic Acid Production
Acetate kinase enzyme**	Beans	Acetic Bacteria	Antioxidant, Reduce
Glucanase**	Beans	Acetic Bacteria	Polyphenol
Amylase	CBS	Fungic	Ferulic Acid Production
Celluclast® (cellulase)	CPH	Comercial not defined	Pectin Extraction
Cellulase	CPH	Fungic	Methane production

*Origin and application used in the work. **The enzymes produced by Acetic Acid Bacteria vary according to the substrate and fermentation conditions; those described are typically expected from these microorganisms.

In this sense, the obtainment of value-added compounds depends on the hydrolysis of lignocellulosic waste into more assimilable carbohydrates. This involves breaking the glycosidic bonds of cellulose and hemicellulose polysaccharides into monosaccharides and oligosaccharides[8]. Pretreatment, therefore, aims to increase enzyme accessibility to the intracellular sugars in the biomass[9]. One of the key challenges is delignification, which significantly influences the success of bioconversion.

The main pretreatments for cocoa application include Hydrothermal Treatment (HTT) with and without Acid, Alkaline Treatment (AT), Boiling Pretreatment (BP), Soaking in Aqueous Ammonia (SAA), and White Fungal Pretreatment (WFT). HT is a widely validated option that relies on hemicellulose solubilization through autohydrolysis facilitated by water, requiring high temperature and pressure, which creates a corrosive environment and is therefore not a cheap alternative. The other methods tend to be less expensive but present their own biotechnological challenges. For instance, SAA operates at natural temperature and atmospheric pressure, but costs increase without an ammonia recovery route. Considering the need for lower investment, these methods are crucial for the viability of bioconversion in regions with abundant biomass and limited resources.

It is interesting to note that most studies discussing the use of cocoa waste (CW) for enzyme production do not present an integrative process flowchart to fully utilize the biomass. In other words, after obtaining the "high value-added component," the by-products from these processes are not fully reused[10], and consequently, do not have a profitable application. Given the biotechnological potential of this raw material, it is crucial to understand its current applications to establish a process that minimizes waste and, ideally, is cyclic.

Knowing the pretreatment conditions and their importance, it is viable to discuss the state of technology of the mentioned enzymes and the possibilities enhanced by their studies. Regarding pectin extraction, it was demonstrated that cocoa pod husks can be satisfactorily utilized with Celluclast®, achieving commercial pectin parameters. Although the concentration of galacturonic acid could be higher, it is a positive result; however, there are no defined next steps. Another approach uses HTT with acid on CPH to recover the pectin from the liquid fraction and uses the residual for bioethanol production with *Candida tropicalis*, while the solid fraction is also used for the same biofuel but with lower yield. This is due to the deficiency in delignification with the enzymatic hydrolysis, which results in decreased conversion as the CPH solid loading increases. This is caused by limited mass transfer and enzymatic inhibition due to the proportionally higher concentration of lignin[10]. The type of enzyme used was not specified, only the supplier.

Despite HTT being deemed a great treatment solution, with acid as an optimization, achieving notable results for pectin extraction and applying enzymes effectively, it achieves a 60% glucan conversion but is not ideal for large-scale bioethanol production from the solid fraction. Therefore, two avenues of study are proposed: 1. Test the Alkaline and White Fungal treatments in the suggested process chain and evaluate the yield of pectin recovery and the enzymatic activity during hydrolysis. 2. Identify which enzymes were used and assess the feasibility of adding different enzymes to the hydrolysis process, applying white fungus only to the solid biomass after HTT.

Furthermore, cocoa waste (CW) has also been used to improve methane production during the anaerobic digestion of West African grass[11]. In this project, it was evident that the addition of Cocoa Pod ash-extract to the system increased methane production by 13% on its own, by 28% with cellulase, and by 12% with the Trace Elements (TE) solution. The TE solution contained nickel, cobalt, and molybdenum, which are chemical elements required in small quantities for the growth and activity of microorganisms[11]. Without cocoa pod (CP) ash, methane production was actually inhibited by 5%, whereas commercial fungal cellulase alone increased methane production by 18%.

The positive performance of the CPH ash-extract can be attributed to its natural trace nutrients and alkalinity, which contribute to pH stability[11]. These factors create an environment conducive to cellulose degradation by cellulase, enhancing methane production. Additionally, the broad range of bioavailable trace elements in CPH ash-extract made the environment less toxic compared to the conventional TE solution alone. In this context, combining cocoa with other lignocellulosic biomass with different capabilities offers special contributions. For instance, xylanases can be obtained from Solid State Fermentation of lignocellulosic biomass. CBS was fermented with sugarcane bagasse, rice husk, and the fungus *Aspergillus oryzae*, yielding a multi-enzymatic extract primarily composed of xylanase. This extract was then applied to the saccharification of residues, including cocoa pod husk, resulting in effective conversion.

Regarding biofuels, in Thomsen's research on low-cost bioethanol production[12], a difference can be observed in the unit of enzymatic convertibility (expressed as glucan conversion) between what is considered cocoa pods and cocoa husks. Although the graphical values seem close, the conclusion is that CW is not ideal for cellulosic ethanol production under the studied conditions, which included HTT, SAA, BP, and WFT (with *C. subvermispora*). The key point is that WFT and HTT have the best numbers, with WFT being considered profitable for laccase production using a different type of white fungi, which are the only organisms capable of efficiently degrading lignin[13]—a crucial factor for viable bioethanol production.

Hydrothermal Treatment (HTT) is expensive but yielded good results with acid addition after pectin extraction. Therefore, it is worth testing the use of P. sanguineus, along with other financially favorable fungi from this family, to reassess bioethanol production from CBS and CPH. Furthermore, considering the aim of low cost and required technology, treating the waste, splitting the solid and liquid fractions as previously mentioned, and applying another inexpensive treatment option to the solid fraction before conversion to bioproducts should be tested as well. Moreover, the common nomenclature of cocoa pods and cocoa husks, which are often considered the same in many studies, does not provide a clear understanding of the factors justifying the differences in their conversion results. Therefore, the ideal conditions to evaluate the profitability and conversion of each type of cocoa waste involve conducting research with biomass from the same origin, treated, characterized, and standardized in terms of nomenclature.

Regarding lipases, they are considered one of the most important enzymes due to their ability to combine a wide range of substrates with high regio- and/or enantio-specificity and/or selectivity[14], catalyzing numerous reactions. An interesting approach with cocoa is the use of Cross-Linked Enzyme Aggregate (CLEA) to obtain CLEA-lipase, an enzymatic complex where lipases are immobilized, forming structures more stable to pH and temperature variations than free enzymes due to aggregation, which confers more stiffness. Additionally, CLEA-lipase from CPH was demonstrably reusable in cycles, retaining 60% of initial activity even after six batches of reuse in a hydrolysis process of 30 minutes at 37ºC. On the other hand, the catalytic efficiency of free enzymes was higher, while Km values are higher for CLEA, demonstrating its affinity with the substrate. There was no addition of fungus or bacteria in this context.

After all, it is valid to discuss the importance of the viability to increase laccase production with cocoa waste. The cocoa residue provides conditions for *P. sanguineus* to generate laccase to an increasing extent over 7 days, while the fungus by itself had zero production. This effect is justified by the high concentration of copper in this raw material and also by the fiber and lignocellulosic contents. This crude laccase enzyme extract presented kojic acid among the natural compounds and was used for Ethinylestradiol degradation. The bioremediation results were functional, offering an effective and economic alternative not yet tested on a large scale. It does not specify if it is CPH, CBS, or another remnant. In conclusion, while exploring cocoa waste for the production of value-added compounds presents promising opportunities, effectively integrating these efforts into a cohesive biomass utilization framework remains a critical challenge.

4 CONCLUSION

Considering the above, this review concludes that cocoa waste offers a range of biotechnological perspectives with social and economic benefits. The research highlights differences between types of cocoa waste, but often lacks specification or standardization regarding their chemical composition differences. Fungal enzymes, in particular, are highly beneficial for cocoa shells and cocoa pod husks, making them a significant investment due to their content of copper and lignocellulose. It is pertinent to test integrated systems using the pretreatment and enzyme production methods mentioned to establish a viable, cyclic, and cost-effective chain without residues. This approach could enable the development of a biorefinery that effectively enhances the production of other biofuels and products.

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ACKNOWLEDGEMENTS

This review is affiliated with the undergraduate research program of the Science and Food Network Development Project (FAPESB - Bahia Foundation for Research Support), the Federal University of Bahia and the Department of Industrial Microbiology at SENAI CIMATEC.