Review

**Agro-Industrial Avocado (Persea americana) Waste Biorefinery**

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**Abstract:**

Significant problems have arisen in the last years, such as climate change, global warming, and hunger. These complications are correlated with the depletion and exploitation of natural resources and environmental contamination. Due to overcrowding, the list of challenges for the next few years is growing.

A comprehensive approach was made to the agro-industrial production of Avocado (Persea americana) and the management of all its biomass waste. So, bioprocesses and biorefinery can be used to produce high added-value products. A large number of residues are composed of lignin and cellulose. They have many potentials to be exploited sustainably for chemical and biological conversion; physical, chemical, and natural treatments improve the following operations. There are some applications to many fields such as pharmaceutical, medical, material engineering, and environmental remediation. Possible pathways are mentioned to take advantage of Avocado as biofuels, drugs, bioplastics, and even in the environmental part and emerging technologies such as nanotechnology using bioprocesses and biotech. In conclusion, Avocado and its waste could be transformed into high value-
added products in industries above to mitigate global warming and save non-renewable energy.

**Keywords:** biorefinery; bioprocess; agro-industry; high-value products; Avocado

1. Introduction

1.1 Biorefinery and Bioprocesses Approach

For years, the world's population was focused on trade relations and economic development with no concern or planning about what this represented to the environment [1]. Now, significant problems have arisen because of this: Climate change, global warming, and hunger. These complications are correlated with the depletion and exploitation of natural resources and environmental contamination. Due to overcrowding, the list of challenges for the next few years is growing. Those challenges generate instability in current economic models and international relations, so society's main challenge is to find sustainable development by implementing economic changes, such as bio-based industries, to mitigate contamination and global warming [2].

When addressing all possible edges to propose a model or solution to these problems, it was pointed out that one of the strategies that impact all fields is the recovery of agro-industrial waste. This assessment includes the implementation of biorefineries [3]. A biorefinery involves converting biomass using various technologies to obtain an extensive list of high value-added products from a bio-based raw material [4].

The biorefinery concept integrates a complete approach of all routes to convert biomass into new products. Biorefinery complement or replace current oil refineries and improving people’s lives. Many chemicals, fuels, and solvents are produced and refined petroleum products, as shown in Fig. 1 [2,5]. Many biotechnological technologies have been developed to optimize energy consumption and reduce the amount of waste. However, designing and proposing all these new bioprocesses has been too hard to move towards these innovative sustainable industries that integrate concepts such as green chemistry, cleaner production, and zero waste [5]. In which we can obtain non-toxic primary products and recyclable or
easily degradable materials that can be compostable. Besides, it is intended to function as an industrial ecosystem. The waste from one industry can be the raw material, resulting in obtaining minimal waste outputs from the production system [6].

Figure 1. Comparison between traditional oil refineries and biorefineries [7]

In this review, a comprehensive approach was made to the agro-industrial production of Avocado (*Persea americana*). Likewise, a study was carried out to manage agro-industrial waste to obtain products with high-added-value.

1.2 Industrial Production of Avocado

The Avocado or pita (*Persea americana*) is a tree native to Mesoamerica. It is origin in the central region of Mexico and some parts of the north of Guatemala. This species' common name comes from Nahuatl (Ahuacatl), which means tree testicles [8,9]. Avocados have a significant amount of monounsaturated fatty acid (oleic acid), phytosterols, and phenolic
antioxidants [9,10]. According to its taxonomic classification, the Avocado belongs to the Lauraceae family [11]. This is the most critical and unique edible fruit from this family, which has a high commercial value [12,13].

The largest production areas are concentrated in Central and South America but are currently distributed worldwide. Avocado consumption is increasing everywhere [14,15] and has caused many countries to invest heavily in the cultivation of *Persea americana* (Fig. 2) [8]. According to the average production between 1994 and 2019, the top 10 avocado producers were Mexico, Dominican Republic, Indonesia, Colombia, Peru, United States, Brazil, Chile, Kenya, and Malawi in that respective order [16]. Regarding FAO data, the relative production levels of these ten countries average in 1994 and 2019. Mexico is the largest producer, and it is there is a production growth over time. In 2019, avocado production was 7.0 million tons (Fig. 3) [8,16].

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Figure 2. Quantities of avocado production per country in 2019 (created with Faostats) [8,17]
According to Zafar and Sedhu (2018) and FAO data from 2019 [9,17], Costa Rica imports the most Avocado with an average value of 13 thousand tons. Furthermore, according to data from the Ministry of Agriculture and Livestock (MAG), in 2019, the National Plan to Strengthen the Avocado Sector was published [18]. Affording to this public report, currently, the national consumption of Avocado is around 12 thousand tons per year, a number that has been reducing in recent years after reaching its highest point between 2012-2014, with consumption close to 15 thousand tons per year [19].

1.3 Avocado Functional and Nutritional Properties

Avocado has a long list of uses and applications in the food industry. Even though this fruit is mainly consumed in a fresh state, the products derived from the avocados have been changing and improving the process industry's benefit due to its increased production and
seasonality. Thus, the most outstanding demand products are guacamole, followed by avocado drinks and ice cream, all produced by the pulp only [20,21].

This fruit is made up of exocarp, endocarp, and mesocarp (Fig. 4). The last one is the most abundant, representing between 52.9 and 81.3% of the fruit mass used in great demand because of its properties and high nutritional value [22,23]. It counts with vitamins such as A, B, D, and E, with different applications, for example, pharmaceutical, nutritional, and cosmetic industries, because of its variety of oils. On the other hand, the Avocado is highly rich in proteins, fibers, unsaturated fatty acids, and carotenoids, making it an outstanding tropical fruit with multiple human health benefits [23,24].

Regarding the avocado peels and seeds, as they are not often used in the food industry, they become an excessive amount of waste containing essential oils and bioactive compounds, fibers, and carbon source, which represents 21-30% of the avocado mass. According to commerce, they are useless with high potential as raw material for diverse industrial fields because of their properties represented in figure 4 [24,25].

Figure 4. Avocado’s parts and properties [10].
2. Applications of Agro-Industrial Residues

2.1 Avocado Waste and Pretreatments

Generally, agro-industrial waste is principally composed of lignocellulose biomass. Lignocellulose waste has been increasing attention due to its properties: mechanical and thermal, renewability, wide availability, non-toxicity, low cost, and biodegradability for new materials engineering [26]. Avocado waste is not the exception. The waste formed by seeds and peels is full of biopolymers and raw material.

Many residues are composed of lignin, and cellulose gives them many potentials to be exploited sustainably for chemical and biological conversion [26,27]. However, to be used, these must go through pretreatments that make them easier to handle and improve the processes' efficiency. For example, physical, chemical, and biological treatments aim to improve the subsequent operations.

According to the final product, some pretreatments can be highlighted (Fig. 5). Physical treatments involve mechanical treatments such as grinding, extrusion, and maceration [28]. Others are implemented by radiation, such as gamma rays, electron beams, or microwaves [29]. Thermal processes such as pyrolysis, roasting, or steam explosions are considered physical [28,30]. Chemicals focus on the fractionation of biomass by hydrolysis mediated by ionic or organic solvents, alkaline or acidic. Finally, biologicals are based on exoenzyme-mediated decomposition or hydrolysis and saccharification; it must be a solid or liquid substrate fermentation by whole-cell systems or just enzymatic digestion [28,31,32].
2.2 Potential Biorefinery Use of Different Avocado Residues

Before designing a pipeline to seek a final product, it is essential to know how the raw material is compounded; an average avocado fruit weighs approximately 150 to 400 g. The seed and peel weigh about 16% and 11%, respectively. However, these proportions vary among cultivars [1]. The annual amount of biomass waste is estimated at 50 billion tons, and all this potential raw material originates from the food industry and losses in the harvest [25].

Tables 1 and 2 show the dry weight composition of avocado seeds and peels on average because it is crucial to mention that the composition varies by area and growing conditions [25]. Carbohydrates can be used in the energy and fuel industry (e.g., bioethanol) [20]. Also, many chemicals and biopolymers based on starch and cellulose can be obtained. [30,31]. Although seed and peel are being prioritized, pulp residues are also sources of monounsaturated fatty acids and metabolites such as oleic or palmitic acid. Also, they have tocopherols, tocotrienols, phytosterols, carotenoids, and polyphenols with pharmacological potential antibiotic activity in human beings [22,35].
Table 1. Composition analysis of avocado seed, peel, and exhausted pulp dry matter basis [36]

| Parameter       | Value (wt%) |
|-----------------|-------------|
|                 | Seed        | Peel        | Pulp |
| Carbohydrates   | 43-85       | 44-84       | 32   |
| Lipids          | 2-4         | 2-6         | 55   |
| Proteins        | 3-9         | 3-8         | 7.5  |
| Minerals        | 2-4         | 2-6         | 6    |

Table 2. Chemical characterization for peel and seed of Avocado [3].

| Parameter      | Value (wt%) |
|----------------|-------------|
|                | Seed        | Peel |
| Moisture       | 6-8         | 7    |
| Extractives    | 34-35       | 33-37 |
| Cellulose      | 26-28       | 6-7  |
| Hemicellulose  | 24-26       | 46-50 |
| Lignin         | 4           | 2    |

According to table 2, the seed and peel's biochemical composition allows the design of bio-based polymers that have emerged with an advantage over the conventional materials due to their biodegradability and renewable feedstock.

3. High-Value Products Obtained from Avocado Residues

The bio-based polymers approach takes advantage of polysaccharides in the avocado peel. Chemical or microbiological treatments can transform the seed into biomaterials such as polylactic acid, nanocellulose fibers, and poly-hydroxybutyrates (PHBs) [26]. Furthermore,
nanotechnology is increasing and gaining popularity because it plays an essential role in many fields such as pharmaceutical, medical, material engineering, and environmental remediation [37].

The importance of a well-structured biorefinery relies on making the most out of the product residues, in this case, Avocado's peel and stone. Numerous high-value products can be obtained from wastes. For example, according to Colombo & Papetti (2019), the starch waste of the Avocado, which represents about 21% of the stone, can be utilized to produce biopolymers, with the purpose of food packaging or even drug delivery [24]. Furthermore, also biofuels and important metabolites can be achieved and created.

3.1 Biofuels and Bioenergy

It is well known the importance of fossil fuels worldwide nowadays, being the most common source of energy commercialized. But it is also known the limits when it comes to its production. Because of the current situation, which can also be depleted, these fuels' prices are continuously increasing [20].

Considering the significant amount of waste obtained by the avocado industry, specifically the stone, the production of bioenergy and biofuels by this product is highly regarded because of its benefits and distinguished potential, being a possible alternative to fossil fuels soon, as it is shown in figure 6 [38].

Until the day, some studies declare possible the obtention of biodiesel by avocado stone through transesterification. It simplifies the process due to the low content of free fatty acids and the presence of triglycerides [8,38,39]. Colombo & Papetti (2019), in addition to starch, determined that the potential use as a biofuel is high, using pyrolysis and torrefaction, in the case of liquid fuel, and applying a thermal process to reduce a part of the volatile liquid, in case of solid fuel as the product of interest [24].
Figure 6. Flow chart by-products of the avocado fruit [38].
In the bioprocesses approach, biomass conversion into biofuels and biochemicals represents a promising and valuable way to mitigate global warming and diversify energy sources [5,40]. Because of that, sugars from the avocado seed and peel can be used to produce bioethanol through fermentation. It can also be used to make other analytes with industrial importance as hydrocarbons like butanediol. However, there are some challenges. The lignocellulosic raw material conversion to ethanol is not optimal yet (Fig 7) [41].

The latest efforts and researches are working intending to solve this lack of technology. In this sense, Gu and the team brought to the global knowledge the use of supercritical CO₂ (SC-CO₂) explosion pretreatment as a green solvent to treat biomass before enzyme hydrolysis [42]. This solvent is one of the most used because it is natural, non-toxic, non-flammable in its operating conditions [43]. Furthermore, conventional enzyme processes require large amounts of water and energy. On the other hand, SC-CO₂ is designed and optimized to reduce energy use and add value to agro-industrial products as bioethanol [42].

Figure 7. Process flow diagram for cellulosic ethanol production, from the beginning (biomass) to the end (fuel) (Adapted from Karagoz, 2019) [44].
3.2 Drugs and Bioactive Compounds

The avocado fruit has been studied in any branch of science to be taken to the industry. Because of that, it is well known that Avocado contains many properties that make this fruit a potential solution and a possible economical alternative above the present options the industry has today. In this case, characteristics and applications, such as nutritional and medical, are highlighted.

Let's start by reviewing the principal by-products in the pharmaceutical field. The Avocado is remarkable because of its antioxidant activity. According to García-Vargas et al. (2020), the Avocado's peel is an excellent antioxidant source. It was confirmed through different preliminary methods; for example, in this case, their presence was determined by aqueous and ethanolic extracts, getting a positive result, indicating a significant activity, and therefore, proving the avocado peel as a trustworthy source of natural antioxidants [8]. Furthermore, the caffeoylquinic acid, a high-interest product of investigation today, is present in the avocado fruit. It is a clear example of this because of its applications in different industry branches. It is pharmacological due to its activity as an anti-inflammatory, antiviral, anti-cancer, even antidiabetic [45,46]. Another study proved that significant antioxidant activity was present in the seed rather than the pulp [47].

The anti-inflammatory and antimicrobial activities of avocado waste are highly studied today. An important example of this corresponds to the investigation belonging to Athaydes et al. (2019). It exposes the possible use of this product to prevent gastric ulcer disease in mice [48]. In this study, its efficacy relies on lowering mediators' production such as IL-6 and PEG₂, known as pro-inflammatory factors [48,49]. There is a limited amount of information and studies about this type of activity; nevertheless, it is currently a topic of interest because of the evidence that is now becoming available. Another study belonging to Dabas et al. (2019) confirms this anti-inflammatory activity in the Avocado by an essay carried out in vitro. They used an extract of the avocado seed on LPS-induced inflammatory responses of murine macrophages; the report results confirm the previous study's conclusions. The section reduces the production of the inflammatory factors [50] significantly.
Finally, there is a big problem that preoccupies the scientist today, according to the current studies about the fact that resistance of bacteria to all the antibiotics will be the first cause of death in the year 2050 is becoming a real threat to the human health and future treatments [51]. Thus, it is imperative the investigate a different natural antibiotic approach. Consequently, the properties of avocado waste and its antimicrobial activity are highlighted as a possible solution soon. According to Amado et al. (2019), ethanolic extracts of the peer, stone, and pulp, utilizing gram-positive strains such as *Staphylococcus aureus* and *Bacillus cereus* and gram-negative including *Escherichia coli* and *Salmonella typhi*. The study consisted in the determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The results determined that the peel showed a better response to the essay than the pulp. It provides a bactericidal and bacteriostatic effect over the gram-positive—this result demonstrated to be more sensitive gram-negative bacteria to these extracts [52].

On the other hand, Villarreal-Lara et al. (2019) carried out a study that had the objective of analyzing the inhibitory activity of the Avocado's acetogenins used against *Listeria monocytogenes*. The test was made by the disc diffusion method. Then the MIC and MBC were also carried out in the investigation. The results showed a significant inhibition activity against gram-positive and gram-negative. The test with *L. monocytogenes* was also successful, developing a decrease in the bacteria's initial growth [53].

### 3.3 Biopolymers

Biotechnological innovations have developed high value-added processes and products such as biomaterials. Those have a significant impact on regenerative medicine, and the design of medical devices is constantly being studied and improved with nanotechnological tools to enhance human life. New materials or nanotechnological innovations are sought to help become more biostable, biocompatible, and biodegradable through all these efforts. It is necessary convergence between bioactivity and good mechanical properties for the generation of new biomaterials [54].
3.3.1 Polylactic Acid

Many of the materials used for the design of medical devices are widely used plastics, such as polyethylene (PE), polypropylene (PP), that can be modified to improve their properties. The medical devices industry demands the designing and engineering new material made from biomass as a raw material. It seeks to enhance the impact to the environment, biocompatibility, the processibility, and energy savings, as does the production of PLA from lactic acid that solid or liquid phase bioprocesses can generate, can be made with filamentous fungi or gram-positive bacteria such as *Lactobacillus delbruekii* after an excellent raw material pretreatment from avocado waste [41]. The Coban & Demirci (2016) study describes how fermentation with fungi may be most effective, in that case with *Rhizopus oryzae*, due to high concentrations of starch in the remaining biomass. Once the lactic acid has been produced and extracted, PLA polymerization is carried out following the reaction (fig 8) as described by Rasal et al. [55]

![Reaction schemes to produce PLA](image)

Figure 8. Reaction schemes to produce PLA [55]
3.3.2 Polyhydroxybutyrate (PHB) Production

Also, the polyhydroxy butyrate (PHB) production can be considered. PHB is a biodegradable and biocompatible thermoplastic. It belongs to polyhydroxyalkanoate's family (PHAs), demonstrating an enormous potential to replace petroleum-based plastics [57,58]. The synthesis develops their production as an intracellular storage material, also known as inclusion bodies, of a wide variety of organisms such as bacteria, which, according to the investigation of Getachew & Woldesenbet (2016), the genus *Bacillus* sp. has shown to be the better producer of PHB from biomass waste as a raw material. They proved the bacteria with several carbon sources such as cane bagasse, corn cob, teff straw (*Eragrostis tef*), and banana peel. Thus, it is possible to accumulate this plastic from avocado waste material [59].

Also, it is used in nanocomposites PHB associated with other plastics as PLA [60]. It improves its mechanical properties. It makes them stronger and increasing their value; for example, according to Kai et al. (2019), these plastics' combination showed a highly improved tensile strength and elongation [61]. This biopolymer can be positively used for cell growth, tissue scaffolding for nerve and bone regeneration, controlled-release drug delivery, surgical sutures, cartilage and cardiovascular support, thermo-gels, wound dressing [57,62].

3.3.3 Starch Applicability

Furthermore, registered analysis from the seed showed that it contained well over 90% starch content. Starch application in textile showed that it performed as well as a commercial starch sample [63]. The recollection and processing of waste avocado seeds can be a new employment source and provide income generation opportunities. Starch is a renewable biopolymer material that is entirely biodegradable, easy to handle, and widely available in nature. It is used in many industrial applications such as food processing, beverages, paper, chemical, binder and adhesive, fermentation, and textile industries [33,34].

Hendra et al. (2018) used avocado starch to obtain a plastic film. Avocado starch has 73.62% and amylopectin 73.55%, so the avocado seed can potentially be plastic film material. They
are manufacturing this plastic film using the standard methods. For example, heating the mixture of starch filler solution to the temperature approaching gelatinization [64], the starch bioplastics have been weak, hydrophilic, less hydrophobic, and low mechanical properties. So, it is necessary to continue researching these materials to improve their physics properties [65].

3.3.4 Nanocellulose Fibers (NCFs)
Likewise, a critical biopolymer obtained from avocado seed and peel is nanocellulose fibers (NCFs). As described, cellulose is one of the most abundant polymers (table 1 and 2) in biomass waste [66]. Thus, these are an essential source to obtain NCFs. These fibers have many properties due to their high surface area, rheological behavior, water absorption, high bending strength (~10 GPa), Young’s modulus of approximately 150 Gpa. NCFs are used in the food and medical industry because they are not cytotoxic or genotoxic. [26,67,68].

To start with the food industry, the NCFs are used as a stabilizing agent, a functional ingredient in superfoods and food packaging [67]. On the other hand, in the medical industry, they can be used as scaffolds for cellular culture, drug excipient and drug delivery, and enzyme immobilization, similarly, in the design of macroscopic materials such as catheters, skin, and bone repair materials, and antimicrobial materials [68].

4. Environment and Bioremediation
This segment is to describe alternative applications that are not precisely to replace petroleum products. However, it is mentioned as mitigating the impact of traditional refineries and that inherently, with these new tenders, and some petroleum-based products must be supported.

Besides, in the environmental field and the eco-friendly technologies can be mentioned the bioremediation. One of the most common treatments is the bio-stimulation of indigenous microorganisms by adding nutrients in an open system to improve the fungi and bacteria growth capability. Some researchers aim to focus on the effectiveness of the mixture of avocado seed and poultry droppings in remediation of eutrophic soils with crude oil [69,70].
As described above, with around 4 million tons per year of production, Avocado is a high nutritional value that generates a lot of waste [22]. So it is being investigated how to use seed as a precursor for activated carbon synthesis to enhance the efficiency of adsorption processes in soil renewal and water remediation that will add more value to traditional agriculture [71].

5. Nanotechnology

In different areas, the Science world community continues to research and seek to apply the sciences to create new technologies such as nanotechnology. According to Oxford Languages, nanotechnology is defined as "the branch of technology that deals with dimensions and tolerances of less than 100 nanometers, especially the manipulation of individual atoms and molecules" [72]. It opens a range of opportunities to develop new products or mechanisms for designing high value-added products, as listed below.

There are multiple applications in the agro-industrial area, such as in pest control by designing nanomaterials. For example, nanocellulose can be extracted and synthesized from the seed and the peel as a new packing material [73], which is also used as a carrier of antimicrobial agents extracted from the same Avocado [74]. Also, phenols and essential oils are added to nanocomposites to improve food materials' lifespan [75] and the production of nano-emulsifiers with some advantages over conventional emulsifiers [76].

Another most incredible research area is discovering and designing nanoparticles with multiple applications according to their properties. For example, the biosynthesis of cooper silver, gold, and both metals alloy nanoparticles using the peel for their biomedical properties has presented antimicrobial and antioxidant applications, as shown in fig 9 their antimycotic activity [37,77].
Figure 8. Antifungal activities of the biosynthesized Silver (PAAgNPs), Gold (PAAuNPs), and Alloy (PAAg–AuNPs)[77].
6. Conclusions

The integrated biorefinery approach allows many countries to harness all their biomass waste and mitigate waste worldwide by focusing on the avocado industry. Also, to verify that avocado waste is a big problem, it was identified that they have quite an essential biotechnological potential. Avocado and its waste could be transformed into high value-added products in industries such as fuel and energy, materials and plastics, new drugs and antibiotics, and even environmental fields. After searching in the available articles, great approaches were identified in emerging technologies such as nanotechnology, mainly contributing to the medical, environmental, and materials industries. More efforts, research, and investment are needed to make all the proposed technologies and bioprocesses economically viable, and the global reality migrates towards these green processes.

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