Journal of Chemistry

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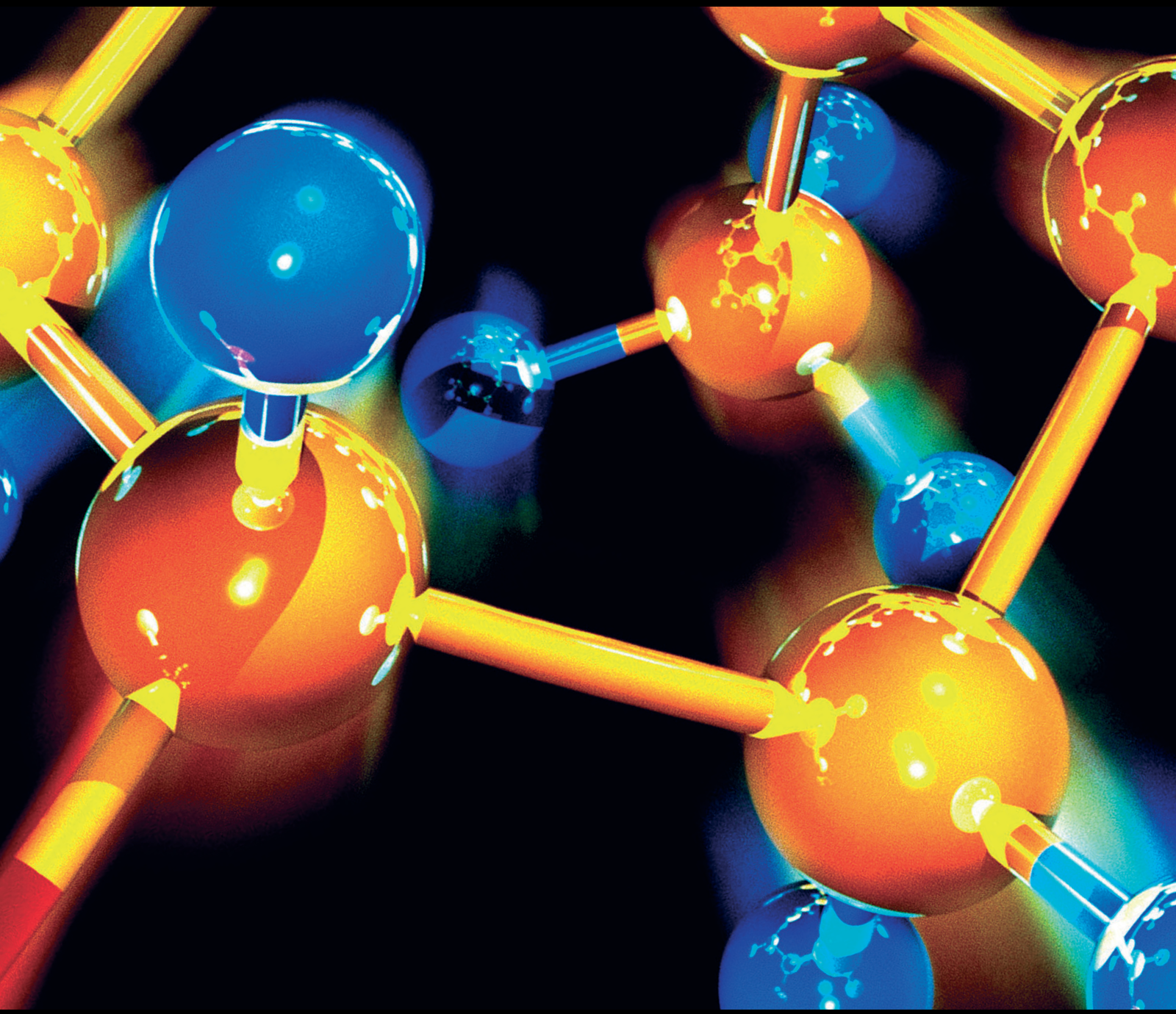

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Review Article

Processing Agroindustry By-Products for Obtaining Value-Added Products and Reducing Environmental Impact

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Over four billion tons of foods are produced annually on the planet, and about a third is wasted. A minimal part of this waste is incinerated or sent to landfills for treatment, avoiding contamination and diseases; the rest is disposed of elsewhere. The current review was aimed at broadening the panorama on the potential of agroindustrial by-products in applications such as biofuels, biomaterials, biocompounds, pharmaceuticals, and food ingredients. It also exposes the main chemical, physical, and biochemical treatments for converting by-products into raw materials with added value through low environmental impact processes. The value of agroindustrial waste is limited due to the scarce information available. There is a need for further research in unexplored areas to find ways of adding value to these by-products and minimizing their contamination. Instead of throwing away or burning by-products, they can be transformed into useful materials such as polymers, fuels, antioxidants, phenols, and lipids, which will effectively reduce food waste and environmental impact.

1. Introduction

The residues or by-products are waste materials that have lost their usefulness or economic value generated in human activities, such as industry, commerce, forest exploitation, livestock, and agriculture [1]. The principal types of by-products are construction materials, mining constituents, and health, radioactive, domestic, commercial, and industrial wastes resulting from the processes of manufacture and industrial activities [2].

In general, wastes comprise exorbitant amounts of garbage transported from domestic or industrial areas to a landfill or a processing plant to prevent environmental impact due to their high degree of contamination. The principal resources affected by these residues are the soil, air, and aquifers. Besides, their disposal has a high cost and does not solve the problem.

The Food and Agriculture Organization of the United Nations reported that a third (approximately 1.3 billion tons annually) of the total food produced for humans is wasted worldwide [3, 4]. Agroindustrial by-products are primarily produced through the food chain in harvest operations, storage, transportation, industrial processing, packaging, wholesale and retail, domestic consumption, and other activities [5]. Nevertheless, many agroindustrial by-products are not reused or treated for proper disposal, negatively impacting human or animal health [6].

The traditional way of disposing of agroindustrial by-products is by landfill storage and incineration outdoor,

which cause more contamination and do not generate valuable products.

The conversion of by-products to useful products or biorefinery must be strategic to obtain a high recovery and low environmental impact [7].

In recent years, scientists and business people worldwide have worked together to identify physical, chemical, or biochemical treatments that give utility to agroindustrial by-products [8], converting them into value-added products, such as fuels, food ingredients, chemicals, biomaterials, pharmaceuticals, and fertilizers [9–11].

This review was aimed at showing the transformation treatments of agroindustrial by-products and the diversity of unconventional sources for gathering raw materials and broadening the panorama on the usefulness and valorization of the by-products.

2. Methodology and Criteria Used for the Search and Selection of References

The methodology used for this review was following the guidelines proposed by Snyder [12]. We used both the systematic literature review and the integrative review approaches. Accordingly, four steps were utilized: (1) the design of the review, (2) the conduction of the review, (3) the analysis of the information, and (4) the review's writing. The databases used for this work were ScienceDirect, Elsevier, Springer, and Google Scholar. The emphasis in the literature search was given to scientific evidence supporting the use of agroindustrial by-products as raw materials to obtain products with characteristics of interest for other industries, the treatments used in their transformation, and their environmental impacts. Furthermore, to reduce the sample size and avoid the articles unrelated to the review, the search was restricted to the last ten years, followed by the debauched checking of abstract and conclusions to choose the appropriate articles.

3. Agroindustrial By-Products

The agricultural products most used in the industry are fruits, vegetables, legumes, and cereals, and their processing generates waste or by-products that have no apparent value. The by-products are derived from primary activities such as agricultural activity (harvesting and pruning) and secondary activities such as the industrial processing of the primary products (Figure 1) [11].

The by-products of primary sources are the residues resulting from harvesting, pruning activities, and collecting products in the field crops. These can be logs, straws, leaves, husks, roots, and seeded pods from crops [11].

These by-products can be considered green or dry according to their moisture content. Greens are high in moisture content and generally decompose in the field. Dry waste has low water content, such as straw, stems, stubble, or leaves, that can be incorporated into the soil, burned, or collected; the collected regularly are used as livestock feed [2, 13].

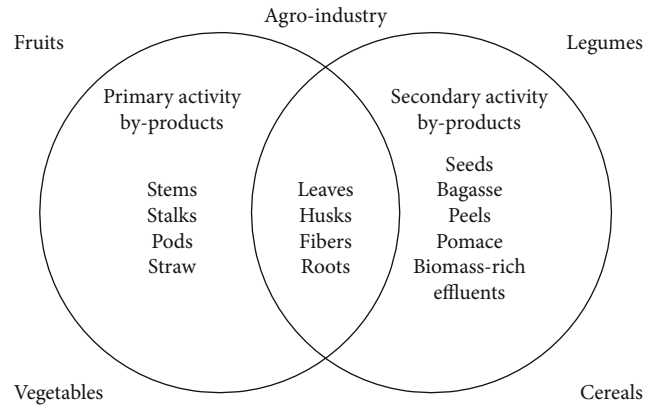

Figure 1: Agroindustry by-products.

On the other hand, by-products resulting from secondary activities are the remnants of the industrial transformation of crops into products. These are mixtures of shells, seeds, roots, bagasse, skins, pulps, and effluents rich in biomass resulting from the industrial process to obtain juices, sauces, jams, and flours, among others [1, 14]. Some by-products can be green or dry, such as leaves, husks, or roots, in which the classification depends on the use of the main product. For example, corn destined to make flour produces primary by-products, such as the leaves and stems of the harvest; however, the corn used to preserve grains maintains the leaves at harvest, but these are discarded in the processing.

The agroindustry produces a large number of by-products each year, and a few of them are traditionally reused in animal feed, crop fertilization, and fuel production, but most are disposed of in landfills or incinerated for disposal [15–17]. However, by-products have a complex and valuable content of chemical and nutritional components, implying that more high-value by-products can be processed for other uses, such as raw materials used in industries as pharmaceutics, food, construction, or medicine [18–20]. The recovery of raw materials of those interesting compounds from by-products depends on the quantities of value-added molecules they have and the treatment that has to be applied to obtain them.

4. Treatments Applied to By-Products

The growing interest in valuing the by-products from agroindustries has led the search for treatments capable of transformation with economic and environmental benefits. From 2006 to date, research related to the use of by-products has increased worldwide, being first-world countries at the top of the list [3].

The pretreatments and treatment processes (processing) applied to by-products use a series of physical, chemical, or biochemical methods to transform organic matter into new products or extract the compounds of interest [9]. Figure 2 shows the principal treatments applied to agroindustrial by-products and the potential biomolecules recovered from them.

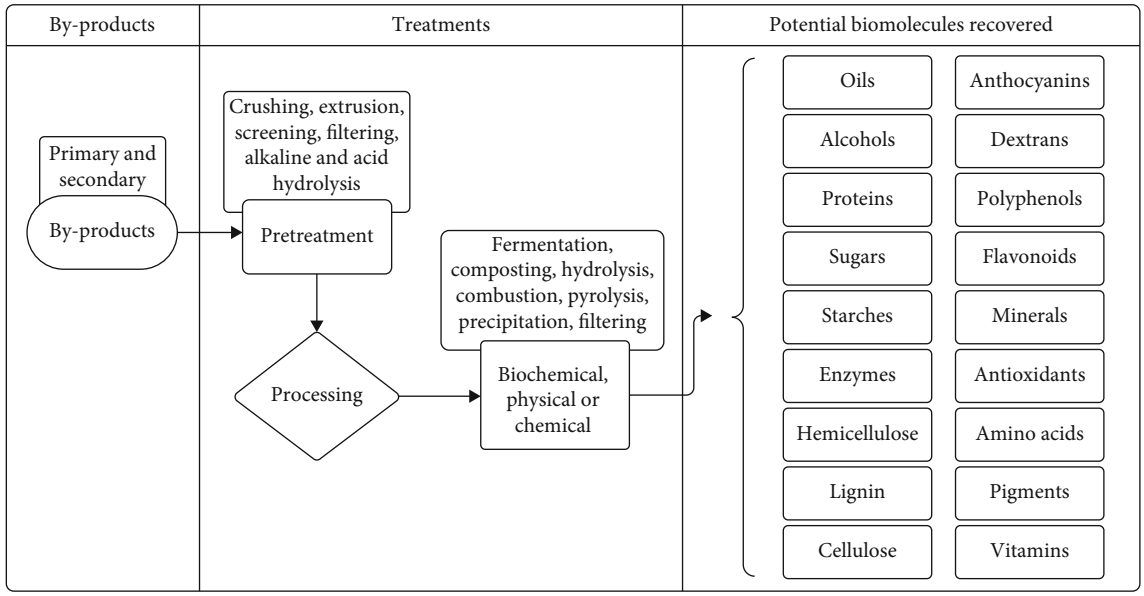

Figure 2: Potential biomolecules recovered from by-products using emerging techniques.

By-product pretreatments are those physical methods such as crushing, screening, and filtering and chemical methods such as alkaline hydrolysis, which facilitate access to reactive molecules, followed by processes to obtain the raw material of interest [21]. Treatment processes such as fermentation, pyrolysis, precipitation, hydrolysis, combustion, composting, and filtration generate the segregation of toxic and lower value compounds from raw materials and transform biomass into new products [22]. The objective of the pretreatment and processing of agroindustrial by-products is to generate value-added compounds useful in food ingredients, pharmaceuticals, fuels, and biological materials [23]. The main processes to which the by-products are subjected to obtain new commercial valued products are described below.

4.1. Biochemical Treatments. Biochemical treatments use the metabolisms of microorganisms such as fungi and bacteria to replace one or more synthetic compounds in chemical reactions. These treatments are also widely used to generate compounds mostly compatible with animals and humans and produce waste with lower contamination than synthetic chemical compounds [7, 24]. Fermentation and composting are the most common of those treatments.

4.1.1. Fermentation. Fermentation is an incomplete oxidation process carried out by fungi or bacteria, which converts the organic matter of complex molecules such as sugars into simple compounds, e.g., alcohols, propionic acid, acetic acid, lactic acid, butyric acid, and galacturonic acid.

Fermentation can be conducted with solid or semisolid materials and water or oxygen limitations [9, 25]. It has been reported that the fermented sweet corn husk and cob and bagasse are utilized to generate animal feed; an experiment that lasted 90 days included 48 fattening steers (264 ± 37.4 kg body weight) randomly allocated to three diets. The average BW gain was significant in steers with a diet containing a mixture of bagasse–vinasse, including pineapple peel silage [26].

Also, grape pomace, a by-product recalcitrant to degradation, when fermented by fungi, reduces phytotoxicity of water-soluble fractions by eliminating monoaromatic compounds. The transformation of grape pomace was evaluated using a steam pretreatment followed by incubation for 90 days with six different fungi. Several of the fungi tested reduced the phytotoxicity of water-soluble fraction from steam-pretreated grape pomace after 90-day incubation; a fraction was applied to lettuce and tomato seeds under germination (83.1 and 90.1 of percent germination). *Ulocladium botrytis* G. gave the most considerable effective phytotoxicity reduction. The resulting product has been used as organic amendment in agriculture [27].

Torres-León et al. [28] reported that solid state from the fermentation of mango seed using the fungus *Aspergillus niger* could mobilize the polyphenolic compounds improving their antioxidants properties. The total phenol content in ethanol extract increased ($p < 0.05$) from 984 mg gallic acid equivalents/100 g to 3288 mg gallic acid equivalents/100 g at 20 h of fermentation. This ethanol extract is particularly promising as a natural antioxidant.

Furthermore, Carpinelli Macedo et al. [29] generated lactic acid (31.6 g/L) from the fermentation of cassava bagasse and corn liquor using *Bacillus amylovorus* and *Lactobacillus acidophilus*.

The review of Mu et al. [30] reported that hydrogen production based on the consumption of lactate has the potential to be the predominant fermentation mechanism when using a variety of potential feedstocks. The latent beneficial effects of lactate-based hydrogen-producing processes are hydrogen production, biomass retention, pH regulation, oxygen depletion, microbial contamination, substrate hydrolysis, and detoxification. They mention that to have

advantageous lactate-driven hydrogen fermentation systems, it is necessary to consider multiple aspects, including the characteristics of the substrate/feed material, the culture conditions, and the biological factors. The application could prevent the frequently reported operational problems in hydrogen-producing bioreactors, such as overgrowth of lactate-producing bacteria and, in the worst case, a process failure.

4.1.2. Composting. Composting is an oxidative biological decomposition process of organic materials generated by the metabolism of existing microorganisms such as psychrotrophic, Saccharomycetales fungal strains, and the bacteria actinobacteria and proteobacteria [31, 32]. Composting is carried out under aerobic conditions and part of the process under thermophilic conditions (>50°C). Vermicompost is a variant of this process that uses the metabolism of worms to transform complex molecules into simple ones.

Various cellulose-rich by-products and phenolic compounds can produce urea, ammonia, and other simple compounds by vermicomposting. The compounds obtained are easily assimilated by crops [33].

The use of grape pomace compost added with hen droppings proved to increase the dry matter in corn more than chemical fertilizer. 0.71 g compost/pot, used as an organic fertilizer in corn grown during twenty days, increased dry matter more than 10% when compared to chemical fertilizer [34]. Besides, the vermicomposting of sugarcane bagasse has been used to obtain lignocellulosic products faster than traditional composting. The waste by-products of the sugarcane industry, bagasse, press-mud, and trash have been subjected to fermentation followed by vermicomposting to shorten stabilization time and improve the quality of the products. Press-mud alone and in combination with other by-products from the sugar processing industries was predecomposed for 30 days by fermenting with a combination of *Pleurotus sajorcaju*, *Trichoderma viride*, *Aspergillus niger*, and *Pseudomonas striatum*. This treatment was followed by vermicomposting for 40 days with the native earthworm, *Drawida willsi*. The effect of adding microbial consortium in the sugarcane by-products reduced the time required for vermicomposting from 40 days to 20 days and produced a nutrient-enriched compost product [35].

Significantly, various by-products rich in cellulose and phenolic compounds can break down to produce urea, ammonia, and other simple compounds that are easier to integrate into the crops [33]. Composting reduces phytotoxicity, eliminates pathogens, weeds, and seeds, and stabilizes the material concerning the demand of nitrogen and oxygen to avoid microbial competition for these elements with plant roots [36].

4.2. Chemical Treatments

4.2.1. Hydrolysis. Hydrolysis is a chemical process in which a molecule is divided into two parts by adding water or other compounds. It usually involves adding of diluted acids or alkaline solutions to dissociate complex molecules or compounds to simple elements.

The acid hydrolysis process is applied to some lignocellulosic by-product fragments of cellulose and hemicellulose, which can be used as a raw material in other products [37]. For example, Martín-Sampedro et al. [38] obtained a stable thermal lignin from *Populus alba* L. using 180°C, 60 min with a liquid/solid ratio of 20 : 1, and 3% *w*/*w* of H_2SO_4.

Abaide et al. [39] reported the production of cellobiose (18.0 g/L), xylose (17.7 g/L), arabinose (3.6 g/L), glucose (1.5 g/L), and levulinic acid (0.7 g/L) sugars. The procedure consisted of the acid application of subcritical water hydrolysis on rice husks at 25 MPa in a semicontinuous mode. The highest reducing sugar yield (18.0 ± 2.9 g/100 g husks) and efficiency (39.5 ± 1.7 g sugars/100 g carbohydrates) were obtained at 220°C and 7.5 g water/g husks.

Tibolla et al. [40] utilized alkaline and acidic hydrolysis on a banana peel, obtaining nanocellulosic materials (average diameter of 3.72 nm). A cellulose compound was isolated from banana peel by applying alkaline treatment and bleaching followed by acid hydrolysis with 0.1, 1.0, or 10% *v*/*v* H_2SO_4.

4.2.2. Precipitation. The precipitation process is utilized to separate products from a solution; a solid and liquid phase is usually obtained due to the chemical reaction between them. Alcohols are precipitating agents, which cause the separation of related products. He et al. [41] recovered insoluble dietary fiber (IDF) from blackcurrant pomace (68.73 ± 0.35 g IDF/100 g), banana peels (54.06 ± 0.30 g IDF/100 g), clementine peel (42.55 ± 0.55 g IDF/100 g), oat hull (74.53 ± 1.14 g IDF/100 g), potato peel (59.38 ± 1.02 g IDF/100 g), and wheat bran (45.6 ± 0.18 g IDF/100 g) by-products utilizing the ethanol washing method. Their results showed that the dietary fiber obtained by the alcoholic extraction contained bound phenolic compounds and had water and oil holding properties, protein absorption, and radical scavenging capacities. These authors also demonstrated the carrier properties and protective effects against heat shock of the six IDFs on three Lactobacillus strains (*Lactobacillus acidophilus* LMG9433T, *Lactobacillus casei* LMG6904T, and *Lactobacillus rhamnosus* LMG25859), observing a better response with the IDF of clementine peel.

Zhang et al. [42] utilized ethanol precipitation to obtain polysaccharides from bamboo. The process was carried out using the bamboo shoot waste that was defatted and decolored with petroleum. The pretreated residues were filtrated, air-dried, and extracted twice with distilled water in an ultrasonic processor. The extract was concentrated using a rotary evaporator under reduced pressure, and ethanol was added to precipitate polysaccharides. Then, the mixture was centrifuged to obtain the precipitated polysaccharides. They concluded that the polysaccharides' physicochemical properties and antioxidant activities were enhanced by the ethanol concentration used to precipitate, 75%.

Campos et al. [43] also applied the precipitation process with a low carrageenan concentration (<0.3% *w*/*v*) to industrial pineapple residues (stems and peels), obtaining a high recovery yield of active bromelain enzyme.

4.2.3. Pyrolysis. Pyrolysis is a process by which organic materials suffer thermal degradation into smaller volatile particles, without oxygen or any other oxidants [11].

By-products are converted into oils, a mixture of gases (methane, hydrogen, carbon dioxide, and carbon monoxide), ash, mainly enriched with carbon, and heat energy [3, 35, 44]. Gurevich Messina et al. [45] applied copyrolysis on peanut shells and cassava starch mixtures obtaining biochar with low water and mineral content and maximizing the bio-oil yield. A mixture composed of 75 w % of starch and 25 w % of peanut shells led to a maximum bio-oil yield (58.2 w %), while its water content was reduced by 3.4% compared with the value expected from the weighted average of the individual results of each component in the mixture. Furthermore, the addition of the starch to the peanut shells led to biochar with less ash content.

Marculescu and Ciuta [46] reported that the pyrolysis applied to grape marc, in a tubular batch reactor with inert conditions (nitrogen 100 cm^3/min) and the heating rate (40-50°C/min) to 500°C, produced three fractions: a liquid (light hydrocarbons), a solid (char and ash), and a volatile (CO_2, CO, H_2, CH_4, C_2H_4, and C_2H_6). Their results demonstrated that the energy provided by the grape marc was 19,729 kJ/kg, energy potential even higher than wood biomass.

Additionally, Kwon et al. [47] applied the pyrolysis process to banana peel, as an alternative transformation treatment of wastes with low environmental impact; they obtained a rich biochar material. To synergistically increase the sustainability of banana peel pyrolysis, in their study, they adopted carbon dioxide as raw material and examined the production of syngas as compared to the process with N_2 in the environment. CO_2-Cofeed pyrolysis expedited the thermal cracking of volatile pyrolysates from the banana peel, resulting in the gas phase homogeneous reaction between CO_2 and the pyrolysates at ≥420°C; this promoted the formation of CO in the temperature region, and more than twenty times of CO was formed in comparison with pyrolysis in the N_2 environment. The researchers demonstrated the environmental benefits of the process due to the use of CO_2 as a reactant.

4.2.4. Direct Combustion. The combustion of agroindustrial wastes consists of the rapid chemical oxidation of biomass, use of oxygen, energy release, and the simultaneous formation of the latest oxidation products of organic matter, CO_2, and water. This process aims to obtain heat energy by direct combustion of dry by-products such as stems, logs, cobs, or straws mainly produced at the harvest stage [48]. Agroindustrial by-products are generally burned for various purposes, including cooking, charcoal production, steam generation, mechanical applications, and electrical energy applications. Due to its lower cost, direct combustion is still considered a dominant technology [9]. Park et al. [48] found that a useful high calorific resource can be obtained by mixing the pepper, rice chaff, and spent coffee ground, molded into pellets.

Also, Ríos-Badrán et al. [49] evaluated the calorific power of sustainable biofuels by manufacturing pellets obtained from rice husks and wheat straws. They found that the pellets made from mixtures of both by-products improved the combustion characteristics having the highest calorific power (4301.10–4573.50 kcal/g) and the most reduced value of ashes compared to those pellets made only of rice husks (3090.64–4049.05 kcal/g). Accordingly, Miranda et al. [50] show that the mixtures of by-products could enhance both the manufacture of the pellet and the thermal properties of the combustion power of olive pomace and forest waste pellets, depending on the proportions of the components and their physicochemical characteristics.

4.3. Physicist Treatments

4.3.1. Filtration. The use of standard and new technology membranes carries out filtration based on selective porous barriers, through which fluids and solutes are selectively transported with the application of transmembrane pressure. Nowadays, micro-, ultra-, and nanofiltration have been proposed not only for the treatment but also for the valorization of agroindustrial by-products. These technologies present advantages such as mild operation conditions and specificity and they are noncontaminant.

The membrane processes are based mainly on some key factors that influence the performance of the process such as the physicochemical composition of the feed stream, the operating parameters, and the membrane features. Accordingly, membrane fouling is the result of specific interactions between the membrane and the properties of the feed components such as molecular size, shape, ionic charge, chemical interactions, and zeta potential, among others, so they must be considered in the process. Furthermore, engineering factors have a prominent influence on membrane fouling, so the temperature, the transmembrane pressure, and the cross-flow velocity have to be considered. Finally, the membrane structure properties of hydrophilicity, hydrophobicity, pore size, and charge influence the process too [51].

These separation processes focus on liquid by-products rich in biomass and phenolic compounds, such as anthocyanins, phenolic acids, and flavonoids. There is currently a trend to apply membrane technology in industrial processes known as the circular economy, in which the reuse of waste plays a crucial role. Therefore, membrane technology (micro-, ultra-, and nanofiltration) applies to processes involving by-products of agribusiness [52].

Cicci et al. [53] applied ultra- and nanofiltration membranes to olive mill wastewater to obtain organic products used as growing media for microalgae. The results of the membrane process demonstrated that the use of membranes seems to be a practical technology to purify organic materials from olive mill wastewater, as long as short-term fouling is under control.

Cassano et al. [54] combined ultra- and nanofiltration membranes, recovering nutraceutical compounds from agrofood wastewater. The authors conclude that the separation capability of the combination offers new opportunities for the formulation of nutraceutical products while reducing the environmental pollution. Their efficiency depends on factors such as membrane material, the molecular weight

of the organic components, and engineering conditions such as pressure, feed flow rate, temperature, and volume, among others.

Also, Castro-Muñoz et al. [55] show in their review detailed analysis on the use of emerging technologies such as pressure-driven membrane filtration processes. They argue that filtration is not only used exclusively to separate by-products and subsequent disposal but can also be used to recover and fractionate high value-added compounds, such as phenolic compounds, from agroindustrial by-products. Also, membrane filtration processes have several benefits: easy scaling, high productivity in permeate flows, low energy requirements, high separation efficiency, simple operation, and the absence of phase transition.

The "nejayote" is a by-product from the alkaline cooking of maize grain in the nixtamalization process and unfortunately contributes to environmental contamination. Castro-Muñoz et al. [56] proposed ultra- and nanofiltration membranes to recover phenolic compounds and fermentable carbohydrates, from the nixtamalization process. The research of these authors demonstrated the potential the nejayote can have in recovering high added value compounds, such as carbohydrates, polyphenols, gums, and calcium components, of interest in the food, pharmaceutical, and biotechnological areas.

4.3.2. Other Physical Processes. Physical processes do not generate chemical changes in by-products; however, they are considered pretreatments. Examples of these are extrusion, grinding, maceration, washing, mixing, heat treatment, and reverse osmosis, among the main ones. Also, before processing, waste should be stored under favorable conditions that do not cause its deterioration [11, 52]. Furthermore, the biochemical, physical, and chemical processes involved in using agroindustrial by-products must ensure the rapid use of waste and be cost-effective in maintaining the added value of the products obtained with such treatments.

Xie et al. [57] utilized a high-pressure process to study the physicochemical properties of pectins obtained from potato peel waste. They found that the high-pressure treatments led to an increase in the galacturonic acid content, as well as in the degree of esterification (Gal+Ara)/Rha ratio, and molecular weight decreases. These results suggest that high-pressure treatments could be an efficient technique to modify pectin from potato peel waste to get thickener or stabilizer agents.

Gouw et al. [58] utilized a heat treatment for increasing the amount and bioaccessibility of phenolics and dietary fiber in four pomace fruits (PF). The process was carried out using the frozen pomace (wet) thawed at room temperature dried in an impingement oven set at 110°C for 3 h and tested in vitro digestion as primary treatment. The results showed all PF have interesting phenolic and dietary fiber content. Authors suggest that their study demonstrated the potential of using FP as fiber-rich and antioxidant functional ingredient.

Also, Arcila et al. [59] utilized the extrusion pretreatment with high or low moisture (15% and 30% w) and high or low screw speed (120 and 250 rpm) to increase the non-starch polysaccharides from wheat bran enabling greater in vitro fermentability by human fecal microbiota.

5. Application of By-Products

As mentioned, by-products contain compounds of excellent chemical and nutritional interest, such as proteins, sugars, enzymes, pigments, flavorings, alcohols, fatty acids, antioxidants, vitamins, minerals, gelling agents, biofuels, antiseptics, and fungicides, among others (Figure 3) [60, 61]. The different treatments mentioned above are applied to by-products for recovering raw materials for its transformation into biofuels, chemicals, biomaterials, pharmaceutical ingredients, and foodstuffs [17]. Below, we will detail the application of the various by-products that have been documented in recent years.

5.1. Biofuels. Biofuel production represents a new economic opportunity to reduce greenhouse gas emissions and improve sustainable energy production. Besides, the possibility of recycling wastes for bioenergy production can reduce oil dependence and have positive impacts on the economy, environment, and society. Biofuels are generated by fermentation and thermochemical processes of biomass [62, 63].

Biofuel production can use substrates such as seeds, grains, or sugars from crops of maize, wheat, rice, and first-generation sugar. However, these conflict with food production; thus, alternative sources rich in lignocellulosic biomass such as by-products are sought [44]. The use of by-products does not enter conflict with staple foods, in addition to be low-cost substrates.

For instance, Maragkaki et al. [64] utilized sewage sludge mixtures with five different residues (95/5 *w*/*w*) to obtain biogas by digestion. Their study shows that the higher methane production was in the following order: crude glycerol > food waste > cheese whey > grape residues > sheep manure. Also, in the research by Scaldaferri and Pasa [65], cashew nutshell liquid was used as a feedstock to produce green diesel (mixed hydrocarbons with 98% efficiency) by cracking it in a batch tank reactor.

Alvarez-Guzmán et al. [66] reported significant efficiency in producing hydrogen and alcohols (ethanol and 2,3-butanediol) using sugarcane molasses instead of wheat straw hydrolysate by dark fermentation with an *Antarctic psychrophilic* GA0F bacterium.

Regarding the use of different substrates, Molinuevo-Salces et al. [67] analyzed the potential of swine manure as substrate and apple pomace as a cosubstrate for biofuel production into biogas based on manure. Different bacterial and yeast strains (strains of *Kluyveromyces marxianus*, *K. lactis*, *Lachancea thermotolerans*, and *Saccharomyces cerevisiae*) were compared to produce bioethanol, obtaining yields of 0.371–0.444 g/g substrate. Also, specific methane yields from bioethanol and biobutanol fermentation residues were 463 and 290 mL CH_4/g substrate, respectively. Methane yield for the codigestion of apple pomace and swine manure was 596 mL CH_4 g/substrate, with an apple pomace percentage of 14.6% and a substrate concentration of 9.38 g/L. The

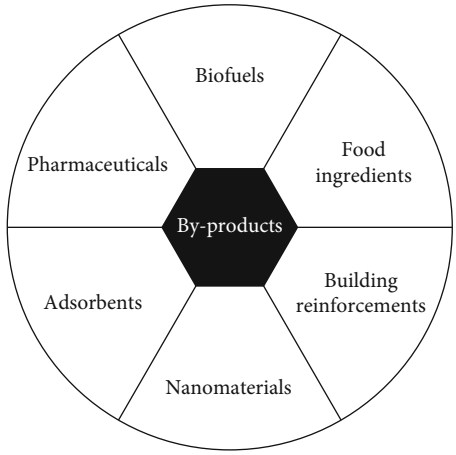

Figure 3: Potential application of by-products.

researchers concluded that apple pomace significantly impacted biofuel production (bioethanol, biomethane, and biobutanol) as cosubstrate.

5.2. Food Ingredients and Pharmaceuticals. The valorization of by-products as pharmaceutical and food ingredients is widely recognized [58–60]. By-products can be used as primary substrates or raw materials to produce multiple medium or high value-added compounds.

He et al. [41] efficiently recovered dietary fiber from blackcurrant pomace, banana peels, clementine peel, oat hull, potato peel, and wheat bran through an ethanol-washing process directed to dried by-products to be applied as probiotics.

On the other hand, cassava bagasse (18% *w*/*v*) and corn liquors (12% *v*/*v*) were pretreated by enzymatic hydrolysis (*Rhizopus oligosporus* amylase) and then fermented with *Lactobacillus amylovorus* and *Lactobacillus acidophilus* to produce lactic acid [29]. Furthermore, natural deep eutectic solvents extracted phenolic compounds from onion seed, olive cake, tomato waste, and pear waste [68]. Wheat bran acid hydrolysate is being used to produce triacylglycerols of oleic acid by fermentation with the yeast *Rhodotorula mucilaginosa* Y-MG1 [69]. Also, sugarcane bagasse, corn stalks, and rice bran irradiated with UV and gamma were used to produce the immunosuppressant mycophenolic acid under solid-state fermentation by the *Penicillium roqueforti* fungus [24].

Nejayote, produced by nixtamalization, is a source of food, nutraceutical, and cosmeceutical ingredients. Ramírez-Jiménez and Castro-Muñoz [70] reported that feruloylated arabinoxylans extracted from nejayote have viscoelastic and nutraceutical properties that can be used as texture enhancer and also as probiotic and insulin regulator. They also identified hydroxycinnamic acids in nejayote (p-coumaric and ferulic acid) that can be utilized as antioxidants in the food and cosmetic industry.

Waste and by-products are currently used as raw materials in fermentation broths to produce valuable compounds. In addition, some pretreatments applied to by-products provide them the chemical properties for their products to be considered efficient ingredients in biotechnological applications. García-Depraect et al. [71] evaluated the effect of long-time refrigeration to effectively preserve the hydrogenogenic biomass for months, supplying readily available inoculants after reactivation. Hydrogen-producing bacteria and lactic acid bacteria had a notable presence all over the whole period of preservation and during reactivation.

Table 1 shows several products with food and pharmaceutical value obtained from agroindustrial by-products.

5.3. Materials: Adsorbents, Nanomaterials, and Building Reinforcements. An emerging field of exploitation of agroindustrial by-products is their use as adsorbents for heavy metals, nanomaterials, and building reinforcements.

Adsorbents for heavy metals are expensive, and the origin of the elements is synthetic. Recently, attention has been given to biobased materials, mainly agroindustrial by-products such as fruits, cereals, legumes, and vegetables, easily accessible in large quantities.

By-products from citrus are rich in lignin, cellulose, and pectin containing many functional groups such as hydroxyl and carboxylic groups that could bind to divalent cations. Meseldzija et al. [72] used lemon peels as a low-cost adsorbent to remove 89% of the copper ions from mining wastewater at natural pH (pH 3). Also, Cunha et al. [73] utilized coconut mesocarp and magnetic cobalt ferrite in xerogel to remove 86% and 49% of cadmium (pH 3) and lead (pH 4) ions, respectively. The coconut mesocarp showed high reuse capacities in three successive reduction cycles. Likewise, Muniz et al. [74] utilized ripe okra and passion fruit seeds as natural coagulants to dairy wastewater for solid-liquid separation in coagulation/flocculation. Both materials could reduce up to 90% of the turbidity. These results suggest that the seeds of both materials could be good sources of coagulant agents that can replace the metallic coagulants and reduce the production of agroindustrial wastes.

On the other hand, cellulose has been widely used to prepare thermosetting and thermoplastic polymers as to replace glass fiber. Cellulose is the best alternative to fiberglass because it offers minor abrasion to the fiber during processing and it can be recycled; besides, it is cheap and available [75].

It has also been possible to extract cellulose up to the nanoscale, showing outstanding characteristics by using chemical methods that reduce its diameter and length [76]. The term "nanocellulose" generally refers to cellulose materials with at least one dimension in the nanometric range [75]. Accordingly, by-products have proven to be a reliable source for the extraction of nanocellulose. It has been reported that applying a chemical (hydrolysis acid) and physical pretreatments (washed, dried, milled, and filtering) to grape pomace resulted in bleached cellulose pulp, and then, through acidic hydrolysis and ultrasound treatments, cellulose nanocrystals were obtained [77]. Also, cellulose nanofibers have been obtained from banana peel bran using chemical (alkaline treatment, bleaching, and acid hydrolysis) and enzymatic treatment (alkaline treatment and hydrolysis with xylanase) [40].

Table 1: By-products with food and pharmaceutical value.

By-products		Value-added products	Source
Cassava	Bagasse	Gluten-free noodle	Fiorda et al. [86]
Sugarcane	Bagasse	Ethanol, lactic acid	de Moraes Rocha et al. [87]; Laopaiboon et al. [88]
Palm	Empty fruit bunch	Xylose	Tan et al. [89]
Pineapple	Peels	Citric acid	Kuforiji et al. [90]
Pineapple	Husk	Dietary fiber	Martins et al. [91]
Tomato	Pomace	Dietary fiber	Martins et al. [91]
Rice	Bran	Dietary fiber	Martins et al. [91]
Pea	Pod	Dietary fiber	Martins et al. [91]
Coffee	Bean	Chlorogenic acids, monosaccharides, disaccharides, oligosaccharides, proteins, minerals, and carboxylic acids	Pérez-Sariñana et al. [92]
Coffee	Husk	Chlorogenic acids, gallic acid, and phenolics	Das Neves et al. [93]; Gemechu [15]
Melon	Peels	Proteins	Silva et al. [94]
Melon	Seeds	Soluble sugars	Silva et al. [94]
Soybean	Seeds	Linoleic acid, linolenic acid	Silva et al. [94]
Grape	Seeds	Linoleic acid, linolenic acid	Silva et al. [94]
Olive	Pomace	Antioxidant	Bermúdez-Oria et al. [95]
Banana	Stalk	Phenolic acids	Arun et al. [96]
Mango	Pulp	Dietary fiber	Martins et al. [91]
Mango	Seeds	Linoleic acid, linolenic acid	Silva et al. [94]

Franco et al. [78] obtained nanocellulose from peach palm residues by ultrafine grinding and chemical delignification. Further, materials dispersed in polymer matrices such as nanocellulose can result in nanocomposites showing enhanced mechanical properties. The use of nanocellulose-based composites as adsorbents for metals is of great interest. For example, Tshikovhi et al. [79] analyzed nanocellulose-based hydrogel by incorporating graphene oxide into the cellulose matrix using NaOH/urea to remove copper(II) ions from an aqueous solution, finding a direct correlation between an increase in the adsorption of copper ions with an increase in graphene oxide/nanocellulose ratio.

Another exciting use of by-products is the obtention of prolamins using chemical solvents. The process generates nanofibers and nanoparticles from grains of corn, sorghum, and wheat [80]. The nanomaterials obtained are conformed principally of prolamins with different vital roles in the food industry and medicine to shield bioactive compounds, emulsion stabilizing, drug delivery systems, and control release of fertilizer.

Besides, cellulose acetate was obtained from corncob through hydrothermal treatment followed by dilute sodium hydroxide (cellulose) and acetylation reactions [22]. Also, lipase, laccase, and amylase have been immobilized into salinized green coconut fiber oxidized by periodate [81].

The replacement of ground-granulated blast furnace slag up to 40% with dehydroxylated corncob produces a geopolymer concrete at ambient curing conditions [82], exhibiting higher strengths than Portland cement concrete [83]. Likewise, Vega-Castro et al. [84] produced polyhydroxyalkanoates from the pineapple peel fermentation process.

Table 2 shows some applications of agroindustrial by-products as nanomaterials, adsorbents, and building reinforcements.

As shown, the raw materials obtained from different agroindustrial by-products have essential applications as carriers of drugs, biofuels, food ingredients, compounds of high nutraceutical value, antioxidants, biopolymers, adsorbent compounds, and concrete reinforcements, among others.

6. Importance of the Use of Agroindustrial By-Products as Raw Materials

The growing demand for products, energy, and environmental policy has considerably increased the interest in recycling, reuse, and recovery of by-products, residues, wastes, and sewage [16]. The use of agroindustrial by-products solves different environmental problems, originated by the generation and disposal of these wastes; it reduces the demand for renewable and nonrenewable natural resources as raw materials. According to the international agenda for 2030, the Sustainable Development Goals aim to halve per capita food waste globally at the retail and consumer level and reduce food losses in production and supply chains, including postharvest losses [4].

One of the aspects that can define the sustainability of development is its environmental impact. The use of

Table 2: By-product applications as nanomaterials, adsorbents, and building reinforcements.

By-products		Use	Source
Açaí	Fibers	Concrete binder	Azevedo et al. [97]
Cauliflower	Cores	Heavy metal adsorbent (Cd^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+})	Landin-Sandoval et al. [98]
Coconut	Shell	Heavy metal adsorbent (Cd^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+})	Landin-Sandoval et al. [98]
Grape	Pomace	Nanofibers as green agent for dendritic silver synthesis	Carbone et al. [99]
Palm	Oil fuel ash	Concrete binder	Nagaratnam et al. [100]
Palm	Shell	CO_2 adsorbent	Ochedi et al. [101]
Rice	Husk	CO_2 adsorbent	Ochedi et al. [101]
Soybean	Pods	Nanofibers as reinforcement of films proteins	González et al. [102]
Soybean	Hulls	Copper ions adsorbent	Wartelle and Marshall [103]
Walnut	Shell	CO_2 adsorbent	Ochedi et al. [101]

agroindustrial by-products reduces this environmental impact and the cost of waste treatment. At the same time, it benefits the corporations economically by adding value to by-products and wastes. Accordingly, "the circular economy" is presented as a system of resource utilization, where reduction (minimum use of raw materials), reuse (maximum reuse of products and components), and recycle (high-quality reuse of raw materials) are the elements based on the production systems [85].

Accordingly, the processing of by-products must adjust to a sustainable system; thus, in the extraction of value-added raw materials, the environmental impact that this process could generate must be considered. As well as the evaluation of the economic viability of the proposed processes is also essential; for this reason, it is necessary to estimate the retail price of the potential product, range of applications, storage costs, transportation, volume and size, flexibility, and location of the by-products [36].

Zihare et al. [6] suggest considering engineering, environmental, and economic criteria to evaluate the impact of the transformation of by-products, helping scientists and investors make sound decisions. Other essential aspects to consider in the transformation of by-products are toxicity (pesticides and mycotoxins), hygiene, safety issues, effects of processing and storage on the functional components of by-products, and interactions with other compounds [15].

7. Discussion

Agroindustrial by-products represent a critical problem for their traditional disposal, but they can function as high-value feedstock in other industries. With the above mentioned, the agroindustrial by-products offer various applications as raw materials in the food, pharmaceutical, and fuel industries, among others. However, the appropriate pretreatments and treatments must be chosen strategically to obtain materials with high nutritional value, later translating into rising economic value.

There is a need to explore the most significant number of physical, chemical, or biochemical treatments in the by-products to standardize the methods for obtaining the products of interest and discern the performance of each product and be able to transfer it to an industrial scale. The combination of treatments is a promising opportunity. In turn, they enhance the reduction of the quantitative environmental impact by using raw materials derived from by-products instead of synthesized raw materials.

The development of extraction and purification systems for raw materials could facilitate access to chemical components with greater sensitivity and specificity. The methods for obtaining biomaterials related to nanomaterials require a more exhaustive analysis to detect by-products with these properties, which could considerably increase the economical and chemical value. The study of the treatments of alternative sources of raw materials for the pharmaceutical, food, fuel, construction, and biomaterials industries is limited in these aspects. It is essential to consider that the recovery of biocompounds from by-products must be a sustainable process that contributes to economic, environmental, and social development.

8. Conclusions

Throughout this in-depth review, pretreatments and treatments such as fermentation, composting, hydrolysis, precipitation, pyrolysis, combustion, and filtration can be used to recover, separate, and fractionate specific compounds; in agreement to their properties, they have potential applications in the biofuels, biomaterials, biocompounds, pharmaceuticals, and food industries from new sources, known as agroindustrial by-products. By-products are on the rise in both producing and transforming countries.

Agricultural production and consumption continue increasing, putting high pressure on natural resources. Food losses in the value chain must be addressed or considered for soil amendments and for the production of environment-friendly by-products.

The circular economy is a production and consumption model that involves sharing, renting, reusing, repairing, renovating, and recycling existing materials and products as many times as possible to create added value. In this way, the life cycle of the products increases. The knowledge of the various applications of agroindustrial by-products as new raw materials with added value in other industries opens the picture to focus the efforts to apply emerging technologies in the recovery of these materials. In practice, it

means reducing waste to a minimum. When a product reaches the end of its useful life, its materials are kept within the economy whenever possible. These can be used productively repeatedly, thus creating additional value. This type of economic model must be implemented by all industries, not just agribusiness, to have less impact on the environment and natural resources.

Furthermore, the high costs of waste disposal make it necessary for industries that use large-scale production processes to focus on by-product recycling. The main concern is the disposal of large amounts discarded and the major environmental problems they can generate. The population worldwide must adopt modern methods and technologies to obtain value-added products such as polymers, fuels, antioxidants, phenols, and lipids, among the main ones from by-products instead of throwing away or burning food waste. In the future, governments may legislate to ensure the use of approaches such as those described herein to reduce water and environmental pollution.

Therefore, researchers must explore suitable treatments to improve the recovery of valuable materials and apply technologies, according to the characteristics of the by-products, to retain functionalities in all processing and storage conditions. Due to the diversity of by-products, specific protocols and statistical methods optimizing the procurement, transformation, and recovery of the products of interest should be proposed. This review should open the picture to encourage using by-products to generate raw materials with high added value through processes that do not affect the environment.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare no conflict of interests, including financial, personal, or otherwise.

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Research Article

Synthesis and Characterization of Feed-Grade Monocalcium Phosphate $Ca(H_2PO_4)_2{\cdot}H_2O$ from Oyster Shell

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Oyster shells are considered as a byproduct or solid waste in mariculture or related food processing areas that face a major disposal problem at the landfill in coastal regions for sustainable development. Oyster shell is composed mostly of $CaCO_3$, and it is also considered as a secondary source of calcium for various applications. In this paper, we extracted the calcium carbonate from oyster shell and used it as the source of calcium for the preparation of feed-grade monocalcium phosphate (MCP). The investigation shows that the heavy metal contents in oyster shells as well as in the synthesized MCP are extremely low, and the synthesized product meets the requirements for the European Union (EU) maximum limits applied for feed additives. The XRD, TG, and IR data analyses confirmed that the synthesized product is monocalcium phosphate.

1. Introduction

Oyster shells are produced mainly in the mariculture and related food processing areas. After harvesting the oyster flesh, the oyster shells are mostly discarded as solid waste. In 2004, about 275,490 tons of oyster shells were produced, 70% of which were wasted in landfills or dumped into waters that may threaten the environment in water, culture medium, and land areas [1].

Various strategies have been proposed to face the problems, such as recycling of the shell wastes rather than disposal as the substitute for aggregate in mortar, building materials, plastic production, water and air treatment, and food supplements [2, 3]. Some others used oyster shells as an alternative calcium source for calcium carbonate instead of natural limestone [1], or the preparation of calcium hydrophosphate, monocalcium phosphate monohydrate, or tricalcium phosphate [4, 5]. In these investigations, the oyster shells are mixed with phosphate species to form the target materials without any proper purification so that most of the impurities, if any, in the starting materials will be retained in the final product.

The use of oyster shells as the calcium source instead of natural calcium carbonate for the preparation of feed-grade calcium phosphate will be interesting and fruitful. The oyster shells which contain a very low content of heavy metals, are generated by living animals, so they are more compatible to the animals as feed supply or additives [6]. The use of oyster shells for the preparation of feed-grade calcium phosphates will add significant value to the waste of mariculture as well as related food processing of bivalve shells.

2. Materials and Methods

2.1. Materials and Reagents. Oyster shells are collected from the Quang Ninh area, on the northeastern seacoast of Vietnam. After rubbing and cleaning with water, the samples are dried and ground to pass through a 160 μm sieve. Phosphoric acid of technical grade is supplied by Duc Giang Chemicals Group. All other chemicals used for the

experiments are reagent grade and commercially available and are used as received without any further purification.

2.2. Preparation Procedure. The precipitated calcium carbonate (PCC) is first prepared from the oyster shell by the modified procedure which is briefly described as follows [7]: the ground oyster shell is heated at 1000°C for 1 h, after cooling to room temperature, the powder is dispersed into water to get a hydrated lime slurry which is then filtered and the hydrated lime solution is bubbled with a flow of carbon dioxide gas until the pH of the formed slurry is about 7.0. The slurry is then filtered and washed with water, then dried at 105°C until the weight remains unchanged to obtain the PCC. The yield of the PCC has not been determined in the experiments.

The monocalcium phosphate monohydrate is prepared from the PCC and phosphoric acid [5, 8]. A typical example can be illustrated as follows: 100 g of phosphoric acid, 85.44% H_3PO_4, were diluted into 70.9 g of water to get a solution of 50% H_3PO_4, and then the obtained solution was heated to 90°C in a water bath. Then, 43.8 g of the precipitated calcium carbonate is gradually added into the solution in small portions, and the slurry was stirred continuously for about 1 hour to form a homogeneous mixture. The product was dried at 95°C to a constant weight and 109.8 g of white powder was obtained. The yield is almost quantitative.

2.3. Analytical Methods. The content of some trace elements such as As, Pb, and Cd in raw materials as well as in the synthesis samples is measured with inductively coupled plasma optical emission spectroscopy (ICP-OES) on Optima 8300 equipment (PERKIN ELMER). The samples with a weight of about 500 mg are added to an excess amount of nitric acid solution and diluted into 50 mL. For the quantification of the elements, an external calibration using the PERKIN ELMER multielement standard, so called *Quality control 21* solution (100 mg/L; 5% HNO_3) was employed. The mass concentrations of the standard solutions were 0 mg/L, 0.2 mg/L, and 1.0 mg/L. To recognize potential spectral disruptions, two different characteristic emission wavelengths were determined for every element, As: 193.696 nm and 188.979 nm; Pb: 220.353 nm and 217.000 nm; and Cd: 228.802 nm and 214.440 nm [9].

The calcium content in the samples is determined by the complexometric method with an ethylenediaminetetraacetic acid standard solution. The phosphorus content is measured by the vanadomolybdophosphoric acid colorimetric method on a Thermo Scientific SPECTRONIC 20D + spectrophotometer at a wavelength of 470 nm using KH_2PO_4 (99.5%, Sigma-Aldrich) as the standard for calibration [10].

The crystal structure of the samples was measured on a Rigaku MiniFlex600 diffractometer using a Cu anode for X-ray generation, $\lambda(\text{CuK}\alpha) = 1.54056$ Å, recorded at room temperature, scanning rate of 2.0°/min, recording intervals of 0.020°, with the two theta angles from 5° to 90°. The morphology of particles in the samples is evaluated with a scanning electron microscopy (SEM) on a Nova NANOSEM 450 equipment.

The thermogravimetric analysis (TG and DTG) of the synthesis samples is measured on a Setaram Labsys Evo S60/58988 (France) thermal analyzer. The sample, with an initial weight of 7.11 mg, is put on an alumina crucible and heated from room temperature to 850°C at a heating rate of 10°C/min, under the flow of air at a flow-rate of 20 mL/min. The weight and heat flow of the sample are recorded during the heat treatment.

The functional groups of the synthesis samples are investigated with FTIR measurements. A small amount of the sample is mixed with KBr, then pelletized (Pike), and scanned in transmission mode on a Jasco FTIR-4200 series spectrophotometer over the wavenumber range of 4000–400 cm^{-1} with a spectral resolution of 4 cm^{-1}. The blank sample is pure KBr.

3. Results and Discussion

3.1. Composition and Characteristics of Oyster Shell, PCC, and the Synthesized Product. The composition of the raw materials for the preparation of the feed-grade additives is crucially important, not only the main ingredient but also the content of the impurities, especially the one of heavy metals which must be lower than certain levels [11, 12].

The X-ray diffraction analysis shown in Figure 1(a) and Figure S1 in the Supporting Information (SI) indicated that the $CaCO_3$ in oyster shell has a calcite structure (JCPDS No. 86-0174) which is in good agreement with other observations [2, 13].

The chemical analysis results show that the calcium carbonate content in the oyster shell sample is about 95.61% which is very close to the reported value of 95.994% $CaCO_3$ for the oyster shell [15]. The thermogravimetric analysis of the oyster shell sample is given in Figure 2(a).

The TG analysis in Figure 2(a) shows the weight loss of 44.58%, which is slightly higher than the expected value of 42.07% for the decomposition of a sample containing 95.61% $CaCO_3$. This may be ascribed to the decomposition of a small amount of organic matter in the oyster shell [16].

In order to investigate the potential applications of oyster shell as a material for the preparation of feed additives, the content of heavy metals including As, Pb, and Cd has been measured with ICP-OES. The results of the determination are shown in the Figure 3(a).

The results in Figure 3(a) show that the dilutions (10-fold) of the samples were measured, and the resulting values were found below the detection limit for these heavy metals. It means that the contents of As, Pb, and Cd in the samples are extremely small. Hence, the upper limits for the contents of these elements were evaluated from the limit of detection (LOD) in the measurement, and the results of the evaluation are shown in Table 1.

For oyster shell, the average upper limits for contents of As, Pb, and Cd are smaller than 27.1, 18.1, and 1.8 ppm, which are also lower than the European Union (EU) maximum limits of 30, 20, and 10 ppm, respectively, for feed additives [11, 12]. It means that the oyster shell meets the EU

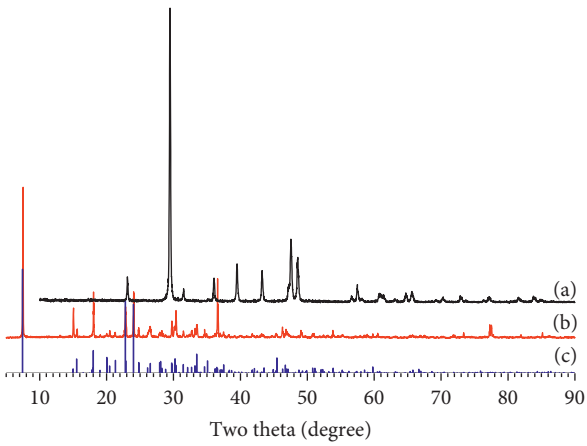

Figure 1: The XRD powder patterns of oyster shell (a), synthesized product (b), and the simulated one of $Ca(H_2PO_4).H_2O$ (c, JCPDS No. 71-0656) represented by the vertical bars, adapted from [14].

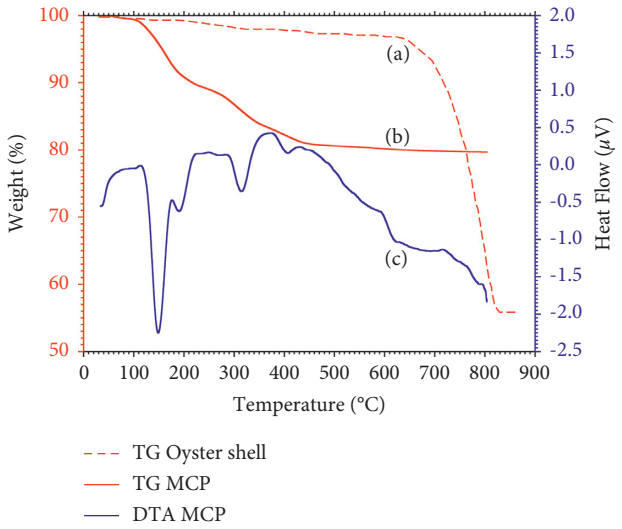

Figure 2: The thermogravimetric analysis (TG, red) and differential thermal analysis (DTA, blue) for the oyster shell (dotted curve, (a)) and the synthesized product (solid curve, (b) and (c)).

requirements for the content of heavy metals in applications or raw materials for the preparation of feed additives.

It is noted that oyster shell also contains some insoluble impurities that cannot be dissolved in nitric acid, even a concentrated one. The investigation shows that the content of the insoluble impurity is about 1.94%, and the XRD analysis indicates that it contains mostly SiO_2 (quartz, JCPDS No. 87-2096) and Al_2O_3 (corundum, JCPDS No. 85-1337), as shown in Figure S2 in the SI. The presence of these impurities may be unwanted for the materials needed to prepare the feed-grade additives like the MCP, so the purification of the oyster shell is required. The purification procedure of the oyster shell is described in Section 2.2 for the transformation of oyster shell into the PCC. The obtained PCC dissolves completely in dilute nitric acid (1 M), and the content of $CaCO_3$ in PCC is about 99.5%. The content of heavy metals in PCC has also been determined, and the results are given in Figure 3(b) which are also found beneath the detection limit for these heavy metals. The evaluated average upper limits for the contents of As, Pb, and Cd are smaller than 26.8, 17.8, and 1.8 ppm, respectively, which are also lower than the European Union (EU) maximum limits. Hence, PCC is a more suitable and preferred material for the preparation of feed-grade additives like MCP. The procedure for the preparation of the MCP is described in Section 2.2.

The chemical analysis of the synthesized product shows that the Ca and P contents in the sample is 15.80 and 24.63%, respectively, which is very close to the values of 15.86 and 24.60% for Ca and P as calculated from the $Ca(H_2PO_4)\cdot H_2O$ formula. The contents of the heavy metals in the synthesized product are also determined, and the results are shown in Figure 3(c).

The results in Figure 3(c) show that the contents of these heavy metals were also extremely small and found below the detection limit. The evaluation results for the upper limits for the content of these elements are given in Table 1. The evaluated average upper limits for the contents of As, Pb, and Cd are also lower than 26.8, 17.8, and 1.8 ppm, respectively, and these values are also smaller than the European Union (EU) maximum limits for feed additives. It means that the preparation of the MCP form PCC derived from oyster shell gives the synthesized product a very small content of heavy metals, and it is suitable for using as a feed additive.

3.2. Crystal Structure of the Synthesized Product. In the system of $CaO\text{-}P_2O_5\text{-}H_2O$, various phosphate phases and species may exist depending on the composition and conditions for the preparation. The structure of the phase(s) formed can be evaluated with the X-ray diffraction (XRD) which may be used as the fingerprint for phase analysis. The XRD patterns of the synthesized sample (b) and the simulated one based on the data of B. Dickens and J. S. Bowen (c, JCPDS No 71-0656) [14] are shown in Figure 1.

The XRD data in Figures 1(b) and 1(c) show that the powder pattern of the sample is consistent with the one of $Ca(H_2PO_4)_2\cdot H_2O$ which contain both Ca and P as confirmed by chemical analysis. Hence, the XRD measurement confirms that the synthesized product is a single phase of $Ca(H_2PO_4)_2\cdot H_2O$ as there are no additional peaks for other phases in the range of measurement.

3.3. Themogravimetric Analysis of the Synthesized Product. The thermal behaviour of the synthesized product can be investigated by thermogravimetric measurement. The TG and DTA patterns of the product are given in Figures 2(b) and 2(c) which show the relative weight and the heat flow of the sample when it is heated from 30 to 800°C. The thermogravimetric analysis data can be divided into different steps corresponding to the decomposition of the MCP and the elimination of water molecules from the sample. Before 100°C, the weight of the sample is almost unchanged and the absorbed water may be small. The weight loss of sample from 100 to 175°C is 7.20% which is close to the value of 7.14% as calculated for the elimination of one lattice water molecule

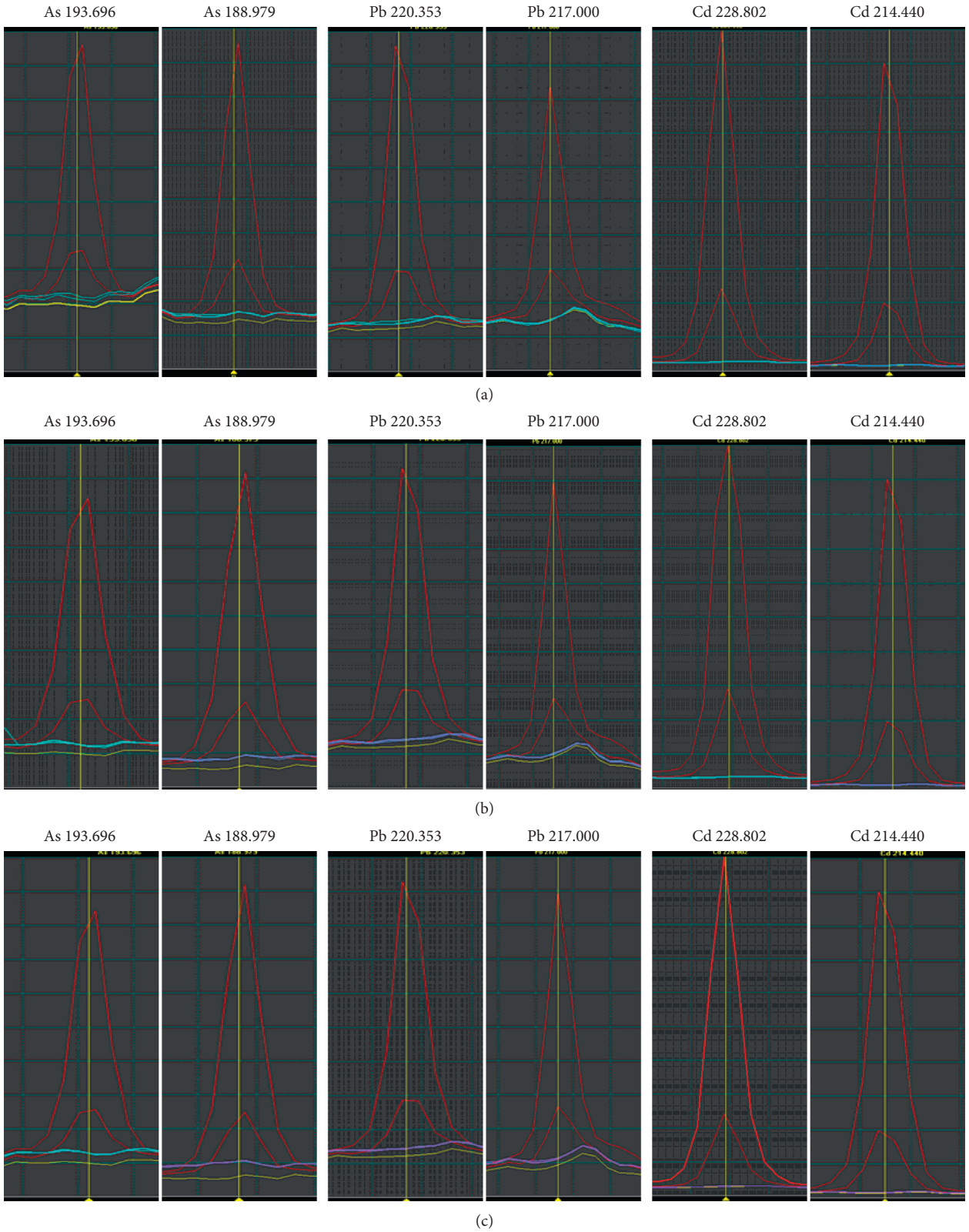

Figure 3: Emission spectra of the 10-fold dilution (turquoise/violet curves) of the oyster shell (a), PCC (b), and MCP (c) samples. The spectra of As are on the left, Pb in the middle, and Cd on the right. The yellow curves for the blank, and the red ones for calibration curves.

Table 1: Upper limits for the As, Pb, and Cd contents in the solid samples derived from the LOD in the solutions.

Sample	Content of heavy elements (ppm)					
	As (188.979 nm)	As (193.696 nm)	Pb (217.000 nm)	Pb (220.353 nm)	Cd (214.440 nm)	Cd (228.802 nm)
LOD, mg/L[(a)]	0.0217	0.0319	0.0064	0.0293	0.0016	0.0020
Oyster shell	<21.9	<32.3	<6.5	<29.7	<1.6	<2.0
PCC	<21.7	<31.9	<6.4	<29.3	<1.6	<2.0
MCP	<21.7	<32.0	<6.4	<29.3	<1.6	<2.0

[(a)]The LOD in the measured solutions is evaluated from the standard deviation (σ) of the blank measurements, LOD = $3 \times \sigma$.

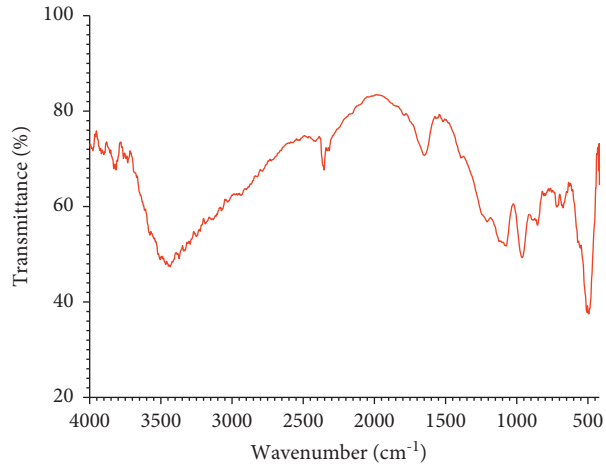

Figure 4: The FTIR spectrum of the synthesized product.

per formula unit of $Ca(H_2PO_4)_2 \cdot H_2O$ as an indication of the large endothermic peak at 150°C on the DTA curve. On further heating, the weight loss of the sample when heated from 175 to about 700°C is 13.80%. The observed value of weight loss is rather close to the value of 14.28% for the removal of 2 water molecules from the intermediate of calcium dihydrogen phosphate to $CaH_2P_2O_7$ and then to stable calcium metaphosphate, $Ca(PO_3)_2$. The decomposition of $Ca(H_2PO_4)_2$ as well as the one of $CaH_2P_2O_7$ is also endothermic, as showed in the corresponding DTA curve. The overall reaction for the thermal decomposition of MCP can be represented by the following equation:

$$Ca(H_2PO_4)_2 \cdot H_2O \longrightarrow {}^{150^\circ C}Ca(H_2PO_4)_2 + H_2O \longrightarrow {}^{200^\circ C}CaH_2P_2O_7 + 2H_2O \longrightarrow {}^{300-500^\circ C}Ca(PO_3)_2 + 3H_2O \quad (1)$$

So, the results of thermogravimetric data analysis confirm that the synthesized product is calcium hydrogen phosphate monohydrate [8].

3.4. The Infrared Analysis of the Synthesized Product. The expected product contains some functional groups, and their presence in the sample can be confirmed by infrared spectroscopy measurement. The Fourier transformation infrared (FTIR) spectrum of the synthesized product is given in Figure 4.

The FTIR spectrum in Figure 4 is rather close to the one reported somewhere and confirms the presence of lattice water molecules as well as the phosphate species in the sample [17]. The observed bands can be assigned as follows:

The broad band centered at 3440, the small bands at 2350 and 1650, and the one at 670 cm^{-1} are assigned for the O-H stretching, the H-O-H rotation, bending, and rocking modes of the lattice water molecules in the monocalcium phosphate monohydrate, respectively.

The small band at 1200 cm^{-1} is ascribed for the P-O-H in-plane bending, and the bands at 950 and 1070 cm^{-1} are for the P-O stretching. The band at 850 and the one centered at 500 cm^{-1} are assigned for the P-O (H) stretching and bending, respectively.

3.5. The Particle Morphology of the Product. The particle morphology of the synthesized product is evaluated with scanning electron microscopy (SEM) measurement. The typical image measured at a magnification of 500 times is shown in Figure 5.

The results in Figure 5 show that particles with a parallelogram-like shape were obtained, which is in good agreement with the results of other work [8].

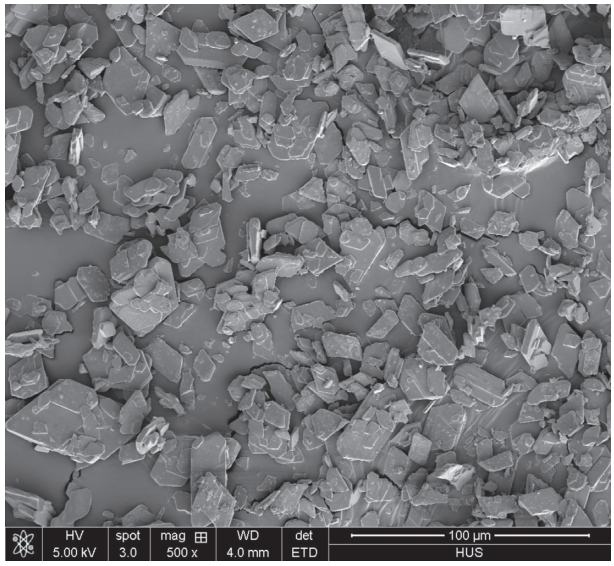

Figure 5: The SEM image of the synthesized product.

Most of the particles are rather small, with a size of about 10–20 μm in length, 5–10 μm in width, and a thickness of a few microns. Small particles may make the dispersion of the product become easier and form more homogeneous ingredients when it is used as feed additives.

4. Conclusions

Oyster shells sometimes are solid waste in mariculture or related food processing areas that face a major disposal problem in coastal regions. Oyster shell also contains a high content of $CaCO_3$ with low content of heavy metals, and it can be recycled and used as a secondary calcium source for the preparation of high-value materials such as feed additives like MCP. The transformation of oyster shell into PCC will remove certain impurities to obtain high quality starting materials for the preparation of MCP. The contents of the heavy metals such as As, Pb, and Cd in oyster shell, PCC, and the synthesized product are all extremely low, and the evaluated values are smaller than the EU maximum limits for feed additives. The synthesized product contains a pure phase of monocalcium phosphate monohydrate, $Ca(H_2PO_4)_2{\cdot}H_2O$, as confirmed by the XRD data, TG, or chemical analyses.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this study.

Acknowledgments

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Supplementary Materials

Figure 1: the powder X-ray diffraction patterns of the oyster shell (black lines) and the simulated ones of calcium carbonate in the calcite modification (blue lines) adapted from JCPDS No. 86-0174. (S1) The observed XRD powder pattern of the oyster shell is similar to the calcite form of $CaCO_3$. Figure S2: the X-ray diffraction patterns of oyster shell residue after digestion with nitric acid. The green vertical lines represent quartz (SiO_2, JCPDS No. 87-2096), and the red lines represent corundum (Al_2O_3, JCPDS No. 85-1337). (*Supplementary Materials*)

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Research Article

Valorization of Albedo Orange Peel Waste to Develop Electrode Materials in Supercapacitors for the Electric Industry

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This work proposes the use of albedo of orange peel in generation of carbon for applications in supercapacitors. For this, a comparison of compositional and electrochemical properties present in the carbons obtained of albedo, flavedo, and the complete orange peel was carried out. The morphology and composition of carbons obtained were analyzed by Field Emission Scanning Electron Microscopy (FESEM), Energy Dispersive X-ray (EDX), X-Ray Diffraction (XRD), and Fourier-transform infrared spectroscopy (FT-IR). The synthetized carbons were not subjected to the activation process by chemical compounds to relate only the properties of orange peel parts with their electrochemical behaviour. All samples were tested by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The carbon obtained of albedo presented a superior specific capacitance (210 F/g) of the rest samples. The value of albedo-based carbon capacitance is comparable with works presented in the literature that used a whole orange peel with chemical activators. In this way, it is possible to obtain large capacitances using only a part of orange peel (albedo). Thus, the importance of this study is that the albedo can be proposed as a material applied to electrodes for supercapacitors while the flavedo can be used in food industry or for oil extraction.

1. Introduction

A big percent of electricity generation in the world depends on fossil fuels. In Mexico, the power plants based on fossil fuels reached 79% of all installed plants in 2019 [1]. However, due to the depletion of fossil fuels, the countries are trying to deliver electricity based on other types of generation. So, the incorporation of alternative energies such as wind, solar, and hydrogen energy has been proposed to be incorporated on microgrids and grids of the electric industry. Even though the alternative energies are a good option, these have a random behavior that requires devices that store energy when it is excessive and deliver it when is necessary. The Energy Storage Systems (ESSs) mostly used are the batteries and supercapacitors [2].

The supercapacitor has the advantage of long cycle life and low cost, and its operation does not cause pollution [2]. Energy storage in supercapacitors is carried out using electrostatic charge accumulation or employing faradic reactions of electroactive species [3]. These devices are constituted by two electrodes (anode and cathode), an electrolyte, and a separator to isolate the electrodes [4]. Electrodes for supercapacitors based on activated carbon have been proposed due to their good electrical properties

and moderate cost and the exploitation of the valorization of organic waste [3]. In this way, several works have reported the synthesis of carbon obtained from organic products, such as sorghum pith [5], banana peel [6], and grapefruit peel [7], where these products are used completely to obtaining carbon with no opportunity to use another part of their waste for another use.

On the other hand, world orange production this year is expected to be approximately 49.4 million tons. According to the USDA, Mexico ranks 2nd in orange production with approximately 4,737 thousand tons per year [8, 9]. These are mainly used in the production of jams, flavoring, and juices. The high demand for orange for such products brings a high generation of waste consisting mainly of its peels that constitute 50–60% wt of the processed fruit [10]. Several works have proposed using whole orange peel to obtain activated carbon for energy storage applications in capacitors. These works highlight their carbon syntheses methods, such as hydrothermal carbonization and one-step [11, 12] and two-step pyrolysis [13, 14], as well as the use of various activators such as $ZnCl_2$ and KOH [11, 12], H_3PO_4 [15], and $(Cu_2(OH)_2CO_3)$ [12].

According to previous information, it is important to take into account that orange peel is composed of a soft white part called mesocarp or albedo (15–25%) and a bright orange outer pate known as flavedo (8–10%) [16]. Albedo is mainly constituted by pectin and 62.5% of cellulose and flavonoids. In comparison, the flavedo is formed by oil glands and pigments or carotenoids [17]. In the literature, works have been found that have used flavedo or albedo to elaborate material applied to supercapacitors [17, 18]. One of those, orange peel flavedo, was subjected to carbonization in an inert atmosphere and then activated utilizing HCl, HCl, and H_2SO_4 to improve its capacitance [17]. From these experiments, it was found that the highest capacitance obtained by CV (Cyclic Voltammetry) (92 F/g) was provided by H_3PO_4 [17], while in another work, the use of all orange components for the construction of a capacitor is proposed [18].

The whole orange peel was carbonized in a single step in the anode (186 F/g); hydrothermal carbonization was performed to the whole orange peel with MnO_2 to synthesize the cathode. At the same time, the albedo was used as a separator between the electrodes. Since few works report the use of orange peel albedo and flavedo and the composition of grapefruit peel is similar, studies of grapefruit albedo with good capacitive characteristics have been found focusing on the activator [19, 20]. Such is the case of [17], where an electrode based on grapefruit albedo doped with nitrogen and $CaCl_2$ was obtained, reaching 245 F/g, as well as the work in [20], where carbon for electrode through a carbonization process in two steps and KOH reaching 292 F/g of specific capacitance were was obtained.

From that mentioned above, it can be concluded that due to the abundance of orange in the world, several authors have proposed its whole peel for the production of carbon and use in supercapacitors [11–15]. Only few works have used flavedo of orange peel as a material for electrodes, focusing their attention on the utilized activators [19, 20]. However, no studies have been carried out on the capacitive behaviour of albedo. In this way, we propose to carry out a comparative study of the capacitive behavior of carbon obtained from whole peel, albedo, and flavedo focusing only on their qualities developed after of the carbonization process. The aim was to permit the use of the part with the best capacitances (albedo) in the industry of electric storage energy and the remaining in the food industry or obtaining oil.

2. Materials and Methods

2.1. Raw Material Preparation. The carbons used in this work were derivated from albedo, the flavedo, and the complete peel of orange (*Citrus sinensis* L.). The raw material (orange peel) was collected from local beverage businesses in Cd. Victoria, Tamaulipas, Mexico. The fruit pulp residue was discarded, while the raw material was washed with soap and water, and it was sun-dried for 48 h. Subsequently, half of the material was conserved whole, and the other half was separated into its albedo (external portion which confers color to the peel) and flavedo (soft internal portion) components. Following this, the three portions were cyclically washed with deionized water (Fermont mark, Mexico) and ethyl alcohol (Innovating Science mark, U.S.A.) three times [21]. The samples were left to dry at room temperature and dehydrated in a muffle at 130°C for 2 hours. Once dehydrated, the samples were stored until their use.

2.2. Preparation of Orange Carbon. The complete orange peel (OPC), albedo (AC), and flavedo (FC) were converted in carbon in a muffle by a thermal process without an inert atmosphere. During the process, oxygen limitation is made through crucibles arrangement and sand (composed of SiO_2 and CaO) which allows at 1000°C by 60 minutes. In this process, chemical activators were not used; this synthesis was realized by duplicated.

2.3. Physical and Morphologic Characterization. Morphology of OPC, AC, and FC was studied with a Field Emission SEM-Philips XL-30 (Eindhoven, the Netherlands) equipment. An SEM/EDX (Philips XL30 series) was used with 20 kV as working voltage using copper holders for the elemental composition. The crystalline structure was determined by X-Ray Diffraction (DRX) with a Philips-X'Pert Pro diffractometer (Eindhoven, the Netherlands); for these tests, a source of CuKα ($\lambda = 0.1542$ nm) at 40 kV 30 mA of operation and a goniometer speed of 0.12°/s was used. The functional groups obtained were studied by fourier-transform infrared spectroscopy (FT-IR) with a WQF-510A Rayleigh FT-IR spectrometer (Beijing, China) equipment at 4000 cm^{-1} to 400 cm^{-1}.

2.4. Electrochemical Behavior. The electrochemical tests (CV and EIS) were performed using a Gill AC (ACM Instruments, England) potentiostat through a three-electrode cell

with 1M of H_2SO_4 as the electrolyte. An Ag/AgCl electrode as a reference, a graphite electrode as a counter electrode and as a working electrode, and a vitreous carbon coated with 10 μL carbon prepared ink were used. The ink was prepared with a 5 mg mix of carbon from each sample (OPC, AC, and FC), 5 μL Nafion solution (Sigma-Aldrich) as a binder, and 5 mL of 2-propanol (Sigma-Aldrich) [22]. The cyclic voltammetry was carried out at speeds of the potential sweep of 10, 15, 20, 25, 30, and 35 mV/s [23–26] in a window potential of −0.2 mV to 1.4 mV [27]. The capacitance calculation through cyclic voltammetry was carried out with the following equation [13–28]:

$$C_p = \frac{\int idV}{2mv \cdot V}, \quad (1)$$

where $\int i\,dV$ is the integrated area under the curve of VC, m (g) is the mass of the carbon, $\Delta V\,(V)$ is the window of potential applied to the test, and v (Vs^{-1}) is the potential sweep speed.

Electrochemical Impedance Tests (EISs) were performed at frequencies from 100,000 [29] to 0.06 Hz using the same cell for CV and electrodes. The results for the elemental composition to the EDS and electrochemical tests are represented as the means of two measurements performed independently, but no statistical analysis was performed.

3. Results and Discussion

3.1. Physical Characteristics of Carbons. Photographs of OPC, FC, and AC are observed in Figure 1. The porous aspect of them and the difference in their brightness can be easily observed. Figure 1(a) shows the OPC with a little porous aspect and an opaque tone. A brighter and porous aspect is observed in the AC (Figure 1(b)) concerning the carbon obtained from the complete peel. The albedo (Figure 1(c)) allowed obtaining a more opaque and porous carbon with respect to FC and OPC, respectively. Manipulating these carbons was more difficult than in the FC, which fell apart when touched with the hands.

3.2. Morphology and Elemental Composition of Carbons. Figure 2 shows the SEM analysis of orange fractions; irregular micrometer-size flakes [7], pores, and small particles on the flakes can be observed on these micrographs. The darkest spaces present in all figures can be due to the porous structure of the obtained material. This structure type favors a better conductivity because they allow greater diffusion of ions, which in turn improves the electrochemical behavior [30]. In the samples of OPC are observed white particles attributed to calcium compound. All the samples present similar aspects; however, in the AC, clearer flakes and holes are more defined.

The results of EDS made to carbons are deployed in Table 1, where the presence of carbon in all samples is evident. Another element found in high percentage was calcium. The composition of the parts of orange peel and the formation of ash are attributed to the combustion of a part of the sample. It is observed that the highest concentration of carbon is found in AC, followed by FC and OPC. In addition, it is observed that the concentration of Ca is the highest in OPC, followed by FC and AC.

3.3. Formation of Compounds. In Figure 3, the compositional analysis of the OPC, AC, and FC by X-ray diffraction is shown. This analysis has corroborated the presence of carbon and calcium as in EDS analysis. The peaks located at 26.1° and 54.0° correspond to the planes (002) and (004) of pristine graphite, rich in carbon [31, 32]. The broad bands located between $2\theta \sim 23°$ and $2\theta \sim 43°$ are characteristic of the (002) and (100) planes of graphitized carbon [33, 34], which only is shown in the AC and FC. Also, the peak located at 23° simultaneously overlaps with the (112) plane of the calcite. Other calcite planes such as (104), (114), (110), (113), (202), (018), (116), and (122) are located at 29.4°, 32.8°, 35.9°, 39.4°, 43.2°, 47.3°, 48.3°, and 57.3°, respectively, according to the chart (ICDD PDF 00-005-0586) [35–37]. The peaks located at 20.6°, 34°, and 37.26° were attributed to $Ca(OH)_2$ (No: 164, PDF 00-004-0733)) [38, 39].

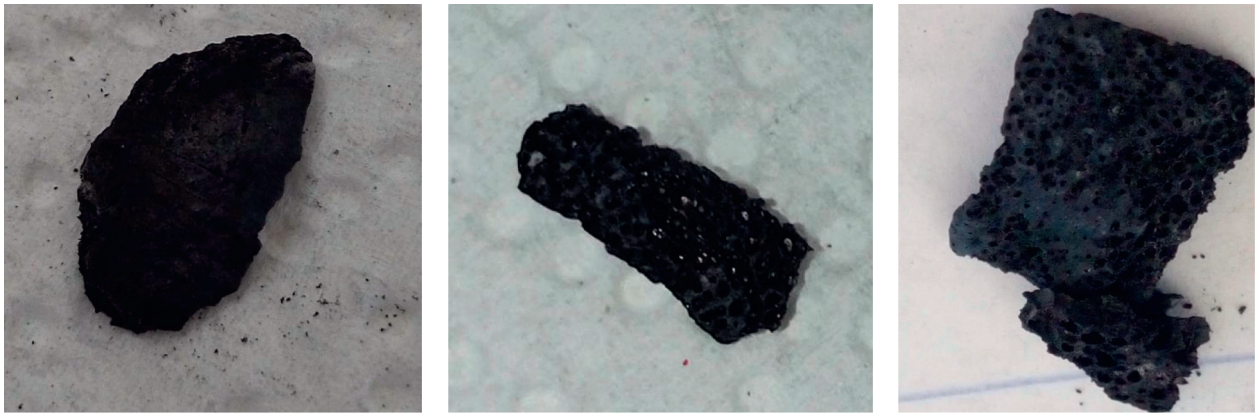
The formation of $CaCO_3$ is the product of the reaction of calcium oxide present in the orange peel, flavedo, and albedo and CO_2, reported at 930.4°C, which obeys equation (2), while the reaction of CaO present in the peels with the water steam generated at temperatures higher than 930.4°C causes the formation of $Ca(OH)_2$ obeying equation (3) [40].

$$CaO_{(s)} + CO_2 \longrightarrow CaCO_{3(s)}, \quad (2)$$

$$CaO + H_2O \longrightarrow Ca\,(OH)_2. \quad (3)$$

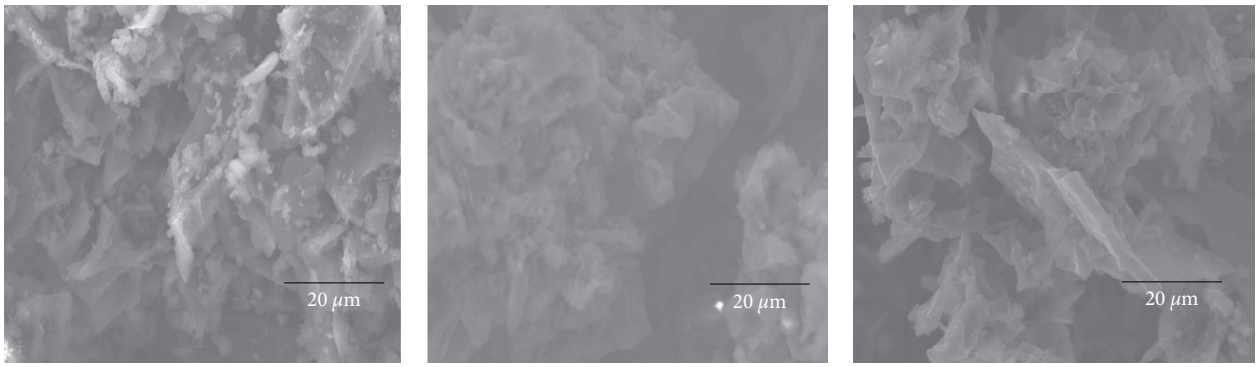
3.4. Functional Groups on the Surface. The spectra of FTIR analysis performed to OPC, FC, and AC are shown in Figure 4. An intense peak at 1447 cm^{-1} and 874 cm^{-1} representing carbonate ion vibrations was observed in all the samples [41]. The peaks located at 2362 cm^{-1} are attributed to contamination on the surface [42]. The peak at 708 cm^{-1} and the band located approximately 3421 and 3419 cm^{-1} correspond to O–H groups [43]. In addition to these peaks, for the OPC, short peaks situated at 2935 cm^{-1}, 2342 cm^{-1} contamination are identified. The peaks at 1242 cm^{-1} and 1041 cm^{-1} correspond to a C–O extension vibration [44, 45], while the peaks at 1731 cm^{-1} and 603 cm^{-1} are attributed to O–H groups.

In the FC sample, the peak localized at 2935 cm^{-1} and 2342 cm^{-1} and 1731 cm^{-1} are vaporized, while the peak localized at 3421 cm^{-1} is smoothed. In this spectrum, the decrease of peaks at 2362 cm^{-1} corresponding to CO2 is noted [42]. Additionally, the intensity of peaks at 1041 cm^{-1}, 603 cm^{-1}, and 874 cm^{-1} is attributed to C–O extension vibration [45], O–H group [43], and carbonate ion vibrations, respectively [41], which are increased. The light increasing at peaks located at 708 cm^{-1} reflects the participation of C–C as a simple bond in the AC sample,



(a) (b) (c)

Figure 1: Aspect of the obtained carbon according to its source of origin. (a) OPC, (b) FC, and (c) AC.



(a) (b) (c)

Figure 2: SEM images at 1000X of (a) OPC, (b) FC, and (c) AC at 1000°C.

Table 1: Elemental composition according to the EDS of orange peel, flavedo, and albedo.

Samples	C	O	Ca	Others
OPC	64.3	5.5	24	6.2
FC	66.73	5.64	23.14	4.5
AC	78	5.66	15	1.34

while the peak of 3419 cm^{-1} corresponding to O–H groups is smoothed [44], the peaks at 2363cm-1 corresponded to CO2 [42] and 1441 and 874 assigned to carbonate ion vibration are maintained as in the other samples.

The oxygen functional groups found on the surface of samples improve the capacitive capacity in materials for supercapacitors and are highly hydrophilic that absorb water easily [46]. In addition, pseudocapacitance is provided by these functional groups in an aqueous acid electrolyte, which ensures the long-term cyclability of the electrodes [24]. In contrast, the hydroxyl/carbonyl groups have shown to well provide capacitance [47].

3.5. Electrochemical Characteristics. Figures 5(a)–5(c) display the VC of OPC, FC, and AC at speeds of 5, 10, 15, 20, 25, 30, and 35 mV/s [24–26]. The potentials shown in the voltagrams were converted to RHE for convenience. All the VC obtained profiles have semirectangular shapes typical of carbon capacitors [48]. This semirectangular shape is maintained with minimal distortion at higher scanning speeds, suggesting rapid transfer of charges without restrictions attributed to a combination of meso- and micropores [49]. Furthermore, a rectangular-shaped distortion can be observed, attributed to the redox reactions originated by the oxygen functional groups. The

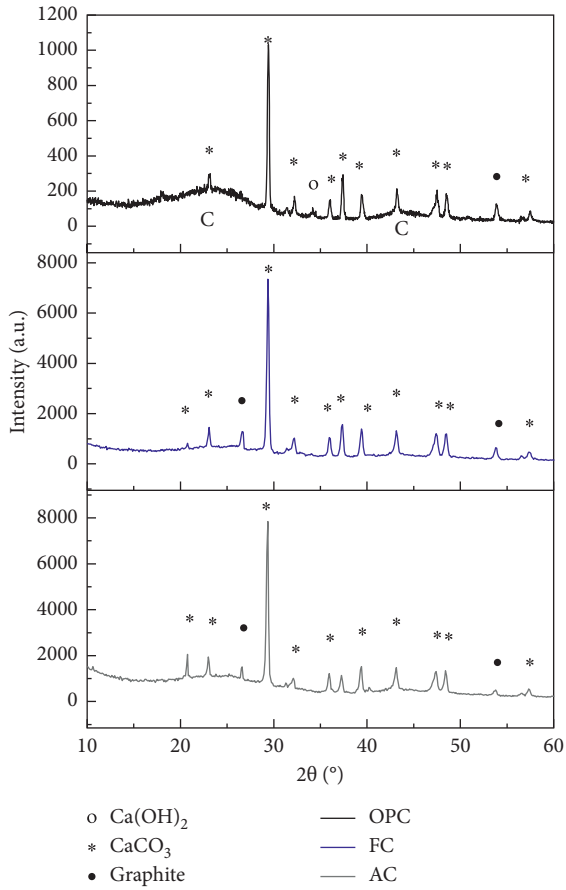


Figure 3: XRD patterns of orange fractions: orange peel, flavedo, and albedo.

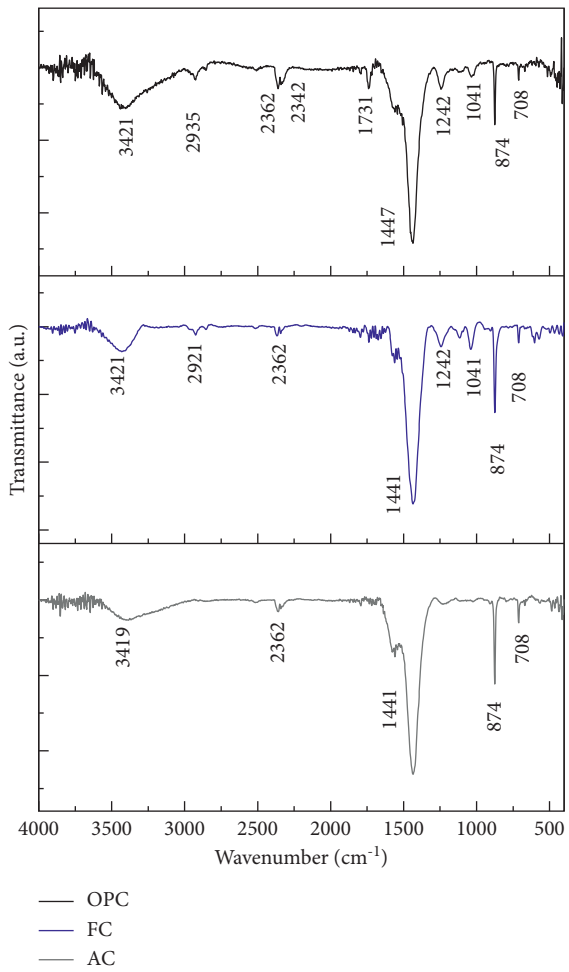


Figure 4: FTIR spectra of carbons based on orange peel (OPC), flavedo (FC), and albedo (AC).

capacitance presented in the material appears to be a contribution of the electrical double layer (EDLC) and pseudocapacitance [50]. The profiles remained above 1.1 V, indicating that the material in a device can be safely operated at that potential [13].

Figure 5(d) compares the voltagrams of the three materials studied at 10 mV/s. The gray line, belonging to the albedo, has a greater dimension of the curves, closely followed by the flavedo (black line) and well above the complete peel (blue line). A closer look at Figure 5(d) indicates more functionality as a capacitor material. The capacitances obtained at 10 mV/s were of 114.8 F/g, 188.7 F/g, and 210.8 F/g for complete OPC, FC, and AC.

Figure 6 shows the trend of capacitance values in the samples at speeds of 10 to 35 mV/s. The highest capacitance values were obtained in the albedo samples, followed by flavedo and much lower in the peel samples. It can be seen that, in the three samples, the capacitance tended to decrease as the speed of the voltage increased. It is observed that the difference between the minimum and maximum speed is approximately 12 F/g.

The calculated capacitances in the samples are within parameters shown at Table 2, where samples of orange peel flavedo and albedo of pomelo are presents, due to which albedo of orange peel has not been used for electrode capacitors. In this way, the importance of this work lies in the fact that there are no reports of obtaining carbon-based orange peel albedo without using activators as a material for anodes in supercapacitors, the proposed method for their production is economical and environment friendly, and the use of nitrogen and was not necessary for mentioning some advantages in comparison of this work, and the alternatives of these subproducts are generated in big amounts in juice industries.

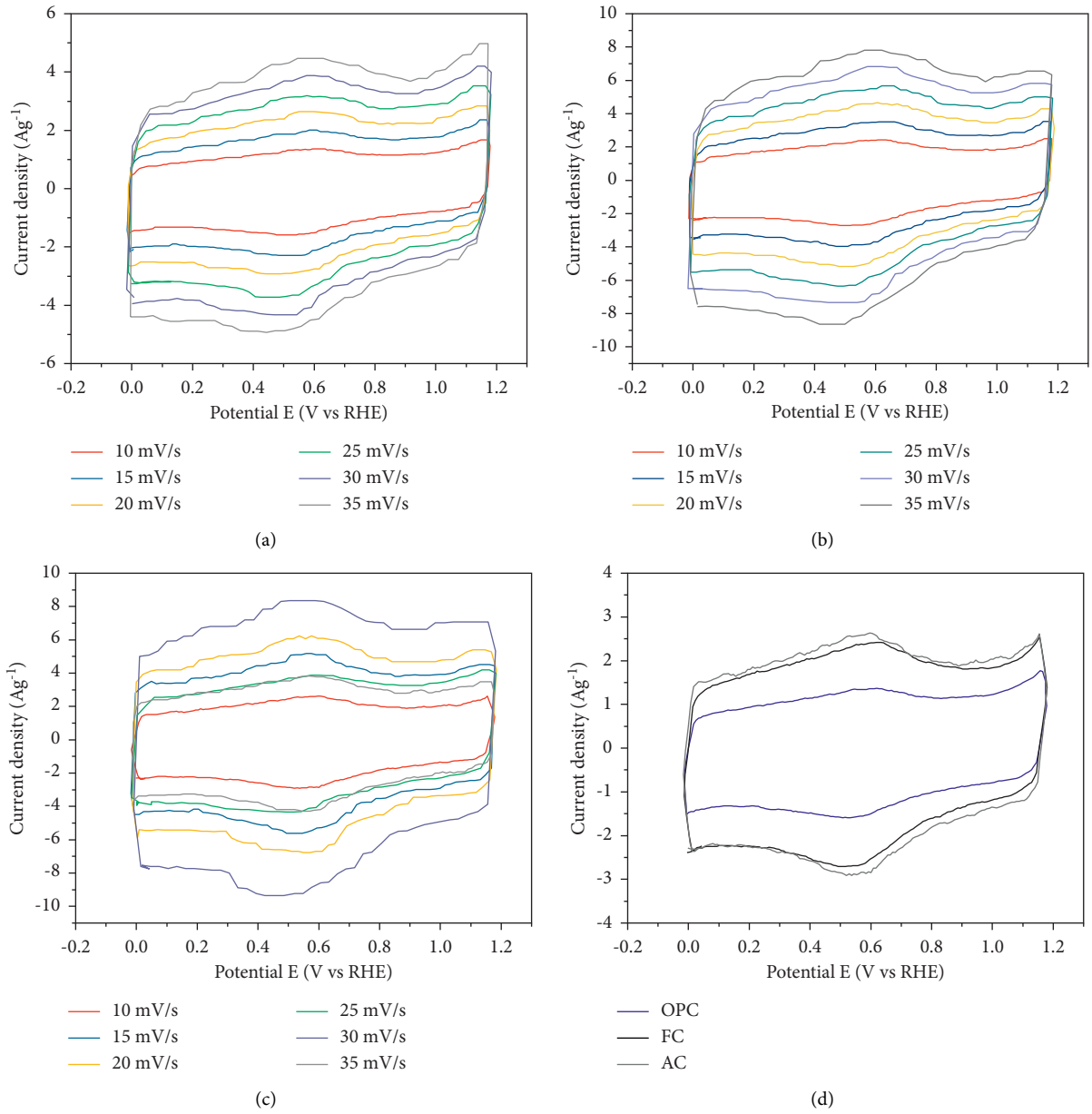


Figure 5: Cyclic voltammetry of (a) OPC, (b) FC, (c) AC, and (d) all samples at 10 mV/s.

The Nyquist diagrams generated from the EIS are shown in Figure 7. The diagrams of the three materials have a similar shape, with no defined semicircles in the high-frequency zone but curves with a tendency to be straight. In high-frequency areas, the absence of semicircles is characteristic of porous carbons, whose charge transfer resistance is minimal [51]. This behavior is related to the easy accessibility of the electrolyte and the electronic transfer through the wide pore fraction [52]. The degree of inclination of the lines is associated with ion diffusion [49]. The inclinations (slopes) of the peel and flavedo curves are close to 45°, while in the albedo, the inclination is close to 90°, which is characteristic of an ideal capacitor [53], which outlines it as the best material of the three studied for supercapacitor applications. The graph shows clearly that the AC presents a steeper slope, traduced as better behavior electrochemical for supercapacitors.

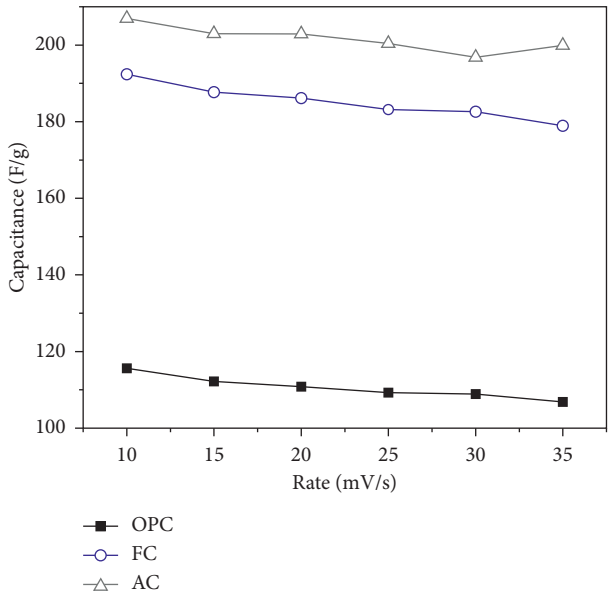


Figure 6: Graph of specific capacitance of OPC, FC, and AC concerning scanning speed.

Table 2: Capacitances of other works and this work about parts of orange peel.

Carbon source	Electrolyte	Specific capacitance (F/g)	Current density (A/g) or sweep velocity (mV/s)	Reference
Flavedo doped with N_2 and activated with H_3PO_4	0.5 mol/L H_3PO_4	92	30 mV/s	[17]
Flavedo doped with N_2 and activated with HCl	0.5 mol/L HCl	28	30 mV/s	[17]
Flavedo doped with N_2 and activated with H_2SO_4	0.5 mol/L H_2SO_4	88	30 mV/s	[17]
Albedo pomelo doped with N_2 PMC	2 mol/L KOH	245	0.5 A/g	[19]

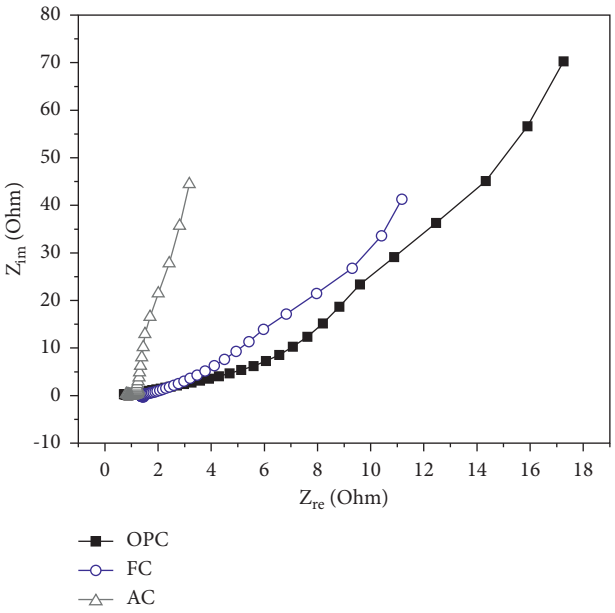


Figure 7: Nyquist diagram of OPC, FC, and AC.

4. Conclusions

This work compared the carbons synthesized from complete orange peel, albedo, and flavedo for applications in supercapacitors. SEM tests show that all carbons obtained from the complete peel, albedo, and flavedo showed characteristic rigid and porous structures. The material presented calcium carbonate incrustations from the pyrolysis reactions obtained at high temperatures. The structural analysis and electrochemical behavior showed that the three types of carbon presented capacitance properties, associated with the rapid diffusion of ions and low charge transfer resistance, attributed to their crystalline structure. The carbon obtained from the albedo showed the best properties as a capacitor.

Data Availability

Data are available on request.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

Acknowledgments

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Review Article

Potential Use of Industrial Cocoa Waste in Biofuel Production

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Worldwide, the wastes derived from food production are generated in elevated volumes annually. In particular, the cocoa industrial wastes represent a source of usable biomass for the elaboration of new products such as food, livestock feed, cosmetics, and chemical products, and they can even be used for the generation of biofuels. The cocoa industrial wastes include cocoa pod husk, mucilage, and bean shells, which contain compounds of interest for different industries. However, the lignocellulose content of these by-products requires a pretreatment to fully utilize them; thus, different biofuels can be produced, depending on the conversion technology used to obtain the highest biomass yield. Recent studies reported the use of cocoa industrial wastes for the production of solid, liquid, and gaseous biofuels; nevertheless, the most common use reported is as a direct combustion source, which is used to supply the same production plants. Therefore, the objective of this work is to carry out a review on the uses of the by-products generated from cocoa for the generation of biofuels, as well as the technological concept applied for the transformation. In addition, the future trends indicate the relevance of using catalysts in production to increase reactions in the conversion of compounds, including the use of statistical models to optimize the processing variables.

1. Introduction

The wastes resulting from the agriculture industry represent 50% of the fresh weight of the total harvested production; in energy terms, this is equivalent to the potential of 90 million tons of oil [1]. In addition, the growing world population demands a greater amount of energy; thus, the shortage of nonrenewable resources is imminent. In addition, the generation of industrial wastes and pollutants represents an ecological and socioeconomic challenge for the population [2]. Therefore, the use of wastes is essential to reduce the environmental impact and create an economic model based on the production of biocomposites [3].

In order to face this environmental problem, the application of the biorefinery concept is a promissory strategy; a biorefinery is defined as the sustainable synergistic processing of biomass on marketable food ingredients, products, and energy [4]. To carry out this process, it is necessary to guarantee the availability of wastes over time, the technical and economic capacity of their production, and the environmental impact produced during the generation cycle [5]. In this sense, residual biomass sources from industrial processing with the potential for the production of biofuels are explored.

In particular, cocoa (*Theobroma cacao* L.) is an umbrophilic crop of great importance in the world due to its applications in the food, cosmetic, and pharmaceutical industries. Cocoa is a tree native to Central and South America; however, the highest production is concentrated in areas with a tropical climate in Africa, Asia, and South America [6–8]. In 2017, the world production of cocoa pods was estimated at 4.2 million tons [9]. Meanwhile, cocoa industrial wastes were estimated at approximately 85% of world cocoa production; these wastes are cocoa pod husk (CPH), mucilage (CM), and bean shell (CBS) [10]. For the cocoa-producing industry, these wastes are a serious problem as they represent economic losses and environmental pollution [6]. In Figure 1, the wastes obtained from the cocoa fruit are shown.

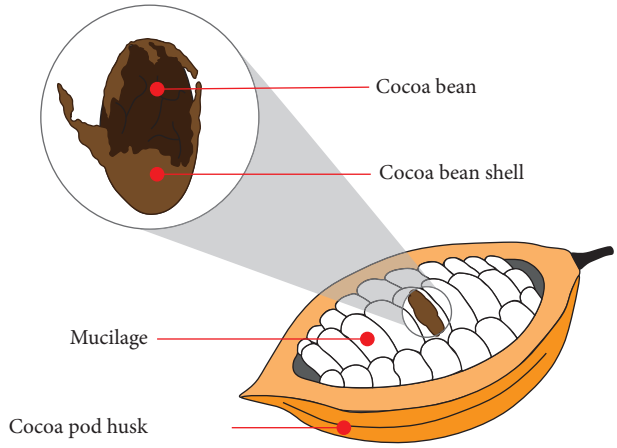

Figure 1: Cocoa industrial wastes.

The whole cocoa pod is a rich source of chemical compounds; however, the beans (main raw material) are the source with the highest content of fat, phenolic compounds, and alkaloids such as theobromine and caffeine [11]. Nevertheless, when the wastes are discarded in the first instance without treatment, they preserve different compounds that can be used to obtain other products through the biorefinery processing scheme [5].

Cocoa wastes contain lignocellulose, which can be used for the production of biofuels; however, it is necessary to carry out a pretreatment to degrade the structure and the fermentable compounds that can be used in a higher proportion. There are different types of pretreatments classified by their nature into chemical, physical, biological, and physicochemical; the choice of pretreatment will depend on the type of biomass and technical and economic aspects in the production of biofuel [12].

In order to reduce the generation of waste in agribusiness, the circular economy seeks options to transform these by-products into sources of bioactive compounds or for the generation of other products [13], as ingredients for food, livestock feed, or as a source for the development of biochemicals, biomaterials, and biofuels [14]. Table 1 shows the chemical composition of cocoa wastes.

The development of technologies that allow an adequate recovery of these wastes has become an economic advantage since it allows achieving the objective of zero waste in the cocoa industry [13].

The amount of cocoa wastes generated each year by the chocolate industry represents a severe health problem in the plantations, as well as an ecological damage, due to the amount of residual organic matter. Thus, the use of wastes is a contribution to minimizing the damage caused in an agroecosystem. Therefore, the objective of this work is to carry out a review on the use of the wastes generated from cocoa for the generation of biofuels, as well as the technological concept applied for the transformation.

2. Basic Concepts

Lignocellulose is an important part of plants; it is mainly found in herbs, trees, stems, husks, and flowers. The main source of lignocellulosic materials is residues from the forestry and agricultural industries. This material is of great importance due to its potential for energy production, which is derived from the composition of the heteromatrix, made up of cellulose, hemicellulose, and lignin that vary in quantity depending on the biomass of origin. [24]. In Table 2, the lignocellulose composition of the cocoa wastes is shown.

Cellulose is the most abundant organic polymer in the world, and it is present in the cell wall of plant cells [30, 31]. However, the proportion depends on the type of biomass, and its range is between 30 and 60% [32]. Cellulose is a linear and crystalline structure formed by microfibers that are linked by hydrogen bonds [33]; this formation is due to the long chains of glucose with approximately 10,000 units, linked together by β-1,4-glucosidic bonds [34, 35].

On the other hand, a hemicellulose is a group of branched polysaccharides that connect cellulose with lignin [30]. Hemicellulose is made up of hexoses (glucose, galactose, and mannose) and pentoses (xylose and arabinose), along with other compounds in smaller quantities [34]. These compounds are linked by β-1,4-glucosidic and sometimes by β-1,3-glucosidic bonds [33]. The main differences between cellulose and hemicellulose are the degree of polymerization, the branching in the main chain in hemicellulose, and the weaker structure in the face of chemical agents [31]. The proportion of this material in the biomass ranges between 25 and 30% [36].

Finally, the lignin is a cross-linked aromatic phenolic polymer made up of phenylpropane units, responsible for the structural rigidity in the outer fibers in plants [30, 33]. This polymer is found in different proportions depending on its origin (softwood, hardwood, or agricultural residue), which can be between 10 and 35% [31]. The three main units that make up lignin are p-coumaryl, coniferyl, and sinapyl alcohol [34]. In Figure 2, the composition of lignocellulose with the structure and interaction of cellulose, hemicellulose, and lignin is shown.

3. Cocoa Industrial Wastes

3.1. Cocoa Pod Husk. Cocoa pod husk (CPH) is the external part of the fruit; therefore, it is the main by-product generated from the processing of cocoa, and it represents 75% of the total weight [19]. For every ton of cocoa beans, an estimated 10 tons of wastes are generated, which are generally considered of none economic value [37]; this waste is generated after harvest, when pulping the fruit and recovering the grains covered by the mucilaginous layer [38].

CPH is made up of three layers, which are the endocarp, mesocarp, and epicarp; however, there are few specialized studies on the use of a part of the cocoa pod [39]. Normally, the most common use of the complete by-product is as fertilizer for the soil of the agroecosystem; nevertheless, there are phytosanitary problems such as pests and fungal diseases when using the CPH without treatment [19].

The chemical composition of CPH allows using this waste for different purposes. Biofuel production is possible using CPH, due to its lignocellulose content [19]. Other compounds present in CPH are fiber, phenols, and minerals such as Ca, K, P, Mg, Na, Zn, Fe, Cu, and Mn [21, 40, 41].

Table 1: Chemical composition of cocoa wastes.

Waste	Moisture (%)	Ash (g/100 g)	Protein (g/100 g)	Fat (g/100 g)	Carbohydrates (g/100 g)	References
CBS	7.05	7.09	15.59	3.00	65.58	[15]
	7.7–10.1	7.3	15–18.1	0.66	17.8	[16]
	7.7	7.3	15	2.02	17.8	[17]
	3.6–13	5.96–11.4	10.3–27.4	1.5–8.5	7.8–70.2	[18]
CPH	6.4–14.1	5.9–13.0	2.1–9.1	5.9–13.0	17.5–47	[16]
	—	6.4–8.4	7–10	1.5–2	32–47	[19]
	—	11.37	11.27	7.15	46.6	[20]
	80.2	9.1	5.9	1.2	57.6	[21]
CM	92.7	7.51–7.58	5.47–5.56	1.91–192	67.99–68.35	[16]
	—	3.7–7.68	0.41–5.56	1.91–354	10.7–68.35	[6]
	80.55	9.34	5.12	0.46	52.43	[22]
	86.38	0.36	0.62	1.45	52.02–70.01	[23]

CBS = cocoa bean shell; CPH = cocoa pod husk; CM = cocoa mucilage.

Table 2: Lignocellulose and pectin content in CPH.

Compound	References				
	[25]	[26]	[27]	[28]	[29]
Cellulose (%)	29.93	23.04	33.02	23.8	32
Hemicellulose (%)	10.94	38.08	37	8.2	9.9
Lignin (%)	11.64	18.19	23.75	33.4	29.5
Pectin (%)	—	—	3.71	6.9	—

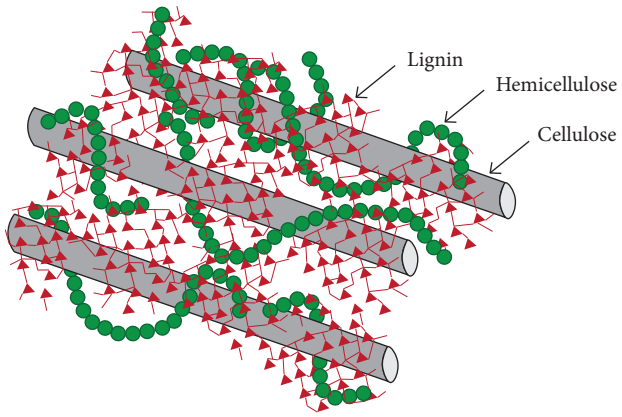

Figure 2: Structure of lignocellulose and interaction between cellulose, hemicellulose, and lignin.

3.2. Cocoa Mucilage. Cocoa mucilage (CM) is the whitish substance that covers the grain; other names as it is known are sweating or pulp [16]. The cocoa pulp juice is a fraction of CM, with a cloudy whitish appearance that is obtained after processing cocoa by means of handling and exerted pressure; this part of CM is widely used in obtaining products such as alcohol, vinegar, soft drinks, citric acid, and cocoa jelly [42].

The generation of CM is the result of the fruit pulping process; after splitting the cocoa pod in half, the beans are extracted with the mucilaginous layer. Immediately, they undergo a controlled fermentation where biochemical reactions are generated that allow the separation of the grain and the mucilage [43]. The amount of mucilage depends on the type of cocoa, maturation, and physical integrity of the fruits, which is reported between 3 and 5% of the total weight of cocoa [44].

CM is composed of spongy cells that contain cellular sap rich in different chemical compounds, containing 10–14% fermentable sugars (such as sucrose, glucose, and fructose), minerals (such as potassium, sodium, calcium, and magnesium), pectin, pentosans, citric acid, salts, cellulose, hemicellulose, and lignin [16, 42, 45].

3.3. Cocoa Bean Shell. Cocoa bean shell (CBS) is a lignocellulosic material obtained from the bean roasting process, and it constitutes 10–17% of the total weight of cocoa beans, although it may vary due to the fermentation process [18]. The importance of this waste lies in the migration of valuable compounds from the grain to the husk during fermentation [46]. The nutritional quality of CBS allows the generation of new products in the industry; in particular, the food, pharmaceutical, cosmetic, and agricultural industries have developed functional products based on this waste [47]. Moreover, CBS contains polysaccharides, phenolic compounds, methylxanthines [14], catechins, epicatechins, procyanidins, caffeine, theophylline, theobromine, and fiber [48–51]. Regarding energy, the potential content has been reported as an ecological source of alternative energy due to its calorific value, slightly higher than wood [6].

4. Biomass Conversion Technologies

The interest in the use of residual biomass from industrial processing has increased in the last decade since the quality of chemical compounds that conserve wastes can be used for the generation of new products with the application of different treatments [52]. The use of biomass in the production of biofuels is essential to reduce the consumption of fossil resources; this determines the development of an economy based on products of biological origin. For this reason, the type of biorefinery process will obtain the highest waste management in the agricultural industry [53].

The biorefinery can be classified by the system or model that is divided into seven categories, according to the type of biomass and technology used, which are conventional biorefinery, green, complete cultures, lignocellulosic raw material, the concept of two platforms, thermochemical, and marine [54]. The biorefinery is a process used for the

generation of different energy products with the application of different technologies for the transformation of biomass [55].

4.1. Physicochemical Conversion. Physicochemical conversion, also known as mechanical extraction, is used to produce liquid biofuels from the seeds of different foods [56]. The main sources used are sunflower and rapeseed; however, used cooking oil, animal fats, and seaweed oil are also used [57]. The oil can be subjected to an esterification or transesterification treatment to produce biodiesel, in which the intention is to transform the triglycerides present in the crude oil into fatty acids and glycerol [58]. In addition, solid waste is generated used for the manufacture of livestock feed [56].

4.2. Biological Conversion. Biological conversion involves microbial action for the decomposition of organic matter [59], and the objective is to produce liquid biofuels and biogas [57]. The most widely used microorganisms are yeasts; however, the use of fungal bacteria and specialized enzymes produces different products of value [58]. This type of technology is adaptable to different biomass sources; therefore, it is a widely used technique around the world [56]. The most relevant processes are fermentation and anaerobic digestion [59].

4.2.1. Anaerobic Digestion. Anaerobic digestion transforms the organic material into biogas, which consists of methane and carbon dioxide and small amounts of hydrogen sulfide [56]; the mechanism of action is activated in the presence of bacteria in a medium lacking oxygen [59]. This process is carried out in biomass with high moisture content (80–90%) [56]. A series of key actions in anaerobic digestion are identified where complex biomass compounds are first hydrolyzed into simpler compounds [57]. Then, acidogenesis occurs that transforms simple compounds into volatile fatty acids, H_2, and CO_2 [59]. Finally, methanogens convert organic acids into CH_4 gas and CO_2 [58].

4.2.2. Fermentation. Fermentation is an anaerobic biological process carried out by yeasts [56], where the goal is to convert sugars like glucose, fructose, and sucrose into ethanol and CO_2 [59]. The sources most used in the generation of bioethanol are sugar cane and corn; however, in Europe, wheat and beet are used more frequently [56]. Biomass can be divided into three types: sugars, starch, and lignocellulosic materials [57].

The most common application is the action of yeasts for the fermentation of starch and generation of ethanol; nevertheless, the use of enzymes for the degradation of cellulose increases the production of ethanol [59]. Recent studies focus on the possibility of applying pretreatment to break the lignocellulosic structure of biomass of forest and agricultural origin for the production of ethanol by fermentation [56]. In addition, glycerol and carboxylic acids can be obtained as by-products from this process. However, the quality and yield will depend on the type of biomass used and other process factors such as temperature, time, and pH [57].

The intention of the production of bioethanol is the gradual replacement of fuels of fossil origin; at present, mixtures of ethanol and gasoline are made (80–20%, respectively) [56]. On the other hand, solid waste after ethanol production can be used as boiler fuel or livestock feed [57]; generally, other treatments such as liquefaction, gasification, or pyrolysis can be used to produce other valuable products [58].

4.3. Thermochemical Conversion. Thermochemical conversion transforms the biomass by breaking bonds for the production of energy, biogas, and bio-oils [58]. The type of biomass must have low moisture content, but high values of organic matter [59]. In addition, the pyrolysis process is considered the basis of thermochemical treatments, due to the formation of solid, liquid, and gaseous residues that are generated [56].

The types of thermochemical treatments are combustion [59], gasification [56], pyrolysis [57], and liquefaction [60]. The choice of the type of treatment depends on different factors such as biomass quality and industry specifications, which include final application, environmental impact, and financial aspects [58]. Industrially, thermochemical processes are more efficient compared to biological processes, in relation to the reaction time and transformation of organic compounds [56].

4.3.1. Combustion. The combustion process is defined as the burning of fuel to release the energy contained in the form of heat of reaction [61]. In this context, combustion converts the chemical energy of biomass into thermal energy [59]; this process generates approximately 90% of the energy obtained through biomass [57]. Combustion transforms carbon into CO_2, while hydrogen into H_2O. Other compounds such as sulfur convert to SO_2 and nitrogen to NO_x [60].

Thermal degradation of biomass occurs in different steps [59]:

(i) Drying of biomass and release of highly volatile materials at a temperature between 25 and 125°C

(ii) Release and burning of volatile materials between 125 and 350°C

(iii) Residual carbon oxidation and burning of volatile materials between 350 and 525°C

4.3.2. Gasification. Gasification is an oxidation process that converts biomass into a gas through the action of a gasifier [59]. When the treatment is carried out by high temperatures above 1200°C, the result is known as synthesis gas composed of H_2 and CO; on the other hand, at low processing temperatures, the product includes CH_4 and CO_2 with impurities of other compounds [56]. The resulting biogas has a calorific value of 4.5–6 MJ/m^3; however, this value depends on the type of biomass from which it comes. The calorific

value of biogas represents 10–50% of the calorific value compared to natural gas [57].

The resulting biogas can be used directly; nevertheless, this product can be transformed, through the application of different processes, into other types of fuels and chemical products [61]. The thermochemical process is more efficient compared to combustion and pyrolysis; in addition, it has been reported that gasification is the best process to generate hydrogen gas from biomass [58].

4.3.3. Pyrolysis. Pyrolysis consists of the application of specific temperatures for the transformation of biomass into solid, liquid, and gaseous products [57]. The types of resulting products are H_2, CH_4, CO, and CO_2 as gaseous compounds, for liquids with tar and oils, as well as solid compounds [58, 61]. This process is carried out in a temperature range of 400–800°C without the presence of oxygen. The quality and performance of the resulting biofuel depend on factors such as time, temperature, biomass composition, and process specifications; forestry and agricultural residues are suitable biomass for the production of biofuels, as a product oil with a calorific value of 38 MJ/kg is obtained [59].

The main application of the pyrolysis process is to obtain charcoal; however, the production of liquid biofuels has increased in the last decade at temperatures of approximately 500°C [56]. The classification of the pyrolysis process is based on the applied temperature range; carbonization occurs between 300 and 500°C, slow pyrolysis around 500°C, fast pyrolysis between 500 and 650°C, and finally, flash pyrolysis at temperatures above 650°C [57].

4.3.4. Liquefaction. Liquefaction is also called hydrothermal liquefaction [56], and it is carried out in an aqueous medium with the application of temperature between 280 and 370°C and high pressure of 10–25 MPa [57]. This type of technology can be used for different types of biomass; nevertheless, algae residues are ideal because this technology omits the drying of the biomass, compared to the pyrolysis process, which means significant savings in energy consumption [61].

The main product obtained from the liquefaction process is bio-oil, which has a calorific value of 30–35 MJ/kg, which can be transformed into different biofuels [62]. In this sense, the liquefaction process involves repolymerization reactions for the transformation of bio-oil, chemical products, solid waste, and gas [58].

5. Biofuels from Cocoa Industrial Wastes

Biofuel is defined as a solid, liquid, or gaseous fuel mainly generated from biomass [63]. These can be classified into first-, second-, and third-generation biofuels. Those of the first generation are obtained from food sources such as sugar, vegetable oils, and starches, while those of the second are those of lignocellulosic biomass and those of the third are those that are produced from aquatic materials such as algae [63]. An advantage of biofuels is that they produce less emissions of SO_2, NO_X, and soot than conventional fossil fuels since they have a minimum content of sulfur, nitrogen, and ash, which represents an advantage when using them [64]. In Figure 3, the general cycle for obtaining biofuels from cocoa industrial wastes is shown.

5.1. Biochar. Biochar is produced with the transformation of biomass by thermochemical treatments [65]. This change produces a solid residue, along with a bio-oil and a gas composed of hydrogen, carbon oxides, and hydrocarbons [66]. The use of CPH in the generation of biochar uses temperatures between 400 and 800°C; however, alternatives to the process with high temperatures are sought because the biochar produced at 500°C presents high pH and decreased performance in general [67].

5.2. Bioethanol. Bioethanol is obtained from the fermentation of sugars by the action of yeasts. In general, starch-rich raw materials are used. The use of lignocellulosic material is possible through a pretreatment that hydrolyzes cellulose into glucose to generate fermentation [68]. The demand for bioethanol has increased the development of technically and economically feasible procedures; these are based on the use of biomass or a fraction of it [69]. Obtaining bioethanol is possible using different raw materials; therefore, first-, second-, and third-generation bioethanol is obtained [70]. Cocoa residues are a good source for the production of bioethanol because the different by-products contain various chemical compounds.

5.3. Biogas. Biogas is a renewable fuel that is produced from the anaerobic digestion of organic material (such as waste and residues from agriculture and the food industry) [71]. During this metabolic pathway, organic carbon is converted through oxidation-reduction reactions into 30–50% carbon dioxide (CO_2, its most oxidized state) and 50–70% methane (CH_4, its lowest form) [72]. Biogas can also contain 0–3% nitrogen, 5–10% water vapor, 0-1% oxygen, and some others corresponding to less than 1%, such as hydrogen sulfide, ammonia, hydrocarbons, and siloxanes. [73]. This fuel can be used to produce electricity, heat, or as a fuel for vehicles, with environmental, climatic, and economic benefits [71].

5.4. Biohydrogen. Biohydrogen is a sustainable fuel produced in its economical form by microbes through fermentation [74]. However, this product can be obtained from different biomass and with various thermochemical processes [75]. There are some complications to producing biohydrogen on a large scale and commercially; the alternatives to increase production efficiency are based on the application of pretreatments to augment the bioavailability of simple sugars in biomass [76]. Biohydrogen is considered the most ecological fuel; the waste from its processing is water vapor; its calorific value (142 MJ/kg) is higher than the value of methane, natural gas, and even gasoline [77].

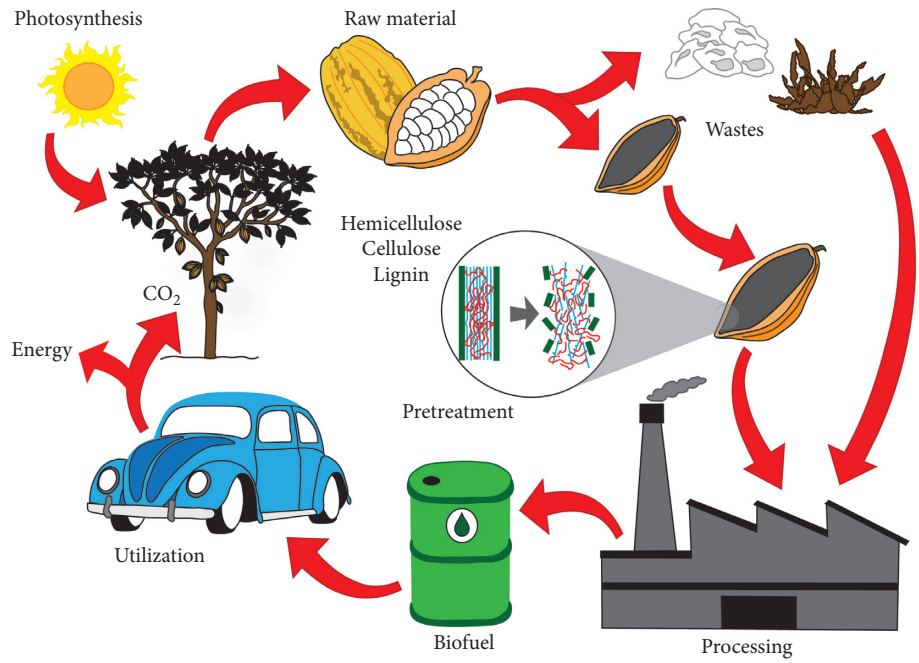

Figure 3: General cycle of obtaining biofuels from cocoa industrial wastes.

5.5. Bio-Oil. Bio-oil is a mixture of organic compounds obtained from the pyrolysis of biomass. Among these compounds are sugar monomers and oligomers, as well as sugar derivatives, such as carboxylic acids, aldehydes, ketones, esters, and alcohols [78]. The abundant presence of carboxylic acids makes the bio-oil corrosive. The water content ranges between 20 and 30% depending on the nature of the biomass, which reduces its calorific value and viscosity; the main applications of bio-oil are low-quality boiler fuel, high-quality car engine fuel after upgrade, sources of value-added chemicals, functional carbon materials, and binders [79]. Some researchers develop methods to upgrade bio-oil into biofuel through hydrodeoxygenation, as well as studying strategies based on biomass pretreatments such as dry torrefaction, wet torrefaction, acid and alkaline treatment, steam explosion, downstream treatment of bio-oil such as emulsification, solvent addition, or filtration [80].

5.6. Liquid Smoke. Liquid smoke is a liquid product that is generated from the decomposition process of organic waste or from the condensation of smoke generated in the burning of biomass [81]. This liquid can be extracted from biomass by rapid pyrolysis. This process is carried out at a medium temperature and with high biomass flows [82]. Rapid pyrolysis of biomass is a thermochemical conversion process to produce biofuels. During this process, heavy tar, light tar (liquid smoke), charcoal, and uncondensed gases are produced [83]. Liquid smoke is also known as wood vinegar, pyrolysis liquid, pyrolysis oil, bio-crude oil, biofuel oil, pyroligneous tar, pyroligneous acid, wood liquid, and wood oil [82]. Table 3 shows studies of the production of biofuels from cocoa by-products.

6. Future Trends of Biofuels from Cocoa By-Products

The trends for the generation of fuels from cocoa lignocellulosic biomass explore the alternatives to use catalysts that accelerate and increase the reactions in the conversion of compounds used in the process. This is because recent studies determine that the use of catalysts produced from agricultural residues is an economically and ecologically feasible alternative [96]. The most promising proposals are based on the use of alkaline, acid, and enzyme catalysts that simplify operations by easily recovering from the mixture [97]. Other aspects to consider in the future are the optimization of the pretreatments for the variables of the process; some investigations use the statistical method of response surface to determine the precise conditions in obtaining biofuels [98].

Some aspects to be resolved in the future are the modernization of the technology used for the conversion and treatments of biomass; these include aspects such as temperature control, industrial capacity, and specialized equipment [63, 99]. In addition, obtaining an adequate and continuous supply of material as a source for the production of biofuels is a particular issue; each time they gain greater relevance due to the development of economies based on this activity, which, in turn, generate new policies, minimize the effects negative to the environment, and avoid biological risks in agroecosystems [100].

The main problem in the production of lignocellulosic biomass biofuels is the staggering at the industrial and commercial level; this is due to the type of technology to be used for the different residues generated from the agroindustry. However, this problem has been addressed by different investigations in recent years with the implementation of strategies to optimize the generation of fuels from lignocellulosic biomass [101].

Table 3: Biofuels generated from cocoa industrial wastes.

Product	Biomass	Process	Results	Reference
Biochar	CPH	Low-temperature pyrolysis with residence times of 30–120 minutes in 30-minute intervals.	Biochar with a calorific value of 17.8 MJ/kg with high potassium content. The resulting biofuel has characteristics similar to lignite.	[40]
Biochar	CPH	Pyrolysis at temperatures of 250, 300, and 350°C. Activation process with HCl to reduce the values of free fatty acids to use cooking oil used in the production of biodiesel.	The activated carbon generated from CPH showed better retention of free fatty acids than the esterification treatment with H_2SO_4.	[84]
Bioethanol	CPH	Fermentation using *Zymomonas mobilis* in a period of 0–8 days. The percentages of microorganisms added were 8–16% v/v.	An alcohol graduation of 10.62% was obtained under 8 day conditions with a concentration of 14% v/v.	[85]
Bioethanol	CM	Fermentation using *Pichia kudriavzevii* for 5 days at a temperature of 30°C.	The result shows that the maximum ethanol concentration was 13.8 g/L.	[86]
Bioethanol	CM	*Saccharomyces cerevisiae* was used for fermentation for 12 days.	The bioethanol production was 4.85%; it is reported as a low concentration for the amount of mucilage.	[87]
Bioethanol	CBS	Fermentation with variation in the amount of yeasts during 6 days of the process; hydrolysis pretreatment with H_2SO_4 at temperatures of 30–80°C in periods of 50–150 minutes.	8.46% bioethanol was produced under pH 8 conditions, with a yeast concentration for fermentation of 0.05 mg/g in 6 days of the process. pH was determined to be the main influencing factor.	[88]
Biogas	CPH	Fermentation process with the application of four pretreatments, acid (H_2SO_4), alkaline (H_2O_2), ground without treatment, and the last one not ground without treatment.	The biogas yields by pretreatment were acid 162.8, alkaline 564.8, ground without treatment 220.8, and unground without treatment 243.3; the highest production was obtained on day 18 of the process.	[89]
Biogas	CPH	The aerobic digestion process together with a hydrothermal pretreatment was used at different temperatures (150–220°C) in times of 5–15 minutes.	The untreated CPH biogas production was estimated at 357l (N)/kgVS, while the biogas production from pretreated biomass was 526.38L(N)/kgVS at 150°C for 15 minutes.	[90]
Biogas	CM	Fermentation process for 25 days with a pretreatment of a NaOH and NaOH-H_2O_2 solution.	The biogas produced was obtained in a concentration of 66.07% with a yield of 0.734 m^3 CH_4/kgVS.	[45]
Biohydrogen	CM	Fermentation process at two temperatures 35°C and 55°C; the organic load of volatile compounds 4, 8, and 12 gVS/L was determined.	The amount of gas produced daily was measured. The reaction was faster at 55 °C, the generation of the product was from the fifth day. The load was determined in 12 gVS/L and the hydrogen production of 703 mL.	[91]
Biohydrogen	CM Pig manure Coffee mucilage	Fermentation of the biomass in a load of 10 gVS/L at a temperature of 35 °C at a pH of 5.5 in a period of time of 12 days.	The amount of biohydrogen produced was 3674.021 mL, which is equivalent to 91.85 mL H_2/gVS.	[92]
Biomethane	CBS Rice straw Hazelnut skin	Fermentation for the production of biomethane from lignocellulosic material with a pretreatment with N-methylmorpholine-N-oxide at a temperature of 120°C for 3 hours.	The effect of the pretreatment was compared against an untreated control sample, in which the biomethane production increased from 199 to 226 ml of CH_4/gVS. This effect was more relevant for rice straw with an 82% increase.	[93]
Bio-oil Biogas	CPH	Pyrolysis at different temperatures from 300°C–600°C in 100°C steps	Bio-oil with a calorific value of 36.23 MJ/kg with characteristics similar to diesel. Biogas with a calorific value of 35.24 MJ/kg presented high values of CO_2, CO, CH_4, H_2S, and H_2O.	[94]
Liquid smoke Biochar	CBS	Pyrolysis with temperatures of 450, 500, and 550°C with an increase of 5–15°C/minute.	The concentration of liquid smoke obtained was 18–23%. The higher speed of heating produced a greater quantity of coal, ash, and water; the calorific power of the product was 22.97 MJ/kg.	[95]

CPH = cocoa pod husk; MJ/kg = megajoule/kilogram; H_2SO_4 = sulfuric acid; HCl = hydrochloric acid; v/v = volume/volume; H_2O_2 = hydrogen peroxide; VS = volatile solids; CO_2 = carbon dioxide; CO = carbon monoxide; CH_4 = methane; H_2S = hydrogen sulfide; H_2O = water; CM = cocoa mucilage; NaOH = sodium hydroxide; L = liter; g = gram; mL = milliliter; pH = potential of hydrogen; mg = milligram; CBS = cocoa bean shell.

7. Conclusions

Food production entails a generation of important wastes for the industry, due to the nutritional quality that they possess even after being considered as nonvalue materials. The challenge is to minimize the negative effect it has on the environment; there are different perspectives to achieve this objective; recovering these wastes and transforming them into new products is an alternative that benefits the integral use of the fruit; this can generate a stable economy around the use of wastes.

The generation of biofuels from by-products of the industry responds to the need to reduce the consumption of fossil fuels and provide a source of sustainable fuels that has the least ecological impact. In this context, cocoa industrial wastes have the potential to be a source of biomass in the production of biofuels, due to their chemical composition, and the amount of waste generated per year worldwide.

Regarding the production of biofuels, CPH was the most versatile by-product for the generation of different biofuels; however, for its better use, it is necessary to apply a pretreatment that allows obtaining rich substrates in compounds for fermentation and subsequent production of liquid and gaseous biofuels. In addition, it contains a significant amount of compounds for the formation of solid biofuels. CBS is the waste that although it contains a higher content of valuable compounds, it contains fewer applications for obtaining biofuels; nevertheless, it is more relevant in other industries such as food, pharmaceuticals, and cosmetics.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Authors' Contributions

All authors actively participate in the complete preparation of the manuscript.

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Research Article

Valorization of *Glycine max* (Soybean) Seed Waste: Optimization of the Microwave-Assisted Extraction (MAE) and Characterization of Polyphenols from Soybean Meal Using Response Surface Methodology (RSM)

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The present study aimed at determining the optimal conditions for extraction of total phenolic compounds from soybean (*Glycine max*) meal, a by-product of the soybean seeds industry, using a green protocol with microwave-assisted extraction (MAE). A face-centered composite design (FCCD) was used for optimization. Based on a screening aimed to determine the factors that significantly influenced the responses, a 50% hydro-ethanolic solution was used with solvent/dry matter ratio (60/1–110/1), power (120–270 W), and time (0–10 min) as factors, while the responses studied were total phenolic and flavonoid contents. FTIR, TLC, DPPH, and FRAP anti-oxidants tests were used to characterize the extracts obtained with optimum conditions. The factors that significantly influenced both responses were the individual effect of all factors, the interaction between solvent/dry matter ratio and extraction time, the quadratic effect of solvent/dry matter ratio, and power for total phenolic content, while only the quadratic effect of power significantly influenced the flavonoid content. The highest contents of phenols (13.09 mg GAE/g) and flavonoid (7.39 mg CE/g) were obtained at 120 W for 0.16 min with a solvent/dry matter ratio of 60/1. ATR-FTIR spectra indicated the presence of polyphenolic compounds in the extract, namely flavonoids. TLC indicated the presence of at least nine compounds in the extract, among which catechin and quercetin were identified with respective Rf of 0.98 and 0.93. DPPH assay showed the anti-oxidant capacity for the extract with an IC_{50} of 194.98 μg/ml. RSM permitted us to develop a green protocol for maximum extraction of polyphenols from soybean seeds waste using less solvent, low power, and a reduced time in MAE.

1. Introduction

Soybean meal is a by-product of the soybean seed industry, which is commonly used for animal feeding [1, 2] or rejected directly in nature, then causing serious problems to municipalities and to the environment [3, 4]. In order to reduce pollution related to soybean seed industry's by-products and wastes, numerous usages have been proposed and tested: broiler breeding [1], growing substrate or source of specific compounds for microorganisms [3, 5], biosurfactants [6], and biodiesel production among others. But soybean waste and meal particularly have not been exploited for the production of polyphenols. Soybean (*Glycine max*) seeds have been reported as one of the richest flavonoid legume sources known nowadays; with up to 3 mg/g dry weight [7], these compounds are not totally destroyed during the different treatments that seeds undergo and may find themselves in soybean meal discarded by industries. Phenolic compounds have multiple applications in nutraceutical and pharmaceutical domains [8]. Flavonoids, for example, and isoflavones precisely are particularly abundant in soybean seeds and have long been exploited for their anti-oxidants, anti-inflammatory, and anti-cancer activities, among others [9]. Exigencies of green chemistry nowadays appeal to

researchers to use "green" processes in science [10]. Recently, the use of supercritical fluid, ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE) methods for extraction of phenols has contributed enough to reduce the cost of production and pollution and improve extraction yield [11, 12]. MAE is said to be the most effective in terms of polyphenol yield, accessibility, greenest, and respect to the environment [13, 14]. Literature has shown that yields of MAE are conditioned by many factors including the nature of the extracting solvent, time of extraction, power of the equipment, dry matter/solvent ratio, and the nature of the matrix [15]. So the determination of experimental conditions for extraction of the highest polyphenolic content from soybean meal using a green protocol will be helpful, since these seeds are among the richest sources of the named compounds. This study aimed at determining the optimal conditions for extraction of phenolic compounds from soybean meal, a soybean seed industry waste product, using a face-centered composite design in the response surface methodology with MAE.

2. Materials and Methods

2.1. Materials

2.1.1. Plant Material. Seeds of *Glycine max* (variety *TGX-1850-10E*) were said to have been obtained from "Institut de RechercheAgricole pour le Developpement" (IRAD), Foumbot (latitude: 5°30′29′N, longitude: 10°38′12′E, and altitude: 1,054 m).

2.1.2. Chemical. Ethanol (95°) and methanol were obtained from the local pharmacy. Ethyl acetate and formic acid were obtained from Sigma.

2.2. Methods

2.2.1. Preparation of Sample Soybean Meal. Soybean meal used was obtained from a local oil refinery situated in Foumbot. The meal was brought to the laboratory in a plastic bag and dried to constant weight at 45°C using an air oven.

2.2.2. Screening of Factors Affecting the Phenol and Flavonoid Contents. On the basis of the literature, variables retained for screening were: time of extraction, dry matter/solvent ratio, proportion of ethanol, and power [16, 17]. Factors influencing the yields of phenols and flavonoids were determined from Table 1.

2.2.3. Extraction of Phenolic Compounds. For each trial, 1 g of soybean meal was mixed in a beaker with the appropriate amount of solvent according to the experimental conditions as given by the chosen design. The mixture was stirred using a magnetic agitator; afterward, it was allowed to rest for 10 minutes at room temperature and put in a microwave oven (SAMSUNG M735) for extraction, under specified conditions. Samples were centrifuged (4,000 rpm/5 min), and the supernatant was collected after filtration through Watman paper no. 1. The solvent was then evaporated in an air oven at 45°C until obtention of the dry extract. Dry extracts were immediately used for the determination of total phenolic and flavonoid contents.

2.2.4. Determination of Total Phenolic Content. The total phenolic content was assessed according to the method proposed by Dohou et al. [1]. Briefly, 0.2 ml of Folin–Ciocalteu reagent (tenfold diluted) was added to a tube containing 0.01 ml of plant extract (5 mg/ml) and 1.39 ml of distilled water. The mixture was allowed to stand for 3 minutes before adding 0.4 ml of sodium carbonate (20% w/v) and then mixed using a vortex. The tube was then incubated at 40°C for 20 min in a water bath, and absorbance was read at 760 nm against a blank using a BIOMATE spectrophotometer. Gallic acid (0.2 g/l) was used to draw a calibration curve. All experiments were carried out in triplicate, and results were expressed as mg of gallic acid equivalent (GAE) per gram of dry extract (mg GAE/g dry weight).

2.2.5. Determination of Flavonoid Content. Flavonoid content was obtained using the method described by Padmadja et *al.* [18]. A volume (0.03 ml) of sodium nitrite (5%) was added to a tube containing 1.49 ml of water and 0.1 ml of extract solution (5 g/ml). After 5 min, a volume (0.003 ml) of aluminum chloride (10%) was added to the tube, and the mixture was allowed to rest for 6 min. Afterward, 0.3 ml of NaOH (1 M) and 0.24 ml of water were introduced, respectively, in the tube and mixed with a vortex before absorbance was read using a BIOMATE spectrophotometer at 510 nm against a blank. Calibration curve was made using catechin. All experiments were made in triplicate, and the results were expressed as mg of catechin equivalent per g of dry extract (mg CE/g of dry weight). Figure 1 depicts the flowchart of the whole work.

2.3. Optimization of the Responses Using the Central Composite Design. Factors such as time of extraction, dry matter/solvent ratio, and power were observed to influence the responses. A face-centered composite design was used, and the studied responses were total phenolic content (Y_1) and flavonoid content (Y_2). Ranges of different factors were taken according to the results of preliminary experiments. Experiments were randomized, and responses were evaluated in triplicate. The proposed model is as follows:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3, \quad (1)$$

where Y is the response (total phenolicor flavonoid content); X_1, X_2, and X_3 are the studied factors; a_0 is the offset term, while a_1, a_2, and a_3 are linear effects; a_{11}, a_{22}, and a_{33} are the quadratic effects; and a_{12}, a_{13}, a_{14}, a_{23}, and a_{34} are interaction effects. All experiments carried out are summarized in Table 2. Ranges are as follows: solvent/seed ratio (ml/g): 59.77/1–110.22/1; power (W): 119.31–270.78 and time (min): 0.04–10.04.

Table 1: Screening plan.

Variable	Experiments	1	2	3
Power	Power (W)	120	180	240
	Time (min)	5	5	5
	Ethanol percentage (%)	20	20	20
	Solvent/dry matter ratio (ml/g)	30/1	30/1	30/1
Time	Power (W)	240	240	240
	Time (min)	2	4	6
	Ethanol percentage (%)	20	20	20
	Solvent/dry matter ratio (ml/g)	30/1	30/1	30/1
Ethanol percentage	Power (W)	240	240	240
	Time (min)	5	5	5
	Ethanol percentage (%)	20	50	80
	Solvent/dry matter ratio (ml/g)	30/1	30/1	30/1
Solvent/dry matter ratio	Power (W)	240	240	240
	Time (min)	5	5	5
	Ethanol percentage (%)	20	20	20
	Solvent/dry matter ratio (ml/g)	20/1	30/1	40/1

2.4. Characterization of the Extract. FTIR, TLC, DPPH, and FRAP anti-oxidant assays were carried out on the polyphenolic-rich extract obtained with the optimum conditions.

2.4.1. Fourier-Transform Infrared Spectroscopy (FTIR). Soybean meal extract was analyzed by a Fourier-transform infrared (FTIR) apparatus coupled with an attenuated total reflectance (ATR) accessory. Spectra were collected at frequency regions of 4,000–400 cm^{-1} using an FTIR spectrometer (Alpha, Bruker, Germany) on a diamond plate at a resolution of 4 cm^{-1}. Two replicates spectra of 50 scans were recorded. Raw spectra were corrected.

2.4.2. Thin-Layer Chromatography (TLC). TLC was performed using a precoated plate covered with 60F250 silica gel (MERCK). Plates of 5 × 8 cm were used and activated at 105°C for 30 min. Two milligram of the extract were dissolved in 10 ml water and centrifuged for 10 min at 3,500 rpm. Ten microliter of supernatant was deposited on the plates using a capillary tube. Two standards were used, namely catechin and quercetin (1 mg dissolved in 50 ml of ethanol, centrifuged, and supernatant used). Development was done for 20 min in a presaturated (30 min) rectangular development chamber. The mobile phase was made of ethyl acetate/formic acid/glacial acetic acid/water (96/11/11/30). After development (6.5 cm), the plate was removed and dried at 45°C in an air oven for 1 min, before visualization under UV light (254 nm). Bands were circled, and Rf calculated.

2.4.3. Anti-Oxidant Assay. DPPH radical scavenging activity of the extract was determined using the method described by Mensor et al. [19], while the ferric reducing assay power (FRAP) was performed as described by Oyaizu [20].

3. Statistical Analysis

Designing and analysis of the results were done using Minitab 18. Experiments were carried out in triplicate. Statistical significance of the model variables was determined at a 5% probability level. Main effects and contour plots were plotted using Sigma Plot v11.0 (c) systat. Data on phenol and flavonoid contents were expressed as mean ± SD and compared using the Bonferroni test with the software SPSS version 22.

4. Results and Discussion

4.1. Screening Factors. Results of the screening factors are indicated in Table 3: usage of high power leads to a reduction in both the total phenolic and flavonoid contents of the extracts. Long cooking time almost induced a linear reduction of the two studied responses. Short times were seen to be best for the highest responses. Variation of ethanol percentage in the extracting solvent showed the highest TPC and TFC at 50%. That observation means water/ethanol (50/50) may be more effective in extracting polyphenol from soybean meal. We noticed the effect of the dry matter/solvent ratio that any increase in the solvent proportion leads to an increase in total phenolic and flavonoid contents.

While testing the influence of any factor, the others were, respectively, kept constant at: power (180 W), time (5 min), solvent/dry matter ratio (30/1), and ethanol proportion (20%).

4.2. Optimization of the Responses Using the Central Composite Design. Results of the screening permitted the optimization of the process using three main factors for extraction of phenolic compounds from soybean meal, namely the microwave power, the cooking time, and the solvent/dry matter ratio. Ethanol proportion was decided to

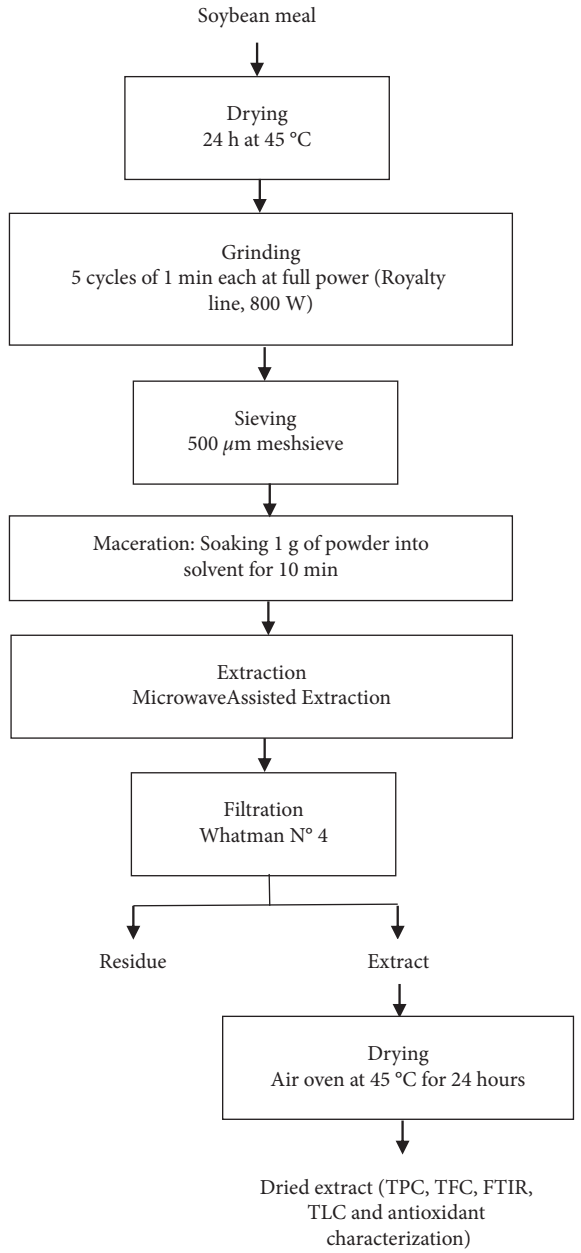

Figure 1: Extraction flow chart of phenolic compounds from soybean meal. TPC: total phenolic content, TFC: total flavonoid content, FTIR: Fourier-transform infrared, and TLC: thin-layer chromatography.

be 50%, since we noticed a maximal production with 50% ethanol proportion (in the solvent) of TPC and TFC during the screening. Also, the literature indicates that dielectric properties of the solvent should be highly taken into consideration when planning to extract phenolic compounds using MAE. Compared to water, ethanol or its mixtures with water have a lower dielectric constant and are more transparent to microwave, thus not well converting them into heat. But its high capacity to dissolve and extract phenolic compounds [21–23] and its greenness oriented the choice of a hydro-ethanolic solution as extracting solvent. Table 4 gives the different factors retained for optimization, in coded and real values with experimental and predicted values of the responses.

4.3. Analysis of Main Effects. The entire experimental plan consisted of 20 trials. The highest total phenolic content (6.87 mg of GAE/g) was obtained at 150 W for 2 min of cooking time with 70 ml of solvent. The lowest content (1.75 mg GAE/g) is observed at 240 W of microwave power with 70 ml of extracting solvent and a heating time of 8 min. Concerning the flavonoid content, the highest value (4.46 mg QE/g) was obtained at 150 W of microwave power,

Table 2: Experimental design in coded and real variables.

Trials	Matrix of coded variables			Matrix of real variables		
	X_1	X_2	X_3	Solvent/meal ratio (ml/g)	Power (W)	Time (min)
1	−1	−1	−1	70	150	2
2	1	−1	−1	100	150	2
3	−1	1	−1	70	240	2
4	1	1	−1	100	240	2
5	−1	−1	1	70	150	8
6	1	−1	1	100	150	8
7	−1	1	1	70	240	8
8	1	1	1	100	240	8
9	−1.68	0	0	59.77	195	5
10	1.68	0	0	110.22	195	5
11	0	−1.68	0	85	119.31	5
12	0	1.68	0	85	270.68	5
13	0	0	−1.68	85	195	0.04
14	0	0	1.68	85	195	10.04
15	**0**	**0**	**0**	**85**	**195**	**5**
16	**0**	**0**	**0**	**85**	**195**	**5**
17	**0**	**0**	**0**	**85**	**195**	**5**
18	**0**	**0**	**0**	**85**	**195**	**5**
19	**0**	**0**	**0**	**85**	**195**	**5**
20	**0**	**0**	**0**	**85**	**195**	**5**

Bold values are replicates of the center points.

Table 3: Conditions and responses for the screening.

Experiment		
	Power	
W (120 W)	1.9	1
W (180 W)	1.3	0.8
W (240 W)	0.8	0.4
	Time (min)	
T (2 min)	2.9	1.3
T (5 min)	1.5	0.7
T (8 min)	1.3	0.6
	Ethanol proportion	
E (20%)	1.9	1.2
E (50%)	4.3	2.4
E (80%)	2.1	1.3
	Dry matter/solvent ratio	
DM/S (30)	1.2	0.4
DM/S (60)	3.9	1.3
DM/S (80)	2.1	1.8

2 min heating time with a solvent/dry matter ratio of 30/1 (ml/g). The lowest flavonoid content (1.25 mg CE/g) is obtained with 70 ml of solvent at 150 W for a boiling time of 2 min. Values ranging from 1.75 to 6.78 mg of GAE/g and 1.25 CE/g to 4.46 CE/g of extract for total phenol and flavonoid content were similar to those obtained by Malenčić et al. [24], Sakthivelu et al. [25], and Josipović et al. [26].

4.3.1. Effect of Solvent Ratio. Figure 2 shows that an increase in the solvent ratio induces an almost linear reduction in the total phenolic content of extracts when going from 60/1 to 80/1 (ml of solvent/g DW). But the responses measured started increasing as the solvent ratio passed from 90:1 to greater values. Such observations may be explained by the type of phenols extracted at each condition, since literature indicates that free or bound phenolic compounds are found in soybean and are not forcibly extracted in the same experimental conditions [16]. Nevertheless, it is well accepted nowadays that high solvent content increases mobility of compounds (mass transfer) from plants matrix, thus explaining the observed increase in the total phenolic content of extracts at a certain solvent ratio, since previous research had already reported that [27]. An increase in the solvent ratio only led to a progressive diminution of the flavonoid content.

Table 4: Matrix of coded and real variables with responses obtained according to experimental conditions and predicted values.

Trials	Matrix of real and coded variables			Responses			
				TPC (mg GAE/g)		TFC (mg CE/g)	
	Solvent	Power	Time	Exp	Pre	Exp	Pre
1	70 (−1)	150 (−1)	2 (−1)	6.87 ± 0.14	6.94	4.46 ± 0.05	4.25
2	100 (1)	150 (−1)	2 (−1)	3.66 ± 0.10	3.87	3.31 ± 0.07	3.20
3	70 (−1)	240 (1)	2 (−1)	4.87 ± 0.09	5.10	2.34 ± 0.02	2.61
4	100 (1)	240 (1)	2 (−1)	1.96 ± 0.17	1.67	1.43 ± 0.05	1.29
5	70 (−1)	150 (−1)	8 (1)	2.09 ± 0.15	2.83	1.25 ± 0.15	1.45
6	100 (1)	150 (−1)	8 (1)	4.24 ± 0.18	4.46	2.11 ± 0.05	1.91
7	70 (−1)	240 (1)	8 (1)	1.75 ± 0.02	1.99	1.70 ± 0.07	1.88
8	100 (1)	240 (1)	8 (1)	2.89 ± 0.09	3.26	1.79 ± 0.05	2.07
9	59.77 (−1,68)	195 (0)	5 (0)	6.21 ± 0.11	5.66	2.65 ± 0.02	2.40
10	110.22 (1.68)	195 (0)	5 (0)	4.24 ± 0.06	4.14	1.55 ± 0.07	1.67
11	85 (0)	119.31 (−1.68)	5 (0)	5.80 ± 0.14	5.27	3.21 ± 0.10	3.42
12	85 (0)	270.68 (1.68)	5 (0)	2.83 ± 0.11	2.71	2.51 ± 0.10	2.18
13	85 (0)	195 (0)	−0.04 (−1.68)	3.15 ± 0.02	3.23	2.80 ± 0.02	2.93
14	85 (0)	195 (0)	10.04 (1.68)	1.84 ± 0.09	1.11	1.48 ± 0.07	1.23
15	**85 (0)**	**195 (0)**	**5 (0)**	2.54 ± 0.04	2.43	1.85 ± 0.02	1.85
16	**85 (0)**	**195 (0)**	**5 (0)**	2.42 ± 0.05	2.43	1.92 ± 0.05	1.85
17	**85 (0)**	**195 (0)**	**5 (0)**	2.51 ± 0.15	2.43	1.84 ± 0.02	1.85
18	**85 (0)**	**195 (0)**	**5 (0)**	2.30 ± 0.05	2.43	1.84 ± 0.02	1.85
19	**85 (0)**	**195 (0)**	**5 (0)**	2.33 ± 0.06	2.43	1.82 ± 0.05	1.85
20	**85 (0)**	**195 (0)**	**5 (0)**	2.39 ± 0.06	2.43	1.84 ± 0.02	1.85

Bold values are replicates of the center points. Pre means predicted value, while Exp means experimental value.

4.3.2. Effect of Power. Figures 2 and 3 depict the influence of power on the total phenolic and flavonoid contents of extracts. We can see from the figures that an increase in the power induces a reduction in the phenol and flavonoid contents of the meal extracts. This could be the consequence of degradation of these compounds exposed to high temperature, since high power in microwave induce a quick elevation of the solvent temperature even when exposure is for a short duration. Đurović et al. [16] made a similar observation.

4.3.3. Effect of Time. From Figures 2 and 3, we can see that any increase in time of exposure also led to a diminution of the TPC and TFC of extracts because of progressive destruction of these thermo-sensitive compounds under long exposure to heat. Previous authors also noticed the same effect [16, 22, 28].

4.4. ANOVA, Regression Equations for the Responses. Table 5 shows the ANOVA and the influence of each independent factor. We can see from the table that all independent factors significantly ($p < 0.05$) influenced both total phenolic and flavonoid contents. Quadratic effects of solvent ratio (X_1X_1) and the power (X_3X_3) significantly affected the total phenolic content of the extract obtained, while only the quadratic effect of the heating power (X_3X_3) significantly influenced the flavonoid content of the extracts. Interaction between solvent ratio and the heating time (X_1X_2) significantly impacted both the total phenolic and the flavonoid content, while only interaction between the time and the boiling microwave power (X_2X_3) significantly affected the flavonoid content of the extracts. We can also see that interaction between solvent ratio and the heating time (X_1X_3) contributed the most in the observed phenolic response (24.74%), followed by the quadratic effect of the solvent ratio (X_1X_1), which contributed up to 22.17% to the final response. Talking about the phenolic content, time (X_3) and interaction between power and time (X_2X_3) contributed the most to the observed response (30.06% and 18.33%, respectively).

The mathematical model predicting the influence of the solvent ratio, boiling time, and working power on the phenol and flavonoid contents of the extracts is given by the following equation:

$$\begin{aligned}\text{TPC} &= 58.24 - 0.795X_1 - 0.1213X_2 - 2.693X_3 \\ &\quad + 0.003883X_1X_1 + 0.000273X_2X_2 - 0.0102X_3X_3 \\ &\quad - 0.000131X_1X_2 + 0.02614X_1X_3 + 0.00186X_2X_3, \\ \text{TFC} &= 19.86 - 0.0876X_1 - 0.0836X_2 - 1.715X_3 \\ &\quad + 0.000297X_1X_1 + 0.000166X_2X_2 + 0.00900X_3X_3 \\ &\quad - 0.000098X_1X_2 + 0.00836X_1X_3 + 0.003824X_2X_3. \end{aligned} \quad (2)$$

5. Assessment of Model Quality and Optimal Conditions

Experimental values show us that these mathematical models can well explain the observed results. According to Joglekar and May [29], a good mathematical model should predict at least 75% of the responses; R^2 should then range from 0.75 to 1. Our results give the determination coefficient for phenols and flavonoid, respectively, to be 0.95 and 0.94,

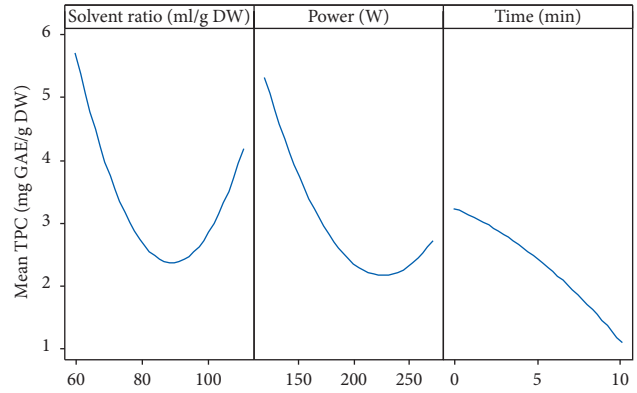

Figure 2: Effects of solvent/raw material ratio, power, and time on TPC.

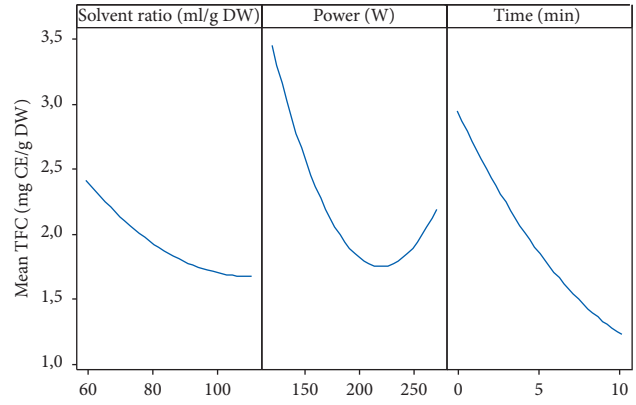

Figure 3: Effects of solvent/raw material ratio, power, and time on TFC.

Table 5: Evaluation of quadratic model: P value, F value, RC, CF (contribution factor, %), AADM, and Bf for phenols and flavonoids.

Source	Total phenolic content				Flavonoid content			
	P value	F value	RC	CF (%)	P value	F value	RC	CF (%)
Solvent ratio (X_1)	**0.005**	12.76	−0.795	6.18	**0.011**	9.74	−0.087	5.52
Power (X_2)	**0.001**	36.45	−0.121	17.65	≤**0.001**	28.32	−0.083	16.04
Time (X_3)	**0.001**	24.96	−2.693	12.09	≤**0.001**	53.08	−1.715	30.06
$X_1 \times X_1$	≤**0.001**	50.79	+0.003	22.72	0.346	0.98	+0.000	0.11
$X_2 \times X_2$	**0.001**	20.27	+0.000	10.24	**0.001**	24.64	+0.000	13.43
$X_3 \times X_3$	0.473	0.56	−0.010	0.27	0.258	1.44	+0.009	0.81
$X_1 \times X_2$	0.601	0.29	−0.000	0.14	0.482	0.53	−0.000	0.30
$X_1 \times X_3$	≤**0.001**	51.09	+0.026	24.74	**0.002**	17.19	+0.008	9.74
$X_2 \times X_3$	0.158	2.33	+0.001	1.13	≤**0.001**	32.37	+0.003	18.33
			Validation of the model					
R^2		0.95				0.94		
AADM		0.00				0.00		
Bf		0.99				0.99		

Bold: individual factors that significantly ($p < 0.05$) influenced the responses.

falling in the good range, which means our second-order polynomial equations, really represented the experimental data. Also, obtaining values of AADM (analysis of the absolute average deviation) and Bf (Bial factor), respectively, equal to 0 and 0.99 for both total phenolic and flavonoid contents, thus confirming the suitability of the models since values were in the normal range (0 for AADM and $0.75 < \text{Bf} < 1.25$ for Bf).

6. Optimization of the Process

After validation of the model, the optimal extraction conditions for total phenolic and flavonoid contents were determined using iso-responses and responses surfaces curves. Iso-responses curves are two-dimensional representations showing the variation of a fixed parameter according to two factors. Figures 4(a)–4(d) illustrate the variation in TPC and

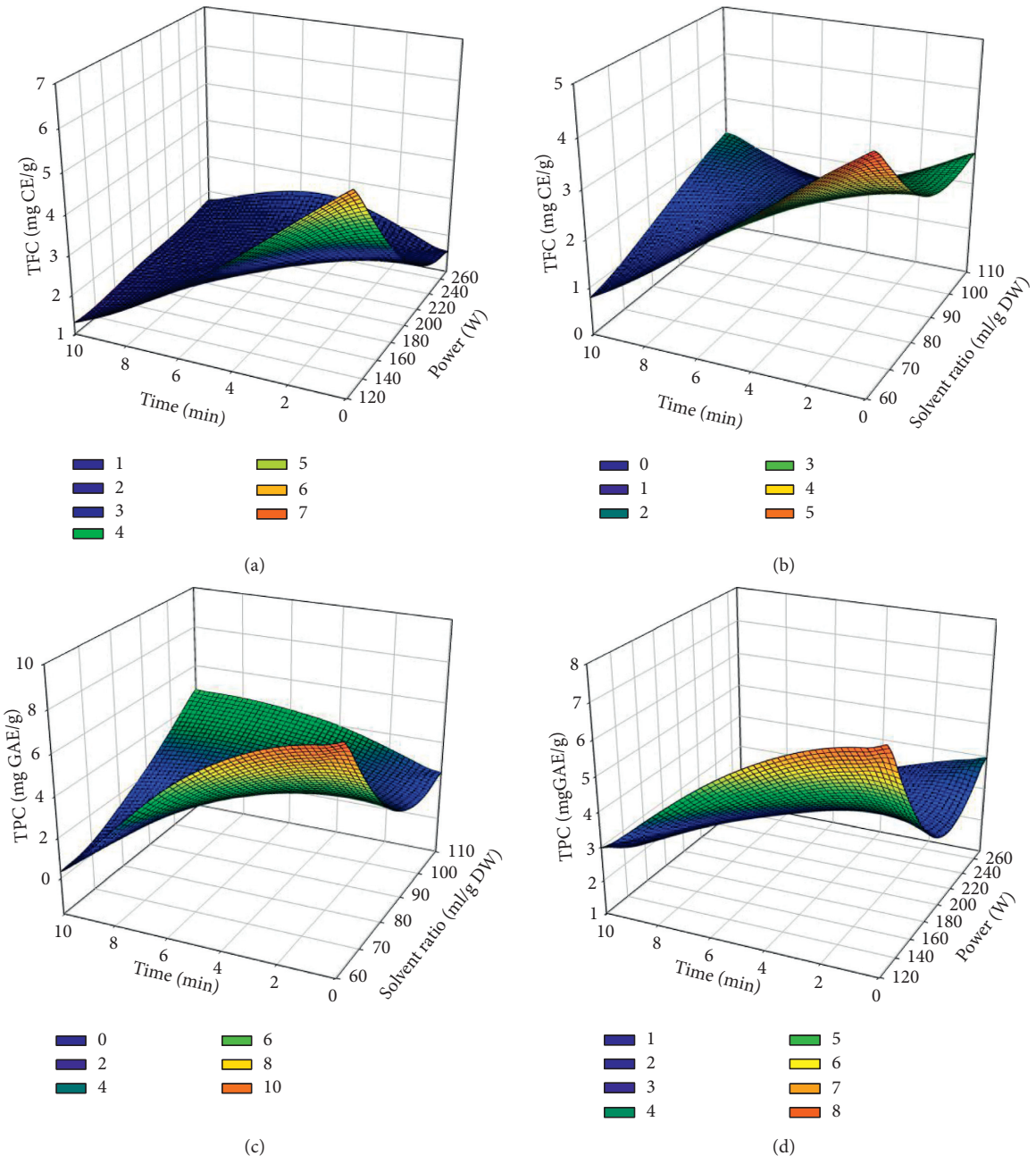

Figure 4: (a, b, c, and d) Response surface curves for TPC and TFC considering the different factors taken 2 by 2.

TFC of soybean meal extracts under the influence of different factors. These figures show that maximum TPC is obtained at 120 W, with a solvent ratio of 60:1 for 0.16 min, and the same for TFC.

7. Confirmation Experiments

In order to confirm the quality of our model to predict the optimal conditions for our responses, experiments were made, replicating optimal conditions and results compared with the predicted maximal values in Table 6. No significant differences were noticed between optimal predicted values and experimental values obtained for TPC and TFC, thus confirming the validity of the predicted optimal values given by the software.

8. Characterization of the Extract

8.1. IR Spectral Analysis. The IR spectra of *Glycine max* seed extract is depicted in Figure 5. Table 7 shows the different absorbance peaks and their assignment. Polyphenols were identified among the compounds present in the extract.

8.2. Thin-Layer Chromatography. Thin-layer chromatography of *Glycine max* seed extract showed a number of nine bands with Rf going from 0.15 to 0.98. Two spots were

Table 6: Experimental, predicted values, and desirability for TPC and TFC in optimal conditions.

	Optimal predicted value	Optimal experimental value	Desirability
Phenol (mg GAE/g)	13.09[a]	12.97 ± 0.05[a]	1.00
Flavonoid (mg CE/g)	7.39[a]	7.42 ± 0.09[a]	1.00

On the same line, values with different letters significantly differ ($p > 0.05$).

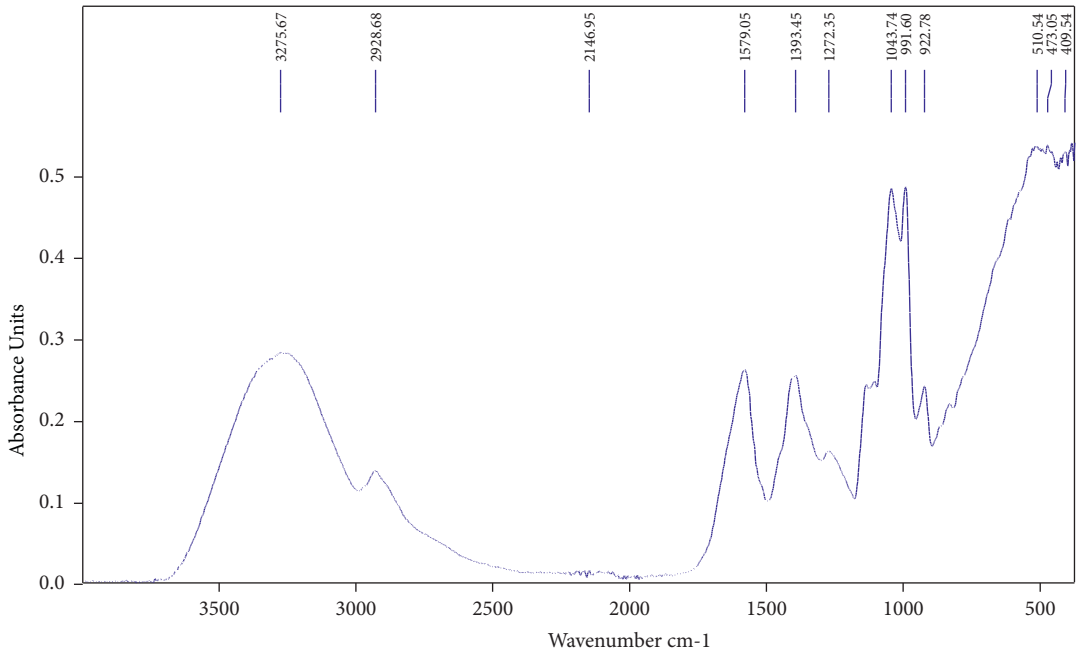

Figure 5: FTIR spectra of *Glycine max* meal extract.

Table 7: Peak wave numbers and assignation.

Wave number (cm^{-1})	Vibration	Assignment	Reference
3275	–C–OH (stretching)	Water and polysaccharides	[30, 31]
2928	–C–H (stretching)	Aliphatic chain: lipids	[30]
		Isoflavones (daidzein and genistein)	[32]
2146	$-NH_2$(stretching)	Free amino acid and/or derivatives	[33]
1579	–C=*C* (skeletal)	Aromatic compounds and flavonoids	
	–C–H (bending)		
1393	–O–H (deformation)	Phenols	[34]
	–C–O (deformation)		
1272	In plane = C–H	Phenols, flavonoids, or aromatic compounds	[35]
1040	Ester –C–O (stretching)	Glycosidic groups	[36]
991		Unidentified	
922	–C–C (stretching)	Alkane lipids, aminiacids, and proteins	
510	Phenol ring (torsion)	Phenols	[37]
473	O–S–O (bending)	SO^{-4} group	[38]
409	In plane –C–OH (bending)	Phenols	[33]

identified as catechin and quercetin, respectively, those with Rf of 0.98 and 0.93. Figure 6 shows the plates with respective spots under UV light and the schematic representation of the plate after development.

TLC revealed the presence of at least nine different compounds or groups of compounds in the obtained extract, with a high relative abundance of quercetin (8). A similar observation was made by Hanan et *al.* [39] who stated that quercetin is the most abundant flavonoid found in soybean seeds. Rf values of all spots observed under UV light are given in Table 8.

8.3. Anti-Oxidant Capacity of the Extract. DPPH and FRAP anti-oxidant capacities of the extract were moderate, with a DPPH scavenging IC_{50} of 194.98 μg/ml and low absorbances at 700 nm in the FRAP test. Moderate DPPH scavenging activity of soybean seeds extracts has already been reported

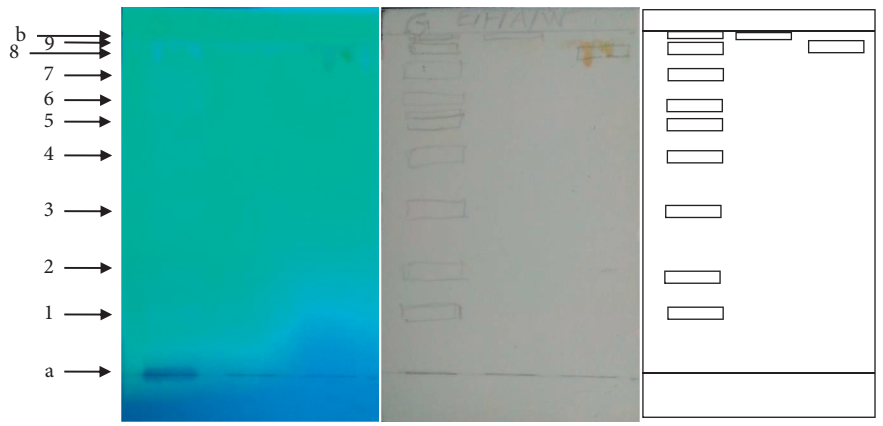

Figure 6: Chromatography plates after development: (a) under UV 254 light, (b) under white light with bands circled, and (c) schematic representation. a = deposit line, b = front line, and 1, 2, 3, 4, 5, 6, 7, 8, and 9 are bands observed under UV light.

Table 8: Rf values of the compounds of the optimized soybean meal extract.

Spots number	1	2	3	4	5	6	7	8	9
Rf	0.15	0.29	0.46	0.72	0.78	0.8	0.89	0.93	0.98
Identification	—	—	—	—	—	—	—	Quercetin	Catechin

Table 9: Percentage of DPPH inhibition and FRAP test absorbances.

Concentration (μg/ml)	DPPH scavenging activity		FRAP
	% Inhibition	IC_{50} (μg/ml)	Absorbance at 700 nm
12.5	39.9		0.10
25	47.11		0.36
50	54.38	194.98	0.47
100	65.09		0.87
200	74.24		1.36

by Djordjevic et al. [40] who found $IC_{50} > 200\ \mu$g/ml. Table 9 shows DPPH and FRAP inhibitory results of the soybean meal extract.

9. Conclusion

The study aimed at determining the optimal conditions for extraction of total phenolic and flavonoid compounds from soybean meal using a green protocol. RSM was used to determine the conditions, and we found out that all factors, namely, solvent ratio, time, and power significantly, influenced both responses. Results suggest that low solvent, power, and time of exposure should be used when attempting to obtain high TPC and TFC extracts from soybean meal with MAE. RSM used in this research permitted us to define the conditions for green extraction of TPC and TFC from soybean waste as: 60/1 solvent/dry matter ratio, 120 W power, and 0.16 min time. FTIR confirmed the presence of polyphenolic compounds in the extract obtained, and TLC permitted to identify catechin and quercetin, while DPPH and FRAP tests showed that the obtained extract possesses moderate anti-oxidant capacities. RSM permitted to obtain a polyphenolic rich extract, containing catechin and quercetin, with anti-oxidant capacities, usable in nutraceutical, food, cosmetic and pharmaceutical industries from seeds waste; thus, soybean meal can be well valorized as good source of polyphenols.

Data Availability

The data are available on request (Woumbo Cerile Ypolyte at woumbocerile@yahoo.fr).

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Woumbo Cerile Ypolyte and Kuate Dieudonné conceived the work, collected seeds, carried out experimentations, analyzed and interpreted data, and wrote the paper. Klang Mathilde Julie followed up the work, verified data analysis, and read the paper for correction. Womeni Hilaire Macaire supervised the work and read the paper.

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Research Article

Optimization of Parameters Using Response Surface Methodology to Develop a Novel Kefir-Like Functional Beverage from Cheese Whey Enriched with Myrtle Juice

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Whey, liquid wastewater from cheese production, is one of the sources of dietary protein and lactose that are still largely unused for human consumption. It is only in recent years that it has aroused the interest of industries and sought as a valuable raw material and thus represents an opportunity for the manufacture of new products. The manufacture of fermented whey drink requires the mixing of whey with fruit juice or an aromatic plant to improve its organoleptic properties and acceptability. Myrtle, an aromatic medicinal plant, known for its health benefits is not well exploited for making dairy products. This is the first report on the development of kefir-myrtle beverage. Three factors were optimized (whey permeates (%), myrtle's juice (%), and kefir grains as inoculum (%)) using a central composite design with response surface methodology. The analyses showed that the number of lactic acid bacteria (LAB) and yeast cells varied from 5.4 to 9.2 $\log_{10}$ CFU/mL and from 4.3 to 6.2 $\log_{10}$ CFU/mL, respectively. A decrease in pH and an increase in the total polyphenol content and antioxidant activity were observed. The analysis of variance indicated the goodness of fit of the model with R^2 from 0.827 to 0.966. The absolute average deviation values of each model were low and ranged from 1.61% to 4.23%. The optimized fermented kefir whey beverage accomplished an overall acceptability of 5.41 (1 to 9 preference scale) and a high number of LAB cells (8.53 $\log_{10}$ CFU/mL). The viability of LAB and yeast cell was maintained at 7.61 and 6.19 $\log_{10}$ CFU/mL, respectively, after 14 days of storage.

1. Introduction

The dairy industry in Tunisia is one of the important food industries in the country. This sector comprises 25 enterprises that processed daily about 3.8 million liters, and 13% of the total volume of produced milk is destined for cheese production [1]. In the past, whey was considered a byproduct, but now it is considered a coproduct. Its valorization is both an economic and ecological issue since it has a high chemical oxygen demand (COD) [2]. Indeed, when whey is discharged into rivers, it generates eutrophication problem and toxicity modifying the physicochemical properties of aquatic ecosystems [3].

The relatively new interest in this byproduct results mainly from its composition rich in proteins, lactose, and water-soluble vitamins and minerals. Furthermore, it represents a well-balanced source of essential amino acids [4].

There are different processes to value whey. Among the most used technologies are drying, evaporation, reverse osmosis, nanofiltration, and ultrafiltration. By its biochemical composition, whey can be also considered an excellent culture medium for microorganisms.

Since lactose is the main component of whey solids, various biotechnological processes have been developed to use whey as a substrate to produce important industrial products having functional properties. For example, it can be

converted into prebiotic such as galactooligosaccharides (GOS), lactulose, lactobionic acid, and tagatose [5]. In addition, lactic acid fermentation of whey makes it rich in GOS [6], which exerts a stimulating effect on the growth of probiotic bacteria [5]. Bioactive peptides manifesting antihypertensive, antioxidant, immunomodulatory, and antimicrobial activities can also be released during fermentation [7].

On the other hand, lactic acid fermentation was known as an important tool to increase the bioavailability of polyphenols in food. To date, kefir grains were largely exploited for dairy and nondairy beverages fermentation. Numerous studies described the health benefits of kefir including antihypertensive, antidiabetic, anti-inflammatory, anticancer, antioxidative, and antihypercholesterolemic properties reviewed by Azizi et al. [8]. These therapeutic's aspects made kefir a suitable proposal for commercial intention.

However, to improve whey fermented beverages' flavor and increase their consumption among young people, they should be mixed with fruits juice [9–12]. In fact, fruit and vegetables are rich in nutrients and phytochemicals such as vitamins, minerals, and phenolic compounds [13]. Due to their various micronutrients, fruits and vegetables are often considered as "functional foods" helping to prevent various diseases such as cancer, obesity, and diabetes [14].

Myrtus communis L. is an aromatic and medicinal plant belonging to the family of Myrtaceae. It is widespread in the Mediterranean regions, such as North Africa and Southern Europe, and also found in South America, Australia, and in some areas of the Himalaya [15]. Myrtle berries have a long history of application in the pharmaceutical and food industries. They contain many biologically active compounds such as phenolic compounds, flavonoids, and anthocyanins [16], which are thought to be responsible for their antioxidant proprieties. They also have various positive effects on human health, and they are used as antiseptic, analgesic, cardiotonic, diuretic, anti-inflammatory, stomachic, nephroprotective, antidote, hemostatic, brain tonic, and antidiabetic properties [16].

Experimental designs can be used as a method for formulating food products. They allow selecting the factors that influence the response, modeling the variations in the system response according to the fluctuations of the factors, and validating experimentally the model described by a mathematical equation. However, a suitable range for each factor is also an important consideration for the accuracy of the final model [17–19].

The aim of this study is to optimize the formula of a functional whey beverage. Optimization was done by a central composite design (CCD) to determine the optimum ratio of whey permeate, myrtle juice, and kefir inoculum on pH, lactic acid bacteria (LAB), and yeast viability, % radical scavenging activity, polyphenol content, and overall acceptability. From this design, the surface plots of considered responses with respective second-order polynomial models were obtained. Finally, the analysis of variance (ANOVA) was employed to judge the adequacy of the model, and the optimized formulation was validated experimentally.

2. Materials and Methods

2.1. Kefir Grains and Raw Material. Kefir grains (KG) used in this study were collected from Tunisian households and preserved by the laboratory of microbial ecology and microbial technology (LETMi, INSAT) [11, 20]. The grains were cultured in sterile cow milk and renewed daily to maintain their viability. Kefir grain is polysaccharides and protein matrixes consisting of a symbiotic community were LAB (10^8 CFU/g) and yeasts (10^5 CFU/g). The predominant LAB in the used grains are related to *Leuconostoc* spp., *Lactobacillus* spp., and *Lactococcus* spp. *Saccharomyces* spp. and *Zygosaccharomyces* spp. are the dominant yeasts [21]. Kefir grains produce 0.6% lactic acid titratable acidity.

Cheese whey was collected from an artisanal cheese maker, and whey permeate was provided by dairy industry. The composition of liquid cheese whey was lactose 5.01% (w/v), proteins 1.22% (w/v), fat 0.34% (w/v), and ash 0.8% (w/v). Whey permeate contains lactose 85% (w/v), proteins 3% (w/v), and ash 7% (w/v).

Lactose, total proteins, the total fat content, and the ash were determined using the HPLC method [22], the Kjeldahl method [23], and a solvent extraction [24] and by heating the samples at 550°C [25], respectively. The pH of the beverages was measured using a pH meter (Mettler-Toledo EL20).

Myrtle fruits (*Myrtus communis*) were collected from the area of Nefza (north-west of Tunisia, latitude 36° 58′ 31″N, longitude 9° 04′ 51″ E, altitude 500 m, far 147 km from Tunis, the capital), purchased from the local market in January 2019. The berries were washed and crushed with a mixer by adding distilled water (8°Brix). The mixture was filtered to obtain a juice that was pasteurized at 70°C for 15 min and used immediately.

2.2. Beverage Formulation Using a Response Surface Methodology (RSM). The solution of cheese whey and whey permeate were sterilized for 20 min at 120°C and then mixed with myrtle juice according to the plan of RSM. Obtained beverages (400 mL) were placed in bottles and inoculated with kefir grains (Figure 1).

Central composite design (CCD) was used to develop a novel kefir-like functional beverage from cheese whey enriched by myrtle juice (MJ). The RSM is a combination of experiment design, statistics, empirical modeling, and mathematical optimization techniques. The number of experiments to be carried out was determined rationally, which avoids redundancies of information. In addition, the implementation of an optimization procedure allows the study of the interactions between the different factors. It is possible that a factor that apparently has no effect on the phenomenon being studied and influences this phenomenon indirectly through an interaction. The construction of the response surfaces is carried out following the adjustment of the model using mathematical functions such as polynomials [26].

The central composite design used for the developing of whey kefir-like beverage consists of nineteen experiments

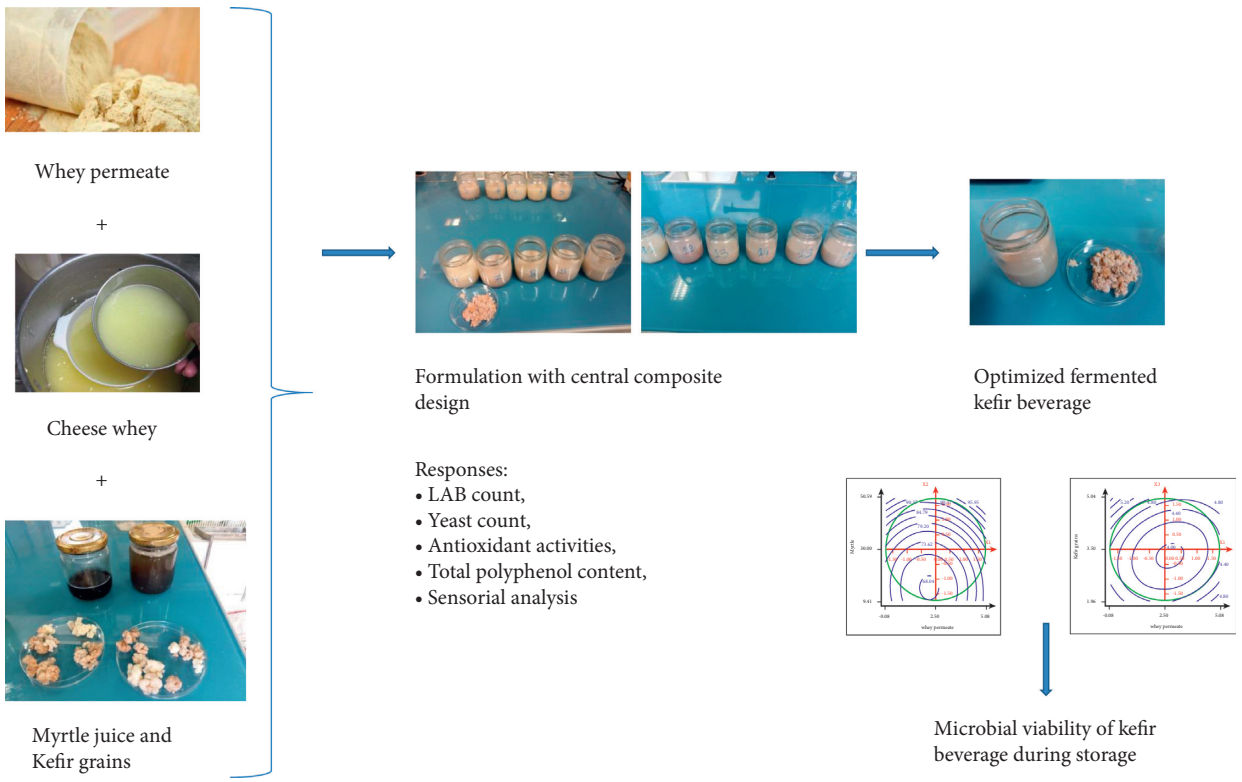

Figure 1: Whey valorization by developing a kefir-like functional beverage from cheese whey, whey permeate, and myrtle juice.

with five replicates at the central points. The independent variables (whey permeate X1, myrtle juice X2, and kefir grains inoculum X3) in the design were assigned into five levels, coded −1.68, −1, 0, 1, and 1.68 as described in Table 1. The responses were pH, lactic acid bacteria viability, yeast viability, total polyphenol content (TPC), antioxidant capacity (DPPH), and overall acceptability (OA). The results obtained from the CCD were used to fit a second-order polynomial equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b22X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3, \quad (1)$$

where Y is the predicted response; X_1, X_2, and X_3 are the independent variables; b_0 the model constant; b_1, b_2, and b_3 are the linear effect of variables; b_{11}, b_{22}, and b_{33} are the squared effect of variables; and b_{12}, b_{13}, and b_{23} are the interaction effect of variables. These coefficients were calculated using software NEMROD-W (version 99901, LPRAI Company).

2.3. Lactic and Yeast Counts. Lactic acid bacteria and yeasts were quantified using conventional culture techniques [27]. LAB were quantified on MRS agar (with cycloheximide 150 μg/mL) and yeasts on PDA supplemented with chloramphenicol (100 mg/L); they were incubated at 37°C and 30°C for 48 h, respectively. The results were expressed as CFU/mL. The numbers of LAB and yeasts were converted to log CFU/mL.

2.4. Polyphenol Content and Free Radical Scavenging Capacity (DPPH Assay). The TPC was determined following the method of Folin-Ciocalteau [28], modified by Karaaslan et al. [29]. The mixture of each beverage (0.03 mL) and distilled water (2.730 mL) was added to the Folin–Ciocalteau reagent (0.15 mL). After adding sodium carbonate (20%), the mixture was left at room temperature for 30 min. The absorbance was measured at 750 nm (Jenway 63200 UV/Vis). The TPC was expressed as mg of gallic acid equivalent/mL of the sample. The correlation coefficient was $R^2 = 0.991$.

The free radical scavenging activity of the samples was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) method according to the method proposed by Balakrishnan and Agrawal with some modifications [30]. Each sample (700 μL) was added to 700 μL DPPH methanolic solution (0.035 mol/L). The mixture was shaken and allowed to stand at room temperature for 30 min. Antioxidant capacity was measured by recording the absorbance at 517 nm using a spectrophotometer (Jenway 63200 UV/Vis). Methanol was used as the blank. All the determinations were performed in triplicate. A mixture of DPPH solution and methanol (instead of the sample) was used as the negative control for this assay. Percentage of DPPH radical scavenging activity was the result of antioxidant activity. The scavenging activity was calculated by the following equation:

$$\text{DPPH scavenging activity}\,(\%) = \frac{(A\,(\text{control}) - A\,(\text{sample}))}{A\,(\text{control})} \times 100. \quad (2)$$

Table 1: Experimental range and levels of the three factors used in the central composite design for beverage formulation.

Level	Independent variables		
	X_1 (whey permeate fortification, % w/v)	X_2 (fruit juice, % w/v)	X_3 (kefir grains, % w/v)
+1.68	5	50	5
+1	4	42	4.4
0	2.5	30	3.5
−1	1	18	2.6
−1.68	0	10	2

2.5. Sensory Evaluation. A total of 40 persons have contributed to a panel test; they were from the food technology department and the dairy industry (students and staff, ranging 24–56 ages). The evaluation was based on the hedonic sensory acceptance of beverage samples using a 9-point hedonic scale. The drinking water was given to tasters to rinse their mouth between each sample. Beverages were evaluated for color, odor, sweetness, acidity, taste, and overall acceptability [31].

2.6. Statistical Analysis. All experiments were carried out in triplicate. All data are reported as the mean ± standard deviation. Statistics were performed with the analysis of variance (ANOVA) procedure (STATIGRAPHICS 202 Centurion XVI software, Statpoint Technologies, Warrenton, USA). Differences were considered significant at $p < 0.05$.

3. Results and Discussion

Whey-based milk drinks can sometimes have unpleasant flavors. The improvement of their organoleptic characteristics can be obtained by the addition of fruit concentrates and/or by fermentation. To obtain a novel kefir-like functional beverage, the whey was fortified by WP, and myrtle juice was then fermented by kefir grains. The effects of whey permeate; myrtle juice and kefir grains' levels on the nutritional and organoleptic characteristics of the obtained beverages were investigated using a CCD. These independent variables (WP (X_1), fruit juice (X_2), and percentage of inoculum (X_3) levels) were prescribed into five levels (−1.68, −1, 0, +1, and +1.68). Tables 2 and 3 present the experimental values of total polyphenol content (mg EGA/mL), radical scavenging activity, LAB and yeasts' viability, pH, and sensory evaluation for each run of the CCD. Results from the 19 runs were fitted to a second-order polynomial equation, and the removal of nonsignificant terms was assigned. When the values of R^2 and R^2adj. are close to 1, the results indicate the adequacy of the fitted models. Yaakob et al. [32] mentioned that R^2 should be at least 80% and Cruz et al. [33] reported that the values of R^2adj. should be over 70%. However, the calculation of R^2 and absolute average deviation values (AAD) together should be better to determine the accuracy of the model. R^2 must be close to 1.0, and the AAD between the predicted and observed data must be as small as possible [34]. AAD values of six predicted models were calculated; they were 4.23% for TPC, 1.61% for antioxidant activities, 2.75% for LAB number, 1.96% for yeasts counts, 2% for pH, and 4.13% for overall acceptability. Therefore, the model illustrates the global variability; it is predictive (Supplementary Materials (available here)).

3.1. Effects of the Variables on Total Phenolic Content and Antioxidant Activity. Myrtle is considered a preventive element against diseases related to oxidative stress. Indeed, it is rich in many chemical compounds including phenolic compounds and essential oils [35]. Many groups of phenolics were identified such as phenolic acids, hydrolyzable tannins (gallotannins), flavonoids, and anthocyanins [36]. Messaoud and Boussaid [37] reported that their antioxidant activities are due to their phenolic compounds. The TPC values of obtained beverages varied from 55.25 ± 0.68 to 90.45 ± 0.07 mg EGA/mL, and the DPPH radical scavenging from 65.25 ± 0.22 to 91.6 ± 0.29% (Table 2). R^2 and adj R^2 for TPC and DPPH radical scavenging activity are close to 1. The AAD values were 4.23% and 1.61%, respectively.

An increase in myrtle juice or whey permeate or kefir inoculum level improves the TPC and DPPH radical scavenging activity ($p < 0.001$; Table 4). It must be mentioned that a p value lower than 0.001 showed that the model is highly significant in the response. The coefficients for linear and quadratic models are also highly significant ($p < 0.01$; Table 4). The highest level of TPC and DPPH radical scavenging activity was obtained with 5.08% WP, 50.59 fruit juice (% w/v), and 5.04% inoculums (Figures 2 and 3).

With regard to interaction, myrtle juice and kefir inoculum's level affect positively the TPC and the antioxidant activity. These results could be due to the metabolic activities of microorganisms in kefir grains. In fact, microbial enzymes break down polyphenol compounds and form aglycones [38]. These latter can be also liberated from their corresponding glycosides, contributing to enhancing the bioavailability of polyphenol [39] and increasing their quantitative amount [40, 41]. Sabokbar et al. [42] showed that the addition of kefir to apple juice enhanced both the total phenolic content and antioxidant activities. During the last years, several works have been interested in the action of lactic acid bacteria and kefir microflora on phenolic compounds [43–46]; they cited many enzymes, which are involved in the hydrolysis mechanisms and which are inducible as a specific stress response.

The improvement of the antioxidant activity after fermentation (Table 5) is in line with other studies [11, 47–51]. The increase of DPPH radical scavenging of fermented beverages indicated that fermentation may produce metabolites with higher and better antioxidant activity. The release of bioaccessible phenolic compounds can be one of

Table 2: Matrix of the central composite design for three variables and the measured responses (DPPH scavenging activity and total phenolic content).

Run	Independent variables			Response variables	
	X_1 (whey permeate, % w/v)	X_2 (myrte juice, % w/v)	X_3 (kefir grains, % w/v)	DPPH scavenging activity (%)	Total phenolic content (mg EGA/mL)
1	1.0	18	2.6	68.40 ± 0.61	57.58 ± 0.28
2	4.0	18	2.6	72.60 ± 0.26	59.75 ± 0.42
3	1.0	42	2.6	71.25 ± 0.36	64.80 ± 0.49
4	4.0	42	2.6	75.60 ± 0.26	63.60 ± 1.04
5	1.0	18	4.4	70.80 ± 0.06	69.97 ± 0.08
6	4.0	18	4.4	73.60 ± 0.30	70.68 ± 0.29
7	1.0	42	4.4	91.36 ± 0.13	85.00 ± 0.04
8	4.0	42	4.4	95.95 ± 0.38	89.45 ± 0.39
9	−0.0	30	3.5	80.45 ± 0.03	82.20 ± 0.91
10	5.0	30	3.5	85.62 ± 0.01	81.69 ± 0.14
11	2.5	10	3.5	69.80 ± 0.04	55.25 ± 0.68
12	2.5	50	3.5	91.60 ± 0.29	90.45 ± 0.07
13	2.5	30	2.0	65.25 ± 0.22	69.30 ± 0.26
14	2.5	30	5.0	89.85 ± 0.20	81.20 ± 0.42
15	2.5	30	3.5	71.50 ± 0.44	63.10 ± 0.13
16	2.5	30	3.5	72.05 ± 0.15	62.50 ± 0.14
17	2.5	30	3.5	71.95 ± 0.43	63.01 ± 0.026
18	2.5	30	3.5	72.42 ± 0.02	62.90 ± 0.35
19	2.5	30	3.5	71.15 ± 0.38	63.05 ± 0.39

Table 3: Matrix of the central composite design for three variables and the measured responses (LAB viability, yeast viability, pH, and overall acceptability).

Run	Independent variables			Response variables		pH	Overall acceptability
	X_1 (whey permeate, % w/v)	X_2 (myrte juice, % w/v)	X_3 (kefir grains, % w/v)	LAB viability ($\log_{10}$ CFU/mL)	Yeast viability ($\log_{10}$ CFU/mL)		
1	1.0	18	2.6	6.28 ± 0.06	4.15 ± 0.01	5.02 ± 0.01	3.90 ± 0.10
2	4.0	18	2.6	6.80 ± 0.06	4.61 ± 0.06	4.88 ± 0.02	4.90 ± 0.20
3	1.0	42	2.6	6.02 ± 0.03	4.85 ± 0.02	4.78 ± 0.06	4.90 ± 0.10
4	4.0	42	2.6	7.39 ± 0.04	5.68 ± 0.09	4.69 ± 0.04	4.40 ± 0.10
5	1.0	18	4.4	6.98 ± 0.04	5.50 ± 0.07	4.45 ± 0.05	4.10 ± 0.20
6	4.0	18	4.4	7.09 ± 0.02	5.17 ± 0.04	4.41 ± 0.04	4.60 ± 0.10
7	1.0	42	4.4	7.10 ± 0.02	5.11 ± 0.03	4.36 ± 0.03	5.60 ± 0.10
8	4.0	42	4.4	9.23 ± 0.03	6.21 ± 0.07	4.21 ± 0.03	5.10 ± 0.20
9	−0.0	30	3.5	5.47 ± 0.09	4.79 ± 0.01	4.54 ± 0.04	4.90 ± 0.10
10	5.0	30	3.5	8.25 ± 0.04	6.02 ± 0.01	4.39 ± 0.03	4.10 ± 0.00
11	2.5	10	3.5	7.90 ± 0.02	5.19 ± 0.03	4.41 ± 0.05	4.70 ± 0.10
12	2.5	50	3.5	8.30 ± 0.03	5.90 ± 0.06	4.19 ± 0.04	5.60 ± 0.26
13	2.5	30	2.0	6.83 ± 0.06	4.39 ± 0.03	4.65 ± 0.01	4.60 ± 0.10
14	2.5	30	5.0	8.67 ± 0.04	5.29 ± 0.04	3.94 ± 0.05	4.50 ± 0.00
15	2.5	30	3.5	7.60 ± 0.07	5.10 ± 0.02	4.35 ± 0.03	4.40 ± 0.36
16	2.5	30	3.5	7.60 ± 0.03	5.31 ± 0.03	4.31 ± 0.02	3.40 ± 0.10
17	2.5	30	3.5	7.41 ± 0.04	5.25 ± 0.03	4.29 ± 0.08	4.10 ± 0.10
18	2.5	30	3.5	7.70 ± 0.03	5.19 ± 0.06	4.30 ± 0.02	3.95 ± 0.05
19	2.5	30	3.5	7.67 ± 0.02	5.26 ± 0.04	4.29 ± 0.02	4.05 ± 0.35

Overall acceptability score (9-point hedonic scale).

the reasons, and their antioxidant capacity is always associated with their health-promoting properties. Hernández-Ledesma et al. [52] reported the antioxidant activity for some peptide chains in whey, which had higher radical scavenging activity than butylated hydroxyanisole (BHA). The antioxidant activity of kefir has also been shown, and it is due to some potential compounds like glutathione, organic acids, and kefiran [8, 51, 53].

The multiple coded equations in terms of coded factors generated for these responses are shown as follows:

Table 4: Analysis terms for the quadratic model representing the investigated responses (TPC, DPPH (%), LAB and yeasts' number, pH, and overall acceptability).

Model terms	Coefficient estimate	*t*.exp	*p* value	Coefficient estimate	*t*.exp.	*p* value
Total phenolic content (mg/mL)				*DPPH scavenging activity (%)*		
b_0	63.199	584.42	***	71.950	325.27	***
b_1	0.386	5.89	**	1.804	13.46	***
b_2	7.620	116.32	***	6.255	46.68	***
b_3	6.545	99.91	***	6.241	46.57	***
b_{11}	5.148	78.57	***	3.220	24.02	***
b_{22}	1.933	29.50	***	2.394	17.86	***
b_{33}	2.781	42.45	***	1.280	9.55	**
b_{12}	0.046	0.54	NS	0.243	1.39	NS
b_{13}	0.524	6.12	**	−0.145	−0.83	NS
b_{23}	2.841	33.20	***	4.632	26.46	***
R^2	0.843			0.966		
Adj.R^2	0.686			0.931		
LAB (log_{10} CFU/mL)				*Yeasts (log_{10} CFU/mL)*		
b^0	7.614	151.07	***	5.226	145.45	***
b^1	0.645	21.12	***	0.302	13.88	***
b^2	0.239	7.83	**	0.265	12.16	***
b^3	0.513	16.80	***	0.309	14.18	***
b^{11}	−0.358	11.71	***	0.042	1.93	NS
b^{22}	0.081	2.64	NS	0.092	4.21	*
b^{33}	−0.043	−1.41	NS	−0.158	−7.24	**
b^{12}	0.359	8.99	**	0.225	7.91	**
b^{13}	0.044	1.10	NS	−0.065	−2.29	NS
b^{23}	0.241	6.05	**	−0.140	−4.92	**
R^2	0.909			0.942		
Adj.R^2	0.818			0.885		
pH				*Overall acceptability*		
b_0	4.299	386.48	***	3.984	26.50	***
b_1	−0.049	−7.31	**	−0.062	−0.68	NS
b_2	−0.080	−11.85	***	0.294	3.23	*
b_3	−0.229	−34.06	***	0.083	0.91	NS
b_{11}	0.108	15.95	***	0.164	1.80	NS
b_{22}	0.049	7.30	**	0.394	4.33	**
b_{33}	0.047	7.04	**	0.182	2.00	NS
b_{12}	−0.008	−0.85	NS	−0.312	−2.63	*
b_{13}	0.005	0.57	NS	−0.062	−0.53	NS
b_{23}	0.017	1.99	NS	0.187	1.58	NS
R^2	0.836			0.827		
Adj.R^2	0.672			0.654		

NS: nonsignificant; * $p < 0.05$, * * $p < 0.01$, and * * * $p < 0.001$.

$$\begin{aligned} \text{TPC} &= 63.199 + 0.386X_1 + 7.620X_2 + 6.545X_3 + 5.148X_1^2 + 1.933X_2^2 + 2.781X_3^2 + 0.524X_{13} + 2.841X_{23}, \\ \text{DPPH\%} &= 71.95 + 1.804X_1 + 6.255X_2 + 6.241X_3 + 3.22X_1^2 + 2.394X_2^2 + 1.28X_3^2 + 4.632X_2X_3. \end{aligned} \quad (3)$$

3.2. Effects of the Variables on pH and LAB and Yeast Cell Viability of Kefir Microorganisms. The effects of independent variables on LAB and yeast cell viability are shown in Table 3. Their number depends on whey permeate, fruit juice, and kefir inoculum level. The linear effects were significantly positive ($p < 0.001$; Table 4). The interaction effects of whey permeate-fruit juice and fruit-kefir grains were also significant ($p < 0.01$; Table 4). An increasing inoculum level allows enhancing LAB and yeasts' number. The same result was observed by Sabokbar and Khodaiyn [9] when they fermented a mixture of pomegranate juice and whey by kefir grains. They reported that the cell density of LAB increased by 1.3 Ulog when they increase the inoculum from 5% to 8%. However, for yeasts' number, nonsignificant difference was observed. M'hir et al. [11] obtained the same result when they prepared a fermented beverage from whey, whey permeate, and date syrup and when they prepared a kefir beverage made with carob, oat flour, and whey permeate [54]. This increase of LAB due to the increase of the level of juice can be explained by the probiotic effect of myrtle (Figure 4). In fact, in the studies of Mangia et al. [55] and Öztürk et al. [56], they showed that myrtle juice exerts a positive effect on lactobacilli.

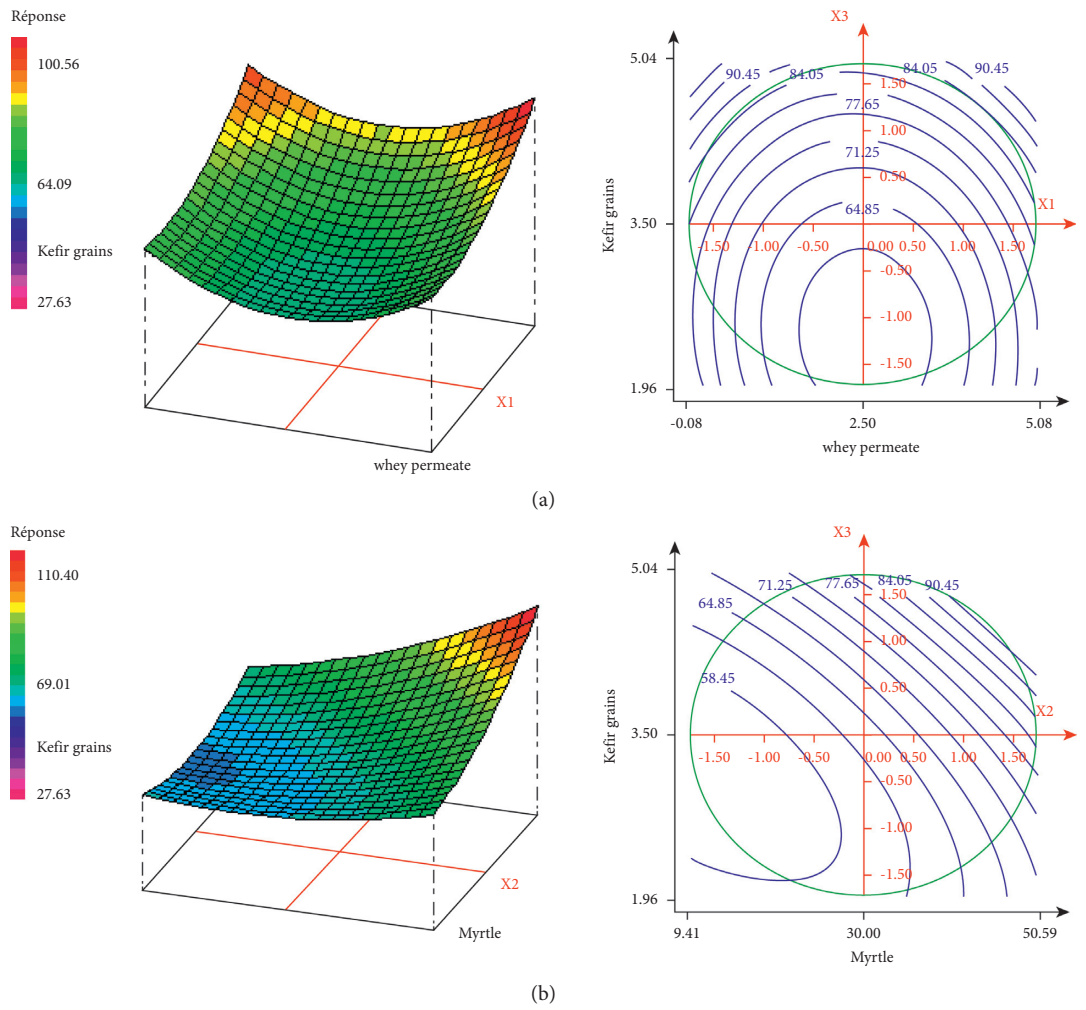

FIGURE 2: Response surface and contour plots representing the effect of WP and KG (a) and the effect of MJ and KG (b) on total polyphenol content (X_1: WP (% w/v); X_2: MJ (% w/v); and X_3: KG (% w/v)).

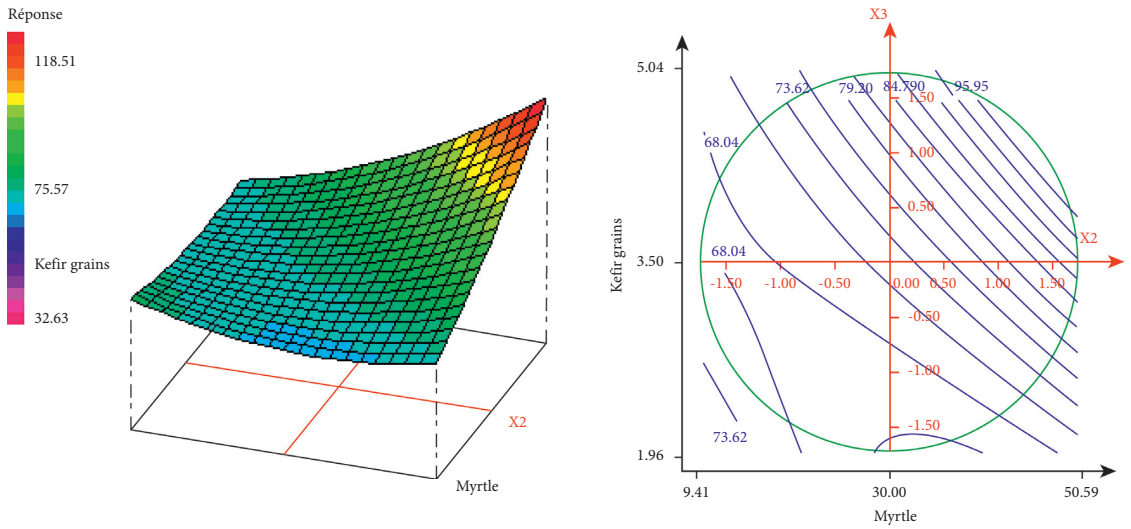

FIGURE 3: Response surface and contour plots representing the effect of MJ and KG on DPPH radical scavenging activity (%; X_2: MJ (% w/v) and X_3: KG (% w/v)).

Table 5: Levels of total phenolic content and antioxidant activities before and after fermentation for each beverage.

Exp	Total phenolic content (mg GAE/mL)		Antioxidant activities (%)	
	Unfermented	Fermented	Unfermented	Fermented
1	$48.75^{b} \pm 0.70$	$57.58^{b} \pm 0.69$	$60.88^{a} \pm 0.51$	$68.40^{b} \pm 0.67$
2	$49.88^{b} \pm 0.17$	$59.75^{c} \pm 0.56$	$63.15^{b} \pm 0.91$	$72.60^{fg} \pm 0.31$
3	$58.46^{de} \pm 0.70$	$64.80^{e} \pm 0.56$	$63.88^{b} \pm 0.45$	$71.25^{de} \pm 0.75$
4	$57.20^{d} \pm 0.96$	$63.60^{de} \pm 0.47$	$65.77^{c} \pm 0.74$	$75.60^{h} \pm 0.43$
5	$52.75^{c} \pm 0.63$	$69.97^{fg} \pm 0.49$	$60.76^{a} \pm 0.62$	$70.80^{cd} \pm 0.28$
6	$59.00^{ef} \pm 0.44$	$70.68^{g} \pm 0.39$	$65.20^{c} \pm 0.42$	$73.60^{g} \pm 0.38$
7	$57.25^{d} \pm 0.89$	$85.00^{i} \pm 0.14$	$69.45^{e} \pm 0.77$	$91.36^{l} \pm 0.70$
8	$58.91^{ef} \pm 0.96$	$89.45^{j} \pm 0.62$	$71.49^{f} \pm 0.41$	$95.95^{m} \pm 0.76$
9	$60^{fg} \pm 0.45$	$82.20^{h} \pm 0.26$	$67.77^{d} \pm 0.35$	$80.45^{i} \pm 0.65$
10	$63.89^{h} \pm 0.28$	$81.69^{h} \pm 0.70$	$71.67^{f} \pm 0.88$	$85.62^{j} \pm 0.65$
11	$45.11^{a} \pm 0.19$	$55.25^{a} \pm 0.34$	$60.78^{a} \pm 0.75$	$69.80^{c} \pm 0.24$
12	$60.68^{g} \pm 0.51$	$90.45^{j} \pm 0.79$	$75.94^{g} \pm 0.53$	$91.60^{l} \pm 0.51$
1 3	$58.30^{de} \pm 0.91$	$69.30^{f} \pm 0.77$	$60.76^{a} \pm 0.36$	$65.25^{a} \pm 0.32$
14	$58.91^{ef} \pm 0.96$	$81.20^{h} \pm 0.26$	$68.25^{de} \pm 0.91$	$89.85^{k} \pm 0.51$
15	$52.10^{c} \pm 0.28$	$63.10^{d} \pm 0.67$	$65.61^{c} \pm 0.57$	$71.50^{def} \pm 0.33$
16	$52.73^{c} \pm 0.40$	$62.50^{d} \pm 0.14$	$65.61^{c} \pm 0.12$	$72.05^{ef} \pm 0.84$
17	$51.77^{c} \pm 0.33$	$63.01^{d} \pm 0.09$	$65.96^{c} \pm 0.15$	$71.95^{def} \pm 0.84$
18	$52.77^{c} \pm 0.45$	$62.90^{d} \pm 0.84$	$65.23^{c} \pm 0.30$	$72.42^{ef} \pm 0.79$
19	$52.05^{c} \pm 0.44$	$63.05^{d} \pm 0.93$	$66.12^{c} \pm 0.12$	$71.15^{de} \pm 0.30$

Values are expressed as mean ± standard deviation (three determinations). Data in the same row with different superscript letters are significantly different at $p < 0.05$.

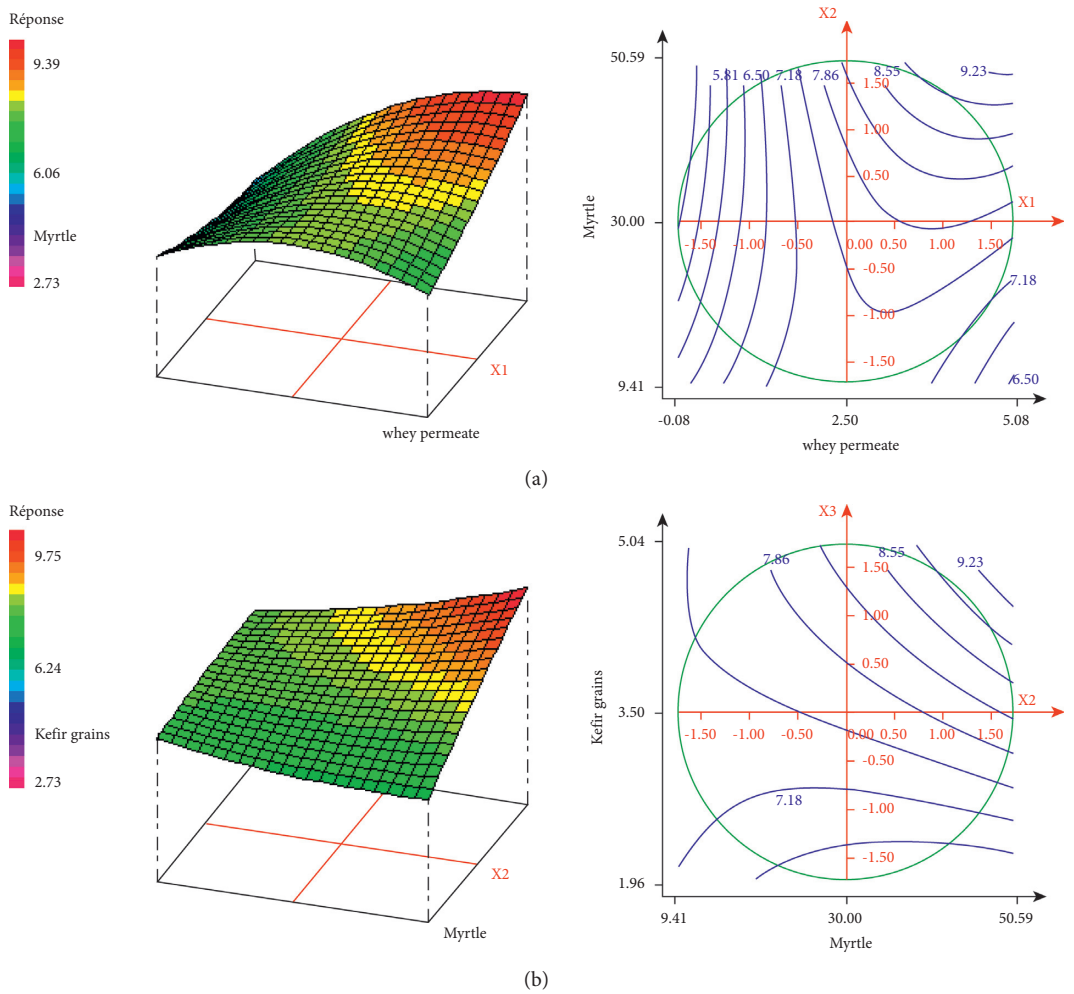

Figure 4: Response surface and contour plots representing the effect of WP and MJ (a) and the effect of MJ and KG (b) on LAB count (X_1: WP (% w/v); X_2: MJ (% w/v); X_3: KG (% w/v)).

The positive interaction between myrtle juice and kefir inoculum on cell density was significant ($p < 0.01$; Table 4). Filannino et al. [45] showed that phenolic compounds can exert a positive effect on LAB growth.

As a consequence of kefir microflora growth, the pH decline during fermentation (pH ranging between 5.02 and 3.94; Table 3) due to lactic acid increase, which can inhibit spoilage and pathogenic bacteria development in the beverage [9]. The pH decrease was significantly related to whey permeate ($p < 0.01$), juice ($p < 0.001$), kefir grains ($p < 0.001$), and their quadratic effects ($p < 0.01$). However, the pH was not significantly influenced by their interaction (Table 4).

The number of viable cells of yeast and LAB of the obtained beverage were in accordance with codex Standard FAO/WHO [57], suggesting at least 10^4 and 10^7 CFU/mL of bacteria and yeast counts, respectively.

The multiple coded equations in terms of coded factors generated for these responses are shown as follows:

$$\begin{aligned}
\text{LAB} &= 7.614 + 0.645X_1 + 0.239X_2 + 0.513X_3 - 0.358X_1^2 + 0.359X_{12} + 0.241X_{23},\\
\text{Yeasts} &= 5.226 + 0.302X_1 + 0.265X_2 + 0.309X_3 + 0.092X_2^2 - 0.158X_3^2 + 0.225X_{12} - 0.14X_{23},\\
\text{pH} &= 4.229 - 0.049X_1 - 0.08X_2 - 0.229X_3 + 0.108X_1^2 + 0.049X_2^2 + 0.047X_3^2.
\end{aligned} \tag{4}$$

3.3. Effects of the Variables on Sensory Evaluation. Sensory analysis is a real lever for the development of new food products. Indeed, the characterization of their organoleptic properties is essential to guarantee their acceptability by consumers.

Only myrtle juice linear and quadratic terms (b_2 and b_{22}) have relevant effects on the overall acceptability (Table 4). As noticed in Figure 5, a significant interaction was detected between whey permeate (X_1) and myrtle juice (X_2). The OA scores ranged from 3.9 ± 0.1 to 5.6 ± 0.26 on a 9-point hedonic scale (Table 3). The most appreciated samples were the beverages from experiment 7 (1% WP, 42% myrtle juice, and 4.4% kefir grains) and 12 (2.5% WP, 50% myrtle juice, and 3.5% kefir grains). The acceptability ratings were highest in the samples containing a high amount of fruit juice. It can be explained by the fact that Myrtle's juice masked the unsavory taste of cheese whey. In the study of Öztürk et al. [56], black and white myrtles improved the taste scores of probiotic goat milk ice cream samples by masking the low pH resulting from fermentation. Koksoy and Kilic [58] showed that acid odor was masked with the fruit aromas resulting in more acceptable drinks for consumption.

The level of juice and whey used in many studies was different; it largely depends on the fruit matrix. For example, Islam et al. [12] suggested that the optimum formula was with 25% whey and 75% pineapple juice. However, Pereira et al. [59] added only 10% of mango fruit to liquid whey protein and concentrated permeates.

Phenolic compounds contained in myrtle juice have also an impact on the obtained beverages' sensory attributes. Indeed, they have an important effect on color, perceived taste, and flavor. Pinto and Vilela [60] reported that different color palettes may influence our taste and flavor perception. The polynomial model for OA is presented by the following equation:

$$\text{OA} = 3.984 + 0.294X_2 + 0.394X_2^2 - 0.312X_1X_2. \tag{5}$$

3.4. Validation. Validation tests were performed in order to determine the LAB and yeasts' cell count, the pH, the TPC, the DPPH antioxidant activity, and OA under optimized condition (2.83% (w/v) at whey permeate, 48.45% (w/v) myrtle juice, and 3.71% (w/v) inoculum). The validation results are demonstrated in Table 6 in which the number of LAB and yeasts, the antioxidant activity, TPC, and the OA were mentioned. TPC showed a lower value than the predicted ones. Taking into account the standard deviations of measured and calculated responses, the results obtained indicated that the experimental values were in good agreement with the predicted values. This suggested that the fitted model is satisfactory and accurate.

3.5. Microbial Viability of Kefir Culture and the Change in Physicochemical Parameters during Storage. The results for the effects of storage temperature at 4°C on the counting of LAB and yeasts and on physicochemical parameters of the developed beverage are shown in Table 7. A reduction in the load of LAB was observed from the first day of storage. LAB were more sensitive to storage than yeasts. Indeed, the loss in the viability of LAB cells was more important than this of yeasts. A very little and nonsignificant decrease of yeasts counts was recorded after 14 days of storage at 4°C. However, the viability is maintained during the two weeks of storage at a high level for all tested microflora as recommended by FAO/WHO 2006 [57]. The number of LAB and yeast viable cells was 7.61 ± 0.14 and 6.19 ± 0.24, respectively, after 2 weeks of storage at 4°C. These results were in agreement with the reports of previous investigators [59, 61]. The microorganisms' viability largely depended both on medium composition and on storage temperature. The pH influence on cell viability has been widely mentioned [62]. Existing exopolysaccharides, as kefiran, might help improve the survival of microorganisms in an acidic or frozen medium by giving a protective envelop that may preserve them from stressful conditions. The resistance of LAB in acidic conditions is due to the action of the proton pump, the change in their cell membrane composition, and other mechanisms [63]. Nualkaekul and Charalampopoulos [64] suggested that *Lactobacillus* probably uses the energy

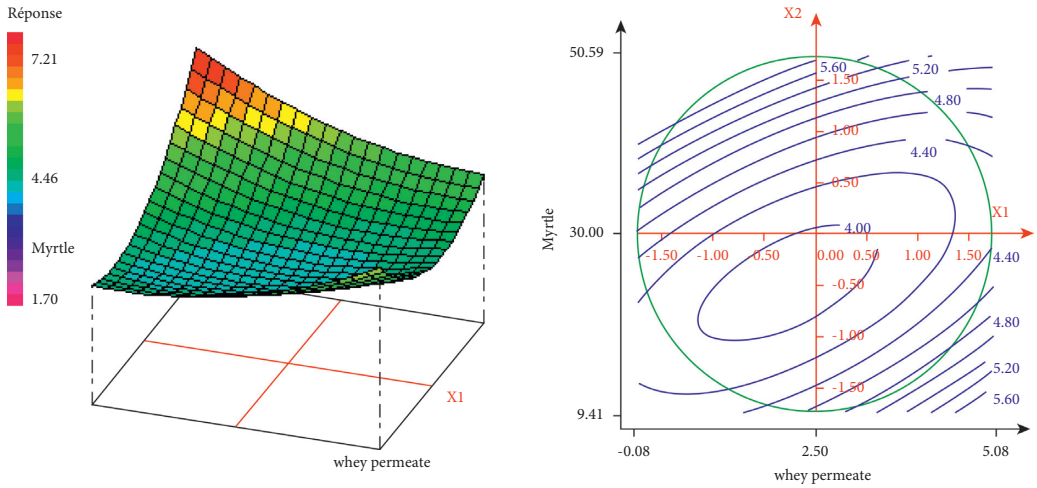

Figure 5: Response surface and contour plots representing the effect of WP and MJ on overall acceptability (X_1: WP (% w/v) and X_2: MJ (% w/v)).

Table 6: Predicted and experimental values of responses under optimum conditions.

Response variables	Experimental	Predicted
TPC (mg GAE/mL)	81.56 ± 0.78	82.77
DPPH (%)	91.61 ± 0.61	91.26
LAB viability ($\log_{10}$ CFU/mL)	8.53 ± 0.30	8.64
Yeast viability ($\log_{10}$ CFU/mL)	6.21 ± 0.27	6.01
pH	4.18 ± 0.02	4.24
OA score	5.41 ± 0.25	5.37

Mean values ± standard deviation (duplicate determination); OA score on 1–9 scale (with 100% d (i): the percentage of calculated desirability).

Table 7: Microbial viability and the change in physicochemical parameters during refrigerated storage.

	LAB ($\log_{10}$ CFU/mL)	Yeast ($\log_{10}$ CFU/mL)	Total polyphenol content (mg EAG/mL)	Scavenging activity (%, DPPH)	Overall acceptability (1–9 scale)	pH
0j	$8.53^{a} \pm 0.60$	$6.21^{a} \pm 0.04$	$81.56^{d} \pm 0.50$	$91.61^{d} \pm 0.63$	$5.41^{ab} \pm 0.12$	$4.18^{d} \pm 0.02$
1j	$7.85^{a} \pm 0.18$	$6.55^{a} \pm 0.09$	$76.13^{c} \pm 0.67$	$88.44^{c} \pm 0.71$	$5.53^{b} \pm 0.17$	$3.99^{c} \pm 0.02$
7j	$7.89^{a} \pm 0.14$	$6.23^{a} \pm 0.16$	$73.86^{b} \pm 0.67$	$86.73^{b} \pm 0.42$	$5.44^{ab} \pm 0.07$	$3.82^{b} \pm 0.02$
14j	$7.61^{a} \pm 0.14$	$6.19^{a} \pm 0.24$	$69.12^{a} \pm 0.15$	$84.57^{a} \pm 0.21$	$5.13^{a} \pm 0.06$	$3.69^{a} \pm 0.01$

Mean ± SD. Data in the same row with different superscript letters are significantly different at $p < 0.05$.

generated through their metabolic activity in order to maintain their viability. During the storage time, the pH value decreased to 3.69 with the progress of the storage period. This acidification through storage could be caused by residual microbial activity. The same result was observed by Islam et al. [12].

The storage has a significant impact on polyphenol content and antioxidant activity. A significant decrease of TPC and of DPPH free radical scavenging activity (%) was observed during storage. The loss of phenolic compounds could be explained by their oxidation.

On day 1 of the postproduction, the sensory properties of the fermented beverage have been slightly improved and then decreased under refrigerated storage. The decrease in product quality was linked to the increase in the contents of organic acids. Indeed, a mild increase in acidity was observed from the second day of storage.

4. Conclusions

The supplementation of cheese whey allowed developing kefir-like beverage having functional proprieties such as high polyphenol content and good antioxidant capacity. The RSM was successfully employed to optimize the LAB and yeast cell counts, the TPC, the antioxidant activity, and the OA with the incorporation of different concentrations of WP, myrtle juice, and kefir grains. The beverage obtained by mixing (2.83% whey permeate and 3.71% kefir grains) gave acceptable sensorial properties. The enumeration of LAB and yeast cells showed that the obtained beverage fulfilled the criterion of probiotic beverage in an acceptable manner even after 14 days of storage at 4°C. Sensory attributes may be ameliorated with further investigations like using myrtle syrup and/or adding another carbon source.

Data Availability

The data used to support the findings of this study are included within the article (Tables 2–7).

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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Supplementary Materials

Analyses of variance of central composite design are provided in Supplementary Materials. (*Supplementary Materials*)

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Research Article

Application of Multivariate Optimization for Phenolic Compounds and Antioxidants Extraction from Moroccan *Cannabis sativa* Waste

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A statistical simplex centroid design methodology was applied to determine the effects of different solvents and their mixtures on the yield, total polyphenol content, 2′2-dipheny-l-picrylhydrazyl (DPPH) radical scavenging activity, and ferric reducing antioxidant power (FRAP) of extracts from the waste of *Cannabis sativa*. The different extractor solvents (ethanol, methanol, water, and hexane) and their binary and ternary combinations were evaluated. The experimental results and their response surface models showed that the highest TPC yield values occur with the binary interaction between water and ethanol around the proportion of (ethanol, 70%; water, 30%). The desirability function showed that the optimal conditions were for TPC extraction ternary mixtures which consisted of 75% ethanol, 12.5% methanol, and 12.5% water. Ternary mixtures including water and binary mixture (ethanol 50% to 75%) yielded extracts with the best DPPH antioxidant activity, whereas pure methanol was the best solvent for extracting molecules with FRAP antioxidant capacity. The desirability function including all responses showed that the optimal solvent mixture consisted of 25% ethanol and 75% methanol.

1. Introduction

Cannabis sativa L. is a dioecious plant belonging to the *Cannabaceae* family. Cannabinoids, flavones, and terpenes are the main phytochemicals found in this plant [1]. This plant was used as a medicine before the Christian era in Asia, principally in India, and after it was introduced to Western medicine in the midst of the 19th century. Recently, the interest in *C. sativa* L. has drastically increased, due to psychoactive and nonpsychoactive compounds, namely, Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD) [2]. This interest had increased in the 1990s through the description of cannabinoid receptors and the identification of an endogenous cannabinoid system in the brain [3]. In the last years, *Cannabis sativa* L. has been the center of the attention of the scientific community and definitely became one of the most studied plants. In Morocco, cannabis cultivation in the northern Rif region is currently undergoing its most significant evolution since the hashish industry emerged in the 1960s and dramatically developed in the 1980s [4]. To separate the golden beige powder called "*Hashish*" resulting from the solidification of the droplets of resin exuding from the tops of the female plants, these plants are first dried, and the resin and trichomes become dusty and more brittle; thus the resin can be separated from the plant material by beat or shake over a thin stretched nylon veil which acts as a sieve [5, 6]. After this process, the plant residue (whole plant fragments) is separated from seeds and discarded.

Phenolic compounds are associated with a high number of biological activities including antioxidant capacity, which may help to protect the cells against the oxidative damage caused by free radicals [7]. Several novel extraction techniques have been developed in an attempt to obtain a more efficient extraction of target compounds by reducing both extraction time and used solvent [8]. The efficient recovery of bioactive polyphenolic phytochemicals, which occur widely in several agri-food residues that may serve as both

food additives and bioactive substances in cosmetics and pharmaceuticals, is one of the higher value options [9]. These secondary metabolites, which usually occur in low concentrations, are medicinally useful. The yields of these metabolites depend on the solvents and methods of extraction [10]. The quantity and quality of plant extracts are depending on the extraction protocol, which includes several factors such as the type of solvent, temperature, pH, the number of extraction steps, liquid-to-solid ratio, and the particle size of the solute contributing to the efficacy of the extraction process [11]. Solvent extraction is the most frequently used technique for the isolation of plant antioxidant compounds, due to the presence of different antioxidant compounds of varied chemical characteristics and polarities. Polar solvents like methanol and ethanol have been extensively employed to extract antioxidant compounds from various plants and plant-based foods [12].

Response surface methodology (RSM) is a well-established tool for the optimization of analytical methods, which is widely applied for the analysis of foods and herbal medicine [13]. The mixture design is a class of response surface experiments whose aim is to develop better or innovative formulations providing optimal requests and to create general conceptions about responses and interactions between independent factors allowing the modelization of the studied interaction [9]. To our knowledge, its use to study the effect of solvent combinations on cannabis waste has not been previously reported.

The goal of this experiment is to perform the lowest number of experiments while gaining the maximum amount of data for the development of an efficient and reproducible model with the desired properties, to obtain clear information on the proportions of individual solvents including ethanol, methanol, water, and hexane in a mixture characterized by its capacity to yield extracts with the highest antioxidant activity and containing the greatest amounts of TPC from *Cannabis sativa* plant residue.

2. Materials and Methods

2.1. Extraction. *Cannabis sativa* plant residue without resin was collected from farmers and was ground into fine powder. The extracts were prepared in triplicate by adding 1 mL of the solvent (pure solvent and mixture) to 50 mg of plant residue powder and subjected to sonication for 30 minutes in an ultrasonic bath at ambient temperature, so the extracts were recovered after centrifugation for 10 minutes at 10,000 rpm and stored in the dark at 4°C.

2.2. Total Phenolic Compounds (TPC). Total phenolic content (TPC) was determined by spectrophotometry, using the colorimetric method, based on the Folin–Ciocalteu reagent [14] with modifications. 50 μL of extract or gallic acid standard solution was mixed with 450 μL of Folin–Ciocalteu reagent solution diluted 10 times. Subsequently, the mixture was shaken on a vortex mixer and left to incubate for 5 minutes at room temperature, and then 450 μL of a Na_2CO_3 solution (75 g·L^{-1}) was added and the mixture was shaken again. After incubation for 2 hours at room temperature their absorption was measured at 760 nm in a UV/visible Jenway 6505 scanning spectrophotometer. The gallic acid calibration curve (standard curve equation, $y = 2.8388x + 0.0556$, $R^2 = 0.9994$) was prepared with a concentration ranging from 0.062 to 1 mg·mL^{-1} in ethanol. The results are expressed in gallic acid equivalent (GAE) g^{-1} dry plant mg. All analyses were done in triplicate.

2.3. DPPH Free Radical Scavenging Activity. The free radical scavenging activity of all extracts was performed following the procedure described by Brand-Williams et al. [15]. Briefly, 25 μL of each extract (with different dilutions) was added to 1 mL of an ethanol solution of DPPH (60 μM). The absorption measurements were made at 515 nm, after the incubation time of 60 minutes at room temperature. The absorption of a blank sample containing the same amount of ethanol and DPPH solution served as a negative control. The percentage inhibition of activity trapping free radicals in each extract was calculated as follows:

$$\%_{\text{Inhibition}} = \left[\left(\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right) \times 100\right]. \quad (1)$$

Triple measurements were made for each sample and the results were expressed in terms of average ± standard error.

2.4. Ferric Reducing Antioxidant Power (FRAP). The reductive capacity of liquid extracts was determined by the reducing power test described by Oyaizu [16]. This method is based on the transformation of ions, Fe^{2+} into Fe^{3+} by polyphenol antioxidants. 0.2 mL of the extract was mixed with 1 mL of 0.2 M phosphate buffer and 1 mL 1% potassium ferricyanide in glass tubes. The prepared reaction mixture was incubated for 20 minutes at 50°C in a water bath. After incubation, 1 mL of 10% trichloroacetic acid solution was added to the reaction mixture. Tubes were centrifuged at 3000 rpm for 10 minutes after 2 mL of the supernatant was mixed with 2 mL of bidistilled water and 0.4 mL of 0.1% ferric chloride solution. The absorbance was measured at a wavelength of 700 nm. The reductive capacity of the extracts is expressed as ascorbic acid equivalent (Acs. E) g^{-1} dry plant. All analyses were done in triplicate.

2.5. Experimental Design and Optimization. Mixture models were revealed to be useful tools in describing the behavior of solvent mixtures to extract bioactive compounds from plants [17]. In the present study, a simplex centroid design with a response surface methodology was employed to determine the effect of interactions among extraction solvents on TPC and antioxidants activities to optimize the extraction conditions. Mixture design experiments were designed and analyzed using the free version StatSoft, Inc. (2011), STATISTICA (data analysis software system), version 10. A total of seven combinations are used. Results were expressed as mean ± SD (RSD (%)) and were analyzed using ANOVA. Significant differences were determined by Tukey's test, with $p < 0.05$ as the significance criterion. The following

polynomial equation of function Xi was fitted for each factor assessed at each experimental point:

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3, \quad (2)$$

where Y is the predicted response and $\beta 1$, $\beta 2$, $\beta 3$, $\beta 12$, $\beta 13$, and $\beta 23$ are constant coefficients for each linear and nonlinear interaction term.

Principal component analysis (PCA) was carried out to understand the influence of the extraction solvents on the antioxidant compounds and identify the relationships between the variables.

3. Results and Discussion

3.1. Solvent Screening. Solvent selection is one of the most important steps in extraction of phenolics and other bioactive compounds from fruits, vegetables, byproducts, and their recovery from plant-based materials [3]. Generally, desired compound extraction efficiency is influenced by multiple parameters, such as temperature, time, and solvent polarity, and their effects may either be independent or interactive [18]. The results of total phenolic contents and the antioxidant activity of the seven pure solvent extracts are shown in (Figure 1). It is obvious to note that the phenolic compounds and antioxidant capacity of *C. sativa* extracts were highly and significantly influenced ($p < 0.05$) by the type of extraction solvent. The same conclusions have been reported by Liyanapathirana and Shahidi [19]. Estimated values of total phenolic content in different extracts yielded using different solvents ranged from 1.90 ± 00 to 19.07 ± 00 mg GAE/g DP, pointing out that ethanol extract was done ten times more TPC than ethyl acetate. Other works corroborating our findings reported TPC results for *C. sativa* ranging from 5.85 to 17.05 mg GAE/g dw [20]. However, higher TPC in inflorescence samples from industrial hemp was quantified in the range of 10.51 to 52.58 mg GAE/g [21].

Ethanol extract exhibited the highest phenolic content followed by methanol extract. Wile, water, and hexane come, respectively, in third and fourth place, whereas dichloromethane was the less efficient solvent for extraction of TPC from *Cannabis sativa* waste. According to [22], the extract obtained by 100% ethanol from *L. aromatica,* showed the highest phenolic content and exhibited the highest total antioxidant activity, reducing power, and DPPH radical scavenging activity.

The seven solvents were also screened using two antioxidant methods. According to the total antioxidant activity (Figure 1), methanol extracts demonstrated the best antioxidant activity (73.47 mg Asc.E/g DP), followed by ethanol. Conversely, dichloromethane extracts exhibited the lowest antioxidant activity. As shown in Figure 1, the DPPH free radical scavenging activity revealed that ethanol and methanol were the best solvents for extracting molecules endowed with antioxidant properties followed by hexane and then water. According to this screening test, methanol, ethanol, water, and hexane were the best solvents for extracting phenolic compounds and other molecules with antioxidant activity, which allowed us to select them to perform the solvent mixture design.

3.2. Mixture Design. RSM is considered the best statistical approach to assess the influence of different experimental factors and their linear and quadratic interactions on analytical responses and to model and optimize the extraction conditions of phenolics that display important biological activities [3]. This approach gathers the maximum amount of information in the minimum number of analyses. The first mixture design was perfumed using hydroalcoholic solvents by mixing water, ethanol, and methanol, whereas the second was done using three organic solvents, ethanol, methanol, and hexane. The results of the effects of the solvent composition on the yield of TPC and antioxidant activities are shown in Table 1.

3.3. Analysis of Variance (ANOVA). To improve the extraction conditions allowing the best antioxidant compounds recovery, the simplex centroid mixture design was used to construct a contour plot by regression model analysis. Experimental data were fitted to the linear, quadratic, and special cubic models. The statistical significance of the model and equation terms was analyzed based on p value (Prob > F). The goodness of fit of the model to justify its robustness was evaluated by the coefficient of determination (R^2) and the adjusted correlation coefficient (Adj-R^2), whereas the model's significance was checked using the F-test.

The analysis of variance (ANOVA) results based at 95% confidence interval, including regression model terms, R^2, F-test, and probability values are depicted in Table 2. In all mixtures, the linear model explained the variance up to good levels, and expanding from linear to a quadratic and special cubic model improved the fit for the regression analysis. Concerning the hydroalcoholic mixtures, the coefficient of determination (R^2) value of the quadratic model was ($R^2 = 1$) and the adjusted R^2 was 0.92 for both TPC and DPPH assays. In the case of FRAP assay, R^2 and adjusted R^2 were equal to 0.96 and 0.95, respectively, which indicated that the model adequately represented the real relationship between the parameters chosen. Furthermore, those two coefficients were equal to 1 ($R^2 = 1$) for the three responses in the special cubic model. Hence, this special cubic model was chosen since it could explain 100% of the variability for TPC, DPPH, and FRAP assays, as well as presenting small p values (p value <0.000001) and high F values (Table 3) indicating that this model was very good in predicting the behavior of the mixtures.

Regarding the mixtures where only organic solvents (ethanol, methanol, and hexane) were used, ANOVA indicated that the quadratic model explained 91%, 100%, and 99% of the total variation of the results, for TPC, DPPH, and FRAP, respectively, but this model was not significant for total phenolic contents (p value =0.08), whereas the special cubic model explained the result's variability at 100% level for all responses, besides

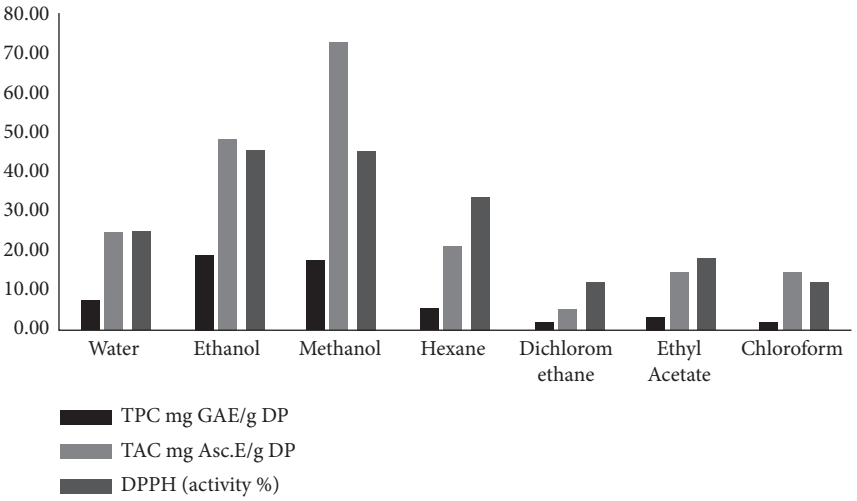

Figure 1: TPC, DPPH, and FRAP of the screened pure solvents.

Table 1: Simplex centroid design and response mean values.

	Ethanol	Methanol	Water	Hexane	TPC	DPPH	FRAP
1	0.00	0.00	0.00	100.00	5.17 ± 0.03	34.06 ± 0.07	21.66 ± 0.36
2	0.00	0.00	100.00	0.00	7.23 ± 0.25	25.03 ± 0.21	25.00 ± 0.09
3	0.00	50.00	0.00	50.00	11.66 ± 0.03	41.03 ± 0.11	55.82 ± 2.44
4	0.00	50.00	50.00	0.00	15.59 ± 0.71	46.87 ± 0.21	37.90 ± 0.77
5	0.00	100.00	0.00	0.00	17.42 ± 0.14	45.48 ± 0.18	73.47 ± 0.38
6	33.33	33.33	0.00	33.33	11.97 ± 0.13	42.45 ± 0.37	40.82 ± 0.59
7	33.33	33.33	33.33	0.00	20.89 ± 0.09ab	53.93 ± 0.15	53.33 ± 1.09
8	50.00	0.00	0.00	50.00	16.79 ± 0.15	36.90 ± 0.11	30.75 ± 0.10
9	50.00	0.00	50.00	0.00	21.03 ± 0.08a	51.90 ± 0.10	45.08 ± 0.12
10	50.00	50.00	0.00	0.00	20 .14 ± 0.30	47.21 ± 0.10	54.23 ± 0.16
11	100.00	0.00	0.00	0.00	19.07 ± 0.04	45.78 ± 0.28	48.90 ± 0.13

Table 2: ANOVA analysis of the three responses.

	Hydroalcoholic mixtures				Organic mixtures			
	F	*p*	*R*-Sqr	Adjusted	*F*	*p*	*R*-Sqr	Adjusted
TPC								
Linear	12.70	0.000363	0.59	0.54	54.55	0.000000	0.86	0.84
Quadratic	448.03	0.000000	1.00	0.99	2.71	0.082195	0.91	0.88
Special cubic	4.85	0.044967	1.00	1.00	1765.19	0.000000	1.00	1.00
DPPH								
Linear	5.63	0.012586	0.38	0.32	106.30	0.000000	0.92	0.91
Quadratic	633.22	0.000000	1.00	0.99	94.30	0.000000	1.00	0.99
Special cubic	187.75	0.000000	1.00	1.00	23.56	0.000256	1.00	1.00
FRAP								
Linear	47.72	0.000000	0.84	0.82	97.26	0.000000	0.92	0.91
Quadratic	17.39	0.000038	0.96	0.95	25.52	0.000004	0.99	0.98
Special cubic	480.67	0.000000	1.00	1.00	63.60	0.000001	1.00	1.00

presenting small p values (p value <0.000001) and high F values (Table 3), indicating that this proposed model provided a very good fit to the data.

The polynomial models describing the correlation between responses obtained for the quadratic model in hydroalcoholic mixtures are as follows:

Table 3: Regression coefficients of second-order polynomial model for the three responses.

	SS	d*f*	MS	*F*	*p*
Model: hydroalcoholic mixtures					
TPC	426.66	6	71.11	683.86	0.000000
DPPH	1605.17	6	267.53	6976.60	0.000000
FRAP	4067.47	6	677.91	2324.71	0.000000
Model: organic mixtures					
TPC	503.617	6	83.94	3226.01	0.000000
DPPH	430.456	6	71.74	1590.37	0.000000
FRAP	5330.570	6	888.43	929.76	0.000000

TPC = 19.04 * ethanol + 17.38 * methanol + 7.20 * water + 8.28 * ethanol * methanol + 32.21 * ethanol * water + 13.75 * methanol * water + 14.18 * ethanol * methanol * water + 0

DPPH = 45.64 * ethanol + 45.34 * methanol + 24.89 * water + 9.01 * ethanol * methanol + 68.68 * ethanol * water + 49.14 * methanol * water + 53.39 * ethanol * methanol * water + 0

FRAP = 48.30 * ethanol + 72.88 * methanol + 24.40 * water − 15.94 * ethanol * methanol + 44.43 * ethanol * water − 33.44 * methanol * water + 235.60 * ethanol * methanol * water + 0

In general, a positive sign for the coefficient in the fitted model indicates the ability of the variable to increase the response, whereas the negative sign indicates the ability of a variable to decrease the response [17]. The equation model showed that the phenolic content and antioxidants activities (DPPH and FRAP) were positively and linearly influenced by ethanol, methanol, and water, respectively, noting that, in linear trials, water resulted in the lowest coefficient, thus, the smallest TPC amounts and the lowest antioxidant activities. Among the binary interactions, water and ethanol interactions resulted in the highest positive effect in all responses, followed by water-methanol interactions for TPC and DPPH, whereas, in the case of FRAP assay, this coefficient was negative in the binary interactions of methanol-ethanol and water-methanol indicating an antagonistic effect between the two solvents. In addition, the ternary mixtures influenced positively the extraction of TPC and antioxidant compounds; this effect was much higher in the FRAP assay.

The regression equations obtained for the special cubic model in organic mixtures correlating the three variables and the analytical responses are as follows:

TPC = 19.07 * ethanol + 17.42 * methanol + 5.17 * hexane + 7.57 * ethanol * methanol + 18.66 * ethanol * hexane + 1.47 * methanol * hexane − 134.86 * ethanol * methanol * hexane

DPPH = 45.78 * ethanol + 45.48 * methanol + 34.06 * hexane + 6.31 * ethanol * methanol − 12.09 * ethanol * hexane + 5.06 * methanol * hexane + 2 0.51 * ethanol * methanol * hexane

FRAP = 48.89 * ethanol + 73.47 * methanol + 21.66 * hexane − 27.83 * ethanol * methanol − 18.13 * ethanol * hexane + 32.99 * methanol * hexane − 155.13 * ethanol * methanol * hexane

Regarding the linear interaction, in contrast to hexane, ethanol exhibited the highest positive effect on TPC extraction and DPPH free radical scavenging activity, while methanol demonstrated the highest positive influence in the FRAP assay. The strongest positive effect of binary interaction was found in ethanol-hexane mixtures for TPC and DPPH and methanol-hexane mixtures for FRAP assay, whereas the ternary mixtures had high and negative influence and displayed an antagonistic effect among the components of the mixture for TPC extraction and FRAP assay of the yielded extracts. These results clearly indicate that the extraction is affected by the polarity of the solvents.

3.4. Contour Plots Analysis

3.4.1. Total Phenolic Compounds. Among the major classes of plant chemicals, namely, terpenoids, phenolics, and alkaloids phenolic compounds including phenolic acids (hydroxybenzoic and hydroxycinnamic acids), polyphenols (hydrolyzable and condensed tannins), and flavonoids are the most important for dietary applications and the most extensively researched [22]. *C. sativa* inflorescence could be considered as a potential novel source of polyphenols intended for nutraceutical formulations [21]. The recovery of phenols depends on the solvent system used since the solvent mixture extracts higher TPC amounts than did pure solvent [23]. Thus, the development of a selective method for the extraction of phenolic compounds is required. The three-dimensional (3D) interaction contour plots are given in Figure 2(a) as a function of the ethanol, methanol, and water interactions. These three-dimensional response surface plots can exhibit how the initial factors affected different responses more clearly.

The interpretation of contour plots in Figure 2(a) demonstrated the total polyphenols extraction using the combination of ethanol and water resulted in the highest antioxidant activities, which can confirm a synergistic effect between these two solvents, in which the interaction between them demonstrates a better result than their isolated actions. Adding water to organic solvents improves extraction rate, but too high water content brought an increased concomitant extraction of other compounds and then lower phenols concentrations [23]. We also noticed that joining water to methanol enhanced its ability to extract phenolic compounds. The highest TPC yield value on the contour graph is seen to occur with the binary interaction between water and ethanol around the proportion of ethanol 70%, water 30%, whereas the best solvent stated by other authors, for extraction of phenolic compounds from hemp, was 50% ethanol [20]. According to Wong et al. [24], increasing ethanol concentration from 20% to 80% was favorable to increase total phenolic content recovery from palm kernel byproducts from 7.8 to 11.5 mg/g GAE.

According to the desirability analysis (results shown in Table 4), the best solvent combination for optimal extraction

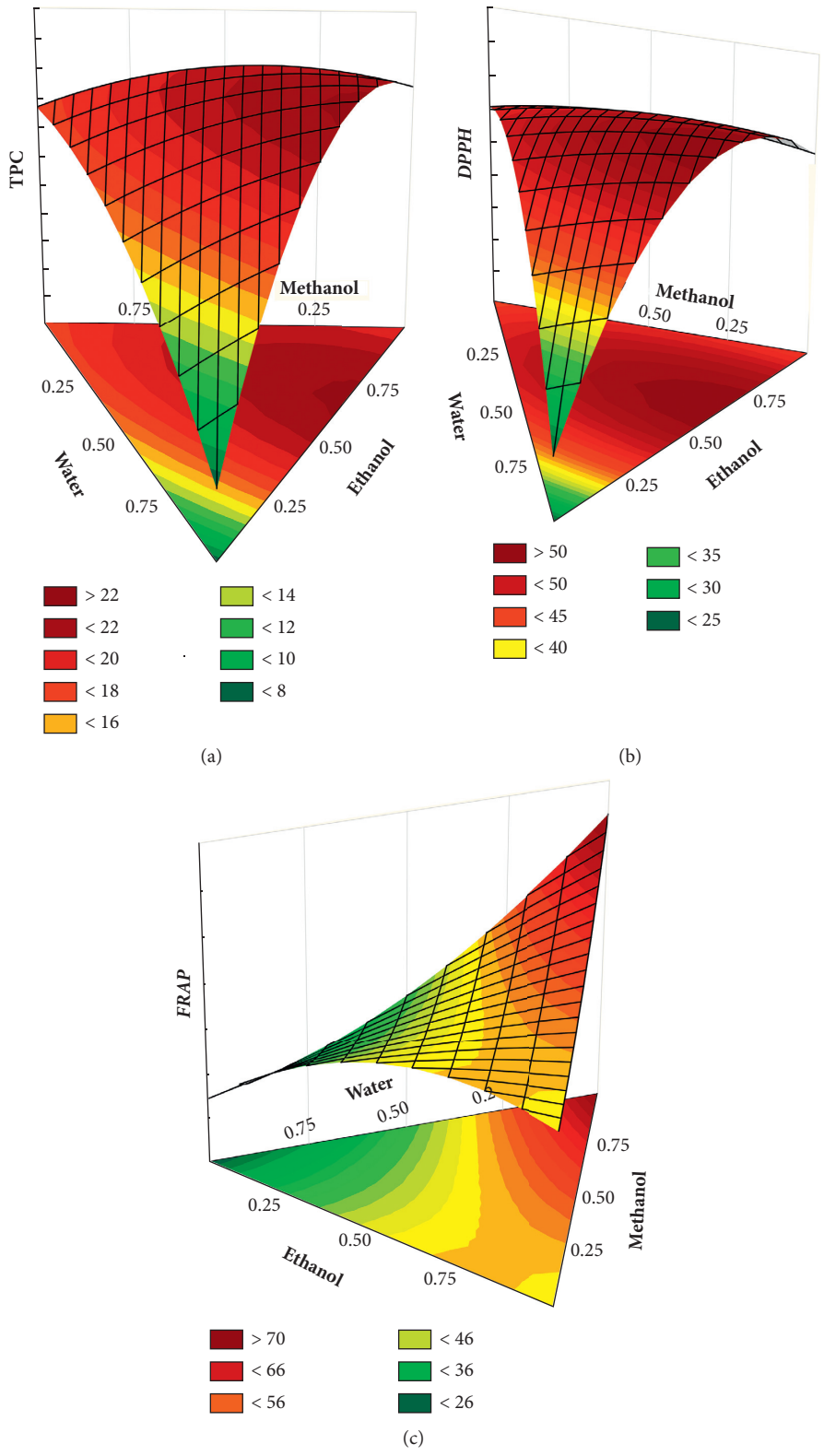

Figure 2: Mixture contour plots of TPC (a), DPPH (b), and FRAP (c) as a function of proportions of ethanol, methanol, and water.

of the phenolic compound is the ternary mixture which consists of 75% ethanol, 12.5% methanol, and 12.5% water.

Total phenolic compound contour plot, extracted using organic solvent mixtures including ethanol, methanol, and hexane, is shown in Figure 3(a). As shown in Figure 3(a), ethanol extracted the highest amounts of TPC, while hexane was the worst solvent for TPC recovery, which rapidly increases when the proportion of the ethanol increases in the

Table 4: Desirability results.

	Hydroalcoholic mixtures			Organic mixtures		
	Ethanol	Methanol	Water	Ethanol	Methanol	Hexane
TPC	75	12.5	12.5	50	50	0
DPPH	50	16.667	33.33	50	50	0
FRAP	0	100	0	0	100	0

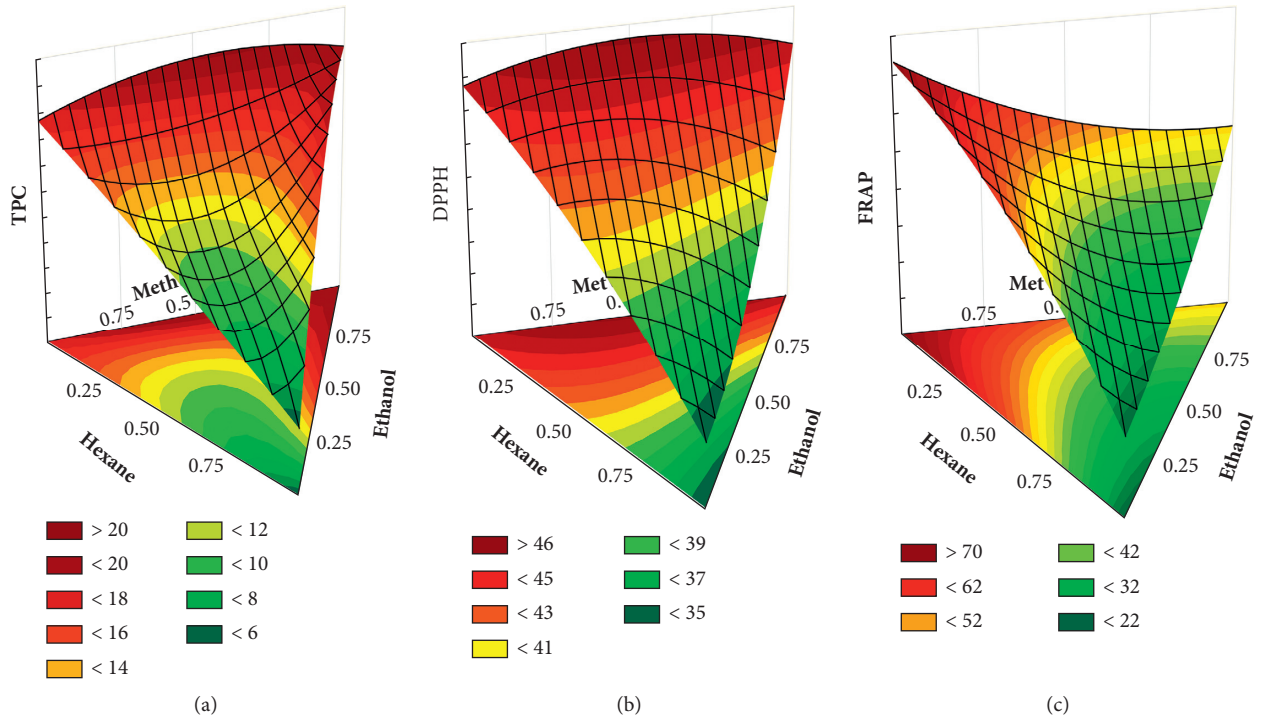

Figure 3: Mixture contour plots of TPC (a), DPPH (b), and FRAP (c) as a function of proportions of ethanol, methanol, and water.

binary mixture. The same observation can be noticed also when adding methanol to hexane, but with less effectiveness. Thus, adding ethanol and methanol to hexane increases its capacity to extract TPC. Desirability analysis (Table 4) revealed the equivalent binary interaction to be the optimal solvent mixture in the absence of water.

3.4.2. DPPH Free Radical Scavenging Activity. Generally, plants bioactive compounds with higher polar character can be easily extracted with water; however highly hydroxylated phenolic compounds, such as catechins, are more soluble in alcohols such as ethanol and methanol [25]. The DPPH assay is employed to test the ability of compounds to act as free radical scavengers and is frequently used to evaluate the antioxidant capacity of foods. The recovery of antioxidants from plant residues is interesting from a technological point of view as valuable components of nutraceuticals in food and pharmaceutical preparations or the cosmetics industry [26].

To investigate the effects of solvents and their interactive effects on antioxidant activity measured by DPPH assay of the extracts, the three-dimensional plots were depicted in Figures 2(b) and 3(b). Concerning the hydroalcoholic mixtures, as evident in Figure 2(b), the DPPH activity values of water extract increased when the methanol or ethanol concentration increases, indicating that adding these organic solvents to water rises its capacity to extract phenolic compounds, although it starts to decrease when ethanol or methanol concentration in the solvent mixture exceeds 75%. The solvent mixtures yielding extracts with the highest DPPH free radical scavenging activity occur between the equivalent ternary mixture, the equivalent binary mixture water-ethanol, and the binary mixture (water 25%, ethanol 75%). Moreover, the optimum solvent mixture given by the desirability analysis (Table 4) was a ternary mixture that consisted of 50% ethanol, 16.667% methanol, and 33.33% water.

Similarly, in the organic mixtures (Figure 3(b)) adding methanol or ethanol to hexane increases its capacity to extract molecules with DPPH free radical scavenging activity. However, in this case the quadratic interaction between methanol and hexane was more effective. The best solvent mixture for yielding extract with optimal DPPH antioxidant activity was the equivalent binary mixture of methanol and ethanol.

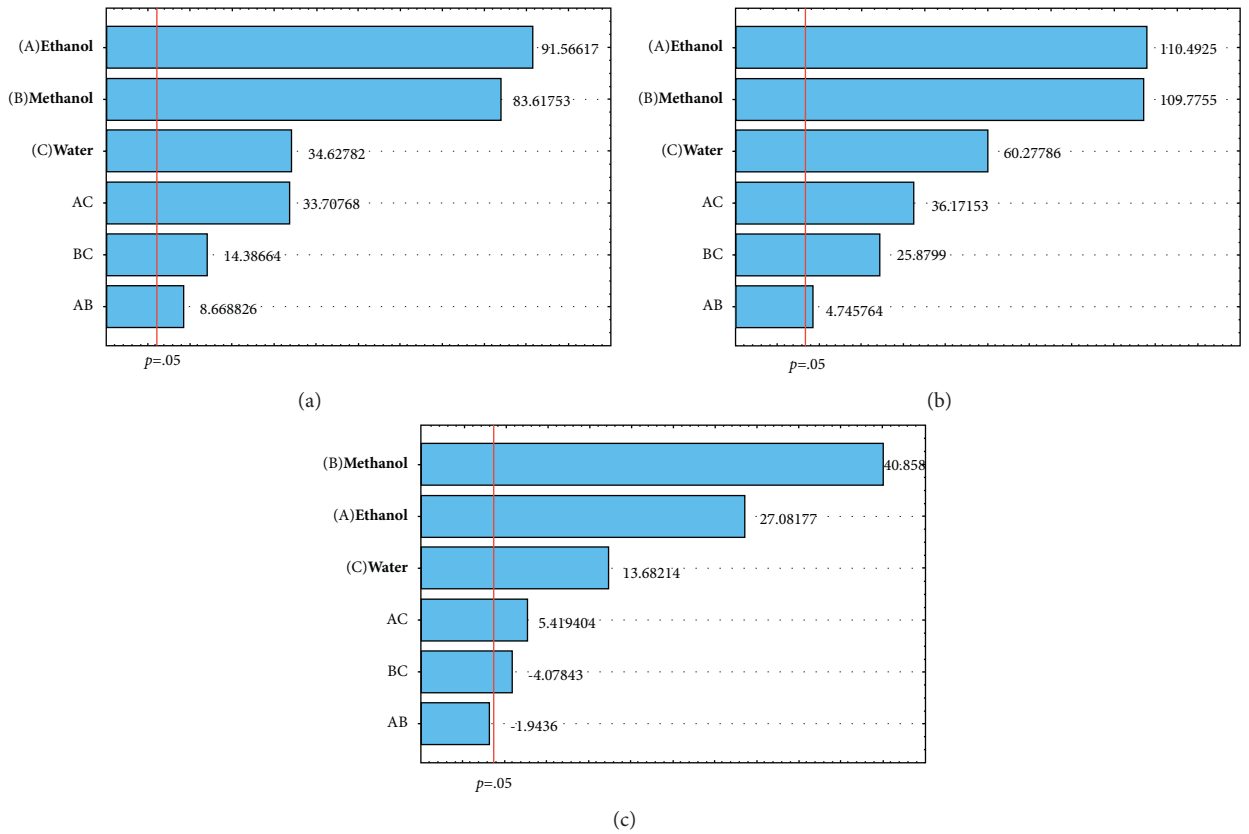

Figure 4: Analysis of the Pareto chart of the standardized effects for total phenolic content (a), DPPH (b), and FRAP (c) assays.

3.4.3. FRAP Assay. The reductive capacity of the extract may serve as a reflection of its antioxidant activity. The response surface plots of FRAP antioxidant assay are depicted in Figures 2(c) and 3(c), illustrating the combined impact of extraction solvents for hydroalcoholic and organic mixtures, respectively. Unlike methanol, the water and hexane were the less effective solvent. From Figures (2(c) and 3(c)), it can be clearly observed that when the methanol proportion increased in the mixtures of the three solvents (ethanol, methanol, and water), FRAP values increased significantly. The same sightings have been observed when using hexane instead of water. FRAP desirability analysis demonstrated that pure methanol was the optimal solvent for extracting bioactive compounds with FRAP antioxidant activity. Regarding FRAP activity, methanol proportion was one of the most important variables affecting extraction from *Thymelaea hirsuta* L [27]. According to the planned mixture design performed to extract total anthocyanins, phenolic compounds, and antioxidants from x´kijit peels, methanol was the solvent producing extracts with the highest extraction yields of total phenolic compounds and other bioactive compounds, exhibiting the highest antioxidant capacity [28].

3.5. Pareto Chart Analysis. To examine the relative importance of the main effects and their interactions using statistical significance ($p < 0.05$), a standardized Pareto chart was employed.

According to Figures 4(a) and 4(b), the result showed the effect of the independent variables (hydroalcoholic mixtures) and their interactions on TPC recovery and DPPH antioxidant activity. We notice for the two responses that all parameter was statistically significant with 95% confidence and had a positive effect on the two responses and was ranked as follows: ethanol > methanol > water > ethanol * water > methanol * water > ethanol * methanol, whereas in the case of FRAP assay (Figure 4(c)), methanol had the greatest significant positive effect followed by ethanol. The binary interaction methanol * water had a low significant negative effect, meaning that adding water to methanol slightly lowered the recovery on molecules having a FRAP antioxidant activity.

Concerning organic solvent (figures not shown), the Pareto analysis revealed that all parameter was statistically significant with 95% confidence. In the linear interactions, mostly ethanol exhibited the highest positive influence, followed by methanol; likewise, the quadratic interactions influenced positively the TPC recovery, but with smaller impact, in contrast to ternary interaction which had a negative effect. In the DPPH assay, methanol and ethanol greatly influenced extractions of antioxidant molecules, followed by hexane with lesser effect. The interaction of ethanol-methanol showed a significant negative influence. Methanol was the pure solvent that highly influenced FRAP antioxidant molecules, followed by ethanol.

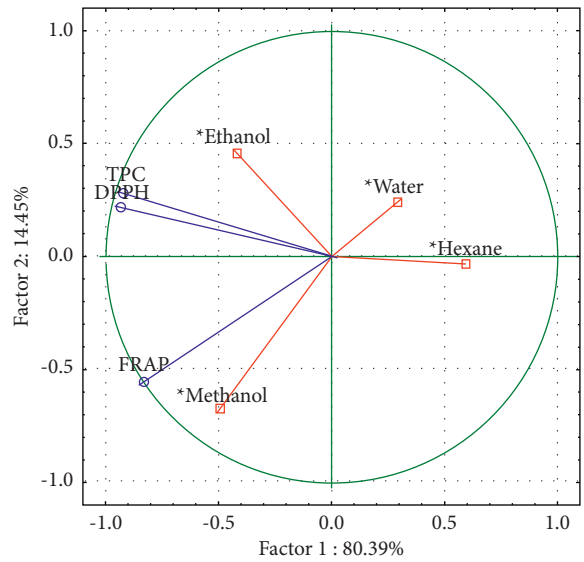

Figure 5: PCA analysis.

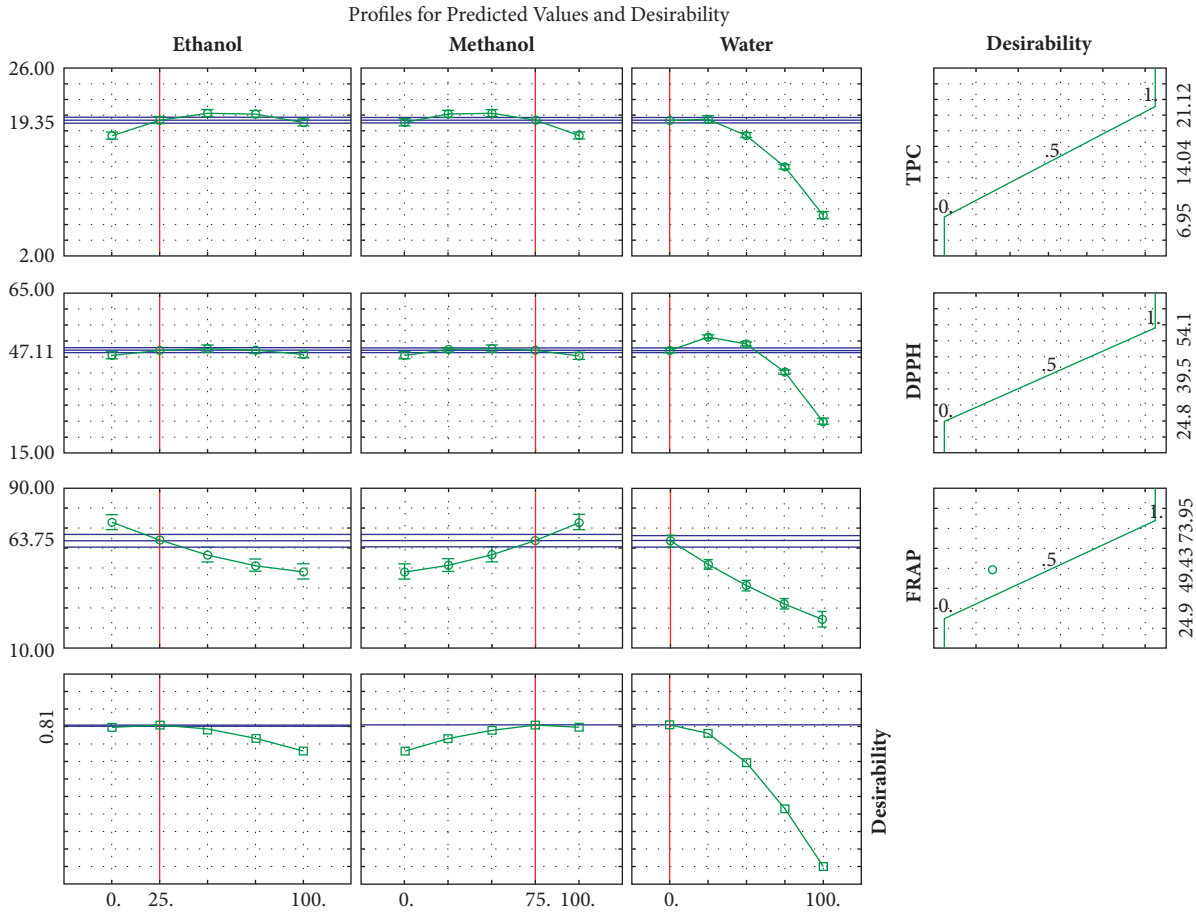

Figure 6: Extraction optimization using the desirability function.

3.6. PCA Analysis. Multivariate analysis can summarize the variability of a complex data set and present it in the most interpretable form, such as principal components. PCA results (Figure 5) show that 94.84% of the variability in the data is accounted by the first two principal components PC1 (Factor 1) and PC2 (Factor 2) which was high enough.

Furthermore, the first three PCs explained 100% of the variance in the data, which was high enough to represent all the variables. In this analysis, if two vectors subtend a small angle to each other, this means that the two variables they represent are strongly correlated. The score plot for PC1 versus PC2 clearly shows a strong positive correlation between TPC and DPPH was observed, indicating that fractions with the highest potency in scavenging DPPH radical presented the highest TPC. The two parameters were in turn positively correlated with ethanol concentration in the solvent extractor and negatively correlated to hexane. On the other hand, FRAP shows a positive correlation with methanol concentration in extraction solvent and was negatively correlated with water content, indicating that those ferric reducing molecules have more affinity to methanol than water and ethanol.

3.7. Desirability Analysis. Optimization using the desirability function was performed in order to maximize the total phenolic compounds and also to maximize the antioxidant capacity (DPPH and FRAP) in the extracts. Results are depicted in Figure 6 for hydroalcoholic mixtures. The final result for the simultaneous optimization including all responses, using the desirability function, suggested that the solvent mixture consisting of 25% ethanol and 75% methanol was the most adequate solvent to achieve the best solution for this combination of variables. However, pure methanol was the solvent when mixtures were performed with the three organic solvents.

4. Conclusions

This is the first report on the optimization of phenolic antioxidants from *Cannabis sativa* residue, in which we revealed that this plant's waste is a useful source for bioactive compounds. The methods can be utilized for further isolation of active fraction/compounds from the cannabis waste. The highest TPC yield values occur with the binary interaction between water and ethanol around the proportion of ethanol, 70%, water, 30%, whereas the best DPPH scavenging activity is obtained with extraction using ternary mixtures including water and binary mixture (ethanol 50% to 75%). Meanwhile, pure methanol was the best solvent for extracting molecules with FRAP antioxidant capacity.

Data Availability

The table data used to support the findings of this study are included within the article.

Conflicts of Interest

The author declares that he has no conflicts of interest.

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