fluids

MDPI

Article

Bioactive Fractions Isolated from By-Products of the Guava (*Psidium guajava*) and Mango (*Mangifera indica* L.) Agri-Food Industry

Leidy J. Cerón-Martínez [1], Andrés M. Hurtado-Benavides [1], Alfredo Ayala-Aponte [2], Liliana Serna-Cock [3] and Diego F. Tirado [4,*]

[1] Facultad de Ingeniería Agroindustrial, Universidad de Nariño, Pasto 52001, Nariño, Colombia; leidyceron07@gmail.com (L.J.C.-M.); ahurtado@udenar.edu.co (A.M.H.-B.)
[2] Escuela de Ingeniería de Alimentos, Universidad del Valle, Cali 760031, Valle del Cauca, Colombia; alfredo.ayala@correounivalle.edu.co
[3] Facultad de Ingeniería y Administración, Universidad Nacional de Colombia, Sede Palmira, Palmira 763533, Valle del Cauca, Colombia; lserna@unal.edu.co
[4] Dirección Académica, Universidad Nacional de Colombia, Sede de La Paz, La Paz 202017, Cesar, Colombia
* Correspondence: dtiradoa@unal.edu.co

Abstract: Valorizing agri-food industrial waste is essential for a circular economy, yielding high-value products, waste reduction, technological solutions, employment opportunities, and enhanced food security. This work shows the valorization of seeds generated as residues from the agri-food industries of guava *pera* (*Psidium guajava*) and Tommy Atkins mango (*Mangifera indica* L.), through extraction with supercritical carbon dioxide ($scCO_2$). After the optimization of the initial solid condition of the raw material (i.e., particle size and moisture content), $scCO_2$ pressure and temperature were established through the response surface methodology (RSM) to obtain an oily extract with the highest content in bioactive compounds of commercial relevance, as well as with a high antioxidant capacity. The total amount of oily extract in guava and mango seeds was 14% and 9%, respectively, while the maximum recovery of supercritical extract was 95% from guava seeds at 38 MPa and 50 °C, and 88% from mango seeds at 37 MPa and 63 °C. Bioactive fractions rich in squalene, γ-tocopherol, α-tocopherol, campesterol, β-sitosterol, and stigmasterol were obtained. The best supercritical extraction conditions, in terms of the bioactive fractions richest in minor compounds, were at 17 MPa and 50 °C for guava seeds and at 23 MPa and 63 °C for mango seeds. At these conditions, the highest antioxidant capacities were also found for the extracts. Thus, these bioactive fractions could be used in a variety of products in the cosmetic, food, pharmaceutical, and medical activities due to the beneficial properties of the identified compounds in health as antioxidants, anti-inflammatories, and cholesterol reducers.

Keywords: circular economy; green engineering; safer solvent; supercritical CO_2; waste valorization; sustainability

Citation: Cerón-Martínez, L.J.; Hurtado-Benavides, A.M.; Ayala-Aponte, A.; Serna-Cock, L.; Tirado, D.F. Bioactive Fractions Isolated from By-Products of the Guava (*Psidium guajava*) and Mango (*Mangifera indica* L.) Agri-Food Industry. *Fluids* **2023**, *8*, 256. https://doi.org/10.3390/fluids8090256

Academic Editors: Maša Knez Marevci, D. Andrew S. Rees and Darija Cör Andrejč

Received: 24 July 2023
Revised: 1 September 2023
Accepted: 14 September 2023
Published: 21 September 2023

1. Introduction

It is difficult to accurately estimate the amount of agri-food industrial waste produced in the world, as it can vary according to factors such as the type of agriculture and industrial practices used, as well as fluctuations in crop yields and market demands. However, according to a report by the Food and Agriculture Organization (FAO) of the United Nations (UN), it is estimated that, globally, about one-third of all food produced for human consumption is lost or wasted each year. This is equivalent to approximately 1.3 billion tons of food, including waste before and after consumption [1].

The amount of agri-food industrial waste produced can also vary according to the specific region. For example, in developed countries, a significant amount of food waste is

produced at the consumption level, while in developing countries such as Colombia, more waste is produced at the production and distribution levels due to poor infrastructure and inadequate storage facilities [1,2].

In general, it is clear that food waste and food loss are major problems that require attention and action at the global level, but also at the local level, so the valorization of agri-food industrial waste is essential from the perspective of the circular economy; as it represents a way to obtain products with high added value, reduce waste, and even generate technological solutions. In addition, the valorization of agri-food industrial wastes can have social benefits by providing new job opportunities, especially in rural areas, which are abundant in Colombia, where many agricultural activities are carried out [3]. Thus, it can contribute to food security [4]. Not to mention that agri-food industrial residues appear to be an affordable and abundant source of bioactive compounds of biological interest; and supercritical carbon dioxide ($scCO_2$) extraction has proven to be a good strategy to obtain them [5,6].

$scCO_2$ has positioned itself as a successful alternative solvent for the extraction of lipophilic components from plant materials, ensuring the obtention of a clean and safe high-quality extract [6]. Its features, such as inertness, non-toxicity, and mild conditions to reach the supercritical state (i.e., 31.1 °C and 7.4 MPa), along with the possibility of recirculation, have allowed its implementation in large-scale commercial production in various pharmaceuticals, cosmetic, and food applications [6,7].

There is great interest in the utilization of tropical fruit wastes due to their wide availability. Fresh guava and mango are often used in the food industry to produce juices, pulps, jams, and other food products, while their seeds are a by-product of their industrial processing, which should be processed in a valuable way rather than simply discarded, due to the high content of commercially interesting compounds they contain. In this regard, previous works of our research group reported the effect of $scCO_2$ operating conditions on the extraction yield and fatty acid profile of supercritical extracts from guava (*Psidium guajava*) [8] and mango (*Mangifera indica* L.) [5] seeds. On this occasion, this work aims to show the high added value of extracts from both agri-food industrial wastes, considering their bioactive profile.

2. Materials and Methods

2.1. Reagents

This study used n-hexane (99%, Sigma-Aldrich, Cali, Colombia), methanol (HPLC grade, ≥99.9%, Sigma-Aldrich, Cali, Colombia), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, 97%, Sigma-Aldrich, Cali, Colombia), dichloromethane (HPLC grade, Honeywell, Charlotte, NC, USA), cholesterol (≥99%, Sigma-Aldrich, Cali, Colombia), anhydrous sodium sulfate (Na_2SO_4, Sigma-Aldrich, Cali, Colombia), 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma-Aldrich, Cali, Colombia), helium (≥99.99%, CRYOGAS, Cali, Colombia), and CO_2 (99.9%, CRYOGAS, Cali, Colombia). All materials were used as received.

2.2. Raw Material Conditioning

Before extraction, guava (*P. guajava*; *pera* variety) and mango (*M. indica* L.; Tommy Atkins variety) seeds were dried with a tray dryer (CST-800, FIQ S.A.S., Bogotá, Colombia) at 50 °C for 10 h up to a moisture content of 7% *w*/*w*. Subsequently, the dried seeds were milled (hammer mill, TRAPP, Jaraguá do Sul, Brazil) until 0.6 mm (ASTM E11 series sieve, mesh 0.10–0.80 mm, PS-35 series 1182, Pinzuar, Bogotá, Colombia).

2.3. Effect of Extraction Conditions on Extract Composition

The $scCO_2$ extractions were performed in a Waters® SFE 500 system (Waters Acquires Thar Instruments, Pittsburgh, PA, USA). A schematic representation of the supercritical equipment used in this study is shown in Figure 1. Full details of the equipment, its set-up, and operation have been described in a previous work [5].

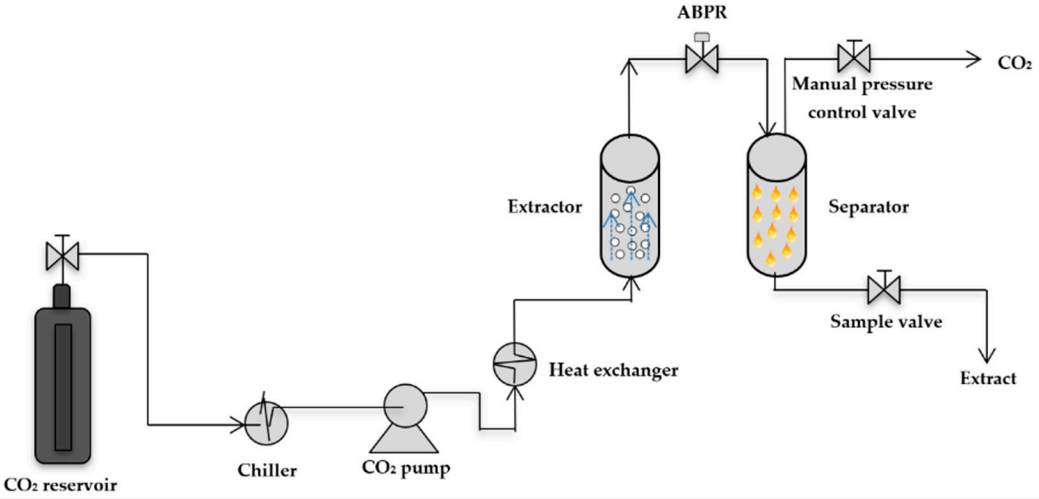


Figure 1. Schematic of the equipment used for supercritical fluid extraction. Taken from Cerón-Martínez et al. [5].

To carry out an extraction in the equipment shown in Figure 1, 0.2 kg of the raw material was placed into the extractor, forming a fixed bed. Upon sealing the extractor, CO_2 was fed until the desired operating conditions were achieved. Then, the automatic back pressure regulator (ABPR) was opened to maintain a continuous flow of 30 g CO_2/min for 150 min. After the ABPR, the CO_2 was depressurized, its solvent power decreased, and the oily extract obtained was collected and stored at 4 °C in pre-weighed amber vials for analysis. The percentage of extract recovery was calculated as the ratio of extract obtained to the total amount of oily extract contained in the seed, which was determined by Soxhlet extraction.

A rotating compound central design (RCCD) with factorial design 2^2, central points, and star points was used. By the response surface methodology (RSM), an extract with a high content of squalene, phytosterols, and tocopherols, along with a high antioxidant capacity was obtained. For the above, the independent factors studied, with their operating ranges, were pressure (X_1; 20 MPa to 35 MPa for guava and mango seeds) and temperature (X_2; 40 °C to 60 °C for guava seeds and 55 °C to 70 °C for mango seeds).

Conventional Solvent Extraction

To obtain the total extract yield and for comparative purposes to $scCO_2$ extraction, Soxhlet extractions were performed using 0.01 kg of dried and milled seeds. The sample was transferred to a cellulose cartridge and inserted into a Soxhlet set-up equipped with a 250 mL flask. Then, 100 mL of n-hexane was added and the whole set-up was heated for 8 h using a heating jacket. Subsequently, the extract was concentrated using a Rotavapor RE-121 (BUCHI, Buchegg, Switzerland). Traces of solvent were removed in an oven at 103 °C for 2 h; finally, the extract was stored under refrigeration at 4 °C until further analysis [5].

2.4. Determination of Minor Compounds

Minor compounds were quantified according to the methodology described by Narváez-Cuenca et al. [9], with modifications. For this, a 100 µL extract was used and diluted to 1 mL in dichloromethane. The extracts were shaken in a vortex for 30 s and Na_2SO_4 was added. A total of 1.0 µL was injected into a Shimadzu QP2010S gas chromatograph (Shimadzu Scientific Corporation, Long Beach, CA, USA) equipped with a split/spitless injector, an SHXRI-5MS column (30 m × 0.25 mm × 0.25 µm, Shimadzu Scientific Corporation, Long Beach, CA, USA), and a QP2010S mass detector with an electron impact ionization source operating at 70 eV in full scan mode. Dichloromethane extract (1 µL) was injected in split mode (1:10 split ratio) at 270 °C. Helium was used as carrier gas at 1 mL/min. The

separation of the compounds was carried out by temperature ramping in the oven. The oven temperature was maintained at 100 °C for 2 min and then increased to 300 °C at a rate of 10 °C/min for 20 min. The temperature was then kept at 300 °C for 15 min before returning to the initial conditions and equilibration for at least 5 min. The system was controlled by Shimadzu LabSolutions GC-MS Ver 2.7 software (Shimadzu Scientific Corporation, Long Beach, CA, USA). Tentative identification of the minor compounds in the extracts was performed by comparing their retention indices and their MS spectra in the National Institute of Standards and Technology (NIST) database. Thresholds of a retention index deviation of less than 3% and a mass spectral match of greater than 85% were applied. Quantification was performed using the internal standard method with cholesterol as the internal standard. Each measurement was replicated three times.

2.5. Antioxidant Capacity of Extracts

Measurements were taken by the DPPH radical scavenging activity assay [6]. The principle of the method relies on the fact that the scavenging of free radicals by samples or standards causes a reduction in the absorbance of DPPH. Briefly, 0.2 mL of a methanolic solution of the sample to be analyzed was added to 3.8 mL of a freshly prepared methanolic solution containing 60 µM of DPPH. The sample was diluted in methanol as many times as necessary. This solution showed an intense violet color with an absorption maximum at 517 nm. The reaction mixture was kept at room temperature in a dark chamber for 30 min. The color change from dark violet to light yellow was measured at 517 nm in a spectrophotometer (GENESYS 10S Series UV-Visible Spectrophotometers, Waltham, MA, USA). A standard curve was prepared under similar conditions using the synthetic reference antioxidant Trolox diluted in methanol, and the antioxidant capacity of the extracts was calculated in millimoles Trolox equivalent per gram of sample (mM TE/g).

2.6. Statistical Analysis

The experimental procedure was replicated three times to ensure robustness and accuracy. Each replicate was analyzed twice ($n = 3 \times 2$) to account for any variability and obtain reliable results. For optimization, as well as the analysis of experimental data and the creation of the design matrix, STATGRAPHICS Centurion XV.II (Statpoint Technologies, Inc., Warrenton, VA, USA) was used. The data underwent analysis of variance (ANOVA), and the statistical significance of the factors was established at a 5% significance level ($p \leq 0.05$).

3. Results and Discussions

3.1. Extraction Recovery

The total amount of oily extract contained in guava and mango seeds, determined by Soxhlet extraction with n-hexane, was (14.1 ± 0.1)% and (9.1 ± 0.4)%, respectively. The maximum supercritical extract recovery was 95% from guava seeds at 38 MPa and 50 °C (see Table 1), and 88% from mango seeds at 37 MPa and 63 °C (see Table 2). In visual appearance, the supercritical extracts exhibited a semi-solid consistency at ambient conditions and displayed a light white hue, contrasting with the Soxhlet extracts. Notably, the supercritical extraction method yielded products of superior purity compared to the conventional Soxhlet technique. The enhanced extraction yield obtained with the organic solvent can be attributed to the relatively lower selectivity of n-hexane. These differences are due to the fact that Soxhlet extraction, unlike supercritical extraction, extracts neutral and polar lipids [5], in addition to pigments such as chlorophyll [6].

Table 1. Minor compounds (mg/g extract) in oily extracts from guava seeds.

Pressure (MPa)	Temperature (°C)	Squalene	γ-Tocopherol	α-Tocopherol	Campesterol	β-Sitosterol	Recovery (%) *
20.0 (−1.00)	40 (−1.00)	0.3 ± 0.0	0.6 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	11.0 ± 0.2	25.5 ± 1.8
20.0 (−1.00)	60 (+1.00)	0.8 ± 0.0	1.6 ± 1.0	0.5 ± 0.1	0.4 ± 0.1	15.7 ± 0.4	16.3 ±1.5
35.0 (+1.00)	40 (−1.00)	0.4 ± 0.0	0.7 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	8.8 ± 1.0	72.6 ± 2.5
35.0 (+1.00)	60 (+1.00)	0.2 ± 0.0	0.5 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	6.4 ± 0.1	90.5 ± 2.6
16.9 (−1.41)	50 (0.00)	1.5 ± 0.1	1.1 ± 0.2	0.5 ± 0.1	0.7 ± 0.1	18.5 ± 0.1	13.8 ± 0.4
38.1 (+1.41)	50 (0.00)	0.2 ± 0.0	0.6 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	6.3 ± 1.0	95.3 ± 3.0
27.5 (0.00)	36 (−1.40)	0.5 ± 0.1	1.1 ± 0.1	0.2 ± 0.0	0.0 ± 0.0	9.6 ± 0.3	56.1 ± 1.9
27.5 (0.00)	64 (+1.41)	0.5 ± 0.1	0.7 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	7.3 ± 0.2	38.7 ± 2.5
27.5 (0.00)	50 (0.00)	0.3 ± 0.0	0.7 ± 0.1	0.3 ± 0.1	0.0 ± 0.0	9.9 ± 0.2	82.7 ± 1.8
Soxhlet extraction (n-hexane)		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.1 ± 0.2	100.0

* Ratio of the amount of extract obtained to the total amount of extractor in seeds.

Table 2. Minor compounds (mg/g extract) in oily extracts from mango seeds.

Pressure (MPa)	Temperature (°C)	Squalene	α-Tocopherol	Campesterol	Stigmasterol	β-Sitosterol	Recovery (%) *
25.0 (−1.00)	55 (−1.00)	3.3 ± 0.6	0.2 ± 0.0	0.7 ± 0.1	1.7 ± 0.3	5.6 ± 0.1	55.1 ± 2.3
25.0 (−1.00)	70 (+1.00)	4.3 ± 0.4	0.6 ± 0.1	0.8 ± 0.0	1.9 ± 0.0	5.8 ± 0.0	38.4 ± 1.6
35.0 (+1.00)	55 (−1.00)	1.6 ± 0.1	0.0 ± 0.0	0.2 ± 0.0	0.7 ± 0.1	2.0 ± 0.0	76.4 ± 2.7
35.0 (+1.00)	70 (+1.00)	1.5 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	0.7 ± 0.1	2.2 ± 0.3	82.0 ± 3.4
22.9 (−1.41)	63 (0.00)	7.6 ± 0.5	0.4 ± 0.0	0.8 ± 0.1	1.8 ± 0.1	4.7 ± 0.1	26.3 ± 2.5
37.1 (+1.41)	63 (0.00)	2.6 ± 0.4	0.1 ± 0.0	0.5 ± 0.0	1.1 ± 0.3	3.9 ± 0.5	87.7 ± 3.3
30.0 (0.00)	52 (−1.41)	1.5 ± 0.3	0.0 ± 0.0	0.3 ± 0.0	0.9 ± 0.0	2.9 ± 0.2	69.0 ± 3.9
30.0 (0.00)	73 (+1.41)	4.3 ± 0.4	0.2 ± 0.0	0.6 ± 0.0	1.3 ± 0.0	3.9 ± 0.0	71.1 ± 3.0
30.0 (0.00)	63 (0.00)	2.1 ± 0.4	0.1 ± 0.1	0.3 ± 0.1	0.7 ± 0.1	2.3 ± 0.4	80.3 ± 3.1
Soxhlet extraction (n-hexane)		1.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.1	2.0 ± 0.5	100.0

* Ratio of the amount of extract obtained to the total amount of extractor in seeds.

3.2. Minor Compounds in Guava Seed Extracts

Table 1 shows the minor compounds identified in the oily extracts from guava seeds obtained with $scCO_2$ and n-hexane.

As shown in Table 1, for the case of the supercritical extracts, squalene, sterols, and tocopherols could be identified, with the sterol β-sitosterol being the most abundant minor compound in the extracts, found in the range of 6.3 mg/g extract to 18.5 mg/g extract. Another sterol identified in the supercritical extracts was campesterol (0.0 mg/g extract–0.7 mg/g extract), with a total sterol content ranging from 6.3 mg/g extract to 19.2 mg/g extract. The sterols reported in Table 1 have also been identified in supercritical extracts obtained under operating conditions similar to those used in this work from guanabana seeds (1.4 mg/g extract–2.8 mg/g extract) [10] and blackberry seeds (4.0 mg/g extract–7.6 mg/g extract) [11], with higher sterol fractions in the extracts obtained in this study.

In a previous work [9], the minor compounds reported in Table 1 were also identified in supercritical extracts from guava seeds. However, the contents reported in this work were higher, despite the use of milder operating conditions. For example, in the work of Narváez-Cuenca et al. [9], a higher pressure and temperature (i.e., 36 MPa and 52 °C), thus higher energy expenditure, were used to achieve supercritical extracts rich in the minor compounds in Table 1. Whereas in this work, the bioactive fractions richer in minor compounds were obtained at a lower pressure and temperature (i.e., 17 MPa and 50 °C).

Recent previous studies [5,6,12], in addition to the classical literature [13], indicate that the initial solid conditions of the raw material, embodied in this work as particle size and moisture content, condition greatly the mass transfer during supercritical extraction, since the transport of the supercritical solvent through the solid phase depends on these: solid particles exceeding a diameter of 0.80 mm act as barriers, impeding the effective penetration of the solvent and the solute solubilization. On the other hand, particles smaller than 0.4 mm pose a potential risk of creating preferential channels and blockages. Additionally, an excess of water can significantly hamper mass transfer throughout the extraction process. Thus, in previous experiments, our research group endeavored to find the optimal particle size and moisture content for the raw material to be subjected

to supercritical extraction, i.e., 0.6 mm and 7% w/w moisture. Therefore, it is assumed that the better results in terms of bioactive fractions rich in minor compounds in this work compared to the literature could be due to the optimized initial solid conditions of the raw material subjected to supercritical extraction in this study. However, it should be kept in mind that drying and particle size reduction are energy intensive unit operations, so optimization of these stages should be considered in case it is desired to bring this process to a commercial scale.

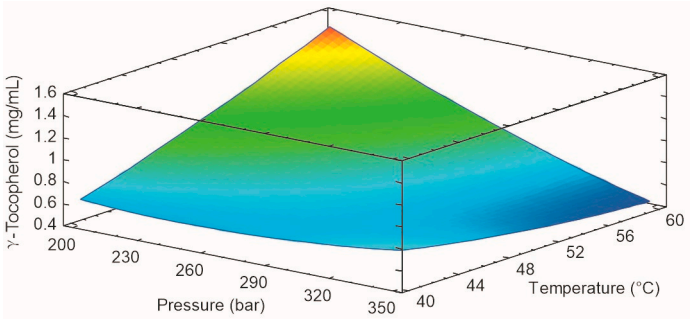
The oily extracts obtained with n-hexane showed significant differences in composition compared to the supercritical extracts. As shown in Table 1, only β-sitosterol was identified in the Soxhlet extract, which had a statistically lower concentration ($p \leq 0.05$) compared to the supercritical extracts. The above was clearly due to the higher selectivity of $scCO_2$ and milder operating conditions during supercritical extraction [6]. It is well known that Soxhlet extraction uses high temperatures during long extraction times, which increases the possibility of thermal decomposition of biological compounds of interest such as squalene, sterols, and tocopherols. The above gives evidence of the advantages of supercritical fluid extraction in obtaining bioactive fractions rich in minor compounds of high biological value, compared to conventional extraction.

Finally, it should be noted that pressure had a statistically significant effect ($p \leq 0.05$) on the enrichment of the extract in squalene, γ-tocopherol, α-tocopherol, and campesterol. However, no statistically significant effect ($p > 0.05$) of pressure on the extraction of β-sitosterol was observed, which supports the idea that β-sitosterol may have been found to be present in the extract obtained with n-hexane. Meanwhile, the effect of temperature was not so clear and only had a significant effect ($p \leq 0.05$) during the extraction of α-tocopherol.

MSR Optimization

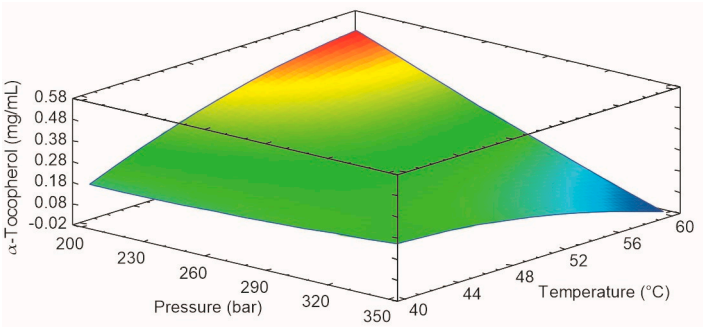
Figure 2 shows the response surfaces for the concentration of γ-tocopherol (see Figure 2a) and α-tocopherol (see Figure 2b) as a function of pressure and temperature. The remaining compounds in Table 1 were not evaluated due to the lack of significant fit ($p < 0.05$) of the models obtained for these compounds. It is possible that a study involving more factors will allow obtaining a model with a better fit.

According to Figure 2, pressure at the levels evaluated exerted a negative effect on the concentration of these compounds. Increasing the pressure from 20 MPa to 35 MPa in the supercritical extraction process resulted in a decrease in the concentration of the tocopherols contained in the extracts from guava seeds. However, the effect of temperature was positive on the concentration of these compounds, since, by increasing the temperature from 40 °C to 50 °C, there was an increase in the concentration of tocopherols.



(**a**)

Figure 2. *Cont.*



(**b**)

Figure 2. Response surfaces for the concentration of (**a**) γ-tocopherol and (**b**) α-tocopherol in supercritical extract from guava seeds.

Temperature plays a very important role in supercritical extraction processes, since its increase can reduce oil extraction yields due to a reduction in $scCO_2$ density, but in this case, it improved the extraction of bioactive compounds, possibly due to its positive impact on diffusion and kinetics; not to mention that temperature could also have helped to improve the cellular opening of the vegetable matrix [6,14].

According to Figure 2, a high extraction temperature led to an increase in the diffusion coefficients of tocopherols, which was reflected in the increased solubility of these compounds in $scCO_2$ [15]. Low extraction pressure and high extraction temperature favored the concentration of tocopherols in guava seed oil. This effect of pressure and temperature on minor compounds has also been reported during supercritical extraction from blackberry seeds [11] and pomegranate seeds [16].

As per Figure 2, the best pressure and temperature conditions to obtain the highest concentration of tocopherols in guava seed oil were found around 17 MPa and 64 °C, i.e., at low pressure and high extraction temperature. According to Figure 2, under these conditions, a maximum concentration of 2.0 mg γ-tocopherol/g extract and 0.7 mg α-tocopherol/g extract could be reached.

3.3. Minor Compounds in Mango Seed Extracts

Table 2 reports the minor compounds identified in the oily extracts from mango seeds obtained with $scCO_2$ and n-hexane. According to Table 2, squalene was the compound present in the highest proportion in the supercritical extracts (1.5 mg/g extract–7.6 mg/g extract).

Olive oil is considered to be a high squalene source, with up to 7 mg squalene/g olive oil [17]. In the supercritical extracts from mango seeds in this study, at 23 MPa and 63 °C, bioactive fractions of squalene with up to 7.6 mg squalene/g extract were achieved, so these bioactive fractions can be considered to be high in squalene. This places supercritical extracts of mango seeds as a good alternative source of this compound with potential practical uses in the cosmetic, food, medical, and pharmaceutical businesses.

Additionally, according to Table 2, the lipid fractions obtained with $scCO_2$ from mango seeds had total sterols from 2.9 mg/g extract to 8.5 mg/g extract; where β-sitosterol was notably the major sterol (2.0 mg/g extract–5.8 mg/g extract), followed by stigmasterol (0.7 mg/g extract–1.9 mg/g extract) and campesterol (0.2 mg/g extract–0.8 mg/g extract). Finally, and concerning the tocopherol content, α-tocopherol was identified in a concentration range of 0.0 mg/g extract–0.4 mg/g extract (see Table 2).

In mango seed extracts obtained by Soxhlet extraction, only β-sitosterol, squalene, and stigmasterol were identified, in descending order (see Table 2). The concentration of these compounds was statistically lower ($p \leq 0.05$) than that obtained in the oil extracted at 22.9 MPa and 63 °C; conditions at which the highest concentration of these compounds was achieved by supercritical extraction. As in the supercritical extraction from guava seeds (see Table 1), the compositional advantage of supercritical extraction over conventional extraction was also evident for mango seeds (see Table 2).

Finally, ANOVA showed that pressure had a statistically significant effect ($p \leq 0.05$) on the content of all the minor compounds reported in Table 2. Temperature on the other hand only exerted a statistically significant effect ($p \leq 0.05$) on squalene content. Finally, the lack-of-fit test was not significant ($p > 0.05$), so the statistical models were adequate to describe the observed concentration data of minor compounds identified in oily extracts from mango seeds.

MSR Optimization

Response surface plots for squalene (see Figure 3a), α-tocopherol (see Figure 3b), campesterol (see Figure 3c), stigmasterol (see Figure 3d), and β-sitosterol (see Figure 3e) concentration as a function of pressure and temperature are shown in Figure 3.

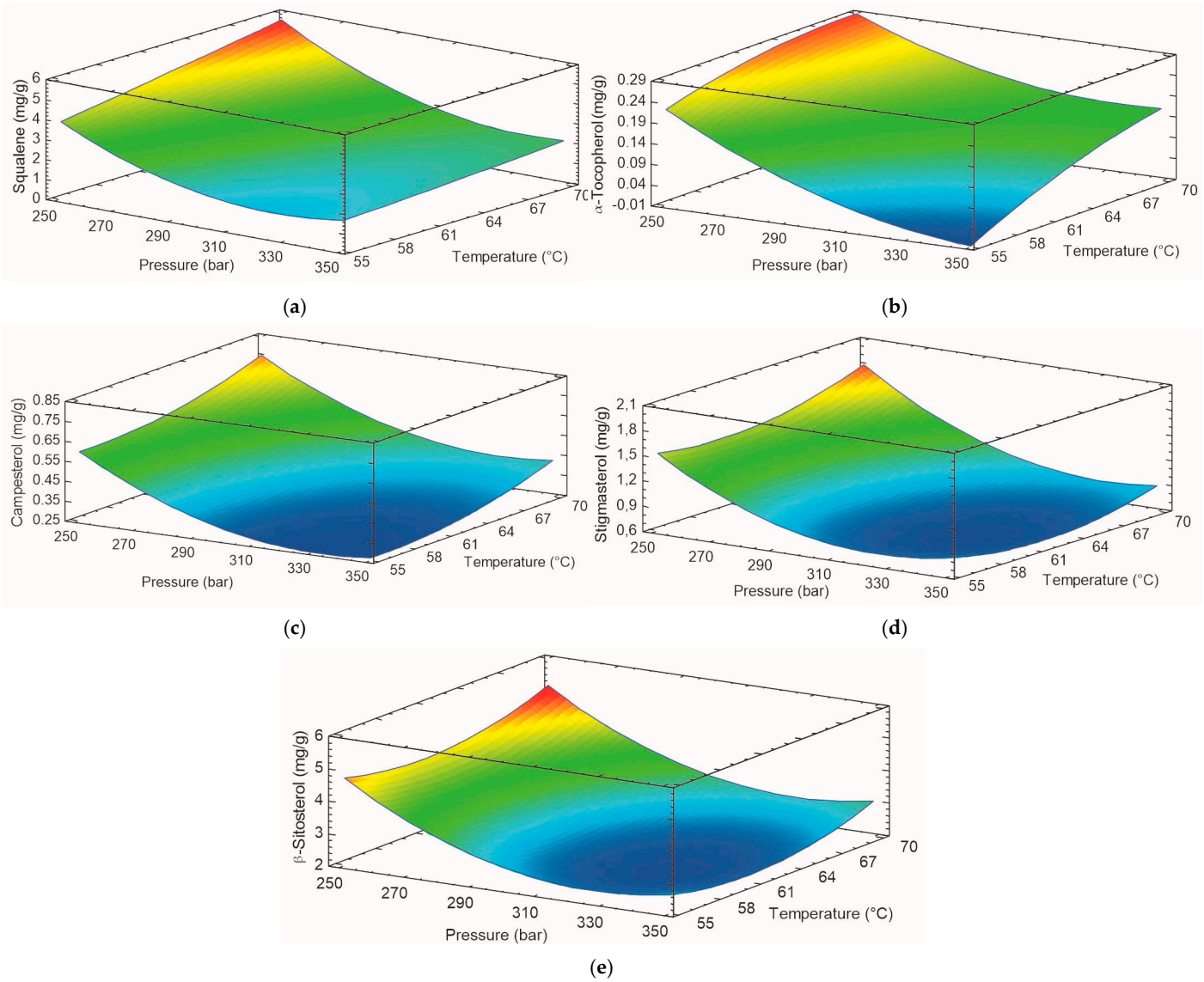


Figure 3. Response surfaces for (**a**) squalene, (**b**) α-tocopherol, (**c**) campesterol, (**d**) stigmasterol, and (**e**) β-sitosterol in supercritical extract from mango seeds.

Figure 3 shows that, as in the case of supercritical extraction from guava seeds (see Figure 2), pressure exerted a negative effect on the content of these minor compounds; therefore, as pressure increased at the levels evaluated, the concentration of these compounds decreased. This was displayed because the increase in pressure caused an increase in molecular interactions. After all, the average intermolecular distance decreased as the

density increased [18]. Additionally, the difference in solubility of squalene, sterols, and tocopherols identified in oily fractions of mango seeds could be affected at high extraction pressures (where higher oil yields were achieved), due to the simultaneous extraction of multiple substances, which could interfere with the extraction of these compounds [19]. High concentrations of squalene and tocopherols at low pressures (15 MPa) have already been reported during supercritical extraction from amaranth [20].

According to Figure 3, temperature had the opposite effect to pressure. That is, the concentration of the minor compounds increased with increasing temperature at the levels evaluated. This was exhibited because the increase in temperature caused an increase in the vapor pressure of the minor compounds, as well as in their diffusion coefficient [5], which was reflected in a higher solubility of the compounds in $scCO_2$ [14].

Finally, according to Figure 3, the $scCO_2$ pressure and temperature levels that allowed obtaining the highest concentrations of the minor compounds in bioactive fractions from mango seeds were found around 23 MPa bar and 73 °C. Under these conditions, a maximum concentration of 8.0 mg squalene/g extract (see Figure 3a), 0.36 mg α-tocopherol/g extract (see Figure 3b), 1.0 mg campesterol/g extract (see Figure 3c), 2.6 mg stigmasterol/g extract (see Figure 3d), and 7.2 mg β-sitosterol/g extract (see Figure 3e) could be achieved.

3.4. Antioxidant Activity of Extracts

Table 3 shows the results of the antioxidant activity of bioactive fractions from guava and mango seeds obtained with $scCO_2$ and by Soxhlet extraction. When extracted with $scCO_2$, the values of the antioxidant activity of oily extracts of guava seeds ranged from 222 µmol Trolox/100 g extract to 281 µmol Trolox/100 g extract. This antioxidant activity was lower than that found in mango seed fractions, which ranged from 214 µmol Trolox/100 g extract to 317 µmol Trolox/100 g extract, as can be corroborated in Table 3. The extract with the highest antioxidant capacity was found at 17 MPa and 50 °C (281 µmol Trolox/100 g extract) for guava seeds and at 23 MPa and 63 °C (317 µmol Trolox/100 g extract) for mango seeds. At these conditions, the richest bioactive fractions were also found, as can be corroborated in Tables 1 and 2.

Table 3. Antioxidant activity (µmol Trolox/100 g) of guava and mango seed extracts.

Guava Seed Extracts			Mango Seed Extracts		
Pressure (MPa)	**Temperature (°C)**	**µmol Trolox/100 g**	**Pressure (MPa)**	**Temperature (°C)**	**µmol Trolox/100 g**
20.0 (−1.00)	40 (−1.00)	243 ± 4^{bcde}	25.0 (−1.00)	55 (−1.00)	236 ± 85^{b}
20.0 (−1.00)	60 (+1.00)	262 ± 5^{ef}	25.0 (−1.00)	70 (+1.00)	263 ± 5^{c}
35.0 (+1.00)	40 (−1.00)	226 ± 5^{bc}	35.0 (+1.00)	55 (−1.00)	215 ± 2^{ab}
35.0 (+1.00)	60 (+1.00)	235 ± 4^{bcd}	35.0 (+1.00)	70 (+1.00)	226 ± 8^{b}
16.9 (−1.41)	50 (0.00)	281 ± 9^{f}	22.9 (−1.41)	63 (0.00)	317 ± 8^{e}
38.1 (+1.41)	50 (0.00)	222 ± 6^{b}	37.1 (+1.41)	63 (0.00)	225 ± 7^{ab}
27.5 (0.00)	36 (−1.40)	224 ± 3^{b}	30.0 (0.00)	52 (−1.41)	263 ± 3^{c}
27.5 (0.00)	64 (+1.41)	254 ± 4^{de}	30.0 (0.00)	73 (+1.41)	281 ± 3^{cd}
27.5 (0.00)	50 (0.00)	248 ± 4^{cde}	30.0 (0.00)	63 (0.00)	291 ± 4^{de}
Soxhlet extraction		177 ± 6^{a}	Soxhlet extraction		200 ± 2^{a}

Different letters (a, b, c, d, e, and f) in the same column represent statistically significant differences at 5% significance.

Table 3 also shows that the extracts obtained with n-hexane had lower antioxidant capacity than the extracts obtained at any of the supercritical conditions ($p \leq 0.05$). However, as in the supercritical bioactive fractions, such antioxidant capacity was higher in the bioactive fractions of mango seeds, which were the richest in bioactive compounds (see Table 2). The above results are since there was a direct relationship between the content

of minor compounds and the antioxidant capacity of the extracts. Other cases have been reported in the literature in which the highest antioxidant activity corresponded to fractions with higher bioactive content [6,21]. Finally, the antioxidant activities of guava and mango seed extracts in this study were similar to that reported for olive oil (300 μmol Trolox/100 g oil), which is well known for its high antioxidant capacity [22].

4. Conclusions and Future Perspectives

Supercritical bioactive fractions rich in squalene, sterols, and tocopherols were obtained from guava (*Psidium guajava*) and mango (*Mangifera indica* L.) seeds. The obtained supercritical extracts were of even higher quality than olive oil, a foodstuff well known for its nutritional properties. The sterol β-sitosterol was the most abundant minor compound in both extracts, followed by squalene.

Squalene is a polyunsaturated triterpene, a biochemical precursor of cholesterol and other steroids. The main source of squalene is shark liver oil, although it can also be found in plant sources such as olives, wheat germ, amaranth, and rice bran [23]. Several beneficial health effects are attributed to this compound, as several studies have obtained results demonstrating certain biological activities: anticarcinogenic, antioxidant, drug carrier, detoxifier, skin moisturizer, an inhibitor of the development of various tumors, and emollient activities [23,24]. In addition, this compound is used as an ingredient in the formulation of skin care products due to its photoprotective effect [25], which shows the potential use of the extracts obtained in bioprospecting. Likewise, β-sitosterol is of major commercial interest due to in vitro and in vivo studies providing evidence that this compound inhibits tumor cell growth. Such studies indicated that β-sitosterol may act as a chemopreventive agent for colon and breast cancer [26,27]. In general, the health-beneficial properties of the in this work have been widely reported in several studies [28,29]; and the most important property is related to its ability to reduce blood cholesterol levels due to the promotion of high density lipoprotein (HDL) production and its potent antioxidant activity.

For their part, the tocopherols found in the extracts of this work act as biological scavengers of free radicals in the human body and participate in the prevention of various diseases [30,31].

In this study, we have observed superior outcomes concerning bioactive fractions abundant in minor compounds, as compared to the existing literature. This achievement can be attributed to the optimization of the initial solid conditions of the raw material, as applied in our supercritical extraction methodology. Nevertheless, it is crucial to recognize that the processes of drying and particle size reduction demand a significant amount of energy [6].

The minor compounds found in this work have several beneficial properties and could be used in different businesses (e.g., food, cosmetic, medical, and pharmaceutical); they could even be exploited by local entrepreneurs. Colombia has a large population of low-income farmers, who need to boost their economy and use local natural resources to do so. In this case, they would not only be using a local natural resource, but also a waste product to improve their food safety.

Due to the biocidal and virucidal power of $scCO_2$ [32], the bioactive fractions obtained are sterile, which facilitates the subsequent direct packaging of the product obtained in any of the aforementioned businesses. It would have more advantages in the food sector, which is the one with the most restrictive microbiological marketing restrictions. Additionally, the products marketed will not only have the green label for only using a GRAS (i.e., generally recognized as safe) solvent such as CO_2, but will also have a natural label.

All of the above demonstrate the potential of the obtained oily extracts as ingredients for food, cosmetic, medical, and pharmaceutical activities. Best of all, these compounds come from an agri-food waste, which means that the raw material would be free or at most, very low cost, not to mention that waste is avoided.

Author Contributions: Conceptualization, A.M.H.-B., A.A.-A. and D.F.T.; Data curation, L.J.C.-M., A.M.H.-B. and D.F.T.; Formal analysis, L.J.C.-M., A.M.H.-B., A.A.-A., L.S.-C. and D.F.T.; Funding acquisition, A.M.H.-B., A.A.-A. and D.F.T.; Investigation, L.J.C.-M., A.M.H.-B., A.A.-A. and D.F.T.; Methodology, L.J.C.-M.; Project administration, A.M.H.-B. and A.A.-A.; Resources, A.M.H.-B., A.A.-A., L.S.-C. and D.F.T.; Software, L.J.C.-M.; Supervision, A.M.H.-B., A.A.-A. and D.F.T.; Validation, A.M.H.-B., A.A.-A. and D.F.T.; Visualization, D.F.T.; Writing–original draft, D.F.T.; Writing–review and editing, D.F.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research study was financially supported by the Fondo de Ciencia, Tecnología e Innovación del Sistema General de Regalías (SGR)-Colombia (project reference 2013000100001) and the Universidad Nacional de Colombia (project reference 58097).

Data Availability Statement: The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. FAO. *The State of Food and Agriculture 2019. Moving Forward on Food Loss and Waste Reduction*; FAO: Rome, Italy, 2019.
2. Yaashikaa, P.R.; Senthil Kumar, P.; Varjani, S. Valorization of agro-industrial wastes for biorefinery process and circular bioeconomy: A critical review. *Bioresour. Technol.* **2022**, *343*, 126126. [CrossRef] [PubMed]
3. Thavorn, J.; Muangsin, V.; Gowanit, C.; Muangsin, N. Research on shelf-life extension technologies for food sustainability: An assessment of scientific activities and networks. *Sci. World J.* **2022**, *2022*, 7120662. [CrossRef] [PubMed]
4. Fikire, A.H.; Zegeye, M.B. Determinants of rural household food security status in North Shewa zone, Amhara region, Ethiopia. *Sci. World J.* **2022**, *2022*, 9561063. [CrossRef] [PubMed]
5. Cerón-Martínez, L.J.; Hurtado-Benavides, A.M.; Ayala-Aponte, A.; Serna-Cock, L.; Tirado, D.F. A pilot-scale supercritical carbon dioxide extraction to valorize Colombian mango seed kernel. *Molecules* **2021**, *26*, 2279. [CrossRef] [PubMed]
6. Tirado, D.F.; Fuente, E.D.L.; Calvo, L. A selective extraction of hydroxytyrosol rich olive oil from alperujo. *J. Food Eng.* **2019**, *263*, 409–416. [CrossRef]
7. Vladić, J.; Jakovljević Kovač, M.; Pavić, V.; Jokić, S.; Simić, S.; Paiva, A.; Jerković, I.; Duarte, A.R. Towards a greener approach for biomass valorization: Integration of supercritical fluid and deep eutectic solvents. *Antibiotics* **2023**, *12*, 1031. [CrossRef] [PubMed]
8. Cerón, L.J.; Hurtado, A.M.; Ayala, A.A. Efecto de la presión y la temperatura de extracción con CO2 supercrítico sobre el rendimiento y composición de aceite de semillas de guayaba (*Psidium guajava*). *Inf. Tecnológica* **2016**, *27*, 249–258. [CrossRef]
9. Narváez-Cuenca, C.E.; Inampues-Charfuelan, M.L.; Hurtado-Benavides, A.M.; Parada-Alfonso, F.; Vincken, J.P. The phenolic compounds, tocopherols, and phytosterols in the edible oil of guava (Psidium guava) seeds obtained by supercritical CO_2 extraction. *J. Food Compos. Anal.* **2020**, *89*, 103467. [CrossRef]
10. Dorado, D.J.; Hurtado-Benavides, A.M.; Martínez-Correa, H.A. Extracción con CO2 supercrítico de aceite de semillas de guanábana (*Annona muricata*): Cinética, perfil de ácidos grasos y esteroles. *Inf. Tecnológica* **2016**, *27*, 37–48. [CrossRef]
11. Pantoja-Chamorro, A.L.; Hurtado-Benavides, A.M.; Martínez-Correa, H.A. Evaluación del rendimiento, composición y actividad antioxidante de aceite de semillas de mora (*Rubus glaucus*) extraído con CO2 supercrítico. *Inf. Tecnológica* **2017**, *28*, 35–46. [CrossRef]
12. Viguera, M.; Marti, A.; Masca, F.; Prieto, C.; Calvo, L. The process parameters and solid conditions that affect the supercritical CO_2 extraction of the lipids produced by microalgae. *J. Supercrit. Fluids* **2016**, *113*, 16–22. [CrossRef]
13. Brunner, G. *Gas Extraction*; Topics in Physical Chemistry; Steinkopff: Heidelberg, Germany, 1994; Volume 4, ISBN 978-3-662-07382-7.
14. Tirado, D.F.; Calvo, L. The Hansen theory to choose the best cosolvent for supercritical CO_2 extraction of β-carotene from Dunaliella salina. *J. Supercrit. Fluids* **2019**, *145*, 211–218. [CrossRef]
15. Bashipour, F.; Ghoreishi, S.M. Response surface optimization of supercritical CO_2 extraction of α-tocopherol from gel and skin of Aloe vera and almond leaves. *J. Supercrit. Fluids* **2014**, *95*, 348–354. [CrossRef]
16. Liu, G.; Xu, X.; Gong, Y.; He, L.; Gao, Y. Effects of supercritical CO2 extraction parameters on chemical composition and free radical-scavenging activity of pomegranate (*Punica granatum* L.) seed oil. *Food Bioprod. Process.* **2012**, *90*, 573–578. [CrossRef]
17. Smith, T.J. Squalene: Potential chemopreventive agent. *Expert Opin. Investig. Drugs* **2000**, *9*, 1841–1848. [CrossRef]
18. López-Padilla, A.; Ruiz-Rodriguez, A.; Reglero, G.; Fornari, T. Study of the diffusion coefficient of solute-type extracts in supercritical carbon dioxide: Volatile oils, fatty acids and fixed oils. *J. Supercrit. Fluids* **2016**, *109*, 148–156. [CrossRef]
19. Bhattacharjee, P.; Chatterjee, D.; Singhal, R.S. Supercritical carbon dioxide extraction of squalene from Amaranthus paniculatus: Experiments and process characterization. *Food Bioprocess Technol.* **2012**, *5*, 2506–2521. [CrossRef]
20. Kraujalis, P.; Venskutonis, P.R. Supercritical carbon dioxide extraction of squalene and tocopherols from amaranth and assessment of extracts antioxidant activity. *J. Supercrit. Fluids* **2013**, *80*, 78–85. [CrossRef]
21. Şahin, S.; Bilgin, M.; Dramur, M.U. Investigation of oleuropein content in olive leaf extract obtained by supercritical fluid extraction and soxhlet methods. *Sep. Sci. Technol.* **2011**, *46*, 1829–1837. [CrossRef]

22. Sousa, A.; Casal, S.; Malheiro, R.; Lamas, H.; Bento, A.; Pereira, J.A. Aromatized olive oils: Influence of flavouring in quality, composition, stability, antioxidants, and antiradical potential. *LWT Food Sci. Technol.* **2015**, *60*, 22–28. [CrossRef]
23. Reddy, L.H.; Couvreur, P. Squalene: A natural triterpene for use in disease management and therapy. *Adv. Drug Deliv. Rev.* **2009**, *61*, 1412–1426. [CrossRef] [PubMed]
24. Kim, S.-K.; Karadeniz, F. Biological importance and applications of squalene and squalane. *Adv. Food Nutr. Res.* **2012**, *65*, 223–233. [PubMed]
25. Das, B.; Yeger, H.; Baruchel, H.; Freedman, M.; Koren, G.; Baruchel, S. In vitro cytoprotective activity of squalene on a bone marrow versus neuroblastoma model of cisplatin-induced toxicity. *Eur. J. Cancer* **2003**, *39*, 2556–2565. [CrossRef]
26. Ju, Y.H.; Helferich, W.G.; Clausen, L.M.; Allred, K.F.; Almada, A.L. β-Sitosterol, β-sitosterol glucoside, and a mixture of β-sitosterol and β-sitosterol glucoside modulate the growth of estrogen-responsive breast cancer cells in vitro and in ovariectomized athymic mice. *J. Nutr.* **2004**, *134*, 1145–1151. [CrossRef] [PubMed]
27. Debele, T.A.; Mekuria, S.L.; Tsai, H.-C. Synthesis and characterization of redox-sensitive heparin-β-sitosterol micelles: Their application as carriers for the pharmaceutical agent, doxorubicin, and investigation of their antimetastatic activities in vitro. *Mater. Sci. Eng. C* **2017**, *75*, 1326–1338. [CrossRef]
28. Jones, P.J.; AbuMweis, S.S. Phytosterols as functional food ingredients: Linkages to cardiovascular disease and cancer. *Curr. Opin. Clin. Nutr. Metab. Care* **2009**, *12*, 147–151. [CrossRef]
29. Flakelar, C.L.; Prenzler, P.D.; Luckett, D.J.; Howitt, J.A.; Doran, G. A rapid method for the simultaneous quantification of the major tocopherols, carotenoids, free and esterified sterols in canola (Brassica napus) oil using normal phase liquid chromatography. *Food Chem.* **2017**, *214*, 147–155. [CrossRef]
30. Brigelius-Flohé, R.; Kelly, F.J.; Salonen, J.T.; Neuzil, J.; Zingg, J.-M.; Azzi, A. The European perspective on vitamin E: Current knowledge and future research. *Am. J. Clin. Nutr.* **2002**, *76*, 703–716. [CrossRef]
31. Jokić, S.; Vidović, S.; Zeković, Z.; Kuzmanović, S.P.; Jevrić, L.; Marić, B. Chemometric analysis of tocopherols content in soybean oil obtained by supercritical CO_2. *J. Supercrit. Fluids* **2012**, *72*, 305–311. [CrossRef]
32. Dacal-Gutiérrez, A.; Tirado, D.F.; Calvo, L. Inactivation of Clostridium spores in honey with supercritical CO_2 and in combination with essential oils. *Processes* **2022**, *10*, 2232. [CrossRef]