



# Article Analysis of Five-Extraction Technologies' Environmental Impact on the Polyphenols Production from *Moringa oleifera* Leaves Using the Life Cycle Assessment Tool Based on ISO 14040

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Abstract: The present study examines, compares, and documents the environmental impact of five extraction techniques through Life Cycle Assessment (LCA). The material used was Moringa oleifera freeze-dried leaves and the assessment was based on their polyphenol content Three out of the five examined techniques are referred to in the literature as "green" techniques, namely Pulsed Electric Field (PEF), Microwave-Assisted Extraction (MAE), and Ultrasound-Assisted Extraction (UAE). The other two examined were conventional extraction techniques and, specifically, boiling water and maceration; the latter served as a control in this study. The analysis utilized special software (SimaPro ecoinvent) for the "cradle to gate" LCA, along with a sensitivity analysis of the model examining the variation in the environmental impact based on the origin of the source of electricity (renewable sources such as photovoltaic arcs), aiming to highlight the optimal technology choice. This LCA study's Functional Unit (FU) was one gram (g) of extracted total polyphenols (dry) produced by a case-specific number of extraction cycles for each technology under assessment (considering their technical efficiency depicted as polyphenols yields), measured by the Folin-Ciocalteu method and expressed as mg Gallic Acid Equivalents per g of dry Moringa oleifera leaves. The study outcome indicates that PEF and MAE deliver the best environmental scores. The main contributing parameters are the Moringa oleifera leaves and the amount and origin of electricity used to make 1 FU. These parameters are dominant in the categories of freshwater ecotoxicity, marine ecotoxicity, human carcinogenic toxicity, and human non-carcinogenic. The better performance of these two techniques is due to the more efficient extraction with reduced electricity consumption, which can become even more environmentally friendly if replaced with renewable sources such as photovoltaic arcs.

**Keywords:** life cycle assessment; green technologies; environmental impact; pulsed electric field; extraction; polyphenols

# 1. Introduction

Biofunctional molecules in the form of natural products of plant origin (such as polyphenols) are a class of chemicals (in the form of a crude mixture, processed fraction, or pure molecules) that exhibit biological activity in living organisms [1–4]. During the last decades, a trend has developed in some parts of the economy's secondary sector (food, pharmaceutical, cosmetic, and chemical industries) concerning the replacement of synthetic, semi-synthetic, or isolated components based on fossil fuels such as petroleum products with "green alternatives", such as those of vegetable or animal origin [5–7].

Even more so, a "green" environmentally friendly production methodology is preferable for this purpose. Various natural products, for example, polyphenols, are chemical



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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). substances isolated from plants with the help of extraction techniques or extraction systems (**S** in this study). Until recently, the extraction of desired components (polyphenols, flavonoids, pigments, carotenoids, essential oils, and others) from plant material required the use of high temperatures and energy or the use of large amounts of toxic organic solvents with a corresponding burden on the environment, high processing costs, and difficulty removing their residues from the final product [5,8]. However, the environmental impact and the "green extraction technology" definition inspection for complex extraction technologies can only be demonstrated and documented through comparative measurements of the respective technology's environmental performance.

Environmental assessments and agriculture or agri-food product improvements use LCA extensively. A plethora of studies illustrates the importance of LCA in the agri-food sector during the last two decades. On the Greek island of Aegina, three alternative production methods were evaluated for pistachio cultivation, assessing energy usage and the associated environmental effects [9]. To evaluate actions for promoting energy saving and reducing water consumption, comparative LCA and sensitivity analysis was also carried out for pistachio, almond, and apple production [10]. Additionally, LCA has been used to contrast various agri-product cultivation techniques. For instance, Longo et al. [11] used the methodology to compare the energy and environmental performance of conventional and organic apples, looking at the input of raw materials and energy sources, the farming process, the post-harvest procedures, and the distribution of the apples to the end users. When considering vinification, bottling, packaging, distribution, and waste disposal practices, LCA has also been used to suggest an improved way to lower the  $CO_2$  emissions of wine production in Italy [12]. The approach can also serve as a tool to examine local policies for sustainable production and consumption patterns and learn more about how to assess the environment in a large agricultural production region [13,14]. The management of agri-product cultivation in a defined agricultural system, such as for the production of apples, is a helpful technique to assess how environmental consequences are influenced [15]. In another case, LCA has also been applied to explain the managerial choices made within agricultural holdings with a view to their sustainable development [16]. The method has also been combined with data envelopment analysis to evaluate the eco-efficiency of intensive rice cultivation in Japan [17].

Even though progress has been reported in LCA studies concerning the software and databases available for use, several tools have also been developed to assist LCA practitioners who continue to struggle with communicating and interpreting results [18]. However, regardless of the plethora of agri-food or food-related LCA studies, there is a lack of LCA studies targeting the analysis of specific food constituents of interest, such as polyphenols.

The present study serves the purpose to examine and compare the environmental impact of five extraction techniques for polyphenols from Moringa oleifera leaves based on a previous study [19]. Three of these techniques are referred to in the literature as green extraction techniques, namely Pulsed Electric Field (PEF) [20-22], Microwave-Assisted Extraction (MAE) [23,24], and Ultrasound-Assisted Extraction (UAE) [25]. The other two are referred to as conventional extraction techniques and include Boiling Water (BW) and Maceration (M, serves as a Control, C, in this study). The reference study [19], which serves as the data source for this study's LCA, utilizes a prototype bench-scale PEF unit and lab-scale equipment to (a) investigate the feasibility of producing high-quality liquid extracts from Moringa oleifera dried ground leaves and (b) demonstrate and document the respective technology's environmental performance through comparative measurements. Our analysis utilized special software (SimaPro ecoinvent) for the "cradle to gate" Life Cycle Assessment (LCA) [26–29], along with a sensitivity analysis of the model examining the variation in the environmental impact. The variation in the environmental impact was based on the origin of the source of electricity, aiming to highlight the optimal technology choice under the knowledge that PEF technology revealed an optimal polyphenol yield, as reported by Bozinou et al. [19].

The Pulsed Electric Field (PEF) technology is a relatively new alternative technology and complies with the requirements of green chemical engineering for long-term production systems and can be utilized in batch and continuous flow applications [30]. PEF is a technology in which the components of the produced extracts are unaltered compared with other treatments (heat treatment, extraction with solvents, and others) [31–38]. As a result, (a) the final product maintains, to a large extent, its desired characteristics such as aroma, oxidation state, color, and others, (b) no toxic solvents are used, and c) a large part of the microorganisms can be killed, resulting in an extract with a low microbial load [39,40]. PEF technology uses high-intensity pulsed electric fields to cause plasma membrane permeability, allowing transmembrane transport of otherwise impermeant molecules [41] or even disrupting the cell membrane, caused when electric fields pierce microbial membranes [42]. Technically, as seen during all our previous work, PEF treatment integrates the application of small electric field pulses of low intensity and minimal duration, with minimal thermal effects (temperature rise to max. +1 °C) on the final product.

## 2. Materials and Methods

## 2.1. Materials and Sample Preparation

## 2.1.1. Plant Material and Extraction Process

This study utilizes freeze-dried ground Moringa oleifera leaves as explained thoroughly in a previous study [19]. In particular, the leaves were cultivated at and collected from the Agioi Apostoloi area (at  $39^{\circ}22'39.4''$  N and  $21^{\circ}54'00.6''$  E and elevation of 100 m, according to Google Earth Pro version 7.3.2.5491 (64 bit) (Google, Inc., Mountain View, CA, USA) of Karditsa County (central Greece)). The leaves from the plants were harvested on the morning of 25 October 2018, while the trees were only two months old. In all extraction methods, the ratio of dried ground leaves (~0.5 mm diameter and 8% moisture content) used was 5% (w/v). More specifically, 1.25 g of freeze-dried leaves was mixed with 25 mL of Double-Distilled (DD) water and, immediately after each extraction treatment, the mixture of water and plant material was filtered through filter paper. In order to have the same final water volume, the residue in the filter was rinsed with DD water until a final volume of 25 mL of the filtered extract was reached [9].

#### 2.1.2. Chemicals

Distilled water and ethanol were of HPLC grade. The Folin–Ciocalteu reagent was purchased from Panreac (Barcelona, Spain). Anhydrous sodium carbonate (>99%) and Gallic acid monohydrate were purchased from Penta (Prague, Czech Republic).

# 2.2. Determination of the Total Polyphenol Content (TPC)

The detailed procedure used for the TPC determination is described adequately in a previous study [19]. The TPC was determined as mg of Gallic Acid Equivalents (GAE) per g of the freeze-dried *Moringa oleifera* leaves, using the following Equation (1):

$$TPC (mg GAE/g dm) = \frac{C_{TP} \times V}{w}$$
(1)

where  $C_{\text{TP}}$  is the concentration of total polyphenols (in mg/L), *V* is the volume of the extraction medium (in L) and *w* is the weight of the *Moringa oleifera* sample (in g of dry material).

## 2.3. Environmental Impact Assessment

The comparative analysis of the environmental impact of the extraction technologies (PEF and others) on the plant material *Moringa oleifera* leaves was carried out with the help of the SimaPro (ecoinvent) computer program (Release 9.0.0.30, PRé Sustainability B.V.). The environmental impact assessment concerns the part of the life cycle assessment of polyphenols from *Moringa oleifera* leaves produced by five different extraction methods (systems).

The comparative study was defined as a "cradle to gate" examination of a system, meaning the part from the production of *Moringa oleifera* leaves to the preparation of a beneficial product (polyphenols). The study focused on five *Moringa oleifera* leaf extraction systems that produce 1 g dry weight of polyphenols (Functional Unit) as a product. These systems are System 1 (S1): Pulsed Electric Field extractor (PEF); System 2 (S2): Microwave Extractor (MAE); System 3 (S3): Ultrasound Extractor (UAE); System 4 (S4): Boiling Water extractor (BW); System 5 (S5): Control extractor (C, static maceration).

The study began with the "Goal and Scope" definition (1st Stage) of the Life Cycle Assessment framework—LCA (Figure 1).



**Figure 1.** General schematic structure of Life Cycle Assessment (LCA) according to ISO 14040:2006 [18] and 14044:2006 [29].

Then, through the data collected from sources such as questionnaires, the literature, and databases of the SimaPro software, a detailed calculation and analysis regarding the functional unit for each system (inventory analysis) followed. This part of the study (2nd Stage) comprises the "Life Cycle Inventory analysis—LCI", as demonstrated in Figure 1.

The following step was the impact assessment of the LCI results on 18 midpoint and 3 endpoint indicators (single scores) with the Recipe 2016 endpoint (H) V1.03/World (2010) H/A/single score method [43] to visualize and quantify the results of the environmental performance of the five *Moringa oleifera* polyphenol production systems. This part of the study (3rd Stage) composes the Life Cycle Impact Assessment (LCIA), as presented in Figure 1. This method was chosen above other well-known LCIA models (such as CML, Eco-Indicator, and Eco-Scarcity) because it is one of the most updated methods from temporal and geographical standpoints, as well as in terms of completeness.

Finally, the sensitivity analysis of the model followed, examining the variation in the environmental impact based on the origin of the source of electricity (renewable sources such as photovoltaic arcs). The final report includes the interpretation of all the above steps and the interconnected results. The aim is to highlight the environmental advantages of using PEF technology compared with other technologies under the knowledge that PEF technology revealed an optimal polyphenol yield [19].

#### 2.3.1. Goal of the Study

The intended application is the environmental performance assessment of the PEF extraction (project PEF EXTRACTION in this study) through a comparative study of different laboratory extraction techniques (systems). The study utilizes published scientific data as recently reported by Bozinou et al. [19].

The study is a cradle-to-gate research (where cradle corresponds to the origin of the materials, such as *Moringa oleifera* leaves in terms of cultivation and water supply, and gate corresponds to the ready-to-deliver produced material, such as *Moringa oleifera* leaf polyphenols). It aims to identify the main inputs (resources) for the output creation (products, emissions) through the comparison of five extraction techniques, calculating their potential environmental impact and concluding in their environmental performance. The contribution of the specific study is the preparation of an aqueous extract (containing the polyphenols) from leaves of the cultivated *Moringa oleifera* tree. The mass of polyphenols (measured in grams of Gallic Acid Equivalents) is the parameter of interest.

The five extracts obtained from the *Moringa oleifera* leaves extraction systems were of 25 mL liquid aqueous extract each, containing a variable amount of polyphenols demonstrating antioxidant activity, as documented by Bozinou et al. [19]. It is worth mentioning that according to the previous study, the polyphenol content of the aqueous extracts is proportionally correlated with antioxidant activity. The aqueous solvent serves as a dissolution medium for the polyphenols. Therefore, the functional unit should be related to the actual production performance of the respective system in terms of polyphenols mass obtained.

On the other hand, the mixture of polyphenols and auxiliary solvent (water) can be considered a semi-finished product for further processing (such as drying). For purposes of quantitative measurement, the preparation of 1 g of polyphenols designates a mass functional unit (defined in Gallic Acid Equivalents).

Water (as a solvent in the final product) is considered a co-product of lower importance in the present focus.

For the sensitivity analysis, 1 Kg (=1000 g) of polyphenols will portray a functional unit for better visualization of the results.

#### 2.3.3. Product Systems under Study

The product manufacturing systems under study are five laboratory technologies with different characteristics, with their reference flows presented in Table 1.

System Number	<b>S</b> 1	S2	<b>S</b> 3	<b>S</b> 4	<b>S</b> 5
Targeted polyphenol extraction (g)	1.00	1.00	1.00	1.00	1.00
Used <i>Moringa oleifera</i> leaves per extraction cycle (g)	1.25	1.25	1.25	1.25	1.25
Yielded mass of polyphenols per extraction cycle (g)	0.046	0.042	0.033	0.036	0.032
Number of extraction cycles needed	21.60	23.75	29.94	27.55	31.35
Total mass of <i>Moringa oleifera</i> leaves (g)	27.00	29.70	37.43	34.44	39.18
$H_2O$ needs as solvent per extraction cycle (g)	34.70	38.50	35.50	40.40	34.60
Total mass of H <sub>2</sub> O (g)	751.62	914.49	1062.87	1112.95	1084.64

Table 1. Reference flow for Systems 1–5 for 1 FU (1 g) from Moringa oleifera leaves 8% moisture.

## 2.3.4. System Boundaries

The study is a "cradle-to-gate" examination of a system that includes incoming raw materials such as *Moringa oleifera* leaves and energy (such as electricity) but not waste scenarios such as disposal of *Moringa oleifera* extracts and spent leaves, which are supposed to be composted by producers and used as bio-fertilizer. The operation of the five systems takes place in Central Greece near the plantation of *Moringa oleifera* trees. From each extraction system, two water stream types arise and cannot be assessed as co-derivatives, because they do not participate in the functional unit, thus they are excluded from the system boundaries. The first type of water is solvent water in which polyphenols are dissolved. The second type of water is wastewater contained as moisture in *Moringa oleifera* leaves (8%), water absorbed by the spent leaves, and water that has evaporated. The second type is part of the solid waste management case. All the above are summarized in Figure 2.



**Figure 2.** System boundaries (**S**1–5) and 1 Functional Unit (FU) production flow diagram for this cradle-to-gate study (where DMPM stands for dry ground *Moringa oleifera* leaves with 8% moisture content). Inputs (energy and mass): DMPM, electric equipment, transportation units, electrical energy, water, auxiliary equipment and materials. Outputs (waste and emissions): polyphenols (FU) product, water (co-derivative), waste (water and spent leaves), thermal energy.

#### 2.3.5. System Functionality (Facts and Assumptions)

In Figure 2, DMPM represents the dried ground *Moringa oleifera* leaves containing 8% *w/w* moisture. Each system's total amount of DMPM was used to produce 1 FU variety.

The product of interest is polyphenols (solid phase measured in grams of Gallic Acid Equivalents). The final product prepared (polyphenols) is in the form of an aqueous solution. Water as a carrier has no active properties. It is considered a co-derivative and thus not included in the system boundaries.

A filtration step takes place immediately after the extraction step and is considered part of the manufacturing process. The filtration step for the preparation of FU is the same in each system and includes a non-mechanical (gravity) process with filter paper and standard laboratory glassware that are banned in the modeling due to their negligible contribution to each system.

Each system produces waste (*Moringa oleifera* spent leaves and absorbed water) in varying amounts. Another non-hazardous waste (such as paper) is not included in the modeling.

Thermal energy is removed from each system. The Control and PEF systems do not generate thermal energy ( $\Delta T \approx 0$  °C). However, the UAE, MAE, and BW systems produce measurable amounts of heat released into the environment, as presented in Table 2, where the process duration and temperature logging data collected via experimentation for each system are presented. However, in SimaPro (Recipe), waste heat modeling is not foreseen, so this emission was not included in the model.

	<b>S1</b>	S2	<b>S</b> 3	<b>S</b> 4	<b>S</b> 5
Extraction duration (min)	40	2	15	2	40
Temperature start (°C)	25	25	25	100	25
Temperature end (°C)	25	80	36	88	25
ΔT (°C)	0	55	11	12	0

**Table 2.** Process duration and temperature logged data for Systems 1–5 and 1 FU (1 g) of *Moringa oleifera* leaves 8% moisture. The temperature at which the extraction started was ambient ( $25 \degree$ C).

#### 2.3.6. LCA Methodology, Impact Categories, and Data Requirements

The emissions data from the ecoinvent database were used to calculate the LCIA factors. The ReCiPe midpoint hierarchist (18 midpoint indicators) and ReCiPe endpoint hierarchist (3 endpoint indicators, single scores) methods were used to calculate the impacts. Foreground data were obtained from the findings of Bozinou et al. [19] and via electricity, fuel consumption calculations, and bibliography, while background data were obtained from the LCI database ecoinvent (Europe and global data) of the SimaPro ecoinvent program.

## 3. Results and Discussion

## 3.1. Inventory Analysis (Inputs/Outputs)

The description of the inventory analysis follows that of the goal and scope, as designated in Figure 1. All the inputs/outputs used for preparing 1 FU were analyzed at the inventory analysis step. Initially, the production of 1 g of *Moringa oleifera* leaves at 8% was modeled. Then, the material content (weight of plastic, metal, glass) of each extractor participating in each system was roughly analyzed. Afterward, the production of 1 FU from each system (S1–S5) was modeled by analyzing the inputs (such as electricity and water) and outputs (such as heat emission and compost). The input data were then used by SimaPro integrated LCI (Life Cycle Inventory) ecoinvent database for further calculations. For convenience and better report handling, all the results for the inventory analysis step with the origin and details regarding the data collected for inventory analysis are presented in Tables S1–S11 in the Supplementary Material.

#### 3.2. Impact Assessment

The impact assessment (LCIA, Life Cycle Impact Assessment) consisted of 18 midpoint indicators for each system (S1–S5) for the production of 1000 g polyphenols (=1000 \* FU) for better visualization of the results and the ReCiPe method 2016 midpoint (H) V1.03/WORLD (2010) H [43]. The 18 indicators (impact categories) are: (1) global warming; (2) stratospheric ozone depletion; (3) ionizing radiation; (4) ozone formation, human; (5) fine particulate matter form; (6) ozone formation, terrestrial; (7) terrestrial acidification; (8) freshwater eutrophication; (9) marine eutrophication; (10) terrestrial ecotoxicity; (11) freshwater ecotoxicity; (12) marine ecotoxicity; (13) human carcinogenic toxicity; (14) human non-carcinogenic; (15) land use; (16) mineral resource scarcity; (17) fossil resource scarcity; (18) water consumption.

Due to differences in the various LCA study components (such as Functional Units, system boundaries, product allocation, laboratory or industrial scale, scaling-up aspects, and impact assessment methods), our results are not comparable with those of other studies. The only citation relative to our unique research is from Barjoveanu et al. [44] and is provided only for contradistinction; any further comparisons would be incorrect.

In the following sections, the figurative analysis from the software is presented and analyzed for each one of the systems under study. For matters of convenience and better report handling, apart from the resulting figures for the PEF system (S1) that are presented here, the rest of the figures for the remaining four systems (S2–S5) are given in the Supplementary Material as Figures S1–S12.

# 3.2.1. System 1 (PEF)

In System 1 (PEF), according to Figure 3 (red arrows), it is shown that the parameters that contribute the most to the environmental impact of the manufactured FU (1 Kg polyphenols) are the electricity (for the extraction and cutting/drying of the leaves) and the *Moringa oleifera* leaves. The effect of these parameters, presented in Figure 4, shows that they contribute more in all categories (see in yellow (electricity) and blue (*Moringa oleifera* leaves)). In Figure 5, after the data normalization, one can see that the effect of these parameters is dominant in the categories of freshwater ecotoxicity, marine ecotoxicity, human carcinogenic toxicity, and human non-carcinogenic.



**Figure 3.** Data model for System **S1** (PEF) from SimaPro (ecoinvent) Release 9.0.0.30, PRé Sustainability B.V. software.



■ PEF5 (S1) polyphenols ■ PEF5 (S1) extractor new ■ Tap water {Europe without Switzerland}| market for | Cut-off, S ■ Electricity, low voltage {GR}| market for | Cut-off, S ■ Moringa leaves 8% moisture (DMPM)

Figure 4. Environmental impact data of the manufactured FU for System S1 (PEF).



■ PEF5 (S1) polyphenols ■ PEF5 (S1) extractor new ■ Tap water {Europe without Switzerland} | market for | Cut-off, S ■ Electricity, low voltage {GR} | market for | Cut-off, S ■ Moringa leaves 8% moisture (DMPM)

**Figure 5.** Normalized environmental impact data of the manufactured FU for System **S**1 (PEF). Plot (**A**) for values greater than 5 and plot (**B**) for values less than 5.

#### 3.2.2. System 2 (MAE)

Similarly, in System 2 (MAE), according to Figure S1 (red arrows), it is shown that the parameters that contribute the most to the environmental impact of the preparation of FU (1 Kg polyphenols) are electricity (for extracting and cutting/drying the leaves) and *Moringa oleifera* leaves. The effect of these parameters, presented in Figure S2, shows that they contribute more in all categories (see in orange (electricity) and blue (*Moringa oleifera* leaves)). In Figure S3, after the data normalization, one can see that the effect of these parameters is dominant in the categories of freshwater ecotoxicity, marine ecotoxicity, human carcinogenic toxicity, and human non-carcinogenic.

#### 3.2.3. System 3 (UAE)

Likewise, in System 3 (UAE), according to Figure S4 (red arrows), it is shown that the parameters that contribute the most to the environmental impact of the preparation of FU (1 Kg polyphenols) are electricity (for extracting and cutting/drying the leaves) and *Moringa oleifera* leaves. The effect of these parameters, presented in Figure S5, shows that they contribute more in all categories (see in yellow (electricity) and blue (*Moringa oleifera* leaves)). In Figure S6, after the data normalization, one can see that the effect of these parameters is dominant in the categories of freshwater ecotoxicity, marine ecotoxicity, human carcinogenic toxicity, and human non-carcinogenic. The relative contribution of electricity in this system is much more apparent (higher participation in the bar graphs than in the PEF and MAE systems) and is mainly due to the longer extraction time (thus more electricity consumption) required.

## 3.2.4. System 4 (BW)

Similarly, in System S4 (BW), according to Figure S7 (red arrows), it is shown that the parameters that contribute the most to the environmental impact of the preparation of FU (1 Kg polyphenols) are electricity (for extracting and cutting/drying the leaves) and *Moringa oleifera* leaves. The effect of these parameters, presented in Figure S8, shows that they contribute more in all categories (see in orange (electricity) and blue (*Moringa oleifera* leaves)). In Figure S9, after the data normalization, one can see that the effect of these parameters is dominant in the categories of freshwater ecotoxicity, marine ecotoxicity, human carcinogenic toxicity, and human non-carcinogenic. The relative contribution of electricity in this system is much more apparent (higher participation in the bar graphs

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than in the PEF and MAE systems) and is mainly due to the greater electricity consumption required to heat the solvent. According to the bar graphs, the environmental impact behavior of System **S**4 (BW) is close to that of System **S**3 (UAE).

## 3.2.5. System 5 (C)

In System **S**5 (C), according to Figure S10 (red arrows), it is shown that the parameters that contribute the most to the environmental impact of the preparation of FU (1 Kg polyphenols) are the electricity for cutting/drying the leaves and the *Moringa oleifera* leaves. System 5 does not involve electricity for the extraction (static maceration in a glass container at ambient temperature). The effect of these parameters, presented in Figure S11, shows that electricity (yellow) contributes the most in all categories. In Figure S12, after the data normalization, one can see that the effect of this parameter is dominant in the categories of freshwater ecotoxicity, marine ecotoxicity, human carcinogenic toxicity, and human non-carcinogenic.

## 3.3. Comparison of the Environmental Performance of S1–S5 Systems

In the previous analysis, the *Moringa oleifera* leaf material and the electricity parameter proved the main parameters that determine the environmental impact of the five systems in the 18 midpoint categories (impact categories).

The analysis took place by selecting the electricity category "Electricity", low voltage (GR)/market for/cut-off, S, which refers to low voltage, mixed source electricity supply in Greece (electrical network).

Then, a comparison between the environmental performance of the five systems took place according to the ReCiPe 2016 endpoint (H) V1.03/world (2010) H/A/single score method. This method offers a grouped and aggregated assessment (measured in Pt units, where Pt is a dimensionless eco-indicator defined as 1 millimeter of the annual environmental load of an average European citizen) of all previous performances in three main general categories of damage (damage assessment): human health, ecosystems, resources (see Figure 6).



**Figure 6.** Comparison of the environmental performance of 5 systems (processes) using Greece's electrical grid. The comparison method utilized is the ReCiPe 2016 endpoint (H) V1.03/world (2010) H/A/single score method.

According to Figure 6, Systems S1 (PEF), S2 (MAE), and S5 (C) show a lower environmental impact in the three general categories than Systems S3 (UAE) and S4 (BW) for the production of 1 FU (or 1000 \* FU). This illustration confirms the conclusions of the previous analysis (midpoint) regarding the similarity of Systems S3 and S4.

# 3.4. Comparison of the Environmental Performance of S1-S5 Systems: A Sensitivity Study

For the sensitivity of the study, the electricity category "Electricity": low voltage (GR)/market for/cut-off, S was replaced with the category electricity, low voltage (GR)/ electricity production, photovoltaic 3 kWp slanted-roof installation, multi-Si, panel, mounted/cut-off, S for "green" electricity from photovoltaic arcs. In the same way (end-point), the impact of the performance of the systems on the three main damage categories was calculated (see Figure 7).



**Figure 7.** Comparison of the environmental performance of the 5 systems (processes) using photovoltaic energy (sensitivity analysis). The comparison method utilized is the ReCiPe 2016 endpoint (H) V1.03/world (2010) H/A/single score method.

As shown in Figure 7, the photovoltaic energy use (to provide green electricity) reduces the score in Pt units in all three categories compared with Figure 6. Additionally, for 1 FU production, Systems S1 (PEF) and S2 (MAE) perform better than Systems S3 (UAE) and S4 (BW) and also somewhat better than System S5 (C).

The reason for the better performance of S1 (PEF) and S2 (MAE) over S5 (C) is further analyzed in Tables S12–S15, provided in the Supplementary section, where the process contribution for the production of 1000 \* FU (1 Kg polyphenols) of System S5 (C) with and without sensitivity analysis, S1 (PEF) with sensitivity analysis, and S2 (MAE) with sensitivity analysis is presented for the study of single scores (endpoint). The main contributions presented in Tables S12–S15 are due to *Moringa oleifera* leaves and electricity and its origin (electrical grid *vs.* photovoltaic). For clarity, the summarized Pt units and their main contributions from Tables S12–S15 are presented in the following table (Table 3).

Table 3. Summarized Pt units and their main contributions.

	Total Pt	Electrical Grid (Pt)	Photovoltaic (Pt)	Moringa oleifera Leaves (DMPM)
<b>S</b> 5-Control (no sensitivity analysis)	57.255323	26.461639	_	27.883349
<b>S</b> 5-Control (with sensitivity analysis)	32.986533	-	2.192848	27.883349
<b>S</b> 1-PEF (with sensitivity analysis)	26.718349	-	3.316455	19.211269
<b>S</b> 2-Microwave (with sensitivity analysis)	26.818446	-	2.931145	21.127833

Based on the data presented in Table 3, it becomes evident that replacing the electricity source with photovoltaic arcs reduces the overall environmental impact score (eco-index Pt) for **S**5 (C) from 57.255323 to 32.986533 (26.461639 vs. 2.192848 for electric energy). In addition, the overall scores of System S1 (PEF) with sensitivity analysis and S2 (MAE) with sensitivity analysis appear lower than that of S5 (C) with sensitivity analysis. In particular, the overall scores were 26.718349, 26.718349, and 32.986533, respectively. This result is justified by the lower electricity consumption and the lower utilization of Moringa oleifera leaves in PEF (19.211269 g) and MAE (21.127833 g) compared with C (27.883349 g) for the production of one FU. That is, the S1 (PEF) and S2 (MAE) systems produce one FU more efficient (in terms of mass) and friendlier (in terms of environmental criteria) than S5 (C) (and thus the S2 (UAE) and S4 (BW) systems). The S1 (PEF) and S2 (MAE) systems have very similar overall performance (26.718349 and 26.818446, respectively) and prove that for the specific application (such as the extraction of polyphenols from Moringa oleifera leaves), the "green" electroporation and microwave technologies are more environmentally friendly than the conventional ones (static Maceration, Boiling Water) but also the alternative "green" technique with ultrasound.

#### 4. Conclusions and Recommendations

From this cradle-to-gate Environmental Impact Assessment study, it is concluded that for the application in question (extraction of polyphenols from *Moringa oleifera* leaves), the extraction techniques PEF (electroporation) and MAE (microwave radiation) show a better environmental score than the techniques of static Maceration, Boiling Water, and UAE (ultrasound). The main parameters contributing to the environmental impact were *Moringa oleifera* leaves and the amount and origin of electricity used to make 1 FU (or 1000 \* FU). The better performance of these two techniques is due to the more efficient extraction with reduced electricity consumption, which can become even more environmentally friendly if replaced with renewable sources such as photovoltaic arcs.

In order to assess the full life cycle impact of any product, a cradle-to-grave LCA is required, including, by definition, the upstream processes, downstream manufacturing, use stage, recycling, and end-of-life processes. In addition, scaling up to a semi- or full-production scale can reveal more aspects of the analysis that might not be trivial. Thus, it is recommended that such unique studies will continue to challenge the scientific community toward a better, greener, and friendlier environmental future.

Finally, one can invert the typical input–output direction for food-related LCA research, particularly for food components such as polyphenols that are health-beneficial. Consuming food serves many different purposes, including giving the body the energy it needs and providing other nutrients that are good for health. A balanced diet that quantifies the food items and their sources is necessary for a healthy body. Therefore, a choice of the Functional Unit for studies on food goods in the future might be the balanced diet that aids in stabilizing the production, distribution, and consumption of foods, thereby improving food security and lowering health risks.

**Supplementary Materials:** The following supporting information can be downloaded at: https: //www.mdpi.com/article/10.3390/su15032328/s1, Table S1: LCI of 1 g *Moringa oleifera* leaves production (8% moisture); Table S2: LCI for the PEF (S1) extractor used; Table S3: LCI for the Microwave (S2) extractor used; Table S4: LCI for the Ultrasound (S3) extractor used; Table S5: LCI for the Boiling Water (S4) extractor used; Table S6: LCI for the Control (S5) extractor used; Table S7: LCI for the production of 1 g of polyphenols using PEF (S1); Table S8: LCI for the production of 1 g of polyphenols using Microwave (S2); Table S9: LCI for the production of 1 g of polyphenols using Ultrasound (S3); Table S10: LCI for the production of 1 g of polyphenols using Boiling Water (S4); Table S11: LCI for the production of 1 g of polyphenols using Maceration - Control (S5); Table S12: Process contribution for the system S5 (C) without sensitivity analysis; Table S13: Process contribution for the system S5 (C) with sensitivity analysis; Table S14: Process contribution for the system S1 (PEF) with sensitivity analysis; Table S15: Process contribution for the system S2 (MAE) with sensitivity analysis; Figure S1: Data model for system S2 (MAE) from SimaPro (ecoinvent) Release 9.0.0.30, PRé Sustainability B.V. software; Figure S2: Environmental impact data of the FU manufactured for the system S2 (MAE); Figure S3: Normalized environmental impact data of the FU manufactured for the system S2 (MAE); Figure S4: Data model for system S3 (UAE) from SimaPro (ecoinvent) Release 9.0.0.30, PRé Sustainability B.V. software; Figure S5: Environmental impact data of the FU manufacture for the system S3 (UAE); Figure S6: Normalized environmental impact data of the FU manufactured for the system S3 (UAE); Figure S7: Data model for system S4 (BW) from SimaPro (ecoinvent) Release 9.0.0.30, PRé Sustainability B.V. software; Figure S8: Environmental impact data of the FU manufacture for the system S4 (BW); Figure S9: Normalized environmental impact data of the FU manufacture for the system S4 (BW); Figure S10: Data model for system S5 (C) from SimaPro (ecoinvent) Release 9.0.0.30, PRé Sustainability B.V. software; Figure S11: Environmental impact data of the FU manufacture for the system S5 (C); Figure S12: Normalized environmental impact data of the FU manufacture for the system S5 (C); Figure S12: Normalized environmental impact data of the FU manufacture for the system S5 (C); Figure S12: Normalized environmental impact data of the FU manufacture for the system S5 (C); Figure S12: Normalized environmental impact data of the FU manufacture for the system S5 (C).

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# References

- 1. Teplova, V.V.; Isakova, E.P.; Klein, O.I.; Dergachova, D.I.; Gessler, N.N.; Deryabina, Y.I. Natural Polyphenols: Biological Activity, Pharmacological Potential, Means of Metabolic Engineering (Review). *Appl. Biochem. Microbiol.* **2018**, *54*, 221–237. [CrossRef]
- Lalas, S.I.; Athanasiadis, V.; Karageorgou, I.; Batra, G.; Nanos, G.D.; Makris, D.P. Nutritional Characterization of Leaves and Herbal Tea of *Moringa oleifera* Cultivated in Greece. *J. Herbs Spices Med. Plants* 2017, 23, 320–333. [CrossRef]
- 3. Pandey, K.B.; Rizvi, S.I. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid. Med. Cell. Longev.* **2009**, *2*, 270–278. [CrossRef]
- 4. Pérez-Jiménez, J.; Neveu, V.; Vos, F.; Scalbert, A. Identification of the 100 richest dietary sources of polyphenols: An application of the Phenol-Explorer database. *Eur. J. Clin. Nutr.* **2010**, *64*, S112–S120. [CrossRef]
- Azmir, J.; Zaidul, I.S.M.; Rahman, M.M.; Sharif, K.M.; Mohamed, A.; Sahena, F.; Jahurul, M.H.A.; Ghafoor, K.; Norulaini, N.A.N.; Omar, A.K.M. Techniques for extraction of bioactive compounds from plant materials: A review. *J. Food Eng.* 2013, 117, 426–436. [CrossRef]
- Khoddami, A.; Wilkes, M.A.; Roberts, T.H. Techniques for analysis of plant phenolic compounds. *Molecules* 2013, 18, 2328–2375. [CrossRef]
- Talmaciu, A.I.; Volf, I.; Popa, V.I. A Comparative Analysis of the "Green" Techniques Applied for Polyphenols Extraction from Bioresources. *Chem. Biodivers.* 2015, 12, 1635–1651. [CrossRef]
- Liberal, Â.; Molina, A.K.; Pereira, C.; Dias, M.I.; Ferreira, I.C.F.R.; Barros, L. Chapter 4 Solid-liquid extraction of polyphenols. In *Technologies to Recover Polyphenols from AgroFood By-products and Wastes*, 1st ed.; Pintado, M.E., Saraiva, J.M.A., Alexandre, E.M., Eds.; Academic Press: London, UK, 2022; Volume 1, pp. 73–112. [CrossRef]
- 9. Bartzas, G.; Komnitsas, K. Life cycle analysis of pistachio production in Greece. Sci. Total Environ. 2017, 595, 13–24. [CrossRef]
- 10. Bartzas, G.; Vamvuka, D.; Komnitsas, K. Comparative life cycle assessment of pistachio, almond and apple production. *Inf. Process. Agric.* **2017**, *4*, 188–198. [CrossRef]
- 11. Longo, S.; Mistretta, M.; Guarino, F.; Cellura, M. Life Cycle Assessment of organic and conventional apple supply chains in the North of Italy. *J. Clean. Prod.* 2017, 140, 654–663. [CrossRef]
- 12. Iannone, R.; Miranda, S.; Riemma, S.; De Marco, I. Improving environmental performances in wine production by a life cycle assessment analysis. *J. Clean. Prod.* 2016, 111, 172–180. [CrossRef]
- 13. Cellura, M.; Longo, S.; Mistretta, M. Life Cycle Assessment (LCA) of protected crops: An Italian case study. *J. Clean. Prod.* 2012, 28, 56–62. [CrossRef]

- 14. Tsangas, M.; Gavriel, I.; Doula, M.; Xeni, F.; Zorpas, A.A. Life Cycle Analysis in the Framework of Agricultural Strategic Development Planning in the Balkan Region. *Sustainability* **2020**, *12*, 1813. [CrossRef]
- 15. Mouron, P.; Nemecek, T.; Scholz, R.W.; Weber, O. Management influence on environmental impacts in an apple production system on Swiss fruit farms: Combining life cycle assessment with statistical risk assessment. *Agric. Ecosyst. Environ.* **2006**, 114, 311–322. [CrossRef]
- 16. Ferrara, C.; De Feo, G. Life Cycle Assessment Application to the Wine Sector: A Critical Review. *Sustainability* **2018**, *10*, 395. [CrossRef]
- 17. Masuda, K. Measuring eco-efficiency of wheat production in Japan: A combined application of life cycle assessment and data envelopment analysis. *J. Clean. Prod.* **2016**, *126*, 373–381. [CrossRef]
- Sohn, J.; Bisquert, P.; Buche, P.; Hecham, A.; Kalbar, P.P.; Goldstein, B.; Birkved, M.; Olsen, S.I. Argumentation Corrected Context Weighting-Life Cycle Assessment: A Practical Method of Including Stakeholder Perspectives in Multi-Criteria Decision Support for LCA. Sustainability 2020, 12, 2170. [CrossRef]
- 19. Bozinou, E.; Karageorgou, I.; Batra, G.; Dourtoglou, V.G.; Lalas, S.I. Pulsed electric field extraction and antioxidant activity determination of *Moringa oleifera* dry leaves: A comparative study with other extraction techniques. *Beverages* **2019**, *5*, 8. [CrossRef]
- Ho, S.; Mittal, G.S. High voltage pulsed electrical field for liquid food pasteurization. *Food Rev. Int.* 2000, *16*, 395–434. [CrossRef]
   Wouters, P.C.; Alvarez, I.; Raso, J. Critical factors determining inactivation kinetics by pulsed electric field food processing. *Trends*
- Food Sci. Technol. 2001, 12, 112–121. [CrossRef]
  Jaeger, H.; Balasa, A.; Knorr, D. Food Industry Applications for Pulsed Electric Fields. In *Electrotechnologies for Extraction from*
- *Food Plants and Biomaterials*, 1st ed.; Lebovka, N., Vorobiev, E., Eds.; Springer: New York, NY, USA, 2009; Volume 1, pp. 181–216. [CrossRef]
- Chan, C.H.; Yusoff, R.; Ngoh, G.C.; Kung, F.W.L. Microwave-assisted extractions of active ingredients from plants. J. Chromatogr. A 2011, 1218, 6213–6225. [CrossRef]
- 24. Chemat, F.; Fabiano-Tixier, A.S.; Vian, M.A.; Allaf, T.; Vorobiev, E. Solvent-free extraction of food and natural products. *TrAC Trends Anal. Chem.* **2015**, *71*, 157–168. [CrossRef]
- 25. Chemat, F.; Tomao, V.; Virot, M. Chapter 5 Ultrasound-Assisted Extraction in Food Analysis. In *Handbook of Food Analysis Instruments*, 1st ed.; Otles, S., Ed.; CRC Press: Boca Raton, FL, USA, 2008; Volume 1, pp. 85–104. [CrossRef]
- ISO 14040:2006; Environmental Management—Life Cycle Assessment—Principles and Framework. International Organization for Standardization: Geneva, Switzerland, 2006. Available online: https://www.iso.org/standard/37456.html (accessed on 27 August 2021).
- ISO 14044:2006; Environmental Management—Life Cycle Assessment—Requirements and Guidelines. International Organization for Standardization: Geneva, Switzerland, 2006. Available online: https://www.iso.org/standard/38498.html (accessed on 27 August 2021).
- Hospido, A.; Davis, J.; Berlin, J.; Sonesson, U. A review of methodological issues affecting LCA of novel food products. *Int. J. Life Cycle Assess.* 2010, 15, 44–52. [CrossRef]
- Cucurachi, S.; Van Der Giesen, C.; Guinée, J. Ex-ante LCA of Emerging Technologies. *Procedia CIRP* 2018, 69, 463–468. [CrossRef]
   Chemat, F.; Rombaut, N.; Meullemiestre, A.; Turk, M.; Perino, S.; Fabiano-Tixier, A.S.; Abert-Vian, M. Review of Green Food
- Processing techniques. Preservation, transformation, and extraction. *Innov. Food Sci. Emerg. Technol.* 2017, 41, 357–377. [CrossRef]
  Briante, R.; La Cara, F.; Febbraio, F.; Patumi, M.; Nucci, R. Bioactive derivatives from oleuropein by a biotransformation on *Olea europaea* leaf extracts. *J. Biotechnol.* 2002, 93, 109–119. [CrossRef]
- 32. Bobinaitė, R.; Pataro, G.; Lamanauskas, N.; Šatkauskas, S.; Viškelis, P.; Ferrari, G. Application of pulsed electric field in the production of juice and extraction of bioactive compounds from blueberry fruits and their by-products. *J. Food Sci. Technol.* **2015**, 52, 5898–5905. [CrossRef]
- 33. Pataro, G.; Carullo, D.; Ferrari, G. Effect of PEF pre-treatment and extraction temperature on the recovery of carotenoids from tomato wastes. *Chem. Eng. Trans.* **2019**, *75*, 139–144. [CrossRef]
- Hendrawan, Y.; Larasati, R.; Wibisono, Y.; Umam, C.; Sutan, S.M.; Choviya Hawa, L. Extraction of Phenol and Antioxidant Compounds from Kepok Banana Skin with PEF Pre-Treatment. *IOP Conf. Ser. Earth Environ. Sci.* 2019, 305, 012065. [CrossRef]
- Luengo, E.; Alvarez, I.; Raso, J. Improving Carotenoid Extraction from Tomato Waste by Pulsed Electric Fields. Front. Nutr. 2014, 1, 12. [CrossRef]
- Martín-García, B.; Tylewicz, U.; Verardo, V.; Pasini, F.; Gómez-Caravaca, A.M.; Caboni, M.F.; Dalla Rosa, M. Pulsed electric field (PEF) as pre-treatment to improve the phenolic compounds recovery from brewers' spent grains. *Innov. Food Sci. Emerg. Technol.* 2020, 64, 102402. [CrossRef]
- Pataro, G.; Carullo, D.; Bakar Siddique, M.A.; Falcone, M.; Donsì, F.; Ferrari, G. Improved extractability of carotenoids from tomato peels as side benefits of PEF treatment of tomato fruit for more energy-efficient steam-assisted peeling. *J. Food Eng.* 2018, 233, 65–73. [CrossRef]
- 38. Sukardi; Pulungan, M.H.; Purwaningsih, I.; Sita, P.F. Extraction of phenolic compounds from basil (*Ocimum americanum* L.) leaves with pretreatment using pulsed electric field (PEF). *IOP Conf. Ser. Earth Environ. Sci.* **2020**, 475, 012056. [CrossRef]
- 39. Grahl, T.; Märkl, H. Killing of microorganisms by pulsed electric fields. Appl. Microbiol. Biotechnol. 1996, 45, 148–157. [CrossRef]
- 40. Álvarez, I.; Pagán, R.; Condón, S.; Raso, J. The influence of process parameters for the inactivation of *Listeria monocytogenes* by pulsed electric fields. *Int. J. Food Microbiol.* **2003**, *87*, 87–95. [CrossRef] [PubMed]

- 42. Brodelius, P.E.; Funk, C.; Shillito, R.D. Permeabilization of cultivated plant cells by electroporation for release of intracellularly stored secondary products. *Plant Cell Rep.* **1988**, *7*, 186–188. [CrossRef]
- Huijbregts, M.A.J.; Steinmann, Z.J.N.; Elshout, P.M.F.; Stam, G.; Verones, F.; Vieira, M.; Zijp, M.; Hollander, A.; van Zelm, R. ReCiPe2016: A harmonised life cycle impact assessment method at midpoint and endpoint level. *Int. J. Life Cycle Assess.* 2017, 22, 138–147. [CrossRef]
- 44. Barjoveanu, G.; Pătrăuțanu, O.-A.; Teodosiu, C.; Volf, I. Life cycle assessment of polyphenols extraction processes from waste biomass. *Sci. Rep.* **2020**, *10*, 13632. [CrossRef] [PubMed]

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