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An Integrated Approach to Develop Innovative, Sustainable, and Effective Cosmetic Ingredients: The Case Report of Fatty-Acids-Enriched Wild Strawberry Waste Extract

Marta Faggian [1], Silvia Lucchetti [1], Sara Ferrari [2], Gabriele De Nadai [2], Stefano Francescato [2], Giovanni Baratto [2], Nicola De Zordi [3], Silvia-Maria Stanic [1], Gregorio Peron [4], Stefania Sut [5], Alessandra Semenzato [5] and Stefano Dall'Acqua [1,5,*]

[1] Unired srl, Via Niccolò Tommaseo 69, 35131 Padova, Italy; marta.faggian@unired.it (M.F.); silvia.lucchetti@unired.it (S.L.)
[2] Unifarco spa, Via Cal Longa 62, 32035 Belluno, Italy
[3] Società Agricola Moldoi—S.A.M srl, Loc. Maras Moldoi 151/a, 32037 Belluno, Italy; nicola.dezordi@societaagricolamoldoi.it
[4] Department of Molecular and Translational Medicine, University of Brescia, Viale Europa 11, 25123 Brescia, Italy; gregorio.peron@unibs.it
[5] Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Via Marzolo 5, 35131 Padova, Italy
* Correspondence: stefano.dallacqua@unipd.it

Abstract: The sourcing of raw materials with low environmental impact, e.g., "upcycled" ingredients from short supply chains, has currently become necessary, and agri-food waste represents a very attractive hub to produce innovative cosmetic extracts. In this paper, an integrated approach considering all the different steps, starting from material selection, extraction, chemical characterization, biological activity evaluation, and environmental impact calculation, was adopted to obtain innovative, sustainable, and effective cosmetic raw materials from food waste. As case report, a supercritical CO_2 extract obtained from wild-strawberry-processing waste after jam production ($WSWSCO_2$ extract) was developed. The fatty acids profile of the waste material and $WSWSCO_2$ extract was investigated via a GC–MS method, and mainly polyunsaturated fatty acids (PUFAs) such as linoleic and linolenic acids were detected. Furthermore, the ability of the $WSWSCO_2$ extract to inhibit 5α-reductase type 1 expression in skin fibroblasts was assessed, confirming significant efficacy at the dose of 5 mg/mL. Finally, in view of the eco-sustainability approach, the environmental impact related to $WSWSCO_2$ extract was calculated using a life cycle assessment (LCA) analytical approach, considering different parameters and indicators (e.g., carbon footprint) and verifying the eco-friendly approach in extract development and production. Although further research is needed, for example, to check the full composition of the extract and its effect on skin cells, these results suggest that the $WSWSCO_2$ extract may represent an innovative and sustainable ingredient for cosmetic applications especially in topical preparation for the treatment of some androgenic-related discomfort, such as acne and androgenic alopecia, reflecting the potentiality of the holistic and pioneering approach related to ingredient development presented in this study for the cosmetic sector.

Keywords: wild strawberry; upcycling; supercritical carbon dioxide (SCO_2); 5α-reductase; cosmetic ingredient

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1. Introduction

In recent years, sustainability has received growing interest from industries and consumers due to the ongoing global environmental crisis and the recommendation and issues settled by European Green Deal. The cosmetic industry is a growing economic sector that generates significant levels of consumption in natural resources and is often under

strict control for its selection and use of raw materials, for its environmental impact, and for the safety issues of the final products [1,2]. Furthermore, environmental issues are highly considered by consumers, and there is an increasing demand for products presenting high levels of safety for the skin and also being environmentally sustainable [3].

There is a need to design products and processes that minimize their environmental footprint. In this context, cosmetic companies have implemented several strategies to improve their sustainability. At first, implementing the packaging using reusable, recyclable, or biodegradable materials but also taking into consideration the design of products considering a life cycle thinking [1]. The life cycle assessment (LCA) methodology is spreading in cosmetic industries as an evaluation tool to estimate the environmental impact of final products towards a green transition also in terms of raw materials selection and manufacturing steps [4].

Another significant topic related to the ecological transition of cosmetics is the use of alternative "sustainable" raw materials. For this reason, bio-waste is studied and, in some cases, used to obtain new raw materials, and the sourcing of new raw materials from waste presenting reduced environmental impact, such as "upcycled" ingredients from short and circular supply chains, has become necessary [5,6]. Meanwhile, large amounts of food waste originate from the food industry, which ends up in landfills, participating in the global emission of carbon and anthropogenic methane, and contributing to the development of global warming as well as being a cost for the disposal. Furthermore, the recovery of by-products from already existing supply chains reduces the need for the specific cultivations of botanicals for extraction purposes, saving soil and water for food and for other productions [3,7].

Several food wastes have a large potential to be used in the cosmetic industry thanks to their richness in antioxidants, polyphenols, proteins, minerals, vitamins, carotenoids, lignans, polysaccharides, natural-derived polymers, and other active substances. Reducing or eliminating waste from the food industry and developing processes that can turn it into valuable products has become an urgent goal to save natural resources and to reduce methane and carbon dioxide emissions, thus helping to boost a circular economy [8].

In this perspective, our research group started a collaboration with a local company, Rigoni di Asiago (Vicenza, Italy), which is one of the main Italian producers of jams and marmalades and cultivates several organic berries (e.g., raspberry, blackberry, blackcurrant, strawberry) in a large scale, generating a huge quantity of bio-waste (about 80 tons/year) made by seeds with residue of pulp and peel. Since berries seeds are important source of vegetal oils containing valuable fatty acids, sterols, tocopherols, and other lipophilic compounds [9], an eco-friendly process using supercritical fluid extraction based on carbon dioxide (SCO_2) was developed to valorize processing waste from fruits and obtain upcycled oils for cosmetic purposes.

SCO_2 extraction has been identified as favorable and performing technique for the recovery of lipophilic actives from natural sources for nutraceutical, pharmaceutical, and cosmetic applications thanks to its low oxidative and thermal impact [10]. SCO_2 extraction allows for obtaining high-quality oils that often do not require further refining; it preserves the unaltered original properties and excludes contamination by residual liquid solvents [11,12].

In our previous work [10], we developed SCO_2 extracts in laboratory scale, starting from raspberry, blueberry, wild strawberry, pomegranate, blackberry, and blackcurrant waste from Rigoni di Asiago. Certified organic biomasses were selected to guarantee the quality of the material and ensure absence of contaminants admitted in conventional agriculture, such as pesticides. We evaluated the qualitative and quantitative phytochemical composition of the obtained SCO_2 extracts, focusing on fatty acids profile, and the results showed the oil extracted from the waste (peel and seeds) of wild strawberries as the most promising for future industrial applications, due to the high extraction yields and the high amount of polyunsaturated fatty acids (PUFAs) obtained in the preliminary prototypes [10].

Wild strawberry or alpine strawberry (*Fragaria vesca* L.) is a perennial herbaceous plant of the rose family that grows naturally throughout northern Europe and that produces

edible fruits. Wild strawberry fruit is flavored, collected, and grown for domestic use and for the industrial production of jam and liquors [13]. Wild strawberry fruits contain monosaccharides, vitamins (C, B1, B2, K), and organic acids (malic, citric, and salicylic) and are a rich source of macro- and micronutrients (calcium, potassium, phosphorus, iron, magnesium, and manganese) [14]. Seeds are part of the residual biomass of wild strawberry fruits' processing waste and contain about 20% of fatty fraction rich in PUFAs, with a low n-6/n-3 fatty acids ratio, which supports the valuable characteristics of wild strawberry seeds oils [15].

PUFAs are essential macronutrients that are attracting attention as potential agents for maintenance of skin health and treatment of skin disorders, particularly those mediated by solar ultraviolet radiation (UVR), including sunburn, cancer, photosensitivity, and photoaging [16].

Furthermore, long-chain PUFAs exhibit potential in diminishing inflammatory processes, which could be beneficial for the management of inflammatory skin diseases, such as atopic dermatitis, psoriasis, and acne [17]. As a source of major lipid components of the stratum corneum, the obtained wild strawberry waste extract could be used as a valuable moisturizing agent for cosmetics products, especially as an emollient, and to repair the moisture barrier of the skin [17]. Indeed, linoleic acid (C18:2) contributes to the composition of ceramides that are crucial for the structure of epidermal barriers, prevent trans-epidermal water loss, and protect from environmental factors and barrier permeability problems [18]. PUFAs are also metabolized to octadecanoids, eicosanoids, docosanoids, endocannabinoids, and related bioactive lipid species, known to mediate inflammatory and immune reactions in many tissues, including skin [19].

It is also reported in the literature that unsaturated fatty acids can inhibit 5α-reductase, a fundamental enzyme in androgens pathway. After birth, 5α-reductase type 1 is expressed in more tissues, including the liver, the skin, the scalp, and the prostate, while 5α-reductase type 2 is expressed in prostate, seminal vesicles, epididymis, liver, and, to a lesser extent, in scalp and skin [20,21]. The biological function of 5α-reductase is the irreversible reduction in testosterone to more potent dihydrotestosterone (DHT) [22]. An overexpression of 5α-reductase enhances cellular DHT and may cause androgen-dependent disorders, including androgenic alopecia, hair loss, hirsutism, and acne [23,24]. A large number of treatments have been tested for these diseases, including pharmacological 5α-reductase inhibitors like finasteride and dutasteride [25,26]. However, the use of these drugs may imply common adverse effects, such as sexual dysfunction, infertility, mood disorders, gynecomastia, and raised cardiovascular morbidity/risk factors [27,28] that are less acceptable in case of skin or hair disorders compared to more serious pathological conditions. For this reason, the use of topical preparation should be recommended and safer.

In this research, an integrated methodological approach to obtain innovative, sustainable, and effective ingredients for cosmetic application is presented. This approach provides the selection of food waste material, the eco-friendly extraction of the selected waste, and the in-depth analysis of the obtained extract in terms of different aspects such as chemical composition, biological activity, as well as the environmental impact of its production.

A supercritical CO_2 extract was obtained from wild strawberry waste ($WSWSCO_2$ extract) provided by an Italian jam manufacturer and used as case study in this research, with the aim to present this methodological approach. The extract has been produced in industrial scale and was characterized for its fatty acid profile, compared to vegetal biomass employed for extraction. The $WSWSCO_2$ extract was then tested for its ability to inhibit 5α-reductase with positive results, suggesting interesting application as an innovative and sustainable cosmetic ingredient for the treatment of androgenic-related skin and hair disorders. The environmental impact of $WSWSCO_2$ extract production was analyzed using the life cycle assessment (LCA) approach and considering different indicators, such as carbon footprint (kg CO_2 equivalent/kg extract), and a set of parameters related to the distribution of energy, water, and ecological and toxicological information to check the effective sustainability of the production.

2. Materials and Methods

2.1. Plant Material

Wild strawberry waste was provided by Rigoni di Asiago, Foza (VI), Italy (45.89717667191054, 11.628357781638497). The waste was composed of wild strawberry seeds and residues of pulp and peel, obtained after jam-manufacturing processes. Wild strawberry waste was immediately frozen after the production and maintained frozen during transportation, then defrost and dried under vacuum at 38 °C before SCO_2 extraction. In total, 14 kg of wild strawberry waste were processed by SCO_2 extraction.

2.2. SCO_2 Extraction Procedure

The $WSWSCO_2$ extract was produced by Società Agricola Moldoi, Sospirolo, (BL) Italy. (46.127662827254056, 12.058158511033051). Supercritical extraction of wild strawberry waste was performed with a TH22-10 ×2 supercritical CO_2 extraction apparatus (Toption Instrument Co., Ltd., Xi'an, China). Briefly, the plant was equipped with two extraction vessels of 10 L and two separators of 5 L. The carbon dioxide (Siad SpA, Trieste, Italy; 99.99% purity, food grade) was carried with a high-pressure liquid pump (Toption Instrument Co., Ltd.). First, 7 kg of milled wild strawberry waste ($\leq$40 mesh) was weighed into the stainless-steel extraction basket, which was loaded onto the jacketed extraction vessel. The flow rate of the supercritical solvent was set at 1 L/min. The extraction pressure was set to 350 bar, while the extraction temperature was set at 50 °C. The separation procedure was set to 50 bar and 40 °C. The extraction was carried on until the amount of extract collected over 1 h decreased to under 0.1% of the raw material. The extraction pressure and the flow rate were maintained constant using a backpressure regulator. During the supercritical carbon dioxide extraction, water (bound moisture from plant material) was co-extracted, then decanted, and the crude extract was collected and stored. The entire procedure was repeated with the last 7 kg. The crude extracts were collected and weighed, and the yield was calculated as g extract/100 g dry material. The extraction led to a $WSWSCO_2$ extract with ponderal yield of 20%.

2.3. GC–MS Analysis of Wild Strawberry Fatty Acids in Waste and SCO_2 Extract

Prior to GC–MS analysis, the fatty acids in wild strawberry waste and $WSWSCO_2$ extract were converted to the corresponding methyl esters. In total, 3 g of crushed wild strawberry waste and 500 mg of $WSWSCO_2$ extract were added to 2 mL of dichloromethane, 15 mL of methanol, 2 drops of concentrated sulfuric acid, and 30 mg of methyl pentadecanoate (Sigma-Aldrich, Milan, Italy), which was used as internal standard for quantification purpose. Waste and extract samples were heated under reflux for 24 h and 1.5 h, respectively. The sample was then cooled on ice and extracted using 2 mL of diethyl ether. Phase separation between methanol and diethyl ether was achieved by adding 10 mL of water saturated with NaCl. Finally, the diethyl ether layer was recovered and analyzed using GC–MS.

The GC system was an Agilent 7820A equipped with an autosampler and coupled to an Agilent 5977B MS (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation was achieved on an Agilent 88% -(cyanopropyl)aryl-polysiloxane (HP-88) column (100 m, 0.25 mm i.d., 0.2 μm), setting the following oven temperature gradient: 0–5 min, 120 °C, then to 240 °C at 3 °C/min; isocratic for 10 min. Total run time: 55 min. Helium was used as carrier gas, and the column flow was 1 mL/min. Heater inlet was set at 300 °C, and injection volume was 1 μL. MS parameters were the following: MS source temperature: 230 °C; quadrupole temperature: 150 °C. Data were acquired in the mass range 40–650 Da. Fatty acids identification was performed by comparison of the retention times and mass spectra of analytes with reference standards and comparing the data from the NIST database (ver. 2014).

2.4. In Vitro Modulation of the Expression of 5α-Reductase Gene in Skin Fibroblasts

The assay is carried out on primary fibroblasts (HSF: human skin fibroblasts, ATCC code CRL-2522, batch 70005437). Cells are cultured in MEM containing 10% FBS and antibiotics at 37 °C and 5% CO_2. Cells are seeded in 6-well plates and allowed to grow for 24 h. After that, the cultures are treated for 24 h with 10 ng/mL testosterone. After this incubation, fresh medium is added and supplemented with $WSWSCO_2$ extract at sub-toxic concentrations (5 and 0.5 mg/mL). The sample was dissolved in ethanol and then diluted in the culture medium. The concentrations were defined on the basis of a preliminary cytotoxicity assay. Untreated cells were used as negative control, while cells treated with Saw palmetto (*Serenoa repens*) extract (Ph. Eur. Reference Standard) were used as positive control (10 μg/mL).

Every sample has been tested in triplicates. After 48 h of exposure, total RNA was purified from cells and dissolved in 50 μL of sterile purified water, and its concentration was determined via spectrophotometer reading. In total, 200 ng of total RNA were retro-transcribed into cDNA using random primers and the following protocol: 42 °C per 10 min, 37 °C per 2 h, 85 °C per 5 min.

Changes in gene expression profile were analyzed with a RealTime PCR technique, using a TaqMan assay. Ad hoc specific commercially available primers were purchased from Applied Biosystem (Thermo Scientific, Waltham, MA, USA). 5α-reductase type 1 (SRD5A1 gene) primers pair sequence was designed within the exon. The TaqMan probe principle relies on the 5′–3′ exonuclease activity of Taq polymerase to cleave a dual-labeled probe during hybridization to the complementary target sequence and fluorophore-based detection. As in other quantitative PCR methods, the resulting fluorescence signal permits quantitative measurements of the accumulation of the product during the exponential stages of the PCR. Changes in gene expression profile were measured using a comparative C_T method ($\Delta\Delta C_T$ method). The sample data were normalized to the level of expression of actin as a housekeeping gene.

Then, the difference ΔC_T between the C_T value of the target (5α- reductase) and the C_T of the housekeeping gene (actin) was assessed.

$\Delta C_T = C_T$ (target) $- C_T$ (housekeeping)

ΔC_T was calculated for each sample.

The untreated sample was considered as the reference (calibrator) and used for each comparation.

$\Delta\Delta C_T$ was assessed as the difference between the ΔC_T of each sample and the ΔC_T of the untreated sample (calibrator).

$\Delta\Delta C_T = \Delta C_T$ sample $- \Delta C_T$ untreated sample (calibrator)

$\Delta\Delta C_T$ was then used to calculate fold change values:

Fold change = $2^{-\Delta\Delta CT}$.

A fold change $\leq$0.8 together with a p value < 0.05, compared to untreated cells, is an index of gene target modulation. This value is compared to the untreated cells to give a judgment on the sample activity.

2.5. Life Cycle Assessment (LCA) Analysis of $WSWSCO_2$ Extract

A cradle-to-gate LCA analysis of the $WSWSCO_2$ extract was performed according to the PEF methodology (PEF Environmental Footprint 3.1). The analysis focused mostly on the production process of $WSWSCO_2$ extract, from the upcycled raw material to the final product. The data quality of the analysis is guaranteed since all the information needed was collected directly from the companies involved: Rigoni di Asiago provided data for the cultivation/production phase, and Società Agricola Moldoi provided the foreground data (energy and water consumption in the production process). Secondary data about transport (considering distance and way of transport) were taken from the database Ecoinvent v3.10. The elaboration of data was performed through SimaPro v9.6 software. The obtained data were elaborated according to the Product Environmental Footprint Category Rules Guidance (PEFCR v6.3—May 2018), and the environmental

impact of the extract were split into 16 environmental indicators. The most relevant impact category is climate change, as known as global warming potential, which alone contributes 21% to the overall environmental impact for the raw material.

In Table 1 are listed all the environmental impact indicators, their units of measure, and their contribution to the overall impact.

Table 1. Environmental impacts, their units of measure, and their contribution to the overall impact according to Annex A—Product Environmental Footprint Category Rules ((PEFCR) Guidance.

Impact Category	Indicator	Unit	Final Weighting Factors (Scaled to 100)
Climate change	Radiative forcing as Global Warming Potential (GWP100)	kg CO_2 eq	21.06
Ozone depletion	Ozone Depletion Potential (ODP)	kg CFC-11 eq	6.31
Human toxicity, cancer	Comparative Toxic Unit for humans (CTUh)	CTUh	2.13
Human toxicity, non-cancer	Comparative Toxic Unit for humans (CTUh)	CTUh	1.84
Particulate matter	Impact on human health	disease incidence	8.96
Ionizing radiation, human health	Human exposure efficiency relative to U235	kBq U235 eq	5.01
Photochemical ozone formation, health	Tropospheric ozone concentration increase	kg NMVOC eq	4.78
Acidification	Accumulated Exceedance (AE)	mol H+ eq	6.20
Eutrophication, terrestrial	Accumulated Exceedance (AE)	mol N eq	3.71
Eutrophication, freshwater	Fraction of nutrients reaching freshwater end compartment (P)	fresh water: kg P eq	2.80
Eutrophication, marine	Fraction of nutrients reaching marine end compartment (N)	fresh water: kg N eq	2.96
Ecotoxicity, freshwater	Comparative Toxic Unit for ecosystems (CTUe)	CTUe	1.92
Land use	Soil quality index 85	Dimensionless (pt)	7.94
	Biotic production	kg biotic production	
	Erosion resistance	kg soil	
	Mechanical filtration	m^3 water	
	Groundwater replenishment	m^3 groundwater	
Water use	User deprivation potential (deprivation-weighted water consumption)	m^3 world eq	8.51
Resource use, minerals and	Abiotic resource depletion (ADP ultimate reserves)	kg Sb eq	7.55
Resource use, fossils	Abiotic resource depletion—fossil fuels (ADP-fossil)	MJ	8.32

kg CO_2 eq: kilograms of carbon dioxide equivalent; kg CFC-11 eq: kilogram of Trichlorofluoromethane equivalent; CTUh: Comparative Toxic Unit for humans; kBq U235 eq: kilogram Becquerel of Uranium 235 equivalent; kg NMVOC eq: kilogram of non-methane volatile organic compounds equivalent; mol H+ eq: equivalent of mole of H+; mol N eq: equivalent of moles of nitrogen; kg P eq: equivalent of kilograms of phosphorus; kg N eq: equivalent of kilograms of nitrogen; m^3 world eq: equivalent amount of water used; kg Sb eq: equivalent of kilograms of antimony; MJ: Mega Joule; pt: point.

3. Results

3.1. GC–MS Analysis of Wild Strawberry Fatty Acids in Waste and SCO_2 Extract

The fatty acids profiles of wild strawberry waste and $WSWSCO_2$ extract are reported in Table 2. The fatty acids amount in supercritical CO_2 extract is almost 50 times higher compared to waste, as expected, confirming the efficacy of the extraction approach. Both in waste and in supercritical CO_2 extract, unsaturated fatty acids (UFAs) are prevalent compared to saturated fatty acids (SFAs), with linoleic (C18:2) and linolenic acid (C18:3) being the most representative compounds, followed by oleic acid (C18:1). Palmitic acid (C16:0) and stearic acid (C18:0) are major saturated fatty acids. In addition, SFA:UFA ratio is 0.11 and 0.16 in waste and supercritical CO_2 extract, respectively.

Table 2. Fatty acids profiles of wild strawberry (WS) waste and wild strawberry supercritical CO_2 ($WSWSCO_2$) extract. Both full names and abbreviations of each identified compound are reported in this Table. ND: not detected.

R.T (min)	Abbreviation	Fatty Acid Identification	WS Waste mg/g	$WSWSCO_2$ Extract mg/g
14.51	C12:0	Lauric acid	ND	0.63 ± 0.03
19.18	C14:0	Myristic acid	ND	0.61 ± 0.08
23.88	C16:0	Palmitic acid	0.88 ± 0.04	23.71 ± 0.85
25.05	C16:1	Palmitoleic acid	ND	0.84 ± 0.04
26.15	C17:0	Heptadecanoic acid	ND	0.82 ± 0.03
28.35	C18:0	Stearic acid	0.51 ± 0.03	27.63 ± 0.96
29.42	C18:1 cis (n9)	Oleic acid	1.80	105.10 ± 1.14
29.55	C18:1 trans (n9)	Elaidic acid	ND	4.11 ± 0.31
31.15	C18:2 cis (n6)	Linoleic acid	4.63 ± 0.31	242.32 ± 1.76
32.48	C20:0	Arachidic acid	ND	17.54 ± 0.79
33.10	C18:3 (n6)	Linolenic acid	6.31 ± 0.45	331.30 ± 1.44
33.35	C20:1 (n9)	cis-11 Eicosenoic acid	ND	4.05 ± 0.24
35.01	C20:2 (n6)	cis-11,14 Eicosadienoic acid	ND	1.27 ± 0.11
36.25	C22:0	Behenic acid	ND	5.81 ± 0.03
39.82	C24:0	Lignoceric acid	ND	30.72 ± 0.64
		Total	14.13	796.45
		Total saturated (SFA)	1.39	108.31
		Total unsaturated (UFA)	12.74	688.14
		Total n6	10.94	574.89
		Total n9	1.80	113.26
		SFA:UFA	0.11	0.16

An exemplificative chromatogram of fatty acids profile of wild strawberry waste is reported in Figure 1.

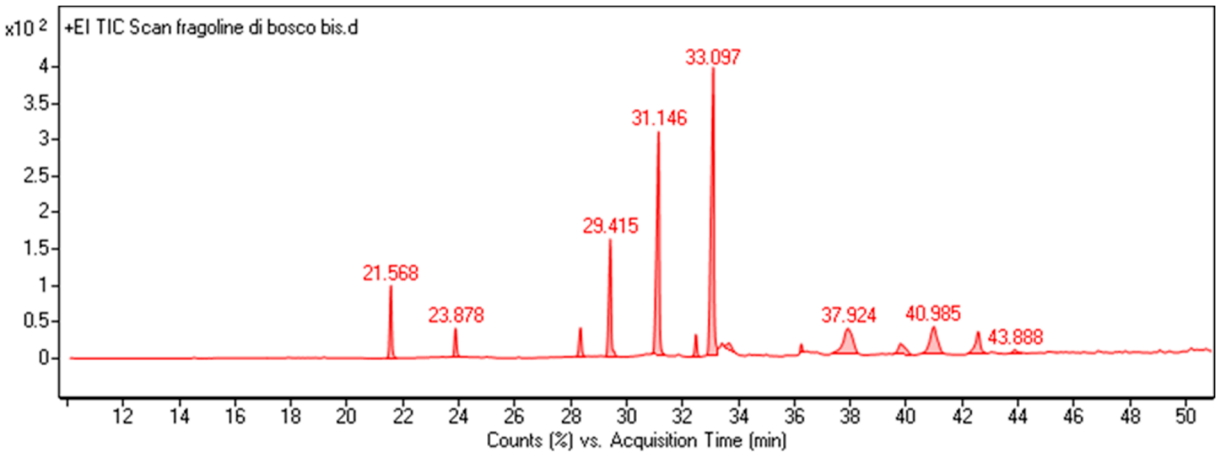

Figure 1. GC–MS chromatogram of wild strawberry seeds fatty acids.

3.2. In Vitro Modulation of the Expression of 5α-Reductase Gene in Skin Fibroblasts

The results of the in vitro assay are reported in Figure 2. The expression of gene in the untreated control cells (negative control—CN) has been arbitrary setted to 1 and corresponds to the full expression of the enzyme, without any interference. Cells were then treated with $WSWSCO_2$ extract (0.5 and 5 mg/mL) and the positive control *Serenoa repens* (saw palmetto) extract (10 µg/mL). The assay confirms that $WSWSCO_2$ extract can significantly inhibit 5α-reductase type I (SRD5A1) gene expression levels after 48 h of treatment at the concentration of 5 mg/mL. At the lowest concentration (0.5 mg/mL), the inhibition is not statistically significant, compared to CN. Compared to wild strawberry and considering the concentration tested, the positive control (CP) *Serenoa repens* extract is almost 500 times more effective than $WSWSCO_2$.

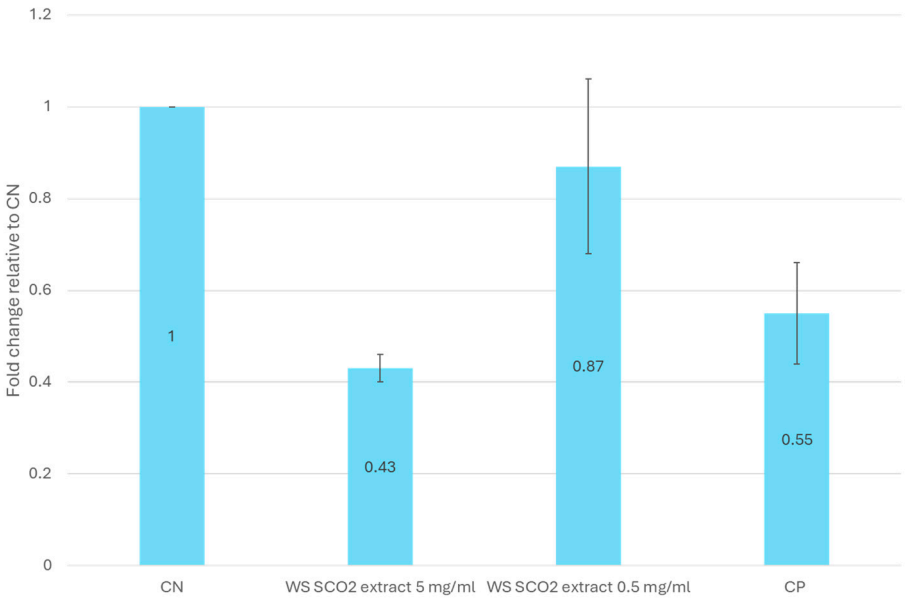

Figure 2. Inhibition of 5α-reductase gene expression in skin fibroblasts. $WSWSCO_2$ (wild strawberry waste supercritical CO_2 extract), CN (negative control—not-treated cells), CP (positive control—saw palmetto extract).

3.3. Life Cycle Assessment (LCA) Analysis of $WSWSCO_2$ Extract

The results of the LCA analysis related to the production of $WSWSCO_2$ extract from the frozen biomass waste provided by Rigoni di Asiago to the final extract is reported in Table 3. To compare all the environmental indicators with the same unit of measurement,

a conversion was applied according to the Annex B.1 of the PEFCR guidance v6.3. Every indicator refers to 1 kg of final product/extract.

Table 3. LCA analysis of $WSWCO_2$ extract, considering conversion to mPt value, according to Annex B.1 of the "Product Environmental Footprint Category 2 Rules (PEFCR) Guidance v6.3.

Indicator	Unit	Value
Global Warming Potential 100a	mPt	1.13
Ozone depletion	mPt	1.27×10^{-1}
Ionizing radiation	mPt	7.74×10^{-2}
Photochemical ozone formation	mPt	1.17×10^{-2}
Particulate matter	mPt	2.32×10^{-1}
Human toxicity, non-cancer	mPt	4.99×10^{-2}
Human toxicity, cancer	mPt	1.74×10^{-2}
Acidification	mPt	1.16×10^{-1}
Eutrophication, freshwater	mPt	1.09×10^{-2}
Eutrophication, marine	mPt	4.47×10^{-2}
Eutrophication, terrestrial	mPt	2.88×10^{-3}
Ecotoxicity, freshwater	mPt	2.30×10^{-1}
Land use	mPt	5.12×10^{-5}
Water use	mPt	1.11×10^{-1}
Resource use, fossils	mPt	4.05×10^{-2}
Resource use, minerals and metals	mPt	4.99×10^{-4}

mPt: milliPoint.

The environmental profile of the $WSWSCO_2$ extract shows low values of land use, water use, eutrophication, and ecotoxicity.

The total value of Global Warming Potential for $WSWSCO_2$ extract is 2.8 kg CO_2 eq/kg product. This value can be divided into 3 different useful insights: the value of raw material production is 0 kg CO_2 eq., the value of the raw material transportation from Rigoni di Asiago to Società Agricola Moldoi is 0.026 kg CO_2 eq., and the value of the supercritical CO_2 extraction is 2.75 kg CO_2 eq.

4. Discussion

With the aim to recover actives for cosmetic applications, our research group has been focusing in recent years on selection of different food waste materials, which have been processed, characterized, and studied in terms of chemical composition and efficacy, to create innovative and sustainable ingredients in respect of circular and local economy. The projects on beeswax by-product and saffron petals are briefly summarized here: Beeswax by-product (BBR) is a waste derived from honey production and is composed by honey, resins, and other constituents. Our analysis revealed the presence of carbohydrates, hydrocarbons, and minerals, as well as a polyphenolic composition similar to propolis and significant antibacterial activity towards Klebsiella pneumoniae, Salmonella enterica, Enterococcus faecalis, methicillin-resistant Staphylococcus aureus, and Pseudomonas aeruginosa [29]. Saffron petals are considered a waste of saffron (*Crocus sativus* L.) cultivation. From this material, an innovative and green extraction technique was performed, employing mixtures of polyols, lactic acid, and betaine as solvents, to develop saffron petal extracts enriched in active compounds such as quercetin and kaempferol derivatives, with demonstrated anti-tyrosinase activity for cosmetic applications [30]. Besides food waste for the cosmetic sector

in particular, our research group has also been working on green extraction approaches to many other food and bio-waste materials, such as larch [31] and picea bark [32].

In the current research, a concept was further developed compared to previous studies: the aim in this case was not only to valorize a food waste but also to establish an integrated pathway comprising different steps (material selection, green extraction, chemical characterization, biological activity, and environmental impact calculation) for the development of an up-cycled active cosmetic ingredient and propose it as innovative model of study for the cosmetic sector, to be virtually applied also to many other wastes and to combine quality and efficacy in view of sustainability.

In this pilot study, the strawberry waste was selected as a starting material for this purpose. Considering that strawberry waste is mostly composed of seeds, a rich source of polyunsaturated fatty acids [33], supercritical CO_2 was chosen as lipophilic green extraction technique to obtain a wild strawberry waste ($WSWSCO_2$) extract in a liquid oily form.

The $WSWSCO_2$ extract developed in this study was investigated, considering the fatty acids composition, its ability to inhibit the activity of 5α-reductase enzyme, and the environmental impact of its production.

Regarding the fatty acids profile, unsaturated fatty acids are prevalent both in $WSWSCO_2$ and the vegetal biomass. These results confirm literature data on wild strawberry seeds fatty acids, which reported unsaturated fatty acids oleic, linoleic, and linolenic acids are the most abundant compared to saturated stearic acid [34]. Grajzer et al. [15] evaluated the fatty acids composition of wild strawberry oils obtained by cold pressing and supercritical CO_2 fractions collected at different timepoints of extraction. The lowest amount of polyunsaturated linolenic acid (30%) was found in oil obtained by cold pressing, while in supercritical CO_2 fractions, higher values were detected, up to 43%. Furthermore, monounsaturated oleic acid was higher in oil obtained by cold pressing, while in CO_2 fractions was detected the lowest amount (12%), suggesting an influence in fatty acid composition depending on the extraction method and a preservation of polyunsaturated compounds with supercritical CO_2 extraction technique.

Moreover, our analysis confirms the preservation of fatty acids profiles and relative abundance both in waste and extract, suggesting that supercritical CO_2 extraction could be a valuable approach to recover and maintain the native fatty acid composition from plants.

As the next step, the in vitro modulation of the expression of 5α-reductase gene in skin fibroblasts was assessed for $WSWSCO_2$ compared to saw palmetto extract as the positive control. $WSWSCO_2$ extract at the concentration of 5 mg/mL is able to inhibit the expression levels of 5α-reductase type 1, being 500 times less active than saw palmetto. This is somewhat expected because many studies report the efficacy of saw palmetto in 5α-reductase inhibition, and this ingredient is commonly found in food supplements as well as pharmaceutical preparations for the treatment of benign prostatic hypertrophy and prostate cancer and, for this reason, was chosen as the positive control in our study [35–38]. Other mechanisms of actions related to saw palmetto have also been proposed, involving anti-androgenic, anti-proliferative, and anti-inflammatory effects [39]. Saw palmetto preparations are available in many dosage forms, including hard capsules containing dried extract and soft gel containing oily extract, typically standardized in fatty acids and phytosterols [40]. Among these fatty acids, the major constituents are lauric, oleic, myristic, and linoleic acids that are reported to be effective inhibitors of 5α-reductase isozymes (type 1 and 2) and involved in saw palmetto mechanisms of action [41].

Abe et al. studied the binding activities of major fatty acids (lauric, oleic, palmitic, myristic, and linoleic) contained in saw palmetto extract for benign prostatic hypertrophy—pharmacologically relevant (α1-adrenergic, muscarinic, 1,4-DHP calcium channel antagonist) receptors. In addition, the effects of saw palmetto and its fatty acids on 5α-reductase activity in rat liver were also examined. Considering the results, linoleic acid presented the greatest receptor-binding activity and inhibitory effect on 5α-reductase compared to other free fatty acids contained in saw palmetto [42]. Inhibition of 5α-reductase activity was reported also for linolenic acid, and the presence of unsaturated bonds was demonstrated to

enhance the inhibitory activity [43]. This may suggest an important role of polyunsaturated fatty acids for pharmacodynamic mechanisms of inhibition of 5α-reductase related to the saw palmetto phytocomplex. Compared to saw palmetto, the $WSWSCO_2$ extract developed in this study contains a major amount of unsaturated fatty acids, and, for this reason, the inhibitory activity against 5α-reductase was investigated. However, saw palmetto was more effective compared to wild strawberry, suggesting a positive impact of entire fatty acids profile, the amount and the contribution of each fatty acid, and the entire lipidic phytocomplex in the biological activity.

Anyway, the $WSWSCO_2$ extract at the concentration of 5 mg/mL is able to inhibit the expression levels of 5α-reductase type 1 and can be included in topical cosmetic preparation for the treatment of skin and hair disorders related to androgen disfunctions, considering the active concentration (5 mg/mL) as preliminary applicative indication for the inclusion of the extract in final cosmetic formulations. Further research is obviously needed to confirm the stability of the extract in the final formulation and the compatibility with other ingredients as well as other more reproducible evaluations of its effect on skin.

In the final step, the environmental impact of the $WSWSCO_2$ extract was calculated. The life cycle assessment (LCA) study is the most used ISO-standardized methodology, providing several insights and permitting the evaluation of environmental impacts of products and services. This kind of analysis considers the consumption of materials, the energy used (quantity and quality), and the emissions to the environment involved in the production chain of the object. There are two types of this analysis; one is called "from cradle to grave", and it considers all the phases, either from the cultivation of vegetable raw materials or from the synthesis of synthetic raw materials to the final product used by consumers. The second one is called "from cradle to gate", and it considers only a few parts of the process, from the cultivation/synthesis of the raw material to the production of the final material. In this study, the second approach was used to calculate the environmental impact of the supercritical CO_2 extraction of wild strawberry [44]. The $WSWSCO_2$ extract is characterized by low values of specific parameters (land use, water use, eutrophication, and ecotoxicity) related to the origin of the starting material that derived from the upcycled wastes of the food transformation industry. This means that the total impact of strawberry fruit production is allocated to the manufacture of strawberry jam made by Rigoni di Asiago and does not impact extract production.

The Global Warming Potential of the extract presents low values related to impact of raw material cultivation and transport, as explained above. The main contribution of Global Warming Potential is mostly associated with the energy employed by supercritical CO_2 extraction, which alone represents the 99% of the impact. However, it should be considered that supercritical extraction approach is less toxic compared to conventional extraction with lyophilic organic solvents [12] and is performs better in terms of yield for the same starting material (wild strawberry) compared to cold pressing, as reported by Grajzer et al [15]. For this reason, supercritical CO_2 extraction is confirmed as an effective and green extraction technique for the recovery of fatty acids from wild strawberry waste.

5. Conclusions

Our research demonstrates that agri-food waste could be an important source for new active cosmetic ingredients. $WSWSCO_2$ extract is characterized by a high amount of PUFAs and significantly reduces the expression of mRNA codifying for the 5α-reductase type 1 enzyme implicated in various skin disorders, such as *acne vulgaris*, seborrhoea, and alopecia. The $WSWSCO_2$ extract represents an innovative upcycled ingredient, with low environmental impact and interesting possibilities of application in skin- and hair-care products.

In conclusion, this study opens the way for more in-depth research on the use of agri-food waste as valuable sources to obtain innovative and sustainable raw materials for cosmetic applications. In particular, the integrated approach related to new ingredient development presented in this study considers different important aspects to assess not only the quality and efficacy of the obtained waste-derived raw material but also allows for

an objective evaluation of its sustainability, representing an interesting model of work for raw material producers in cosmetic field. It will be interesting and stimulating to follow the progress of this approach, also considering other types of green extractions to recover a wide profile of bio-actives from wastes and test the efficacy on various cell lines, with sustainability as key point.

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