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Co-Fermentation of Dandelion Leaves (*Taraxaci folium*) as a Strategy for Increasing the Antioxidant Activity of Fermented Cosmetic Raw Materials—Current Progress and Prospects

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Abstract

In response to the growing interest in natural cosmetic raw materials with antioxidant and moisturising properties, this study focuses on the use of dandelion leaves (*Taraxaci folium*) in the co-fermentation process involving selected strains of *Saccharomyces cerevisiae* and *Lactobacillus rhamnosus* MI-0272. The aim of the study was to develop an innovative method of co-fermentation of dandelion leaves using waste beet molasses and organic cane biomolasses as substrates to produce lactic acid (LA), which is the main component of fermented cosmetic raw materials (FCRMs). The scope of the research included the determination of antioxidant activity using the DPPH (AA-DPPH) and ORAC (AA-ORAC) methods, determination of total polyphenol content (TPC) using the Folin–Ciocalteu method, assessment of lipophilicity by measuring the log P partition coefficient, assessment of wettability (contact angle), and statistical analysis. The key results indicated that the developed method allows for up to a fivefold reduction in fermentation time, enabling the production of FCRMs with the highest antioxidant activity (AA-DPPH = 3.0 ± 0.1 mmol Tx/L (Trolox equivalents per litre); AA-ORAC = 0.55 ± 0.02 mmol Tx/L) and the highest polyphenol content (TPC = 3589 ± 25 mg gallic acid equivalents per litre (GA/L)), with LA content (determined by GC-MS) up to 37 g/L. In addition, the analysis of the relationship between lipophilicity and membrane wettability showed that the hydrophilic antioxidants contained in FCRMs (log P = −0.9) can accumulate in the aqueous layers of the epidermis, suggesting their potential local protective and antioxidant effects. The results obtained confirm the potential of the developed technology in the production of modern cosmetic raw materials with antioxidant properties. Further research should include qualitative and quantitative analysis of phenolic acids contained in FCRMs and evaluation of the effectiveness of cosmetic preparations containing FCRMs in vivo.

Keywords: antioxidant bioferments; cosmetic fermentation; *Saccharomyces cerevisiae*; *Lactobacillus rhamnosus* MI-0272; DPPH assay; ORAC assay; Folin–Ciocalteu assays; hydrophilic antioxidants; lactic acid

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1. Introduction

Dandelion (*Taraxacum officinale*), a representative species of the *Taraxacum* genus, is a perennial herbaceous plant widely distributed in the temperate zones of Europe, Asia,

and North America. It is characterised by a rosette of basal leaves, hollow stems containing milky sap, and distinctive yellow inflorescences composed exclusively of ligulate flowers. This plant is commonly found in meadows, pastures, and roadsides, showing high resistance to environmental conditions and the ability to spread rapidly [1,2]. From a taxonomic perspective, *T. officinale* belongs to the order Asterales and is one of the best-known species within the genus *Taraxacum* [3]. Folk medicine, including European phytotherapy and Chinese medicine, has used dandelion for centuries due to its ease of cultivation [4]. Traditionally, dandelion has been used as a diuretic, cholagogue, anti-inflammatory, and detoxifying agent [5]. Various parts of this plant, including the roots, leaves, and flowers, were used to support liver function, stimulate digestion, and treat skin conditions. Today, this raw material is valued for its antioxidant, antibacterial, hepatoprotective, and antirheumatic properties, resulting from its rich phytochemical composition [6,7]. Dandelion is distinguished by its diverse and rich phytochemical profile, making it a valuable source of bioactive compounds for pharmaceutical and cosmetic applications [6,8,9]. This popular medicinal and edible plant contains flavonoids (composed of two benzene rings connected by a three-carbon bridge and containing conjugated double bonds and hydroxyl groups) and their glycosidic forms linked to sugar residues (so-called glycosides), as well as phenolic acids (including ferulic acid, caffeic acid, *p*-hydroxyphenylacetic acid, dicaffeoylquinic acid, hydroxycinnamic acid, chicoric acid, and chlorogenic acid), followed by tannins—polyphenolic compounds subdivided into hydrolysable tannins (esters of ellagic and gallic acid) and condensed tannins (polymeric proanthocyanidins). Additionally, dandelion contains amino acids (involved in key metabolic pathways and exhibiting neuroprotective effects), coumarins, lignans, phytosterols, terpenes, glycoproteins, oligosaccharides, and alkaloids [6,10–12]. Dandelion extracts are increasingly used as active ingredients in cosmetic formulations, dietary supplements, and functional foods. Examples of products available on the market include herbal teas, cleansing tonics, anti-ageing creams, liver support preparations, and detox capsules, promoted as natural remedies with multiple biological effects [7,13,14]. Particular attention was paid to the antioxidant activity of flavonoids (naringenin, delphinidin, quercetin, apigenin, and luteolin) and their glycoside forms (prunin, myrtillin, quercetin-3-rhamnoside, apigenin-7-glucoside, and luteolin-7-glucoside) derived from dandelion. These compounds can act as electron donors, effectively stabilising free radicals and reactive oxygen species (ROS), exhibiting significant antioxidant activity. However, the poor bioavailability of flavonoid compounds with antioxidant potential found in dandelion may limit their penetration through the skin and reduce protection against auto-oxidation and photodamage to deeper cells when applied to the skin. On the other hand, in the case of the glycosidic forms of flavonoids, which are characterised by a more complex structure and higher molecular weight than aglycones, their bioavailability may be even lower [15–18]. To increase the bioavailability of flavonoids and their glycoside forms, dandelions are fermented, resulting in fermented dandelion extracts (bioferments) used in the food industry as functional foods and food additives, as well as in the cosmetics industry as cosmetic raw materials for creating new cosmetic formulations. The growing popularity of dandelion bioferments is attributed to their broad spectrum of activity, in particular, increased antioxidant activity associated with the formation of phenolic compounds during fermentation, biocompatibility, and the absence of cytotoxic effects on skin fibroblasts. The production of fermented extracts involves not only the extraction of active ingredients contained in this plant raw material (as is the case with extracts obtained by ultrasound-assisted extraction methods) but also the enzymatic conversion of macromolecular components with a high molecular weight (which are difficult to penetrate the skin) into more bioavailable small-molecule products (easier to penetrate the skin) [15,18–21].

Recently, dandelion fermentation processes involving lactic acid bacteria (LAB) have become one of the key biotechnological methods used in the processing of plant raw materials to obtain fermented cosmetic raw materials (FCRMs), which are used in both the food and cosmetics industries [22–27]. Fermentation carried out with the participation of single LAB strains, such as *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, or *Pediococcus acidilactici*, initiates a series of metabolic changes, including the hydrolysis of flavonoid glycosides and the release of free phenolic acids with a lower molecular weight [28]. These changes result in an increase in antioxidant activity (AA) and total phenolic content (TPC) in fermented plant extracts [29]. The optimal parameters for the fermentation process include an incubation temperature of 30–37.5 °C, a fermentation time of 24 h to 21 days, an initial pH of the fermentation mixture in the range of 5.0–6.5, an inoculum concentration of 1–5 log colony forming units per millilitre (CFU)/mL, and a plant material content in the range of 5–10 weight percent (wt%). In addition, the presence of simple sugars in the fermentation mixture (such as glucose or sucrose) is an important raw material for LAB bacteria, enabling the production of lactic acid (LA), which is the main metabolite of lactic acid fermentation [30,31]. LA, which forms naturally during the fermentation of plant materials, plays an important role not only as a pH regulator and preservative but also as an active cosmetic ingredient. In cosmetology, it has moisturising, keratolytic, and skin-lightening properties, making it widely used in cosmetic products for skin care (toners, chemical peels, anti-ageing and moisturising creams, and exfoliating preparations) [22,32,33]. The presence of LA in FCRMs further increases their functional value, supporting antioxidant activity and improving the application properties of cosmetic formulations. Furthermore, as a natural ingredient that exhibits synergistic effects with other fermentation metabolites (such as phenolic acids and bioactive peptides), LA contributes to the increased biological activity of FCRMs, which are an alternative to conventional active ingredients in new-generation cosmetics [34–37]. The addition of lipase after fermentation was intended to hydrolyse the cell walls of LAB, thereby limiting their further growth and metabolic activity and preventing undesirable changes in the chemical profile of the preparation [38]. Lipase promotes the breakdown of lipid structures present in microorganism cells, which can lead to the release of additional bioactive compounds. This improves the quality and functionality of the final product [39].

Despite the numerous benefits of subjecting plant raw materials and unfermented extracts to fermentation to obtain FCRMs, characterised by increased bioavailability of active compounds, higher antioxidant activity and increased content of phenolic compounds, this process is associated with significant technological limitations [40,41]. The most important ones include prolonged fermentation time (lasting up to 21 days to obtain optimal biological parameters of FCRMs), difficulties in scaling the process from laboratory to industrial level, variability in the activity of LAB strains depending on the type of plant material, and the need for precise selection of fermentation parameters, such as plant material concentration, initial simple sugar content, inoculum quantity, and process duration [20,28,34]. These factors have a significant impact on the efficiency of fermentation and the properties of the final fermented products, which require the process to be designed depending on the application of the FCRMs.

The use of co-fermentation of dandelion leaves with the participation of *Lactobacillus rhamnosus* MI-0272 and *Saccharomyces cerevisiae* strains, using waste beet molasses and organic cane biomolasses as substrates, allows for the effective production of FCRMs with high antioxidant activity, LA and polyphenol content, while reducing fermentation time. The aim of this study was to develop an innovative method of co-fermentation of dandelion leaves as an alternative to classic fermentation with single LAB strains. Co-fermentation utilises the synergistic action of bacteria (releasing phenolic compounds

in glycoside forms) and yeast (creating an environment conducive to bacterial growth and increasing their enzymatic activity), which makes it possible to obtain a cosmetic raw material with the desired biological properties [42–44]. This process enables the biotransformation of bioactive compounds into more bioavailable forms by releasing them from glycoside complexes, increasing their water solubility, and improving their chemical stability. The increased bioavailability of the active substances contained in FCRMs results from the fact that during fermentation, the glycoside forms of flavonoids (with a complex structure and a higher molecular weight) undergo hydrolysis to free flavonoids, which are then metabolised to more bioavailable phenolic acids (with a low molecular weight). For comparison purposes, fermentation of dandelion flowers (*Taraxaci flos*) and roots (*Taraxaci radix*) was also carried out based on the standard methodology described in our previous studies [10,15,16]. *Taraxaci flos*, in the context of cosmetic and pharmaceutical applications, due to their phytochemical composition (which determines their antioxidant and antimicrobial properties and protective effect on the skin), are often considered more valuable parts of the dandelion than the flowers (which contain carotenoids and flavonoids, but in much smaller quantities than the leaves) and roots (rich in inulin and sesquiterpenes, but with lower antioxidant potential and bioavailability after fermentation) [18,45].

2. Materials, Laboratory Equipment, and Methods

2.1. Materials and Laboratory Equipment

Dandelion leaves, roots, and flowers were procured from EkoBLIK (Karczew, Poland), beetroot molasses was sourced from "Cukrownia Kluczewo" Stargard (Poland), and BIO cane molasses (NatVita) was acquired from Mirków (Poland). The total content of 6-carbon sugars (Brix) in molasses was determined using the refractometric method.

Probiotical (Novara, Italy) supplied the LAB strains (*L. rhamnosus* MI-0272, *L. reuteri* MI_0168, *L. salivarius* LY_0652, *L. brevis* LY_1120, *L. acidophilus* MI-0078, *L. plantarum* MI-0102, and *L. rhamnosus* Y-0457), while *S. cerevisiae* was acquired from Pol-Aura (Różnowo, Poland). OXOID (M.R.S. BROTH, Rogosa, Sharpe, Basingstoke, UK) provided a LAB medium (CM0359).

DPPH (2,2-diphenyl-1-picrylhydrazyl) was procured from Sigma Aldrich (St. Louis, MO, USA). Merck (Darmstadt, Germany) supplied the Folin–Ciocalteu reagent, iron (II) sulphate heptahydrate, iron (II) sulphate, and gallic acid. Chempur (Piekary Śląskie, Poland) supplied n-octanol, octane, dipotassium hydrogen phosphate, sodium phosphate dibasic dihydrate, barium hydroxide, magnesium (II) sulphate heptahydrate, orthophosphoric acid, potassium hydroxide, sodium hydroxide, iron (III) chloride hexahydrate, ammonium chloride, calcium chloride dihydrate, potassium dihydrogen phosphate, methanol, and 96% ethanol. Thermo Scientific (Białystok, Poland) supplied Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Tx 97%), AAPH (2,2′-azobis(2-methylpropionamidine) dichlorohydrate, 98%), and Lipase AY30, while fluorescein sodium salt (purity) was acquired from Angene (Białystok, Poland).

The following laboratory equipment was used in the study: KRUSS Optronic DR301-95 digital refractometer (A. Krüss Optronic GmbH, Hamburg, Germany); GC-MS-QP2020 Nexis gas chromatograph coupled with a mass spectrometer (Shimadzu Corporation, San Jose, CA, USA), equipped with an autosampler; FSF-031S ultrasonic bath (Chemland, Stargard, Poland) with a frequency of 40 kHz and temperature control; GENESYS™ UV-Vis spectrophotometer (Thermo Scientific, Thermo Fisher Scientific, Waltham, MA, USA); Eppendorf 5810R laboratory centrifuge (Eppendorf AG, Hamburg, Germany); TECAN Infinite® F50 microplate reader (Tecan Group Ltd., Männedorf, Switzerland); and a laboratory grinder (MUL-TISERW-Morek, Brzeźnica, Poland).

2.2. Preparing Fermented Cosmetic Raw Materials (FCRMs) from Dandelion Leaves, Roots, and Flowers

The preparation of FCRMs (FCRM-1:FCRM-20) from dandelion leaves, roots, and flowers followed a common four-stage protocol, with composition and process parameters specific to each raw material given in Table 1.

Table 1. The conditions for the fermentation and co-fermentation of dandelions to obtain FCRM-1:FCRM-20.

Name FCRM	Plant Part	Molasses Type and Content [g (wt%)]	Water [g (wt%)]	Plant Material [g (wt%)]	LAB Strain [g (wt%)]	Yeast Strain [g (wt%)]	LAB/Yeast Ratio	Mineral Salts [g (wt%)]	Lipase [g (wt%)]	Fermentation Temp [°C]	Time [Days]
[1] FCRM-1	leaf	Beet and cane 2.5/2.5 (2.8/2.8)	75 (83)	0.05 (0.06)	9 (10)	1 (1.1)	9:1	-	0.001 (0.01)	29.5–31.5	7
[1] FCRM-2	leaf	Beet and cane 2.5/2.5 (2.7/2.7)	75 (82)	2 (2.2)	9 (10)	1 (1.1)	9:1	-	0.001 (0.01)	29.5–31.5	7
[1] FCRM-3	leaf	Beet and cane 2.5/2.5 (3.1/3.1)	75 (92)	0.25 (0.31)	0.9 (0.9)	0.1 (0.1)	9:1	-	0.001 (0.01)	29.5–31.5	3
[1] FCRM-4	leaf	Beet and cane 2.5/2.5 (2.4/2.4)	75 (71)	0.25 (0.24)	22.5 (21)	2.5 (2.4)	9:1	-	0.001 (0.01)	29.5–31.5	5
[1] FCRM-5	leaf	Beet and cane 0.25/0.25 (0.2/0.2)	75 (74)	0.25 (0.25)	22.5 (22)	2.5 (2.5)	9:1	-	0.001 (0.01)	29.5–31.5	4
[1] FCRM-6	leaf	Beet and cane 7.5/7.5 (6.5/6.5)	75 (65)	0.25 (0.22)	22.5 (20)	2.5 (2.2)	9:1	-	0.001 (0.01)	29.5–31.5	6
[2] FCRM-7	roots	Cane 6 (12)	30 (60)	0.1 (0.2)	10 (20)	-	-	2/1/1 (4/2/2)	0.07 (0.14)	37.5 ± 0.5	10
[3] FCRM-8	roots	Cane 6 (12)	30 (60)	0.2 (0.4)	10 (20)	-	-	2/1/1 (4/2/2)	0.07 (0.14)	37.5 ± 0.5	10
[4] FCRM-9	roots	Cane 6 (12)	30 (60)	0.3 (0.6)	10 (20)	-	-	2/1/1 (4/2/2)	0.07 (0.14)	37.5 ± 0.5	10
[5] FCRM-10	roots	Cane 6 (12)	30 (60)	0.4 (0.8)	10 (20)	-	-	2/1/1 (4/2/2)	0.07 (0.14)	37.5 ± 0.5	10
[6] FCRM-11	roots	Cane 6 (12)	30 (60)	0.5 (1)	10 (20)	-	-	2/1/1 (4/2/2)	0.07 (0.14)	37.5 ± 0.5	10
[7] FCRM-12	roots	Cane 6 (12)	30 (60)	1 (1.9)	10 (20)	-	-	2/1/1 (4/2/2)	0.07 (0.14)	37.5 ± 0.5	10
[8] FCRM-13	roots	Cane 6 (12)	30 (60)	1.5 (2.9)	10 (20)	-	-	2/1/1 (4/2/2)	0.07 (0.14)	37.5 ± 0.5	10
[3] FCRM-14	flowers	Cane 18 (5.42)	300 (90)	12 (3.6)	10 (3)	-	-	2/1/1 (0.6/0.3/0.3)	0.02 (0.006)	37.5 ± 0.5	15
[2] FCRM-15	flowers	Cane 18 (5.42)	300 (90)	12 (3.6)	10 (3)	-	-	2/1/1 (4/2/2)	0.02 (0.006)	37.5 ± 0.5	14
[5] FCRM-16	flowers	Cane 18 (5.42)	300 (90)	12 (3.6)	10 (3)	-	-	2/1/1 (4/2/2)	0.02 (0.006)	37.5 ± 0.5	10
[4] FCRM-17	flowers	Cane 18 (5.42)	300 (90)	12 (3.6)	10 (3)	-	-	2/1/1 (4/2/2)	0.02 (0.006)	37.5 ± 0.5	14
[6] FCRM-18	flowers	Cane 18 (5.42)	300 (90)	12 (3.6)	10 (3)	-	-	2/1/1 (4/2/2)	0.02 (0.006)	37.5 ± 0.5	15
[7] FCRM-19	flowers	Cane 18 (5.42)	300 (90)	12 (3.6)	10 (3)	-	-	2/1/1 (4/2/2)	0.02 (0.006)	37.5 ± 0.5	15
[8] FCRM-20	flowers	Cane 18 (5.42)	300 (90)	12 (3.6)	10 (3)	-	-	2/1/1 (4/2/2)	0.02 (0.006)	37.5 ± 0.5	11

[1] *Lactobacillus rhamnosus MI-0272* and *Saccharomyces cerevisiae*; [2] *Lactobacillus reuteri*; [3] *Lactobacillus salivarius*; [4] *Lactobacillus brevis*; [5] *Lactobacillus acidophilus*; [6] *Lactobacillus plantarum*; [7] *Lactobacillus rhamnosus MI-0272*; [8] *Lactobacillus rhamnosus LY-0457*.

2.2.1. Raw Material Preparation

In the first stage, i.e., before the extraction process, dried and chopped dandelion leaves were ground in a laboratory grinder and sieved through laboratory sieves with a diameter of 0.20 to 0.50 mm, with the fraction of raw material collected in a receiver with a diameter of <0.20 mm being used for extraction (which ensures the most efficient use of the raw material and provides a larger surface area for contact with the microorganisms used in the process) [34].

2.2.2. Inoculum Preparation

In the second stage, LAB inoculum is produced (as described in detail in Supplement S1.1.) using the *L. rhamnosus* MI-0272 strain, which enables the most efficient production of LA. The introduced bacterial strain was propagated for 44 h at the optimal temperature range (i.e., 35.5–37.5 °C), conducive to their activity and colonisation, thus obtaining the inoculum [10,15]. Next, the *S. cerevisiae* yeast inoculum was prepared according to the procedure described in Supplement S1.1. and then cooled to a temperature of 25.5–28.5 °C before further use [46].

2.2.3. Co-Fermentation/Fermentation Process

And in the end, non-fermented dandelion extract (NDE) co-fermentation was carried out using an inoculum consisting of the *L. rhamnosus* MI-0272 strain (responsible for the most efficient production of LA) and *S. cerevisiae* yeast (in a ratio of 9:1) at a temperature range of 29.5–31.5 °C (ensuring the activity of both bacterial and yeast strains) [44]. Waste beet molasses and cane biomass (in a 1:1 ratio) were used as raw materials for the production of LA, in which the total content of six-carbon sugars (Brix) was determined using a refractometer [47]. Before starting the co-fermentation process, unfermented extracts from dandelion leaves were obtained. For this purpose, a mixture of molasses and deionised water was introduced into 300 mL glass bioreactors. The mixture was stirred intensively for 30–45 s at a speed of at least 3500 rpm, i.e., until the molasses was completely dissolved in water, after which the plant material was added. Extraction was carried out using an ultrasonic-assisted method with an ultrasonic bath (40 kHz) with a thermostat at a controlled temperature of 29.5–31.5 °C for 7 min, thus obtaining unfermented dandelion extracts (NDE). The NDE was then subjected to co-fermentation in 300 mL glass bioreactors, controlling the amount of molasses mixture added (0.5–13.0 wt%) so that the initial 6-carbon sugar content in the fermentation mixture was in the range of 0.3–9.0 wt%. (Brix of beet molasses = 69%, Brix of bio molasses from sugar cane = 70%), the amount of plant material at 0.1–2.2 wt%, the amount of LAB inoculum at 1.1–22.3 wt%, the amount of yeast inoculum at 0.1–2.53 wt%, and the content of deionised water at 65.1–92.3 wt% and enzyme (lipase) 0.01 wt% (Table 1). The use of a mixture of beet and cane molasses in appropriate proportions relative to dandelion leaves and water (determined based on preliminary own research that has not yet been published) as a source of carbon and nitrogen provides optimal growth conditions for the *L. rhamnosus* MI-0272 strain, which has high nutritional requirements. Beet molasses provides digestible forms of nitrogen, mainly in the form of amino acids (such as betaine, glutamic acid, aspartic acid, and alanine) and ammonium salts (e.g., ammonium sulphate), which eliminates the need to add additional mineral salts (necessary in the case of fermentation carried out exclusively with cane molasses) [33]. Due to the synergistic action of LAB and *S. cerevisiae* yeast (in proportions considered most favourable based on preliminary studies that have not yet been published), it was possible to significantly shorten the fermentation time (up to seven times) compared to classic fermentation methods using a single LAB strain, while maintaining the desired functional properties of the fermentation products (FCRM-1-FCRM-20). Co-fermentation

was completed at the stage of obtaining the highest LA and TPC content, conducting two independent experiments (n = 2), and the results were averaged—Table S2. Finally, the obtained fermented cosmetic raw material was filtered through a glass funnel, centrifuged (at 3800–4200 rpm for 7–11 min), and sterilised in an ultrasonic bath (at 80 °C for 25–45 min), thus obtaining a ready-to-use FCRM that can be used as an active ingredient in cosmetic preparations. The undoubted advantages of the *L. rhamnosus* MI-0272 strain in LA production have led to its use in the co-fermentation of dandelion leaves, enabling the production of FCRMs, in which LA is the main product. As a result, it has been successfully used in co-fermentation, eliminating the need for additional mineral salts (which were necessary in the case of fermentation using bio molasses from sugar cane) [20,42].

Fermentation of dandelion roots to obtain FCRM-7:FCRM-13 was carried out using a mixture of LAB strains and cane molasses as a source of 6-carbon sugars. Biomolasses were used as raw material to produce LA, in which the total content of six-carbon sugars (Brix) was determined using a refractometer. The bacterial strains *L. reuteri*, *L. salivarius*, *L. brevis*, *L. acidophilus*, *L. plantarum*, and *L. rhamnosus* were propagated for 48 h at 37.5 °C to obtain the inoculum [15]. LAB capable of producing LA are non-spore-forming, catalase-negative, relatively anaerobic, and non-motile. This group includes Gram-positive rods of varying lengths from the genus *Lactobacillus*, whose species characteristic is tolerance to low pH values in the fermentation environment [32,42,48]. A detailed description of the preparation of FCRMs from dandelion roots can be found in Supplement S1.2. (Obtaining fermented cosmetic raw materials (FCRMs) from dandelion roots). Fermentation was finished when the greatest LA and TPC content was achieved, after which two separate tests (n = 2) were conducted, and the findings were averaged—Table S2. After fermentation, the obtained FCRMs were first filtered using a glass funnel and then centrifuged (5 min, 166 Hz, 10,000× *g*), followed by additional filtration using sterile syringe filters with a porosity of 0.45 µm, designed for sterilising aqueous solutions [20].

The fermentation of dandelion flowers to obtain FCRM-14:FCRM-20 was carried out using cane biomolasses as a raw material for LA production (in which the total content of six-carbon sugars was determined using a refractometer) and using single strains of LAB: *L. reuteri* MI_0168, *L. salivarius* LY_0652, *L. brevis* LY_1120, *L. acidophilus* MI-0078, *L. rhamnosus* MI-0272, *L. plantarum* MI-0102, and *L. rhamnosus* LY-0457 [42]. Each strain was individually propagated for 48 h at 37.5 °C, resulting in seven separate inocula [20]. A detailed description of the procedure for preparing FCRMs from dandelion flowers can be found in Supplement S1.3. Fermentation was complete when the highest levels of LA and TPC were reached, and the results were averaged from two different tests (n = 2)—Table S2. After completion of the process, the obtained FCRMs were filtered through a glass funnel, centrifuged (5 min, 166 Hz, 10,000× *g*), and additionally filtered using sterile syringe filters with a pore size of 0.45 µm (intended for sterilising filtration of aqueous solutions) [20].

As part of the research, different inoculum concentrations were used for the co-fermentation of leaves and the fermentation of dandelions' roots and flowers to obtain a series of bioferments labelled FCRM-1 to FCRM-20. The selection of inoculum concentrations was based on the results of preliminary studies, which allowed for the determination of the most favourable conditions for microbial activity and the efficiency of metabolite production, especially LA. During the co-fermentation of leaves and the fermentation of roots and flowers of dandelion, the course of these processes was monitored by controlling the LA content and acidity [42] of both the initial fermentation mixtures and the final bioferments (Table S2). The samples were taken at 24-h intervals to determine the LA content using GC-MS. At the same time, the total polyphenol content (TPC) was also monitored every 24 h using a spectrophotometric method and the Folin–Ciocalteu reagent. In addition, these processes were monitored by controlling the pH of both the initial fermentation mix-

tures and the final bioferments. The pH values for the initial fermentation mixtures ranged from 6 to 7, while the pH values for the final FCRMs from dandelion FCRM-1:FCRM-20 ranged from 3 to 4. After the co-fermentation of the leaves and the fermentation of the roots and flowers of the dandelion, lipase was added to the mixture to hydrolyse the cell walls of the bacteria, which also limited the further growth of microorganisms.

2.3. Determination of Lactic Acid by GC-MS

The GC-MS analysis was conducted on a Shimadzu GC-MS-QP2020 Nexis (Shimadzu, San Jose, CA, USA) equipped with a Shimadzu SH-I-5MS column (30 m × 0.25 mm × 0.25 µm), consistent with a previous study [6]. The detailed procedure is described in Supplement S1.4. Each sample was analysed three times. The LA content in FCRM was determined based on a calibration curve using octane as an internal standard [32]. The results are presented as mean ± standard deviation (SD).

2.4. Determination of Total Polyphenol Content Using the Folin–Ciocalteu Method

The total polyphenol content (TPC) in FCRMs was determined using the Folin–Ciocalteu method, using a GENESYS 50 spectrophotometer (Thermo Scientific) and a wavelength of λ = 750 nm, with gallic acid as a standard [40]. The detailed procedure is described in Supplement S1.5.

The total polyphenol content was determined using the following Formula (1):

$$TPC = \frac{[(C_{FCRM} - C_{B.S.}) \cdot V_S]}{V_{FCRM}} \cdot 100\% \quad (1)$$

where

TPC—total polyphenol content by the Folin–Ciocalteu method [mmol/L],
C_{FCRM}—concentration of polyphenols in tested FCRM [mmol/L],
$C_{B.S.}$—concentration of polyphenols in the blank sample [mmol/L],
V_S—total volume of solution introduced into volumetric flasks [L],
V_{FCRM}—volume of FCRM introduced into volumetric flasks [L].

The results were expressed as mmol GA/L FCRM based on the calibration curve ($y = 0.0075x$, $R^2 = 0.997$). All measurements were performed three times, and the results are presented as mean ± standard deviation (SD) [32].

2.5. Determination of Antioxidant Activity: DPPH Assay

The antioxidant activity of FCRMs was evaluated using the DPPH free radical scavenging technique, following the protocol established by Kucharska et al. [20]. Measurements were conducted using a Thermo Scientific GENESYS 50 spectrophotometer at a wavelength of λ = 517 nm. Trolox (Tx) served as the reference chemical. A detailed description of the method can be found in the Supplement, Section S1.6. Three independent tests were conducted. The results are presented as mean ± standard deviation (SD), while antioxidant activity (AA-DPPH) was expressed as mmol Tx/L FCRM, based on the calibration curve: $y = -1.0321x + 1.1342$ ($R^2 = 0.997$) [40].

2.6. Determination of Antioxidant Activity: ORAC Assay

A modified ORAC method was used to determine the ability to absorb oxygen radicals [49]. A Trolox solution with a concentration of 0.1 mmol/L was used as a positive control, while a negative control (blank test) consisted of 35 µL of phosphate buffer (pH 7.4) instead of the tested FCRM sample. A detailed description of the method can be found in the Supplement, Section S1.7.

First, the surface areas were calculated using the following Formula (2):

$$AUC = 1 + + \ldots + f_n + 1/f_0 \,;\; AUC = \sum_{i=0}^{n} f_i \,/f_0 \tag{2}$$

where

AUC—fluorescence area,

f_n—fluorescence value at time n,

f_i—fluorescence value at a given time,

f_0—initial fluorescence value.

Next, the ORAC antioxidant capacity was calculated using the following Formula (3):

$$ORAC = C_{Tx} * \frac{AUC_{FCRM} - C_{B.S.}}{C_{Tx} - C_{B.S.}} \tag{3}$$

where

$ORAC$—antioxidant capacity of fermented cosmetic raw material [mmol Tx/L],

C_{Tx}—concentration of Trolox solution (0.1 mmol/L),

AUC—area of fluorescence intensity decreases for the tested FCRM samples, blank samples, and Trolox solution.

All measurements were performed three times, and the results are presented as mean ± standard deviation (SD). The antioxidant activity (AA-ORAC) for the tested FCRMs was expressed as the concentration of Trolox, in mmol Tx/L, as a reference substance.

2.7. Lipophilicity Assessment

The lipophilicity assessments of FCRMs were conducted using a previously established protocol [42] and Equation (4), utilising the Thermo Scientific GENESYS 50 device (Thermo Fisher Scientific, Norristown, PA, USA) [20], as follows:

$$P = \frac{C_0}{C_w} = \frac{C_0 - C_w}{C_w} = \frac{S_0 - S}{S} = \frac{\int_{\Delta_1}^{\Delta_2} A^0 d\Lambda - \int_{\Delta_1}^{\Delta_2} A d\Lambda}{\int_{\Delta_1}^{\Delta_2} A d\Lambda} \tag{4}$$

where

C—concentration of total compounds in the n-octanol layer and in the water layer,

S—area occupied by the compound in the UV-Vis spectrum,

A—absorbance,

0—concentration of total compounds in the n-octanol layer and in the aqueous layer, concentration of total compounds in the n-octanol layer and in the aqueous layer/initial area occupied by the compound in the UV-Vis spectrum/initial absorbance,

Λ—wavelength.

A detailed description of the method can be found in the Supplement, Section S1.8. [50].

2.8. Wettability

The effect of FCRMs on surface wettability was assessed by measuring the contact angle with a drop shape analyser, Kruss 165 DSA100 (Filderstadt, Germany), according to a previously used procedure [42]. A drop of fermented cosmetic raw material (3 µL) was placed on a STRAT-M® membrane with a layer thickness of 320 µm (Sigma-Aldrich, Darmstadt, Germany), which is a substitute for human skin [51]. The contact angle was measured (from ten different locations) 5 s after placing the FCRM drop on the synthetic membrane, using the deposited drop technique and DSA4 software, version 1.14. Results are presented as the mean ± standard deviation (SD).

2.9. Statistical Analysis

To assess the impact of the co-fermentation process on antioxidant activity and total phenolic content in FCRMs and NDEs, a statistical analysis was performed, including descriptive statistics and one-way analysis of variance (ANOVA). For each method of determining antioxidant activity (AA-DPPH, AA-ORAC) and total phenolic content (TPC), mean values, variances, and ANOVA test results were compiled, comparing the two study groups, i.e., FCRMs and NDEs. In the case of significant differences, Tukey's test was used to identify pairs of groups that differed statistically significantly.

Statistical calculations were performed using Statistica 13 PL software (StatSoft, Krakow, Poland). The results are presented as mean values. Differences were considered statistically significant at a significant level of $\alpha < 0.05$.

3. Results

3.1. Methods for Obtaining Fermented Cosmetic Raw Materials and Evaluation of Lactic Acid Production During Dandelion Fermentation

A method for obtaining FCRMs from dandelion leaves (FCRM 1–6) was developed, based on the use of an aqueous solution of molasses (beet and cane in a 1:1 ratio), to which leaves were added in various proportions. Ultrasound-assisted extraction was then carried out to obtain unfermented leaf extracts (NDE 1–6). Detailed information on the proportions of the ingredients and the conditions of the co-fermentation process is presented in Table 1, while the full procedure is described in the Supplementary Materials (S1.1. Preparing fermented cosmetic raw materials (FCRMs) from dandelion leaves).

Figure 1 shows the results of the LA content of FCRMs and NDEs.

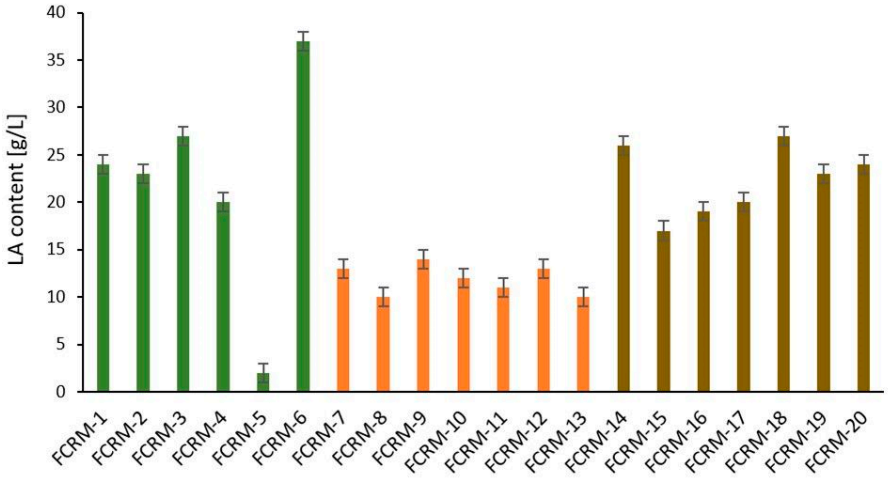


Figure 1. LA content in FCRMs from dandelion leaves—FCRM-1:FCRM-6 (green); dandelion roots—FCRM-7:FCRM-13 (orange); and dandelion flowers—FCRM-14:FCRM-20 (brown).

The fermentation processes of plant raw materials using a single inoculant consisting of a single strain of the *L. genus* (i.e., *L. reuteri* MI_0168, *L. salivarius* LY_0652, *L. brevis* LY_1120, *L. acidophilus* MI-0078, *L. plantarum* MI-0102, *L. rhamnosus* MI-0272, and *L. rhamnosus* Y-0457) or mixtures thereof take a very long time (up to 15 days) to produce FCRMs containing the maximum content of both LA (a natural skin moisturiser) and phenolic acids (responsible for the increased antioxidant activity of the fermented cosmetic raw material)—Table 1. Therefore, this study proposes a method that primarily shortens the fermentation time of plant raw materials such as dandelion. The research showed that a mixture of molasses can be successfully used in the fermentation of dandelion leaves using LAB strains, which eliminated the need for additional mineral salts (which were necessary in the case of dandelion flower fermentation using cane molasses)—Table 1. In addition, by conducting

a co-fermentation process using two inoculants, *L. rhamnosus* MI-0272 and *S. cerevisiae*, the fermentation time was reduced by up to 5 times, i.e., from 15 days (in the case of fermentation carried out in the presence of *L. salivarius* LY_0652) to 3 days, obtaining LA at a comparably high level (i.e., 27 ± 1 g/L in the case of FCRM-3) as in the case of fermentation carried out using a single inoculant (26 ± 1 g/L in the case of FCRM-14)—Figure 1. No LA was found in any of the unfermented extracts NDE 1–6, while during 10 days of fermentation of dandelion roots using 11.4–11.7 wt% cane molasses content, plant material content of 0.2–1.9 wt%, inoculum content consisting of a mixture of LAB strains of 19–19.5 wt%, and fermented dandelion extracts FCRM 7–13 containing LA from 10 ± 1 g/L to 14 ± 1 g/L were obtained—Figure 1, Table S1.

Figure 2 presents the fermentation of dandelion flowers in the presence of 7 individual strains of LAB.

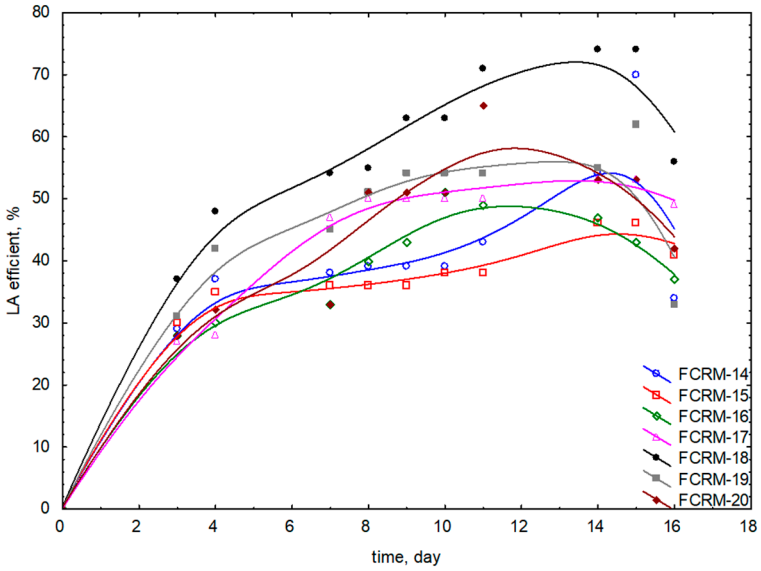


Figure 2. LA production efficiency during the fermentation of dandelion flowers in the presence of 7 individual strains of LAB: FCRM-14—*L. salivarius* LY_0652, FCRM-15—*L. reuteri* MI_0168, FCRM-16—*L. acidophilus* MI-0078, FCRM-17—*L. brevis* LY_112, FCRM-18—*L. rhamnosus* MI-0272, FCRM-19—*L. plantarum* MI-0102, FCRM-20—*L. rhamnosus* LY-0457.

All FCRM 14–20 showed higher LA content (17–27 g/L) than previously reported values for ferments involving *Rhodotorula glutinis* and *L. casei* (15.05 g/L) [26], confirming the effectiveness of the LAB strains used in this study. The LA content in FCRM-14:FCRM-20 depended on the LAB strain used in the fermentation process. LA was produced in the highest amount (i.e., 27 ± 1 g/L) during fermentation carried out in the presence of *L. rhamnosus* MI-0272 (FCRM-18) and was produced with 74% efficiency after 15 days of the process. Similarly high amounts of LA, i.e., 26 ± 1 g/L and 23 ± 1 g/L, were observed during fermentation carried out using *L. salivarius* LY_0652 (FCRM-14) and *L. plantarum* MI-0102 (FCRM-19), with LA yields of 70% and 62%, respectively. In contrast, the process carried out in the presence of strains such as *L. rhamnosus* LY-0457 (FCRM-20) and *L. reuteri* MI_0168 (FCRM-15) enabled maximum LA yield to be achieved after 11 days (65% yield) and 14 days (46%), respectively. This suggests that the fermentation activity of these LAB strains was higher but shorter in duration, which may be due to faster substrate (molasses) depletion or greater sensitivity to acidification of the fermentation environment [52]. The *L. brevis* LY_112 strain enabled the production of LA in FCRM-17 with a 53% yield after 14 days of fermentation, which indicates its moderate efficiency in LA production [53]. However, in the case of the process conducted in the presence of *L. acidophilus* MI-0078 (FCRM-16), although this strain enabled maximum LA yield (51%) to be achieved earlier

(i.e., on the 10th day of fermentation), it showed a rapid decline in yield after that time, which may indicate limited tolerance to fermentation conditions [54]. The decrease in LA efficiency after reaching its maximum is consistent with the mechanism of inhibition of LA production resulting from the acidification of the cytoplasm by undissociated LA. This phenomenon leads to a disruption of the driving force of protons and a reduction in cellular energy, which inhibits bacterial growth [55]—Figures 1 and 2.

3.2. Antioxidant Activity and Total Polyphenol Content of Fermented Cosmetic Raw Materials

Table 2 shows the results of the antioxidant activity and total polyphenol content of FCRMs and NDEs from dandelion leaves.

Table 2. The results of the antioxidant activity (AA-DPPH and AA-ORAC) and total polyphenol content (TPC) of FCRMs and NDEs.

Name FCRM	AA-DPPH [mmol Tx/L]	AA-ORAC [mmol Tx/L]	TPC [mg GA/L]
FCRM-1/NDE-1	1.0 ± 0.1/0.6 ± 0.1	0.44 ± 0.01/0.14 ± 0.01	1251 ± 12/252 ± 8
FCRM-2/NDE-2	3.0 ± 0.1/1.9 ± 0.1	0.55 ± 0.02/0.19 ± 0.01	3589 ± 25/576 ± 10
FCRM-3/NDE-3	0.8 ± 0.1/0.2 ± 0.1	0.46 ± 0.01/0.17 ± 0.01	1595 ± 11/621 ± 13
FCRM-4/NDE-4	1.3 ± 0.1/0.4 ± 0.1	0.54 ± 0.02/0.18 ± 0.01	2256 ± 32/381 ± 12
FCRM-5/NDE-5	1.2 ± 0.1/0.8 ± 0.1	0.54 ± 0.02/0.19 ± 0.01	2256 ± 32/186 ± 6
FCRM-6/NDE-6	1.5 ± 0.1/0.5 ± 0.1	0.45 ± 0.01/0.16 ± 0.01	1603 ± 30/722 ± 14

Mean ± SD (n = 3).

Figure 3 shows the results of the antioxidant activity (AA-DPPH and AA-ORAC) and the total polyphenol content (TPC) of FCRMs and NDEs from *T. folium*.

The results presented in Figure 3 clearly indicate that co-fermentation of dandelion leaf extracts in the presence of two inoculants (*L. rhamnosus* MI-0272 and *S. cerevisiae*) increases both antioxidant activity (AA-DPPH and AA-ORAC) and total polyphenol content (TPC) of FCRMs. All FCRM 1–6 were characterised by higher antioxidant activity compared to their corresponding NDE 1–6—Figure 3, Table 2.

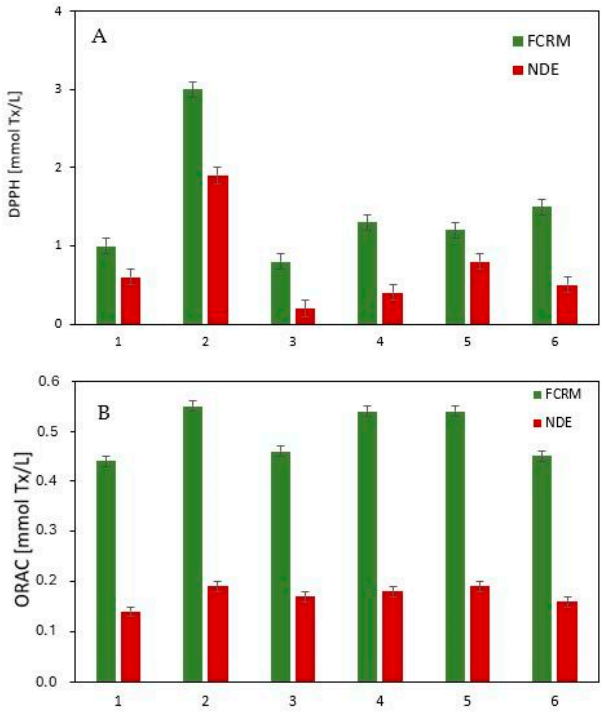


Figure 3. *Cont.*

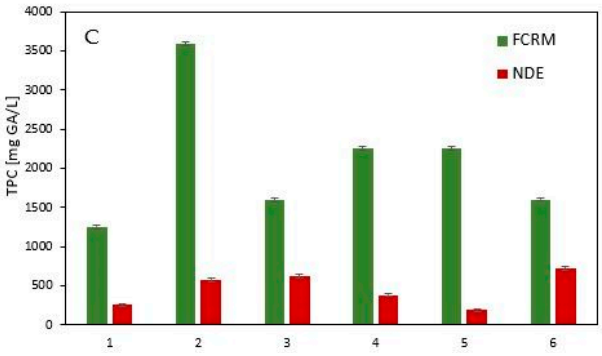


Figure 3. Antioxidant activity and total polyphenol content of FCRM-1:FCRM-6 (green) and (NDE-1:NDE-6 (red) from dandelion leaves; (**A**)—antioxidant activity measured by the DPPH method (AA-DPPH); (**B**)—antioxidant activity measured by the ORAC method (AA-ORAC); (**C**)—total polyphenol content (TPC).

Table 3 shows the results of the antioxidant activity and the total polyphenol content of FCRMs from dandelion roots.

Table 3. The results of the antioxidant activity (AA-DPPH and AA-ORAC) and the total polyphenol content (TPC) of FCRMs from dandelion roots.

Name FCRM	AA-DPPH [mmol Tx/L]	AA-ORAC [mmol Tx/L]	TPC [mg GA/L]
FCRM-7	1.7 ± 0.1	0.49 ± 0.01	90 ± 1
FCRM-8	1.9 ± 0.1	0.51 ± 0.01	114 ± 1
FCRM-9	1.6 ± 0.1	0.50 ± 0.01	106 ± 1
FCRM-10	1.2 ± 0.1	0.48 ± 0.01	106 ± 1
FCRM-11	1.2 ± 0.1	0.49 ± 0.01	102 ± 2
FCRM-12	1.2 ± 0.1	0.49 ± 0.01	109 ± 2
FCRM-13	1.3 ± 0.1	0.50 ± 0.01	111 ± 2

Mean ± SD (n = 3).

Antioxidant activity tests (using the DPPH free radical reduction technique) of FCRMs obtained by fermenting dandelion roots showed that sample FCRM-8 had the highest antioxidant potential (1.9 ± 0.1 mmol Tx/L). FCRM-7 and FCRM-9 had slightly lower AA values, i.e., 1.7 ± 0.1 mmol Tx/L and 1.9 ± 0.1 mmol Tx/L, respectively. Furthermore, the antioxidant activity of FCRM 9–13 estimated by the DPPH method was slightly lower and remained at a comparable level of 1.2–1.3 ± 0.1 mmol Tx/L (TPC) of FCRMs. All FCRM 1–6 were characterised by higher antioxidant activity compared to their corresponding NDE 1–6—Table 3.

The results of antioxidant activity using the ORAC method for FCRMs from dandelion roots showed that regardless of the tested FCRM, the AA-ORAC values remained at a comparable level (0.48 ± 0.01 to 0.51 ± 0.01 mmol Tx/L), with the highest oxygen radical absorption capacity, i.e., 0.51 ± 0.01 mmol Tx/L, being characterised by FCRM-8 obtained using the following process parameters: cane molasses content 11.7 wt%, dandelion root content 0.4 wt%, and inoculum content 19.5 wt%. The fermentation parameters used also favoured the highest TPC production (114 ± 1 mg GA/L), responsible for the highest antioxidant activity value using the DPPH technique of FCRM-8—Table 3.

Table 4 shows the results of the antioxidant activity and the total polyphenol content of FCRMs from dandelion flowers.

Table 4. The results of the antioxidant activity (AA-DPPH and AA-ORAC) and the total polyphenol content (TPC) of FCRMs from dandelion flowers.

Name FCRM	AA-DPPH [mmol Tx/L]	AA-ORAC [mmol Tx/L]	TPC [mg GA/L]
FCRM-14	1.1 ± 0.1	0.49 ± 0.01	2380 ± 12
FCRM-15	1.3 ± 0.1	0.49 ± 0.01	2419 ± 11
FCRM-16	1.2 ± 0.1	0.49 ± 0.01	2397 ± 12
FCRM-17	1.6 ± 0.1	0.50 ± 0.01	2555 ± 10
FCRM-18	1.5 ± 0.1	0.49 ± 0.01	2511 ± 12
FCRM-19	1.6 ± 0.1	0.50 ± 0.01	2536 ± 13
FCRM-20	1.2 ± 0.1	0.49 ± 0.01	2380 ± 11

Mean ± SD (n = 3).

The results of antioxidant activity using the ORAC method for FCRMs from dandelion flowers showed that regardless of the tested FCRM, the AA-ORAC values remained at a comparable level (0.49 ± 0.01 to 0.50 ± 0.01 mmol Tx/L), with the highest oxygen radical absorption capacity, i.e., 0.50 ± 0.01 mmol Tx/L, being characterised by FCRM-17 (obtained during fermentation carried out in the presence of the *L. reuteri* MI_0168 strain for 14 days) and FCRM-19 (obtained during fermentation in the presence of the *L. plantarum* MI-0102 strain for 15 days). The fermentation parameters used, such as cane molasses content of 5.3 wt% with an initial sugar content of 3.7 wt%, dandelion flower content of 1.5 wt%, inoculum content of 3 wt%, lipase content of 0.02 wt%, and water content of 89 wt%, favoured an increase in the total polyphenol content for these fermented raw materials, where the TPC level was 2555 ± 10 mg GA/L and 2536 ± 13 mg GA/L, respectively. Furthermore, the highest degree of DPPH free radical reduction was also observed for FCRM-17 and FCRM-19 (1.6 ± 0.1 mmol Tx/L), slightly lower for FCRM-18 (1.5 ± 0.1 mmol Tx/L). The polyphenolic compounds present in fermented raw materials obtained from dandelion flowers have a significant effect on the ability to neutralise free radicals in the DPPH test (Table 4).

Tables 5–10 present a summary and the results of the analysis of variance (ANOVA) for the values obtained in the antioxidant activity (AA-DPPH and AA-ORAC) and total phenolic content (TPC) tests in FCRMs and NDEs obtained by co-fermentation.

Table 5. Summary statistics for antioxidant activity measured by the DPPH method (AA-DPPH).

Group	Count	Sum	Average	Variance
AA-DPPH (FCRM-1:FCRM-6)	6	8.8	1.47	0.62
AA-DPPH (NDE-1:NDE-6)	6	4.4	0.733	0.37

Table 5, containing statistics for antioxidant activity assessed by the DPPH method (AA-DPPH) in two groups of samples, i.e., FCRM-1:FCRM-6 and NDE-1:NDE-6 from dandelion leaves, shows that the FCRM group has a higher average antioxidant activity value (1.47) and greater variability (variance 0.62) compared to the NDE group, for which the average is 0.733 and the variance is 0.37, suggesting a beneficial effect of the co-fermentation process of dandelion leaves on the antioxidant activity of FCRMs using the DPPH method.

Table 6. ANOVA results for antioxidant activity measured by the DPPH method (AA-DPPH).

Source of Variation	SS	df	MS	F	*p*-Value	F Crit
Between groups	1.61	1	1.61	3.26	0.101	4.96
Within groups	4.95	10	0.49			
Total	6.56	11				

SS (Sum of Squares)—sum of squares of deviations, determining variability between and within groups; df (Degrees of Freedom)—number of degrees of freedom for each category; MS (Mean Square)—mean square calculated as SS/df; F—F statistic value indicating the ratio of variability between groups to variability within groups; *p*-value—*p* value determining the level of statistical significance; $p < 0.05$ indicating significant differences; F Crit—critical F value for a given level of significance (if F > F Crit, the differences are statistically significant).

Table 6 presenting the results of the analysis of variance (ANOVA) for antioxidant activity estimated using the DPPH method indicates that the differences between the groups (FCRM-1:FCRM-6 and NDE-1:NDE-6) are not statistically significant ($p = 0.101$, F < F Crit), because the *p*-value exceeds the accepted significance level ($\alpha = 0.05$) and the F-statistic value (3.26) is lower than the critical F-value (4.96).

Table 7. Summary statistics for antioxidant activity measured by the ORAC method (AA-ORAC).

Group	Count	Sum	Average	Variance
AA-ORAC (FCRM-1:FCRM-6)	6	2.98	0.497	0.0027
AA-ORAC (NDE-1:NDE-6)	6	1.03	0.172	0.00038

Table 7 presents a summary of data for two groups of samples analysed using the ORAC method, where group FCRM-1:FCRM-6 showed a significantly higher average antioxidant activity value using the ORAC method (0.497) and greater variability (variance 0.0027) compared to the NDEs group, for which the average is 0.172 and the variance value is 0.00038, which may indicate greater antioxidant efficacy of FCRMs as estimated by the ORAC method.

Table 8. ANOVA results for antioxidant activity measured by the ORAC method (AA-ORAC).

Source of Variation	SS	df	MS	F	*p*-Value	F Crit
Between groups	0.317	1	0.32	208	$5.07 \cdot 10^{-8}$	4.96
Within groups	0.015	10	0.0015			
Total	0.332	11				

SS (Sum of Squares)—sum of squares of deviations, determining variability between and within groups; df (Degrees of Freedom)—number of degrees of freedom for each category; MS (Mean Square)—mean square calculated as SS/df; F—F statistic value indicating the ratio of variability between groups to variability within groups; *p*-value—*p* value determining the level of statistical significance; $p < 0.05$ indicating significant differences; F Crit—critical F value for a given level of significance (if F > F Crit, the differences are statistically significant).

Table 8 shows that the differences between the analysed groups (FCRMs and NDEs) are statistically significant, which is confirmed by the very high F statistic (208) and the extremely low *p* value ($5.07 \cdot 10^{-8}$), well below the significance level of 0.05. This means that the antioxidant activity measured by the ORAC method differs significantly between the studied groups.

Table 9. Summary statistics for total phenolic content (TPC) in analysed samples.

Group	Count	Sum	Average	Variance
TPC (FCRM-1:FCRM-6)	6	12,550	2092	697,642
TPC (NDE-1:NDE-6)	6	2738	456	46,504

Table 9 shows that the TPC group (FCRM-1:FCRM-6) of fermented dandelion leaf extracts is characterised by a significantly higher average polyphenol content (2092) and greater variability (variance 697642) compared to the group of unfermented dandelion leaf extracts TPC (NDE-1:NDE-6), for which the average is 456 and the variance is 46504. These results indicate significant differences in the content of phenolic compounds among the analysed groups.

Table 10. ANOVA results for total phenolic content (TPC) in analysed samples.

Source of Variation	SS	df	MS	F	*p*-Value	F Crit
Between groups	8,022,945	1	8,022,945	21.6	0.000917	4.96
Within groups	3,720,733	10	372,073			
Total	11,743,678	11				

SS (Sum of Squares)—sum of squares of deviations, determining variability between and within groups; df (Degrees of Freedom)—number of degrees of freedom for each category; MS (Mean Square)—mean square calculated as SS/df; F—F statistic value indicating the ratio of variability between groups to variability within groups; *p*-value—*p* value determining the level of statistical significance; $p < 0.05$ indicating significant differences; F Crit—critical F value for a given level of significance (if F > F Crit, the differences are statistically significant).

The results presented in Table 10 show that the differences in total polyphenol content (TPC) between the analysed groups are statistically significant, which is confirmed by the high F statistic (21.6) and the very low *p* value (0.000917), well below the significance level of 0.05. This means that TPC values differ significantly between fermented and unfermented dandelion leaf extracts.

3.3. Lipophilicity Assessment

Figure 4 shows examples of UV-Vis spectra of FCRMs obtained during the co-fermentation of leaves and fermentation of dandelion roots.

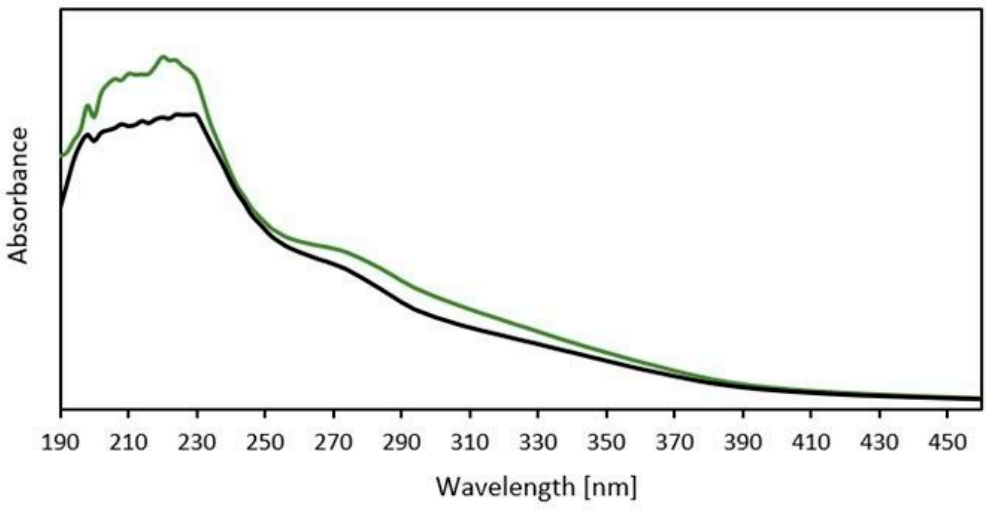


Figure 4. *Cont.*

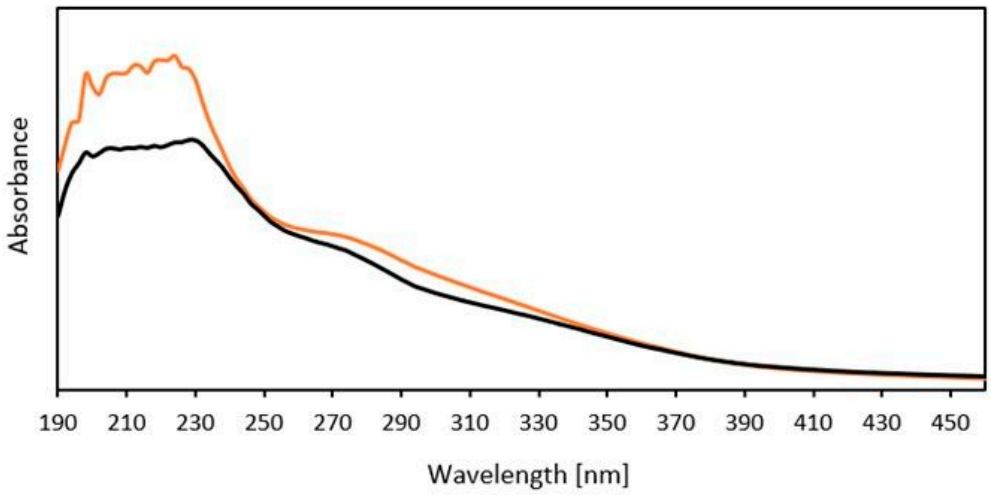


Figure 4. Example UV-Vis spectra of FCRMs; above—FCRM-6: initial UV-Vis spectrum of FCRM-6 (green, C_1) and UV-Vis spectrum of FCRM-6 in equilibrium with n-octane (black, C_0); below—FCRM-13: initial UV-Vis spectrum of FCRM-13 (orange, C_1) and UV-Vis spectrum of FCRM-13 in equilibrium with n-octane (black, C_0).

The assessment of the lipophilicity of FCRMs indicates their clearly hydrophilic nature, which is confirmed by the log P partition coefficient value of -0.9 for both FCRM-6 and FCRM-13 (Figure 4). Such a low log P value promotes the solubility of FCRMs in the aqueous phase rather than in the lipid phase and results from the presence of highly polar compounds in FCRMs [56].

The hydrophilicity of the tested FCRMs is related to the presence of numerous hydroxyl and carboxyl groups, which are characteristic of phenolic compounds and LA—the main products of fermentation involving LAB. The presence of these functional groups increases the ability of molecules to form hydrogen bonds with water, which translates into their good solubility in an aqueous environment [57].

3.4. Wettability

Figure 5 shows photographs of examples of droplets of FCRMs obtained during the co-fermentation of leaves and the fermentation of dandelion roots.

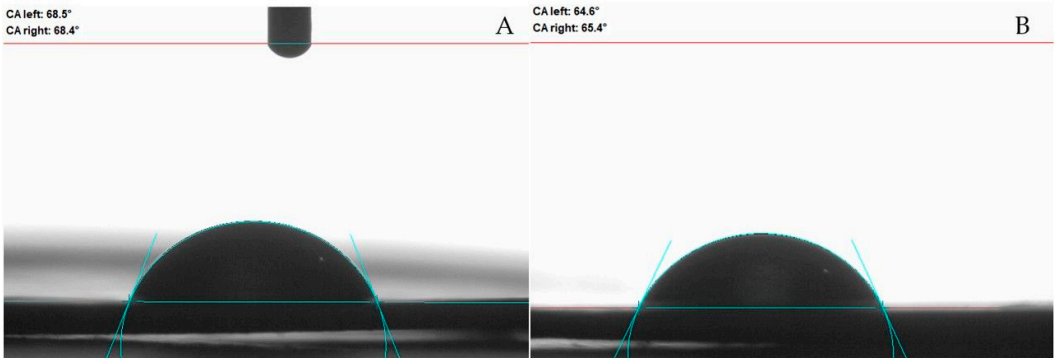


Figure 5. Contact angle (c.a.) of: (**A**): FCRM-6 and (**B**): FCRM-13.

The wettability of FRCMs, measured by the contact angle using the contact drop method, further confirms the hydrophilic nature of FCRMs from dandelion leaves (FCRM-6) and dandelion roots (FCRM-13). The average wetting angles for FCRM-6 are 68.5 ± 0.1° and for FCRM-13 65.0 ± 0.1°, which indicates good wettability of the membrane surface.

These values are typical for bioferments with high hydrophilicity and correlate with the results of the log P analysis [58]—Figure 5.

4. Discussion

The proposed method of co-fermentation of dandelion leaves using two inoculants, *L. rhamnosus* MI-0272 and *S. cerevisiae*, which utilises a mixture of molasses (i.e., waste beet molasses and bio cane molasses) as a substrate for obtaining LA, enabled the fermentation process to be shortened by up to 5 times while maintaining high LA yield, which reached a maximum of 37 ± 1 g/L (Tables S1 and 1, Figures 1 and 2). LA, the main product of LAB fermentation, plays a key role as a natural moisturising factor (NMF) for the skin. Its presence in the stratum corneum affects the skin's ability to bind and retain water, which is essential for maintaining its proper barrier function [59]. Disorders in skin composition, especially NMF deficiency, are directly related to the occurrence of dryness and roughness of the skin, as well as increased susceptibility to irritation and inflammation [60]. Therefore, LA is widely used in cosmetic formulations as an active ingredient with moisturising properties that support epidermal renewal [61]. The use of LA-rich FCRMs (Figure 1) with skin-moisturising properties and polyphenols with antioxidant properties (Figure 3) in the cosmetics industry results in cosmetic raw material ingredients with a broad spectrum of action (from moisturising to protecting against oxidative stress to supporting skin regeneration). Furthermore, the use of a mixture of molasses eliminated the need to add additional mineral salts to the fermentation medium, which are essential for LAB growth [62]. The addition of mineral salts to the fermentation medium proved necessary in the case of the fermentation of dandelion flowers and roots, where cane molasses was used as a substrate for LA production. Furthermore, analysis of the results indicates that the LA content in FCRM-1:FCRM-20 depends significantly on the type of dandelion part used (leaves, roots, flowers), the fermentation method used (single inoculant fermentation vs. co-fermentation with two inoculants) and the process parameters (Table 1). Co-fermentation of dandelion leaves with LAB and yeast demonstrated the greatest potential for effective LA production. This approach not only increased LA yield but also significantly reduced process time. Fermentation of dandelion flowers also resulted in bioferments with a high LA content but required a longer time and the use of additional mineral salts, which increased the complexity and cost of the process. This study indicates that although flowers can be a valuable raw material, their fermentation may be less technologically efficient compared to leaves. The absence of LA in unfermented extracts confirms that its presence is the result of microbial activity. These results emphasise the importance of carefully selecting both the plant parts and the fermentation parameters to obtain bioferments with the desired functional and cosmetic properties (Tables S1 and 1; Figures 1 and 2). The study showed that the use of co-fermentation to obtain FCRM 1–6 from dandelion leaves significantly increases not only the LA content but also the TPC content in relation to NDE 1–6. Furthermore, the increase in total polyphenol content estimated by the Folin–Ciocalteu method contributes to an increase in antioxidant activity using the DPPH (AA-DPPH) technique. Co-fermentation allows maximum AA-DPPH and TPC values to be achieved up to 4 times faster than fermentation using a single inoculant. This evidence suggests that the fermentation activity of bacterial and yeast strains was higher than that of individual LAB strains. The highest degree of DPPH free radical reduction and the highest polyphenol content were observed in the case of NDE-2 co-fermentation (for which TPC = 576 ± 10 mg GA/L and AA-DPPH 1.9 ± 0.1 mmol Tx/L) carried out using a mixture of molasses (with a content of 5.4 wt%), i.e., waste beet molasses (2.5 g) and bio cane molasses (2.5 g), using an initial sugar content of 3.8 wt% and obtaining FCRM-2 with a total polyphenol content (TPC) of 3589 ± 25 mg GA/L and AA-DPPH antioxidant

activity of 3.0 ± 0.1 mmol Tx/L. In contrast, FCRM-4 (with a TPC content of 2256 ± 32 mg GA/L) and FCRM-6 (with a TPC content of 1603 ± 30) showed ≥3 times higher antioxidant activity than NDE-4 and NDE-6, i.e., 3.0 ± 0.1 mmol Tx/L and 1.5 ± 0.1 mmol Tx/L, respectively. In addition, NDE-3 co-fermentation lasting only 3 days made it possible to obtain FCRM-3, which was characterised by a 4-fold higher DPPH radical scavenging capacity compared to the DPPH scavenging capacity of NDE-3, while the total polyphenol content estimated by the Folin–Ciocalteu method increased more than 2.5-fold (i.e., from 621 ± 13 mg GA/L to 1595 ± 11 mg GA/L). The high antioxidant activity of FCRMs by the DPPH method indicates the presence of effective hydrogen donors in FCRMs, such as phenolic acids, which are typical products of the fermentation of plant raw materials using LAB [31]. Co-fermentation can lead to the formation of new polyphenolic compounds with antioxidant potential or can increase the bioavailability of existing ones [63]. The increase in TPC after co-fermentation may be the result of the enzymatic breakdown of complex phenolic compounds, e.g., glycoside forms of flavonoids with a complex structure and higher molecular weight, into free flavonoids, which are then metabolised into more bioavailable compounds, i.e., phenolic acids (Figure 3, Tables 2–4) [64]. A comparison of the fermentation results of dandelion flowers and roots reveals significant differences in antioxidant activity using the DPPH and ORAC methods and in the total content of phenolic compounds, which can be attributed to the different phytochemical composition of these parts of the plant. FCRM-7:FCRM-13 obtained from dandelion roots were characterised by moderate antioxidant activity and relatively low phenolic compound content (Table 3). Despite ORAC values indicating the ability to neutralise oxygen radicals, the TPC level was significantly lower than in the case of FCRM-14:FCRM-20 from flowers. The highest antioxidant activity (AA-DPPH) and TPC values for FCRMs from roots were recorded for FCRM-8, which may suggest that the appropriate fermentation parameters were used (Table 1). In contrast, bioferments from dandelion flowers showed a significantly higher content of phenolic compounds and higher antioxidant activity (AA-DPPH), confirming their potential as a cosmetic raw material (Table 4). FCRM-17 and FCRM-19 stood out in terms of both their ability to reduce DPPH free radicals and their TPC levels, which may be due to appropriately selected fermentation parameters that promote the release and transformation of macromolecular compounds into small-molecule products (Table 1). A comparison of the antioxidant activity results obtained using the DPPH method for the FCRMs indicates that the effectiveness of the fermentation process in improving antioxidant properties depends both on the type of dandelion part used (leaves, roots, flowers) and on the fermentation method and process parameters used (Tables 2–4). The greatest increase in antioxidant activity compared to unfermented extracts was observed in FCRMs obtained from dandelion leaves, especially when co-fermentation with two inoculants was used, which also made it possible to obtain bioferments with a significantly increased content of phenolic compounds, which translates into their higher antioxidant properties. FCRMs obtained from dandelion flowers also showed high antioxidant activity using this method, with a significantly longer fermentation time and in the presence of mineral salt additives. FCRMs obtained from dandelion roots were characterised by lower AA-DPPH values, which may result from the limited content of phenolic compounds present in this part of the plant and the lower efficiency of fermentation under the process conditions used. The results of antioxidant activity determined by the ORAC method for FCRMs indicate that the effectiveness of the fermentation process in improving the ability to neutralise free radicals depends on the type of dandelion used, the fermentation method applied and the process parameters. FCRMs obtained from dandelion leaves showed the highest antioxidant potential, increasing the ability of bioferments to capture oxygen radicals but also contributing to an increase in the content of phenolic compounds. FCRMs obtained

from dandelion flowers showed similar AA-ORAC values, regardless of the fermentation parameters used. This suggests that this part of the plant is rich in active ingredients and that fermentation does not affect their conversion into more bioavailable small molecules. FCRMs obtained from dandelion roots were characterised by lower antioxidant activity according to the ORAC method, which may be related to the limited content of phenolic compounds present in this part of the plant and the lower efficiency of fermentation under the conditions used in the study (Figure 3). The observed differences in the antioxidant activity exhibited by FCRMs, assessed using the DPPH and ORAC methods, result from the different chemical reaction mechanisms on which these tests are based. While ORAC reflects the ability to neutralise radicals under conditions more like physiological conditions, DPPH preferentially detects compounds by reducing properties in FCRMs. The differences in the results obtained by these methods may therefore result not only from FCRMs obtained from different parts of the dandelion, but also from their specific reactivity towards different types of radicals [65].

Similar observations regarding the increase in LA and phenolic compounds in a two-step method for obtaining fermented dandelion extracts (FDEs) used as functional foods have been reported by other authors [22], confirming the effectiveness of this method in enhancing the antioxidant properties of fermented raw materials [22]. The use of *L. plantarum*, *L. fermentum*, *L. rhamnosus*, and *L. casei* strains in this two-stage fermentation method allowed us to produce fermented dandelion extracts with varying LA content, with the highest concentration observed in the case of fermentation with *L. rhamnosus*. The presence of LA in fermented plant extracts is important evidence of the fermentation activity of the microorganisms used, which can produce this metabolite [66]. The varying concentrations of LA confirm the effectiveness of the fermentation process and indicate the significant influence of fermentation conditions (such as time, temperature, pH, and inoculum concentration) on LA production efficiency [67]. Furthermore, the presence of LA in bioferments indicates both preservative and pH-regulating properties, while also maintaining functional effects such as increasing skin hydration, reducing transepidermal water loss (TEWL), and improving the permeability of active ingredients through the stratum corneum; these factors make LA a valuable ingredient in cosmetic formulations [32,68]. The biotechnological method of natural LA production is an alternative to synthetic moisturising ingredients, contributing to the development of more sustainable and safer cosmetic products. Furthermore, the possibility of standardising the LA content of bioferments paves the way for their wider use as controlled raw materials in the cosmetics industry, which may be important for both manufacturers and consumers looking for products with proven efficacy and safety [69,70]. The increased content of the phenolic acids (chlorogenic acid, chicoric acid, and caffeic acid) contributed to an increase in the antioxidant activity of FDE, estimated using the DPPH method. The degree of DPPH free radical reduction increased by approximately 30% (in the case of bioferments obtained using *L. plantarum* strains) and by over 50% (in the case of bioferments obtained using *L. casei* strains), making this strain particularly promising in the context of functional food production [22].

Study results published by other authors [23] indicate that fermentation of *T. officinale* extracts with *L. plantarum* and *L. casei* strains leads to a significant increase in antioxidant activity, assessed using the DPPH method. The presented study used the solid-state fermentation (SSF) method as an effective way to obtain fermented dandelion extracts (used as functional foods and food additives) with increased antioxidant activity and improved flavonoid bioavailability [23]. The studies showed that the content of aglycones (formed because of enzymatic conversion of glycosidic forms of flavonoids) in the fermented extract was over 65% higher (i.e., 183.72 $\pm$ 2.24 mg/g) than in the case of unfermented dandelion extract (109.49 $\pm$ 1.05 mg/g), which in the case of FDE increased the antioxidant activity

estimated by the DPPH technique. The antioxidant activity values for FDE and NDE were IC_{50} = 0.075 and IC_{50} = 0.088 mg/mL, respectively [23].

In the literature, we have also described other methods of obtaining fermented plant extracts (FDEs) using seed coats (pomace), as well as from the extract, oil, and seeds of *S. marianum*, which are completely safe for health (i.e., non-cytotoxic to skin cells) and effective on the skin [15,22]. The studies included the assessment of antioxidant activity (AA) determined by the DPPH method, the content of phenolic compounds (TPC) determined by the Folin–Ciocalteu method, and lactic acid yield (LA_w) determined by the GC-MS method. In addition, the studies also demonstrated the anti-ageing potential of cosmetic preparations containing fermented extracts from defatted S. marianum seeds as cosmetic raw materials. The studies showed that FCRMs obtained by biotechnological methods in the fermentation process (lasting 14 or even 21 days) of appropriate plant raw materials using LAB strains (i.e., *L. reuteri* MI_0168, *L. salivarius* LY_0652, *L. brevis* LY_1120, *L. acidophilus* MI-0078, *L. rhamnosus* MI-0272, and *L. plantarum* MI-0102) and industrial waste products (i.e., vegetable molasses used as a raw material for LA production), due to their higher TPC content, exhibited increased antioxidant activity compared to their unfermented counterparts [40]. However, the presence of LA, which acts as a natural preservative (widely used in the food industry and, more recently, increasingly in the cosmetics industry) and is a NMF, can extend the shelf life of cosmetic formulas containing FDE as active ingredients (while preventing lipid oxidation in these cosmetic products), as well as increase skin hydration by reducing transepidermal water loss (TEWL) [16]. The observed differences in antioxidant activity and total phenolic content indicate a significant influence of the type of raw material subjected to fermentation on the antioxidant properties of the obtained bioferments. Fermented oil extract from *Silybum marianum* showed particularly high antioxidant activity, while the fermentation of non-defatted seeds resulted in increased LA yield, which may be related to the presence of natural sugars and lipids supporting the metabolism of LA bacteria. The presence of these components may have created a more favourable fermentation environment, supporting the growth of microorganisms and their enzymatic activity [71]. The appropriate selection of plant raw materials and their processing forms (e.g., defatting, oil extraction) in the design of bioferments with targeted biological properties is important in the context of cosmetic applications, where the antioxidant activity of bioferments is crucial for the functionality of a cosmetic product with antioxidant properties [72]. It is also worth noting that even in the case of defatted seeds, which had the lowest AA and TPC values, fermentation contributed to a significant improvement in these parameters compared to the unfermented extract. All analysed fermented extracts showed the presence of LA, which was not found in any of the unfermented extracts. The presence of this compound not only proves the effectiveness of the fermentation process but also increases the functional value of the extracts—both as a natural preservative and as an ingredient supporting skin hydration [20,40].

The log P values and contact angle are crucial for assessing the physicochemical properties of active ingredients in cosmetic formulations, especially in terms of skin permeability and accumulation in the skin. The wetting angle reflects the ability of a substance to spread on the skin surface [73]. A lower wetting angle indicates better wetting, which may facilitate contact between the active ingredient and the skin surface and promote its absorption. High wettability is particularly beneficial for hydrophilic ingredients, which can then act more effectively locally, e.g., as antioxidants that protect skin cells from oxidative stress caused by UV radiation, environmental pollution, or ageing processes [74]. In the context of bioferments with high antioxidant activity, the relationship between log P and the wetting angle allows us to predict their behaviour in cosmetic formulations and how they interact with the skin. Ingredients with a suitably selected lipophilicity and wettability profile can

be deliberately targeted at specific layers of the skin, enabling the design of products with targeted actions— surface, epidermal or deep [75]. The relationship between the lipophilicity (Figure 4) of FCRMs (exhibiting high antioxidant activity) and the wettability of a membrane substitute for human skin (Figure 5) is important in the context of using RCRMs as active ingredients in cosmetic formulations. The hydrophilic substances contained in FCRMs (due to their polarity and ability to form hydrogen bonds) show high affinity for the aqueous environment, which promotes their accumulation in epidermal layers such as the granular and spinous layers. The accumulation of these hydrophilic substances in the layers of the epidermis with a higher water content enables local antioxidant action, which is important in the context of protecting skin cells from oxidative stress caused by UV radiation, environmental pollution, or ageing processes [76,77].

Statistical analysis showed that for the parameters of total phenolic content (TPC) and antioxidant activity determined by the ORAC method (AA-ORAC), the F-statistic values were significantly higher than the critical F-value, and the *p*-values were significantly lower than the accepted significance level ($\alpha = 0.05$). Such high F values and very low *p*-values indicate statistically significant differences between fermented and unfermented samples, which confirms the impact of the co-fermentation process on the increase in antioxidant activity and the content of FCRMs' phenolic compounds. In the case of antioxidant activity assessed by the DPPH (AA-DPPH) method, the F statistic value was slightly lower than the critical value, and the *p*-value slightly exceeded the significance level. This means that the differences between the groups were not statistically significant, but the higher average AA-DPPH value in the fermented samples suggests a positive effect of fermentation. Based on the results of the ANOVA analysis, it can be assumed that dandelion leaf extracts subjected to co-fermentation are characterised by significantly higher antioxidant activity (AA-ORAC) and phenolic compound content (TPC). Additionally, the observed higher mean values for AA-DPPH in fermented samples support the hypothesis of a beneficial effect of fermentation on the antioxidant properties of extracts (Tables 5–10).

5. Conclusions

The aim of this study was to develop an innovative method of co-fermentation of dandelion leaves as an alternative to classic fermentation using single LAB strains. The research conducted confirmed the effectiveness of the proposed method, enabling the production of FCRMs with potential cosmetic applications. The proposed method of co-fermentation of dandelion leaves using two inoculants (*L. rhamnosus* MI-0272 and *S. cerevisiae*), with the use of beet and cane molasses (which are substrates for LA production), increased the fermentation rate several times, shortening the time needed to obtain the highest content of LA (a natural moisturising factor) and polyphenols (responsible for the antioxidant activity of FCRMs), which indicates the possibility of conducting fermentation to obtain FCRMs with skin moisturising properties.

The studies showed that the hydrophilic antioxidants contained in fermented dandelion leaf raw materials can accumulate in the granular and spinous layers of the epidermis. Their presence in these skin layers may result in local antioxidant activity, which is important in the context of protecting skin cells from oxidative stress, which plays a key role in skin ageing processes. The results of the study suggest the potential use of fermented dandelion extracts as active ingredients in cosmetic preparations with protective and anti-ageing properties.

The increased content of phenolic compounds (TPC) in FCRMs compared to their unfermented counterparts translates into a significantly higher ability to neutralise free radicals using the DPPH method. This confirms that the co-fermentation process intensifies the biotransformation of phenolic compounds, increasing the antioxidant activity of the

final fermented product. The statistical analysis confirmed significant differences between fermented and unfermented extracts, both in terms of TPC content and antioxidant activity determined by the ORAC method, which clearly indicates the beneficial effect of the co-fermentation process.

The presented research constitutes a preliminary analysis of the co-fermentation process of dandelion leaves, confirming its high potential as a source of active ingredients for cosmetic applications. Therefore, further research should focus on the complete qualitative and quantitative characterisation of phenolic compounds present in fermented extracts, as well as on the development and evaluation of cosmetic formulations containing FCRMs. It will be particularly important to conduct in vivo studies to assess the effectiveness of the preparations on the skin and confirm their protective and anti-ageing properties under application conditions.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/app15169021/s1, S1.1. Preparing fermented cosmetic raw materials (FCRMs) from dandelion leaves; S1.2. Obtaining fermented cosmetic raw materials (FCRMs) from dandelion roots, S1.3. Obtaining fermented cosmetic raw materials (FCRMs) from dandelion flowers; S1.4. Determination of LA by GC-MS; S1.5. Determination of total polyphenol content using the Folin–Ciocalteu method; S1.6. Determination of Antioxidant Activity: DPPH Assay; S1.7. Determination of Antioxidant Activity: ORAC Assay; S1.8. Lipophilicity assessment; Table S1. The results of the LA content (LA_c) of FCRMs; Table S2. The results of the acidity of the tested fermented cosmetic raw materials from dandelion leaves (FCRM-1:FCRM-6), dandelion roots (FCRM-7:FCRM-13), and dandelion flowers (FCRM-14:FCRM-20).

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