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Birch Bark Dry Extract by Supercritical Fluid Technology: Extract Characterisation and Use for Stabilisation of Semisolid Systems

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Abstract: Triterpene compounds like betulin, betulinic acid, erythrodiol, oleanolic acid and lupeol are known for many pharmacological effects. All these substances are found in the outer bark of birch. Apart from its pharmacological effects, birch bark extract can be used to stabilise semisolid systems. Normally, birch bark extract is produced for this purpose by extraction with organic solvents. Employing supercritical fluid technology, our aim was to develop a birch bark dry extract suitable for stabilisation of lipophilic gels with improved properties while avoiding the use of toxic solvents. With supercritical carbon dioxide, three different particle formation methods from supercritical solutions have been tested. First, particle deposition was performed from a supercritical solution in an expansion chamber. Second, the Rapid Expansion of Supercritical Solutions (RESS) method was used for particle generation. Third, a modified RESS-procedure, forming the particles directly into the thereby gelated liquid, was developed. All three methods gave yields from 1% to 5.8%, depending on the techniques employed. The triterpene composition of the three extracts was comparable: all three gave more stable oleogels compared to the use of an extract obtained by organic solvent extraction. Characterizing the rheological behaviour of these gels, a faster gelling effect was seen together with a lower concentration of the extract required for the gel formation with the supercritical fluid (SCF)-extracts. This confirms the superiority of the supercritical fluid produced extracts with regard to the oleogel forming properties.

Keywords: supercritical carbon dioxide; RESS; birch bark; triterpenes; gel formation; rheology; amplitude sweep

1. Introduction

For over two hundred years, birch bark and its pentacyclic triterpene constituents such as betulin (BE), lupeol (LU), betulinic acid (BA), oleanolic acid (OA) and erythrodiol (ER) have been a subject of research [1,2]. In 1788, betulin was the first of these triterpenoids to be isolated from the outer bark of birch by sublimation [3]. Since then, the physicochemical properties of these substances and the extraction from birch bark have been reinvestigated several times [1,2,4]. During the last fifteen years, several pharmacological effects have additionally been discovered for these triterpenoids (Table 1).

Table 1. Pharmacological effects of triterpenoids obtained from bark of birch. BE: betulin, BA: betulinic acid, OA: oleanolic acid, LU: Lupeol.

Pharmacological Effects of Triterpenoids Contained in Birch Bark	References
antibacterial, antiviral, anti-parasite	BE: [5,6]; BA: [7–9]; BE/BA: [10,11]; BE/BA/OA: [12]; LU/BA: [13]; OA/BA: [14]
hepatoprotective	BE: [15]; BE/BA: [16]; OA: [17–20]; LU: [21]; BA: [22]
antitumor	BE: [23,24]; BA: [25,26] BE/BA: [27,28]; LU: [29]; OA: [18,19,30]
wound healing	extract: [31–33]; BA: [34]; OA: [35,36]
anti-inflammatory	BE: [5,37]; LU: [38,39]; OA: [18,40]

Apart from these pharmacological effects, birch bark extract is able to act as a gelling agent and Pickering emulsifier [41–43]. Together with its anti-inflammatory and wound healing effects, this makes birch bark dry extract interesting for the stabilisation of semisolid systems, such as gels and creams, acting as an active ingredient and an excipient in parallel. A common method for obtaining birch bark dry extract or particular triterpenoids is the extraction of birch bark with organic solvents [41,44–47]. Some other production methods like Ultrasonic Assisted Extraction (UAE) [48] and sublimation [49] are described in the literature. Most of these methods require either high temperature or toxic organic solvents or even both. To gain a dry extract, the organic solvents have to be removed in an additional step. High process temperature causes thermal stress, which can influence extract quality. Extraction using supercritical fluids as extraction solvent could be an alternative [41,50–52]. Carbon dioxide (CO_2) is often used as a solvent for Supercritical Fluid Extraction (SFE) due to its low critical point (304.13 K/7.38 MPa) [53,54]. Different techniques can be used to obtain particles from the supercritical solution. It is possible to deposit the particles out of a liquid carbon dioxide phase after expanding the supercritical solution into a separator, or to spray the supercritical solution through a nozzle into ambient air in a vessel [55,56]. This Rapid Expansion of Supercritical Solutions (RESS) enables the production of very small particles [54,57,58]. To our knowledge, there are no reports focussing on the improvement of stabilisation of semisolid systems by birch bark extract particles produced with supercritical fluid extraction. The aim of our study is, therefore, to develop methods for the extraction of birch bark with supercritical carbon dioxide on a high-pressure lab-scale extraction unit employing different means of particle deposition. This includes the deposition of a particular extract directly into a lipophilic phase like jojoba oil to produce a gel in a single step process, combining both, extraction and gel formation. The extracts were characterized by analysing the leading compounds content, the surface area and the dispersive surface energy of the particles and the rheological properties of the gels obtained.

2. Materials and Methods

2.1. Reference Material

A dry extract obtained from Birken AG (Niefern-Oeschelbronn, Germany) was analysed for comparison reasons. This extract was prepared by organic solvent extraction employing n-heptane with a patented method [43].

2.2. Extraction

Initially, 50.0 to 200.0 g of chopped and dried birch bark (kindly provided by Birken AG, Niefern-Oeschelbronn, Germany) was placed in a high-pressure extraction unit with an extraction volume of 3.3 L (SITEC GmbH, Maur/Zurich, Switzerland). Supercritical carbon dioxide (Westfalen AG, Muenster, Germany) was conveyed for three hours via a high-pressure pump to the incubator to obtain a solution of the components in supercritical CO_2. Monitoring of the extraction parameters was done for mass flow and density with a mass flow meter (MassFlo 2100 di 1.5, Danfoss, Ebersbach, Germany) located between the high-pressure pump and the entrance of the extraction vessel, temperature (SITEC Thermocouple Pt100, SITEC GmbH, Maur/Zurich, Switzerland) and pressure (SITEC pressure

transducer, SITEC GmbH, Maur/Zurich, Switzerland). The extraction conditions were optimized in pilot experiments in the same setup, obtaining an extraction yield of about 5.8% ± 1.4%, and were found to be a pressure of 35.0 MPa and a temperature of 333.2 K (Supplementary Materials, Table S1). Three ways of separating the extract from the supercritical solution were tested:

- Supercritical fluid extract (SCF-E): The supercritical solution was expanded through an automatic valve (pneumatic) into a separator at 4.0 MPa. Under these conditions, the supercritical CO_2 separated into a liquid and a gaseous phase. In the liquid phase, the extract accumulated and when the pressure was completely released, particles deposed and could be removed as a dry extract (Figure 1a). In parallel, the CO_2 was recycled by the high-pressure pump and refed to the incubator. For this setup, the mass flow of CO_2 was between 12.5 and 15.0 kg/h, and the CO_2 density at 35 MPa and 333.2 K was 850.0 ± 2.0 kg/m^3.
- RESS-Extract (R-E): The supercritical solution here was expanded through a capillary nozzle (diameter: 150 µm) into a vessel where the precipitated particles could be collected (Figure 1b). To avoid the formation of ice near the nozzle due to a massive temperature drop caused by the expansion of the supercritical solution (Joule-Thompson effect), the CO_2 mass flow was reduced to 1.0–2.0 kg/h for this setup. Additionally, the vessel was heated so that the mean deposition temperature remained at 317.5 ± 3.5 K. In the expansion unit, the pressure was maintained at 4.4 ± 0.3 MPa. A manual micro valve controlled the CO_2 flow through the nozzle. The pressure in the incubation chamber was kept at 35 MPa and the temperature at 333.2 K, as in the SCF-E experiments. The CO_2 density was measured at 888.3 ± 7.2 kg/m^3, being slightly higher due to the reduced mass flow compared to the SCF-E conditions.
- A third extract was prepared by a modified RESS-procedure (MR). Here, the supercritical solution was expanded through the nozzle again, but directly into a lipophilic phase (jojoba oil) to form a gel in a single step production method, including particle deposition and gel formation (Figure 1c). For this method, the nozzle and the jojoba oil (20.0 g) were placed in a cylindrical plastic container (diameter: 6 cm; height: 11 cm) with a perforated lid. Due to the smaller expansion volume of this container, the setup required an improved heating to avoid the formation of ice during expansion. Therefore, the vessel was filled with water (about 328 K) and the container immersed into the heated water to enhance heat transmission. The conditions in the extraction unit were as with RESS.

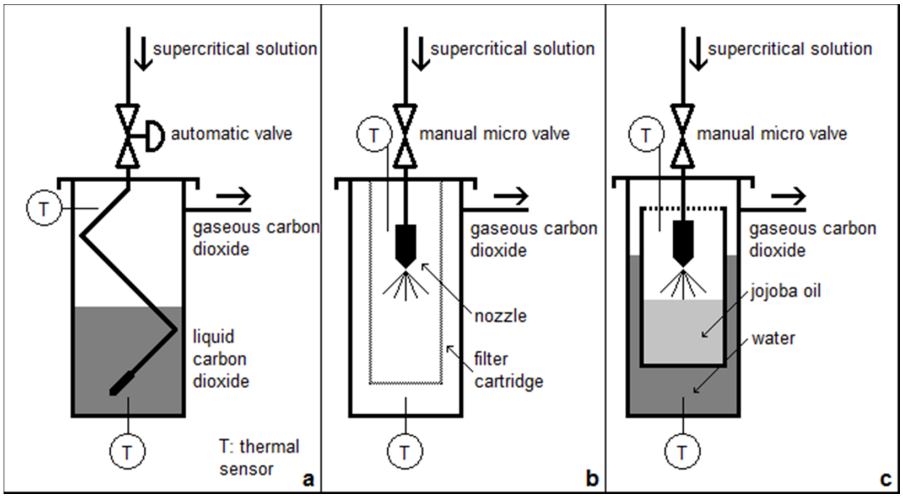

Figure 1. Different particle deposition methods from supercritical solutions. (**a**) Supercritical fluid extract by phase separation (SCF-E); (**b**) Rapid Expansion of Supercritical Solutions (RESS)-procedure (RESS); (**c**) modified RESS-procedure (MR).

2.3. HPLC Analysis

To quantify their triterpene composition, extracts were analysed by High Performance Liquid Chromatography (HPLC) (Shimadzu, Kyoto, Japan). Extracts and triterpene references were dissolved in isopropanol. Validation based on ICH Guideline Q2(R1) was performed. A sample volume of 10 µL was injected to a Nucleosil® C18 RP column (Macherey-Nagel, Dueren, Germany). For separation of five triterpenoids (BE, BA, OA, ER, LU), a gradient system was used as mobile phase (Table 2) and peaks were detected at 210 nm.

Table 2. Time program for High Performance Liquid Chromatography (HPLC) analysis of triterpenoids contained in birch bark.

Time (min)	Mode	Ratio Acetonitrile/Water	Flow Rate (mL/min)
0–13	isocratic	75/25	1.2
14–18	gradient	up to 90/10	1.2
19–35	isocratic	90/10	1.2
36–40	gradient	down to 75/25	1.2
41–45	isocratic	75/25	1.2

2.4. Gel Production

Birch bark dry extract and jojoba oil were dispersed with an Ultra-Turrax® (Ika Labortechnik, Staufen, Germany) for two minutes at 8000 rpm to obtain an oleogel. Gels produced directly by RESS-method had no further treatment.

2.5. Rheology

An amplitude sweep (AS) was performed at 296.15 K with a 25-mm plate-plate setup and a gap of 0.8 mm at a Physica MCR 501 rheometer (Anton Paar, Graz, Austria). For the AS, gels were sheared with a logarithmic ramp (oscillation; deformation 0.01–1000; frequency 1/s) after pre-shearing and equilibration time (300 s).

Evaluation of the experiments was performed by the parameters for gel strength, the mean storage modulus value in the linear visco-elastic region (LVE) was calculated. Another parameter to characterise the gel structure was the dissipation factor (tan δ) in the LVE. All data were generated from the original measurements by the use of Rheoplus/32 V3.40 (Anton Paar, Graz, Austria).

2.6. Surface Analysis

Specific surface area, according to Brunauer, Emmett and Teller (BET), was determined with a SA 3100 (Beckman Coulter, Brea, CA, USA) by nitrogen gas adsorption. Samples were degassed at ~313 K for 240 min under vacuum and then measured at a temperature of ~77 K in liquid nitrogen.

Dispersive surface energy measurements were made by inverse gas chromatography (iGC) using a SMS iGC (Surface Measurement Systems Ltd., London, UK). Special iGC columns of 3 mm in diameter were packed with ~0.120 g of sample for supercritical fluid extracts and 0.090 g for organic solvent extract. Different alkanes (hexane, heptane, octane, nonane and decane) were injected at infinite dilution (0.03 p/p°) and their peaks as well as the appropriate retention times were detected with a flame ionization detector (FID). Methane was used to determine the dead volume. IGC Advanced Analysis Macro V1.41 was applied for evaluation using Microsoft Excel 2010 and 2013 (Surface Measurement Systems Ltd., London, UK/Microsoft Corporation, Albuquerque, NM, USA).

3. Results

3.1. Birch Bark Extraction with Supercritical Carbon Dioxide

The RESS extraction method and the extraction method using a separator showed differences in the extraction yield. Performing the RESS method, the mass flow had to be limited to 1.53 ± 0.09 kg/h to avoid the formation of ice. This resulted in an extraction yield of 0.90% ± 0.09% (Figure 2), related to the initial amount of birch bark. To evaluate the dependence of the extraction yield at the selected working conditions (35 MPa and 333.2 K) from the mass flow, we reduced the mass flow in the production of the SCF-E to 5.09 ± 0.06 kg/h and compared the outcome to the normally used flow of 13.78 ± 0.46 kg/h (Figure 2).

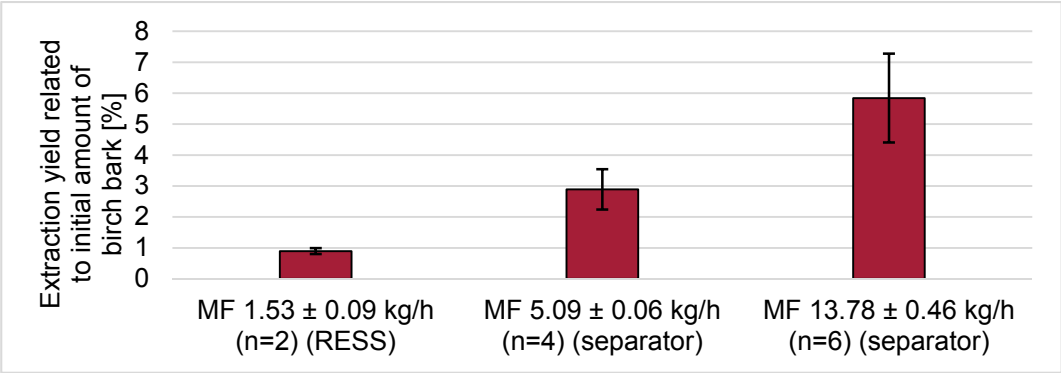

Figure 2. SCF extraction yield (birch bark extract) depending on carbon dioxide mass flow (MF) by different extraction and deposition methods (RESS/separator); *n*: number of independent productions, mean ± SD; mean ± span width for RESS.

The modified RESS process enabled the gel production by spraying the supercritical solution directly into jojoba oil; therefore, process yields with respect to the drug mass could not be determined.

3.2. Triterpene Composition of Birch Bark Extracts

Triterpene composition of birch bark powder is reported to be about 85% betulin, 5% betulinic acid, 3% oleanolic acid, 0.7% lupeol and 6.3% unidentified triterpens, detected by gas chromatography (all % mass) [43]. In our extracts the triterpens were detected by HPLC. Supercritical fluid extracts (n = 18) and RESS extracts (n = 3) were produced under standard extraction conditions and their triterpene content was compared to an organic solvent extract as reference (Figure 3). Extracts produced by supercritical fluid technology showed a difference in the content of betulin, lupeol, and the non identified substances (other). There were only minor differences for betulinic acid, oleanolic acid and erythrodiol. The betulin content in the SCF-E (63.08% ± 8.17%) and the RESS-E (24.36% ± 4.47%) was lower compared to the OS-E (81.60%). Lupeol and the other substances showed an opposing trend (Lupeol: SCF-E: 15.82% ± 4.64%; RESS-E: 32.11% ± 4.23%; OS-E: 2.08%). This indicates an obviously better solubility for lupeol in $scCO_2$ compared to betulin. A low CO_2 mass flow during the RESS extraction and therefore a longer equilibration time increased this effect.

To produce extracts with a certain triterpene composition, a single batch of birch bark material has been extracted ten times (fractional RESS extraction). These ten fractions (F1–F10) were analysed by HPLC to compare their content of betulin, lupeol and unidentified substances. The first RESS-fraction (F1) showed similar contents for betulin, lupeol and other substances. For the following fractions we saw an ongoing shift in composition to a higher betulin content and a lower lupeol content, probably due to different solubilities of the triterpenes in supercritical carbon dioxide. The other substances showed a behaviour similar to lupeol (Figure 4). With this fractional extraction, the production of extracts with an individual triterpene composition was possible.

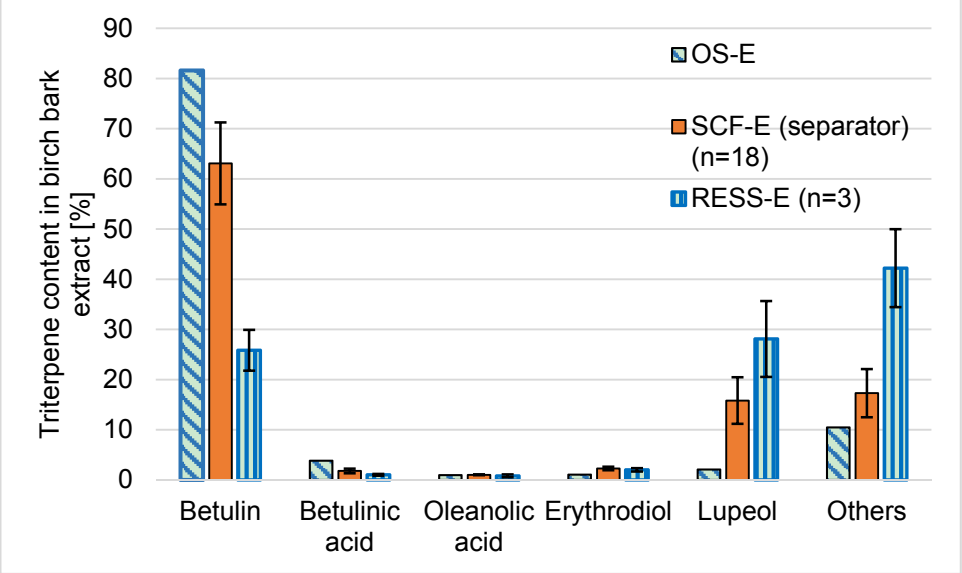

Figure 3. Triterpene composition of birch bark extract obtained by different extraction methods; mean ± SD.

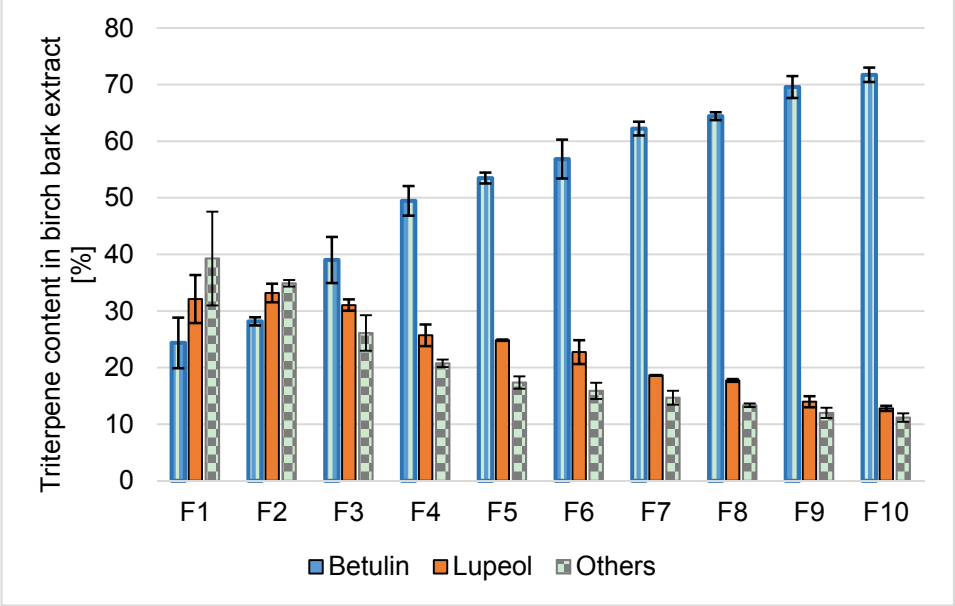

Figure 4. Triterpene composition of fractional RESS extracts; n = 2–3, mean ± SD or span width.

3.3. Rheological Characterisation of Gels Stabilised with Birch Bark Extracts

Jojoba oil stabilised with OS-E, SCF-E, R-E and by the modified RESS (MR) procedure extract showed gel characteristics (examples in Figure 5), as indicated by the storage modulus (G') being larger than the loss modulus (G") in the linear viscoelastic region of the flow curve.

A comparison between gels, stabilised by the different types of extract, confirmed a higher gel strength for those gels stabilised with extracts obtained by supercritical fluid technology (Figure 6) compared to gels formed with OS-E. The OS-E gel, SCF-E gel and RESS-E gel were stabilised with 6% of dry extract, whereas the extract content of the gels produced by the modified RESS-M process remains undefined. All three supercritical fluid-based particle deposition methods gave extracts that lead to gels with a gel strength of more than 8500 Pa. The OS-E was only able to form gels with a gel strength

of less than 1000 Pa (Figure 6). Different extract surface properties resulting from the fast particle deposition from the supercritical solution could be the reason for the ability to form stronger gels.

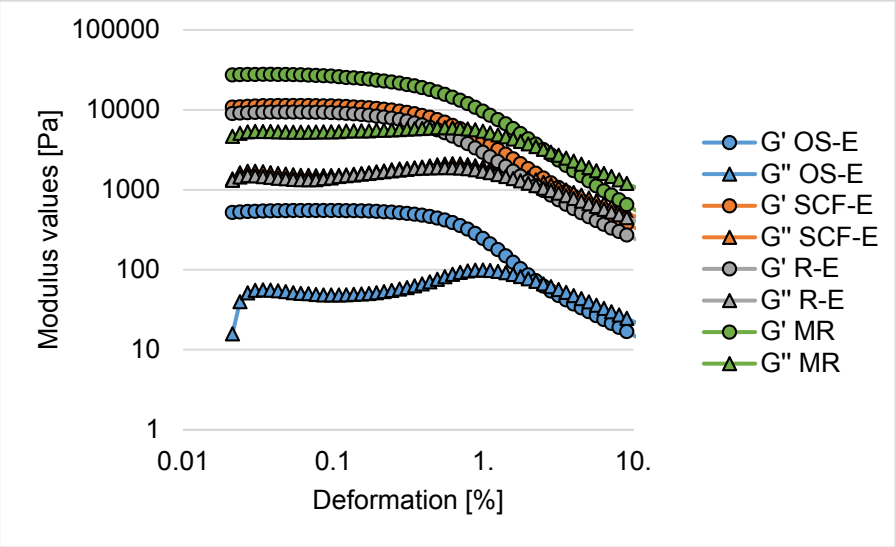

Figure 5. Amplitude sweep of gels stabilised by different types of birch bark extract.

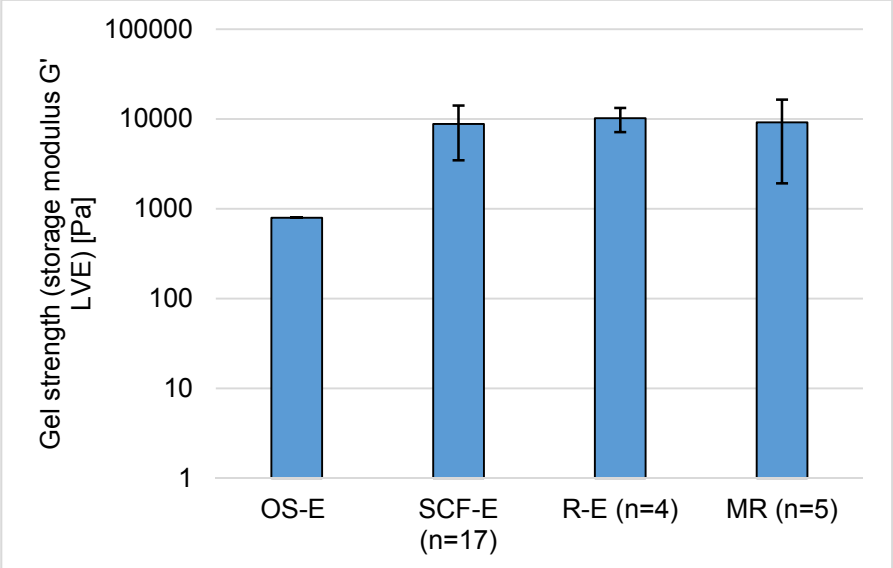

Figure 6. Gels stabilised by different types of birch bark extract (gel strength: amplitude sweep (AS) after 24 h); mean ± SD.

To investigate the gelling speed of the gels an amplitude sweep was performed every 30 min, starting immediately after the gel production. For the SCF-E gel, the G' values started and remained at a higher level compared to the OS-E gel. As a reference for almost complete gelling, an amplitude sweep after 24 h was performed. The SCF-E gel, however, reached this reference point already within six hours, whereas the OS-E gel only reached 28% of the reference value (Figure 7). This experiment could not be performed with the two RESS-extracts due to the design of the RESS-system.

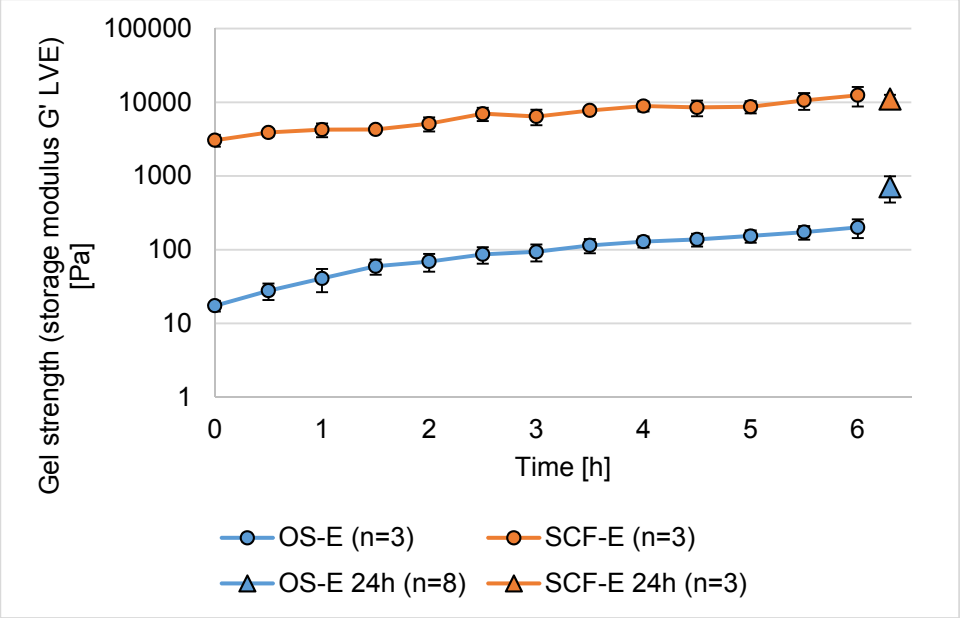

Figure 7. Gel strength (amplitude sweep) during the first six hours of gel formation for organic solvent and supercritical fluid extract gel (six percent extract in oil); mean ± SD.

For a gel, a dissipation factor less than one is typical. Studying the dissipation factor during the first six hours of gel formation (AS; LVE) confirmed differences between a SCF-E gel and an OS-E gel. Directly after gel production, the SCF-E gel showed a dissipation factor of 0.12 ± 0.02, which slightly increased during six hours. The OS-E gel started at 0.38 ± 0.04, the value decreased over time (Figure 8). The SCF-E particles obviously were able to form a stable particulate network direct after homogenising and therefore faster, compared to the OS-E particles. Again, this might hint at differences in extract particle size and surface properties between the different types of extract, which enables a faster formation of a network structure in the oil. After three hours, both types of gels were on the same dissipation level.

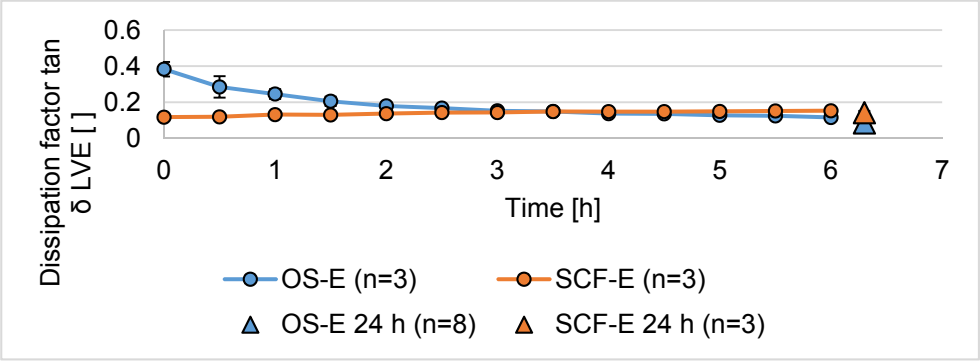

Figure 8. Dissipation factor (amplitude sweep) during the first six hours of gel formation for organic solvent and supercritical fluid extract gel (six percent extract in oil); mean ± SD.

To study the minimum extract content required for gel formation, gels with different amounts of birch bark extract were produced. A decrease of extract content caused a decrease in gel strength, especially for the OS-E gel. With two percent OS-E it was hardly possible to produce a gel, and gel strength dropped under 3 Pa, whereas one percent SCF-E was enough to obtain a gel with over 250 Pa (Figure 9).

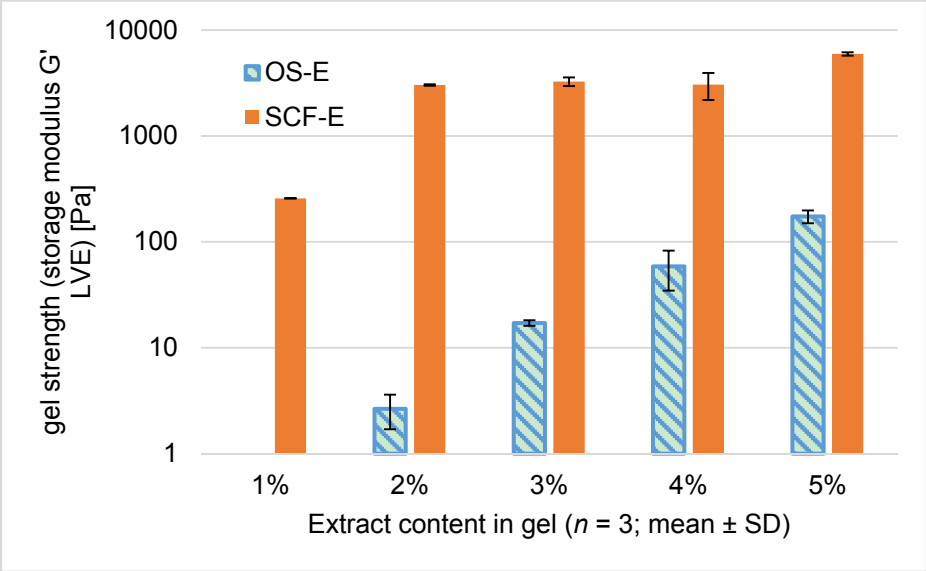

Figure 9. Gel strength (amplitude sweep) as a function of extract content in gels, stabilised by organic solvent and supercritical fluid extract; $n = 3$, mean ± SD.

The dissipation factor for the SCF-E gel remained constant over an extract content range from one to five percent. For the OS-E gel a smaller amount of extract had a negative influence on the gel character. Less than three percent of OS-E lead to a gel with a dissipation factor close to one (Figure 10). Below this concentration, the viscous properties dominate over the elastic properties and we have no gel any more. The minimum extract concentration, necessary for gelling, was lower for the SCF-E gel compared to the OS-E gel. This indicates a more effective gel stabilisation by supercritical fluid extracts.

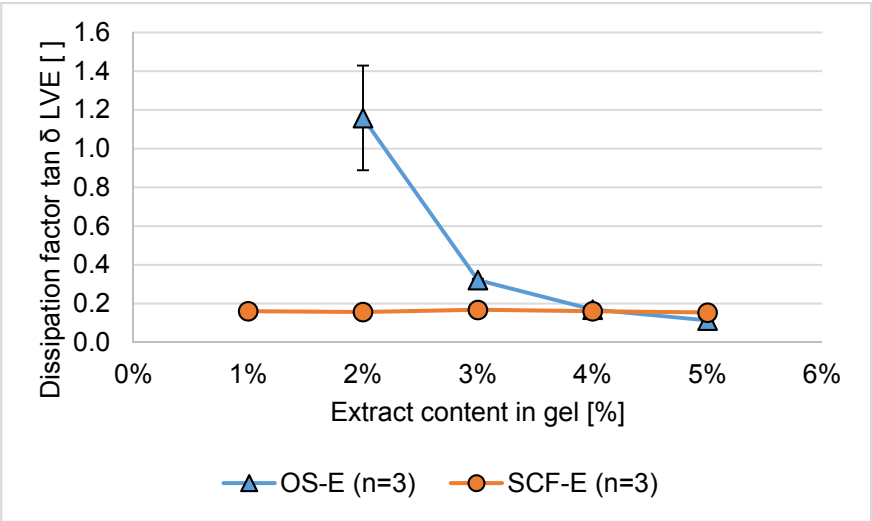

Figure 10. Dissipation factor (amplitude sweep) as a function of extract content in gels, stabilised by organic solvent and supercritical fluid extract; mean ± SD.

3.4. Surface Analysis of Birch Bark Extracts

Extract surface properties could play a crucial role in the stabilization of the semisolid systems. This becomes obvious when comparing pairs of extracts (Table 3) with a similar specific surface area (OS-E and SCF-E1; SCF-E2 and SCF-E3) which give gels with different strength. The supercritical fluid extracts showed specific surface areas from 15 m^2/g to 75 m^2/g, making a pure correlation between

surface area and gel strength unlikely. Surface energy, however, could be another crucial parameter for gel formation. Four extracts were, therefore, analysed by inverse gas chromatography (iGC) for their dispersive surface energy (Table 3). It is striking that all supercritical fluid extracts showed a similar dispersive surface energy which is different from the organic solvent extract. This is a hint for a different crystallisation behaviour out of supercritical solutions, compared to precipitation out of organic solvents. The observed difference in the chemical composition, however, might influence this finding too.

Table 3. Surface characterisation of birch bark extract obtained by different extraction methods.

Type of Extract	Specific Surface Area (BET) (m^2/g)	Dispersive Surface Energy (mJ/m^2)	Gel Strength (Pa)
OS-E	29.6 ± 0.3	62.42 ± 0.33	716 ± 278
SCF-E1	27.9 ± 1.3	51.59 ± 0.47	4486 ± 888
SCF-E2	37.3 ± 0.1	51.67 ± 0.59	2211 ± 245
SCF-E3	36.2 ± 2.0	51.27 ± 0.65	11,081 ± 1540

4. Discussion

Birch bark extraction with supercritical carbon dioxide, as an alternative to conventional organic solvent extraction, turned out to be a promising method. Employing supercritical fluid extraction and subsequent separation of the extract in a separation vessel provided acceptable extraction yields, being comparable to other SCF-methods [51]. RESS-extraction by spraying the supercritical solution through a nozzle was successful, but showed a poor extraction yield due to the limited carbon dioxide mass flow. This low yield might, however, also be affected by the entrainment of submicron particles by the CO_2 stream. A direct production of a gel by spraying the supercritical solution through a nozzle directly into jojoba oil (modified RESS procedure) would here be preferential and turned out to be possible, giving stable gels. The control of this process has, however, to be improved by adaption of the pilot plant with regard to the risk of ice formation during expansion.

Repetitive extraction of the same material with supercritical carbon dioxide provided extracts with a different triterpene composition. Early fractions contained less betulin and more lupeol, compared to an organic solvent extract. The betulin content increased with further extraction steps whereas the lupeol content and the content of unidentified substances decreased. By this method, the supercritical fluid extraction allows the production of extracts with different triterpene compositions. This might give extracts with different pharmacological and stabilizing properties. The more important finding is, however, that obviously lupeol and the unidentified compounds dissolve faster in supercritical carbon dioxide than betulin, giving an extract composition which is, at least in part, different from the organic solvent extract.

Extracts from supercritical fluid extraction showed better and faster gelling abilities, compared to the organic solvent extract. Gels challenged by an amplitude sweep for rheological characterization showed much higher modulus values when the stabilisation was performed with supercritical fluid extracts from all tested particle deposition methods compared to the gel stabilised by the organic solvent extract. For these gels, the gel character, indicated by a dissipation factor less than one, was detected instantly after the gel production, whereas the gel stabilised by organic solvent extract needed several hours to reach the same level. The minimum extract content, necessary for gelling, was lower for the supercritical fluid extracts. This indicates a more effective formation of the network structure in these gels.

The supercritical fluid extracts differ from the organic solvent extract in several ways. First of all, the specific area of the particles is higher, compared to the organic solvent extract. This could be a reason for a better particle to particle interaction and an improved interaction of the liquid phase (jojoba oil) with the particle surface and thereby the formation of the gel network. The dispersive surface energy, however, was less when compared to the organic solvent extract. That might be due to

the micromorphology of the supercritical carbon dioxide-treated particles, having an obviously more polar surface structure. This would improve the contact between the SCF extract particles and the jojoba oil. Grysko and Daniels [59] have shown that the gel formation by birch bark extracts depends on polar interactions between the oil and the gel forming agent, supporting this view. The lack of a concentration related gel strength for the SCF-extracts might, however, be due to the fact that even at the lowest concentration the gel strength is already almost maximal. This confirms the usability of the supercritical carbon dioxide for the formation of birch bark extracts with superior gel forming properties compared to the organic solvent extract.

5. Conclusions

Birch bark dry extract is an interesting product, unique insofar as it is usable both as an active ingredient to improve wound healing and as an oleogel stabilizing agent. So far, the extract is produced by organic solvent extraction with all the well-known disadvantages. The production of the extract with a more environment-friendly method could be beneficial. Supercritical CO_2 is widely accepted to replace solvents. Producing dry extracts from birch bark by either supercritical fluid extraction or the RESS-technique leads to extracts which show gel-forming properties superior to the organic solvent extract. This is true with respect to the gel formation speed as for the strength of the gels obtained. In addition, a one-step, extraction, particle generating and gel forming technique, such as the modified RESS process (MR), might even be of further advantage for the production technology of birch bark oleogels.

Supplementary Materials: Supplementary materials are available online at http://www.mdpi.com/2076-3417/7/3/292/s1.

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

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